Contents:

This page contains links to Virology teaching material for University of Cape Town Molecular & Cell Biology Department courses MCB2016F (2nd year BSc) and MCB3024S (3rd year). They are free for anyone to use, the only stipulation being acknowledgement by lecturers / teachers of the source of the material.

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Note:

This tutorial draws heavily on print published as well as electronically published work, as well as on things I and others have written that have not been published.

This was originally written as a supplement to my **Introduction to Virology** 7-lecture course to second-year Microbiology major students (MIC202S) in the **Microbiology Department** at the **University of Cape Town.** It now constitutes the basis of a 7-lecture course in introductory microbiology (**MCB2016F**) and a 20-lecture course (**MCB3024S, Defence and Disease**) given to 3rd year students.

I hope you find it useful.

However, please do NOT download and use material in any other form than it is presented here, without permission.

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This Version: 16th May, 2008

What is a Virus?

DEFINITIONS

VIRIONS

OTHER VIRUS-LIKE AGENTS

Viruses

Viruses may be **defined** as **acellular organisms** whose **genomes** consist of **nucleic acid**, and which obligately **replicate inside host cells** using **host metabolic machinery** and **ribosomes** to form a **pool of components** which assemble into particles called **VIRIONS**, which serve to **protect the genome** and to **transfer it to other cells**.

They are distinct from other so-called **VIRUS-LIKE AGENTS** such as **VIROIDS and PLASMIDS and PRIONS**

Alternative definitions:

S Luria *et al*. AJ Cann

And EP Rybicki, ©2008:

A virus is an infectious acellular entity composed of compatible genomic components derived from a pool of genetic elements.

The concept of a virus as an organism challenges the way we define life:

- viruses do not **respire**,
- nor do they display **irritability**;
- they do not **move**
- and nor do they **grow**,
- however, they do most certainly **reproduce**, and may adapt to new hosts.

By older, more **zoologically and botanically** biased criteria, then, **viruses are not living**. However, this sort of argument results from a **"top down"** sort of definition, which has been modified over years to take account of **smaller and smaller things** (with fewer and fewer legs, or leaves), until it has met the ultimate **"molechisms" or "organules"** - that is to say, **viruses** - and has proved inadequate.

If one **defines life** **from the bottom up** - that is, from the **simplest forms capable of displaying the most essential attributes of a living thing** - one very quickly realises that the only real criterion for life is:

The ability to replicate

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and that only systems that contain nucleic acids - in the natural world, at least - are capable of this phenomenon. This sort of reasoning has led to a new definition of organisms:

"An organism is the unit element of a continuous lineage with an individual evolutionary history."

The key words here are **UNIT ELEMENT**, and **INDIVIDUAL**: the thing that you see, now, as an organism is merely the **current slice in a continuous lineage**; the individual evolutionary history denotes the **independence of the organism over time**. Thus, **mitochondria and chloroplasts and nuclei and chromosomes** are not organisms, in that together they constitute a continuous lineage, but separately have no possibility of survival, despite their independence before they entered **initially symbiotic**, and then **dependent** associations.

The concept of replication is contained within the concepts of individual viruses constituting continuous lineages, and having an evolutionary history.

Thus, given this sort of lateral thinking, **viruses become quite respectable as organisms:**

- they most definitely **replicate**,
- their **evolution** can (within limits) be **traced quite effectively**, and
- they are **independent in terms of not being limited to a single organism as host**, or even necessarily to a single species, genus or phylum of host.

More views on viruses and their definition:

It's Life, Jim, but not as we know it...

Posted by **ajcann** on July 24, 2007

Todays post is a welcome return of guest blogger:

Ed Rybicki, Department of Molecular and Cell Biology, University of Cape Town, South Africa.

It's Life, Jim, but not as we know it...

Which could well apply to viruses, my very own favourite organisms - after all, **they don't respire, grow, excrete** or any of those other good things that classical organisms supposedly do - but that's not the point of this piece. Rather, I'd like to speculate on an interesting convergence of articles I've seen recently, on (1) extrasolar planets, (2) water on same, and (3) the possibility of novel lifeforms. Some of this is an accident of the irregular timing of receipt of my New Scientist and Nature subscriptions,

It's Life, Jim, but not as we know it… « MicrobiologyBytes via kwout

A feeling for the molechism*

Posted by **ajcann** on June 26, 2007

Todays post is a welcome return of guest blogger:

Ed Rybicki, Department of Molecular and Cell Biology, University of Cape Town, South Africa.

I think it's permissible, after working on your favourite virus for over 20 years, to develop some sort of feeling for it: you know, the kind of insight that isn't directly backed up by experiment, but that may very well be right. Or not - but in either case, it would take a deal of time and a fair bit of cash to prove or disprove, and would have sparked some useful discussion in the meantime. And then, of course, the insights you have into (insert favourite virus name here) - if correct - can usually be extended into the more general case, and if you are sufficiently distinguished, people may actually take them on board, and you will have contributed to Accepted Wisdom.

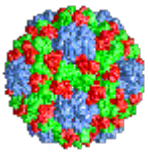
I can't pretend - at least, outside of my office - to any such Barbara McClintock-like distinction; however, I have done a fair bit of musing on my little sphere of interest as it relates (or not) to the State of the Viral Universe, and I will share some of these rambles now with whoever is interested.

A feeling for the molechism* « MicrobiologyBytes via kwout

DEFINITIONS

VIRIONS

OTHER VIRUS-LIKE AGENTS



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Introduction to Molecular Virology

Origins of Viruses

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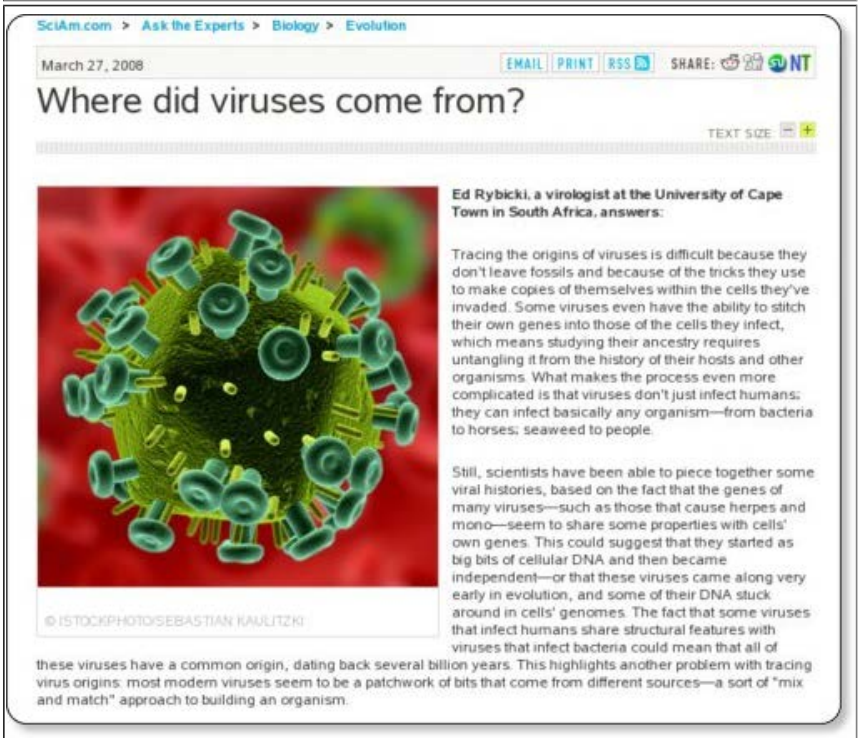
Virus Origins Lite: see this article in Scientific American	Virus Origins Serious: see this article in ViroBlogy
	
Where did viruses come from?: Scientific American via kwout	ViroBlogy via kwout

SciAm.com > Ask the Experts > Biology > Evolution

March 27, 2008 EMAIL PRINT RSS SHARE:

Where did viruses come from?

TEXT SIZE

Ed Rybicki, a virologist at the University of Cape Town in South Africa, answers:

Tracing the origins of viruses is difficult because they don't leave fossils and because of the tricks they use to make copies of themselves within the cells they've invaded. Some viruses even have the ability to stitch their own genes into those of the cells they infect, which means studying their ancestry requires untangling it from the history of their hosts and other organisms. What makes the process even more complicated is that viruses don't just infect humans; they can infect basically any organism—from bacteria to horses; seaweed to people.

Still, scientists have been able to piece together some viral histories, based on the fact that the genes of many viruses—such as those that cause herpes and mono—seem to share some properties with cells' own genes. This could suggest that they started as big bits of cellular DNA and then became independent—or that these viruses came along very early in evolution, and some of their DNA stuck around in cells' genomes. The fact that some viruses that infect humans share structural features with viruses that infect bacteria could mean that all of these viruses have a common origin, dating back several billion years. This highlights another problem with tracing virus origins: most modern viruses seem to be a patchwork of bits that come from different sources—a sort of "mix and match" approach to building an organism.

© ISTOCKPHOTO/SEBASTIAN KAULITZKI

ViroBlogy

Up-to-date Virology posts for students at UCT

« Who do you bind to, my lovely? So there is light at the end of the tunnel »

From what did viruses evolve or how did they initially arise?

This was originally written as an Answer to a Question posted to Scientific American Online; however, as what they want to publish bears no resemblance to what I wrote, I shall post the original here.

The answer to this question is not simple, because, while viruses all share the characteristics of being obligate intracellular parasites which use host cell machinery to make their components which then self-assemble to make particles which contain their genomes, they most definitely do not have a single origin, and indeed their origins may be spread out over a considerable period of geological and evolutionary time. Viruses infect all types of cellular organisms, from Bacteria through Archaea to Eukarya; from *E. coli* to mushrooms; from amoebae to human beings – and virus particles may even be the single most abundant and varied organisms on the planet, given their abundance in all the waters of all the seas of planet Earth. Given this diversity and abundance, and the propensity of viruses to swap and share successful modules between very different lineages and to pick up bits of genome from their hosts, it is very difficult to speculate sensibly on their deep origins – but I shall outline some of the probable evolutionary scenarios.

Virus Origins

The **probably multiple origins of viruses** are lost in a sea of conjecture and speculation, which results mostly from their nature: **no-one has ever detected a fossil virus as a particle**; they are too small and probably too fragile to have withstood the kinds of processes that led to fossilisation, or even to preservation of short stretches of nucleic acid sequences in leaf tissues or insects in amber.

As a result, we are **limited to studying viruses that are isolated in the present**, or from material that is **at most a few decades old**. The new science (or art) of virus molecular systematics is, however, shedding a great deal of light on the distant relationships of, and in some cases on the **presumed origins of**, many important groups of viruses. This is as a result of the **sequencing** of all or part of the genomes of representatives of many of the known varieties of viruses, including the **largest (pox- and herpesviruses)** and the **smallest (gemini- and other ssDNA viruses)**. If viral genomes are compared with each other and with cellular sequences, **presumed patterns of evolution / divergence** of the genomes can be reconstructed.

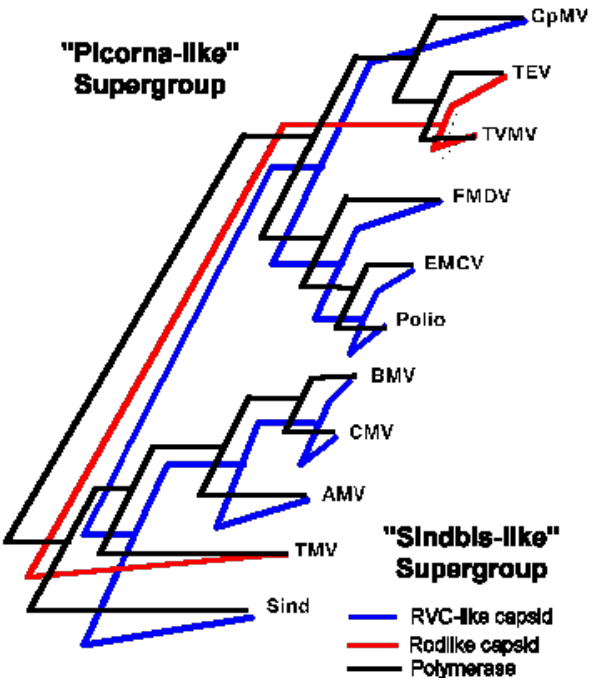
Geminiviruses, for example, are a diverse group of viruses - with **different genera** having **different numbers of genes and genome components** - that **presumably have a common origin** - and one that may be **traceable back to beyond 200 Myr BP**, if one takes into account **geographical diversity**, and **genetic divergence of vectors** and of **plant hosts** (see Rybicki, 1994). Divergence of these viruses is illustrated here.

Potyviruses are also a **putatively ancient family of viruses**, with genera that have **different numbers of genome components** and whose **gene pool is not shared by all members**: a representative **coat protein tree** is shown **here**. **Bymoviruses** have **two genome components** and are **fungus-transmitted**; all the rest have a **single genome component**. **Potyviruses** are **aphid-transmitted; rymo-** and **triticiviruses** are **mite-transmitted**, and **ipomoviruses** are **whitefly-transmitted**. They are supposed to descend from a **fungal virus**, probably over 200 Myr ago (AJ Gibbs, pers. comm.).

If one were to go far back into evolutionary time, a case could be made for **descent from a single ancestor** of at least the **replicase-associated functions of all viruses with positive-sense and negative-sense single-strand RNA genomes**; likewise, **large DNA viruses like pox- and herpesviruses** and **phycodnaviruses** could be presumed to have **"degenerated"** (if one believes viruses to be degenerate organisms, which I for one do not...) **from cellular organisms**, given that their enzymes share more sequence similarity with sequences from cells than with other viruses or anything else.

Retroviruses, pararetroviruses, retrotransposons and retroposons all probably share a **common origin of the reverse transcription function**, which in turn may be a **living relic** of the enzyme that enabled the switch from a presumably **RNA-based genetics to DNA-based heredity**.

The main message to come from **"deep" phylogenetic studies** is that **early virus evolution was almost certainly modular**: that is, that certain **"core modules"** that proved to be successful - like the **retrovirus pol gene**, and the **picornavirus-like protease-Vpg-polymerase module** - appear in a number of **different contexts**. Thus, certain animal viruses - like **picornaviruses and alphaviruses** - have relatives among **plant viruses** which do not necessarily share the **same morphology**, number of **genome components**, or even **genome organisation or number of gene**s.



For example, small spherical **picornaviruses** (ssRNA, 1 component, infect animals) are related to **comoviruses** (small spherical, 2 component, plant) and ***Potyviridae*** (filamentous, 1 or two genome components, plant), as part of a **"Picorna-like Supergroup"**. Similarly, **Sindbis** (ssRNA, enveloped spherical, single RNA, animals) virus is related to **Bromoviridae** (naked spherical, 3 component, plant) and **Tobamoviruses** (naked rod, 1 component, plant), in a **"Sindbis (or Alpha-) virus-like Supergroup"**. This is covered well here. A diagram illustrating **one way of looking at this phenomenon** is shown here: it shows **overlaid phylogenetic relationships** of different components of some well-known viruses, illustrating how **different components of a group of viruses may have different "gene trees"**. In this case, it shows how **individual viruses in two different "supergroups" of viruses** - defined in terms of **polymerase** affinities - have **different gene trees** when **capsid proteins** and **polymerase relationships** are taken into account. Thus, for ss(+)RNA viruses at least, going back beyond a certain level results in a **severe blurring** of perceived relationships, as well-defined families of viruses appear to share **essential core components** with other well-defined families / orders (see also here), while having nothing else in common.

It is very quickly apparent from sequence studies that there can have been **no single origin of viruses** as organisms. For instance, there is **no obvious way** one can relate viruses of the size and complexity of the ***Poxviridae*** [double-stranded linear DNA,130-375 kb, 150-300 genes] with viruses like the **tobamoviruses** [ss linear RNA, 6-7 kb, 4 genes], or either of these with the ***Geminiviridae*** [ss circular DNA, 2.7 - 5.4 kb, 3-7 genes]. Thus, there can be **no simple "family tree" for viruses**; rather, their evolutionary descent must resemble a number of scattered "**bushes**". Viruses as a class of organism must be therefore be considered to be **polyphyletic** in origins: that is, having a number of **independent origins**, almost certainly at **different times**, usually from **cellular organisms**.

What they have in common is a role as the ultimate **"stripped-down" parasites**:

organisms which can **only undergo a life cycle inside the cells of a host organism**, using at the very least the **metabolic enzymes and pathways and ribosomes** of that host to produce **virion components which get assembled into infectious particles**.

Virus Origins

Virus Evolution

Virus Evolution

One major assumption has to be made, if one is ever to make any sense of the sorts of relationships emerging from molecular phylogenetic studies. This is:

THAT VIRUSES CO-EVOLVE WITH THEIR HOSTS, LIKE ANY GOOD PARASITE.

Fortunately, there appears to be quite a lot of justification for it, especially from studies of viruses such as **papillomaviruses**, **endogenous retrovirus-like sequences** in animal genomes, and **herpesviruses**. For example, the divergence of **primates** and of **birds related to chickens** have been traced by comparing the **types and sequences of retroviral-derived sequences** in their genomes. It has also been repeatedly shown that the **closest relatives of human papillomavirus types** infecting **particular tissue types** (eg: cutaneous wart types, genital mucosal types) are **those viruses infecting similar tissue types in other primates**, indicating that these **tissue preferences were well established before the divergence of humanoid apes from the primate line**.

It is quite useful here to consider the **timeline of evolution of life** from its **beginnings in water,** as well as the **timeline of colonisation of dry land by organisms**, seeing as our **knowledge of viruses** is limited very largely to **ones infecting terrestrial organisms**. This process went much as follows (see also here):

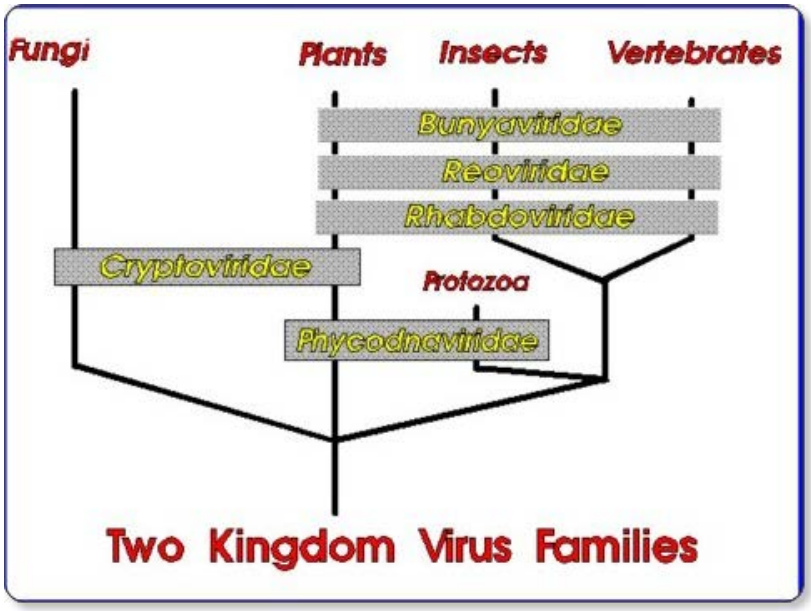
- **4000 Myr**: life originated in the sea / shallow ponds
- **3500 Myr:** bacteria emerged from the seas to colonise the land
- **1000 Myr**: plants / fungi slowly colonised inland of coastal margins
- **700 Myr**: insects / arthropods crawled out to feed on plants
- **350 Myr**: vertebrates adapted to air breathing / survival on land

Viruses of nearly all the **major classes of organisms - animals, plants, fungi and bacteria / archaea** - probably **evolved with their hosts in the seas**, given that **most of the evolution of life on this planet has occurred there**. This means that **viruses also probably emerged from the waters** with their different hosts, during the **successive waves of colonisation** of the terrestrial environment. Thus,

- viruses of **terrestrial bacteria** probably **derive from those of the original colonisers**;
- most **viruses of land plants** are probably evolved from **those in the green algae** that emerged +/- 1000 Myr ago;
- most viruses of **terrestrial arthropods** derive from the **first founders**;
- most viruses of **terrestrial vertebrates** derive from those that came ashore with the **first air-breathers**.

This would explain why **viruses in the different broad types of hosts are generally so different**: they have had **aeons to adapt to each "life niche**" since **divergence from the respective common ancestor**. Thus, **bacteria / archaea and eukaryote**s share no virus types, as they have been **diverged so long**. However, there are some **marked similarities** between **viruses infecting plants and those infecting vertebrates** (see here), and even more between **those infecting arthropods and those in vertebrates**. A useful reference point for these sorts of comparisons is the Tree of Life Home Page; in particular their 16S ribosomal RNA gene tree, or alternatively the simpler tree here. Thus, as it can be seen that as **Metazoa (all animals)** are far more similar to one another than they are to **Viridaeplantae (all green plants)**, it is reasonable to assume that **their viruses would be more similar to one another** as well. It is also instructive to note that the Tree of Life includes no virus trees / bushes yet...!

A complicating factor in the picture of viruses co-evolving with their hosts over millennia is the fact that **viruses apparently can - and obviously do - make big jumps in hosts** every now and then. It seems obvious, for example, that **arthropods** are almost certainly the **original source** for a number of **virus families infecting insects and mammals** - such as the ***Flaviviridae*** - and probably also of **viruses infecting insects and other animals and plants** - such as the ***Rhabdoviridae* and *Reoviridae*** - as well (see also here). For example, picornaviruses of mammals are **very similar structurally and genetically** to a **large number of small RNA viruses of insects** and to at least **two plant viruses**, and - as the insect viruses are **more diverse** than the mammalian viruses - **probably had their origin in some insect that adapted to feed on mammals (or plants) at some distant point in evolutionary time**.



2king.gif (GIF Image, 640x480 pixels) via kwout

Clues as to how this happens are given by the fact that **flock house virus** - a small naked isometric **ssRNA virus of insects** - can **replicate in plants** when introduced into leaves, but only at the site of infection. This gives it a **survival advantage** over other similar viruses which cannot replicate in plants, as these are now no longer a **passive, but an active reservoir** for the **re-infection of insects**. For example, **leafhopper A virus** and ***Rhopalosiphum padi* [aphid] virus** are both **stable enough** and are injected into plants at **sufficient concentration** by their hosts, to make the plants into **"circulative non-propagative" vectors** of the viruses. The addition of a **movement function** - a **"module" peculiar to plant viruse**s - would make **flock house virus** into a **fully-fledged plant virus** as well; loss of ability to infect insects would then **limit it to plants.**

It is obvious that a **number of such cross-overs** have occurred: there are **reoviruses** which only infect plants; **flaviviruses** which only infect humans; **picornaviruses** which only infect animals or plants, and so on. An interesting **"emerging virus" is tomato spotted wilt virus** - genus **Tospovirus**, family ***Bunyaviridae*** - which is postulated to have **relatively recently acquired the ability to infect plants** as well as its **thrips vector insect**, as well as obtaining a **movement** function, and is now a **major pathogen of a wide range of plant species**.

Another corollary of considering cellular **organismal evolution** as a marker of **virus evolution** is that the **terrestrial virus gene pool** must constitute **only a subset of the virus lineages currently present in the oceans**: if all of the **major lines of terrestrial organisms** are descended from **subsets of parallel-developing lineages in the oceans**, then the **terrestrial ancestral gene pool is smaller than the oceanic**. Assuming **virus diversity parallels host diversity**, therefore the **ancestral terrestrial virus gene pools are also smaller than their oceanic equivalents**.

Recent findings that an **astonishing diversity** (as well as quantity) of viruses **exists in seawater** may support this conclusion: it has been proposed that **viruses may well be one of the prime limiting factors to microbial and planktonic growth in seawater**, given each millilitre contains so many viral particles. The finding that only about **10% of bacteria found in seawater** (as represented by clonal populations of PCR-generated 16S ribosomal RNA gene fragments) actually **resemble any known taxon of bacteria**, also bolsters the view that we may only have **uncovered a small proportion of the viruses on this planet.** If one considers that **green plants** represent only a **small proportion of the eukaryotic algal gene pool**, and that we know of **hundreds of terrestrial plant viruses**, whereas there is only one **properly characterised** group of viruses from algae (***Phycodnaviridae***), then the potential diversity still to be uncovered must be huge.

See below for further discussion on this topic.

« Hepatitis B and C viruses Todays Video: Deadly »

Marine viruses — major players in the global ecosystem

Posted by ajcann on September 21, 2007

Viruses are by far the most abundant 'lifeforms' in the oceans and are the reservoir of most of the genetic diversity in the sea. The estimated 10^30 viruses in the ocean, if stretched end to end, would span farther than the nearest 60 galaxies. Every second, approximately 10^23 viral infections occur in the ocean. These infections are a major source of mortality, and cause disease in a range of organisms, from shrimp to whales. As a result, viruses influence the composition of marine communities and are a major force behind biogeochemical cycles. Each infection has the potential to introduce new genetic information into an organism or progeny virus, thereby driving the evolution of both host and viral assemblages. Probing this vast reservoir of genetic and biological diversity continues to yield exciting discoveries.

Marine viruses - major players in the global ecosystem
Nature Reviews Microbiology 2007 5, 801-812

This entry was posted on September 21, 2007 at 7:12 am and is filed under Biology, Environment, Microbiology, Science, Virology. You can follow any responses to this entry through the RSS 2.0 feed. You can leave a response, or trackback from your own site.

Marine viruses — major players in the global ecosystem « MicrobiologyBytes via kwout

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Virus Origins

Virus Evolution

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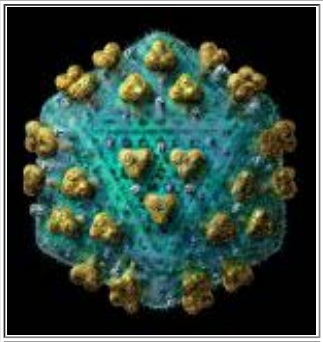
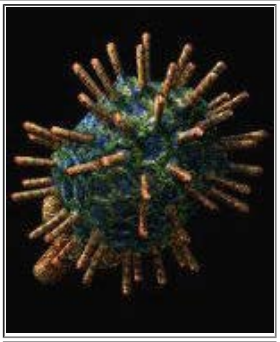
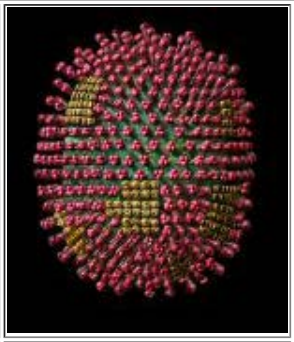
Introduction to Molecular Virology

WHAT ARE VIRIONS?

VIRIONS are **virus particles**: they are the **INERT CARRIERS** of the genome, and are **ASSEMBLED** inside cells, from **virus-specified components**: they do not **GROW**, and do not form by **DIVISION**.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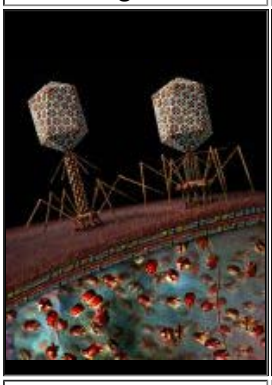
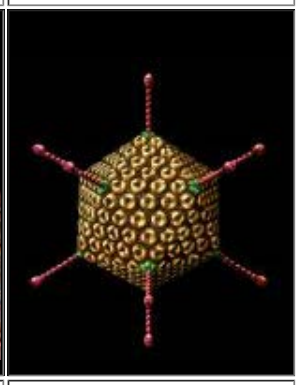
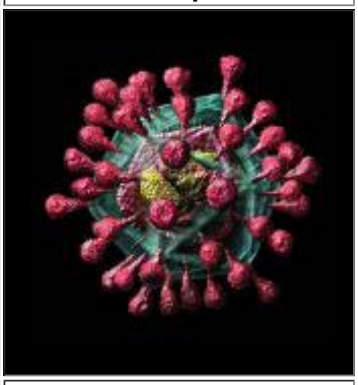
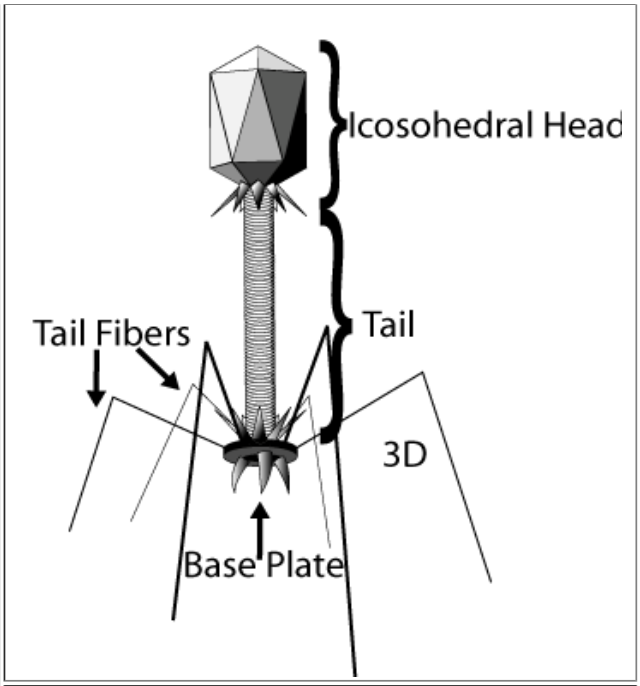
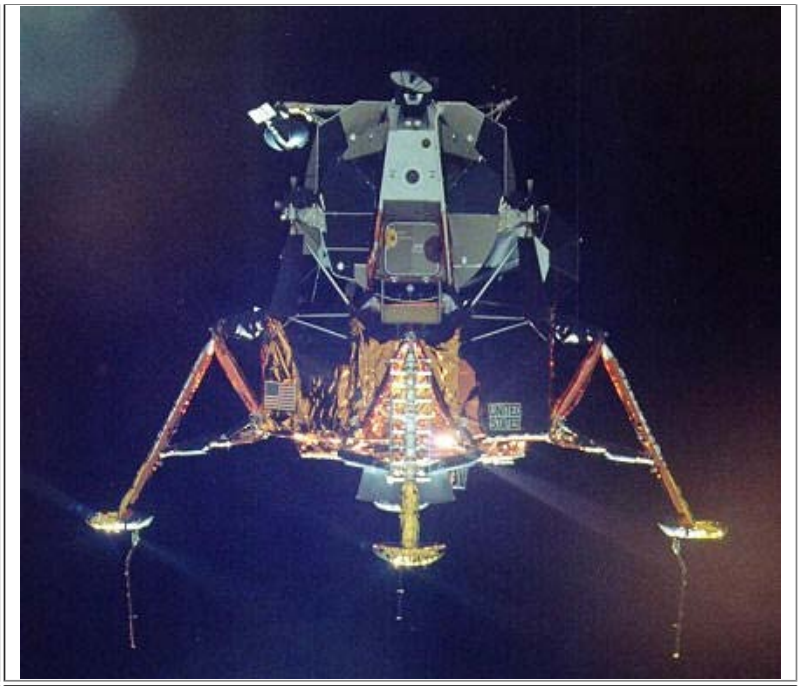
				
Tobacco mosaic	**Filamentous phage**	**T4 phage**	**Adenovirus**	**Coronavirus**
All pictures copyright Russell Kightley www.rkm.com.au				

They may be regarded as the **EXTRACELLULAR PHASE** of the virus: they are exactly analogous to **"spacecraft"** in that they **take viral genomes from cell to cell**, and they **protect the genome** in inhospitable environments in which **the virus cannot replicate**.

Note the strong resemblance between a bacterial virus - T2, T4 or T6 phage of *E coli*, which evolved possibly billions of years ago - and the only human-crewed spacecraft to have landed on another planet.

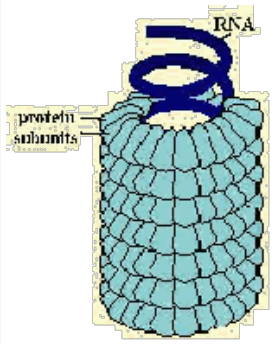
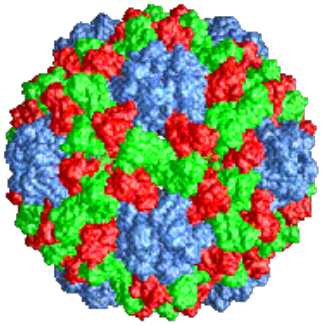
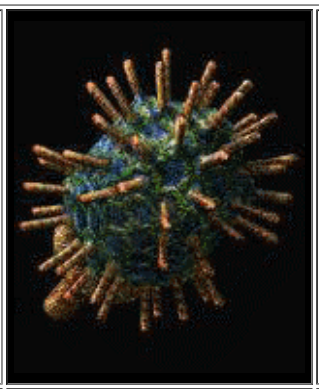
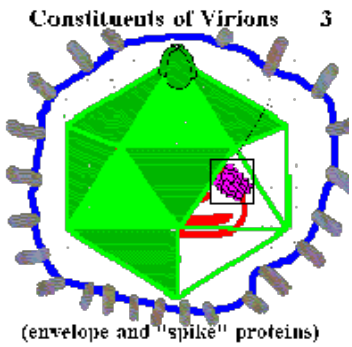
	
T-Even phage structure - copied from Wikipedia	**Lunar excursion module (LEM) of Apollo 16: courtesy NASA**

Basically, all virions have:

- **genomic nucleic acid**: this may be **RNA or DNA, ss or ds**, and may be **all or part** of the genome (see also **here**)
- **protein** associated with the **genome**: this may be in the form of a **helically-assembling** or **isometrically-assembling** **SIMPLE NUCLEOCAPSID** only, or
- **more complex structures with two or more layers of protein**, with **nucleoprotein** innermost.

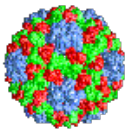
If the virions are simple **NUCLEOPROTEINS** - that is, **contain only nucleic acid and protein** - then they are usually composed **ONLY** of virus-specified components. However, **certain host components may be "trapped" within virions**, such as **POLYAMINES**: these are **polycationic compounds which serve to neutralise charge on the viral nucleic acid** as it is packed into the **CAPSID**, or protein coat. **PAPOVAVIRUSES** may in addition **encapsidate host histones** associated in **NUCLEOSOME** complexes with virus genomic DNA.

The essentials of virion structure may be seen by clicking on the images below.

			
Simple helical structures	**Simple isometric structures**	**Compound structures**	**Enveloped and complex virions**

Excellent electron micrographs of virions - and an **explanation of their basic structure** - may be seen **at the University of Cape Town's Medical Microbiology Division teaching pages.**

For other **excellent representations of virus structure**, go to the **University of Wisconsin's Institute for Molecular Virology**, where **Jean-Yves Sgro** has assembled a gallery of **3-D image reconstructions of different viruses**, together with explanations of structure.



Tutorial Contents

"What is a Virus?"

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Emerging Viruses

What is a little frightening is that **apparently new diseases are still erupting into our human environment at regular intervals**: new and often extremely virulent viruses, such as those causing **Ebola, Marburg** and **Crimean-Congo haemorrhagic fevers**, **hantavirus pulmonary syndrome** and **Korean haemorrhagic disease**, and of course, **HIV1 and 2**. Particularly interesting are those viruses that very recently have appeared out of nowhere, such as the **"equine morbillivirus"** and **bat lyssavirus** reported recently **from Australia, and both apparently having a reservoir in bats**.

These "emerging" and even "re-emerging" viruses are a **great cause for concern internationally**, and the subject of a **great deal of concentrated research**. News on **these and other viruses** may be found on our site **here**. There are also a number of essays on emerging viruses available on this server, via this page: these include a new one on plant viruses as emerging pathogens.

Other sites:

Perhaps the best general-purpose site is the ProMED releases on emerging diseases. These are also collected and (searchably) archived at their Web site. The releases cover all infectious diseases, and of animals as well as of plants. It was particularly fascinating to follow the slowly developing concern over the ongoing incidences of monkeypox infection in central Democratic Republic of the Congo (formerly Zaire), and the emergence of an avian influenza as a potential threat to human health in Hong Kong. More recently, the West Nile Virus scare in the NE USA has been a subject for intense coverage: just when **New Yorkers and their neighbours** were enjoying their summer, **West Nile virus** pops out of the **avian and mosquito populations again**....the public debate on the outbreak can be read at a **special Newsday page**. The **CNN site** carries a **special story on dengue fever**, incidentally, as well as **updates on West Nile in the US Northeast**. Anyone who wants to read **WHO** releases on outbreaks, incidentally, may read them **here** - and they don't mention West Nile! And just recently, my local supermarket was busy reassuring people that they **don't stock British lamb**, to calm fears of **new variant CJD infection via sheep meat**. Also from the **very excellent ProMED** we hear that there has also been a scare about a **cluster of nvCJD cases in the UK**, and whether or not this presages the start of a major outbreak.

Other major sites for information on hot viruses are the Centers for Disease Control and Prevention (CDC) and World Health Organisation sites: the information to be gleaned from here is definitely authoritative, if not particularly current, and more than a little dry (as befits official releases). However, both CDC and WHO issue regular updates during especially severe disease outbreaks, which can be the only definitive news to emerge in real time, especially if the outbreak is somewhere particularly rural or Third World. The WHO publishes a Weekly Epidemiological Record, as an Adobe Acrobat (pdf) file; this is also available via email. The CDC publishes an extremely useful journal entitled "Emerging Infectious Diseases", available electronically as HTML or as a pdf file, or in hard copy: this is a valuable source of authoritative data/descriptions on/of viruses - including the only publication I know of to date which comes close to showing an animal reservoir for Ebola Zaire. EID also contains the definitive account of the Kikwit outbreak.

Other sources of "hard" news are few and far between - however, a regular scan of the Web using one of the many search engines can turn up the odd useful snippet; so can a regular scan of the better online newspapers, such as the Electronic Telegraph (UK), the Nando Times (USA), and the Electronic Mail and Guardian (SA).

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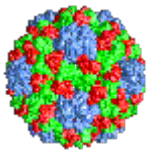
Essays on Virology Topics:

- "Emerging and Re-Emerging Viruses" by Ms Alison Jacobson, Dept Microbiology, UCT
- "Hantaviruses, with Emphasis on Four Corners [now Sin Nombre] Hantavirus" by Brian Hjelle, University of New Mexico
- "Emerging and Re-Emerging Virus Diseases: A Challenge for Biotechnology" by Ed Rybicki, University of Cape Town
- "Problems in the Surveillance and Control of Viral Diseases with Special Reference to the Developing World" by Dr Fred Murphy, University of California at Davis
- NEW! "Plant Viruses as Emerging Pathogens" by Ed Rybicki and Gerhard Pietersen, UCT and ARC-PPRI, Roodeplaat



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Virus Classification

Introduction	Taxonomic Criteria	Hosts of Viruses
Cross-Kingdom Viruses	Cross-Phylum Viruses	Other Resources

Introduction

A **universal system for classifying viruses**, and a unified taxonomy, has been established by the **International Committee on Taxonomy of Viruses (ICTV)** since 1966. The system makes use of a series of ranked taxons, with the:

- - **Order (-*virales*)**being the highest currently recognised.
  - - then **Family (-*viridae*)**
    - - **Subfamily (-*virinae*)**
      - - **Genus (-*virus*)**
        - - **Species** (eg: *Tobacco mosaic virus*)

For example, the **Ebola** virus from Kikwit is classified as:

- Order *Mononegavirales*

- **Family *Filoviridae***

- **Genus *Filovirus***

- **Species: *Ebola virus Zaire***

The current (2005) Eighth Report of the ICTV lists more than **5,400 viruses in 1938 species, 287 genera, 73 families and 3 orders.**

Orders include:

- ***Caudovirales***, tailed dsDNA phages
- ***Mononegavirales***, viruses with enveloped virions with similar ss(-)RNA genomes
- ***Nidovirales***, viruses with enveloped globular virions with similar ss(+)RNA genomes

These are presumed to constitute the highest level of "**monophyletic**" groups of viruses, with a **common ancestry, so far recognised**.

A **comprehensive alphabetical list of viruses** appears at the **ICTVdb** site **here**. This includes virus-like agents.

A B C D F G H I L M N O P Q R S T U V

Unassigned Viruses Satellites Viroids Prions

Virus Orthography, or spelling and usage conventions

- The **names** of **all** taxonomic groups - orders, families, subfamilies, genera and species - are **written in italics with the first letter capitalised** (see examples above).
- **Proper nouns** in names should be **capitalised** as well: e.g. ***Semliki Forest*** *virus.*

This format refers only to official taxonomic entities: these are concepts, while viruses are real.

Vernacular or common-use forms of names are neither capitalised nor written in italics. Thus, while *Tobacco mosaic virus* refers to the species and *Tobamovirus* to the taxonomic genus, **tobacco mosaic viruses** or **tobamoviruses** are the entities that you work with.

Taxonomic Criteria

The most important criteria are:

Host Organism(s): eukaryote; prokaryote; vertebrate, etc.

Particle Morphology: filamentous; isometric; naked; enveloped

Genome Type: RNA; DNA; ss- or ds-; circular; linear

- although a **number of other criteria** - such as

- **disease symptoms,**
- **antigenicity,**
- **protein profile,**
- **host range,** etc.

- are important in **precise** identification, **consideration of the above three criteria** - and in many cases, just **morphology** - are sufficient in most cases to allow identification of a virus down to **familial** if not **generic** level.

An interactive virus identification key was developed at this site: test it and see how the criteria rapidly allow identification.

Hosts of viruses

These include all classes of cellular organisms described to date:

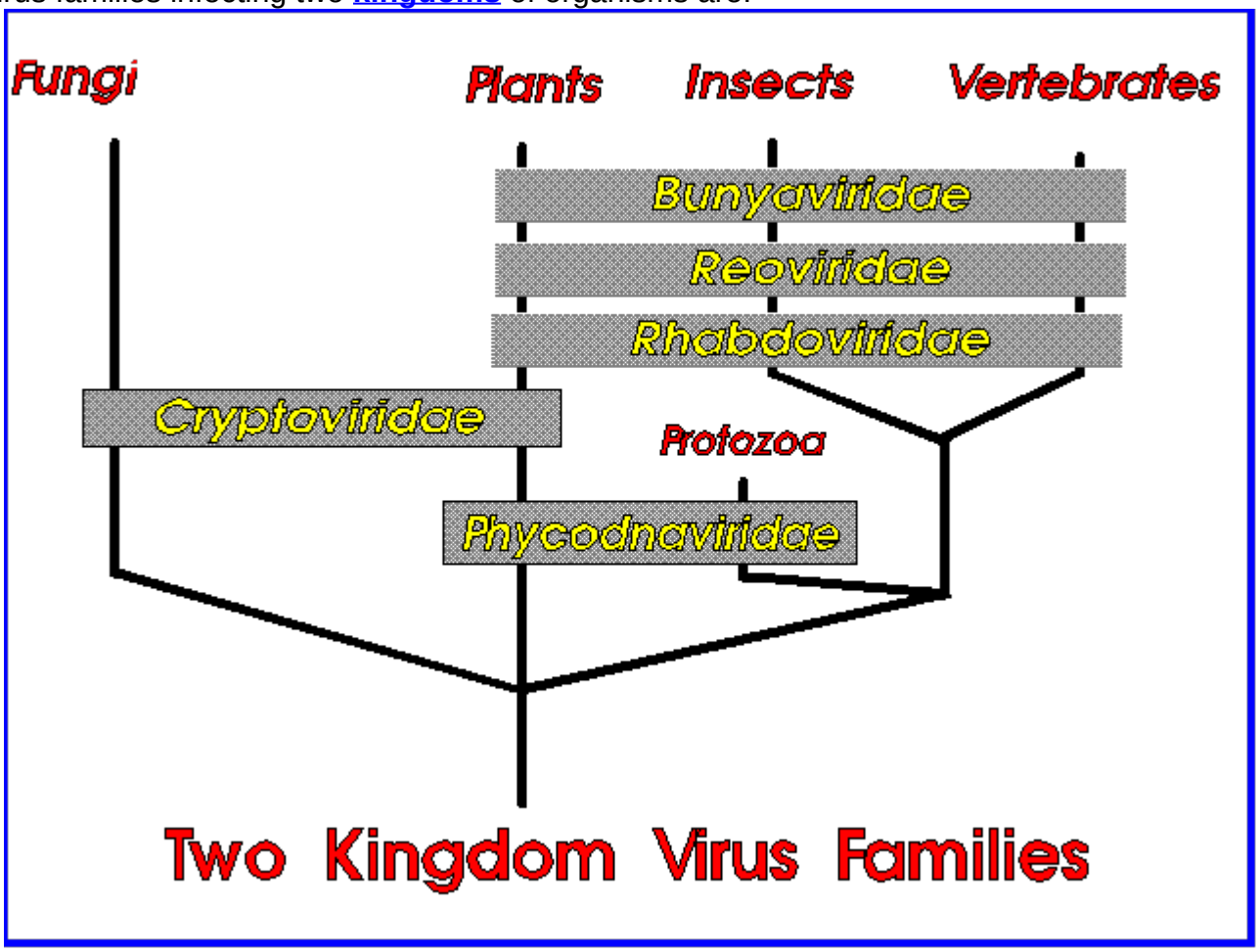
Prokaryotes:	**Archaea**	**Bacteria**	**Mycoplasma**	**Spiroplasma**
Eukaryotes:	**Algae**	**Plants**	**Protozoa**	**Fungi**
Invertebrates			**Vertebrates**	

This page links to a comprehensive listing of **viruses by host infected**.

Cross-Kingdom Viruses

Certain virus families / groupings cross "kingdom" or phylum boundaries.

For example, virus families infecting two kingdoms of organisms are:



- Bunyaviruses: animals and plants
- Partitiviruses: plants and fungi

- Reoviruses: animals and plants
- Rhabdoviruses: animals and plants
- Phycodnaviruses: protozoa and plants
- Picornavirus-like viruses: plants and animals
- Totiviruses: protozoa / fungi and insects - tentative

The reasons for this are probably caught up in their evolution and cospeciation with their hosts (see also here).

For example, bunyaviruses and rhabdoviruses probably originated in their present form in insects, and were spread to plants and other animals after insects emerged from the seas and preyed on other terrestrial hosts.

The dsDNA cryptoviruses most probably originated in fungi, and were spread to land plants after these emerged into the terrestrial environment, as fungi began to parasitise them.

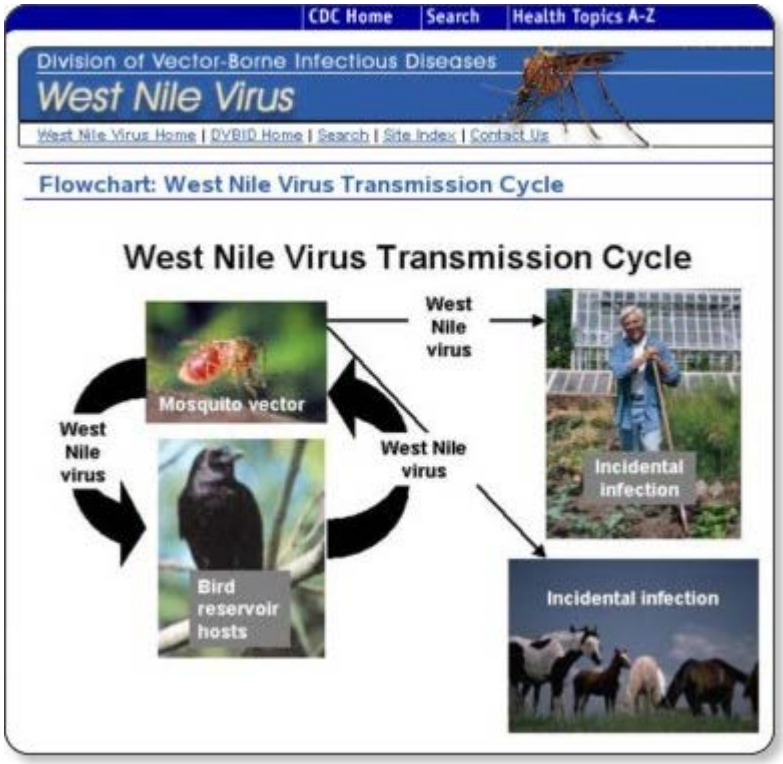
The phycodnaviruses - all of which are found in aquatic environments - could well have started out in the progenitors of protozoa and aquatic / marine plants.

Cross-Phylum Viruses

Virus families infecting across different phyla (all infecting insects and vertebrates) are:

- Flaviviruses
- Iridoviruses
- Parvoviruses
- Poxviruses
- Togaviruses

These viruses almost certainly spread from insects to vertebrates, after vertebrates joined insects on dry land and insects began to feed on them. An example here is West Nile flavivirus, which infects mosquitoes and birds, and can be transmitted to humans and other animals as well.



CDC: West Nile Virus - Flowchart: West Nile Virus Transmission Cycle via kwout

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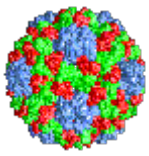
Other Resources:

An excellent resource for virus taxonomy is the **International Committee on Virus Taxonomy (ICTV) Database**, the **ICTVdB**, maintained by **Cornelia Büchen-Osmond** and **others**.

A very good set of pages can be found at the **MicrobiologyBytes.com** site.

The ICTV virus database (ICTVdb) has a mirror Web site: visit it at the **National Center for Biotechnology Information** server.



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Introduction to Molecular Virology

Genome Diversity

The range of virus genomes **as found in virions** includes:

DNA						RNA			
double-stranded			single-stranded			double-stranded		single-stranded	
linear	circular		linear	circular		linear		linear*	
single	single	multiple	single	single	multiple	single	multiple	(+)sense**	(-)sense
								single / multiple	single / multiple

* = only **viroids**, some **satellite RNAs** and **satellite viruses** like **Hepatitis delta virus** (HDV) have circular ssRNA genomes

** = mRNA-sense or translatable. (-)sense is complementary to (+)sense and must be transcribed to give mRNA

Additionally, these genomes may be:

- **circularly permuted linear single-component dsDNA**
- single or multiple component **(-)ssRNA genomes** which may be **partially translatable or ambisense**;
- **(+)sense ssRNA genomes replicating via reverse transcription** to and transcription from longer-than-genome-length dsDNA, and
- **dsDNA genomes replicating via transcription** to and **reverse transcription** from longer-than-genome-length positive-sense ssRNA.

Virus genomes range in size from around 1800 nucleotides (ssDNA circoviruses) up to 1.2 million nucleotides (dsDNA, Acanthamoeba polyphaga mimivirus)

Viruses are the only organisms on this planet to still have **RNA** as their sole genetic material. They are also the only autonomously replicating organisms to have **single-stranded DNA**.

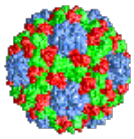
In contrast, **prokaryotes** have **mainly single-component circular** (occasionally multiple) or **linear dsDNA (*Streptomyces*, *Helicobacter*)** while all **eukaryotes have multi-component linear dsDNA**, and all the genomes replicate **via the classic semi-conservative route**.

Viral genome types have inspired a **classification based on what is found in virions**, coupled with

their replication strategy. This is the **Baltimore Classification,** and will be discussed here in a broader treatment of **Virus Replication.**

See also the Leicester material here.

An overview of the taxa Recognized in the Universal System of Virus Taxonomy in terms of their genome content may be found here.



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Introduction to Molecular Virology

Virus Entry Into Cells

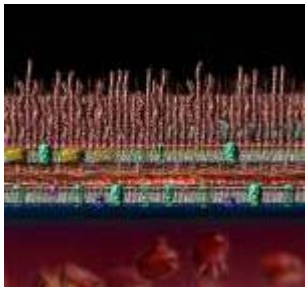
This depends largely upon the **TYPE** of the cell, and of the virus: **the cell type has a great deal of influence on the strategy the virus uses to gain access**; in turn, **specific virus types may employ different strategies to gain access to the same cell type**. However, the **greatest commonality in strategy** is probably observed between viruses infecting a **single broad type of host, defined by the nature of their cell walls**: these may be defined as:

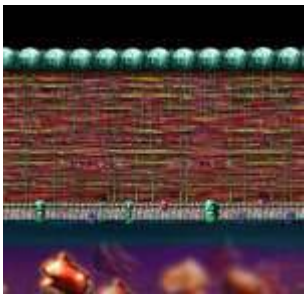
- **bacterial (eg: Eubacteria, Archaea)**
- **animal (eg: all animal cells)**
- **plant-like (eg: green multicellular plants, algae, fungi).**

Bacterial cell

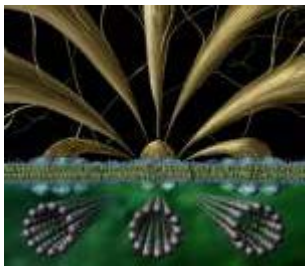
Animal cell

Plant cell


Gram-negative cell wall


Gram-positive cell wall

Animal cell wall


Plant cell wall

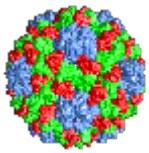
Pictures copyrighted by Russell Kightley

Links which explain the basic structures of these kinds of cells may be found at Wikipedia.

The material here will be divided into the following headings:

BACTERIAL CELL ENTRY

EUKARYOTE CELL ENTRY
Animal Cell Entry
Plant Cell Entry
Fungal Cell Entry



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Genomic Replication Strategies of Viruses

The old terms "eclipse phase" or "latent period" describe that part of a virus life cycle when no infectious virus can be extracted from cells which had just been exposed to infectious virions: a good illustration of the concept in terms of a virus assay experiment is shown **here**.

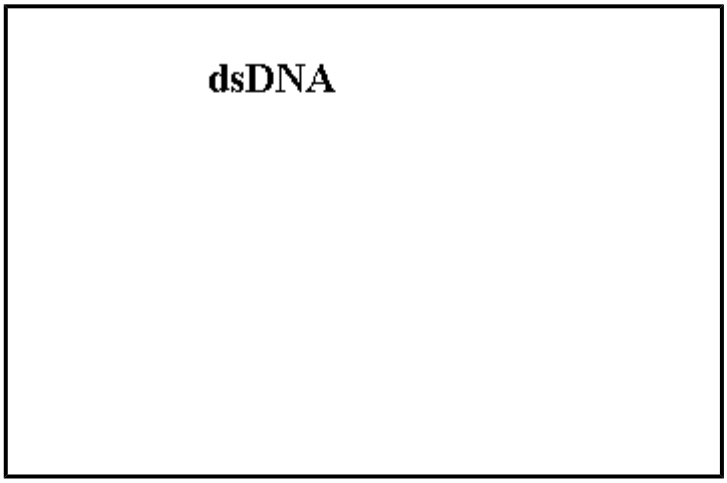
What happens once a virus is uncoated, or partially uncoated, depends largely upon what sort of virus it is. The **Baltimore Classification** of viruses by their genome types and replication strategies makes it fairly easy to predict the broad sort of strategy that a virus with a given genome will employ in order to get replicated. This classification was originally devised by David Baltimore; it originally only had six categories, but the discovery of **"DNA retroviruses"** or **PARARETROVIRUSES** in the 1980s has necessitated a new Class VII.

To understand the classification, one must understand how cells replicate their genomes, and express mRNAs, and proteins. This is conveniently explained by the "Watson-Crick Central Dogma", which states that:

INFORMATION FLOW IN CELLS GOES FROM:

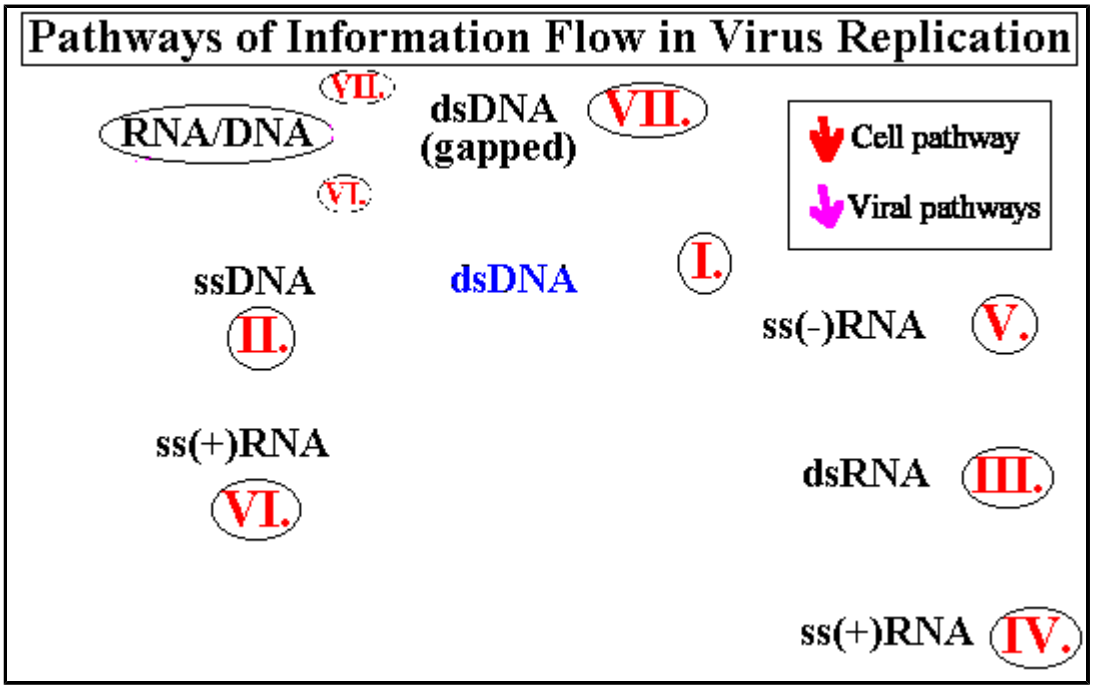
- DNA TO DNA (REPLICATION)
- DNA TO RNA (TRANSCRIPTION)
- AND RNA TO PROTEIN (TRANSLATION)

This may conveniently be described in terms of a diagram showing "information flow":



Note: this is an animated GIF file - wait until the whole cycle finishes (+/- 5 sec)

By contrast, viral replication is far more complicated in terms of information flow:



Note: this is an animated GIF file - wait until the whole cycle finishes (+/-20 sec)

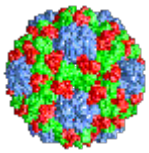
The Baltimore Classification of Viruses According to Their Genome Types and Their Replication Strategies

The classes are:

I	dsDNA viruses replicating via DNA (semi-conservative)
II	ssDNA viruses replicating via DNA (semi-conservative)
III	dsRNA viruses replicating via (+)RNA (conservative?)

IV	**ssRNA viruses with (+)-sense genomes replicating via RNA (semi-conservative)**
V	**ssRNA viruses with (-)sense genomes replicating via RNA (semi-conservative)**
VI	**"diploid" ssRNA viruses which replicate via reverse transcription with a greater-than-genome-length dsDNA intermediate**
VII	**dsDNA viruses which replicate via reverse transcription with a greater-than-genome-length ssRNA intermediate.**

See also section on "RNA Virus Replication"



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What is a Virus?

DEFINITIONS
VIRIONS
OTHER VIRUS-LIKE AGENTS

Viruses

Viruses may be **defined** as **acellular organisms** whose **genomes** consist of **nucleic acid**, and which obligately **replicate inside host cells** using **host metabolic machinery** and **ribosomes** to form a **pool of components** which assemble into particles called **VIRIONS**, which serve to **protect the genome** and to **transfer it to other cells**.

They are distinct from other so-called **VIRUS-LIKE AGENTS** such as **VIROIDS and PLASMIDS and PRIONS**

Alternative definitions:

S Luria *et al*. AJ Cann

And EP Rybicki, ©2008:

A virus is an infectious acellular entity composed of compatible genomic components derived from a pool of genetic elements.

The concept of a virus as an organism challenges the way we define life:

- viruses do not **respire**,
- nor do they display **irritability**;
- they do not **move**
- and nor do they **grow**,
- however, they do most certainly **reproduce**, and may adapt to new hosts.

By older, more **zoologically and botanically** biased criteria, then, **viruses are not living**. However, this sort of argument results from a **"top down"** sort of definition, which has been modified over years to take account of **smaller and smaller things** (with fewer and fewer legs, or leaves), until it has met the ultimate **"molechisms" or "organules"** - that is to say, **viruses** - and has proved inadequate.

If one **defines life** **from the bottom up** - that is, from the **simplest forms capable of displaying the most essential attributes of a living thing** - one very quickly realises that the only real criterion for life is:

The ability to replicate

---

and that only systems that contain nucleic acids - in the natural world, at least - are capable of this phenomenon. This sort of reasoning has led to a new definition of organisms:

"An organism is the unit element of a continuous lineage with an individual evolutionary history."

The key words here are **UNIT ELEMENT**, and **INDIVIDUAL**: the thing that you see, now, as an organism is merely the **current slice in a continuous lineage**; the individual evolutionary history denotes the **independence of the organism over time**. Thus, **mitochondria and chloroplasts and nuclei and chromosomes** are not organisms, in that together they constitute a continuous lineage, but separately have no possibility of survival, despite their independence before they entered **initially symbiotic**, and then **dependent** associations.

The concept of replication is contained within the concepts of individual viruses constituting continuous lineages, and having an evolutionary history.

Thus, given this sort of lateral thinking, **viruses become quite respectable as organisms:**

- they most definitely **replicate**,
- their **evolution** can (within limits) be **traced quite effectively**, and
- they are **independent in terms of not being limited to a single organism as host**, or even necessarily to a single species, genus or phylum of host.

More views on viruses and their definition:

It's Life, Jim, but not as we know it...

Posted by ajcann on July 24, 2007

Todays post is a welcome return of guest blogger:

Ed Rybicki, Department of Molecular and Cell Biology, University of Cape Town, South Africa.

It's Life, Jim, but not as we know it...

Which could well apply to viruses, my very own favourite organisms - after all, they don't respire, grow, excrete or any of those other good things that classical organisms supposedly do - but that's not the point of this piece. Rather, I'd like to speculate on an interesting convergence of articles I've seen recently, on (1) extrasolar planets, (2) water on same, and (3) the possibility of novel lifeforms. Some of this is an accident of the irregular timing of receipt of my New Scientist and Nature subscriptions,

It's Life, Jim, but not as we know it… « MicrobiologyBytes via kwout

A feeling for the molechism*

Posted by ajcann on June 26, 2007

Todays post is a welcome return of guest blogger:

Ed Rybicki, Department of Molecular and Cell Biology, University of Cape Town, South Africa.

I think it's permissible, after working on your favourite virus for over 20 years, to develop some sort of feeling for it: you know, the kind of insight that isn't directly backed up by experiment, but that may very well be right. Or not - but in either case, it would take a deal of time and a fair bit of cash to prove or disprove, and would have sparked some useful discussion in the meantime. And then, of course, the insights you have into (insert favourite virus name here) - if correct - can usually be extended into the more general case, and if you are sufficiently distinguished, people may actually take them on board, and you will have contributed to Accepted Wisdom.

I can't pretend - at least, outside of my office - to any such Barbara McClintock-like distinction; however, I have done a fair bit of musing on my little sphere of interest as it relates (or not) to the State of the Viral Universe, and I will share some of these rambles now with whoever is interested.

A feeling for the molechism* « MicrobiologyBytes via kwout

Definitions

by EP Rybicki (1999). An Electronic Introduction to Molecular Virology. Buglet Electronic Press, Cape Town.

Acellular Organisms:
acellular: not composed of cells (= "bodies of protoplasm made discrete by an enveloping plasma membrane");
Penguin Dictionary of Biology, 9th Edition, 1994

Genome:
The total genetic complement of a virus. This may be composed of RNA or DNA, and be single- or double-stranded. It may also be fragmented.

Viruses 1:

"Viruses are entities whose genomes are elements of nucleic acid that replicate inside living cells using the cellular synthetic machinery, and cause the synthesis of specialised elements [virions] that can transfer the genome to other cells".

SE Luria, JE Darnell, D Baltimore and A Campbell (1978). General Virology, 3rd Edn. John Wiley & Sons, New York, p2 of 578.

Viruses 2:

"Virus are submicroscopic, obligate intracellular parasites...[and]

- **Virus particles (virions) are formed from the assembly of pre-formed components;**
- **Virus particles themselves do not "grow" or undergo division;**
- **Viruses lack the genetic information which encodes apparatus necessary for the generation of metabolic energy or for protein synthesis (eg: ribosomes)".**

AJ Cann (1997). Principles of molecular virology, 2nd Edition. Academic Press, San Diego.

Organism:

"An organism is the unit element of a continuous lineage with an individual evolutionary history."

SE Luria, JE Darnell, D Baltimore and A Campbell (1978). General Virology, 3rd Edn. John Wiley & Sons, New York, p4 of 578.

Definitions of Life:

Classical Newer Versions

Classical Properties of Living Organisms:

- Reproduction
- Nutrition
- Respiration
- Irritability
- Movement
- Growth
- Excretion

More modern definitions include the storage and replication of genetic information as nucleic acid, and the presence of or potential for, enzyme catalysis

(Penguin Dictionary of Biology, 9th Edition, 1994)

Newer Definitions of Life

Life may (somewhat irreverently) be defined in general terms as:

"The phenomenon associated with the replication of self-coding informational systems",

or more specifically as:

"The phenomenon associated with the replication of nucleic acids".

- Rybicki EP, 1996.

Another more serious view:

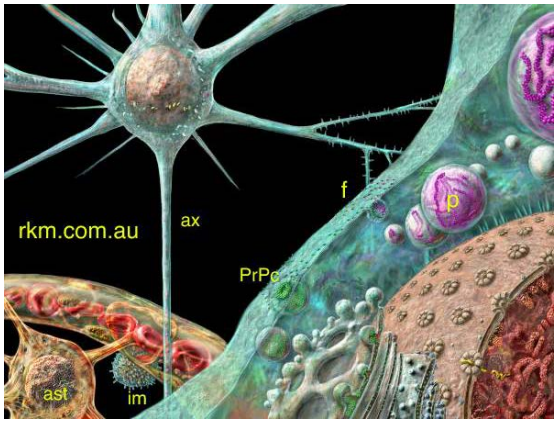
"Life can be viewed as a complex set of processes resulting from the actuation of the instructions encoded in nucleic acids. In the nucleic acid of living cells these are actuated all the time; in contrast, in a virus they are actuated only when the viral nucleic acid, upon entering a host cell, causes the synthesis of virus-specific proteins. Viruses are thus "alive when they replicate in cells, while outside cells viral particles are metabolically inert and are no more alive than fragments of DNA."

- Dulbecco R and Ginsberg HS, 1980. Virology, p.854-855 (originally published as a section in Microbiology, 3rd Edn., Davis et al., Harper and Row, Hagerstown).

Other Autonomous or Semi-Autonomously Replicating Genomes

There are a number of types of genomes which have some sort of independence from cellular genomes: these include "retrons" or **retrotransposable elements**, bacterial and fungal (and eukaryotic organelle) plasmids, satellite nucleic acids and satellite viruses which depend on helper viruses for replication, and viroids. A new class of agents - PRIONS - appear to be "proteinaceous infectious agents" (see also here for an ICTV description, here for some local information and more links).

Retrons	**Plasmids**	**Satellite Nucleic Acids**
Satellite Viruses	**Viroids**	**Prions**



Depiction of Prions infecting Neurons: from Russell Kightley Media

Plasmids

Plasmids may **share a number of properties** with viral genomes - **including modes of replication**, as in **ss circular DNA plasmids and viruses** - but are **not pathogenic** to their host organisms, and are **transferred by conjugation** between cells rather than by **free extracellular particles**.

Satellite Nucleic Acids

Certain viruses have associated with them nucleic acids that are **dispensable in that they are not part of the genome**, which **have no (or very little) sequence similarity** with the viral genome, yet **depend on the virus for replication**, and are **encapsidated by the virus**. These are mainly **associated with plant viruses** and are generally **ssRNA**, both linear and circular - however, a **circular ssDNA satellite** of a plant **geminivirus** has recently been found.

Satellite Viruses

There are also **viruses which depend for their replication on** HELPER VIRUSES: a good example is **tobacco necrosis satellite virus (sTNV)**, which has a **small piece of ssRNA which codes only for a capsid protein**, and depends for its replication on the presence of TNV. Another good example is the **hepatitis delta agent with its circular ssRNA genome.** The **adeno-associated viruses** (AAVs) are also satellite viruses dependent on the linear dsDNA **adenoviruses** for replication, but which have **linear ssDNA genomes** and appear to be **degenerate or defective parvoviruses.**

Viroids

Viroids are **small naked circular ssRNA genomes** which appear **rodlike** under the EM, which are **capable of causing diseases in plants**. They **code for nothing but their own structure**, and are **presumed to replicate by somehow interacting with host RNA polymerase**, and to **cause pathogenic effects by interfering with host DNA/RNA metabolism** and/or transcription. A **structurally similar disease agent** in humans is the **hepatitis B virus-dependent hepatitis delta agent**, which additionally codes for a structural protein.

Retroid Elements and Retroviruses

Retroviridae [ssRNA(+) viruses replicating via a longer-than-genome-length dsDNA intermediate], ***Hepadnaviridae***, **caulimoviruses and badnaviruses** [family **Caulimoviridae**, gapped circular dsDNA viruses replicating via longer-than-genome-length RNA intermediates] all share the unlikely attribute of **the use of an enzyme complex consisting of a RNA-dependent DNA polymerase/RNAse H in order to replicate**. They share this attribute with several **retrotransposons**, which are eukaryotic transposable cellular elements with striking similarities with retroviruses [such as the yeast Ty element, the mammalian LINE-1 elements, and the Drosophila copia element]; and with **retroposons**, which are eukaryotic elements which transpose via RNA intermediates, but share **no obvious genomic similarity with any viruses other than the reverse transcriptase**.

Bacteria such as *E coli* also have reverse- transcribing transposons -known as *retrons* - but these are very different to any of the eukaryotic types while **preserving similarities in certain of the essential reverse transcriptase sequence motifs**.

All of these elements are collectively known as **RETROELEMENTS**; the fact that the reverse transcriptases of all of them have some amino acid identity suggests a common evolutionary origin.

Several reviewers have pointed out that **just such an enzyme as reverse transcriptase** would have been necessary for the **transition from what is widely believed to have been an RNA world** - that is, where all the extant organsisms had RNA genomes - to the present world in which **all cellular organisms have DNA genomes**.

Viruses with RNA genomes which use RNA-dependent RNA polymerases for their replication may be the **only remnants of that pre-DNA era;** however, **cellular elements and viruses which use reverse transcriptase** may share a **common origin as cell-derived "modules"** coding for a reverse transcriptase, which **evolved to become retrons and retroposons and retrotransposons**. Addition of structural proteins may have allowed evolution of **retroviruses**.

The evolution of the DNA retroviruses - *Hepadnaviridae*, caulimo- and badnaviruses - is more obscure; it appears as though these **arose from retrotransposon-like sequences**, but this probably occurred near the origin of of these types of element as they are **so diverse in sequence and genome organisation**.

It is believed that retrotransposons may contribute substantially to the evolution of their hosts. Evidence for this has been obtained by studying human LINE-1s (Long Interspersed Nuclear Elements) - a group of retrotransposable elements which make up approximately 15 % of the human genome. The vast majority of LINE-1s are no longer retrotransposition competent and it is believed that in humans only between 30 and 60 full length LINE-1s are currently active. There is strong evidence from sequences in the sequence databases to suggest that active LINE-1s play an important role in "exon shuffling" (belived to be the major mechanism of macro-evolution whereby entirely new genes are created by reshuffling the components of older genes). The most compelling evidence that LINE-1s do facilitate exon shuffling, however, is the experimental demonstration that they are not only able to move large amounts of non-LINE-1 exonic DNA but also insert this DNA into unrelated expressed genes to obtain chimeras which encode active hybrid gene products.

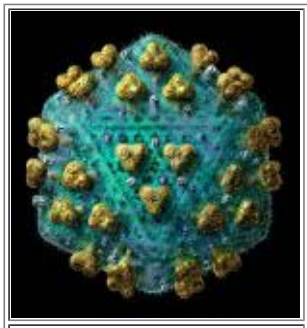
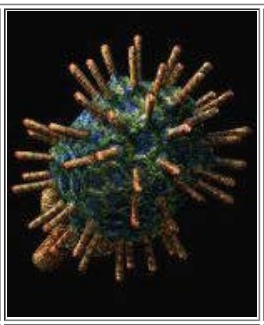
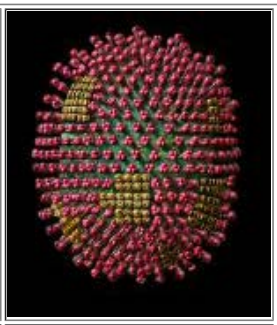
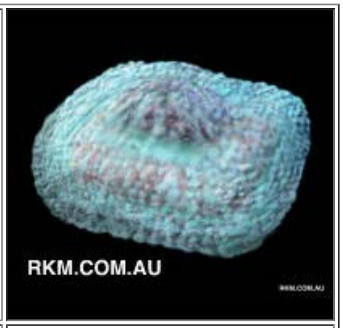
References:

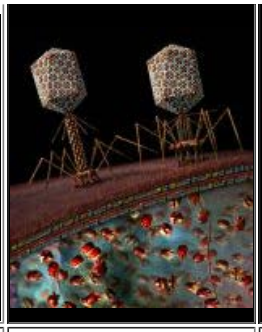
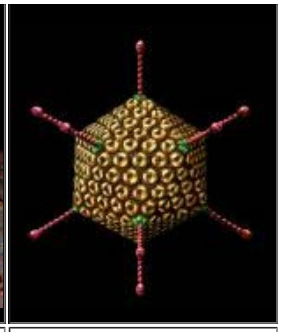
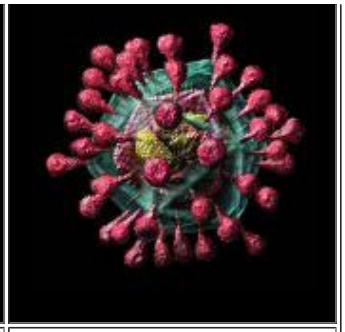
Chapter 7: "Evolution by transposition" (pp. 172-203) in "Fundamental of Molecular Evolution" by Wen-Hsiung Li and Dan Graur; Sinauer Associates, Inc., Sunderland, Mass., 1991

Moran, J. V., R. J. DeBerardinis, and H. H. Kazazian. 1999. Exon shuffling by L1 Retrotransposition. Science 283:1530-1534.

WHAT ARE VIRIONS?

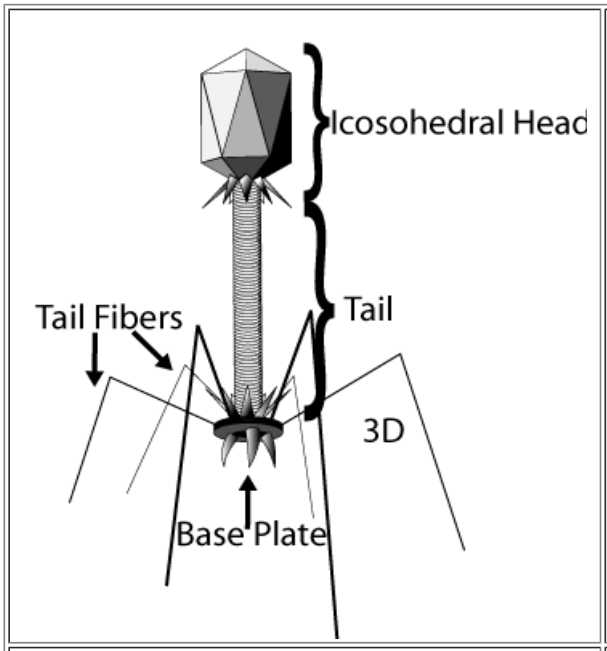
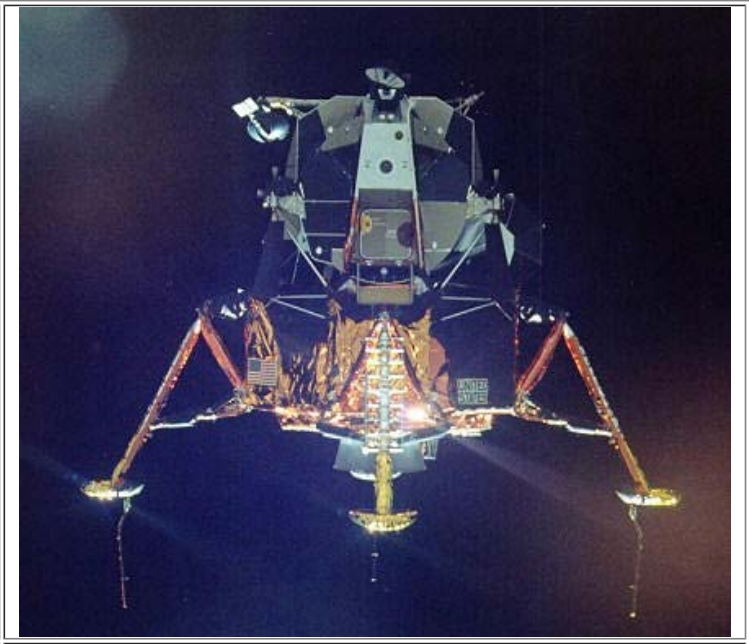
VIRIONS are **virus particles**: they are the **INERT CARRIERS** of the genome, and are **ASSEMBLED** inside cells, from **virus-specified components**: they do not **GROW**, and do not form by **DIVISION**.

				
HIV	**Phage Φ29**	**Phage P22**	**Influenza**	**Smallpox**

				
Tobacco mosaic	**Filamentous phage**	**T4 phage**	**Adenovirus**	**Coronavirus**
All pictures copyright Russell Kightley www.rkm.com.au				

They may be regarded as the **EXTRACELLULAR PHASE** of the virus: they are exactly analogous to **"spacecraft"** in that they **take viral genomes from cell to cell**, and they **protect the genome** in inhospitable environments in which **the virus cannot replicate**.

Note the strong resemblance between a bacterial virus - T2, T4 or T6 phage of *E coli*, which evolved possibly billions of years ago - and the only human-crewed spacecraft to have landed on another planet.

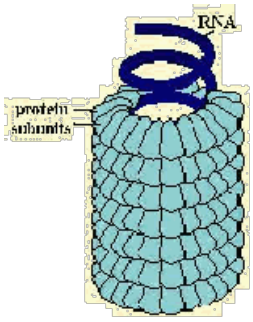
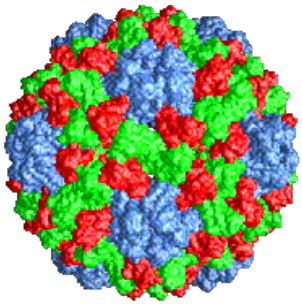
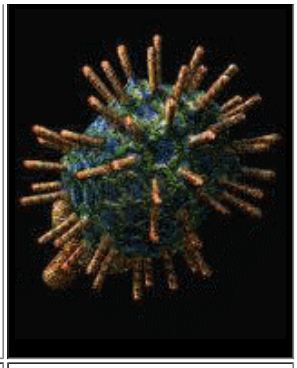
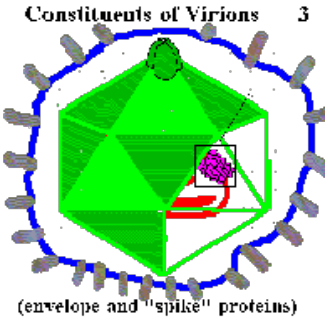
	
T-Even phage structure - copied from Wikipedia	**Lunar excursion module (LEM) of Apollo 16: courtesy NASA**

Basically, all virions have:

- **genomic nucleic acid**: this may be **RNA or DNA, ss or ds**, and may be **all or part** of the genome (see also **here**)
- **protein** associated with the **genome**: this may be in the form of a **helically-assembling** or **isometrically-assembling** **SIMPLE NUCLEOCAPSID** only, or
- **more complex structures with two or more layers of protein**, with **nucleoprotein** innermost.

If the virions are simple **NUCLEOPROTEINS** - that is, **contain only nucleic acid and protein** - then they are usually composed **ONLY** of virus-specified components. However, **certain host components may be "trapped" within virions**, such as **POLYAMINES**: these are **polycationic compounds which serve to neutralise charge on the viral nucleic acid** as it is packed into the **CAPSID**, or protein coat. **PAPOVAVIRUSES** may in addition **encapsidate host histones** associated in **NUCLEOSOME** complexes with virus genomic DNA.

The essentials of virion structure may be seen by clicking on the images below.

			
Simple helical structures	Simple isometric structures	Compound structures	Enveloped and complex virions

Excellent electron micrographs of virions - and an **explanation of their basic structure** - may be seen **at the University of Cape Town's Medical Microbiology Division teaching pages.**

For other **excellent representations of virus structure**, go to the **University of Wisconsin's Institute for Molecular Virology**, where **Jean-Yves Sgro** has assembled a gallery of **3-D image reconstructions of different viruses**, together with explanations of structure.

Helical Nucleocapsids

This is one of the **SIMPLEST FORMS** of viral capsid: the **protein** is **"wound on"** to the **viral nucleic acid** (generally **ssRNA**, though **M13** and other filamentous phage virions contain **circular ssDNA**) in a simple **HELIX**, like a screw (see the diagram for tobacco mosaic virus, below).

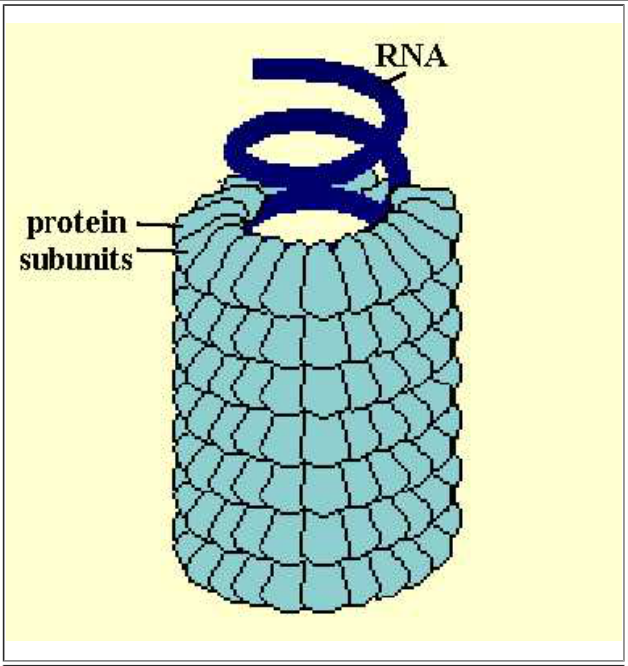
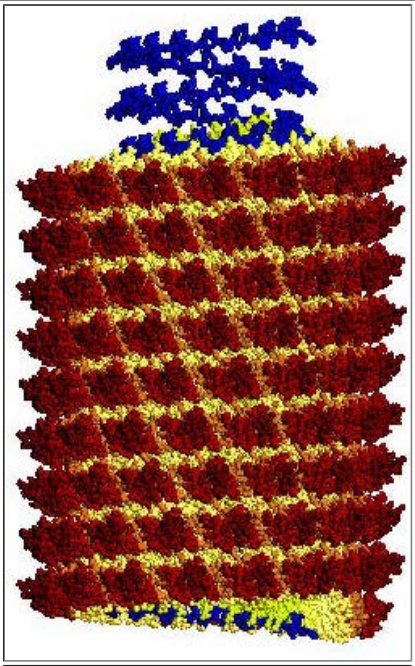
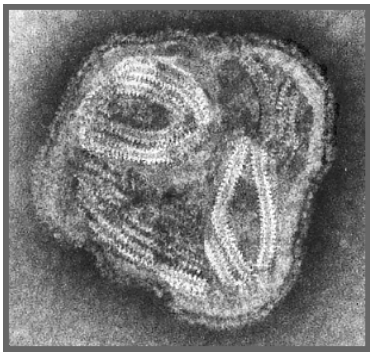
	

Diagram of TMV structure: protein subunits are all in equivalent crystallographic positions, related by a right-hand "screw translation"	**Image reconstruction of TMV**
courtesy L Stannard	**8th Report ICTV**

In the case of **TMV** this is the **entire virion**: this is also the case for all **RODLIKE and FILAMENTOUS** virions where no membranes are involved. This includes all ***Tobamoviridae*, *Potyviridae*, and *Closteroviridae***, but NOT ***Filoviridae***, like Ebola virus (see **here**).



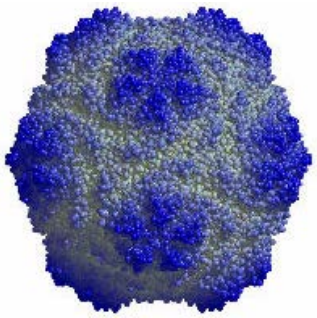
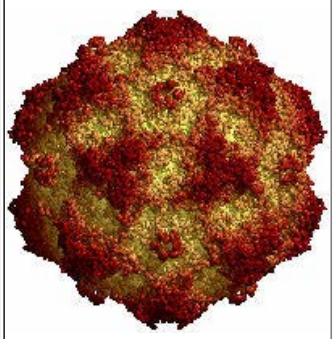
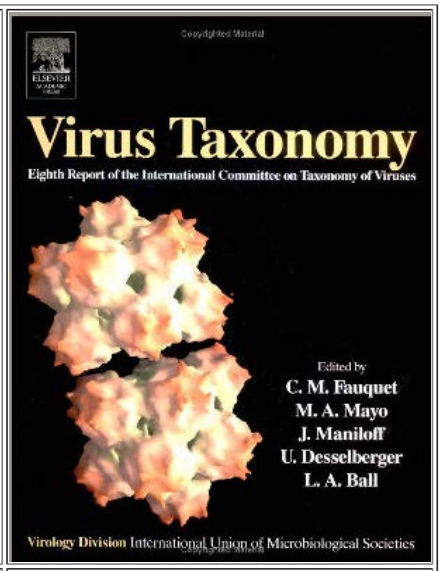
In other cases, **filamentous helical nucleocapsids** may be **enclosed within matrix protein** and a **membrane studded with spike proteins**: excellent examples of this are **PARAMYXOVIRIDAE**, detailed images of which can be found **here**, at Linda Stannard's site, and **for ORTHOMYXOVIRIDAE here** in this tutorial.

Isometric Nucleocapsids

These are built up according to **simple structural principles**, as **amply outlined here**, and in **more detail here**. Put simply, nearly **all isometric virions** are constructed around a **BASIC ICOSAHEDRON**, or solid with 20 equilateral trinagles for faces. It suffices to say that the **"quasi-icosahedral"** capsid is possibly **Nature's most popular means of enclosing viral nucleic acids**; they come in many sizes, from **tiny T=1 structures** (Nanoviruses, eg: banana bunchy top virus; 18 nm diameter) to **huge structures** such as those of **Iridoviridae** or **Phycodnaviridae** (over 200 nm diameter).

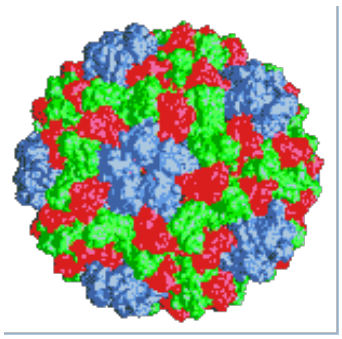
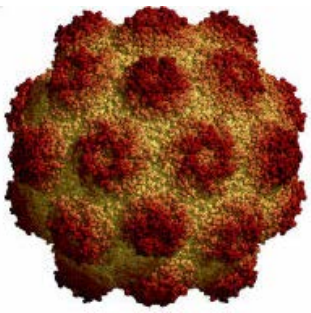
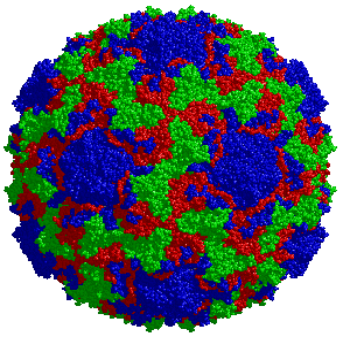
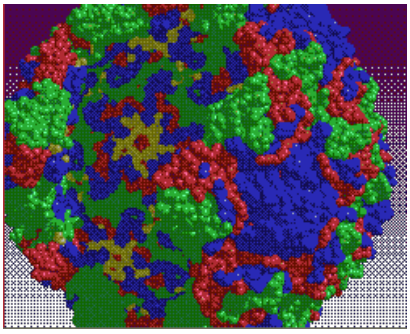
The simplest virions are those of the viruses with the smallest genomes: these are virions such as those of the **ssRNA satellite tobacco necrosis virus** (sTMV), the ssDNA **canine parvovirus** (CPV) and **porcine circovirus** (PCV), and **microviruses** infecting E coli and other bacteria (eg: φX174 phage): these all have a **simple icosahedral T=1 surface lattice structure**. Some examples are shown below. **All structural subunits of these capsids are in the same positional state, or have the same interactions with their neighbours.**

A unique derivative structure is that of **geminivirus** virions, which have **two incomplete T=1 icosahedra** joined at the missing vertex.

		
Satellite tobacco necrosis virus	**Canine parvovirus**	**Geminivirus: Cover of the ICTV 8th Report**
Images from the ICTV 8th report; derived from the Virus World site, for non-profit educational use		

An **example of a more complex structures** are illustrated below. The animated GIF to the left shows **Cowpea chlorotic mottle (CCMV) virion surface structure (courtesy J-Y Sgro)**, which is composed of **180 copies of a single coat protein molecule, in a T=3 surface lattice**. The different colours in the CCMV picture represent **different "positional states" of the capsid protein**: subunits around **5-fold** rotational axes of symmetry are **BLUE**, and cluster as **PENTAMERS**; subunits around **3-fold axes** are **RED** and **GREEN** to reflect their different **2-fold symmetries**; they cluster as **HEXAMERS** around **"local 6-fold axes".**

Another example is that of **turnip yellow mosaic virus (TYMV):** this has **exactly the same basic structure**, with a **180 copies of a single type of coat protein subunit**, with the **pentamer-hexamer clustering** appearing more pronounced

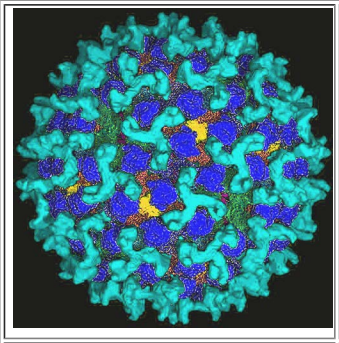
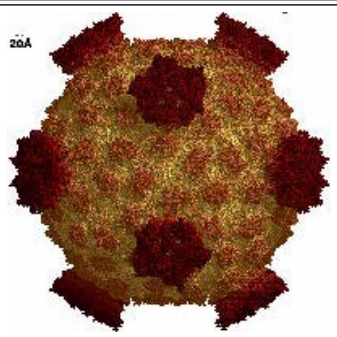
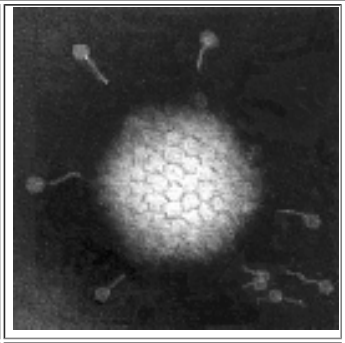
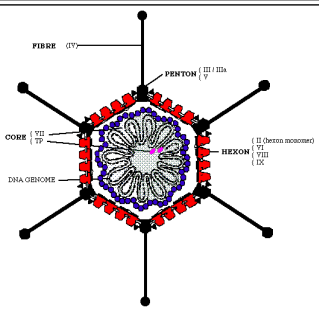
	
CCMV capsid	TYMV capsid
courtesy J-Y Sgro	ICTV 8th report
	
Rhinovirus R16	Rhinovirus R16
courtesy J-Y Sgro	

A **more complex capsid** - that of the common-cold-causing ***Rhinovirus*** R16 (family: ***Picornaviridae***), with **60 copies of 4 proteins in a T=3 structure** - is shown on the **bottom left and right** (animation modified from one by **J-Y Sgro**) and **right**. The **right** image shows a capsid with a cutaway, to reveal internal structure. **BLUE** subunits around 5-fold axes are **VP1**; **RED** and **GREEN** are **VP3** and **VP2** respectively; **YELLOW** subunits (seen only internally) are **VP4**. The VP4 subunits are formed by autocatalytic cleavage of **VP0** (into **VP2** and **VP4**) upon binding of a "**procapsid**" with **viral genomic ssRNA.**

Note the similarity between the CCMV and R16 structures - despite one having a single CP, and the other having 3 structural CP subunits.

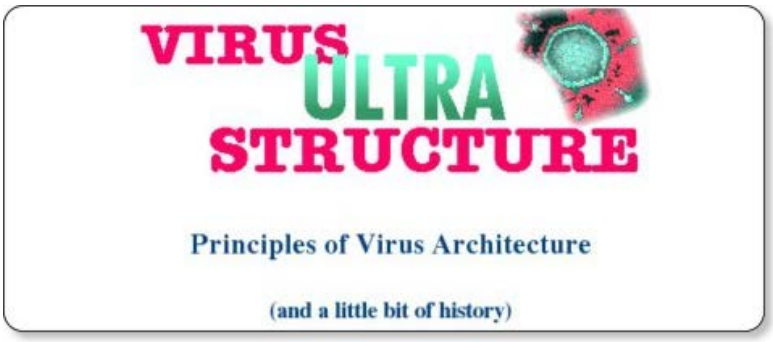
See here for further details of picornaviruses, here for a scheme showing picornavirus assembly, here for a scheme outlining polyprotein processing of picornaviruses, and here for material on picornaviruses from the University of Leicester course.

More complex capsids are generally found for viruses with **larger genomes**, whether composed of RNA or DNA. These include virions such as those of **reoviruses** and **adenoviruses**, both of which have complex or multilayered naked isometric capsids.

			
Bluetongue orbivirus (*Reoviridae*)	**Mammalian reovirus core with spikes**	**Electron micrograph of an adenovirus**	**Link to diagram of an adenovirus structure**
courtesy AJ Cann	**ICTV 8th Report**	**courtesy L Stannard**	**courtesy AJ Cann**

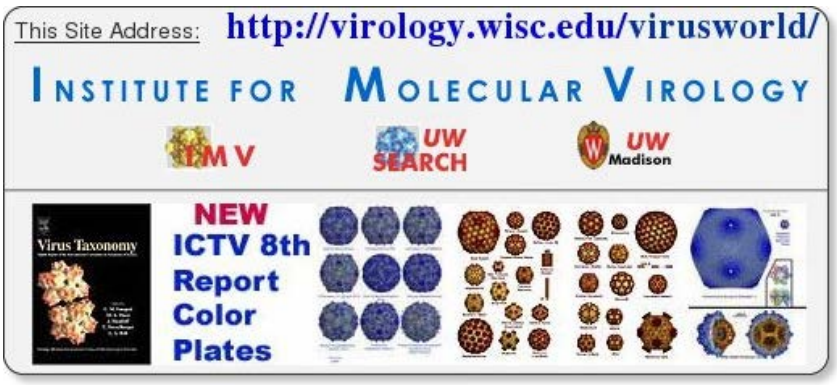
You may like to look here for structures from the University of Calgary material.

More complex structures may be seen in electron micrographs, and diagrams explaining icosahedral and quasi-icosahedral structure at Linda Stannard's Web site.



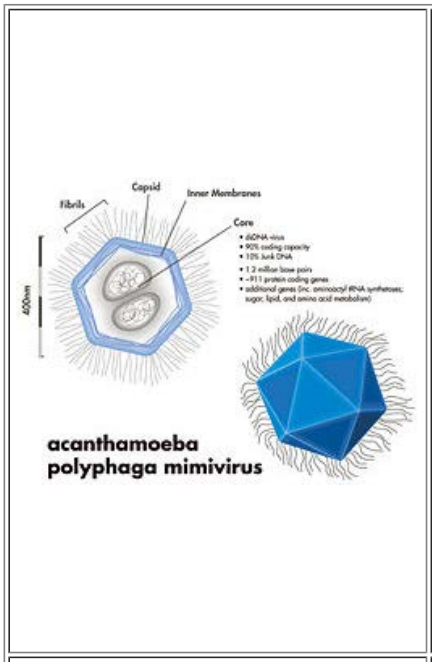
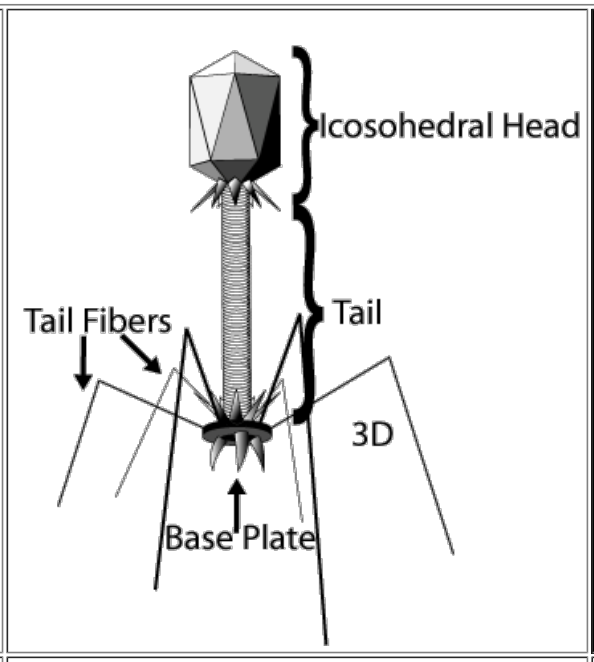
Principles of Virus Architecture via kwout

The "Virus World" site has an excellent set of high-resolution image reconstructions from physical data of non-enveloped simpler isometric viruses.



Virus World via kwout

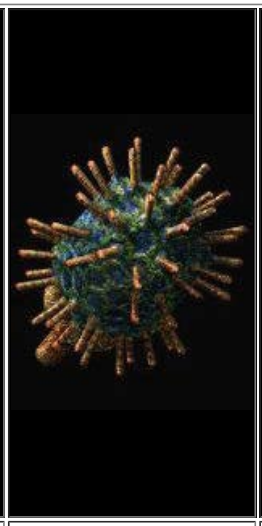
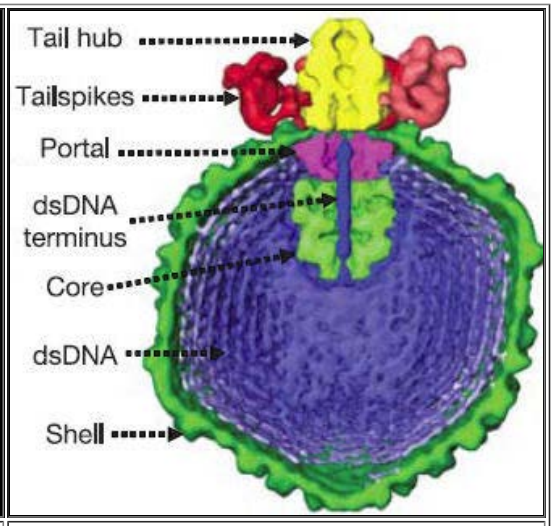
Complex or Compound Virions

		
Mimivirus. The huge regular icosahedral capsid contains lipid membranes and other structures	**T-even phage particle: note elongated isometric head (icosahedron with hexamer expansion net), helical tail, and base plate and neck structures**	**Depiction of a orthopoxvirus virion. Note presence of two lipid bilayers and amorphous structure**

Link to Wikipedia	Link to Wikipedia	copyright Russell Kightley

Most **bacteriophages** - or more properly, **archaeal and bacterial** viruses - have a more complex structure than the simple isometric and helical nucleocapsids typical of many plant and animal viruses. Even viruses with relatively small genomes may have more than one type of architecture associated with their virions, normally in the shape of some kind of **TAIL** structure attached to an isometric **HEAD**. The **T-even** viruses - part of the **"T4-like virus" genus, family *Myoviridae*** - have the general structure shown centre, above: this is an **isometric head** attached via a **connector assembly** to a **contractile helically-constructed tail** with a rigid **core**, with **tail fibres** and **baseplate. See here for explanation of how the virus gains entry to enterobacterial cells.**

λ-like viruses - family ***Siphoviridae*** **- have thinner non-contractile tails** and **isometric heads,** with **tail fibres.** The **P22-like and Φ29-like viruses** in family ***Podoviridae*** and ***Salmonella*** **phage Epsilon 15** have **isometric** heads and **short, thick tails**.

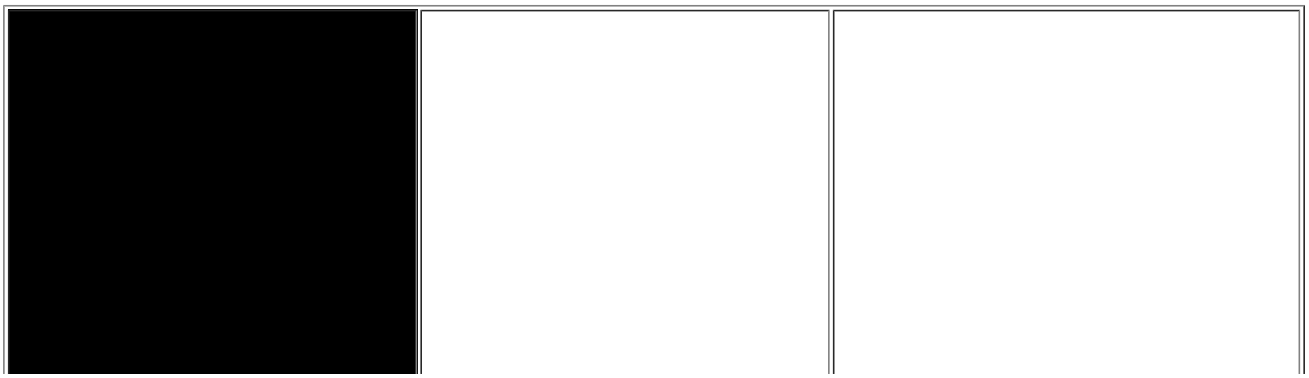



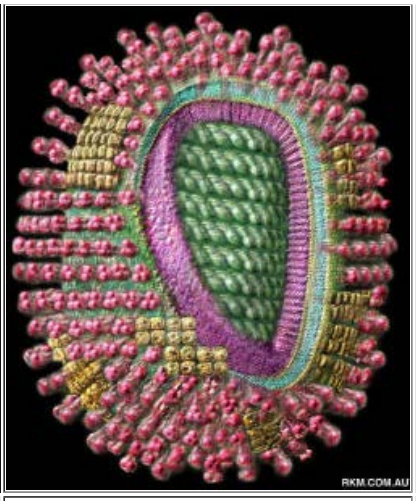
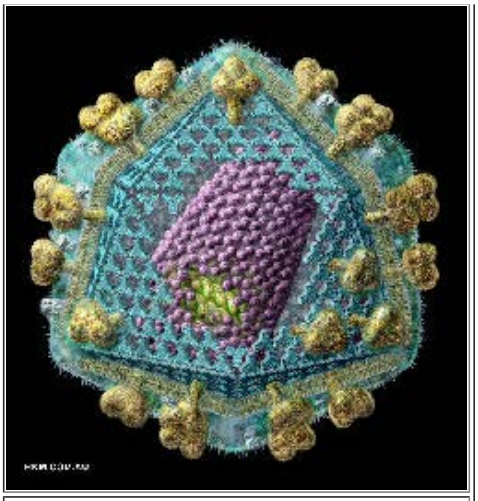
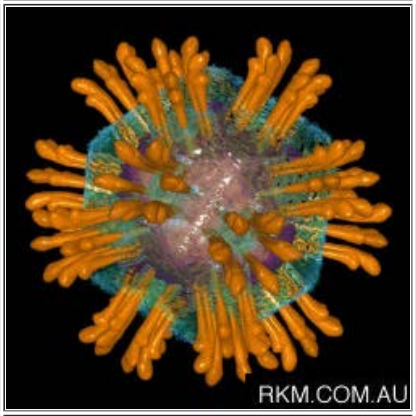
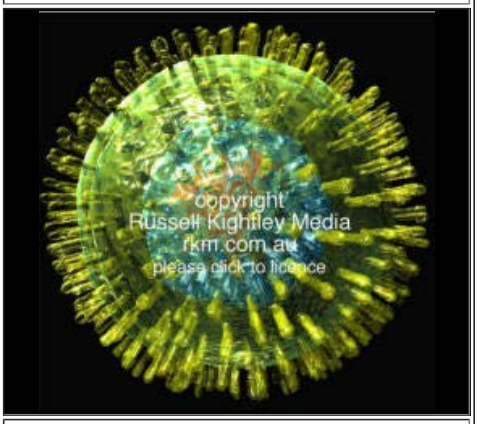
Enterobacteria phage λ	Enterobacteria phage P22	Bacillus phage Φ29	Salmonella phage Epsilon 15
copyright Russell Kightley			Reprinted by permission from Macmillan Publishers Ltd: Jiang W, et al. Nature. 2006 Feb 2;439(7076):612-6 copyright 2006

Enveloped Virions

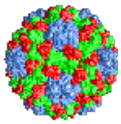
SOME virions additionally have:

- **ENVELOPES**, or **lipid bilayer MEMBRANES**: these always have **viral proteins** inserted in them (almost always **GLYCOPROTEINS**); these are often referred to as **SPIKES**.
- There is usually an **inner layer** of **protein** (usually referred to as **MATRIX** protein) inside the envelope, usually in contact with the **(nucleo)CAPSID** protein. See below for examples (West Nile virus, *Flaviridae*; influenza virus, *Orthomyxoviridae;* HIV, *Retroviridae;* SARS coronavirus; Hepatitis C flavivirus. The nucleoprotein can be helical (Ortho- and Paramyxoviridae) or isometric



		
Cutaway depiction of West Nile flavivirus: note regular arrangement of envelope protein, underlying membrane and isometric capsid	**Cutaway depiction of influenza virion: note helical nucleoprotein, matrix and envelope layers and spikes**	**Cutaway depiction of HIV virion: note conical nucleoprotein, isometric matrix and envelope layers and spikes**
		
SARSCoV (SARS coronavirus). Note elongated membrane spikes and underlying isometric capsid	**Hepatitis C flavivirus (HCV): note elongated, regularly-spaced spikes and isometric structure**	**Herpesvirus: note many envelope glycoproteins, "tegument" inside membrane, and isometric capsid**
copyright Russell Kightley (www.rkm.com.au)		

Enveloped virions may additionally incorporate **host membrane proteins** in their envelopes: while this may be a **side effect of the "budding" process** by which viral nucleoprotein complexes acquire envelopes, it may also in some cases be a **mechanism for avoidance of host immune systems** (eg: **hepatitis B virus** incorporates **host serum albumin** into its capsid; **HIV virions** may incorporate **host MHC** proteins).



Tutorial Contents

"What is a Virus?" | Isometric Virions | Helical Nucleocapsids | Compound structures

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Virus Classification

Introduction	Taxonomic Criteria	Hosts of Viruses
Cross-Kingdom Viruses	Cross-Phylum Viruses	Other Resources

Introduction

A **universal system for classifying viruses**, and a unified taxonomy, has been established by the **International Committee on Taxonomy of Viruses (ICTV)** since 1966. The system makes use of a series of ranked taxons, with the:

- - **Order (*-virales*)**being the highest currently recognised.
  - - then **Family (*-viridae*)**
    - - **Subfamily (*-virinae*)**
    - - **Genus (*-virus*)**
    - - **Species** (eg: *Tobacco mosaic virus*)

For example, the **Ebola** virus from Kikwit is classified as:

- Order *Mononegavirales*

- **Family *Filoviridae***

- **Genus *Filovirus***

- **Species: *Ebola virus Zaire***

The current (2005) Eighth Report of the ICTV lists more than **5,400 viruses in 1938 species, 287 genera, 73 families and 3 orders.**

Orders include:

- ***Caudovirales***, tailed dsDNA phages
- ***Mononegavirales***, viruses with enveloped virions with similar ss(-)RNA genomes
- ***Nidovirales***, viruses with enveloped globular virions with similar ss(+)RNA genomes

These are presumed to constitute the highest level of "**monophyletic**" groups of viruses, with a **common ancestry, so far recognised**.

A **comprehensive alphabetical list of viruses** appears at the **ICTVdb** site **here**. This includes virus-like agents.

A B C D F G H I L M N O P Q R S T U V

Unassigned Viruses Satellites Viroids Prions

Virus Orthography, or spelling and usage conventions

- The **names** of **all** taxonomic groups - orders, families, subfamilies, genera and species - are **written in italics with the first letter capitalised** (see examples above).
- **Proper nouns** in names should be **capitalised** as well: e.g. ***Semliki Forest** virus.*

This format refers only to official taxonomic entities: these are concepts, while viruses are real.

Vernacular or common-use forms of names are neither capitalised nor written in italics. Thus, while *Tobacco mosaic virus* refers to the species and *Tobamovirus* to the taxonomic genus, **tobacco mosaic viruses** or **tobamoviruses** are the entities that you work with.

Taxonomic Criteria

The most important criteria are:

Host Organism(s): eukaryote; prokaryote; vertebrate, etc.

Particle Morphology: filamentous; isometric; naked; enveloped

Genome Type: RNA; DNA; ss- or ds-; circular; linear

- although a **number of other criteria** - such as

- **disease symptoms,**
- **antigenicity,**
- **protein profile,**
- **host range,** etc.

- are important in **precise** identification, **consideration of the above three criteria** - and in many cases, just **morphology** - are sufficient in most cases to allow identification of a virus down to **familial** if not **generic** level.

An **interactive virus identification key** was developed at this site: test it and see how the criteria rapidly allow identification.

Hosts of viruses

These include all classes of cellular organisms described to date:

Prokaryotes:	**Archaea**	**Bacteria**	**Mycoplasma**	**Spiroplasma**
Eukaryotes:	**Algae**	**Plants**	**Protozoa**	**Fungi**
Invertebrates			**Vertebrates**	

This page links to a comprehensive listing of **viruses by host infected**.

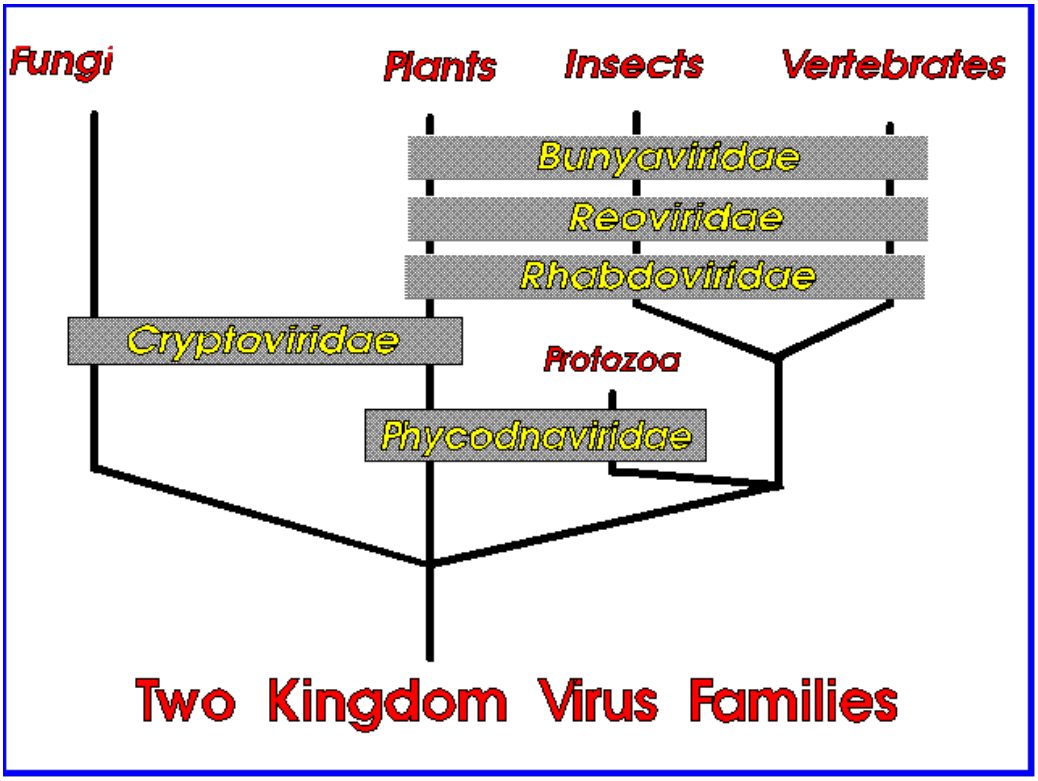
Cross-Kingdom Viruses

Certain virus families / groupings cross "kingdom" or phylum boundaries.

For example, virus families infecting two **kingdoms** of organisms are:

- Bunyaviruses: animals and plants
- Partitiviruses: plants and fungi
- Reoviruses: animals and plants
- Rhabdoviruses: animals and plants
- Phycodnaviruses: protozoa and plants
- Picornavirus-like viruses: plants and animals
- Totiviruses: protozoa / fungi and insects - tentative

The reasons for this are probably caught up in their evolution and cospeciation with their hosts (see also here).



For example, bunyaviruses and rhabdoviruses probably originated in their present form in insects, and were spread to plants and other animals after insects emerged from the seas and preyed on other terrestrial hosts.

The dsDNA cryptoviruses most probably originated in fungi, and were spread to land plants after these emerged into the terrestrial environment, as fungi began to parasitise them.

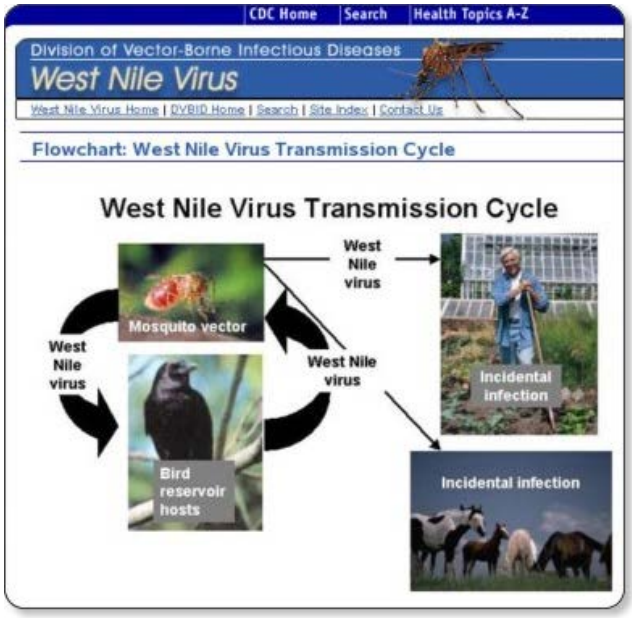
The phycodnaviruses - all of which are found in aquatic environments - could well have started out in the progenitors of protozoa and aquatic / marine plants.

Cross-Phylum Viruses

Virus families infecting across different phyla (all infecting insects and vertebrates) are:

- Flaviviruses
- Iridoviruses
- Parvoviruses
- Poxviruses
- Togaviruses

These viruses almost certainly spread from insects to vertebrates, after vertebrates joined insects on dry land and insects began to feed on them. An example here is West Nile flavivirus, which infects mosquitoes and birds, and can be transmitted to humans and other animals as well.



CDC: West Nile Virus - Flowchart: West Nile Virus Transmission Cycle via kwout

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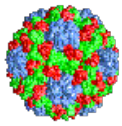
Other Resources:

An excellent resource for virus taxonomy is the **International Committee on Virus Taxonomy (ICTV) Database**, the **ICTVdB**, maintained by **Cornelia Büchen-Osmond** and **others**.

A very good set of pages can be found at the **MicrobiologyBytes.com** site.

The ICTV virus database (ICTVdb) has a mirror Web site: visit it at the **National Center for Biotechnology Information** server.



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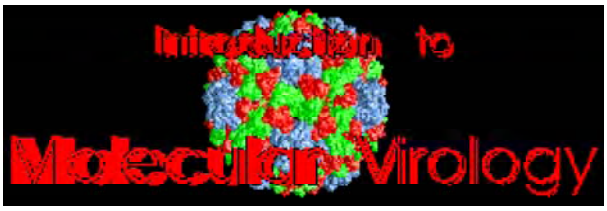

Contents:

This page contains links to Virology teaching material for University of Cape Town Molecular & Cell Biology Department courses MCB2016F (2nd year BSc) and MCB3024S (3rd year). They are free for anyone to use, the only stipulation being acknowledgement by lecturers / teachers of the source of the material.

Course Material	Level (yr)	Recommended Reading Mater
What is a Virus?	2	Origins of Viruses
Virions	2	Emerging Viruses and other Virolog Essays
Classification of Viruses	2	
Viral Genome Diversity	2/3	External Resources
Links to Virus Ultrastructure and Viruses in Cell Culture	3	Mirrored Virology Course From University of Calgary
Virus Entry Into Cells	3	Virology resources at the University Leicester
Replication of Viruses	3	Other Teaching Material Links
- Replication of RNA Viruses	3	
MCB2016F STUDY NOTES VERSION	2	
MCB3024S STUDY NOTES VERSION	3	
MCB3024S Extra Material	3	Highly Recommended General Microbiology News
The Viroblogy Site		MicrobiologyBytes

Note:

This tutorial draws heavily on print published as well as electronically published work, as well as on things I and others have written that have not been published.

This was originally written as a supplement to my **Introduction to Virology** 7-lecture course to second-year Microbiology major students (MIC202S) in the **Microbiology Department** at the **University of Cape Town.** It now constitutes the basis of a 7-lecture course in introductory microbiology (**MCB2016F**) and a 20-lecture course (**MCB3024S, Defence and Disease**) given to 3rd year students.

I hope you find it useful.

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This Version: 16th May, 2008

What is a Virus?

DEFINITIONS
VIRIONS
OTHER VIRUS-LIKE AGENTS

Viruses

Viruses may be **defined** as **acellular organisms** whose **genomes** consist of **nucleic acid**, and which obligately **replicate inside host cells** using **host metabolic machinery** and **ribosomes** to form a **pool of components** which assemble into particles called **VIRIONS**, which serve to **protect the genome** and to **transfer it to other cells**.

They are distinct from other so-called **VIRUS-LIKE AGENTS** such as **VIROIDS and PLASMIDS and PRIONS**

Alternative definitions:

S Luria *et al*. AJ Cann

And EP Rybicki, ©2008:

A virus is an infectious acellular entity composed of compatible genomic components derived from a pool of genetic elements.

The concept of a virus as an organism challenges the way we define life:

- viruses do not **respire**,
- nor do they display **irritability**;
- they do not **move**
- and nor do they **grow**,
- however, they do most certainly **reproduce**, and may adapt to new hosts.

By older, more **zoologically and botanically** biased criteria, then, **viruses are not living**. However, this sort of argument results from a **"top down"** sort of definition, which has been modified over years to take account of **smaller and smaller things** (with fewer and fewer legs, or leaves), until it has met the ultimate **"molechisms" or "organules"** - that is to say, **viruses** - and has proved inadequate.

If one **defines life** **from the bottom up** - that is, from the **simplest forms capable of displaying the most essential attributes of a living thing** - one very quickly realises that the only real criterion for life is:

The ability to replicate

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and that only systems that contain nucleic acids - in the natural world, at least - are capable of this phenomenon. This sort of reasoning has led to a new definition of organisms:

"An organism is the unit element of a continuous lineage with an individual evolutionary history."

The key words here are **UNIT ELEMENT**, and **INDIVIDUAL**: the thing that you see, now, as an organism is merely the **current slice in a continuous lineage**; the individual evolutionary history denotes the **independence of the organism over time**. Thus, **mitochondria and chloroplasts and nuclei and chromosomes** are not organisms, in that together they constitute a continuous lineage, but separately have no possibility of survival, despite their independence before they entered **initially symbiotic**, and then **dependent** associations.

The concept of replication is contained within the concepts of individual viruses constituting continuous lineages, and having an evolutionary history.

Thus, given this sort of lateral thinking, **viruses become quite respectable as organisms:**

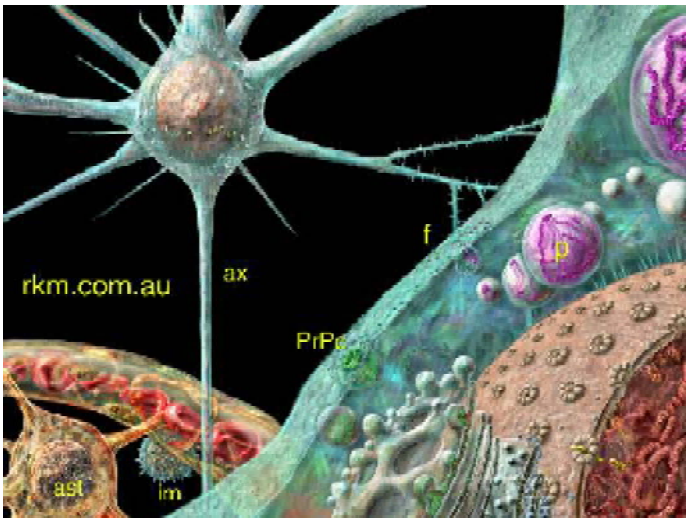
- they most definitely **replicate**,
- their **evolution** can (within limits) be **traced quite effectively**, and
- they are **independent in terms of not being limited to a single organism as host**, or even necessarily to a single species, genus or phylum of host.

More views on viruses and their definition:

Other Autonomous or Semi-Autonomously Replicating Genomes

There are a number of types of genomes which have some sort of independence from cellular genomes: these include "retrons" or **retrotransposable elements**, bacterial and fungal (and eukaryotic organelle) plasmids, satellite nucleic acids and satellite viruses which depend on helper viruses for replication, and viroids. A new class of agents - PRIONS - appear to be "proteinaceous infectious agents" (see also here for an ICTV description, here for some local information and more links).

Retrons	**Plasmids**	**Satellite Nucleic Acids**
Satellite Viruses	**Viroids**	**Prions**



Depiction of Prions infecting Neurons: from Russell Kightley Media

Plasmids

Plasmids may **share a number of properties** with viral genomes - **including modes of replication**, as in **ss circular DNA plasmids and viruses** - but are **not pathogenic** to their host organisms, and are **transferred by conjugation** between cells rather than by **free extracellular particles**.

Satellite Nucleic Acids

Certain viruses have associated with them nucleic acids that are **dispensable in that they are not part of the genome**, which **have no (or very little) sequence similarity** with the viral genome, yet **depend on the virus for replication**, and are **encapsidated by the virus**. These are mainly **associated with plant viruses** and are generally **ssRNA**, both linear and circular - however, a **circular ssDNA satellite** of a plant

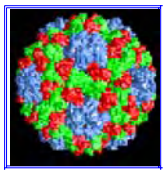
geminivirus has recently been found.

Satellite Viruses

There are also **viruses which depend for their replication on** HELPER VIRUSES: a good example is **tobacco necrosis satellite virus (sTNV)**, which has a **small piece of ssRNA which codes only for a capsid protein**, and depends for its replication on the presence of TNV. Another good example is the **hepatitis delta agent with its circular ssRNA genome.** The **adeno-associated viruses** (AAVs) are also satellite viruses dependent on the linear dsDNA **adenoviruses** for replication, but which have **linear ssDNA genomes** and appear to be **degenerate or defective parvoviruses.**

Viroids

Viroids are **small naked circular ssRNA genomes** which appear **rodlike** under the EM, which are **capable of causing diseases in plants**. They **code for nothing but their own structure**, and are **presumed to replicate by somehow interacting with host RNA polymerase**, and to **cause pathogenic effects by interfering with host DNA/RNA metabolism** and/or transcription. A **structurally similar disease agent** in humans is the **hepatitis B virus-dependent hepatitis delta agent**, which additionally codes for a structural protein.



Back to "What is a Virus"?

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Retroid Elements and Retroviruses

Retroviridae [ssRNA(+) viruses replicating via a longer-than-genome-length dsDNA intermediate], ***Hepadnaviridae***, **caulimoviruses and badnaviruses** [family **Caulimoviridae**, gapped circular dsDNA viruses replicating via longer-than-genome-length RNA intermediates] all share the unlikely attribute of **the use of an enzyme complex consisting of a RNA-dependent DNA polymerase/RNAse H in order to replicate**. They share this attribute with several **retrotransposons**, which are eukaryotic transposable cellular elements with striking similarities with retroviruses [such as the yeast Ty element, the mammalian LINE-1 elements, and the Drosophila copia element]; and with **retroposons**, which are eukaryotic elements which transpose via RNA intermediates, but share **no obvious genomic similarity with any viruses other than the reverse transcriptase**.

Bacteria such as *E coli* also have reverse- transcribing transposons -known as *retrons* - but these are very different to any of the eukaryotic types while **preserving similarities in certain of the essential reverse transcriptase sequence motifs**.

All of these elements are collectively known as **RETROELEMENTS**; the fact that the reverse transcriptases of all of them have some amino acid identity suggests a common evolutionary origin.

Several reviewers have pointed out that **just such an enzyme as reverse transcriptase** would have been necessary for the **transition from what is widely believed to have been an RNA world** - that is, where all the extant organsisms had RNA genomes - to the present world in which **all cellular organisms have DNA genomes**.

Viruses with RNA genomes which use RNA-dependent RNA polymerases for their replication may be the **only remnants of that pre-DNA era;** however, **cellular elements and viruses which use reverse transcriptase** may share a **common origin as cell-derived "modules"** coding for a reverse transcriptase, which **evolved to become retrons and retroposons and retrotransposons**. Addition of structural proteins may have allowed evolution of **retroviruses**.

The evolution of the DNA retroviruses - *Hepadnaviridae*, caulimo- and badnaviruses - is more obscure; it appears as though these **arose from retrotransposon-like sequences**, but this probably occurred near the origin of of these types of element as they are **so diverse in sequence and genome organisation**.

It is believed that retrotransposons may contribute substantially to the evolution of their hosts. Evidence for this has been obtained by studying human LINE-1s (Long Interspersed Nuclear Elements) - a group of retrotransposable elements which make up approximately 15 % of the human genome. The vast majority of LINE-1s are no longer retrotransposition competent and it is believed that in humans only between 30 and 60 full length LINE-1s are currently active. There is strong evidence from sequences in the

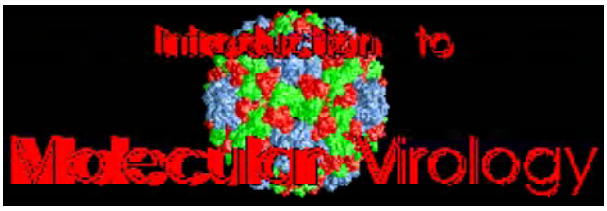
sequence databases to suggest that active LINE-1s play an important role in "exon shuffling" (belived to be the major mechanism of macro-evolution whereby entirely new genes are created by reshuffling the components of older genes). The most compelling evidence that LINE-1s do facilitate exon shuffling, however, is the experimental demonstration that they are not only able to move large amounts of non-LINE-1 exonic DNA but also insert this DNA into unrelated expressed genes to obtain chimeras which encode active hybrid gene products.

References:

Chapter 7: "Evolution by transposition" (pp. 172-203) in "Fundamental of Molecular Evolution" by Wen-Hsiung Li and Dan Graur; Sinauer Associates, Inc., Sunderland, Mass., 1991

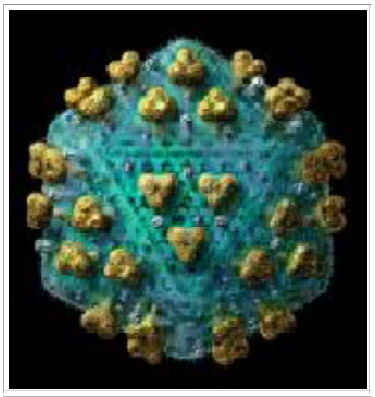
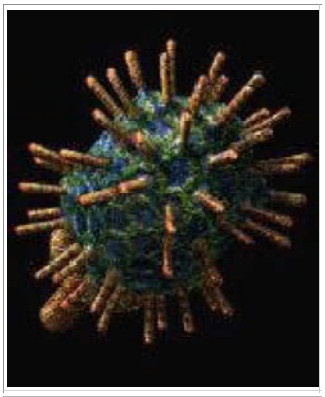
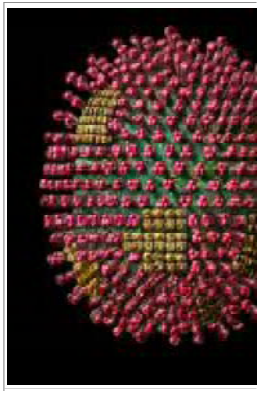
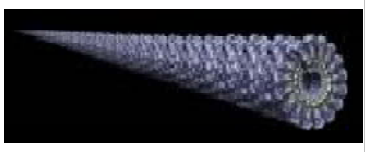
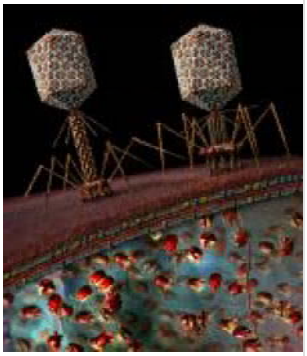
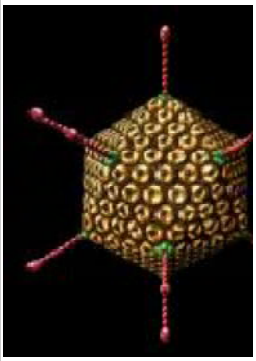
Moran, J. V., R. J. DeBerardinis, and H. H. Kazazian. 1999. Exon shuffling by L1 Retrotransposition. Science 283:1530-1534.

Return to Origins of Viruses



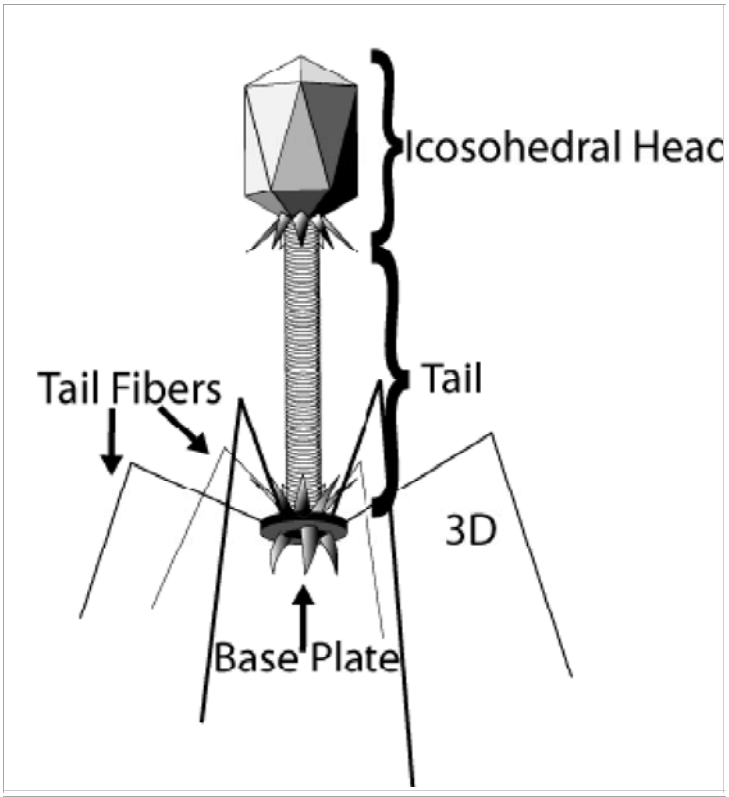
WHAT ARE VIRIONS?

VIRIONS are **virus particles**: they are the **INERT CARRIERS** of the genome, and are **ASSEMBLED** inside cells, from **virus-specified components**: they do not **GROW**, and do not form by **DIVISION**.

			
HIV	**Phage Φ29**	**Phage P22**	**Influenza**
			
Tobacco mosaic	**Filamentous phage**	**T4 phage**	**Adenovirus**
			All pictures copyright Russell Kightley www.rkm.com

They may be regarded as the **EXTRACELLULAR PHASE** of the virus: they are exactly analogous to **"spacecraft"** in that they **take viral genomes from cell to cell**, and they **protect the genome** in inhospitable environments in which **the virus cannot replicate**.

Note the strong resemblance between a bacterial virus - T2, T4 or T6 phage of *E coli*, which evolved possibly billions of years ago - and the only human-crewed spacecraft to have landed on another planet.



T-Even phage structure - copied from Wikipedia

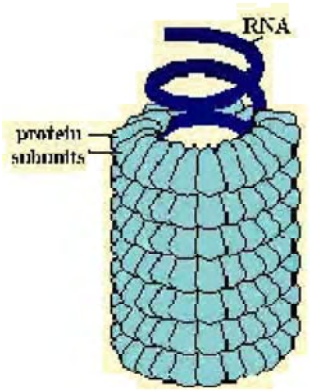
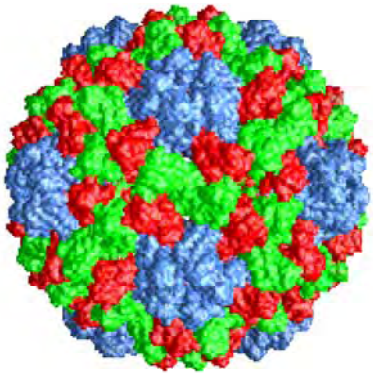
Lunar excursion module (LEM) o NASA

Basically, all virions have:

- **genomic nucleic acid**: this may be **RNA or DNA, ss or ds**, and may be **all or part** of the genome (see also **here**)
- **protein** associated with the **genome**: this may be in the form of a **helically-assembling** or **isometrically-assembling** **SIMPLE NUCLEOCAPSID** only, or
- **more complex structures with two or more layers of protein**, with **nucleoprotein** innermost.

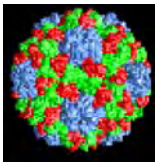
If the virions are simple **NUCLEOPROTEINS** - that is, **contain only nucleic acid and protein** - then they are usually composed **ONLY** of virus-specified components. However, **certain host components may be "trapped" within virions**, such as **POLYAMINES**: these are **polycationic compounds which serve to neutralise charge on the viral nucleic acid** as it is packed into the **CAPSID**, or protein coat. **PAPOVAVIRUSES** may in addition **encapsidate host histones** associated in **NUCLEOSOME** complexes with virus genomic DNA.

The essentials of virion structure may be seen by clicking on the images below.

			
Simple helical structures	Simple isometric structures	Compound structures	E

Excellent electron micrographs of virions - and an **explanation of their basic structure** - may be seen **at the University of Cape Town's Medical Microbiology Division teaching pages.**

For other **excellent representations of virus structure**, go to the University of Wisconsin's Institute for Molecular Virology, where **Jean-Yves Sgro** has assembled a gallery of **3-D image reconstructions of different viruses**, together with explanations of structure.



Tutorial Contents

"What is a Virus?"

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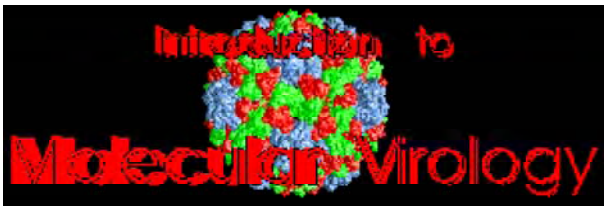


Virus Classification

Introduction	Taxonomic Criteria	Hosts of Viruses
Cross-Kingdom Viruses	Cross-Phylum Viruses	Other Resources

Introduction

A **universal system for classifying viruses**, and a unified taxonomy, has been established by the **International Committee on Taxonomy of Viruses (ICTV)** since 1966. The system makes use of a series of ranked taxons, with the:

- - **Order (-*virales*)**being the highest currently recognised.
  - - then **Family (-*viridae*)**
    - - **Subfamily (-*virinae*)**
      - - **Genus (-*virus*)**
        - - **Species** (eg: *Tobacco mosaic virus*)

For example, the **Ebola** virus from Kikwit is classified as:

- Order *Mononegavirales*

- **Family *Filoviridae***

- **Genus *Filovirus***

- **Species: *Ebola virus Zaire***

The current (2005) Eighth Report of the ICTV lists more than **5,400 viruses in 1938 species, 287 genera, 73 families and 3 orders.**

Orders include:

- ***Caudovirales***, tailed dsDNA phages
- ***Mononegavirales***, viruses with enveloped virions with similar ss(-)RNA genomes
- ***Nidovirales***, viruses with enveloped globular virions with similar ss(+)RNA genomes

These are presumed to constitute the highest level of "**monophyletic**" groups of viruses, with a **common ancestry, so far recognised**.

A **comprehensive alphabetical list of viruses** appears at the **ICTVdb** site **here**. This includes virus-like agents.

A B C D F G H I L M N O P Q R S T U V

Unassigned Viruses Satellites Viroids Prions

Virus Orthography, or spelling and usage conventions

- The **names** of **all** taxonomic groups - orders, families, subfamilies, genera and species - are **written in italics with the first letter capitalised** (see examples above).
- **Proper nouns** in names should be **capitalised** as well: e.g. ***Semliki Forest*** *virus.*

This format refers only to official taxonomic entities: these are concepts, while viruses are real.

Vernacular or common-use forms of names are neither capitalised nor written in italics. Thus, while *Tobacco mosaic virus* refers to the species and *Tobamovirus* to the taxonomic genus, **tobacco mosaic viruses** or **tobamoviruses** are the entities that you work with.

Taxonomic Criteria

The most important criteria are:

Host Organism(s): eukaryote; prokaryote; vertebrate, etc.

Particle Morphology: filamentous; isometric; naked; enveloped

Genome Type: RNA; DNA; ss- or ds-; circular; linear

- although a **number of other criteria** - such as

- **disease symptoms,**
- **antigenicity,**
- **protein profile,**
- **host range,** etc.

- are important in **precise** identification, **consideration of the above three criteria** - and in many cases, just **morphology** - are sufficient in most cases to allow identification of a virus down to **familial** if not **generic** level.

An **interactive virus identification key** was developed at this site: test it and see how the criteria rapidly allow identification.

Hosts of viruses

These include all classes of cellular organisms described to date:

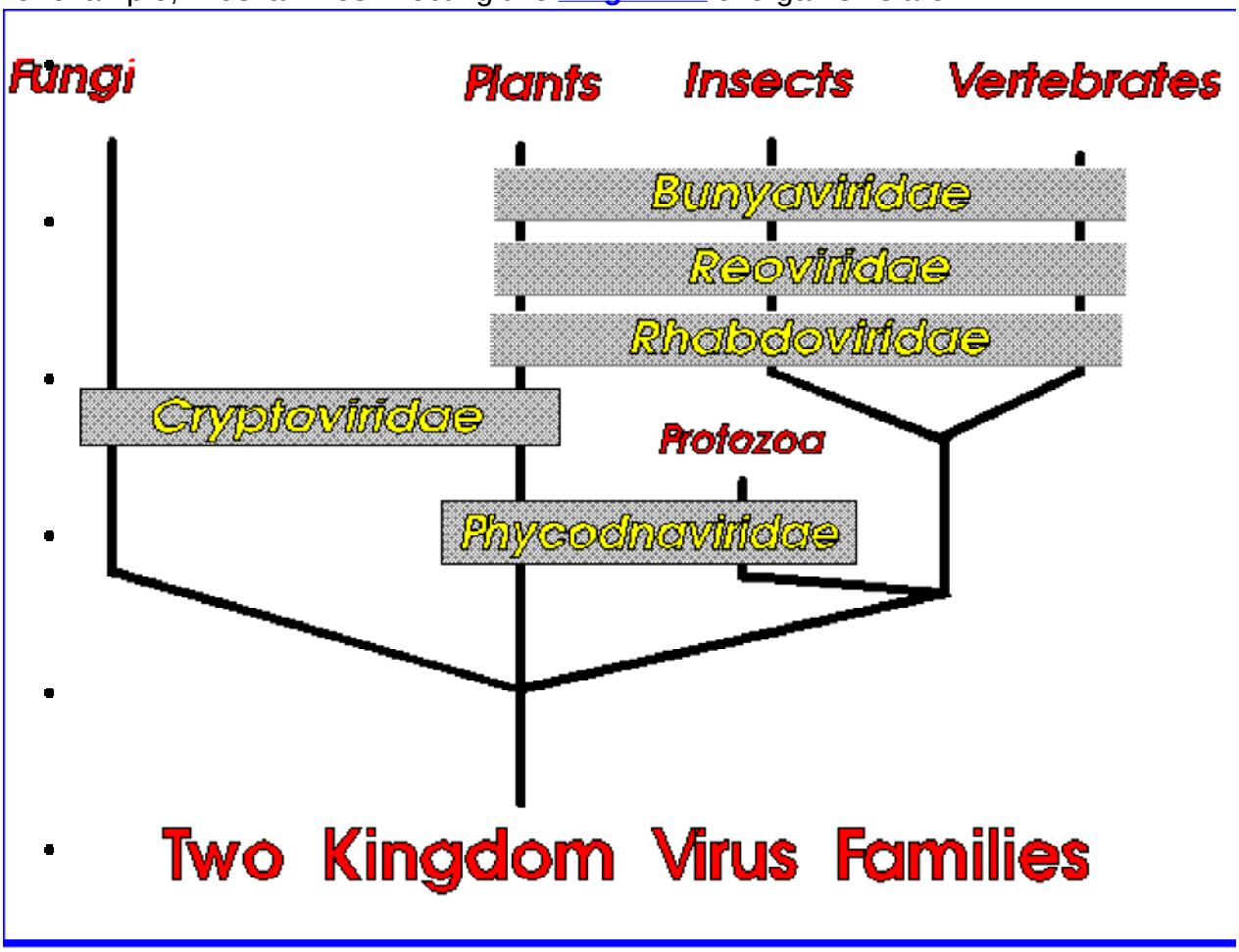
Prokaryotes:	**Archaea**	**Bacteria**	**Mycoplasma**	**Spiropla**
Eukaryotes:	**Algae**	**Plants**	**Protozoa**	**Fung**
Invertebrates			**Vertebrates**	

This page links to a comprehensive listing of **viruses by host infected**.

Cross-Kingdom Viruses

Certain virus families / groupings cross "kingdom" or phylum boundaries.

For example, virus families infecting two **kingdoms** of organisms are:



plants
and animals
- Totiviruses: protozoa / fungi and insects - tentative

The reasons for this are probably caught up in their evolution and cospeciation with their hosts (see also here).

For example, bunyaviruses and rhabdoviruses probably originated in their present form in insects, and were spread to plants and other animals after insects emerged from the seas and preyed on other terrestrial hosts.

The dsDNA cryptoviruses most probably originated in fungi, and were spread to land plants after these emerged into the terrestrial environment, as fungi began to parasitise them.

The phycodnaviruses - all of which are found in aquatic environments - could well have started out in the progenitors of protozoa and aquatic / marine plants.

Cross-Phylum Viruses

Virus families infecting across different phyla (all infecting insects and vertebrates) are:

- Flaviviruses
- Iridoviruses
- Parvoviruses
- Poxviruses
- Togaviruses

These viruses almost certainly spread from insects to vertebrates, after vertebrates joined insects on dry land and insects began to feed on them. An example here is West Nile flavivirus, which infects mosquitoes and birds, and can be transmitted to humans and other animals as well.

Introduction to Molecular Virology

What is a Virus?

DEFINITION

VIRIONS

OTHER VIRUS-LIKE AGENTS

Viruses

Viruses may be **defined** as **acellular organisms** whose **genomes** consist of **nucleic acid**, and which obligately **replicate inside host cells** using **host metabolic machinery** and **ribosomes** to form a **pool of components** which assemble into particles called **VIRIONS**, which serve to **protect the genome** and to **transfer it to other cells**.

They are distinct from other so-called **VIRUS-LIKE AGENTS** such as **VIROIDS and PLASMIDS and PRIONS**

Alternative definitions:

Viruses 1:

"Viruses are entities whose genomes are elements of nucleic acid that replicate inside living cells using the cellular synthetic machinery, and cause the synthesis of specialised elements [virions] that can transfer the genome to other cells".

SE Luria, JE Darnell, D Baltimore and A Campbell (1978). General Virology, 3rd Edn. John Wiley & Sons, New York, p2 of 578.

Viruses 2:
"Virus are submicroscopic, obligate intracellular parasites...[and]

- **Virus particles (virions) are formed from the assembly of pre-formed components;**
- **Virus particles themselves do not "grow" or undergo division;**
- **Viruses lack the genetic information which encodes apparatus necessary for the generation of metabolic energy or for protein synthesis (eg: ribosomes)".**

AJ Cann (1997). Principles of molecular virology, 2nd Edition. Academic Press, San Diego.

The concept of a virus as an organism challenges the way we define life:

- viruses do not **respire**,
- nor do they display **irritability**;
- they do not **move**
- and nor do they **grow**,
- however, they do most certainly **reproduce**, and may adapt to new hosts.

By older, more **zoologically and botanically** biased criteria, then, **viruses are not living**. However, this sort of argument results from a **"top down"** sort of definition, which has been modified over years to take account of **smaller and smaller things** (with fewer and fewer legs, or leaves), until it has met the ultimate **"molechisms" or "organules"** - that is to say, **viruses** - and has proved inadequate.

Classical Properties of Living Organisms:

- Reproduction
- Nutrition
- Respiration
- Irritability
- Movement
- Growth
- Excretion

More modern definitions include the storage and replication of genetic information as nucleic acid, and the presence of or potential for, enzyme catalysis

(Penguin Dictionary of Biology, 9th Edition, 1994)

NEWER DEFINITIONS OF LIFE

Life may (somewhat irreverently) be defined in general terms as:

"The phenomenon associated with the replication of self-coding informational systems",

or more specifically as:

"The phenomenon associated with the replication of nucleic acids".

- Rybicki EP, 1996.

Another more serious view:

"Life can be viewed as a complex set of processes resulting from the actuation of the instructions encoded in nucleic acids. In the nucleic acid of living cells these are actuated all the time; in contrast, in a virus they are actuated only when the viral nucleic acid, upon entering a host cell, causes the synthesis of virus-specific proteins. Viruses are thus "alive when they replicate in cells, while outside cells viral particles are metabolically inert and are no more alive than fragments of DNA."

- Dulbecco R and Ginsberg HS, 1980. Virology, p.854-855 (originally published as a section in Microbiology, 3rd Edn., Davis et al., Harper and Row, Hagerstown).

Copyright Ed Rybicki, October 1995; April, June,1998

If one **defines life** **from the bottom up** - that is, from the **simplest forms capable of displaying the most essential attributes of a living thing** - one very quickly realises that the only real criterion for life is:

The ability to replicate

and that only systems that contain nucleic acids - in the natural world, at least - are capable of this phenomenon. This sort of reasoning has led to a new definition of organisms:

Organism:
"An organism is the unit element of a continuous lineage with an individual evolutionary history."

SE Luria, JE Darnell, D Baltimore and A Campbell (1978). General Virology, 3rd Edn. John Wiley & Sons, New York, p4 of 578.

The key words here are **UNIT ELEMENT**, and **INDIVIDUAL**: the thing that you see, now, as an organism is merely the **current slice in a continuous lineage**; the individual evolutionary history denotes the **independence of the organism over time**. Thus, **mitochondria and chloroplasts and nuclei and chromosomes** are not organisms, in that together they constitute a continuous lineage, but separately have no possibility of survival, despite their independence before they entered **initially symbiotic**, and then **dependent** associations.

The concept of replication is contained within the concepts of individual viruses constituting continuous lineages, and having an evolutionary history.

Thus, given this sort of lateral thinking, **viruses become quite respectable as organisms:**

- they most definitely **replicate**,
- their **evolution** can (within limits) be **traced quite effectively**, and
- they are **independent in terms of not being limited to a single organism as host**, or even necessarily to a single species, genus or phylum of host.

Definitions

by EP Rybicki (1999). An Electronic Introduction to Molecular Virology. Buglet Electronic Press, Cape Town.

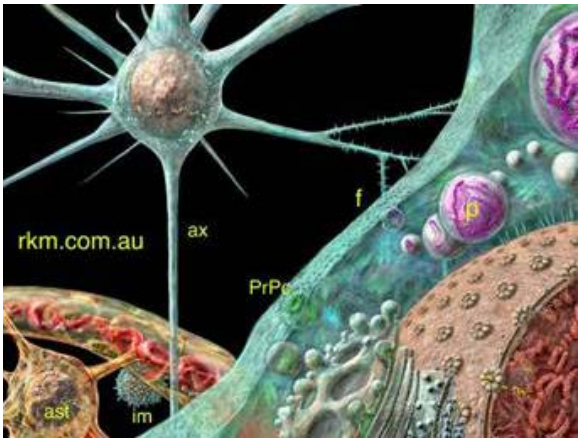
Acellular Organisms:
acellular: not composed of cells (= "bodies of protoplasm made discrete by an enveloping plasma membrane");
Penguin Dictionary of Biology, 9th Edition, 1994

Genome:
The total genetic complement of a virus. This may be composed of RNA or DNA, and be single- or double-stranded. It may also be fragmented.

Other Autonomous or Semi-Autonomously Replicating Genomes

There are a number of types of genomes which have some sort of independence from cellular genomes: these include "retrons" or **retrotransposable elements**, bacterial and fungal (and eukaryotic organelle) plasmids, satellite nucleic acids and satellite viruses which depend on helper viruses for replication, and viroids. A new class of agents - PRIONS - appear to be "proteinaceous infectious agents" (see also here for an ICTV description, here for some local information and more links).

Retrons	**Plasmids**	**Satellite Nucleic Acids**
Satellite Viruses	**Viroids**	**Prions**



Depiction of Prions infecting Neurons: from Russell Kightley Media

Plasmids

Plasmids may **share a number of properties** with viral genomes - **including modes of replication**, as in **ss circular DNA plasmids and viruses** - but are **not pathogenic** to their host organisms, and are **transferred by conjugation** between cells rather than by **free extracellular particles**.

Satellite Nucleic Acids

Certain viruses have associated with them nucleic acids that are **dispensable in that they are not part of the genome**, which **have no (or very little) sequence similarity** with the viral genome, yet **depend on the virus for replication**, and are **encapsidated by the virus**. These are mainly **associated with plant viruses** and are generally **ssRNA**, both linear and circular - however, a **circular ssDNA satellite** of a plant **geminivirus** has recently been found.

Satellite Viruses

There are also **viruses which depend for their replication on** HELPER VIRUSES: a good example is **tobacco necrosis satellite virus (sTNV)**, which has a **small piece of ssRNA which codes only for a capsid protein**, and depends for its replication on the presence of TNV. Another good example is the **hepatitis delta agent with its circular ssRNA genome.** The **adeno-associated viruses** (AAVs) are also satellite viruses dependent on the linear dsDNA **adenoviruses** for replication, but which have **linear ssDNA genomes** and appear to be **degenerate or defective parvoviruses.**

Viroids

Viroids are **small naked circular ssRNA genomes** which appear **rodlike** under the EM, which are **capable of causing diseases in plants**. They **code for nothing but their own structure**, and are **presumed to replicate by somehow interacting with host RNA polymerase**, and to **cause pathogenic effects by interfering with host DNA/RNA metabolism** and/or transcription. A **structurally similar disease agent** in humans is the **hepatitis B virus-dependent hepatitis delta agent**, which additionally codes for a structural protein.

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Retroid Elements and Retroviruses

Retroviridae [ssRNA(+) viruses replicating via a longer-than-genome-length dsDNA intermediate], ***Hepadnaviridae***, **caulimoviruses and badnaviruses** [family **Caulimoviridae**, gapped circular dsDNA viruses replicating via longer-than-genome-length RNA intermediates] all share the unlikely attribute of **the use of an enzyme complex consisting of a RNA-dependent DNA polymerase/RNAse H in order to replicate**. They share this attribute with several **retrotransposons**, which are eukaryotic transposable cellular elements with striking similarities with retroviruses [such as the yeast Ty element, the mammalian LINE-1 elements, and the Drosophila copia element]; and with **retroposons**, which are eukaryotic elements which transpose via RNA intermediates, but share **no obvious genomic similarity with any viruses other than the reverse transcriptase**.

Bacteria such as *E coli* also have reverse- transcribing transposons -known as *retrons* - but these are very different to any of the eukaryotic types while **preserving similarities in certain of the essential reverse transcriptase sequence motifs**.

All of these elements are collectively known as **RETROELEMENTS**; the fact that the reverse transcriptases of all of them have some amino acid identity suggests a common evolutionary origin.

Several reviewers have pointed out that **just such an enzyme as reverse transcriptase** would have been necessary for the **transition from what is widely believed to have been an RNA world** - that is, where all the extant organsisms had RNA genomes - to the present world in which **all cellular organisms have DNA genomes**.

Viruses with RNA genomes which use RNA-dependent RNA polymerases for their replication may be the **only remnants of that pre-DNA era;** however, **cellular elements and viruses which use reverse transcriptase** may share a **common origin as cell-derived "modules"** coding for a reverse transcriptase, which **evolved to become retrons and retroposons and retrotransposons**. Addition of

structural proteins may have allowed evolution of **retroviruses**.

The evolution of the DNA retroviruses - *Hepadnaviridae*, caulimo- and badnaviruses - is more obscure; it appears as though these **arose from retrotransposon-like sequences**, but this probably occurred near the origin of of these types of element as they are **so diverse in sequence and genome organisation**.

It is believed that retrotransposons may contribute substantially to the evolution of their hosts. Evidence for this has been obtained by studying human LINE-1s (Long Interspersed Nuclear Elements) - a group of retrotransposable elements which make up approximately 15 % of the human genome. The vast majority of LINE-1s are no longer retrotransposition competent and it is believed that in humans only between 30 and 60 full length LINE-1s are currently active. There is strong evidence from sequences in the sequence databases to suggest that active LINE-1s play an important role in "exon shuffling" (belived to be the major mechanism of macro-evolution whereby entirely new genes are created by reshuffling the components of older genes). The most compelling evidence that LINE-1s do facilitate exon shuffling, however, is the experimental demonstration that they are not only able to move large amounts of non-LINE-1 exonic DNA but also insert this DNA into unrelated expressed genes to obtain chimeras which encode active hybrid gene products.

References:

Chapter 7: "Evolution by transposition" (pp. 172-203) in "Fundamental of Molecular Evolution" by Wen-Hsiung Li and Dan Graur; Sinauer Associates, Inc., Sunderland, Mass., 1991

Moran, J. V., R. J. DeBerardinis, and H. H. Kazazian. 1999. Exon shuffling by L1 Retrotransposition. Science 283:1530-1534.

WHAT ARE VIRIONS?

VIRIONS are **virus particles**: they are the **INERT CARRIERS** of the genome, and are **ASSEMBLED** inside cells, from **virus-specified components**: they do not **GROW**, and do not form by **DIVISION**.

They may be regarded as the **EXTRACELLULAR PHASE** of the virus: they are exactly analogous to **"spacecraft"** in that they **take viral genomes from cell to cell**, and they **protect the genome** in inhospitable environments in which **the virus cannot replicate**.

Excellent examples of virions - and an **explanation of their basic structure** - may be seen **here, at the University of Cape Town's Medical Microbiology Department home page.**

For other **excellent representations of virus structure**, go to the **University of Wisconsin's Institute for Molecular Virology**, where **Jean-Yves Sgro** has assembled a gallery of **3-D image reconstructions of different viruses**, together with explanations of structure.

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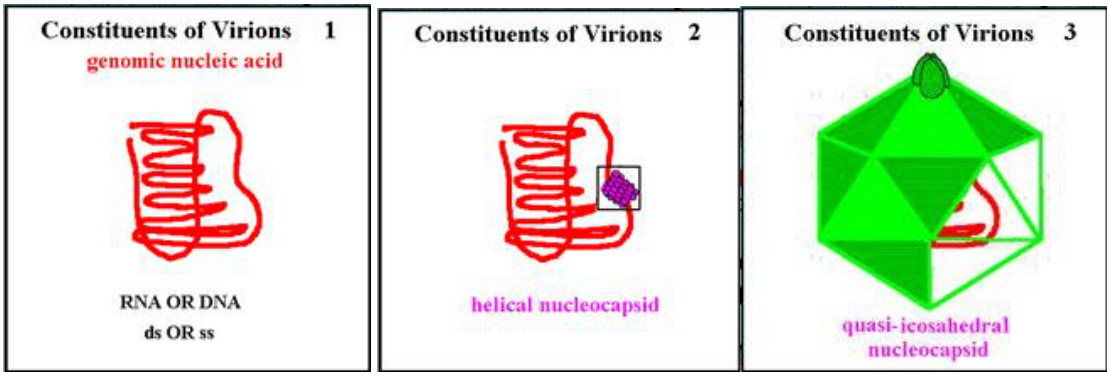
Basic Virion Constituents

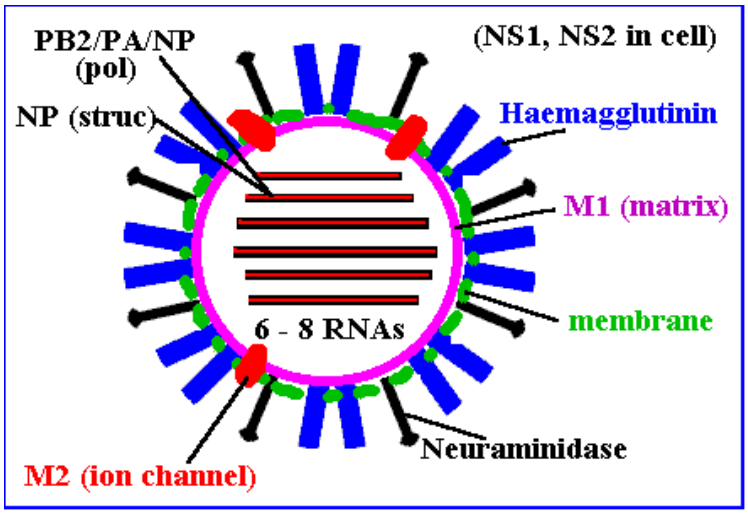


Basically, all virions have:

- **genomic nucleic acid**: this may be **RNA or DNA, ss or ds**, and may be **all or part** of the genome (see also **here**)
- **protein** associated with the **genome**: this may be in the form of a **helically-assembling** or **isometrically-assembling** **SIMPLE NUCLEOCAPSID** only, or may constitute **more complex structures with two or more layers of protein**, with nucleoprotein innermost.

SOME virions additionally have:

- **ENVELOPES**, or **lipid bilayer MEMBRANES**: these always have **viral proteins** inserted in them (almost always **GLYCOPROTEINS**); these are often referred to as **SPIKES**.
- There is usually an **inner layer** of protein (usually referred to as **MATRIX** protein) inside the envelope, usually in contact with the **CAPSID** protein. See **here** for an example (influenza virus, *Orthomyxoviridae*).

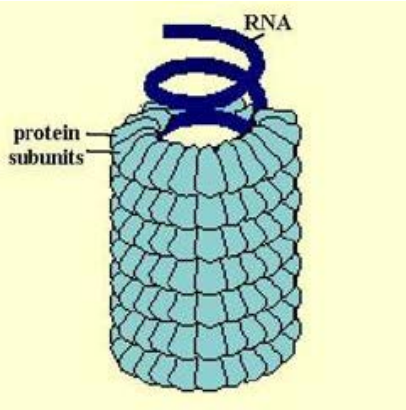


If the virions are simple **NUCLEOPROTEINS** - that is, **contain only nucleic acid and protein** - then they are usually composed **ONLY** of virus-specified components. However, **certain host components may be "trapped" within virions**, such as **POLYAMINES**: these are **polycationic compounds which serve to neutralise charge on the viral nucleic acid** as it is packed into the **CAPSID**, or protein coat. **PAPOVAVIRUSES** may in addition **encapsidate host histones** associated in **NUCLEOSOME** complexes with virus genomic DNA.

More complex virions - those with **membranes**, and/or **large genomes** - may additionally incorporate **host membrane proteins** in their envelopes: while this may be a **side effect of the "budding" process** by which viral nucleoprotein complexes acquire envelopes, it may also in some cases be a **mechanism for avoidance of host immune systems** (eg: hepatitis B virus incorporates host serum albumin into its capsid).

Helical Nucleocapsids

This is one of the **SIMPLEST FORMS** of viral capsid: the **protein** is **"wound on"** to the **viral nucleic acid** (generally **ssRNA**, though **M13** and other filamentous phage virions contain **circular ssDNA**) in a simple **HELIX**, like a screw (see the diagram for tobacco mosaic virus, below).



courtesy L Stannard

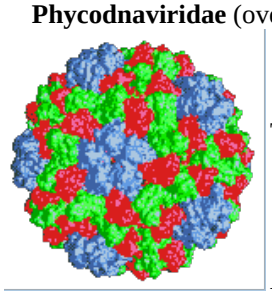
In the case of **TMV** this is the **entire virion**: this is also the case for all **RODLIKE and FILAMENTOUS** virions where no membranes are involved. This includes all **Tobamoviridae, Potyviridae, and Closteroviridae**, but NOT **Filoviridae**, like Ebola virus (see **here**).

In other cases, **filamentous helical nucleocapsids** may be **enclosed within matrix protein** and a **membrane studded with spike proteins**: excellent examples of this are **PARAMYXOVIRIDAE**, images of which can be found here, at Linda Stannard's site.

Isometric Nucleocapsids

These are built up according to **simple structural principles**, as **amply outlined here**, and in **more detail here**. Put simply, nearly **all**

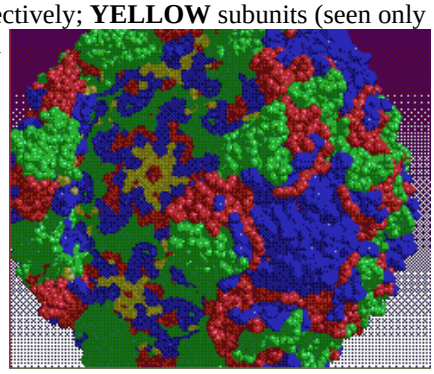
isometric virions are constructed around a **BASIC ICOSAHEDRON**, or solid with 20 equilateral trinagles for faces. It suffices to say that the **"quasi-icosahedral"** capsid is possibly **Nature's most popular means of enclosing viral nucleic acids**; they come in many sizes, from **tiny T=1 structures** (Nanoviruses, eg: banana bunchy top virus; 18 nm diameter) to **huge structures** such as those of **Iridoviridae** or **Phycodnaviridae** (over 200 nm diameter). A **good example of a simple structure** is illustrated below in the animated GIF: this shows **cowpea chlorotic mottle (CCMV) virion surface structure (courtesy J-Y Sgro)**, which is composed of **180 copies of a single coat protein molecule**.



The different colours in the picture represent **different "positional states" of the capsid protein**: subunits around **5-fold** rotational axes of symmetry are **BLUE**, and cluster as **PENTAMERS**; subunits around **3-fold axes** are **RED** and **GREEN** to reflect their different **2-fold symmetries**; they cluster as **HEXAMERS** around **"local 6-fold axes".**

Another recent example - that of **turnip yellow mosaic virus (TYMV)** - is given **here**. This has **exactly the same basic structure**, with a **single type of coat protein subunit**, only the **pentamer-hexamer clustering** is more pronounced

A **more complex capsid** - that of the common-cold-causing ***Rhinovirus*** R16 (family: ***Picornaviridae***), with **60 copies of 4 proteins in a T=3 structure** - is shown below (animation modified from one by **J-Y Sgro**). This shows a capsid with a cutaway, to reveal internal structure. **BLUE** subunits around 5-fold axes are **VP1**; **RED** and **GREEN** are **VP3** and **VP2** respectively; **YELLOW** subunits (seen only internally) are **VP4**. The VP4 subunits are formed by autocatalytic cleavage of **VP0** (into **VP2** and **VP4**) upon binding of a "**procapsid**" with **viral genomic ssRNA.**



See here for further details of picornaviruses, here for a scheme showing picornavirus assembly, and here for a scheme outlining polyprotein processing of picornaviruses, and here for material from the Leicester course.

More complex structures may be seen at Linda Stannard's Web site.

VIRUS CLASSIFICATION

CONTENTS

Introduction	Other Resources	Taxonomic Criteria
Hosts of Viruses	Cross-Kingdom Viruses	Cross-Phylum Viruses

Introduction

A **universal system for classifying viruses**, and a unified taxonomy, has been established by the **International Committee on Taxonomy of Viruses (ICTV)** since 1966. The system makes use of a series of ranked taxons, with the:

- - **Order (-*virales*)**being the highest currently recognised.
  - - then **Family (-*viridae*)**
    - - **Subfamily (-*virinae*)**
      - - **Genus (-*virus*)**
        - - **Species** (eg: tobacco mosaic virus)

For example, the **Ebola** virus from Kikwit is classified as:

- Order Mononegavirales

- Family *Filoviridae*

- Genus *Filovirus*

- Species: Ebola virus Zaire

Other Resources:

An excellent resource for virus taxonomy is the **International Committee on Virus Taxonomy (ICTV) Database**, the **ICTVdB**, maintained at the **Australian National University** by **Cornelia Büchen-Osmond** and **Mike Dallwitz**.

It is organised as a collection of several pages, accessible as an **alphabetical list of virus families** (index list mirrored locally); from **1: Adenoviridae**, to **80: Viroids**, as well as according to **nucleic acid content and strandedness** and according to **general viral host range**. Data have been entered in DELTA format, and clicking on any familial or lower taxon gives an immediate output from the database:

for example, clicking on " **03. Arenaviridae** ... **03.0.1.1.003 Lassa virus**" gives us a full description of the virus and its genome, with **links to genome database accessions**, references, and pictures if available. **An excellent place to start if you know nothing about a given disease agent.**

A good illustration of classification of viruses is the **Index Virum** page.

The ICTV proper has a new Web site too: visit it at the **National Center for Biotechnology Information** server.

The most important taxonomic criteria are:

Host Organism(s)

Particle Morphology

Genome Type

- although a number of other criteria - such as **disease symptoms, antigenicity, protein profile, host range,** etc. - are important in precise identification, consideration of the above three criteria are sufficient in most cases to allow identification of a virus down to familial if not generic level.

An **interactive virus identification key** is currently under development at this site: test it and see how the criteria rapidly allow identification.

A key developed by Claude Fauquet and included on the **ICTV 6th Report** is available at the **ICTV Web site** at the NCBI; an **updated and HTMLised version** of it is also available here.

Another site offering a virus identification resource is the **Bioinformatics Centre** at the **University of Pune, India** : their resource allows the probabilistic identification of unknown animal viruses, as well as suggesting tests to increase accuracy of identification.

Hosts of viruses include all classes of cellular organisms described to date:

General host range

Prokaryotes:	**Archaea**	**Bacteria**	**Mycoplasma**	**Spiroplasma**
Eukaryotes:	**Algae**	**Plants**	**Protozoa**	**Fungi**
Invertebrates			**Vertebrates**	

Certain virus families / groupings cross "kingdom" or phylum boundaries.

For example, virus families infecting two **kingdoms** of organisms are:

- Bunyaviridae (animals and plants)
- Partitiviridae (plants and fungi)
- Reoviridae (animals and plants)
- Rhabdoviridae (animals and plants)
- Phycodnaviridae (protozoa and plants)
- (Picornaviridae) (plants and animals - tentative)
- (Totiviridae) (protozoa / fungi and insects - tentative)

Virus families infecting across different phyla (all infecting insects and vertebrates) are:

- Flaviridae
- Iridoviridae
- Parvoviridae
- Poxviridae
- Togaviridae

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AN ILLUSTRATED TUTORIAL.

Laboratory diagnosis of viral infections frequently requires the isolation of the virus in cell cultures. Cell monolayers are inoculated with a suitable clinical specimen, and then observed for cytological changes that indicate virus growth.

Cytopathic changes

The term "*cytopathic effect*" (CPE) is frequently applied to virus-induced cellular changes that are visible by light microscopy. These changes include swelling or shrinkage of cells, the formation of multinucleated giant cells (syncytia), and the production of "inclusions" (made visible by staining) in the nucleus or cytoplasm of the infected cell.

The most efficient way to demonstrate cellular changes is by staining with chromatic dyes.
Cell monolayers are fixed and then exposed to basic and acidic dyes that accentuate the nature and location of the changes.

The use of **haemotoxylin** (basic dye) and **eosin** (acidic dye) is often referred to as
H&E staining.

The gross appearance of the cellular changes, and the location and nature of the "inclusions" - i.e. *basophilic* or *eosinophilic* - can in many instances be used as a diagnostic criterion to identify the causative virus. These will be illustrated for some of the viruses commonly isolated in cell culture:-

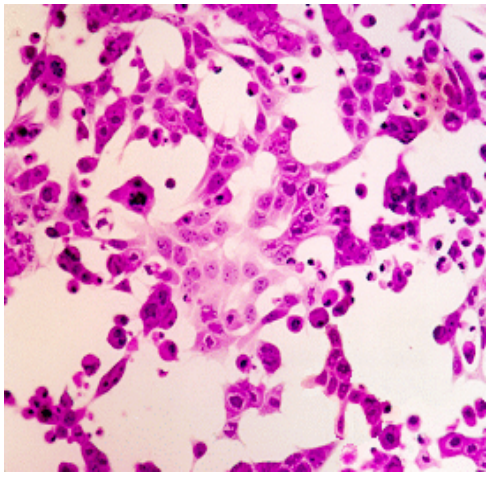
- Adenovirus - example of basophilic intranuclear inclusions
- Reovirus - example of eosinophilic intra-cytoplasmic inclusions

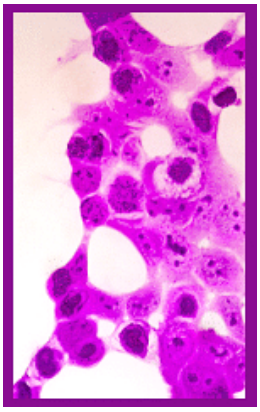
See also

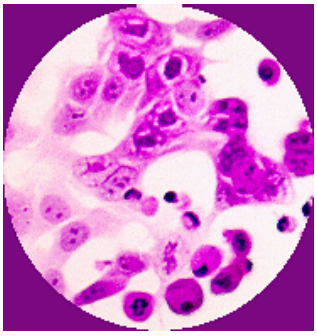
Syncytia:	The herpes group of viruses:
Measles virus **Respiratory syncytial virus** **Mumps virus**	**herpes simplex virus** **human cytomegalovirus** **varicella zoster virus**

Adenovirus

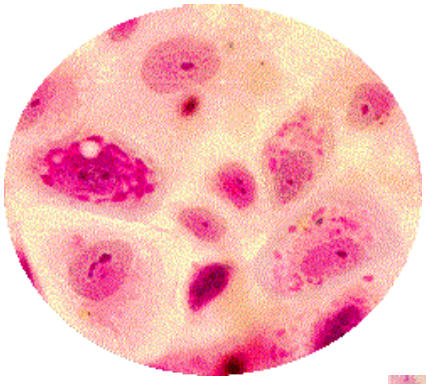
Cells infected with adenovirus have an affinity for haematoxylin (a purplish-blue dye). Infected cells become rounded and the cell sheet disintegrates. Dark basophilic inclusions within the nuclei represent accumulated viral proteins at the site of virus assembly.



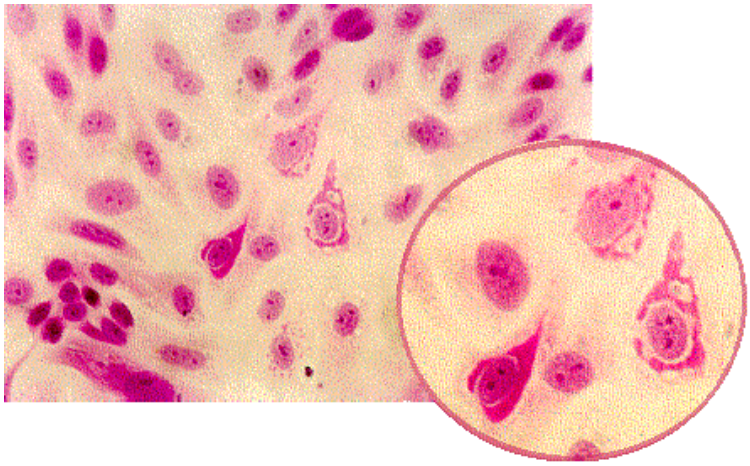




Reovirus



Replication of reovirus particles occurs in the cytoplasm of the cell, and in the final stages of assembly the virus particles bud through the endoplasmic reticulum membrane. Cytoplasmic sites of accumulated viral protein are stained with eosin (deep pink).



Go to:

Syncytia:
The herpes group of viruses:

Return to: Medical Microbiology Homepage

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Department of Medical Microbiology, University of Cape Town.
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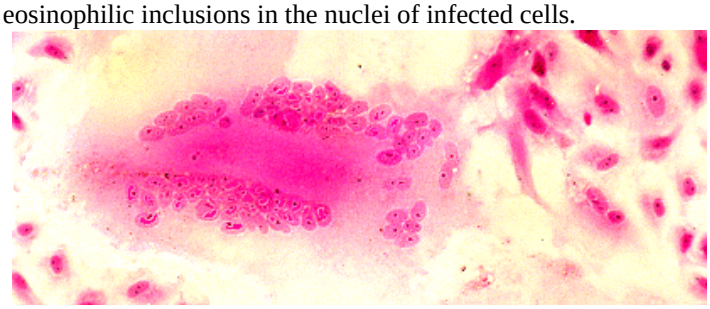
SYNCYTIA

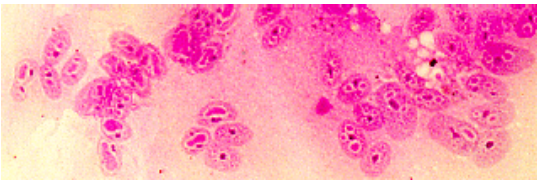
Many enveloped viruses possess a fusion protein in their envelopes. This confers the ability of the virion to fuse with the host cell membrane and thus allow entry of the infectious genomic material into the cell cytoplasm. During replication of the virus, expression of the fusion protein at the cell membrane can result in the fusion of neighbouring cells, and the formation of multi-nucleate cells or syncytia.

Measles virus

Very large syncytia can be formed during replication of measles virus in cell culture.
An additional distinguishing feature of measles is the presence of distinct eosinophilic inclusions in the nuclei of infected cells.

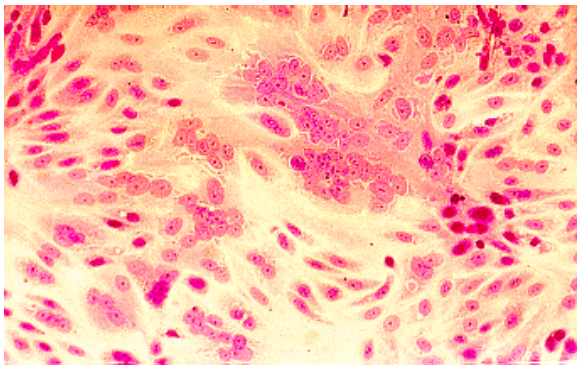
In the syncytium shown on the right, multiple nuclei are clustered around an eosinophilic cytoplasmic mass that probably represents the Golgi compartments of the fused cells.



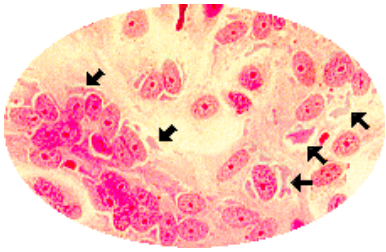


Intra-nuclear inclusions are clearly visible.

Respiratory syncytial virus

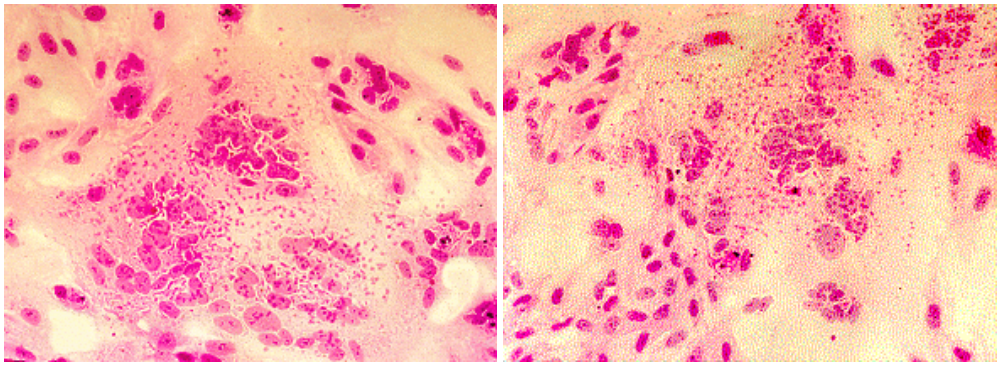


As its name implies, respiratory syncytial virus (RSV) causes large syncytia.
Nuclei do not contain inclusions, but pale eosonophilic inclusions can often be seen in the cytoplasm (see below).



Mumps virus

Mumps virus CPE is indistinguishable from that of RSV (shown above).
However, mumps virus encodes a **haemagglutin** protein which is incorporated in the virus envelope, and appears at the cell surface from which progeny virions will bud. If erythrocytes (red blood cells) are added to infected cell sheets, they will adhere to the cell surface.
This process is termed **haemadsorption**
and allows differentiation between mumps and RSV infections.



Haemadsorption of erythrocytes on the surface of cells infected with mumps virus

Go to:
The herpes group of viruses:
CPE homepage

Return to: Medical Microbiology Homepage

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Department of Medical Microbiology, University of Cape Town.
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Entrance, Entertainment and Exit: The Virus Life Cycle

Viruses have a defined "life cycle" as do any other type of organisms; however, given that they are obligate intracellular parasites, this cycle revolves around:

- **getting into a host cell**
- **replicating there, and**
- **getting out again.**

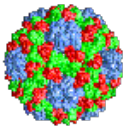
For eighteen years now I have taught this cycle under the heading

"Entrance, Entertainment, and Exit*",

as this is the best mnemonic I know to remind one of the process. Other courses tend to label these steps as (for example)

Virus Entry Into Cells
Replication of Viruses
Assembly and Release of Virions

These pages link frequently to the University of Leicester Microbiology Dept. Virology 335 course material, mirrored at this site: use the "back" button on your browser to return from a link.



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* = from the Pink Floyd song **"The Grand Vizier's Garden Party"** (Roger Waters), on the album "**Ummagumma**". Which also contains the delightful "**Several Species of Small Furry Animal Gathered Together in a Cave and Grooving With a Pict**", which title is only a little shorter than the unforgettable Hoagy Carmichaels' "**I'm a Cranky Old Yank in my Clanky Old Tank, on the Streets of Yokohama With My Honolulu Mama, Doing Those Beat-O, Street-O, Flat on my Seat-O, Hirohito Blues**"...but I digress.

Click **here** for Pink Floyd midi files to brighten your browsing.

Introduction to Molecular Virology

VIRUS ENTRY INTO CELLS

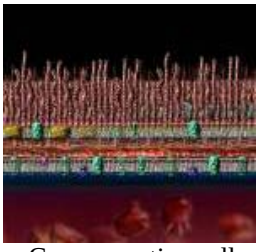
This depends largely upon the **TYPE** of the cell, and of the virus: **the cell type has a great deal of influence on the strategy the virus uses to gain access**; in turn, **specific virus types may employ different strategies to gain access to the same cell type**. However, the **greatest commonality in strategy** is probably observed between viruses infecting a **single broad type of host, defined by the nature of their cell walls**: these may be defined as:

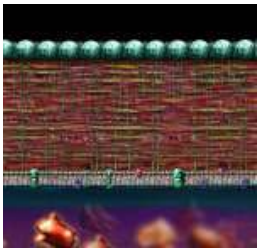
- **bacterial (eg: Eubacteria, Archaea)**
- **animal (eg: all animal cells)**
- **plant-like (eg: green multicellular plants, algae, fungi).**

Bacterial cell

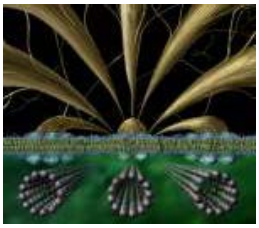
Animal cell

Plant cell


Gram-negative cell wall


Gram-positive cell wall

Animal cell wall

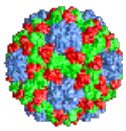

Plant cell wall

Pictures courtesy of Russell Kightley

Links which explain the basic structures of these kinds of cells may be found at the ESG Biology Hypertextbook Site at MIT and at the WWW Cell Biology Course Site.

The material here will be divided into the following headings:

1. **BACTERIAL CELL ENTRY**
2. **EUKARYOTE CELL ENTRY**
   a. **Animal Cell Entry**
   b. **Plant Cell Entry**
   c. **Fungal Cell Entry**



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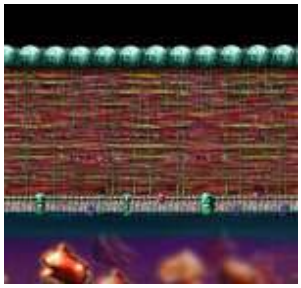
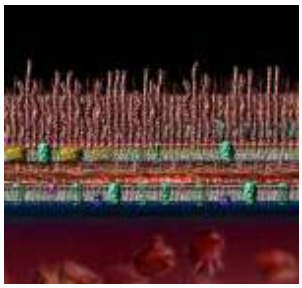
BACTERIAL CELL ENTRY

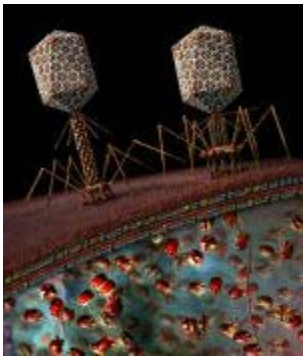
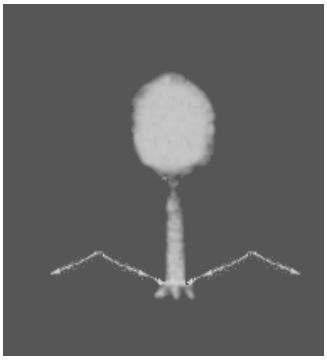
Bacterial cell walls are strong and relatively thick, to protect them from osmotic lysis and predation, and to give them shape. GRAM-POSITIVE cells have a single internal lipid bilayer, and a thick PEPTIDOGLYCAN cell wall.

Gram-positive and gram-negative cell walls
(courtesy Russell Kightley)

GRAM-NEGATIVE cells have an internal membrane, a thin peptidoglycan layer, another membrane, and often a polysaccharide-based CAPSULE.

Bacterial viruses (BACTERIOPHAGES) have therefore to have some means of breaching a quite formidable barrier if they are to enter the cell. They also generally have SPECIFIC RECEPTOR SITES on the bacteria, to which SPECIFIC ATTACHMENT PROTEINS bind: these receptor sites may be **lipopolysaccharides, cell wall proteins, teichoic acid, or flagellar or pilus proteins**.

Phage T4 - *Enterobacteria phage T4*, genus "T4-like Viruses", family *Myoviridae*, or viruses with 34-170 kbp dsDNA genomes, isometric heads and contractile tails - infects the **gram-negative bacterium *E coli*.** It has one of the more complex entry mechanisms, involving an **active injection process**. This is shared by others of the so-called **T-even phages** of the family ***Myoviridae***. The process is shown in the still image above left (courtesy Russell Kightley), and in the animated graphic above right (derived from EM images taken by L Stannard, Dept Medical Microbiology, UCT).

Click here for an non-animated view of the process if your browser does NOT support GIF animation.

The **phage tail fibres** are the **attachment sites**; these individually bind the bacterial cell surface - specifically to certain lipopolysaccharides and to the surface outer membrane protein OmpC. This is **REVERSIBLE** binding, and is probably due to **electrostatic interactions** as it is Mg2+ and Ca2+ dependent. After **TAIL FIBRE** binding has consolidated, the **BASEPLATE** then settles down onto the surface and binds firmly to it. After this occurs, a CONFORMATIONAL CHANGE takes place in baseplate and sheath protein structures, and the **TAIL SHEATH CONTRACTS**, pushing the **TAIL CORE** through the cell wall, possibly in an **ATP-driven process: this is aided by a lysozyme activity associated with the baseplate assembly**. This is an **IRREVERSIBLE** process. DNA is then extruded from the phage head. This is then used for initial transcription and virus expression.

Phage lambda - *Enterobacteria phage λ*, genus *"λ-like Viruses"*, family *Siphoviridae*, a tailed phage with an isometric head and a 49 kbp dsDNA genome - attaches to the maltose receptor on the surface of the *E coli* cell. Although the tail is non-contractile, a DNA injection mechanism similar to that of T-even phages allows entry of DNA into the cell, leaving the capsid behind.

Lambda phage infecting *E coli*
(courtesy Russell Kightley)

MS2 phage - *Enterobacteria phage MS2*, genus *Levivirus*, family *Leviviridae* - **an isometric single-stranded RNA-containing virus infecting *E coli*** - attaches to **PILIN** (the building block of PILI) via its single attachment or **A PROTEIN**. The A protein is **covalently linked** to the 5'-end of the genomic RNA; thus, when the pilus is retracted into the cell, **the A protein and RNA are pulled with it**, leaving the empty capsid outside.

Here is an animated diagram of a DNA-containing enveloped isometric phage entering a gram-negative bacterium.

Tutorial Contents	Virus Entry	Bacterial Entry	Animal Cell Entry	Plant&Fungal Cell Entry

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VIRUS ENTRY INTO CELLS

EUKARYOTE CELL ENTRY

a. Animal Cell Entry

- **Animal Cells and Vesicle Transport**

- **Direct Cell Membrane Fusion**
- **Entry via Endocytotic Vesicle**
- **Nuclear Targetting**

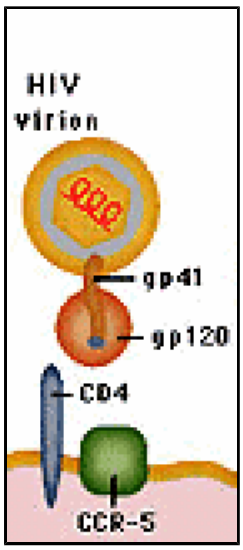
Animal Cells and Vesicle Transport

Eukaryote cells do not have cell walls; they have only lipid bilayer cell membranes (=**PLASMALEMMA**) with associated embedded proteins. They also have a very highly developed INTERNAL MEMBRANE COMPLEX, consisting of **endoplasmic reticulum**, **Golgi apparatus, nuclear membrane**, and various **specialised vesicles** such as **LYSOSOMES**, which are involved in intracellular digestion. Eukaryote cells have an intricate system of **vesicle transport** centred on the **Golgi apparatus**; this involves export of protein(s) and vesicles from the ER to the Golgi; production of EXPORT vesicles containing proteins from this for fusion with the cell membrane (=**EXOCYTOSIS**); production of LYSOSOMES to fuse with **ENDOSOMES** for digestion of material internalised by **RECEPTOR-MEDIATED** or non-specific **ENDOCYTOSIS** (PHAGOCYTOSIS for particulates; PINOCYTOSIS for liquid). Other CYTOSKELETON-DIRECTED vesicle trafficking involves targetting of vesicles back to the Golgi and to the NUCLEAR MEMBRANE.

The initially-formed **ENDOCYTOTIC** vesicles resulting from receptor-mediated endocytosis are coated with **CLATHRIN**, a structural protein which promotes curvature of the membrane at **COATED PITS** - which become vesicles as the protein continues to assemble and impresses the membrane into a sphere. Clathrin is recycled back to the cell membrane after endocytosis, either because of interaction of the vesicle with a lysosome, or due to another process.

Direct Cell Membrane Fusion

Viruses take advantage of these processes in a number of ways, after they have attached to the cell surface via **binding to a specific receptor**. The simplest is **DIRECT MEMBRANE FUSION**, where the virion membrane fuses with the cell membrane, and the virion nucleoprotein complex is delivered into the cell cytoplasm directly. This is generally a **pH-independent process**, and requires only that the **membrane be fluid** (ie: temperature in the physiological range), and generally that some divalent cations be present.

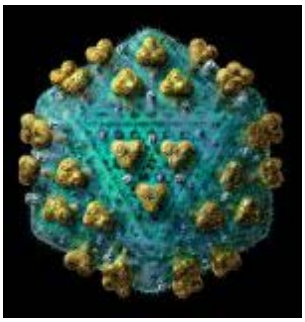


The entry process for **HIV** is shown in the graphic (from the Univ Leicester material, copyright Dr AJ Cann).

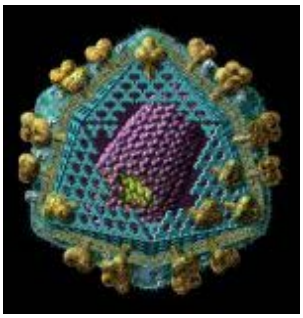
Click here for an non-animated view of the process if your browser does not support GIF animation.

Here the virion attachment protein - **gp120** - (see also **here**) attaches initially to the **CD4** protein on a helper T-cell. The gp120 undergoes conformational change due to binding, and binds the **accessory receptor** - **CCR-5,** a **chemokine** receptor in this case, although there are others - as well. **gp41** - a cleavage product of a **gp160** precursor, and a part of the **"spike protein"** of the viral membrane - is then able to bind into the cell membrane, via a hydrophobic domain. A condensation of the gp41 structure - formation of a "6 helix bundle" - causes close juxtaposition of cell and viral membranes, which promotes membrane fusion and **nucleoprotein entry into the cell. An excellent depiction of this process is given on the Roche site.**

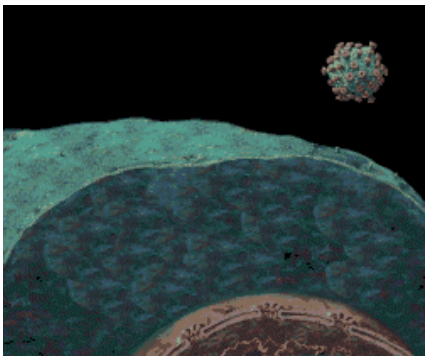
The entire HIV life cycle is shown here: graphics kindly provided by Russell Kightley.



HIV virion: outer view showing gp160



HIV virion structure: cutaway showing matrix and nucleoprotein

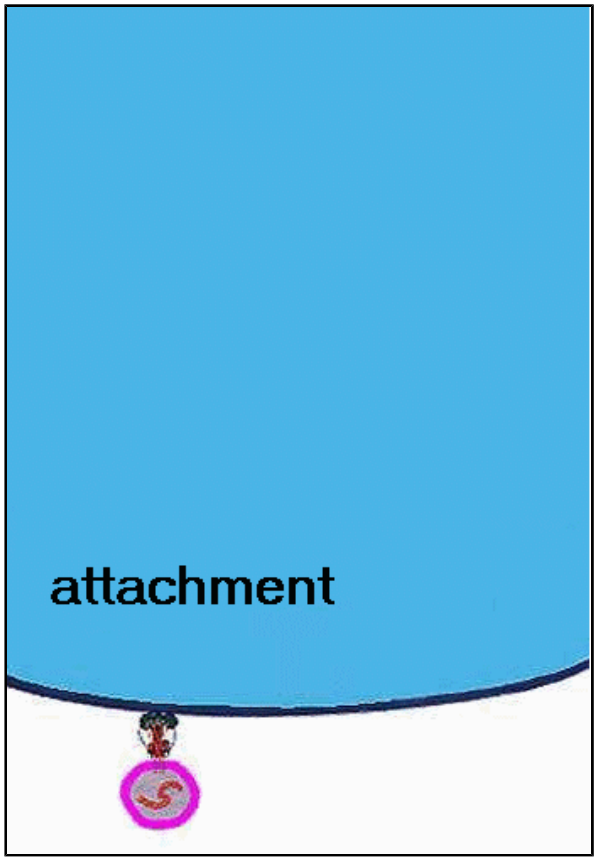


HIV cell entry and replication animation

PARAMYXOVIRIDAE are also able to enter cells in this fashion; in fact, an F or fusion (glyco)protein purified from enveloped virions of Sendai virus is often used to artificially fuse cells in *in vitro* experiments.

Entry via Endocytotic Vesicle

Another means of cell entry via membrane interactions is typified by **influenza virus**: this attaches to cell membranes via its**HAEMAGGLUTININ** (HA) protein - a trimeric attachment protein - which binds **NEURAMINIC ACID** residues on cellular glycoproteins. The process is demonstrated in the accompanying graphic (from the Univ Leicester material, copyright Dr Shaun Heaphy).



The virion binds **reversibly** to the cell surface via **one or a few attachment proteins**. Receptor consolidation results in curvature of the membrane around the particle, and **eventual formation of an endocytotic vesicle** by a "zipper" process.

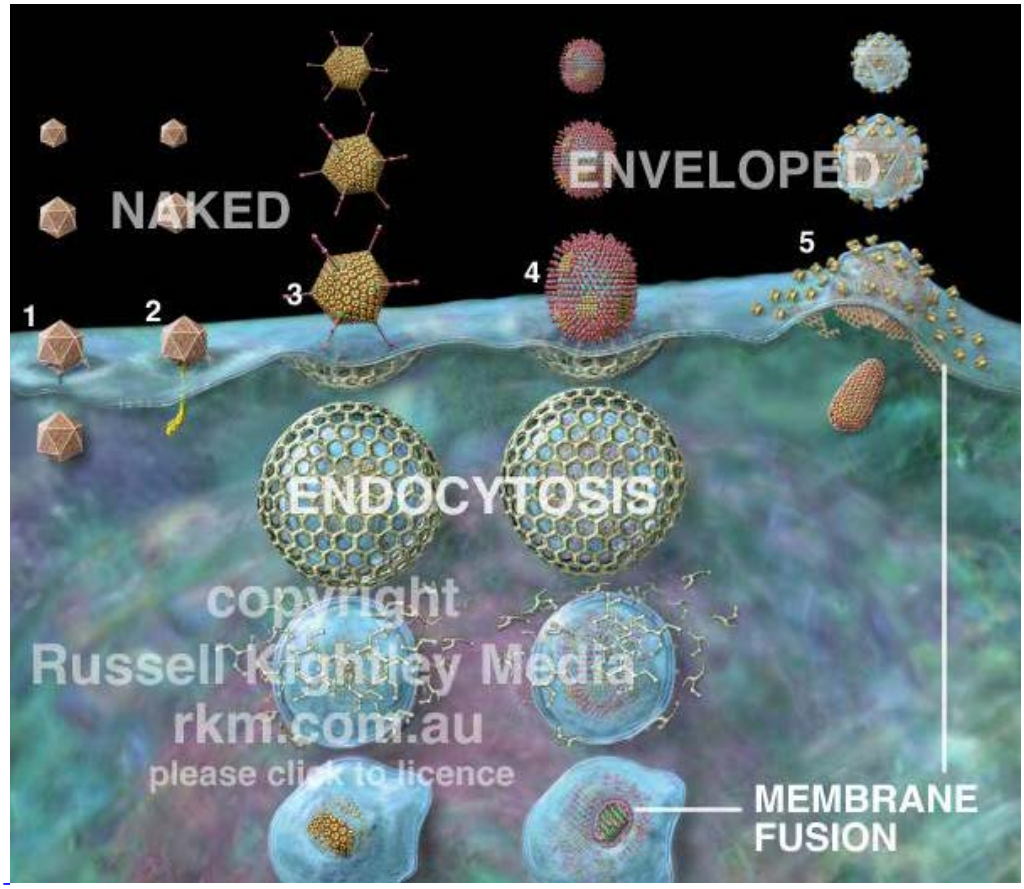
Click here for an non-animated view of the process if your browser does not support GIF animation.

A **cell surface protease cleavage** divides the HA into HA1 and HA2: this causes a conformational change which exposes a hydrophobic **"fusion domain"** on HA2. A further **pH-induced structural change** (with lysosome fusion, pH drops), allows **insertion of part of HA2** into the vesicle membrane. Another conformational change caused by **membrane interaction** results in "**apposition**" of membranes and fusion, allowing escape from the vesicle of the viral nucleoprotein, into the host cell cytoplasm. This is generally known is **pH-dependent entry**.

Many enveloped viruses share this mode of entry; so too do many non-enveloped viruses, such as the complex dsDNA **adenoviruses** (***Adenoviridae)*** and the simpler ssRNA **picornaviruses** (***Picornaviridae).*** The former appear to enter via an endocytotic vesicle, then to interact with the vesicle membrane - probably because of structural alterations due to the pH shift - so as to expel a partially-uncoated viral core structure into the cytoplasm. This is then targetted to the nucleus, where the DNA appears to enter via a nuclear pore.

Picornaviruses do something similar: that is, the capsid becomes rearranged as a result of pH-induced structural transitions, whereby the VP4 internal protein is lost and the surface becomes more lipophilic, and interacts with a vesicle membrane so as to allow exit of the RNA into the cytoplasm. A more complex process may also occur which allows **RNA to enter the cell from externally-bound virions**.

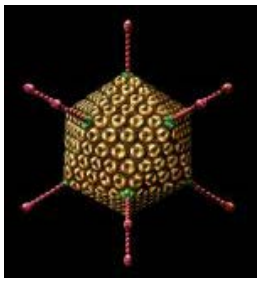
These mechanisms are summarised in this diagram: click here to link to source page.



Reoviridae - isometric dsRNA non-enveloped viruses with up to 12 genome components - enter by endocytosis, are partially uncoated in lysosomes, and then core particles enter the cytoplasm by an unknown process, perhaps similar to what occurs with adenoviruses. Core particles then function as virus transcription "factories"

Nuclear Targetting

A basic requirement in the infection of Eukarya would be that **DNA genomes end up in the nucleus** - except for **Pox-, Phyco-** and some **Baculoviridae** - and that **RNA genomes end up in the cytoplasm** - except for myxoviruses - as these are the sites where the respective viruses may be expected to replicate their genomes. DNA is its own nuclear targetting signal in that naked DNA is seen to be moved into cell nuclei if introduced into cells; however, most DNA viruses which require a nuclear replication stage seem to have some specific means of targetting partially-disassembled virions to the cell nucleus. **Nucleoproteins with nuclear localisation signals (NLS)** are very often involved.



Adenoviruses - large naked isometric virions with linear dsDNA of 30-38kbp - appear to be transported by means of the **hexon protein** of the partially degraded naked capsid that is released into the cytoplasm, interacting with **microtubules**. This reaches a nuclear pore and allows escape of viral DNA plus certain viral polypeptides into the nucleus.

Parvoviruses - small naked isometric viruses with linear ~5 kb ssDNA genomes - enter host cells by receptor-mediated endocytosis, escape from endosomal vesicles to the cytoplasm, and then replicate in the nucleus. Canine parvovirus virions at least use the motor protein **dynein** to be transported along microtubules to the nucleus.

Herpesviruses enter by fusion with the cell membrane, and the core particles migrate to nuclear pores, and release DNA there.

Poxviruses - which have "intracellular" single-enveloped and "extracellular" double-enveloped forms - may enter by **direct cell fusion** (pH-independent) or **lysosomal vesicle fusion** (pH-dependent and inhibitable by lysomotropic agents), with the latter perhaps predominating. Once core virions are in the cytoplasm, they uncoat further to expose a nucleoprotein complex which is first transcriptionally and later, replicationally, active.

Tutorial Contents	Virus Entry	Bacterial Entry	Animal Cell Entry	Plant&Fungal Cell Entry

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(Unless otherwise stated)

VIRUS ENTRY INTO CELLS

EUKARYOTE CELL ENTRY

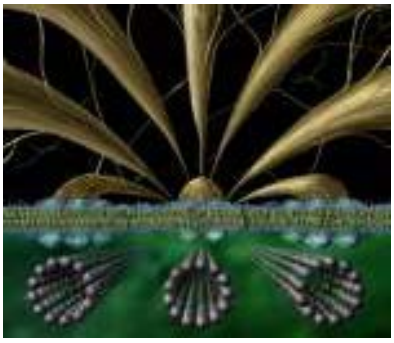
b. Plant Cell Entry
c. Fungal Cell Entry

Plant Cell Entry

Plant cells, while **superficially similar to animal cells in basic construction** as far as all organelles except **chloroplasts** and often extensive **vacuoles** are concerned (see here), have **one large and fundamental difference to animal cells**, which profoundly affects the way in which they are infected by viruses, and how viruses move between them.

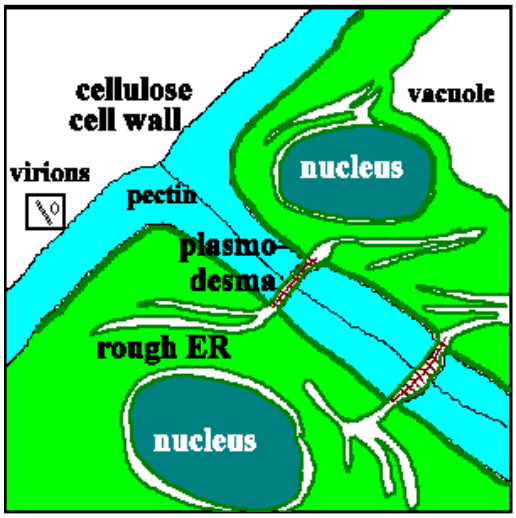
This is their possession of thick, rigid, cellulose-based cell walls.

Plant cell


Detail of cellulose

Plasmodesmata

courtesy of Russell Kightley



Every cell is separated from every other cell by cell walls, whose dimensions are far larger than the size of the average virion (ie: >1 micron, cf. TMV = 300 nm). This means that plant cells are effectively inaccessible to viruses, even given mechanisms of injection similar to the T-even phages. Live plant cells **interconnect only via specific discontinuities in the cellulose walls**: the most numerous of these are **plasmodesmata**, which are **complex structures filled with membrane-derived processes continuous with the endoplasmic reticulum**. These are **"gated" intercellular channels**, which limit the passage of molecules between cells, and **certainly do not admit particles as large as virions**. Plant viruses have therefore **evolved specific movement functions**, mediated by one or more virus-specified proteins, which interact with the plasmosdesmatal machinery so as to **increase the "pore" size**, and allow specific transport of viral nucleoprotein complexes. **All plant-infecting viruses possess one or more movement-related protein** (MP) genes: these are very varied, although there are distinct groups of them, and **they appear to derive from host plant genes for chaperonins and plasmodesmata-associated proteins.**

Plant viruses, therefore - **which are almost overwhelmingly ssRNA +ve sense and non-enveloped - do not appear to specifically interact with host cell membranes or cell walls, as do bacterial and animal viruses**; even when the plant-infecting virus also infects an animal (eg: plant **rhabdoviruses** and **bunyaviruses**) and presumably behaves normally in the other host, and even though **apparently plant cells are capable of phagocytosis / endocytosis**. The mechanisms employed to enter cells rather appear to be **passive carriage through breaches in the cell wall** in the first instance, followed by later **cell-to-cell spread** in a plant by means of **specifically-evolved "movement" functions**, and perhaps spread via **conductive tissue** as whole virions.

The "**passive carriage**" referred to above could mean:

- 1) a purely **mechanical** injury that **breaches the cell wall** and transiently breaches the plasma membrane of underlying cells;
- 2) similar gross injury due to the **mouthparts of a herbivorous arthropod**, such as a beetle;
- 3) **injection directly into cells** through the piercing mouthparts of sap-sucking insects or nematodes;
- 4) carriage into plant tissue on or in association with **cells of a fungal parasite**;
- 5) **vertical transmission** through infected seed or by vegetative propagation;
- 6) transmission via **pollen**; and
- 7) **grafting** of infected tissue onto healthy tissue.

ONE EXCEPTION to the above rule are some of the **PHYCODNAVIRIDAE**, which have **large (>300 kb) dsDNA genomes**, and **very large (130 - 200 nm), complex virions**. Exemplars which infect algae appear to have **specific enzymes at the surface of virions** which

may **degrade the CHITIN cell wall of the alga** , to allow interaction of the particle with the cell membrane directly. **Some of these viruses also appear to be able to "inject" their DNA**, much as phages do, and to enter a lysogenic state.

The mode of transmission of viruses affects their concentration and localisation in plants. For example, **mechanically** transmitted viruses (eg: *Bromoviridae*, *Tobamoviruses*) **tend to reach very high concentrations** in most tissues of a plant (up to 4g / kg plant): this is necessary for survival, as it guarantees that a large number of virions will be present for onward transmission by whatever non-specific means presents itself. **Viruses which are introduced into plants via insect vectors** with piercing mouthparts, on the other hand, tend to be **limited in their multiplication to phloem elements**, which are preferred target tissues for insect feeding. Consequently, these viruses (eg: *Luteoviruses*, *Geminiviridae*) reach only very low concentrations (mg / kg) in whole plants.

---

c. Fungal Cell Entry

There are certain superficial similarities between plants and FUNGI with respect to the cell wall; however, in the latter case, cell walls are composed of CHITIN, a different complex polysaccharide, and fungal hyphae are often effectively **"tubes" with no cross-walls**.

No fungal viruses appear to have any specific mechanisms for gaining entry to fungal cells; indeed, it is extremely difficult to demonstrate the infectivity of virus-like particles, and it is only since the advent of the GENE GUN or biolistic transformation apparatus, that many viruses have been shown to be infectious at all - **by being "shot" into fungal cells adsorbed onto metal particles**.

It is probable that most fungal viruses - like the plant-infecting ***CRYPTOVIRUS*** genus of the mainly fungus-infecting ***Partitiviridae*** - are only transmitted by "grafting", or the physical connection of infected to healthy cells by **anastomosis.** Thus, **fungal mating is a good means of transmission, as it results in the mixing of cell contents of different hyphae**.

Tutorial Contents	Virus Entry	Bacterial Entry	Animal Cell Entry	Plant&Fungal Cell Entry

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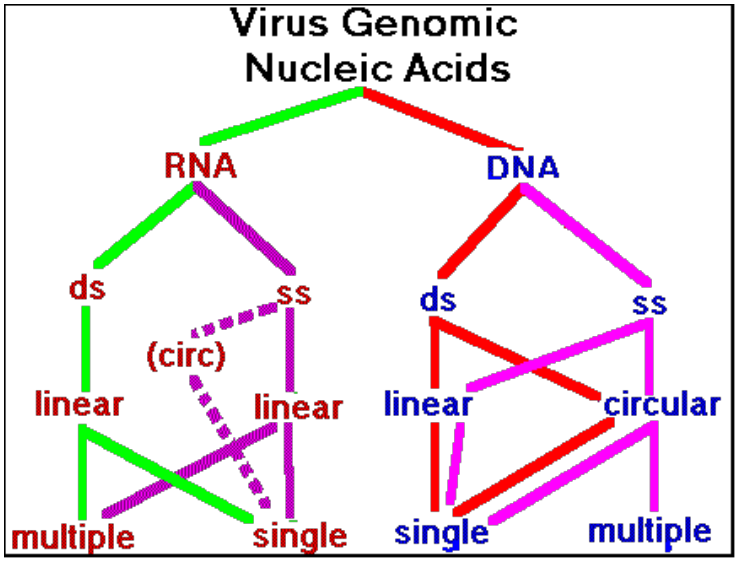
(Unless otherwise stated)

GENOME DIVERSITY



DNA						RNA			
double-stranded			single-stranded			double-stranded		single-stranded	
linear	circular		linear	circular		linear		linear (circular)*	
single	single	multiple	single	single	multiple	single	multiple	(+)sense	(-)sense
								single / multiple	single / multiple

* = viroids only

Viruses are the only organisms on this planet to still have **RNA** as their sole genetic material. They are also the only autonomously replicating organisms to have **single-stranded DNA**. The range of virus genomes as found in virions encompasses:

- **single-component dsDNA**, linear or circular (occasionally circularly permuted linear);
- single, double or multi-component **circular ssDNA**;
- single-component **linear ssDNA**;
- single or multi-component **dsRNA**;
- single or multiple component **ssRNA genomes** which may be
  - totally **"positive"**(or messenger) polarity,
  - totally **"negative"** (or anti-messenger) polarity, or
  - **partially positive negative-sense**;
- "diploid" **positive-sense ssRNA genomes which replicate via reverse transcription** to and transcription from longer-than-genome-length dsDNA, and
- nicked and/or partially **dsDNAs which replicate via transcription** to and reverse transcription from longer-than-genome-length positive-sense ssRNA.

In contrast, **prokaryotes** have **mainly single-component circular** (occasionally multiple) or **linear dsDNA (*Streptomyces*, *Helicobacter*)** while all **eukaryotes have multi-component linear dsDNA**, and all the genomes replicate **via the classic semi-conservative route**.

Viral genome types have inspired a **classification based on what is found in virions**, coupled with their replication strategy. This is the **Baltimore Classification,** and will be discussed in a broader treatment of **Virus Replication.**

An overview of the taxa Recognized in the Universal System of Virus Taxonomy in terms of their genome content may be found here.

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GENOMIC REPLICATION STRATEGIES OF VIRUSES

The old terms "eclipse phase" or "latent period" describe that part of a virus life cycle when no infectious virus can be extracted from cells which had just been exposed to infectious virions: a good illustration of the concept in terms of a virus assay experiment is shown **here**.

What happens once a virus is uncoated, or partially uncoated, depends largely upon what sort of virus it is. The **Baltimore Classification** of viruses by their genome types and replication strategies makes it fairly easy to predict the broad sort of strategy that a virus with a given genome will employ in order to get replicated. This classification was originally devised by David Baltimore; it originally only had six categories, but the discovery of **"DNA retroviruses"** in the 1980s necessitated a new Class VII.

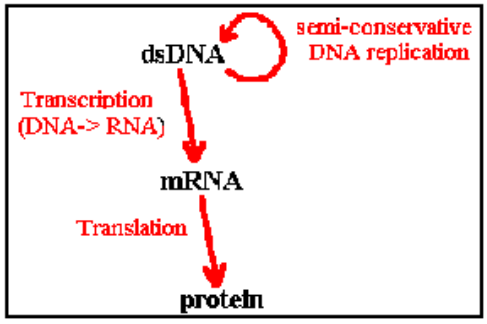
To understand the classification, one must understand how cells replicate their genomes, and express mRNAs, and proteins. This is conveniently explained by the "Watson-Crick Central Dogma", which states that:

INFORMATION FLOW IN CELLS GOES FROM:

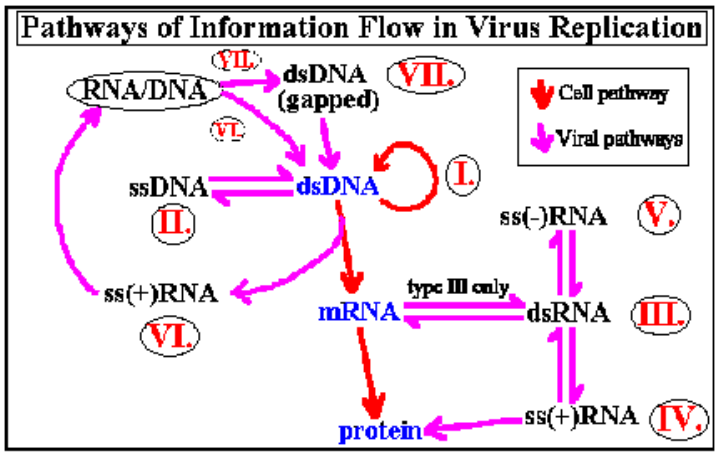
- DNA TO DNA (REPLICATION)
- DNA TO RNA (TRANSCRIPTION)

- AND RNA TO PROTEIN (TRANSLATION)

This may conveniently be described in terms of a diagram showing "information flow":



By contrast, viral replication is far more complicated in terms of information flow:



The Baltimore Classification of Viruses According to Their Genome Types and Their Replication Strategies

The classes are:

I	**dsDNA viruses replicating via DNA (semi-conservative)**
II	**ssDNA viruses replicating via DNA (semi-conservative)**
III	**dsRNA viruses replicating via (+)RNA (conservative?)**
IV	**ssRNA viruses with (+)-sense genomes replicating via RNA (semi-conservative)**
V	**ssRNA viruses with (-)sense genomes replicating via RNA (semi-conservative)**
VI	**"diploid" ssRNA viruses which replicate via reverse transcription with a greater-than-genome-length dsDNA intermediate**
VII	**dsDNA viruses which replicate via reverse transcription with a greater-than-genome-length ssRNA intermediate.**

See also section on "RNA Virus Replication"

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(Unless otherwise stated)

CLASS I:

Steps in Replication

1. Primary transcription by host enzymes

2. Translation of early (=regulatory) proteins

3. Viral genomic DNA replication (usually by host enzymes)

4. Late transcription (usually mediated by viral proteins)

5. Synthesis of late (=structural) proteins

6. Assembly of structural protein and DNA into virions

CLASS II:

Steps in Replication

1. Conversion into dsDNA (=host repair process?)

2. Early transcription (by host enzymes)

3. Translation of (regulatory) protein and "rolling circle" ssDNA replication

4. Late transcription (usually mediated by viral proteins)

5. Synthesis of late (=structural) proteins

6. "Sequestering" of viral genomic ssDNA

7. Assembly into virions

CLASS III:

Steps in Replication

1. Primary transcription in virion core in cytoplasm by viral RDRP, and export of (+)sense RNA to cytoplasm

2. Translation of (+)sense RNA, accumulation of viral proteins

3. Assembly of (+)sense RNA and viral proteins into immature virions

4. Transcription of (+)sense RNA into dsRNA in virions by viral RDRP

5. Secondary transcription of dsRNA

6. Final assembly / maturation of virions

CLASS IV:

Steps in Replication

1. Translation of virion RNA as mRNA (early products=RDRP)

2. Synthesis of (-)sense RNA on (+)sense template by RDRP (=formation of replicative complex, RC)

3. Synthesis of (+)sense RNA, mRNA and (-)sense RNA

4. Translation of (+)sense and mRNA, synthesis of structural protein (which biases RC to produce (+)sense RNA?)

5. Assembly of structural protein and (+)sense RNA and maturation of virions

---

CLASS V:

Steps in Replication

1. Primary transcription of virion (-)sense RNA by RDRP in virion core in cytoplasm, production (mainly) mRNA and (+)sense RNA, formation replicative complex (RC)

2. Translation mRNAs, accumulation of products

3. Virion proteins interact with RC, bias it towards production of full-length (+)sense RNA and therefore of genomic (-)sense RNA

4. Secondary transcription from progeny (-)sense RNA, translation, accumulation structural proteins

5. Nucleocapsid assembly and maturation, budding of nucleocapsid through host membrane containing viral envelope proteins

---

CLASS VI:

Steps in Replication

1. Reverse transcription in cytoplasm, using tRNA primer, of virion ss(+)RNA by virion-associated reverse transcriptase (RT), into intermediate RNA/DNA complex

2. Conversion of RNA/DNA complex into linear and circular proviral dsDNA forms with long terminal repeats (LTRs) by RT; import into nucleus

3. Integration of linear proviral DNA into host cell DNA, by means of integrase function of RT

4. Replication and trasncription as for host DNA, using host enzymes

5. Modification of transcription by (early) viral products (bias to production of genome-length (+)sense RNA?)

6. Translation, accumulation of (late) structural protein, assembly with (+)sense genomic RNA into viral nucleoprotein, budding through membrane containing viral envelope (glyco)proteins

---

CLASS VII:

Steps in Replication

1. Entry of viral DNA into nucleus, conversion of "gapped" genomic DNA into cccDNA by host repair synthesis

2. Transcription by host RNA pol into mRNA(s) and longer-than-genome-length "genomic" (+)sense RNA

3. Translation of mRNA and (+)sense RNA in cytoplasm, accumulation of viral products

4. Interaction of viral proteins with (+)sense RNA, assembly of provirions, and reverse transcription of RNA inside virions by viral RT to RNA/DNA complex

5. Conversion of RNA/DNA complex to circular, gapped dsDNA by virion RT

6. Final virion maturation, arrest of further DNA polymerase activity in virion (budding for hepadnaviruses)

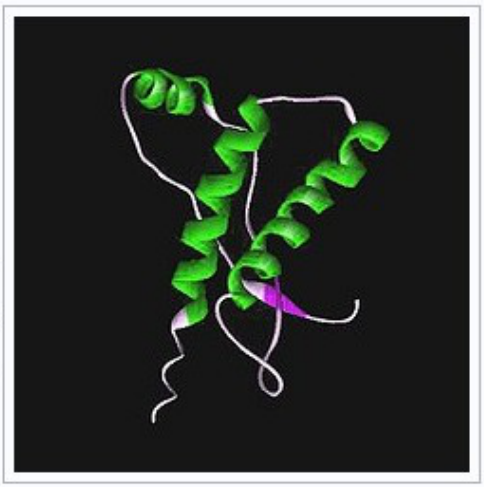
Other Autonomous or Semi-Autonomously Replicating Genomes

There are a number of types of genomes which have some sort of independence from cellular genomes: these include "retrons" or **retrotransposable elements**, bacterial and fungal (and eukaryotic organelle) plasmids, satellite nucleic acids and satellite viruses which depend on helper viruses for replication, and viroids. A new class of agents - PRIONS - appear to be "proteinaceous infectious agents".

Retrons	Plasmids	Satellite Nucleic Acids
Satellite Viruses	Viroids	Prions

What the heck are prions for?

Posted by ajcann on May 21, 2008



Prions are proteinaceous infectious agents that were first discovered because of their role in the etiology of transmissible spongiform encephalopathies (TSEs), a set of fatal neurological disorders that include Creutzfeldt-Jakob disease, scrapie and bovine spongiform encephalitis. TSEs arise from progressive misfolding of the endogenous cellular prion protein (Prp [PrPC]) into disease-associated scrapie form (PrPSc), which, in turn, disrupts normal cellular function and results in the formation of aggregates and amyloid-like plaques. Although there has been a clear association between PrPSc with these disease states, the cellular function of PrPC remains incompletely understood. PrPC is expressed across the entire central nervous system and at particularly high levels in the hippocampus, striatum, and frontal cortex, with apparently wide subcellular distribution, including synaptic sites. A synaptic role of PrPC is consistent with evidence from PrP-null mice showing deficits in spatial learning, altered long-term potentiation and increased excitability of hippocampal neurons. Several studies have also suggested that PrPC may provide neuroprotection. For example, cultured hippocampal neurons obtained from PrP-null mice show an increased apoptosis during oxidative stress. Mice lacking PrPC show increased neuronal damage after ischemic stroke, whereas protection is evident upon the viral-based overexpression of PrPC in rats. Finally, in several in vivo models of seizure activity, PrP-null mice showed increased mortality, likely as a consequence of hyperexcitability leading to excitotoxicity. The cellular and molecular basis for these effects remains unknown. Recent research shows that PrPC exerts a neuroprotective role by inhibiting excitotoxic cell death and that PrPC is a modulator of synaptic function and, consequently, neuronal excitability.

What the heck are prions for? « MicrobiologyBytes via kwout

Plasmids

Plasmids may **share a number of properties** with viral genomes - **including modes of replication**, as in **ss circular DNA plasmids and viruses** - but are **not pathogenic** to their host organisms, and are **transferred by conjugation** between cells rather than by **free extracellular particles**.

Satellite Nucleic Acids

Certain viruses have associated with them nucleic acids that are **dispensable in that they are not part of the genome**, which **have no (or very little) sequence similarity** with the viral genome, yet **depend on the virus for replication**, and are **encapsidated by the virus**. These are mainly **associated with plant viruses** and are generally **ssRNA**, both linear and circular - however, a **circular ssDNA satellite** of a plant **geminivirus** has recently been found.

Satellite Viruses

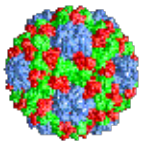
There are also **viruses which depend for their replication on** HELPER VIRUSES: a good example is **tobacco necrosis satellite virus (sTNV)**, which has a **small piece of ssRNA which codes only for a capsid protein**, and depends for its replication on the presence of TNV. Another good example is the **hepatitis delta agent with its circular ssRNA genome.** The **adeno-associated viruses** (AAVs) are also satellite viruses dependent on the linear dsDNA **adenoviruses** for replication, but which have **linear ssDNA genomes** and appear to be **degenerate or defective parvoviruses.**

Viroids

Viroids are **small naked circular ssRNA genomes** which appear **rodlike** under the EM, which are **capable of causing diseases in plants**. They **code for nothing but their own structure**, and are **presumed to replicate by somehow interacting with host RNA polymerase**, and to **cause pathogenic effects by interfering with host DNA/RNA metabolism** and/or transcription. A **structurally similar disease agent** in humans is the **hepatitis B virus-dependent hepatitis delta agent**, which additionally codes for a structural protein.

From Wikipedia: Putative secondary structure of the **PSTV** (potato spindle tuber **viroid**).
black - secondary structure of the viroid red - GAAAC sequence common to all viroids yellow - central conservative sequence

Author: Jakub Friedl (user kyknos), 2005



Back to "What is a Virus"?

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Definitions

by EP Rybicki (1999). An Electronic Introduction to Molecular Virology. Buglet Electronic Press, Cape Town.

Acellular Organisms:
acellular: not composed of cells (= "bodies of protoplasm made discrete by an enveloping plasma membrane");
Penguin Dictionary of Biology, 9th Edition, 1994

Genome:
The total genetic complement of a virus. This may be composed of RNA or DNA, and be single- or double-stranded. It may also be fragmented.

Viruses 1:

"Viruses are entities whose genomes are elements of nucleic acid that replicate inside living cells using the cellular synthetic machinery, and cause the synthesis of specialised elements [virions] that can transfer the genome to other cells".

SE Luria, JE Darnell, D Baltimore and A Campbell (1978). General Virology, 3rd Edn. John Wiley & Sons, New York, p2 of 578.

Viruses 2:

"Virus are submicroscopic, obligate intracellular parasites...[and]

- **Virus particles (virions) are formed from the assembly of pre-formed components;**
- **Virus particles themselves do not "grow" or undergo division;**
- **Viruses lack the genetic information which encodes apparatus necessary for the generation of metabolic energy or for protein synthesis (eg: ribosomes)".**

AJ Cann (1997). Principles of molecular virology, 2nd Edition. Academic Press, San Diego.

Organism:

"An organism is the unit element of a continuous lineage with an individual evolutionary history."

SE Luria, JE Darnell, D Baltimore and A Campbell (1978). General Virology, 3rd Edn. John Wiley & Sons, New York, p4 of 578.

Definitions of Life:

Classical **Newer Versions**

Classical Properties of Living Organisms:

- Reproduction
- Nutrition
- Respiration
- Irritability
- Movement
- Growth
- Excretion

More modern definitions include the storage and replication of genetic information as nucleic acid, and the presence of or potential for, enzyme catalysis

(Penguin Dictionary of Biology, 9th Edition, 1994)

"Just as physicists recognise light either as electromagnetic waves or as particulate photons, depending on the context, so biologists can profitably regard viruses both as exceptionally simple microbes and as exceptionally complex chemicals".

- R Dulbecco and HS Ginsberg, 1980. Virology (originally published as a section of Microbiology, 3rd. Edn., by Davis et al., Harper and Row, Hagerstown); p. 855.

Return to What is a Virus

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Life may (somewhat irreverently) be defined in general terms as:

"The phenomenon associated with the replication of self-coding informational systems",

or specifically as:

"The phenomenon associated with the replication of nucleic acids".

- Rybicki EP, 1996.

Another more serious view:

"Life can be viewed as a complex set of processes resulting from the actuation of the instructions encoded in nucleic acids. In the nucleic acid of living cells these are actuated all the time; in contrast, in a virus they are actuated only when the viral nucleic acid, upon entering a host cell, causes the synthesis of virus-specific proteins. Viruses are thus "alive when they replicate in cells, while outside cells viral particles are metabolically inert and are no more alive than fragments of DNA."

- Dulbecco R and Ginsberg HS, 1980. Virology, p.854-855 (originally published as a section in Microbiology, 3rd Edn., Davis et al., Harper and Row, Hagerstown).

References and Notes

Classical Properties of Living Organisms:

- Reproduction
- Nutrition
- Irritability
- Movement
- Growth

(return)

Definition of a Virus:

SE Luria, JE Darnell, D Baltimore and A Campbell (1978). General Virology, 3rd Edn. John Wiley & Sons, New York, p2 of 578.

(return)

Definition of an Organism:

SE Luria, JE Darnell, D Baltimore and A Campbell (1978). General Virology, 3rd Edn. John Wiley & Sons, New York, p4 of 578.

(return)

Computer Viruses as Life Forms:

Steven Hawking, of black holes fame, apparently believes that computer viruses should count as life: they are obligate parasites which exploit the "metabolism" of the host computer they infect, they replicate in the form of their source code [=genome], and they newest and nastiest can mutate while they do so (Weekend Argus, 6-7 August, 1994).

Computer viruses infecting cells...Russell Kightley Media

(return)

Fossil DNA:

Short stretches of DNA - no more than 500 nucleotides - have been amplified up *in vitro* by the technique of polymerase chain reaction or PCR, from mites entombed in amber up to 200 MYr BP, and from fossilised leaves up to 60 MYr old. This DNA can be sequenced and compared to that of morphologically related modern organisms.

(return)

Virus Taxonomy Reference:

FA Murphy and DW Kingsbury (1990). Virus Taxonomy. Chapter 2 in Fields Virology, 2nd. Edn. (BN Fields et al, Eds.) Raven Press, New York.

EG Strauss, JH Strauss and AJ Levine (1990). Virus Evolution. Chapter 9 in Fields Virology, 2nd. Edn. (BN Fields et al, Eds.) Raven Press, New York.

(return 1)

(return 2)

Origin of Eukarya:

Eukarya probably arose from cells which probably most closely resembled Archaea-like organisms (Archaebacteria), about 1.4 billion years ago. Their key differences from Prokarya and Archaea - the possession of nuclear membranes and mitochondria - are difficult to explain. The former may be a simple adaptation to localise functions specific to DNA replication and transcription, and may have happened independently in at least one other of the Bacteria. The latter, however, is postulated to have had its origins in an endosymbiotic association of a free-

living bacterium with another with aerobic respiration, possibly related to the present-day *Agrobacterium, Rhizobium and Rickettsias*: the latter would have been present in the cytoplasm of the former, and would gradually have lost its cell wall (though remaining enveloped), and much of its genome (much of whose function was taken over by the host), though retaining its own DNA replication, circular genome structure, and ribosomal RNA and protein genes. This would have been the origin of true Eukarya, all of whom would descend from this pioneering symbiosis.

Another endosymbiosis which became permanent happened at least once (and possibly several times) much later on in evolution, with the entry into symbiosis of a primitive member of the Eukarya and a photosynthetic bacterium: this was possibly an ancestral relative of *Prochloron*, which has similar chlorophyll, but may have been a cyano-(blue-green)bacterium. As happened with mitochondria, the chloropast percursor lost its wall, and much of its genome; however, as it occurred much more recently in evolutionary time, chloroplast genomes are usually much larger than mitochondrial genomes. Several eukaryotic marine algae have chloroplasts which appear to have different origins than those of all land plants: these may have been independent acquisitions. Still others have complicated multi-membrane layered chloroplasts which appear to have vestigial nuclei: these are probably derived from primitive photosynthetic eukaryotes which formed endosymbiotic associations with other eukaryotes.

Reference:

Prescott et al., Microbiology: 2nd. Edn.

Strauss *et al.*, Virus Evolution, in Fields *et al.*, Virology, 2nd. Edn.

(return)

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IN THE BEGINNING:

Was the Cosmic Bubble
And the Bubble was without form
And brightness was upon the face of the void

A few milliseconds later
Expansion had occurred
And the Universe was lumpy
As it is, lo, unto this day
As is confirmed by satellites

And the plasma cooled, and the atoms associated
And upon the surface of the newly-formed planets
Yea, and perhaps even also in the interstellar spaces
Surface catalysis caused polymerisations
And on Earth lightning, and deepsea thermal vents
And volcanoes, and hot springs
All of these were energy sources for the cooking of the primal soup

And nucleotides came to be, and short oligoribonucleotides,
And amino acids, and lipids
Bilayers and multilayers did the lipids form
And so did they entrap primitive ribozymes
Which did replicate themselves crudely according to their kinds
And mutated, and made more ribozymes
And to each a different function:

So there were the polymerases
And the proto-ribosomes
And the spliceosomes
And the primitive tRNAs

And the reverse transcriptase, and eventually also the DNA,
which was a partner to the RNA
And a better information store, though less generally useful

So it came to be that DNA usurped the role of RNA
And RNA was cast out into the proto-cytoplasm
Where it was a messenger, and an enzyme
And a helpmeet to the DNA

Though some of it escaped as viruses
Renegade genomes, beyond the cell
In the outer darkness
Where there is wailing, and gnashing of teeth
(For no obvious reason)

And the DNA waxed fruitful
And it multiplied bounteously,according to its kinds
And it made new kinds, by mutation
As the polymerases were not so accurate yet

And Urkaryote begat Bacteria and Archaea and Eukarya
And the Archaea and Eubacteria did excise all or most of their introns
And stayed small

While Eukarya kept theirs, and grew
(Though there is lately some mystery about eocytes)

And divers Bacteria did enter Eukarya
Endosymbiotes they became
Which would later greatly occupy **Lyn Margulis**
And begat more unto their kind, and evolved
Mitochondria and chloroplasts did they become
And possibly peroxisomes also

And the kinds did multiply all over the face of the planet
And maybe also on other planets, as claimed by NASA
And perhaps also in the void
And did change the face of it, and the air above the ground
To an oxygen/nitrogen atmosphere they did change it
By photosynthesis, mainly
And this did allow more energetic metabolic processes to occur

Which allowed more complex organisms

Which meant that, after three billenia
Animals could come to be
And it was so
That slimy things did crawl, with legs
Out of a slimy sea

And although large bolides did rain down upon the Earth
Yea, and comets
So as to cause ice ages and mass extinctions
So did the DNA keep multiplying and evolving
To make ever more complex organisms did it evolve

Until it came to pass
That apes did come down from the trees
In the dry Pleistocene did the apes walk
And swim also, If one is to believe **Elaine Morgan**
And "The Descent of Woman"

But I digress...

And they evolved to fit the new niche
Larger brains did they acquire
And upright carriage
And other problems

And lo, they did search for Meaning
And Truth

And Food and Sex also

And they did become confused
So they invented religion
To explain that which happened

And it came to govern them, so that they were not free to think
And were reluctant to take their clothes off
Which is the cause of some of the problems

But Mother Gaea will fix it, eventually....

(apologies to Samuel Taylor Coleridge for the crib from "The Rime of the Ancient Mariner")

This appeared in its entirety on the screen of a computer which had accidentally been left on overnight in a locked office sometime in 1992. In the absence of other claims, and because he refuses to believe in Divine Dictation, this is

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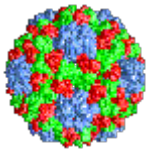
back up to slimy things...



RETURN TO VIRUS ORIGINS

Koonin EV, Dolja VV (1993)

Evolution and Taxonomy of Positive-Strand RNA Viruses. Implications of Comparative Analysis of Amino Acid Sequences.

Crit Rev Biochem Molec Biol 28:546-547

"The grouping revealed by phylogenetic analysis may provide the basis for revision of virus classification, and a phylogenetic taxonomy of positive-strand RNA viruses is outlined. Some of the phylogenetically-derived divisions of positive-strand RNA viruses include also double-stranded RNA viruses indicating that in certain cases the type of genome nucleic acid may not be a reliable taxonomical criterion for viruses.

Hypothetical evolutionary scenarios for positive-strand RNA viruses are proposed. It is hypothesised that all positive-strand RNA viruses and some related double-stranded RNA viruses could have evloved from a common ancestor virus that contained genes for RNA-dependent RNA polymerase, a chymotrypsin-related protease that functioned also as the capsid protein, and possibly an RNA helicase."

Return to Origins of Viruses

Evolution of Viruses With Single-Stranded Negative Sense RNA Genomes

The evolution of viruses with single-stranded negative-sense RNA genomes appears more clear than that of their ss(+)sense and dsRNA counterparts. In fact, the phylogenetic affinities of the families Paramyxoviridae, Rhabdoviridae and Filoviridae have been recognised by their being grouped toether by the ICTV into the Order Mononegavirales, of viruses possessing linear, non-segmented ssRNA(-) genomes with a similar gene order [3' non-translated region - core proteins - envelope proteins - polymerase - 5' non-translated region], a single 3' promoter, and helical nucleocapsids.

It is assumed that these virus families obviously descend from a common ancestor; however, the sequence affinities of especially the polymerase genes but also the nucleocapsid genes of viruses in the families Orthomyxoviridae and Bunyaviridae with the Mononegavirales, indicate that a common ancestor for all ssRNA(-) viruses is not unlikely.

Reference:

Virus Taxonomy - Sixth report of the International COmmittee on Taxonomy of viruses (Murphy et al., Eds.). Springer-Verlag, Wien, 1995.

Return to Origins of Viruses

Retroid Elements and Retroviruses

Retroviridae [ssRNA(+) viruses replicating via a longer-than-genome-length dsDNA intermediate], Hepadnaviridae, caulimoviruses and badnaviruses [gapped circular dsDNA viruses replicating via longer-than-genome-length RNA intermediates] all share the unlikely attribute of the use of an enzyme complex consisting of a RNA-dependent DNA polymerase/RNAse H in order to replicate. They share this attribute with several retrotransposons, which are eukaryotic transposable cellular elements with striking similarities with retroviruses [such as the yeast Ty element, and the Drosophila copia element]; and with retroposons, which are eukaryotic elements which transpose via RNA intermediates, but share no obvious genomic similarity with any viruses other than the reverse transcriptase. Bacteria such as E coli also have reverse- transcribing transposons -known as retrons - but these are very different to any of the eukaryotic types while preserving similarities in certain of the essential reverse transcriptase sequence motifs.

All of these elements are collectively known as RETROELEMENTS; the fact that the reverse transcriptases of all of them have some amino acid identity suggests a common evolutionary origin.

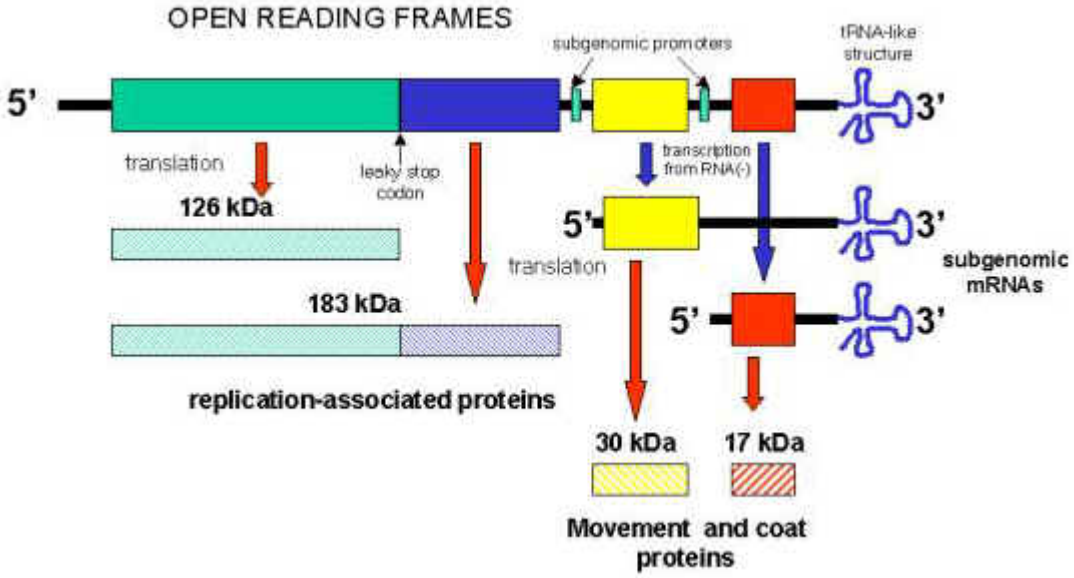
Several reviewers have pointed out that just such an enzyme as reverse transcriptase would have been necessary for the transition from what is widely believed to have been an RNA world - that is, where all the extant organsisms had RNA genomes - to the present world in which all cellular organisms have DNA genomes. Viruses with RNA genomes which use RNA-dependent RNA polymerases for their replication may be the only remnants of that pre-DNA era; however, cellular elements and viruses which use reverse transcriptase may share a common origin as cell-derived "modules" coding for a reverse transcriptase, which evolved to become retrons and retroposons and retrotransposons. Addition of structural proteins may have allowed evolution of retroviruses. The evolution of the DNA retroviruses - Hepadnaviridae, caulimo- and badnaviruses - is more obscure; it appears as though these arose from retrotransposon-like sequences, but this probably occurred near the origin of of these types of element as they are so diverse in sequence and genome organisation.

Reference:

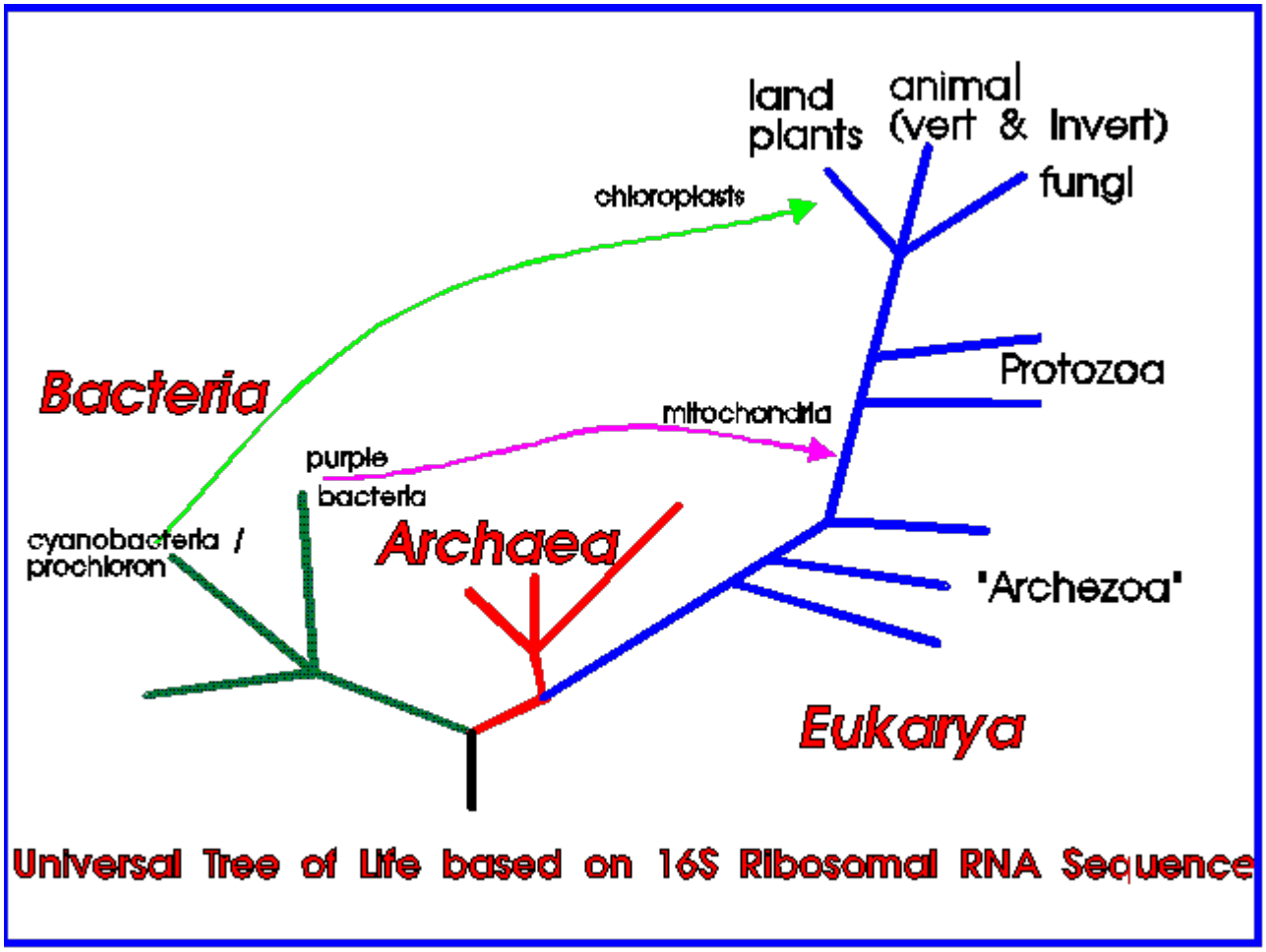
Chapter 7: "Evolution by transposition" (pp. 172-203) in "Fundamental of Molecular Evolution" by Wen-Hsiung Li and Dan Graur; Sinauer Associates, Inc., Sunderland, Mass., 1991

Return to Origins of Viruses

Scheme showing the expression strategy of tobamoviruses: initial translation produces a replicase which then synthesises subgenomic mRNAs from a (-) strand template for expression of ORFs near the viral genomic 3' end.



Legend First Previous Next Last


land plants
animal (vert & invert)
fungi
chloroplasts
Protozoa
Bacteria
mitochondria
purple bacteria
cyanobacteria / prochloron
Archaea
'Archezoa'
Eukarya
Universal Tree of Life based on 16S Ribosomal RNA Sequence

Picornaviridae:

(return to Main)

(For a more General Reference: Picornaviridae and Their Replication, CHAPTER 20. Roland R. Rueckert. Virology. Second Edition, edited by B.N. Fields, D.M. Knipe et al. Raven Press, Ltd., New-York - 1990)

This has been modified from the version found on the Wisconsin Virology WWW server (without permission...!)

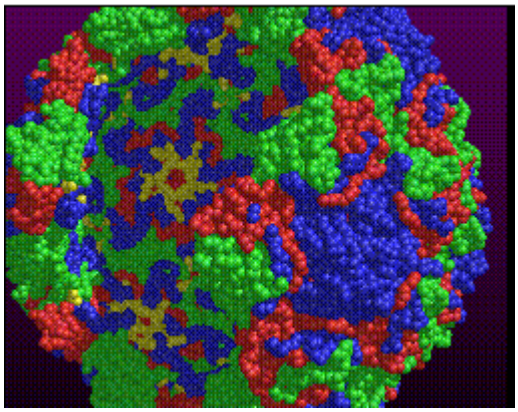
Classification

The Picornaviridae family: *pico*rna*. 'PICO' means small, 'RNA' signifies that these are single standed positive sense RNA viruses..

The picornavirus family is divided in 5 Genera and further subdivided into members.

- Enterovirus
  - Human polioviruses 1-3 (serotypes=3)
  - Human hepatitis virus A (serotypes=1)
  - Theiler's murine encephalomyelitis (serotypes=1)
- Rhinovirus
  - Human rhinoviruses 1-100 (serotypes=100)
  - Bovine rhinoviruses 1-2 (serotypes=2)
- Aphthovirus
  - Foot-and-mouth disease virus 1-7 (serotypes=7)
- Cardiovirus
  - Encephalomyocarditis (EMC), Mengo (serotypes=1)
- Unassigned
  - Equine rhinoviruses 1-2, Cricket paralysis... (serotypes>3)

Structure:



This is an image reconstruction from X-ray crystallographic data of the r16 common cold virus: this is a member of the Rhinovirus genus of the Picornaviridae. Click on picture, then on the ">>" of the AAPLAY control line to animate: this will display the inner and outer capsid structures. VP1 is blue and clustered around the twelve 5-fold rotational axes; VP2 and 3 are green and red respectively, and cluster around the twenty local 6-fold rotational axes. VP4 can only be seen internally, where it clusters around 5-fold axes. Considerably more detail on structure is available below.

Relationships:

Picornaviruses also have a very similar genomic organisation to comoviruses - despite the latter having two genomic components instead of one - and can feasibly proposed to be evolutionarily related to comoviruses, albeit distantly. Still more distant is a relationship with potyviruses: these share only a "core" replicase-related sequence, and have filamentous particles rather than spherical.

Polyproteins The single RNA is translated into a polyprotein that is subsequently cleaved. The way the proteins cleave themselves out is slightly different amongst the different genera. For our structural purposes suffice it to say that the coat proteins are products of the cleavage of the P1 precursor protein.

```
       <-------- P1>
       ----------------------------------------------------------------
Vpg---|  VP0   |  VP3  |  VP1 |2A| 2B |    2C   |3A|3B|  3C  |    3D  |
       ----------------------------------------------------------------
```

P1 - > VP0 + VP3 + VP1 and then VP0 -> VP2 + VP4 once the virus has assembled.

Vpg (virion protein genome) is attached to the 5'end of the RNA.

Coat Proteins

In schematics and color graphics and movie representations the convention:

- VP1 = blue
- VP2 = green
- VP3 = red
- VP4 = yellow

is used.

Coat Protein Mass

VP1	VP2	VP3	VP4	P1
32,300	28,500	26,200	7,200	94,200

VP1-3 has the now famous wedge-like protein fold motif shared by most virus structures solved by X-ray crystallography. It is created by a common CORE made up of 8 antiparallel Beta-strands forming a Beta-barrel structure. Proteins amongst viruses mainly differ in the loop insertions between the strands. These insertions accounts for a lot of the virus external appearance and also are the target structures for antibodies in the case of an animal virus. (see below). Interestingly there is no sequence conservation in the Beta strands structures of viruses although the structure is very well conserved.

Here is a representation of the 8-stranded Beta barrel. The beta strands orientation is marked with the sybols: V,^,> and The *** represent the 2 Alpha- helices. The helix between loop EF and strand F is in the back.

```
                          -
                         (   `-
                        ***    \     EF
            ___>____C__ ***     \
  BC    (             \ ***      \
         \            |_______     |
          _\<_____h __________|_____________ HI ( \ *** | ' \ _\__\____E________>_|_/
\_
        \ \  \         ***    |     COOH--\     \    GH
  DE     \ \  \          |   /              \     \
        (_\_\<_\__f___/ / \ | FG \ \ \ \ |_************* \ | V ^ V ^ *************) | | G D I B
| | | \ \ \ \ / | | \_\__\__\_______________>---/--/---'
                \  \  \                  /  /
  CD             \__\__\_____________<_/ / \ \ / \__\____________>_/
```

```
\
|
NH2
```

The VP4 protein is much shorter and is found only on the interior surface of the virus. It results from the cleavage of VP0 into VP2 and VP4 which occurs after assembly. In poliovirus the N-terminus is myristylated.

Icosahedral Capsid

60 of each of the coat proteins assemble into an icosymmetric structure with icosahedral symmetry. Picornaviruses have a triangulation number of T=1 but this is also refered to as a P=3 for Pseudo-T=3 structure. A T=3 structure occurs when all 180 (3 protein for each of the 60 facets) are chemically identical but have to assume slightly different neighbouring environment: The proteins which are around the 5-fold axis have 4 neighbours while the ones at the 3-fold axis have 5 neighbours.

```
                 _________________
                /\               /\
               /  \             /  \
              /    \           /    \
             /      \         /      \
            /        \       /        \
           /          \ /              \  T=1:
          /         - V -               \  A 20 facets
         /        _ /  /|\  \ _          \  Icosahedron.
        /      _ /    / | \    \ _       \   Contains
       /   _ /        / 60  \       \ _   \      5-fold vertices.
      /_ -           /___|___\          - _\ T=3:
      \  -  _                 20       _ -  /     Each of the 20
       \     \ _              |     - /     /      facets can be
        \        \ _          |   - /      /      subdivided into
         \          \ _ ^ _ /             /       60 total triangles.
          \           |                   /      Each of these 60
           \         / \                 /      triangles contains
            \       /   \               /      approximately one
             \     /     \             /      each of the VP coat proteins.
              \   /       \           /
               \/_________________\/
```

```
                VP1  |   VP1
                   5                          Within each of the 60
                .  '/ \` .                     triangles fits about 1 each of
              ,    /    \   `.                  VP1-3. The symmetry axes are
        ,    VP1 /      \ VP1   `  :  `    \     labeled 5,2,3. p3 is a pseudo
    ,             /  VP1  \          `  .  `  \    3-fold axis within the triangle.
,                /         \           `     .\    Around the icosahedral
                /          / \         ` VP3  '    5-fold are five VP1s.
               /          /   \        `. . ./    But around the 3-fold
              /          /     \      /           are 3 VP2s and 3 VP3s
     VP3     /_______p3/         \   /     /      alternating.
            /           \         \ /              In Plant viruses with T=3
 \         /             \   VP3   \ VP2 /          symmetry the similarity is
          /      VP2      \         \                with VP1=A, VP2=C, VP3=B.
    \    /                 \         \   /   VP3
VP2__3/___________2_____\_________\3 __
      \             \                 |
       \             \               / \  VP2
   VP2  \  VP3       \    VP2       /   \
         \            \            /  VP3  \
          \            \_________/
           \           /        /
                      /
```

In T=3 plant viruses each protein is synthezised independently while in the polioviruses they arise from the cleavage of P1 as illustrated above. It is worh noting that the VP proteins issued from a single P1 precursor do not form a triangular face as the previous drawing might suggest. Instead they form what is usually refered to as the

```
                                 *Biological* asymmetric
                   5             unit as opposed to the *crystallographic*
                  / \             asymmetric unit. An asymmetric unit is
                 /   \             a set of minimum non-redundant information
                /     \            necessary to reconstruct an icosaheron from
               /       \           it using only the icosahedral symmetry.
              /         \           The complete icosahedron is indeed
       _______/   VP1    /         mathematically obtained wether one uses
```

```
    /      /         /
   /      /         /
  /      /_______  /
 / VP3  /        \
/      /          \
\     /    VP2     \
 \   /             \
  \3/_______________\
                 2
```

a triangular asymmetric unit or this biological unit in which the VP3 area has only been switched to the right!

Canyon and Drug Binding in VP1 Hydrophobic Pocket

The VP1 proteins form a small cylindrical protrusion at the 5-fold axis. Around that protrusion is a depression sometimes refered to as the 'canyon'. This canyon is the receiving site for the cellular receptor which has been co-crystallized in this position by the crystallographers at Purdue University, and also observed there by cryo-EM reconstruction.

Here is a projection of the canyon onto one of the 60 triangular facets. The scales to the left shows the position in Angstroms along the X axis and range from 4 to 42. The bottom scale shows the size in Angstroms for the Y axis ranging from 01 at the right to 61 at the left; (the axis choice was slected by the crystallographers). The canyon appears as numbers within a background of + symbols. Numbers range from 1 to 4, 4 showing the deepest part of the canyon. The rim of the canyon is arbitrarily set at 137 Angstroems from the center of the particle. The output was prepared by the program V-suf (Rossmann and Palmenberg (1988), Virology 164,373-382)

```
 X                              5-fold
                                  *
 42                              1.1
 40                            +++++++
 38                           1++++++++
 36                          +1+++++++1+
 34                        ++++++1++++++++
 32                       +1+++++++++++++
 30                       +11++1++++++++++
 28                     12253++11112++++++++.
 26                    ++++++3433311+++++++1++
 24                   ++++++++++311+++++++3+++
 22                 ++++++++++++++3233222232111+++
 20               ++++++++++++++++22233443333331++++
 18              ++++++++++++++++++33222334++++++++
 16             ++++++++++++++++()++12111122++++++++++
 14            ++++++++++++++++++++++++1++++++++++++++
 12           .+1++++++++++++++++++++21+++++++++++++++.
 10         ++++++++++++++++++++++++1++++++++++++++++++
  8        ++++++++++++++++++++++++++++++++++++++++++++++
  6       +++++++++++++++++++++++++++++++++++++++++++++++++
  4      ++++++++++++++++++++++++++++++++++++++++++++++++++++ 3-fold
_____________________________________________________________________
     66555555555544444444443333333333222222222211111111111000000000 - Y values
     10987654321098765432109876543210987654321098765432109876543210987654321
```

The VP1 protein has also the peculiarity of having a hydrophobic pocket accessible from the surface via a small 'pore' entrance. It is indicated by the symbol () in the above drawing (approximate position). In some of the virus structures resolved by X-ray crystallography a substance can be found in this hydrophobic cavity. The nature of the compound is not known. It is refered to as a Sphingolipid in the case of poliovirus and as a sugar in the case of rhinovirus 1a, but crystallographers only see some 'extra' electron density. The pharmaceutical company Sterling Winthrop has synthezised compounds, often refered to as WIN-drugs, that diffuse readily in the VP1 pocket. (see e.g. Heinz et al. J. Virol. (1989) vol.63, pp 2476-2485, Genetic and Molecular Analyses of Spontaneous Mutants of Human Rhinovirus14 That Are Resistant to an Antiviral Compund). Other companies also study similar compounds (Jansen, Chalone, Sandoz) but none of them are ready for commercial use.

WIN 52035

```
 ___      ____
|   N    /    \              O____N
|    \\__<()>-O--  /\   __/   ||
|___O/    \____/    \/  \/  \\___||__CH3

Oxazoline  Phenoxy   Aliphatic  Isoxazole       Chain
```

The effect of the compound is to raise the canyon floor and prevent attachement to the cellular receptor that now cannot fit properly in the canyon and / or impair the uncoating of the capsid upon cell entry. The presence of such a compound in the structure confers thermal stability to the virion.

It is suggested that this is a trigger mechanism that allows the virus to survive in vivo. For instance poliovirus is stable in acid conditions and survives inside the stomach while rhinovirus does not.

Antigenic Sites

Antigenic sites have been determined by escape mutations

> Rueckert et al. (1986), Virus Attachement and Entry Into Cells, American Society for Microbiology: Location of four neutralization antigens on the three- dimensioan; surface of a common-cold picornavirus, human rhinovirus14;
>
> Rossmann et al. (1985), Nature, 317,145-153. Structure of the human common cold virus and functional relationship to other picornaviruses)

Viruses were resistant to a panel of murine monoclonal antibodies. "Mutations were clustered in the serotype-variable regions of the amino acid sequences for the virus capsid proteins". These antigenic areas are found to protrude out of the surface of the virus. Refer also to the above diagram, showing the depth of the canyon...

Four neutralization Immunogens (NIm) sites have been resolved by cross-neutralization tests. Sites are labeled NIm IA, IB, II, and III. The ** symbols approximate the size of the area. IB is a smaller area than IA.

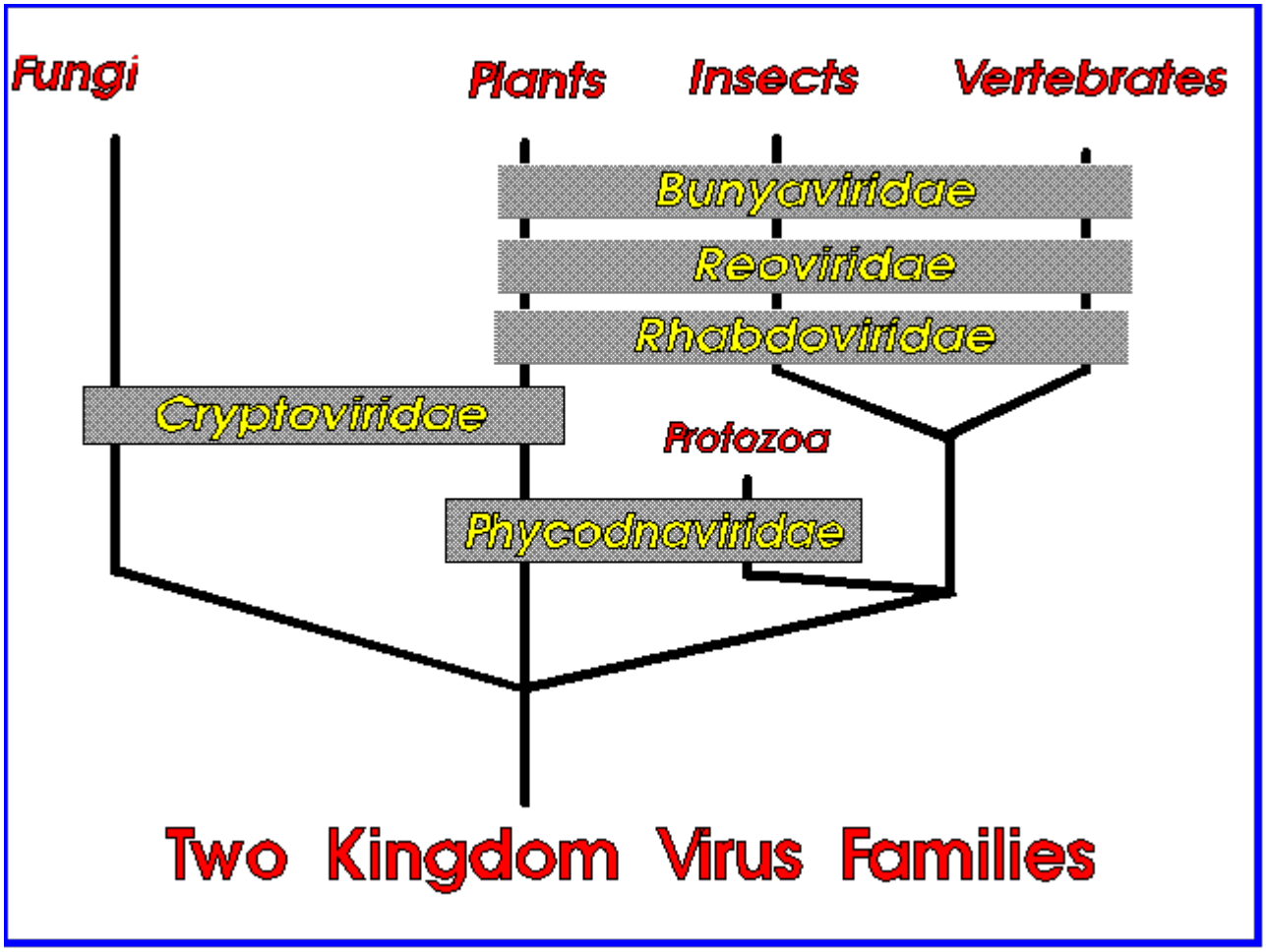
```
                 5
                / \
               / IA\
              /  ** \
             /       \
            /   IB   \
           /         / \
          /         /   \
         /         /     \
        /   *     /       \
       /   ** *  \        \
      /    II ** \     *    \
     /            \   III*   \
  3/______________\__________\3
                 2
```

```
IA::(VP1): Q83,K85,D138,S139
  IB::(VP1):D91,E95

     II::(VP2):E136,S158,A159,E161,V162
         (VP1):E210

        III::(VP3):N72,R75,E78,G203
             (VP1):K287
```

The major antigenic site is on the BC loop in VP1 and VP3 but in the EF loop in VP2.


Fungi
Plants
Insects
Vertebrates
Bunyaviridae
Reoviridae
Rhabdoviridae
Cryptoviridae
Protozoa
Phycodnaviridae
Two Kingdom Virus Families

Bunyaviridae

Members of the Family:

Genera:

- **Bunyavirus**: Bunyamwera supergroup
- **Phlebovirus**: Sandfly fever and Uukuniemi group
- **Nairovirus**: Nairobi sheep disease group
- **Hantavirus**: Hantaan group
- **Tospovirus**: Tomato spotted wilt virus group (plants)

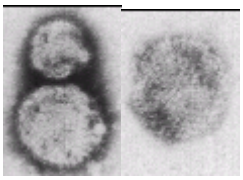
Host Range:

Hosts of Bunyaviridae were previously known to include various arthropods and/or warm or cold-blooded vertebrates: lately this has been widened to include many species of plants, and perhaps also thrips (Thysanoptera; exopterygote insects).

Transmission:

Largely by mosquitoes, ticks, phlebotomine flies, and other arthropods - which accounted for the group being included in the so-called "Arboviruses". Transovarial anmd venereal transmission has been demonstrated for some mosquito-borne viruses. Aerosol infection can occur - for example, with Four Corners virus - either incidentally, or as the principal mode of transmission. No arthropod vector has been shown for Hantaviruses.

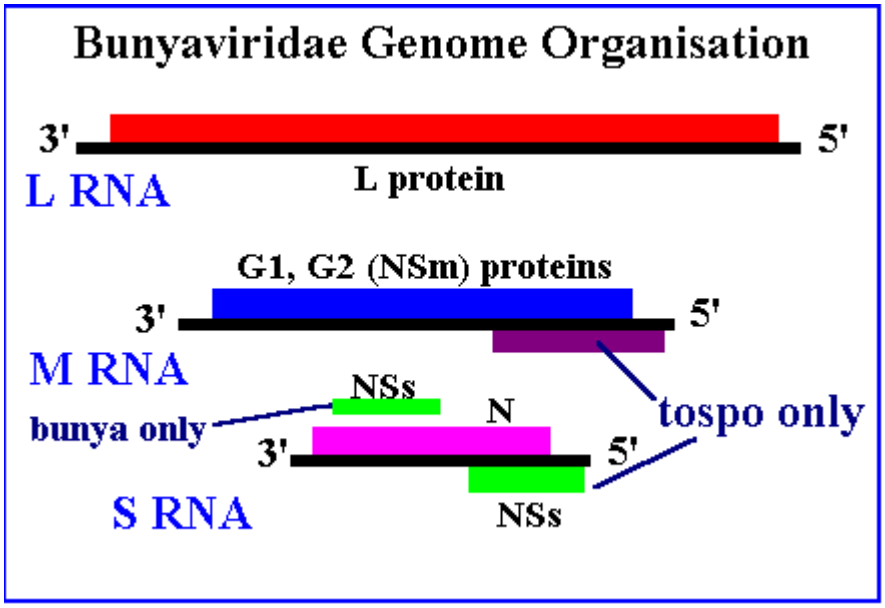
Virions:



(Thanks to Brian Hjelle, MD, of the Department of Pathology, University of New Mexico School of Medicine, for the EMs of Four Corners Hantavirus)

Viruses in the taxonomic family Bunyaviridae have multicomponent, largely negative-sense RNA genomes (ambisense), encapsidated in spherical / pleomorphic enveloped particles containing helical nucleocapsids. Particles have a sedimentation coefficient of 350-500 Svedbergs, a buoyant density in CsCl of 1.2 g per cc, and are sensitive to lipid solvents and detergents.

Nucleic Acids:



The genome consists of three molecules of -ve or ambisense RNA: these are L (large, 6.5 - 14.4 kb), M (medium, 3.2 - 6.3 kb) and S (small, 0.8 - 2.0 kb). The 5' and 3' ends are hydrogen-bonded, and RNAs and nucleocapsids are circular.

Proteins:

There are usually 4 proteins associated with virions: these are 2 external membrane-associated glycoproteins (G1, G2), and nucleocapsid protein (N), and a large (L) protein which is presumably a transcriptase, as nucleocapsids have such an activity.

Effects on Vertebrate Cells:

Some induce cell fusion at low pH. Most - except for Hantaviruses - cause cytopathic effects. Some have ion-dependent haemagglutination activity.

Expression of Genome:

The viruses replicate in the cytoplasm of infected cells; after transcription from genomic RNAs which occurs (presumably) in partially disassembled capsids. Host RNAs are known to prime viral mRNA synthesis. L RNA encodes the L protein; a single ORF in the M RNA encodes the G glycoproteins, which are cotranslationally cleaved to G1 and G2. The S RNA encodes the N protein, and - in some cases - a non structural NS. The latter RNA is most likely to be ambisense; that is, containing ORFs in both (-)ve and (+)ve senses. See the accompanying graphic, kindly provided by Brian Hjelle, MD.

The viruses mature by budding into smooth-surfaced vesicles in or near the Golgi region, but may also bud through the plasma membrane.

Adapted from **CH Calisher, Bunyaviridae**, pp. 273-274 in:

Classification and Nomenclature of Viruses - Fifth Report of The International Committee on Taxonomy of Viruses, Eds. RIB Francki, CM Fauquet, DL Knudson and F Brown; Archives of Virology Supplementum 2, Springer-Verlag, Wien, 1991.

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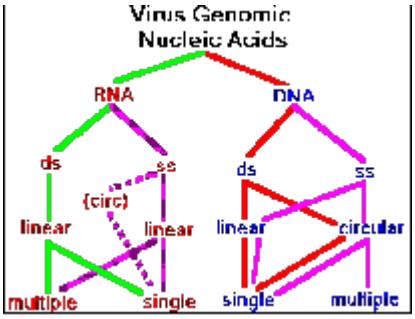
Introduction to Molecular Virology

Genome Diversity and Genomic Replication Strategies



Viruses are the only organisms on this planet to still have **RNA** as their sole genetic material. They are also the only autonomously replicating organisms to have **single-stranded DNA**. The range of virus genomes as found in virions encompasses:

- **single-component dsDNA**, linear or circular (occasionally circularly permuted linear);
- single, double or multi-component **circular ssDNA**;
- single-component **linear ssDNA**;
- single or multi-component **dsRNA**;
- single or multiple component **ssRNA genomes** which may be
  - totally **"positive"**(or messenger) polarity,
  - totally **"negative"** (or anti-messenger) polarity, or
  - **partially positive negative-sense**;
- "diploid" **positive-sense ssRNA genomes which replicate via reverse transcription** to and transcription from longer-than-genome-length dsDNA, and
- nicked and/or partially **dsDNAs which replicate via transcription** to and reverse transcription from longer-than-genome-length positive-sense ssRNA.

In contrast, **prokaryotes** have only **single-component circular (mainly) or linear (Streptomyces, Helicobacter) dsDNA** while all **eukaryotes have multi-component dsDNA**, and all the genomes replicate **via the classic semi-conservative route**.

The Baltimore Classification of Viruses According to Their Genome Types and Their Replication Strategies

These various types of virus genomes can be broken down into **seven fundamentally different groups**, which obviously require different basic strategies for their replication. **David Baltimore**, who originated the scheme, has given his name to the so-called **"Baltimore Classification"** of virus genomes.

To understand the classification, one must understand how cells replicate their genomes, and express mRNAs, and proteins. This is conveniently explained by the "Watson-Crick Central Dogma", which states that

INFORMATION FLOW IN CELLS GOES FROM:

DNA TO DNA (REPLICATION)

DNA TO RNA (TRANSCRIPTION)

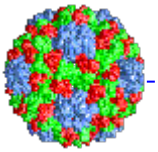
AND RNA TO PROTEIN (TRANSLATION)

This may conveniently be described in terms of a diagram as shown below:

Briefly, the types are classified by **type of nucleic acid in virions** (see also **genomes.gif**), and **replication strategy**. The types are:

- **I: dsDNA viruses**
- **II: ssDNA viruses**
- **III: dsRNA viruses**
- **IV: ssRNA viruses with (+)-sense genomes**
- **V: ssRNA viruses with (-)sense genomes**
- **VI: "diploid" ssRNA viruses which replicate via greater-than-genome-length dsDNA**
- **VII: dsDNA viruses which replicate via greater-than-genome-length ssRNA.**

(return to Top)



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Helical Nucleocapsids

This is one of the **SIMPLEST FORMS** of viral capsid: the **protein** is **"wound on"** to the **viral nucleic acid** (generally **ssRNA**, though **M13** and other filamentous phage virions contain **circular ssDNA**) in a simple **HELIX**, like a screw (see the diagram for tobacco mosaic virus, below).

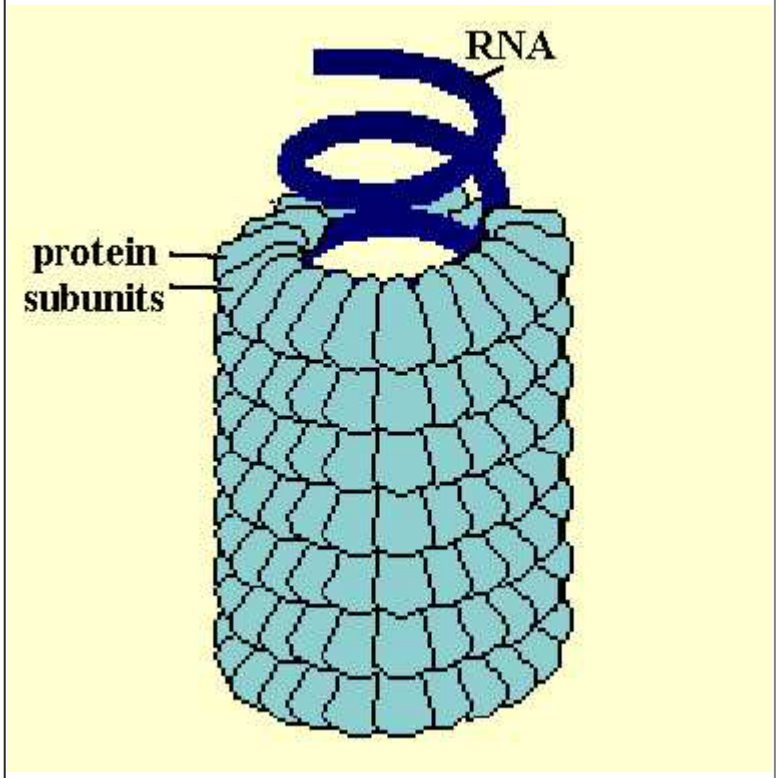


Diagram of TMV structure: protein subunits are all in equivalent crystallographic positions, related by a right-hand "screw translation"

courtesy L Stannard

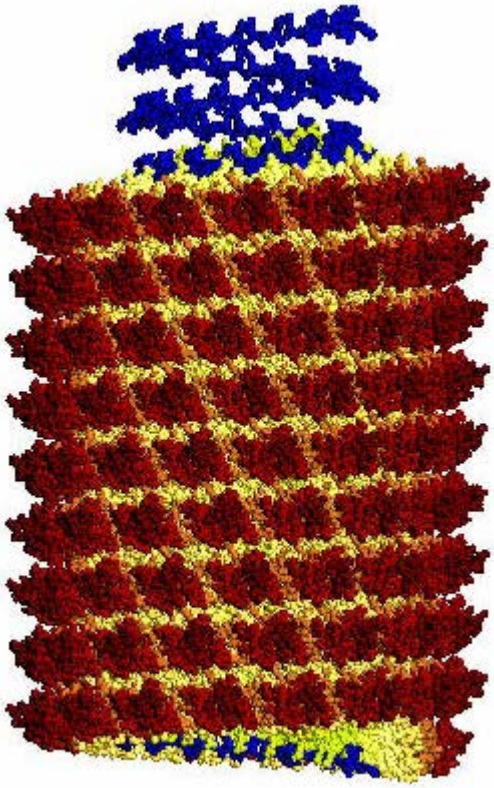


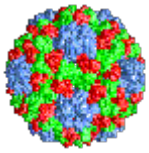
Image reconstruction of TMV

8th Report ICTV

In the case of **TMV** this is the **entire virion**: this is also the case for all **RODLIKE and FILAMENTOUS** virions where no membranes are involved. This includes all ***Tobamoviridae*, *Potyviridae*, and *Closteroviridae***, but NOT ***Filoviridae***, like Ebola virus (see **here**).

In other cases, **filamentous helical nucleocapsids** may be **enclosed within matrix protein** and a **membrane studded with spike proteins**: excellent examples of this are **PARAMYXOVIRIDAE**, detailed images

of which can be found **here**, at Linda Stannard's site, and **for ORTHOMYXOVIRIDAE** **here** in this tutorial.



"What is a Virus?" **Basic Virion Constituents** **Isometric Nucleocapsids**

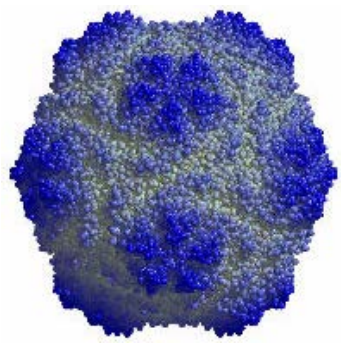
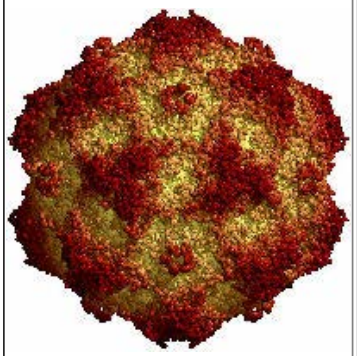
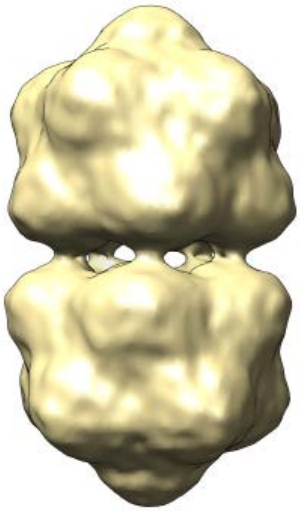
Tutorial Contents

Isometric Nucleocapsids

These are built up according to **simple structural principles**, as **amply outlined here**, and in **more detail here**. Put simply, nearly **all isometric virions** are constructed around a **BASIC ICOSAHEDRON**, or solid with 20 equilateral trinagles for faces. It suffices to say that the **"quasi-icosahedral"** capsid is possibly **Nature's most popular means of enclosing viral nucleic acids**; they come in many sizes, from **tiny T=1 structures** (Nanoviruses, eg: banana bunchy top virus; 18 nm diameter) to **huge structures** such as those of **Iridoviridae** or **Phycodnaviridae** (over 200 nm diameter).

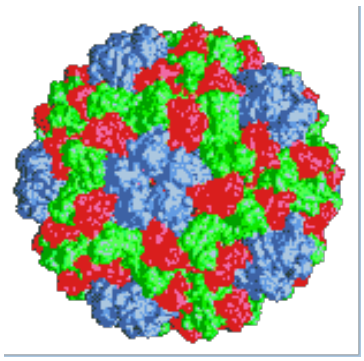
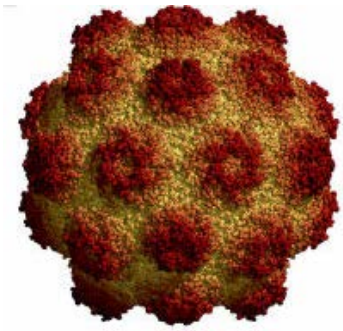
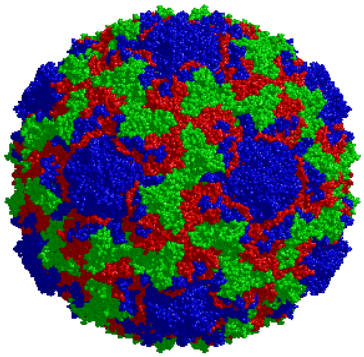
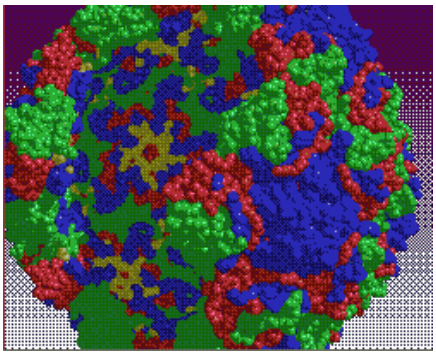
The simplest virions are those of the viruses with the smallest genomes: these are virions such as those of the **ssRNA satellite tobacco necrosis virus** (sTMV), the ssDNA **canine parvovirus** (CPV) and **porcine circovirus** (PCV), and **microviruses** infecting E coli and other bacteria (eg: φX174 phage): these all have a **simple icosahedral T=1 surface lattice structure**. Some examples are shown below. **All structural subunits of these capsids are in the same positional state, or have the same interactions with their neighbours.**

A unique derivative structure is that of **geminivirus** virions, which have **two incomplete T=1 icosahedra** joined at the missing vertex, with a twist.

		
Satellite tobacco necrosis virus	**Canine parvovirus**	**Maize streak mastrevirus**
Images from the ICTV 8th report; derived from the Virus World site, for non-profit educational use		**copyright Kyle Dent, UCT**

An **example of a more complex structures** are illustrated below. The animated GIF to the left shows **Cowpea chlorotic mottle (CCMV) virion surface structure (courtesy J-Y Sgro)**, which is composed of **180 copies of a single coat protein molecule, in a T=3 surface lattice**. The different colours in the CCMV picture represent **different "positional states" of the capsid protein**: subunits around **5-fold** rotational axes of symmetry are **BLUE**, and cluster as **PENTAMERS**; subunits around **3-fold axes** are **RED** and **GREEN** to reflect their different **2-fold symmetries**; they cluster as **HEXAMERS** around **"local 6-fold axes".**

Another example is that of **turnip yellow mosaic virus (TYMV):** this has **exactly the same basic structure**, with a **180 copies of a single type of coat protein subunit**, with the **pentamer-hexamer clustering** appearing more pronounced

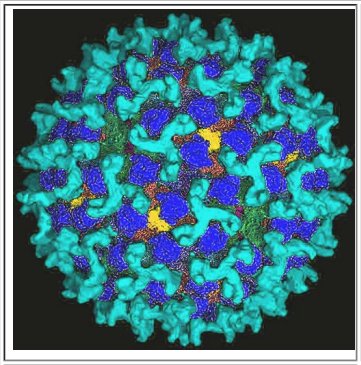
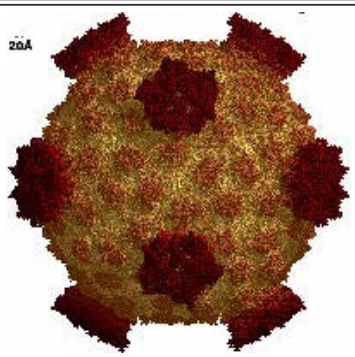
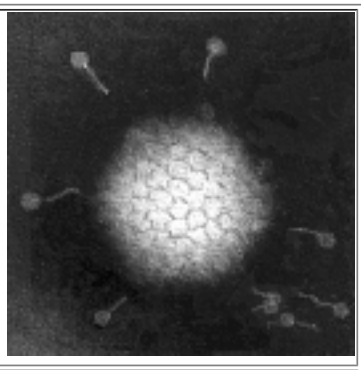
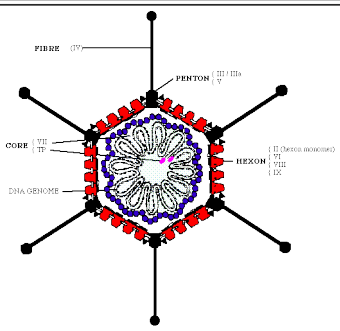
	
CCMV capsid	TYMV capsid
courtesy J-Y Sgro	ICTV 8th report
	
Rhinovirus R16	Rhinovirus R16
courtesy J-Y Sgro	

A **more complex capsid** - that of the common-cold-causing ***Rhinovirus*** R16 (family: ***Picornaviridae***), with **60 copies of 4 proteins in a T=3 structure** - is shown on the **bottom left and right** (animation modified from one by **J-Y Sgro**) and **right**. The **right** image shows a capsid with a cutaway, to reveal internal structure. **BLUE** subunits around 5-fold axes are **VP1**; **RED** and **GREEN** are **VP3** and **VP2** respectively; **YELLOW** subunits (seen only internally) are **VP4**. The VP4 subunits are formed by autocatalytic cleavage of **VP0** (into **VP2** and **VP4**) upon binding of a "**procapsid**" with **viral genomic ssRNA.**

Note the similarity between the CCMV and R16 structures - despite one having a single CP, and the other having 3 structural CP subunits.

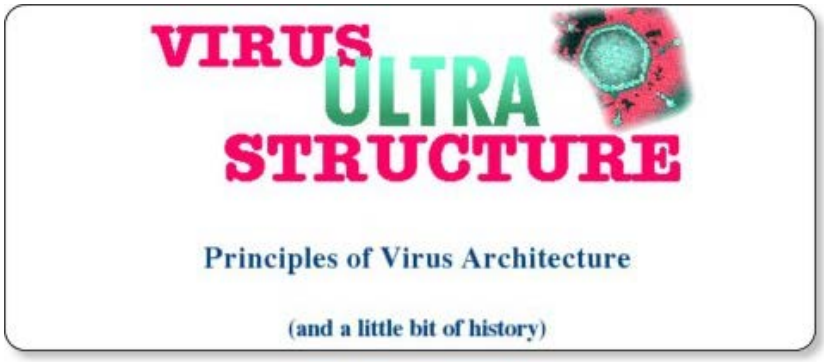
See here for further details of picornaviruses, here for a scheme showing picornavirus assembly, here for a scheme outlining polyprotein processing of picornaviruses, and here for material on picornaviruses from the University of Leicester course.

More complex capsids are generally found for viruses with **larger genomes**, whether composed of RNA or DNA. These include virions such as those of **reoviruses** and **adenoviruses**, both of which have complex or multilayered naked isometric capsids.

			
Bluetongue orbivirus (*Reoviridae*)	**Mammalian reovirus core with spikes**	**Electron micrograph of an adenovirus**	**Link to diagram of an adenovirus structure**
courtesy AJ Cann	ICTV 8th Report	**courtesy L Stannard**	**courtesy AJ Cann**

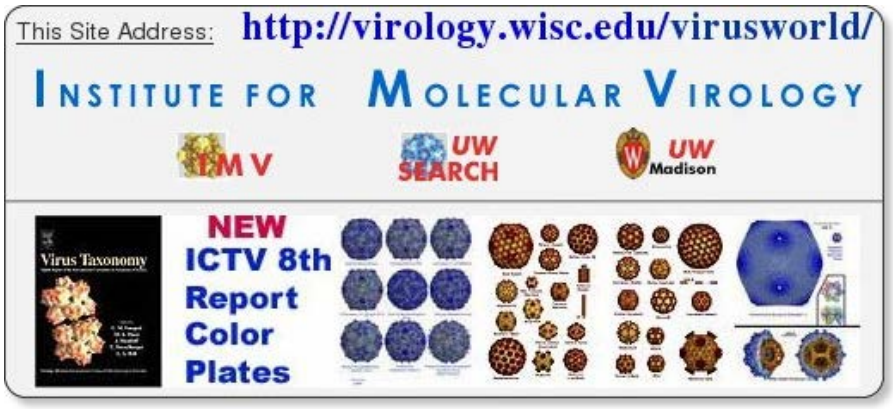
You may like to look here for structures from the University of Calgary material.

More complex structures may be seen in electron micrographs, and diagrams explaining icosahedral and quasi-icosahedral structure at Linda Stannard's Web site.

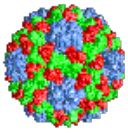


Principles of Virus Architecture via kwout

The "Virus World" site has an excellent set of high-resolution image reconstructions from physical data of non-enveloped simpler isometric viruses.



Virus World via kwout



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"What is a Virus?" **Enveloped Virions** **Helical Nucleocapsids** **Compound structures**

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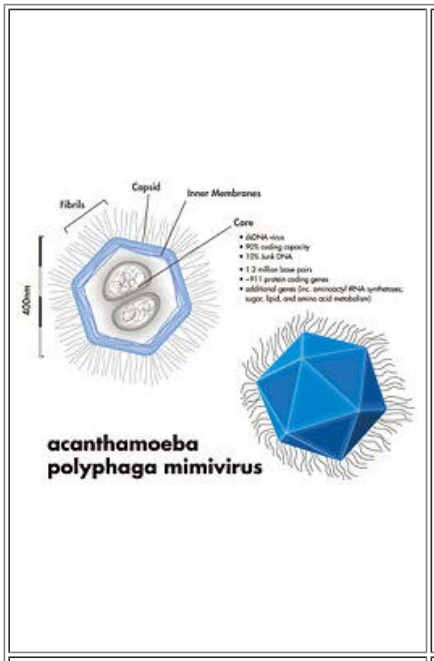
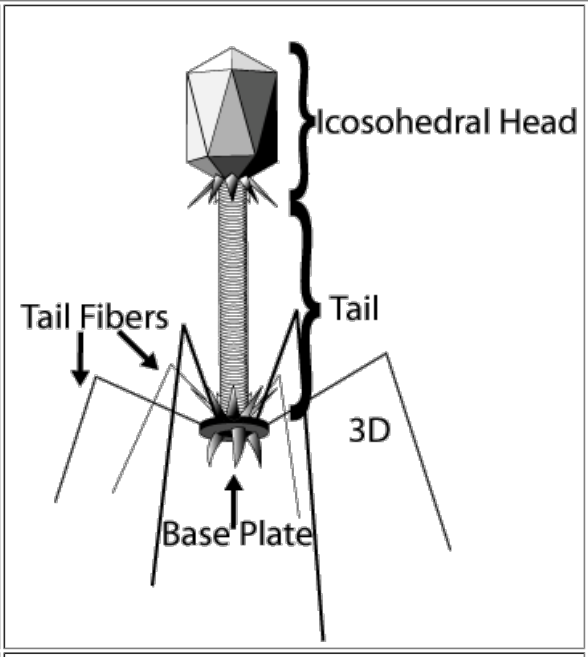
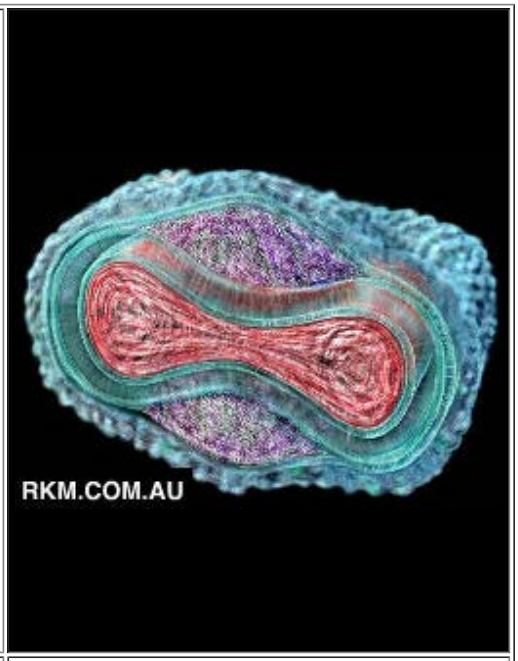
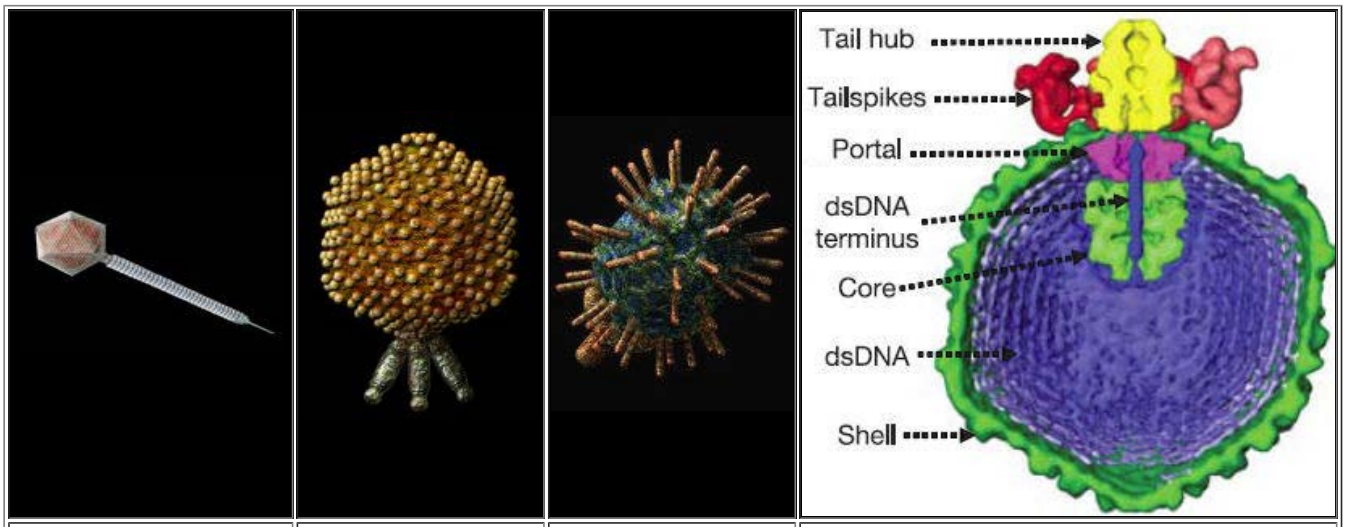
Complex or Compound Virions

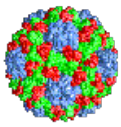
		
Mimivirus. The huge regular icosahedral capsid contains lipid membranes and other structures	**T-even phage particle: note elongated isometric head (icosahedron with hexamer expansion net), helical tail, and base plate and neck structures**	**Depiction of a orthopoxvirus virion. Note presence of two lipid bilayers and amorphous structure**
Link to Wikipedia	**Link to Wikipedia**	**copyright Russell Kightley**

Most **bacteriophages** - or more properly, **archaeal and bacterial** viruses - have a more complex structure than the simple isometric and helical nucleocapsids typical of many plant and animal viruses. Even viruses with relatively small genomes may have more than one type of architecture associated with their virions, normally in the shape of some kind of **TAIL** structure attached to an isometric **HEAD**. The **T-even** viruses - part of the **"T4-like virus" genus, family *Myoviridae*** - have the general structure shown centre, above: this is an **isometric head** attached via a **connector assembly** to a **contractile helically-constructed tail** with a rigid **core**, with **tail fibres** and **baseplate. See here for explanation of how the virus gains entry to enterobacterial cells.**

λ-like viruses - family ***Siphoviridae*** **- have thinner non-contractile tails** and **isometric heads,** with **tail fibres.** The **P22-like and Φ29-like viruses** in family ***Podoviridae*** and ***Salmonella*** **phage Epsilon 15** have **isometric** heads and **short, thick tails**.



Enterobacteria phage λ	Enterobacteria phage P22	Bacillus phage Φ29	Salmonella phage Epsilon 15
copyright Russell Kightley			Reprinted by permission from Macmillan Publishers Ltd: Jiang W, et al. Nature. 2006 Feb 2;439(7076):612-6 copyright 2006



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"What is a Virus?" | Helical Nucleocapsids | Isometric Virions | Enveloped Virions

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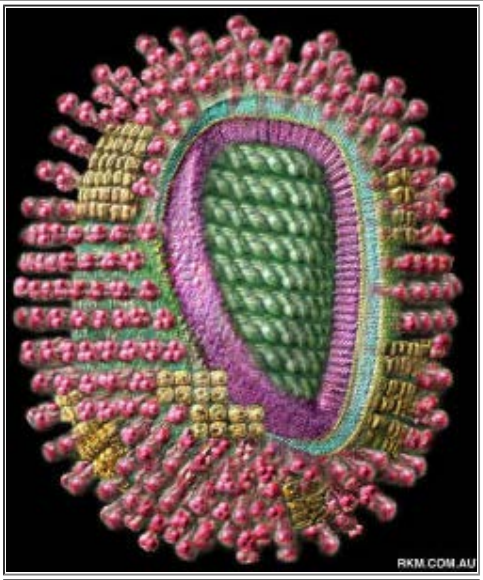
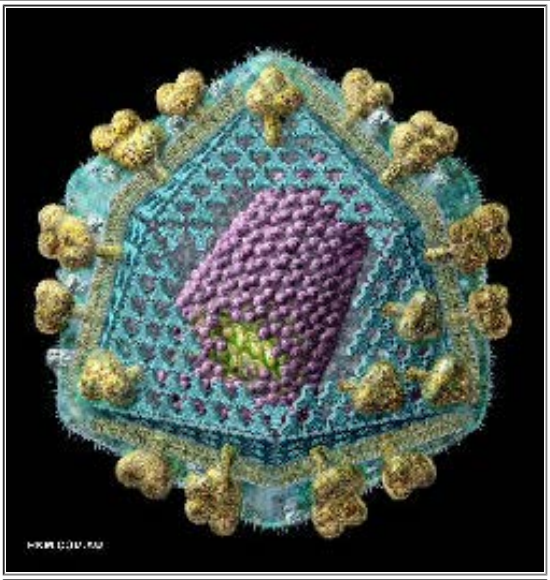
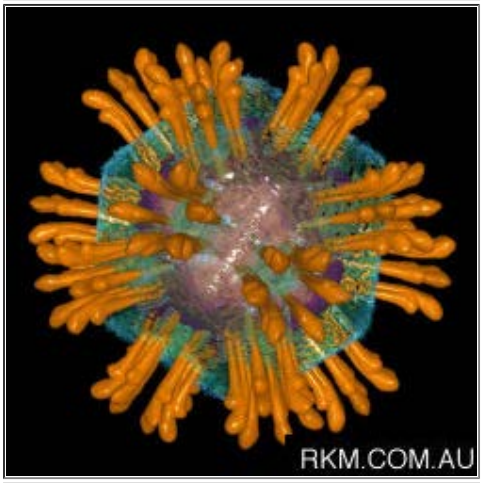
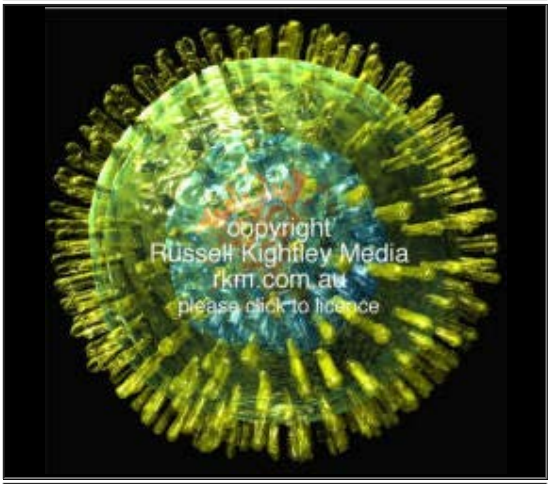
Introduction to Molecular Virology

Enveloped Virions

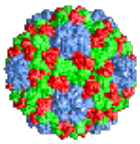
SOME virions additionally have:

- **ENVELOPES**, or **lipid bilayer MEMBRANES**: these always have **viral proteins** inserted in them (almost always **GLYCOPROTEINS**); these are often referred to as **SPIKES**.
- There is usually an **inner layer** of **protein** (usually referred to as **MATRIX** protein) inside the envelope, usually in contact with the **(nucleo)CAPSID** protein. See below for examples (West Nile virus, *Flaviridae*; influenza virus, *Orthomyxoviridae;* HIV, *Retroviridae;* SARS coronavirus; Hepatitis C flavivirus. The nucleoprotein can be helical (Ortho- and Paramyxoviridae) or isometric

		
Cutaway depiction of West Nile flavivirus: note regular arrangement of envelope protein, underlying membrane and isometric capsid	**Cutaway depiction of influenza virion: note helical nucleoprotein, matrix and envelope layers and spikes**	**Cutaway depiction of HIV virion: note conical nucleoprotein, isometric matrix and envelope layers and spikes**
		
SARSCoV (SARS coronavirus). Note elongated membrane	**Hepatitis C flavivirus (HCV): note elongated, regularly-**	**Herpesvirus: note many envelope**

spikes and underlying isometric capsid	**spaced spikes and isometric structure**	**glycoproteins, "tegument" inside membrane, and isometric capsid**
copyright Russell Kightley (www.rkm.com.au)		

Enveloped virions may additionally incorporate **host membrane proteins** in their envelopes: while this may be a **side effect of the "budding" process** by which viral nucleoprotein complexes acquire envelopes, it may also in some cases be a **mechanism for avoidance of host immune systems** (eg: **hepatitis B virus** incorporates **host serum albumin** into its capsid; **HIV virions** may incorporate **host MHC** proteins).



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"What is a Virus?" | **Isometric Virions** | **Helical Nucleocapsids** | **Compound structures**

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AUSTRALIAN HORSE DISEASE

In response to misinformation on the Net, Dr Paul Morley posted the following:

```
To: virology@net.bio.net
From: Morley@admin3.usask.ca (Paul Morley)
Subject: Re: horse disease in Australia
Date: Thu, 8 Dec 1994 09:28:37

This was thought to be caused by a
morbillivirus.  I have attached a posting from the chief veterinary officer of
Australia to the OIE.

Paul Morley
Morley@admin3.usask.ca
Western College of Veterinary Medicine, University of Saskatchewan, Canada
```

---

12 October 1994

Dr Jean Blancou
Director General
Office International des Epizooties
PARIS
Dear Dr Blancou

Acute Equine Respiratory Syndrome in Brisbane, Queensland

I would like to inform you of a condition which has occurred in the period from 7 to 26 September 1994, during which fourteen thoroughbred horses died or were euthanased after becoming seriously ill with an **"acute equine respiratory syndrome"**.

The condition has been restricted to properties directly associated with 1 stable in the suburb of Hendra in Brisbane The situation as of 12 October 1994 is summarised in the following table:

Location	Description	No. of horses recovered	No. dead or euthanased	No. in-contact Healthy	Total
* Hendra 1 (Brisbane)	IP1	4	11	9	24
* Hendra 2 (Brisbane)	neighbour to IP1	1	1	10	12
* Hendra 3 (Brisbane)	neighbour to IP1	0	0	7	7
Cannon Hill (Brisbane)	holding associated with IP1	0	1	14	15
Kenilworth (150 kms north of Brisbane)	holding associated with IP1	1	1	300	302
Samford	holding associated with IP1	1	0	20	21

*These 3 stables are immediately adjacent to each other

Although there are 300 horses on this holding, the 2 affected horses from IP1 have not been in-contact with the other horses on this spelling property.

All negative to Serum Neutralisation test

The 7 recovered horses are positive on SNT

Extensive investigations have been undertaken by government veterinary authorities and private veterinary experts and known endemic and exotic diseases of horses have been excluded.

Clinical Signs

Of the 21 cases known to date, 14 have presented as an acute respiratory syndrome with death in 1 to 3 days. A further 4 animals have been clinically affected but recovered, while 3 have been virtually inapparent infections. Clinical cases have been inappetant and pyrexic (up to 410C) with shallow respiration and most having a frothy nasal discharge varying from clear to blood tinged. Mucous membranes have usually been dark to cyanotic and sometimes slightly jaundiced. Several animals were reported to have dependent oedema (angle of jaw, legs, sheath), ataxia and head pressing. Terminal animals have usually died in extremis with a copious frothy nasal discharge.

Pathology

The common feature at gross necropsy has been severe lung congestion and oedema with blood-stained stable froth in the airways. Other findings have been petechial and ecchymotic haemorrhages in various organs as well as slight jaundice. Microscopically, lesions are those of an acute interstitial pneumonia with damage to endothelial linings of small blood vessels, haemorrhage and foci of early necrosis.

Aetiology

The Australian Animal Health Laboratory (AAHL) and the Animal Research Institute, Queensland have isolated the same virus from 5 cases. Since the initial virus isolation, all further work with potentially infective material has been undertaken at AAHL. Studies at AAHL have shown this virus (AERS virus) belongs to the Paramyxoviridae family and may be a member of the Morbillivirus genus. Evidence for this includes:

- electron microscopy shows a nucleocapsid with a herring bone pattern typical of the Paramyxoviridae;
- AERS virus haemagglutinates guinea pig red cells but results are variable with red cells from other species;
- AERS virus does not possess neuraminidase which is consistent with the Morbillivirus genus;
- nucleotide sequencing studies suggest that the virus is a Morbillivirus but work is continuing in this area;
- immunofluorescent antibody studies on virus infected monolayers using antibodies to turkey rhinotracheitis, rinderpest, Newcastle disease, respiratory syncitial, mumps, measles, canine distemper, and parainfluenza 3 viruses were negative;
- immuno electron microscopy shows virus nucleocapsids to bind to labelled antibody to the isolated virus.
- Antibodies to parainfluenza types 1, 2 and 3 produce low level binding.

Transmission tests in 4 horses at AAHL have shown the isolated virus is the cause of the syndrome. Evidence for this includes:

- no bacterial pathogen or toxin could be detected; African horse sickness, equine influenza, equine herpes virus, equine viral arteritis and the equine viral encephalitides were eliminated;
- AERS virus was isolated from the lungs of 5 to 6 cases;
- specific antibody to AERS virus is present in 4 recovered cases and 3 in-contacts believed to have suffered a very mild and transient illness, but not in other horses;
- positive transmission tests to 4 horses, 2 of which received pure virus culture, with subsequent recovery of the virus at autopsy.

Natural History

The incubation period in the natural cases was mostly 8 to 11 days with a maximum possible of 16 days. With the AAHL transmission tests, the incubation period was 3-12 days. Two suspect human cases had a putative incubation period of 5-8 days.

All the other cases (20) in horses and the suspect human cases can be linked to the original index case which was first observed sick on 7 September and died on 9 September.

From the pathogenesis, clinical picture and pattern of spread, natural transmission is most likely direct via frothy nasal discharges as a consequence of close contact or mechanical transfer. Aerosol transmission seems unlikely - the upper respiratory tract does not have lesions and coughing is not a feature of the syndrome.

Available evidence suggests that this virus is not highly contagious under conditions of natural transmission.

To date there is no evidence as to the possible source of the virus.

Serological Surveillance Results

AAHL has developed a Serum Neutralisation and an Immunofluorescence test and to date 1265 samples have been collected for testing. Results of 587 tests finalised to date, have shown that the only positive horses have been those located on, or originating from, the infected Hendra racing stable in suburban Brisbane. All horses within a 1 kilometre radius of this infected premises are being tested for presence of antibodies to this virus. Further results are expected within the next 48 hours. Blood samples have been submitted from areas outside the proclaimed Infected Area with all results to date being negative.

Movement Controls

Based upon surveillance results, the infected area has been reduced from a broad area of southeast Queensland to a 5 kilometre around the index premises and premises associated with direct movement of horses from the index stable. Limited horse race meetings have resumed in Brisbane and based on serological survey results indicating no seroconversion in horses not on quarantined premises all movement restrictions, except those applying to quarantine premises, have been lifted from midnight on 11 October 1994.

Public Health

The trainer of the horses also became affected with a severe respiratory condition and died on the 27 September 1994. The trainer, a stablehand, and the consulting veterinarian have seroconverted to the isolated virus. The stablehand exhibited influenza-like symptoms, but the veterinarian remained in normal health.

Blood samples from 18 other humans associated with this suburban location have remained sero-negative.

Transmission is believed to have been caused by very close contact with infected horses, for example hands in the mouth, and close contact with infected saliva.

The Queensland Director of Public Health has assured the general public that there is no public health risk from the equine virus.

I will keep you informed as further information becomes available.

Yours sincerely

GARDNER MURRAY

Australian Chief Veterinary Officer

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RETURN TO MAIN

ProMED Digest #205
From: douglaI@prose.dpi.qld.gov.au (Ian Douglas)
Date: Thu, 24 Oct 1996 18:29:06 -0400
Subject: PRO/AH> Equine morbillivirus - Australia: bat reservoir (07)

EQUINE MORBILLIVIRUS - AUSTRALIA: BAT RESERVOIR (07)
==========

From: "Douglas, Ian"
Date: Thu, 24 Oct 1996 16:32:00 +1000

Subject: A new bat paramyxovirus

Queensland Department of Primary Industries (DPI) scientists, led by Dr Peter Young, have achieved a major breakthrough in solving the equine morbillivirus mystery with the identification of flying foxes (fruit bats), genus Pteropus, as the natural host of the virus.

A paramyxovirus has been isolated from animals representing three species of fruit bat. The DPI team, including Kim Halpin and Hume Field have shown that this bat paramyxovirus is identical to equine morbillivirus by electron microscopy, immunofluorescence, neutralisation tests and sequencing of the matrix protein gene.

Previous studies by the research team have shown that the antibody prevalence rate in flying foxes is about 15% (Emerging Infectious Diseases Vol 2, 239-240). However there is no indication that the virus has caused infection of people who have had close contact with these animals over long periods of time. Nor is there any indication of spread to other animals, except for the two known occasions when infection occurred in horses, and subsequently in humans in close contact with infected horses.

The DPI research team will now concentrate on discovering the method of transmission of bat paramyxovirus among flying foxes. This information should provide clues to the mechanism of spill-over to other animals.

These results are being prepared for publication. Requests for further information on this work should be directed to:

Dr Peter Young - youngp@prose.dpi.qld.gov.au".

K J Dunn
Executive-Director, Animal and Plant Health Service and
Chief Veterinary Officer (Queensland)

[- In the previous 48 hours we have had a series of journalists' reports come into ProMED but we requested an authoritative report before posting. Thanks, Ian. MHJ -]

.....mhj

From: pharm6@op1.up.ac.za (Jonathan Taylor)
Date: Thu, 24 Oct 1996 18:57:34 -0400
Subject: PRO/AH> FIV in African lions

FELINE IMMUNODEFICIENCY VIRUS IN AFRICAN LIONS
==========

Date: Wed, 23 Oct 1996 15:57:40 GMT+2
From: "Jonathan Taylor"
Via: "Veterinary professionals list"
Subject: Re: FIV in Africa

> Date sent: Mon, 21 Oct 1996 11:29:33 -0400 (EDT)
> From: "Susan G. Wynn, DVM"
> To: "Veterinary professionals list"
> Subject: FIV in Africa

> I had a client tell me that an African gamekeeper informed her that most
> of the lions in Africa are FIV positive. She does field work for some
> wildlife preserve in Kenya, so I cannot discount this story completely.
> Does anyone know anything about this?
>

A sizeable population of the lions in the Kruger Park are FIV positive, but whether this is clinically significant in the short life span of a wild lion is unknown.

Probably a more pressing problem is that some of the lion in the Southern part of the park have been diagnosed with TB, probably infected by Cape Buffalo, infected in turn by cattle bordering the park.

- ---
Jonathan Taylor
Onderstepoort
South Africa

[- One might wonder whether FIV might have contributed to the recently reported outbreaks of lethal canine morbillivirus infections ("distemper") in African lions as well. MHJ -]
.....mhj

From: mjn@mailpc.brs.gov.au
Date: Thu, 24 Oct 1996 19:42:23 -0400
Subject: PRO/AH> Equine morbillivirus - Australia: bat reservoir (08)

EQUINE MORBILLIVIRUS - AUSTRALIA: BAT RESERVOIR (08)
==========

From: "Nunn, Mike"
Date: Tue, 22 Oct 96 14:28:00 PDT

For people with access to the Worldwide web, Ian Douglas' information sheet and earlier information on EMV are available in Adobe .pdf format from:

www.dpi.qld.gov.au/ahwb/ahwbmain.htm

- ---
Mike

Dr M.J. Nunn
Principal Veterinary Officer
Bureau of Resource Sciences
P (06) 272 4036
F (09) 271 6697

[- The following gives additional information and updated background relating to the previous posting "Equine morbillivirus - Australia (07)". MHJ]

[Ian Douglas' internet] AUSTHORSE posting of 16 October:

Some time ago I posted material relating to the research into the causative agent of the Acute Equine Respiratory Syndrome AERS - the virus referred to as the equine morbillivirus (EMV).

The Queensland DPI has today released results from its search for the virus's natural host.

I attach the text of an information sheet prepared to provide an update, for your information.

This important research result has come from work led by Dr Peter Young, leader of the QDPI Animal and Plant Health Service research team, based at the Animal Research Institue, Yeerongpilly, Brisbane..

Ian Douglas
Principal Veterinary Officer

A NEW BAT PARAMYXOVIRUS OF FLYING FOXES - LIKELY NATURAL HOSTS FOR EMV

The Animal and Plant Health Service has isolated a new bat paramyxovirus from several flying foxes. The similarity of this virus and the virus associated with the fatal -Acute Equine Respiratory Syndrome (AERS)-, first described in September, 1994, suggests that the flying fox is the natural host of AERS. This information sheet updates previous advices concerning AERS research.

Investigations

Experimental challenge of a range of animals with EMV showed that the disease could be reproduced artificially in cats and guinea pigs. A survey of 500 cats from the Brisbane metropolitan area showed that none tested positive for EMV.

A retrospective study of horse specimens submitted to veterinary laboratories in Queensland going back as many years as possible, has looked for cases that may have been caused by EMV but which were not diagnosed as such at the time. No cases have been found.

Since 1994, over 5000 animals throughout Queensland, including a range of wildlife species, have been tested for evidence of EMV. Most of those tested were horses. In addition, horses suffering from sudden illness or death with signs that may have been caused by EMV have been investigated with negative results.

It seems clear that EMV infection of horses is not a usual occurrence in this State. It may be that these two foci of infection in horses are the only previous occurrences. An on-going watch for possible future outbreaks will be maintained.

All testing conducted has, with the exception of flying foxes, failed to show any evidence of EMV infection in locations or in animal species other than those initially detected (horses and humans at Hendra and Mackay).

Work with Flying Foxes

Animal and Plant Health Service researchers found that about 15% of animals tested, from all four species of flying fox present in Australia, carried antibodies to EMV.

This antibody reactivity with the equine morbillivirus confirmed that the bats had been previously infected by a member of the Paramyxovirus group of viruses which is the same family as that to which EMV belongs and also suggests that the bat virus is closely related to EMV.

It was not possible, based on antibody presence alone, to establish the degree of relatedness of the bat paramyxovirus (BPV) to the EMV virus. To do this it was necessary to isolate BPV and compare it with EMV.

This was achieved in September 1996 when the APHS research team, working at the Animal Research Institute, isolated a paramyxovirus from uterine fluids from an injured female flying fox that had miscarried twin foetuses. The Grey headed flying fox (Pteropus poliocephalus) was found in the Brisbane area. An apparently identical virus has subsequently been isolated from three other flying foxes.

The first virus isolated reacts strongly with human and horse serum samples that have antibodies to EMV. Similar changes to cell cultures are produced by BPV and EMV and the viruses appear identical on electron microscopy. Preliminary studies have shown that the genetic sequence of the newly isolated virus is similar to the EMV isolates. These results demonstrate that BPV and EMV are the same virus.

Flying foxes are dispersed widely throughout the country and overseas and are native species. As only two outbreaks of EMV infection have been recorded it appears that spill-over of this virus to other species is a rare event.

It is likely that flying foxes do not pose a significant risk to people. The three human cases that are recorded all contacted the virus from horses. Until more is known about this bat paramyxovirus it would be advisable for those handling flying foxes to observe normal good hygiene and care.

With this break-through achieved, our future work on the natural history of the infection can advance. The Animal and Plant Health Service will look to solve the final piece of the puzzle, how the virus jumped from bat to horse on two occasions. The possibility that the seasonal occurrence of infection may be related to breeding cycles in flying foxes and to pregnancy in the two mares that first contacted the virus will be researched.

.....mhj

Emerging Plant Viruses Causing Disease Problems

Ed Rybicki and Gerhard Pietersen
October 2000

This material is excerpted and modified from:
"Rybicki EP, Pietersen G (1999) Plant virus disease problems in the developing world. Advances in Virus Research 53: 128-175"

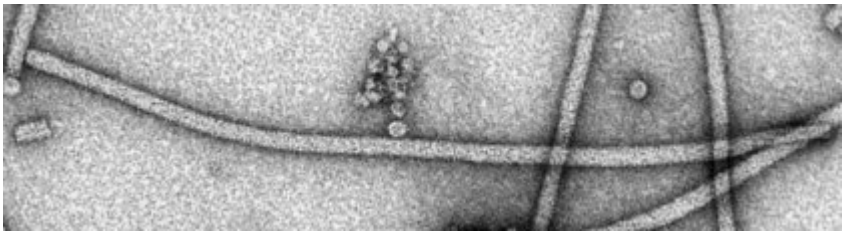
There are three major groups of viruses that seem to be most important among new disease-causing agents worldwide, and that could reasonably be termed "emerging" in terms of apparently being new viruses causing new and serious diseases. The most important of these are in the taxonomic families *Potyviridae* and *Geminiviridae*, the *Tospovirus* genus of the *Bunyaviridae*, and perhaps also pararetroviruses like *Banana streak virus* (BSV).

- *Potyviridae*
- *Geminiviridae*
- *Tospovirus*
- *Banana streak virus*

Potyviridae

The *Potyviridae* are the largest single taxonomic group of plant viruses, and the subject of more scientific papers per year than any other plant virus taxonomic group except perhaps the geminiviruses. It seems that potyviruses, and specifically aphid-transmitted viruses of the genus *Potyvirus*, are one of the most successful groups of plant pathogens in the world. The genus has been claimed to be almost as ancient as flowering plants, and has a worldwide distribution throughout higher plants. New potyviruses seem to appear with new crop introductions just about anywhere these occur. In South Africa, for example, *Passiflora edulis* (passionfruit) cultivation in the 1980s was severely limited by diseases, many of which appeared linked to a mixture of potyvirus-like agents. One of these viruses was characterized here in detail and found to be not the passionfruit woodiness virus (PWV) characterized in Australia and the Far East, but a distinct species now known to be a strain of cowpea aphid-borne mosaic virus (CABMV). It is very interesting that a similar disease in Brazil seems to be caused by a closely-related strain of the same virus, whereas woodiness disease in Australasia and Asia is caused by distinct species of viruses.

Another intriguing aspect of emerging potyviral plant diseases is the appearance of hitherto latent virus infections. A well-documented case investigated here was the appearance in cultivated specimens of the horticulturally important flowering bulbs *Ornithogalum* and *Lachenalia* species of at least two distinct potyviruses. One of these, *Ornithogalum mosaic virus* (OrMV), first described in South African-derived bulbs in Holland, was found in plant specimens collected in localities where the plants were endemic. However, it caused apparent or overt infections only when plants were kept in cultivation.


Particle of *Ornithogalum mosaic virus* (OrMV)

Geminiviridae

Viruses in the family *Geminiviridae*, and specifically the whitefly-transmitted begomoviruses, are presently associated with severe diseases in tomatoes the world over, with cotton disease in India and elsewhere, and with

disease problems and food shortages due to disease in cassava in central Africa. Infections with *Bean golden mosaic virus* (BGMV) are apparently the single largest limiting factor to bean production in Central America.

Polston and Anderson (1997) covered the Caribbean basin and the Central American region in an excellent review of the emergence of whitefly-transmitted geminiviruses (WTGs) in tomatoes. They estimated that 20 - 100% of crop loss throughout these areas has been caused by epidemics of WTGs, ranging from *Tomato golden mosaic* (TGMV) to *Tomato yellow leaf curl* (TYLCV) to *Potato yellow mosaic* (PYMV). They listed 17 distinct viruses known to affect tomatoes in this region, and this is probably a conservative estimate, as more are constantly being found.

Much of the apparently emerging nature of the virus diseases is due to the worldwide spread of a new "silverleaf" or B biotype of the vector *Bemisia tabaci*, which is now being touted as the new species *Bemisia argentifolii*. The new vector has a much wider range of preferences for feeding than older vector types, which has apparently resulted in the spread of viruses that normally infect only weed or endemic plant species into adjacent, previously untargeted crop species. Roye *et al.* (1997) surveyed geminiviruses in weeds (notably *Sida* and *Wissadula* species) in Jamaica and showed a population of viruses distinct from crop-infecting geminiviruses - and one perhaps poised to enter crops once a suitable vector biotype appears. Although the problem also exists in the developed world , and most notably in the southern United States and Europe, it can be dealt with, to at least some extent, by changing cultivation practices. The same option does not exist in the developing world, however, due to the expensive nature of the measures (screen houses, heavy spraying regimes). Thus, the inexorable spread of the vector into these areas will almost certainly mean heavy crop losses.

This may have already happened in cassava in Uganda and western Kenya; however, the disease problem there is also associated with a natural recombinant virus (between *African cassava mosaic virus*, ACMV, and *East African cassava mosaic virus*, EACMV) with increased virulence. The scale of the problem was aptly illustrated by an anonymous agricultural researcher from Uganda, who said (to E.P. Rybicki, in 1997) that there was no cassava disease problem in eastern Uganda, as there was no more cassava to be infected!

Severe leaf distortion and mosaic and leaf loss in cassava in western Kenya caused by *African cassava mosaic virus / East African cassava mosaic virus* recombinant. Inset: healthy leaf. Photos by EP Rybicki, 1997

Begomovirus diseases also seem to exemplify a phenomenon first noted with potyviruses; unrelated or distinct virus species in the genus cause very similar disease syndromes in the same crop plants, usually in widely separated geographical areas but sometimes in the same small area. Thus, TYLCV-Israel, -Sardinia, and -Thailand are distinct species of virus despite causing the same disease; however, so are the several viruses causing cotton leaf curl disease in India and Pakistan, and the several distinct viruses causing tomato leaf curl in India. This is undoubtedly related to their worldwide distribution and also to their apparent antiquity. Geminivirus variation appears linked to geographic separation and host plant genetic divergence, as well as to continental drift (Rybicki, 1994). All these

factors indicate that the youngest age for the family *Geminiviridae* is hundreds of millions of years and that of begomoviruses is at least tens of millions of years. This diversity and ubiquity guarantee that a wide variety of virus genotypes exists, probably as well-adapted and essentially symptomless infections in endemic species or weeds, and that any change in transmission characteristics of the vector will result in appearance of the virus in crop plants almost instantly.

Whereas begomoviruses have been making their presence felt as they spread out of endemic hosts into cultivated species, viruses in the genus *Mastrevirus* continue to emerge into crop species in Africa. Maize streak disease (MSD) was first described in 1901, so can hardly be considered as emerging. However, more recent findings indicate that a hitherto unsuspected and distinct group of *Maize streak virus* (MSV) strains cause disease in wheat and some grasses, rather than the disease being caused by the same closely-related group of virus isolates and strains as are found in maize (Rybicki *et al*., 1998; see also here). Sugarcane streak virus(es) (SSVs) also still seem to be present in Africa. Whereas the better characterized variants SSV-Natal and SSV-Mauritius have not been a problem in southern Africa for over 30 years, and especially not in commercially-grown sugarcane varieties, distinct mastrevirus species have recently been characterized from mildly diseased "traditional" material from Egypt (Bigarré *et al*., 1999).

A ray of hope in the struggle to combat TYLCV-like diseases is the fact that genetic engineering approaches using pathogen-derived resistance appear to be quite promising. Tomato plants transgenic for the TYLCV coat protein appear to be resistant to the virus (Kunik *et al*., 1994). Additionally, transgenic *Nicotiana benthamiana* plants expressing antisense RNA to the *Rep* gene were resistant to TYLCV infection (Bendahmane and Gronenborn, 1997). These developments, coupled with traditional and marker-assisted breeding techniques, may allow rapid dissemination of improved material to farmers.

Tospoviruses

The genus *Tospovirus* (family *Bunyaviridae*) is an extremely important group of plant viruses capable of infecting a large range of important crops. The type member, *Tomato spotted wilt virus* (TSWV), has one of the broadest host ranges among plant viruses. Tospoviruses are transmitted exclusively by thrips in a circulative propagative manner, meaning that the virus multiplies in the vector. TSWV was thought to be the only member of this genus until recently, and has had a serious impact on many crop species worldwide. It causes severe outbreaks in a large variety of crops grown in tropical and subtropical climate zones. Since 1990, other related but distinct viruses in this genus have been identified. The following members or tentative members are described: TSWV, *Groundnut ringspot virus* (GRSV), *Tomato chlorotic spot virus* (TCSV), *Impatiens necrotic spot virus* (INSV), *Watermelon silver mottle virus* (WSMV), and *Groundnut bud necrosis virus* (GBNV).

Owing to the worldwide spread of the Western flower thrip (*Frankliniella occidentalis* Pergande), the most efficient vector of tospoviruses, TSWV and some of the other tospoviruses are increasing being reported as causing problems in developing countries. These "new" tospoviruses often originate in developing countries, and some appear to occur only in these countries. For example, GRSV was first detected on peanuts during a survey of the viruses of this crop in South Africa, and isolate SA-05 now serves as the type isolate of GRSV. Thus far, this virus has been detected only on peanuts in South Africa, where it is relatively common, and on tomatoes in Brazil and Argentina. TCSV was first reported from Brazil, and GBNV was first detected in India.

Research on this important virus group has increased dramatically, and new tospoviruses are constantly being found. It could be that only the lack of resources in developing countries for the diagnosis of hard-to-identify viruses prevents even more reports of new tospoviruses.

Banana Streak Virus / Pararetroviruses

Banana streak virus (BSV) is a pararetrovirus - that is, a virus with circular dsDNA in virions which replicates via a longer-than-genome-length ssRNA(+) intermediate, via reverse transcription (see here). BSV may be considered an emerging problem in light of recent findings suggesting, first, that it occurs in an integrated form in *Musa* (see here); second, that it has been found in this form in all *Musa* germplasm tested thus far; third, that it is seed transmitted; and fourth, that the integrated virus can be activated to form the episomal form through stress. Stress can possibly include tissue culture propagation. Thus, it may be that initial BSV infections may largely be the result

of plant stress, which means that BSV infections could occur anywhere, at any time, in the absence of any vectored introduction. It may be that genetic engineering in the form of a "knock-out" of the integrated viral genome can come to the rescue, at least of major commercial varieties.

Contents:

Topic	Level	Topic	Level
What is a Virus?	MCB205F	Emerging Viruses	MCB205F/ MCB311S
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Classification of Viruses	MCB205F	Response and Evaluation Form	All
Viral Genome Diversity	MCB205F	Mirrored Virology Course From University of Leicester	All
Virus Ultrastructure: by Linda Stannard	MCB311S	Other Teaching Material Links	All
Entrance, Entertainment and Exit: Virus Entry Into Cells Replication of Viruses	MCB311S	NetMind	
Origins of Viruses	MCB311S	Note on Use	

A mirror of the Virology BS335 course

may no longer be offered thanks to changes in policy by the University of Leicester

despite the best efforts of AJ Cann

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Note:

This tutorial draws heavily on print published as well as electronically published work, as well as on things I and others have written that have not been published.

This was originally written as a supplement to my **Introduction to Virology** 7-lecture course to second-year Microbiology major students (MIC202S) in the **Microbiology Department** at the **University of Cape Town.** It now constitutes a full 30-lecture course (**Molecular and General Virology**) given to 3rd year students.

I hope you find it useful!! However, please do NOT download and use material in any other form than it is presented here, without permission.

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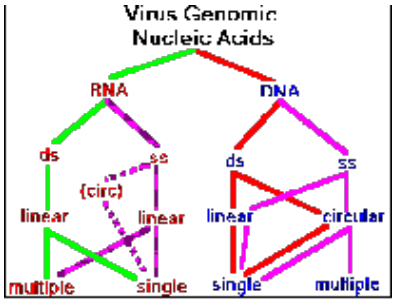
unless otherwise stated

This Version: 7th May, 2003

Introduction to Molecular Virology

Genome Diversity



Click the Figure for a graphical depiction of the many different types of viral genome found in virions.

DNA						RNA					
double-stranded			single-stranded			double-stranded		single-stranded			
linear	circular		linear	circular		linear		linear (circular)*			
single	single	multiple	single	single	multiple	single	multiple	(+)sense		(-)sense	
								single	multiple	single	multiple

* = viroids only

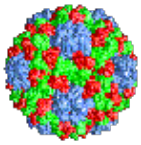
Viruses are the only organisms on this planet to still have **RNA** as their sole genetic material. They are also the only autonomously replicating organisms to have **single-stranded DNA**. The range of virus genomes as found in virions encompasses:

- **single-component dsDNA**, linear or circular (occasionally circularly permuted linear);
- single, double or multi-component **circular ssDNA**;
- single-component **linear ssDNA**;
- single or multi-component **dsRNA**;
- single or multiple component **ssRNA genomes** which may be
  - totally **"positive"**(or messenger) polarity,
  - totally **"negative"** (or anti-messenger) polarity, or
  - **partially positive negative-sense**;
- "diploid" **positive-sense ssRNA genomes which replicate via reverse transcription** to and transcription from longer-than-genome-length dsDNA, and
- nicked and/or partially **dsDNAs which replicate via transcription** to and reverse transcription from longer-than-genome-length positive-sense ssRNA.

In contrast, **prokaryotes** have **mainly single-component circular** (occasionally multiple) or **linear dsDNA (*Streptomyces*, *Helicobacter*)** while all **eukaryotes have multi-component linear dsDNA**, and all the genomes replicate **via the classic semi-conservative route**.

Viral genome types have inspired a **classification based on what is found in virions**, coupled with their replication strategy. This is the **Baltimore Classification,** and will be discussed in a broader treatment of **Virus Replication.**

An overview of the taxa Recognized in the Universal System of Virus Taxonomy in terms of their genome content may be found here.



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Viruses sorted according to general host categories

Viruses sorted according to host categories

Algal Viruses

VC Order; Family; Subfamily	VirusCode Genus Name	VirusCode	Type Species Name
00.109. *Marnaviridae*	00.109.0.01. *Marnavirus*	00.109.0.01.001.	*Heterosigma akashiwo RNA virus*
00.051. *Phycodnaviridae*	00.051.0.01. *Chlorovirus*	00.051.0.01.001.	*Paramecium bursaria Chlorella virus 1*
	00.051.0.02. *Prasinovirus*	00.051.0.02.001.	*Micromonas pusilla virus SP1*
	00.051.0.03. *Prymnesiovirus*	00.051.0.03.001.	*Chysochromulina brevifilum virus PW1*
	00.051.0.04. *Phaeovirus*	00.051.0.04.001.	*Ectocarpus siliculosus virus 1*
	00.051.0.05. *Raphidovirus*	00.051.0.05.001.	*Heterosigma akashiwo virus 01*
	00.051.0.06. *Coccolithovirus*	00.051.0.06.001.	*Emiliania huxleyi virus 86*

Archaea Viruses

VC Order; Family; Subfamily	VirusCode Genus Name	VirusCode	Type Species Name
00.095. *Guttaviridae*	00.095.0.01. *Guttavirus*	00.095.0.01.001.	*Sulfolobus newzealandicus droplet-shaped virus*
00.028. *Fuselloviridae*	00.028.0.01. *Fusellovirus*	00.028.0.01.001.	*Sulfolobus spindle-shaped virus 1*
00.038. *Lipothrixviridae*	00.038.0.01. *Alphalipothrixvirus*	00.038.0.01.001.	*Thermoproteus tenax virus 1*
	00.038.0.02. *Betalipothrixvirus**	00.038.0.02.001.	*Sulfolobus islandicus filamentous virus*
	00.038.0.03. *Gammalipothrixvirus*	00.038.0.03.001.	*Acidianus filamentous virus 1*
02. *Caudovirales*			
02.043. *Myoviridae*	02.043.0.01. "T4-like viruses"	02.043.0.01.001.	*Enterobacteria phage T4*
	02.043.0.06. "ϕH-like viruses"	02.043.0.06.001.	*Halobacterium phage ϕH*
02.066. *Siphoviridae*	02.066.0.08. "ϕC31-like viruses"	02.066.0.08.001.	*Streptomyces bacteriophage ϕC31*
	02.066.0.06. "ψM1-like viruses"	02.066.0.06.001.	*Methanobacterium phage yM1*
00.083. *Rudiviridae*	00.083.0.01. *Rudivirus*	00.083.0.01.001.	*Sulfolobus islandicus rod-shaped virus 2*
00.102. _	00.102.0.01. *Salterprovirus*	00.102.0.01.001.	*His 1 virus*

Bacterial Viruses/Bacteriophages

VC Order; Family; Subfamily	VirusCode Genus Name	VirusCode	Type Species Name
00.020. *Corticoviridae*	00.020.0.01. *Corticovirus*	00.020.0.01.001.	*Pseudoalteromonas phage PM2*
00.021. *Cystoviridae*	00.021.0.01. *Cystovirus*	00.021.0.01.001.	*Pseudomonas phage ϕ6*
00.035. *Inoviridae*	00.035.0.01. *Inovirus*	00.035.0.01.001.	*Enterobacteria phage M13*
	00.035.0.02. *Plectrovirus*	00.035.0.02.001.	*Acholeplasma phage MV-L51*
00.037. *Leviviridae*	00.037.0.01. *Levivirus*	00.037.0.01.001.	*Enterobacteria phage MS2*
	00.037.0.02. *Allolevirus*	00.037.0.02.001.	*Enterobacteria phage Qß*
00.042. *Microviridae*	00.042.0.01. *Microvirus*	00.042.0.01.001.	*Enterobacteria phage ϕX174*
	00.042.0.02. *Spiromicrovirus*	00.042.0.02.001.	*Spiroplasma phage 4*
	00.042.0.05. *Bdellomicrovirus*	00.042.0.05.001.	*Bdellovibrio phage MAC 1*
	00.042.0.04. *Chlamydiamicrovirus*	00.042.0.04.001.	*Chlamydia phage 1*
02. *Caudovirales*			
02.043. *Myoviridae*	02.043.0.01. "T4-like viruses"	02.043.0.01.001.	*Enterobacteria phage T4*
	02.043.0.02. "P1-like viruses"	02.043.0.02.001.	*Enterobacteria phage P1*

	02.043.0.03.	"P2-like viruses"	02.043.0.03.001.	*Enterobacteria phage P2*
	02.043.0.04.	"Mu-like viruses"	02.043.0.04.001.	*Enterobacteria phage Mu*
	02.043.0.05.	"SPO1-like viruses"	02.043.0.05.001.	*Bacillus phage SPO1*
00.053. *Plasmaviridae*	00.053.0.01.	*Plasmavirus*	00.053.0.01.001.	*Acholeplasma phage L2*
02. *Caudovirales*				
02.054. *Podoviridae*	02.054.0.01.	"T7-like viruses"	02.054.0.01.001.	*Enterobacteria phage T7*
	02.054.0.02.	"ϕ29-like viruses"	02.054.0.02.001.	*Bacillus phage ϕ29*
	02.054.0.03.	"P22-like viruses"	02.054.0.03.001.	*Enterobacteria phage P22*
	02.054.0.04.	"N4-like viruses"	02.054.0.04.001.	*Enterobacteria phage N4*
02.043. *Siphoviridae*	02.066.0.01.	"λ-like viruses"	02.066.0.01.001.	*Enterobacteria phage λ*
	02.066.0.02.	"T1-like viruses"	02.066.0.02.001.	*Enterobacteria phage T1*
	02.066.0.03.	"T5-like viruses"	02.066.0.03.001.	*Enterobacteria phage T5*
	02.066.0.04.	"L5-like viruses"	02.066.0.04.001.	*Mycobacterium phage L5*
	02.066.0.05.	"c2-like viruses"	02.066.0.05.001.	*Lactococcus phage c2*
	02.066.0.06.	"ψM1-like viruses"	02.066.0.06.001.	*Methanobacterium phage ψM1*
	02.066.0.07.	"N15-like viruses"	02.066.0.07.001.	*Enterobacteria phage N15*
	02.066.0.08.	"ϕC31-like viruses"	02.066.0.08.001.	*Streptomyces bacteriophage ϕC31*
00.068. *Tectiviridae*	00.068.0.01.	*Tectivirus*	00.068.0.01.001.	*Enterobacteria phage PRD1*

Fungal Viruses

VC Order; Family; Subfamily	VirusCode	Genus Name	VirusCode	Type Species Name
00.008. *Barnaviridae*	00.008.0.01.	*Barnavirus*	00.008.0.01.001.	*Mushroom bacilliform virus*
00.106. *Chrysoviridae*	00.106.0.01.	*Chrysovirus*	00.106.0.01.001.	*Penicillium chrysogenum virus*
00.033. *Hypoviridae*	00.033.0.01.	*Hypovirus*	00.033.0.01.001.	*Cryphonectria hypovirus 1*
00.098. *Metaviridae*	00.098.0.01.	*Metavirus*	00.098.0.01.001.	*Saccharomyces cerevisiae Ty3 virus*
00.096. *Narnaviridae*	00.096.0.01.	*Narnavirus*	00.096.0.01.001.	*Saccharomyces 20S RNA narnavirus*
	00.096.0.02.	*Mitovirus*	00.096.0.02.001.	*Cryphonectria mitovirus 1*
00.049. *Partitiviridae*	00.049.0.01.	*Partitivirus*	00.049.0.01.001.	*Atkinsonella hypoxylon virus*
00.097. *Pseudoviridae*	00.097.0.01.	*Pseudovirus*	00.097.0.01.001.	*Saccharomyces cerevisiae Ty1 virus*
	00.097.0.02.	*Hemivirus*	00.097.0.02.001.	*Drosophila melanogaster copia virus*
00.060. *Reoviridae*	00.060.0.12.	*Mycoreovirus*	00.060.0.12.001.	*Mycoreovirus 1*
00.063. _	00.063.0.01.	*Rhizidiovirus*	00.063.0.01.001.	*Rhizidiomyces virus*
00.075. *Totiviridae*	00.075.0.01.	*Totivirus*	00.075.0.01.001.	*Saccharomyces cerevisiae virus L-A*

Invertebrate Viruses

VC Order; Family; Subfamily	VirusCode	Genus Name	VirusCode	Type Species Name
00.082. *Ascoviridae*	00.082.0.01.	*Ascovirus*	00.082.0.01.001.	*Spodoptera frugiperda ascovirus 1a*
00.006. *Baculoviridae*	00.006.0.01.	*Nucleopolyhedrovirus*	00.006.0.01.001.	*Autographa californica multiple nucleopolyhedrovirus*
	00.006.0.02.	*Granulovirus*	00.006.0.02.001.	*Cydia pomonella granulovirus*
00.009. *Birnaviridae*	00.009.0.03.	*Entomobirnavirus*	00.009.0.03.001.	*Drosophila X virus*
00.101. *Dicistroviridae*	00.101.0.01.	*Cripavirus*	00.101.0.01.001.	*Cricket paralysis virus*
00.105. _	00.105.0.01.	*Iflavirus*	00.105.0.01.001.	*Infectious flacherie virus*

00.036.	*Iridoviridae*	00.036.0.01.	*Iridovirus*	00.036.0.01.001. *Invertebrate iridescent virus 6*
		00.036.0.02.	*Chloriridovirus*	00.036.0.02.001. *Invertebrate iridescent virus 3*
00.098.	*Metaviridae*	00.098.0.01.	*Metavirus*	00.098.0.01.001. *Saccharomyces cerevisiae Ty3 virus*
		00.098.0.02.	*Errantivirus*	00.098.0.02.001. *Drosophila melanogaster Gypsy virus*
		00.098.0.03.	*Semotivirus*	00.098.0.03.001. *Ascaris lumbricoides Tas virus*
00.103.	*Nimaviridae*	00.103.0.01.	*Whispovirus*	00.103.0.01.001. *White spot syndrome virus 1*
00.045.	*Nodaviridae*	00.045.0.01.	*Alphanodavirus*	00.045.0.01.001. *Nodamura virus*
00.050.	*Parvoviridae*			
00.050.2.	*Densovirinae*	00.050.2.01.	*Densovirus*	00.050.2.01.001. *Junonia coenia densovirus*
		00.050.2.02.	*Iteravirus*	00.050.2.02.001. *Bombyx mori densovirus*
		00.050.2.03.	*Brevidensovirus*	00.050.2.03.001. *Aedes aegypti densovirus*
		00.050.2.04.	*Pefudensovirus*	00.050.2.04.001. *Periplaneta fuliginosa densovirus*
00.055.	*Polydnaviridae*	00.055.0.01.	*Ichnovirus*	00.055.0.01.001. *Campoletis sonorensis ichnovirus*
		00.055.0.02.	*Brachovirus*	00.055.0.02.001. *Cotesia melanoscela bracovirus*
00.058.	*Poxviridae*			
00.058.2.	*Entomopoxvirinae*	00.058.2.01.	*Alphaentomopoxvirus*	00.058.2.01.001. *Melolontha melolontha entomopoxvirus*
		00.058.2.02.	*Betaentomopoxvirus*	00.058.2.02.001. *Amsacta moorei entomopoxvirus 'L'*
		00.058.2.03.	*Gammaentomopoxvirus*	00.058.2.03.001. *Chironomus luridus entomopoxvirus*
00.097.	*Pseudoviridae*	00.097.0.02.	*Hemivirus*	00.097.0.02.001. *Drosophila melanogaster copia virus*
00.060.	*Reoviridae*	00.060.0.06.	*Cypovirus*	00.060.0.06.001. *Cypovirus 1*
		00.060.0.11.	*Idnoreovirus*	00.060.0.11.001. *Idnoreovirus -1*
01.	*Mononegavirales*			
01.062.	*Rhabdoviridae*	01.062.0.01.	*Vesiculovirus*	01.062.0.01.001. *Vesicular stomatitis Indiana virus*
		01.062.0.02.	*Lyssavirus*	01.062.0.02.001. *Rabies virus*
		01.062.0.03.	*Ephemerovirus*	01.062.0.03.001. *Bovine ephemeral fever virus*
		01.062.0.04.	*Cytorhabdovirus*	01.062.0.04.001. *Lettuce necrotic yellows virus*
		01.062.0.05.	*Nucleorhabdovirus*	01.062.0.05.001. *Potato yellow dwarf virus*
		01.062.0.06.	*Novirhabdovirus*	01.062.0.06.001. *Infectious hematopoietic necrosis virus*
00.070.	*Tetraviridae*	00.070.0.01.	*Betatetravirus*	00.070.0.01.001. *Nudaurelia capensis β virus*
		00.070.0.02.	*Omegatetravirus*	00.070.0.02.001. *Nudaurelia capensis ω virus*

Plant Viruses

VC Order; Family; Subfamily		VirusCode Genus Name		VirusCode	Type Species Name
00.088.	-	00.088.0.01.	*Benyvirus*	00.088.0.01.001.	*Beet necrotic yellow vein virus*
00.010.	*Bromoviridae*	00.010.0.01.	*Alfamovirus*	00.010.0.01.001.	*Alfalfa mosaic virus*
		00.010.0.02.	*Ilarvirus*	00.010.0.02.001.	*Tobacco streak virus*
		00.010.0.03.	*Bromovirus*	00.010.0.03.001.	*Brome mosaic virus*
		00.010.0.04.	*Cucumovirus*	00.010.0.04.001.	*Cucumber mosaic virus*
		00.010.0.05.	*Oleavirus*	00.010.0.05.001.	*Olive latent virus 2*
00.011.	*Bunyaviridae*	00.011.0.05.	*Tospovirus*	00.011.0.05.001.	*Tomato spotted wilt virus*
00.015.	*Caulimoviridae*	00.015.0.01.	*Caulimovirus*	00.015.0.01.001.	*Cauliflower mosaic virus*
		00.015.0.02.	*Soymovirus*	00.015.0.02.001.	*Soybean chlorotic mottle virus*
		00.015.0.03.	*Cavemovirus*	00.015.0.03.001.	*Cassava vein mosaic virus*

		00.015.0.04.	*Tungrovirus*	00.015.0.04.001.	*Rice tungro bacilliform virus*
		00.015.0.05.	*Badnavirus*	00.015.0.05.001.	*Commelina yellow mottle virus*
		00.015.0.06.	*Petuvirus*	00.015.0.06.001.	*Petunia vein clearing virus*
00.111.	-	00.111.0.01.	*Cheravirus*	00.111.0.01.001.	*Cherry rasp leaf virus*
00.017.	*Closteroviridae*	00.017.0.01.	*Closterovirus*	00.017.0.01.001.	*Beet yellows virus*
		00.017.0.02.	*Crinivirus*	00.017.0.02.001.	*Lettuce infectious yellows virus*
		00.017.0.03.	*Ampelovirus*	00.017.0.03.001.	*Grapevine leafroll-associated virus 3*
00.018.	*Comoviridae*	00.018.0.01.	*Comovirus*	00.018.0.01.001.	*Cowpea mosaic virus*
		00.018.0.02.	*Fabavirus*	00.018.0.02.001.	*Broad bean wilt virus 1*
		00.018.0.03.	*Nepovirus*	00.018.0.03.001.	*Tobacco ringspot virus*
00.108.	-	00.108.0.01.	*Endornavirus*	00.108.0.01.001.	*Vicia faba endornavirus*
00.056.	*Flexiviridae*	00.056.0.01.	*Potexvirus*	00.056.0.01.001.	*Potato virus X*
		00.056.0.02.	*Mandarivirus*	00.056.0.02.001.	*Indian citrus ringspot virus*
		00.056.0.03.	*Allexivirus*	00.056.0.03.001.	*Shallot virus X*
		00.056.0.04.	*Carlavirus*	00.056.0.04.001.	*Carnation latent virus*
		00.056.0.05.	*Foveavirus*	00.056.0.05.001.	*Apple stem pitting virus*
		00.056.0.06.	*Capillovirus*	00.056.0.06.001.	*Apple stem grooving virus*
		00.056.0.07.	*Vitivirus*	00.056.0.07.001.	*Grapevine virus A*
		00.056.0.08.	*Trichovirus*	00.056.0.08.001.	*Apple chlorotic leaf spot virus*
00.027.	-	00.027.0.01.	*Furovirus*	00.027.0.01.001.	*Soil-borne wheat mosaic virus*
00.029.	*Geminiviridae*	00.029.0.01.	*Mastrevirus*	00.029.0.01.001.	*Maize streak virus*
		00.029.0.02.	*Curtovirus*	00.029.0.02.001.	*Beet curly top virus*
		00.029.0.03.	*Begomovirus*	00.029.0.03.001.	*Bean golden yellow mosaic virus*
		00.029.0.04.	*Topocuvirus*	00.029.0.04.001.	*Tomato pseudo-curly top virus*
00.032.	-	00.032.0.01.	*Hordeivirus*	00.032.0.01.001.	*Barley stripe mosaic virus*
00.034.	-	00.034.0.01.	*Idaeovirus*	00.034.0.01.001.	*Raspberry bushy dwarf virus*
00.039.	*Luteoviridae*	00.039.0.01.	*Luteovirus*	00.039.0.01.001.	*Barley yellow dwarf virus–PAV*
		00.039.0.02.	*Polerovirus*	00.039.0.02.001.	*Potato leafroll virus*
		00.039.0.03.	*Enamovirus*	00.039.0.03.001.	*Pea enation mosaic virus-1*
00.098.	*Metaviridae*	00.098.0.01.	*Metavirus*	00.098.0.01.001.	*Saccharomyces cerevisiae Ty3 virus*
00.093.	*Nanoviridae*	00.093.0.01.	*Nanovirus*	00.093.0.01.001.	*Subterranean clover stunt virus*
		00.093.0.02.	*Babuvirus*	00.093.0.02.001.	*Banana bunchy top virus*
00.094.	-	00.094.0.01.	*Ophiovirus*	00.094.0.01.001.	*Citrus psorosis virus*
00.089.	-	00.089.0.01.	*Ourmiavirus*	00.089.0.01.001.	*Ourmia melon virus*
00.049.	*Partitiviridae*	00.049.0.03.	*Alphacryptovirus*	00.049.0.03.001.	*White clover cryptic virus 1*
		00.049.0.04.	*Betacryptovirus*	00.049.0.04.001.	*White clover cryptic virus 2*
00.087.	-	00.087.0.01.	*Pecluvirus*	00.087.0.01.001.	*Peanut clump virus*
00.086.	-	00.086.0.01.	*Pomovirus*	00.086.0.01.001.	*Potato mop-top virus*
00.057.	*Potyviridae*	00.057.0.01.	*Potyvirus*	00.057.0.01.001.	*Potato virus Y*
		00.057.0.02.	*Rymovirus*	00.057.0.02.001.	*Ryegrass mosaic virus*
		00.057.0.03.	*Bymovirus*	00.057.0.03.001.	*Barley yellow mosaic virus*
		00.057.0.04.	*Macluravirus*	00.057.0.04.001.	*Maclura mosaic virus*
		00.057.0.05.	*Ipomovirus*	00.057.0.05.001.	*Sweet potato mild mottle virus*
		00.057.0.06.	*Tritimovirus*	00.057.0.06.001.	*Wheat streak mosaic virus*
00.097.	*Pseudoviridae*	00.097.0.01.	*Pseudovirus*	00.097.0.01.001.	*Saccharomyces cerevisiae Ty1 virus*
		00.097.0.03.	*Sirevirus*	00.097.0.03.001.	*Glycine max SIRE1 virus*

00.060.	*Reoviridae*	00.060.0.07.	*Fijivirus*	00.060.0.07.001. *Fiji disease virus*
		00.060.0.08.	*Phytoreovirus*	00.060.0.08.001. *Wound tumor virus*
		00.060.0.09.	*Oryzavirus*	00.060.0.09.001. *Rice ragged stunt virus*
01.	*Mononegavirales*			
01.062.	*Rhabdoviridae*	01.062.0.04.	*Cytorhabdovirus*	01.062.0.04.001. *Lettuce necrotic yellows virus*
		01.062.0.05.	*Nucleorhabdovirus*	01.062.0.05.001. *Potato yellow dwarf virus*
00.112.	_	00.112.0.01.	*Sadwavirus*	00.112.0.01.001. *Satsuma dwarf virus*
00.065.	*Sequiviridae*	00.065.0.01.	*Sequivirus*	00.065.0.01.001. *Parsnip yellow fleck virus*
		00.065.0.02.	*Waikavirus*	00.065.0.02.001. *Rice tungro spherical virus*
00.067.	_	00.067.0.01.	*Sobemovirus*	00.067.0.01.001. *Southern bean mosaic virus*
00.069.	_	00.069.0.01.	*Tenuivirus*	00.069.0.01.001. *Rice stripe virus*
00.071.	_	00.071.0.01.	*Tobamovirus*	00.071.0.01.001. *Tobacco mosaic virus*
00.072.	_	00.072.0.01.	*Tobravirus*	00.072.0.01.001. *Tobacco rattle virus*
00.074.	*Tombusviridae*	00.074.0.01.	*Tombusvirus*	00.074.0.01.001. *Tomato bushy stunt virus*
		00.074.0.02.	*Carmovirus*	00.074.0.02.001. *Carnation mottle virus*
		00.074.0.03.	*Necrovirus*	00.074.0.03.001. *Tobacco necrosis virus A*
		00.074.0.04.	*Dianthovirus*	00.074.0.04.001. *Carnation ringspot virus*
		00.074.0.05.	*Machlomovirus*	00.074.0.05.001. *Maize chlorotic mottle virus*
		00.074.0.06.	*Avenavirus*	00.074.0.06.001. *Oat chlorotic stunt virus*
		00.074.0.07.	*Aureusvirus*	00.074.0.07.001. *Pothos latent virus*
		00.074.0.08.	*Panicovirus*	00.074.0.08.001. *Panicum mosaic virus*
00.074.	*Tymoviridae*	00.077.0.03.	*Maculavirus*	00.077.0.03.001. *Grapevine fleck virus*
		00.077.0.01.	*Tymovirus*	00.077.0.01.001. *Turnip yellow mosaic virus*
		00.077.0.02.	*Marafivirus*	00.077.0.02.001. *Maize rayado fino virus*
00.078.	_	00.078.0.01.	*Umbravirus*	00.078.0.01.001. *Carrot mottle virus*
00.092.	_	00.092.0.01.	*Varicosavirus*	00.092.0.01.001. *Lettuce big-vein associated virus*

Protozoa Viruses

VC Order; Family; Subfamily		VirusCode Genus Name		VirusCode	Type Species Name
00.110.	_	00.110.0.01.	*Mimivirus*	00.110.0.01.001.	*Acanthamoeba polyphaga mimivirus*
00.097.	*Pseudoviridae*	00.097.0.02.	*Hemivirus*	00.097.0.01.002.	*Drosphila melanogaster copia virus*
00.075.	*Totiviridae*	00.075.0.02.	*Giardiavirus*	00.075.0.02.001.	*Giardia lamblia virus*
		00.075.0.03.	*Leishmaniavirus*	00.075.0.03.001.	*Leishmania RNA virus 1 - 1*

Vertebrate Viruses

VC Order; Family; Subfamily		VirusCode Genus Name		VirusCode	Type Species Name
00.001.	*Adenoviridae*	00.001.0.01.	*Mastadenovirus*	00.001.0.01.001.	*Human adenovirus C*
		00.001.0.02.	*Aviadenovirus*	00.001.0.02.001.	*Fowl adenovirus A*
		00.001.0.03.	*Atadenovirus*	00.001.0.03.001.	*Ovine adenovirus D*
		00.001.0.04.	*Siadenovirus*	00.001.0.04.001.	*Frog adenovirus*
00.107.	_	00.107.0.01.	*Anellovirus*	00.107.0.01.001.	*Torque teno virus*
00.003.	*Arenaviridae*	00.003.0.01.	*Arenavirus*	00.003.0.01.001.	*Lymphocytic choriomeningitis virus*
03.	*Nidovirales*				
03.004.	*Arteriviridae*	03.004.0.01.	*Arterivirus*	03.004.0.01.001.	*Equine arteritis virus*
00.002.	*Asfarviridae*	00.002.0.01.	*Asfivirus*	00.002.0.01.001.	*African swine fever virus*

00.005. *Astroviridae*	00.005.0.01. *Mamastrovirus*	00.005.0.01.001. *Human astrovirus*
	00.005.0.02. *Avastrovirus*	00.005.0.02.001. *Turkey astrovirus*
00.009. *Birnaviridae*	00.009.0.01. *Aquabirnavirus*	00.009.0.01.001. *Infectious pancreatic necrosis virus*
	00.009.0.02. *Avibirnavirus*	00.009.0.02.001. *Infectious bursal disease virus*
01. *Mononegavirales*		
01.081. *Bornaviridae*	01.081.0.01. *Bornavirus*	01.081.0.01.001. *Borna disease virus*
00.011. *Bunyaviridae*	00.011.0.01. *Orthobunyavirus*	00.011.0.01.001. *Bunyamwera virus*
	00.011.0.02. *Hantavirus*	00.011.0.02.001. *Hantaan virus*
	00.011.0.03. *Nairovirus*	00.011.0.03.001. *Dugbe virus*
	00.011.0.04. *Phlebovirus*	00.011.0.04.001. *Rift Valley fever virus*
00.012. *Caliciviridae*	00.012.0.01. *Vesivirus*	00.012.0.01.001. *Vesicular exanthema of swine virus*
	00.012.0.02. *Lagovirus*	00.012.0.02.001. *Rabbit hemorrhagic disease virus*
	00.012.0.03. *Norovirus*	00.012.0.03.001. *Norwalk virus*
	00.012.0.04. *Sapovirus*	00.012.0.04.001. *Sapporo virus*
00.016. *Circoviridae*	00.016.0.01. *Circovirus*	00.016.0.01.001. *Porcine circovirus - 1*
	00.016.0.02. *Gyrovirus*	00.016.0.02.001. *Chicken anemia virus*
03. *Nidovirales*		
03.019. *Coronaviridae*	03.019.0.01. *Coronavirus*	03.019.0.01.001. *Infectious bronchitis virus*
	03.019.0.02. *Torovirus*	03.019.0.02.001. *Equine torovirus*
82.022. _	82.022.0.01. *Deltavirus*	82.022.0.01.001. *Hepatitis delta virus*
00.026. *Flaviviridae*	00.026.0.01. *Flavivirus*	00.026.0.01.001. *Yellow fever virus*
	00.026.0.02. *Pestivirus*	00.026.0.02.001. *Bovine viral diarrhea virus 1*
	00.026.0.03. *Hepacivirus*	00.026.0.03.001. *Hepatitis C virus*
01. *Mononegavirales*		
01.025. *Filoviridae*	01.025.0.01. *Marburgvirus*	01.025.0.01.001. *Lake Victoria marburgvirus*
	01.025.0.02. *Ebolavirus*	01.025.0.02.001. *Zaire ebolavirus*
00.084. _	00.084.0.01. *Hepevirus*	00.084.0.01.001. *Hepatitis E virus*
00.030. *Hepadnaviridae*	00.030.0.01. *Orthohepadnavirus*	00.030.0.01.001. *Hepatitis B virus*
	00.030.0.02. *Avihepadnavirus*	00.030.0.02.001. *Duck hepatitis B virus*
00.031. *Herpesviridae*	00.031.0.01. *Ictalurivirus*	00.031.0.01.001. *Ictalurid herpesvirus 1*
00.031.1. *Alphaherpesvirinae*	00.031.1.01. *Simplexvirus*	00.031.1.01.001. *Human herpesvirus 1*
	00.031.1.02. *Varicellovirus*	00.031.1.02.001. *Human herpesvirus 3*
	00.031.1.03. *Mardivirus*	00.031.1.03.001. *Gallid herpesvirus 2*
	00.031.1.04. *Iltovirus*	00.031.1.04.001. *Gallid herpesvirus 1*
00.031.2. *Betaherpesvirinae*	00.031.2.01. *Cytomegalovirus*	00.031.2.01.001. *Human herpesvirus 5*
	00.031.2.02. *Muromegalovirus*	00.031.2.02.001. *Murid herpesvirus 1*
	00.031.2.03. *Roseolovirus*	00.031.2.03.001. *Human herpesvirus 6*
00.031.3. *Gammaherpesvirinae*	00.031.3.01. *Lymphocryptovirus*	00.031.3.01.001. *Human herpesvirus 4*
	00.031.3.02. *Rhadinovirus*	00.031.3.02.001. *Saimiriine herpesvirus 2*
00.036. *Iridoviridae*	00.036.0.03. *Ranavirus*	00.036.0.03.001. *Frog virus 3*
	00.036.0.04. *Lymphocystivirus*	00.036.0.04.001. *Lymphocystis disease virus 1*
	00.036.0.05. *Megalocytivirus*	00.036.0.05.001. *Infectious spleen and kidney necrosis virus*
00.045. *Nodaviridae*	00.045.0.02. *Betanodavirus*	00.045.0.02.001. *Striped jack nervous necrosis virus*
00.046. *Orthomyxoviridae*	00.046.0.01. *Influenzavirus A*	00.046.0.01.001. *Influenza A virus*
	00.046.0.02. *Influenzavirus C*	00.046.0.02.001. *Influenza C virus*

	00.046.0.03.	*Thogotovirus*	00.046.0.03.001.	*Thogoto virus*
	00.046.0.04.	*Influenzavirus B*	00.046.0.04.001.	*Influenza B virus*
	00.046.0.05.	*Isavirus*	00.046.0.05.001.	*Infectious salmon anemia virus*
00.099. *Papillomaviridae*	00.099.0.02.	*Alphapapillomavirus*	00.099.0.02.001.	*Human papillomavirus – 32*
	00.099.0.03.	*Betapapillomavirus*	00.099.0.03.001.	*Human papillomavirus – 5*
	00.099.0.04.	*Gammapapillomavirus*	00.099.0.04.001.	*Human papillomavirus – 4*
	00.099.0.05.	*Deltapapillomavirus*	00.099.0.05.001.	*European elk papillomavirus*
	00.099.0.06.	*Epsilonpapillomavirus*	00.099.0.06.001.	*Bovine papillomavirus – 5*
	00.099.0.07.	*Zetapapillomavirus*	00.099.0.07.001.	*Equine papillomavirus – 1*
	00.099.0.08.	*Etapapillomavirus*	00.099.0.08.001.	*Fringilla coelebs papillomavirus*
	00.099.0.09.	*Thetapapillomavirus*	00.099.0.09.001.	*Psittacus erithacus timneh papillomavirus*
	00.099.0.10.	*Iotapapillomavirus*	00.099.0.10.001.	*Mastomys natalensis papillomavirus*
	00.099.0.11.	*Kappapapillomavirus*	00.099.0.11.001.	*Cottontail rabbit papillomavirus*
	00.099.0.12.	*Lambdapapillomavirus*	00.099.0.12.001.	*Canine oral papillomavirus*
	00.099.0.13.	*Mupapillomavirus*	00.099.0.13.001.	*Human papillomavirus – 1*
	00.099.0.14.	*Nupapillomavirus*	00.099.0.14.001.	*Human papillomavirus - 41*
	00.099.0.15.	*Xipapillomavirus*	00.099.0.15.001.	*Bovine papillomavirus - 3*
	00.099.0.16.	*Omicronpapillomavirus*	00.099.0.16.001.	*Phocoena spinipinnis papillomavirus*
	00.099.0.17.	*Pipapillomavirus*	00.099.0.17.001.	*Hamster oral papillomavirus*
01. *Mononegavirales*				
01.048. *Paramyxoviridae*				
01.048.1. *Paramyxovirinae*	01.048.1.01.	*Respirovirus*	01.048.1.01.001.	*Sendai virus*
	01.048.1.02.	*Morbillivirus*	01.048.1.02.001.	*Measles virus*
	01.048.1.03.	*Rubulavirus*	01.048.1.03.001.	*Mumps virus*
	01.048.1.04.	*Henipavirus*	01.048.1.04.001.	*Hendra virus*
	01.048.1.05.	*Avulavirus*	01.048.1.05.001.	*Newcastle disease virus*
01.048.2. *Pneumovirinae*	01.048.2.01.	*Pneumovirus*	01.048.2.01.001.	*Human respiratory syncytial virus*
	01.048.2.02.	*Metapneumovirus*	01.048.2.02.001.	*Avian metapneumovirus*
00.050. *Parvoviridae*				
00.050.1. *Parvovirinae*	00.050.1.01.	*Parvovirus*	00.050.1.01.001.	*Minute virus of mice*
	00.050.1.02.	*Erythrovirus*	00.050.1.02.001.	*Human parvovirus B19*
	00.050.1.03.	*Dependovirus*	00.050.1.03.001.	*Adeno-associated virus - 2*
	00.050.1.04.	*Amdovirus*	00.050.1.04.001.	*Aleutian mink disease virus*
	00.050.1.05.	*Bocavirus*	00.050.1.05.001.	*Bovine parvovirus*
00.052. *Picornaviridae*	00.052.0.01.	*Enterovirus*	00.052.0.01.001.	*Poliovirus*
	00.052.0.02.	*Rhinovirus*	00.052.0.02.001.	*Human rhinovirus A*
	00.052.0.03.	*Hepatovirus*	00.052.0.03.001.	*Hepatitis A virus*
	00.052.0.04.	*Cardiovirus*	00.052.0.04.001.	*Encephalomyocarditis virus*
	00.052.0.05.	*Aphthovirus*	00.052.0.05.001.	*Foot-and-mouth disease virus*
	00.052.0.06.	*Parechovirus*	00.052.0.06.001.	*Human parechovirus*
	00.052.0.07.	*Erbovirus*	00.052.0.07.001.	*Equine rhinitis B virus*
	00.052.0.08.	*Kobuvirus*	00.052.0.08.001.	*Aichi virus*
	00.052.0.09.	*Teschovirus*	00.052.0.09.001.	*Porcine teschovirus*
00.047. *Polyomaviridae*	00.047.0.01.	*Polyomavirus*	00.047.0.01.001.	*Simian virus 40*
00.058. *Poxviridae*				
00.058.1. *Chordopoxvirinae*	00.058.1.01.	*Orthopoxvirus*	00.058.1.01.001.	*Vaccinia virus*

	00.058.1.02.	*Parapoxvirus*	00.058.1.02.001.	*Orf virus*
	00.058.1.03.	*Avipoxvirus*	00.058.1.03.001.	*Fowlpox virus*
	00.058.1.04.	*Capripoxvirus*	00.058.1.04.001.	*Sheeppox virus*
	00.058.1.05.	*Leporipoxvirus*	00.058.1.05.001.	*Myxoma virus*
	00.058.1.06.	*Suipoxvirus*	00.058.1.06.001.	*Swinepox virus*
	00.058.1.07.	*Molluscipoxvirus*	00.058.1.07.001.	*Molluscum contagiosum virus*
	00.058.1.08.	*Yatapoxvirus*	00.058.1.08.001.	*Yaba monkey tumor virus*
00.060. *Reoviridae*	00.060.0.01.	*Orthoreovirus*	00.060.0.01.001.	*Mammalian orthoreovirus*
	00.060.0.02.	*Orbivirus*	00.060.0.02.001.	*Bluetongue virus*
	00.060.0.03.	*Rotavirus*	00.060.0.03.001.	*Rotavirus A*
	00.060.0.04.	*Coltivirus*	00.060.0.04.001.	*Colorado tick fever virus*
	00.060.0.05.	*Aquareovirus*	00.060.0.05.001.	*Aquareovirus A*
	00.060.0.10.	*Seadornavirus*	00.060.0.10.001.	*Banna virus*
00.061. *Retroviridae*				
00.061.1. *Orthoretrovirinae*	00.061.1.01.	*Betaretrovirus*	00.061.1.01.001.	*Mouse mammary tumor virus*
	00.061.1.02.	*Gammaretrovirus*	00.061.1.02.001.	*Murine leukemia virus*
	00.061.1.03.	*Alpharetrovirus*	00.061.1.03.001.	*Avian leukosis virus*
	00.061.1.05.	*Deltaretrovirus*	00.061.1.05.001.	*Bovine leukemia virus*
	00.061.1.06.	*Lentivirus*	00.061.1.06.001.	*Human immunodeficiency virus 1*
	00.061.1.08.	*Epsilonretrovirus*	00.061.1.08.001.	*Walleye dermal sarcoma virus*
00.061.2. *Spumaretrovirinae*	00.061.2.07.	*Spumavirus*	00.061.2.07.001.	*Simian foamy virus*
01. *Mononegavirales*				
01.062. *Rhabdoviridae*	01.062.0.01.	*Vesiculovirus*	01.062.0.01.001.	*Vesicular stomatitis Indiana virus*
	01.062.0.02.	*Lyssavirus*	01.062.0.02.001.	*Rabies virus*
	01.062.0.03.	*Ephemerovirus*	01.062.0.03.001.	*Bovine ephemeral fever virus*
	01.062.0.06.	*Novirhabdovirus*	01.062.0.06.001.	*Infectious hematopoietic necrosis virus*
03. *Nidovirales*				
03.104. *Roniviridae*	03.104.0.01.	*Okavirus*	03.104.0.01.001.	*Gill-associated virus*
00.073. *Togaviridae*	00.073.0.01.	*Alphavirus*	00.073.0.01.001.	*Sindbis virus*
	00.073.0.02.	*Rubivirus*	00.073.0.02.001.	*Rubella virus*

Unassigned Viruses				
			VirusCode	**Type Species Name**

Subviral Agents

VC Order; Family; Subfamily	**VirusCode Genus Name**		**VirusCode**	**Type Species Name**
Viroids				
80. *Viroid*				
80.002. *Avsunviroidae*	80.002.0.01.	*Avsunviroid*	80.002.0.01.001.	*Avocado sunblotch viroid*
	80.002.0.02.	*Pelamoviroid*	80.002.0.02.001.	*Peach latent mosaic viroid*
80.002. *Pospiviroidae*	80.001.0.01.	*Pospiviroid*	80.001.0.01.001.	*Potato spindle tuber viroid*
	80.001.0.02.	*Hostuviroid*	80.001.0.02.001.	*Hop stunt viroid*
	80.001.0.03.	*Cocadviroid*	80.001.0.03.001.	*Coconut cadang-cadang viroid*
	80.001.0.04.	*Apscaviroid*	80.001.0.04.001.	*Apple scar skin viroid*
	80.001.0.05.	*Coleviroid*	80.001.0.05.001.	*Coleus blumei viroid 1*
Satellites				
81. *Satellites*				

81.001. *Satellite Virus*
81.002. *Satellite Nucleic Acids*

Prion	
90. *Prion*	90.001. *Mamalian Prions*
	90.002. *Fungal Prions*

Additional References

ICTV Species Lists
ICTVdB taxon description
ICTVdB Picture Gallery

Comments to ICTVdB Management
Last Modified 04-10-45624 by Cornelia Büchen-Osmond
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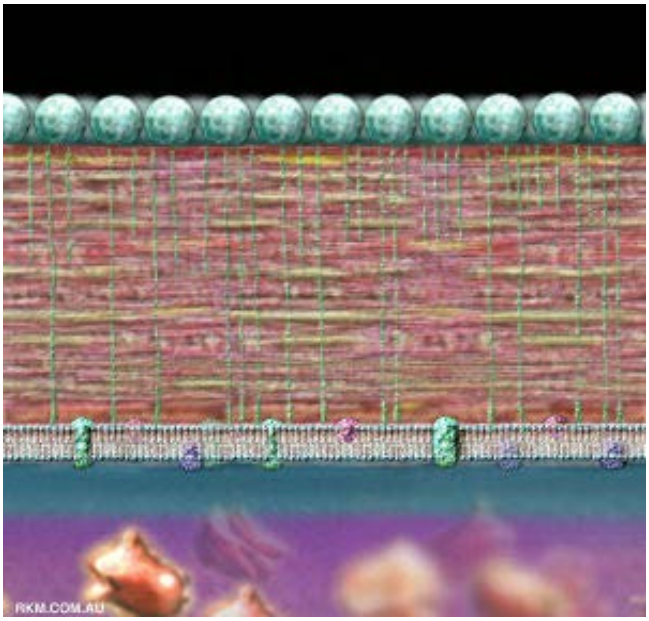
Virus Entry Into Cells

BACTERIAL CELL ENTRY

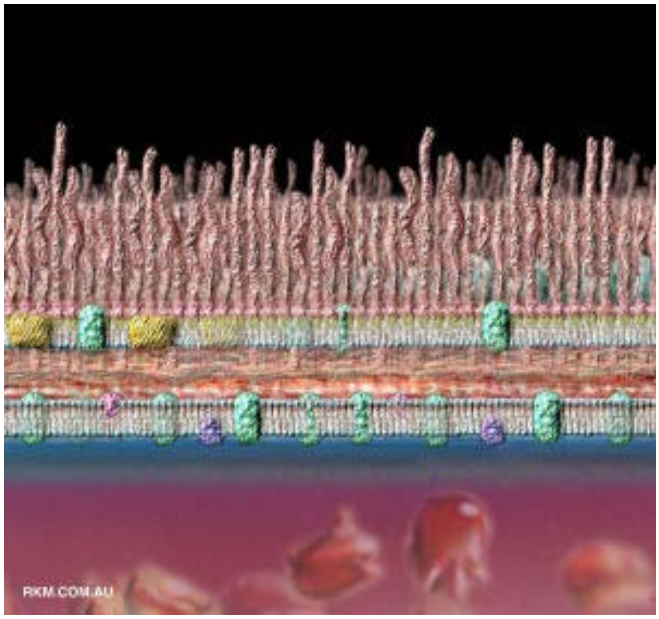
Phage Molecular Motors

Bacterial cell walls are **strong and relatively thick**, to protect them from **osmotic lysis and predation**, and to give them **shape**. **GRAM-POSITIVE** cells have a **single internal lipid bilayer**, and a thick **PEPTIDOGLYCAN** cell wall.

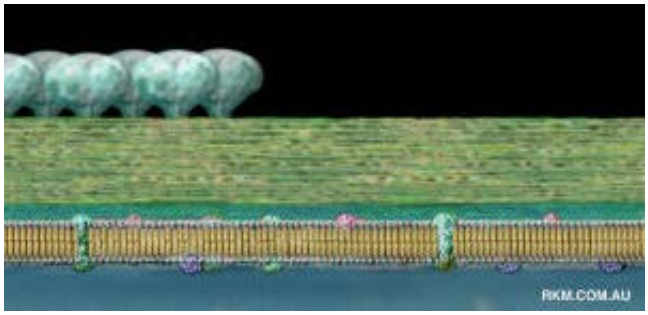
GRAM-NEGATIVE cells have an **internal membrane**, a thin peptidoglycan layer, another membrane, and often a **polysaccharide-based CAPSULE**.

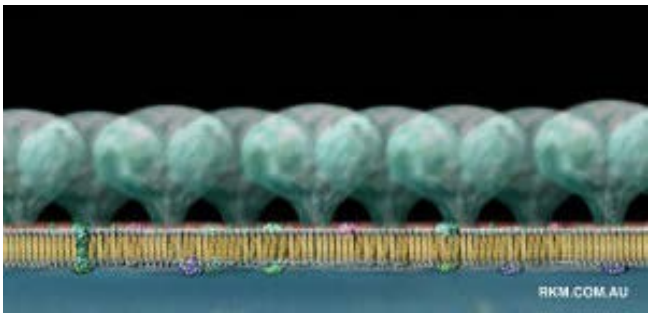


Eubacteria Gram-positive cell walls



Eubacteria Gram-negative cell walls

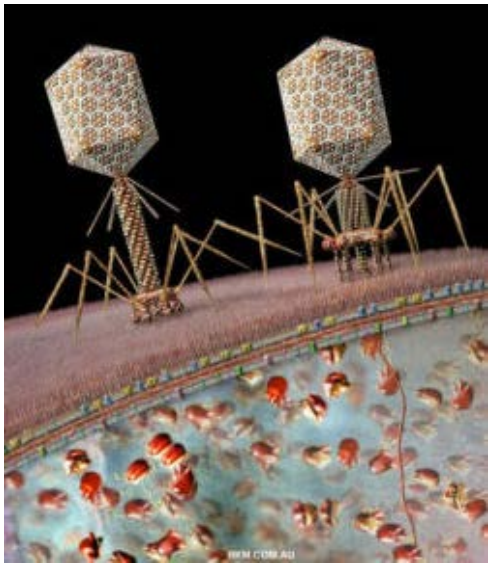




Archaea Gram-positive and -negative cell walls **A generic bacterial cell**

copyright Russell Kightley

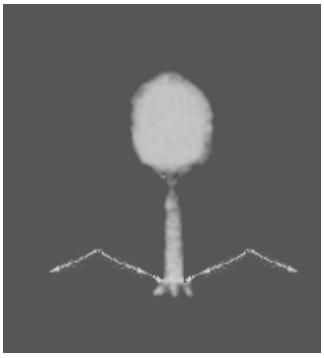
Bacterial viruses (**BACTERIOPHAGES**) have therefore to have some means of breaching a quite formidable barrier if they are to enter the cell. They also generally have **SPECIFIC RECEPTOR SITES** on the bacteria, to which **SPECIFIC ATTACHMENT PROTEINS** bind: these receptor sites may be **lipopolysaccharides, cell wall proteins, teichoic acid, or flagellar or pilus proteins**.



Phage T4 - *Enterobacteria phage T4*, genus "T4-like Viruses", family *Myoviridae*, or viruses with 34-170 kbp dsDNA genomes, isometric heads and contractile tails - infects the **gram-negative bacterium *E coli.*** It has one of the more complex entry mechanisms, involving an **active injection process**. This is shared by others of the so-called **T-even phages** of the family ***Myoviridae***. The process is shown in the still image above left (courtesy Russell Kightley)

The animated graphic showing T4 phage attaching and injecting its DNA was derived from negative-stained electron micrograph images taken by Linda Stannard, (then Dept Medical Microbiology, UCT).

See here for a high-resolution video, and here for a recent YouTube video

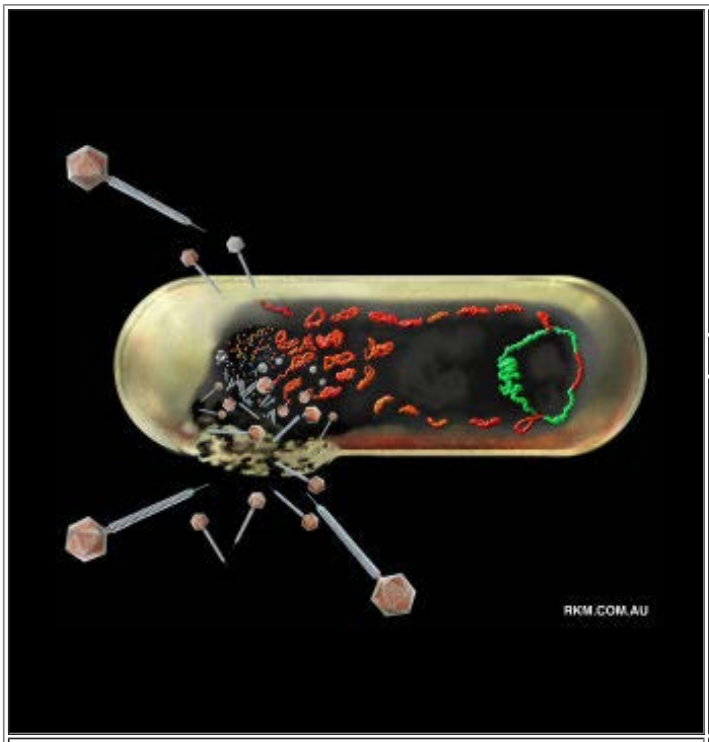


Click here for an non-animated view of the infection process if your browser does NOT support GIF animation.

Click here for a fanciful YouTube version

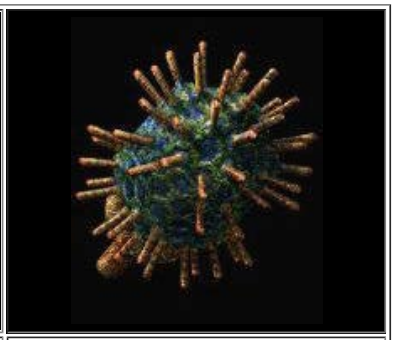
The **phage tail fibres** are the **attachment sites**; these individually bind the bacterial cell surface - specifically to certain lipopolysaccharides and to the surface outer membrane protein OmpC. This is **REVERSIBLE** binding, and is probably due to **electrostatic interactions** as it is Mg2+ and Ca2+ dependent. After **TAIL FIBRE** binding has consolidated, the **BASEPLATE** then settles down onto the surface and binds firmly to it as a result of conformational changes in the **SHORT TAIL FIBRES**. After this occurs, a conformational change takes place in the **TAIL SHEATH, which then CONTRACTS**, pushing the **TAIL CORE** through the cell wall, possibly in an **ATP-driven process: this is aided by a lysozyme activity associated with the tip of the tail tube**. This is an **IRREVERSIBLE** process. **DNA is then extruded** from the phage head. This is then used for initial transcription and virus expression.

Phage lambda - *Enterobacteria phage λ*, genus *"λ-like Viruses"*, family *Siphoviridae,* a tailed phage with an isometric head and a 49 kbp dsDNA genome - attaches to the **maltose receptor** on the surface of the *E coli* cell via the J protein in the tail tip. The receptor is a **porin**, responsible for transport of maltose across the outer membrane. Although the **tail is non-contractile**, a **DNA injection mechanism** similar to that of T-even phages allows **entry of DNA into the cell**, via a sugar transport protein (ptsG) in the inner membrane, **leaving the capsid behind.**



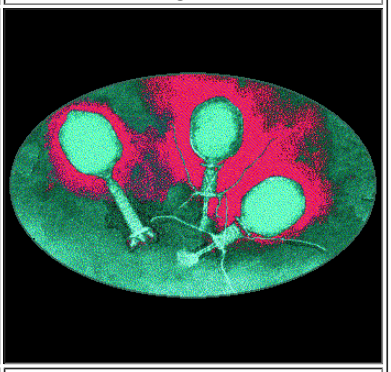
Lambda phage infecting *E coli* (copyright Russell Kightley)

M13-type (fd) phage



Phage Φ29

Phage P22

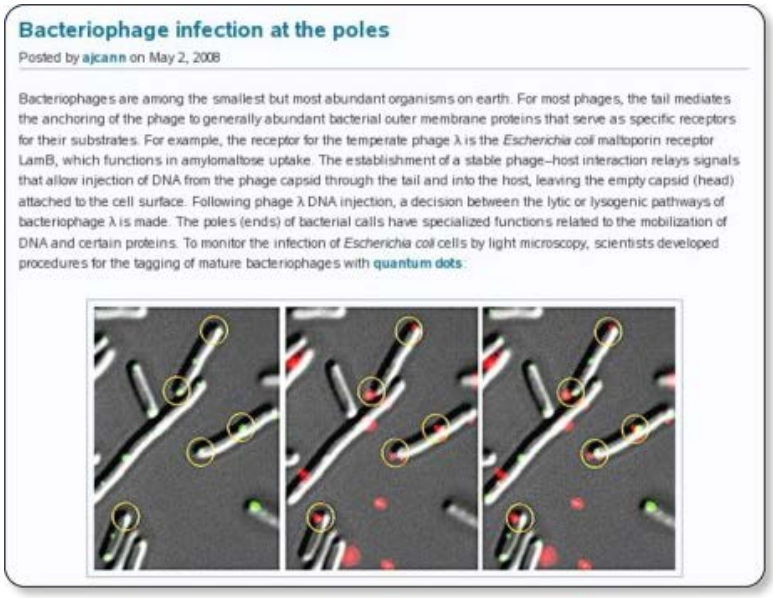


T4 phage particles

MS2 phage - *Enterobacteria phage MS2,* genus *Levivirus,* family *Leviviridae* - **an isometric single-stranded RNA-containing virus infecting *E coli*** - attaches to the **PILIN** (the building block of PILI) of the F(ertility) pili via its single attachment or **A PROTEIN**. The A protein is **covalently linked** to the 5'-end of the genomic RNA; binding pilin causes **cleavage of the A protein** and releases it from the capsid. Tthus, when the pilus is retracted into the cell, **the A protein and RNA are pulled with it**, leaving the empty capsid outside.

Here is an animated diagram of a DNA-containing enveloped isometric phage entering a gram-negative bacterium.

Interestingly, it is possible that where phages attach may have an effect of the outcome - specifically whether or not the infection is **lytic** or **lysogenic**. Click on the links below to see a recent **MicrobiologyBytes** post.



Bacteriophage infection at the poles

Posted by ajcann on May 2, 2008

Bacteriophages are among the smallest but most abundant organisms on earth. For most phages, the tail mediates the anchoring of the phage to generally abundant bacterial outer membrane proteins that serve as specific receptors for their substrates. For example, the receptor for the temperate phage λ is the *Escherichia coli* maltoporin receptor LamB, which functions in amylomaltose uptake. The establishment of a stable phage–host interaction relays signals that allow injection of DNA from the phage capsid through the tail and into the host, leaving the empty capsid (head) attached to the cell surface. Following phage λ DNA injection, a decision between the lytic or lysogenic pathways of bacteriophage λ is made. The poles (ends) of bacterial cells have specialized functions related to the mobilization of DNA and certain proteins. To monitor the infection of *Escherichia coli* cells by light microscopy, scientists developed procedures for the tagging of mature bacteriophages with quantum dots.

Bacteriophage infection at the poles « MicrobiologyBytes via kwout

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Phage Molecular Motors

The **injection of DNA** from bacterial viruses into cells is usually enabled by a "**molecular motor**" housed at the **junction of the tail and head** of the virus particle: this is also responsible for **filling pre-assembled phage heads** with DNA in the infected cell as part of the virion assembly and maturation process. Thus the motors are **nanomachines** which can run in both directions.

This is generally an **ATP-driven process**, and in this is similar to other cellular molecular motors such as **myosin, kinesin and dynein** in eukaryotes.

Figure 1

Structure of epsilon15 bacteriophage.

a, 200-kV CCD image of epsilon15 particles embedded in vitreous ice.

b, Surface rendering of the 20A ° resolution three-dimensional map of entire epsilon15 bacteriophage reconstructed without symmetry imposition.

The capsid (dark green) exhibits good icosahedral symmetry as indicated by the icosahedral lattice (grey).

c, d, The structural components of epsilon15 bacteriophage are annotated in the central section density (c) and the cut-away surface view (d) of the three-dimensional density map.

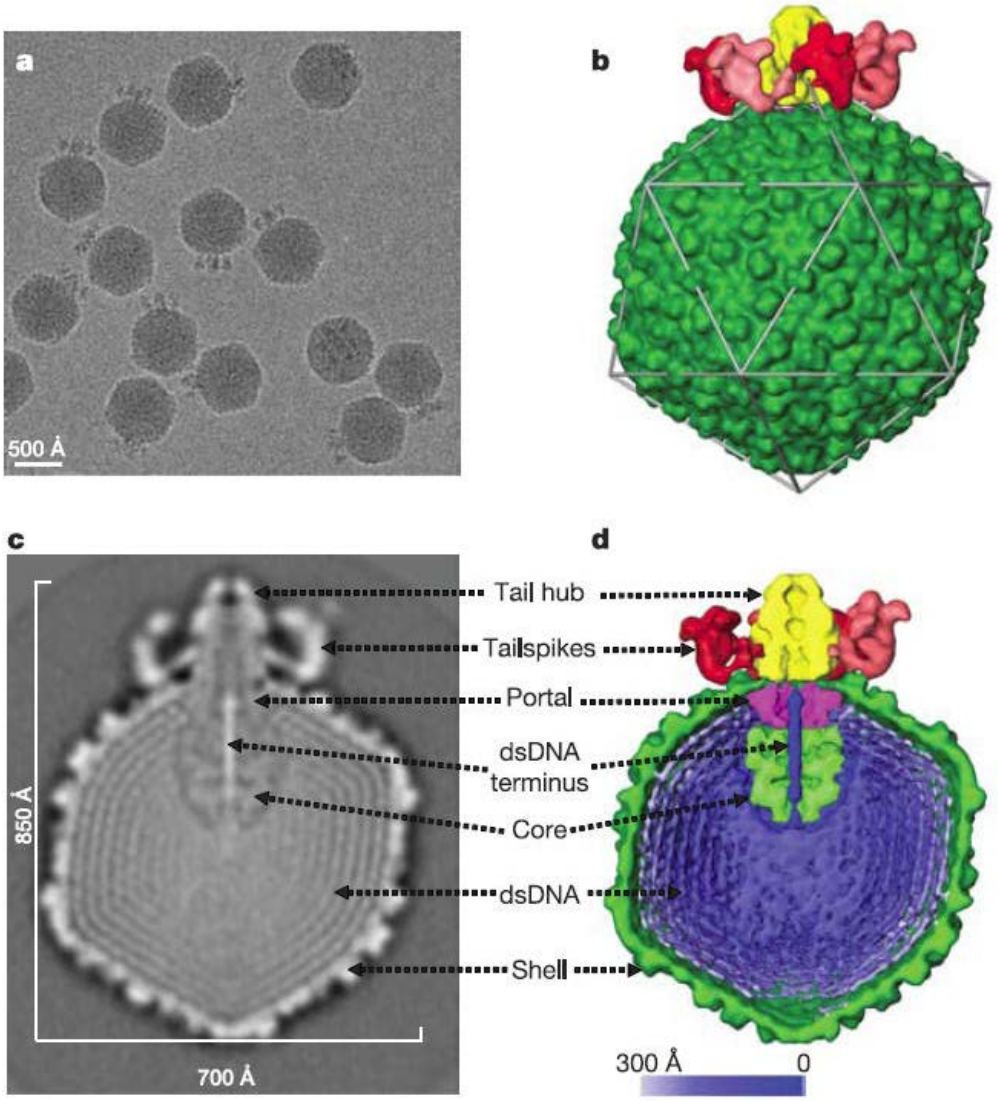


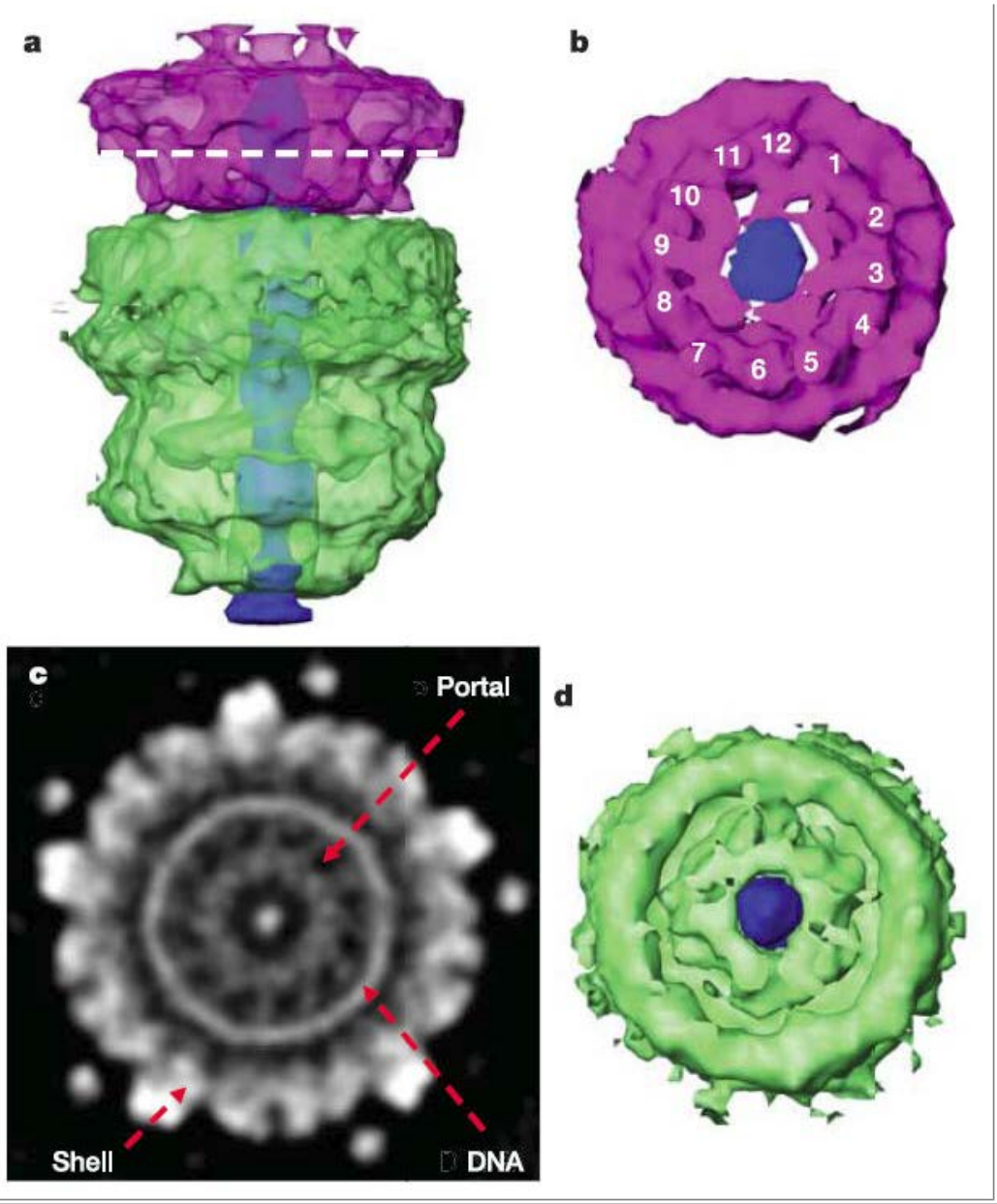
Figure 3

Structure of the portal complex and internal core.

a, Side view of the portal complex (pink), internal core (light green) and the putative straight dsDNA terminus (dark blue). The portal complex and internal core are coloured semi-transparently to show that their central channels are filled with the putative dsDNA terminus.

b, Bottom view of the portal complex with the nearly 12-fold structural features labelled around the filled central channel.

c, The apparent 12-fold arrangement of densities for the portal complex in a section image of the three-dimensional map. The location of the section is indicated by the dashed line in a.



d, Top view of the internal core (light green) showing the central channel filled with the putative dsDNA terminus (dark blue).

Reprinted by permission from Macmillan Publishers Ltd: Jiang W, Chang J, Jakana J, Weigele P, King J, Chiu W. *Structure of epsilon15 bacteriophage reveals genome organization and DNA packaging/injection apparatus*. Nature. 2006 Feb 2;439(7076):612-6 **copyright 2006**

Tutorial Contents	Virus Entry	Bacterial Entry	Animal Cell Entry	Plant&Fungal Cell Entry

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(Unless otherwise stated)

Virus Entry Into Cells

EUKARYOTE CELL ENTRY

Animal Cell Entry

- Animal Cells and Vesicle Transport
- Direct Cell Membrane Fusion
- Entry via Endocytotic Vesicle
- Fusion Proteins
- Nuclear Targetting

Animal Cells and Vesicle Transport

Eukaryote cells do not have cell walls; they have only lipid bilayer cell membranes (=**PLASMALEMMA**) with associated embedded proteins. They also have a very highly developed INTERNAL MEMBRANE COMPLEX, consisting of **endoplasmic reticulum**, **Golgi apparatus, nuclear membrane**, and various **specialised vesicles** such as **LYSOSOMES**, which are involved in intracellular digestion. Eukaryote cells have an intricate system of **vesicle transport** centred on the **Golgi apparatus**; this involves export of protein(s) and vesicles from the ER to the Golgi; production of EXPORT vesicles containing proteins from this for fusion with the cell membrane (=**EXOCYTOSIS**); production of LYSOSOMES to fuse with **ENDOSOMES** for digestion of material internalised by **RECEPTOR-MEDIATED** or non-specific **ENDOCYTOSIS** (**PHAGOCYTOSIS** for particulates; **PINOCYTOSIS** for liquid). Other CYTOSKELETON-DIRECTED vesicle trafficking involves targetting of vesicles back to the Golgi and to the NUCLEAR MEMBRANE.

The initially-formed **ENDOCYTOTIC** vesicles resulting from receptor-mediated endocytosis are coated with **CLATHRIN**, a structural protein which promotes curvature of the membrane at **COATED PITS** - which become vesicles as the protein continues to assemble and impresses the membrane into a sphere. Clathrin is recycled back to the cell membrane after endocytosis, either because of interaction of the vesicle with a lysosome, or due to another process.

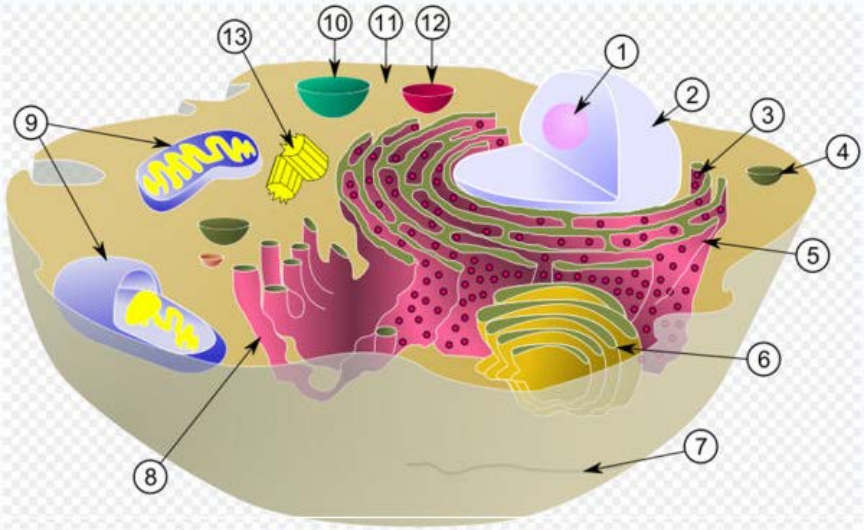
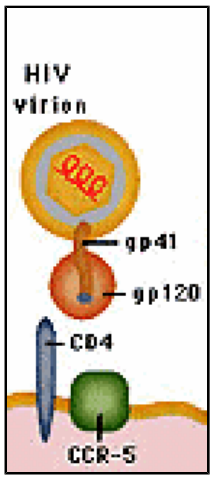


Diagram of a typical animal cell. Labelled organelles are:

1. Nucleolus
2. Nucleus
3. Ribosome
4. Vesicle
5. Rough endoplasmic reticulum
6. Golgi apparatus
7. Cytoskeleton
8. Smooth endoplasmic reticulum
9. Mitochondrion
10. Vacuole
11. Cytoplasm
12. Lysosome
13. Centriole

Direct Cell Membrane Fusion

Viruses take advantage of these processes in a number of ways, after they have attached to the cell surface via **binding to a specific receptor**. The simplest is **DIRECT MEMBRANE FUSION**, where the virion membrane fuses with the cell membrane, and the virion nucleoprotein complex is delivered into the cell cytoplasm directly. This is generally a **pH-independent process**, and requires only that the **membrane be fluid** (ie: temperature in the physiological range), and generally that some divalent cations be present.

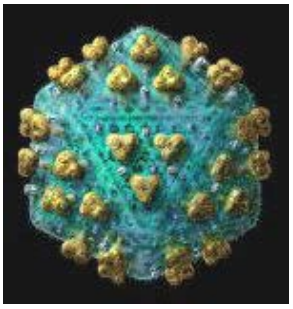


The entry process for **HIV** is shown in the graphic (from the Univ Leicester material, copyright Dr AJ Cann).

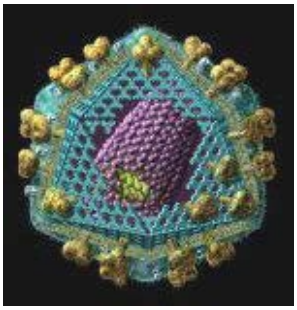
Click here for an non-animated view of the process if your browser does not support GIF animation.

Here the virion attachment protein - **gp120** - (see also **here**) attaches initially to the **CD4** protein on a helper T-cell. The gp120 undergoes conformational change due to binding, and binds the **accessory receptor - CCR-5,** a **chemokine** receptor in this case, although there are others - as well. **gp41** - a cleavage product of a **gp160** precursor, and a part of the **"spike protein"** of the viral membrane - is then able to bind into the cell membrane, via a hydrophobic domain. A condensation of the gp41 structure - formation of a "6 helix bundle" - causes close juxtaposition of cell and viral membranes, which promotes membrane fusion and **nucleoprotein entry into the cell.**

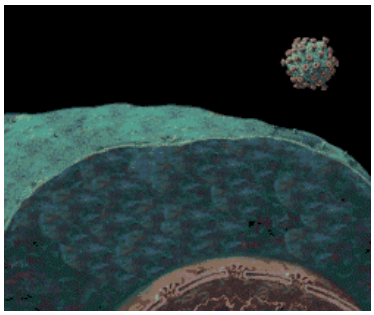
The entire HIV life cycle is shown here: graphics kindly provided by Russell Kightley.



HIV virion: outer view showing gp160



HIV virion structure: cutaway showing matrix and nucleoprotein



HIV cell entry and replication animation

Another depiction of the infection process is given on the Roche site.

PARAMYXOVIRUSES are also able to enter cells in this fashion; in fact, an F or fusion (glyco)protein purified from enveloped virions of **Sendai virus** is often used to artificially fuse cells in *in vitro* experiments.

HERPESVIRUSES also are able to enter cells by direct cell membrane fusion, and to cause cell fusion (multinucleate giant cells or syncytia)

Entry via Endocytotic or Other Vesicle

Another means of cell entry via membrane interactions is typified by **influenza virus**: this attaches to cell membranes via its **HAEMAGGLUTININ** (HA) protein - a trimeric attachment protein - which binds **NEURAMINIC ACID** residues on cellular glycoproteins. This and the neuraminidase (NA) protein - which exists as as a tetramer, present to about 20% of total envelope protein - contact the MATRIX (M1) protein which constitutes the internal shell. This in turn interacts with the viral nucleoprotein (vRNP) complex.

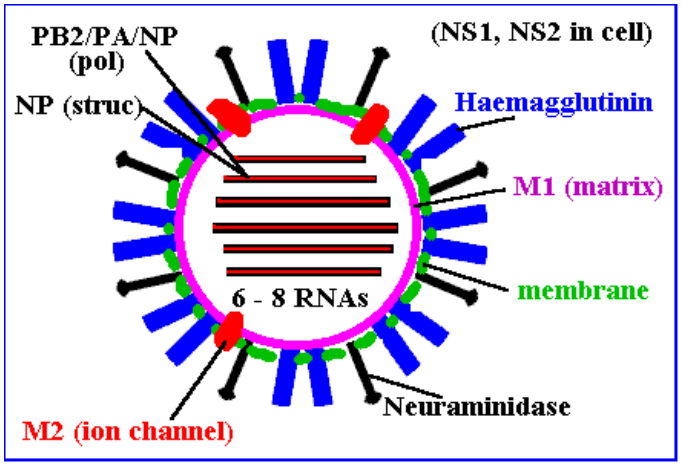
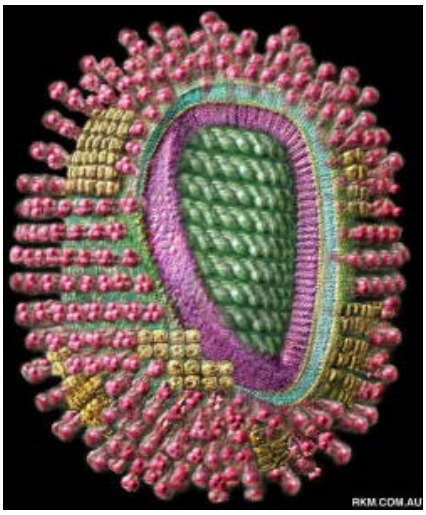


Diagram showing components of the influenza virion



Cutaway model of the influenza virion

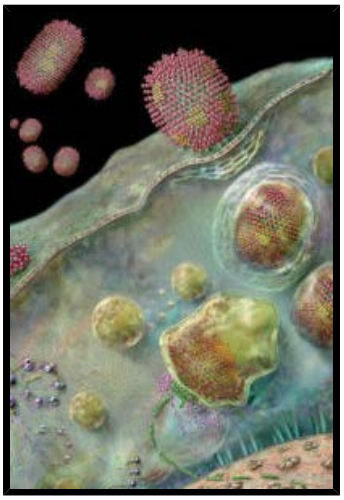
Haemagglutinin trimer

The virion binds **reversibly** to the cell surface via **one or a few attachment proteins**. Receptor consolidation - recruitment of more receptors, resulting in more binding - results in curvature of the membrane around the particle, and **eventual formation of an endocytotic vesicle** by a "zipper" process.

Click here for a view of the process - from Dr Sean Heaphy, Univ Leicester.

A **cell surface protease cleavage** divides the HA into HA1 and HA2: this causes a conformational change which exposes a hydrophobic **"fusion domain"** on HA2. A further **pH-induced structural change** (with lysosome fusion, pH drops), allows **insertion of part of HA2** into the vesicle membrane. Another conformational change caused by **membrane interaction** results in "**apposition**" of membranes and fusion, allowing escape from the vesicle of the viral nucleoprotein, into the host cell cytoplasm. This is generally known is **pH-dependent entry**. Another version of the process, depicting the whole influenza virus life cycle, is shown below left (picture derived from original by Russell Kightley).

The **M2 membrane protein** - present at a low copy number of <100 in the influenza virus membrane - is a homotetramer which, at low pH, is the smallest **ion channel protein** yet discovered, making it a **VIROPORIN**. This is activated in the low-pH environment of an endosome, and allows the passage of protons (H^+) into the virion: this lowers the pH, causes conformational changes in M1 which disrupt interactions between it and the vRNP, which allows its release into the cytoplasm free of the encapsulating M1 protein matrix. The vRNP then moves to the nucleus - unusual for an RNA virus - by means of **NUCLEAR LOCALISATION SIGNALS** in the NP contacting the cell transport machinery (see also here).



Influenza virus entry into cells via endocytotic vesicle

Fusion Proteins

All enveloped viruses appear to share the fusion mode of entry, whether they fuse with the cell membrane directly or with the membrane around an internalised vesicle. This is mediated by three identified classes of envelope glycoproteins, which nevertheless share a very similar mechanism for inducing fusion.

Class I fusion proteins are found in **retroviruses** (HIV, leukaemia viruses), **myxoviruses** (influenza) **coronaviruses** (SARSCoV) and **paramyxoviruses** (mumps, measles), among others. The "spikes" are composed of **three identical protein subunits, largely alpha-helical in structure,** assembled as trimers, with subunits generated from a precursor that is **cleaved into two pieces**. The carboxy-terminus of one piece is anchored to the viral membrane; the new amino terminus has a characteristic stretch of 20 hydrophobic amino acids: this is the **fusion peptide**. Class I proteins all have a **trimeric helical coiled-coil rod** adjacent to the fusion peptide: this may act as a template for the refolding of protein segments during fusion, when the "**six helix bundle**" forms.

Interfering with this process is the basis of a highly successful new class of HIV therapeutics: **entry inhibitors**. See here for a movie on how one particular product - T20 or Fuzeon, from Trimeris - works. Another view of HIV chemotherapy in general can be seen here.

See here for an excellent short movie on how HIV enters its host cells.

Class II fusion proteins have distinctly different structural features: they predominantly have a **β-sheet-type structure** and are not cleaved during biosynthesis; the "fusion peptide" portion that inserts into the target membrane is thought to be an internal hydrophobic fusion loop. They are found in **dengue**, **tick-borne encephalitis**, **yellow fever** and other **flaviviruses** **(see also here)**, and **Semliki Forest virus** and other **alphaviruses**, among others.

The proteins have **three principal domains**:

- **domain I** begins at the amino terminus,
- **domain II** contains the internal fusion loop, and
- **domain III** is at the carboxy terminus.

The **dimeric** protein binds to one or a few cellular receptors; receptor binding consolidates and the **virus is internalised**. The acidic pH inside endosomes causes **domain II to swing upward**, allowing monomers to rearrange laterally. The **fusion loop** inserts into the host-cell membrane, enabling **trimer** formation of the viral glycoprotein. **Domain III** shifts and rotates to create contacts, **bending the membrane**. The formation of further contacts leads to unrestricted **hemifusion** and the final, most stable form of the protein. As with Class I proteins, **several viral envelope spikes** are involved in the formation of a single fusion pore.

See here for a PowerPoint presentation depiction of the process for Class I and Class II proteins (UCT MCB3024S only).

A graphic depiction of the process of membrane fusion is shown below.

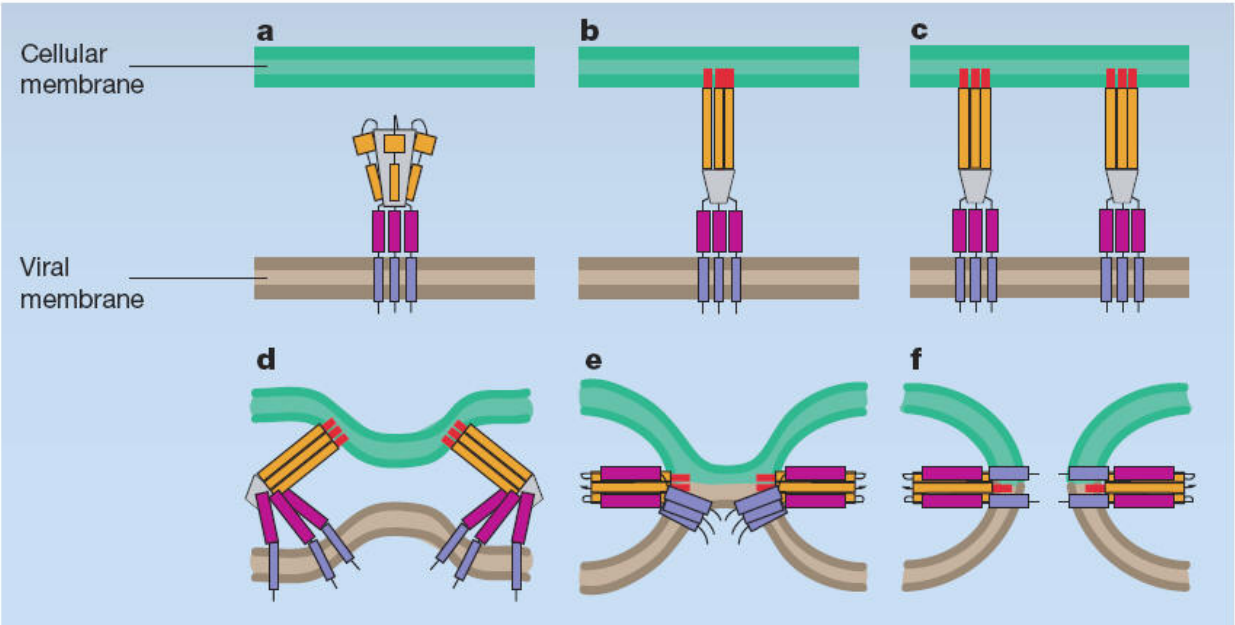


Figure 1 Proposed mechanism for membrane fusion by class I fusion proteins. a, The metastable conformation of a trimeric generalized fusion protein, with helical domain A in orange, helical domain B in pink, and the transmembrane domain in purple. b, After binding to a receptor on the cellular membrane, or on exposure to the low pH found in intracellular compartments (endosomes), the protein forms an extended conformation and the hydrophobic fusion peptide (red) inserts into the target membrane. c, Several trimers are thought to be involved. d, Protein refolding begins. The free energy thereby released causes the membranes to bend towards each other. e, Formation of a restricted hemifusion stalk allows the lipids in the outer leaflets of the membranes to mix. f, Protein refolding completes, forming the final, most stable form of the fusion protein, with the fusion peptide and transmembrane domain anti-parallel to each other but in the same membrane. Only a and f have been observed by crystallography, but biochemical data support many of the proposed steps.

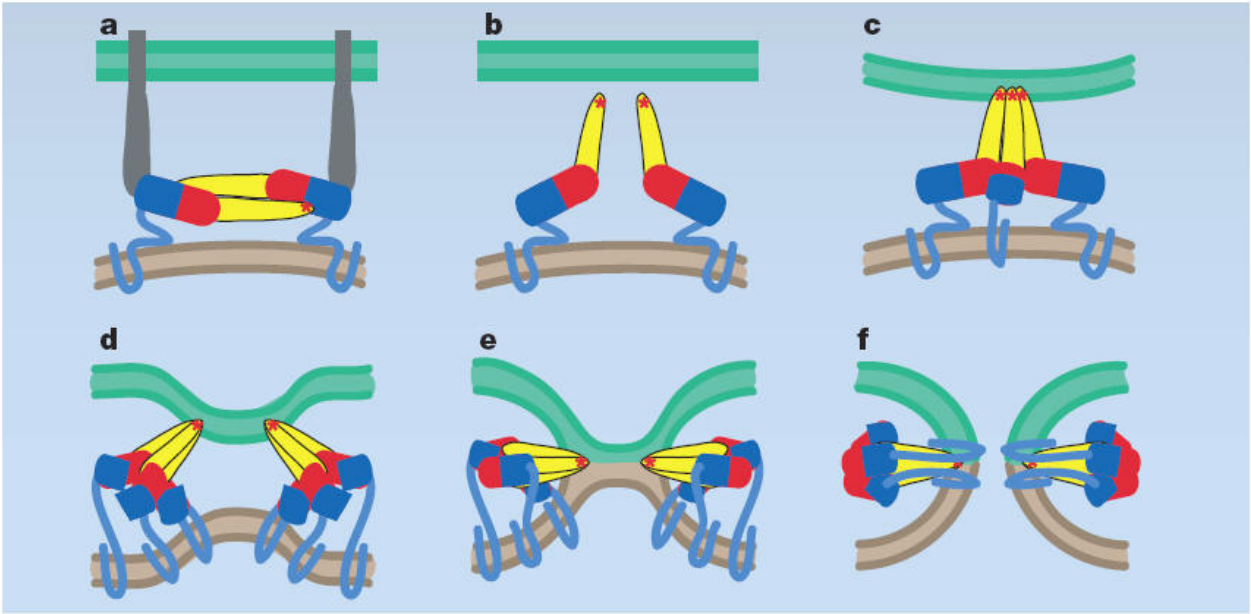


Figure 2 Proposed mechanism for fusion by class II proteins, incorporating the new results[1–3]. a, The dimeric E protein binds to a cellular receptor (grey) and the virus is internalized to endosomes. Membrane fusion, which will release the virus into the body of the cell, takes place within endosomes. Domain I is in red, domain II in yellow, and domain III in light and dark blue (the lighter blue shows a predicted but unsolved structure). b, The acidic pH inside endosomes causes domain II to swing upward, permitting E monomers to rearrange laterally. c, The fusion loop (red dot) inserts into the outer leaflet of the host-cell membrane, enabling trimer formation. d, The formation of trimer contacts extends from the top to the bottom of the molecule. Domain III shifts and rotates to create contacts, bending the membrane. e, The formation of further contacts leads to unrestricted hemifusion. f, The final most stable form of the protein. Only a and f have been observed by crystallography but biochemical data support many of the other steps.

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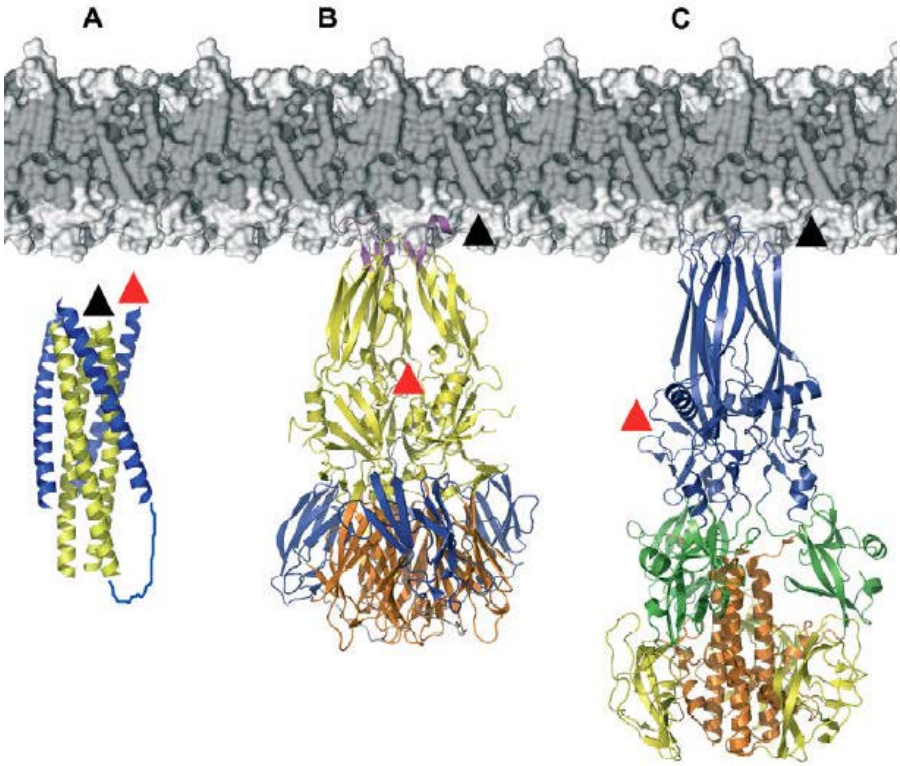
Class III fusion proteins also form **trimers of hairpins** as a fusion structure by combining two structural elements. These proteins are characteristic of **rhabdoviruses** like **rabies** and **vesicular stomatitis viruses**, and **herpesvirus** gB attachment glycoproteins. Similar to **Class I proteins**, the **post-fusion trimer** has a **central α-helical trimeric core**; however, the **fusion** domains have **two fusion loops at the tip of an elongated β-sheet**, similar to **Class II fusion proteins**.

The following graphic clearly shows the three different forms:

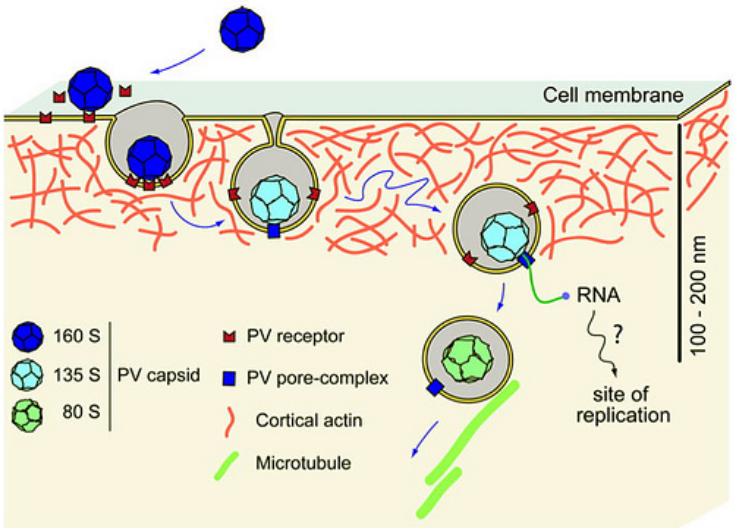
copied with permission from Weissenhorn W, Hinz A, Gaudin Y. Virus membrane fusion. FEBS Lett. 2007 May 22;581(11):2150-5

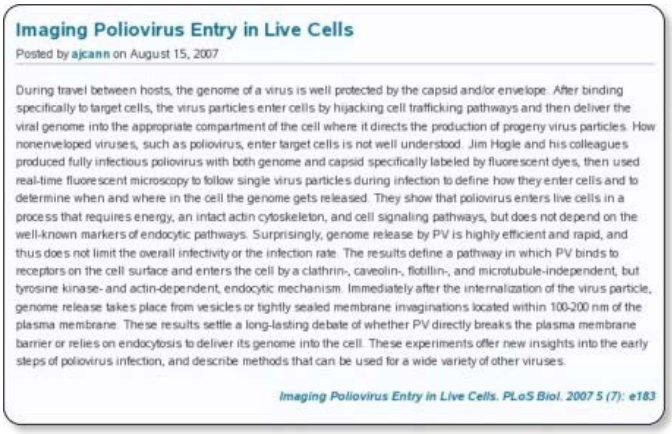


Ribbon diagrams of representative structures of class I, II and III fusion proteins in their proposed post-fusion conformations. The positions of both membrane anchors at the tip of the elongated structures are indicated by black (fusion peptide, fp) and red (transmembrane, TM) arrows. (A) HIV-1 gp41 core structure; (B) Flavivirus fusion protein E and (C) VSV glycoprotein G.

Most **non-enveloped viruses**, such as the complex dsDNA **adenoviruses** (***Adenoviridae)*** and the simpler ssRNA **picornaviruses** (***Picornaviridae*)*,*** also enter cells via vesicles***.*** The former appear to enter via an endocytotic vesicle, then to interact with the vesicle membrane - probably because of structural alterations due to the pH shift - so as to expel a partially-uncoated viral core structure into the cytoplasm. This is then targetted to the nucleus, where the DNA appears to enter via a nuclear pore.

Picornaviruses do something similar: that is, the capsid becomes rearranged as a result of pH-and/or receptor binding induced structural transitions, whereby the VP4 internal protein is externalised and the virion surface becomes more lipophilic, and interacts with a vesicle membrane to form a pore so as to allow exit of the RNA into the cytoplasm. A more complex process may also occur which allows **RNA to enter the cell from externally-bound virions**. Recent developments have allowed researchers to track picornavirus RNA and capsid separately following cell entry.





Imaging Poliovirus Entry in Live Cells

Posted by ajcann on August 15, 2007

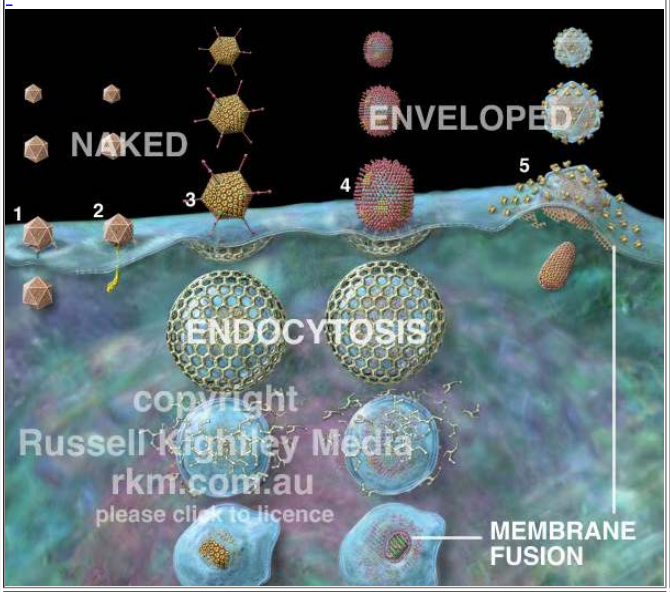
During travel between hosts, the genome of a virus is well protected by the capsid and/or envelope. After binding specifically to target cells, the virus particles enter cells by hijacking cell trafficking pathways and then deliver the viral genome into the appropriate compartment of the cell where it directs the production of progeny virus particles. How nonenveloped viruses, such as poliovirus, enter target cells is not well understood. Jim Hogle and his colleagues produced fully infectious poliovirus with both genome and capsid specifically labeled by fluorescent dyes, then used real-time fluorescent microscopy to follow single virus particles during infection to define how they enter cells and to determine when and where in the cell the genome gets released. They show that poliovirus enters live cells in a process that requires energy, an intact actin cytoskeleton, and cell signaling pathways, but does not depend on the well-known markers of endocytic pathways. Surprisingly, genome release by PV is highly efficient and rapid, and thus does not limit the overall infectivity or the infection rate. The results define a pathway in which PV binds to receptors on the cell surface and enters the cell by a clathrin-, caveolin-, flotillin-, and microtubule-independent, but tyrosine kinase- and actin-dependent, endocytic mechanism. Immediately after the internalization of the virus particle, genome release takes place from vesicles or tightly sealed membrane invaginations located within 100-200 nm of the plasma membrane. These results settle a long-lasting debate of whether PV directly breaks the plasma membrane barrier or relies on endocytosis to deliver its genome into the cell. These experiments offer new insights into the early steps of poliovirus infection, and describe methods that can be used for a wide variety of other viruses.

Imaging Poliovirus Entry in Live Cells. PLoS Biol. 2007 5 (7): e183

Imaging Poliovirus Entry in Live Cells « MicrobiologyBytes via kwout

These mechanisms are summarised in the diagram below left: click here or on the box below to link to the source page.

Note that "naked" particle entry is probably an artifact of very high multiplicity of infection used when investigating the phenomenon of cell entry in tissue-cultured cells.



2. NAKED VIRUS - GENOME INJECTION: virus attaches to cell surface and releases its genome which penetr created in the plasma membrane. (Bacteriophages, which attack bacterial cells, also inject their genomes and m see our diagram of T4 phages injecting).

3. NAKED VIRUS - ENDOCYTOSIS: virus attaches to cell surface receptor molecules and sinks into a clathri closes off creating a clathrin coated vesicle (drawn as a cage like sphere) and so the contained virus partic molecular cage soon dissociates into component triskelions (the propeller like objects) which leave a vesicle. T contained virion to an endosome. At some stage thereafter, the viral components are released. The virus shown i

4. ENVELOPED VIRUS - ENDOCYTOSIS & MEMBRANE FUSION: virus enters cell by receptor mediated end with the endosome membrane and so the virus components are released. The virus shown here is an influenza v illustration.

5. ENVELOPED VIRUS - MEMBRANE FUSION: virus enters the cell when its outer membrane fuses with the pl contents are then spilled into the cytoplasm of the cell. This example is HIV, which is unusual in having a cor spherical). Please see our HIV illustrations.

GENERAL POINTS: This diagram is only intended as an overview. The viruses are depicted to show general pri protein capsid, whilst ENVELOPED VIRUSES are cloaked in cell membrane, added during budding from the h genetic material vary with the type of virus. Some viruses release their genetic material almost immediately (se cell nucleus still contained in a protein vehicle (see entry 5).

FOR INFORMATION ON VIRUSES ENTERING CELLS please see virus entry into animal cells from Ed Rybicki

viral entry into animal cells; image by Russell Kightley Media via kwout

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Reoviridae - isometric dsRNA non-enveloped viruses with up to 12 genome components - enter by endocytosis, are partially uncoated in lysosomes, and then core particles enter the cytoplasm by an unknown process, perhaps similar to what occurs with adenoviruses. **Core particles then function as virus transcription "factories"**

SEE ALSO the animated graphics of viral entry for individual families of viruses from the University of Calgary site mirror

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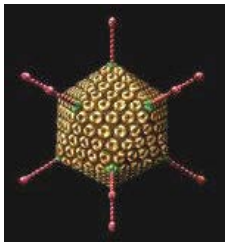
Nuclear Targetting

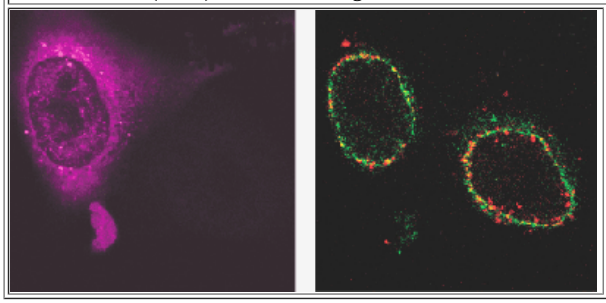


A basic requirement in the infection of Eukarya would be that **DNA genomes end up in the nucleus** - except for **Pox-, Phyco-** and some **Baculoviridae** - and that **RNA genomes end up in the cytoplasm** - except for myxoviruses - as these are the sites where the respective viruses may be expected to replicate their genomes. DNA is its own nuclear targetting signal in that naked DNA is seen to be moved into cell nuclei if introduced into cells; however, most DNA viruses which require a nuclear replication stage seem to have some specific means of targetting partially-disassembled virions to the cell nucleus. **Nucleoproteins with nuclear localisation signals (NLS)** are very often involved.

Adenoviruses - large naked isometric virions with linear dsDNA of 30-38kbp - appear to be transported by means of the **hexon protein** of the partially degraded naked capsid that is released into the cytoplasm, interacting with **microtubules**. This reaches a nuclear pore and allows escape of viral DNA plus certain viral polypeptides into the nucleus.

A relatively recent finding (in 2001) was that adenovirus 2 (Ad2) docks to the nuclear pore after microtubule transport via the nuclear-pore complex (NPC) receptor CAN/Nup214. The virus then disassembles slowly and transfers its genetic material through the nuclear pore while remaining attached to the NPC. The process does not occur, however, without the linker histone H1 protein. H1 probably binds a surface-exposed acidic amino acid cluster on hexon proteins, thereby probably destabilising the capsid and triggering viral disassembly. In normal cells, H1 that leaks out of the nucleus is retrieved from the cytoplasm by the proteins importin $_{\beta}$ and importin 7: the disassembly of Ad2 requires the same two importins, leading to speculation that hexon-bound H1 is recognised by importin $_{\beta}$ and importin 7, and the complex is brought partially into the nucleus, thus triggering a gradual disassembly of the virus particle at the NPC. This allows the DNA that exits particles to directly enter the nucleus.

Laura Bonetta (2001) Virus knocking at nucleus door. *Nature Medicine* **7**, 1284



The photographs show two human lung carcinoma cells, one of which was injected with anti-histone H1 antibodies. The injected cell can be seen in the photograph on the left, stained with an injection marker present in the antibody solution (purple). The photograph on the right shows the two cells stained with antibodies to the NPC (green) and Ad2 DNA (red). In the uninjected cell, Ad2 DNA can be seen bound to NPC on the nuclear membrane but also inside the nucleus; in the cell injected with anti-H1 antibodies all Ad2 DNA remains bound to the NPC.

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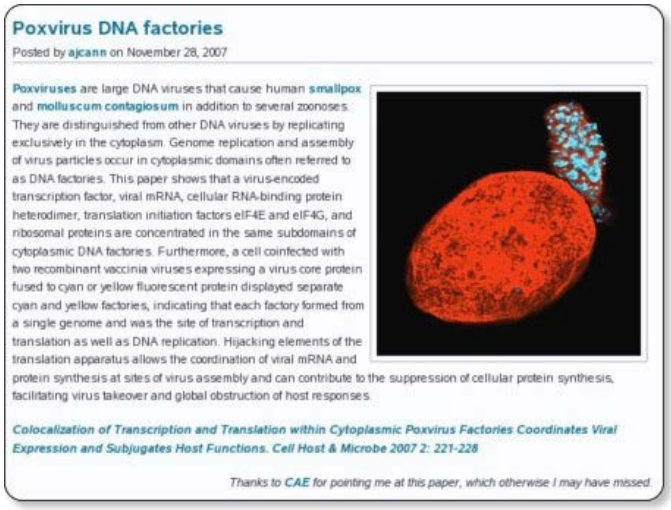
Parvoviruses - small naked isometric viruses with linear ~5 kb ssDNA genomes - enter host cells by receptor-mediated endocytosis, escape from endosomal vesicles to the cytoplasm, and then replicate their DNA in the nucleus. Canine parvovirus virions at least use the motor protein **dynein** to be transported along microtubules to the nucleus.

Herpesviruses generally enter by fusion with the cell membrane - by a process that involves several envelope glycoproteins acting in concert - and the core particles migrate to nuclear pores, and release DNA there.

Poxviruses - which have "intracellular" single-enveloped and "extracellular" double-enveloped forms - may enter by **direct cell fusion** (pH-independent) or **lysosomal vesicle fusion** (pH-dependent and inhibitable by lysomotropic agents), with the latter perhaps predominating. Once core virions are in the cytoplasm, they uncoat further to expose a nucleoprotein complex which is first transcriptionally and later, replicationally, active.



Poxvirus DNA factories

Posted by ajcann on November 28, 2007

Poxviruses are large DNA viruses that cause human **smallpox** and **molluscum contagiosum** in addition to several zoonoses. They are distinguished from other DNA viruses by replicating exclusively in the cytoplasm. Genome replication and assembly of virus particles occur in cytoplasmic domains often referred to as DNA factories. This paper shows that a virus-encoded transcription factor, viral mRNA, cellular RNA-binding protein heterodimer, translation initiation factors eIF4E and eIF4G, and ribosomal proteins are concentrated in the same subdomains of cytoplasmic DNA factories. Furthermore, a cell coinfected with two recombinant vaccinia viruses expressing a virus core protein fused to cyan or yellow fluorescent protein displayed separate cyan and yellow factories, indicating that each factory formed from a single genome and was the site of transcription and translation as well as DNA replication. Hijacking elements of the translation apparatus allows the coordination of viral mRNA and protein synthesis at sites of virus assembly and can contribute to the suppression of cellular protein synthesis, facilitating virus takeover and global obstruction of host responses.

Colocalization of Transcription and Translation within Cytoplasmic Poxvirus Factories Coordinates Viral Expression and Subjugates Host Functions. Cell Host & Microbe 2007 2: 221-228

Thanks to CAE for pointing me at this paper, which otherwise I may have missed.

Poxvirus DNA factories « MicrobiologyBytes via kwout

Tutorial Contents	**Virus Entry**	**Bacterial Entry**	**Animal Cell Entry**	**Plant&Fungal Cell Entry**

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(Unless otherwise stated)

Virus Entry Into Cells

EUKARYOTE CELL ENTRY

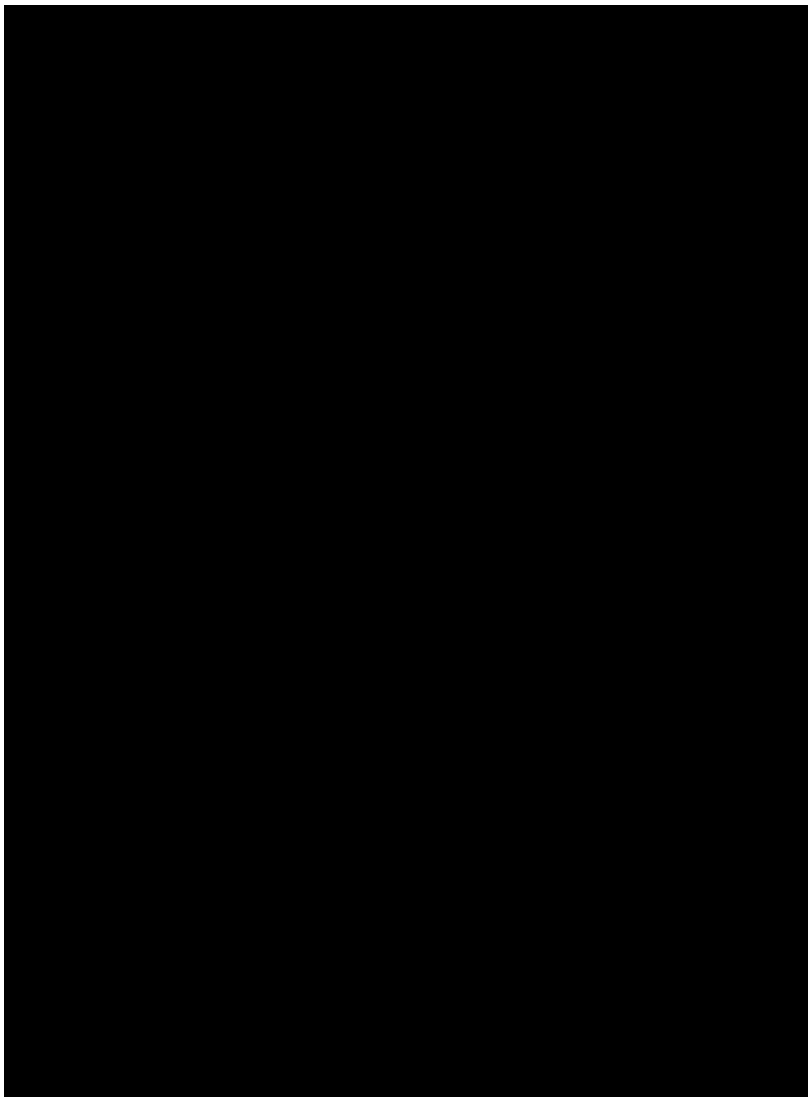
Plant Cell Entry

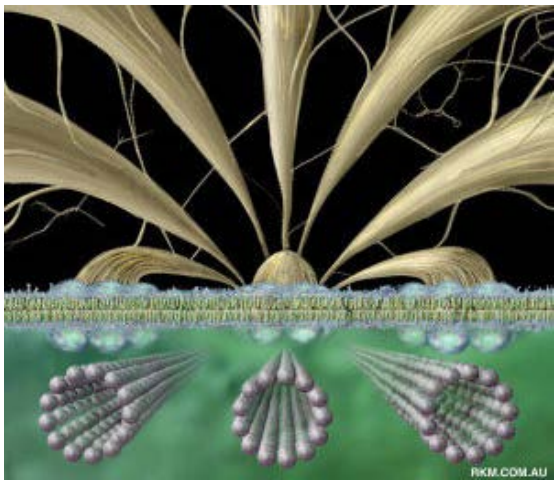
Fungal Cell Entry

Plant Cell Entry

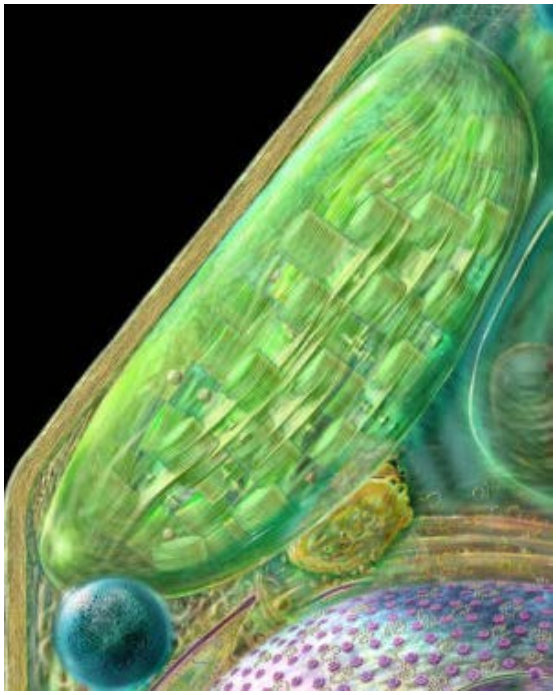
Plant cells, while **superficially similar to animal cells in basic construction** as far as all organelles except **chloroplasts** and often extensive **vacuoles** are concerned (see here), have **one large and fundamental difference to animal cells**, which profoundly affects the way in which they are infected by viruses, and how viruses move between them.

This is their possession of thick, rigid, cellulose-based cell walls.



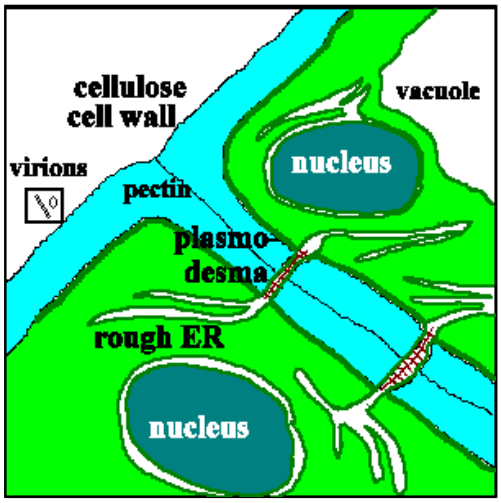


Plant cell section, showing nucleus, chloroplasts and vacuole

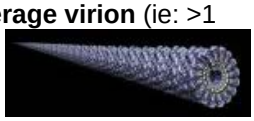


Detail of cellulose and chloroplast

copyright Russell Kightley



Every cell is separated from every other cell by thick cell walls, whose **dimensions are far larger than the size of the average virion** (ie: >1 micron, cf. TMV = 300 nm). This means that plant cells are effectively inaccessible to viruses, even given mechanisms of injection similar to the T-even phages. Live plant cells **interconnect only via specific discontinuities in the cellulose walls**: the most numerous of these are **plasmodesmata**, which are **complex structures filled with membrane-derived processes continuous with the endoplasmic reticulum**. These are **"gated" intercellular channels**, which limit the passage of molecules between cells, and **certainly do not admit particles as large as virions**. Plant viruses have therefore **evolved specific movement functions**, mediated by one or more virus-specified proteins, which interact with the plasmosdesmatal machinery so as to **increase the "pore" size**, and allow specific transport of viral nucleoprotein complexes. **All plant-infecting viruses possess one or more movement-related protein** (MP) genes: these are very varied, although there are distinct groups of them, and **they appear to largely derive from host plant genes for chaperonins and plasmodesmata-**



associated proteins.

Plasmodesmata

Plant viruses, therefore - **which are almost overwhelmingly ssRNA +ve sense and non-enveloped** - **do not appear to specifically interact with host cell membranes or cell walls, as do bacterial and animal viruses**; even when the plant-infecting virus also infects an animal (eg: plant **rhabdoviruses** and **bunyaviruses**) and presumably behaves normally in the other host, and even though **apparently plant cells are capable of phagocytosis / endocytosis**. The mechanisms employed to enter cells rather appear to be **passive carriage through breaches in the cell wall** in the first instance, followed by later **cell-to-cell spread** in a plant by means of **specifically-evolved "movement" functions**, and perhaps spread via **conductive tissue** as whole virions.

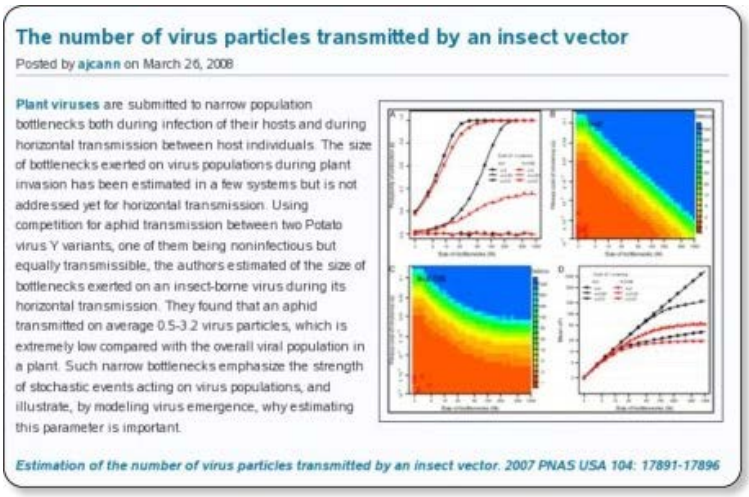
See here for a new model of plant virus movement

The "**passive carriage**" referred to above could mean:

- 1) a purely **mechanical** injury that **breaches the cell wall** and transiently breaches the plasma membrane of underlying cells;
- 2) similar gross injury due to the **mouthparts of a herbivorous arthropod**, such as a beetle;
- 3) **injection directly into cells** through the piercing mouthparts of sap-sucking insects or nematodes;
- 4) carriage into plant tissue on or in association with **cells of a fungal parasite**;
- 5) **vertical transmission** through infected seed or by vegetative propagation;
- 6) transmission via **pollen**; and
- 7) **grafting** of infected tissue onto healthy tissue.

The mode of transmission of viruses affects their concentration and localisation in plants. For example, **mechanically** transmitted viruses (eg: **bromoviruses**, **tobamoviruses**) **tend to reach very high concentrations** in most tissues of a plant (up to 4g / kg plant): this is necessary for survival, as it guarantees that a large number of virions will be present for onward transmission by whatever non-specific means presents itself. **Viruses which are introduced into plants via insect vectors** with piercing mouthparts, on the other hand, tend to be **limited in their multiplication to phloem elements**, which are preferred target tissues for insect feeding. Consequently, these viruses (eg: **luteoviruses**, **geminiviruses**) reach only very low concentrations (**mg / kg**) in whole plants.

See also here for insect transmission:



The number of virus particles transmitted by an insect vector

Posted by ajcann on March 26, 2008

Plant viruses are submitted to narrow population bottlenecks both during infection of their hosts and during horizontal transmission between host individuals. The size of bottlenecks exerted on virus populations during plant invasion has been estimated in a few systems but is not addressed yet for horizontal transmission. Using competition for aphid transmission between two Potato virus Y variants, one of them being noninfectious but equally transmissible, the authors estimated of the size of bottlenecks exerted on an insect-borne virus during its horizontal transmission. They found that an aphid transmitted on average 0.5-3.2 virus particles, which is extremely low compared with the overall viral population in a plant. Such narrow bottlenecks emphasize the strength of stochastic events acting on virus populations, and illustrate, by modeling virus emergence, why estimating this parameter is important.

Estimation of the number of virus particles transmitted by an insect vector. 2007 PNAS USA 104: 17891-17896

The number of virus particles transmitted by an insect vector « MicrobiologyBytes via kwout

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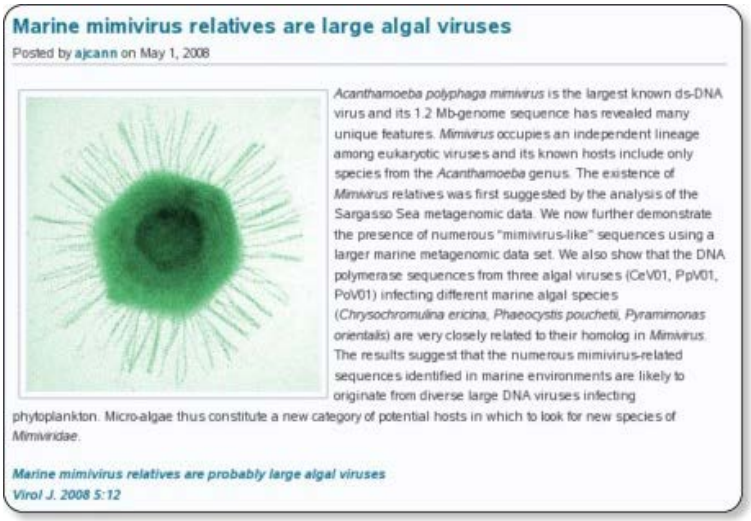
An EXCEPTION to the above rules are some of the **PHYCODNAVIRIDAE**, which infect **marine and aquatic algae and some protozoans**, which have **large (>300 kb) dsDNA genomes**, and **very large (130 - 200 nm), complex virions**. Exemplars which infect algae appear to have **specific enzymes at the surface of virions** which may **degrade the CHITIN cell wall of the alga**, to allow interaction of the particle with the cell membrane directly. **Some of these viruses also appear to be able to "inject" their DNA**, much as phages do, and to enter a **lysogenic** state.

A useful Web page describing phycodnaviruses and other **large-genome DNA viruses** - though dated - is **GiantVirus.Org.**

See also these posts in MicrobiologyBytes:



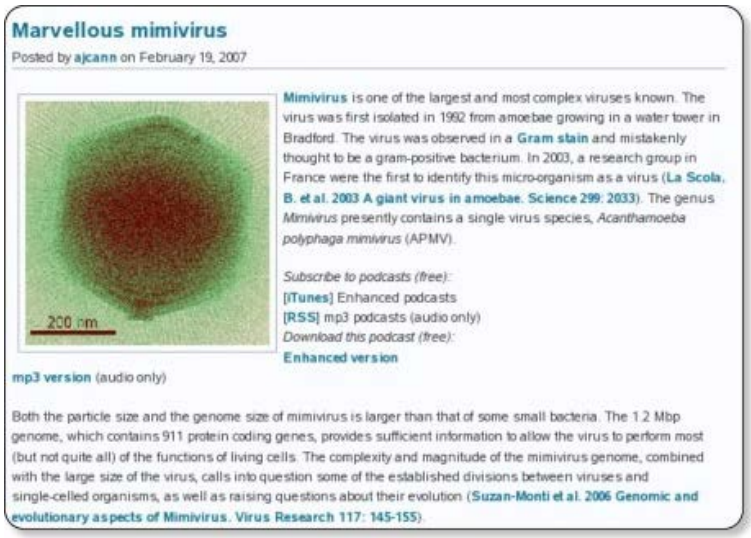
Marine mimivirus relatives are large algal viruses

Posted by ajcann on May 1, 2008

Acanthamoeba polyphaga mimivirus is the largest known ds-DNA virus and its 1.2 Mb-genome sequence has revealed many unique features. *Mimivirus* occupies an independent lineage among eukaryotic viruses and its known hosts include only species from the *Acanthamoeba* genus. The existence of *Mimivirus* relatives was first suggested by the analysis of the Sargasso Sea metagenomic data. We now further demonstrate the presence of numerous "mimivirus-like" sequences using a larger marine metagenomic data set. We also show that the DNA polymerase sequences from three algal viruses (CeV01, PpV01, PoV01) infecting different marine algal species (*Chrysochromulina ericina*, *Phaeocystis pouchetii*, *Pyramimonas orientalis*) are very closely related to their homolog in *Mimivirus*. The results suggest that the numerous mimivirus-related sequences identified in marine environments are likely to originate from diverse large DNA viruses infecting phytoplankton. Micro-algae thus constitute a new category of potential hosts in which to look for new species of *Mimiviridae*.

Marine mimivirus relatives are probably large algal viruses
Virol J. 2008 5:12

Marine mimivirus relatives are large algal viruses « MicrobiologyBytes via kwout



Marvellous mimivirus

Posted by ajcann on February 19, 2007

Mimivirus is one of the largest and most complex viruses known. The virus was first isolated in 1992 from amoebae growing in a water tower in Bradford. The virus was observed in a **Gram stain** and mistakenly thought to be a gram-positive bacterium. In 2003, a research group in France were the first to identify this micro-organism as a virus (**La Scola, B. et al. 2003 A giant virus in amoebae. Science 299: 2033**). The genus *Mimivirus* presently contains a single virus species, *Acanthamoeba polyphaga mimivirus* (APMV).

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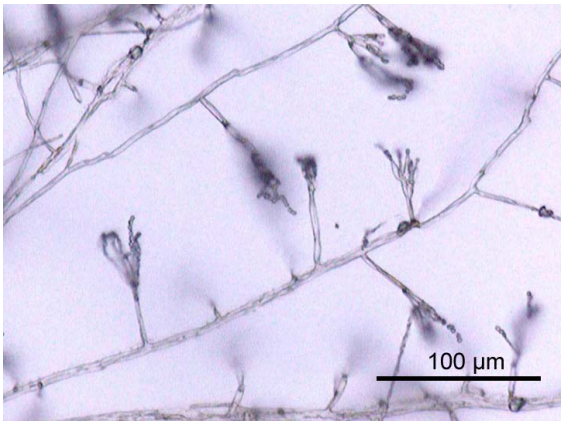
Both the particle size and the genome size of mimivirus is larger than that of some small bacteria. The 1.2 Mbp genome, which contains 911 protein coding genes, provides sufficient information to allow the virus to perform most (but not quite all) of the functions of living cells. The complexity and magnitude of the mimivirus genome, combined with the large size of the virus, calls into question some of the established divisions between viruses and single-celled organisms, as well as raising questions about their evolution (**Suzan-Monti et al. 2006 Genomic and evolutionary aspects of Mimivirus. Virus Research 117: 145-155**).

Marvellous mimivirus « MicrobiologyBytes via kwout

Fungal Cell Entry



Fungal hyphae - of *Penicillium* sp.

There are certain superficial similarities between plants and FUNGI with respect to the cell wall; however, in the latter case, cell walls are composed of CHITIN, a different complex polysaccharide, and fungal hyphae are often effectively **"tubes" with no cross-walls**.

No fungal viruses appear to have any specific mechanisms for gaining entry to fungal cells; indeed, it is extremely difficult to demonstrate the infectivity of virus-like particles, and it is only since the advent of the **GENE GUN** or biolistic transformation apparatus, that many viruses have been shown to be infectious at all - **by being "shot" into fungal cells adsorbed onto metal particles**.

It is probable that most fungal viruses - like the plant-infecting ***CRYPTOVIRUS*** genus of the mainly fungus-infecting ***Partitiviridae*** - are only transmitted by "grafting", or the physical connection of infected to healthy cells by **anastomosis.** Thus, **fungal mating is a good means of transmission, as it results in the mixing of cell contents of different hyphae**. Otherwise, transmission would be **vertical**, or to progeny via spore formation.

It is claimed - in the ICTV 8th Report article on *Narnaviridae*, which include the chestnut blight fungus-infecting mitoviruses - that most fungal viruses have no extracellular stage. **Indeed, narnaviruses do not even make particles.**

Tutorial Contents	Virus Entry	Bacterial Entry	Animal Cell Entry	Plant&Fungal Cell Entry

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(Unless otherwise stated)

Newer Definitions of Life

Life may (somewhat irreverently) be defined in general terms as:

"The phenomenon associated with the replication of self-coding informational systems",

or more specifically as:

"The phenomenon associated with the replication of nucleic acids".

- Rybicki EP, 1996.

Another more serious view:

"Life can be viewed as a complex set of processes resulting from the actuation of the instructions encoded in nucleic acids. In the nucleic acid of living cells these are actuated all the time; in contrast, in a virus they are actuated only when the viral nucleic acid, upon entering a host cell, causes the synthesis of virus-specific proteins. Viruses are thus "alive when they replicate in cells, while outside cells viral particles are metabolically inert and are no more alive than fragments of DNA."

- Dulbecco R and Ginsberg HS, 1980. Virology, p.854-855 (originally published as a section in Microbiology, 3rd Edn., Davis et al., Harper and Row, Hagerstown).

Retroid Elements and Retroviruses

Retroviridae [ssRNA(+) viruses replicating via a longer-than-genome-length dsDNA intermediate], ***Hepadnaviridae***, **caulimoviruses and badnaviruses** [family **Caulimoviridae**, gapped circular dsDNA viruses replicating via longer-than-genome-length RNA intermediates] all share the unlikely attribute of **the use of an enzyme complex consisting of a RNA-dependent DNA polymerase/RNAse H in order to replicate**. They share this attribute with several **retrotransposons**, which are eukaryotic transposable cellular elements with striking similarities with retroviruses [such as the yeast Ty element, the mammalian LINE-1 elements, and the Drosophila copia element]; and with **retroposons**, which are eukaryotic elements which transpose via RNA intermediates, but share **no obvious genomic similarity with any viruses other than the reverse transcriptase**.

Bacteria such as *E coli* also have reverse- transcribing transposons -known as *retrons* - but these are very different to any of the eukaryotic types while **preserving similarities in certain of the essential reverse transcriptase sequence motifs**.

All of these elements are collectively known as **RETROELEMENTS**; the fact that the reverse transcriptases of all of them have some amino acid identity suggests a common evolutionary origin.

Several reviewers have pointed out that **just such an enzyme as reverse transcriptase** would have been necessary for the **transition from what is widely believed to have been an RNA world** - that is, where all the extant organsisms had RNA genomes - to the present world in which **all cellular organisms have DNA genomes**.

Viruses with RNA genomes which use RNA-dependent RNA polymerases for their replication may be the **only remnants of that pre-DNA era;** however, **cellular elements and viruses which use reverse transcriptase** may share a **common origin as cell-derived "modules"** coding for a reverse transcriptase, which **evolved to become retrons and retroposons and retrotransposons**. Addition of structural proteins may have allowed evolution of **retroviruses**.

The evolution of the DNA retroviruses - *Hepadnaviridae*, caulimo- and badnaviruses - is more obscure; it appears as though these **arose from retrotransposon-like sequences**, but this probably occurred near the origin of of these types of element as they are **so diverse in sequence and genome organisation**.

It is believed that retrotransposons may contribute substantially to the evolution of their hosts. Evidence for this has been obtained by studying human LINE-1s (Long Interspersed Nuclear Elements) - a group of retrotransposable elements which make up approximately 15 % of the human genome. The vast majority of LINE-1s are no longer retrotransposition competent and it is believed that in humans only between 30 and 60 full length LINE-1s are currently active. There is strong evidence from sequences in the sequence databases to suggest that active LINE-1s play an important role in "exon shuffling" (belived to be the major mechanism of macro-evolution whereby entirely new genes are created by reshuffling the components of older genes). The most compelling evidence that LINE-1s do facilitate exon shuffling, however, is the experimental demonstration that they are not only able to move large amounts of non-LINE-1 exonic DNA but also insert this DNA into unrelated expressed genes to obtain chimeras which encode active hybrid gene products.

References:

Chapter 7: "Evolution by transposition" (pp. 172-203) in "Fundamental of Molecular Evolution" by Wen-Hsiung Li and Dan Graur; Sinauer Associates, Inc., Sunderland, Mass., 1991

Moran, J. V., R. J. DeBerardinis, and H. H. Kazazian. 1999. Exon shuffling by L1 Retrotransposition. Science 283:1530-1534.

Return to Origins of Viruses

Helical Nucleocapsids

This is one of the **SIMPLEST FORMS** of viral capsid: the **protein** is **"wound on"** to the **viral nucleic acid** (generally **ssRNA**, though **M13** and other filamentous phage virions contain **circular ssDNA**) in a simple **HELIX**, like a screw (see the diagram for tobacco mosaic virus, below).

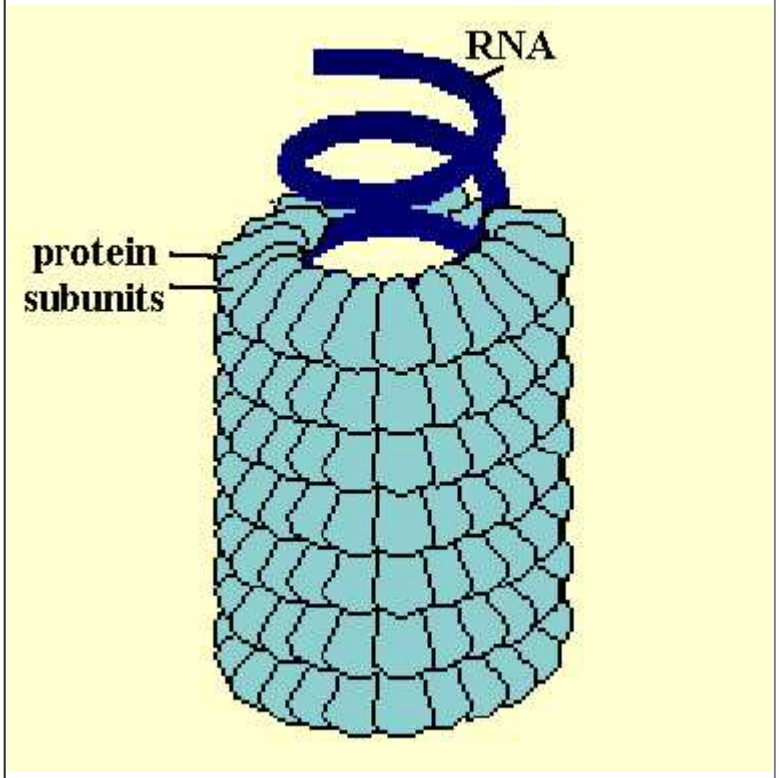


Diagram of TMV structure: protein subunits are all in equivalent crystallographic positions, related by a right-hand "screw translation"

courtesy L Stannard

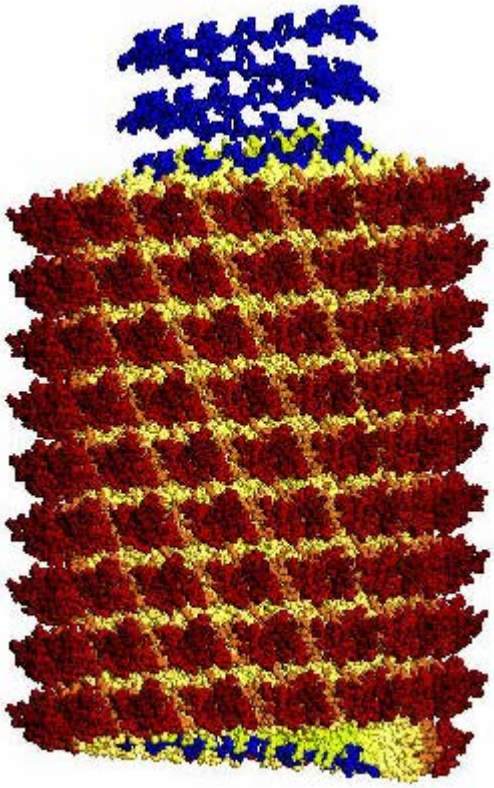
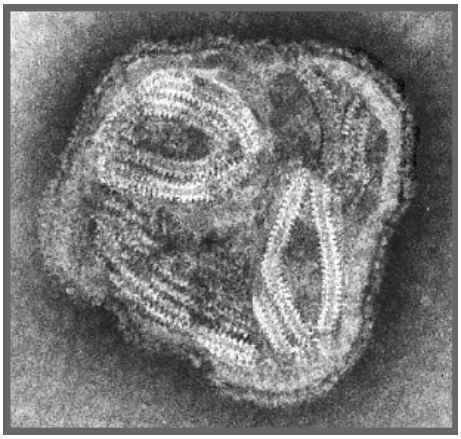


Image reconstruction of TMV

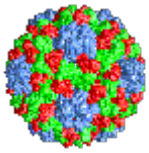
8th Report ICTV

In the case of **TMV** this is the **entire virion**: this is also the case for all **RODLIKE and FILAMENTOUS** virions where no membranes are involved. This includes all ***Tobamoviridae*, *Potyviridae*, and *Closteroviridae***, but NOT ***Filoviridae***, like Ebola virus (see **here**).

In other cases, **filamentous helical nucleocapsids** may be **enclosed within matrix protein** and a **membrane studded with spike proteins**: excellent examples of this are **PARAMYXOVIRIDAE**, detailed images



of which can be found **here**, at Linda Stannard's site, and **for ORTHOMYXOVIRIDAE** **here** in this tutorial.



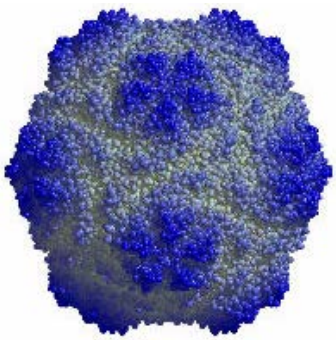
"What is a Virus?" **Basic Virion Constituents** **Isometric Nucleocapsids**
Tutorial Contents

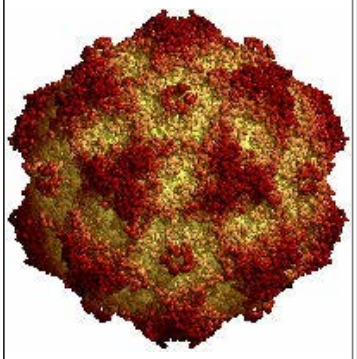
Isometric Nucleocapsids

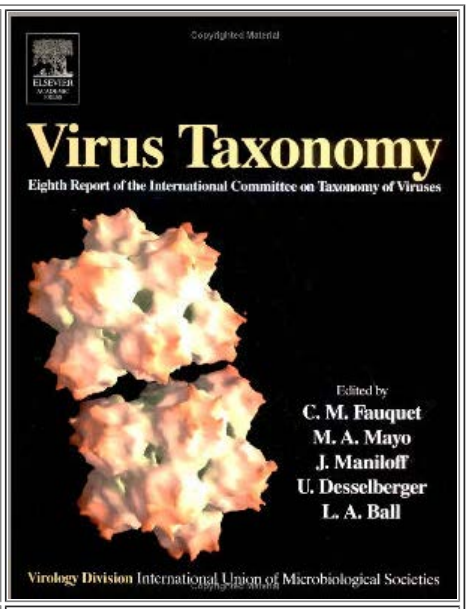
These are built up according to **simple structural principles**, as **amply outlined here**, and in **more detail here**. Put simply, nearly **all isometric virions** are constructed around a **BASIC ICOSAHEDRON**, or solid with 20 equilateral trinagles for faces. It suffices to say that the **"quasi-icosahedral"** capsid is possibly **Nature's most popular means of enclosing viral nucleic acids**; they come in many sizes, from **tiny T=1 structures** (Nanoviruses, eg: banana bunchy top virus; 18 nm diameter) to **huge structures** such as those of **Iridoviridae** or **Phycodnaviridae** (over 200 nm diameter).

The simplest virions are those of the viruses with the smallest genomes: these are virions such as those of the **ssRNA satellite tobacco necrosis virus** (sTMV), the ssDNA **canine parvovirus** (CPV) and **porcine circovirus** (PCV), and **microviruses** infecting E coli and other bacteria (eg: φX174 phage): these all have a **simple icosahedral T=1 surface lattice structure**. Some examples are shown below. **All structural subunits of these capsids are in the same positional state, or have the same interactions with their neighbours.**

A unique derivative structure is that of **geminivirus** virions, which have **two incomplete T=1 icosahedra** joined at the missing vertex.



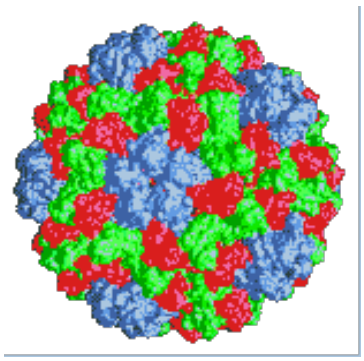
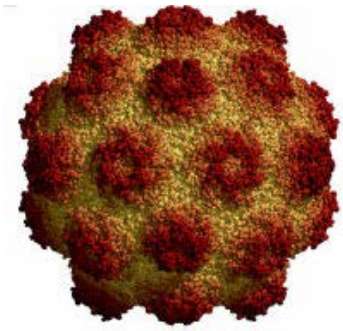
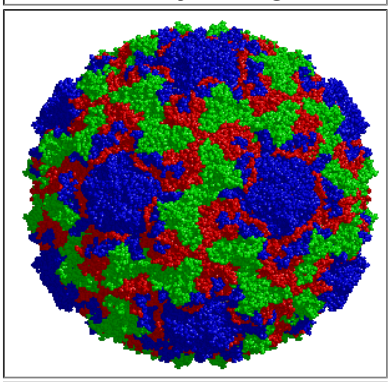
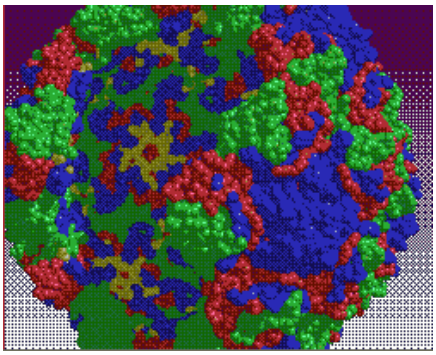




Satellite tobacco necrosis virus	Canine parvovirus	Geminivirus: Cover of the ICTV 8th Report
Images from the ICTV 8th report; derived from the Virus World site, for non-profit educational use		

An **example of a more complex structures** are illustrated below. The animated GIF to the left shows **Cowpea chlorotic mottle (CCMV) virion surface structure (courtesy J-Y Sgro)**, which is composed of **180 copies of a single coat protein molecule, in a T=3 surface lattice**. The different colours in the CCMV picture represent **different "positional states" of the capsid protein**: subunits around **5-fold** rotational axes of symmetry are **BLUE**, and cluster as **PENTAMERS**; subunits around **3-fold axes** are **RED** and **GREEN** to reflect their different **2-fold symmetries**; they cluster as **HEXAMERS** around **"local 6-fold axes".**

Another example is that of **turnip yellow mosaic virus (TYMV):** this has **exactly the same basic structure**, with a **180 copies of a single type of coat protein subunit**, with the **pentamer-hexamer clustering** appearing more pronounced

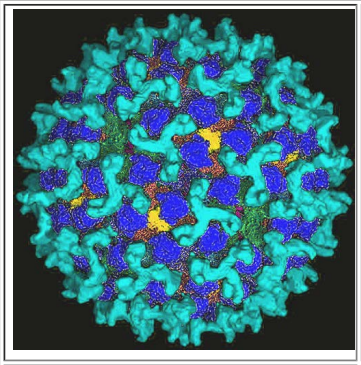
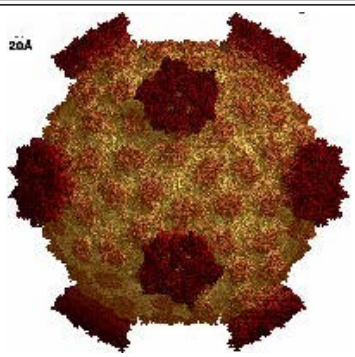
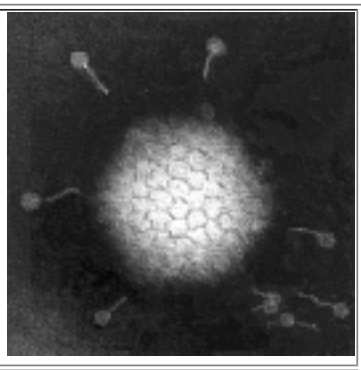
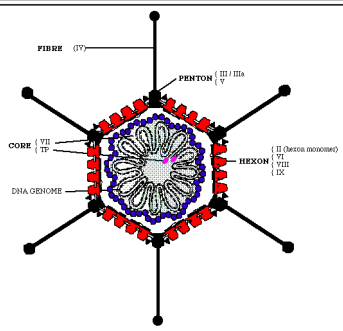
	
CCMV capsid	TYMV capsid
courtesy J-Y Sgro	ICTV 8th report
	
Rhinovirus R16	Rhinovirus R16
courtesy J-Y Sgro	

A **more complex capsid** - that of the common-cold-causing ***Rhinovirus*** R16 (family: ***Picornaviridae***), with **60 copies of 4 proteins in a T=3 structure** - is shown on the **bottom left and right** (animation modified from one by **J-Y Sgro**) and **right**. The **right** image shows a capsid with a cutaway, to reveal internal structure. **BLUE** subunits around 5-fold axes are **VP1**; **RED** and **GREEN** are **VP3** and **VP2** respectively; **YELLOW** subunits (seen only internally) are **VP4**. The VP4 subunits are formed by autocatalytic cleavage of **VP0** (into **VP2** and **VP4**) upon binding of a "**procapsid**" with **viral genomic ssRNA.**

Note the similarity between the CCMV and R16 structures - despite one having a single CP, and the other having 3 structural CP subunits.

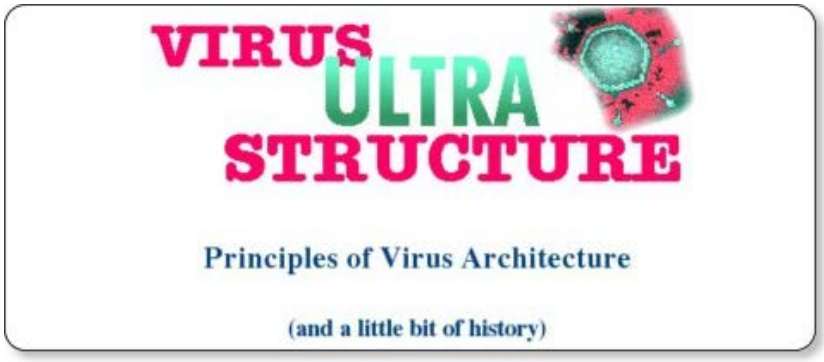
See here for further details of picornaviruses, here for a scheme showing picornavirus assembly, here for a scheme outlining polyprotein processing of picornaviruses, and here for material on picornaviruses from the University of Leicester course.

More complex capsids are generally found for viruses with **larger genomes**, whether composed of RNA or DNA. These include virions such as those of **reoviruses** and **adenoviruses**, both of which have complex or multilayered naked isometric capsids.

			
Bluetongue orbivirus (*Reoviridae*)	**Mammalian reovirus core with spikes**	**Electron micrograph of an adenovirus**	**Link to diagram of an adenovirus structure**
courtesy AJ Cann	**ICTV 8th Report**	**courtesy L Stannard**	**courtesy AJ Cann**

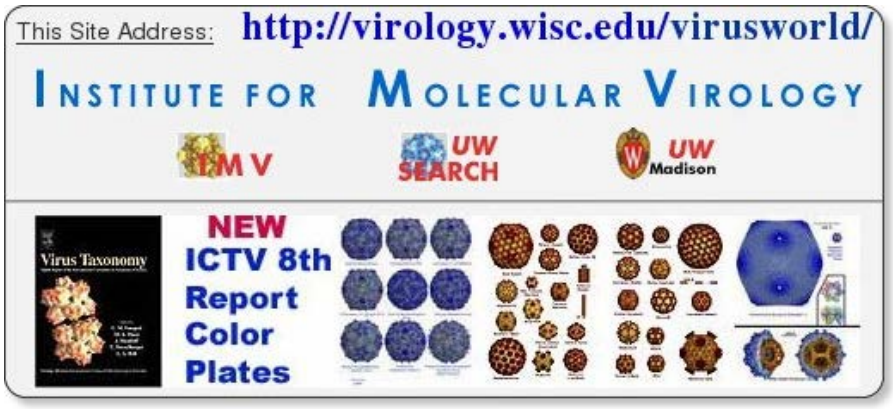
You may like to look here for structures from the University of Calgary material.

More complex structures may be seen in electron micrographs, and diagrams explaining icosahedral and quasi-icosahedral structure at Linda Stannard's Web site.

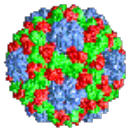


Principles of Virus Architecture via kwout

The "Virus World" site has an excellent set of high-resolution image reconstructions from physical data of non-enveloped simpler isometric viruses.



Virus World via kwout



Tutorial Contents

"What is a Virus?" **Enveloped Virions** **Helical Nucleocapsids** **Compound structures**

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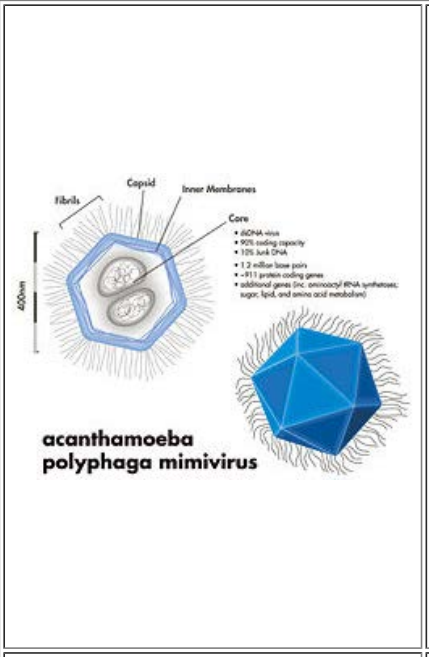
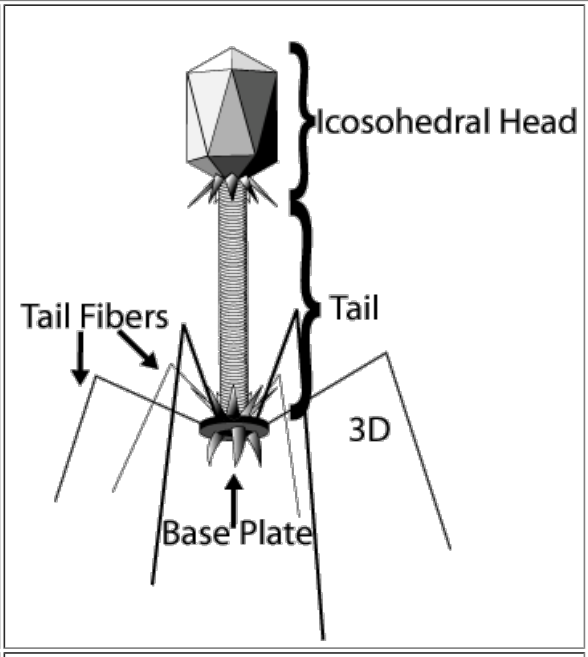
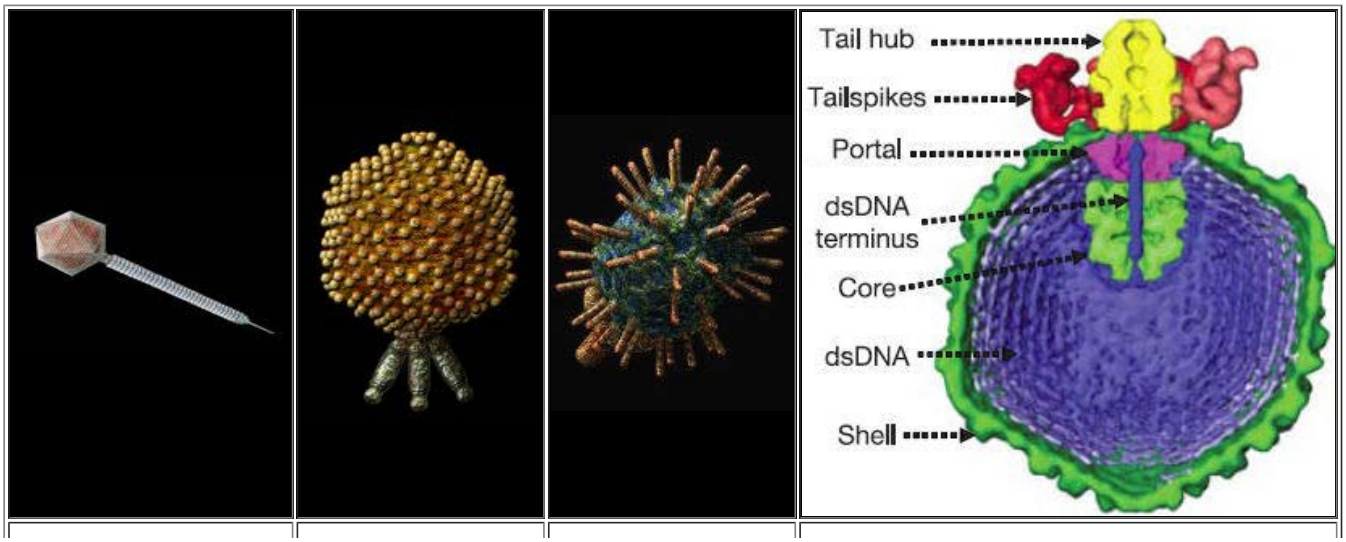
Introduction to Molecular Virology

Complex or Compound Virions

		
Mimivirus. The huge regular icosahedral capsid contains lipid membranes and other structures	**T-even phage particle: note elongated isometric head (icosahedron with hexamer expansion net), helical tail, and base plate and neck structures**	**Depiction of a orthopoxvirus virion. Note presence of two lipid bilayers and amorphous structure**
Link to Wikipedia	**Link to Wikipedia**	**copyright Russell Kightley**

Most **bacteriophages** - or more properly, **archaeal and bacterial** viruses - have a more complex structure than the simple isometric and helical nucleocapsids typical of many plant and animal viruses. Even viruses with relatively small genomes may have more than one type of architecture associated with their virions, normally in the shape of some kind of **TAIL** structure attached to an isometric **HEAD**. The **T-even** viruses - part of the **"T4-like virus" genus, family *Myoviridae*** - have the general structure shown centre, above: this is an **isometric head** attached via a **connector assembly** to a **contractile helically-constructed tail** with a rigid **core**, with **tail fibres** and **baseplate. See here for explanation of how the virus gains entry to enterobacterial cells.**

λ-like viruses - family ***Siphoviridae*** **- have thinner non-contractile tails** and **isometric heads,** with **tail fibres.** The **P22-like and Φ29-like viruses** in family ***Podoviridae*** and ***Salmonella*** **phage Epsilon 15** have **isometric** heads and **short, thick tails**.



Enterobacteria phage λ	Enterobacteria phage P22	Bacillus phage Φ29	Salmonella phage Epsilon 15
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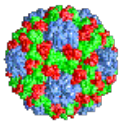

Tutorial Contents

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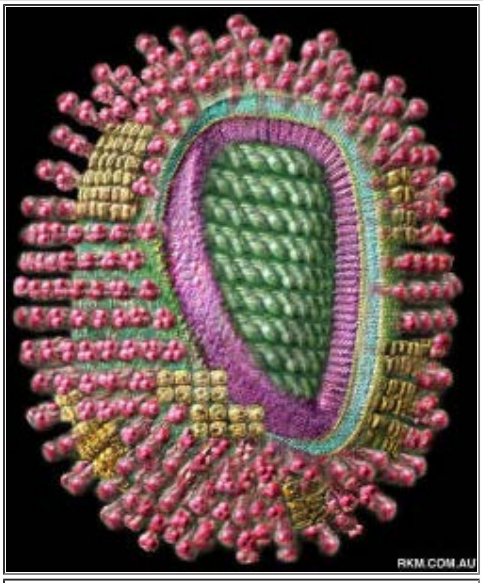
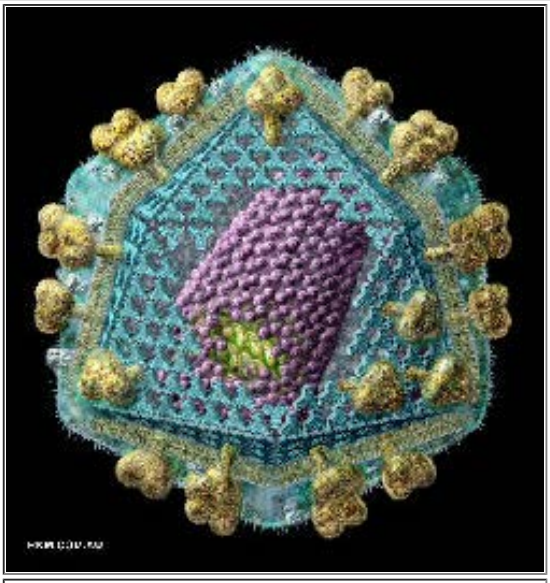
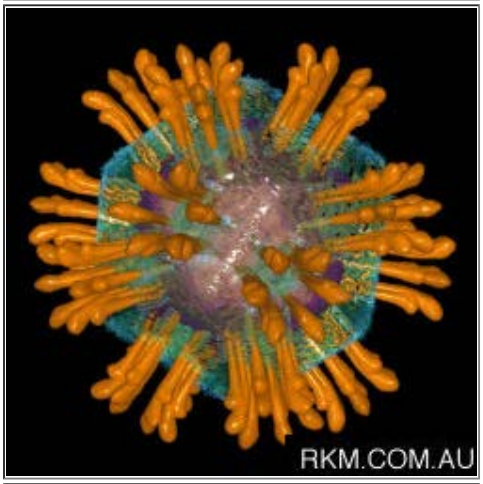
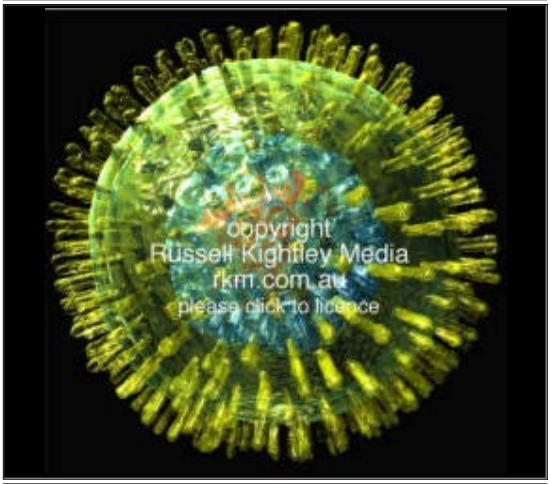
Introduction to Molecular Virology

Enveloped Virions

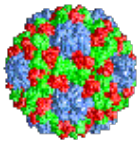
SOME virions additionally have:

- **ENVELOPES**, or **lipid bilayer MEMBRANES**: these always have **viral proteins** inserted in them (almost always **GLYCOPROTEINS**); these are often referred to as **SPIKES**.
- There is usually an **inner layer** of **protein** (usually referred to as **MATRIX** protein) inside the envelope, usually in contact with the **(nucleo)CAPSID** protein. See below for examples (West Nile virus, *Flaviridae*; influenza virus, *Orthomyxoviridae;* HIV, *Retroviridae;* SARS coronavirus; Hepatitis C flavivirus. The nucleoprotein can be helical (Ortho- and Paramyxoviridae) or isometric

		
Cutaway depiction of West Nile flavivirus: note regular arrangement of envelope protein, underlying membrane and isometric capsid	**Cutaway depiction of influenza virion: note helical nucleoprotein, matrix and envelope layers and spikes**	**Cutaway depiction of HIV virion: note conical nucleoprotein, isometric matrix and envelope layers and spikes**
		
SARSCoV (SARS coronavirus).	**Hepatitis C flavivirus (HCV):**	

Note elongated membrane spikes and underlying isometric capsid	**note elongated, regularly-spaced spikes and isometric structure**	**Herpesvirus: note many envelope glycoproteins, "tegument" inside membrane, and isometric capsid**
copyright Russell Kightley (www.rkm.com.au)		

Enveloped virions may additionally incorporate **host membrane proteins** in their envelopes: while this may be a **side effect of the "budding" process** by which viral nucleoprotein complexes acquire envelopes, it may also in some cases be a **mechanism for avoidance of host immune systems** (eg: **hepatitis B virus** incorporates **host serum albumin** into its capsid; **HIV virions** may incorporate **host MHC** proteins).



Tutorial Contents

"What is a Virus?" | **Isometric Virions** | **Helical Nucleocapsids** | **Compound structures**

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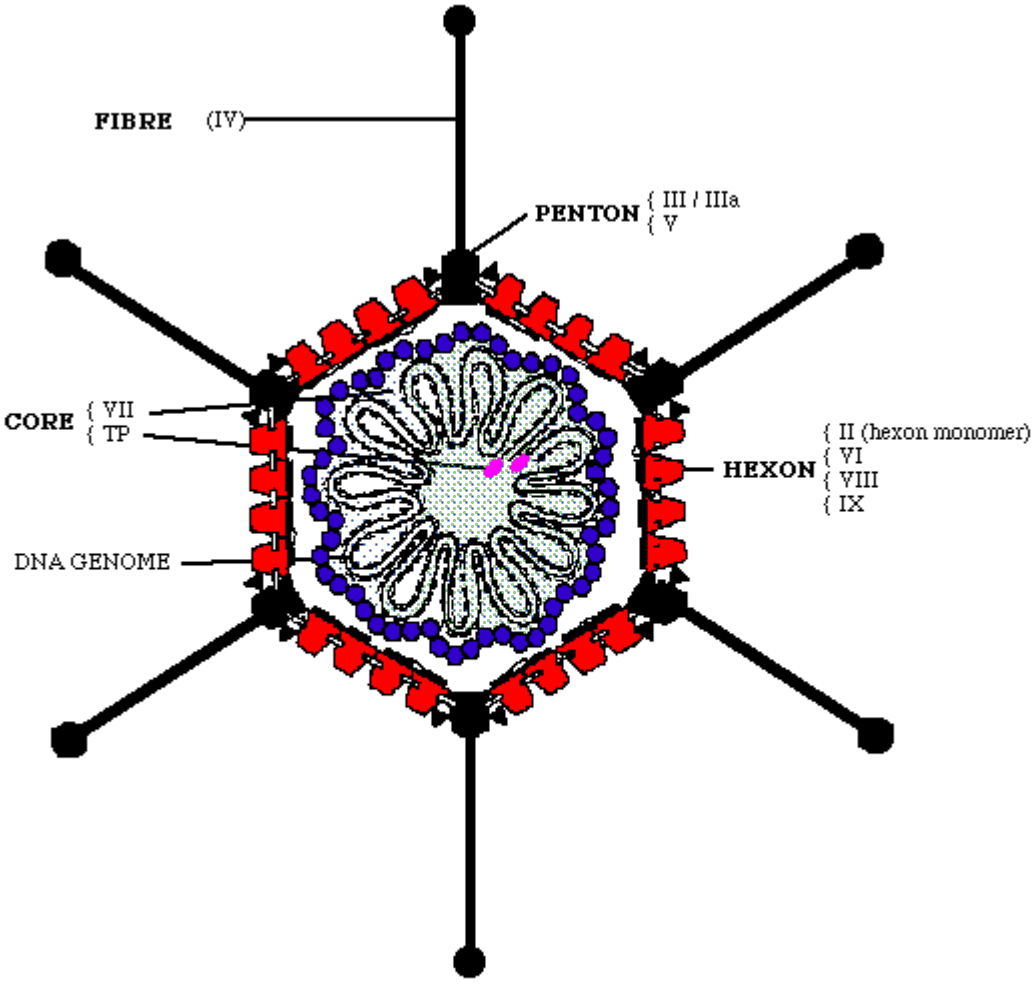

RESEARCH SCHOOL OF BIOLOGICAL SCIENCES

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First... to learn the nature of things

NATURAM PRIMUM COGNOSCERE RERUM

Description generated automatically from DELTA-format data including sections links in Table of Contents. Description may not cover all sections listed and therefore some links may not be functional at present.

59. Prions

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Taxonomy

Virus Code. 59. *Virus Accession number* 59000000.
Synonym(s): subviral agents of spongiform encephalitis.

Comments: Prions are small, proteinaceous infectious particles that resist inactivation by procedures which affect nucleic acids. To date, no detectable nucleic acids of any kind and no virus-like particles have been associated with prions. Prions cause scrapie and other spongiform encephalopathies of animals and humans.

Virus infects vertebrates.

Description is on taxonomic level of family.

Properties of Virion

Morphology

Microsomal membranes visible in infected brain cells. Virions not enveloped. Microsomal fractions from infected brain tissues enriched for prion infectivity contain numerous membrane vesicles; detergent extraction and limited proteolysis of brain microsomes generate rod-shaped particles. The rods are smooth, almost ribbon-like, and infrequently are twisted. The rods resemble purified amyloid, both ultrastructurally and histochemically. The rods are not considered the infectious entity since large PrP 27-30 polymers are not required for infectivity. Nucleocapsids rod-shaped (smooth, almost ribbon-like); (25-)165(-550) nm long; 11 nm in diameter.

Taxonomic Structure

Taxonomic structure of the family.

References

The following references are cited in the *Sixth ICTV Report*: rf_prion.htm.

Data sources and contributors.

The above description has been compiled from the data presented in the *Sixth ICTV Report* by Prusiner SB, Baldwin M, Collinge J, DeArmond SJ, Marsh R, Tateishi J, Weissmann C.

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Author of this webpage: C. Büchen-Osmond
Developer of the software to create this webpage: M. J. Dallwitz
ICTV and DELTA related References

Note: The ICTV database is under development and is subject to changes and corrections. If you have questions about this project please contact us. Your comments and help will be appreciated.

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Created: April 1995 *Last updated:* 8 April 1998.

Retroid Elements and Retroviruses

Retroviridae [ssRNA(+) viruses replicating via a longer-than-genome-length dsDNA intermediate], ***Hepadnaviridae***, **caulimoviruses and badnaviruses** [family **Caulimoviridae**, gapped circular dsDNA viruses replicating via longer-than-genome-length RNA intermediates] all share the unlikely attribute of **the use of an enzyme complex consisting of a RNA-dependent DNA polymerase/RNAse H in order to replicate**. They share this attribute with several **retrotransposons**, which are eukaryotic transposable cellular elements with striking similarities with retroviruses [such as the yeast Ty element, the mammalian LINE-1 elements, and the Drosophila copia element]; and with **retroposons**, which are eukaryotic elements which transpose via RNA intermediates, but share **no obvious genomic similarity with any viruses other than the reverse transcriptase**.

Bacteria such as *E coli* also have reverse- transcribing transposons -known as *retrons* - but these are very different to any of the eukaryotic types while **preserving similarities in certain of the essential reverse transcriptase sequence motifs**.

All of these elements are collectively known as **RETROELEMENTS**; the fact that the reverse transcriptases of all of them have some amino acid identity suggests a common evolutionary origin.

Several reviewers have pointed out that **just such an enzyme as reverse transcriptase** would have been necessary for the **transition from what is widely believed to have been an RNA world** - that is, where all the extant organsisms had RNA genomes - to the present world in which **all cellular organisms have DNA genomes**.

Viruses with RNA genomes which use RNA-dependent RNA polymerases for their replication may be the **only remnants of that pre-DNA era;** however, **cellular elements and viruses which use reverse transcriptase** may share a **common origin as cell-derived "modules"** coding for a reverse transcriptase, which **evolved to become retrons and retroposons and retrotransposons**. Addition of structural proteins may have allowed evolution of **retroviruses**.

The evolution of the DNA retroviruses - *Hepadnaviridae*, caulimo- and badnaviruses - is more obscure; it appears as though these **arose from retrotransposon-like sequences**, but this probably occurred near the origin of of these types of element as they are **so diverse in sequence and genome organisation**.

It is believed that retrotransposons may contribute substantially to the evolution of their hosts. Evidence for this has been obtained by studying human LINE-1s (Long Interspersed Nuclear Elements) - a group of retrotransposable elements which make up approximately 15 % of the human genome. The vast majority of LINE-1s are no longer retrotransposition competent and it is believed that in humans only between 30 and 60 full length LINE-1s are currently active. There is strong evidence from sequences in the sequence databases to suggest that active LINE-1s play an important role in "exon shuffling" (belived to be the major mechanism of macro-evolution whereby entirely new genes are created by reshuffling the components of older genes). The most compelling evidence that LINE-1s do facilitate exon shuffling, however, is the experimental demonstration that they are not only able to move large amounts of non-LINE-1 exonic DNA but also insert this DNA into unrelated expressed genes to obtain chimeras which encode active hybrid gene products.

References:

Chapter 7: "Evolution by transposition" (pp. 172-203) in "Fundamental of Molecular Evolution" by Wen-Hsiung Li and Dan Graur; Sinauer Associates, Inc., Sunderland, Mass., 1991

Moran, J. V., R. J. DeBerardinis, and H. H. Kazazian. 1999. Exon shuffling by L1 Retrotransposition. Science 283:1530-1534.

Return to Origins of Viruses

Last Updated: Saturday, October 4, 1945624.

Prion Diseases

This document describes infectious agents which (almost certainly) do not have a nucleic acid genome. It seems that a protein alone is the infectious agent. The infectious agent has been called a **prion**. A prion has been defined as **"small proteinaceous infectious particles which resist inactivation by procedures that modify nucleic acids"**. The discovery that proteins alone can transmit an infectious disease has come as a considerable surprise to the scientific community.
Prion diseases are often called **spongiform encephalopathies** because of the post mortem appearance of the brain with large vacuoles in the cortex and cerebellum. Probably most mammalian species develop these diseases. Specific examples include:

- **Scrapie**: sheep
- **TME** (transmissible mink encephalopathy): mink
- **CWD** (chronic wasting disease): muledeer, elk
- **BSE** (bovine spongiform encephalopathy): cows

Humans are also susceptible to several prion diseases:

- **CJD**: Creutzfeld-Jacob Disease
- **GSS**: Gerstmann-Straussler-Scheinker syndrome
- **FFI**: Fatal familial Insomnia
- **Kuru**
- **Alpers Syndrome**

These original classifications were based on a clinical evaluation of a patients family history symptoms and are still widely used, however more recent and accurate molecular diagnosis of the disease is gradually taking the place of this classification.

The **incidence** of sporadic CJD is about 1 per million per year.
GSS occurs at about 2% of the rate of CJD.

It is estimated that 1 in 10,000 people are infected with CJD at the time of death.
These figures are likely to be underestimates since prion diseases may be misdiagnosed as other neurological disorders.

The diseases are characterised by **loss of motor control, dementia, paralysis wasting and eventually death, typically following pneumonia**. Fatal Familial Insomnia presents with an untreatable insomnia and dysautonomia. Details of pathogenesis are largely unknown.

Visible end results at post-mortem are **non-inflammatory lesions, vacuoles, amyloid protein deposits and astrogliosis**.

GSS is distinct from CJD, it occurs typically in the 4th-5th decade, characterised by cerebellar ataxia and concomitant motor problems, dementia less common and disease course lasts several years to death. (Originally thought to be familial, but now known to occur sporadically as well).
CJD typically occurs a decade later has cerebral involvement so dementia is more common and patient seldom survives a year (originally thought to be sporadic, but now known to be familial as well).
FFI pathology is characterised by severe selective atrophy of the thalamus.
Alpers syndrome is the name given to prion diseases in infants.

Scrapie was the first example of this type of disease to be noticed and has been known about for many hundreds of

years. There are two possible methods of transmission in sheep:

1. Infection of pasture with placental tissue carrying the agent followed by ingestion,or direct sheep-lamb transmission i.e. an acquired infection.

2. Parry showed considerable foresight by suggesting that it is not normally an infectious disease at all but a genetic disorder. He further suggested that selective breeding would get rid of the disease.

Humans might be infected by prions in 2 ways:

1. Acquired infection (diet and following medical procedures such as surgery, growth hormone injections, corneal transplants) i.e. infectious agent implicated.

2. Apparent hereditary mendelian transmission where it is an autosomal and dominant trait. This is not prima facie consistent with an infectious agent.

This is one of the features that single out prion diseases for particular attention. They are **both** infectious **and** hereditary diseases (?see below). They are also sporadic, in the sense that there are also cases in which there is no known risk factor although it seems likely that infection was acquired in one of the two ways listed above.

Kuru is the condition which first brought prion diseases to prominence in the 1950s. Found in geographically isolated tribes in the Fore highlands of New Guinea. Established that ingesting brain tissue of dead relatives for religious reasons was likely to be the route of transmission. They ground up the brain into a pale grey soup, heated it and ate it. Clinically, the disease resembles CJD. Other tribes in the vicinity with same religious habit did not develop the disease. It is speculated that at some point in the past a tribe member developed CJD, and as brain tissue is highly infectious this allowed the disease to spread. Afflicted tribes were encouraged not to ingest brain tissue and the incidence of disease rapidly declined and is now almost unknown.

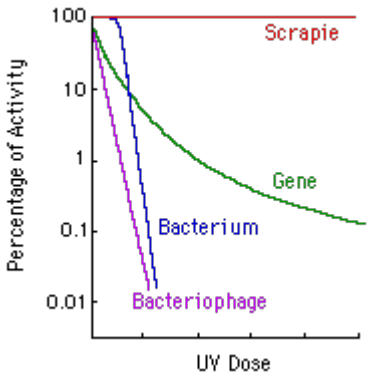
Evidence suggests that a prion is a modified form of a normal cellular protein known as **PrPc** (for cellular), a normal host protein encoded by a single exon of a single copy gene. This protein is found predominantly on the surface of neurones attached by a glycoinositol phospholipid anchor, and is **protease sensitive**. Thought to be involved in synaptic function.
The modified form of PrPc which may cause disease i.e. the prion is known as **PrPsc** (for scrapie) which is **relatively resistant to proteases** and accumulates in cytoplasmic vesicles of diseased individuals.

It has been proposed that PrPsc when introduced into a normal cell causes the conversion of PrPc into PrPsc. Process is unknown but it could involve a chemical or conformational modification.

Several lines of evidence support protein only model of infection:

1. Nucleic acid is not necessary for infectivity:

- unusually small target size for ultraviolet and ionising radiation:



- the low ratio of nucleic acid to infectious material.

- resistance of infectivity to agents which modify or damage nucleic acids but infectivity is susceptible to reagents which destroy proteins:

Stabilities of the scrapie agent and viriods (PSTV):

Chemical Treatment:	Concentration	PSTV	Scrapie
Et2PC	10-20mM	(-)	+
NH2OH	0.1-0.5mM	+	-
Psoralen	10-500µg/ml	+	-
Phenol	Saturated	-	+
SDS	1-10%	-	+
Zn2+	2mM	+	-
Urea	3-8M	-	+
Alkali	pH 10	(-)	+
KSCN	1M	-	+
Enzymatic Treatment:	Concentration	PSTV	Scrapie
RNAse A	0.1-100µg/ml	+	-
DNAse	100µg/ml	-	-
Proteinase K	100µg/ml	-	+
Trypsin	100µg/ml	-	+

+ = inactivated; - = no change in infectivity

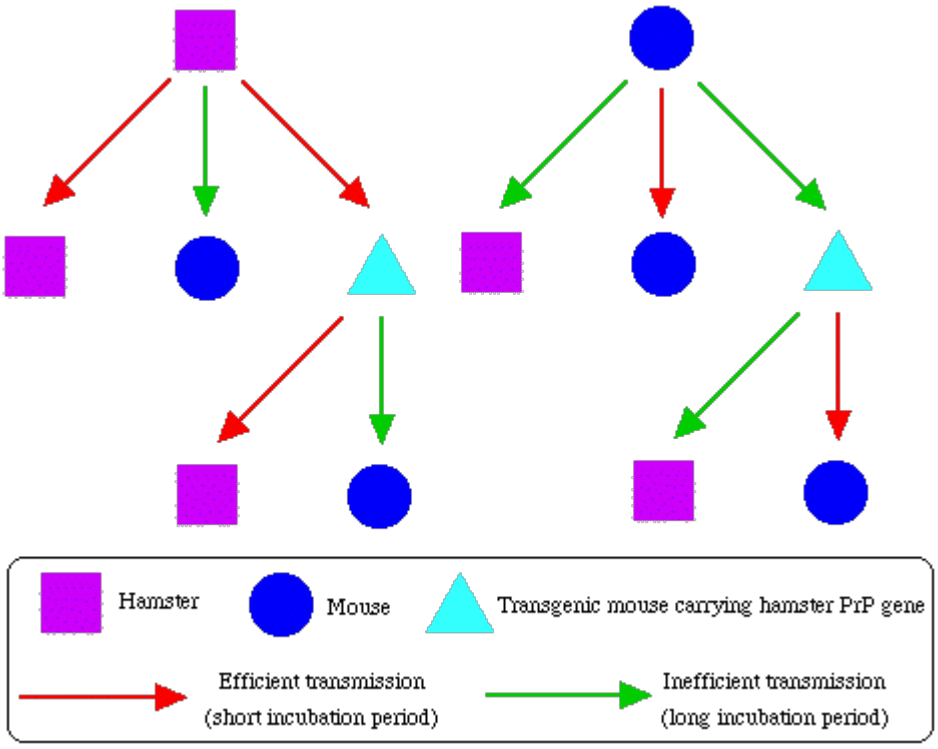
- failure to identify a scrapie specific nucleic acid either in prion preparations or infected brains using a variety of sophisticated techniques.

2. PrPsc is associated with scrapie infectivity:

- purification of scrapie infectivity results in preparations highly enriched for PrPsc
- purification of PrPsc results in enrichment of scrapie activity
- purification of PrPsc by SDS-PAGE also recovers infectivity
- PrPsc can be denatured and renatured without loss of infectivity

3. Susceptibility of a host to prion infection is co-determined by the prion inoculum and the PrP gene:

- disease incubation time for a single prion isolate various between mouse strains, this variation depends on the Sinc gene, this is very closely linked to or coincident with the PrP gene itself, suggesting some forms of PrPc may be more easily converted to PrPsc than others.
- when prions are transmitted from one species to another disease develops only after a very long incubation period, if at all, but on serial passage in the new species the incubation time often decreases dramatically and then stabilises. This species barrier can be overcome by introducing a PrP transgene from the prion donor i.e. hamster PrPc but not murine PrPc is a suitable substrate for conversion to hamster PrPsc by hamster prions and vice versa:



4. Mutated gene can cause susceptibility to disease without apparent infection:

- Homozygosity at the polymorphic amino acid position 129 of PrP protein predisposes an individual to acquired and sporadic CJD
- 2 unrelated GSS families have the same double mutation i.e. 178 D-N; 200 E-K
- disease tightly linked to a P-L mutation at 102 in some familial GSS cases. 100 controls did not have this mutation nor did another 15 sporadic victims of CJD
- other familial cases have been shown to carry this or several other mutations e.g. 117-A-V; 198 F-S; N178; V129 = CJD; N178 M129 = FFI

One possibility, **the genetic explanation**, was that these mutations enhance the rate of spontaneous conversion of PrPc to PrPsc which permits disease manifestation within the lifetime of an individual. Suggests that some sporadic incidents can be accounted for by somatic mutation of the PrP gene. **An alternative explanation is that these mutations confer susceptibility to infection**

5. Crucial experiment:

Mice carrying a murine transgene with the 102P-L GSS mutation spontaneously develop a lethal scrapie like disease. Brains also contain infectious prions because transmission to recipient animals has been demonstrated. Mice lacking the PrPc gene develop normally, no evident physiological or behavioural problems. Suggests that loss of PrPc function is unlikely to be the cause of disease rather accumulation of PrPsc is responsible. When inoculated with prions they do not develop disease . There are obvious implications here for the livestock and pharmaceutical industries and treatment of familial cases. Animals expressing reduced levels of PrPc are also resistant to infection - perhaps more immediately relevant to human disease. Animals overexpressing PrPc are more likely to develop prion diseases.

Evidence against the prion model:

The existence of many different strains of scrapie (at least 15: latency, lesion patterns differ) which can be propagated in the same inbred mouse strain. These can transmit serially without changes in properties in the same mouse strain homozygous for a single PrP genotype. Cannot argue that there a distinct PrPc is converted to distinct PrPsc. Must mean that a common PrPc is corrupted in a different way, seems improbable but there is now experimental support for it.

Are prion diseases genetic disorders?

Is the genotype of an animal or human the direct cause of a prion disease or a susceptibility factor? It has long been known that some genotypes of sheep often develop scrapie, e.g. in the UK the genotypes:

- Ala136Ala, Arg154Arg, Gln171Gln
- Val136Val, Arg154Arg, Gln171Gln

are almost always identified in scrapie infected sheep. In contrast only one sheep with Ala136Ala,Arg154Arg,Arg171Arg has been identified with scrapie, these animals are resistant to both a scrapie and BSE challange. Surprisingly (if the genetic explanation is correct), these scrapie-susceptible genotypes are common in Australia and New Zealand which are thought to be free of scrapie. Antipodean sheep have also been bought back to the UK and maintained in quarantine conditions and not developed scrapie. In other words, the genotype does not confer scrapie on the animal but susceptibility to scrapie infection. **Scrapie would appear to be an infectious disease, not a genetic one.** This observation may have implications for families carrying a mutant prion gene.

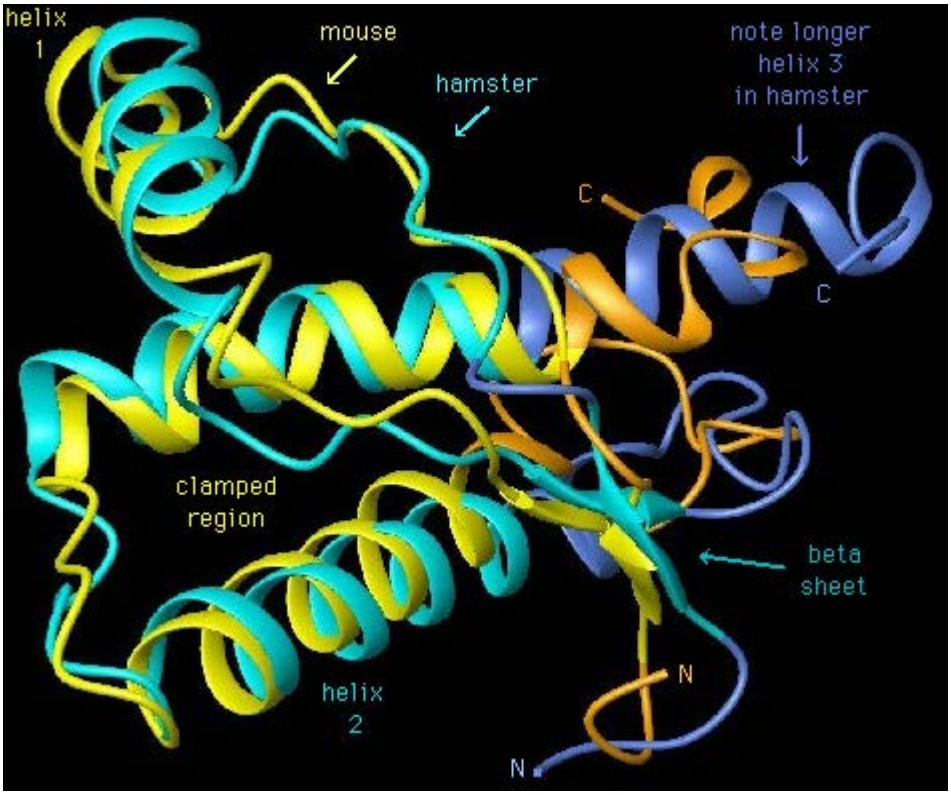
So how can a protein be infectious?

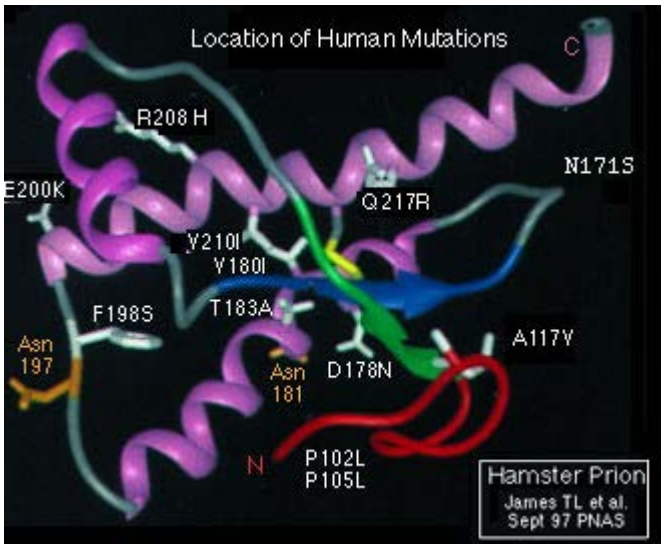
PrPsc is a protease resistant form of PrPc, both are extensively post translationally modified. No chemical differences between the two forms of the protein have been detected. Clearly there must be some difference. One great problem is that infectivity ratio is about 100,000:1, so infectious agent even if derived from PrPc may not be PrPsc and it could be chemically modified.

However, a more likely possibility is that the difference between PrPc and PrPsc is conformational. 3D structure of part of the murine PrPc expressed in E. coli has recently been determined. As expected from spectroscopy measurements PrPc is predominantly alpha helical and contains almost no beta sheet. The structure of PrPsc has not yet been determined but is predicted to be predominantly B- sheet. Proposed therefore that this protein can adopt 2 quite different stable conformations. The safe PrPc form is normally adopted but rarely it can switch to the PrPsc form. Mutations favour this switch. Propose that PrPsc is transdominant and converts PrPc to PrPsc in an exponential fashion. Precedents for this model do exist. There is a yeast mutant phenotype which doesn't correlate to any mutational difference in the gene structure but does correlate to a different protein structure. The Ure2p protein converts to an inactive conformation. Mutations in the tumour suppressor protein p53 which are associated with the onset of neoplastic disease have a different conformation to the normal protein. When normal protein is incubated with mutant protein its conformation is altered to the mutant form.

The first image below shows the structure of part of the hamster and mouse PrPc molecules superimposed. The close similarity in structures is obvious, as is the preponderance of alpha helical structure.The second shows the position of various mutations important for prion disease development *in humans* modelled on the hamster structure PrPc.Many of these mutations are positioned such that they could disrupt the secondary structure of the molecule.

Both images from Dr Thomas Pringle of the Sperling Foundation Official Mad Cow Disease Home Page

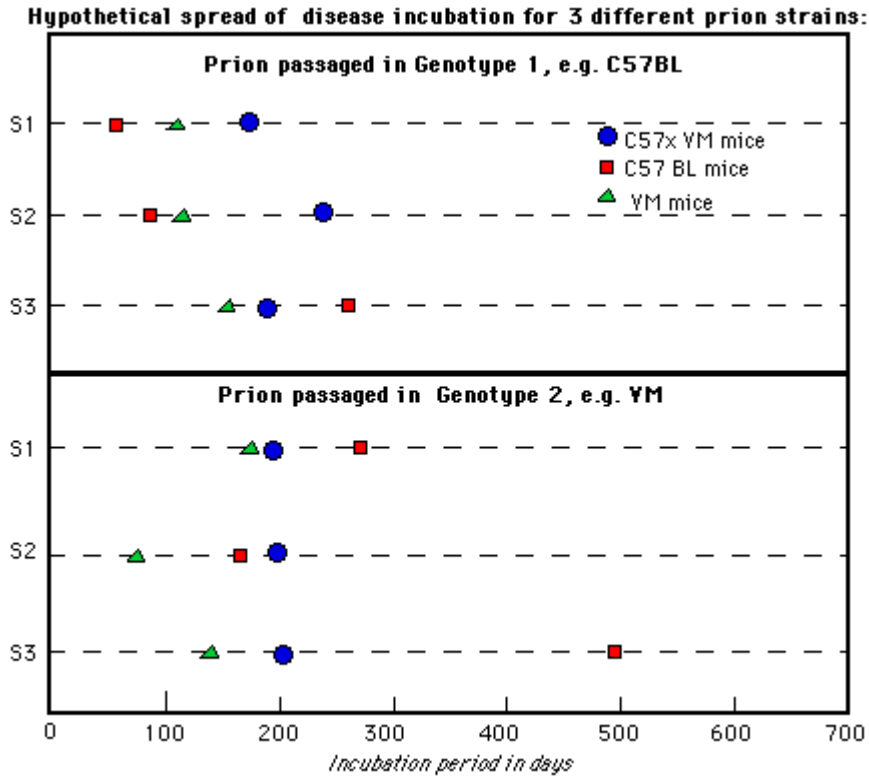




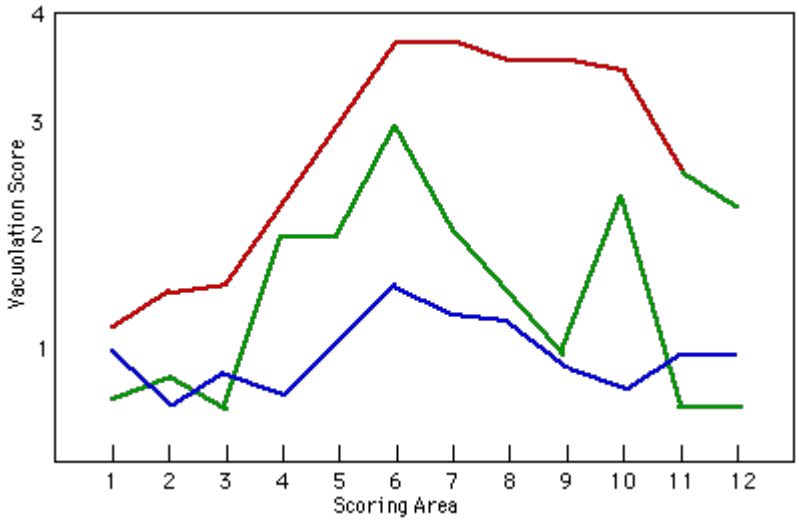
Strain Typing:

The existence of multiple strains of prion agents by definition means that the agents carry strain specific information. The virino hypothesis states that this is a small nucleic acid molecule. The prion hypothesis states that it is due to differences in the chemical or tertiary structure of the prion protein.
Strain discrimination relies on the use of mice of defined genotype and having different PrP gene sequences. Measurement of parameters such as incubation period in these mice and the pathological changes occurring in the brain are made.

Measurement of Incubation period: Precise quantities of brain homogenates from clinically affected animals are injected intracerebrally into mice. Animals are then assessed regulary for the definite appearance of disease using a defined set of observations. This measurement is very precise with errors of less than 2% of the mean:



Pathological changes: Strains show very different and reproducible differences in the pattern of vacuolar degeneration of different inbred mice. These effects are scored in a variety of different brain sections. Up to 12 topologically specific sites of the brain are scored for vacuolation e.g. cortex, cerebellum, brain stem etc. This semiquantitative assessment or lesion profile is then plotted. Individual strains have a characterisitc and highly reproducible lesion profile in a given mouse genotype e.g. Using this technique it is very clear that the recent outbreaks of new TSE diseases in various animals is caused by a prion with very similar characteristics ie BSE and quite distinct from previously recognized scrapie prion strains. Strain typing has also demonstrated beyond reasonable doubt in the last few months that nv-CJD or v-CJD are caused by a strain very much like the BSE agent. Some people now call this disease Human BSE:



BSE and NV-CJD:

This section will continue to be overtaken by events. (The time of writing is 20th August 1998.)
Many people have been concerned since the recognition of the BSE epidemic in 1987 that it may pose a risk to humans.
Strain typing studies show one major strain. BSE can transmit to many species experimentally e.g. sheep, pigs and macaque monkeys.
Strain typing studies have confirmed that domestic cats, big cat and exotic ungulates have been infected by eating

tainted beef.
About 2 years ago a new variant of CJD was recognized in the British population. At the time of writing 26 cases have been confirmed. People have developed this form of CJD at strikingly early ages 17-55. The neuropathology and early clinical symptoms are also very distinct from 'classical' CJD. The CJD Surveillance Unit at Edinburgh and the committee charged with giving the government recommendations to deal with this threat to human health, SEAC (Spongiform Encepahalopathy Advisory Committee) have concluded that the most likely cause is ingestion of infected beef.
Strain typing studies of NV-CJD as described above have confirmed that this is very probably the case..
Earlier experiments involving the production of a "protease fingerprint" of PrPsc showed that 10 or so of the NV-CJD cases so far analysed had a unique form and distribution characteristic of a BSE infection and completely different to 'classical' CJD.
It seems therefore that BSE has infected humans by oral ingestion.
An epidemic? It is currently unclear whether the incidence of NV-CJD will remain very low or become very high.
It is likely that a large proportion of the UK population has been exposed to the infectious agent.
The size of the epidemic will depend on infectious dose exposure via the gastric route which may be cumulative size of the species barrier: for a more detailed discussion of these points, click here.

Infection and Pathogenesis

Ingested prions may be absorbed across the gut wall at Peyers patches. These are a part of the MALT, or mucosal associated lymphoid tissue. It is thought that the MALT presents microorganisms to the immune system in a contained and ideal fashion, facilitating a protective immune response. Prions could be taken up in the same way. Lymphoid cells then phagocytose the particle and travel to other lymphoid sites such as nodes, the spleen and tonsils. The prion can replicate at these sites. Many of these sites are innervated and eventually the prion gains access to a nerve and then propagates back up the axon to the spinal cord and eventually the brain.

- SCID mice are resistant to a prion challenge, confirming the importance of the lymphoid system.
- PrP null mice ie those in which both alleles have been disrupted, $PrP^{o/o}$, cannot be infected.
- $PrP^{o/o}$mice carrying a $PrP^{+/+}$ brain graft can develop pathology following an intracerebral injection but only in the graft.
- $PrP^{o/o}$ mice + graft + a reconstituted $PrP^{+/+}$ lymphoreticular system are resistant to a prion challenge.

This indicates another compartment in addition to the brain and the LRS must express PrP, if a peripheral prion challenge is to be successful. That compartment is probably a nerve. Mature B lymphocytes are also now known to be required for the development of the disease following infection from a peripheral route.

Tg PrP mice and knockout mice have been fantastically informative in prion research. In a further series of recent experiments $PrP^{o/o}$ mice have had a hamster PrP transgene incorporated. This transgene has been put under the transcriptional control of either the glial fibrillary acidic protein promoter, or the neuron specific enolase promoter such that hamster PrP is only expressed in either the glial cells or neuronal cells. Following an intracerebral prion challenge both groups of animals can replicate the prion agent and develop disease pathology. So either glial cells or neural cells can propagate the disease independently. The fact that PrPsc intracerebral injection alone in $PrP^{o/o}$ mice, does not cause pathology means that cells must be making PrP for a pathological result. It is known that cytokine levels are elevated in the later stages of a prion infection. Astrocytes and other glial cells produce these and presumably have a key role in pathogenesis. This conclusion is supported by the fact that murine retroviruses are known which infect glial cells and result in a spongiform degeneration of the brain.

Treatment:

PrP overexpression facilitates the development of prion diseases. It may therefore follow that agents which reduce PrP expression will delay the onset of prion diseases.
One can speculate that chemicals which bind to and stabilise the PrPc conformation may be beneficial.
Similarly agents destabilising the PrPsc conformation may be effective.
Agents which interfere with the putative PrPc-PrPsc interaction might similarly be effective.

A number of reagents showing affinity for amyloid proteins are known e.g congo red. As our knowledge of the structure of PrP increases, the chances of rationally deducing effective therapeutics based on these ideas increases.

Finally we have seen that PrP expression is required for pathology. Chemicals affecting the endocytosis, exocytosis, intracellular trafficking and degradation of proteins and in particular PrP may also be effective. Amphotericin for instance is reported to delay prion disease in hamsters (although it apparently has little effect in humans).

Further Information:

Books about:

Prions | Mad Cow Disease | BSE (technical)

- **References**
- **How Now Mad Cow:** Online tutorial.

BSE: Latest News:
Visit The Microbiology Newsroom

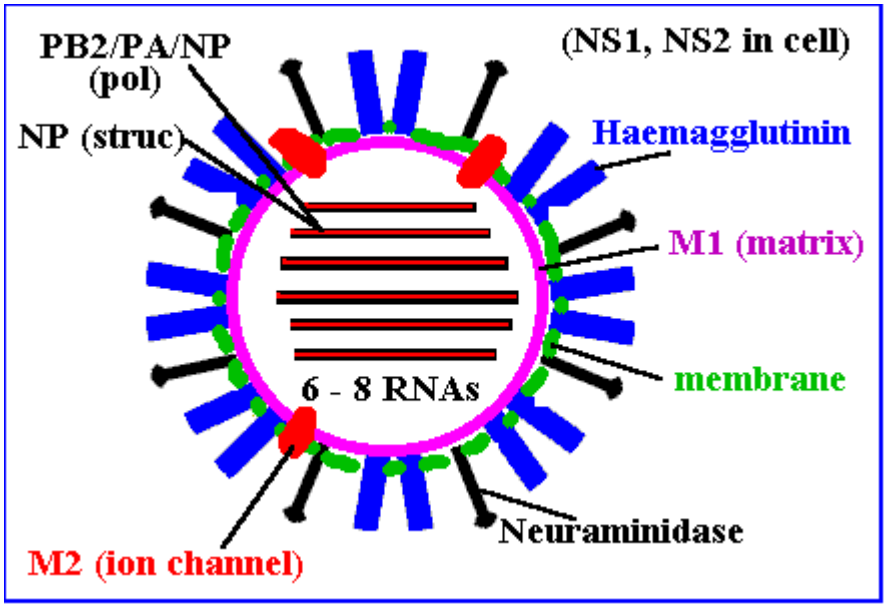
Latest publications on BSE/CJD:

Click Here

Other BSE links:

- John Pattison: The Emergence of Bovine Spongiform Encephalopathy & Related Diseases. Emerging Infectious Diseases 4(3):390-394, 1998.
- Ministry of Agriculture, Fisheries & Food (MAFF)
- I.A.O.
- United States Department of Agriculture
- Official Mad Cow Disease Home Page
- C.A.B. INTERNATIONAL
- BMJ's BSE-CJD Homepage
- **Politics**
- **Search for more information on this topic**.

© Dr Shaun Heaphy, 1998.


PB2/PA/NP
(pol)
(NS1, NS2 in cell)
NP (struc)
Haemagglutinin
M1 (matrix)
membrane
6 - 8 RNAs
Neuraminidase
M2 (ion channel)

Last Updated: Saturday, October 4, 1945624.

Picornaviruses

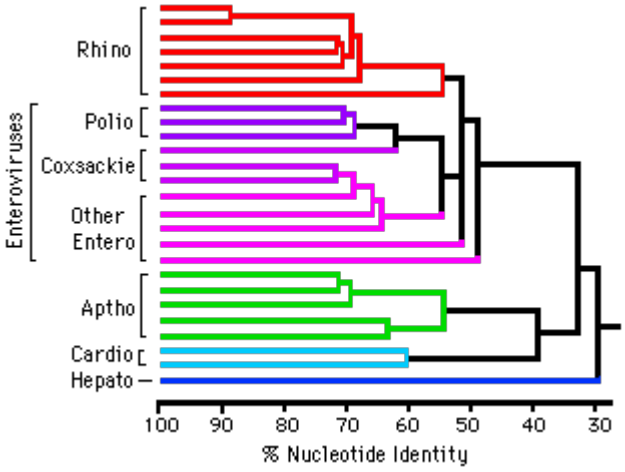
Introduction:

Picornaviruses are among the most diverse (more than 200 serotypes) and 'oldest' known viruses (temple record from Egypt ca. 1400 B.C.). FMDV was one of the first viruses to be recognised - Loeffler and Frosch 1898. Poliomyelitis as a viral disease was first recognised by Landsteiner and Popper, 1909 (though the virus was not isolated until the 1930's.
Name: 'Pico *(Greek very small)* RNA Viruses'.

Classification:

Originally based on physical properties (particle density & pH-sensitivity) & serological relatedness, more recently based on nucleotide sequence. There are 5 genera:

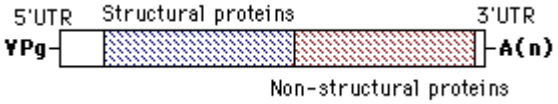
Aphthovirus	7 serotypes
Cardiovirus	2 serotypes
Enterovirus	111 serotypes
Hepatovirus	2 serotypes (1 human, 1 simian)
Rhinovirus	105 serotypes
Unassigned	3 serotypes
Total:	~230 viruses



Genome:

The genome consists of one s/s (+)sense RNA molecule of between 7.2kb (HRV14) to 8.5kb (FMDV). A number of features are conserved in all Picornaviruses:

- Genomic RNA is infectious (~1x10^6-fold less infectious than intact particles, although infectivity is increased if the RNA is introduced into cells by transfection) - CHARACTERISTIC OF (+)SENSE RNA VIRUSES !!!
- There is a long (600-1200 base) untranslated region at the 5' end (important in translation, virulence and possibly encapsidation and a shorter 3' untranslated region (50-100 bases) - important in (-)strand synthesis. The 5' UTR contains a 'clover-leaf' secondary structure known as the **IRES: Internal Ribosome Entry Site** (see below).
- The rest of the genome encodes a single 'polyprotein' of between 2100-2400 aa's.
- Both ends of the genome are modified, the 5' end by a covalently attached small, basic protein VPg (~23 aa's), the 3' end by polyadenylation (polyadenylic acid sequences are not genetically coded, there is a 'polyadenylation signal' upstream of the 3' end as in eukaryotic mRNAs):



Structure:

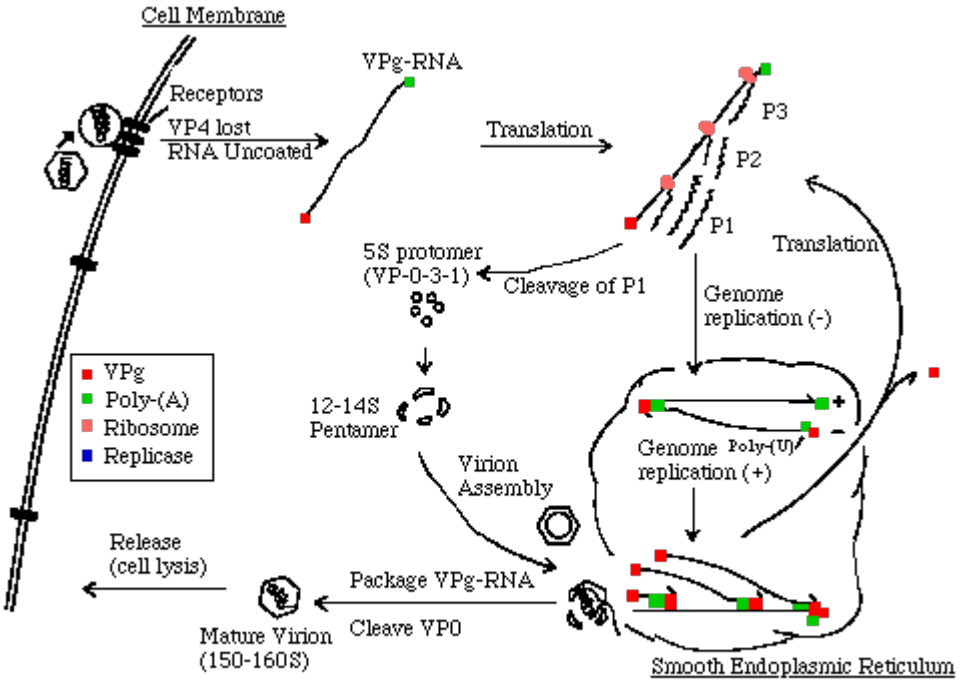
The capsid consists of a densely-packed icosahedral arrangement of 60 protomers, each consisting of 4 polypeptides, VP1, 2, 3 and 4 - all derived from cleavage of the original protomer VP0, with (pseudo) T=3 packing. The particle is 27-30nm in diameter (depending on type and degree of desiccation), while the length of the genome (stretched-out) is ~2,500nm therefore the genome is tightly packed into the capsid, together with sodium or potassium ions or polyamines (in rhinoviruses) to counteract the negative charges on the phosphate groups. To view an electron micrograph of negatively-stained picornavirus particles, click here.

To view a computer generated animation of a picornavirus capsid, click here. This image is based on the real atomic co-ordinates of rhinovirus 16 and shows a view inside the capsid. In this video:

- VP1: is in blue
- VP2: is in green
- VP3: is in red
- VP4: is in yellow (only visible on the inside of the particle)

Replication:

We know a great deal about Picornavirus replication due to single-step growth curve type experiments performed at high multiplicity of infection. Replication occurs entirely in the cytoplasm - it can occur even in enucleated cells and is not inhibited by actinomycin D.



Receptors:

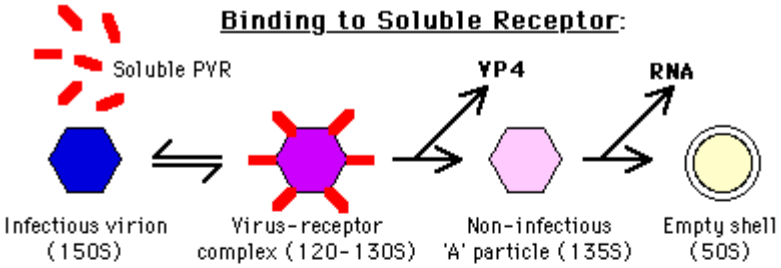
The cellular receptors for several different groups of picornaviruses have been identified using a number of different techniques over the last few years:

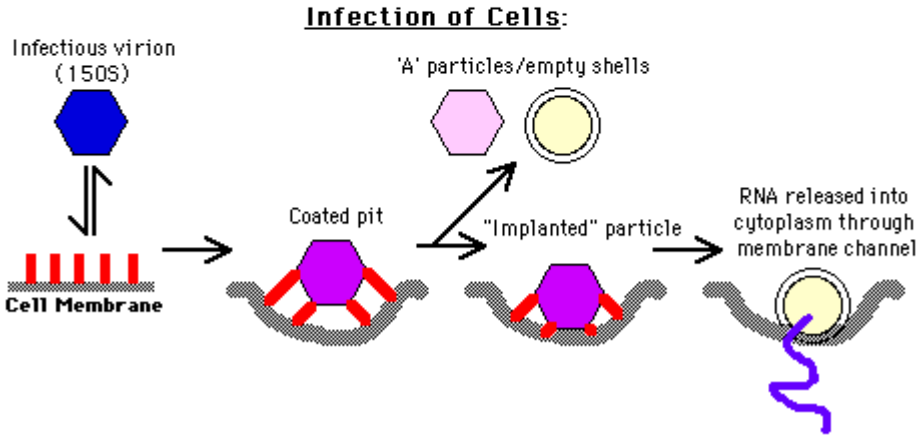
- Binding competition between different viruses
- MAbs which block virus binding
- Fluorescently labelled virus (Echovirus)

Virus:	# Serotypes:	Receptor:	Description:
Human Rhinovirus	**91**	ICAM-1 (Intracellular Adhesion Molecule 1)	Immunoglobulin-like molecule; 5 domains
Human Rhinovirus	**10**	LDLR (Low Density Lipoprotein Receptor)	
Poliovirus	**3**	CD155	Immunoglobulin-like molecule; 3 domains
Coxsackie A	**3**	ICAM-1	
Echo	**2**	VLA-2	Integrin-like molecule
Echo	**6**	DAF (Decay Accelerating Factor)	???
EMCV	**1**	VCAM-1 (Vascular Cell Adhesion Molecule)	???

Uncoating:

After adherence to the receptor, the virus can be eluted again, but if this happens, the particle undergos conformational changes due to the loss of VP4 and infectivity is lost - this is also the first stage in uncoating:



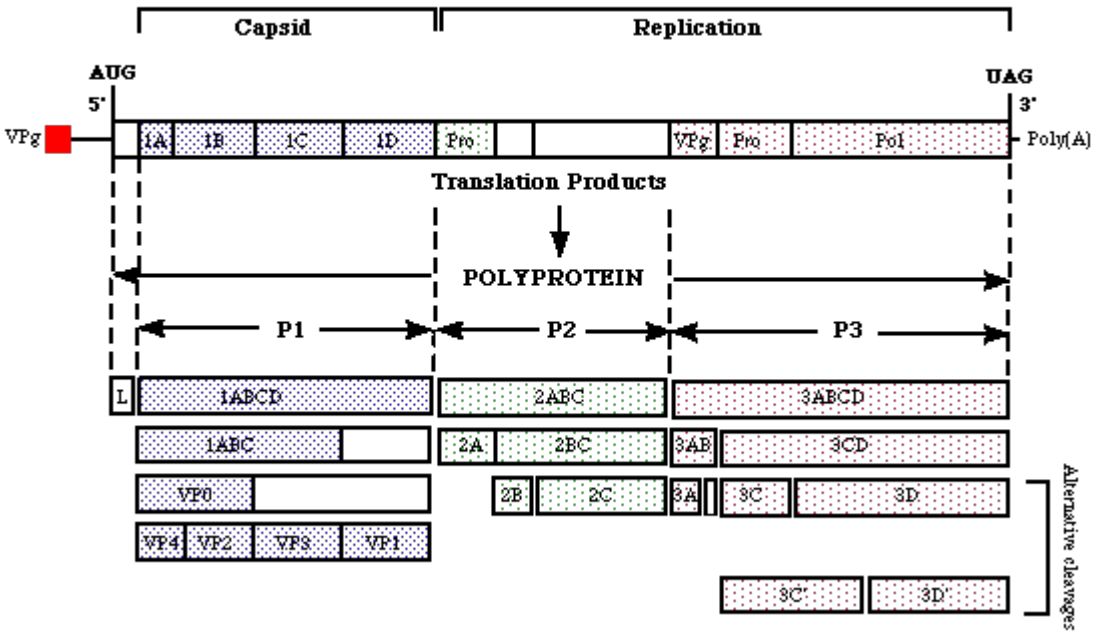


Translation:

The kinetics of Picornavirus replication are rapid, the cycle being completed in from 5-10 (typically 8) hours. Genomic RNA is translated directly by polysomes, but ~30 min after infection, cellular protein synthesis declines sharply, almost to zero, this is called **'SHUTOFF'** - the primary cause of c.p.e:

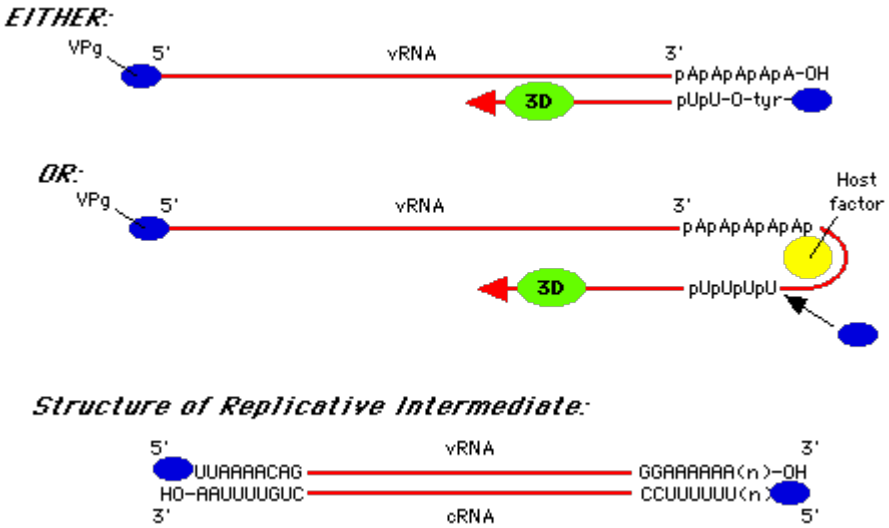
Time after Infection:	Event:
~1-2h	Sharp decrease in cellular macromolecular synthesis; margination of chromatin (loss of homogeneous appearance of nucleus)
~2.5-3h	Start of viral protein synthesis; vaculoation of cytoplasm, beginning close to nucleus & spreading outwards
~3-4h	Permeabilization of plasma membrane
~4-6h	Virus assembly in cytoplasm (crystals sometimes visible)
~6-10h	Cell lysis; release of virus particles

Shutoff appears to be due to cleavage of the 220kD **'cap-binding complex' (CBC)** involved in binding the m7G cap structure at the 5' end of all eukaryotic mRNAs during initiation of translation. This is carried out by **poliovirus protein 2A.**
The 5' UTR contains the **IRES:** Internal Ribosome Entry Site or 'landing pad'. Normally, translation is initated when ribosomes bind to the 5' methylated cap then scan along the mRNA to find the first AUG initiation codon. The IRES overcomes this & allows picornavirus RNAs to continue to be translated after degradation of CBC.
The polyprotein is initially cleaved by P2A into P1 & P2P3. Further cleavage events are carried out by 3C - the main picornavirus protease. All of these cleavages are highly specific (drug target!):



Genome Replication:

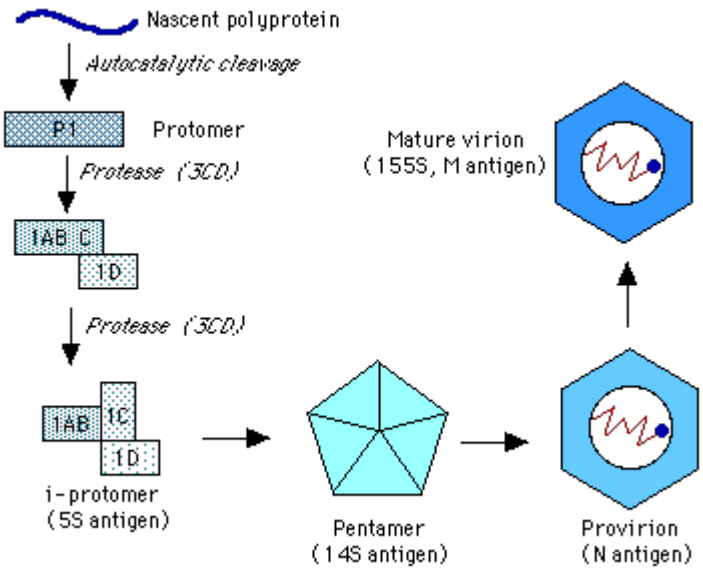
One of the products made is the virus RNA-dependent RNA polymerase (**3D**), which copies the genomic RNA to produce a (-)sense strand. This forms the template for (+)strand (genomic) RNA synthesis, which occurs via a multi-stranded replicative intermediate complex (RI). In vitro transcription studies have suggested 2 possible models by which genome replication might occur:



The (-)ve sense cRNA serves as a template for multiple (+)ve sense strands, some of which are translated, others which form vRNA.

Assembly:

RNA is believed(?) to be packaged into preformed capsids, although the molecular interactions between the genome & the capsid responsible for this process are not clear. Empty capsids (defective) are common in all Picornavirus infections. The capsid is assembled by cleavage of the P1 polyprotein precursor into a protomer consisting of VP0,3,1 which join together enclosing the genome:



Maturation:

Maturation (& infectivity) relies on an internal autocatalytic (?) cleavage of VP0 into VP2 + VP4.

Release:

Release (in most cases) on the virus from the cytoplasm occurs when the cell lyses - probably a 'preprogrammed' event which occurs a set time after the cessation of 'housekeeping' macromolecular synthesis at shutoff. (Hepatitis A virus is relatively non-lytic & sets up a more persistent infection).

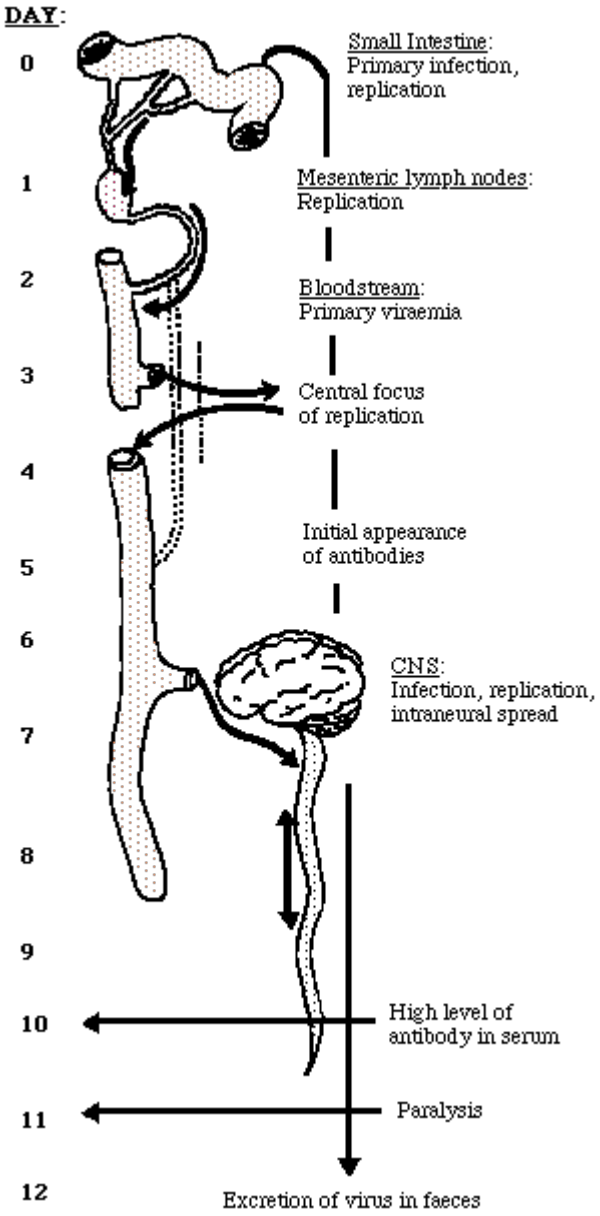
Enteroviruses

Enterovirus infections are common in humans; seasonal peak in autumn; frequently undiagnosed:

Polioviruses	3 serotypes
Coxsackieviruses group A	23 serotypes
Coxsackieviruses group B	6 serotypes
Echoviruses	31 serotypes
Enteroviruses	38 serotypes
Total:	111 serotypes

Polioviruses:

To view a high resolution computer-generated image reconstruction of a poliovirus particle, click here. Note the icosahedral symmetry which is clearly visible in this image. These are the prototypic Picornaviruses; there are 3 distinct serotypes. They cause poliomyelitis (flaccid muscular paralysis).
As with all the Enteroviruses, they are transmitted by the faecal-oral route.



Primary site of infection is **lymphoid tissue** associated with the oropharynx and gut (GALT).

Virus production at this site leads to a **transient viraemia**, following which the virus may infect the **CNS**. This is of interest because of this apparent 'dual tropism' of the virus for two distinct cell types - lymphoid/ epithelial cell in the gut and neurons in the CNS - different receptors, etc.

Replication of the virus in the CNS occurs in the 'grey matter', particularly motor neurons in the anterior horns of the spinal cord and brain stem. Distinctive 'plaques' produced in the grey matter are due to lytic replication of the virus & probably inflammation caused by an over-enthusiastic immune response.

~1% of people infected with the most virulent strains experience paralysis (99% asymptomatic infections). Death is usually due to respiratory failure by paralysis of the intercostal muscles and diaphragm.
Effective polyvalent vaccines are available against polioviruses - OPV/IPV. In 1988, the World Health Assembly established the year 2000 for achieving global poliomyelitis eradication. By 1994, the Americas were certified as polio-free. All other regions are making steady progress towards this goal. The WHO reports that global eradication of poliomyelitis is still on course for the year 2000.

Books about Poliovirus: click here

Coxsackieviruses:

Algonquin indian name of village in N.Y. where first isolated (Daldorf and Sickles/suckling mice/1948). Two groups, based on pathology in suckling mice:

Group A: Cause acute myositis (muscular inflammation) with inflammation and necrosis. 24 serotypes.
Group B: Cause degenerative 'plaques' in brain, muscle and pancreas (model for induced diabetes in mice). 6 serotypes.

In man, these viruses show a seasonal, epidemic pattern of infection (mostly sub-clinical), associated with meningitis, paralysis (usually less severe than acute poliomyelitis), myocarditis, etc. Common infections worldwide (inc. UK) - no effective treatment/prophylaxis!

Echoviruses:

Enteric **C**ytopathic **H**uman **O**rphan viruses; not linked to any human disease (hence 'orphan'). 32 serotypes (echo 10 = reovirus 1; echo 28= HRV1A). Common cause of asymptomatic (?) enteric infections.

'New' Enteroviruses:

Since 1969, 'new' Enteroviruses have been assigned numbers, not names:

Type:	Illness:
68	Pneumonia
69	None (?)
70	Acute haemorrhagic conjunctivitis (1969-1974 pandemic)
71	Meningitis
72	Hepatitis A virus (now a separate genus: Hepatovirus)

Rhinoviruses

Cause of 'the common cold' (but not the only one!). ~105 serotypes (hence repeated infections). Relatively fragile viruses (c.f. Enteroviruses), with optimum growth temperature of 33°C (URT infection). Extensive human volunteer studies show no evidence for susceptibility when exposed to cold/wet conditions (!) although general immune status is probably important. Little c.p.e. Many types grow very poorly in vitro. Replicate in ferrets - other animal reservoirs? Symptoms due to damage to cilliated epithelium in URT. Little consequence in itself, but predisposes to secondary bacterial infections - a major problem in infants and elderly. In addition, a major economic pest worldwide (lost working days).
No effective prophylaxis or treatment; (Pliny the Younger recommended 'kissing the hairy muzzle of a mouse') - in spite of extensive molecular knowledge. There is little or no cross-protection between serotypes. Protection relies on levels of secreted Ab in URT - may be relatively short-lived (e.g. a few years rather than life-long).
To view a computer generated animation of a rhinovirus particle, click here. This image is based on the real atomic co-ordinates of rhinovirus 14. The antigenic sites on the surface of this particle are highlighted in purple. Note how the distribution of the antigenic sites on the capsid emphasises its icosahedral symmetry.
Medscape Article: What's New With Common Colds? Complications and Management.

Apthoviruses

This is the group of viruses responsible for foot-and-mouth disease (FMD) - a major economic pest worldwide, especially in S.America and Australasia. Controlled largely by vaccination (inactivated vaccine - occasional vaccine-linked outbreaks) or slaughter of infected animals. They are physically quite distinct from other Picornaviruses:

- **Acid-labile** - below pH 7.0.
- **Antigenically** - 7 serotypes, (A; C; O; SAT1,2,3; Asia-1), location of antigenic sites on capsid quite different from Enteroviruses.
- **Genome** - Larger than other Picornaviruses, ~ 8.5kb; 5' non-translated region contains poly-C tract of ~100-170nt - function not known (encapsidation?).

To view a high resolution computer-generated image reconstruction of a FMDV particle, click here.

Cardioviruses

One serotype. Includes encephalomyocarditis virus (EMCV) (model infection of mice), mengovirus, Maus-Elberfield virus, Columbia virus - all considered to be strains of EMCV (really a mouse virus, but can infect man,

elephants, squirrels...). Genome size ~7.8kb; 5' non-translated region contains poly-C tract of ~100-170nt (like Apthoviruses).

- Pretend to be poliovirus!
- References: Click here
- Search for more information on this topic
- Search **MEDLINE** for the latest publications on this topic:

Click Here

Return to BS335 HomePage

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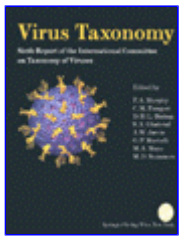
List of ICTV approved Virus Families and Genera

This list of the taxonomic structure of virus families and genera sorted according to their *general host range* has been compiled from



Classification and Nomenclature of Viruses

Sixth Report of the International Committee on Taxonomy of Viruses (1995)
F.A. Murphy, C.M. Fauquet, D.H.L. Bishop, S.A. Ghabrial, A.W. Jarvis, G.P. Martelli, M.A. Mayo, M.D. Summers (eds.) Archives of Virology/Supplement 10, 586 pp. Springer Verlag, Wien New York.

The taxonomic structure of virus families and genera are listed in three different ways to facilitate the search for a particular virus family or genus:

Family and genus names sorted

1. in alphabetical order
2. according to their nucleic acid content and strandedness
3. according to their general host range

Throughout this document taxa are listed according to their general host range. Each type species name is preceded by the name of the order, family and genus it belongs to. Names of orders, families and genera are in italic script, if they are approved b y the ICTV. Taxa names in quotes (and not in italic script) indicate that this taxon has not an ICTV international approved name. Species (vernacular) names are given in regular script.

To access documents that contain *The Taxonomic Structure* of the family and/or genus to which the taxon belongs click on the highlighted name.

Taxonomic Structure of Families and Genera listed according to their General Host

---

Categorie of hosts found in virus taxa:

- Algae
- Archaea
- Bacteria
- Fungi
- Invertebrates
- Mycoplasma
- Plants
- Protozoa
- Spiroplasma
- Vertebrates

To access a complete lists of species belonging to a family or genus click on the respective highlighted family/genus name.

Algae

Back to list of host categories

- Family: *Phycodnaviridae*
  - Genus: *Phycodnavirus*
  - *Type species:* Paramecium bursaria Chlorella virus 1

Archaea

Back to list of host categories

- Family: *Fuselloviridae*
  - Genus: *Fusellovirus*
  - *Type species:* Sulfolobus virus 1

- Family: *Lipothrixviridae*
  - Genus: *Lipothrixvirus*
  - *Type species:* Thermoproteus virus 1

Bacteria

Back to list of host categories

- Family: *Corticoviridae*
  - Genus: *Corticovirus*
  - *Type species:* Alteromonas phage PM2

- Family: *Cystoviridae*
  - Genus: *Cystovirus*
  - *Type species:* Pseudomonas phage f6

- Family: *Inoviridae*
  - Genus: *Inovirus*
  - *Type species:* coliphage fd

- Family: *Leviviridae*

- Genus: *Levivirus*
- *Type species:* enterobacteria phage MS2
- Genus: *Allolevivirus*
- *Type species:* enterobacteria phage Qß

- Family: *Microviridae*
  - Genus: *Microvirus*
  - *Type species:* coliphage fX174
  - Genus: *Bdellomicrovirus*
  - *Type species:* Bdellovibrio phage MAC1
  - Genus: *Chlamydiamicrovirus*
  - *Type species:* Chlamydia phage 1

- Family: *Myoviridae*
  - Genus: "T4 -like phages"
  - *Type species:* coliphage T4

- Family: *Podoviridae*
  - Genus: "T7 -like phages"
  - *Type species:* coliphage T7

- Family: *Siphoviridae*
  - Genus: lambda-like Phages"
  - *Type species:* coliphage lambda

- Family: *Tectiviridae*
  - Genus: *Tectivirus*
  - *Type species:* enterobacteria phage PRD1

Fungi

Back to list of host categories

- Family: *Barnaviridae*
  - Genus: *Barnavirus*
  - *Type species:* mushroom bacilliform virus

Family: *Hypoviridae*
- Genus: *Hypovirus*
- *Type species:* Cryphonectria hypovirus 1-EP713

- Family: *Partitiviridae*
  - Genus: *Partitivirus*
  - *Type species:* Gaeumannomyces graminis virus 019/6A
  - Genus: *Chrysovirus*
  - *Type species:* Penicillium chrysogenum virus

  - Genus: *Rhizidiovirus*
  - *Type species:* Rhizidiomyces virus

- Satellites
  - ssRNA Satellite Viruses
  - *Type species:* tobacco necrosis satellite

- Family: *Totiviridae*
  - Genus: *Totivirus*
  - *Type species:* Saccharomyces cerevisiae virus L-A

Invertebrates

Back to list of host categories

- Family: *Baculoviridae*
  - Genus: *Nucleopolyhedrovirus*
  - *Type species:* Autographa californica nucleopolyhedrovirus
  - Genus: *Granulovirus*
  - *Type species:* Plodia interpunctella granulovirus

- Family: *Birnaviridae*
  - Genus: *Entomobirnavirus*
  - *Type species:* Drosophila X virus

- Family: *Iridoviridae*
  - Genus: *Iridovirus*
  -

Type species: Chilo iridescent virus
- Genus: *Chloriridovirus*
- *Type species:* mosquito iridescent virus

- Family: *Nodaviridae*
  - Genus: *Nodavirus*
  - *Type species:* Nodamura virus

- Family: *Parvoviridae*
  - Subfamily: *Densovirinae*
    - Genus: *Densovirus*
    - *Type species:* Junonia coenia densovirus
    - Genus: *Iteravirus*
    - *Type species:* Bombyx mori densovirus
    - Genus: *Contravirus*
    - *Type species:* Aedes aegypti densovirus

- Family: *Polydnaviridae*
  - Genus: *Ichnovirus*
  - *Type species:* Campoletis sonorensis virus
  - Genus: *Bracovirus*
  - *Type species:* Cotesia melanoscela virus

- Family: *Poxviridae*
  - Subfamily: *Entomopoxvirinae*
    - Genus: *Entomopoxvirus A*
    - *Type species:* Melolontha melolontha entomopoxvirus
    - Genus: *Entomopoxvirus B*
    - *Type species:* Amsacta moorei entomopoxvirus
    - Genus: *Entomopoxvirus C*
    - *Type species:* Chironomus luridus entomopoxvirus

- Family: *Reoviridae*
  - Genus: *Cypovirus*
  - *Type species:* Bombyx mori cypovirus 1

- Satellites
  - ssRNA Satellites

- Family: *Tetraviridae*
    - Genus:
    - *Type species:*
    - Genus: "Nudaurelia capensis omega-like viruses"
    - *Type species:* Nudaurelia capensis omega virus

Mycoplasma

Back to list of host categories

- Family: *Inoviridae*
    - Genus: *Plectrovirus*
    - *Type species:* Acholeplasma phage L51

- Family: *Plasmaviridae*
    - Genus: *Plasmavirus*
    - *Type species:* Acholeplasma phage L2

Plants

Back to list of host categories

    - Genus: *Badnavirus*
    - *Type species:* commelina yellow mottle virus

- Family: *Bromoviridae*
    - Genus: *Alfamovirus*
    - *Type species:* alfalfa mosaic virus
    - Genus: *Ilarvirus*
    - *Type species:* tobacco streak virus
    - Genus: *Bromovirus*
    - *Type species:* brome mosaic virus
    - Genus: *Cucumovirus*
    - *Type species:* cucumber mosaic virus

- Family: *Bunyaviridae*
    - Genus: *Tospovirus*
    -

Type species: tomato spotted wilt virus

- Genus: *Capillovirus*
- *Type species:* apple stem grooving virus

- Genus: *Carlavirus*
- *Type species:* carnation latent virus

- Genus: *Caulimovirus*
- *Type species:* cauliflower mosaic virus

- Genus: *Closterovirus*
- *Type species:* beet yellows virus

- Family: *Comoviridae*
  - Genus: *Comovirus*
  - *Type species:* cowpea mosaic virus
  - Genus: *Fabavirus*
  - *Type species:* broad bean wilt virus 1
  - Genus: *Nepovirus*
  - *Type species:* tobacco ringspot virus

- Genus: *Dianthovirus*
- *Type species:* carnation ringspot virus

- Genus: *Enamovirus*
- *Type species:* pea enation mosaic virus

- Genus: *Furovirus*
- *Type species:* soil-borne wheat mosaic virus

- Family: *Geminiviridae*
  - Genus: *Mastrevirus* (Subgroup I Geminivirus)
  - *Type species:* maize streak virus
  - Genus: *Curtovirus* (Subgroup II Geminivirus)
  -

Type species: beet curly top virus
- Genus: *Begomovirus* (Subgroup III Geminivirus)
- *Type species:* bean golden mosaic virus

- Genus: *Hordeivirus*
- *Type species:* barley stripe mosaic virus

- Genus: *Idaeovirus*
- *Type species:* raspberry bushy dwarf virus

- Genus: *Luteovirus*
- *Type species:* barley yellow dwarf virus

- Genus: *Machlomovirus*
- *Type species:* maize chlorotic mottle virus

- Genus: *Marafivirus*
- *Type species:* maize rayado fino virus

- Genus: *Necrovirus*
- *Type species:* tobacco necrosis virus

- Family: *Partitiviridae*
  - Genus: *Alphacryptovirus*
  - *Type species:* white clover cryptic virus 1
  - Genus: *Betacryptovirus*
  - *Type species:* white clover cryptic virus 2

- Genus: *Potexvirus*
- *Type species:* potato virus X

- Family: *Potyviridae*
  - Genus: *Potyvirus*
  - *Type species:* potato virus Y
  - Genus: *Rymovirus*
  -

Type species: ryegrass mosaic virus
- Genus: *Bymovirus*
- *Type species:* barley yellow mosaic virus

- Family: *Reoviridae*
  - Genus: *Fijivirus*
  - *Type species:* Fiji disease virus
  - Genus: *Phytoreovirus*
  - *Type species:* wound tumor virus
  - Genus: *Oryzavirus*
  - *Type species:* rice ragged stunt virus

- Order: *Mononegavirales*
  - Family: *Rhabdoviridae*
    - Genus: *Nucleorhabdovirus*
    - *Type species:* potato yellow dwarf virus

  - Satellites
    - ssRNA Satellite Viruses
    - *Type species:* tobacco necrosis satellite

  - Family: *Sequiviridae*
    - Genus: *Sequivirus*
    - *Type species:* parsnip yellow fleck virus
    - Genus: *Waikavirus*
    - *Type species:* rice tungro spherical virus

    - Genus: *Sobemovirus*
    - *Type species:* Southern bean mosaic virus

    - Genus: *Tenuivirus*
    - *Type species:* rice stripe virus

    - Genus: *Tobamovirus*
    - *Type species:* tobacco mosaic virus

    - Genus: *Tobravirus*
    - *Type species:* tobacco rattle virus

  - Family: *Tombusviridae*
    - Genus: *Carmovirus*

- *Type species:* carnation mottle virus
- Genus: *Tombusvirus*
- *Type species:* tomato bushy stunt virus

- Genus: *Trichovirus*
- *Type species:* apple chlorotic leaf spot virus

- Genus: *Tymovirus*
- *Type species:* turnip yellow mosaic virus

- Genus: *Umbravirus*
- *Type species:* carrot mottle virus

- Unassigned Viruses

- Viroids

  Type species: potato spindle tuber viroid

Protozoa

Back to list of host categories

- Family: *Totiviridae*
  - Genus: *Giardiavirus*
  - *Type species:* Giardia lamblia virus
  - Genus: *Leishmaniavirus*
  - *Type species:* Leishmania RNA virus 1-1

Spiroplasma

Back to list of host categories

- Family: *Microviridae*
  - Genus: *Spiromicrovirus*
  - *Type species:* Spiroplasma phage 4

Vertebrates

Back to list of host categories

- Family: *Adenoviridae*
  - Genus: *Mastadenovirus*
  - *Type species:* human adenovirus 2
  - Genus: *Aviadenovirus*
  - *Type species:* fowl adenovirus 1

  - Genus: "African swine fever-like viruses"
  - *Type species:* African swine fever virus

- Family: *Arenaviridae*
  - Genus: *Arenavirus*
  - *Type species:* lymphocytic choriomeningitis virus

  - Genus: *Arterivirus*
  - *Type species:* equine arteritis virus

- Family: *Astroviridae*
  - Genus: *Astrovirus*
  - *Type species:* human astrovirus 1

- Family: *Birnaviridae*
  - Genus: *Aquabirnavirus*
  - *Type species:* infectious pancreatic necrosis virus
  - Genus: *Avibirnavirus*
  - *Type species:* infectious bursal disease virus

- Family: *Bunyaviridae*
  - Genus: *Bunyavirus*
  - *Type species:* Bunyamwera virus
  - Genus: *Hantavirus*
  - *Type species:* Hantaan virus
  - Genus: *Nairovirus*
  - *Type species:* Nairobi sheep disease virus
  - Genus: *Phlebovirus*
  - *Type species:* sandfly fever Sicilian virus

- Family: *Caliciviridae*
  - Genus: *Calicivirus*
  - *Type species:* vesicular exanthema of swine virus

- Family: *Circoviridae*

        - Genus: *Circovirus*
        - *Type species:* chicken anemia virus

    - Family: *Coronaviridae*
        - Genus: *Coronavirus*
        - *Type species:* avian infectious bronchitis virus
        - Genus: *Torovirus*
        - *Type species:* Berne virus

        - Genus: *Deltavirus*
        - *Type species:* hepatitis delta virus

- Order: *Mononegavirales*
    - Family: *Filoviridae*
        - Genus: *Filovirus*
        - *Type species:* Marburg virus

    - Family: *Flaviviridae*
        - Genus: *Flavivirus*
        - *Type species:* yellow fever virus
        - Genus: *Pestivirus*
        - *Type species:* bovine diarrhea virus
        - Genus: "Hepatitis C - like viruses"
        - *Type species:* hepatitis C virus

    - Family: *Hepadnaviridae*
        - Genus: *Orthohepadnavirus*
        - *Type species:* hepatitis B virus
        - Genus: *Avihepadnavirus*
        - *Type species:* duck hepatitis B virus

    - Family: *Herpesviridae*
        - Subfamily: *Alphaherpesvirinae*
        - Genus: *Simplexvirus*
        - *Type species:* human herpesvirus 1
        - Genus: *Varicellovirus*
        - *Type species:* human herpesvirus 3
        - Subfamily: *Betaherpesvirinae*
        - Genus: *Cytomegalovirus*
        - *Type species:* human herpesvirus 5
        - Genus: *Muromegalovirus*

Type species: mouse cytomegalovirus 1
- Genus: *Roseolovirus*
- *Type species:* human herpesvirus 6
- Subfamily: *Gammaherpesvirinae*
  - Genus: *Lymphocryptovirus*
  - *Type species:* human herpesvirus 4
  - Genus: *Rhadinovirus*
  - *Type species:* ateline herpesvirus 2

- Family: *Iridoviridae*
  - Genus: *Ranavirus*
  - *Type species:* frog virus 3
  - Genus: *Lymphocystivirus*
  - *Type species:* flounder virus
  - Genus: "Goldfish virus -like viruses"
  - *Type species:* goldfish virus 1

- Order: *Mononegavirales*
  - Family: *Filoviridae*
  - Family: *Paramyxoviridae*
  - Family: *Rhabdoviridae*

- Family: *Orthomyxoviridae*
  - Genus: *Influenzavirus A, B*
  - *Type species:* influenza A virus
  - Genus: *Influenzavirus C*
  - *Type species:* influenza C virus
  - Genus: "Thogoto-Like viruses "
  - *Type species:* Thogoto virus

- Family: *Papovaviridae*
  - Genus: *Polyomavirus*
  - *Type species:* murine polyomavirus
  - Genus: *Papillomavirus*
  - *Type species:* cottontail rabbit papillomavirus (Shope)

- Order: *Mononegavirales*
  - Family: *Paramyxoviridae*
    - Subfamily: *Paramyxovirinae*
      - Genus: *Paramyxovirus*
      - *Type species:* human parainfluenza virus 1
      - Genus: *Morbillivirus*
      -

Type species: measles virus
- Genus: *Rubulavirus*
- *Type species:* mumps virus
- Subfamily: *Pneumovirinae*
  - Genus: *Pneumovirus*
  - *Type species:* human respiratory syncytial virus

- Family: *Parvoviridae*
  - Subfamily: *Parvovirinae*
    - Genus: *Parvovirus*
    - *Type species:* mice minute virus
    - Genus: *Erythrovirus*
    - *Type species:* B19 virus
    - Genus: *Dependovirus*
    - *Type species:* adeno-associated virus 2

- Family: *Picornaviridae*
  - Genus: *Enterovirus*
  - *Type species:* poliovirus 1
  - Genus: *Rhinovirus*
  - *Type species:* human rhinovirus 1A
  - Genus: *Hepatovirus*
  - *Type species:* hepatitis A virus
  - Genus: *Cardiovirus*
  - *Type species:* encephalomyocarditis virus
  - Genus: *Aphthovirus*
  - *Type species:* foot-and-mouth disease virus O

- Family: *Poxviridae*
  - Subfamily: *Chordopoxvirinae*
    - Genus: *Orthopoxvirus*
    - *Type species:* vaccinia virus
    - Genus: *Parapoxvirus*
    - *Type species:* orf virus
    - Genus: *Avipoxvirus*
    - *Type species:* fowlpox virus
    - Genus: *Capripoxvirus*
    - *Type species:* sheeppox virus
    - Genus: *Leporipoxvirus*
    - *Type species:* myxoma virus

- Genus: *Suipoxvirus*
- *Type species:* swinepox virus
- Genus: *Molluscipoxvirus*
- *Type species:* Molluscum contagiosum virus
- Genus: *Yatapoxvirus*
- *Type species:* Yaba monkey tumor virus

- Prions: Agents of Spongiform Encephalopathies
  - *Type species:* scrapie agent

- Family: *Reoviridae*
  - Genus: *Orthoreovirus*
  - *Type species:* reovirus 3
  - Genus: *Orbivirus*
  - *Type species:* bluetongue virus 1
  - Genus: *Rotavirus*
  - *Type species:* simian rotavirus SA11
  - Genus: *Coltivirus*
  - *Type species:* Colorado tick fever virus
  - Genus: *Aquareovirus*
  - *Type species:* golden shiner virus

- Family: *Retroviridae*
  - Genus: "mammalian type B retroviruses"
  - *Type species:* mouse mammary tumor virus
  - Genus: "mammalian type C retroviruses"
  - *Type species:* murine leukemia virus
  - Genus: "avian type C retroviruses"
  - *Type species:* avian leukosis virus
  - Genus: "type D retroviruses"
  - *Type species:* Mason-Pfizer monkey virus
  - Genus: "blv-htlv retroviruses"
  - *Type species:* bovine leukemia virus
  - Genus: *Lentivirus*
  - *Type species:* human immunodeficiency virus 1
  - Genus: *Spumavirus*
  - *Type species:* human spumavirus

- Order: *Mononegavirales*

Family: *Rhabdoviridae*

- Genus: *Vesiculovirus*
- *Type species:* vesicular stomatitis Indiana virus
- Genus: *Lyssavirus*
- *Type species:* rabies virus
- Genus: *Ephemerovirus*
- *Type species:* bovine ephemeral fever

- Satellites
  - dsDNA Satellites
  - ssRNA Satellites

- Family: *Togaviridae*
  - Genus: *Alphavirus*
  - *Type species:* Sindbis virus
  - Genus: *Rubivirus*
  - *Type species:* rubella virus

- Unassigned Viruses

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Course BS335: Lecture Notes

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Dr Alan Cann:

- **Virus Families**

- Viral Immunopathogenesis
- Pathogenesis of AIDS
- Plant Virology
- Emerging Viruses

Dr Shaun Heaphy:

Virus Transformation of Cells:
- Mechanisms of Cell Transformation
- RNA Transforming Viruses
- DNA Transforming Viruses
- Tumour Suppressor Proteins

Novel Infectious Agents:
- Viroids and virusoids
- Prions

General Information:

- Antiviral drugs
- The Big Picture Book of Viruses (Courtesy of David M. Sander)
- Virus Structure
- Virus Replication
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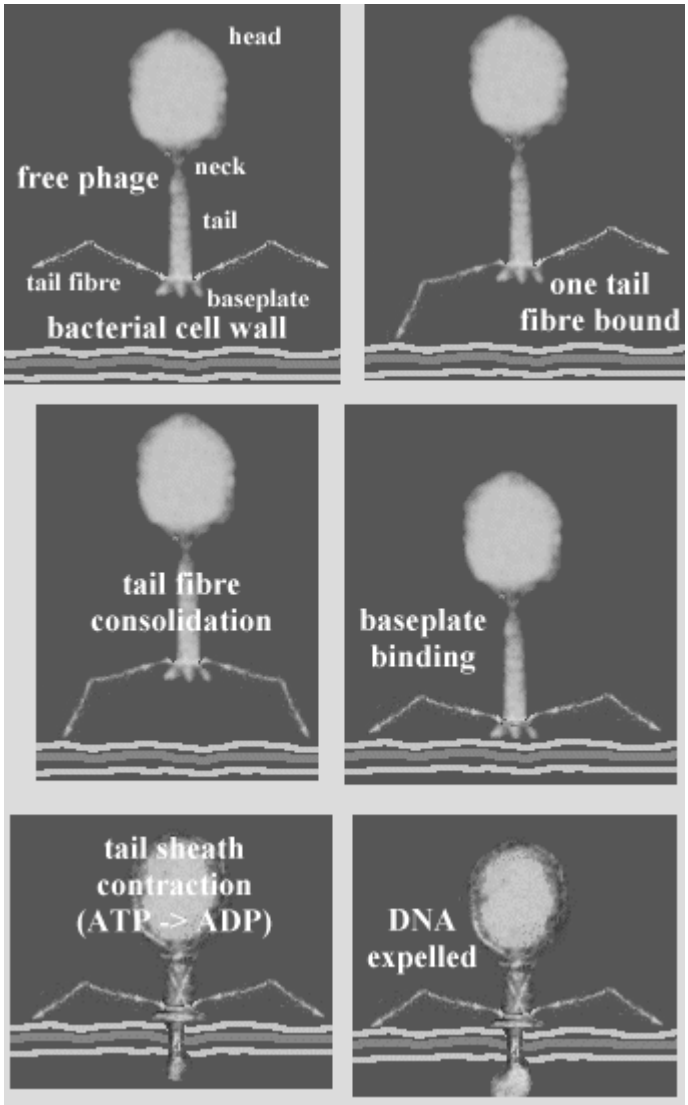

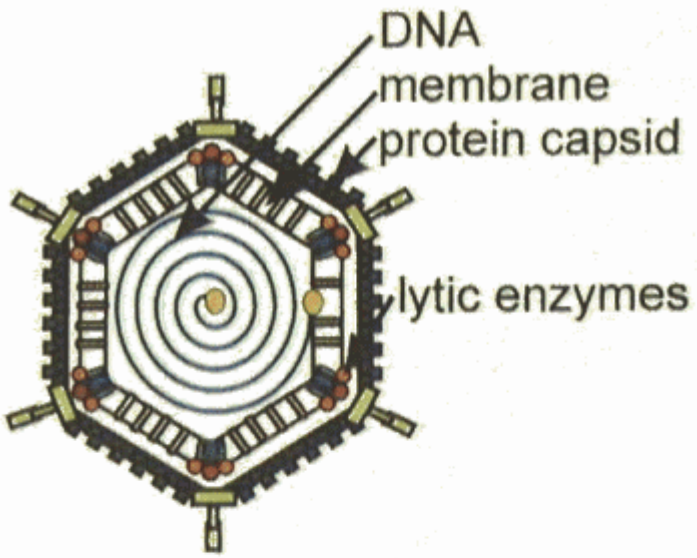
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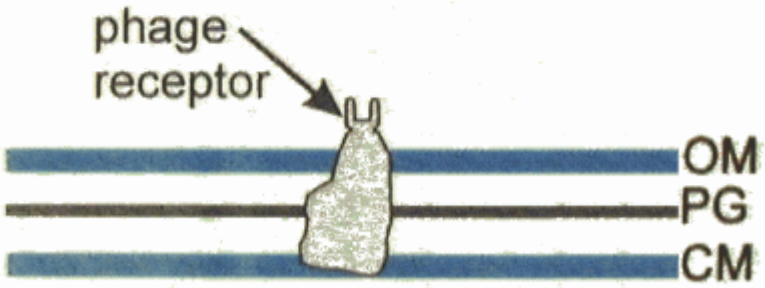
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RNA plant and animal virus replication

Ed Rybicki, October 2000

- Introduction
- Virus taxa involved
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- Genome expression and replication
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Introduction

The world of **RNA plant and animal viruses** is a wide and complex one: they may have

- **double-stranded (ds)**, or
- **messenger-sense (+) or**
- **anti-messenger-sense (-) or even ambisense ((+) and (-) in the same genome) single-stranded (ss) genomes**,

and have **single or multiple genome components**; in simple naked, or complex enveloped virions. **It is quite feasible that RNA genomes of viruses are the only extant lineal descendants of the primaeval RNA world, given that they still replicate as the progenitor genome is supposed to have - that is, by use of a template-specific RNA-dependent RNA polymerase (RdRp), which is also known as an RNA replicase.** **See here for a brief description of types of RNA viruses and their genomes**.

This section will cover **the essentials of the life cycles of a range of RNA plant and animal viruses** in a comparative manner, **from entry into, to release from the host cell**, highlighting similarities among and differences between the main groups of viruses mentioned above, with specific examples where relevant.

Virus taxa involved

Plant and animal virus taxa to be covered, and their broad properties, are shown in **Table 1**. It can readily be seen that although there are a number of genera and families that are unique to either type of host, there are also a number of viruses in the same families that have different hosts.

These viruses seem to be connected in that if they do not also infect arthropods, then they are related to viruses that do.

Plants, fungi and arthropods were the first complex organisms to colonise the terrestrial environment; a close association therefore developed between them **before any chordates emerged from the oceans**, to which arthropods then subsequently adapted as new hosts - meaning that similar arthropod-derived viruses could have

adapted to very dissimilar alternative hosts. **It is interesting that most plant viruses are ssRNA(+) and non-enveloped, while most animal viruses are enveloped**: this could mean that early terrestrial plants contained mainly ssRNA(+) viruses, which then developed in isolation until terrestrial arthropods emerged. **It may also mean that envelopes confer no survival advantage on plant viruses**.

Other material on **virus evolution** may be found here.

All RNA viruses have linear genomes, without significant terminal repeat sequences, and all employ RdRps. These are **template-specific, but do not have proofreading ability**, and do **not make use of RNA primers for replication**, as do all DNA polymerases. **The RdRps also all specifically recognise different origins of replication at the 3'-termini of both (+) and (-) sense RNAs, whatever the type of genome**.

Entry into cells

There is a fundamental difference in mechanisms employed to enter host cells between viruses infecting animal cells and viruses infecting plants. This is because animal cells are separated by barriers far less formidable than the thick, rigid and impermeable cell walls consisting of cellulose and pectin that separate plant cells from one another. **This is covered in a general way here**.

Plant viruses

Because **plant cell walls are so thick** compared to the sizes of the viruses infecting them (>10 m m compared to largely <1 m m), plant viruses have not evolved mechanisms similar to those of **bacteriophages** for entering their host cells. The only ways that viruses can enter plant cells to cause a primary infection are via:

1) a purely **mechanical** injury that breaches the cell wall and **transiently breaches the plasma membrane** of underlying cells;
2) similar **gross injury** due to the mouthparts of a **herbivorous arthropod**, such as a beetle;
3) **injection** directly into cells through the **piercing mouthparts of sap-sucking insects or nematodes**;
4) **carriage into** plant tissue on or in association with cells of a **fungal parasite**;
5) **vertical transmission through infected seed** or by vegetative propagation;
6) transmission via **pollen**; and
7) **grafting** of infected tissue onto healthy tissue.

For example, the **ssRNA(-)** viruses *Tomato spotted wilt virus* (TSWV) and *Crimean-Congo haemorrhagic fever virus* (CCHFV) - both in family ***Bunyaviridae*** (see Table 1) - **share a common particle morphology, and infect the cells of their respective arthropod hosts in similar ways**: that is, by a **specific attachment** and a **fusion or phagosomal** uptake mechanism. CCHFV also infects the the cells of its mammalian hosts similarly (see below). However, **TSWV infects plant cells by injection directly into cells via the piercing mouthparts of its insect vector**, the Western flower thrips, and not via membrane interactions.

Once virions are in the cytoplasm, they are generally **uncoated** to some extent by a variety of processes, including simple dissociation and/or enzyme-mediated partial degradation of the particles, to release the viral genome as a **naked RNA or as a nucleoprotein complex**.

Animal viruses

The initial phase of cell entry starts **when attachment proteins on the virion surface attach to specific receptors on the cell surface**. Both attachment proteins and receptors are normally **glycoproteins**; the cellular proteins can be things like transplantation markers (MHC proteins), adhesins, or simply sialyloligosaccharides (sugars attached to glycoproteins) in the case of ortho- and paramyxoviruses. The attachment is normally **temperature- and pH-dependent**, and is due to the same sorts of molecular structural complementarities - "lock and key" fit - as occur with enzyme-substrate and antibody-antigen binding.

There are essentially **two different paths** that are followed for entry into the cell: these are

- **receptor-mediated endocytosis, and**
- **direct membrane fusion.**

The first is perhaps the primary means of viral cell entry, and is simply a subversion of a normal cellular process. Virus particles become attached at multiple sites to cellular receptors, as these consolidate within the plasma membrane. If these complexes migrate to coated pits, they are **internalised as clathrin-coated vesicles** as part of normal **endocytosis**. These vesicles quickly fuse with **endosomes** and then **lysosomes**, which renders their internal environment considerably more acidic and introduces a host of **degradative proteases, lipases, and other enzymes.** The pH shift generally triggers conformational changes in the attachment protein complexes, which in the case of enveloped virions, may expose lipophilic "fusion domains" that allow fusion of the viral envelope and the vesicle membrane. This has been show to occur with **orthomyxoviruses**, for example. In the case of non-enveloped virions, pH-induced conformational changes in the capsid may cause increased **hydrophobicity / lipophilicity,** which will allow interactions with the vesicle membrane that can cause pore formation. This is known to occur with **picornaviruses**. In either case, **the result is the entry of an RNA-protein complex (=nucleoprotein or nucleocapsid) or of naked RNA into the cytoplasm**, which is the most important part of the uncoating process.

Direct membrane fusion as a mode of entering cells is possible only with enveloped viruses, and is common among **paramyxoviruses**. The viruses require a **fusion-promoting protein** on their virion surfaces, which, in the presence of consolidated receptor-attachment protein binding, promotes fusion of cell and virion membranes and the release of the nucleoprotein into the cell cytoplasm. **This is a pH-independent process**, and may occur at the cell surface, or within an **endosomal** vesicle.

Genome expression and replication

The replication of these viruses is intimately involved with the expression of their genomes: **all of the viruses** must produce **all or most of the components of an RdRp**, and often other proteins as well, in order to transcribe full-length complementary RNA molecules from RNA templates (**see Figure 1**). Whereas **modes of entry of the viruses split largely along host lines**, the exact type of genome of the virus determines the mode(s) of expression and replication. For example,

- **ssRNA(+) genomes may be wholly or partially translated upon entry into the cell** to produce the RdRp, followed by synthesis of full-length complementary RNA(-) and then of full-length RNA(+), and often also of subgenomic mRNA(s).
- **ssRNA(-) genomes must be accompanied into the cell by RdRp for subgenomic mRNAs to be transcribed** before translation is possible.
- **dsRNA genomes are transcribed conservatively** from within virion-derived nucleoprotein complexes, and new genomes are transcribed by newly synthesised RdRp from the mRNAs so liberated.

Additionally, **all plant-infecting viruses possess one or more movement-related protein** (MP) genes: these are very varied, although there are distinct groups of them, and **they appear to derive from host plant genes for chaperonins and plasmodesmata-associated proteins** (Melcher, 2000).

ssRNA(+) genomes

All of these viruses have wholly or partially translatable genomes, and as a result **are usually infectious as naked RNA**. Apart from this common feature, there are few other evolutionary similarities. After partial or complete uncoating upon entry into the cytoplasm, the genomic RNA is recognised by the translation initiation factors and ribosomal subunits and **translation of the open reading frame (ORF) nearest the 5' end of the RNA(s) is initiated**. If there are still proteins bound to the RNA, which in the case of plant viruses is most likely, **this process efficiently strips them off**. While all ssRNA(+) genomes have at least one ORF accessible for translation, and those with multicomponent genomes will have more than one, expression of any with more than one ORF per genome segment **will suffer from the limitation that the eukaryotic translation machinery is**

heavily biased to expressing only the 5'-proximal ORF. Thus, these viruses have evolved two main strategies for expressing their whole genomes. These are:

- **expression of the whole of a genome component as a single ORF**, which is then proteolytically processed to yield smaller protein products;
- **expression of 5'-distal ORFs via subgenomic mRNAs**.

The first strategy is typical of a group of viruses including the *Picornaviridae* and *Potyviridae*: these viruses make use of **co- and post-translational cleavages** by virus-coded endoproteinases, as well as of sequential and sometimes alternative cleavages, in order to make a large number of proteins as well as to regulate their own replication (**see Figure 2**) (Rueckert, 1996). The strategy results in **near-equimolar amounts of the different proteins being made**; because this is undesirable in the case of RdRp, **the replicase complex for these viruses is "single-use"**, in that a freshly-synthesised RdRp polyprotein is needed to initiate replication on each new template. Another means of removing excess components is employed by potyviruses, which **export their so-called "nuclear inclusion body" (NIa and NIb) or replicase protein subunits to the nucleus** to be sequestered as insoluble aggregates. Comoviruses and nodaviruses resemble picornaviruses that have been cut into two segments; the bymoviruses in family *Potyviridae* similarly resemble a cleaved potyvirus genome. **All of the viruses in a picornavirus-like supergroup** (picorna-, poty-, como-, calici- and other viruses) **use an RdRp that makes use of a protein as a primer for both (+) and (-) sense RNA production**: this is part of the precursor RdRp and is cleaved off as elongation of the initial complex occurs, to become a 5'-genome-linked protein, usually known as Vpg. **Viruses in this supergroup tend to have 3'-polyadenylated (polyA) genome segments**, with the polyA sequences being part of the genome and copied into polyU in (-) sense RNA. The processed polyprotein strategy is shared by flaviviruses, whose genomes also contain a single large ORF; however, these virus genomes are not polyadenylated, and do not have a Vpg.

The second strategy is **typical of two supergroups of viruses usually termed the alpha-like and carmo-like viruses** in terms of sequence and genome organisation affinities (Strauss *et al.*, 1996), and is exemplified by the **generic tobamoviruses**. The genome of *Tobacco mosaic virus* (TMV; **see Figure 3**) is initially expressed by means of **translation of two proteins from its single 5'-proximal ORF**: a 126 kDa protein is expressed ten times more abundantly than a 183 kDa protein, which is the product of **read-through of a stop codon** near the 3'-end of the large ORF. **Both proteins are replicase components**, along with at least one host protein (Lewandowski and Dawson, 2000). Further expression only occurs after synthesis of full-length RNA(-) from the RNA(+) template, and **transcription of subgenomic mRNAs** encompassing one or more of the 3'-proximal ORFs from this by internal initiation of transcription at **RNA "promoter" sequences**. These "promoters" differ from origins of replication in that **they are not copied into mRNA transcripts**, which are therefore not replicatable although they contain the 3'-origin. **This allows temporal separation of early (=regulatory) and late (=structural) genes**. The virus genomes usually have a cell mRNA-like 5'-cap structure (7-methyl guanosine triphosphate, m^7Gppp), and **many have a complex 3'-terminal structure**, often resembling a tRNA (and aminoacylatable), and otherwise a series of **pseudoknots**. This serves both to protect the 3'-end from exonucleases and as a **specific RdRp recognition site**.

Some viruses utilise a mix of both strategies: for example, togaviruses (eg: *Sindbis virus*, genus *Alphavirus*) translate a **proteolytically processed polyprotein that includes a RdRp**; subsequent replication results in **subgenomic mRNA production from the 3'-half of the genome**, and production of **another processable polyprotein**. Viruses with segmented genomes may produce single proteins from single segments, or, in the case of bromoviruses or tobraviruses, for example, may have both monocistronic and multicistronic segments. In any case, all subgenomic mRNAs will be 3'-coterminal, as there appear to be no mechanisms for transcription termination. Coronavirus mRNAs appear to **all have the same 5'-terminal leader sequence of 50-80 bases**, indicating a more complicated form of transcription than recognition of internal promoter sequences in RNA(-) molecules.

Replication in all cases involves an initial transcription of full-length RNA(-) from an infecting RNA(+) template, and transcription from this of RNA(+), and perhaps also subgenomic mRNA. Replication complexes are usually closely associated with membrane complexes derived from the ER or perhaps nuclear membranes, and **free RNA(-) is not found**. In some cases it has been shown that coat protein (CP) helps regulate the expression of RNA(+), in that cp^- mutants accumulate approximately equal amounts of both senses of RNA, while normal viruses accumulate much more RNA(+), especially as the CP concentration increases late in infection. dsRNA forms of viral genomes and of subgenomic RNAs can be isolated from infected cells for many ssRNA(+) viruses, including

most plant viruses, some picorna-like insect viruses, and coronaviruses: **this may be how some dsRNA viruses originated** (see **below**).

ssRNA(-) viruses

These viruses seem to be an evolutionarily recent development, as they infect only higher eukaryotes, like arthropods, vertebrates, and higher plants. **The viruses infecting plants probably do so as a result of close association of insects and host plants in recent evolutionary times**; most of these still also infect an insect vector / alternative host. The group includes the only taxonomic order among RNA(-) viruses: this is the order *Mononegavirales*, including the families *Orthomyxoviridae*, *Paramyxoviridae*, and *Filoviridae*, **all of which have single-component genomes and share a basic genome arrangement and significant sequence similarities** (**Figure 4**). **Nearly all of the RNA(-) viruses in Table 1 share a similar major RdRp subunit (L-type protein gene)**; there are also similarities in their nucleoproteins (N or NP genes).

Replication of all the viruses commences with the transcription by virion-associated RdRp of usually monocistronic mRNAs from genomic RNA(s) in the newly uncoated nucleoprotein complexes (**see Figure 1**). For the **segmented** genomes of bunya- and orthomyxoviruses, **this usually means a single mRNA per segment**; for the non-segmented **mononegaviruses**, this means **multiple transcription initiation and termination events** on a full-length RNA(-), at **intergenic repeated sequences**, with transcription apparently usually initiating at the genomic 3'-end with **synthesis of a 50-base leader**. Transcripts are capped and polyA tailed; **the RdRp complex (consisting of L, N / NP and other proteins) adds caps**, while **tails** are apparently added by **RdRp stuttering at short polyU repeats** at the end of genes. Independent transcription events in paramyxoviruses **allow control of level of expression**: these viruses transcribe far more mRNAs for structural protein genes at the 3'-end of the genome than for regulatory genes at the 5'-end, **possibly due to the progressive failure of the RdRp complex** at reinitiating multiple times down the length of the RNA(-) (Lamb and Kolakofsky, 1996).

Production of full-length RNA(+) rather than of mRNAs is **triggered by binding mainly of newly-synthesised viral N** (nucleoprotein) **but also of P (RdRp minor subunit) proteins** to the 5'-leader sequence, somehow causing the RdRp to ignore all termination and polyadenylation signals. The RNA(+) is then used as template for RNA(-) transcription: this also has a 5'-leader, **which is also recognised as an assembly origin by N protein**. Thus, concomitant genome or anti-genome synthesis and nucleoprotein assembly occur, **with a bias for (-) strand synthesis**, possibly due to preferential recognition of the (+) strand 3'-origin.

The **trisegmented bunyaviruses** (**see Figure 5**) have an interesting **"cap stealing" strategy:** virion-associated L or replicase protein **cleaves cellular mRNAs 12-18 nt from their 5'-ends**, and uses the capped leaders to prime transcription of non-polyadenylated mRNA on the three virion L, M and S (-) RNAs. **These mRNAs are shorter than the genome segments**, as transcription is apparently terminated by hairpin loops. It is not certain how bunyaviruses switch from mRNA to full-length RNA(+) transcription; however, **the N protein may act similarly to the way it does in mononegaviruses**. Two genera of the *Bunyaviridae* also have **at least one ambisense RNA**: phleboviruses and tospoviruses transcribe a mRNA from the 3'-end of the S segment RNA(+); the plant-infecting tospoviruses in addition have an ambisense M RNA, with the extra gene (5'-end of M RNA(-)) **being involved in movement functions in plants** (Schmaljohn, 1996).

The 8 component orthomyxoviruses are unusual in a number of respects, including having the **most segmented genome among ssRNA(-) viruses**, and the fact that **both transcription and replication occur in the nucleus**. Transcription occurs by the **same cap-stealing mechanism as for bunyaviruses**, but with **termination and polyadenylation occurring as for mononegaviruses**, with RdRp stuttering at short polyU repeats at the end of genes. The RdRp is also different to those of the other viruses, with **3 viral subunits** (PB1-2 and PA). The switch from primer-dependent mRNA synthesis to RNA(+) and RNA(-) synthesis occurs after protein synthesis, **possibly due to free NP binding**. There is evidence of **temporal regulation of expression**, with regulatory proteins being made in greatest amounts at early times, and structural proteins later: **this is due to selective replication of specific template RNA(-) into mRNA** (Lamb and Krug, 1996).

Arenaviruses have a 2-component genome, each segment of which **also has a (+) sense ORF at the 5'-end of RNA(-)**, which is transcribed from RNA(-) as an mRNA. **Arenavirus transcription also makes use of some kind of priming**, possibly by short capped oligoribonucleotides, and mRNAs are subgenomic and not polyadenylated: transcription is apparently terminated by intergenic stem-loop structures. **Transcription occurs from both full-**

length RNA(-) and RNA(+) templates, with "early" products including the L and N proteins and "late" products including a membrane GP protein and a Z protein. **There is a clear switch between transcription and replication**, but although the N protein may be involved, this is not proven (Southern, 1996).

Commonalities in expression and replication of ssRNA(-) viruses appear to include **distinct transcription and replication functions for the RdRp**, probably triggered by **binding of the virion nucleoprotein** (N or NP) subunits. Thus, **both RNA(-) and RNA(+) may be found complexed with N proteins in replication complexes**. As for ssRNA(+) viruses, glycoproteins (GPs) are generally expressed as are cellular trans- or outer membrane proteins; that is, they have signal sequences that result in translocation into the rough ER during translation, and are subsequently glycosylated according to signals perceived by the cellular machinery.

dsRNA viruses

While it is tempting to speculate that these viruses are the monophyletic survivors of a pre-DNA dsRNA genome era, the truth is that, although there is **very wide diversity among dsRNA viruses**, at least some of them may descend from ssRNA(+) viruses. Two distinct groups of dsRNA viruses have polymerase affinities with alpha-like and poty-like viruses respectively (Smart *et al.*, 1999; Gibbs *et al.*, 2000). **Thus, the viruses are certainly polyphyletic in origin**, and there is almost certainly a wide variety of mechanisms used for expression and replication. However, many of the viruses have not been well studied, so details are lacking.

Reoviruses are the best-studied dsRNA viruses. Representatives of the family infect **plants, animals, and insects**, and many infect an insect vector as well as an animal or plant alternate host. **The viruses all have a double capsid structure**, the outer layer of which is stripped off, partly due to proteolysis, during endocytotic entry. **Naked core particles in the cytoplasm are able to transcribe capped and non-polyadenylated genome-segment-length monocistronic mRNAs**, via an RdRp activity associated with the insides of the hollow spike structures at 5-fold rotational axes of symmetry. These are extruded into the cytoplasm as they are synthesised, and are translated. **Viral products accumulate as viroplasms**: associations of viral structural and polymerase proteins and mRNAs result in **assembly of immature particles, inside which mRNAs are transcribed to give RNA(-) molecules** with which they become base-paired. **This is the best-characterised example of conservative replication for any organism**. New "core" particles also produce mRNAs, but these appear to be largely uncapped (Nibert *et al.*, 1996).

Partitiviruses appear to follow much the same genome expression strategy as reoviruses, in that monocistronic mRNAs are transcribed, which can act as templates for RNA(-) transcription (Strauss *et al.*, 2000).

Birnaviruses have 2-component monocistronic genomes, with 5'-Vpgs, and transcribe genome-length capped mRNAs in virions in the cytoplasm, which then serve as template for the newly synthesised RdRp. **Unlike viruses discussed above, one segment (A) encodes a polyprotein**, which is cleaved to give virion proteins VP1, VP2 and VP3, while the other segment (B) produces a polymerase with a capping function (Roner, 1999).

***Trichomonas vaginalis* viruses are unusual among dsRNA viruses** in having **single-component genomes with multiple ORFs** (Bessarab *et al.*, 2000). Details are sketchy, but there are similarities with the larger totiviruses. These virus genomes have two large overlapping ORFs, and express a protein from the 5'-proximal ORF, and a larger fusion protein from both ORFs by means of a ribosomal frameshift. **Partitiviruses may derive from totiviruses**, as their polymerase sequences show some similarity (Ghabrial, 1998).

A newly characterised group, tentatively named the endornaviruses, were formerly regarded as **dsRNA plasmids of plants**. They resemble the potyvirus-resembling **hypoviruses** in lacking particles, but may be transmitted by seed or by grafting, and **may have their origin within the alpha-like virus cluster**: their 10kb dsRNAs have a single ORF with recognisable helicase and polymerase motif similarities (Gibbs *et al.*, 2000). **Presumably these exist as RdRp-associated replicative intermediates and multiply semi-conservatively, like ssRNA(+) viruses**.

Assembly and exit

The processes of assembly of the virions are as varied as their structures; however, **there is a logical divide between those with membranes, and those without**. The former tend to be considerably more complex than the latter, which may be as simple as a nucleoprotein composed of a single type of protein. **There is a commonality between all of the viruses in that their core nucleoproteins assemble either as helices (usually) or as isometric particles** (Harrison *et al.*, 1996). This assembly is usually a simple process, but often very **specific**, and is **driven by increasing concentrations of genomic or pre-genomic RNA and of structural protein**. Assembly takes place in the cytoplasm for all except the orthomyxoviruses, which assemble nucleoproteins containing N, PB1, PB2, and PA proteins in the nucleus, from where they are exported to the cytoplasm after association of the complexes with the M_1 or matrix protein. **The interaction of protein and RNA may be promoted by their sequestration in inclusion bodies or viroplasms**, which are often associated with elaborations of internal ER-derived membranes.

For some of the **simple naked isometric viruses**, **specific nucleation of assembl**y at low CP concentration is followed by **complete nucleocapsid assembly as CP concentration increases**. For **picornaviruses**, however, there is a complex assembly process. One model of the process involves assembly of a complete RNA-free provirion. This then undergoes autolytic protein cleavage due to its associating with genomic RNA, which is then encapsidated due to a complicated structural reorganisation (**see here for an animation**). In another model, viral RNA is complexed with smaller protein aggregates, which are then further processed. **Reoviruses** also have a complex assembly process, starting with the mRNA-protein complex, which becomes a RNAse-sensitive double capsid that does not contain nonstructural (NS) proteins. This synthesises RNA(-) strands, and then undergoes some structural changes to become RNAse-insensitive and to have NS proteins associated with it. **Virions may collect in amorphous or paracrystalline arrays** inside infected cells: **plant viruses especially may accumulate at very high concentration**s. Release of such virions may be induced by **virus-induced cell lysis**, such as is the case with some picornaviruses. However, in most cases **release is by cell death followed by membrane degradation**.

For most of the more complex virions, such as those of the mononegaviruses and coronaviruses, **nucleocapsid or nucleoprotein assembly is followed by association of these with matrix (M) proteins**. In the case of rhabdoviruses, **soluble M protein appears to condense the loosely helical nucleoprotein aggregate into a more compact form** resembling the virion interior (Wagner and Rose, 1996). With this virus and others, **membrane-bound M protein binds specifically with nucleoproteins**, first to localise the complexes to **membrane sites** that include the plasmalemma, and internal compartments such as the Golgi apparatus, ER, and elaborations of these. Increasing the number of M protein-nucleoprotein interactions causes recruitment of M proteins, which causes the membrane to fold around them in the start of the act of "budding". **Membrane glycoproteins (GP)** are an essential part of all enveloped viruses; **the cytoplasmic stubs of these also interact with and are recruited by the M proteins** (where present) to provide a virus-specific exterior to the budding virion. **Virions can bud without glycoproteins in some cases** (eg: coronaviruses); however, **these are non-infectious**. Localised patches of membrane in infected cells may have M and GPs associated with each other, which are then specifically bound by free nucleoprotein complexes.

Bunyaviruses, arenaviruses, and togaviruses do not have M proteins: instead, these virus nucleocapsids have a d**irect interaction with the cytoplasmic portion of transmembrane GPs**. In the case of bunyaviruses the GPs are embedded in intracellular vesicles: cytoplasmic NPs then bud into the vesicles by association with the cytoplasmic portions, to produce enveloped virions within the vesicles.

Release of enveloped virions is a simple consequence of the final act of assembly. When a membrane containing M and/or GPs has completely folded around a nucleoprotein, it produces a vesicle: if this is external to the cell, then it has budded; if it is inside another vesicle, such as a post-Golgi vesicle, then the fusion of this with the plasmalemma in the normal course of cellular vesicle trafficking will result in extracellular budding.

Virions of ortho- and paramyxoviruses have **haemagglutinin glycoproteins** (HAs) that bind **sialyloligosaccharides**: as these HAs may contain the same sugars, both viruses also have **virion-associated neuraminidases** (NAs), which enzymatically destroy the receptors to **negate the possibility of virions binding to one another** during or after budding (Lamb and Krug, 1996; Lamb and Kolakofsky, 1996).

Plant virus genomes may also move from cell to cell via plasmodesmata, or complex membrane-lined channels that penetrate the cell wall: **this is a complex process involving virus-coded MP(s)** which specifically bind viral RNA, and in many cases involves **transport of a nucleoprotein complex which is not an assembled capsid**. It is possible that genomes of plant reoviruses and other dsRNA plant viruses may **move as ssRNA(+) nucleoprotein complexes rather than as dsRNA**.

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Glossary:

capsid: protein shell made from virus-specified protein, usually contains viral genome

virion: particle containing all or part of the virus genome, always composed of a nucleocapsid, may also have extra shells of protein and/or a host-derived membrane envelope containing viral glycoproteins

nucleoprotein: protein-RNA complex (usually unstructured)

nucleocapsid: capsid protein-RNA complex (usually with a regular structure)

ssRNA: single stranded RNA

dsRNA: double-stranded RNA

ssRNA(+): single-stranded RNA of messenger polarity (can be translated directly)

ssRNA(-): single-stranded RNA of anti-messenger polarity(has to be transcribed to express protein)

ambisense RNA: RNA with both messenger and anti-messenger sense polarity

RdRp: RNA-dependent RNA polymerase (also called replicase)

Legends to Figures:

Figure 1.

Pathways of information flow for RNA viruses. Double-stranded (dsRNA) viruses replicate conservatively via a full-length RNA(+) which is transcribed from dsRNA by a virion-associated virus-specific RNA-dependent RNA-polymerase (RdRp). The RNA(+) then acts as mRNA, for synthesis of viral proteins, then as a template for RNA(-) synthesis, to which it base-pairs. Single-stranded (ssRNA) (+)-sense virus genomes initially act as mRNAs, and translate a RdRp component. The RNA(+) then replicates via a full-length RNA(-), which is caught up in replicative complexes and is never free. This is used as template for mRNA transcription if this occurs, by the viral RdRp. Viruses with ssRNA(-) genomes replicate by means of a virion-associated RdRp taken into the cell. This initially acts as a transcriptase, to make subgenomic mRNAs. The genome replicates via transcription of full-length RNA(+). Translation is a cell-specific process; all transcription and replication is done by virus-specific RdRps.

Figure 2.

A scheme describing polyprotein translation and processing for picornaviruses (modelled on poliovirus; see Rueckert, 1996). Vpg = 5'-genome-linked protein; polyA = polyadenylate sequence at 3'-end.

A. Ribosomes initiate at the AUG start codon nearest the 5'-end of the single long open reading frame (ORF; shown in yellow), and translate until the termination codon at the end of the long ORF. Co-translational proteolytic cleavages occur due to two viral proteases: the 2A and 3C proteins. The 2A activity begins autolytically in *cis*; it then acts in *trans;* the 3C cleavages are similar (shown by blue and yellow arrows respectively). The first 2A cleavage produces the P1 structural protein precursor, which is subsequently processed by the 3A protease.
B. Scheme showing the full extent of processing. Regulatory and structural proteins are shown. P1, P2, etc: polyprotein designations. ? = cleavage by unknown mechanism.

   P1 can assemble into pentamers: these are then processed via successive cleavages into VP0, VP3, and VP1, after which 12 pentamers may assemble into icosahedral procapsids. Complete assembly requires genomic RNA, and is accompanied by an apparently autolytic cleavage of VP0 into VP4 and VP2. Some of the P2 and other polyprotein processing accompanies replication: the RdRp may initially include a "3ABCD" complex; initiation of new transcription on a nucleotide covelently bound to the 3B moiety is accompanied by its cleavage from the rest of the complex, to form the Vpg. The remainder of the replicase is capable only of elongation, and cannot initiate replication again.

Figure 3.

Depiction of the expression strategy of the *Tobacco mosaic virus* (TMV) genome. Red arrows indicate host-dependent translation; blue arrows indicate transcription from an RNA(-) template. Solid boxes are open reading frames (ORFs); these are shown in different colours. Hatched boxes indicate proteins. The 5'-ends of the viral RNA and the mRNAs have a 7-methyl guanosine triphosphate (m^7Gppp) cap structure; the 3'-tRNA-like sequence of all RNAs is shown as a cloverleaf structure.

The ORF nearest the 5'-end of genomic RNA is translated into a 126 kDa protein. A "leaky" stop codon allows infrequent translational readthrough (1/10 times) to give a 183 kDa protein product. These two proteins together with (a) host protein(s) constitute the RdRp and replicase. This transcribes a full-length RNA(-) from genomic RNA(+). The RdRp can also transcribe the RNA(-) into RNA(+), and, by recognition of two or more "RNA promoter" sequences within the RNA(-) sequence, into at least two nested subgenomic mRNAs: all products of transcription from RNA(-) share the same 3'-terminus. Only the 5'-proximal ORF of each mRNA is translated.

Figure 4.

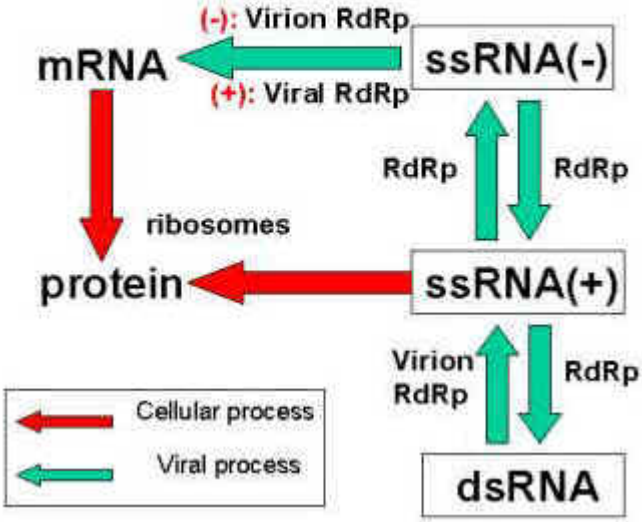
Depiction of gene order and function and designations in viruses of the order *Mononegavirales*. The genomic 3'-end is a free -OH group; the 5'-end is phosphorylated but uncapped. The leader sequence is about 50 bases long and conserved in viruses in the same genus. Intergenic sequences are conserved within a virus, and include polyU sequences of 4-7 bases. The different open reading frames (ORFs) are shown in different colours. The gene order is

conserved among viruses in the order, as is the function. N/NP are nucleoproteins which bind viral (-) and (+)-sense RNA; NS proteins are non-structural and involved in aspects of regulation; M or matrix protein is bound by assembled nucleocapsids and binds the cytoplasmic portion of the G/GP membrane glycoproteins, which span the cell-derived envelope in the assembled virions; the L protein is the main component of the RdRp, and is incorporated into virions.

Figure 5.

Depiction of genome components and expression strategy of bunyaviruses. All viruses have three genomic ssRNA(-) components: these are L, M and S, coding for polymerase (L), glycoproteins (G1, G2) and non-structural (NSm), and nucleoprotein (N) and non-structural (NSs) proteins, respectively. Different open reading frames (ORFs) are shown in diferent colours. Genus-specific ORFs are indicated: only bunyaviruses have an extra NSs (small non-sructural protein) ORF internal to the N ORF; tospo- and phleboviruses have an ambisense (both (+) and (-) sense ORFs) S component, with an mRNA being transcribed off the RNA(+) form of the genome. Tospoviruses in addition have an ambisense M component, with the extra 5'-ORF coding for a host-derived movement protein (MP).

Pathways of Information Flow for RNA Viruses



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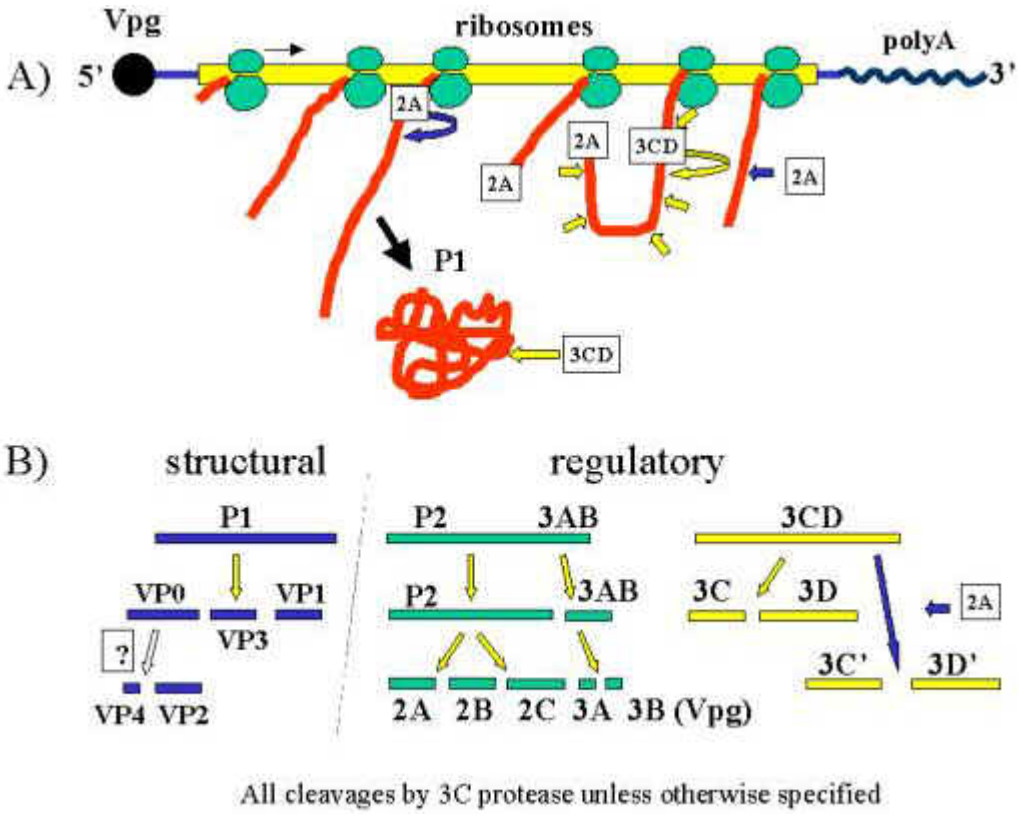
Scheme showing picornavirus genome expression strategy.
A: Depiction of genome translation and co-translational proteolytic cleavages
B: Detail of proteolytic processes showing final products and intermediates



All cleavages by 3C protease unless otherwise specified

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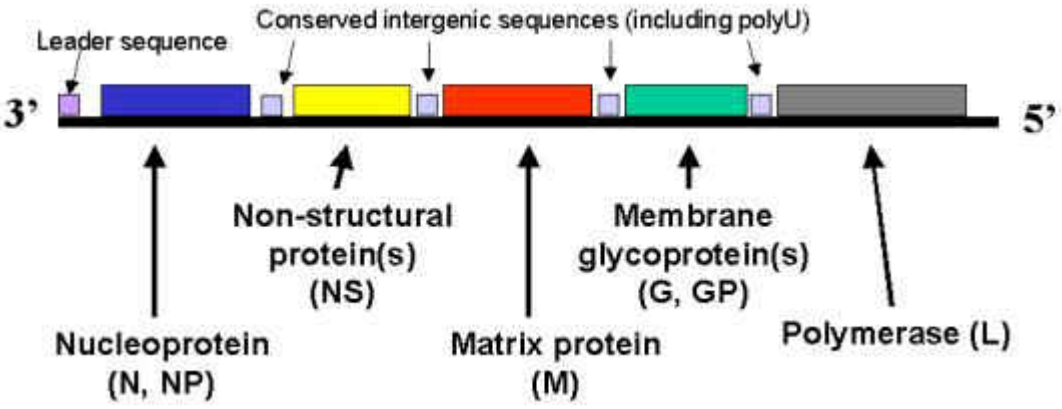
Gene order and function and designations in viruses of the order *Mononegavirales*.

Key: N/NP are nucleoproteins which bind viral (-) and (+)-sense RNA; NS proteins are non-structural and involved in aspects of regulation; M or matrix protein is bound by assembled nucleocapsids and binds the cytoplasmic portion of the G/GP membrane glycoproteins, which span the cell-derived envelope in the assembled virions; the L protein is the main component of the RdRp, and is incorporated into virions.



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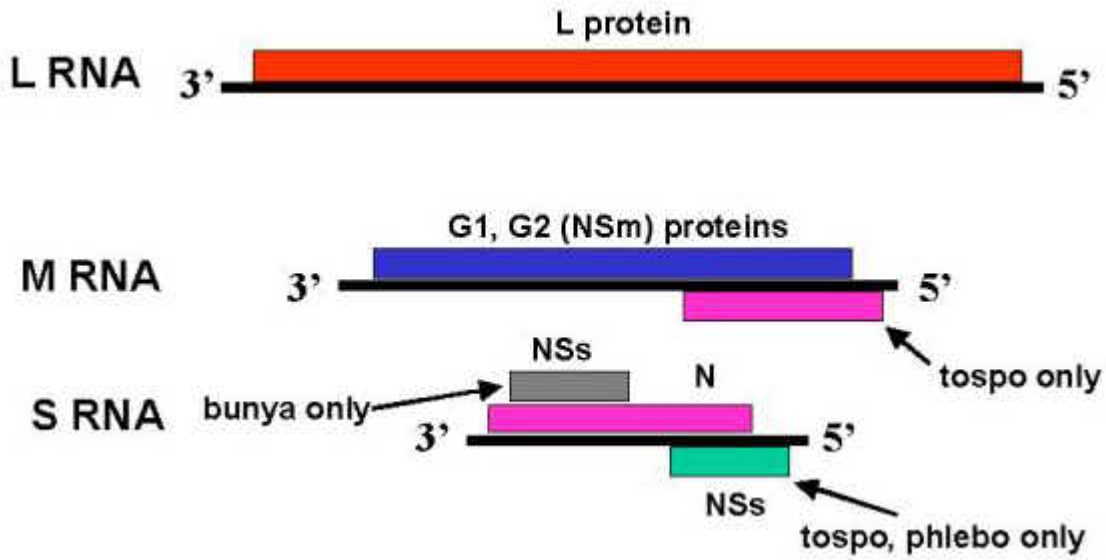
Genome components and expression strategy of bunyaviruses

ORFs are shown in different colours: those above the lines are in the (-) sense, those below in the (+) or mRNA sense

. Explanation: ORFs are polymerase (L), glycoproteins (G1, G2) and non-structural (NSm), and nucleoprotein (N) and non-structural (NSs) proteins



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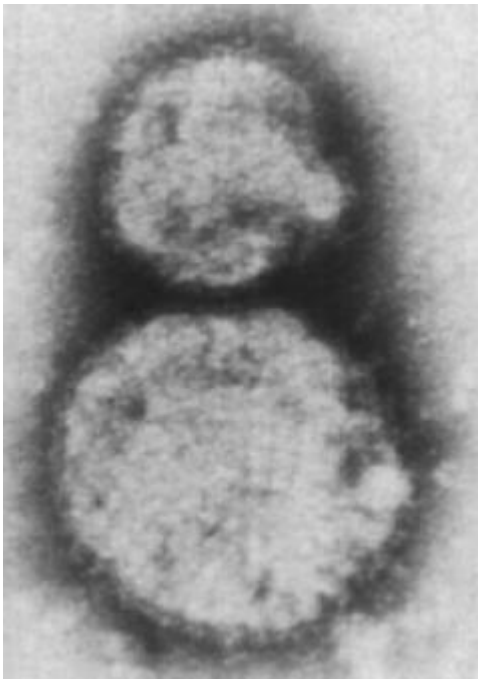

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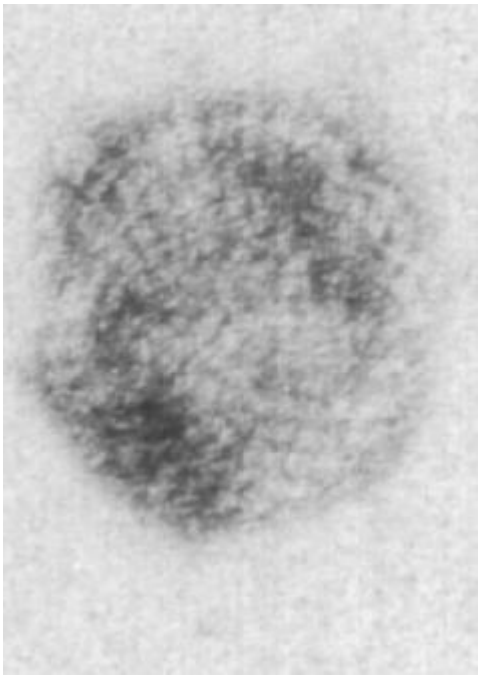
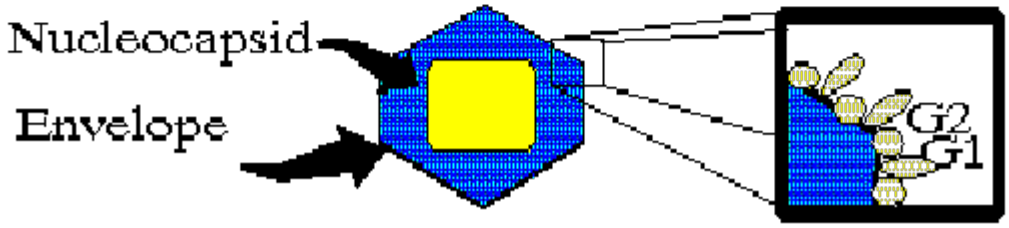
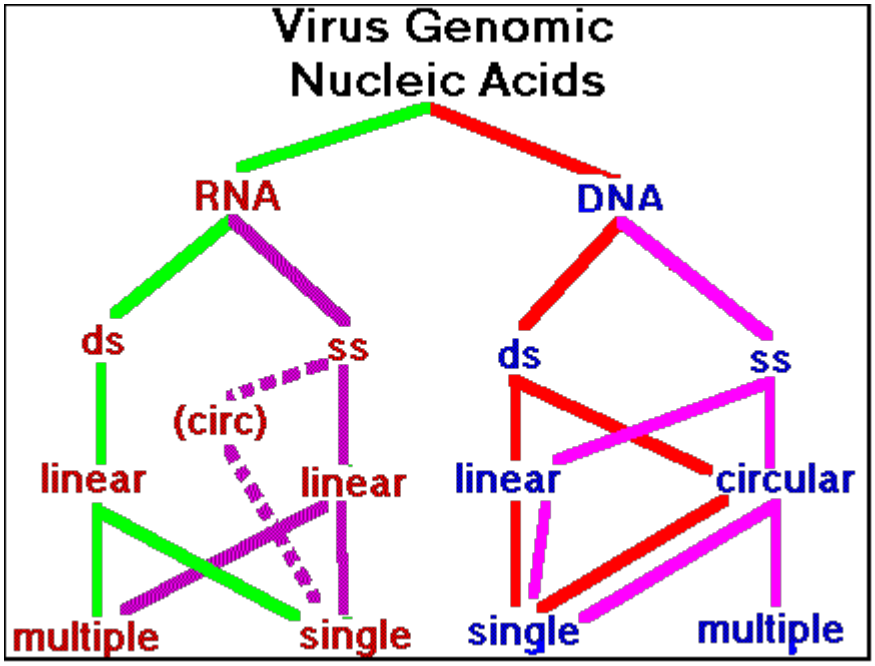


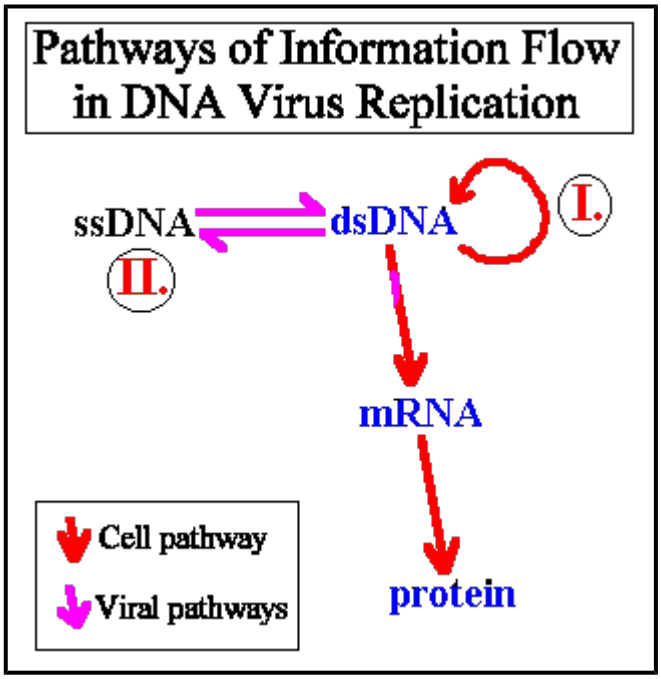
	Segment	Size (nt)	Encodes
	L	~6500	
ORF-L			Polymerase
	M	~3700	
ORF-G1/G2			Envelope
	S	~2000	
ORF-N			Nucleocapsid
ORF-NSs			?



genomes.gif %d×%d pixels



DNA Genome Diversity and Genomic Replication Strategies



Move mouse pointer over circled numbers; click to go to relevant sections

DNA Genome Types:

A) Type I: dsDNA

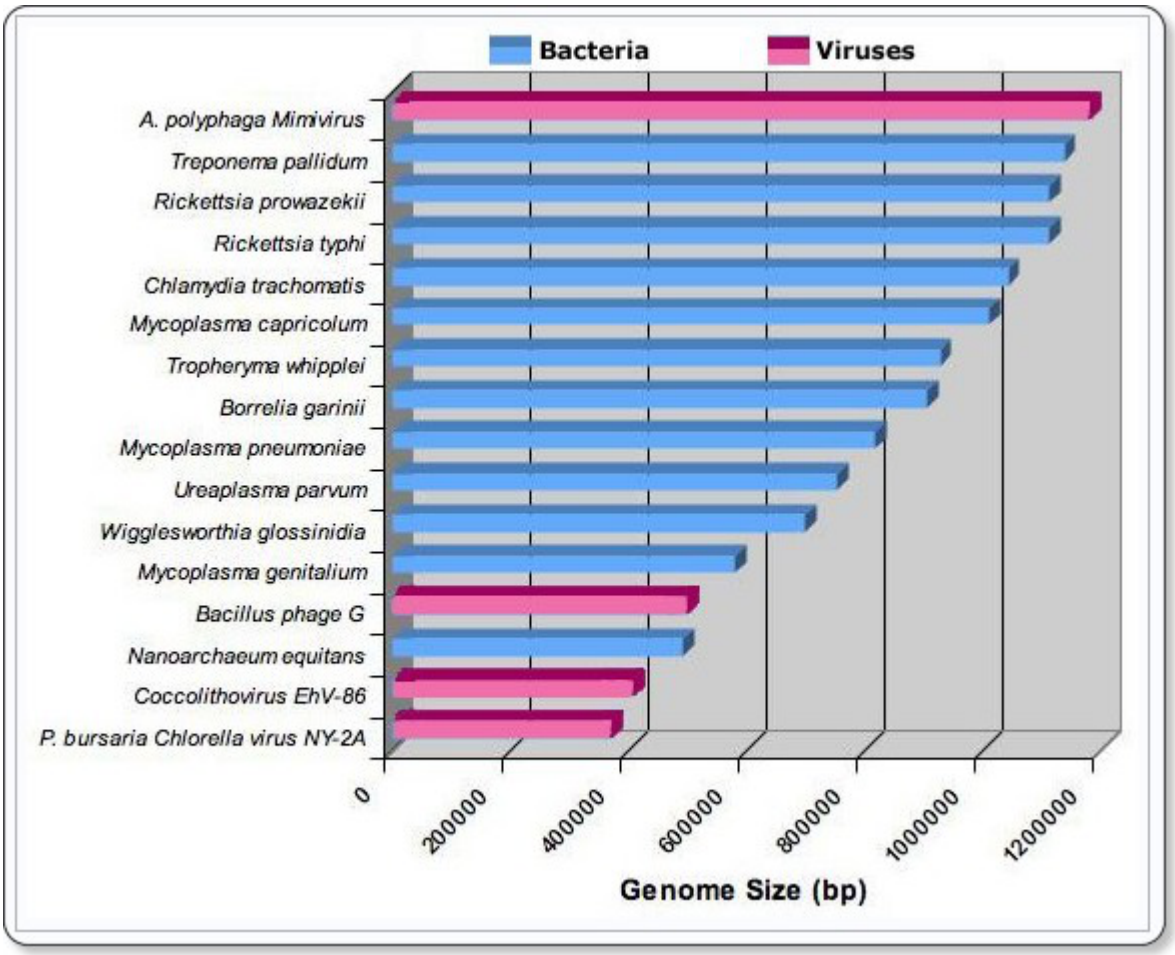
Replication Scheme Diagram

dsDNA viruses include:

- viruses infecting **Eubacteria** and **Archaea**, or phages (eg. the ***Podoviridae***, phages of *E coli*);
- viruses of **higher animals** (***Pox-, Herpes-, Adeno-, Papovaviridae***);
- viruses of **insects** (***Baculo-*** and ***Irido- and Polydnaviridae***);
- and viruses of **eukaryotic algae** (***Phycodnaviridae***);
- viruses of **fungi**

These range in size from:

- 5-8 kb (***Papovaviridae***)
- through 30-40 kb (**lambda phage** and ***Adenoviridae***)
- to about 200 kb (***Baculoviridae***)
- to over 300 kb (***Herpes- and Pox-*** and ***Irido-*** and ***Phycodnaviridae***)
- through ~400 kb (***Phycodnaviridae***)
- up to over 1 million bases (**Mimivirus**).



Marvellous mimivirus « MicrobiologyBytes via kwout

By contrast, the smallest prokaryote genome size is about 500 kb (*E coli* = 4000 kb)

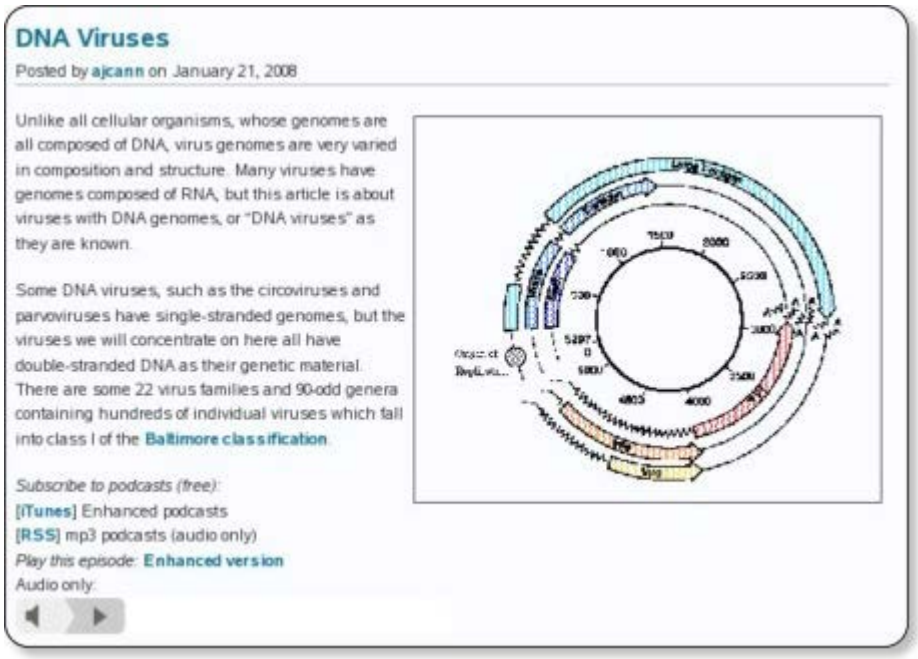
They may have:

- **circular** genomes (***Papova-*** and ***Baculo-*** and ***Polydnaviridae***);
- **linear** genomes (***Adeno-*** and ***Herpesviridae***, some **phages**);
- have **circularly permuted linear** genomes (**phage T4**, some ***Iridoviridae***);
- or **linear genomes with covalently closed ends** (***Pox-*** and ***Phycodnaviridae***).

All viruses except ***Polydnaviridae*** have **single-component genomes**; the latter have **multiple components ranging in size from 2 - 20 kb**, and the number which constitute an individual genome is not known.

Replication of the viruses is in all cases by the **semi-conservative method favoured by cellular genomes**; however, smaller circular genomes (eg. ***Papovaviridae*)** replicate by means of **bidirectional**

replication forks from a single origin, like some plasmids. Among the viruses of **Eukarya**, replication mainly **occurs in the nucleus**, using **cellular enzymes** such as polymerases, methylases, etc. However,the replication of **Poxviruses,** some **Baculoviruses** (granulosis group), and some of the replication of **iridoviruses**, takes place in **virus-specified "inclusion bodies" in the cytoplasm**, using **viral-coded** enzymes, most important of which are **DNA-dependent DNA polymerases**.



DNA Viruses « MicrobiologyBytes via kwout

Reference:

Virus Taxonomy: Sixth report of the International Committee on Taxonomy of Viruses (FA Murphy *et al.*, Eds.); Springer-Verlag, Wien, 1995.

(return to Top)

B) Type II: ssDNA

Replication Scheme Diagram

ssDNA viruses include organisms infecting:

- bacteria ("bacteriophages", eg. ***Inoviridae, Microviridae***),
- mammals (***Circoviridae***, ***Parvoviridae*** click for image),
- birds (**circovirus**-like organisms),
- and plants (***Geminiviridae***, banana bunchy top-like viruses or **Nanoviruses**).

They can have:

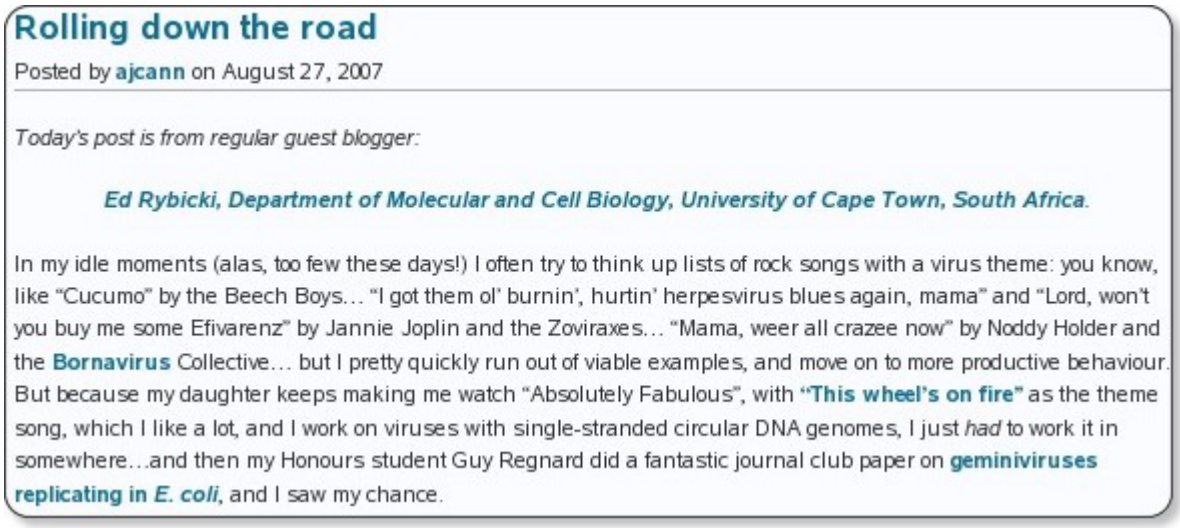
- linear single-component genomes (***Parvoviridae***),
- circular single-component genomes (***Microviridae, Inoviridae***, ***Circoviridae***, some ***Geminiviridae***),

- circular two-component genomes (some ***Geminiviridae***),
- or circular multicomponent (>3) genomes (**Nanoviruses**).

The genomes are all relatively small:

- the ***Circoviridae*** have genomes of about 3 kb;
- ***Parvoviridae*** have genomes of 4-5 kb;
- the ***Geminiviridae*** about 2.7 - 5.4 kb (depending on whether are mono- or bi-component);
- the ***Microviridae*** (including the famous phiX 174) about 4.5 kb;
- the ***Inoviridae*** and the **Nanoviruses** about 5-6 kb.

Replication of all of the viruses requires formation of a **"replicative form" (RF) double- stranded DNA intermediate**: this is formed soon after infection, almost certainly by the host cell DNA polymerases engaging in **"repair" of the ssDNA**.



Rolling down the road

Posted by **ajcann** on August 27, 2007

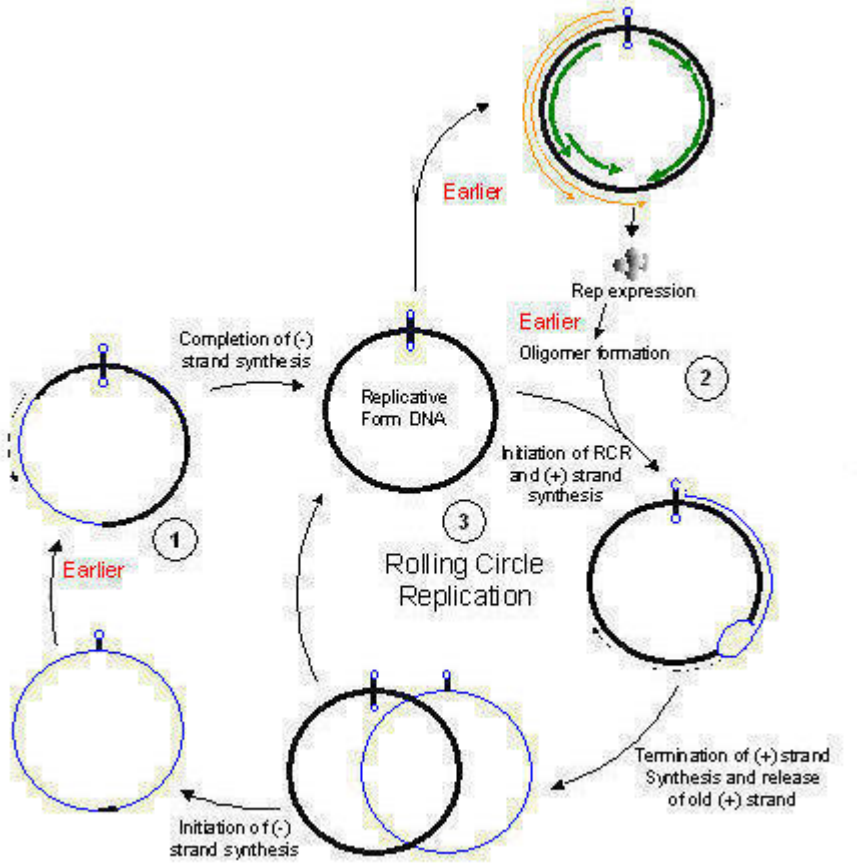
Today's post is from regular guest blogger:

Ed Rybicki, Department of Molecular and Cell Biology, University of Cape Town, South Africa.

In my idle moments (alas, too few these days!) I often try to think up lists of rock songs with a virus theme: you know, like "Cucumo" by the Beech Boys… "I got them ol' burnin', hurtin' herpesvirus blues again, mama" and "Lord, won't you buy me some Efivarenz" by Jannie Joplin and the Zoviraxes… "Mama, weer all crazee now" by Noddy Holder and the **Bornavirus** Collective… but I pretty quickly run out of viable examples, and move on to more productive behaviour. But because my daughter keeps making me watch "Absolutely Fabulous", with **"This wheel's on fire"** as the theme song, which I like a lot, and I work on viruses with single-stranded circular DNA genomes, I just *had* to work it in somewhere…and then my Honours student Guy Regnard did a fantastic journal club paper on **geminiviruses replicating in *E. coli***, and I saw my chance.

Rolling down the road « MicrobiologyBytes via kwout

	The replication machinery of **geminiviruses** and **nanoviruses** from plants, **circoviruses**, **anelloviruses** and even **parvoviruses** from animals, and the aforementioned phages and even plasmids, all has a common origin - which may extend to the mobilisation mechanism used by bacteria like *Agrobacterium tumefaciens*. A general scheme for ssDNA virus replication can be seen here. **Geminiviruses**, like other ssDNA entities, have a *rep* gene, producing a **Rep** protein: in plants, this is expressed from a double-stranded replicative intermediate form of the genome (RF), and **cleaves the genome (+) strand at a specific *ori* sequence**, binds to the free 5′ end, and then **mediates ligation of the newly-**



displaced (+) strand. The accumulation of ssDNA seems to depend on the production of coat protein, which probably sequesters nascent ss(+)DNA, as CP^- mutants produce no ssDNA.

There is a fair degree of specificity in all this, with viruses having a specified host range, and Reps having specificity for a narrow range of virus genomes.

In the case of **circular genomes in Eukarya** (circoviruses, eg. porcine circovirus; also psittacine beak-and-feather disease agent, chicken infectious anaemia agent; ***Geminiviridae***; BBTRV), these get converted into **cccds-(plasmid-like)-DNA in the nucleus**, and become associated with nuclear proteins and complexes such as nucleosomes.

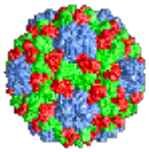
Parvoviruses have an interesting strategy for replicating their genomes, which uses **internal or self-complementarity of genome ends** to get around the problem of how to replicate a **linear DNA genome.** A **virus-specific process** is required both to **nick RF DNA, and to sequester newly-formed genomic ssDNA into assembling particles**: in the case of **Parvoviridae** the first is done by a NS1 protein (which binds the new 5'-terminus resulting from the nick) and the second by the coat protein; in ***Geminiviridae*** it appears as if the first is done by a similarly-acting **Rep** protein, and the second also by the coat protein.

Reference:

Parvoviridae: Fields Virology (2nd Edn), Chapter 62

Virus Taxonomy: Sixth report of the International Committee on Taxonomy of Viruses (FA Murphy *et al.*, Eds.); Springer-Verlag, Wien, 1995.

dsDNA viruses	ssDNA viruses	dsRNA viruses	ssRNA (+)-sense viruses	ssRNA (-)sense viruses	ssRNA retroid viruses	dsDNA retroid viruses



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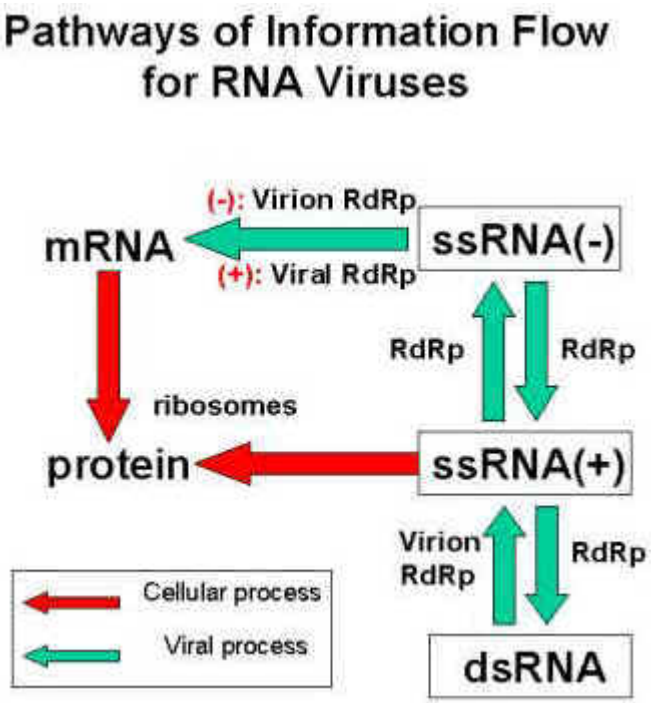
dsDNA viruses	ssDNA viruses	dsRNA viruses	ssRNA (+)-sense viruses	ssRNA (-)sense viruses	ssRNA retroid viruses	dsDNA retroid viruses

RNA Genome Diversity and Genomic Replication Strategies



See also section on "RNA Virus Replication"

RNA Genome Types:

A) Type III: dsRNA

Replication Scheme Diagram

Type III viruses include:

- enveloped phages (***Cystoviridae***),
- the animal- plant- and insect-infecting ***Reoviridae***,
- the vertebrate- and invertebrate-infecting ***Birnaviridae***
- the ***Totiviridae***, in primitive Eukarya (fungi and protozoa, and perhaps in insects)
- ***Partitiviridae***, which mainly infect fungi, and the
- **Cryptoviruses** (family ***Partitiviridae***), which occur in plants, transmissible only via seed or pollen.

The viruses have:

- **single**-component (***Totiviridae***)
- **two**-component (***Birna-, Crypto-*** and ***Partitiviruses***)
- **three**-component (***Cystoviridae***) and
- **multi**-component (***Reoviridae***, 10-12 segments) genomes

All multiple component genomes are encapsidated in a single particle.

Virus genome sizes:

- ***Partitiviridae***: 4-9 kb
- ***Birnaviridae***: about 6 kb
- ***Totiviridae***: 4-7 kb
- ***Hypoviridae***: 10-13 kb
- ***Cystoviridae***: 13 kb
- ***Reoviridae***: 20-27 kb

All genomes apparently replicate by a **conservative mechanism**, wherein **ds input RNA is transcribed by viral enzyme to mRNA**, which both codes for protein, and **acts as template for second strand synthesis**.

Reference:

Virus Taxonomy: Sixth report of the International Committee on Taxonomy of Viruses (FA Murphy *et al.*, Eds.); Springer-Verlag, Wien, 1995.

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B) Type IV: (+)ve-sense ssRNA

See also section on "RNA Virus Replication"

Replication Scheme Diagram

Class IV viruses include:

- Viruses infecting Eubacteria **(*Leviviridae*)**
- Viruses of insects **(*Noda-, Tetraviridae*)**
- Viruses of fungi **(*Barnaviridae*)**
- Viruses of plants **(*Bromo-, Como-, Poty-, Sequi- and Tombusviridae*, and 19 unassigned genera)**
- Viruses of vertebrates **(*Astro-, Calici-, Corona-, Flavi-, Picorna- and Togaviridae*, and genus Arterivirus)**

The viruses have:

- **single-component** genomes with **single ORFs** (***Poty-, Picorna-, Sequiviridae***)
- **single components** with **multiple ORFs** (***Toga-, Caliciviridae*, Tobamovirus**)
- **two components** with **single ORFs** (***Como-, Noda-, Tetri- and Potyviridae*** genus **Bymovirus**)

- **two components** with **multiple ORFs** (**Tobra-, Furo-, Enamovirus**)
- **three components** with **multiple ORFs (Hordeivirus, *Bromoviridae*)**

Genome sizes range from:

- less than 5 kb (***Levi-, Barna-, Tombusviridae***), to
- 7-12 kb (***Astro-, Como-, Picorna-, Calici-, Bromo-, Poty-, Sequi-, Toga-, Flaviviridae,*** **Tobraviruses**)
- to 13 kb (**Arteriviruses**) - 15.5 kb (**Closteroviruses**)
- to 20 - 30 kb (***Coronaviridae***)

Genome expression strategies range from :

- **expressing a single polyprotein** from **each genome component** and **proteolytically** processing it (***Picorna-, Poty-, Sequi-, Como-, Nodaviridae***)
- **expressing proteins from 5'-proximal ORFs** and from **subgenomic messengers** (**Tobamoviruses, *Bromoviridae***)
- a **mixture** of the two strategies (***Togaviridae,*** **Tymoviruses, *Caliciviridae***)

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C) Type V: (-)ve-sense ssRNA

See also section on "RNA Virus Replication"

Replication Scheme Diagram

Type V viruses include:

- viruses infecting vertebrates only **(*Arena-, Orthomyxo-, Paramyxoviridae*)**;
- viruses infecting vertebrates and arthropods **(*Bunya-, Rhabdoviridae*)**;
- viruses infecting plants and arthropds (**Bunya-, *Rhabdoviridae***);
- viruses infecting plants only **(Tenuiviruses)**

The viruses have:

- single-component genomes with multiple ORFs **(*Filo-, Paramyxo-, Rhabdoviridae*)**
- two-component ambisense genomes **(Arenaviruses)**
- three-component, occasionally ambisense genomes (***Bunyaviridae*, Tenuivirus (4)**)
- six to eight component genomes **(*Orthomyxoviridae*)**

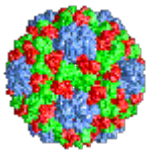
The families ***Paramyxo-, Rhabdo and Filoviridae*** are grouped together in the **Order Mononegavirales**, due to similarities in **virion structure, gene order and transcription.**

Virus genome sizes:

- single component, 11-19 kb: **Mononegavirales**
- 6 - 8 component, 10-14 kb: ***Orthomyxoviridae***
- 3 component, 11-20 kb: ***Bunyaviridae***

Class V viruses are probably a **late evolutionary development**, as they **occur only among higher Eukarya** (arthropods and vertebrates): **viruses infecting plants** probably do so as a result of **close association of insects and host plants in recent evolutionary times**.

dsDNA viruses	ssDNA viruses	dsRNA viruses	ssRNA (+)-sense viruses	ssRNA (-)sense viruses	ssRNA retroid viruses	dsDNA retroid viruses



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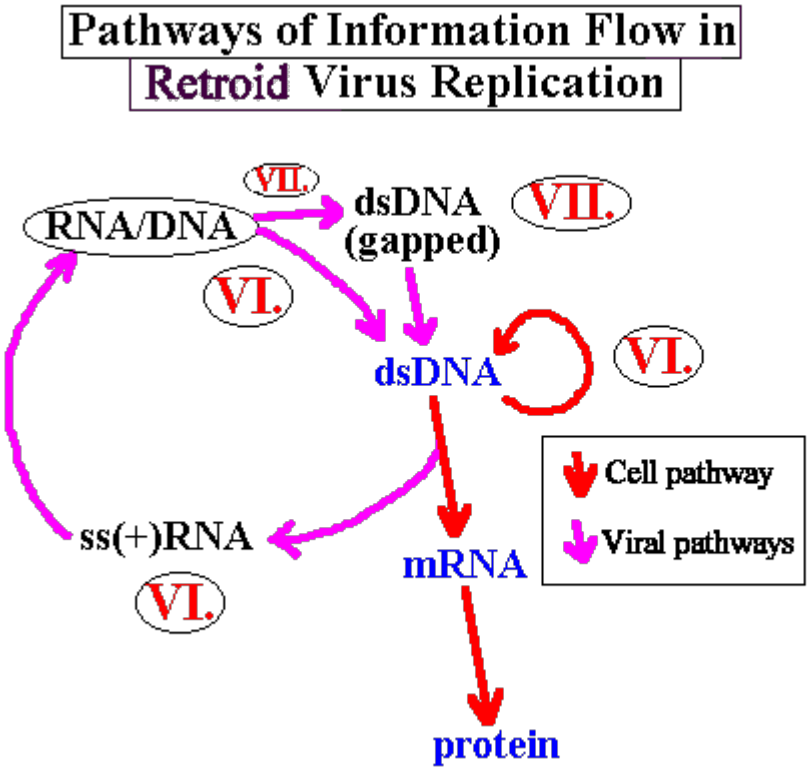
Retroid RNA/DNA Genome Diversity and Genomic Replication Strategies

NB: more material is also available here on retroid elements generally: these include retrotransposons, retroposons and retrons.



Retroid Genome Types:

A) Type VI: diploid ssRNA genomes replicating via longer-than-genome-length dsDNA intermediates

Replication Scheme Diagram

Class VI viruses include:

Only viruses of vertebrates in the family *Retroviridae*

These are (by genus):

- mammalian type B retroviruses (eg: mouse mammary tumour virus, MMTV)
- mammalian type C retroviruses (Moloney murine leukaemia virus, MuLV)
- avian type C viruses (avian leukaemia virus, ALV)
- type D viruses (Mason-Pfizer monkey virus, MPMV)
- BLV-HTLV viruses (human T-lymphotropic virus, HTLV)
- Lentivirus (HIV, SIV)
- Spumavirus (human spumavirus)

The virus genomes are:

- diploid ssRNA (held together by protein)
- messenger sense
- capped and poly-A tailed
- with 4 basic coding regions:

gag-pro-pol-env

Retroviridae replicate via a **dsDNA, longer-than-genome-length intermediate (provirus)**, which is **integrated covalently into the host cell chromosomal DNA**.

Conversion of RNA to DNA, and integration into host DNA, is done by the **viral-coded RNA-dependent DNA polymerase**, which also has RNAse H activity, and DNA-dependent DNA pol activity, as well as encoding the integrase function. Transcription of viral mRNA - which is spliced to allow expression of 3'-proximal ORFs - is by **host RNA pol II**.

An animation of the initial replication process - as culled from the Leicester material - is shown here; a still image is shown here.

An animation of the infection process - also from the Leicester material - is shown here.

An animation of the HIV infection process is shown here.

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B) Type VII: dsDNA genomes replicating via longer-than-genome-length ssRNA intermediates

Replication Scheme Diagram

Class VII viruses include:

Viruses with encapsidated circular dsDNA genomes (usually gapped or nicked), which replicate via **longer-than-genome-length messenger-sense ssRNA intermediates**. Such as:

- viruses infecting vertebrates **(*Hepadnaviridae*)**
- isometric viruses infecting plants **(Caulimoviruses)**
- bacilliform viruses infecting plants **("Badnaviruses")**
- and now an infectious *gypsy*-type retrotransposon of *Petunia* **(petunia vein-clearing virus)**

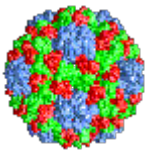
The viruses have:

- partially double-stranded non-covalently closed circular DNA, 3.2 kb (*Hepadnaviridae*)

circular dsDNA with single-strand break(s) in both strands, about 8 kb (Caulimoviruses and Badnaviruses and petunia virus)

All the viruses are probably derived from the same origin as each other, which they share with **Retroviridae:** for this reason Class VII viruses have been proposed as **"Pararetroviridae".**

dsDNA viruses	ssDNA viruses	dsRNA viruses	ssRNA (+)-sense viruses	ssRNA (-)sense viruses	ssRNA retroid viruses	dsDNA retroid viruses



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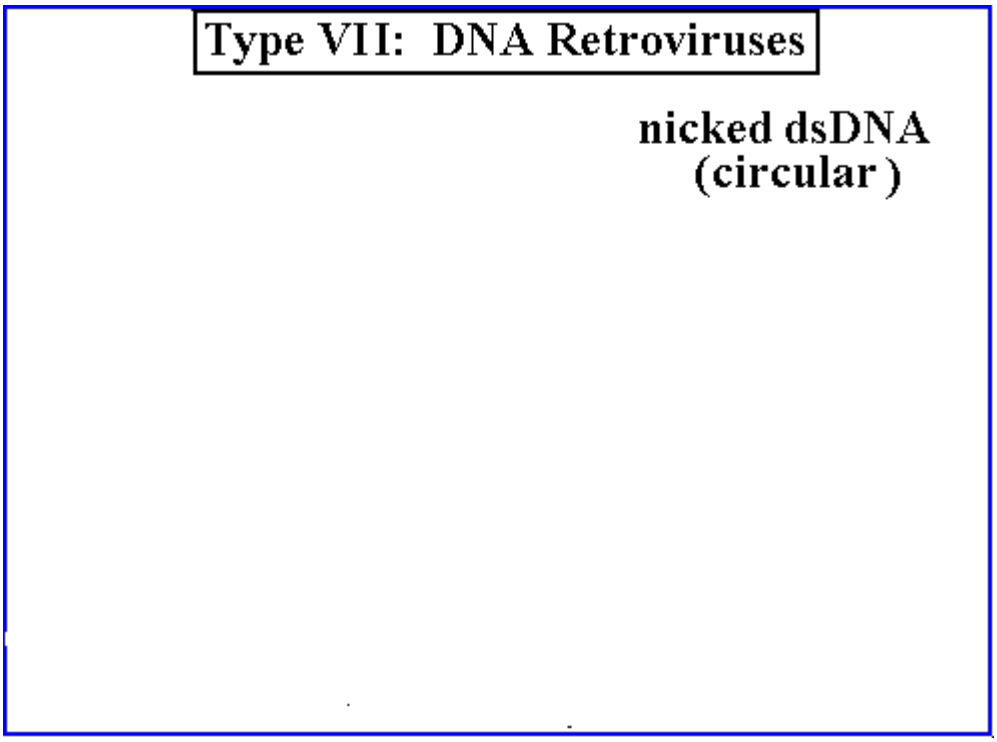
Replication Strategy of dsDNA Viruses Replicating via a Longer- Than-Genome-Length RNA Intermediate



Note: look at status bar when mouse is over numbers in picture for explanations of steps

Note: this is an animated GIF, which takes about 30 sec to cycle through completely.

Steps in Replication

1. Entry of viral DNA into nucleus, conversion of "gapped" genomic DNA into cccDNA by host repair synthesis

2. Transcription by host RNA pol into mRNA(s) and longer-than-genome-length "genomic" (+)sense RNA

3. Translation of mRNA and (+)sense RNA in cytoplasm, accumulation of viral products

4. Interaction of viral proteins with (+)sense RNA, assembly of provirions, and reverse transcription of RNA inside virions by viral RT to RNA/DNA complex

5. Conversion of RNA/DNA complex to circular, gapped dsDNA by virion RT

6. Final virion maturation, arrest of further DNA polymerase activity in virion (budding for hepadnaviruses)

Return to Genomes

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Evidence that badnavirus infection in Musa can originate from integrated pararetroviral sequences.

Ndowora T, Dahal G, LaFleur D, Harper G, Hull R, Olszewski NE, Lockhart B

Department of Plant Pathology, University of Minnesota, St. Paul, Minnesota, 55108, USA.

Virology 1999 Mar 15;255(2):214-20

When some virus- and disease-free Musa spp. (banana and plantain) are propagated by tissue culture, the resulting plants develop infections with banana streak badnavirus (BSV), a pararetrovirus. In sharp contrast to the virion DNA recovered from natural infections, the virion DNA from tissue culture-associated infections of different Musa spp. was highly similar if not identical. Although BSV does not employ integration during the infection cycle, BSV DNA was found to be integrated into the Musa genome. While one integration consisted of a partial BSV genome, a second contained more than one complete genome that was almost identical to BSV recovered from tissue culture-derived plants. The arrangement of this integrated BSV DNA suggests that it can yield an infectious episomal genome via homologous recombination. This report documents the first instance of integrated DNA of a nonintegrating virus giving rise to an episomal viral infection and identifies tissue culture as a possible trigger for the infection, raising the question of whether similar activatable viral sequences exist in the genomes of other plants and animals.

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Student Feedback Questionnaire for Microbiology MIC305S Molecular Virology Module

This anonymous questionnaire seeks information about your experience of this module. It would be greatly appreciated if you would answer the questions accurately as such feedback is important if the module is to be improved for future years. Please read the statements below and indicate how strongly each applies to you.

Click on the "Submit Answers" button at the end of the questionnaire when you are finished.

General Statements:

1. The module objectives were made clear:

- ○ Strongly Agree
- ○ Agree
- ○ Neutral
- ○ Disagree
- ○ Strongly Disagree

2. I feel that the module achieves its objectives:

- ○ Strongly Agree
- ○ Agree
- ○ Neutral
- ○ Disagree
- ○ Strongly Disagree

3. The module teaching was well structured:

- ○ Strongly Agree
- ○ Agree
- ○ Neutral
- ○ Disagree
- ○ Strongly Disagree

4. The reference material was readily available:

- ○ Strongly Agree
- ○ Agree
- ○ Neutral
- ○ Disagree
- ○ Strongly Disagree

6. The lecturer was accessible and helpful:

- ○ Strongly Agree
- ○ Agree
- ○ Neutral
- ○ Disagree
- ○ Strongly Disagree

7. I would recommend the module to other students:

- ○ Strongly Agree
- ○ Agree
- ○ Neutral
- ○ Disagree
- ○ Strongly Disagree

8. There was good coordination between different parts of the module:

- ○ Strongly Agree
- ○ Agree
- ○ Neutral
- ○ Disagree
- ○ Strongly Disagree

9. I followed up the teaching with outside reading:

- ○ Strongly Agree
- ○ Agree
- ○ Neutral
- ○ Disagree
- ○ Strongly Disagree

10. The form of the assessment was clear to me:

- ○ Strongly Agree
- ○ Agree
- ○ Neutral
- ○ Disagree
- ○ Strongly Disagree

11. The lectures stimulated my interest:

- ○ Strongly Agree
- ○ Agree
- ○ Neutral
- ○ Disagree
- ○ Strongly Disagree

12. The lectures were well organized and presented:

- ○ Strongly Agree
- ○ Agree
- ○ Neutral
- ○ Disagree
- ○ Strongly Disagree

13. The lectures were well paced:

- ○ Strongly Agree
- ○ Agree
- ○ Neutral
- ○ Disagree
- ○ Strongly Disagree

14. The lectures increased my understanding:

- ○ Strongly Agree
- ○ Agree
- ○ Neutral
- ○ Disagree
- ○ Strongly Disagree

15. The animations were a useful part of the module:

- ○ Strongly Agree
- ○ Agree
- ○ Neutral
- ○ Disagree

○ Strongly Disagree

Please comment on their utility and how they could be improved.

Name up to three good features of the module:

Name up to three ways in which this module could be improved:

What was most interesting to you?

Please add any other comments about the module:

Submit Reset

Thank you.

Ed Rybicki

Entrance, Entertainment and Exit:

The Virus Life Cycle

Viruses have a defined "life cycle" as do any other type of organisms; however, given that they are obligate intracellular parasites, this cycle revolves around:

- **getting into a host cell**
- **replicating there, and**
- **getting out again.**

For eighteen years now I have taught this cycle under the heading

"Entrance, Entertainment, and Exit*",

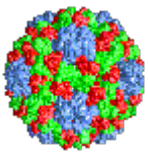
as this is the best mnemonic I know to remind one of the process. Other courses tend to label these steps as (for example)

Virus Entry Into Cells

Replication of Viruses

Assembly and Release of Virions

These pages link frequently to the University of Leicester Microbiology Dept. Virology 335 course material, mirrored at this site: use the "back" button on your browser to return from a link.



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* = from the Pink Floyd song **"The Grand Vizier's Garden Party"** (Roger Waters), on the album "**Ummagumma**". Which also contains the delightful "**Several Species of Small Furry Animal Gathered Together in a Cave and Grooving With a Pict**", which title is only a little shorter than the unforgettable Hoagy Carmichaels' "**I'm a Cranky Old Yank in my Clanky Old Tank, on the Streets of Yokohama With My Honolulu Mama, Doing Those Beat-O, Street-O, Flat on my Seat-O, Hirohito Blues**"...but I digress.

Click **here** for Pink Floyd midi files to brighten your browsing.

MCB3011S Course Notes and Handouts

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Virus Entry Into Cells

Virus Movement in Plants

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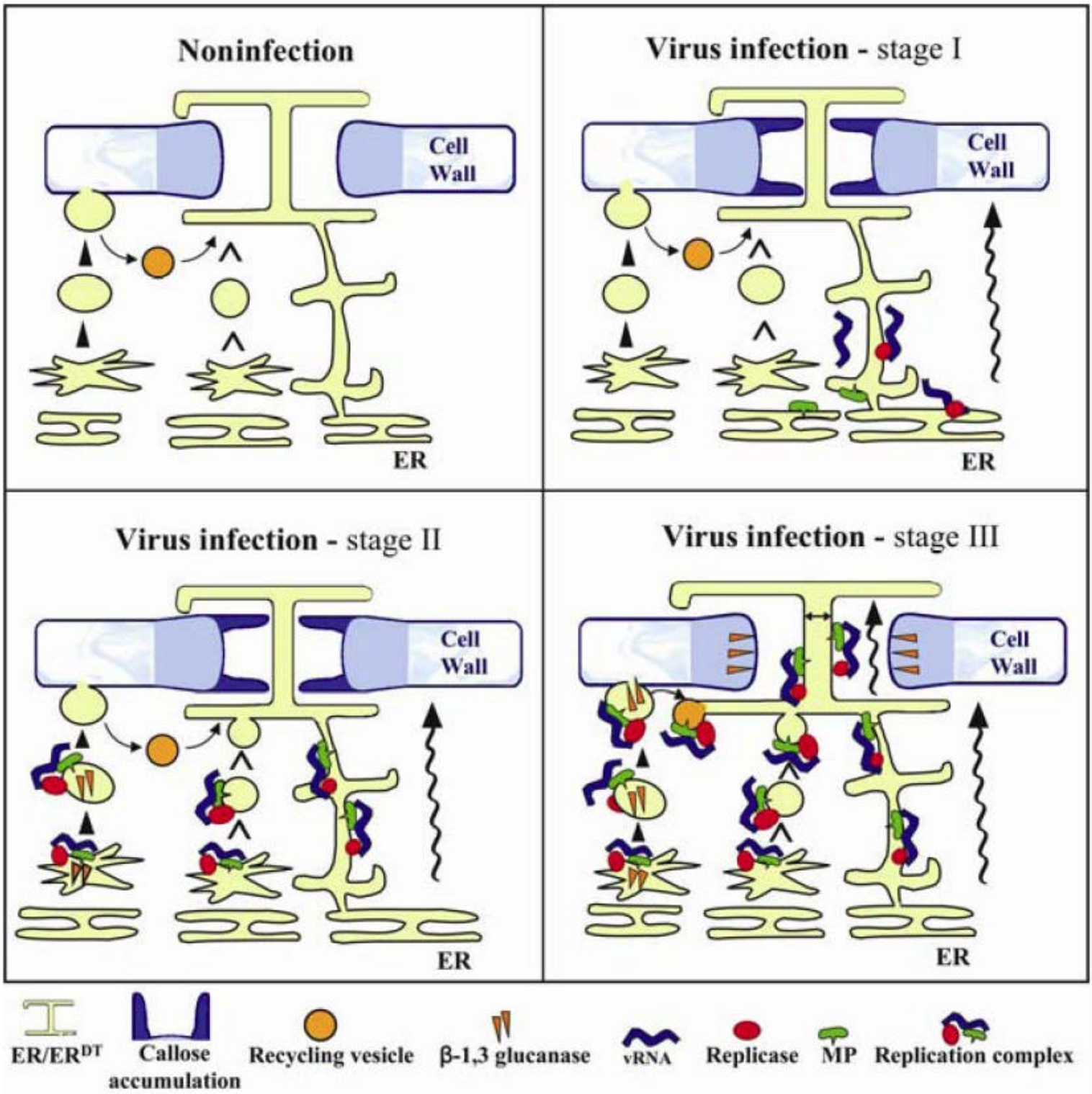
Guenoune-Gelbart D, Elbaum M, Sagi G, Levy A, Epel BL. Tobacco mosaic virus (TMV) replicase and movement protein function synergistically in facilitating TMV spread by lateral diffusion in the plasmodesmal desmotubule of Nicotiana benthamiana. Mol Plant Microbe Interact. 2008 Mar;21(3):335-45



A model of cell-to-cell spread of *Tobacco mosaic virus*. Stage I: Upon infection, viral RNA (vRNA) initiates synthesis of replicase, plus and minus vRNA stands, subgenomic RNAs and movement protein (MP), and coat protein. In response to infection, callose accumulates in the wall region surrounding the plasmodesmata (Pd) restricting the cytoplasmic sleeve. Stage II: MP, an integral endoplasmic reticulum (ER) membrane protein, functions as a protein raft binding

vRNA on its cytoplasmic domains forming a replication complex (VRC) that may also contain replicase. Intracellular trafficking of the VRC to the cortical ER is either by diffusion in the ER lipids (squiggle arrow) or by vesicular trafficking (open arrowheads). Stress-induced class I beta-1,3,glucanase traffics in the lumen of VRC vesicles to plasma membrane (PM) with requisite docking protein (filled arrowheads). Stage III: Cycling vesicles containing beta-1,3-glucanase cargo fuse to PM and deliver beta-1,3-glucanase to the cell wall (filled arrowheads). Callose is hydrolyzed, allowing Pd to dilate. Vesicles with attached VRC recycle back to the cortical ER, in which vesicles fuse to cortical ER (curved arrow). VRC diffuses through the Pd to adjacent cells by diffusion in ERdesmotubule continuum (squiggle arrow) motivated by the concentration gradient between a viral-infect cell and adjacent noninfected cells.

Plant & Fungal Cell Entry

Last Updated: Saturday, October 4, 1945624.

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General:

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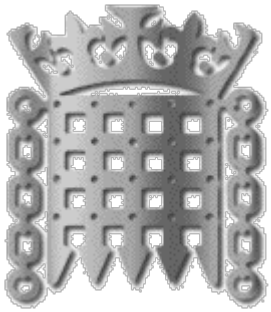

The Rt. Hon. Stephen Dorrell,
Minister of State for Health
Her Majesty's Government
3rd December 1995.

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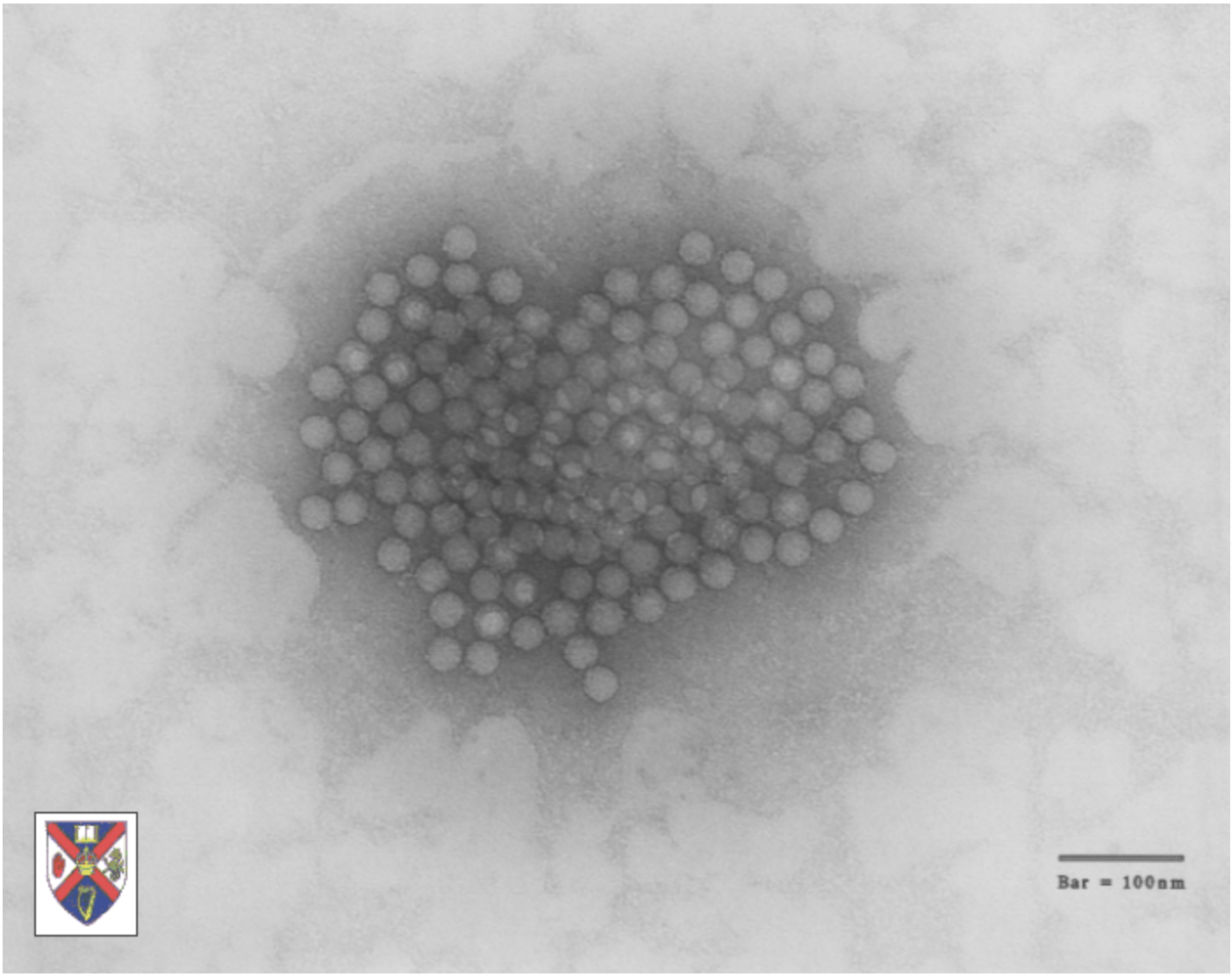
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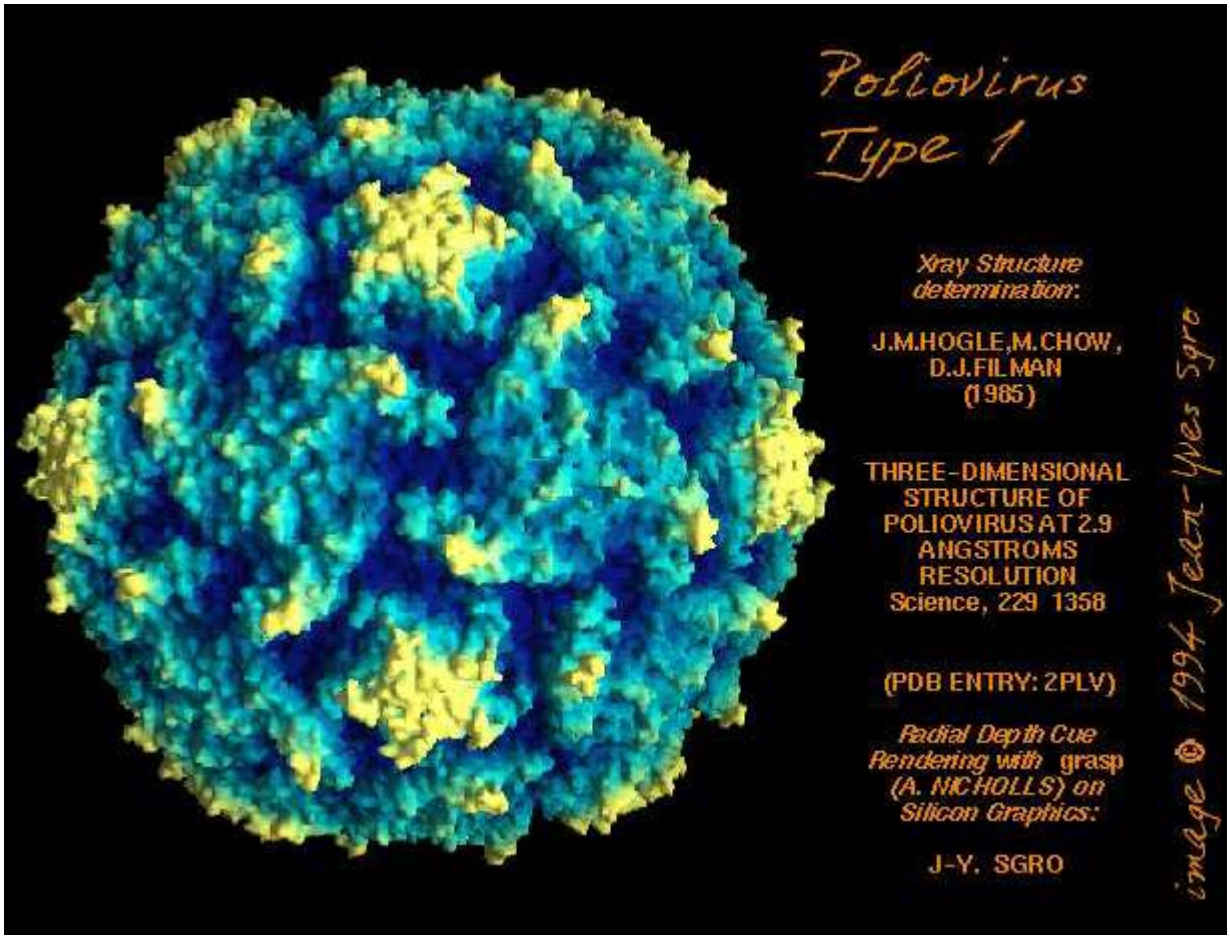

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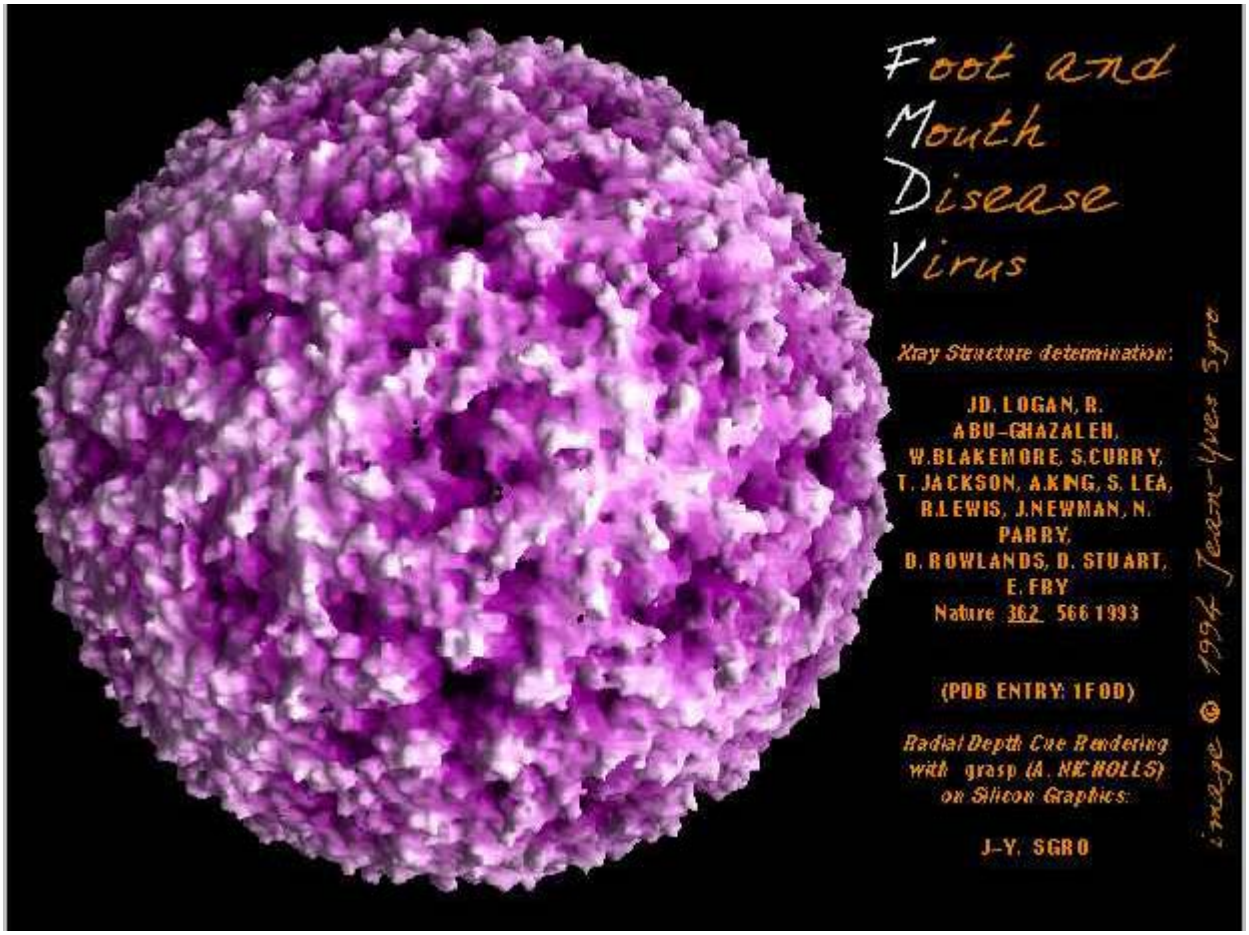

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Information on Virus Families

Click on any of the links below to access detailed information on virus families described on Course 335:

DNA Viruses:	RNA Viruses:
Adenoviruses	Coronaviruses
Iridoviruses	Filoviruses
Herpesviruses	Orthomyxoviruses
Papovaviruses	Paramyxoviruses
Parvoviruses	Picornaviruses
Poxviruses	Retroviruses
Viral Hepatitis	Rhabdoviruses
"Arboviruses" Arenaviruses, Bunyaviruses, Flaviviruses, Togaviruses	
Diarrhoea Viruses Astroviruses, Caliciviruses, Reoviruses (inc. Rotaviruses)	

- Adenoviruses
- 'Arboviruses' Arenaviruses, Bunyaviruses, Flaviviruses, Togaviruses
- Coronaviruses
- Diarrhoea Viruses Astroviruses, Caliciviruses, Reoviruses (inc. Rotaviruses)
- Filoviruses
- Iridoviruses
- Herpesviruses
- Orthomyxoviruses
- Papovaviruses
- Paramyxoviruses
- Parvoviruses
- Picornaviruses
- Poxviruses
- Retroviruses
- Rhabdoviruses
- Viral Hepatitis

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Course BS335: Viral Immunopathology

Detailed notes for these lectures can be found in:

Cann A.J: Principles of Molecular Virology. Academic Press, 2nd Edition, 1997 Chapter 6.

Additional online information is available at the link below:

- MBChB: Infection and Immunity

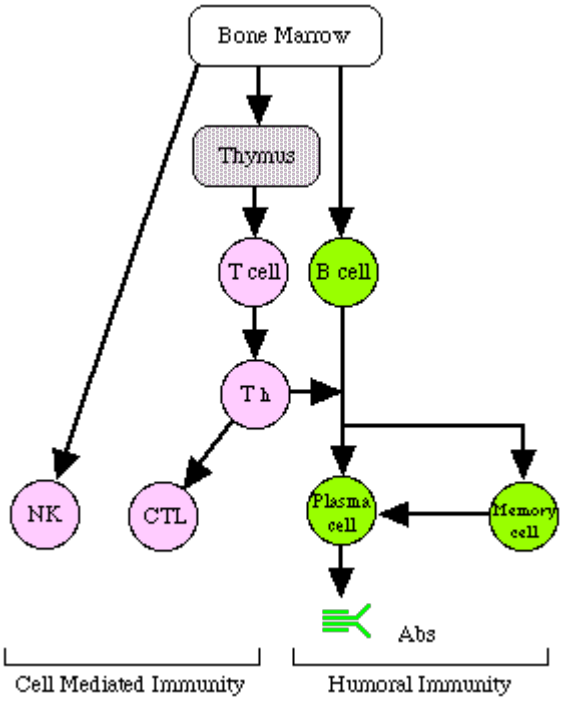
Introduction:

The ultimate goal of anti-viral therapy - to completely restore all functions to the infected cell - is normally unattainable, since irreversible damage to cellular functions occurs very early in most virus infections. The more realistic goal for anti-viral therapy is to inhibit or halt:

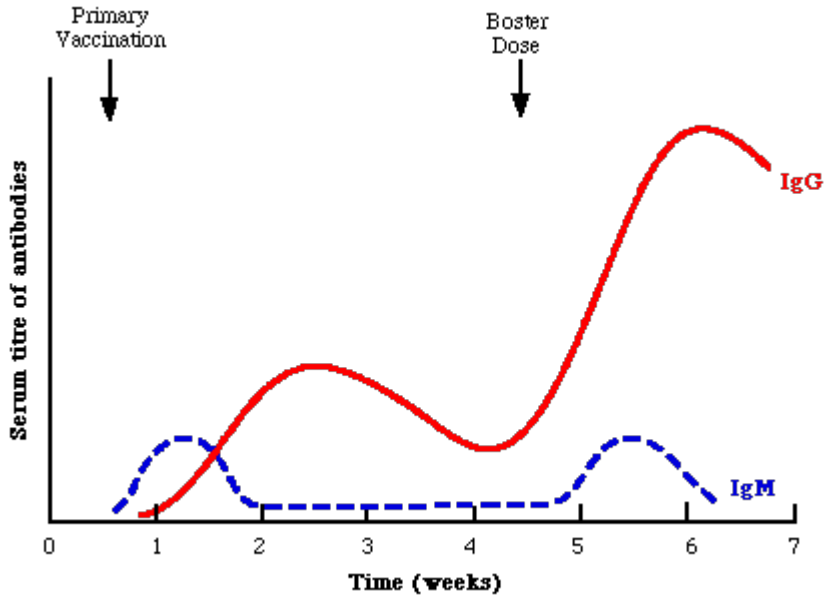
- virus replication
- spread of the virus to uninfected cells

How many effective drugs against viruses are currently available to the clinician? (a handful). Therefore, immunity is far more important in viral infections than therapy.

Overview of the Immune System:



Humoral Immunity:



In addition to neutralization by antibodies, **complement** plays an important role in virus infections. **Virolysis** is the direct lysis of virus particles by antibodies + complement.

Cell Mediated Immunity (CMI)

The Role of MHC:

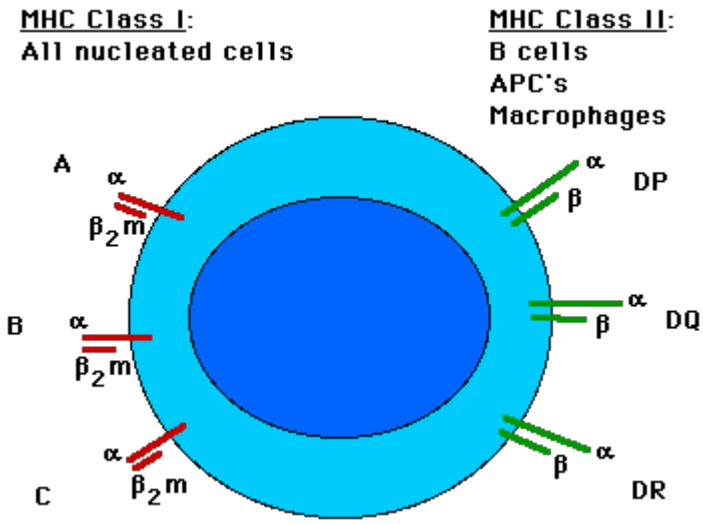
The molecular basis of antigen recognition by T cells is well understood. The TcR recognises short antigen-derived peptide sequences presented in association with self MHC class I or MHC class II molecules at the surface of an antigen presenting cell (APC).

T cell recognition, therefore, involves direct cell-cell contact between the antigen-specific TcR on the T lymphocyte

and an MHC compatible cell which presents the processed antigen in association with surface MHC molecules. The finding that self MHC molecules are involved in the recognition of antigen by T lymphocytes led to the concept of "MHC restriction" of T cell responses, and pointed to the important role that products of the major histocompatibility complex play in the cell mediated immune response. The major histocompatibility complex consists of a cluster of genes, most of which encode products with immunologically related functions.

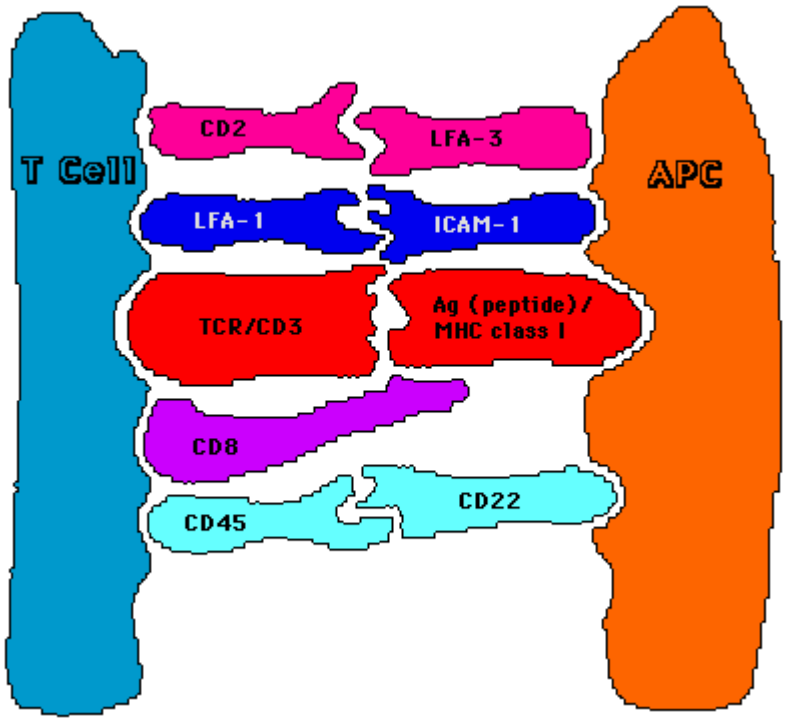
In humans, the MHC is located on the short arm of chromosome 6 and spans approximately 4 megabases of DNA. It can be divided into three regions termed class I, class II and class III:

- The **class III** region contains genes which encode a number of complement components and the tumour necrosis factor cytokines, amongst other molecules.
- **MHC class I molecules** consist of a polymorphic, MHC-encoded, membrane-spanning heavy chain, and a monomorphic light chain, beta2-microglobulin.
- **MHC class II molecules** consist of a heterodimer of two MHC-encoded, membrane-spanning proteins, the alpha and beta polypeptide chains of the MHC class II molecule.



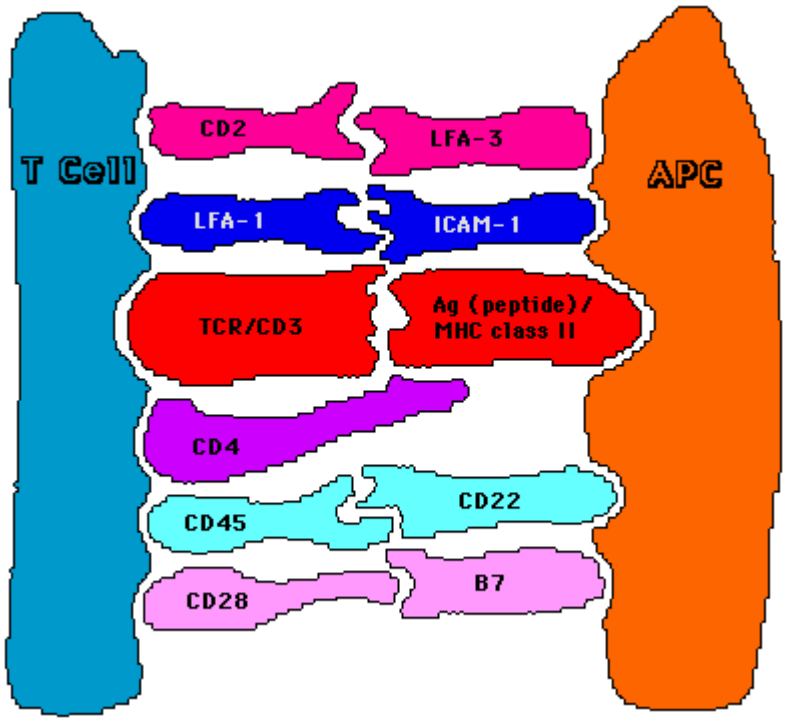
In addition to the interaction between MHC/antigen and the TcR, MHC class I and class II molecules also bind to the CD8 and CD4 T cell surface molecules, respectively.

Thus, MHC class I molecules present antigen to CD8+ T cells:



Animation: Degradation and transport of antigens that bind MHC class I molecules
(needs Macromedia Shockwave Flash Player)

MHC class II molecules present antigen to CD4+ T cells:



Animation: Degradation and transport of antigens that bind MHC class I molecules
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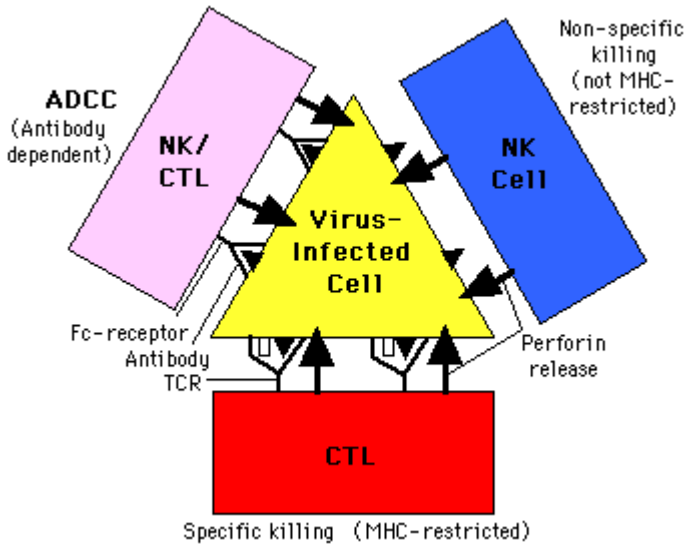
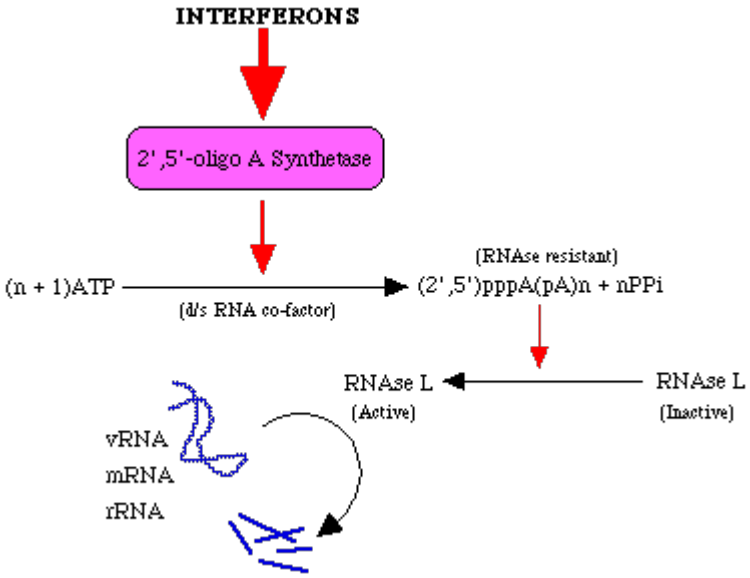


Interferons:

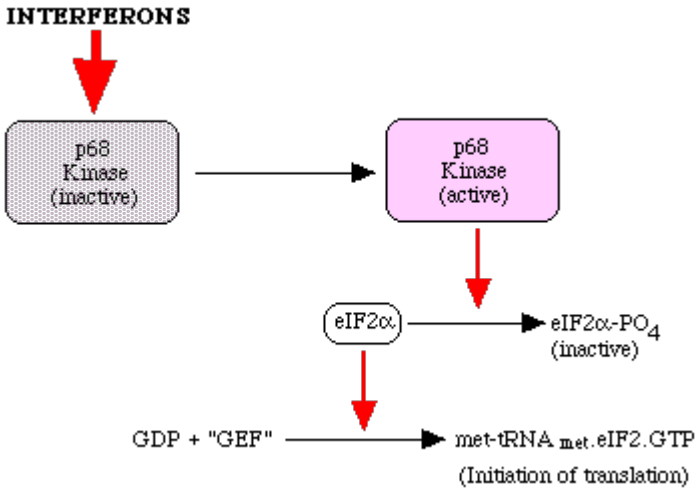
Mechanisms of Antiviral Activity

There are 3 general mechanisms which are understood in some detail plus a number of others (in some cases specific to certain viruses) which are less well understood.

1. **2,5-oligo A:**



2. **68kD RNA-activated protein kinase - PKR:**



3. **Mx gene**

Other actions: (Mechanisms unknown):

- Inhibits penetration and uncoating of SV40 and other viruses, possibly by altering the composition/structure of the cell membrane.
- IFN inhibits primary transcription of many virus genomes e.g. SV40, HSV.
- Inhibits cell transformation by retroviruses.

Therapeutic Uses of IFN:

Viral Infections:	
Chronic active hepatitis:	HBV, HCV
Condylomata accuminata:	(Genital warts - papilloma)
Tumours:	
Hairy cell leukaemia	
Kaposi's sarcoma:	HHV-8 in AIDS patients(?)

Congenital conditions:
Chronic granulomatous disease - IFN gamma reduces bacterial infections.

Conclusion:

IFN is a powerful weapon against viruses, but acts as a blunderbuss, not a magic bullet! As its cell-regulatory activities are becoming better understood, it is finding increasing use as a cancer treatment - e.g. IFNalpha/HCL. The long term prospects for antiviral use are uncertain. Huge amounts of alpha-IFN are currently being used to treat HBV/HCV patients, but it is not clear how this treatment works & only a small proportion of patients respond to treatment.

- References: Virus Immunology.
- References: Interferons.
- **Medscape Article:** What's New With Common Colds? Complications and Management.
- Search for more information on this topic.

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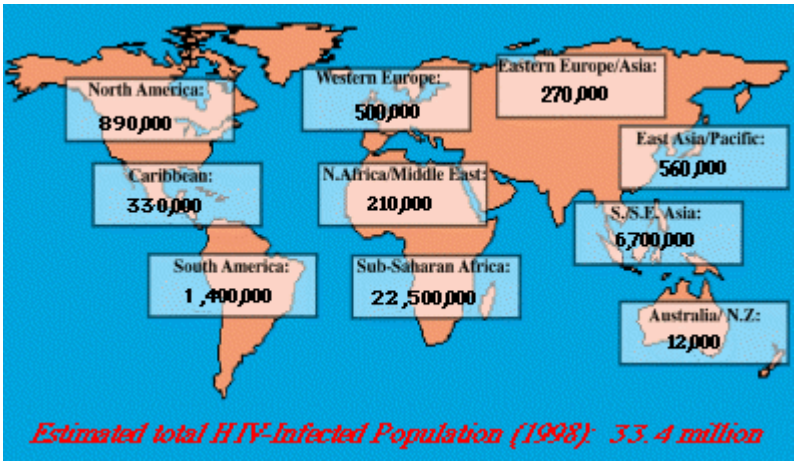
Contents:

Part 1: The Pathogenesis of AIDS (including therapy)

Part 2: Who gets AIDS?

The Pathogenesis of AIDS



Latest statistics from WHO

The pathogenesis of AIDS is dependent on the biology of HIV, e.g:

- 'Trojan horse' mechanism - virus escapes recognition by replication inside monocytes, from where it can spread to other tissues and other hosts.
- Latency - Lentiviruses do not show true latency (unlike Herpes viruses or lambda) but do have the capacity to control the expression of their genome by means of virus-encoded trans-acting regulatory proteins (tat and rev).
- Antigenic variation - new variants continually arise. In other Lentiviruses such as CAEV, each new antigenic variant results in a flare up of disease. May also occur in HIV and contribute to decline of immune system - 'ratcheting' mechanism due to increasing virus load?

Some of the immune abnormalities in HIV infection include:

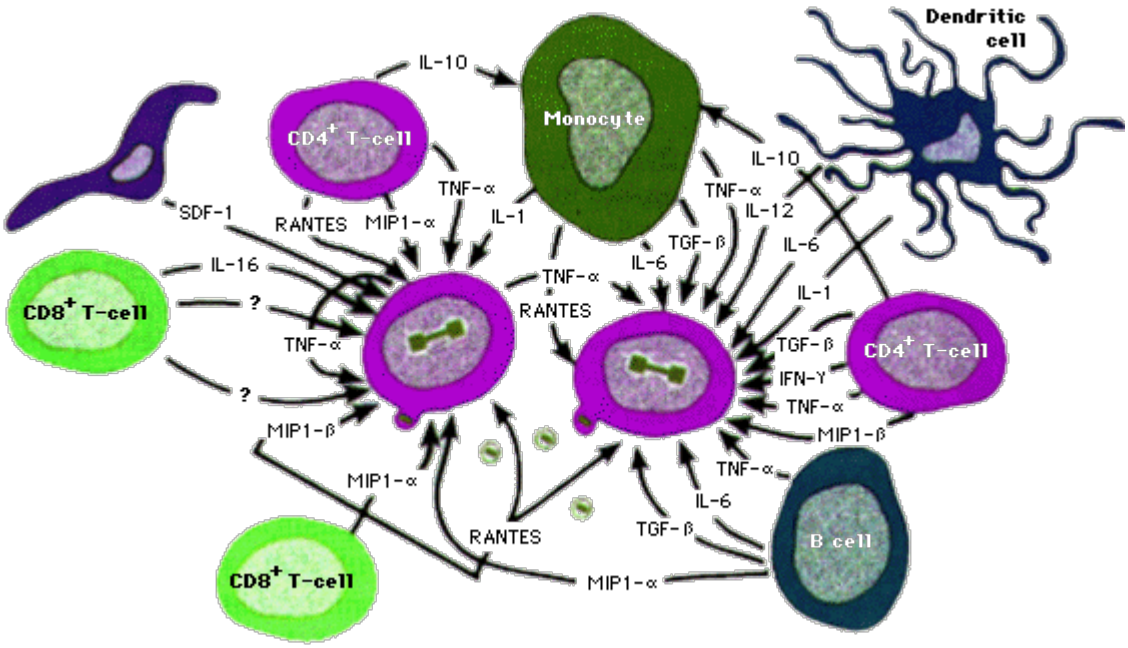
- Altered cytokine expression
- Decreased CTL and NK cell function
- Decreased humoral and proliferative response to antigens and mitogens
- Decreased MHC-II expression
- Decreased monocyte chemotaxis
- Depletion of CD4+ cells
- Impaired DTH reactions
- Lymphopenia
- Polyclonal B-cell activation

It is not clear how much of the pathology of AIDS is directly due to the virus and how much is caused by the

immune system itself. There are numerous models which have been suggested to explain how HIV causes immune deficiency:

Direct Cell Killing:

This was the first mechanism suggested, based on the behavior of certain laboratory isolates of HIV. Subsequent experiments suggested that there is not sufficient virus present in AIDS patients to account for all the damage seen, although killing of CD4+ cells may contribute to the overall pattern of pathogenesis seen in AIDS. Indirect effects of infection, e.g. disturbances in cell biochemistry and lymphokine production may also affect the regulation of the immune system:



However, the expression of virus antigens on the surface of infected cells leads to **indirect killing by the immune system** (NK/CTL/ADCC) - effectively a type of autoimmunity. Recently, this hypothesis has been resurrected as a result of more accurate quantitation of virus load and replication kinetics in infected individuals (see below).

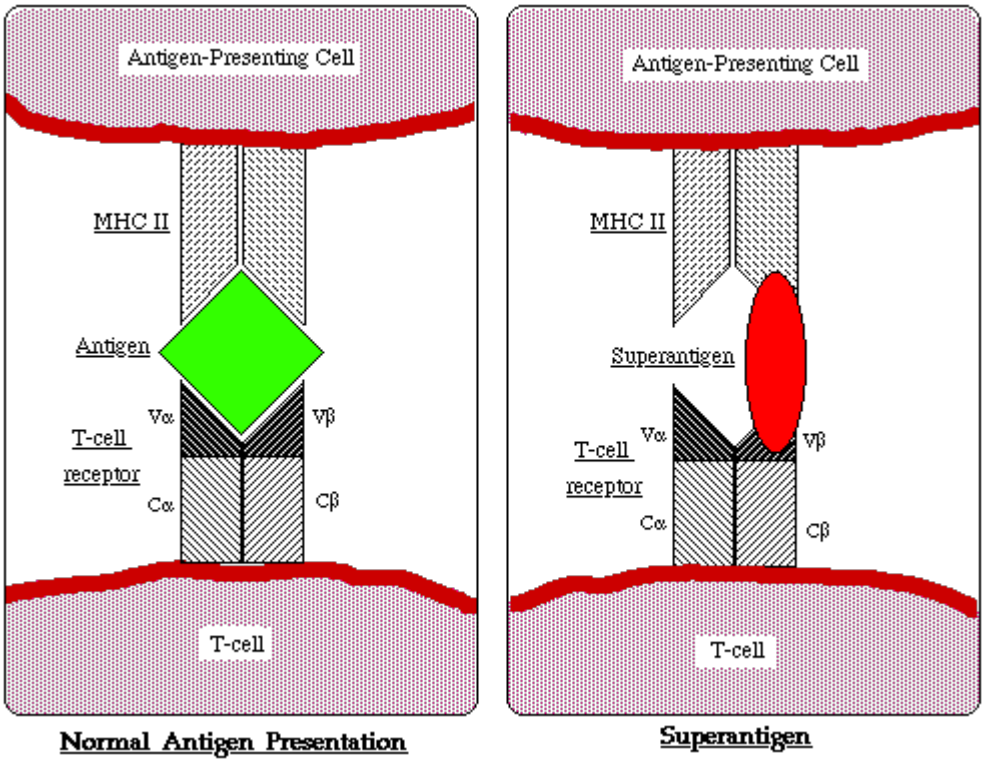
Antigenic Diversity:

This theory holds that the continual generation of new antigenic variants eventually swamps and overcomes the immune system, leading to its collapse.
There is no doubt that new antigenic variants of HIV constantly arise during the long course of AIDS, in a similar way to CAEV infection of goats. It has not been completely established how this might lead to the collapse of the immune system, but it is envisaged that there might be a 'ratchet' effect, with each new variant contributing to the slight but irreversible decline in immune function.
N.B: Because of the way virus infections are handled by the immune system, it is probable that variation of T-cell epitopes on target proteins recognised by CTLs are at least (probably more) important than B-cell epitopes which generate the antibody response to a foreign antigen. It has recently been reported that at least some variants can inhibit the CTL response to wild-type HIV (Meier, U. et al. Cytotoxic T lymphocyte lysis inhibited by viable HIV mutants. Science 270: 1360-62, 1995).
A mathematical model has been constructed which simulates antigenic variation during the course of infection. When primed with all of the known data about the state of immune system during HIV infection, it provides a startling accurate depiction of the course of AIDS (Nowak MA, et al. Antigenic diversity thresholds and the development of AIDS. Science 254: 963-969, 1991).
Medscape Article: Genotypic Variation and Molecular Epidemiology of HIV.

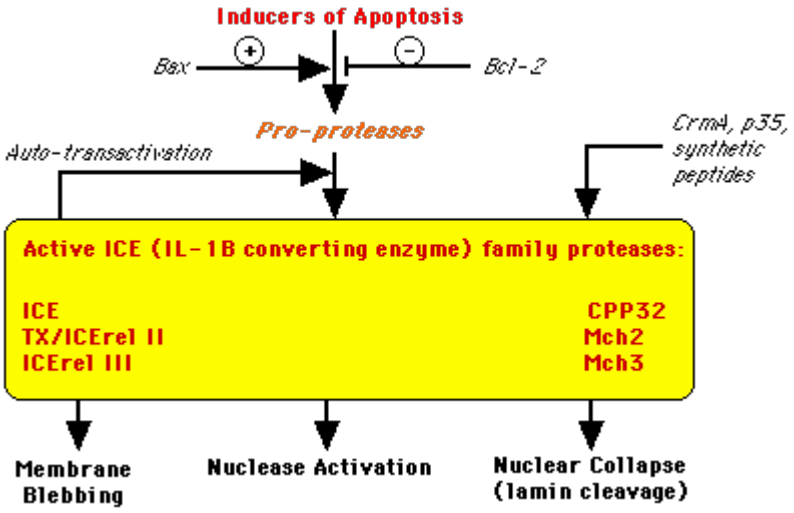
The Superantigen Theory:

Superantigens are molecules which short-circuit the immune system, resulting in massive activation of T-cells

rather than the usual, carefully controlled response to foreign antigens. It is believed that they do this by binding to both the variable region of the beta-chain of the T-cell receptor (V-beta) and to MHC II molecules, cross-linking them in a non-specific way:



This results in polyclonal T-cell activation rather than the usual situation where only the few clones of T-cells responsive to a particular antigen presented by the MHC II molecule are activated. The over-response of the immune system produced results in autoimmunity, as rare clones of T-cells which recognise self antigens are activated, and immune suppression, as the activated cells subsequently die or are killed by other activated T-cells. It is possible that such superantigens might also induce **apoptosis** (pronounced "apo-tosis"), or 'programmed cell killing' (Cohen JJ. Apoptosis. Immunol. Today 14: 126-136, 1993):



It has been reported that in some AIDS patients, certain clones of T-cells bearing particular Vb T-cell receptor rearrangements are depleted or absent. This is precisely what would be expected if some clones of cells were being eliminated by the presence of a superantigen. However, unlike other retroviruses (e.g. mouse mammary tumour virus (MMTV) and the murine leukaemia virus (MuLV) responsible for murine acquired immunodeficiency syndrome [MAIDS]) no superantigen has been conclusively identified in HIV, despite intensive investigation. Thus the practical relevance of superantigens in AIDS remains in some doubt. However, it is possible that exposure to superantigens produced by opportunistic infection(s) might play an important role in AIDS.

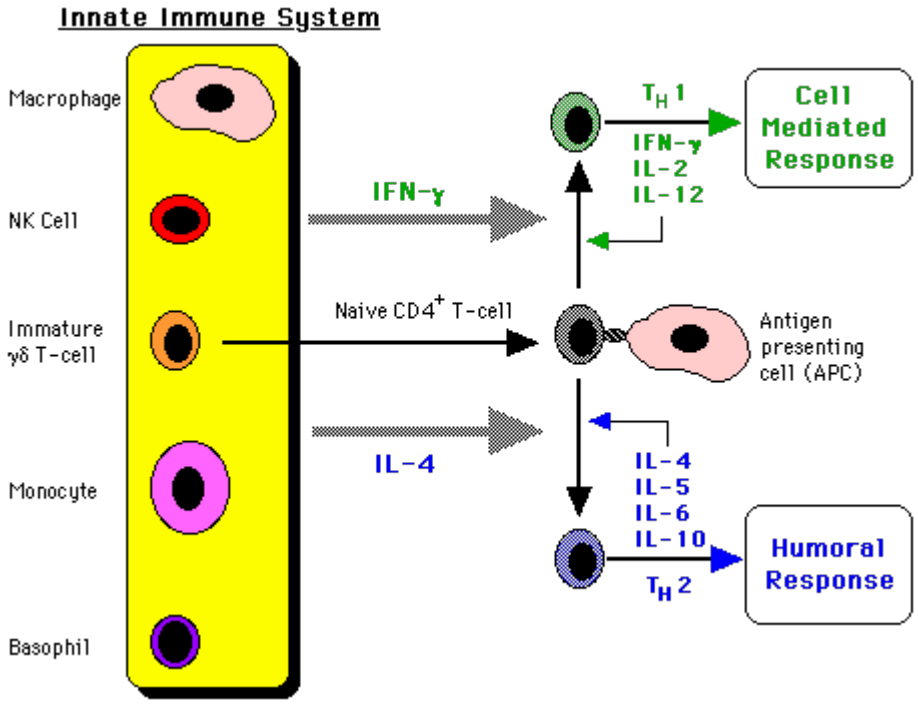
Receptor Signalling:

There have been several reports that HIV binding to CD4 induces an intracellular signal which may have a detrimental effect on cells. None of these have been completely convincing. However, there is a second component to the HIV receptor which is required for cell entry: chemokine receptors such as CXCR4 & CCR5. Although HIV-mediated signal transduction is not required for fusion & entry, at least some HIV isolates induce a signal when the envelope protein binds to CCR5 (Weissman et al, Nature 389: 981-985, 1997). HIV disease is characterized (in

part) by persistent immune activation. Envelope-mediated signalling through binding to chemokine receptors could contribute to cellular activation. HIV replicates only in activated cells, so this activity promotes replication directly & may also assist in the spread of the virus to uninfected cells by inducing the migration of activated cells to sites of virus replication via chemotaxis. These signals may also contribute indirectly to the pathogenesis of the infection by inducing apoptosis or anergy.
Neuronal apoptosis is a feature of HIV-1 infection in the brain, contributing to dementia. gp120 from some strains of HIV binds with high affinity to CXCR4 expressed on hNT neurons. Both gp120 and the Cys-X-Cys chemokine SDF-1[alpha] can directly induce apoptosis in hNT neurons in the absence of CD4 and in a dose-dependent manner. Thus the HIV-1 envelope glycoprotein may elicit apoptotic responses through chemokine receptors.

TH1-TH2 Switch:

Early in HIV infection, TH1-responsive T-cells predominate and are effective in controlling (but not eliminating) the virus. At some point, a (relative) loss of the TH1 response occurs and TH2 HIV-responsive cells predominate:



The hypothesis is therefore that the TH2-dominated humoral response is not effective at maintaining HIV replication at a low level and the virus load builds up, resulting in AIDS.

N.B. This is a theoretical proposal, and has not yet been proved, but is shaping our understanding of the immune response to many different pathogens, not just HIV (Clerici M, Shearer G. A TH1-TH2 switch is a critical step in the etiology of HIV infection. Immunol. Today 14: 107-111, 1993).

Virus Load and Replication Kinetics:

Recent reports involving accurate quantitation of the amount of virus in infected patients have revealed that much more virus is present than originally thought. Using quantitative PCR methods to accurately measure the amount of virus present in HIV-infected individuals and determining how these levels change when patients are treated with compounds which inhibit virus replication, it has been shown that:

- Continuous and highly productive replication of HIV occurs in all infected individuals, although the rates of virus production vary by up to 70-fold in different individuals
- The average half-life of an HIV particle/infected cell in vivo is 2.1 days
- Up to $2x10^9$ HIV particles are produced each day
- An average of $2.6x10^9$ new CD4+ cells are produced

Thus, contrary to what has recently been thought, there is a very dynamic situation in HIV-infected subjects

involving continuous infection, destruction and replacement of CD4+ cells, with billions of new cells being infected and killed each day. These data suggest a return to cellular killing (although predominantly immune-mediated rather than virus-mediated) as a direct cause of the CD4+ cell decline in AIDS. For reasons which are not yet clear, this is a (marathon) race between virus production, destruction and cellular regeneration which, after many years, most individuals loose, resulting in the absolute decline of the CD4 segment of the immune system and the development of full-blown AIDS.

Conclusions:

The ultimate mechanism by which HIV infection causes AIDS remains unknown, but recent reports strongly implicate immune-mediated killing of virus-infected cells as the major factor in the pathogenesis of this disease. These new ideas are informing future thinking about possible therapeutic intervention in HIV-infected individuals.

Therapy of HIV Infection:

Several distinct classes of drugs are now used to treat HIV infection:

1. **Nucleoside-Analog Reverse Transcriptase Inhibitors (NRTI)**. These drugs inhibit viral RNA-dependent DNA polymerase (reverse transcriptase) and are incorporated into viral DNA (they are chain-terminating drugs).
   - Zidovudine (AZT = ZDV, Retrovir) first approved in 1987
   - Didanosine (ddI, Videx)
   - Zalcitabine (ddC, Hivid)
   - Stavudine (d4T, Zerit)
   - Lamivudine (3TC, Epivir)
2. **Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)**. In contrast to NRTIs, NNRTIs are not incorporated into viral DNA; they inhibit HIV replication directly by binding non-competitively to reverse transcriptase.
   - Nevirapine (Viramune)
   - Delavirdine (Rescriptor)
3. **Protease Inhibitors**. These drugs are specific for the HIV-1 protease and competitively inhibit the enzyme, preventing the maturation of virions capable of infecting other cells.
   - Saquinavir (Invirase) first approved in 1995
   - Ritonavir (Norvir)
   - Indinavir (Crixivan)
   - Nelfinavir (Viracept)

- National Institute of Allergy & Infectious Disease: Division of Acquired Immune Deficiency Syndrome
- **Medscape Article:** Antiretroviral Drugs to Fight AIDS: "The **combination** of ZDV, 3TC, and ritonavir during early-stage disease produces significant and sustained reduction of viral load as well as an increase in $CD4^+$ cell count. The same combination in late-stage disease ($CD4^+$ <50 cells/mm^3) lowers mortality rates."
- **Medscape Article:** Gene Therapy for HIV/AIDS: Where Are We Going?

Part 2: Who gets AIDS?

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Plant Viruses

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ssDNA Plant Viruses
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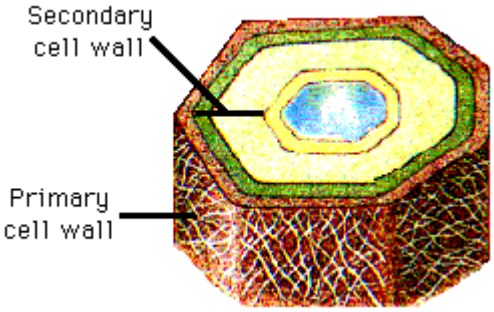
ssRNA Plant Viruses

This document is in no way intended to be a complete or even thorough discussion of plant viruses. Much more detailed information is available elsewhere!

Virus Infection of Plants

In economic terms, viruses are only of importance if it is likely that they will spread to crops during their commercial lifetime, which of course varies greatly between very short extremes in horticultural production and very long extremes in forestry. Some estimates put total worldwide damage due to plant viruses as high as US$ 6 x 10^{10} per year.



Plant viruses face special problems initiating an infection. The outer surfaces of plants are composed of protective layers of waxes and pectin, but more significantly, each cell is surrounded by a thick wall of cellulose overlying the cytoplasmic membrane.

To date, no plant virus is known to use a specific cellular receptor of the type that animal and bacterial viruses use to attach to cells. Rather, plant viruses rely on a mechanical breach of the integrity of a cell wall to directly introduce a virus particle into a cell. This is achieved either by the vector associated with transmission of the virus or simply by mechanical damage to cells. After replication in an initial cell, the lack of receptors poses special problems for plant viruses in recruiting new cells to the infection.

Transmission of Plant Viruses

There are a number of routes by which plant viruses may be transmitted:

- **Seeds:** These may transmit virus infection either due to external contamination of the seed with virus particles, or due to infection of the living tissues of the embryo. Transmission by this route leads to early outbreaks of disease in new crops which are usually initially focal in distribution, but may subsequently be transmitted to the remainder of the crop by other mechanisms (below).

- **Vegetative propagation/grafting:** These techniques are cheap and easy methods of plant propagation, but provide the ideal opportunity for viruses to spread to new plants.

- **Vectors:** Many different groups of living organisms can act as vectors and spread viruses from one plant to another:
    - Bacteria (e.g. *Agrobacterium tumefaciens* - the Ti plasmid of this organism has been used experimentally to transmit virus genomes between plants)
    - Fungi
    - Nematodes
    - Arthropods: Insects - aphids, leafhoppers, planthoppers, beetles, thrips, etc.
    - Arachnids - mites

- **Mechanical:** Mechanical transmission of viruses is the most widely used method for experimental infection of plants and is usually achieved by rubbing virus-containing preparations into the leaves, which in most plant species are particularly susceptible to infection. However, this is also an important natural method of transmission. Virus particles may contaminate soil for long periods and may be transmitted to the leaves of new host plants as wind-blown dust or as rain-splashed mud.

Transmission of plant viruses by insects is of particular agricultural importance. Extensive areas of monoculture and the inappropriate use of pesticides which kill natural predators can result in massive population booms of insects such as aphids.

Plant viruses rely on a mechanical breach of the integrity of a cell wall to directly introduce a virus particle into a cell. This is achieved either by the vector associated with transmission of the virus or simply by mechanical damage to cells.

Transfer by insect vectors is a particularly efficient means of virus transmission. In some instances, viruses are transmitted mechanically from one plant to the next by the vector and the insect is merely a means of distribution, flying or being carried on the wind for long distances (sometimes hundreds of miles). Insects which bite or suck plant tissues are, of course, the ideal means of transmitting viruses to new hosts. This is known as non-propagative transmission. However, in other cases (e.g. many plant rhabdoviruses) the virus may also infect and multiply in the tissues of the insect (propagative transmission) as well as those of host plants. In these cases, the vector serves as a means not only of distributing the virus, but also of amplifying the infection:

Group III geminivirus are transmitted by insect vectors (leafhoppers or whiteflies) and their genomes consist of two circular, single-stranded DNA molecules. These viruses cause a great deal of crop damage in plants such as tomatoes, beans, squash, cassava and cotton and their spread may be directly linked to the inadvertent world-wide

dissemination of a particular biotype of the whitefly *Bemisia tabaci* . This vector is an indiscriminate feeder, encouraging rapid and efficient spread of viruses from indigenous plant species to neighbouring crops.

Multipartite Plant Viruses

- **Segmented** virus genomes are those which are divided into two or more physically separate molecules of nucleic acid, all of which are then packaged into a single virus particle.
- Although **multipartite** genomes also segmented, each genome segment is packaged into a separate virus particle.

Family:	Segments:
Geminivirus (group III) (single-stranded DNA)	Bipartite
Comovirus (single-stranded RNA)	Bipartite
Furovirus (single-stranded RNA)	Bipartite
Tobravirus (single-stranded RNA)	Bipartite
Partitiviridae (double-stranded RNA)	Bipartite
Bromoviridae (single-stranded RNA)	Tripartite
Hordeivirus (single-stranded RNA)	Tripartite

These discrete particles are structurally similar and may contain the same component proteins, but often differ in size depending on the length of the genome segment packaged. In one sense, multipartite genomes are, of course, segmented, but this is not the strict meaning of these terms as they are used here.

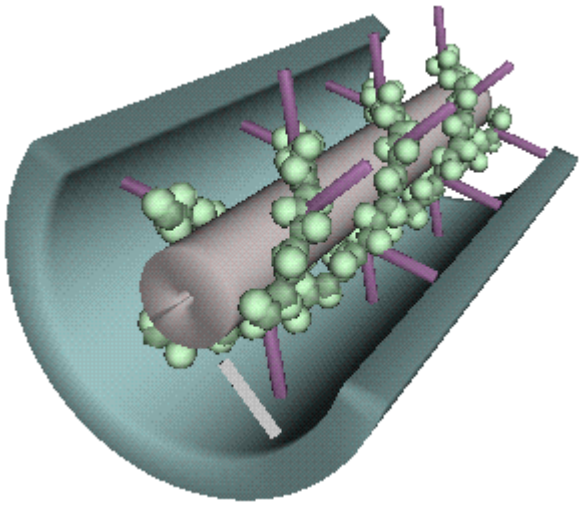
Genome segmentation reduces the probability of breakages due to shearing, thus increasing the total potential coding capacity of the genome. However, the disadvantage of this strategy is that all the individual genome segments must be packaged into each virus particle, or the virus will be defective as a result of loss of genetic information. Separating the genome segments into different particles (the multipartite strategy) removes the requirement for accurate sorting, but introduces a new problem in that all the discrete virus particles must be taken up by a single host cell to establish a productive infection. This is perhaps the reason multipartite viruses are only found in plants. Many of the sources of infection by plant viruses, such as inoculation by sap-sucking insects or after physical damage to tissues, result in a large inoculum of infectious virus particles, providing opportunities for infection of an initial cell by more than one particle.

Pathogenesis of Plant Virus Infections

Initially, most plant viruses multiply at the site of infection, giving rise to localized symptoms such as necrotic spots on the leaves. Subsequently, the virus may be distributed to all parts of the plant either by direct cell-to-cell spread or by the vascular system, resulting in a systemic infection involving the whole plant. However, the problem these viruses face in reinfection and recruitment of new cells is the same as they face initially - how to cross the barrier of the plant cell wall.

Plant cell walls necessarily contain channels called **plasmodesmata** which allow plant cells to communicate with each other and to pass metabolites between them.

However, these channels are **too small** to allow the passage of



virus particles or genomic nucleic acids.

Many (if not most) plant viruses have evolved specialized movement proteins which modify the plasmodesmata. One of the best known examples of this is the 30k protein of tobacco mosaic virus (TMV). This protein is expressed from a sub-genomic mRNA and its function is to modify plasmodesmata causing genomic RNA coated with 30k protein to be transported from the infected cell to neighbouring cells. Other viruses, such as cowpea mosaic virus (CPMV - Comovirus family) have a similar strategy but employ a different molecular mechanism. In CPMV, the 58/48k proteins form tubular structures allowing the passage of intact virus particles to pass from one cell to another.

Typically, virus infections of plants might result in effects such as growth retardation, distortion, mosaic patterning on the leaves, yellowing, wilting, etc. These macroscopic symptoms result from:

- **necrosis** of cells, caused by direct damage due to virus replication
- **hypoplasia**, i.e. localized retarded growth frequently leading to mosaicism (the appearance of thinner, yellow areas on the leaves)
- **hyperplasia**, which is excessive cell division or the growth of abnormally large cells, resulting in the production of swollen or distorted areas of the plant.

Plants might be seen as sitting targets for virus infection - unlike animals, they cannot run away. However, plants exhibit a range of responses to virus infections designed to minimize their effects. Initially, infection results in a 'hypersensitive response', manifested as the synthesis of a range of new proteins, the 'PR' (**p**athogenesis **r**elated) protein'. Although this system is poorly understood, at least some of these proteins have been characterized and have been shown to be proteases, which presumably destroy virus proteins, limiting the spread of the infection. There is some similarity here between this response and the production of interferons by animals. In addition, systemic resistance to virus infection is a naturally occurring phenomenon in some strains of plant, e.g. the tobacco *N* gene encodes a cytoplasmic protein with a nucleotide binding site which interferes with the TMV replicase. This is clearly a highly desirable characteristic and is highly prized by plant breeders, who try to spread this attribute to economically valuable crop strains. There are probably many different mechanisms involved in systemic resistance, but in general terms there is a tendency towards increased local necrosis as substances such as proteases and peroxidases are produced by the plant to destroy the virus and to prevent its spread and subsequent systemic disease. An example of this is the N gene, which when present in plants causes TMV to produce a localized, necrotic infection rather than the systemic mosaic symptoms normally seen.

Amazing: Plants can even warn each other that viruses are coming! Don't believe it? Read:
Shulaev, P. et al. Airborne signallingby methyl salicylate in plant pathogen resistance. Nature 385: 718-721 (1997).

Virus-resistant plants have been created by the production of transgenic plants expressing recombinant virus

proteins or nucleic acids which interfere with virus replication without producing the pathogenic consequences of infection, e.g:

- Virus coat proteins: these have a variety of complex effects, including:
  - Inhibition of virus uncoating
  - Interference of expression of the virus at the level of RNA ("gene silencing" by 'untranslatable' RNAs)
- Intact or partial virus replicases which interfere with genome replication
- Antisense RNAs
- Defective viral genomes
- Satellite sequences
- Catalytic RNA sequences (ribozymes)
- Modified movement proteins

This is a very promising technology which offers the possibility of substantial increases in agricultural production without the use of expensive, toxic and ecologically damaging chemicals (fertilizers, herbicides, or pesticides), but is at present still in its infancy.

Another developing aspect of plant biotechnology is the construction of **CVPs - C**himaeric **V**irus **P**articles - recompinant viruses such as:

- cowpea mosaic virus - an icosahedral member of the Comovirus family
  or
  - potato X virus - a rod-shaped member of the Potexvirus family

which express short antigenic peptides on their surface. Several grams of CVPs can be produced cheaply & easily per kilogram of plant leaves. This is a very promising vaccine technology.

Emergent Plant Viruses

Rarely, there seems to be an example of an emergent virus which has acquired extra genes and as a result of new genetic capacity, becomes capable of infecting new species. A possible example of this phenomenon is seen in tomato spotted wilt virus (TSWV). TSWV is a Bunyavirus with a very wide plant host range, infecting over 600 different species from 70 families. In recent decades, this virus has been a major agricultural pest in Asia, the Americas, Europe Africa. Its rapid spread has been due to dissemination of its insect vector (the thrip *Frankinellia occidentalis*) and diseased plant material. TSWV is the type species of the Tospovirus genus and has a similar morphology and genomic organization to the other Bunyaviruses. However, TSWV undergoes **propagative transmission** and it has been suggested that it may have acquired an extra gene in the M segment via recombination, either from a plant or from another plant virus. This new gene encodes a **movement protein**, conferring the capacity to infect plants and cause extensive damage.

- Search for more information on this topic.
- References.
- Plant Virus Servers/Information
- ICTV Approved Plant Virus Families and Genera (Prepared by Dr. Zhongguo Xiong, University of Arizona).
- Molecular Plant Virology Course notes from Dr Zhongguo Xiong, University of Arizona.

Books about Plant Virology: click here

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Emerging Viral Diseases

General:

- For the very latest news on emerging viruses, visit **The Microbiology Newsroom**.
- ? Probable Human Infection with a Newly Described Virus in the Family Paramyxoviridae ?
- W.H.O Disease Outbreak News
- Influenza: An Emerging Disease
- Emerging Infectious Disease: WWW Journal.
- Addressing Emerging Infectious Disease Threats
- Emerging & Re-emerging Viruses: An Essay
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- Hantaviruses, with emphasis on Four Corners Hantavirus
- Hantavirus Pulmonary Syndrome
- Serendipity, Science, and a New Hantavirus
- Stalking The Deadly Hantavirus: A Study In Teamwork

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Filoviruses:

- "Marburg and Ebola"
- Dr. Frederick A.Murphy Talks about the Ebola Virus
- Dr. Donald P.Francis Talks to Teachers About the Ebola Outbreak (Genentech, Inc.)
- Regulation of a Runaway Replicator by Myles Axton
- Ebola Virus Hemorrhagic Fever: General Information (Centers for Disease Control)

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Viral Transformation of Cells

Cancer can be viewed as a genetic disease. It is due to discrete changes in the cellular genome which in some cases are heritable. Virus are probably responsible for about 15% of human cancers and as a risk factor are second only to tobacco. This set of notes is concerned with the phenomenon of cell transformation by viruses. It will discuss the process of transformation, RNA tumour viruses, DNA tumour viruses and viruses which cause tumours in humans e.g. EBV, papilloma, Hepatitis B and HTLV-1.

Transformation:

can be defined as "**the introduction of inheritable changes in a cell causing changes in the growth phenotype and immortalisation**".

The cell cycle, **(G0)-G1-S-G2-M**, is highly regulated in multicellular organisms. The G0/G1 boundary is a particularly important control point because this acts as a commitment to cell division. Tumour formation results from a failure of these regulatory mechanisms; tumour cells continue to divide under circumstances in which their normal cellular counterparts do not. Transformation of cells in culture is the in vitro counterpart of the process by which tumour induction in animals (by viruses) occurs. Many of the properties which can be used to differentiate a transformed cell from a non transformed control are also observed when tumour cells are compared with their normal counterparts. For example:

Growth:

- high/indefinite saturation density
- different, usually reduced serum requirement
- growth in agar-anchorage independent
- tumour formation when injected into animals
- no contact inhibition of movement
- less oriented growth
- growth on normal cell monolayers

Surface:

- increase agglutinability of plant lectins
- changes in glycoproteins and glycolipids
- loss of tight junctions
- foetal antigen expression
- different staining properties
- increased rate of nutrient transfer
- increased secretion of proteases

Intracellular:

- disruption of cytoskeleton
- altered amounts of signalling molecules (cyclic nucleotides, phosphoinositides)

This list highlights regulatory mechanisms acting on normal cells which can be broadly divided into 3 groups:

1. Those involving cell-cell contact i.e. contact inhibition of growth.
   Normal cells exhibit it, transformed cells do not.
2. Those involving dependence on exogenous growth factors.
   Normal cells show such a dependence, transformed cells do not.
3. In some cell types, anchorage dependent growth constitutes a further group of regulatory properties.

Normal cells exhibit it, transformed cells do not.

These features suggest the basis for selecting cell transformants and assaying transformation. Most cell lines are already transformed and by definition not suitable for transformation assays.

Primary cells have a limited life span in culture. They reach a crisis phase and die after about 50 generations. It is clearly possible to use primary cells in transformation assays but the availability of a characterized cell line behaving in a consistent and identifiable manner is preferable.

Todaro and Green developed a mouse fibroblastic cell line called NIH 3T3 which has been extensively used for studying transformation by cellular and viral oncogenes and other agents. They plated mouse fibroblasts at a low density and replated them every 3 days at this low density ensuring that the cells were never in contact. After more than a year they had cells which were capable of indefinite growth in culture but which still exhibited contact inhibition of growth. They were also aneuploid (chromosomal number is not haploid x N). They undergo spontaneous transformation in vitro in the absence of exogenous agents and exhibit a low tumouroginicity when introduced into mice. It is reasonable to conclude that they have accumulated a mutation or series of mutations required for expression of the transformed phenotype. These features of NIH3T3 cells (which behave "normally" in many ways) make it suitable for use in "transformation assays" because only a limited number of further mutations (perhaps 1) tip the cell over the edge into a fully transformed state. This feature is obviously useful in assays for cellular oncogenes.

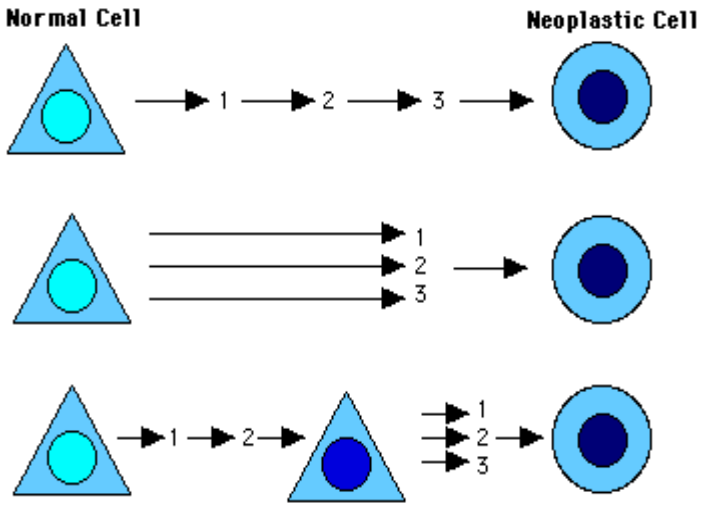
Assays for Cell Transformation.

Any differences between transformed and non transformed cells can be used to assay the transforming ability of viruses or chemicals e.g. morphology, - increased refractility or differentiation. Different assays may give rise to transformants with different properties. Three assays are commonly used, each exploiting a different selection pressure:

- Focus forming assay. The first assay for viral transformation devised by Rubin and Temin. In mid 1950s Rous sarcoma virus, an avian retrovirus, was found to induce morphological changes and extend the life of chick embryo fibroblast cells in culture. Transformation was rapid and quantitative. Transformation was detected as foci of dense morphologically altered cells in monolayers of chick embryo fibroblasts. This type of assay which depends on the loss of contact inhibition has subsequently been used for a number of DNA viruses e.g. polyoma and SV40 in NIH3T3 cells. It depends on the loss of contact inhibition following transformation so cells must obviously show this feature initially.
- Anchorage independent growth in agar or a methyl cellulose suspension. Primary fibroblasts and many fibroblastic lines must attach to a solid surface before they can divide i.e. anchorage dependence. They fail to grow when suspended in a viscous fluid or gel. Possible to select transformed cells on the basis of growth in the absence of a solid substratum. This is usually considered the most stringent assay. BHK21 cells transformed with polyoma virus show anchorage independent growth.
- Reduced serum requirement The reduced serum requirement of transformed cells can be exploited e.g. NIH 3T3 cells will not grow in media with less than 5% serum. SV40 transformants could be selected for by plating in 0.5% serum. This assay exploits the requirement of cells for growth factors.

Transformation by individual genes:
The development of high efficiency transfection protocols in the 1970s was an important technical advance. This led to the observation that transfected DNA could confer on the cell biochemically detectable characteristics. This prompted studies of the transfection of cellular DNA from transformed cells into normal cells which demonstrated that such DNA was capable of eliciting transformation. Using these techniques a considerable number of cellular and viral oncogenes were identified. NIH3T3 cells were often used in these studies because they take up DNA efficiently. NIH3T3 cells do not detect all classes of transforming genes - only 50% of tumour derived DNAs gave rise to transformants. This is due either to their recessive nature or to a lack of activity in the NIH3T3 system particularly. It seems evident that the way in which these cells were established would influence the types of oncogenes they could be used to detect. Thus although the cell line exhibits growth control it is capable of indefinite growth which could be considered an essential feature of any transformation process.

Mechanism of Transformation:



In vivo and epidemiological studies suggest that transformation is a **multistep process** involving **initiation, promotion and progression** events. Chemically induced carcinogenesis (e.g. a polycyclic hydrocarbon) on mouse skin results in an initiation event which is the activation of a cellular ras gene. The initiation event is irreversible and phenotypically undetectable. Application of a phorbol ester such as TPA promotes clonal expansion of cells with the ras mutation. This can occur 12 months after the initial ras activation. There is an initial period of reversibility in this clonally expanded cell population but finally irreversible genetic changes occur leading to the formation of a malignant tumour. The promotion and progression stages involve further cellular mutations.

The molecular basis of transformation in NIH3T3 cells is similar. The (viral) v-myc gene from the avian retrovirus MC29 and activated (cell) c-N-ras derived from a human bladder tumour cooperate to transform NIH3T3 cells. Neither are sufficient on their own. It is possible to transfect the oncogenes together or sequentially in either order transform the cells.

In contrast to this, some DNA viruses are apparently capable of a single step transformation. The multifunctionality of their viral oncogenes goes some way to explaining. There are cases where a single step transformation does a take place. An example presented below are the transducing retroviruses.

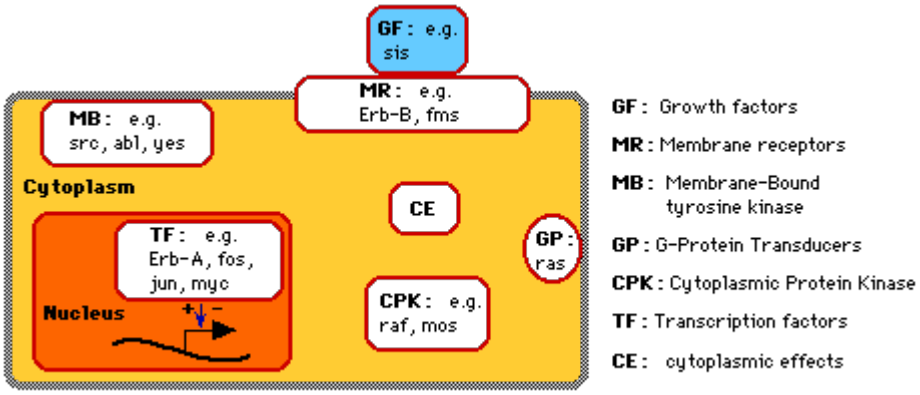
There is also a class of cellular genes known as tumour suppressors e.g. p53 and the retinoblastoma gene product Rb. These encode proteins that exert a negative control on cell replication. Interference with the function of these genes seems to be a major factor in some types of DNA viral oncogenesis. In many cell types progression from a low grade non malignant to a high grade malignant tumour involves mutations in the p53 gene. The function of these genes is discussed here.

How do viruses transform cells?

The frequency of infection by agents such as EBV, HBV or HTLV-1 are far greater than the incidence of the cancers with which they are linked. Viruses may act in concert with other factors in the evolution of cancer. The other factors might be genetic, immunological or environmental. Cell transformation by viruses is accompanied by the persistence of all or a part of the viral genome. It is also accompanied by the continual expression of a limited number of viral genes. Viral oncogenes disrupt the normal cellular gene expression and signal transduction pathways. The signal transduction pathway is responsible for altering cellular gene expression in response to a wide range of external and internal signals. It is a complex net of regulatory proteins which means that a given gene product can be (de)activated by many different stimuli and that a single stimuli can (de)activate many different genes. Viral oncogenes disrupt the normal functioning of this net. They do this by virtue of altered structures or overproduction. The end result is an altered transcriptional profile which favours cell division. This can be divided this into 4 broad groups, with many known examples of oncogenes for each grouping:

1. Cell surface where receptors interact with growth factors and components of the extracellular matrix.
2. Transmembrane signalling apparatus including the cytoplasmic domain of receptors and submembranous components that are functionally linked to surface receptors. conveying signals from the outside of the cell to the interior.
3. Cytosolic elements i.e. soluble proteins and second messengers.

4. Nuclear proteins including DNA binding proteins and factors which interact directly and indirectly in gene regulation and replication.



At the biochemical level, communication between compartments often involves **protein phosphorylation**. Thus the initial step in transmembrane signalling involves the interaction of an extracellular ligand (peptide hormone or growth factor with its receptor to trigger protein phosphorylation within the cell. This phosphorylation is catalysed by the cytoplasmic domain of the receptor. Other components that might be involved include submembranous kinases and G proteins, these latter are not integral but associate with the membrane. In this way events happening outside of the cell are signalled to the cytoplasm. Cytosolic protein factors are often serine/threonine protein kinases some of which may be activated directly by events happening at the membrane, e.g. protein kinase C family. They also include small soluble second messengers which again may be generated at the membrane e.g. cyclic nucleotides and inositol phosphates.

Nuclear proteins comprise a large and functionally diverse group includes proteins which bind directly to cis-acting regulatory elements in DNA. Proteins which form large multifunctional complexes in transcriptional regulation and DNA replication e.g. T3 receptor and steroid hormone receptors. Many of these factors are phospho-proteins whose activity again may be regulated in part by phosphorylation. If any of these become permanently switched on, either because of a dosage effect or a mutation then the normal pathway is bypassed and transformation may result.

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RNA TUMOUR VIRUSES

Oncogenic Retroviruses.

Divided into 3 major groups based on their likely mechanism of oncogenesis:

1) Acutely transforming or transducing:

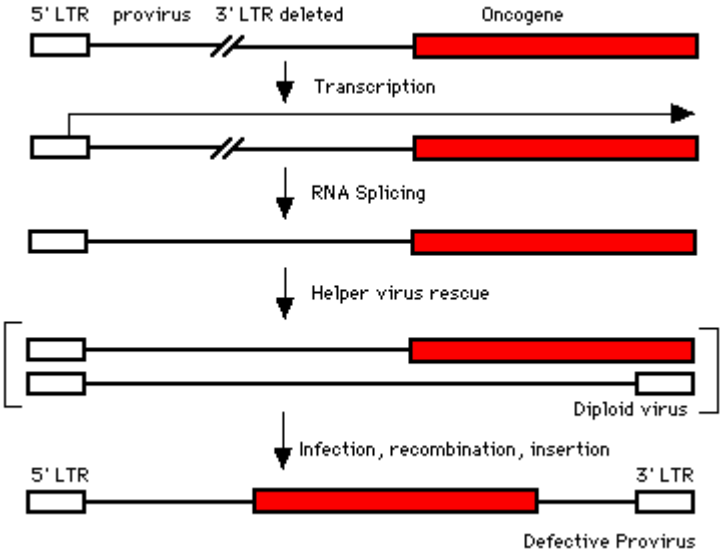
e.g. Rous Sarcoma Virus. These contain viral oncogenes (v-onc). They are generally replication defective and induce polyclonal tumours at approaching 100% efficiency within days as a result of single step carcinogenesis. In nature they exhibit a low horizontal spread and no vertical spread. The v-onc is transduced from a host cell genome (c-onc) into the genome of an initially replication competent retrovirus. The v-onc can differ from the c-onc in a number of ways:

- Lack introns
- May be truncated e.g. v-src
- May contain point mutations e.g. v-ras
- Frequently find fusions between a viral gene and the oncogene e.g. gag-onc or env-onc
- Expressed at abnormally high levels under LTR control

These alterations can profoundly alter the effects v-oncs have on growth. More than 40 oncs have been discovered. More than half have been found in these acutely transforming viruses. Not all found in retroviruses. Clearly only a subset of the total oncogene pool. Range is limited by transducing method and perhaps gene size. There are various ways of trying to impose order on this incompletely understood system, e.g. site of action as explained in the last lecture on cell transformation:

Simian Sarcoma Virus	v-sis	Growth Factor
Avian Erthyroblastosis Receptor	v-erbB	Growth Factor Receptor
Rous sarcoma virus	v-src	Tyrosine kinase
Kirsten murine sarcoma virus	v-kras	G protein transducer
Moloney murine sarcoma virus	v-mos	serine/threonine kinase
MC29 avian myelocytoma virus	v-myc	transcription factor

How are cellular oncogenes acquired?



A plausible model which can be reproduced in culture is that integration occurs close to and upstream of an oncogene. The provirus has deleted 3' LTR. Transcription will therefore go through the oncogene, introns removed and because it has a 5' packaging signal the RNA can be packaged. A second "helper virus" infects the cell to enable this RNA to be packaged into a virion particle. As retroviruses are diploid a chimaeric virus may result. During next round of infection non-homologous recombination may take place during reverse transcription giving rise to a provirus with two LTRs. The resulting retrovirus is defective because 3' coding information is lost.. But it can transform a cell. Infectious transforming virus is only released if functional helpers are present. Examples include:

Simian sarcoma virus and v-sis:
Identified in a fibrosarcoma of a woolly monkey. Characterised by DNA sequencing and antibody immune precipitations as a 38K protein product. Not phosphorylated and no kinase activity. Env-sis fusion product, involving 5' end of env. Data base comparisons showed a striking homology to human platelet derived growth factor (PDGF) beta-chain, about 88%. PDGF has a & beta-chains which forms both homo and heterodimers. SSV transformed cells produce large amounts of the homodimer which is functionally indistinguishable from the real thing. About 10% is secreted and the rest is firmly associated with cellular membrane fractions, probably due to the fusion with env. This suggests an autocrine function in which continual stimulation of the PDGF receptor results in eventual transformation. One discrepancy is that SSV transformed cells can grow in soft agar while PDGF stimulated normal cells cannot. This could be because:

1. The fusion protein has slightly different properties to the normal protein
2. The intracellular interaction of the homodimer with internal receptors might evoke a different response from that elicited by exogenous PDGF.

Cells transformed by many cytoplasmic oncogenes also produce to excess various growth factors. This suggests that the external stimulus provided by these factors is important in producing and maintaining the transformed phenotype.

Avian erythroblastosis virus and v-erbB gene (EGF receptor gene):
Induces erythroblastosis and sarcoma in chicken, transforms fibroblasts and erythroid cells in vitro. The v-erbB gene codes for an epidermal growth factor receptor (EGFR). The helper virus is a non-defective ALV

EGFR has 4 domains:

1. N-terminal extracellular EGF binding domain
2. Transmembrane segment
3. Tyrosine kinase catalytic domain
4. C-terminal kinase regulatory domain

The kinase activity is likely to be the primary effector function. EGF binding stimulates this activity resulting in autophosphorylation of EGFR and subsequent phosphorylation of cellular polypeptides. The v-erbB gene is truncated at both 5' and 3' ends. The 5' truncation alone will cause erythroblastosis; the 3' deletion is required for fibroblast transformation. The biochemical details of its activation are unknown but are presumably related to the

loss of the C-terminal regulatory domain.

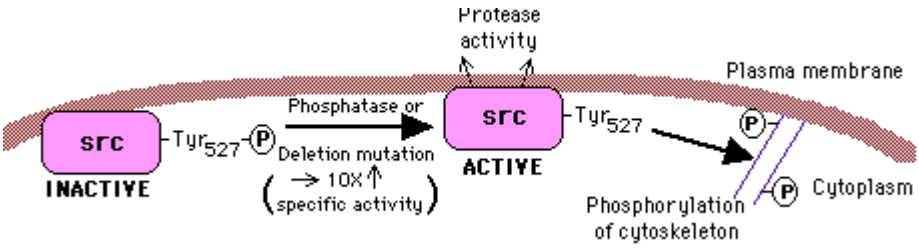
Known substrates for phosphorylation include GAP a GTPase activating protein; Phosphatyl-3-kinase and phospholipase C (thereby influencing inositoyl phosphate mediated signally events); and Grb2 a protein I shall discuss in a few moments. Proteins containing SH_2 domains are also substrates, see below. These can serve as molecular adaptors in signalling.

Rous Sarcoma virus and the src gene:

First isolated in 1911 by Rous, capable of transmitting a sarcoma between Plymouth Rock chickens. It usually requires a replication competent virus such as avian leukosis virus for transmission. It has an env deletion. Unusually for this class of viruses, replication competent viruses can be isolated, but they are unstable. Src was eventually identified as gene responsible for transformation by using a combination of approaches e.g. genetic analysis of t.s. mutants and non-conditional (deletion) mutants. Position of the gene in the virus was then determined by recombinational analysis and oligonucleotide mapping. The gene product was identified by immune precipitation using sera from sarcoma bearing rabbits. It is a phosphoprotein with a molecular weight of 60K. Unlike many v-oncs this is not expressed as a fusion with normal virus polypeptide. The corresponding cellular gene and protein product turns out to be a tyrosine protein kinase. The mammalian genome contains many (>30) tyrosine kinase genes in a single gene family. The catalytic domains show extensive homology. There is considerable divergence in the other receptor binding domain. The kinase activity is turned on when it associates with an activated receptor.

c-src is located on the inner surface of the plasma membrane, anchored to the membrane by a myristilated N-terminal glycine residue. It concentrates at focal points between cells, where it is associated with the cytoskeleton. src contains a number of domains N-SH_3-SH_2-Kinase domain-Regulatory domain-C (SH stands for src homology region). These are found in a wide variety of proteins but were first recognized in src. SH_2 domains bind phosphotyrosine residues, SH_3 domains bind proline rich sequences in many different proteins enabling proteins like src to potentially bind to a very wide range of other proteins.

Activation of src weakens cell contacts and induces membrane ruffling which is associated with increased cell motility.Fibronectin a protein associated with the cytoskeleton as well as several other associated proteins are phosphorylated by src. This is due to the src mediated activation of a protein called focal adhesion kinase or FAK. src activation also increases secretion of plasminogen activator, this elevates plasmin levels which degrades proteins in the extracellular matrix again increasing cell motility



What are the differences between c and v-src ?

- v-src has a series of point mutations but these are not conserved in various strain.
- v-src is also close to viral LTR and contains no introns but over expression of c-src gene by a heterologous promoter or homologous substitution in a RSV does not result in complete transformation.

By elimination it seems that a 900 base deletion from the 3' end of v-src is the cause. The result of this deletion is that the 19 C-terminal amino acids v-src are lost and replaced by 12 amino acids derived from the 3'UTR of c-src. This leads to a simple (but not complete) biochemical explanation. Src is normally inactive. The kinase activity of src is down regulated by phosphorylation of a tyrosine 527. This is thought to bind intramolecularly to the SH_2 domain resulting in an inactive conformation.c-src gains activity if it is dephosphorylated by in vitro phosphatase treatment. v-src lacks this tyrosine residue and is permanently activated. There is good evidence for this model from other experiments, e.g:

- Other transducing retroviruses which carry src have C-terminal truncations which result in the loss of tyrosine 527

- The activation of other tyrosine-kinase oncogenes such as v-yes and v-fgr also involve C-terminal truncations and the loss of the phosphorylatable tyrosine residue
- Site directed mutagenesis of the src tyrosine 527 gives it expression level dependent transforming ability in the 3T3 assay.

Moloney sarcoma virus and mos:
Isolated from a rhabdomyosarcoma in mice. The virus will transform cultured fibroblasts. Expressed as a env-mos fusion protein in the soluble portion of cytoplasm. It is a serine/threonine protein kinases. c-mos is normally expressed at very low levels in a cell (<1 mos MRNA per cell). c-mos gene has the same transforming power as the v-mos gene which suggests that transformation may be a consequence of overexpression by the viral LTR.

MC29 and myc:
Avian myelocytomatosis virus, of which MC29 is the prototype, caries the v-myc gene as a gag-myc fusion. c-myc is a DNA binding phosphoprotein found in the nuclear matrix. It binds to a consensus DNA sequence CACGTG as a heterodimer with another protein known as max.A variety of cells express it during the proliferative phase. Both the mRNA and protein have a very short half life consistent with a regulatory role in the cell cycle. The gene is turned off normally; unregulated expression is important in transformation as evidenced by transformation assays and transgenic animal experiments. Again, the properties of v-onc may be explained just by:

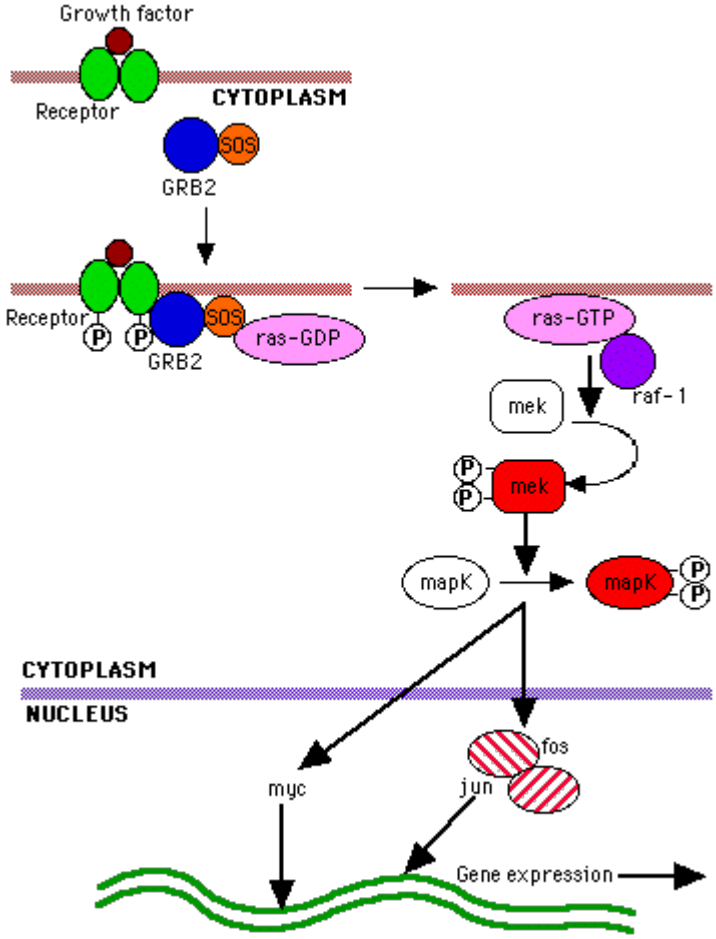
1. High level expression driven by the LTR
2. By a variety of point mutations found in v-myc.
3. Other nuclear oncogenes also identified including myb, jun, fos and ski.

Presumably, all are involved in switching on transcription of genes involved with cell growth. One signal transduction pathway which is particularly well understood is exemplified by:

Kirsten and Harvey murine sarcoma virus and ras:
Isolated from sarcomas that developed in rats. This virus has transduced a ras oncogene. Generally the transduced ras gene is not fused to a viral gene and contains no deletions. Activation is explained in terms of:

- High levels of expression driven by the viral LTR
- Point mutations which occur at 2 specific positions in p21 ras i.e. 12 and 59 which reduce GTPase activity

The ras family of proteins are located on cytoplasmic face of the cell membrane. They have a GTPase activity and a homology with G proteins. These proteins serve to couple extracellular signals via a receptor to intracellular enzymes. Antibodies raised to the GTP binding domain reverse the transformed phenotype when injected into cells confirming the importance of the GTPase activity. **Transduction Pathway:**



A growth factor binds to a receptor-resulting in receptor dimerisation and trans-phosphorylation.
A protein (GRB2-SOS) dimer complex binds to the receptor (growth factor receptor binding protein and son of sevenless).
Ras associates with SOS, a guanine exchange factor, and exchanges GDP for GTP. Ras is like a switch, it is on when bound to GTP, off when bound to GDP.
Raf-1 a protein kinase associates with SOS and is activated.
This complex phosphorylates MEK which in turn phosphorylates MAPK (mitogen activated protein kinase.
MEK can phosphorylate several oncogenes including myc, jun and ets, which are transcriptional factors. Get co-ordinated changes in gene expression, positive factors stimulated, -ve factors inhibited.
This highly conserved pathway which is present in man Drosophila and yeast has many steps. There are alternative activators and MAPK may be phosphorylated by kinases other than MEK.

Of the factors described in this pathway so far only ras mutations appear in human cancers.

2. Replication competent viruses and insertional mutagenesis

Exemplified by avian leukosis viruses. The initial event is thought to be integration near a cellular gene - generally myc. Viruses of this type are replication competent and the animals are chronically viraemic. Tumours appear after a long multistaged latent period (in contrast to transducing retroviruses) and are clonal in origin. So although many cells are infected, only one progresses to a tumour.

When immunologically incompetent newly hatched chicks are infected with ALV a large number of cells in the bursa of fabricus are infected. Within one month of this a preneoplastic transformation occurs with the formation of about 100 follicles containing immature blast cells. A few of these form nodules 5-10mm in size after several months. During natural bursal regression disseminated metastases derive from these nodules.

It was discovered that in most bursal lymphomas induced by ALV the cellular myc gene was activated, apparently because proviral integration occurs near the myc locus. Myc RNA is elevated 50x compared to normal cells. The myc transcripts varied in length but generally contained U5 sequences derived from the 3' LTR This suggests that the elevated myc transcription is due to the activity of the 3' LTR promoter elements. Simplest interpretation is

therefore that there is abnormal activation of the c-myc gene. This is an example of promoter insertion. There are also cases where insertion is 3' of the myc gene or where insertion is in the wrong orientation in which case the activation effect is postulated to be due to enhancer insertion. This conclusion seems warranted because in all known cases of this the enhancer sequences are maintained.

These experiments helped to establish the insertional model of oncogenesis.

However the story is more complicated than you might think and provides more evidence for the multistage development of these tumours. Other experiments using the NIH3T3 system and DNA from bursal tumours showed that the "transforming DNA" was not myc (failure to detect myc activity in these cells is hardly surprising as they are already immortalized) but Blym-1. This codes for a 8,000MW protein homologous to the N-terminus of transferrin. The clear inference is that myc activation precedes Blym-1-1 activation. The function of Blym-1 is unknown.

Mouse mammary tumour virus:
MMTV also has a long induction period for tumourigenesis. The transformation event is rare despite 50% of mammary epithelial cells being infected. Three major unlinked and unrelated loci are transcriptionally activated in many MMTV tumours int1, int2 and int3. Int-1 is involved in normal mouse neural development. In normal cells int-1 transcripts are not detectable. In transformed cells there are about 10 copies of mRNA per cell. Int-1 can transform a mammary epithelial cell line. Transgenic mice expressing int-1 under the control of the MMTV LTR show a high frequency of adenocarcinomas developing from hyperplastic mammary glands. An enhancer element of the LTR is thought to cause overexpression of int-1 since it is always located downstream or in the opposite transcriptional sense upstream. Int2 shows homology to fibroblastic growth factor, potential autocrine activator. Int3 is another protein involved in cell development.

3. Replication competent viruses with transacting functions, e.g. HTLV-1

Transactivating non-defective oncoviruses:

- Human T Cell Leukaemia virus types I and II.
- Bovine leukaemia virus or BLV is a closely related virus.

The genome organization is specific to this group, i.e. 5'-LTR-gag-pol-env-rex-tax-LTR-3'

HTLV-1 is associated with specific lymphoid malignancies and is endemic in Japan, the Caribbean and Africa. Discovered after intense efforts to isolate retroviruses by R. Gallo. The virus was isolated from an adult T cell malignancy. The malignancy is always a monoclonal CD4+ T cell. Molecular probes show that HTLV-1 is present in almost all cases of adult T cell leukaemia. There is also a strong sero-epidemiological correlation between the disease and the virus. Transmission is thought to occur by cell-cell contact and not by free virus during the intimate exchange of body fluids:

- Perinatal transmission via milk lymphocytes during breast feeding
- Lymphocytes contained in semen during sexual intercourse
- Unscreened blood transfusions
- Sharing of blood contaminated needles by intravenous drug users.

In Japan, about 1% population is infected. Clustering results in much higher rates of infections in some regions, e.g. Okinawa 35%, Kyushu province 10%. The virus is becoming increasing common in W.Europe and N. America particularly amongst intravenous drug users and homosexuals. About 1-3% of infected individuals will eventually develop this aggressive leukaemia after an incubation period which is usually several decades long. Following an asymptomatic period, the patient may develop pre-ATL, in which they remain asymptomatic but there is a clonal expansion of T-cells which can be morphologically-altered. 50% of patients with pre ATL progress to chronic/smouldering ATL. This is manifested by skin lesions (mycosis fungoides) and high leukocyte counts. They then progress to acute ATL within several months. The survival time is measured in months.

White blood cell counts are elevated, there is a dominant malignant clone of morphologically altered T cells. Symptoms include lymphadenopathy, hepatosplenomegaly, and hypercalcaemia. Patients are also immunocompromised and present with fungal infections, pneumocystis pneumonia and cytomegalovirus infection.

Despite a wide spread disseminated immune response the infection is never cleared. Treatment is reserved for subacute and acute phases. However, there are no treatments of demonstrated value.

HTLV can transform T lymphocytes in culture. Transformation is defined as the ability to grow indefinitely in the absence of exogenous T cell growth factor (TCGF) or interleukin 2 (IL-2). HTLV-1 is not thought to have a preferred integration site which might lead to cellular gene activation. No single activated oncogene can be detected in a transformation assay. It is currently assumed that the virus augments T cell proliferation and thus provides an expanded T cell population in which a malignant event can occur.

There is some molecular data which may be relevant to transformation. Transactivation of transcription by an HTLV gene product called tax has been observed. Tax is essential for viral replication. Viruses which do not contain a functional tax gene are not infectious. They make only trace amounts of RNA suggesting a role in transcriptional activation. Direct evidence of this has been obtained using a transient transfection assay with a LTR-enzyme reporter gene. Tax can transactivate other host promoters, e.g. an NFkB-like transcription factor characteristic of activated T cells which binds to an upstream element of the TCGF gene. Mice transgenic for tax develop multiple mesenchymal proliferative disorders.

HTLV-1 positive individuals also have a 20% lifetime risk of developing tropical spastic paraparesis. This is characterized by uncoordinated motor control. The pyramidal tracts of the spinal chord which carry motor neurones house the virus and show degenerative changes. The cause of this syndrome is unclear.

Until recently the only RNA tumour viruses belonged to the family Retroviridae. Now it seems that a member of the Flaviviridae family can also cause tumours. Hepatitis C virus was identified in 1989 as the cause on 90% of non-A non- B hepatitis infections. It is an enveloped virus and contains a single stranded RNA genome of 9.5kb. The virus is undoubtedly transmitted by blood contact. Perinatal and sexual transmission may also occur. Infection may be asymptomatic for decades but the majority of infections eventually result in hepatitis and hepatocellular carcinoma. Conditions for growth in vitro have not yet been established and little is known about the pathology and cancer caused by the virus.

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DNA TRANSFORMING VIRUSES.

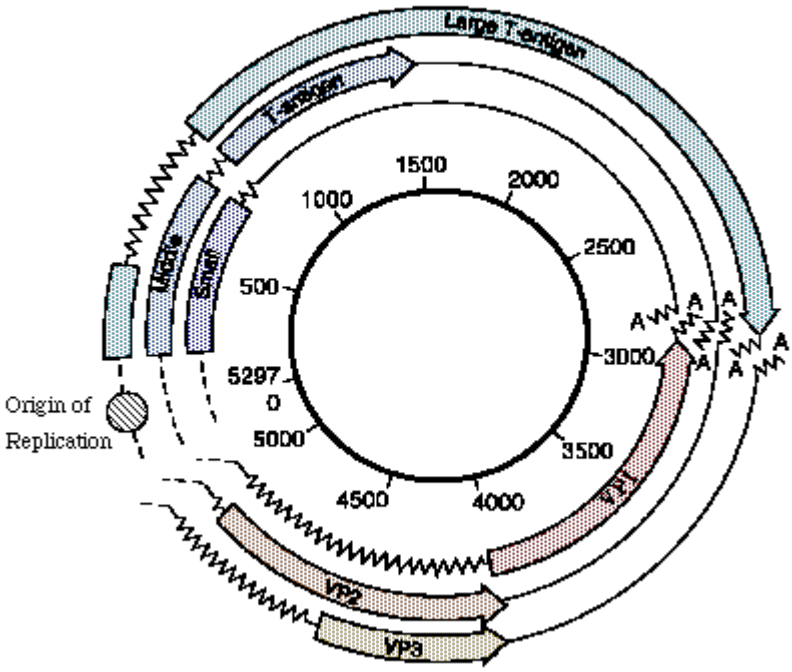
The genes responsible for transformation in these viruses have no cellular homologues. They are true viral genes required for viral growth. Most cells in the body are quiescent and not synthesizing DNA for replication. DNA viruses need to make DNA in order to replicate and many of them are partially dependent on the cell to do this. They therefore modify gene expression in the host cell to favour DNA synthesis and cell replication e.g adenovirus in terminally differentiated epithelial cells, these cells must be pushed into S phase for viral replication. The normal result of an infection is a lytic cycle resulting in the death of the cell.The aberrant result of a non-permissive infection is rarely (10^{-5}) transformation. This inefficiency reflects the lack of a specific mechanism for genome integration. The viral proteins responsible for transformation generally have a nuclear location. They have proved to be an interesting tool with which to study cell growth control mechanisms.

Papovaviruses:

Have small genomes ~5-10kb and have been extensively studied. The family includes SV40, and polyoma virus. These viruses can interact with cells in one of 2 ways:

- Lytic pathway in permissive cells leading to production of virus particles and cell death.
- non-lytic pathway in non--permissive cells which leads to transformation.

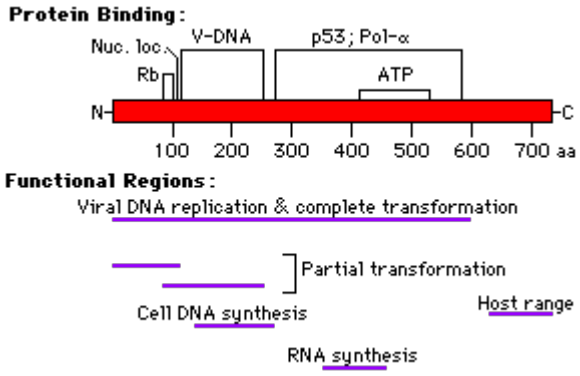
SV40 was originally identified as a non-cytopathic virus in rhesus monkeys which could produce a lytic infection in green monkey cells and cause tumours in hamsters. The possibility that transformation was a result of integration was ruled out early on and attention focused on viral (onco)genes.



SV40 codes for 2 "T" or tumour antigens 94K and 17K. They are the result of differential splicing of a single transcript. Small T seems to be dispensable for virus growth in culture and for transformation. Large T acts as a switch from early to late transcription for the virus.

Studies show that large T is multifunctional in nature, both with respect to its viral functions and its transforming functions.

Properties of SV40 Large T antigen:



- 98% nuclear
- DNA binding
- DNA independent ATPase
- ATP dependent DNA helicase
- Binds to DNA polymerases, AP2, p53, Rb
- Undergoes phosphorylation, acylation, adenylation, polyADP ribosylation, glycosylation.

Molecular genetic analysis suggests that N-terminal 140 or so amino acids of large T is involved in transformation. Specifically a region between 105-114. Sequence homology with E1A transformation domain from adenovirus. Binds to Rb. Mutants in this region are defective in induction of focus formation. p53 binding site is located at the C-terminus. Nuclear transport defective mutants of large T have been analysed, when such a mutation is transfected into primary cells it does not transform suggesting a nuclear function. However, transformation of NIH 3T3 cells is as efficient as the wild type gene. Here it is assumed to be functioning in the cytoplasm. i.e. consistent with a multistep model of transformation.

Mechanism of action is probably via an interaction with tumour suppressor genes. Rb-p105 is a phosphorylated nuclear protein which modulates transcription. Rb gene identified in individuals who develop retinoblastoma early in life. p53 also modulates transcription. T antigen association with these 2 proteins is postulated to overcome the inhibition of cell growth.

Adenoviruses:

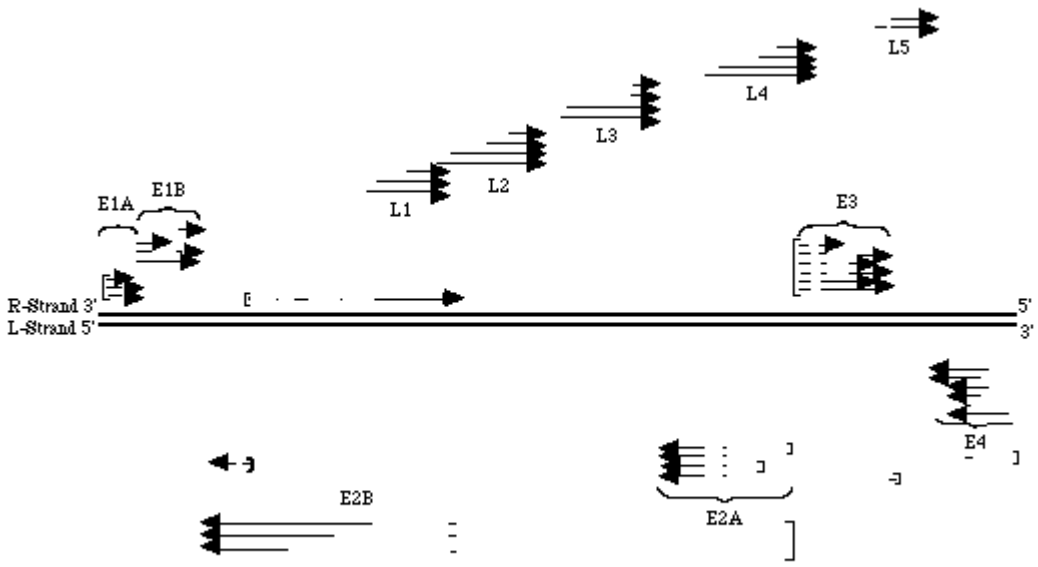


Fig 5.16 These are large d/s DNA viruses with genomes in excess of 100kb. Human serotypes transform rat cells and some cause tumours in hamsters but natural infections have not been shown to cause transformation. The viral genome integrates into cellular DNA. The 3' 2800bp of the genome is involved in transformation, and is known as

E1 region (3' end r strand). Two transcripts are associated with this region, E1A and E1B. Both give rise to multiple RNAs and proteins:

- E1A effects partial transformation on its own
- E1B does not effect transformation but it does increase levels of E1A.

The E1A protein is nuclear and phosphorylated at multiple serine residues, acidic and proline rich.

3 functions have been assigned to specific domains in the proteins:

- Stimulation of DNA synthesis
- Transcriptional repression/induction of mitosis
- Transactivation of transcription

Transactivation is not due to direct DNA binding but association with other transcription factors such as AP1. As with the T antigen of SV40, there is great interest in its interaction with Rb protein and other cellular proteins.

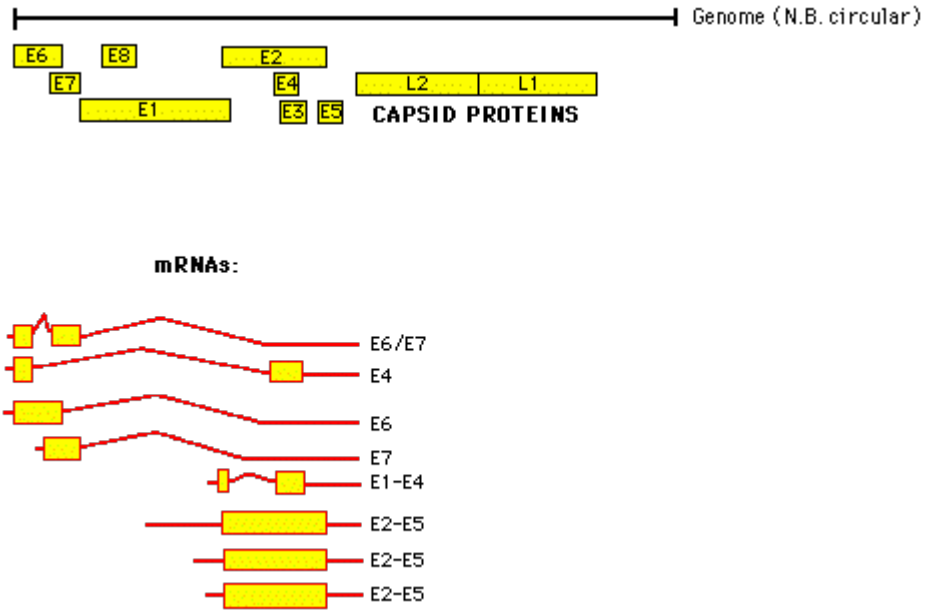
The E1b protein can interact with p53.

Papillomaviruses:

Genuine human tumour viruses which may be responsible for 10% of human cancers.

Diagnosis and treatment: A wart is seldom misdiagnosed, most regress spontaneously. Importance depends on location, skin wart may be unsightly but a wart on the vocal chord may be life threatening. Traditional therapy is application of caustic agent, cryotherapy, 5 flurouracil and surgery. Interferon therapy useful for genital warts of less use for oral warts. Prevention, spread by infectious virions contacting minor epithelial abrasions. No vaccine imminent, infection not thought to invoke a strong and lasting immune response. Genital infection best avoided by safe sex practices: it is estimated that about 80% of the adult population are infected many subclinically. The fact that only a small number of "high risk" infections leads to cancer suggests that HPV infection alone is not sufficient to cause cancer but is one significant step towards it (90% of all cervical cancers contain HPV DNA). Infants which passage through an infected birth canal may manifest juvenile onset laryngeal papilloma most before the age of 5, life threatening. Tends to recur and patients require surgery frequently, some every 2 weeks. Immunosuppressed transplant patients also prone to clinically troublesome warts.

Papillomaviruses have 8 kb genomes, are epitheliotropic and cause benign proliferations and also cancer. More than 70 pathogenic human strains have so far been identified. 2 main groups, cutaneous and mucosal, a substantial number infect the anogenital tract and may cause penile, vulval, and anal warts and penile vulval and cervical cancer. Transmission of these papilloma viruses is sexual and the diseases are sexually transmitted. These infections are very rare in virgins. Infection is directly correlated with the number of partners a couple has had unprotected sex with. The oral mucosa similarly sensitive to these genital viruses. Several viruses are exclusively infect the oral mucosa. 90% of all cervical cancers contain HPV DNA.



The E6 and E7 genes of HPV 16/18 are expressed as proteins in human tumours and tumour derived cell lines. E6/7 DNA can transform NIH3T3 cells. E7 is a cytoplasmic serine phosphoprotein, and has been shown to be a transcriptional transactivator and transforming protein. E7 binds Rb protein-presumably moves to nucleus. E6 binds to a cellular protein called E6-Ap. This complex then binds to p53. E6-Ap is a ubiquitin ligase. Cellular enzymes load it with activated ubiquitin molecules which are transferred to p53. Ubiquitin loading of p53 targets it for degradation by proteasome mediated proteolysis. The forced entry into S-phase in conjunction with genomic instability, resulting from p53 degradation may lead to malignancy. E6/7 proteins from 'low risk' HPV strains appear not to associate with Rb and p53. Expression of E6/7 antisense constructs reduces cell growth indicating that E6/7 may not only participate in initiation but also maintain the proliferative and malignant phenotype.

E7 can also interact with transcription factors such as jun and fos, upregulating Ap1 responsive promotors

Other observations suggest that E6-7 gene expression is necessary but not sufficient for malignant growth:

- Cervical cancer takes years to develop from lesions which already contain HPV
- In vitro immortalized cells are not malignant in early passages and fusion of (HPV+) carcinoma cells with normal cells results in a normal phenotype despite having E6/7 gene expression.
- Various studies show that E6/7 gene expression is suppressed in normal cells, occurs by inhibition of transcription.
- Location of viral DNA seems to be important in the deregulation of HPV gene expression. Cancer cells have a higher proportion integrated than premalignant cells. Integration often disrupts the E2 ORF. This controls HPV gene expression.
- DNA methylation may also play a role. Malignant cells have more HP18 methylation than non-malignant clonal cells (methylated genes normally inactivated so significance is not apparent).

Herpesviruses:

Most herpesviruses are not proven to be responsible for any human cancers, although they may be a cofactor with papilloma for cervical cancer. However, HHV8 has recently been implicated as the causal factor for Kaposi's sarcoma, KS. The virus is present in tumour cells, tracks tightly to and precedes the presence of disease. Latent infection but a switch to the lytic phase may be responsible for the formation of lesions. KS is a disfiguring skin cancer which is particularly common in AIDS patients. It is a peculiar neoplasm containing many different cell types. Possibly paracrine in origin. Spindle cells are dominant but there are also infiltrating inflammatory cells and vascular elements. In the immunocompetent the tumour is indolent and localised. In the immunocompromised it is more widespread, disfiguring and possibly fatal. Restoration of the immune system can result in arrest and even remission. The spindle cells are not fully transformed but do grow and secrete proinflammatory and angiogenic factors. HHV8 appears to be the agent responsible for promoting spindle cell growth. This virus is a member of the lymphotropic subfamily of the herpes virus, as is EBV. As well as being present in KS, the viral genome can also be

found in:

- AIDS related B cell lymphoma
- Castleman's Disease (another lymphoproliferative disease)

1-5% of spindle cells are lytically infected, the rest are latently infected. 1-2% of US blood donors are infected. The incidence of HHV8 coinfection of HIV infected individuals is

- HIV+ men 25-30%
- HIV+ women 3-4%
- HIV+ haemophiliacs 2-3%

This incidence closely correlates with the incidence of KS in these patients as well. Suggests that the virus may be sexually transmitted. Can certainly be found in both semen and saliva. Viral gene products possibly implicated in pathogenesis include IL6 and CC chemokines, as well as a chemokine receptor. The virus also contains several oncogenes including GPCR, (G protein coupled receptor) which transforms NIH3T3 cells. They then produce VEGF (vascular endothelial growth factor), a powerful angiogenic factor. KSHV have pirated many genes from the eukaryotic genome, which then mutate quickly, to develop properties different to the cellular counterpart. A more speculative link with multiple myeloma (clonal expansion of antibody producing plasma cells) has also been proposed, 2500 cases in UK pa. HHV8 infection of dendritic cells results in IL6 secretion which then expands the bone marrow plasma cells ie. an unusual bystander effect. 100% patients infected with the virus. 25% of patients with MGUS(monoclonal gammopathy of undetermined significance), the precursor condition are also infected with the virus, 200 000 patients in the UK. Exactly the proportion who develop myeloma. Does the virus determine disease progression ?

Epstein Barr Virus (EBV) is linked to at least 4 different types of tumour:

- Burkitt's lymphoma (African manifestation)
- Nasopharyngeal cancer (common Chinese & SE Asian manifestation)
- B cell lymphomas in immune suppressed individuals (organ transplantation or HIV)
- Hodgkin's lymphoma, clonal form exists in 40% of afflicted individuals

90% of adults in W.Europe and N. America carry EBV. Infection persists for life with virus regularly shed by salivary gland. Salivary fluid transmits the virus e.g. infected eating utensils and kissing. Definition of transformation in this system is the conversion of non-dividing B lymphocytes from (EBV-) individuals to indefinitely proliferating lymphoblasts. A link with Burkitt's lymphoma was first established when virus isolated from cultured Burkitt's cells, and linked to the disease sero-epidemiologically. EBV DNA can be isolated from tumours, viral specific antigens can be detected in the tumours, infection with EBV induces a lymphoma like disease in new world primates. In vitro EBV immortalization of B cells further supports the link.

The mechanism by which the virus transforms cells is still uncertain. The transforming gene(s) have not been unambiguously identified (large virus). Possible candidates include EBNA-1 (Epstein Barr nuclear antigen) and EBNA-2. EBNA-1 associates with a transcriptional enhancer element in the EBV origin of replication. Also suggested that EBNA-1 activates the lymphoid recombinase RAG genes. Activation of these genes may be required for viral integration into the host genome. It could also result in chromosomal rearrangements and translocations. EBNA-1 Tg mice have an increased incidence of lymphoma. EBNA-2 is a transcriptional transactivator for both viral and cellular genes. It upregulates the LMP-1and LMP-2 genes, as well as markers for B cell activation e.g CD23 as well as CD21, the EBV receptor. Another candidate is LMP, the latent membrane protein which acts as a constitutively activated receptor. It has some similarities to the mas oncogene. It is the only latent viral gene product which can transform cell lines in vitro. It induces the expression of many cellular genes. LMP-1 interacts with cellular proteins that transduce signals from the TNF family of receptors. It prevents p53 induced apoptosis and bypasses Rb function. Seems to act as a constitutively activated receptor via NFKB. A consistent finding in BL is a chromosomal translocation involving immunoglobulin (Ig) genes usually on chromosome 14 to sequences within or adjacent to the c-myc locus on chromosome 8. Mice transgenic for this c-myc rearrangement develop B cell hyperplasia and monoclonal B cell lymphomas. Obviously implicate these events in the development of Burkitt's lymphomas. The precise mechanism is unknown, perhaps a clonal expansion increases the probability of further neoplastic mutations as I have previously described. A link with malaria may be due to expansion of the B cell population which follows this protozoan infection.

The contribution of EBV in nasopharangeal cancers, Hodgkin's lymphoma and B lymphomas in immune suppressed individuals remains to be elucidated, other cofactors must be involved.

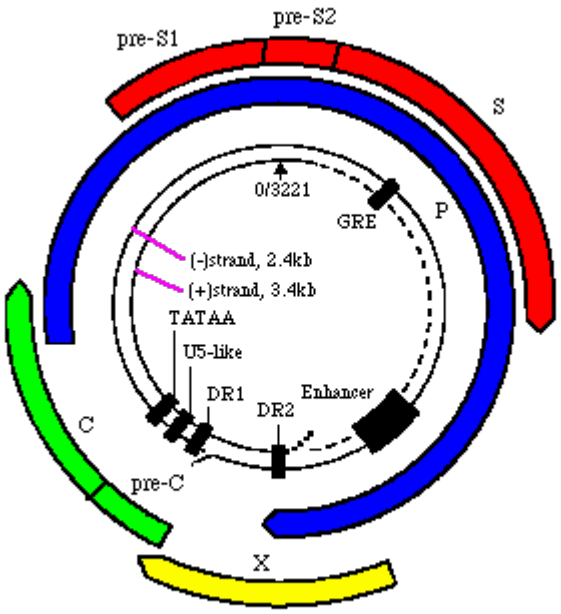
There is no effective chemotherapy for EBV infections. BL multifocal at time of diagnosis so chemo is therapy of choice. Remission induced in 80% of patients treated.NPC is a malignancy of the squamous epithelium in the posterior nasopharynx. NPC is treated by surgery and radiotherapy. Survival rates good if it has not reached the supraclavicular fossal lymph nodes. If it has then less than 20% survive 5 years. Good example of the interplay of viral, genetic and environmental factors. Consumption of salted fish in endemic regions is high. These are thought to contain high levels of phorbol esters and nitrosamines. Genetic studies have shown a loss of heterozygosity in several chromosomal regions thought to contain a susceptibility locus. Most carcinomas have a prolonged dysplasia-carcinoma in situ phase. But not in NPC, viral gene expression is thought to result in a rapid clonal expansion of cells and invasive neoplasia.In post transplant lymphoma a reduction in immunosupression therapy may induce regression.

Mutations are not found in Rb or p53 in EBV associated tumours. Presumably viral gene expression interfers with the function of these proteins in another way

Hepatitis B Virus

More than 200 million infected worldwide. 2 million die annually of which 700 000 are due to development of a monoclonal hepatocellular carcinoma (HCC). HBV is sexually transmitted but much more infectious than HIV. 30% of the infected individuals develop a mild acute infection within a few months. Symptoms are managed during the acute phase. It is not known what can be done to prevent the development of a chronic infection which may be asymptomatic or incapacitating.

HCC develops in 2-10% of infected patients after several decades of chronic liver disease and cirrhosis. Accounts for 30% of viral induced cancers and 5% of all cancers. The risk of ceveloping HCC is increased 100 fold following chronic infection with this virus. There is a seroepidemiological correlation between HCC and HBV infection throughout the world, except curiously for Greece. Viral transcripts can also be identified in transformed cells.



The HBV genome persists in the great majority of tumour cells. ORF X which codes for a transactivator protein is maintained. Transfection of ORF-X into NIH3T3 cells induces tumours in nude mice. Liver cancer develops in mice transgenic for ORF-X. In humans a pleiotropic transactivation of cellular genes by ORF-X and Pre S2 is thought to activate PKC/Raf signalling pathways. This activates the AP1 and NFKB transactivator functions which in turn control genes relevant for proliferation. Also find modifications in the short arm of C11 and the long arm of C13 in HCC. Perhaps tumour suppressing genes are located at these positions ? Point mutations in p53 have been

reported in HCC. Continuous liver cell regeneration may allow the accumulation of mutational events that lead to the emergence of an HCC clone.

A range of hepadnaviruses also infect birds e.g. woodchuck hepatitis virus-induces cancer a year or so after exposure. In these animals no cirrhosis develops and there is little hepatitis. But the viral DNA is rearranged and the tumour is monoclonal. This suggests that specific viral functions are more important for initiation and maintenance of proliferative changes than regenerative events. Cis activation of c-myc and N-myc is almost always observed.

Control: Medical workers are susceptible. Stable virus, mucosal and epidermal routes of entry. Infection can be prevented by avoiding direct contact with body fluids. A vaccine is available, not routinely administered in the UK ! But this virus will remain a serious public health problem for years to come, despite the vaccine. Therapy is liver resection, transplantation, or ethanol injection into the tumour.

References: Click here.

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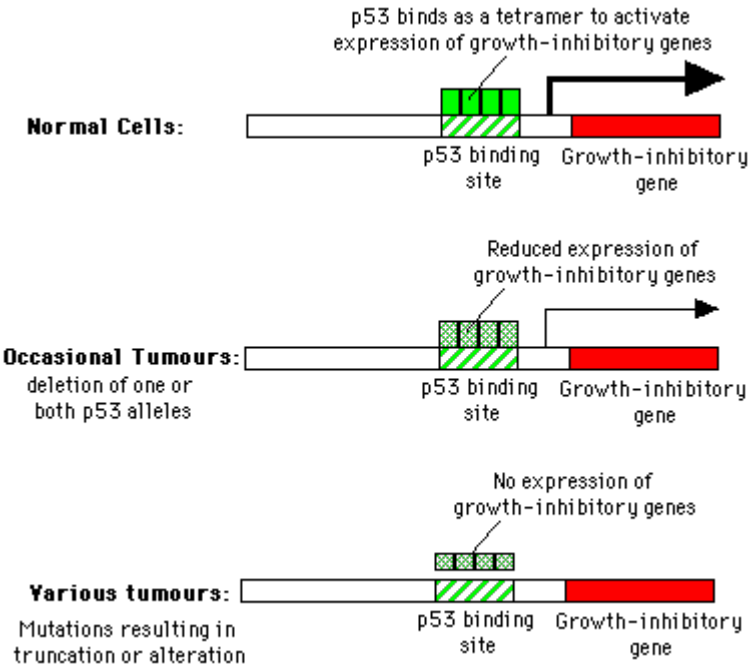
Tumour Suppressor Proteins.

Likely to be as important as oncogenes in cancer pathogenesis. It has been a long standing hope that despite the biological heterogeneity of cancer cells there would be common denominators for understanding, treating and preventing these diseases. The tumour suppressor proteins may play this role.

p53 Tumour Suppressor Protein:

p53 acts in late G1 phase. It prevents the cell progressing to the S phase i.e. is antiproliferative. p53 function almost always disrupted in high grade tumour cells. The protein contains an N-terminal acidic transactivator domain, this in combination with the nuclear localization of p53 suggests a role in transcriptional control. Much direct evidence to support this conclusion. p53 is a tetrameric protein, it binds specifically to promoter element and specifically transactivates gene expression.



p53 mutations are common in many tumour cells, causing the protein to tend to lose its DNA binding and transactivation properties. Mutations also cause a conformational change in the protein (detected by using antibodies). This conformational change is transdominant and can be transmitted to "conformationally normal p53". Mixed oligomers lose their ability to transactivate transcription.

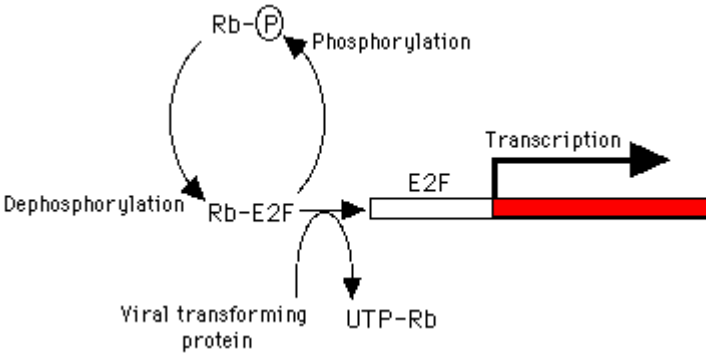
The normal conformation is antiproliferative. In addition to these intragenic mutations I have described numerous instances where viral proteins directly perturb p53 function by sequestering it or in one case by degrading it. Can also be disrupted by a cellular protein made by the MDM2 gene, identified by virtue of its amplification in spontaneously transformed cell lines, product binds to p53 and prevents transactivation. Not an experimental curiosity this gene is amplified in a majority of human sarcomas. A simple model for p53 function proposed in the figure transactivates genes involved in down regulating cell growth or invasion. Expression is lost in a number of ways, loss of one or both alleles so p53 concentration drops. Nonsense mutations so oligomerization domain at C-terminus is lost or missense transdominant mutations. missense mutation in one allele usually results in the loss of the other allele through mitotic recombination resulting in the absence of any wt protein e.g. colon, bladder, brain lung and liver.

p53 also modulates the function of WAF-1 a 21kd protein which inhibits G1 cyclins. These are a class of proteins which control the cell cycle in the G1 phase.

In mice or humans harbouring germline mutations in p53, development is normal. Suggests p53 not involved in the

normal control of the cell cycle. Develop tumours soon after birth. Thought that it plays an import role in controlling growth in stressed cells. For example, both X-ray or drug treatment of cells results in p53 expression and cell growth is arrested until the stress is removed and any DNA damage is repaired. So p53 is a molecular policeman which makes sure that the a cell is not stressed and that the DNA is in good shape. If it isn't p53 will not allow replication until the damage is repaired or it will trigger apoptosis. DNA tumour viruses have to knock out p53 function because they need the cells in S phase and this damage response must not be triggered. Tumour cells may progress through a stress phase e.g. anoxia or anueploidy and then inevitably select for cells with p53 disfunction.

RB1 is thought to act as a brake on the advancement of cells from G0/G1 into S phase. It is another target for all of the DNA viruses which has to be taken out if replication is to occur. Both copies of the gene need to be lost for oncogenesis, found in retinoblastomas, sarcomas, breast and bladder cancers etc. Best characterised cellular protein interacting with Rb is E2F, this is a transactivator. E2F activates proteins used in S phase such as TK and DNA pol alpha simplistic view is that Rb sequesters this protein in G1 and prevents transactivation. Mutation or phosphorylation results in loss of function. Hypophosphorylated in G1, hyperphosphorylated by cyclin dependent kinases at other times. Viral proteins bind to the hypo state preventing E2F sequestration suggesting that this is largely responsible for tumour suppression. Rb also inhibits RNA pol I and RNA pol III transcription:



Not all tumour suppressor proteins act directly at the level of transcription. The tumour suppressor gene involved in neurofibromatosis (NF) encodes a protein which may exert negative control over ras. NF is a genetic disease characterised by tumours originating in the neural crest. Neurofibromin is a GTPase activating protein and converts active GTPras to inactive GDPras, effectors of ras not yet known. Not all tumour suppressor genes code for proteins.

References: Click here.

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Viroids and Virusoids

Subject of these two lectures are viroids, virusoids and prions. These are unusual infectious agents characterised by having a very small genome and in the case of prions, possibly no genome at all. If you are only interested in prions, click here.

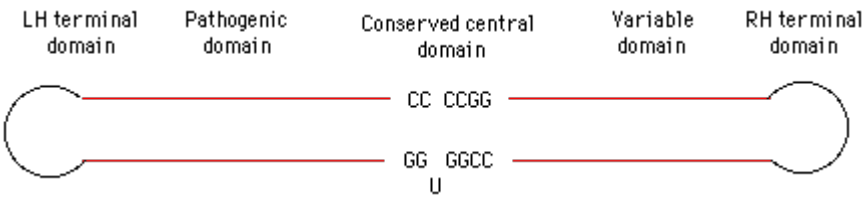
Viroids are common plant pathogens which are a serious economic problem.
25 different viroid sequences have been determined and numerous variants identified :

Group A:	e.g. avocado sunblotch viroid, peach latent mosaic viroid	
Group B:	Subgroup B1:	potato spindle tuber viroid, coconut cadang cadang viroid, tomato plant macho viroid
	Subgroup B2:	citrus bent leaf viroid, pear blister canker viroid

The RNA genomes of viroids are 246-375 nucleotides in length and share many similarities:

- They are all single stranded covalent circles
- There is extensive intramolecular base pairing
- A DNA-directed RNA polymerase makes both plus and minus strands
- Replication does not depend on the presence of a helper virus
- No proteins are encoded

The structure of a group B viroid is indicated schematically below:



The classification described above is based on analysis of the central conserved region (CCR). Group A viroids are clearly different to those described above . They lack a CCR and possess a ribozyme activity (a ribozyme is a catalytic RNA molecule, in this case RNA cleavage is the ribozyme activity). Additionally it is speculated that Group A viroids may replicate in chloroplasts whereas Group B viroids replicate in the nucleus and nucleolus. 3 enzymic activities are required for viroid replication, an RNA polymerase, an RNAse and an RNA ligase.

Group A viroids probably replicate via a symmetric rolling circle mechanism, whereas Group B viroids probably use an asymmetric mechanism. By this I mean the +ve infecting circular RNA strand of a viroid serves as a template to make a large linear multimeric -ve strand. RNA pol II is probably the enzyme which does this. Group B viroids with an asymmetric replication pathway then make +ve RNA from this long linear molecule. A host RNAse activity cleaves the +ve strand into unit viroid lengths. This molecule is then ligated to form a circular viroid. In Group A viroid replication the long -ve RNA is self cleaved by the associated ribozyme activity. The RNA circularizes to form a -ve circle. A second rolling circle event makes a long linear +ve strand which is again cleaved by the ribozyme activity. The short viroid RNA is then ligated to the circular form.

There thus seems to be fundamental differences between the two groups of viroids, presumably reflecting different origins.

Probably there is more than one mechanism responsible for viroid pathogenesis. Recent evidence suggests that one pathway is due to viroid RNA activating a plant RNA activated protein kinase, or PKR. (analogous to the PKR enzyme activated by viral RNAs in mammalian cells). Protein synthesis is reduced and this causes pathogenic effects.In the case of potato spindle tuber viroid, there is a good correlation between a strains pathogenicity and its ability to activate PKR in vitro.

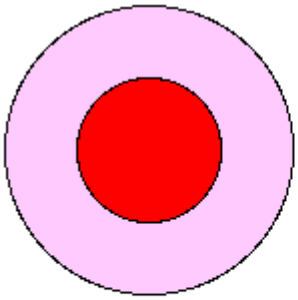
Virusoids and Satellites

Virusoids or satellite RNAs are also several hundred nucleotides long circular and single stranded. They depend on a helper virus for replication. This helper virus also encapsidates them. e.g:

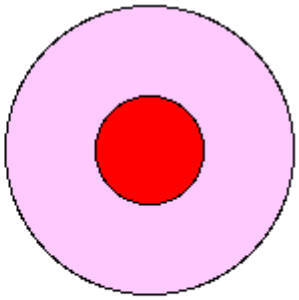
- barley yellow dwarf virus satellite RNA: Helper - Luteovirus.
- tobacco ringspot virus satellite RNA: Helper - Nepovirus
- subterranean clover mottle virus satellite RNA: Helper - Sobemovirus

Virusoids replicate in the cytoplasm using an RNA-dependent RNA polymerase. This enzymic activity is common in plants but not found in animal cells.
It is not known if viroids and virusoids are the progenitors of modern viruses or have degenerated from other more complicated viruses. They can be spread by vegetative propagation, within seeds or by direct inoculation either by insects or man.

There are similar infectious agents which infect animals, e.g. newt satellite 2 transcript. One such agent infecting humans is the **hepatitis delta virus (HDV)**.
HDV was first identified in the 1970s in Australia as a nuclear antigen, the delta antigen. Subsequently, it was found to be the cause of a particularly virulent form of hepatitis known as type D hepatitis.
Common in indigenous natives of S.America, method of transmission not understood. Can be transmitted perinatally. In the West, transmission is associated with drug abuse and transfusion of blood products. It seems prudent to assume that it can also be transmitted sexually. No specific treatment.
The delta antigen is associated with a defective pathogen which is obligatorily associated with Hepatitis B helper virus:



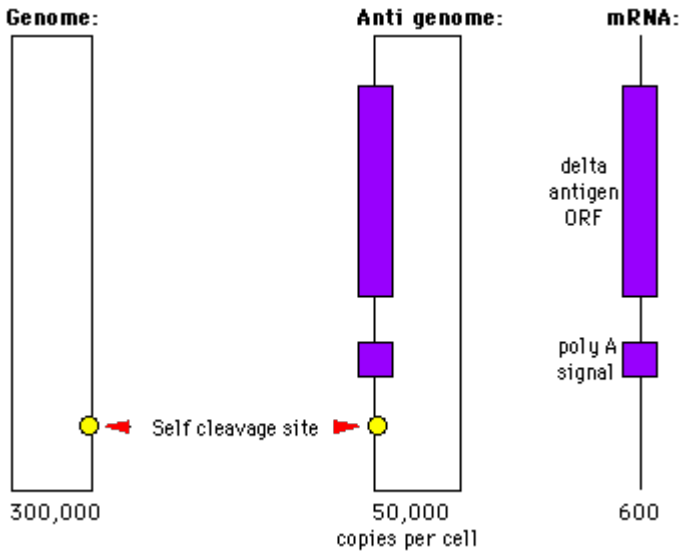
Infectious HBV, 40nM outer envelope + surface antigens, 27nm nucleocapsid with core protein, Reverse Transcriptase and HBV DNA



Infectious HDV, 40nM outer envelope+ HBV surface antigens. 19nm nucleocapsid with 60 copies of delta antigen and HDV genomic RNA.

This virus has a circular single stranded RNA genome of about 1700 nucleotides. It has a ribozyme (RNA cleavage) activity. This is the smallest known genome for an animal virus.
RNA and delta antigen (195 AA) are packaged in a Hepatitis B particle. No DNA intermediate has been detected during the replication phase and it is thought that replication occurs by RNA directed RNA synthesis using a DNA dependent RNA polymerase. Certain parts of the genome and the pattern of replication is of course similar to a viroid. One difference is that mRNA and a protein - the delta antigen - are made.
The encapsidated RNA is (-)ve sense strand so (+)strand RNA synthesis to make the mRNA is required. These RNAs are nuclear associated and in any case circular RNAs are not good templates for protein synthesis. About 10% of the antigenomic strand is cleaved and polyadenylated and serves as a 800nt mRNA. RNAse activities tend to be exonucleolytic rather than endonucleolytic. The fact that the genomic strands are circular probably contributes to the agents stability.

The different RNAs made by this virus are indicated in the diagram shown below: The genomic strand is 70% Watson Crick base paired and rod like in gross structure.



The delta antigen is a 22kd nuclear phosphoprotein essential for replication and particle formation. It is basic and associates specifically with the RNA genome thereby stabilising it. Two forms are made differing by 19 amino acids at the C-terminus. The large form is a dominant inhibitor of genome regulation and directs genome packaging into Hep B viral particles. This packaging is due to farnesylation of a Cys residue 4 AA from the C-terminus. Recently a host protein interacting with the delta antigen has been identified. In fact sequence similarities suggest it is a cellular homolog of the delta antigen. This suggests that it may be able to modulate viral replication. It may also suggest that HDV originated from a viroid like element which then "captured" a cellular transcript. Probably the genomic strand transferred onto the mRNA for the cell homolog of the delta antigen. This was copied into the antigenomic strand and stabilised as part of the genome.
These agents all have a small genome. They replicate, spread, and if appropriate, form particles, in much the same way as all of the other viruses you will hear about during this course. One could almost say that there is a continuum between naked replicating transmissible RNAs, depender packaged transmissible RNAs, simple viruses and complicated viruses like the herpesviridae and poxviridae. Organelles and obligate cellular bacterial parasites like rickettsiae would seem to represent a different lineage completely (because of the independent capacity for protein synthesis).

The next group of infectious agents I shall turn to are the prions, which are very different.

Books about Viroids: click here

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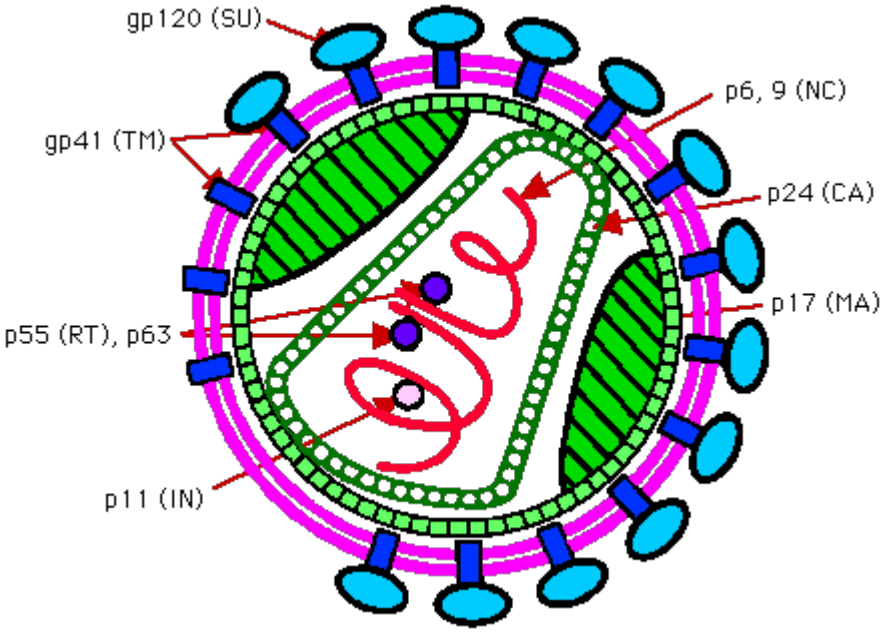
Virus Structure

Human immunodeficiency virus (HIV-1) will be discussed in detail here as an example of virus structure. In some cases, comparisons between HIV-1 structure and that of other viruses will be made, either because other viruses are better understood or to illustrate variations in these processes.

Schematic representation of the structure of HIV:



HIV is a fairly complex virus, although by no means the most complicated known. The virus is thought to contain 2 identical copes of a positive sense (i.e. mRNA) single-stranded RNA strand about 9,500 nucleotides long. These may be linked to each other to form a genomic RNA dimer.

The RNA dimer is in turn associated with a basic **nucleocapsid** (NC) protein (p9/6). By analogy with other RNA viruses, this nucleoprotein filament may be helical, although this has not actually been determined in the case of HIV.

The ribonucleoprotein particle is encapsidated by a capsid made up of a **capsid** protein (CA), p24. The capsid environment also contains other viral proteins such as **integrase** and **reverse transcriptase**. It also contains a wide variety of other macromolecules derived from the cell including tRNAlys3, which serves as a primer for reverse transcription. The capsid has an icosahedral structure.

The capsid is in turn encapsidated by a layer of **matrix protein (MA)**, p17. This matrix protein is associated with a lipid bilayer or envelope. The matrix protein may be:

- a continuous shell attached to the envelope as in HIV
- noncontinuous but associated with envelope
- separate from the envelope.

The HIV **envelope** is derived from the host cell plasma membrane and is acquired when the virus buds through the cell membrane. An envelope is a common feature in animal viruses but uncommon in plant viruses. In the case of herpesviruses, the envelope is derived from the nuclear membrane. Other viruses such as vaccinia derive an envelope from the golgi body. A viral envelope contains the lipid and protein constituents of the membrane from which it is derived. In addition it also contains viral proteins often forming spikes or peplomers. The major HIV

protein associated with the envelope is **gp120/41**. This functions as the viral antireceptor or attachment protein. gp41 traverses the envelope, gp120 is present on the outer surface and is noncovalently attached to gp41. The precursor of gp120/41 (gp160) is synthesized in the endoplasmic reticulum and is transported via the golgi body to the cell surface.

Other types of virus structure are known:

1. Naked helical nucleocapsids, e.g. tobacco mosaic virus (TMV).
2. Naked icosahedral capsids, e.g. members of the picornavirus family.
3. Encapsidated nucleocapsids, two concentric shells of protein surrounding a nucleoprotein filament, e.g. the orbivirus bluetongue. This virus is not enveloped.

Rhabdoviruses have a similar structure to that described for HIV, they are enveloped and have a matrix protein. However the capsid is a helical nucleofilament.

Helical Nucleocapsids:

TMV, an RNA virus, is a particularly well understood example of virus structure. Protein subunits can be placed around the circumference of a circle to form a disc. If the discs are stacked, then a tube is created with room for the nucleic acid down the middle. A closer examination of these virus structures shows that the coat proteins are not arranged cylindrically but helically. This is because of the propensity for nucleic acids to adopt helical structures. By arranging the protein subunits helically then equivalent bondings between the proteins and nucleic acid can be made- except for the two end subunits. All known filamentous viruses are helical. Typically they are 15-19nm wide. The length depends on the size of the genome but 300-500nm is within the normal range. The structure of TMV can be described in terms of the number of subunits per turn of the helix i.e. 16.3. The pitch or rise per turn of the helix i.e. 2.28nm and the axial rise per subunit i.e. 0.14nm.

It is possible that the nucleoprotein filament of HIV has a similar structure. Nucleocapsid proteins are usually basic (+) proteins to neutralize and facilitate the packaging of acidic (-) nucleic acid.

Icosahedral Nucleocapsids:

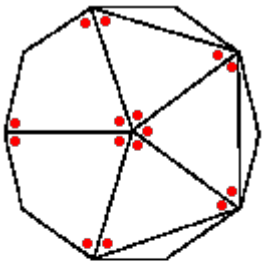
Electron microscopy suggests that many viruses are roughly spherical. A detailed examination shows that they are actually icosahedral. Icosahedral viruses are very common plant and animal viruses. The HIV capsid layer is thought to have an icosahedral structure. At the moment precise details of the HIV capsid structure are not known

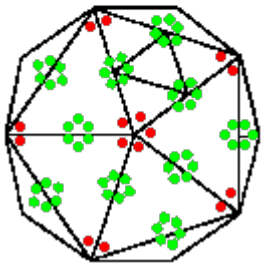
but some general considerations are described below:

The subunits of the capsid are located around the vertices or face of an icosahedron. An icosahedron has 20 equilateral triangles arranged around the face of a sphere. It is defined by having 2, 3 and 5 fold axis of symmetry.

There are several reasons why viruses adopt icosahedral symmetry.
One is that triangulating a dome into 20 is the best way of producing a shell of equivalently bonded identical structures. It is the minimum free energy structure. The real situation is more complicated than this, because all known viruses have more than 20 subunits. 60 subunits can obviously be arranged symmetrically around an icosahedron. Only a very few viruses have such a small number of subunits e.g. ØX174. This may be because it is difficult to maintain an integral particle with a small number of subunits. Viruses generally fit 60 x N subunits into their capsids. N is sometimes called the triangulation number and values of 1,3,4,7,9,12 and more are permitted. However more than 60 subunits cannot be arranged in an equivalent fashion around an icosahedron.

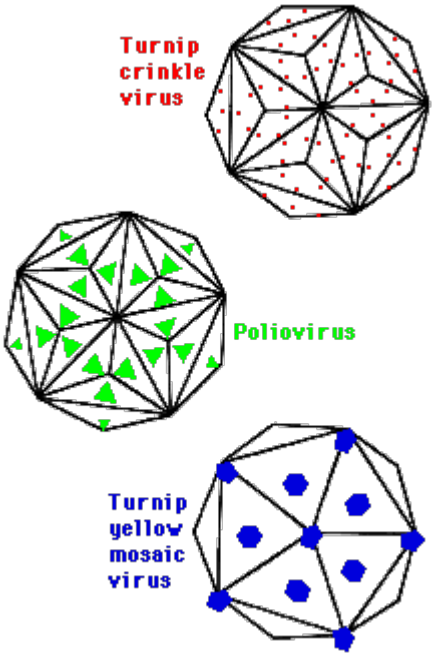
Consider a T=4 virus:





The minimum free energy solution is to divide each triangle into 4 further triangles and place a subunit at each corner. There will be 12 x 20 subunits, i.e. 240 with 12 pentamers and 30 hexamers. They are obviously not equivalent, but 180 are and the remaining 60 are making similar contacts. They are said to be **quasi equivalent**.

Protein subunits are not spaced independently but cluster because this maximizes the intermolecular interactions which stabilize the particle. To illustrate this consider a particle with 180 subunits, 3 kinds of clustering are possible:



- In turnip crinkle virus they cluster at the centre of edges giving 90 capsomers composed of dimers
- In poliovirus they cluster at the centre of the triangle giving rise to 60 morphological structures or capsomers composed of trimers
- In turnip yellow mosaic virus they cluster at the point of the triangles giving 20 hexamers and 12 pentamers, in all 32 capsomers.

One consequence of this clustering is that bonds between subunits in a capsomer are stronger than bonds between capsomers which means they can be isolated for functional and structural studies.

Why subunit construction ?

Necessity: A triplet codon has a MW approximating to 1000 and codes for an amino acid of average MW 150. So at

best a nucleic acid can only code for 15% of its weight as a protein. As viruses are composed of 50-90% by weight protein there must be more than one protein and subunit construction is essential.

Self Assembly: Seminal experiments in 1955 by Fraenkel Conrat and Williams. It was shown that TMV virus spontaneously formed when mixtures of purified coat protein and its genomic RNA were incubated together. This means that the structure that TMV adopts is self-ordered and therefore corresponds to a free energy minimum.

Fidelity: DNA, RNA and protein synthesis are all subject to occasional error By using a smaller protein and hence gene, it means there is less chance of an error occurring.

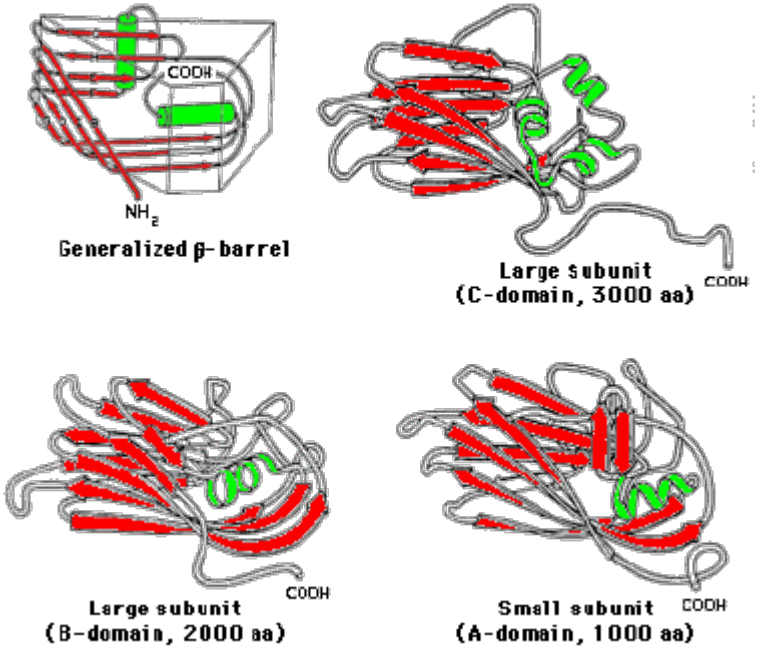
Economy: The correct structure can be formed with the minimum of waste since if a subunit is synthesised or folded incorrectly then only a small unit has to be discarded.

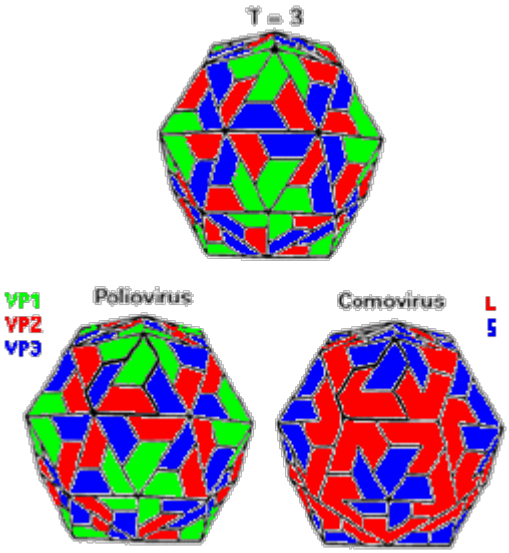
Complexity: There are physical constraints which prevent the tight packing of say an octahedron or tetrahedron. Put crudely, the holes between the subunits would be too big and the particle too leaky. Small number of contacts would be insufficient for stability.

The larger the number of subunits the more stable the virus becomes. The larger the virus particle and the bigger and more complex its genome can be.

Structure of bean pod mottle virus (BPMV):

The structure of several viruses have now been determined using X-ray crystallography. We will consider one example to illustrate the simplicity of their structures.





BPMV is an icosahedral comovirus. It has a bipartite RNA single stranded genome of positive sense. It is a T=3 virus like the picornaviruses. Many of these have evolved from a common ancestor and the proteins have a similar fold.

In a T=3 virus such as poliovirus there are 3 different subunits per 60 triangles. BPMV is subtly different. It is composed of 60 triangular units. Each unit is composed of 3 antiparallel beta-barrel proteins.

60 copies each of coat protein of two types:

S: 22Kd
L: 42Kd

These are made as a polyprotein, C-B-A. A is cleaved off. S(A) has 1 beta-barrel structure and L(C-B) has 2. This gives 180 subunits if you take the beta-barrel as the subunit. Each domain is 180-190aa long. Subunit structures have been determined. B and C are the L subunit covalently linked, interface stabilized by hydrophobic interactions. Helix in A domain interacts with a helix in B. Quaternary structure is clearly spherical due to polypeptide protrusions. It forms a wedge 50Ä long 17Ä at narrow end and 30Ä wide at large end. Two shades of green are the large subunit, small subunit is blue.

It is also possible to map some of the RNA chain in this structure, i.e. about 7 bases. Looks like A-form RNA and it's magnitude is such that this must occur some 60 times in the structure. This suggests that the RNA itself has icosahedral symmetry. Contacts between the RNA and protein are visible, in a pocket between the C and B domains. The interactions are electrostatic and van der Waals bonds.

Summary:

The structure of HIV is complex. Its overall shape is thought to be icosahedral. The glycoprotein spikes showed a skewed icosahedral symmetry which is thought to reflect the icosahedral structure of matrix. The triangulation number is 71(!). The capsid is also icosahedral and may contain 1890 subunits with a triangulation number of 63(!). The nucleocapsid may adopt a helical nucleofilament structure or may also be icosahedral (not known).

What have we learned from studying virus structures ?

Knowledge of the biomolecular interactions which occur in these large structures is of fundamental importance. It may now be possible to design small compounds which bind to the surface of a virus and prevent it from entering a cell. Most animal viruses initiate infection and entry into host cells by attaching to receptors on the host cell membrane. Clearly the viral receptor attachment site must remain conserved. However the immune system may react to the surface of a virus and produce neutralizing antibodies. This can result in the continual changing of the antigenic surface of a virus that chronically infects a host. There is an obvious conflict here. Now known that the surfaces of a number of viruses e.g. polio, have deep clefts, canyons or pits. Residues in these canyons are often

much more conserved than surrounding surface residues. It is therefore thought that these clefts may constitute the receptor binding site and that residues at the bottom of the canyon are inaccessible to antibodies and therefore under no pressure to change. Some evidence supports this hypothesis:

- influenza antireceptor site is located in a cleft.
- insect viruses which are not subject to an immune response do not have canyons, the receptor ligand can even be a protrusion from the surface.

References: Click here.

Search for more information on this topic.

Books about Virus Structure: click here

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Virus Replication

Human immunodeficiency virus (HIV-1) will be discussed in detail here as an example of virus replication. In some cases, comparisons between HIV-1 replication and that of other viruses will be made, either because other viruses are better understood or to illustrate variations in these processes.

General Considerations

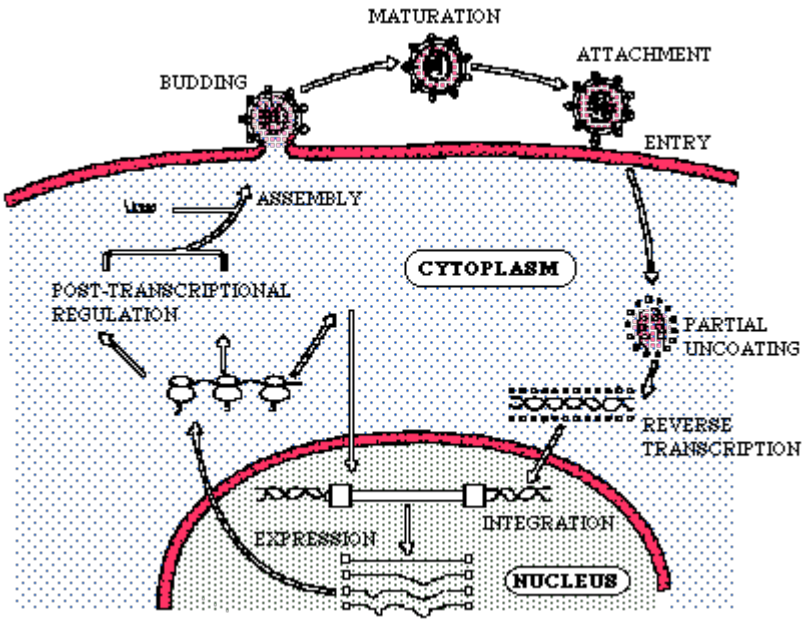
The infectivity/particle ratio in picornaviruses can be as low as 0.1%, i.e. only 1 in 1000 virion particles are infectious. In rotaviruses, the ratio is about 0.2%. Virus particles may be defective for innumerable reasons. One consequence is that the study of replication is made more difficult. Information about the reproductive cycle of viruses comes largely from synchronously infected cells.

Shortly after infection and for several hours, only low amounts of parental infectious material can be identified, this is the so called eclipse phase. Genome replication has been initiated but progeny virus are not yet released. There is then a maturation phase when viral material accumulates exponentially in the cell or surrounding medium. After a few hours cells infected with lytic viruses become metabolically disordered and viral production ceases. Titres then slowly drop. Cells infected with non lytic viruses can continue to produce viral particles indefinitely. This reproductive cycle may vary from 6-8h in picornaviruses to more than 40h in some herpesviruses. Yields in cells infected with polio can be greater than 100,000 copies of virus per infected cell.

Infection of a cell may be:

- productive, the cells are permissive for viral replication and virion progeny are released
- abortive, the cells are non permissive for a viral function and virion particles not produced.
- restrictive, the cell is transiently permissive and a few virus are produced. Viral production then ceases but the genome persists. Examples include Epstein Barr and herpes simplex. May still have serious consequences for the host.

The replication cycle of HIV is shown:



All viruses have to penetrate, replicate and exit a cell. The details of how they do this obviously vary. The Baltimore classification is a useful way to order this information, see **Course 224**.

Virus replication can be divided into (arbitrary) phases:

Initiation:

- attachment
- penetration
- uncoating

In which the genetic material of the virus is introduced into the cell, often accompanied by essential protein cofactors.

Replication:

- genome synthesis
- RNA production
- protein synthesis

The genomic size, composition and organization of viruses shows tremendous diversity. There is no single pattern of replication. But all make proteins with 3 sets of functions to:

- ensure replication of the genome
- package the genome into virus particles
- alter the metabolism of the infected cell so that viruses are produced.

In a few instances, e.g. parvoviruses, it is cellular enzymes which replicate the viral genome and these are just assisted by viral proteins. But in most cases viral proteins are responsible for genome replication, albeit with the assistance of cellular proteins. It is viral proteins that are responsible for the third and final stage, i.e: **Release:**

- assembly
- maturation
- exit from cell

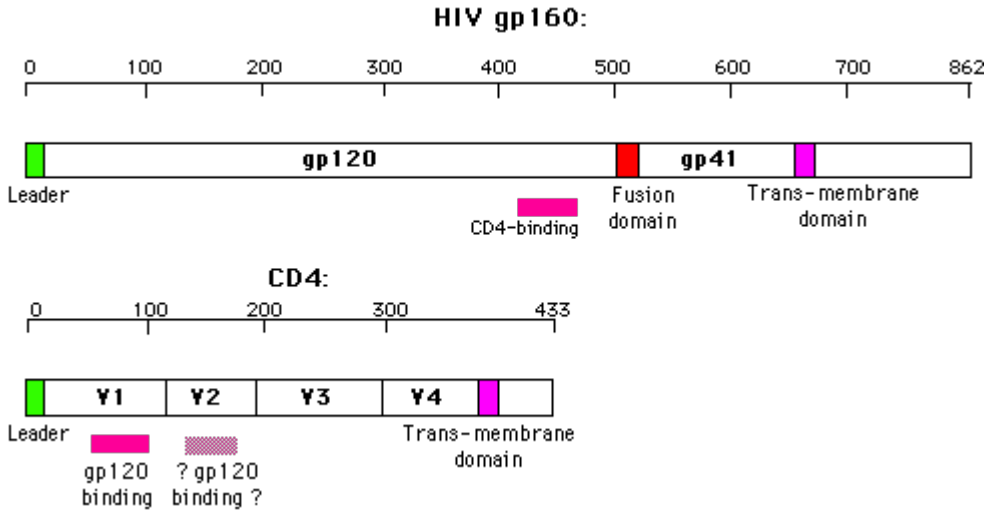
Initiation Phase:

Attachment, Penetration and Uncoating

The virion protein or antireceptor binds to a cell surface receptor. A classic example of this process is the haemaglutinin antireceptor of influenza virus. Complex viruses such as pox or herpes may have more than one antireceptor. Furthermore, the antireceptor may have several domains which react with different receptors.

Cellular receptors are largely glycoproteins. The interaction of the receptor and antireceptor requires counter ions to reduce electrostatic repulsions and is temperature and energy independent. In some cases, attachment leads to irreversible changes in the structure of the virion. The influenza haemaglutinin protein bind to glycoproteins carrying neuraminic (sialic) acid. The virus also has a neuraminidase on its surface and can detach from the cell by hydrolysing neuraminic acid from the glycoprotein.

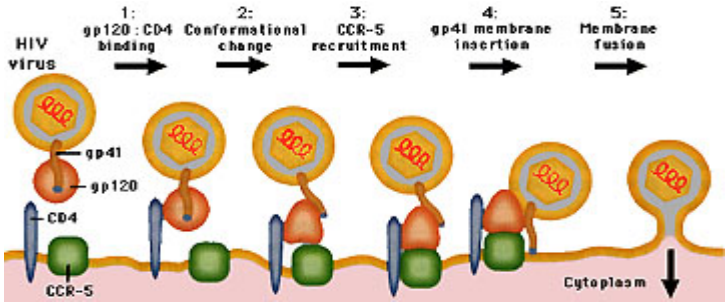
The antireceptor of HIV is the gp120 envelope glycoprotein:



This is initially synthesized as a precursor, gp160. A cellular proteases cleave this into gp120 and gp41. gp41 and 120 remain associated as a heterodimer and it is this association which retains the 120 moiety with the virus, since gp41 has the transmembrane domain. The proteolysis event is essential for virus infectivity.

The receptor for HIV is CD4 antigen, which binds gp120 with an affinity constant in the nanomolar range. Binding has been demonstrated by coprecipitation experiments using antibodies to either protein.

Definitive proof that CD4 is the HIV receptor was obtained by Maddon and Weiss. They showed that CD4 negative human cells (e.g. HeLa cells) cannot be infected with HIV. Expression of CD4 in these cells following transfection with a cloned CD4 gene made them permissive. However, additional structures are also necessary, since transfection of mouse cells with CD4 does not render them permissive for HIV infection. These second receptor components have recently been identified as members of the beta-chemokine family, in particular CXCR-4 and CCR-5.



The CD4 molecule has 4 regions which resemble the variable regions of antibody molecules. It is a member of the immunoglobulin superfamily of molecules. The binding site for gp120 has been mapped using blocking antibodies and site-directed mutagenesis. It maps to the first variable region of the protein from amino acids 16-84. Additional amino acids of the second variable domain may also contribute to binding. Crystal structure of this protein has now been solved.

Sequences important for binding CD4 have also been mapped on gp120. A region in the C-terminal half from amino acids 397-439 associates with it. A deletion of 12 amino acids in this region or site substitutions abolish binding.

More detailed understanding of the interactions of these two proteins is now dependent on knowing the tertiary structure of gp120. This is particularly important because it is a potential therapeutic site.

After attachment, penetration, an energy dependent step, occurs quickly. There are several ways in which this occurs:

1. Endocytosis of the entire particle resulting in accumulation of virus particles inside a cytoplasmic vesicle. This is said to be obligatory for unenveloped viruses such as polio (but see below). Some enveloped viruses, e.g. orthomyxoviruses, also use this method. It is a pH-dependent process

2. Fusion of the cellular membrane with the virion envelope and direct release of the capsid into the cytoplasm examples include paramyxo and herpes viruses as well as HIV. This is pH-independent.

3. Rarely, translocation of the virus particle directly into the cytoplasm

The precise biophysical details of the fusion process remain unknown. Some viruses may enter the cell in more than one way. With other viruses the evidence for an entry mechanism is not always absolutely conclusive.

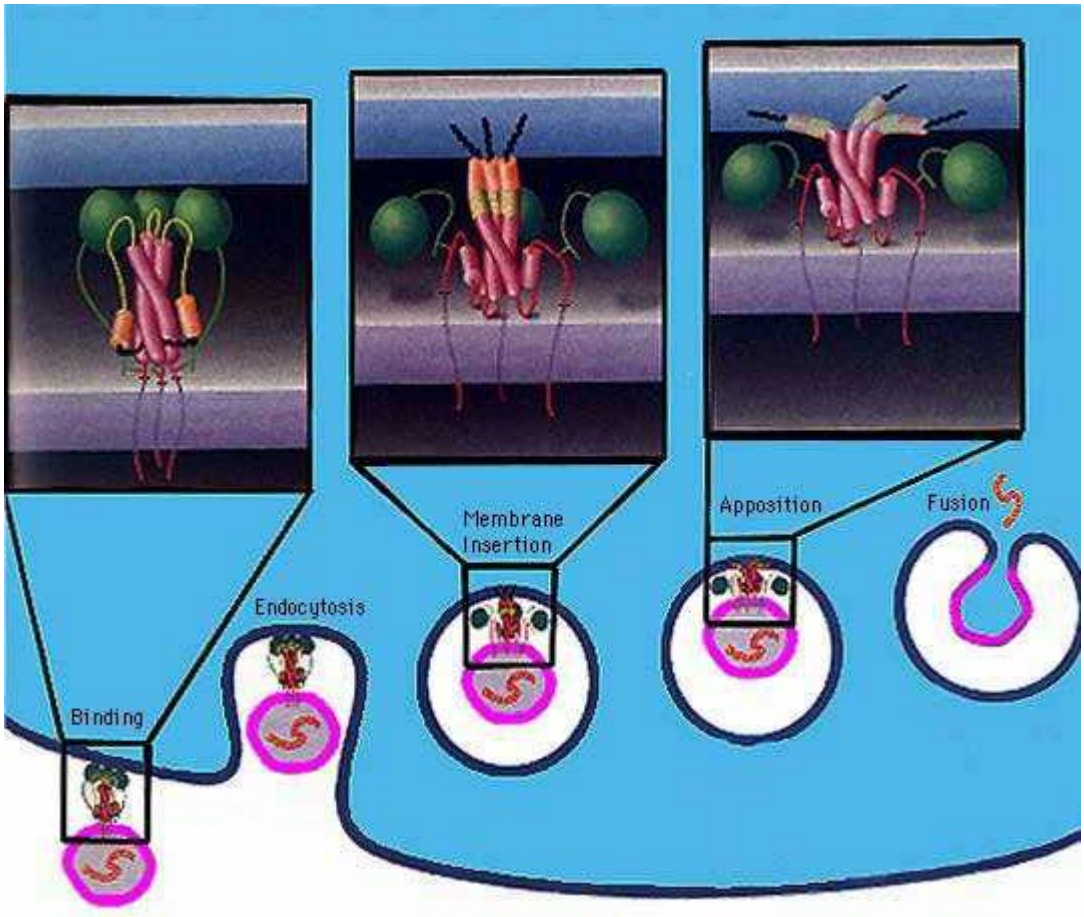
The pH-independent method is used by HIV and Sendai virus (paramyxovirus) and involves direct fusion of the viral envelope with the cell membrane and subsequent release of the capsid into the cytoplasm. (Confusingly, it seems that other retroviruses may be internalized by a pH-dependent process.) The envelope glycoprotein in many viruses including HIV exists as a heterodimer with the extra cellular component (gp120) interacting with the receptor and the transmembrane component (gp41) inducing fusion with the host membrane. The transmembrane protein of enveloped viruses generally contains a stretch of hydrophobic amino acids at the N-terminus implicated in membrane fusion. HIV is no exception and mutations in this region abrogate cell fusion. Anything which disrupts the hydrophobicity disrupts fusion. Mutations which enhance hydrophobicity actually enhance the fusogenic potential of viruses. This process does not result in the formation of an intracellular membrane bound particle, the capsid is released directly into the cytoplasm.

Evidence that viruses infect cells by pH-independent mechanisms can be obtained in several different ways:

- EM evidence shows direct fusion with the plasma membrane
- virus particles remain infectious in alkaline media
- In the case of HIV, a truncated CD4 with cytoplasmic domain deleted still functions as a receptor despite not being internalized in response to phorbol esters.

The pH-dependent process involves **endocytosis** of a virus. Orthomyxoviruses, such as influenza, enter cells in this way. The receptor bound virus is internalized into the cell by endocytosis. Endocytosed vesicles form bodies called endosomes. These have an acidic pH which induces a conformational change in the envelope protein resulting in the exposure of a hydrophobic fusion domain. This facilitates fusion of viral and endosomal membranes and release of the capsid particle into the cell.

The antireceptor for influenza virus is haemaglutinin. The structure of this protein in several forms has been determined by X ray crystallography. The virus contains 2 other membrane proteins, the neuraminidase and M2 proteins.



Haemaglutinin is made as a precursor protein with 549 amino acid residues. It is glycosylated and stabilized by several disulphide bonds. The antireceptor is anchored to the viral membrane by HA2. HA2 has a hydrophobic stretch from 185-211 which is inserted into the viral membrane . The N-terminal sequence forms a trimeric structure of 3 x HA2 held together by hydrophobic bonds. The HA1 chain forms a pocket at the top of this structure which accommodates neuraminic acid of a glycoprotein receptor (ubiquitous).

Native haemaglutinin is cleaved by a cell surface protease after amino acid 328 into HA1 and HA2 (221 amino acids). The two polypeptide chains remain tightly associated after this cleavage. They are held together by both non covalent and covalent bonds. Cleavage results in a large conformational change which exposes a fusion like domain at the new N-terminus of HA2, strongly conserved, hydrophobic. The virus may be vesicle bound at this stage.

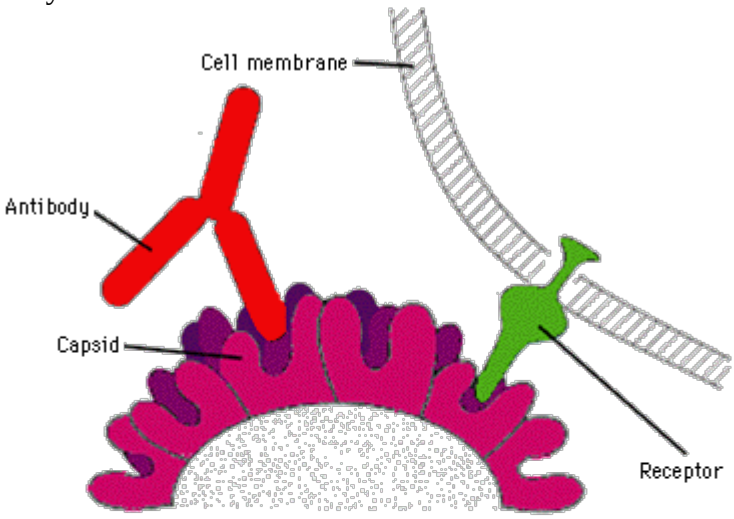
From the picture above you can see that the viral and cell membranes are some distance apart (100Ä) after cleavage. The fusion domain is also inaccessible in the interior of HA2. Additional conformational changes induced by pH 5-6 in the endosome bring the fusion domain out of the interior of HA. An extended loop sequence changes conformation to a coiled coil and projects the fusion domain into the cell membrane. The two membranes are now linked. HA2 sequences then melt into the lipid bilayer of the cell drawing the viral membrane closer. HA2 known to induce lipid mixing of different bilayers, but mixing of cell contents cannot take place until membrane fusion occurs.

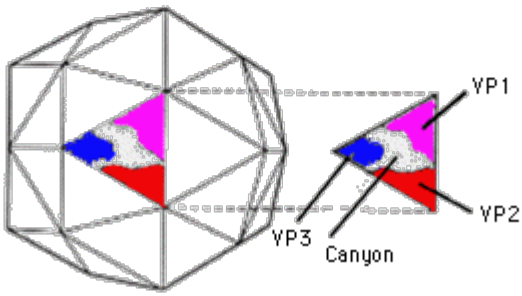
A number of chemical agents can help identify which method of uptake is being employed:

- Lysosomotropic agents such as ammonium chloride, chloroquine (weak bases) can be used to distinguish between pH-dependent penetration and pH-independent penetration because they inhibit the former and not the latter. They are thought to act by acting as weak buffers in endosomes and preventing acidification.
- Carboxylic ionophores such as monensin and nigericin allow protons to equilibrate between the endosomal and cytoplasm compartments thus preventing acidification
- DCCD (dicycohexylcarbodiimide) inhibits the ATPases which pump protons into the vesicle.

Entry of unenveloped viruses

The uptake of unenveloped viruses was, until recently, always considered to involve endocytosis into an endosome, i.e. to be pH-dependent and sensitive to ionophores and lysosomotropic agents. An example is the poliovirus which belongs to the picornavirus family. This is an icosahedral T=3 virus.





The capsid consists of 4 polypeptides, VP1, 2, 3 and 4.
VP4 is buried in the capsid and associated with the viral RNA.
VP1, 2 and 3 form the external capsid. Five VP1 protein subunits at the 5 fold axis of symmetry form a canyon in the capsid which is the recognition site for the receptor.

The polio receptor belongs to IgG superfamily (as does CD4), it has a MW of 45Kd. It has an amino terminal Ig-like domain which binds to the virus, a transmembrane domain and a cytoplasmic domain (which is dispensible for function.There are several thousand copies of the receptor present on a cell.

After binding to the receptor, the virus is endocytosed into an endosome. At a low pH, poliovirus becomes more lipophilic and can associate with the endosome membrane possibly forming a pore. Presumably the pH shift results in exposure of hydrophobic domains in the capsid proteins. During this process VP4 is lost from the particle; VP2 may also be lost. This gives the particle increased flexibility. The genomic RNA is ejected through an endosomal pore into the cytoplasm.

It is possible that poliovirus can also enter the cytoplasm directly in a pH-independent process. It has been demonstrated that the purified viral receptor converts the 160S poliovirus particle into a 135S form lacking VP4. This further dissociates to an 80S complex in which the RNA has been lost. It has also been observed that an "engineered" receptor molecule which lacks the cytoplasmic domain and in its place contains a GPI anchor preventing internalization still confers susceptibility to infection. It is possible that the hydrophobic amino terminus of VP1 is exposed on the surface of the capsid during the transition from a 160S complex to a 135S complex and that the capsid then fuses directly with the cell membrane.

Uncoating.

This is the general term applied to events after penetration which allow the virus to express its genome . It is poorly understood.

In reoviruses, the capsid only ever partially disintegrates and replication takes place in a structured particle.

In poxviruses, host factors induce the disruption of the virus. The release of DNA from the core depends upon viral factors made after entry.

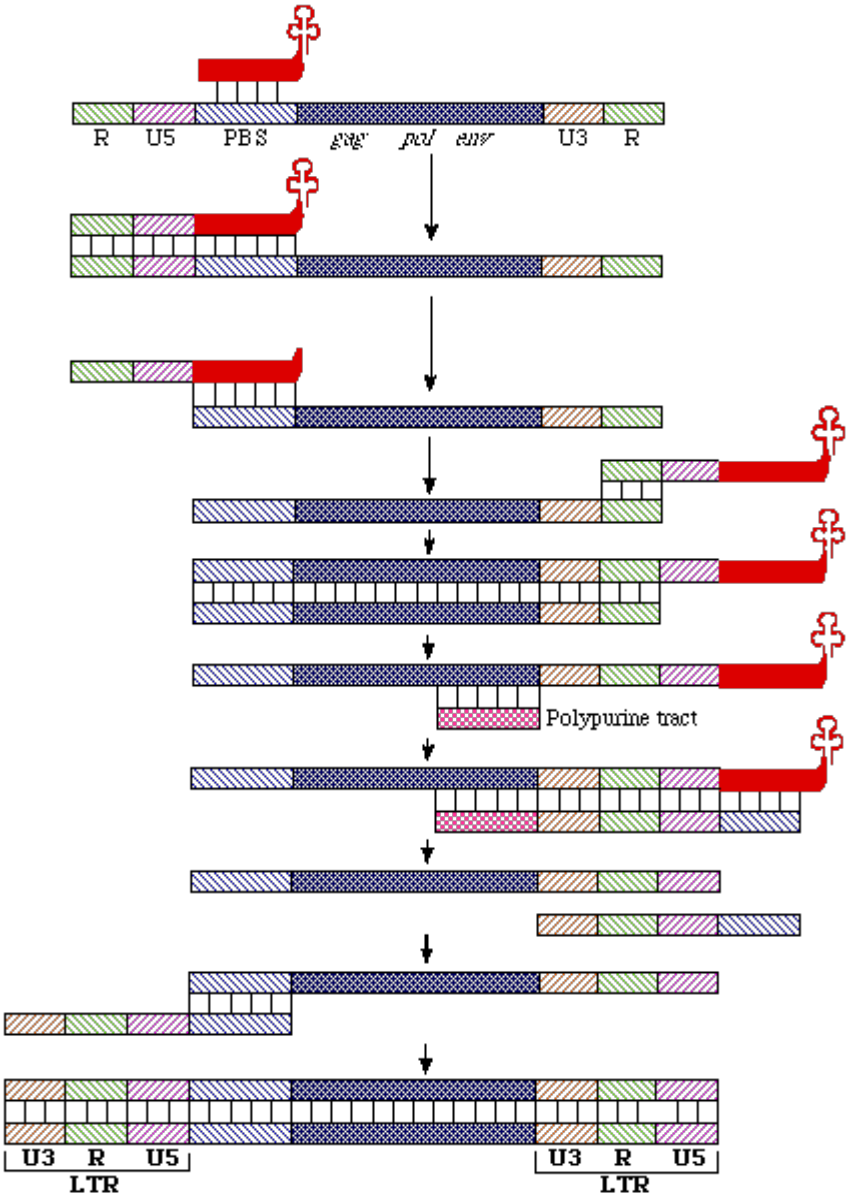
Orthomyxo, paramyxo and picornavirus all lose the protective envelope or capsid upon entry into the cytoplasm. In the influenza virus an envelope viral protein called M2 may allow endosomal protons into the virion particle resulting in its partial dissolution and permitting replication. M2 is a polypeptide of 97aa It forms a tetrameric channel forming structure within the viral envelope. Amantadine and rimantidine are anti-influenza drugs which function in part by inhibiting M2. In cells treated with rimantidine the nucleocapsid remains associated with matrix protein and does not go to the nucleus.

In herpesviruses, adenoviruses and papovaviruses, the capsid is eventually routed along the cytoskeleton to nuclear envelope.

Replication.

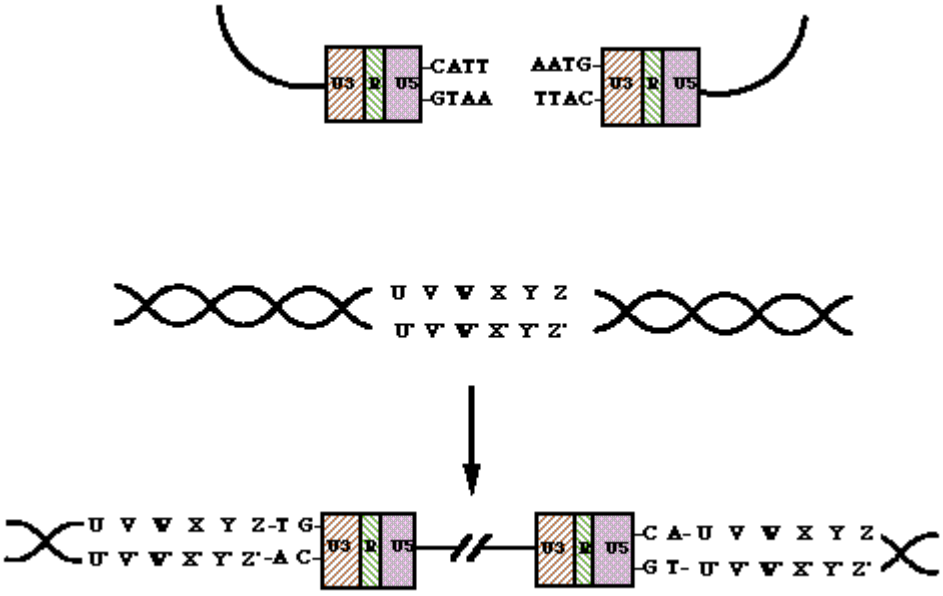
The diversity of viral genomes and general replication strategies was discussed in **Course 224**. They will discussed again in more detail elsewhere in this course. If you are not familiar with the Baltimore classification of viruses then re-read those notes.

In retroviruses, the matrix protein probably stays associated with cytoplasmic membrane after entry. Reverse transcription ie copying the RNA genome into a double stranded DNA form is thought to take place in a structured remnant of the capsid in the cytoplasm. The enzyme concerned is reverse transcriptase. It is both an RNA and DNA directed DNA polymerase. It also has an associated RNAse activity. The process is illustrated below:



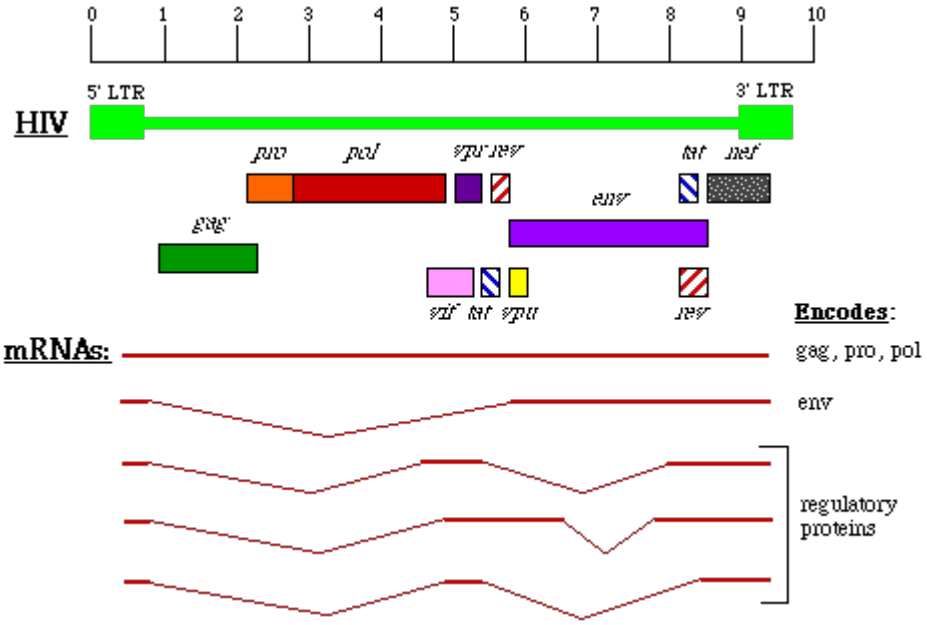
It results in the formation of a double-stranded proviral DNA which is longer than the viral RNA by one copy of U3, R and U5. In reality, the procedure is more complicated than shown. There are probably more than 2 strand jumps because retroviral RNA is often broken or nicked. At every nick of necessity the RT complex has to switch to another RNA strand (remember that there are two present).

The proviral DNA is transported to the nucleus and integrated into the cellular DNA. HIV is not thought to show any obvious preferences in the sites of integration. The viral enzyme catalyzing this process is called integrase.



A linear form of the proviral DNA is integrated. This reaction is unique to retroviruses. Two bases are first removed from the linear DNA at the 3' end to give a 3' recess. A staggered cut 6 nucleotides apart is then made in the genomic DNA and the resulting 5' phosphates are ligated to the 3' OH groups. This leaves a gapped intermediate which is repaired. The reaction can take place in vitro in the absence of ATP. Cleavage always occurs at the same place in the LTR. The reaction does not go to completion in quiescent T cells.

Organization of the HIV genome:



~9.5 kilobases long with 3 large open reading frames, *gag* , *pol* and *env* , common to all retroviruses. there are also several smaller reading frames. Four are accessory in the sense that they can be dispensed with in culture:

- vif - infectivity ?
- vpr - transcription ?
- vpu - env chaperone ?
- nef - indirect effect on transcription?

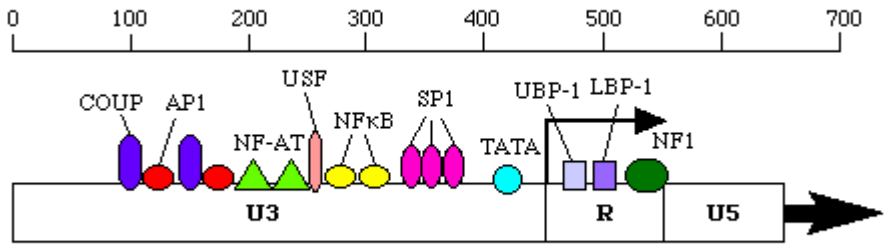
Tat and rev are essential viral proteins involved in the control of gene expression. The gag protein made from an unspliced genomic message. The *pol* gene overlaps the gag gene by 241 nucleotides. The reading frame of pol is -1 with respect to *gag* . In about 10% of cases a gag-pol fusion protein is expressed because of a -1 ribosomal slippage.

This occurs within a sequence of 6U residues, U UUU UUA and means that 1 base is read twice. The process is called ribosomal frameshifting and occurs in other viral families, pol also contains an aspartate protease activity. This cleaves the gag/pol fusion protein. It further cleaves pol to liberate integrase, reverse transcriptase and free protease.

gp120 (env) is made from a singly spliced message.

The tat and rev genes both have 2 exons, partially overlap each other in different reading frames, made from doubly spliced messages.

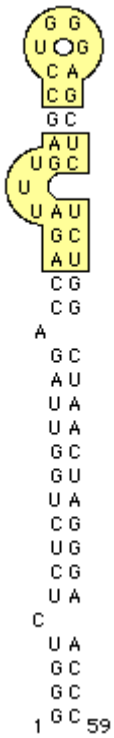
The LTR functions as a highly compressed promoter. Following integration the virus behaves as a resident cellular gene:



Transcription is a complicated process involving the interplay of cis-acting regulatory sequences in the LTR and trans-acting proteins made by the cell and the virus.

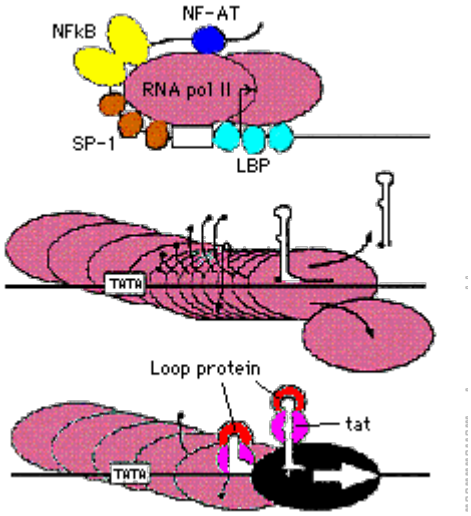
RNA polymerase II acts at a promoter in concert with accessory transcription factors (TFs). These bind specifically to sequences flanking the promoter and in some cases to RNA polymerase itself. A cell may have hundreds of TFs. Some sites in the LTR function as binding sites for general transcription factors such as the TATA binding factor (TFIID) whilst others bind tissue specific transcription factors e.g. NFkB. NFkB is a particularly important activator in T cells. The presence of many TF binding sites in the viral LTR explains why it is activated in many different cells by many different stimuli. Cellular transcription factors include EBP-1, NFkB and SP1, CTF/NF1, LBP-1.

LTR function simply requires a threshold level of transcription. Significant levels of HIV gene expression are only seen in the presence of tat protein . Tat increases or transactivates mRNA production up to a 100X. Tat function requires an RNA sequence known as TAR which is present at the immediate 5' end of all mRNAs:



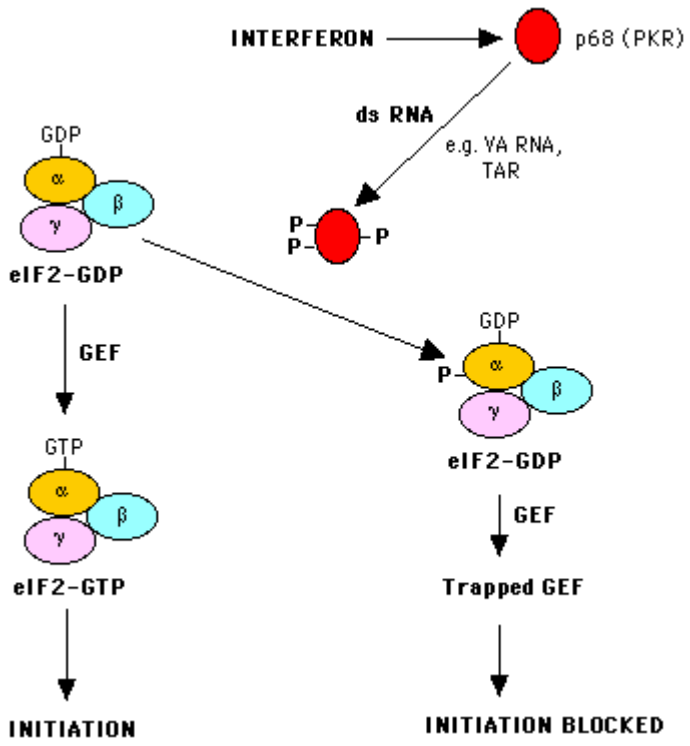
Tar is thought to form a stem-loop structure. Experiments show that tat protein binds directly to TAR RNA with a Kd of about 1nM. One molecule of tat binds to RNA. Mutagenesis experiments show that binding is dependent on the presence of a U bulge in the TAR element. TAR is only functional as an RNA element. TAR only functions in a position immediately 3' of the site of transcription initiation. This RNA-dependent enhancer activity of tat is unique in eukaryotic gene expression.

Other experiments show that the HIV promoter does not assemble a particularly efficient RNA polymerase complex. The initiation rate is slow and elongation efficiency (processivity) is poor. It is thought that tat binding to TAR recruits other elements to the transcriptional complex which enhance processivity:



Initially in an HIV infection small amounts of tat and rev mRNA accumulate due to the low endogenous activity of the promoter in the absence of tat. This allows small amounts of tat to be made which then promotes more transcription and more tat in a positive feedback. The rev protein controls this positive feedback. Over a period of hours although transcription rates remain high the RNA made shifts from small double spliced messages to a single spliced message which encodes env and eventually to an unspliced genomic message which encodes gag and pol. Rev is responsible for this shift in the transcriptional pattern. It down regulates tat and rev RNA in a negative feedback. The rev response involves a cis element of about 250 bases present in *env* . This for obvious reasons has been called the rev-response element or RRE, again it is only functional as an RNA element. Rev binds directly to the RRE with a Kd of 1nM. The position of the RRE explains why the double spliced messages are not rev sensitive, i.e. because they are spliced out. Rev has been suggested to control either RNA splicing or nuclear-cytoplasmic transport or translation. Recently it has been shown to associate with nucleoporin proteins that are thought to be found in the nuclear pore complex.

Protein translation is another control point during viral replication. Interferon is made in response to viral infection. It switches on more than 30 other genes. **2'5' polyadenylate synthetase** makes 2'-5' linked polyadenylates from ATP . These activate a protein called **RNAse L** which degrades cellular and viral RNAs. Many viruses produce double stranded RNA during replication. These can activate another enzyme known as **PKR**. PKR is a protein kinase. When it is activated it phosphorylates the alpha factor of protein initiation factor eIF2:



This then sequesters a guanine nucleotide exchange factor which is essential for the initiation of protein synthesis and is present in very low amounts. This results in the cessation of host cell protein synthesis prevents virus replication: Many viruses have evolved strategies to down regulate PKR:

- Reoviruses and various pox viruses make proteins which sequester the activating RNAs
- Pox viruses also makes a "suicide"pseudosubstrate for PKR
- Poliovirus expresses a protease which degrades it
- Influenza induces synthesis of a cellular repressor
- EBV and adeno virus make RNAs which bind to the enzyme but do not activate it and prevent other RNAs from activating it.

HIV may behave like EBV and adenovirus. The TAR sequence which is double-stranded binds to PKR but does not activate it. TAR is ideally placed to do this because it is at the 5' end of all HIV mRNAs and PKR is associated with ribosomes. This gives rise to the possibility that preferential translation of HIV mRNA may occur in cells where host protein synthesis has largely been shut off.

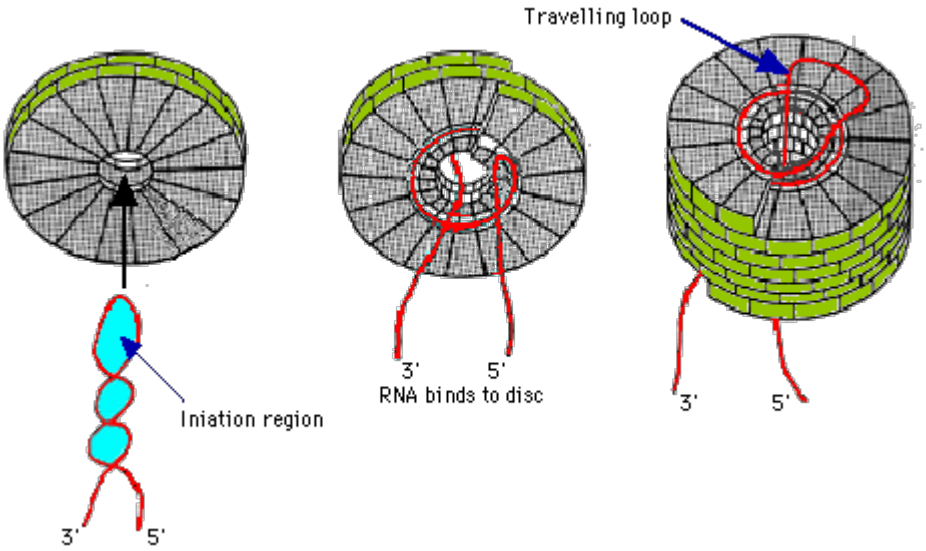
Assembly, maturation and release.

Some viruses make morphogenetic factors which are not structurally part of the virus but whose presence is required for normal assembly. These are sometimes called molecular chaperones. Cellular **chaperones** may also take part in this process.

Other viruses self-assemble. It has been known for more than 40 years that a mixture of TMV proteins and RNA will assemble and make infectious virus in the test tube. This suggests that the TMV structure is a minimum free energy state for its constituent parts.

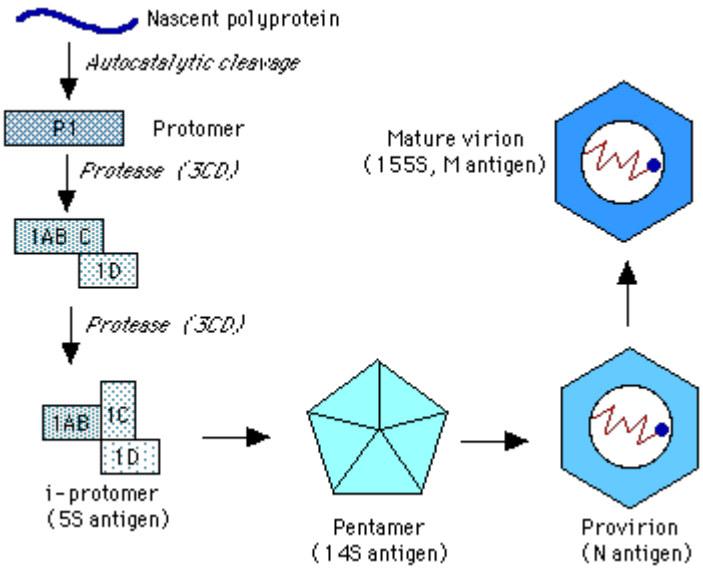
Symmetry is important in viral assembly. It reduces ambiguities in the assembly process and makes it easier. If the coat protein had to form different contacts in different places within the virus structure it would lead to ambiguities in assembly.

In the assembly of TMV the coat protein first forms a disc structure with 17 subunits per ring. This is close to the 16.34 subunits per turn found in the virus. Close examination of electron micrographs shows that the discs are not actually symmetrical, they have a pronounced polarity:



The disc then interacts with a high affinity binding site on TMV RNA. This converts the disc to a helical "locked washer". Further discs or aggregates of coat protein add to this structure, and also switch to the locked washer. RNA is entrapped in the middle of the disc. Studies show that disc binding is initiated at a unique internal site and that subsequent growth occurs in both directions but at very unequal rates. The major direction of assembly is 3'-5'. This might be expected since the initial nucleation site is close to the 3' end of the virus. RNA is drawn into the assembling structure in a travelling loop. Growth in the 5' direction is fast because a disc can add straight to the protein filament and RNA is drawn up through it. Growth in the 3' direction is slower because the RNA has to threaded through the disc before it can add to the structure. It seems probable that many helical capsids and nucleocapsids will form in a fashion similar to this.

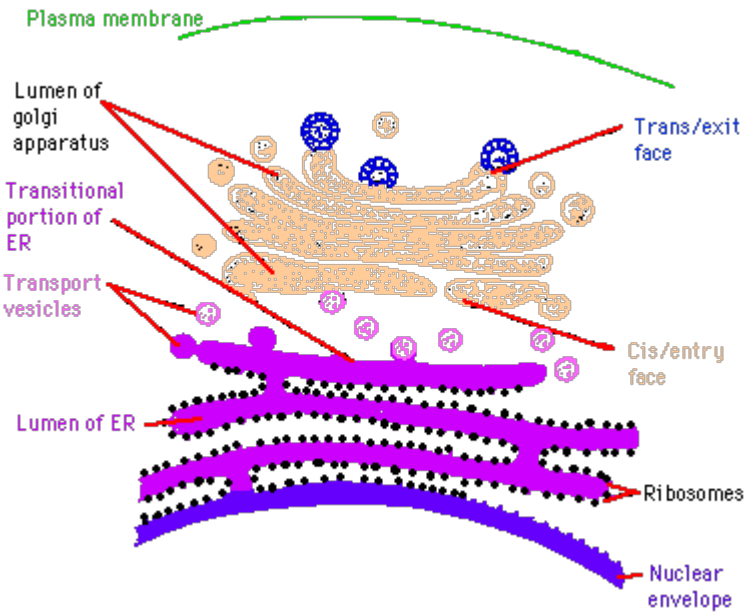
Poliovirus forms a procapsid in the cytoplasm. This procapsid nucleic acid which must have specific signals which ensure that only the genomic strand is encapsidated associate with this nascent particle:



The nascent polyprotein undergoes several cleavage steps to give the "Monomer-protomer". Five protomers associate to form a pentamer. Twelve pentamers associate to form the icosahedral capsid. RNA associates with the pentamers. This assembly process is self-driven and concentration dependent. Monomers are more stable as pentamers and pentamers are more stable as icosahedral structures. Assembly occurs at lower protein concentrations in the presence of cell extracts than in their absence. This suggests that cellular factors help in this process. RNA association with the pentamer may facilitate icosahedral formation.Viruses which are not enveloped like polio usually depend upon disintegration of the cell for their release. The cell often disintegrates because viral replication has prevented normal house keeping functions.

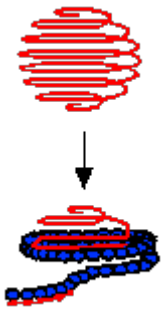
Budding

Some viruses, e.g. herpesviruses, assemble in the nucleus. They acquire an envelope as they pass through the inner nuclear membrane.



They then accumulate, between the inner and outer lamella of the nucleus. This is topologically equivalent to the cisternae of the cytoplasmic reticulum. From here they pass in a vesicle to the cell surface. These viruses are protected from the cytoplasm. This budding process is invariably cytolytic.

Other enveloped viruses acquire their lipid bilayer from the plasma membrane. Capsid assembly can take place in the cytoplasm or in the case of HIV at the plasma membrane. The HIV gag fusion protein p55 is inserted into the lipid membrane by because of a myristyl group at the N-terminus. A patch of gag proteins form associated with the membrane either because of protein-protein interactions or because of association with the viral RNA. The approximately spherical virus may form because of a curvature induced by the gag protein association or because of the helical nature of the RNA. The RNA becomes extensively associated with the nucleocapsid moiety of gag.



The nascent virion then buds through the membrane. The fact that 5% of gag is in the form of a gag-pol fusion which contains reverse transcriptase, integrase and protease ensures that proteins required for infection are present in the virion particle. The relatively high concentration of protease results in its activation and the cleavage of the gag and pol proteins and the eventual formation of the mature and infectious virus which was discussed at the beginning of this document. The fact that the env glycoprotein has a symmetry related to an icosahedron suggests that it may interact with the matrix protein underlying the lipid bilayer and that its incorporation into the virion particle does not just depend upon the envelope glycoprotein being present in the lipid bilayer. Other macromolecular constituents of the cytoplasm are also incorporated into the virion unspecifically. The reverse transcriptase primer may be specifically associated with either the viral RNA or the gag protein. This process does not necessarily result in cell lysis and death.

References: Click here.

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Virus Vectors & Gene Therapy: Problems, Promises & Prospects

David Peel

MBChB Special Study Module Project Report
Department of Microbiology & Immunology
University of Leicester.

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1. Introduction: Viruses as Vectors
2. Retrovirus Vectors
3. Adenovirus Vectors
4. Adeno-Associated Virus
5. Herpes Simplex Virus
6. Non-Viral Methods of DNA Transfer
7. Comparison of Vectors
8. Strategies for Gene Therapy: Cancer & Clinical Trials
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Introduction:

The cumulative research directed towards understanding the molecular biology underlying all aspects of disease & development has yielded a wealth of information. As gene products & their interactions with the cellular environment have been characterised, so the possibility of treating disease by using DNA as a drug has arisen. All proteins are coded for by DNA, & diseases ultimately result from the expression of one or more abberent proteins, e.g. an oncogene or pathogen protein, or the lack of a functional form. In theory therefore, all diseases could be treated by expression of the appropriate protein in the effected cells. Conceptually the most simple disease to treat would be a monogenic recessively inherited disease, such as haemophilia (Snyder et al, 1997), whereby the functional form of the gene would be added to the cell restoring it to a normal phenotype. However, research is underway to treat monogenic dominantly inherited diseases such as hypercholesteroleamia (Gerad & Collen, 1997),

& acquired genetic diseases such as cancers (Roth & Cristiano, 1997). Regulation of cellular proliferation e.g. to prevent atherosclerosis following angioplasty (Kim et al, 1997), or to promote of cellular repair following trauma to the CNS (Federoff et al, 1992) & protection from infectious disease (Caruso & Bank, 1997) are also currently being investigated.

Gene therapy potentially represents one of the most important developments to occur in medicine, but before this can be realised certain technical problems common to all methods of gene delivery must be overcome. In order to modify a specific cell type or tissue, the therapeutic gene must be efficiently delivered to the cell, in such a way that the gene can be expressed at the appropriate level & for a sufficient duration. Two broad approaches have been used to deliver DNA to cells, namely viral vectors & non-viral vectors, which have different advantages as regards efficiency, ease of production & safety. This paper will review these methods & then discuss the genetic strategies used to achieve prolonged tissue specific expression of the therapeutic gene.

Viruses as Vectors:

Viruses are obligate intra-cellular parasites, designed through the course of evolution to infect cells, often with great specificity to a particular cell type. They tend to be very efficient at transfecting their own DNA into the host cell, which is expressed to produced new viral particles. By replacing genes that are needed for the replication phase of their life cycle (the non-essential genes) with foreign genes of interest, the recombinant viral vectors can transduce the cell type it would normally infect. To produce such recombinant viral vectors the non-essential genes are provided in trans, either integrated into the genome of the packaging cell line or on a plasmid. As viruses have evolved as parasites, they all elicit a host immune system response to some extent. Though a number of viruses have been developed, interest has centred on four types; retroviruses (including lentiviruses), adenoviruses, adeno-associated viruses & herpes simplex virus type 1.

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Table 1: Familial and important generic taxa of plant and animal RNA viruses and important properties

Genome	Taxon	Host(s)[3]	Genome segments	Morphology, Envelope Y/N
ssRNA(-)	*Arenaviridae**	V	2	Spherical, Y
	Bornaviridae[1]	V	1	Spherical, Y
	*Bunyaviridae**	V, P Arth	3	Spherical, Y
	Delta virus	V	1	Spherical, N
	Filoviridae[1]	V	1	Elongated Bacilliform, Y
	Ophiovirus[2]	P	3	Filamentous, N
	Orthomyxoviridae	V	8	Spherical, Y
	Paramyxoviridae[1]	V	1	Spherical, Y
	Rhabdoviridae[1]	V, P, I	1	Bacilliform, Y
	Tenuivirus[2]	P	4	Filamentous, N
ssRNA(+)	*Arteriviridae*[4]	V	1	Spherical, Y
	Astroviridae	V	1	Isometric, N
	Bromoviridae	P	3	Isometric, Bacilliform, N
	Caliciviridae	V	1	Isometric, N
	Closteroviridae	P	1	Filamentous, N

	Comoviridae	P	2	Isometric, N
	Coronaviridae[4]	V	1	Spherical, Y
	Flaviridae	V, I	1	Spherical, Y
	Furoviridae	P	2	Rodlike, N
	Luteoviridae	P	1	Isometric, N
	Necrovirus	P	1	Isometric, N
	Nodaviridae	I	2	Isometric, N
	Picornaviridae	V	1	Isometric, N
	Potexvirus	P	1	Filamentous, N
	Potyviridae	P	1-2	Filamentous, N
	Sequiviridae	P	1	Isometric, N
	Sobemovirus	P	1	Isometric, N
	Tetraviridae	I	1	Isometric, N
	Tobamovirus	P	1	Rodlike, N
	Tobravirus	P	1	Rodlike, N
	Togaviridae	V, I	1	Spherical, Y
	Tombusviridae	P	1	Isometric, N
	Tymovirus	P	1	Isometric, N
dsRNA	*Birnaviridae*	V, Arth	2	Isometric, N
	Partitiviridae	P, Fungi	2-4	Isometric, N

	Reoviridae	V, I, P	10-12	Isometric, N
	Totiviridae	Protozoa	1	Isometric, N
	Hypoviridae	P, Fungi	1	None

Legend:

* = ambisense: genomes: mostly (-), but partly (+)-sense; 1 = Order *Mononegavirales*; 2 = resemble bunyavirus nucleoproteins; 3 = V(ertebrate), I(nsect), P(lant), Arth(ropod); 4 = order *Nidovirales*. After Murphy (Murphy, 1996) and Pringle (Pringle, 1999).

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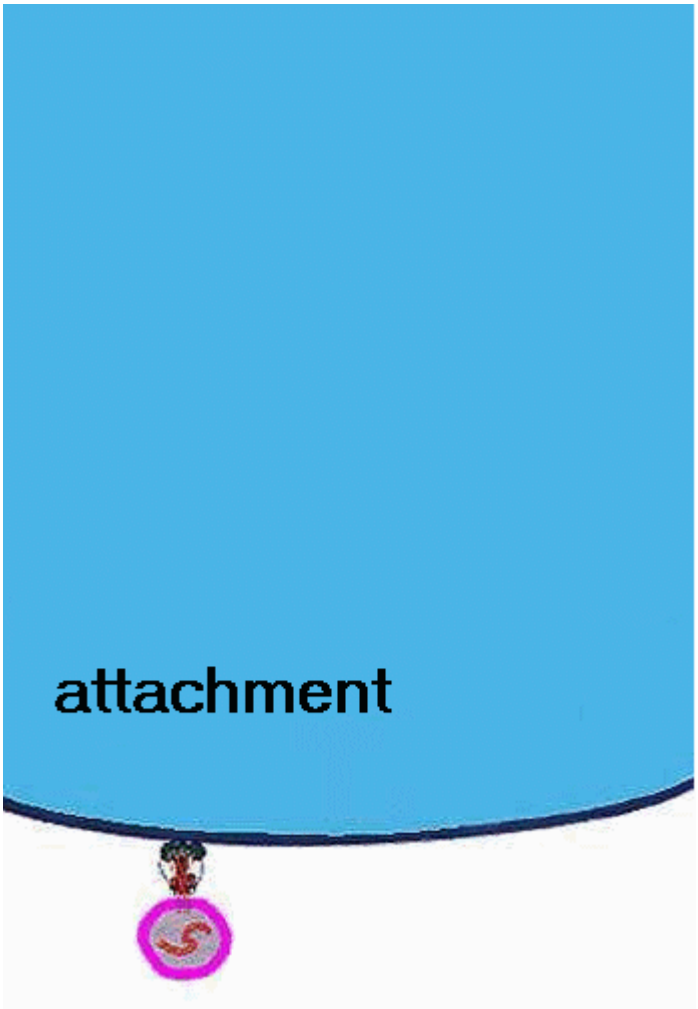

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Paramyxoviruses

The family is divided into 3 genera:

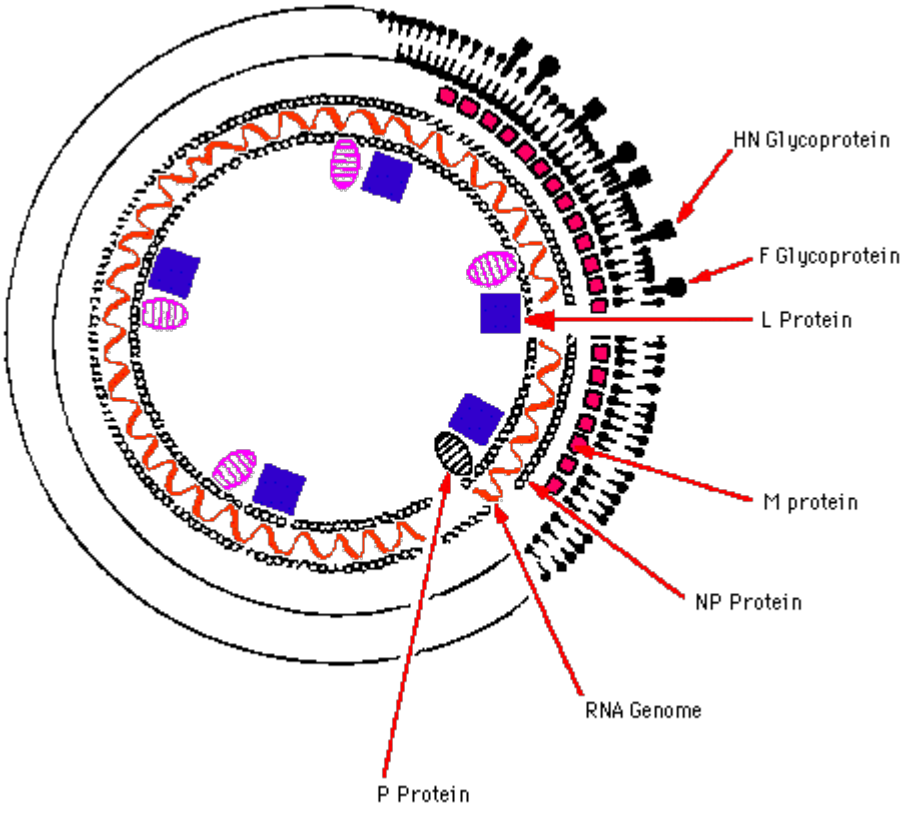
Paramyxovirus: Parainfluenzavirus 1-4; Mumps
Pneumovirus: Respiratory Syncytial Virus (RSV)
Morbillivirus: Measles; Canine Distemper Virus.

Parainfluenzaviruses and RSV produce acute respiratory diseases (c.f. influenza), Morbilliviruses and Mumps systemic disease - diversity! They also differ from Orthomyxoviruses genetically - non-segmented genome with little genetic variation (c.f. influenza).

Morphology:

Glycoproteins - do not form such prominent spikes as on influenza virus:

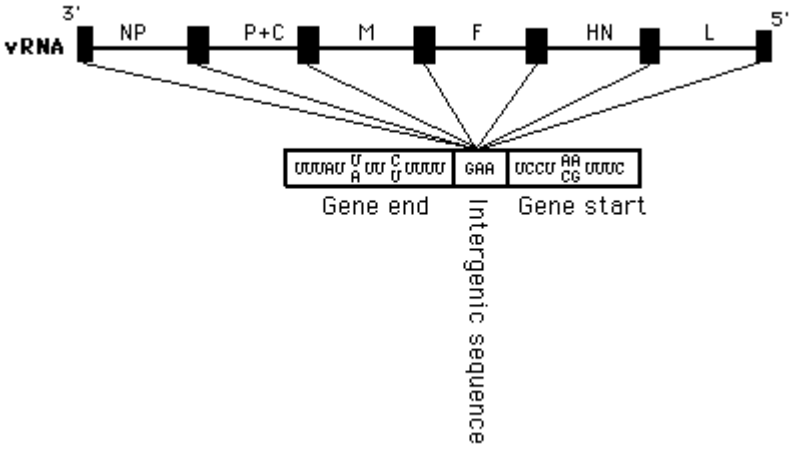
HN - haemagglutinin + neuraminidase activities;
Measles - referred to as **H** protein - no neuraminidase activity;
RSV - **G** protein - neither activity.
F - consists of 2 disulphide-linked subunits (**F1** + **F2**) - responsible for cell fusion + haemolytic function.
Other proteins:
The **M** (matrix) protein lines the inner surface of the envelope.
NP - nucleoprotein.
L and **P** - polymerase activity



To view a negatively-stained electron micrograph of paramyxovirus particles, click here - or here for material from Cape Town.

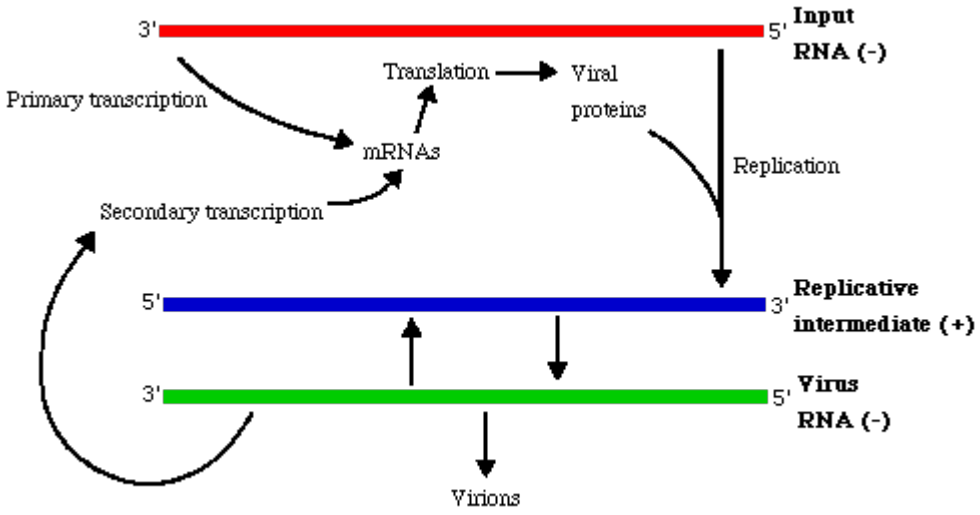
Genome:

Non-segmented (-)sense RNA, 17-20kb. The linear arrangement of genes (6) are separated by repeated sequences, a polyadenylation signal at the end of the gene, the intergenic sequence GAA followed by a translation start signal at the beginning of the next gene.



Replication:

Very similar for all viruses in this group. Unlike influenza, all the action occurs in the cytoplasm. However, the overall strategy very similar to influenza, although unlike influenza, Paramyxovirus replication is resistant to actinomycin D.
A large excess of nucleocapsids are produced in infected cells, which form characteristic cytoplasmic inclusion bodies. Syncytium formation is quite common (F glycoprotein).



Pathogenesis:

Parainfluenzaviruses 1-4:

Cause acute respiratory infections of man ranging from relatively mild influenza-like illness to bronchitis, croup (narrowing of airways which can result in respiratory distress) and pneumonia; common infection of children. Transmitted by aerosols, virus is usually limited to U.R.T. (no viraemia). Infections of L.R.T. (e.g. in very young

children) lead to more serious symptoms. Little serological variation, therefore rare infection in adults.

Mumps:

Recognised by the ancient Greeks, virus first isolated in 1934. Haemagglutination is a valuable assay technique for this virus. Humans are believed to be the only natural reservoir for the virus (possibly primates). Transmission via saliva and respiratory secretions; less infectious than measles/chickenpox - more adult cases. Typically causes painful swelling of parotid glands 16-18 days after infection. This is preceded by primary replication of the virus in epithelial cells of the U.R.T. and local lymph nodes, followed by viraemia. In children, mumps is usually self-limited, but in adults (post-puberty) a proportion of cases have more serous sequalae: orchitis (20-30% of males - rarely resulting in sterility); meningitis, encephalitis, pancreatitis, myocarditis, nephritis - <1% adult cases.
Treatment: none (passive immunization has been used).
Prevention: one invariant serotype therefore vaccines are viable - both formalin-inactivated and live attenuated exist, the latter now being widely used- see below.

Measles:

One of the most infectious diseases known! $>10^6$ deaths p.a. in children in the third world - now part of the W.H.O expanded programme of immunization. Childhood infection almost universal, protection resulting from this is probably lifelong. Both man and wild monkeys are commonly infected, but the virus can also infect rodents (in wild?). In culture, produces characteristic intranuclear inclusion bodies and syncytial giant cells. Transmission and initial stages of disease similar to mumps, but this virus can also infect via the eye and multiply in the conjunctivae. Viraemia following primary local multiplication results in widespread distribution to many organs.
Symptoms: After a 10-12 day incubation period, dry cough, sore throat, conjunctivitis (virus may be excreted during this phase!!!), followed a few days later by the characteristic red, maculopapular rash and Koplik's spots - raised red spots with white centres in the mouth. Towards the end of the disease, there is extensive, generalized virus infection in lymphoid tissues and skin. Complications include bronchopneumonia and otitis media (with or without secondary bacterial infections) (relatively common), and encephalitis (~1:2000 cases). Subacute schlerosing pan encephalitis (SSPE) results from a rare (~1 : $3x10^5$ cases of measles), chronic infection in which the virus multiplies in the brain with the expression of a limited repertoire of virus genes, resulting in neurodegenerative disease.
Treatment: None
Prevention: Both live and killed vaccines exist. Vaccination with the live attenuated vaccine has been practiced in the US since the 1960's with a dramatic decline in the incidence of the disease (210), but has only been used more recently in the UK. Trivalent live attenuated vaccine (MMR) usually given - all of these viruses best avoided during pregnancy!
Medscape Article: "Genetic Diversity of Wild-Type Measles Viruses: Implications for Global Measles Elimination Programs"

Books about Measles Virus: click here

RSV:

First isolated in 1956 and subsequently recognised as a major cause of L.R.T. disease in infants and young children. Infects man, monkeys and some rodents with disease production, but inapparent infections (resulting in spread of virus) may occur in many mammals. In culture, causes characteristic syncytial masses - hence the name. Highly infectious, transmission by respiratory secretions. Primary multiplication occurs in epithelial cells of U.R.T. producing a mild illness. In ~50% children less than 8 months old, virus subsequently spreads into the L.R.T. causing bronchitis, pneumonia and croup. Has been suggested as a possible factor in cot death. Prevention: Currently no vaccine! Also, infection does not result in lasting protection (c.f. mumps, measles) therefore repeated infections ('colds') occur throughout life - usually without serious consequences in adults.

? Probable Human Infection with a Newly Described Virus in the Family Paramyxoviridae ?

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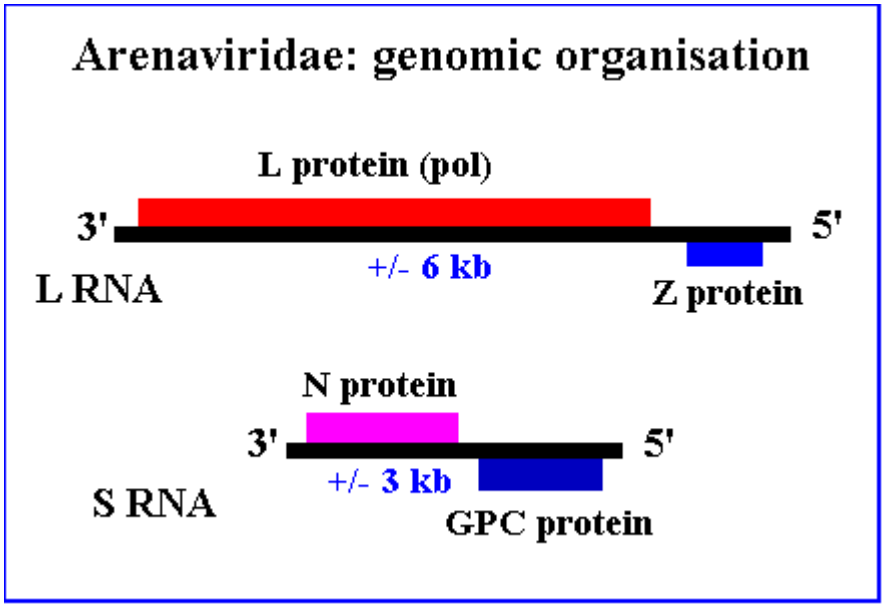
Arenaviridae: genomic organisation
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3'
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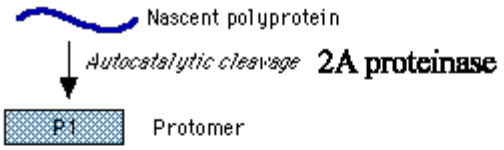

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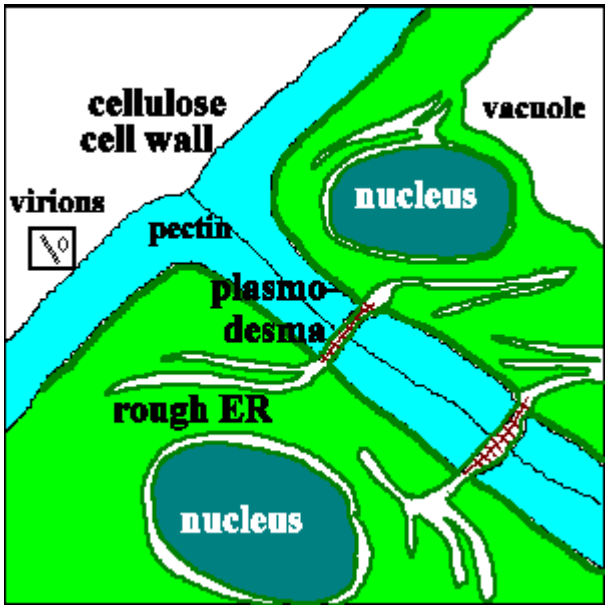
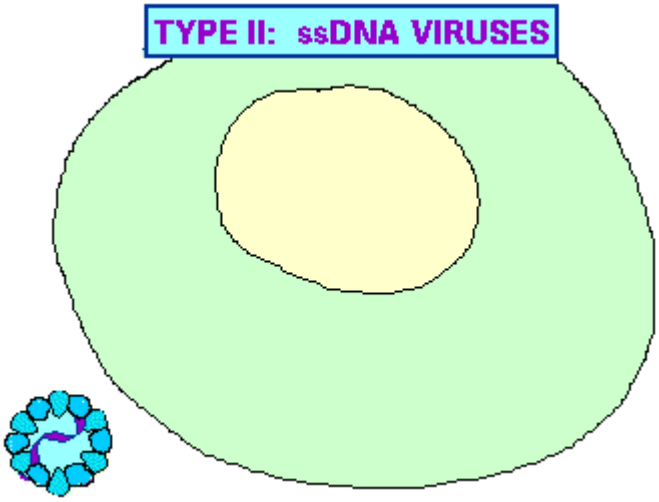


Replication Strategy of ssDNA Viruses



Note: this is an animated GIF, which takes about 30 sec to cycle through completely.

Steps in Replication

1. Conversion into dsDNA (=host repair process?)
2. Early transcription (by host enzymes)
3. Translation of (regulatory) protein and "rolling circle" ssDNA replication
4. Late transcription (usually mediated by viral proteins)
5. Synthesis of late (=structural) proteins
6. "Sequestering" of viral genomic ssDNA
7. Assembly into virions

Return to DNA Genome Types

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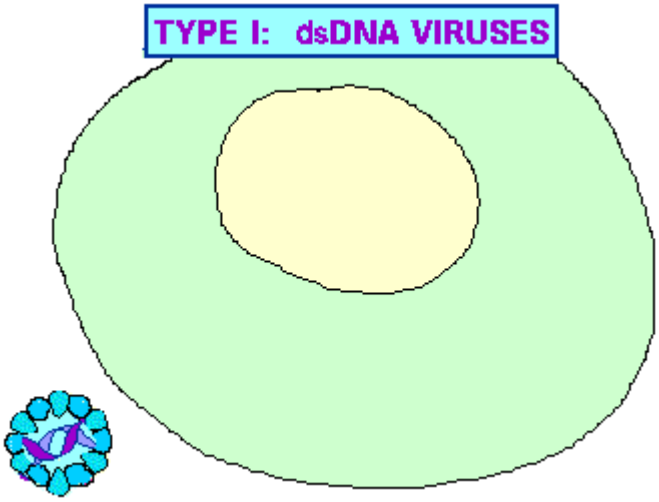
Replication Strategy of dsDNA Viruses



Note: this is an animated GIF, which takes about 30 sec to cycle through completely.

Steps in Replication

1. **Primary transcription by host enzymes**
2. **Translation of early (=regulatory) proteins**
3. **Viral genomic DNA replication (usually by host enzymes)**
4. **Late transcription (usually mediated by viral proteins)**
5. **Synthesis of late (=structural) proteins**
6. **Assembly of structural protein and DNA into virions**

Return to DNA Genomes

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Poxviruses

Introduction:

The largest and most complex viruses known! Have been known about for centuries - the characteristic "pocks" produced by variola virus (Smallpox) giving their name to all forms of infectious disease "a dose of the pox". Smallpox first appeared in China and the Far East at least 2000 years ago.

The Pharaoh Ramses V (left) died of smallpox in 1157 B.C. The disease reached Europe in 710 A.D. and was transferred to America by Hernando Cortez in 1520 - 3.5 million Aztecs died in the next 2 years. In the cities of 18th century Europe, smallpox reached plague proportions and was a feared scourge - highly infectious. Five reigning European monarchs died from smallpox during the 18th century.

Smallpox has now been eradicated - the last naturally occurring outbreak of smallpox was in Somalia on 26th October 1977.

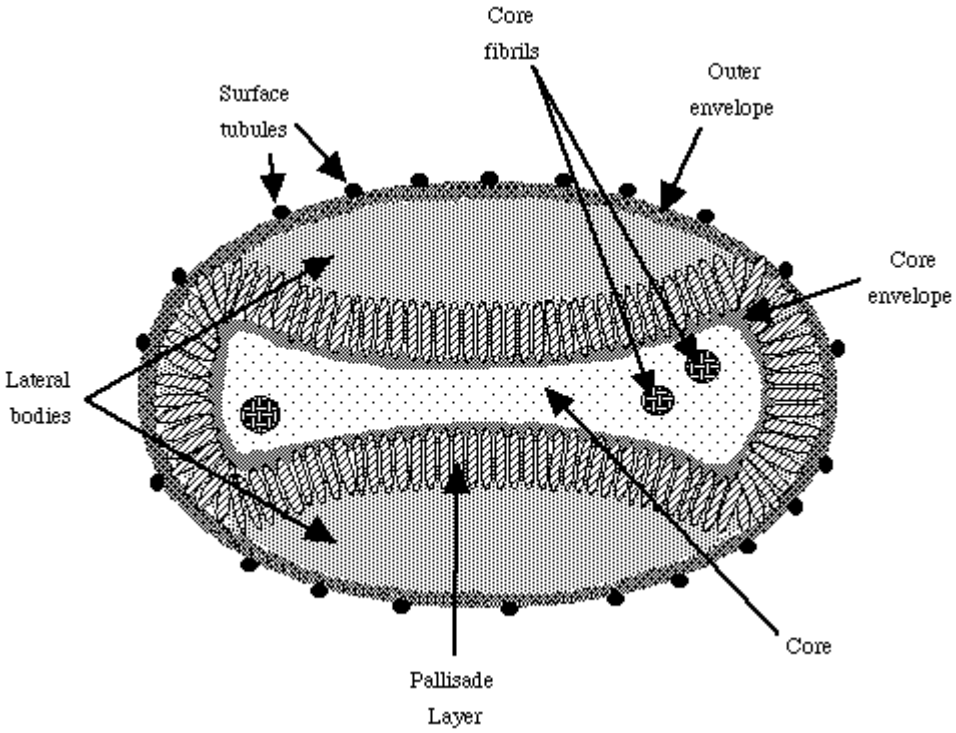
Taxonomy:

Subfamily:	Genus:	Members:
Chordopoxvirinae	Avipoxvirus	fowlpox virus
	Capripoxvirus	sheeppox virus
	Leporipoxvirus	myxoma virus
	Molluscipoxvirus	Molluscum contagiosum
	Orthopoxvirus	vaccinia virus
	Parapoxvirus	orf virus
	Suipoxvirus	swinepox virus
	Yatapoxvirus	Yaba monkey tumor virus
	Entomopoxvirus A	Melolontha melolontha entomopoxvirus

Entomopoxvirinae	Entomopoxvirus B	Amsacta moorei entomopoxvirus
	Entomopoxvirus C	Chironomus luridus entomopoxvirus

Morphology:

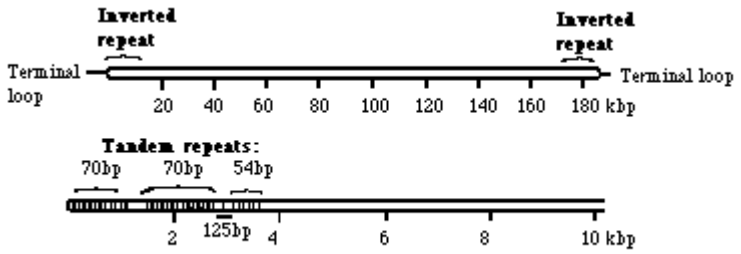
Oval or "brick-shaped" particles 200-400nm long - can be visualized by the best light microscopes (just). The external surface is ridged in parallel rows, sometimes arranged helically. The particles are extremely complex, containing many proteins (more than 100) and detailed structure is not known.
The extracellular forms contain 2 membranes (**EEV** - extracellular enveloped virions), intracellular particles only have an inner membrane (**IMV** - intracellular mature virions).



Thin sections in E.M. reveal that the outer surface is composed of lipid and protein which surrounds the core, which is biconcave (dumbbell-shaped), with 2 "lateral bodies" (function unknown). The core is composed of a tightly compressed nucleoprotein. To view an electron micrograph of a negatively-stained poxvirus particle click here.
Antigenically, poxviruses are very complex, inducing both specific and cross-reacting antibodies - hence ability to vaccinate against one disease with another virus (below). There are at least 10 enzymes present in the particle, mostly concerned with nucleic acid metabolism/genome replication.

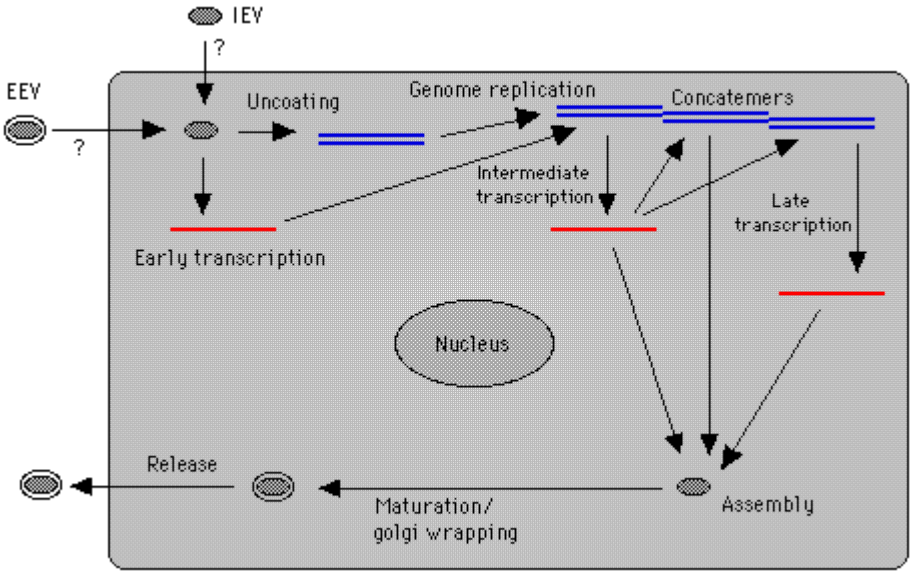
Genome:

Linear, d/s DNA of 130-300kbp. Ends of genome consist of a terminal hairpin loop (no free ends) with several tandem (i.e. direct) repeat sequences (this arrangement is found at the ends of chromosomes from a number of different organisms). The ends of the genome form direct repeats called inverted terminal repeats (ITRs). Several poxvirus genomes have been sequenced. Most of the essential genes are located in the central part of the genome, while non-essential (in tissue culture) genes are located at the ends. There are ~200 genes in the genome.



Replication:

Occurs in the **cytoplasm** - the virus is sufficiently complex to have acquired all the functions necessary for genome replication (c.f. Herpesviruses). There is some contribution from the cell but it is not clear what this is - poxvirus gene expression and genome replication occur in enucleated cells, but maturation is blocked.



Receptors are not known, but probably >1 on different cell types. For Vaccinia, one of these is probably the EGF receptor (epidermal growth factor).
Penetration is complex and may also involve >1 mechanism.
Uncoating occurs in two stages, removal of the outer membrane as the particle enters the cell and in the cytoplasm, the particle (minus its outer membrane) is further uncoated and the core passes into the cytoplasm.
Gene expression is carried out (exclusively?) by viral enzymes associated with the core and is divided into 2 phases:

Early genes: ~50% genome, expressed before genome replication
Late genes: expressed after genome replication; late promoters are dependent on DNA replication for activity.

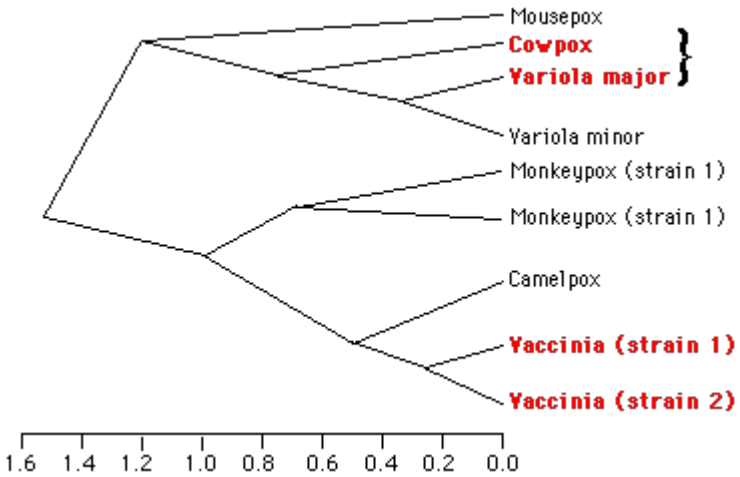
Poxvirus gene expression has been studied in detail because of the interest in the use of Vaccinia virus as a vector for expression of heterologous genes.
Genome replication is believed to involve self-priming, leading to the formation of high m.w. concatemers (isolated from infected cells) which are subsequently cleaved and repaired to make virus genomes. The many virus-encoded enzymes involved in replication (e.g. thymidine kinase - *tk*) offer potential targets for chemotheraputic agents.
Assembly occurs in the cytoskeleton; the events involved in putting together such a complex particle are not understood, but probably involve interactions with the cytoskeleton (e.g. actin-binding proteins). Inclusions are formed in the cytoplasm which mature into virus particles. Actin 'comet tails' form which shoot IEV through the cytoplasm to the cell surface, and possibly into adjacent cells - these have been timed moving at 3µm/min. This may provide an alternative mechanism for cell to cell spread (c.f. EEV).
Overall, replication of this large, complex virus is rather quick ~12h.

Vaccinia virus shows considerable resistance to the antiviral effects of interferons. One of the early genes of this virus, **K3L**, encodes a protein which is homologous to eIF-2a which inhibits the action of PKR. In addition, the **E3L** protein also binds dsRNA & inhibits PKR activation. As well as these, other poxvirus-encoded proteins interfere with the actions of complement, IL-1 and TNFs. Vaccinia infection of cells can confer "protection" from IFN on other viruses, e.g. Picornaviruses.

Variola and Vaccinia:

At least 9 different poxviruses cause disease in humans, but variola virus (VV) and vaccinia are the best known. VV strains are divided into variola major (25-30% fatalities) and variola minor (same symptoms but less than 1% death rate). "Variolation" = the administration of material from known smallpox cases (hopefully variola minor!!!) to protect recipients - practiced for at least 1000 years (Chinese) but risky - Jenner was nearly killed by variolation in 1756!

The precise origins of vaccinia virus are uncertain:
Edward Jenner, 14th May 1796, used cowpox (Sarah Nemes!) to "vaccinate" 8 year old James Phipps, who he later challenged with VV (ethical?) and showed that he was protected. For more than 100 years, the "vaccine strains" (many origins) were propagated from arm-to-arm, but for at least the last 50 years, Vaccinia has been a distinct virus from cowpox - origin? (In molecular terms, vaccinia is most similar to buffalopox - did the two viruses somehow become mixed at some stage?).



Vaccination was almost universally adopted worldwide around 1800, but it took a major commitment from the WHO in 1965 to achieve eradication.
Eradication of smallpox was possible for 3 reasons:

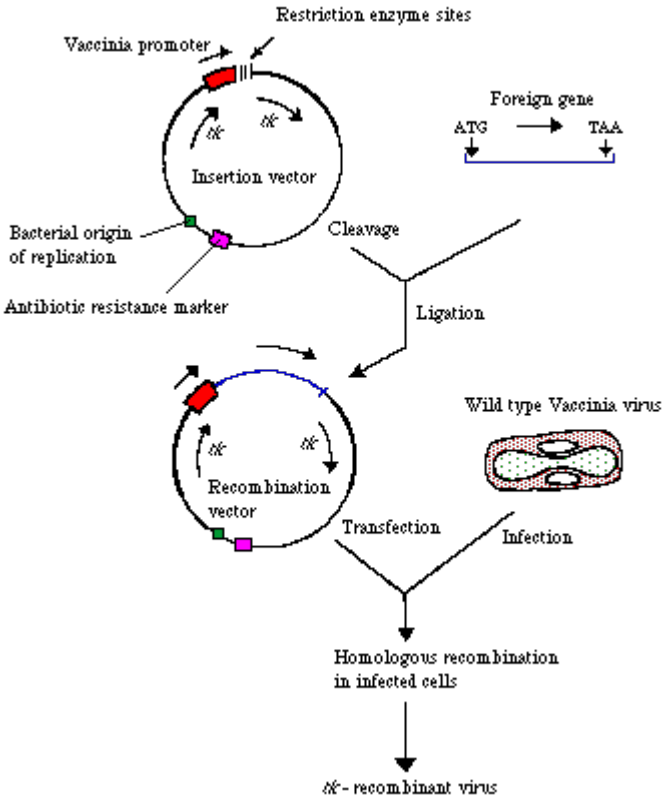
1) There is no other reservoir for VV but man (including primates) (c.f. Arboviruses)
2) VV causes only acute infections, from which the infected person either:
   a) dies
   b) recovers with life-long immunity (c.f. Herpesviruses)
3) Vaccinia virus is an effective immunogen.

Infection with both viruses occurs naturally by the respiratory route (high transmission rate!!!) and is systemic, producing a variety of symptoms, but most notably with VV characteristic pustules and scarring of the skin. Replication of vaccinia is more localized. Administration of vaccinia to immunocompromised hosts results in systemic infection with neurological damage or death.

Online tutorial on smallpox

Unfortunately, monkeypox shows every sign of an emerging virus disesae about to replace smallpox.

Expression Vectors:



Makes use of homologous recombination in infected cells to introduce foreign DNA coupled to VV promoter (e.g. *tk*) into virus genome. Numerous variants have now been made, some which include indicator systems such as beta-galactosidase to give recombinant plaques a blue colour (c.f. M13).
Problems for human vaccines:

- The % of the human population which has already been vaccinated - lifelong protection may result in poor response to recombinant vaccines (?).
- Dangerous in immunocompromised hosts.

  The possible solution to this may be to use Avipoxvirus vectors, e.g. fowlpox or canarypox as 'suicide vectors' - undergo abortive replication in mammalian cells:

- can express high levels of foreign proteins
- no danger of pathogenesis
- no natural immunity in humans

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Last Updated: Saturday, October 4, 1945624.

Herpesviruses

Name comes from the Greek *'Herpein'* - 'to creep' = chronic/latent/recurrent infections. Epidemiology of the common Herpesvirus infections puzzled clinicians for many years. In 1950, Burnet and Buddingh showed that HSV could become latent after a primary infection, becoming reactivated after later provocation. Weller (1954) isolated VZV from chicken pox and zoster, indicating the same causal agent. ~100 Herpesviruses have been isolated, at least one for most animal species which has been looked at. To date, there are 8 known human Herpesviruses.
The family is divided into 3 Sub-families:

Alphaherpesvirinae:	
Simplexvirus	human herpesvirus 1, 2 (HSV-1, HSV-2)
Varicellovirus	human herpesvirus 3 (VZV)
Betaherpesvirinae:	
Cytomegalovirus	human herpesvirus 5 (CMV)
Muromegalovirus	mouse cytomegalovirus 1
Roseolovirus	human herpesvirus 6, 7 (HHV-6, HHV-7)
Gammaherpesvirinae:	
Lymphocryptovirus	human herpesvirus 4 (EBV)
Rhadinovirus	human herpesvirus 8 (HHV-8)

Large (genomes up to 235kbp DNA) and complex viruses ~35 virion proteins. All encode a variety of enzymes involved in nucleic acid metabolism, DNA synthesis and protein processing (protein kinase). The Herpesviruses are widely separated in terms of genomic sequence and proteins, but all are similar in terms of virion structure and genome organization:

Size:	180-200nm
Envelope:	Present; associated glycoproteins.
Tegument:	Protein-filled region between capsid and envelope.
Capsid:	Icosahedral, 95-105nm diameter; 162 hexagonal capsomers.
Core:	Toroidal (DNA around protein), ~75nm diameter.
Genome:	Linear, d/s DNA, 130-230kbp
Replication:	Nuclear.
Assembly:	Nuclear.
Common Antigens:	None!

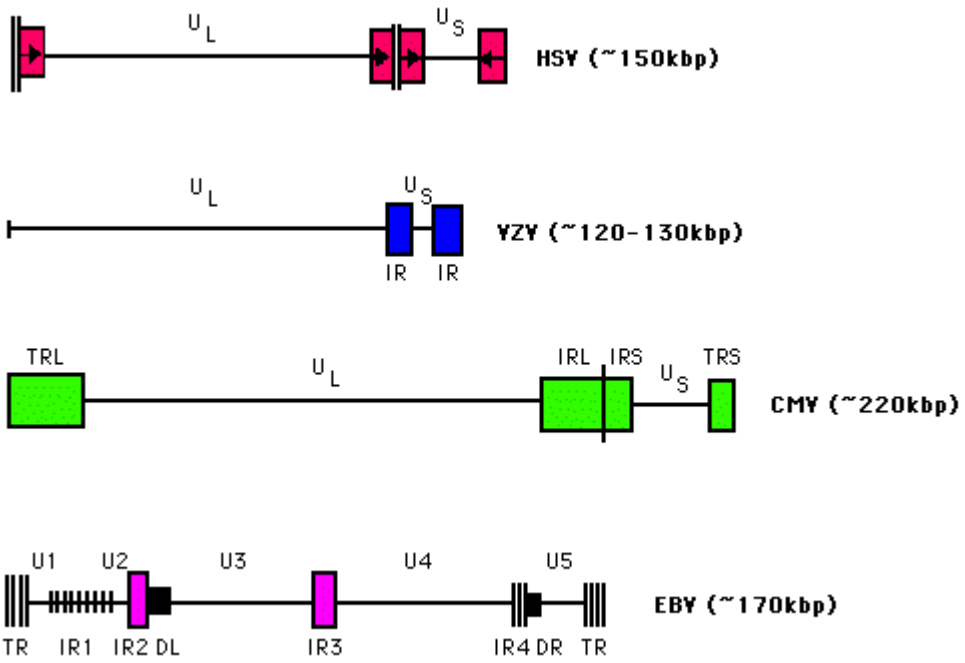
Structure:

The structure of the herpesvirus particle is very complex. The **core** consists of a toroidal shape with the large DNA genome would around a proteinaceous core. The complex **capsid** surrounds the core. Outside the capsid is the **tegument**, a protein-filled region which appears amorphous in electron micrographs. On the outside of the particle

is the **envelope**, which contains numerous glycoproteins (see discussion of Herpes Simplex Virus, below). To view an electron micrograph of negatively-stained herpesvirus particles click here. N.B. All herpesviruses are almost indistinguishable in electron micrographs.

To view an image of the herpesvirus capsid click here.

Genome:



All herpesvirus genomes have a unique long (UL) and a unique short (US) region, bounded by inverted repeats. The repeats allow rearrangements of the unique regions and Herpesvirus genomes exist as a mixture of 4 isomers. Herpesvirus genomes also contain multiple repeated sequences and depending on the number of these, genome size of various isolates of a particular virus can vary by up to 10kbp.

The prototype member of the family is **Herpes Simplex Virus (HSV):** ~160kbp - complete sequence is known. Prior to this, the HSV genome had been mapped extensively by a large number of t.s. mutants. There are 2 antigenic types, HSV-1 and HSV-2 which share antigenic cross-reactivity but different neutralization patterns and tend to produce different clinical symptoms. Man is believed to be the natural host for HSV, but the virus is also capable of infecting various animals, including rodents (good animal models). Human infection is virtually universal - most adults are seropositive.

Replication:

The HSV envelope contains at least **9 glycoproteins**. Most of these appear to be individually dispensable for infectivity. Interaction of glycoprotein(s) with cellular receptors (not known, but probably different receptors on different cell types - a characteristic of the larger, more complex DNA viruses, and probably common cell surface molecules rather than specific proteins) results directly in **fusion** of the envelope with the cell membrane. Endocytosis is not absolutely required, but may occur (alternative route for penetration).

Fusion deposits the capsid in the cytoplasm, where it migrates to the **nucleus**. The core enters via a nuclear pore where the genome is circularized.

Transcription of the large, complex genome is sequentially regulated in a **cascade** fashion. ~50 mRNAs are produced by host cell RNA polymerase II.

Three distinct classes of mRNAs are made:

Alpha - immediate early (IE) mRNAs	5 trans-acting regulators of virus transcription
Beta - (delayed) Early mRNAs	Further non-structural regulatory proteins & minor structural proteins

Gamma - Late mRNAs	Major structural proteins

Gene expression is co-ordinately regulated:

- If translation is blocked shortly after infection, IE mRNAs accumulate in the nucleus, but no other virus mRNAs are transcribed.
- Synthesis of early gene products turns off IE products and initiates genome replication.
- Some of the late structural proteins (gamma-1) are produced independently of genome replication, others (gamma-2) are only produced after replication.

Both IE and E proteins are required for genome replication. A virus-encoded DNA-dependent DNA polymerase and DNA-binding protein are involved in replication, together with a number of enzymes (e.g. thymidine kinase) which alter cellular biochemistry. In addition, cellular proteins are required for genome replication, therefore HSV replication occurs in the nucleus (c.f. Poxviruses).
Viral DNA replication is the target for a number of successful anti-Herpesvirus drugs (e.g. acyclovir, gancyclovir, etc). The pattern of replication is complex, involving at least 3 potential origins of replication, and resulting in the formation of high molecular weight DNA concatemers.
Virus particles (core plus capsid) assemble in the nucleus - genomic concatemers are cleaved and packaged into pre-assembled capsids.
The envelope is acquired from the inner lamella of the nuclear membrane, and particles accumulate in the space within the inner and outer lamellae. How these particles are transported to the cell surface is not clear and may or may not involve the golgi apparatus. Mutations in certain envelope glycoproteins interfere with cytoplasmic transport. Any remaining virus particles are released when the cell lyses (~24h after infection).
HSV infection appears to be a 'wasteful' process, only ~25% of viral DNA/protein produced is incorporated into virions. The rest accumulates in the cell, which eventually dies. This process produces characteristic nuclear inclusion bodies.

Pathogenesis:

Herpes simplex (HSV/ HHV-1/2):

Primary infection occurs through a break in the mucus membranes of the mouth or throat, via the eye or genitals or directly via minor abrasions in the skin. because of the universal distribution of the virus, most individuals are infected by 1-2 years of age; initial infection is usually asymptomatic, although there may be minor local vesicular lesions. Local multiplication ensues, followed by viraemia and systemic infection. There then follows life-long latent infection with periodic reactivation.
During primary infection, the virus enters peripheral sensory nerves and migrates along axons to sensory nerve ganglia in the CNS - allows virus to escape immune response! During latent infection of nerve cells, viral DNA is maintained as an episome (not integrated) with limited expression of specific virus genes required for the maintenance of latency - true latency.
The delicate balance of latency may be upset by various disturbances, physical (injury, U.V, hormones, etc) or psychological (stress, emotional upset - perhaps affecting immune system/hormonal balance).
Reactivation of latent virus leads to recurrent disease - virus travels back down sensory nerves to surface of body and replicates, causing tissue damage:

HSV-1: Primarily associated with oral (cold sores) and ocular lesions
HSV-2: Primarily associated with genital and anal lesions

Although painful, most recurrent infections resolve spontaneously, usually to reoccur later. More serious are herpetic keratitis (ulceration of cornea due to repeated infection which can lead to blindness) and encephalitis (very rare but often fatal). Incidence of genital herpes has increased sharply during the last few decades - sexual promiscuity and oral contraceptives.
No vaccines currently licensed but a number under development - particularly for HSV-2 - a good candidate for post-exposure vaccination.

HSV-1 is under active development as a vector for gene therapy.

Is HSV infection associated with Alzheimer's disease?

Alzheimer's disease affects 10% of people aged over 65 and 20% of those aged over 75. In the UK, this amounts to about 750,000 cases, and 4 million in the USA. A large proportion of elderly people have HSV in their brains, irrespective of whether they have Alzheimer's disease. A particular version (allele) the human ApoE4 gene is known to be a risk factor for the development of Alzheimer's disease, but by no means all those who carry this allele get Alzheimer's. The same ApoE4 allele is a strong risk factor for cold sores caused by HSV infection. Could HSV infection and the ApoE4 allele combine to play a role in Alzheimer's disease?

Itzhaki et al. "Herpes simplex virus 1 in brain & risk of Alzheimer's disease" Lancet 349: 241-244, 1997.
Lin et al. "Alzheimer's disease, herpes virus in brain apolipoprotein E4 and herpes labialis" Alzheimer's Reports 1: 173-178, 1998.

Books about herpes & herpes infection: click here

Latest MEDLINE publications on HSV: Click Here

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Varicella Zoster Virus (VZV / HHV-3):

Gives rise to 2 distinct clinical syndromes:

Varicella (Chicken pox) - (nothing to do with chickens or pox!). Infection normally occurs in childhood (~90% - more?), via respiratory tract or conjunctiva. After multiplication at the inoculation sites, virus spreads to bloodstream and reticuloendothelial system. Secondary multiplication involves skin and mucosa, producing vesicles filled with very high titres of infectious virus! Complications are rare, but may include CNS infection.

Zoster (Shingles) - After primary infection, virus persists in sensory ganglia of CNS. It is not clear if this is a latent or a persistent infection, but 'reactivation' after many years leads to infection and tissue damage to dermatosome served by infected ganglia - most serious when cranial nerves are involved, affecting face/head - can lead to blindness.

Therapy - acyclovir.

Complete sequence of VZV genome is known, ~125kbp. Infects a variety of human and animal cell types in vitro.

Latest MEDLINE publications on VZV: Click Here

Books about Zoster/shingles: click here

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Cytomegalovirus (CMV / HHV-5):

The largest of the Herpesviruses, genome ~240kbp. The kinetics of CMV infection are 'slow' - 7-14 days c.f. 24-48h for HSV. As with some other Herpesviruses, certain parts of the CMV genome have considerable homology with cellular DNA, implying that the virus has acquired cellular genes during evolution. The complete nucleotide sequence is known and expression has been studied in detail. Upstream of the IE genes, there is a promoter/enhancer region which has been characterized in detail and is remarkable for its strength - is often used for heterologous expression of recombinant genes. This is the first region to be transcribed after infection and initiates replication.

CMV infection is common; 60% of the UK population have experienced infection by the age of 40. Most infections are asymptomatic. Apart from during pregnancy and newborn infants exposed in utero, active (as opposed to latent) CMV infection only occurs in people with immune defects, specifically T-cell defects, e.g. AIDS patients and immunosuppressed transplant patients.

Transmission is believed to be by oral/respiratory route. Infection produces enlargement of cells and nuclear inclusion bodies in a wide range of tissues - systemic infection.

In spite of the widespread distribution, CMV-related illness is rare and occurs only in two groups:

- **Immunocompromised:** Evidence that the host immune response (particularly cell-mediated) plays a role in latency comes from the evidence of what occurs on immunosuppression. Latent virus is reactivated and AIDS/transplant patients experience frequent and severe infections with the potential for involvement of many possible organs.
- **Foetal Infections:** Particularly a problem when primary infection of the mother occurs, resulting in congenital abnormalities in a proportion of cases.

Online CMV Experiment:
Mr Campbell's Kidney

Therapy - Gancyclovir.

Latest MEDLINE publications on CMV: Click Here

Books about Cytomegalovirus: click here

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Epstein-Barr Virus (EBV / HHV-4):

Dual cell tropism for human B-lymphocytes (generally non-productive infection) and epithelial cells (productive infection). There is no suitable animal host, but replication/latency has been studied extensively in transformed human cell lines. Widespread worldwide, >90% infection in most human populations (lifelong!). The usual outcome of infection is polyclonal B-cell activation and benign proliferation, which may be sub-clinical or produce infectious mononucleosis (glandular fever). Uniquely among Herpesviruses, there is a well-established relationship between EBV and oncogenesis - Burkitts lymphoma and nasopharyngeal carcinoma. Complete nucleotide sequence (~172kbp) is known, contains many internal repeats. **Therapy/Vaccine** - none.

X-linked lymphoproliferative syndrome (XLP) is a rare condition usually seen in males where on initial infection with EBV (usually around the age of 2-3) results in a hyperimmune response, sometimes causing a fatal form of glandular fever and sometimes cancer of the lymph nodes. Death by the age of 40 is inevitable. XLP is an inherited defect due to a faulty gene on the X chromosome. Females have two X chromosomes, so if one copy is faulty, the other can usually compensate. But males have just one X chromosome and one Y. There is no backup X, hence the predominance of the disease in males. The gene responsible (SH2D1A or SAP) causes a failure in the communication between the cells of the immune system. A protein known as SLAM ("signalling lymphocyte activation molecule") stimulates activity and proliferation of cells in the immune system. SLAM is found on the surface of both B and T cells, effectively coupling them together. When this happens, it signals changes inside the cells that cause them to become active and to develop and proliferate. SAP ("SLAM-associated protein") is produced by T cells, and is an inhibitor of SLAM. This is the gene which is defective in XLP. EBV first infects the throat but shortly afterwards invades the B cells, and triggers them to multiply, partly by increasing the number of SLAM proteins on the surface. Without functional SAP protein, the body is unable to control the B-cell proliferation triggered by infection. T cells recognize the EBV-infected B cells as foreign and instigate a massive inflammatory response. (J.Sayos et al. Nature 395: 462-469, 1998).

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Human Herpesvirus 6 (HHV-6):

(a.k.a. "HBLV"). Isolated in 1986 in lymphocytes of patients with lymphoreticular disorders - tropism for $CD4^+$ lymphocytes. Genome ~160kbp. HHV-6 is now recognised as being a universal human infection. Discovery of the virus solved a longstanding mystery - primary infection in childhood causes "roseola infantum" a.k.a. "fourth disease", a common childhood rash whose cause was previously unknown. Ab titres are highest in children and decline with age. Consequences of childhood infection appear to be mild. Primary infections of adults are rare but have more severe consequences - mononucleosis/hepatitis. HHV-6 infection is a problem in immunocompromised patients.

Latest MEDLINE publications on HHV-6: Click Here

Human Herpesvirus 6 by Stephen Dewhurst, David Skrincosky and Nanette van Loon, Expert Reviews in Molecular Medicine.

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Human Herpesvirus 7 (HHV-7):

First isolated from human $CD4^+$ cells in 1990. Genome ~170kbp, organization similar to but distinct from HHV-6. The complete genome sequence of HHV-7 has been determined and this shows a high degree of conservation of genetic content and encoded amino acid sequences to HHV-6. However, there is only limited antigenic cross-reactivity between the two viruses. At present, there is no clear evidence for the direct involvement of HHV-7 in any human disease, but HHV-7 might be a co-factor in HHV-6-related syndromes ???

Latest MEDLINE publications on HHV-7: Click Here

Human Herpesvirus 7 by Stephen Dewhurst, David Skrincosky and Nanette van Loon, Expert Reviews in Molecular Medicine.

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Human Herpesvirus 8 (HHV-8):

(a.k.a. KSHV). Sequences of an unique herpesvirus were recently identified in 100% of amplifiable samples from AIDS patients with Kaposi's sarcoma (KS) and 15% of non-KS tissue samples from AIDS patients.

There is a strong correlation (>95%) with KS in HIV^+ *and* HIV^- patients. The virus can be isolated from PBMC as well as KS tumour cells; appears to have a less ubiquitous world distribution than other HHVs???
Recent evidence suggests that one of the genes of HHV-8, vGPCR (viral G-protein coupled receptor) acts as a vascular switch, turning on synthesis of a powerful angoigenic agent, vascular endothelial growth factor (VEGF), which is responsible for the development of KS. However, HHV-8 also contains a considerable number of other 'pirated' cellular genes in an 'oncogenic cluster' within the virus genome which may also be involved in the development of malignancy (See Boshoff, C. Nature 391: 24-25, 1998), e.g. the K1 gene (Nature Med. 4:435 1998). In addition to KS, this virus may also cause other tumours such as B-cell lymphomas (±EBV as 'helper'). HHV-8 resembles EBV in that:

- It has a tropism for epithelial and B-cells.
- It is kept under immunological control and only presents a problem during immunosuppression.

HHV-8 & AIDS-Related Neoplasms
Medscape Article: Kaposi's Sarcoma & HHV-8: Implications for Therapy

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Adenoviruses

Introduction:

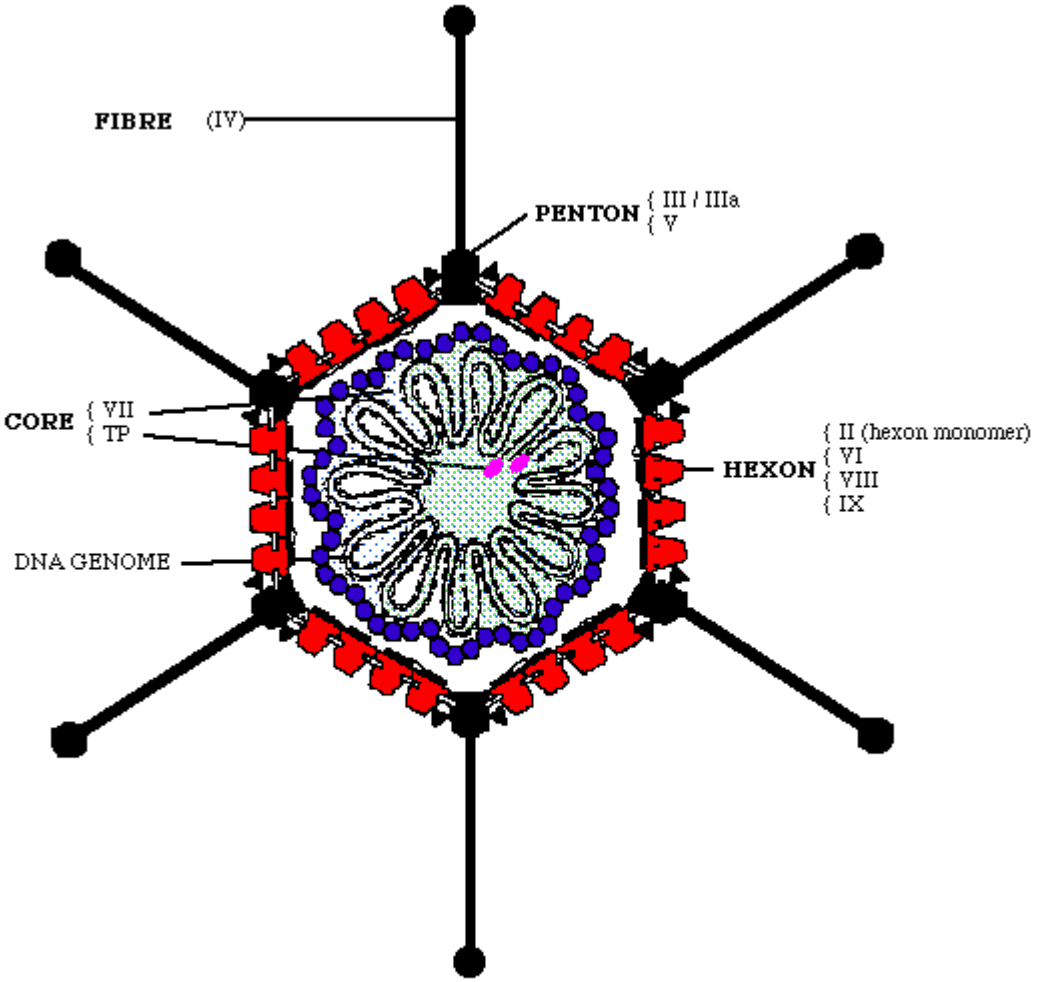
Adenoviruses are a frequent cause of acute upper respiratory tract (URT) infections, i.e. "colds". In addition, they also cause a number of other types of infection. They were first isolated in 1953 by investigators trying to establish cell-lines from adenoidal tissue of children removed during tonsillectomy and from military recruits with febrile illness.
In 1962, some Adenoviruses were shown to cause tumours in rodents - this caused a considerable panic ! (N.B. Adenovirus oncogenesis appears to be associated with abortive infections and has never been observed in humans.) During investigation of the Adenovirus genome and gene expression, many techniques were developed which were subsequently used to examine other viruses/cellular genes - these viruses are an important model system for the understanding of many other viruses. Some characteristic features of Adenoviruses are:

- Widespread in nature, infecting birds, many mammals and man. There are 2 genera, Aviadenovirus (avian) and Mastadenovirus (mammalian)
- Can undergo latent infection in lymphoid tissues, becoming reactivated some time later.
- Several types have oncogenic potential.

Taxonomy:

			Haemagglutination:	
Sub-Group:	**Types:**	**Oncogenic Potential:**	**Rhesus:**	**Rat:**
A	12, 18, 31	HIGH	-	+ / -
B	3, 7, 11, 14, 21, 34, 35	Weak	+	-
C	1, 2, 5, 6	None	-	+ / -
D	8-10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39	None	+ / -	+
E	4	None	-	+ / -
F - G	40, 41	?	-	+ / -

Morphology:



There are at least 10 proteins in the Adenovirus capsid:

Name:	Location:	Known Functions:
II	Hexon monomer	Structural
III	Penton base	Penetration
IIIa	Associated with penton base	Penetration
IV	Fibre	Receptor binding; haemagglutination
V	Core: associated with DNA & penton base	Histone-like; packaging?
VI	Hexon minor polypeptide	Stabilization/assembly of particle?
VII	Core	Histone-like
VIII	Hexon minor polypeptide	Stabilization/assembly of particle?
IX	Hexon minor polypeptide	Stabilization/assembly of particle?
TP	Genome - Terminal Protein	Genome replication

N.B. There is no protein I (!)

All Adenovirus particles are similar: non-enveloped, 60-90nm diameter. They have icosahedral symmetry easily visible in the electron microscope by negative staining and are composed of 252 capsomers: 240 "hexons" + 12 "pentons" at vertices of icosahedron (2-3-5 symmetry).
Individual protomers can be isolated by progressive chemical disruption of purified virus particles. The hexons consist of a trimer of polypeptide II with a central pore; VI, VIII and IX are minor polypeptides also associated with the hexon, thought to be involved in stabilization and/or assembly of the particle. The pentons are more complex; the base consists of a pentamer of peptide III, 5 molecules of IIIa are also associated with the penton base. The

pentons have a toxin-like activity, purified pentons causing c.p.e. in the absence of any other virus components (a unique property). A thin glycoprotein fibre (IV) protrudes form the centre of each penton - responsible for haemagglutination (the RBC receptor molecule is not known).

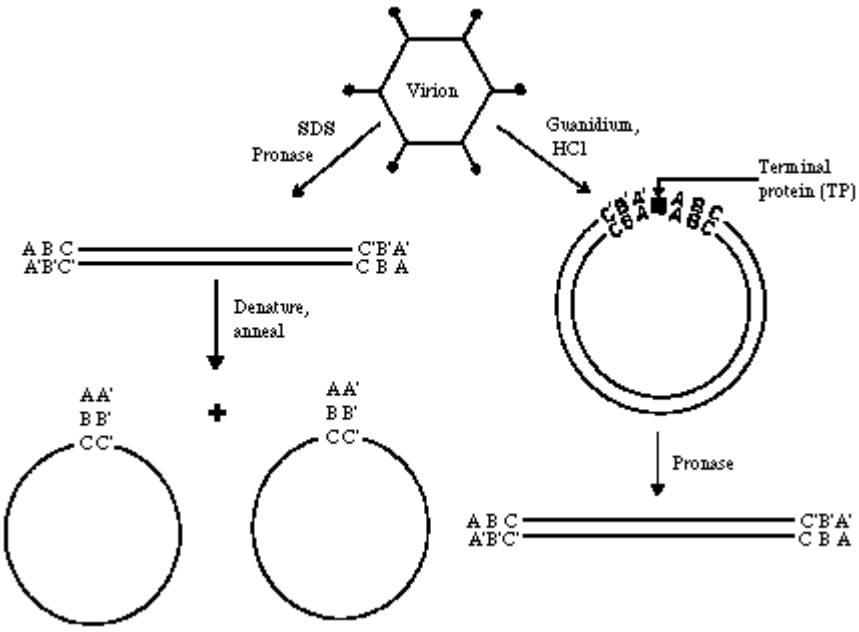
To view a negatively-stained electron micrograph of adenovirus particles, click here. The thin fibres protruding from each vertex of the icosahedral particle are just visible (look closely!) and the triangular faces of the icosahedral particle can be made out.
Image reconstruction of a type 2 adenovirus particle.

The core of the particle contains at least 4 proteins:

- **TP** (Terminal Protein) covalently attached to the 5' ends of the genome strands
- **V** (180 copies/particle) and **VII** (1070 copies/particle) are basic proteins (arginine rich, similar to histones) non-covalently associated with the genome forming a "chromatin-like" substance
- **Mu**, a small (4kD) protein whose location and function are not known

Genome:

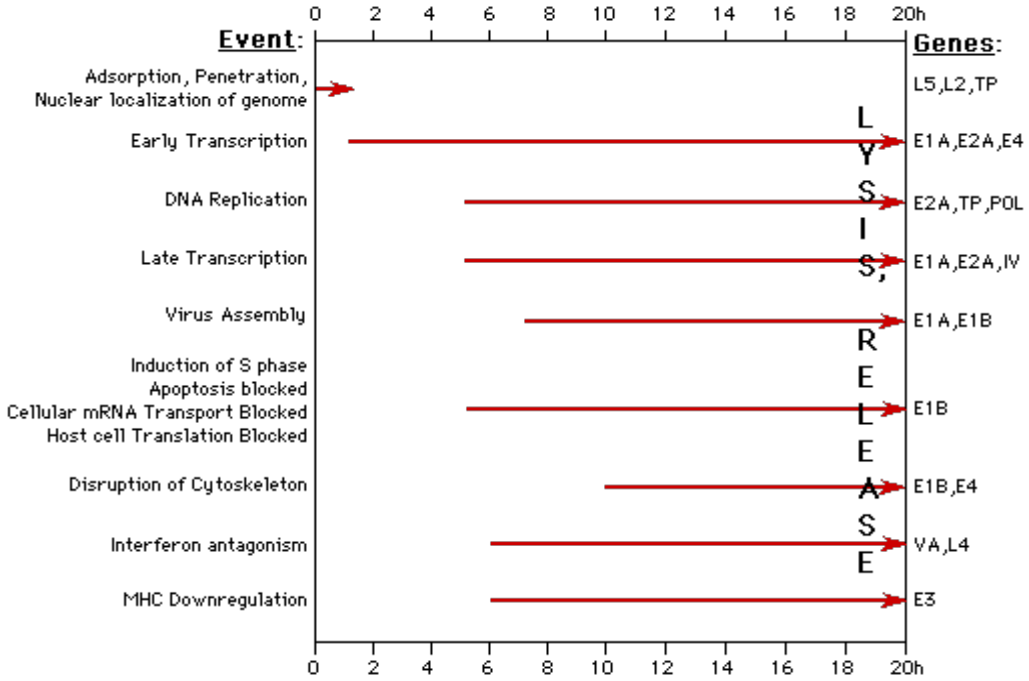
Linear, non-segmented, d/s DNA, 30-38kbp (size varies from group to group) which has the theoretical capacity to encode 30-40 genes. Genome structure (cross-hybridization, restriction map) is one of the characters used to assign viruses to groups (70-95% homology within groups, 5-20% homology between groups).



- The terminal sequences of each strand are inverted repeats, hence the denatured single strands can form "panhandle" structures (100-140bp).
- There is a 55kD protein covalently attached to the 5' end of each strand.

Replication:

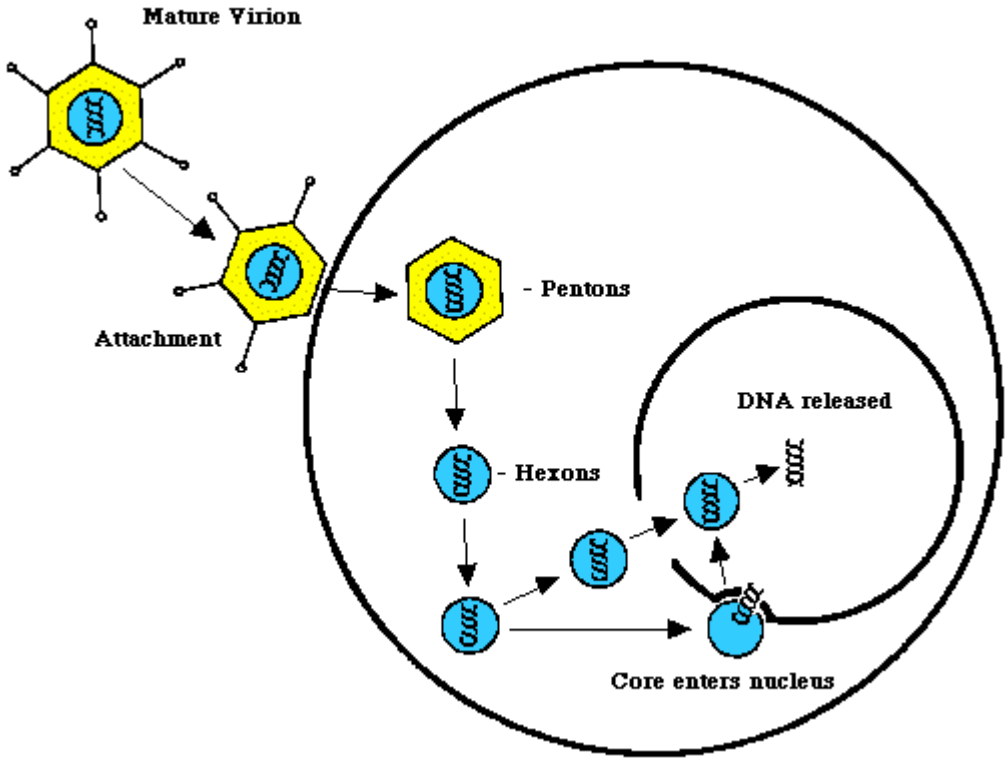
Replication of all Adenoviruses is similar and occurs in the **NUCLEUS**:



Replication is divided into **EARLY** and **LATE** phases, the latter defined as beginning with the onset of DNA replication *(N.B. this division is characteristic of the replication of DNA viruses!).* Attachment to cells is rather slow, taking several hours to reach a maximum.

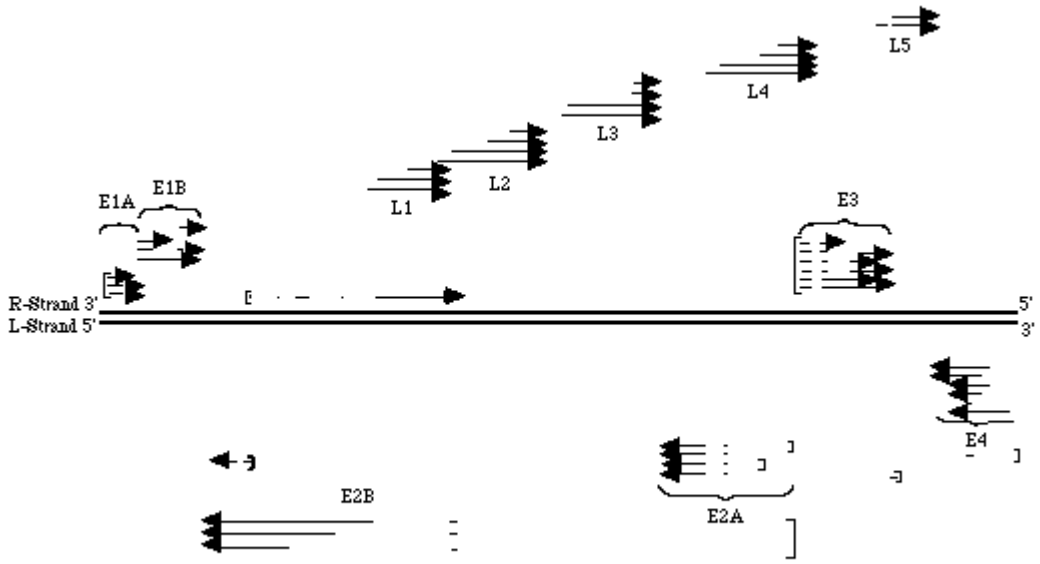
Uptake of the adenovirus particle is a two stage process involving an initial interaction of the fibre protein with a range of cellular receptors, which include the MHC class I molecule & the coxsackievirus-adenovirus receptor. The penton base protein then binds to the integrin family of cell surface heterodimers allowing internalisation via receptor-mediated endocytosis. Most cells express primary receptors for the adenovirus fibre coat protein, however internalisation is more selective.

PENETRATION involves phagocytosis into phagocytic vacuoles, after which the toxic activity of the pentons is responsible for rupture of the phagocytic membrane and release of the particle into the cytoplasm.



Uncoating follows an ordered sequence, first the pentons, releasing a spherical, partially uncoated particle into the cytoplasm. The core migrates to the nucleus where the DNA enters through nuclear pores, whereupon it is converted into a virus DNA-cell histone complex.

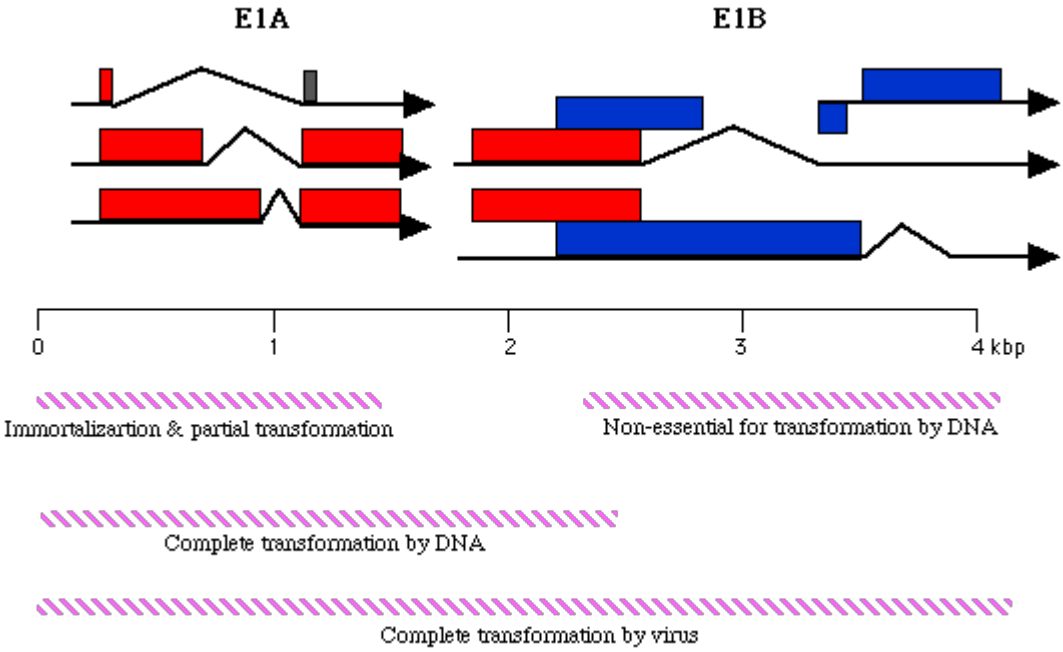
Gene Expression:



Before and independently of genome replication, immediate early and early mRNAs are transcribed from the input DNA. Transcription of the Adenovirus genome is regulated by virus-encoded trans-acting regulatory factors. Products of the immediate early genes regulate expression of the early genes. Early genes are encoded at various locations on both strands of the DNA (l="leftward strand" and r="rightward strand"). Multiple protein products are made from each gene by alternative splicing of mRNA transcripts - splicing was first discovered in Adenoviruses (Sharp, 1977).

Phase:	Genes Transcribed:
Immediate early	E1A
Early	E1B, E2A, E2B, E3, E4, some virion proteins
Late	Late genes, mostly virion proteins

The first mRNA/protein to be made (~1h after infection) is **E1A**. This protein is a trans-acting transcriptional regulatory factor whose precise mode of action is not known (not a DNA-binding "transcription factor") but is necessary for transcriptional activation of early genes. The protein is also capable of activating transcription from a variety of other viral and cellular promoters and shows no sequence-specificity, rather a modification of the cellular environment.
The second protein made is **E1B**. E1A + E1B together (and independently of other virus proteins) are capable of transforming primary cells in vitro (especially Ad5, Ad12).
Transformation is: "A CHANGE IN THE MORPHOLOGICAL, BIOCHEMICAL OR GROWTH PARAMETERS OF THE CELL", which may or may not result in cells which are able to produce tumours in experimental animals (= NEOPLASTIC transformation).

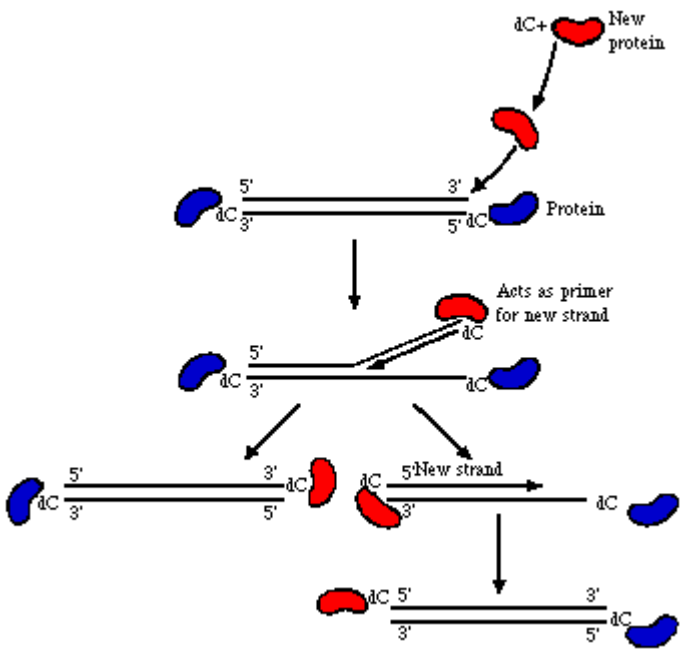


The activities of the two proteins can be dissected out by molecular techniques:

E1A: Can immortalize primary cells in vitro.
E1B: Does not transform cells on its own, but "co-operates" with E1A to stably transform cells.
E1A + E1B: Necessary for full transformation and tumour formation in animals.

E1A has also been shown to bind a cellular protein, **p105-RB**, the product of the retinoblastoma gene (retinoblastomas result when this gene is deleted or damaged, hence it is an "anti-oncogene" or "tumour suppressor"). E1B binds to **p53**, another tumour suppressor involved in the control of the cell cycle. N.B. Binding of DNA virus nuclear proteins to cellular tumour suppressors is a shared mechanism of cell transformation found in several virus families.
Together, these observations indicate that Adenoviruses, in the course of sequestering cellular machinery and altering the intracellular environment to favour viral replication, have profound effects on cellular functions. Viewed in this light, transformation is just an accidental (and rare) outcome of infection. The basis for oncogenesis (c.f. immortalization of cells in vitro - above) is not clear, but it is known that Ad12 E1A turns off class I MHC expression, possibly allowing tumours to escape destruction by CTLs.

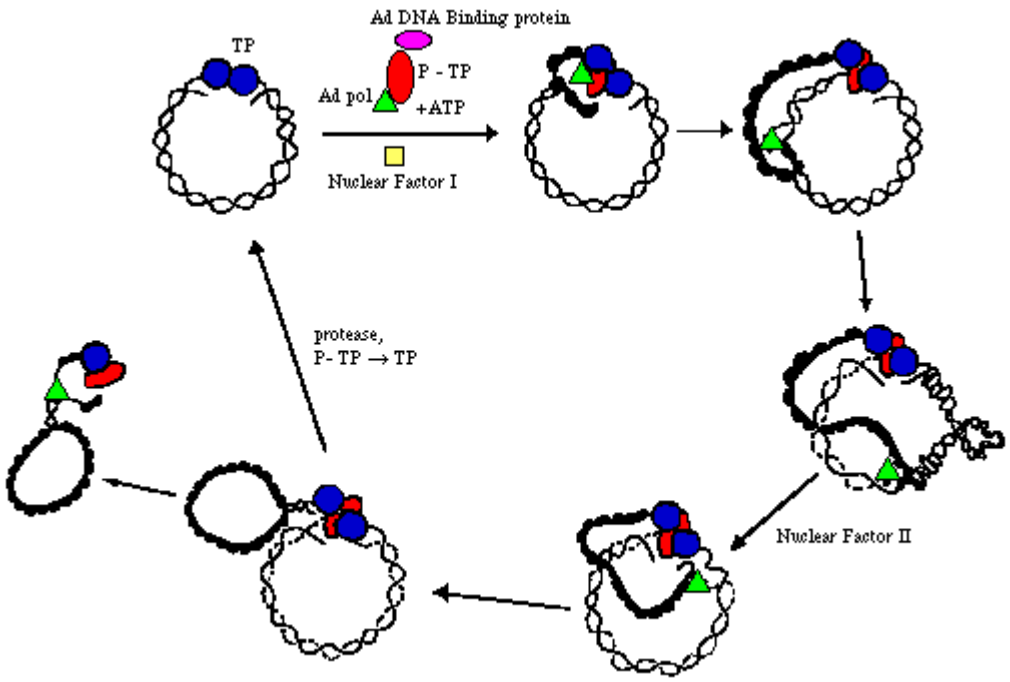
DNA Replication:



Adenovirus DNA replication has been studied extensively both in vivo (t.s. mutants in infected cells) and in vitro (nuclear extracts). At least 3 virus-encoded proteins are known to be involved in DNA replication:

- **TP** (a.k.a. Ad DNA Pro) acts as a primer for initiation of synthesis.
- **Ad DBP** - a DNA-binding protein.
- **Ad DNA Pol** - 140kD DNA-dependent polymerase.

In addition, many cellular proteins in the nucleus also participate in replication of the genome (e.g. NFI, NFII, topoisomerase I).



Late Transcription:

At the onset of DNA replication, the pattern of transcription changes radically from the early to the late genes. There is cis-acting control of this switch, i.e. only newly replicated DNA is used for late gene transcription, but the mechanism controlling this is not understood. The late genes are transcribed from the major late promoter; at least 13 species of mRNA are produced by alternative splicing.
Assembly occurs in the nucleus, but begins in the cytoplasm when individual monomers form into hexon and penton capsomers. Empty, immature capsids are assembled from these protomers in the nucleus, where the core is formed from genomic DNA + associated core proteins. Although host cell macromolecular synthesis ceases earlier in the infection, infected cells remain intact and do not lyse (disruption of cytoskeleton changes shapes of cell - rounds up). Virus particles tend to accumulate in the nucleus and are visible in the microscope as eosinophilic crystals - **INCLUSION BODIES**. These are thought to be the basis of latent infections - reactivation is caused by accidental lysis of infected cells, releasing virus particles from the nuclei - effectively a re-infection. More properly, this type of mechanism of persistence is known as "occult" (hidden) infection, rather than "latent" (c.f. Herpesviruses).
Adenoviruses are known to interact with other viruses, notably defective Parvoviruses (Adeno Associated Viruses). SV40 (Papovavirus) has also been shown to act as a helper virus for Adenoviruses - co-infection overcomes a late block to Adenovirus replication in certain cell types which are normally non-permissive. This may involve functional substitution of SV40 T-antigen for Ad DBP (?). Infectious Adeno-SV40 hybrids have been isolated by rescuing Adenovirus deletion mutants by super-infecting with SV40, indicating functional overlap between the two families.

Pathogenesis:

Certain types of Adenovirus are commonly associated with particular clinical syndromes:

Disease:	At Risk:

Acute Respiratory Illness	Military recruits, boarding schools, etc.
Pharyngitis	Infants
Gastroenteritis	Infants
Conjunctivitis	All
Pneumonia	Infants, military recruits
Keratoconjunctivitis	All
Acute Haemorrhagic Cystitis	Infants
Hepatitis	Infants, liver transplant patients

Note that this list includes relatively common infections (at the top) and some rare infections (bottom). Most Adenovirus infections involve either the respiratory or gastrointestinal tracts or the eye.
Adenovirus infections are very common, most are asymptomatic. Most people have been infected with at least 1 type at age 15. Virus can be isolated from the majority of tonsils/adenoids surgically removed, indicating latent infections. It is not known how long the virus can persist in the body, or whether it is capable of reactivation after long periods, causing disease (it is hard to isolate this occult virus as it may be present in only a few cells). It is known that virus is reactivated during immunosuppression, e.g. in AIDS patients.

Therapy:

None. Inactivated vaccines have been developed and are routinely used for military recruits in some countries (e.g. USA). This is because adolescents and others in close daily contact are at risk for epidemic spread of respiratory infections - risk to general population is so low that vaccination is not a viable proposition.
In recent years, there has been considerable interest in developing Adenoviruses as defective vectors to carry and express foreign genes for therapeutic purposes. One reason for this is that the Adenovirus genome is relatively easily manipulated in vitro (c.f. Retroviruses) and the genes coupled to the MLP are efficiently expressed in large amounts.

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Papovaviruses

This family contains 2 genera of oncogenic DNA viruses:

- Papillomaviruses
- Polyomaviruses

The Family was originally named for its 3 main members:

- **PA**pillomavirus
- **PO**lyomavirus
- Simian **V**acuolating **A**gent (SV40 - a member of the Polyomavirus genus)

Polyomaviruses

Polyomaviruses infect a wide variety of vertebrates (12 members now known). Polyomavirus was isolated by Gross in 1953 while he was studying leukaemia in mice and named because it caused solid tumours at multiple sites. The second member of the family, Simian Vacuolating Virus 40 (SV40) was isolated by Sweet and Hilleman in 1960 in primary monkey kidney cells cultures being used to grow Sabin OPV (!). Two human Polyomaviruses were isolated in 1971:

BK Virus (BKV) - from an immunosuppressed kidney transplant patient
JC Virus (JCV) - from a case of progressive multifocal leukoencephalopathy (PML).

Because of the small size of their genomes, their oncogenic properties and the existence of in vitro systems, Polyomaviruses have been used extensively as models for DNA replication.

Morphology:

Polyomaviruses virions are non-enveloped, ~45nm diameter T=7 icosahedral particles. The complete structure of SV40 has been described. There are 3 capsid proteins, VP1-3, which form 72 pentameric capsomers, 60 hexagonally co-ordinated +12 pentamerically co-ordinated (at the vertices):

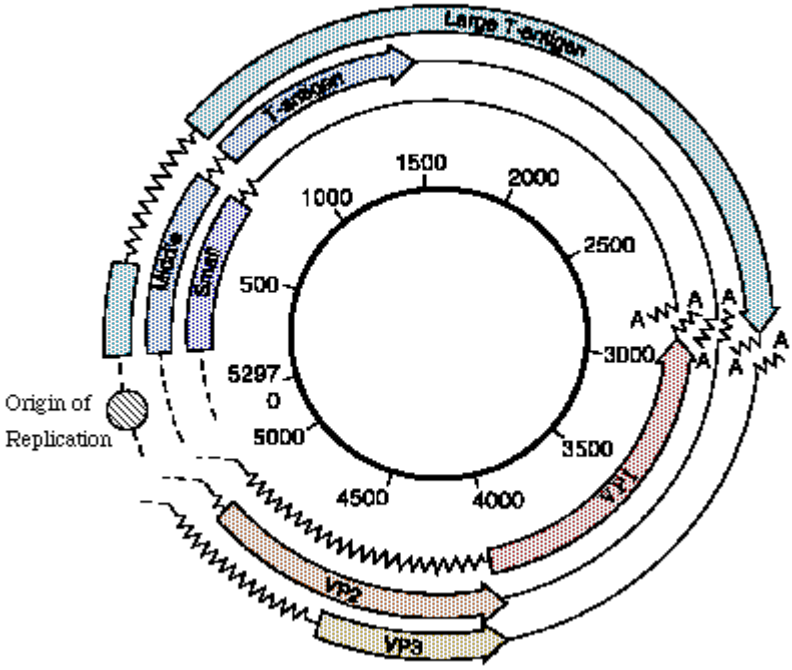
There are 3 capsid proteins, VP1-3, which form 72 pentameric capsomers, 60 hexagonally co-ordinated + 12 pentamerically co-ordinated (at the vertices).

Each virion contains 360 copies of VP1 (i.e. 72 x 5) + 30-60 copies each of VP2 & VP3, i.e. ~1 copy/pentamer. Each copy of VP1 has a sialic acid binding site on the surface & these form the receptor-binding site for the virus; hence the particles have haemagglutinating properties.

VP2/3 have overlapping sequences (see below) - VP2 contains the entire sequence of VP3 at it's C-terminus, +115 aa at the N-terminus. The precise location of VP2/3 is unknown. VP2 is myristylated at its NH2-terminus, which is believed to be important in holding the particle together (c.f. Picornavirus VP4).

Genome:

Polyomavirus genomes are d/s, circular DNA molecules, ~5kbp in size. The entire nucleotide sequence of all the viruses in the family is known and the architecture of the Polyomavirus genome (i.e. number and arrangement of genes and regulatory signals and systems) has been studied in detail.
Within the particles, the DNA assumes a supercoiled form (like plasmid DNA). Four cellular histones H2A, H2B, H3 and H4 are associated with the DNA.
Polyomavirus genomic organization is designed to pack maximal information (6 genes) into minimal space (5kbp). This paradox is achieved by the use of both strands of the genome DNA and overlapping genes:



The origin of replication is surrounded by non-coding regions which control transcription. VP1 is a encoded by a "dedicated" ORF, but the VP2 and 3 genes overlap so that VP3 is contained within VP2. SV40, BKV and JCV encode a small (60-70aa) protein known as the agnoprotein which enhances assembly of virus particles & cell to cell spread.
Polyomaviruses also encode "T-antigens" - proteins which can be detected by sera from animals bearing polyomavirus-induced tumours:

Protein Size (a.a.):			
Virus:	**Large T:**	**Small T:**	**Middle T:**
Polyoma	785	195	421
SV40	708	174	---
BKV	695	172	---
JCV	688	172	---

These proteins have common N-terminal regions but unique COOH-termini derived from alternative splicing patterns.

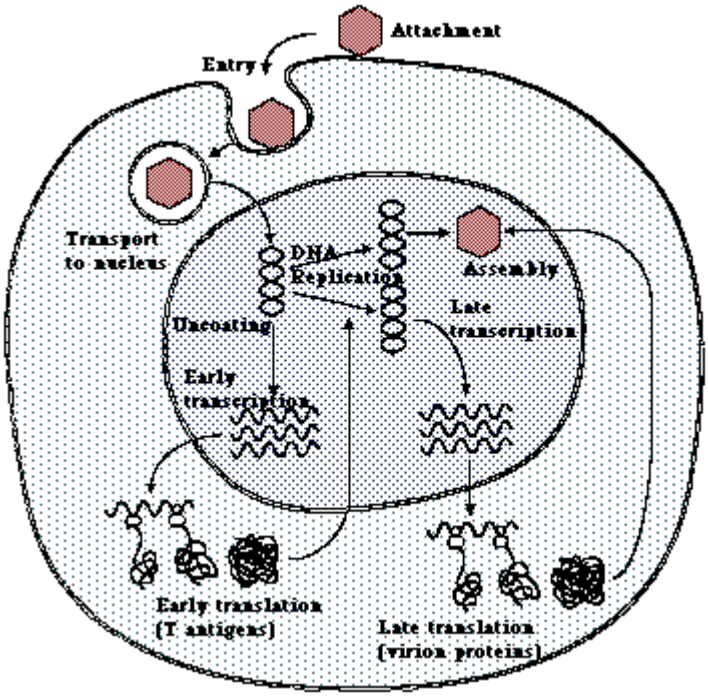
Replication:

PyV genomes are d/s, circular DNA molecules, ~5kbp in size. The entire nucleotide sequence of all the viruses in the family is known & the architecture of the PyV genome (i.e. number & arrangement of genes & regulatory signals & systems) has been studied in detail.
Within the particles, the DNA assumes a supercoiled form (like plasmid DNA). Four cellular histones H2A, H2B, H3 & H4 are associated with the DNA.

The genome is functionally divided into 3 regions:

- **Early:** Expressed early in virus infection, i.e. BEFORE genome replication. Expression of early genes continues during the late stage of infection. Encodes non-structural proteins (i.e. not present in virus particle).
- **Late:** Expressed later in virus infection, i.e. DURING & AFTER genome replication. Encodes structural proteins (i.e. present in virus particle).
- **Regulatory region:** Contains transcriptional promoters & enhancers plus the unique origin of DNA replication.

TEMPORAL CONTROL (i.e. EARLY vs. LATE) OF GENE EXPRESSION IS A COMMON FEATURE OF CLASS I VIRUSES.



Attachment:

SV40 receptor appears to be MHC class I antigen(s).
Receptors for PyV are not known, but appear to contain sialic acid (haemagglutination) & be widespread in many tissue/species.
VP1 (only(?) external protein on virus capsid) responsible for receptor binding (anti-VP1 Abs block binding).

Entry:

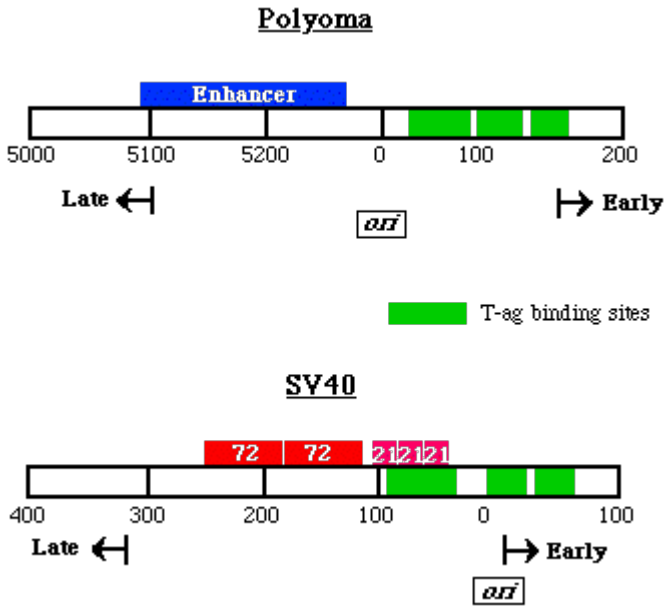
VP2/3 are myristylated & believed to interact with cellular membranes to facilitate entry.
Virions are taken up by endocytosis & is transported to the nucleus by interaction of endocytic vacuoles with the cytoskeleton.

Uncoating:

Virus particles enter by the nuclear pores & uncoating occurs inside the nucleus.
The rest of the replication cycle occurs in the nucleus. VP2/3 mutants defective in uncoating, therefore these proteins are involved in the process, although the details are unknown.

Gene Expression:

Inside the nucleus, the virus mini-chromosome (genome-histone complex) is transcribed by host cell RNA polymerase II to produce early mRNAs.
Because of the relative simplicity of the genome, PyV are heavily dependent on the cell for transcription & genome replication. However, the genome contains cis-acting regulatory signals (surrounding the origin of replication) which direct transcription, & trans-regulatory proteins (the T-antigens) which direct transcription & replication.
Alternative splicing produces 2 (or 3) species of early mRNA/T-antigen:
large-T & small-T (plus middle-T in murine polyomavirus - a membrane protein found in the plasma membrane, important in cell transformation).
Transcription from the early region promoter is autoregulated by binding of large-T antigen to the regulatory region of the genome:



The early gene promoter contains strong enhancer elements which cause it to be active in newly infected cells. The early region proteins are the T-antigens.
Small T-antigen is not essential for virus replication, but indirectly (i.e. interacts with cellular proteins but does not bind directly to virus genome) enhances transcription from the late promoter.
As the concentration of large T-antigen builds up in the nucleus, transcription of the early genes is repressed by direct binding of the protein to the origin region of the virus genome, preventing transcription from the early promoter and causing the switch to the late phase of infection. After DNA replication has occurred, transcription of the late genes occurs from the late promoter and results in the synthesis of the structural proteins, VP1, VP2 and VP3.
The SV40 late promoter is a very strong promoter & is activated by binding of large T-antigen to the 72bp repeats upstream of the transcription start site.
Therefore, the role of the SV40 T-antigen in controlling the transcription of the genome is comparable to that of a 'switch'.

Genome Replication:

Large T-antigen has a complex action & binds to various cellular proteins:

- DNA polymerase alpha & s/s DNA binding protein, both involved in DNA replication
- p53 & p105

SV40 DNA replication is initiated by binding of large T-antigen to the origin region of the genome. The function of T-antigen is controlled by phosphorylation, which decreases the ability of the protein to bind to the SV40 origin. The SV40 genome is very small and does not encode all the information necessary for DNA replication. Therefore, it is essential for the host cell to enter S phase, when cell DNA and the virus genome are replicated together. Protein:protein interactions between T-antigen and DNA polymerase-alpha directly stimulate replication of the virus genome. Inactivation of tumour suppressor proteins bound to T-antigen causes G1-arrested cells to enter S phase, promoting DNA replication.
Therefore, in addition to increasing transcription, another function of T-antigen is to alter the cellular environment to permit virus genome replication.

Assembly/Maturation:

Virus proteins contain 'nuclear localization signals' which results in their accumulation in the nucleus, where they migrate after being synthesized in the cytoplasm.
Assembly occurs in the nucleus. Since the structure of the virus is relatively simple, assembly & maturation of the particle are simultaneous.

Release:

Some virus particles are exported to the cell surface in cytoplasmic vacuoles. The remaining virus is released when the cell lyses (SV40!). Mechanism of cell injury is not clear, but is not a surprise due to the severe interference with normal cellular metabolism & growth that these viruses cause.
The complete replication cycle takes 48-72h (depending on multiplicity of infection).

Pathogenesis:

Infection of cells by PyV can result in two outcomes:

- Productive (lytic) infection
- Non-productive (abortive) infection

The outcome appears to be determined primarily by the cell type infected.
However, after infection, some (unknown) determinant in the intracellular environment (rather than the receptor-V.A.P. interaction) determines the outcome of the infection:

- **Polyomavirus:** Lytic infection of mouse cells, abortive infection of rat/hamster cells.
- **SV40:** Lytic infection of monkey cells, abortive infection of mouse cells.

Transformation is a rare and accidental consequence of the sequestration of tumour suppressor proteins. Inactivation of tumour suppressor proteins bound to T-antigen causes G_1-arrested cells to enter S phase and divide and this is the mechanism which results in transformation. However, the frequency with which abortively infected cells are transformed is low (about $1x10^{-5}$).

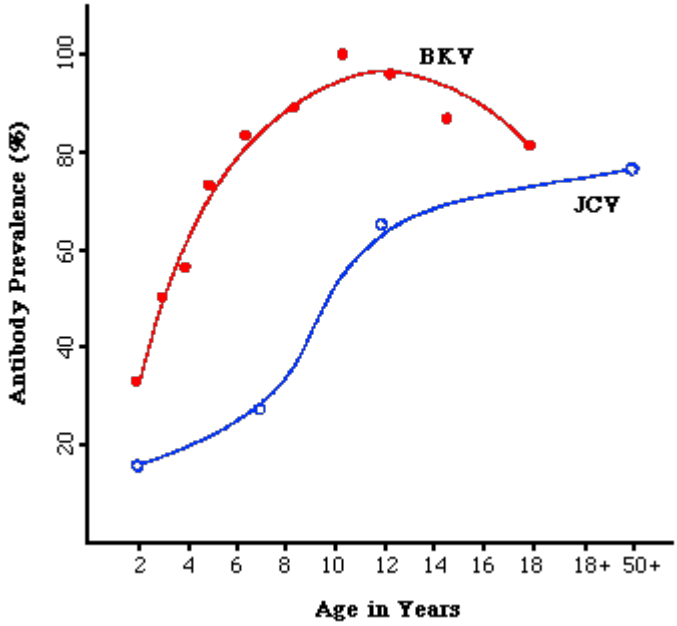
Therefore, T-antigen:

- Alters the cellular environment, affecting the cell cycle & DNA replication, enhancing virus replication (simple genomes!)
- Accidentally may result in cellular transformation

Viral DNA replication is initiated by binding of large T-antigen to the origin, replication then proceeds bidirectionally from this point - **IMPORTANT MODEL FOR CELLULAR DNA REPLICATION / ONCOGENESIS.**

There is conflicting evidence about the pathogenic potential of Polyomaviruses. Between 1955 and 1963, millions of Americans were exposed to SV40 in Sabin OPV. By 1961 between 80-90% of all U.S. children and adolescents under the age of 20 had been injected. There is no evidence that this had any ill effects. Alternatively, 2 viruses

commonly infect man and have been associated with disease.



Site of primary infection is not known, but may be the respiratory tract. The implications of this are that the vast majority of primary infections with these viruses are asymptomatic. However, both viruses are oncogenic when inoculated into newborn hamsters. Once infected, the viruses persist (for life?) and disease appears to be associated with reactivation rather than primary infection. Pregnancy is known to reactivate Polyomaviruses infections, but without any known pathologic consequence.

JC Virus

Associated with **progressive multifocal leukoencephalopathy (PML)**. This is a rare disease, involving plaques of demyelination/inflammation in the CNS. Oligodendrocytes from these lesions (responsible for the synthesis and maintenance of the myelin sheath around neurons) are productively infected with JCV. The disease is seen in two main groups of people:

- the elderly
- immunocompromised hosts (transplant and AIDS patients).

BK Virus: Primary infection is associated with a mild respiratory illness in children. The virus has also been isolated from various human tumours, but a cause and effect relationship has not been demonstrated.

Search **MEDLINE** for the latest publications on polyomaviruses:

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Papillomaviruses

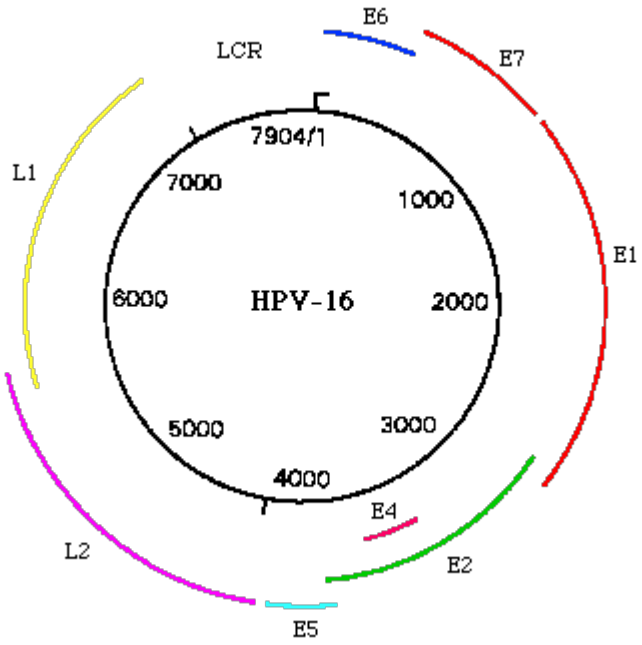
The viral nature of human warts was first identified in 1907; the first Papillomavirus was isolated from rabbits by Richard Shope in 1933. In spite of this early start, human Papillomaviruses (HPV) remained largely unstudied until the advent of molecular virology (cloning) in the 1970s. This is because to date, no HPV grows in vitro. Much of our knowledge comes from Bovine Papillomavirus (BPV) for which animal host systems exist.

Morphology:

Papillomaviruses are small, non-enveloped icosahedral particles ~52-55nm diameter. There are 72 capsomers (60 hexameric + 12 pentameric) arranged on a T = 7 lattice. Apart from the larger size, these appear very similar to Polyomaviruses particles (N.B. no sequence relatedness). There are 2 capsid proteins, 1 major (encoded by the L1 gene) and 1 minor (L2).
To view a negatively-stained electron micrograph of papillomavirus particles, click here.

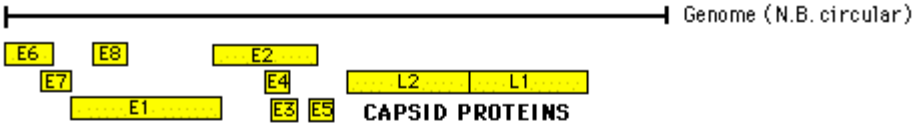
Genome:

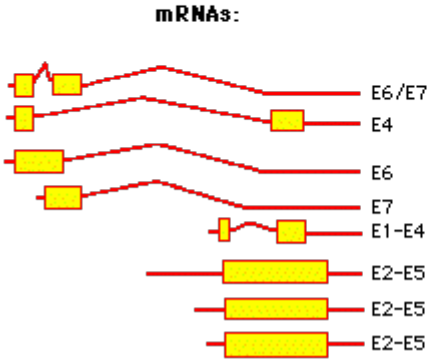
The Papillomavirus genome consists of circular, d/s DNA ~8kbp in size, associated with cellular histones to form a chromatin-like substance. At least 12 different HPV genomes have been sequenced, and the genetic organization is similar to that shown above.



Replication:

Individual isolates are highly species specific. All are tropic for squamous epithelial cells (receptors unknown). The virus infects the basal cells of the dermal layer, and early gene expression can be detected in these cells (in situ hybridization). However, late gene expression, expression of structural proteins and vegetative DNA synthesis is restricted to terminally differentiated cells of the epidermis which implies a link between cellular differentiation and viral gene expression.





Gene:	Function:
E1	Initiation of DNA replication (helicase)
E2	Transcriptional regulation/DNA replication
E3	???
E4	Late NS protein; Disrupts cytoskeleton?
E5	Transforming protein, interacts with growth factor receptors, e.g. PDGF
E6	Transforming protein, binds to p53 leading to degradation
E7	Transforming protein, binds to pRB
E8	???
L1	Major capsid protein
L2	Minor capsid protein

Expression of the Papillomavirus genome is complex because there are:

- multiple promoters (at least 7)
- alternative splicing patterns
- the link between differentiation and gene expression

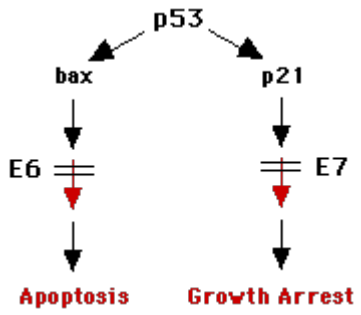
Transcription has been studied in detail by transfection of cloned Papillomavirus DNA into cells. Only one strand of the genome is transcribed. Two classes of proteins are produced:

Early Proteins: Non-structural regulatory proteins, including trans-acting transcriptional regulators (E2, E7).
Late Proteins: The structural proteins L1 and L2.

Transformation: Is complex! Depends on the early gene products. The transforming proteins appear to vary from one virus type to another. There is still some confusion about the function/mechanism of the transforming proteins. The general principle appears to be that two (or more) early proteins co-operate to give a transforming phenotype. Although some viruses can transform cells on their own (e.g. BPV-1), others also appear to require co-operation with an activated cellular oncogene (e.g. HPV-16/ras). More confusingly, in most cases, all or part of the Papillomavirus genome including the putative "transforming genes" is maintained in the tumour cells, whereas in other cases (e.g BPV-4), the virus DNA may be lost after transformation - a "hit-and-run" mechanism.

In HPVs:

- E2 binds to the early promoter and decreases expression of E6/E7; loss of E2 is thus the first stage in transformation.
- E6 binds to p53 via a cellular protein ("p100") and targets it for degradation via the ubiquitin pathway.
- E7 binds pRB and prevents phosphorylation. This would normally result in apoptosis BUT:
- Both E6 and E7 interact with a number of cellular proteins which influence the outcome of infection:



Genome Replication:
The genome is replicated as a multicopy nuclear plasmid (episome). Two mechanisms are involved in genome replication:

1. **Plasmid Replication** - occurs in cells in the lower levels of the dermis. Initially, the virus DNA is amplified to 50-400 copies/diploid genome. After this, it replicates once per cell division, the copy number/cell remaining constant. The E1 protein is involved in this phase of replication.
2. **Vegetative Replication** - occurs in terminally differentiated cells in the epidermis. In terminally differentiated cells (or growth-arrested cells in culture) control of copy number appears to be lost and the DNA is amplified up to very high copy numbers (000's copies/cell).
3. Virus is shed from epidermal cells when these are sloughed off and is transmitted by direct contact (esp. genital warts) and indirect contact.

Pathogenesis:

These viruses are widespread in nature and infect birds and mammals. The usual outcome of infection is the formation of a benign outgrowth of cells, a wart or papilloma. These may occur almost anywhere in or on the body. Skin warts are divided into **flat warts (superficial)** and **plantar warts (deeper)**. **Genital warts (condylomas)** occur in the genital tract and are transmitted by sexual intercourse. Warts can be treated by topical application of caustic substances or freezing, but surgical removal is more reliable, and is required for internal warts e.g. laryngeal. Warts may persist for many years, but may regress spontaneously due to a CTL response. There may be some enhanced risk of skin warts exposed to U.V. light developing into invasive squamous cell carcinoma (very rare). However, there is also considerable interest in Papillomaviruses as a cause of cancer, particularly in recent years the possible association between HPV and cervical carcinoma. At least 58 different HPV have been identified using molecular techniques. In the last few years, a number of types have been suggested to be associated with particular tumours:

Cancer:	Predominant types:	Co-factors:
Skin carcinomas	HPV-5, 8	U.V., genetic?
Lower genital tract cancers	HPV-16, 18, 31, 33	???
Malignant transformation of respiratory papillomas	HPV-6, 11	X-rays

However, these associations are far from certain at the present time:

Prevalence of Human Papillomavirus in Cervical Cancer: A Worldwide Perspective

[J Natl Cancer Inst 87:796-802, 1995]

F. Xavier Bosch, M. Michele Manos, Nubia Muñoz, Mark Sherman, Angela M. Jansen, Julian Peto, Mark H. Schiffman, Victor Moreno, Robert Kurman, Keerti V. Shah, International Biological Study on Cervical Cancer (IBSCC) Study Group

Background: Epidemiologic studies have shown that the association of genital human papillomavirus (HPV) with cervical cancer is strong, independent of other risk factors, and consistent in several countries. There are more than 20 different cancer-associated HPV types, but little is known about their geographic variation.
Purpose: Our aim was to determine whether the association between HPV infection and cervical cancer is consistent worldwide and to investigate geographic variation in the distribution of HPV types.
Methods: More than 1,000 specimens from sequential patients with invasive cervical cancer were collected and stored frozen at 32 hospitals in 22 countries. Slides from all patients were submitted for central histologic review to confirm the diagnosis and to assess histologic characteristics. We used polymerase chain reaction-based assays capable of detecting more than 25 different HPV types. A generalized linear Poisson model was fitted to the data on

viral type and geographic region to assess geographic heterogeneity.
Results: HPV DNA was detected in 93% of the tumors, with no significant variation in HPV positivity among countries. HPV 16 was present in 50% of the specimens, HPV 18 in 14%, HPV 45 in 8%, and HPV 31 in 5%. HPV 16 was the predominant type in all countries except Indonesia, where HPV 18 was more common. There was significant geographic variation in the prevalence of some less common virus types. A clustering of HPV 45 was apparent in western Africa, while HPV 39 and HPV 59 were almost entirely confined to Central and South America. In squamous cell tumors, HPV 16 predominated (51% of such specimens), but HPV 18 predominated in adenocarcinomas (56% of such tumors) and adenosquamous tumors (39% of such tumors).
Conclusions: Our results confirm the role of genital HPVs, which are transmitted sexually, as the central etiologic factor in cervical cancer worldwide. They also suggest that most genital HPVs are associated with cancer, at least occasionally. *Implication:* The demonstration that more than 20 different genital HPV types are associated with cervical cancer has important implications for cervical cancer-prevention strategies that include the development of vaccines targeted to genital HPVs.

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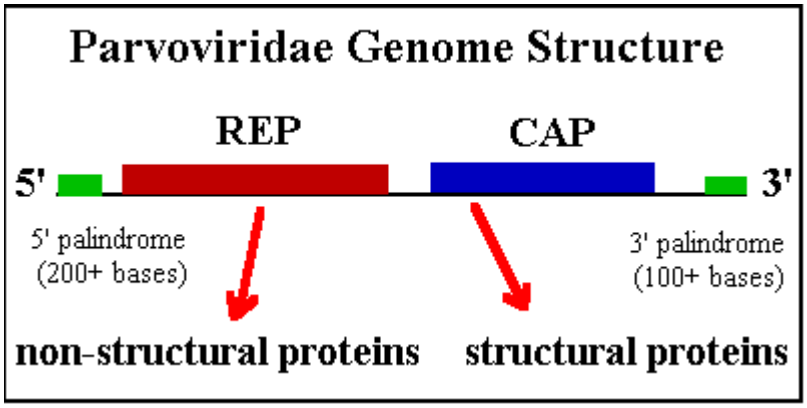


Last Updated: Saturday, October 4, 1945624.

Parvoviruses

Introduction:

Parvoviruses are among the smallest, simplest eukaryotic viruses and were only discovered in the 1960's. They are widespread in nature; human Parvovirus infections were only recognised in the 1980's. Essentially, they fall into two groups, defective viruses which are dependent on helper virus for replication and autonomous, replication-competent viruses. In all, >50 parvoviruses have been identified.

The Family contains 6 genera divided between 2 subfamilies:

Genus:	Type species:	Host organism:
Parvovirinae:		
Parvovirus	Mice minute virus	Vertebrates
Erythrovirus	B19 virus	Vertebrates
Dependovirus	Adeno-associated virus 2	Vertebrates
Densovirinae:		
Densovirus	Junonia coenia densovirus	Invertebrates
Iteravirus	Bombyx mori densovirus	Invertebrates
Contravirus	Aedes aegypti densovirus	Invertebrates

The Dependoviruses were the first to be discovered. Unlike the other 5 genera, these are replication-defective viruses which are entirely dependent helper viruses. The genus consists of 4 types of adeno-associated viruses (AAV1-4) which require Adenoviruses (or Herpesviruses) for helper functions to replicate. The adenovirus genes involved are early (regulatory) rather than late (structural), such as E1A, but it has recently been shown that treatment of cells with U.V, cycloheximide or some carcinogens can replace the requirement for helper virus. Therefore the requirement appears to be for a modification of the cellular environment rather than a specific virus protein - probably affecting transcription of the defective virus genome.

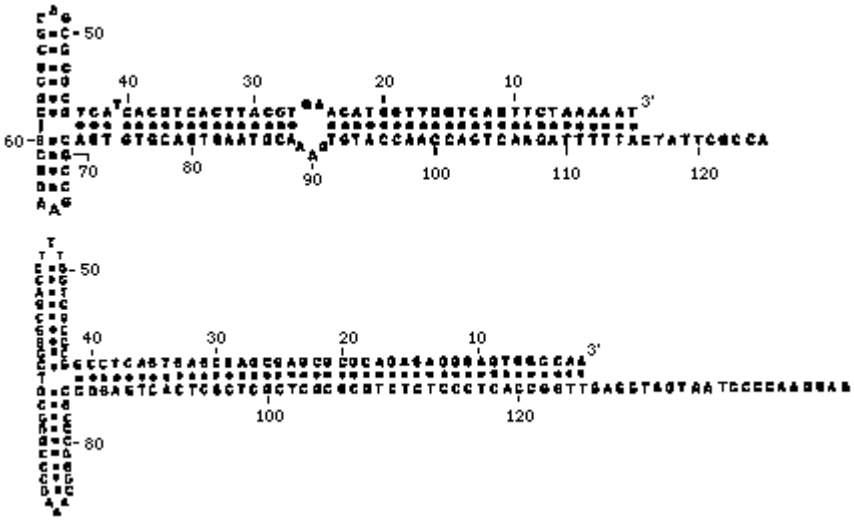
Morphology:

Particles are icosahedral, 18-26nm diameter & consist only of protein (50%) + DNA (50%). There are 3 capsid proteins, VP1-3. VP3 is derived by protease cleavage of VP2. Infectious virions of canine parvovirus contain 60 protein subunits that are predominantly VP-2. The central structural motif of VP-2 has the same topology (an eight-stranded antiparallel beta-barrel) as has been found in many other icosahedral viruses (e.g. picornaviruses).
The capsid confers considerable stability on the virions, which are resistant to inactivation by pH, solvents or high temperatures (1h @ 50°C).

To view a negatively stained electron micrograph of AAV, click here. In this image, the larger adenovirus particles (diameter 60-90nm) are easily distinguishable from the smaller Dependovirus particles (diameter ca. 20nm).
To view a computer-generated image of canine parvovirus reconstructed from crystallographic data, click here.

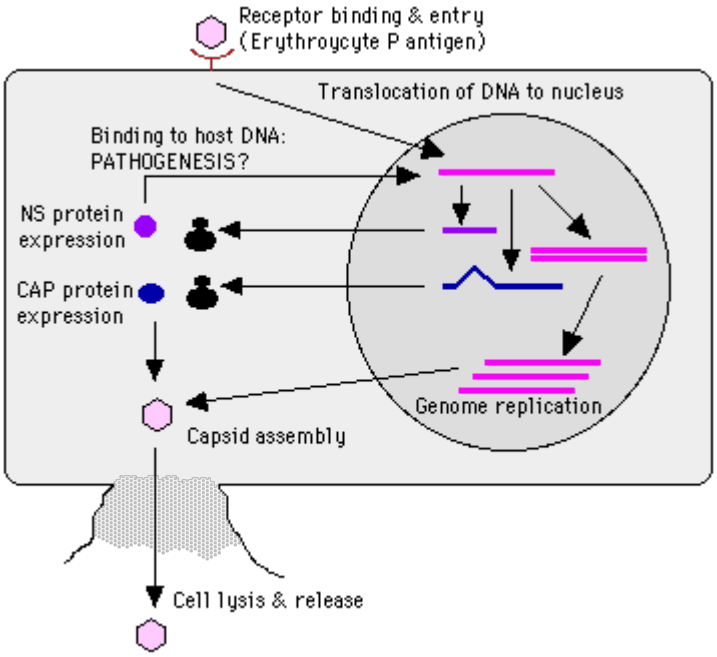
Genome:

Linear, non-segmented, s/s DNA, ~5kb. Most of the strands packaged seem to be (-)sense, but AAVs package equal amounts of (+) and (-) strands, and all seem to package at least a proportion of (+)sense strands. The ends of the genome have palindromic sequences of ~115nt which form "hairpins". These structures are essential for the initiation of genome replication.

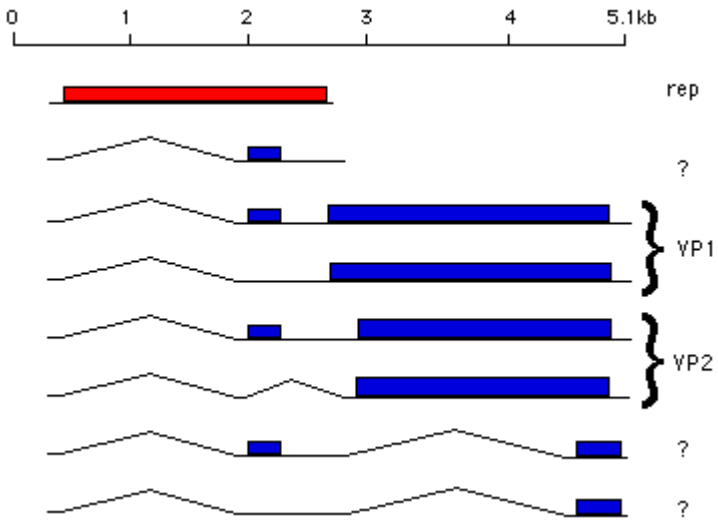


Replication:

The pathogenic human parvovirus B19 is difficult to grow in culture, so comparatively little is known about its biology. The receptor molecule for B19 is tetrahexosoceramide, a glycolipid (erythrocyte P antigen) - explains cell tropism of B19:



Replication occurs in the nucleus & is thought to follow the scheme above. All Parvoviruses are highly dependent on cellular functions for genome replication. The autonomous viruses require the cell to pass through S-phase for replication to occur, but unlike Polyomaviruses (below) cannot turn on host cell DNA synthesis. The defective viruses are even more dependent - require host cell machinery + helper virus for replication.



Expression of the Parvovirus genome is poorly understood. The helper function required by the defective viruses is thought to be involved in transcription of virus genes. Host cell DNA polymerase is necesary for genome replication.

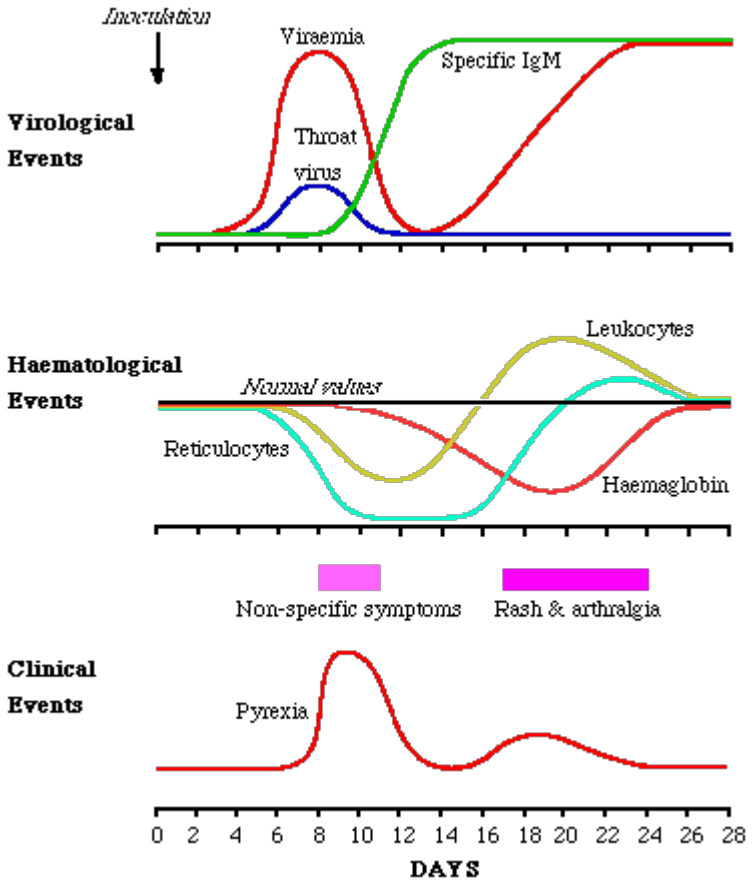
In the absence of helper virus, Dependoviruses can establish a latent infection; in this state, the virus genome is integrated into the host cell DNA - & can be rescued >50 passages later by Adenovirus infection. The rep gene is involved in this process, which is poorly understood. There is evidence for vertical transmission of avian AAV in chickens. Conversely, AAV appears to inhibit cellular transformation by Adenoviruses.

Pathogenesis:

Parvoviruses cause infections in a wide variety of birds and mammals, but 70-90% of most human populations are seropositive. The pathology of Parvoviruses is shaped by their dependence on cellular functions for replication - cell tropism is broad, but since cells must pass through S-phase, they tend to infect rapidly dividing tissues, most commonly:

- the foetus
- the intestinal epithelium
- the haematopoetic system

The only known human Parvovirus is referred to as **B19**. First discovered in 1974, it was only in 1981 that its association with aplastic crisis in children was first realized.



This involves an acute depression in the production of red blood cells from the bone marrow, directly due to infection of reticulocytes. This is a transient event which is usually not of great clinical significance except in patients with other haematological diseases, e.g. sickle-cell disease. This is followed by a rubella-like rash, usually the most obvious symptom of B19 infection. Children are much more likely than adults to develop the rash (not all do), which is known as erythaema infectiosum (EI), "fifth disease" or "slapped cheek disease".
In ~80% patients, there is also arthropathy - temporary arthritis-like joint involvement (particularly in adults).
B19 infection in pregnancy is also associated with early foetal loss, although the probability of this appears to be low (<10%).
Treatment/Prevention: None, although immune globulin has been used in chronic parvovirus infection of immunocompromised patients.

Feline Parvovirus and **Canine Parvovirus** have emerged during the last decade as serious veterinary pathogens. Vaccines against both now exist and are widely used.
Aleutian disease virus (named after strain of mink in which it is most frequently seen) infects mink, causing persistent infections. Control is usually by slaughter.

Gene Therapy:

AAV have recieved considerable attention in recent years as vectors for gene therapy:

- They integrate into cellular DNA at high frequency to establish a stable latent state
- They are not associated with any known (human) disease
- Because of the simplicity of the genome, vectors can be constructed which will not express any (undesirable) viral gene products

but:

- Only ~5kb of DNA can be packaged into the parvovirus capsid & some viruses sequences must be retained for packaging
- Integration into host cells DNA may potentially have damaging consequences (?)

- References: Click here
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Books about Parvoviruses: click here

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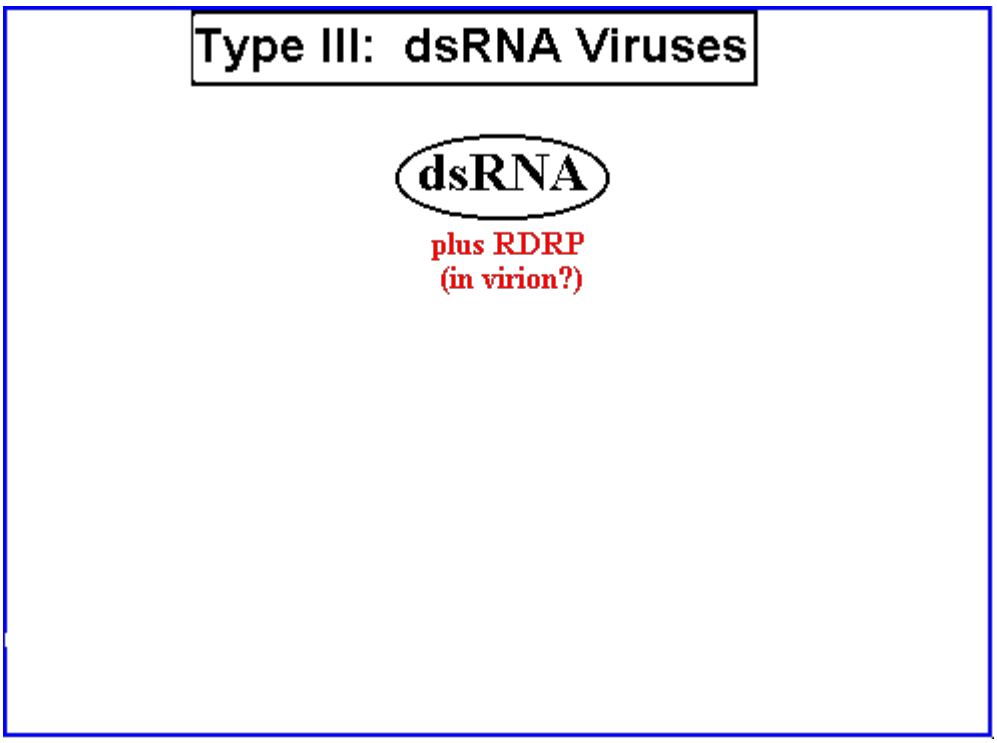
Replication of Parvoviruses

Replication of the linear genome of Parvoviridae (eg. feline parvovirus; human parvovirus B19) involves use of a semi-stable genomic 3'-hairpin structure as a primer, synthesis of new DNA by host delta DNA pol using genomic DNA as template, covalent closure then re-opening of the circular DNA product, and re-initiation at the new 3'-terminus as primer after transient hairpin formation at the new 3' end after "melting" of the newly-formed duplex. A complicated series of steps follows, involving repeated single-strand cleavages and 5'-end binding by virus-coded NS1 protein, and possible topoisomerase action.

The other circular genomes can replicate by a "rolling circle" model: nicking of one strand of the cccDNA RF allows use of an exposed 3'-terminus as a primer, which results in elongation of the "primer" strand, and displacement of the other. Ordinarily this results in greater-than- unit-length ss- and dsDNA forms being found in infected cells.

(return to replication section)

Replication Strategy of dsRNA Viruses



This is an animated GIF file. It takes about 15 sec to cycle all the way through.

Steps in Replication

1. Primary transcription in virion core in cytoplasm by viral RDRP, and export of (+)sense RNA to cytoplasm

2. Translation of (+)sense RNA, accumulation of viral proteins

3. Assembly of (+)sense RNA and viral proteins into immature virions

4. Transcription of (+)sense RNA into dsRNA in virions by viral RDRP

5. Secondary transcription of dsRNA

6. Final assembly / maturation of virions

Return to RNA Genomes

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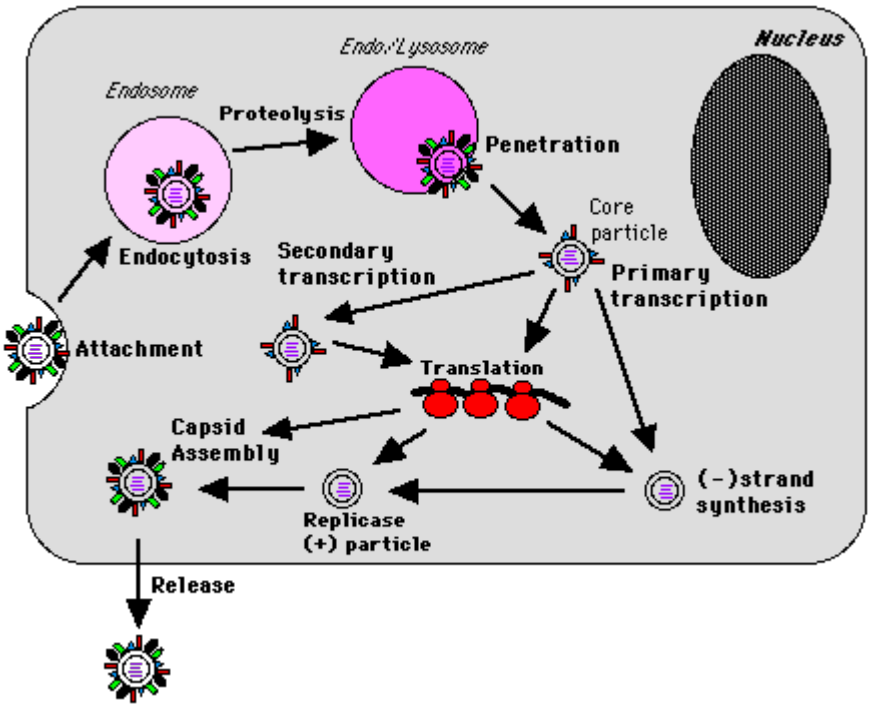

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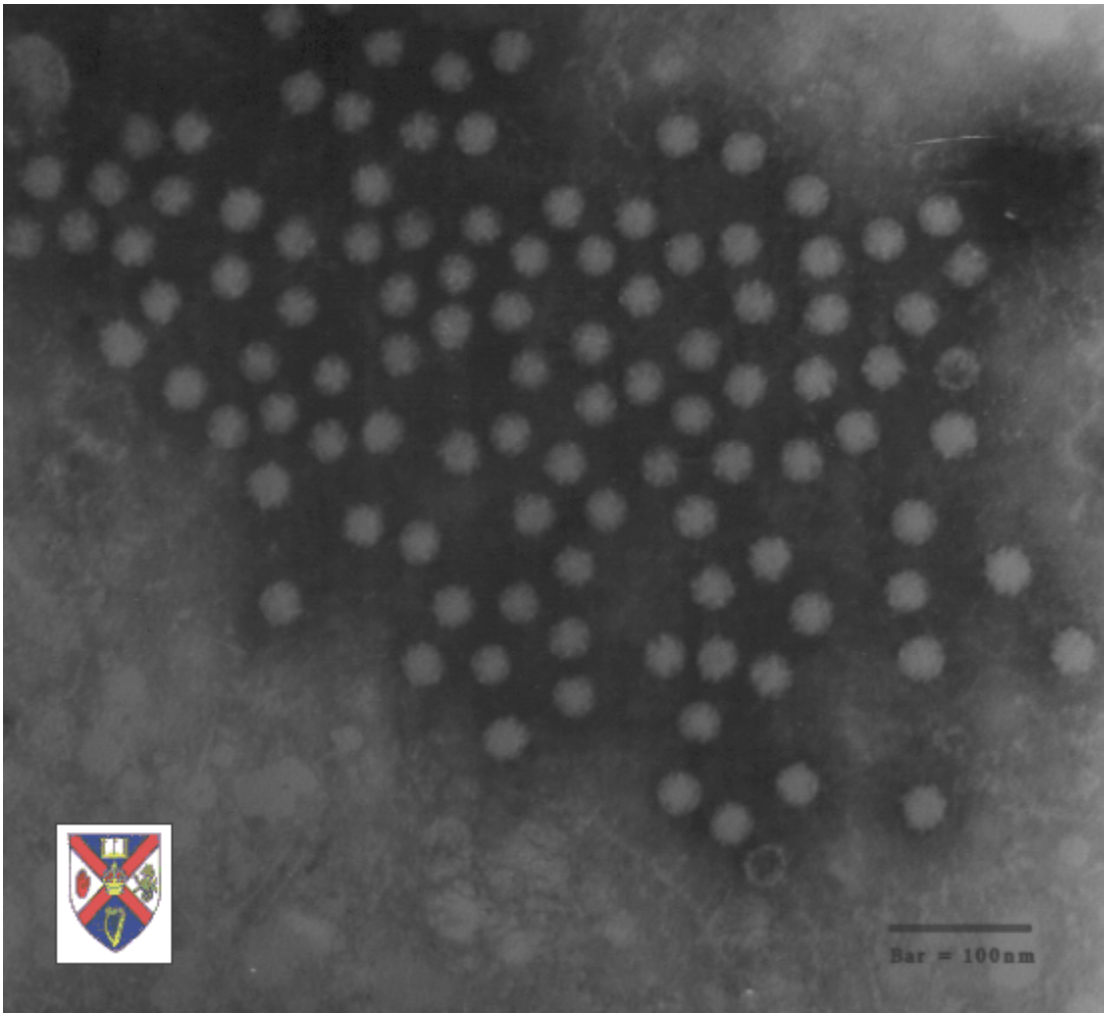
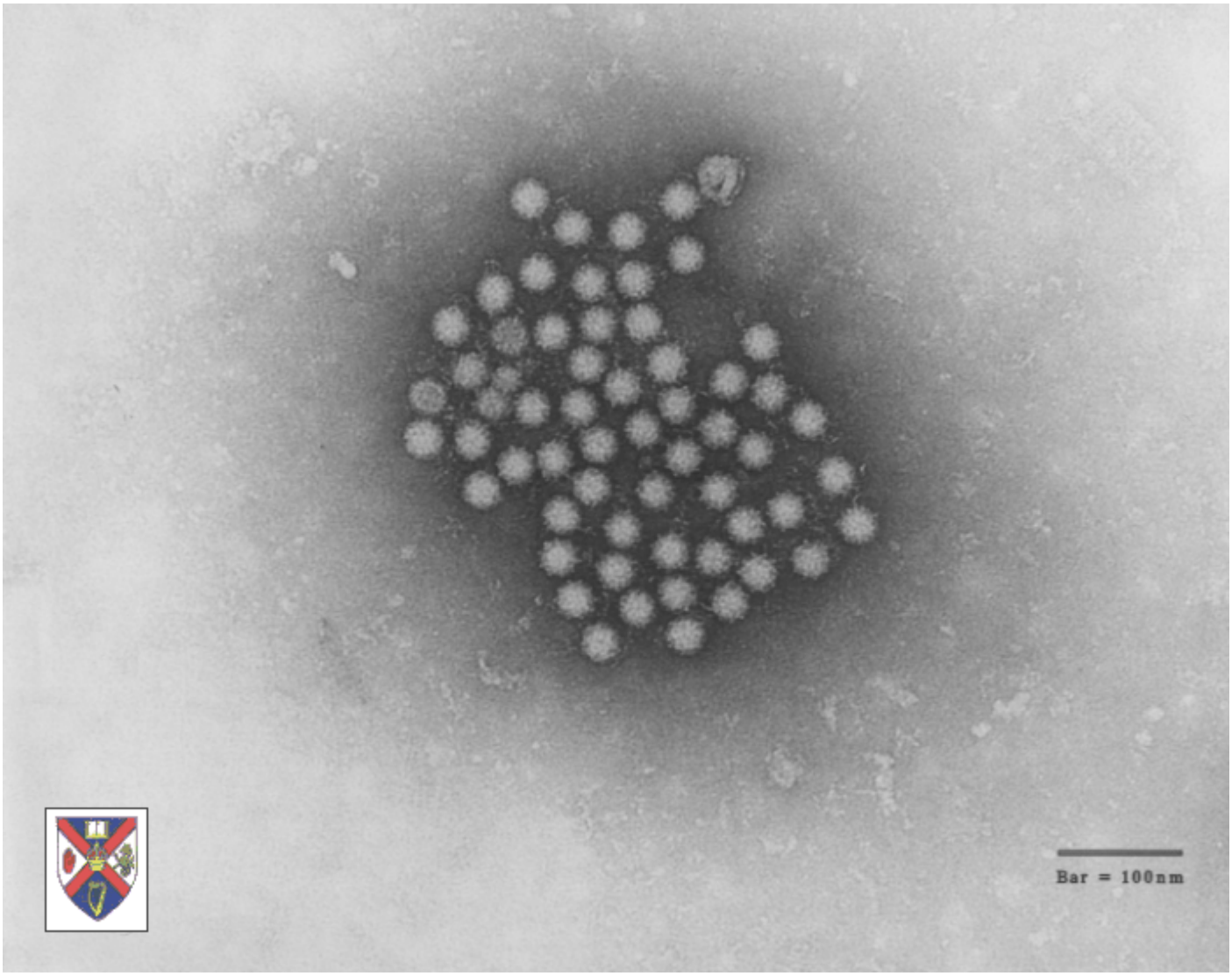



Bar = 100nm

Last Updated: Saturday, October 4, 1945624.

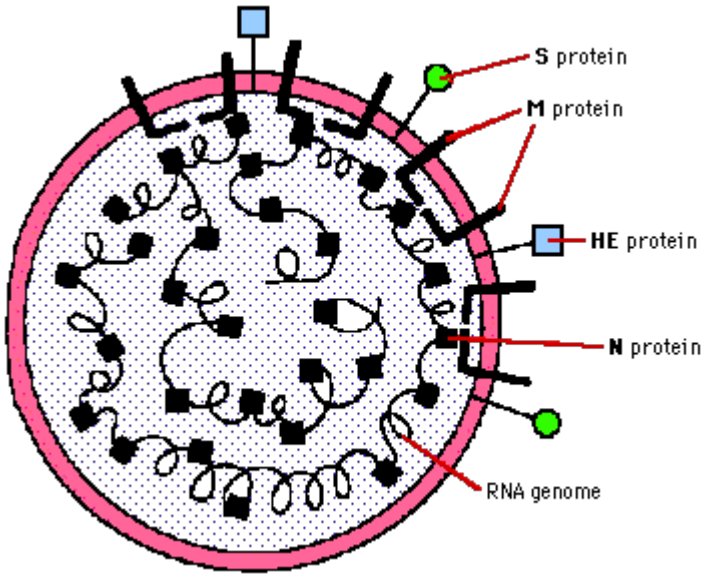
Coronaviruses

Introduction:

First isolated from chickens in 1937. After the discovery of Rhinoviruses in the 1950's, >50% of colds still could not be ascribed to known agents. In 1965, Tyrrell and Bynoe used cultures of human cilliated embryonal trachea to propagate the first human Coronavirus in vitro.
There are now ~13 species in this Family, which infect not only man but cattle, pigs, rodents, cats, dogs and birds (some are serious veterinary pathogens, especially chickens).

Morphology:

Particles are irregularly-shaped, ~60-220nm in diameter, with an outer envelope bearing distinctive, 'club-shaped' peplomers (~20nm long x 10nm at wide distal end). This 'crown-like' appearance gives the family its name. Centre of particle appears amorphous in -ve stained EM preps, the nucleocapsid being in a loosely wound rather disordered state.



The envelope carries two glycoproteins:

- **S** - Spike glycoprotein: receptor binding, cell fusion, major antigen
- **M** - Membrane glycoprotein: transmembrane - budding & envelope formation

In a few types, there is a third glycoprotein:

- **HE** - Haemagglutinin-esterase

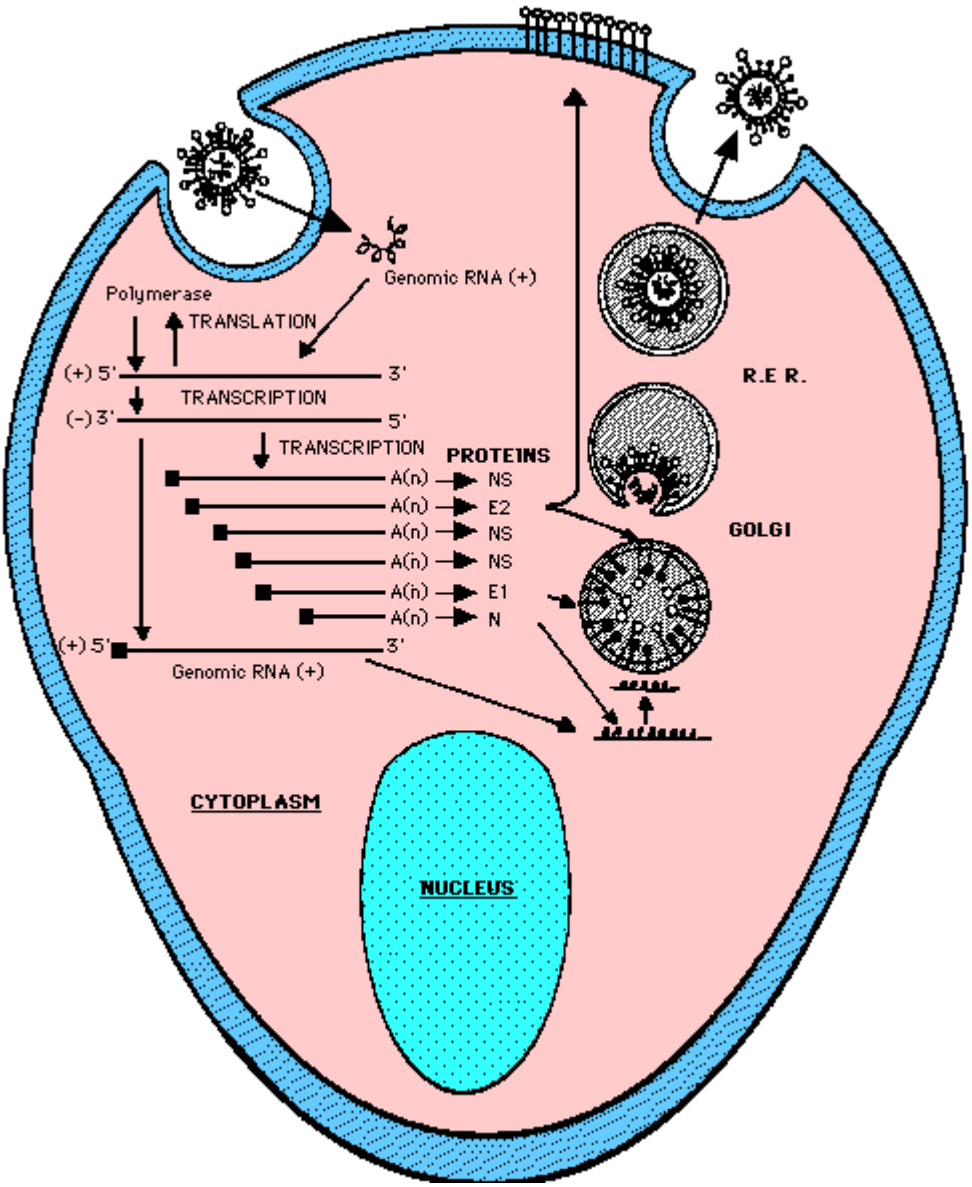
The genome is associated with a basic phosphoprotein, **N**.

To view a negatively-stained electron micrograph of a coronavirus particle, click here.

Genome:

Non-segmented, s/s, (+)sense RNA, 27-31 kb (dependent on virus) - the longest of any RNA virus. Genome has 5' methylated cap and 3' poly-A and functions directly as mRNA (unlike (-)sense RNA viruses, no polymerase in particles!) - but this is a bit more complex than at first sight (below).

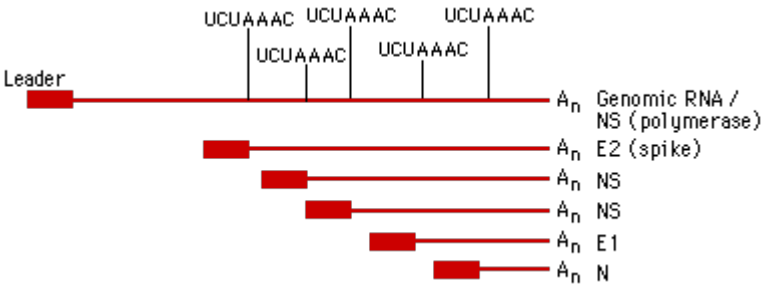
Replication:



Generally, human Corona's do not grow in cultured cells, therefore relatively little is known about them, but 2 strains (229E & OC43) grow in some cell lines & have been used as a model. Replication is slow compared to other enveloped viruses, e.g. 24h c.f. 6-8h for influenza.

Entry occurs via endocytosis & membrane fusion (probably mediated by E2). Replication occurs in the **cytoplasm**.

Initially, the 5' 20kb of the (+)sense genome is translated to produce a viral polymerase, which then produces a full-length (-)sense strand (this step is poorly understood). This is used as a template to produce mRNA as a 'nested set' of transcripts, all with an identical 5' non-translated leader sequence of 72nt & coincident 3' polyadenylated ends:



Each mRNA is monocistronic, the genes at the 5' end being translated from the longest mRNA & so on. These unusual cytoplasmic structures are produced not by splicing (post-transcriptional modification) but by the polymerase during transcription. Between each of the genes there is a repeated **intergenic sequence** - UCUAAAC - which interacts with the transcriptase plus cellular factors to 'splice' the leader sequence onto the start of each ORF.

Assembly occurs by budding into the golgi apparatus, particles being transported to the surface of the cell by the secretory nature of this organelle & released.

Pathogenesis:

These viruses infect a variety of mammals & birds. The exact number of human isolates are not known as many cannot be grown in culture. They cause:

- respiratory infections (common)
- enteric infections (occasional - mostly in infants >12 mo.)
- neurological syndromes (???)

Transmitted by aerosols of respiratory secretions, growth appears to be localized in epithelium of U.R.T. but there is no adequate animal model for the human respiratory coronaviruses. Clinically, most infections cause a mild, self-limited disease (classical 'cold' or upset stomach), but there may be rare neurological complications. Greatest incidence in children in winter, less common in adults. Number of serotypes/extent of antigenic variation unknown. Re-infections appear to occur throughout life (implying multiple serotypes (at least 4 are known) and/or antigenic variation) hence prospects for immunization appear bleak.

- References: Click here
- Search for more information on this topic
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Books about Coronaviruses: click here

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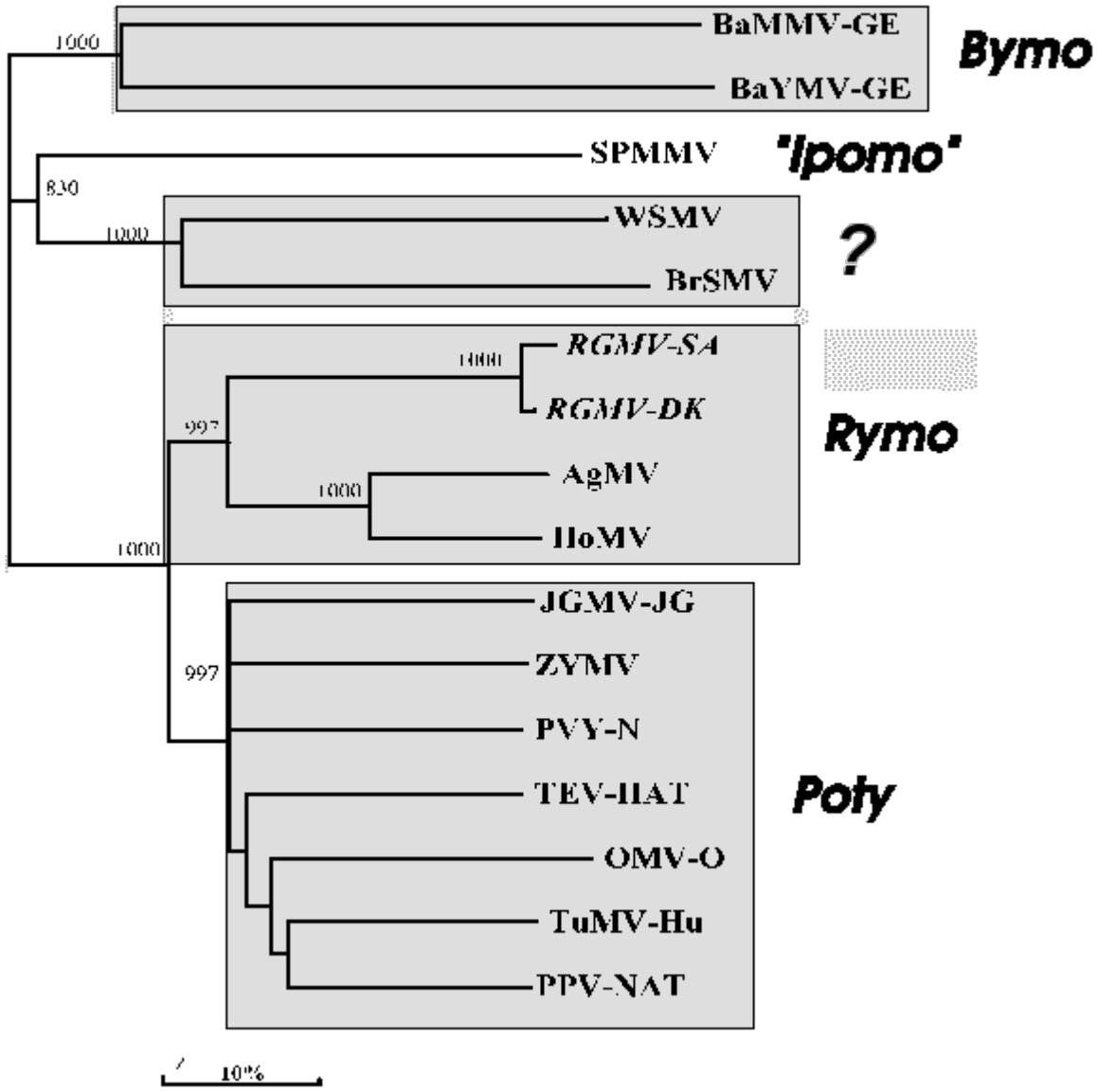
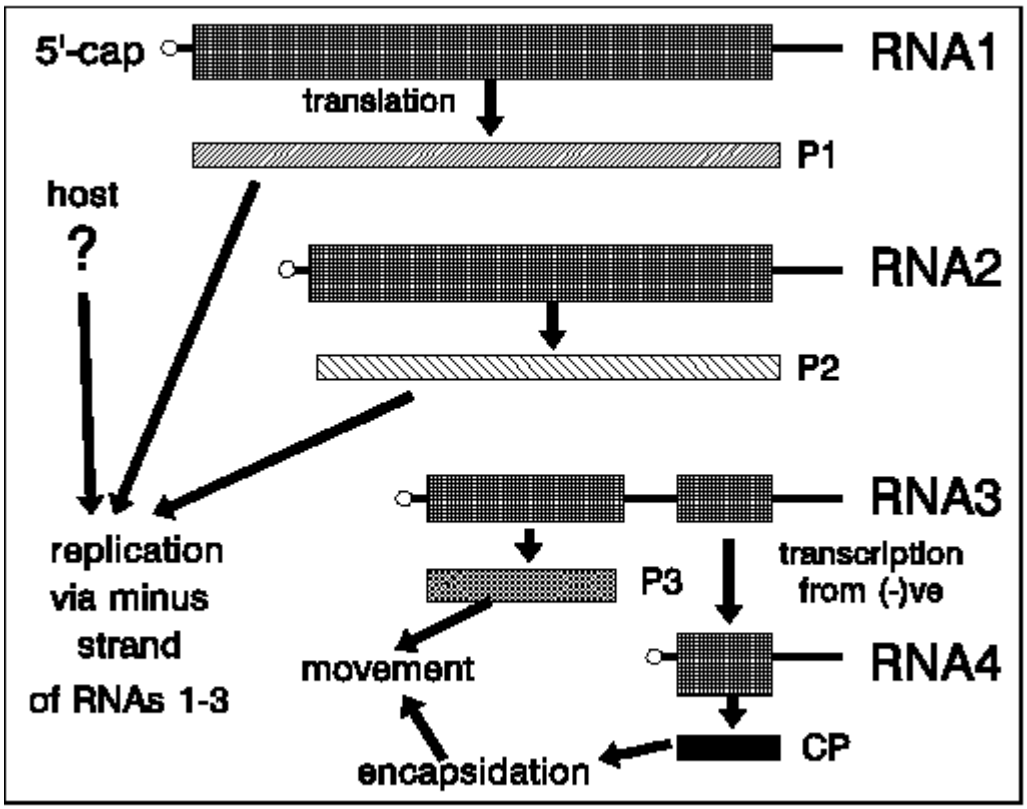

1000
BaMMV-GE
BaYMV-GE
Bymo
SPMMV
"Ipomo"
830
1000
WSMV
BrSMV
?
1000
RGMV-SA
RGMV-DK
997
Rymo
1000
AgMV
HoMV
1000
JGMV-JG
997
ZYMV
PVY-N
TEV-HAT
Poty
OMV-O
TuMV-Hu
PPV-NAT
10%

Bromoviridae Genome and Replication



Last Updated: Saturday, October 4, 1945624.

'Myxoviruses'

Name is from the Greek *'myxa'* = mucus!
Influenza pandemics have been recognised for several centuries. In the last century, *H. influenzae* (and other bacteria) were cited as the causal agent, but in 1933 Smith, Andrewes and Laidlaw isolated the virus in ferrets. In the 1940's (still pre-tissue culture, primitive immunology, few other viruses known), the characteristic property of haemagglutination was observed, followed by the discovery that the virus could be propagated in embryonated hens eggs (after adaptation) - this made influenza one of the best studied viruses during this period. More viruses with similar properties were added to the group, until it was split into 2 families in the 1970s:

Characteristic:	Orthomyxoviridae:	Paramyxoviridae:
Representatives:	**One genus:** Influenzavirus: Influenza virus A, B, C	**Three genera:** Paramyxovirus: Parainfluenzavirus 1-4, Senai virus (mouse), mumps, Newcastle disease virus Morbillivirus: Measles, rinderpest, canine distemper Pneumovirus: Respiratory syncytial virus (RSV)
Size:	Particle: 80-120nm (highly pleiomorphic) Core diameter: 9nm	Particle: 125-250nm (somewhat pleiomorphic) Core diameter: 18nm
Replication:	Nuclear	Cytoplasmic
Genome:	Segmented (-)sense RNA	Non-segmented (-)sense RNA

It is believed that all (-)sense RNA viruses may have evolved from a common ancestor - all replicate their genomes by a common mechanism; Paramyxoviruses and Rhabdoviruses have non-segmented genomes with similar organization and control of gene expression. Although unlike these other families, Orthomyxovirus genomes are segmented, but a detailed study of one of these types explains much about the others.

Orthomyxoviruses

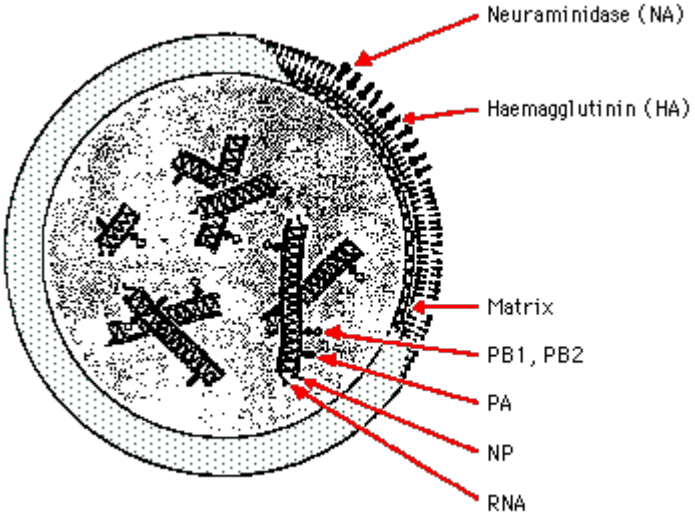
Host Range:

Influenza A viruses infect a wide variety of mammals, including man, horses, pigs, ferrets and birds. Pigs and birds are believed to be particularly important reservoirs, generating pools of genetically/antigenically diverse viruses which get transferred back to the human population via **reassortment** (close contact between pigs and man in the far east; ducks - migration!). The main human pathogen, associated with epidemics and pandemics.

Influenza B viruses infect much man and birds; cause human disease but generally not a severe as A types. Believed to be epidemiologically important - reassortment with type A leads to epidemics. Influenza C viruses infect man alone, but do not cause disease (?). They are genetically and morphologically distinct from A and B types - little studied.

Morphology:

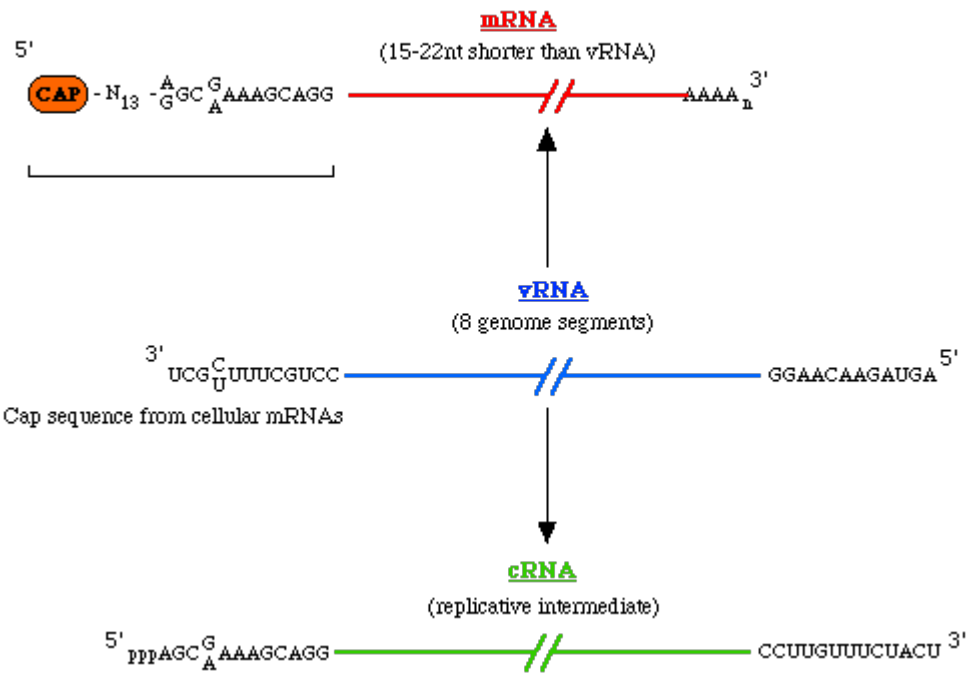
Influenza virus particles are highly pleiomorphic, mostly spherical/ovoid, 80-120nm diameter, but many forms occur, including long filamentous particles (up to 2000nm long x 80-120nm diameter). The outer surface of the particle consists of a lipid envelope from which project prominent glycoprotein spikes of two types, the haemagglutinin, ~135Å trimer (HA), and neuraminidase, ~60Å tetramer (NA). The inner side of the envelope is lined by the matrix protein. The genome segments are packaged into the core. The 'RNP' (RNA + nucleoprotein) is in a helical form with the 3 polymerase polypeptides associated with each segment. The particles are relative labile (half-life a few hours @ R.T.), not resistant to drying, etc.



To view an electron micrograph of negatively-stained influenza virus particles click here.

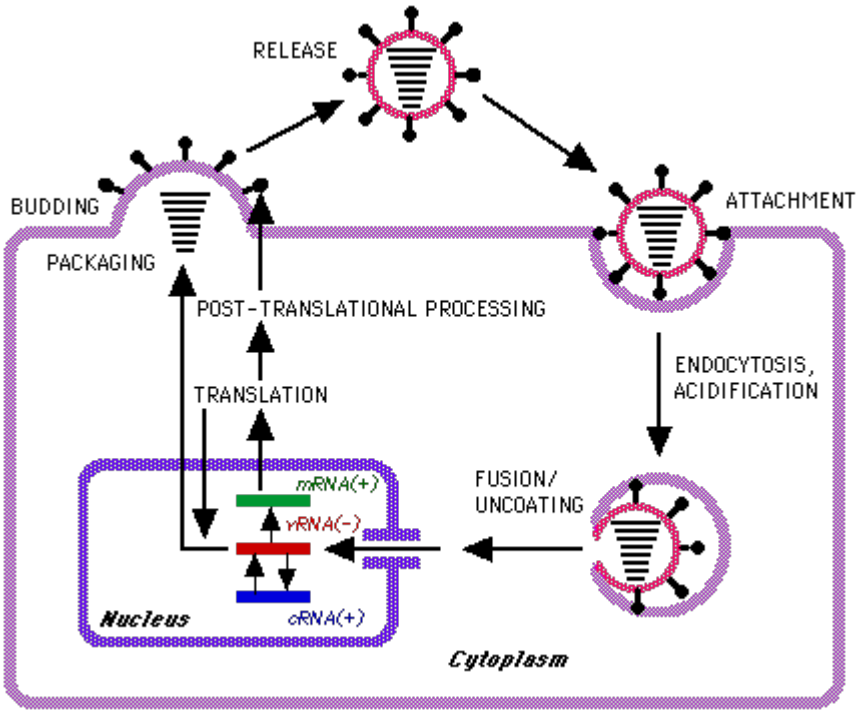
Genome:

Consists of s/s (-)sense RNA in 8 segments (7 in Influenza C). The structure of the influenza virus genome is known in great detail because of the tremendous amount of genetic investigation (conventional and molecular) which has been done. The 5' and 3' terminal sequences of all the genome segments are highly conserved:



Segment:	Size(nt)	Polypeptide(s)	Function
1	2341	PB2	Transcriptase: cap binding
2	2341	PB1	Transcriptase: elongation
3	2233	PA	Transcriptase: protease activity (?)
4	1778	HA	Haemagglutinin
5	1565	NP	Nucleoprotein: RNA binding; part of transcriptase complex; nuclear/cytoplasmic transport of vRNA
6	1413	NA	Neuraminidase: release of virus
7	1027	M1	Matrix protein: major component of virion
		M2	Integral membrane protein - ion channel
8	890	NS1	Non-structural: nucleus; effects on cellular RNA transport, splicing, translation
		NS2	Non-structural: nucleus+cytoplasm, function unknown

Replication:



- Entry into the cell is facilitated by binding of the HA spikes to mucoproteins containing terminal N-acetyl neuraminic acid (NANA = sialic acid) groups. This interaction can be reversed by polysaccharide cleavage by NA spikes - prevents virus being 'sequestered' by inappropriate cell types - NANA is very common on cell surfaces/in mucus and would otherwise swamp the virus particle, effectively 'neutralizing' it.
  Online Experiment: Haemagglutination

- After binding, the particle is engulfed by endocytosis via coated pits into endocytotic vesicles and finally endosomes. These are acidified by the cell; at about pH 5.0, the HA monomers are cleaved by trypsin-like enzymes in the endosome at the base of the 'stem' into the HA1 (upper NH2 portion) and HA2 (trans-membrane COOH portion) polypeptides (linked by disulphide bonds). This cleavage causes a conformational change in the HA spike which activates the membrane-fusion function located in HA2. The combination of the close proximity of the virus envelope and the membrane of the endosome and the active membrane-fusion domain of HA2 results in fusion of the two membranes and passage of the nucleocapsid into the cytoplasm.

- pH activated ion-channels made up of M2 protein are also important in uncoating.

- Specific nuclear targeting sequences in the NP protein result in translocation of the nucleocapsid into the nucleus.

Genome segments are transcribed by the 3 polymerase polypeptides associated with each genome segment:

i) During the initial phase of infection (~2h), active host cell DNA synthesis is required and replication is prevented by U.V, mitomycin C, etc, but not thereafter. The reason is that the initial step in replication is that PB2 attaches to the m7G cap of host mRNAs. This structure is cleaved from the mRNA by PB1, remaining attached to PB2. The cap serves as a primer for RNA synthesis and 11-15 nucleotides (complementary to the conserved sequence at the 3' end of the vRNA) are added by PB1, after which PB2 dissociates from the growing strand (these structures can be isolated from infected cells). PB1 + PA then complete the synthesis of the (+)sense strand.

ii) Two classes of (+)sense RNA are made in infected cells:

- Incomplete, 3' polyadenylated transcripts which are exported to the cytoplasm and serve as **mRNAs** (due to the presence of a specific polyadenylation sequence ~20nt from the 5' end of the (-)sense vRNA template strand)
- **cRNA** = complete, non-polyadenylated (+)sense copies of the (-)sense vRNA (made by readthrough of the polyadenylation signal) which serve as template for the synthesis of progeny (-)sense vRNAs.

d/s (+/-) replicative intermediate structures can be isolated from the nucleus of infected cells.

Most of the proteins made (e.g. HA, NA) remain in the cytoplasm or become associated with the cell membrane. However, the NP protein migrates back into the nucleus, where it associates with newly-synthesized vRNA to form new nucleocapsids. These migrate back out into the cytoplasm and towards the cell membrane (mechanism unclear). The level of free nucleoprotein (NP) is thought to control whether mRNA or cRNA is produced, i.e. later in infection there is lots of NP, mRNA synthesis stops but cRNA synthesis continues. **NP is thus a crucial switch in the replication cycle between expression and assembly.**

~4h after infection, patches of M1 protein form on the cell membrane, which appears to thicken, incorporating HA and NA on the outside of the membrane. The nucleocapsid segments are incorporated into the particle as it buds out through the membrane. NA is thought to have a role in release of budding particles (inhibited by anti-NA Abs).

The **packaging mechanism** responsible for sorting 8 distinct genome segments into each particle is not known. However, it has recently been found that there >8 RNA segments/particle - the exact number is not known and may vary.

Assuming packaging of segments is completely random:

12 segments/particle = 10% of particles would be viable
14 segments/particle = 20% of particles would be viable

However, there is some evidence that the sorting of genome segments is not a purely random process (McCauley et al. Virology 216: 326-337, 1996), so the situation remains confused.

Virus particles are gradually **released** from the surface of the cell over a period of several hours. The cell does not lyse, but eventually dies (due to disturbance of normal cellular macromolecular synthesis?).

Pathogenesis:

Spread is by aerosols - very efficient (occasionally fomites). Even in epidemics, there are 3:1 - 9:1 infections:clinical case - very infectious. Primary infection involves the cilliated epithelial cells of the U.R.T. Necrosis of these cells results in the usual symptoms of the acute respiratory infection (fever, chills, muscular aching. headache, prostration, anorexia). Normally self-limited infection usually lasts 3-7 days (+convalescence). Death from primary Influenza infection is very rare and appears to be determined by host factors rather than 'virulence' of virus. Damage to respiratory epithelium predisposes to secondary bacterial infections which accounts for most deaths.

Enzyme helps make flu virus a killer, study finds.

An extra enzyme helps make some strains of the flu virus especially deadly, researchers reported on Monday. They said knowing about the enzyme might help doctors find people infected with the most dangerous strains, and might provide a target for drugs. Yoshihiro Kawaoka and colleagues at the University of Wisconsin said the most dangerous strain of influenza A makes use of an extra protein that helps it infect cells throughout the body. The influenza A virus has two surface proteins -- hemagglutinin (HA) and neuraminidase (NA). To infect cells, the HA protein has to be cut into two pieces by enzymes known as proteases. The proteases that can do this are normally common in the lungs and throat but not elsewhere -- which is why flu usually is just a respiratory illness. Writing in the Proceedings of the National Academy of Sciences, Kawaoka's team said the worst strain of flu uses a different enzyme, plasmin, to help chop up HA. Plasmin is common throughout the body, meaning this strain can infect all sorts of tissues. They looked at a virus descended from the strain that caused the 1918 pandemic of flu, blamed for killing more than 20 million people around the world. "This is a mechanism that we never knew existed in human viruses," Kawaoka said in a statement. His group tested 10 other strains of flu and did not find the same enzyme being used. He said perhaps doctors could look for this enzyme in a flu outbreak to determine how likely it is to be dangerous. "Now we have additional markers that we can look for when a peculiar outbreak of human or animal flu occurs," he said. "Their findings point us in a direction to better understand the pathology of these more virulent influenza viruses," Dominick Iacuzio, programme officer for influenza at the National Institute for Allergies and Infectious Diseases (NIAID), which funded the study, said.

A novel mechanism for the acquisition of virulence by a human influenza A virus.
Hideo Goto & Yoshihiro Kawaoka.

PNAS USA 95: 10224-10228, 1998:

Cleavage of the hemagglutinin (HA) molecule by proteases is a prerequisite for the infectivity of influenza A viruses. Here, we describe a novel mechanism of HA cleavage for a descendant of the 1918 pandemic strain of human influenza virus. We demonstrate that neuraminidase, the second major protein on the virion surface, binds and sequesters plasminogen, leading to higher local concentrations of this ubiquitous protease precursor and thus to increased cleavage of the HA. The structural basis of this unusual function of the neuraminidase molecule appears to be the presence of a carboxyl-terminal lysine and the absence of an oligosaccharide side chain at position 146 (N2 numbering). These findings suggest a means by which influenza A viruses, and perhaps other viruses as well, could become highly pathogenic in humans.

Prevention/Treatment:

Several anti-influenza drugs already exist. Amantadine and rimantadine are active against influenza A viruses. The action of these closely related agents is complex and incompletely understood, but they are believed to block cellular membrane ion channels.

- The target for both drugs is the matrix protein (M2), but resistance to the drug maps to the haemagglutinin (HA) gene.
- This biphasic action results from the inability of drug-treated cells to lower the pH of the endosomal compartment (a function normally controlled by the M2 gene product), a process which is essential to induce conformational changes in the HA protein to permit membrane fusion.

Due to the important role played by neuraminidase in transmission of the virus throughout the host, it has recently been seen as a prospective target for anti-influenza drugs. The active site of neuraminidase is made up of 11 universally conserved amino acid residues.
Synthetic sialic acid analogues work well against neuraminidase at lower levels than amantadine and rimantadine, but need to be given intranasally, and are more effective as a prophylactics, with little effect after infection.
GS4071 is a novel orally administered inhibitor of neuraminidase called which has been shown to confer decreased severity and duration of symptoms in clinical trials.

Vaccines: Isolated HA gives good serological protection (estimated at 60-80%). Vaccines are produced by reassortment of egg-adapted strains with strains with the required HA type. Large amounts of virus are then grown in embryonated eggs (cheap and efficient), purified and formalin inactivated. Vaccine is given sub-cutaneously: if of a new antigenic type, 2 doses are necessary for adequate protection (i.e. depends on age of patient).

The Snag: In order to give time for adequate vaccine stocks to be produced, a decision must be made, usually in about August, as to which HA type to use for this years vaccine (for the winter season). There is an elaborate and sophisticated epidemiological monitoring system worldwide, which helps these decisions:

- World Health Organization Influenza Surveillance
- Current European Influenza Surveillance Report
- Current USA Influenza Surveillance Report

Unfortunately, because of the capricious nature of influenza virus, the right decision which gives truly effective protection is only made about one year out of two. There is much interest in other types of vaccine - especially genetically engineered/subunits and live attenuated, but neither is currently in use (the molecular basis for attenuation is not clear).

STOP PRESS:

Killer 'flu is coming - or is it ???

In May 1997, a 3-year-old boy died of complications of influenza in the intensive care unit of a Hong Kong hospital. Note that the child did not die from influenza directly, but rather from **Reye's syndrome**, a rare neurological complication of certain virus infections caused when young people with fevers take aspirin. This case was the first isolation of an influenza type-A subtype **H5N1** in a human. Subtype H5 influenza viruses can cause lethal avian influenza, a disease that may decimate flocks of domestic poultry. It is not known how the boy became infected with the virus, however there had been an avian flu epidemic in the region a month earlier.

Dr. Robert G.Webster, Chairman of the Department of Virology and Molecular Biology at St. Jude Children's Research Hospital, Memphis, Tennessee, said:

"Predictions we've been making, is that there will be another **pandemic** - probably before the end of the century. We've been preparing pandemic manuals, what to do when the pandemic comes. And this is the first example of what could develop into it. We don't think it will. But how well have we performed our duties? If we go back and rate ourselves, we haven't done all that well,

because the virus occurred in Hong Kong in May, and it was months before we realized it was there, so we have to get our act together. We will learn from this how to perform better when the real one comes ... You've got a very large, virgin population out there - large enough to start a pandemic."

Is flu on the warpath once again?

Probably not yet, says Nancy Arden, a CDC epidemiologist who's followed the Hong Kong incident. Scientists have not found **H5N1** in more than 4,000 throat samples taken from Chinese respiratory-disease patients since the outbreak. "It appears not to have been spreading among humans", she says. But she notes that "this is the first time that a purely avian strain of influenza has been found to cause the flu in humans, and that's why there was such an intensive investigation." Viruses can't cause pandemics unless they have two qualities, Arden notes. First, they must be able to infect humans, as **H5N1** apparently can. Second, they must be able to spread easily among the human population. For unknown reasons, **H5N1** appears to lack this vital talent.

But could **H5N1** mutate so that it can spread rapidly through the human population?

To date (June 1998) H5N1 has been linked to the deaths of six people including two children, and a dozen others have been infected.

Watch this space ...

- References: Click here
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- Influenza: An Emerging Disease
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Last Updated: Saturday, October 4, 1945624.

Rhabdoviruses

Introduction:

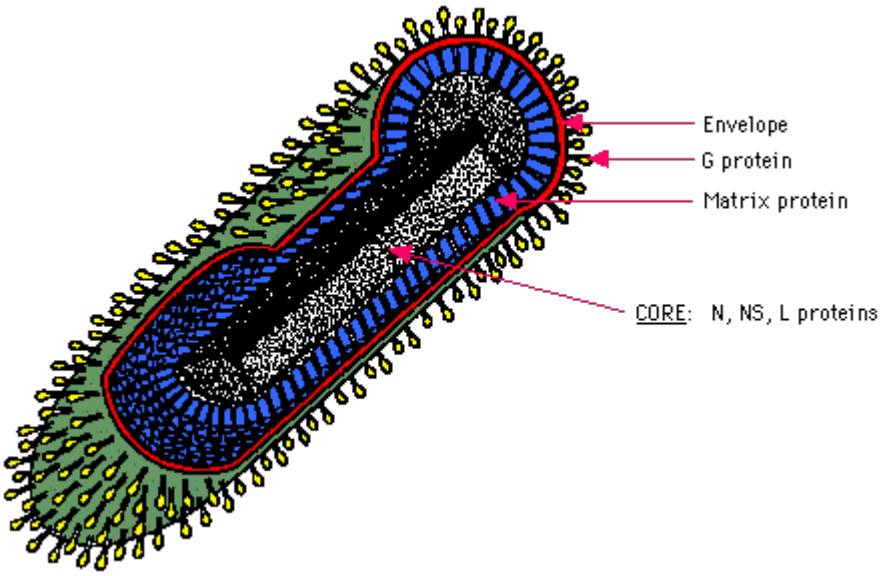
Rhabdo = Greek. 'rod-shaped'.

Genetically, these viruses have non-segmented (-)sense RNA genome reminiscent of Paramyxoviruses. There are >200 Rhabdoviruses known (probably still an underestimate of the total), which infect man (Rabies - the only member of the group to do so 'naturally'), other mammals, fish, insects (some replicate in arthropods and were previously classified as Arboviruses) and plants - versatile! The family is split into 5 genera:

Lyssavirus (Greek. 'frenzy'):	Rabies virus; other mammalian viruses; some insect viruses
Vesiculovirus:	Vesicular Stomatitis Virus (VSV); mostly insect viruses
Ephemerovirus:	Bovine ephemeral fever virus (vertebrates)
Cytorhabdovirus:	Lettuce necrotic yellows virus (plants)
Nucleorhabdovirus:	Potato yellow dwarf virus (plants)

Rabies is an 'ancient' disease, first shown to be of infectious origin in 1808, shown to be of viral etiology by Pasteur in the 1880's (when Pasteur and Koch were developing the germ theory of disease - prior to the firm modern definition of 'viruses' by Beijerinick (1898)). Over a decade, Pasteur carried out the serial passage of Rabies virus in rabbits, and eventually succeeded in isolating an attenuated preparation which was used to treat patients bitten by mad dogs (not without some risks).

Morphology:

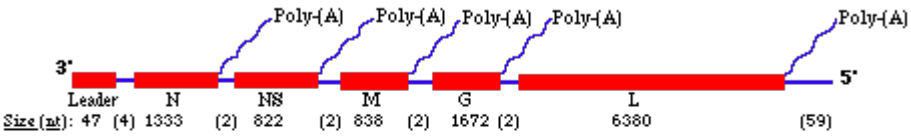


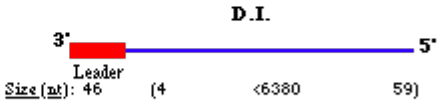
Particles ca. 180 x 70nm with unique bullet-shaped appearance (all rather similar). Enveloped with prominent spikes on surface (**G** protein - haemagglutinates RBCs), but not very variable in appearance. The envelope is lined

by the matrix protein and contains the nucleocapsid (RNA + **N** protein) wound helically inside the core. Two non-structural proteins, **L** and **NS**, are associated with the nucleocapsid and act in concert as the viral polymerase. As with most enveloped viruses, the particle is relatively labile.

Genome:

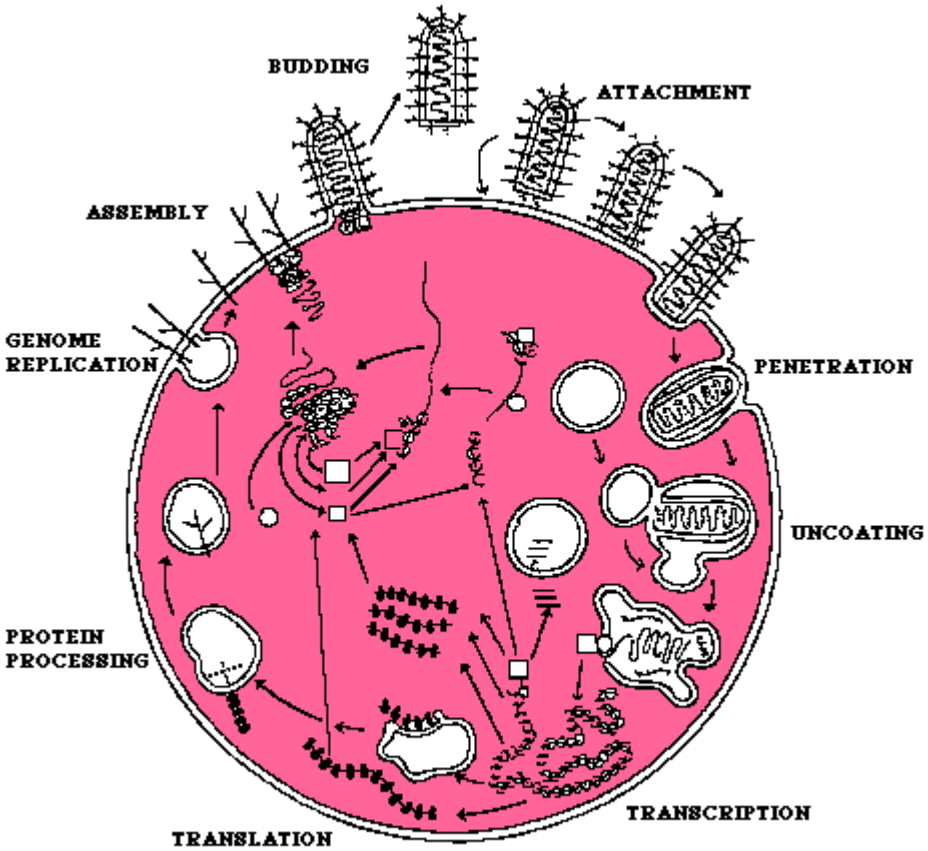
~11kb. ~50nt 'leader' region at the 3' end and ~60 non-translated region at the 5' end of the (-)sense vRNA. Similar to paramyxoviruses, there is a conserved polyadenylation signal at the end of each gene and a short intergenic region between each of the 5 genes. Most of our knowledge of Rhabdoviruses comes from Vesicular Stomatitis Virus (VSV) - very similar to Rabies, but the entire rabies genome has now been cloned, sequenced and expressed (see below).



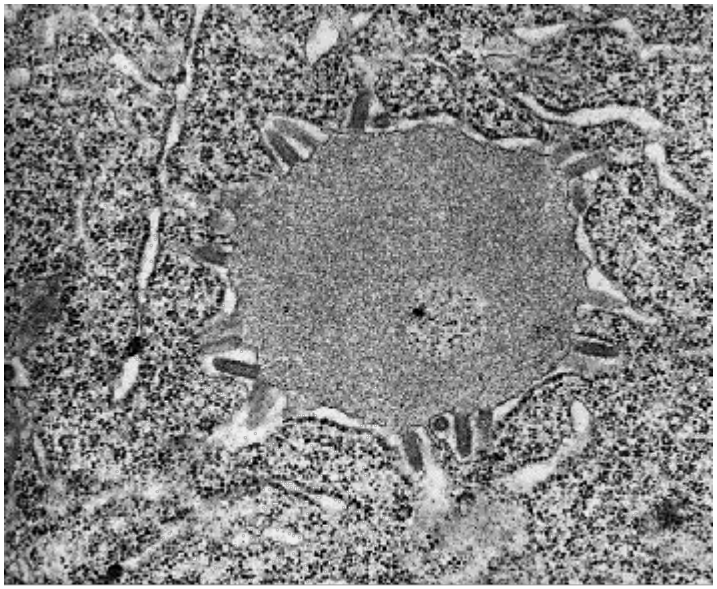


Replication:

Transmission varies depending on virus/host, but most are transmitted by direct contact - e.g. rabies - animal bites or insect vector. There is a long incubation period in vivo, but this is not reflected in the kinetics of virus replication in culture. The G protein spikes bind to receptors on the surface of host cells and the viruses enters the cell by endocytosis and fusion with the membrane of the vesicle (as Paramyxoviruses), mediated by the G protein.



Receptor molecules for Rhabdoviruses are not known, but are believed to be phospholipids rather than specific proteins. Replication occurs in the **cytoplasm** - both the L and NS proteins are necessary for transcription - neither function alone. Five monocistronic mRNAs are produced, capped at the 5' end and polyadenylated at the 3' end and each containing the leader sequence from the 3' end of the vRNA at the 5' end of the message. These mRNAs are made by sequential transcription of the ORFs in the virus genome and it has been shown that the intergenic sequence is responsible for termination and re-initiation of transcription by the polymerase between each gene, thus producing separate transcripts.
Progeny vRNA is made from a (+)sense intermediate, but this step is not well understood. The genome is replicated by the L + P polymerase complex (as in transcription), but additional host cell factors (not known) are also required. It is characteristic of Rhabdoviruses that these events all occur in a portion of the cytoplasm which acts as a virus 'factory' and appears as a characteristic cytoplasmic inclusion body - e.g. Rabies - perinuclear **Negri bodies** (Negri, 1909).



Electron micrograph of rabies virus in brain cells at 64,000X magnification. The bullet-shaped particles surrounding the smooth gray circle are rabies. The circle itself is the Negri body, which can be seen with a light microscope. Courtesy of Dr. F.A. Murphy, UC Davis.

Virions are assembled around the tightly coiled nucleoprotein core, and bud both from cytoplasmic membranes and the outer membrane of the cell (acquiring the M + G proteins as they do so).

Pathogenesis: Rabies:

Entry occurs by wound or abrasion of skin directly into bloodstream (animal bite - e.g. vampire bats in S. America). 1o replication occurs locally in muscle and connective tissue (no symptoms), but virus eventually infects peripheral nerves, then travels (passively?) along neuronal axons to CNS, where it produces severe and fatal encephalitis. Few cases escape these severe consequences. Viraemia and haematogenous spread of virus to CNS has not been shown. Incubation period varies from 3-8 weeks to 1 year depending on size and site of inoculation (e.g. head/face/neck vs. hands or feet).

Epidemiology:

Man is a dead-end infection for the virus, normal hosts are foxes, dogs, cats, bats, skunks, etc. Cattle rabies is a serious economic disease in countries where Rabies is endemic. Pattern is endemic rather than epidemic.

Treatment:

No effective drug treatment, but passive immunization is of value. This is one of the cases where therapeutic (post-exposure) vaccination is important - the aim is not to prevent infection but to moderate the severity of the disease. 3 types of vaccine exist:

- **Inactivated** - as pioneered by Pasteur in the 1880's - used until the 1950's (material from infected animals containing up to 5% nervous tissue - provoked severe immune reactions in recipients, but saved lives). This type of vaccine was then produced by growing the virus in embryonated duck eggs - better, but still produced severe encephalitis in some recipients. Inactivated vaccine is now produced by growing the virus in diploid human fibroblasts - effective and safe but expensive, therefore duck egg-produced material still used in third world.
- **Live attenuated** - has been used to vaccinate domestic animals and livestock. Effective, but not considered sufficiently safe for human use.
- **Recombinant** - recently developed recombinant vaccinia virus / G protein vaccine is being used to eradicate rabies in foxes in Europe (Brochier et al, Nature 354: 520 (1991).

VSV:

Normally causes an epidemic, self-limited disease of cattle which is of some economic importance in countries where it is endemic, e.g. N.America. Similar in appearance to foot & mouth disease! Can infect man, causing a relatively mild febrile illness.

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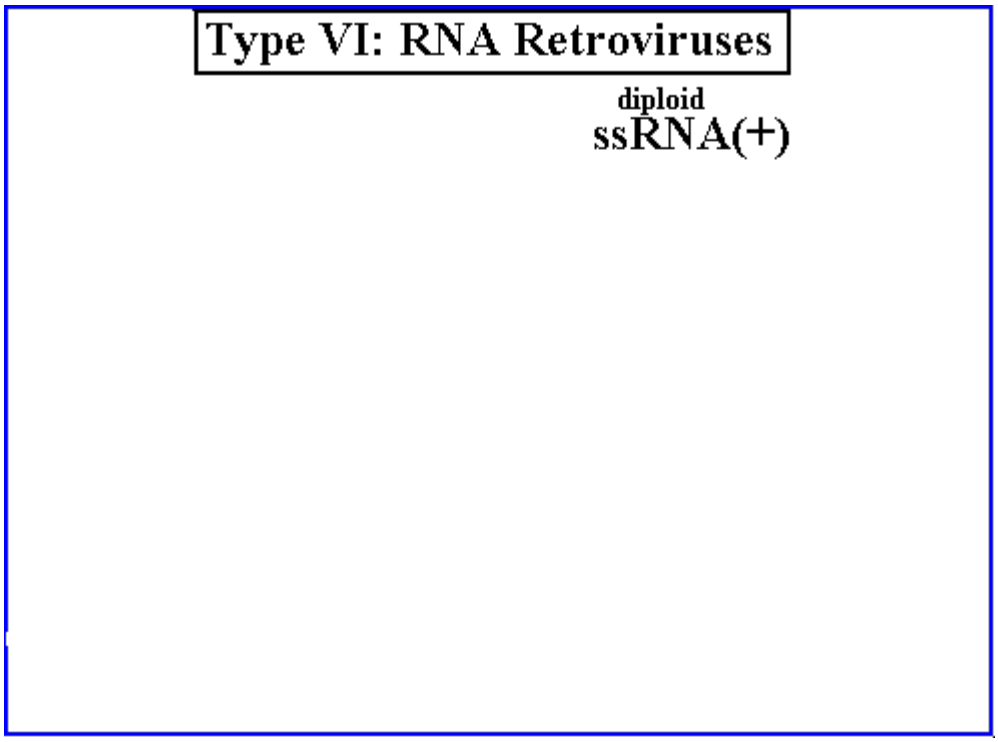
Replication Strategy of ss(+)RNA Viruses Replicating via a Longer-Than-Genome-Length DNA Intermediate



Note: look at status bar when mouse is over numbers in picture for explanations of steps

Note: this is an animated GIF, which takes about 30 sec to cycle through completely.

Steps in Replication

1. Reverse transcription in cytoplasm, using tRNA primer, of virion ss(+)RNA by virion-associated reverse transcriptase (RT), into intermediate RNA/DNA complex

2. Conversion of RNA/DNA complex into linear and circular proviral dsDNA forms with long terminal repeats (LTRs) by RT; import into nucleus

3. Integration of linear proviral DNA into host cell DNA, by means of integrase function of RT

4. Replication and trasncription as for host DNA, using host enzymes

5. Modification of transcription by (early) viral products (bias

to production of genome-length (+)sense RNA?)

6. Translation, accumulation of (late) structural protein, assembly with (+)sense genomic RNA into viral nucleoprotein, budding through membrane containing viral envelope (glyco)proteins

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Return to Genomes

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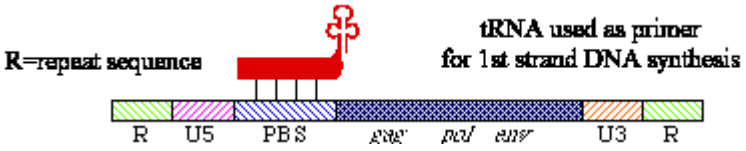

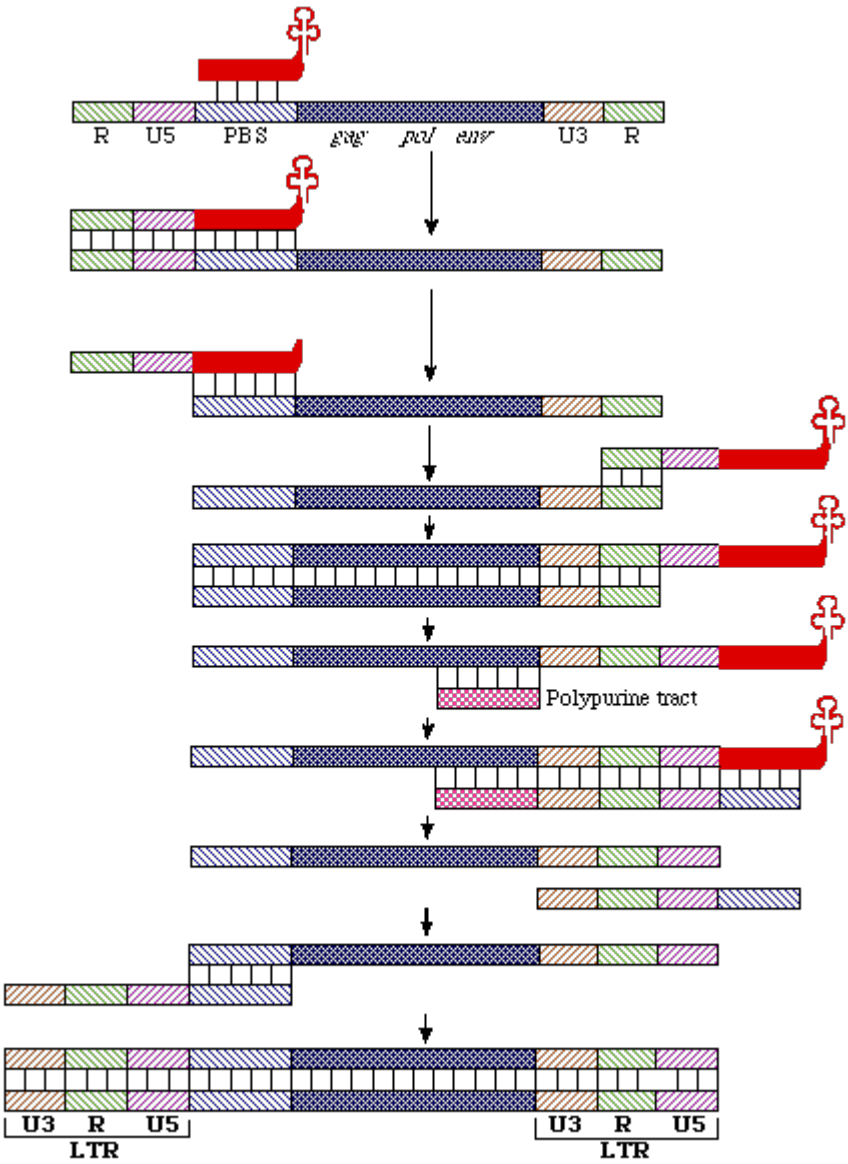
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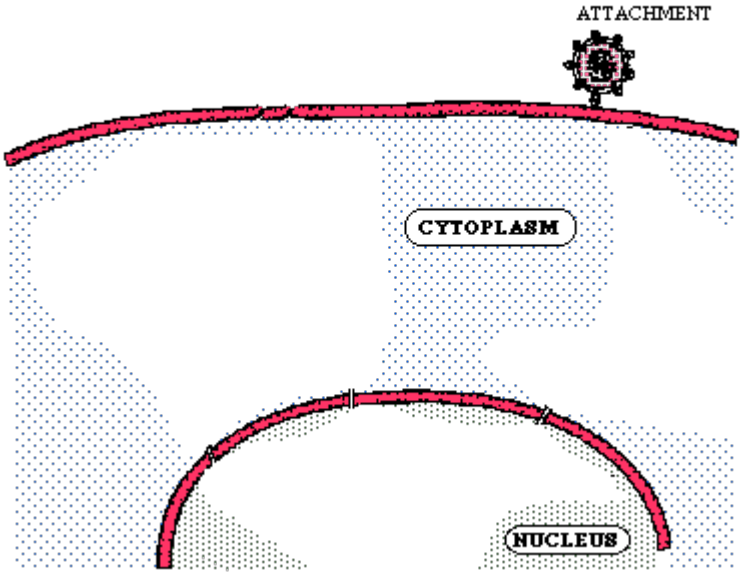

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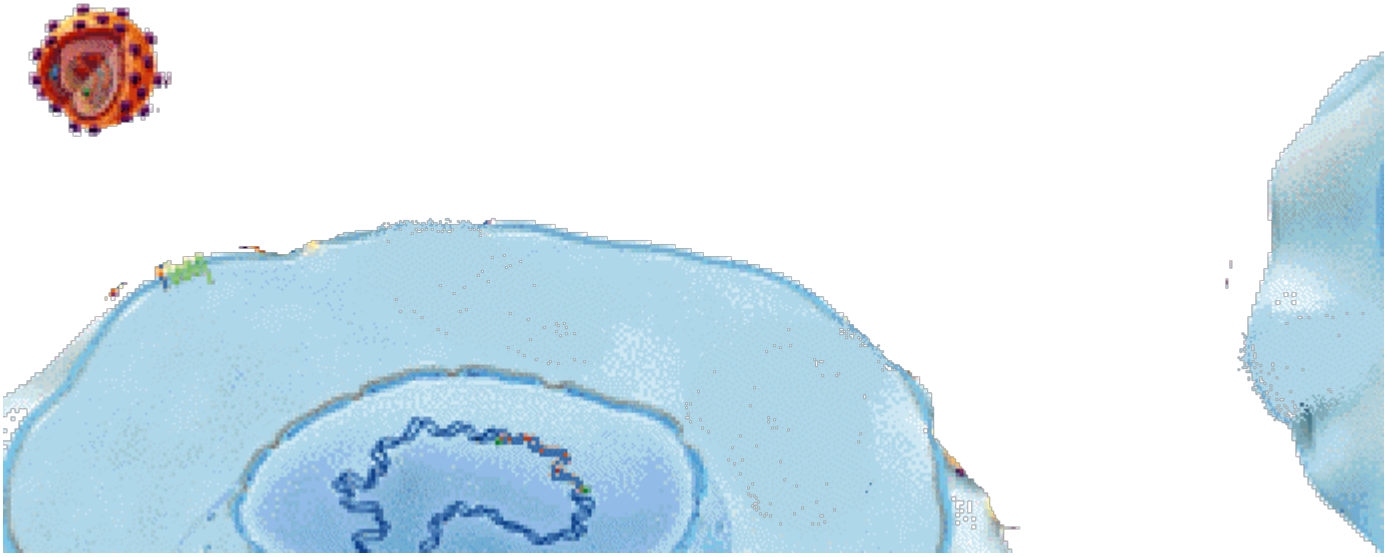

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nv (new variant) CJD is:

Spongiform Encephalopathy Advisory Committee

"A previously unrecognised and consistent disease pattern"
"Although there is no direct evidence of a link, on current data and in the absence of any credible alternative the most likely explanation at present is that these cases are linked to exposure to BSE".

UK Spongiform Encephalopathy Advisory Committee (SEAC) 1996.

"In the past few weeks we believe we may have identified a new clinico-pathological phenotype of CJD which may be unique to the United Kingdom. This raises the possibility of a causative link between BSE and CJD".

Dr Robert Will of the National Creutzfeldt-Jakob Disease Surveillance Unit, in a letter to all the neurologists in the UK, On 21st March 1996.

What is the truth ?

You decide

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Last Updated: Saturday, October 4, 1945624.

Filoviruses

Name comes from the Latin: *filo* = 'threadlike'.

1967: Marburg/Frankfurt, Germany. Laboratory workers preparing primary cell cultures from African green monkeys resulted in an outbreak of a previously unrecognised disease. Highly infectious; 31 cases, 7 deaths (some probably survived due the first theraputic administration(?) of interferon).

1976: Outbreak of a previously unrecognised haemorrhagic fever in Zaire & Sudan 'Ebola disease': 500 diagnosed cases, 460 deaths!

From these two outbreaks, 2 novel viruses (Marburg & Ebola) were isolated - placed in a new family, the **Filoviridae:**

Year	Location	Cases	Mortality	%	Reference
1971	Zaire	1	0	0	Samaranayake & Peiris, 1996
1976	Sudan	360	150	42	Samaranayake & Peiris, 1996
1976	Zaire	318	280	89	Samaranayake & Peiris, 1996
1977	Zaire	2	2	100	Samaranayake & Peiris, 1996
1979	Sudan	34	22	65	Samaranayake & Peiris, 1996
1989	Reston, USA "The Hot Zone"	4	0	0	Sodhi, 1996
1994	Gabon & Cote d'Ivoire	?	?	?	Breman et al, 1997
1995	Zaire	315	246	78	Breman et al, 1997

The Hot Zone by Richard Preston.

The dramatic and chilling story of an Ebola virus outbreak in a surburban Washington, D.C. laboratory, with descriptions of frightening historical epidemics of rare and lethal viruses. More hair-raising than anything Hollywood could think of, because it's all true.

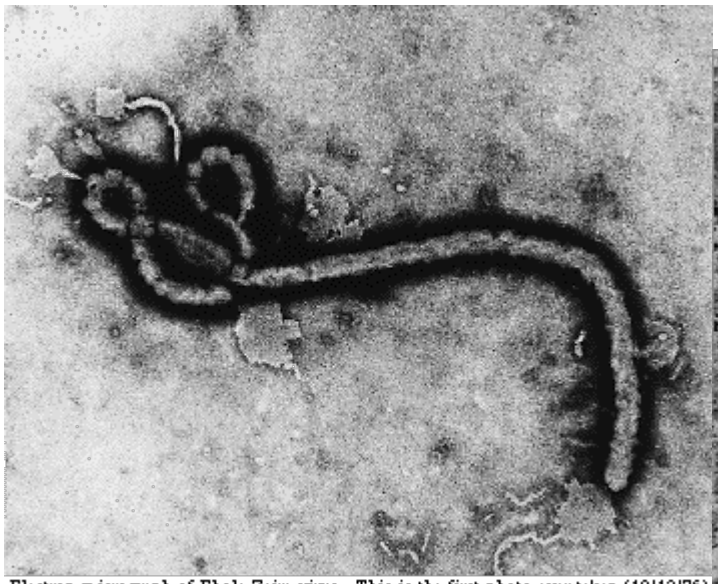
The Coming Plague: Newly Emerging Diseases in a World Out of Balance by Laurie Garrett.

Based on research and interviews with experts in virology, molecular biology, disease ecology, and medicine, an exploration of our battles with microbes examines the current outbreak of infectious diseases and outlines what can be done to prevent the coming plague.

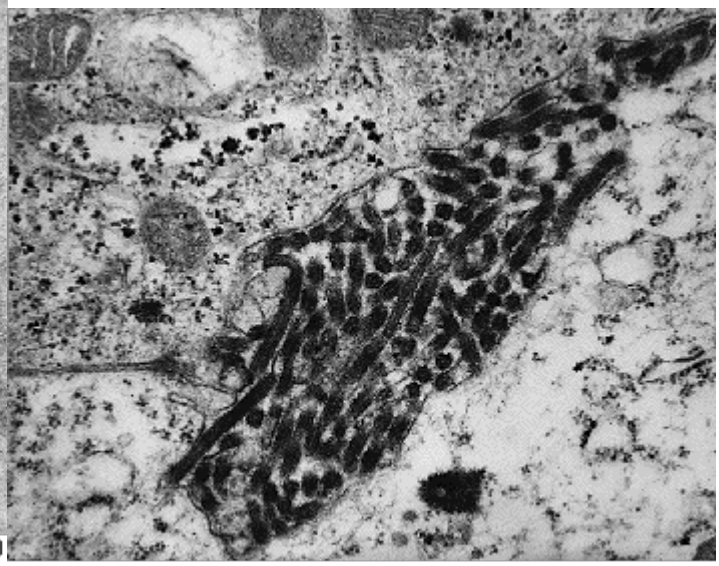
This family of viruses has strong structural & genetic similarities to both Rhabdoviruses & the Paramyxoviruses.

Particles:

Pleiomorphic, elongated, 80nm diameter x 130-14,000nm long - sometimes straight, but may be curved or hooked - 'U' or '6' shaped. Particles are rounded at one end with a distended swelling at the other. The core has a striated appearance similar to that of Rhabdoviruses.



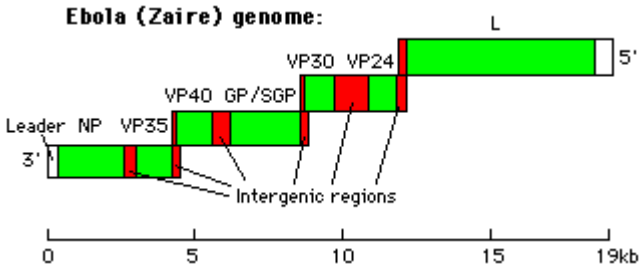
Electron micrograph of Ebola Zaire virus. This is the first photo ever taken (10/13/76) by Dr. F.A. Murphy, now at UC Davis, then at CDC. Diagnostic specimen in cell culture at 160,000X magnification.

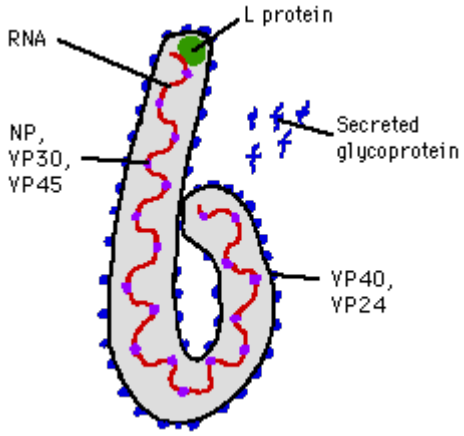


Marburg virus pictured between two human liver cells at 75,00X magnification. Electron micrograph courtesy of Dr. F.A. Murphy, UC Davis.

Genome:

The filovirus genome is s/s, unsegmented, (-)sense RNA, ~19kb. Size varies with length of particle - optimum infectivity for Marburg ~790nm, Ebola ~970nm. Encodes 7 proteins from monocistronic mRNA complementary to vRNA. Signals & expression strategy reminiscent of Rhabdoviruses & Paramyxoviruses, but there are differences, e.g. some filovirus genes overlap:





The ribonucleoprotein complex consists of the nucleoprotein (NP), the structural proteins VP30, VP35 & the polymerase, L. The location of VP40 & VP24 have not been accurately determined, but are thought to be membrane associated. The L protein is the largest protein, & is the virion associated RNA dependent RNA polymerase. The glycoprotein (GP/SGP) is an integral membrane protein & is the only glycosylated virion protein, containing both N- & O-linked oligosaccharides.

Pathogenesis

The clinical manifestations of Ebola virus infection are severe. The incubation period varies between four & sixteen days. The initial symptoms are a severe frontal & temporal headache, generalised aches & pains, malaise, by the

second day the victim will have a fever. Later symptoms include watery diarrhoea, abdominal pain, nausea, vomiting, a dry sore throat, & anorexia. By day seven of the symptoms, the patient will have a maculopapular (small slightly raised spots) rash. At the same time the person will develop thrombocytopenia & haemorrhagic manifestations, particularly in the gastrointestinal tract, & the lungs, but it can occur from any orifice, mucous membrane or skin site. By day twelve the skin starts to peel away from the rash. Ebola causes lesions in almost every organ, although the liver & spleen are the most noticeably affected. Both are darkened & enlarged with signs of necrosis. The cause of death is normally shock, associated with fluid & blood loss into the tissues.

The haemorrhagic & connective tissue complications of the disease are not really understood, but may be related to the fact that the VP40 protein is antigenically related to human cell matrix proteins (abdominal aortic aneurism protein & MFAP-4), leading to autoimmune attack.

Why does the immune system not clear the infection? This may be associated with the two forms of the virus glycoprotein. The glycoprotein gene has a translation stop codon in the middle of it, preventing the synthesis of the full length protein. Approximately twenty percent of the mRNA isolated from infected cells had been edited to contain an extra adenosine in a stretch of seven adenosine residues at positions 1019-1026. This causes a frame shift, allowing the synthesis of the full length protein . The larger protein (130Kd - GP) is membrane associated protein, & the truncated version (approximately 60 Kd - SGP) is secreted.

A possible role for SGP is to protect the virus from the immune system as a decoy antigen. However, SGP binds to neutrophils & interferes with their function. Moreover, GP also appears to be immunosuppressive, further interfering with the response to infection.

Relatively little work has been performed on these viruses because of the difficulties of working with them, but it is now known that:

- They are possibly simian viruses, probably widespread throughout Africa, although no animal reservoir has yet been identified.
- Serological surveys in Africa suggest that asymptomatic human infections occur sporadically (i.e. subjects still alive!).

Filoviruses are a major category of emerging viruses.

- References: Click here

Books about Ebola virus: click here

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Last Updated: Saturday, October 4, 1945624.

Retroviruses

Introduction:

Retroviruses have received much attention in recent years (even before the discovery of the first human retrovirus in 1981), but they have a long history:

- Retroviruses have received much attention in recent years (even before the discovery of the first human retrovirus in 1981), but they have a long history:
- **1904:** Ellerman and Bang, searching for an infectious cause (bacterium) for leukaemia, studied leukaemia in chickens and succeed in transferring the disease from one to another by cell-free tissue filtrates.
- **1905:** Paul Ehrlich proposed his theory of 'immune surveillance':
    - Tumour cells frequently emerge in the organism
    - They are rapidly eliminated by the immune system
- **1911:** Peyton Rous transmitted solid tumours of chickens by transplanting tissue, but also isolated the infectious agent (RSV). This discovery was followed by many other examples of acutely transforming retroviruses, together with the structural characterization of the viruses involved.
- **1960's:** Howard Temin knew that retrovirus genomes were composed of RNA and observed that replication was inhibited by actinomycin D (inhibits DNA synthesis therefore he proposed the concept of reverse transcription (Nobel prize awarded to Baltimore and Temin, 1975).
- **1969:** Huebner and Todaro proposed the viral oncogene hypothesis - the transmission of viral and oncogenic information as genetic elements (rather than as a pathogenic response to a virus) - explains the vertical (germ line) transmission of 'cancers', first observed by Gross, 1951.
- **1970s:** Richard Nixon's 'war on cancer' (post Kennedy space programme - the race to the moon) - failed to find any retroviral agents which cause human cancer (many false alarms - but did pump a lot of money into biomedical research).
- **1981:** Human T-cell leukaemia virus discovered, the first pathogenic human retrovirus.
- **1983:** Human immunodeficiency virus discovered.
- Most of the retroviruses we currently know (many!) infect vertebrates, but as a group, they have been identified in virtually all organisms including invertebrates - evolutionarily successful design!

Taxonomy:

Genus:	Type Species:
Avian type C retroviruses	avian leukosis virus (ALV)
BLV-HTLV retroviruses	bovine leukaemia virus (BLV)
Lentivirus	human immunodeficiency virus (HIV-1)

Mammalian type B retroviruses	mouse mammary tumor virus (MMTV)
Mammalian type C retroviruses	murine leukaemia virus (MLV)
Spumavirus	human spumavirus (HSRV)
Type D retroviruses	Mason-Pfizer monkey virus (MPMV)

Historically, retroviruses were divided into groups based on their morphology in negatively-stained E.M. pictures:

- **A-type:** Also known as 'intracisternal particles'. Non-enveloped, (non-infectious???) immature particles only seen inside cells, believed to result from endogenous retrovirus-like genetic elements.
- **B-type:** Enveloped, extracellular particles with a condensed, acentric core and prominent envelope spikes, e.g. MMTV.
- **C-type:** As B-type, but with a central core and barely visible spikes - e.g. most mammalian and avian retroviruses (MLV, ALV, HTLV, HIV).
- **D-type:** Usually slightly larger (to 120nm) and spikes less prominent, e.g. MPMV.

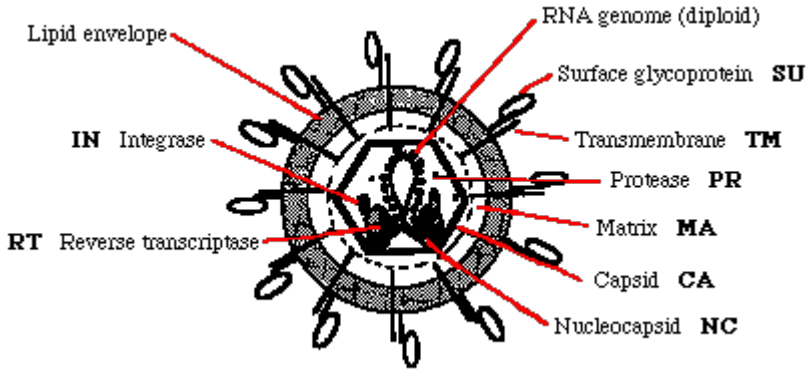
By and large, molecular genetic studies have borne out these morphologic differences, but have also largely replaced them - most comparisons now made on the basis of sequence conservation.

Retrovirus Structure:

There is considerable diversity between various types of retrovirus; the following is a generalized description of the particle. There is a universal nomenclature for retrovirus proteins:

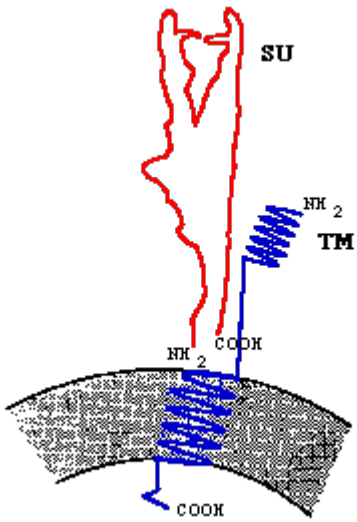
Name:	Protein:	Function:
MA	Matrix	matrix protein (gag gene); lines envelope
CA	Capsid	capsid protein (gag gene); protects the core; most abundant protein in virus particle
NC	Nucleocapsid	capsid protein (gag gene); protects the genome; forms the core
PR	Protease	Essential for gag protein cleavage during maturation
RT	Reverse transcriptase	Reverse transcribes the RNA genome; also has RNAseH activity
IN	Integrase	Encoded by the pol gene; needed for integration of the provirus
SU	Surface glycoprotein	The outer envelope glycoprotein; major virus antigen
TM	Transmembrane protein	The inner component of the mature envelope glycoprotein

All the above proteins are essential for replication; some retroviruses also encode additional essential and non-essential proteins.



Retroviruses have enveloped particles, somewhat variable in size/shape but ~100nm diameter. The envelope carries a virus-encoded glycoprotein, which forms spikes in the membrane. There are certain structural/functional similarities between the envelope glycoprotein and the influenza haemagglutinin (N.B: NO SEQUENCE SIMILARITIES). The mature protein is cleaved into 2 polypeptides:

- the outer envelope glycoprotein (**SU**), the major antigen of the virus, responsible for receptor binding, linked by disulphide bonds to:
- the trans-membrane glycoprotein (**TM**), holds the SU protein in the envelope, responsible for membrane fusion



Inside the membrane is the **matrix (MA)** protein, rather amorphous. This largely obscures the **capsid (CA)**, which is *believed* to be icosahedral. CA is the most abundant protein in the particle (~33% total weight). Inside the capsid is the **core** = RNA genome+NC protein+RT+IN. This is usually a conical, electron-dense structure clearly visible in -ve stained E.M. pictures (matrix and capsid appear amorphous).

Genome:

All retrovirus genomes consist of two molecules of RNA, which are s/s, (+)sense and have 5' cap and 3' poly-(A) (equivalent to mRNA). These vary in size from ~8-11kb. Retrovirus genomes have 4 unique features:

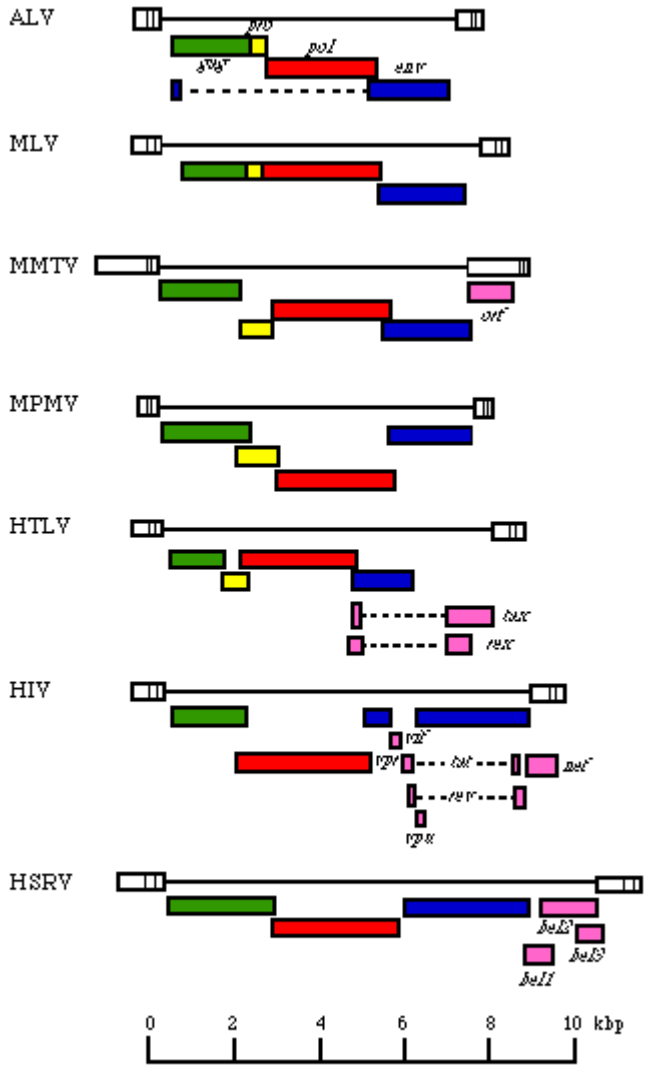
1. They are the only viruses which are truly diploid.
2. They are the only RNA viruses whose genome is produced by cellular transcriptional machinery (without any participation by a virus-encoded polymerase).
3. They are the only viruses whose genome requires a specific cellular RNA (tRNA) for replication.
4. They are the only (+)sense RNA viruses whose genome does **not** serve directly as mRNA immediately after infection.

These two molecules are physically linked as a dimer by hydrogen bonds (co-sediment). In addition, there is a 3rd type of nucleic acid present in all particles, a specific type of tRNA (usually trp, pro or lys) - required for replication

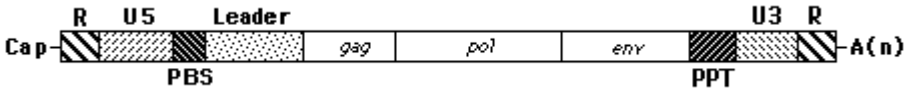
(below).
Gene order in all retroviruses is invariant:

5' - gag - pol - env - 3'

Some retroviruses have additional genes:



Sequence features of retrovirus genomes:



R Region: A short (18-250nt) sequence which forms a direct repeat at the both ends of the genome, which is therefore 'terminally redundant'.

U5: A unique, non-coding region of 75-250nt which is the first part of the genome to be reverse transcribed, forming the 3' end of the provirus genome (below).

Primer Binding Site: 18nt complementary to the 3' end of the specific tRNA primer used by the virus to begin reverse transcription.

Leader: A relatively long (90-500nt) non-translated region downstream of the transcription start site and therefore present at the 5' end of all virus mRNAs.

Polypurine Tract: A short (~10) run of A/G residues responsible for initiating (+)strand synthesis during reverse transcription.

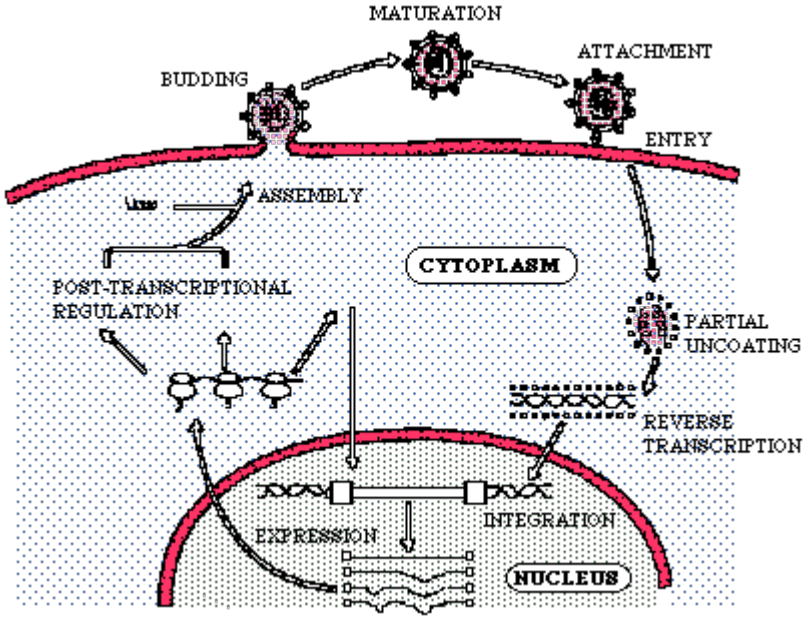
U3: A unique non-coding region of 200-1,200nt which forms the 5' end of the provirus after reverse transcription; contains the promoter elements responsible for transcription of the provirus.

Replication:

To initiate the infection, the SU envelope glycoprotein binds to a specific receptor on the surface of the host target cell. The specificity of this interaction does much to determine the cell-tropism and pathogenesis of different retroviruses, or even different isolates of the same virus (e.g. HIV). Murine retroviruses (MLVs) are sub-divided on the basis of receptor-determined host species specificity:

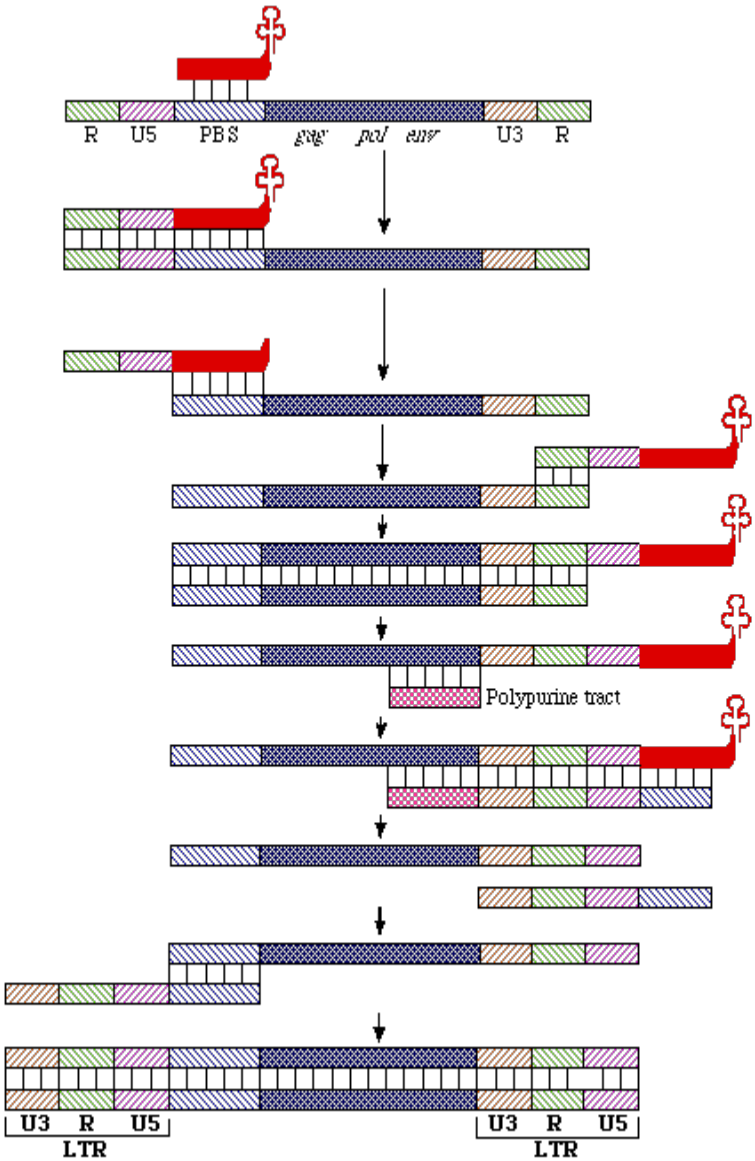
- **Ecotropic:** Infect only mouse cells.
- **Xenotropic:** Infect only non-mouse cells (e.g. rat, hamster).
- **Amphotropic:** Infect both mouse and non-mouse cells.

Interference between an exogenous virus and an endogenous virus of the same receptor specificity results in 'interference groups' of viruses (e.g. ALVs). In recent years, a number of different retrovirus receptor molecules have been identified, e.g. MuLV, HIV.

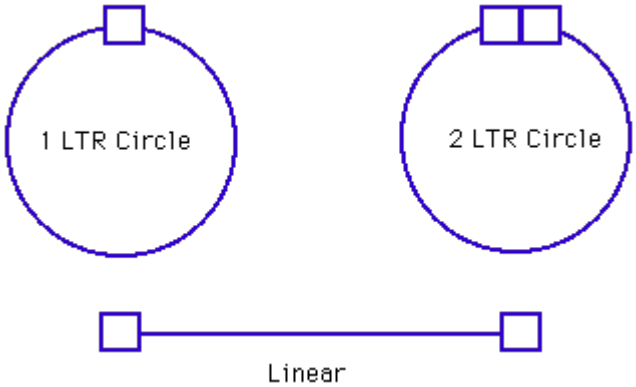


It is probable that receptor binding results in conformational changes in the glycoprotein spike, revealing the (previously masked) fusion domain in the TM protein and resulting in fusion of the virus envelope with the cell membrane. Penetration and uncoating are poorly understood, but it is now known that uncoating is only partial, resulting eventually in a core (nucleocapsid) particle within the cytoplasm. Reverse transcription occurs inside the ordered structure of this core particle - with the reactants (RT + RNA + nucleotides) free in solution, reverse transcription is initiated but cannot be completed, and aborts soon after.

Reverse Transcription:



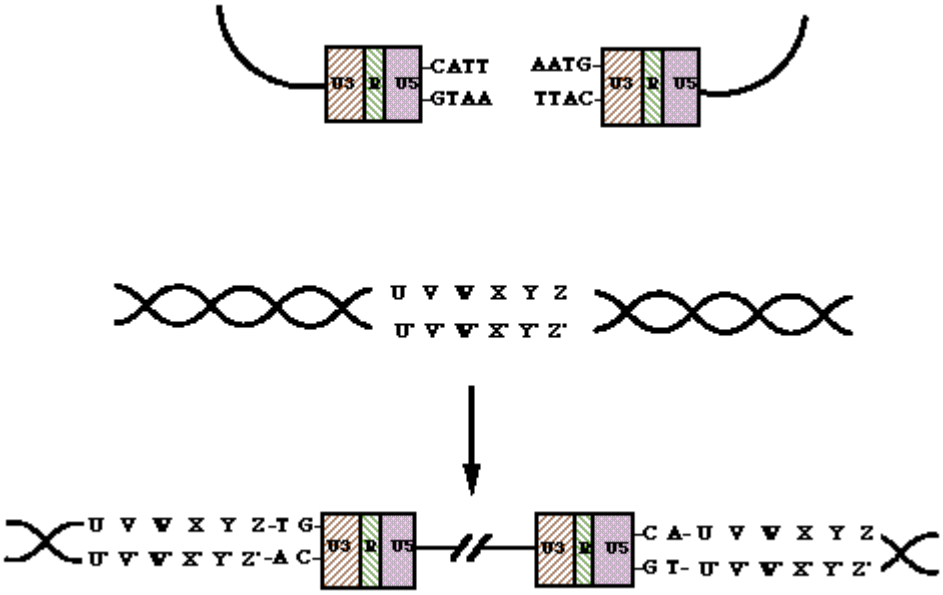
The d/s DNA product formed by this reaction is known as the **provirus** (c.f. 'prophage') and differs from the vRNA in being longer by one U3,R,U5 sequence. As a result, there is a direct repeat of this sequence present at each end of the provirus genome, and these are known as the long terminal repeats (LTRs). Three forms of provirus DNA are found in all infected cells:



It is not clear how these are related to one another, but the circles probably form by intracellular ligation. The linear and 2-LTR circle forms are infectious (unlike the (+)sense vRNA!). Reverse transcription occurs in the cytoplasm, after which the provirus DNA migrates into the nucleus.

Integration:

Catalysed by the IN polypeptide (part of the RTase complex). Integration is a highly specific reaction with respect to the provirus, but random with respect to host cell DNA. Formerly, it was thought that the 2-LTR circle was the substrate for integration, but it is now believed that the linear form (probably the direct product of reverse transcription) is the actual substrate used.



The ends of the LTRs consist of inverted repeats of 4-6 bp. These are brought together to form a cleavage site for IN and are cleaved to form a staggered cut. This molecule is then inserted into the host cell DNA. The net result of the integration process is that:

1. The integrated provirus contains 1 or 2 less bases at the end of each LTR
2. The ends of the integrated LTRs always have the same sequence: 5' - TG...CA - 3'
3. 4-6 bp of host cell DNA flanking the integrated provirus are duplicated.

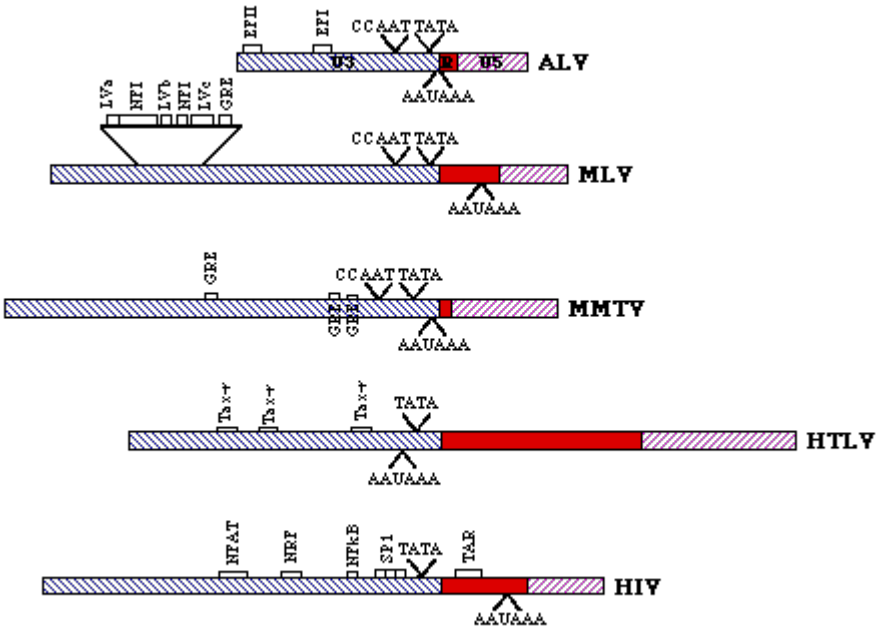
These observations can be explained by a model where a staggered cut (5' overhang) is introduced into both the ends of the LTRs and the host cell DNA, followed by joining of the cut ends and repair of the free 3' ends. Once integrated, the provirus is present for the lifetime of the cell (think about germ-line integration). There is no specific mechanism for excision of the provirus (c.f. lambda), and the infected cell cannot be 'cured'.

Gene expression:

Retroviruses use the cellular transcriptional machinery for expression (although a few encode additional transcriptional and post-transcriptional regulatory factors - HTLV and HIV). Therefore they are expressed like cellular genes. To compress maximal information into a small genome, they make use of a number to 'tricks', such as splicing and ribosomal frameshifting.

LTR Structure:

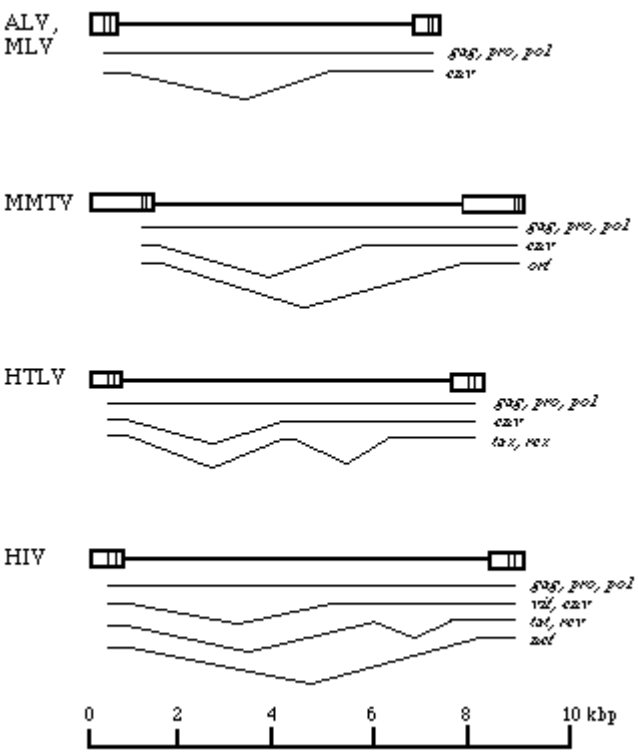
The U3 region of the LTR contains the promoter elements responsible for the initiation of transcription:



In recent years, various LTRs have been intensively studied and dissected by molecular techniques, including:

- nucleotide sequencing (and comparison with cellular promoter elements with known functions)
- nuclease protection studies:
  - S1 protection to determine precise transcription start sites
  - DNAse I protection to determine DNA-binding protein sites
- in vitro transcription studies (reconstitute 'the nucleus' in vitro).

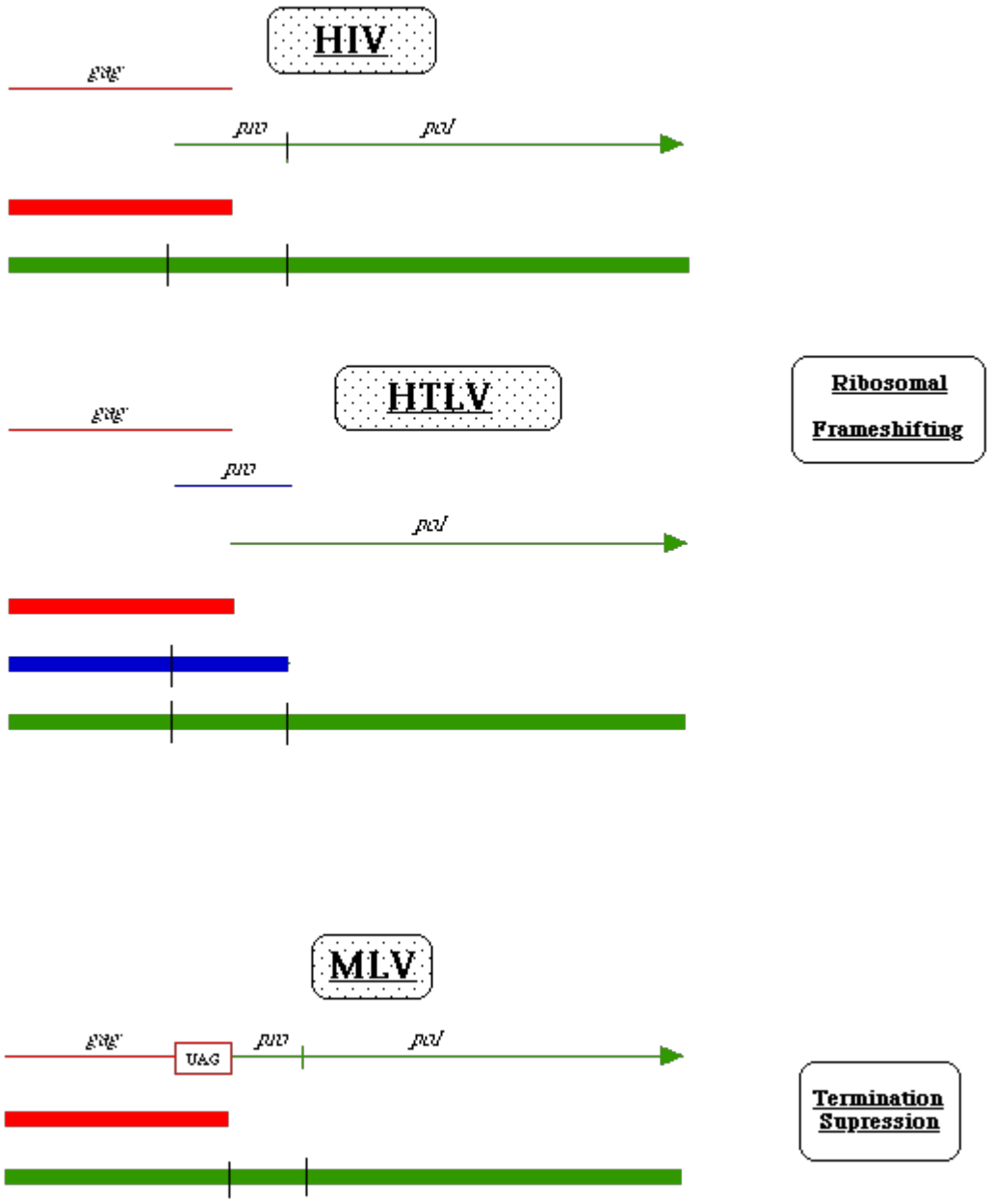
Splicing:



Splicing is regulated by the cellular apparatus which interacts with cis-acting sequences present in the mRNA. The proteins encoded by *gag*, *pol* and *pro* (see below) genes are expressed from a full length genomic RNA (= vRNA). The env protein is expressed from a spliced mRNA. In more complex retro's, e.g. HTLV, Lentiviruses, there are multiply spliced mRNAs are produced. Pattern of splicing in HIV is very complex!

Expression of the protease gene: *pro* overlaps *gag* and/or *pol*, but is still expressed from the same full-length

mRNA. Different viruses have a variety of post-transcriptional strategies to do this:



Assembly:

B- & D-type viruses: Capsid/nucleocapsid assembly occurs in the cytoplasm, later bud out through the cell membrane, acquiring the envelope during this process.
C-type viruses: Assembly occurs at the cell surface. Thickened patches begin to form in the membrane (env proteins on outer surface, gag proteins inside). The genome is packaged as the particle buds out through the membrane.

With both types, **maturation** occurs after the particle has budded, by cleavage events catalysed by the protease. Considerable structural changes occur during this process, resulting in the smooth gag shell of the immature particle being completely rearranged and leading to the condensation of the core visible in mature particles. To see a video animation of this process, click here.
N.B. - some types of retrovirus, notably Lentiviruses, are capable of infecting cells by direct cell-to-cell contact, without the formation of infectious extracellular particles.

To view a negatively stained electron micrograph of HIV particles (C-type), click here.

To view a video animation of HIV replication, click here.

Genetics:

The genetics of retroviruses are complex:

- High mutation rate - reverse transcription is an error-prone process.
- Recombination - occurs during recombination, promoted by the combination of 2 strands of RNA into 1 d/s DNA provirus.
- Interactions with the host cell - insertional mutagenesis, transduction.

Endogenous Retrovirus-Like Genetic Elements: 5-10% of the mammalian genome is composed of retrovirus-like 'retrotransposons' !

Pathogenesis:

Retroviral pathogenesis has concentrated on oncogenesis, but retroviruses also cause a variety of haematopoetic and neurological conditions.

It was recently reported that an ancient retrotransposon insertion is the cause of Fukuyama-type muscular dystrophy, one of the commonest autosomal recessive disorders in Japan (Kobayashi et al, Nature 394: 388-392, 1998). To date this is the only known instance of insertional mutagenesis of the human genome caused by this type of element, but other examples look certain to be discovered in future.

Retroviruses are under active development as vectors for gene therapy.

Pretend to be a Retrovirus!

- References: Click here
- Search for more information on this topic
- Search **MEDLINE** for the latest publications on this topic:

Click Here

Books about Retroviruses: click here

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Last Updated: Saturday, October 4, 1945624.

Viral Hepatitis

Hepatitis = 'inflammation of the liver' and as such is not a single disease (just like 'cancer'). Because of the central role of the liver in metabolism, just about all viruses are capable of involving the liver in some instances, however, there are at least six viruses which specifically seem to infect and damage hepatocytes. No two of these belong to the same family!

Definitions:

- **Acute:** Short term and/or severe.
- **Chronic:** Lingering or lasting - may or may not be severe.
- **Fulminant:** Developing quickly and lasting a short time, high mortality rate.
- **Cirrhosis:** Hardening: may be the result of infection or toxins (e.g. alcohol)
- **Jaundice:** Yellowing of the skin, eyes, etc due to raised levels of bilirubin in the blood due to liver damage.

Non-A, Non-B (NANB) Hepatitis:

Acute, infectious hepatitis was recognised in 1885, but the first infectious agent (HBV) was not identified until the 1960's. HAV was isolated in 1973, but this still left many cases which could not be ascribed to either virus, referred to as 'NANBH'. (N.B. There are many non-infectious causes of hepatitis, e.g. toxins, drugs, autoimmunity).
Now that six 'hepatitis viruses' have been identified, there are few cases of 'non-A-E-hepatitis', but how many more viruses wait to be discovered? Reports continue to circulate concerning the existence of still other hepatitis viruses. Some of the agents are reported to be sensitive to chloroform (i.e. enveloped) while others are not. This may suggest the existence of multiple viruses, as yet undescribed, but this is still uncertain. It is likely that further hepatitis viruses will be described at some time in the future.

- Hepatitis A Virus (HAV)
- Hepatitis B Virus (HBV)
- Hepatitis C Virus (HCV)
- Hepatitis D Virus (HDV)
- Hepatitis E Virus (HEV)
- GBV-C/HGV
- TTV

Books about Viral Hepatitis: click here

- References: Click here
- Search for more information on this topic
- Atlas of Liver Pathology
- Viral Hepatitis

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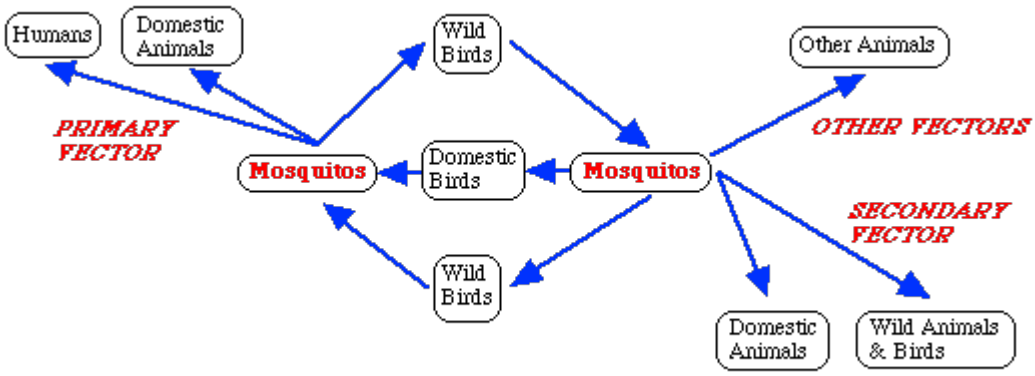
'Arboviruses'

There are a large group (more than 400) of enveloped RNA viruses which are transmitted primarily (but not exclusively) by Arthropod vectors (mosquitoes, sand-flies, fleas, ticks, lice, etc) & which were previously grouped together under the name 'Arboviruses'. More recently, this disordered assemblage has been split into 4 bona fide virus families:

Family:	Genus:	Type Species:	# Serotypes:
Togaviridae	Alphavirus	Sindbis	27 serotypes
	Rubivirus	Rubella	1 serotype
Flaviviridae	Flavivirus	Yellow fever	69 serotypes
	Pestivirus	Bovine viral diarrhoea	3 serotypes
	Hepatitis C virus	HCV	1 serotype (variable!)
Bunyaviridae	Bunyavirus	Bunyamwera	168 serotypes
	Hantavirus	Hantaan	32 serotypes
	Phlebovirus	Sandfly fever	51 serotypes
	Nairovirus	Crimean-Congo haemorrhagic fever	35 serotypes
	Tospovirus	Tomato spotted wilt	2 serotypes
	Unassigned		42 serotypes
Arenaviridae	Arenavirus	Lymphocytic choriomeningitis	17 serotypes

Mostly, these viruses are relatively fragile (e.g. not resistant to desiccation), therefore many are reliant on vector for transmission. This dependency tends to limit them to tropical & sub-tropical regions (some exceptions - rubella, HCV).
They have complex life-cycles & replicate in both the primary hosts, secondary hosts (which may often be dead-ends) & the Arthropod vectors. Therefore, there may be several animal reservoirs for each virus - eradication would be practically impossible & the best approach is to block transmission by human vaccination/eradication of the vector (e.g. mosquitoes):



Medscape Article: Vector-Borne Disease Surveillance and Natural Disasters

Morphology:

All are enveloped:

- **Togaviruses:** Spherical, 65-70nm; Capsid: 240 monomers, icosahedral, T=4; Envelope: 80 trimer spikes, each spike = 3 x E1/E2 heterodimers, T=4 arrangement.
  Image reconstruction of Semliki Forest virus.

- **Flaviviruses:** Spherical, 40-60nm; Capsid: Symmetry indistinct, 2 proteins: nucleocapsid ('C') and matrix ('M'); Envelope: 1 glycoprotein ('E').

- **Bunyaviruses:** Spherical, 80-120nm Nucleocapsid: Helical, 3 segments, 2 proteins - nucleocapsid ('N'), RNA polymerase ('L'); Envelope: 2 glycoproteins, G1 and G2, spikes less obvious.

- **Arenaviruses:** Pleiomorphic, 50-300nm; Nucleocapsid: As Bunyaviruses, helical; Envelope: 2 glycoprotein spikes 'GP1' 'GP2'.

Genome:

- **Togavirus Genomes:** Single-stranded, (+)sense, non-segmented RNA, ~11.7kb, 4-8% total weight of particle. Resembles cellular mRNAs: i.e. 5' cap, 3' poly-A.

- **Flavivirus Genomes:** Single-stranded, (+)sense RNA, ~10.5kb. 5' cap but not polyadenylated. Genetic organization differs from Toga's - structural proteins at 5' end of genome, N.S. at 3' end.

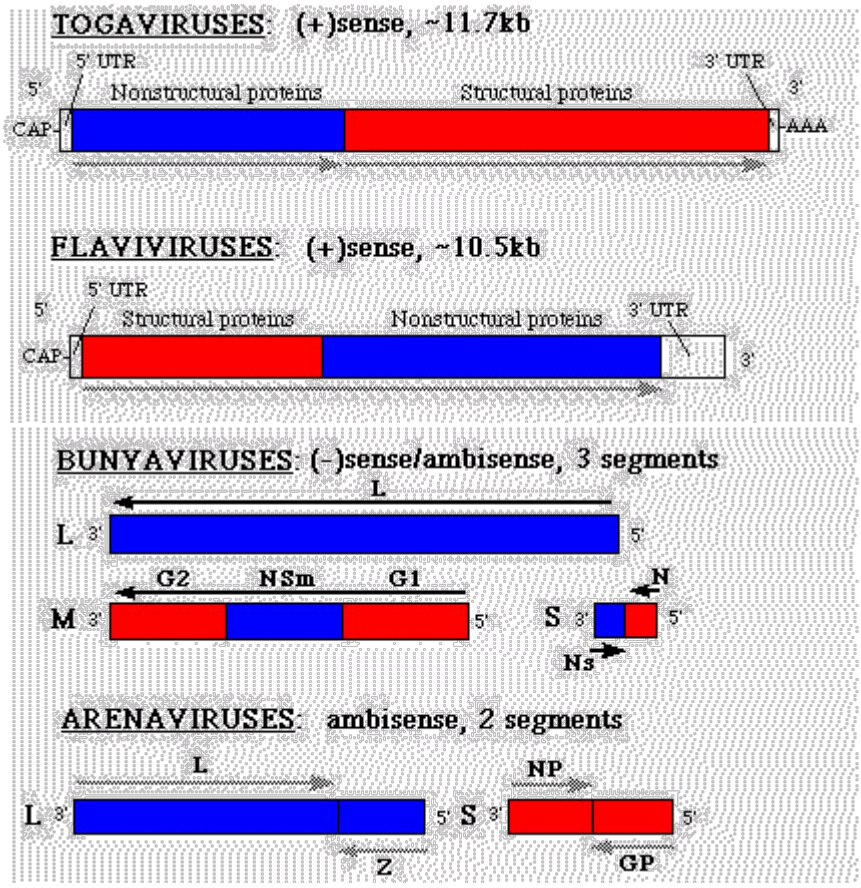
- **Bunyavirus Genomes:** s/s (-)sense RNA; segmented - each virion contains 3 molecules:

  Segment / Coding capacity:
  L ~8.5kb / L, other ?
  M ~5.7kb / G1, G2, NSM
  S ~0.9kb / N, NSS

  All 3 RNA species are linear, but in the virion, appear circular because the ends are held together by base-pairing. Not present in equimolar amounts! 5' ends not capped; 3' ends not polyadenylated; genomic RNA not infectious.
  Phleboviruses: Differ from the other 4 genera (Bunyavirus, Nairovirus, Hantavirus, Tospovirus) in the following respect: Genome segment S organization is different - **AMBISENSE** - 5' end is (+)sense, 3' end (-)sense.

- **Arenavirus Genomes:** Linear, s/s RNA. Two segments - L (~5.7kb) - encodes L and Z proteins and S (~2.8kb); 5' part is (+)sense and encodes N protein; 3' part (-)sense, encodes G protein. BOTH genome segments have an **ambisense** organization. Replication is similar to that of Phleboviruses.

Arbovirus Genomes:



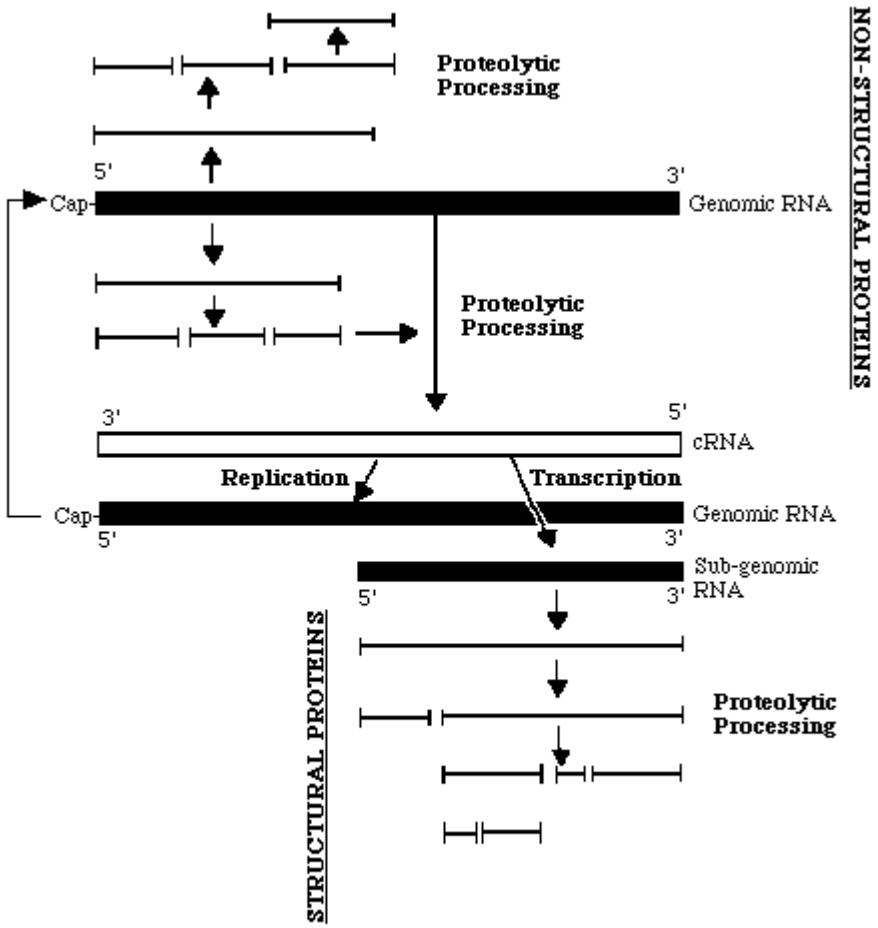
Replication:

Togavirus Replication:

Characteristically, 2 rounds of translation:

i. (+)sense genomic RNA ('49S' = 11.7kb) acts directly as mRNA and is partially translated (5' end) to produce N.S. proteins.
ii. These proteins are responsible for replication, forming a complementary (-)strand, the template for further (+)strand synthesis.
iii. Two species of (+) RNA are synthesized, full length genomic RNA and sub-genomic mRNA ('26S' = 4.1kb).
iv. Translation of the newly synthesized sub-genomic RNA results in production of structural proteins (from 3' end of genome).
v. Assembly occurs at the cell surface, and the envelope is acquired as the virus buds from the cell. Release and maturation almost simultaneous.

Replication occurs in the cytoplasm and is rapid (~4h c.f. 20-30h for Flaviviruses). Cellular receptors are not known - obviously widely distributed. Glycoprotein spikes are responsible for receptor binding - antisera neutralize attachment.



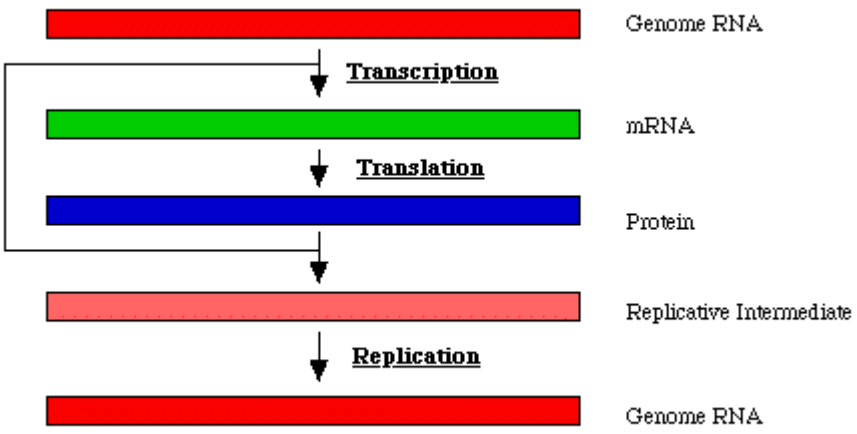
Flavivirus Replication:

Initial stages are similar to Toga's (occurring in cytoplasm), but there are significant differences:

i. The entire virus genome is translated as a single polyprotein which is then cleaved into the mature proteins (as Picornaviruses, c.f. Toga's).
ii. Complementary (-)strand RNA is synthesized by N.S. proteins, used as a template for genomic progeny RNA synthesis.
iii. Assembly occurs during budding, characteristically into cytoplasmic vacuoles rather than the cell surface as Toga's. Release occurs when cell lyses.

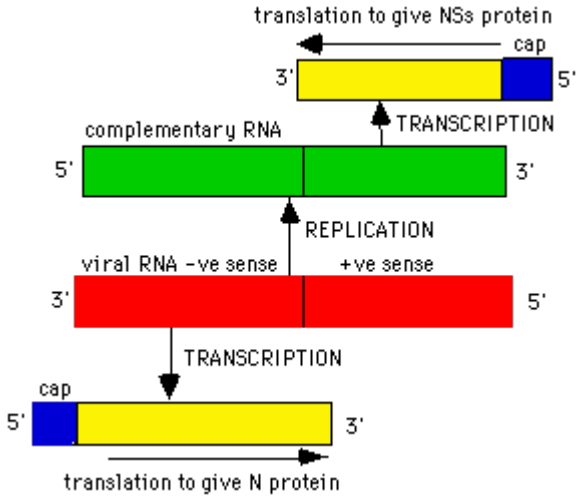
Bunyavirus Replication:

Similar to Orthomyxoviruses:



i) Virus polymerase (L protein) copies genome to form:
 a) mRNA encoding N protein - 5' m7G cap is obtained by cannibalizing host cell mRNAs - like influenza.
 b) (+)sense intermediate
ii) (+)sense intermediate is copied by L protein to form new genomic RNA.
iii) Virus buds into Golgi vacuoles - released when cell lyses (c.f. Toga's - bud from plasmalemma, Flavi's - bud into undifferentiated cytoplasmic vacuoles).

Phleboviruses: follow same basic strategy, but there are 2 rounds of transcription (one before, one after the formation of a 'reverse-sense' RNA intermediate) to cope with the ambisense coding strategy:

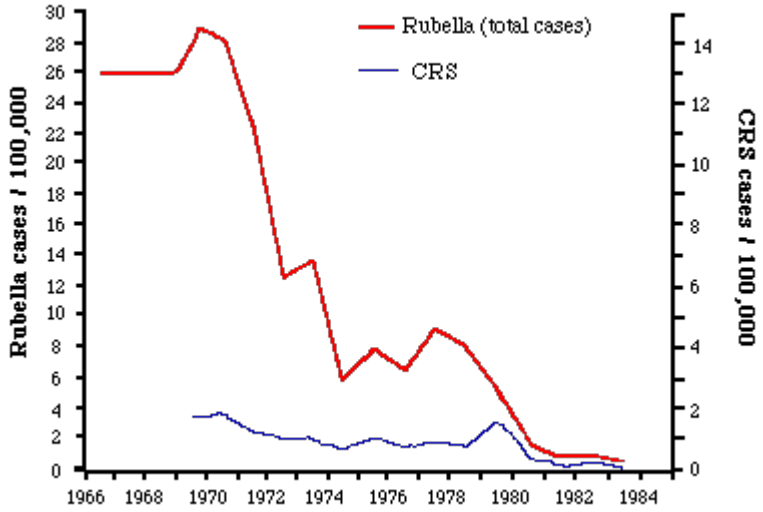


Togaviruses:

Host Range: Broad, grow in both mammalian and insect cell lines, characteristically producing c.p.e. in the former but not the latter (mechanism unclear). Only Alphaviruses are arthropod-borne.
Pathogenesis: Virus is transmitted from the salivary glands of the mosquito to the bloodstream of the vertebrate host. Virus travels to the skin and reticuloendothelial system (spleen and lymph nodes), where the primary infection occurs, then viraemia follows - systemic infection. Can involve CNS (esp. encephalitis), skin/bone marrow/blood vessels (haemorrhagic fevers). Not well understood.
Treatment: A few experimental vaccines, none commonly in use.

Rubella virus:
One species, quite distinct from Alphaviruses. Limited host range - mammalian cells only. First recognised as a distinct disease in 1814 (as opposed to Measles/Scarlet fever). Association with congenital abnormalities recognised by Gregg 1941 (epidemic of congenital cataracts) - the first recognition of a virus as a teratogenic agent (i.e. capable of disrupting normal foetal development). Virus isolated in 1962. Similar to other Toga's, but slightly smaller (particle 60-70nm; genome ~11kb).

Host Range/Transmission: Can grow in a variety of mammalian cells. No known invertebrate host - man is the only reservoir, hence this virus is targeted by W.H.O. for eradication. Transmitted by aerosols - highly contagious.
Pathogenesis: Adult infections frequently sub-clinical! Characteristic pink, continuous maculopapular rash appears in 95% of adolescent patients 14-25 days (av. 18d) after infection - patient is infectious for most of this time. After early viraemia, virus multiplies in many organs, particularly lymph nodes (lymphadenopathy), including the placenta, but symptoms in adults are rare. In children, a mild febrile illness - less severe than measles. Virus crosses placenta and multiplies in the foetus. Up to 85% of infants infected in the first trimester of pregnancy get congenital rubella syndrome (CRS) - low birth weight, deafness, CNS involvement, abortion. The earlier in pregnancy infection occurs, the worse.
U.S. epidemic 1964: 20,000 cases of CRS - the last major epidemic in the USA (pre-vaccine).
Foetus is persistently infected (presumably due to immature immune response) and continues to excrete virus after birth - a risk to doctors, nurses and other patients.
Prevention/Control: A live attenuated vaccine has been used in the USA since the late 1960's and more recently in the UK (as MMR). For women infected during first trimester of pregnancy, theraputic abortion may be recommended.



Flaviviruses:

Host Range: Similar to Toga's. Can survive for long periods in hosts such as ticks by replicating in this host (without damage to the insect). In vitro, replicate in many insect and mammalian cell lines.
Pathogenesis: Similar to Toga's, but produce a wider range of diseases - (fever; arthralgia; rash; haemorrhagic fever; encephalitis). Outcome of infection is influenced by both virus and host-specific factors (age, sex, genetic susceptibility, pre-exposure to same or related agent).

Yellow Fever: (Latin 'flavus' = yellow). One virus, of invariant serotype, first recognised by Walter Reed, 1900 (Panama Canal). Transmitted by mosquitoes.
Pathogenesis: Transient viraemia, primary multiplication in lymph nodes; secondary multiplication occurs in liver (jaundice), spleen, kidneys, heart and bone marrow with much tissue damage. Very debilitating, mortality ~10%. Genetic variation between different human populations results in various severity of disease, but genes involved are not known.
17D - live attenuated vaccine strain (Theiler 1937) - very effective. Has eradicated Yellow Fever from USA - much more difficult to tackle in central and S. America where mosquito control is less effective.

Tutorial on Yellow Fever.

Dengue Fever: First described 1780, virus isolated by Sabin 1944.
At least 4 serotypes; Major health problem worldwide (Asia, Africa and America). Transmitted by mosquitoes - not affected; an infected mosquito may infect others (not via man). Primary infection produces a (relatively) mild, self-limited, febrile illness. Re-infection with a different antigenic type of the virus may result in dengue haemorrhagic

fever: high fever, haemorrhagic shock, myocarditis, encephalitis; mortality ~15% - probably autoimmune mediated. No vaccine yet.
Medscape Article: "Emergence of Dengue Hemorrhagic Fever in the Americas".

Bunyaviruses:

More than 200 species - the largest family of viruses.
Host range: Natural hosts include a variety of Arthropods and mammals. Can replicate extensively in insects - transovarian passage allows overwintering.
Pathogenesis: Varied, because they are a very large group of viruses, but generally: Insect bite results in transient viraemia; replication then occurs in target organs - varies from one virus to another, as does severity (mild to severe).

Rift Valley Fever: First isolated from sheep in E.Africa 1930. In man, produces an acute, 'flu-like illness. Transmitted by mosquitoes from animal reservoirs (e.g. sheep) to man leads to EPIZOOTICS. In the last decade, there have been massive outbreaks of R.V.F. in sub-Saharan Africa - millions of people infected, attack rates of up to 35% - more mosquitoes, more people or more domestic animals ?

Jan 16th, 1998: The U.N. Food and Agriculture Organisation (FAO) warned that an outbreak of Rift Valley Fever in Kenya and Somalia thought to have killed at least 600 people risked spreading to other parts of Africa. "A serious outbreak of Rift Valley Fever in parts of northeastern Kenya and adjacent areas of Somalia constitutes an international emergency," said a statement. According to the latest reports from the World Health Organisation (WHO), the disease has killed 300 people in Kenya and claimed another 300 lives in Somalia. Heavy flooding in parts of Kenya has brought people into closer contact than usual with animals over the past two weeks, the WHO says.
FAO said there was a high risk the disease could spread to other countries in the region. "As well as new areas of Kenya and Somalia the countries most at risk are Sudan, Egypt, Ethiopia, Uganda and Tanzania," the statement said. The organisation warned that because mosquitos could be transported long distances on the wind there was even a risk the disease could cross the Red Sea to the Arabian peninsula. The only effective means of protecting livestock was through preventative immunisation and that vaccination once an epidemic has taken hold usually fails to prevent substantial livestock losses. There is no vaccine for humans.

To find the latest news on Rift Valley Fever,
visit "The Microbiology Newsroom"

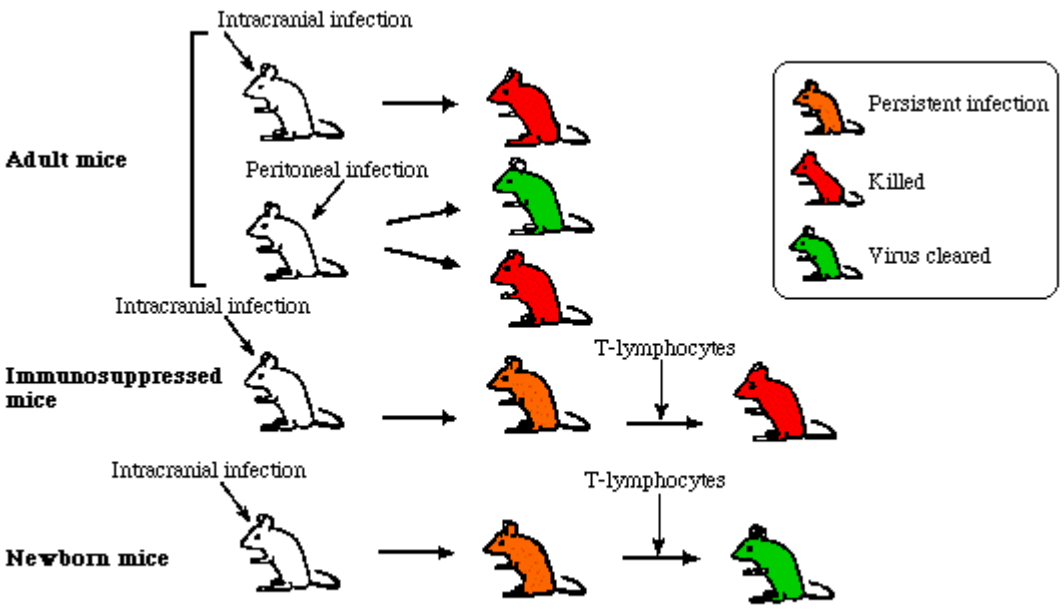
Sand Fly Fever (Phlebotomous Fever): Transmitted by Phlebotomous flies (sand-flies). Common in the Mediterranean - a big problem during WWII. An acute, febrile illness controlled by control of vector with insecticides.

Hantavirus genus: Spread from rodents (reservoir) to man by aerosolized faeces, not insect vector. These are important emergent viruses.

Arenaviruses:

A relatively new family (17 types) which first came to prominence with the identification of a 'new' disease in 1969 (Lassa fever).
Host Range: Rodent viruses - do not appear to require arthropods for spread. Do not infect insect cells.
Pathogenesis: In natural (rodent) hosts, produce chronic (life-long) infections. Also capable of establishing a persistent infection in cell lines - a model for this type of infection.

LCMV: A murine virus which has been studied in detail as a model for chronic virus infection. In mice, virus does not cause much cell damage. Pathogenesis is believed to result from CTL response to virus-infected cells. Rarely infects man, causing a mild disease (occasionally severe with haemorrhaging).



Lassa Fever: Natural host is small Nigerian rodent, *Mastomys natalensis* - transfer to man occurs via droppings (not Arthropods). Human infections (rare) are highly infectious, produce severe, systemic febrile disease with high mortality. This contrast with the rodent infection where there is apparently no pathology (virus is adapted to host).

Many Arboviruses are particularly important in that they are emerging viruses.

Emerging Infections Information Network: Thomas P.C. Monath, M.D. "The Resurgence of Yellow Fever".

- References: Click here
- Spotlight on Arboviral Encephalitis
- Emerging Viral Diseases
- Search for more information on this topic
- Search **MEDLINE** for the latest publications on this topic:

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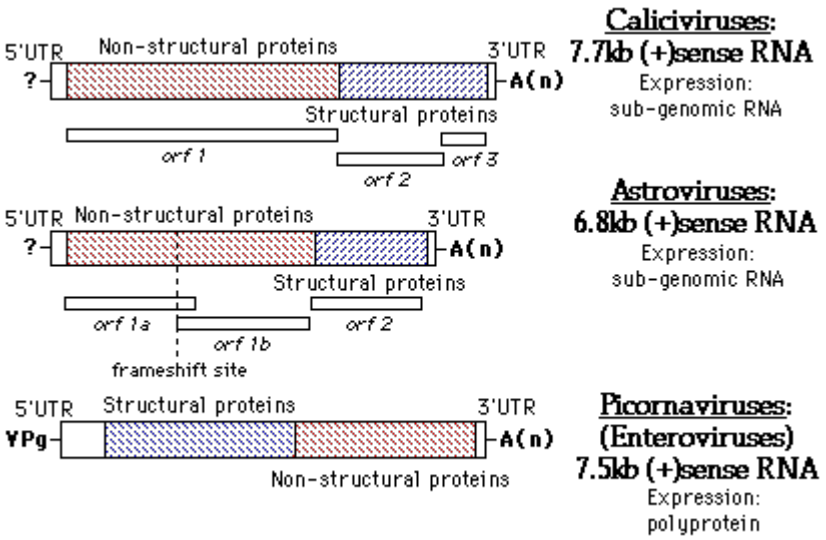
Last Updated: Saturday, October 4, 1945624.

Diarrhoea Viruses: Astroviruses; Caliciviruses; Reoviruses (Rotaviruses)

Diarrhoea is not as dramatic an illness as AIDS. However, the above groups of viruses are of great economic importance, causing millions of lost working days each year, as well as much discomfort. Diarrhoea continues to be a major cause of morbidity and mortality worldwide resulting in an estimated 1000 deaths among children each day, the highest incidence being in developing countries of the world. One well documented source of infection is the consumption of shellfish (polluted by sewage) - and therefore, they also have economic consequences for fishermen and the food industry. Such viruses often cause mini-epidemics in families, hospital wards, etc and are potentially very dangerous to seriously ill hospital patients. More importantly, these viruses contribute to the massive mortality caused by infantile diarrhoea in developing countries and are responsible for uncounted millions of deaths each year.
The human pathogens in these groups have been poorly studied since for the most part, they do not grow in culture. Only recently has molecular information on the nature of their genomes become available. The taxonomy of these viruses is still somewhat confused and they have previously been grouped on the basis of their appearance in the EM (unreliable). Recently, nucleotide sequences of members of each group have been determined, allowing genome organization to be used as a basis for future classification:



Group:	Particle Morphology:	Structural Proteins:
Caliciviruses:		
Norwalk group:	Spherical, without surface definition	1 (~60kD)
"HuCV" (e.g. UK1-4):	Prominent surface hollows, 6-pointed star	1 (~62kD)
Astroviruses:	Spherical, 5/6 pointed star without central depression	3 ? (cleaved from polyprotein precursor, c.f. Picornaviruses)
Parvovirus-like:	Spherical, featureless	??? (DNA genome)
"Small Round Viruses" (SRVs):	Spherical, featureless	??? (RNA genome)

Caliciviruses:

Human caliciviruses (HCV) are commonly identified by electron microscopy in faecal specimens from patients with diarrhoea, although serological assays are now becoming available (cannot be diagnosed on clinical grounds. These viruses cannot be cultured in vitro & there are no animal models available other than experimental transmission to human volunteers. These viruses are very widely distributed worldwide & infection is very common, especially in children - most adults appear to have been infected & may or may not be immune to reinfection. Calicivirus infection is associated with diarrhoea or vomiting lasting 1-4 days (incubation period 1-2 days). The most frequent source of infection appears to be contaminated food/beverages - may cause up to 90% of food-related gastroenteritis outbreaks. Treatment: supportive only (rehydration).

Phylogenetic analysis of the genus Calicivirus in the family Caliciviridae based on the nucleotide and amino acid sequences of human and animal calicivirus 3D RNA-dependent RNA polymerase (approximately 470nt) and capsid hypervariable regions (approximately 1,200nt) to generate phylogenetic trees indicates that there are five separate genogroups:

- small round-structured viruses (SRSV)
- Sapporo-like human caliciviruses (HuCVs)
- hepatitis E virus (HEV)-like HuCVs
- rabbit-like animal aclicivirus (AnCVs)
- vesicular exanthema of swine virus (VESV)-like AnCVs

To view an electron micrograph of calicivirus particles, click here. The cup-like depressions which give this family of viruses their name are just visible if you observe the perimeter of these negatively stained particles.
Medscape Article: Calicivirus Emergence From Ocean Resevoirs: Zoonotic and Interspecies Movements.

Astroviruses:

Astroviruses were first described in 1975 as a result of EM studies of an outbreak of diarrhoea in a maternity unit and a survey into the etiology of diarrhoea in childhood in a Scottish city. Subsequently astroviruses were experimentally transmitted to adult volunteers and adapted to growth in foetal LLCMK2 cells. Restrictions on the availability and use of foetal tissue limited the cultivation of astroviruses and therefore studies were confined to a few laboratories that access to electron microscopes. Surveys conducted using EM showed that astrovirus infection occurs world-wide and accounts for 2-8% of cases of diarrhoea in infants. A recent pilot study investigating the causes of infectious intestinal disease in England based on surveillance in GP practices showed that astroviruses were the most frequent viral cause (Roderick et al, Epidemiol Infect 1995:114: 277-288). A major advance in the ability of laboratories to diagnose astrovirus came as the result of the finding that they could be propagated in a continuous line of colon carcinoma cells CaCO2 (Willcocks et al, Arch Virol, 1990; 113: 73-81). The development of enzyme immunoassays (EIAs) for detecting viruses in stools showed that astrovirus is a significant cause of diarrhoea in developing countries. In Thailand, 8.6% of cases of diarrhoea were associated with astrovirus and a similar rate was found in Guatemala (7.3%). Application of EIA to study outbreaks in day care centres in the USA showed that many more cases were detected by EIA than by electron microscopy, the most widely used diagnostic technique.

The name of the family is derived from their distinctive appearance in electron microscopy - a smooth or slightly indented outer electron-dense shell with an inner, negatively staining 5 or 6 pointed star shaped core. To view a negatively-stained electron micrograph of astrovirus particles, click here.

Clinically, these viruses cause similar symptoms to caliciviruses. Astroviruses have been isolated from birds, cats, dogs, pigs, sheep, cows & man. There are at least 7 human astrovirus serotypes. Like rotaviruses, astrovirus infections occur through the year with peaks in the winter months. Infections have been shown to occur mainly in childhood. A serological survey of 87 with children for antibody prevalence to astrovirus in England found that 67% of the children became seropositive by the age of 4 years and 87% by age 10. Other studies showed that most of the cases of infection were detected in children under five years of age with the majority of the children being under 1 year of age. Outbreaks of astrovirus infection involving children and elderly patients have been described and prolonged excretion documented in immunosuppressed, immunodeficient and AIDS patients. Significantly higher seroprevalence rates of astrovirus have been reported in adults exposed to contaminated water compared with

a control group (Myint et al, Lancet 1995; 343:1640-1). Studies have shown that astrovirus type 1 is the prevalent strain in the UK accounting for 65% of the cases. However this situation may be changing with the emergence of more cases of astrovirus type 4. In contrast, type 2 was the most prevalent in Mexico (31%) and type 1 relatively rare (6%) and there are reports of outbreaks of foodborne astrovirus infection in Japan involving thousands of children and adults associated with astrovirus type 6.

Small Round Structured Viruses (SRSVs):

The status of these other viruses at present uncertain. Undoubtedly, some isolates will be found to be bona fide Caliciviruses or Astroviruses when molecular data is obtained. As to whether there are as yet unidentified families of viruses which cause gastroenteritis - ???

Reoviruses:

Respiratory **E**nteric **O**rphan viruses, i.e. infect the human respiratory and intestinal tracts, *usually* without disease symptoms. First recognised in 1959 - previously (wrongly) classified as echoviruses (Picornaviridae). There are >150 species in the family Reoviridae. They are a diverse group, infecting invertebrates, vertebrates and plants, but are unified by their most unique feature - the composition of their genome - **d/s RNA**.

Genus:	Type Species:	Hosts:
Aquareovirus	golden shiner virus	Fish
Coltivirus	Colorado tick fever virus	Primary host - Insects; Secondary host - mammals
Cypovirus	Bombyx mori cypovirus 1	Insects
Fijivirus	Fiji disease virus	Insects, plants
Orbivirus	bluetongue virus 1	Primary host - Insects; Secondary host - mammals
Orthoreovirus	reovirus 3	Mammals, birds
Oryzavirus	rice ragged stunt virus	Plants
Phytoreovirus	wound tumour virus	Plants
Rotavirus	simian rotavirus SA11	Mammals

General Characteristics:

- 70-85nm diameter, nearly spherical icosahedral particles
- Non-enveloped, capsid = double shell of proteins
- Genome = 10-12 segments d/s RNA
- Replication: occurs in cytoplasm; incomplete uncoating of virions, which possess all the enzymes required for d/s RNA transcription (not in cells!)

Host Range:

This family is ubiquitous in nature - infecting invertebrates, vertebrates and plants. Abs have been found in all species of mammals tested (except whales), implying wide cell tropism/ubiquitous receptor (see below).

Morphology:

All are similar: Non-enveloped, icosahedral capsids (T=13) composed of double protein shell:
Outer shell ~80nm diameter; Inner shell ~60nm diameter. The various genera are serologically unrelated. Proteins:

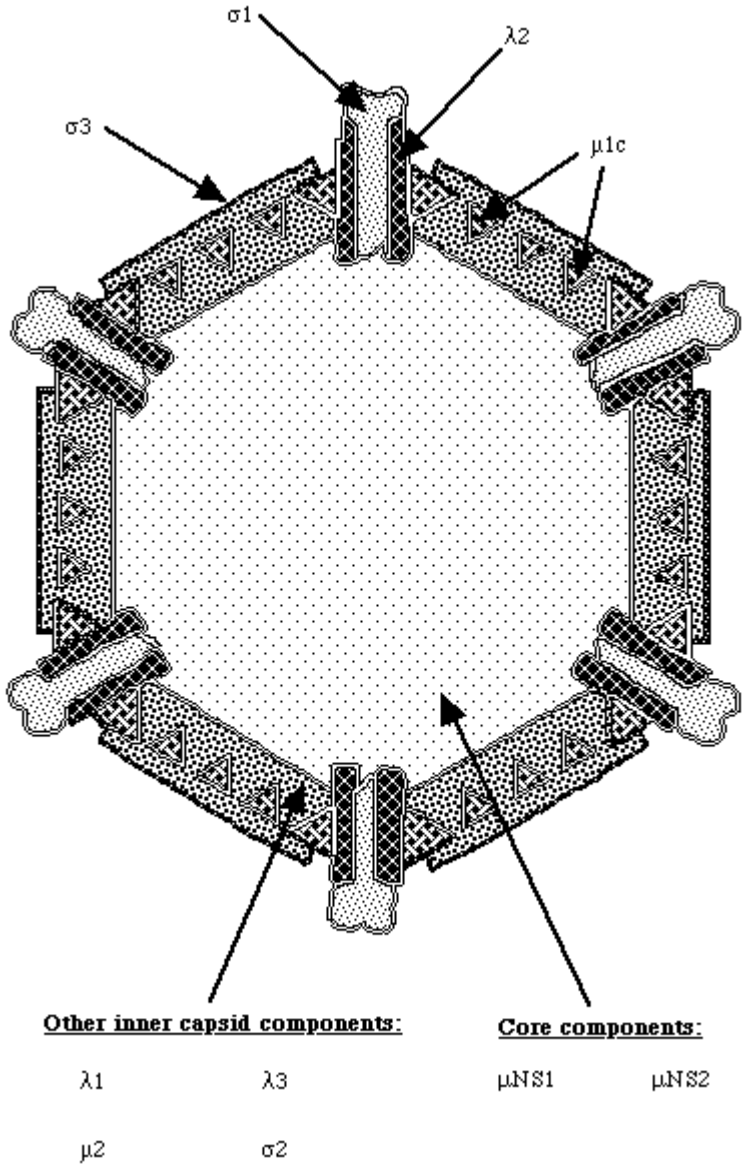
Genus:	Outer capsid:	Core:	Non-structural:
Reoviruses:	s-3, m-1c, s-1 (HA)	l-1, s-2, l-2, l-3, m-2	m-NS, s-NS
Orbiviruses:	VP2 (HA), VP5	VP3, VP7, VP1, VP4, VP6	NS1, NS2, NS3
Rotaviruses:	VP4 (HA), VP7	VP2, VP6, VP1, VP3	NSP1, NSP2, NSP3, NSP4, NSP5, NSP5A

To view a negatively-stained electron micrograph of reovirus particles, click here. Reovirus particles are very stable environmentally (especially waterborne Rotaviruses!). To view a negatively-stained electron micrograph of Rotavirus particles, click here.

The Atomic Structure of the Bluetongue Virus Core. J.M.Grimes et al. Nature 395: 470 (1998).

Orthoreovirus Structure:

More detail is known about the structure/function relationships of the Reovirus capsid than most other viruses. Rotavirus particles have the appearance of a wheel with spokes radiating out from the inner capsid. Orbivirus particles appear smooth.



Genome:

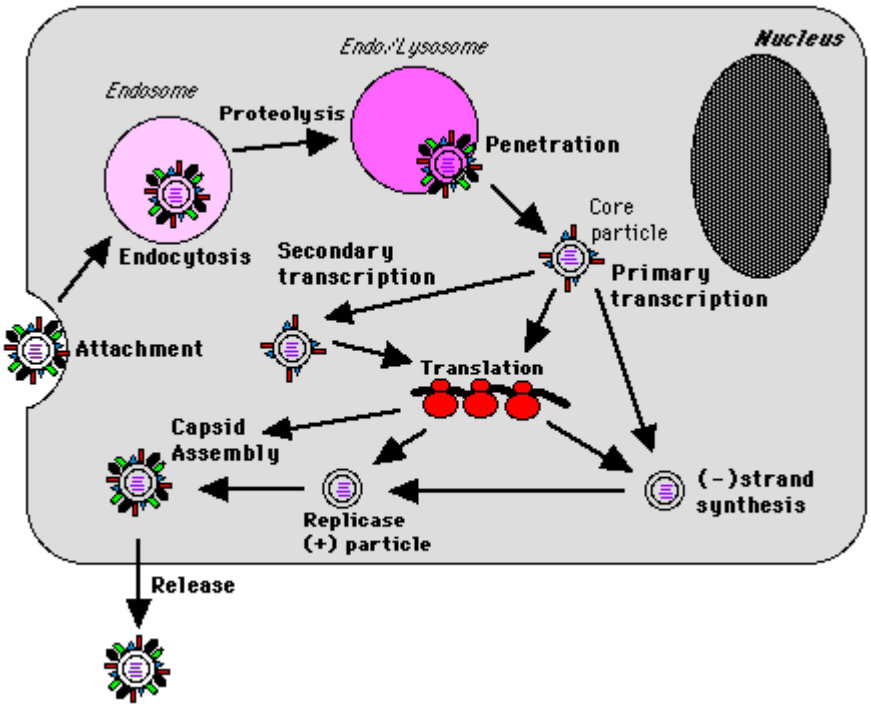
Segmented, d/s RNA - 10 (Reo's) / 11 (Rota's) segments in 3 distinct size classes:

- **L** - encodes proteins designated l ("lambda")
- **M** - encodes proteins designated m ("mu")
- **S** - encodes proteins designated s ("sigma")

These range in size from ~3.9kbp (**L**) - 1kbp (**S**). 30% of the RNA in the particles is in the form of small s/s oligonucleotides, synthesized internally by the viral polymerase - possibly abortive transcripts?

Replication:

Occurs in cytoplasm:



Receptor(s) known to contain sialic acid (haemagglutination, broad cell tropism), but have not been definitively identified.
Particles are internalized & partially uncoated in endolysosomes in the cytoplasm (resistant to protease digestion- if completely uncoated, virus would be destroyed).
Early transcription of the d/s RNA genome by viral polymerase occurs inside this sub-viral particle. The various genome segments are transcribed/translated at different frequencies-the main advantage of a segmented genome? (reassortment?)
RNA is transcribed conservatively - only (-)sense strands are used, resulting in synthesis of (+)sense mRNAs, which are capped inside the core - all this occurs without de novo protein synthesis.
mRNAs leave core & are translated in the cytoplasm.

Primary transcription (depending on factors inside the virus particle) results in capped transcripts which are **not** polyadenylated. At least 7 enzymatic activities are present in reovirus particles to carry out this process (not necessarily all separate peptides:

d/s RNA-dependent RNA polymerase	l-3
RNA triphosphatase	???
guanyltransferase	l-2
methyltransferase 1	l-2?
methyltransferase 2	l-2?
helicase	???

Secondary transcription occurs later in infection in particles produced inside the infected cells & results in uncapped non-polyadenylated transcripts.

The genome is replicated in the cytoplasm in a conservative fashion (c.f. DNA replication) - an excess of (+)sense strands are produced which serve as late mRNAs & as template for (-)sense strand synthesis (i.e. each (-) strand leads to many (+) strands - not one-for-one as semi-conservative replication). The mechanism responsible for segregation of the various genome segments into developing particles is not known.

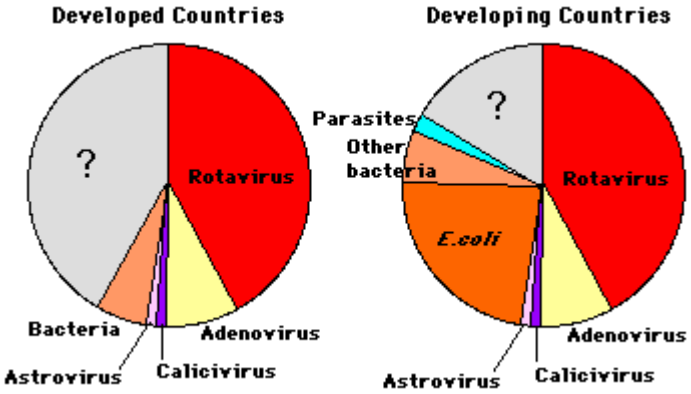
Particles assemble in the cytoplasm 6-7h after infection - inclusion bodies. Rotaviruses bud from the E.R. into internal spaces & are eventually released when the cell lyses.

Pathogenesis:

The majority of human Orthoreovirus infections involve the gastrointestinal & upper respiratory tracts & are asymptomatic, occasionally producing mild febrile illness, or very rarely, serious complications - **orphan viruses**. The majority of adults have Abs.

Rotaviruses:

Infections cause endemic/epidemic gastroenteritis and infantile diarrhoea - second only to U.R.T. infections in infants <24 months - a major cause of death in the 3rd World:



Rotaviruses are the most common cause of severe diarrhoea worldwide. In developing countries, rotavirus infection may cause up to one million deaths each year, accounting for an estimated 20-25% of all deaths due to diarrhoea and 6% of all deaths among children less than five years old (Cook et al, Bull WHO, 1990; 68:171-7). Because of the magnitude of disease associated with rotavirus infections and because public health interventions to provide clean water and improved sanitation are unlikely to decrease the incidence of disease, vaccines are being developed as the first strategy for prevention. The first rotavirus vaccine, a live oral tetravalent vaccine derived from a rotavirus isolated from a rhesus monkey is expected be licensed in the United States and Europe in 1998. For the latest information, visit: Rotavirus Online.
Medscape Article: Emerging Rotavirus Vaccines.

Rotaviruses have three shells - an outer capsid, inner capsid and core which surrounds the 11 segments of double-stranded RNA. Two of the structural proteins, VP7 (the glycoprotein or G protein) and VP4 (the protease cleaved or P protein) make up the outer shell and define the serotype of the virus and are the major antigens involved in virus neutralization.

Although ten VP7 serotypes have been observed in children with diarrhoea, four are considered to be important epidemiologically (G1-G4) and have been included in the make-up of the tetravalent rhesus rotavirus vaccine (Gentsch et al, J Infect Dis 1996; 174: 5306). Similarly, although seven VP4 genotypes have been reported in children, only two (P8 and P4) are common. Several studies have documented that G types 1-4 are the predominant serotypes causing human disease worldwide. However, in Europe, 10% of rotavirus specimens could not be typed as G1 to G4 and may represent uncommon types, while in Britain between 2 and 14% of strains could not be typed. The diversity of strains in these countries may be important for vaccine development. Since reassortant vaccines were engineered to contain the most prevalent G antigens (G1-G4), these vaccines may protect less well against unusual strains circulating in other countries. The lower efficacy of candidate rotavirus vaccines in developing countries is not fully understood and is likely to be due to many factors. One explanation may be disease caused by VP7 serotypes not covered by current vaccine candidates, alternatively the lack of incorporation of a human P type in the tetravalent rhesus rotavirus vaccine.

Rotaviruses cause messy but generally self-limited infections, rare in adults (Abs) and trivial in consequence, but may kill infants unless properly treated - oral rehydration therapy (O.R.T.) with isotonic glucose/mineral salt solution keeps children alive (treats symptoms, not cause). Rotaviruses are also a major cause of mortality in calves, piglets, etc - highly infectious.

Orbiviruses:

Similar in many respects to Orthoreoviruses. There are >130 serotypes of orbivirus which infect birds & mammals, including humans, causing a variety of haemorrhagic fevers, encephalitis & other conditions:

Properties:	Orbivirus:	Reovirus:
Acid sensitivity:	Sensitive	Resistant
Detergent sensitivity:	Partial sensitivity	Resistant
Tissue tropism:	Haematopoetic & epithelial cells	Intestinal tract
Transmission:	Insect vector	Faecal-oral

African Horse Sickness - fever followed by death within hours in >90% affected animals. Transmitted by midges, ticks. Vaccine available.
Bluetongue - Disease of sheep, also infects cattle, producing mild disease; severe economic pest - causes fetal loss. Transmitted by midges (Culicoides). No vaccine yet.
Colorado Tick Fever - Tick transmitted disease endemic in USA. Acute febrile illness, usually without lasting consequences.

- References: Click here
- Search for more information on this topic
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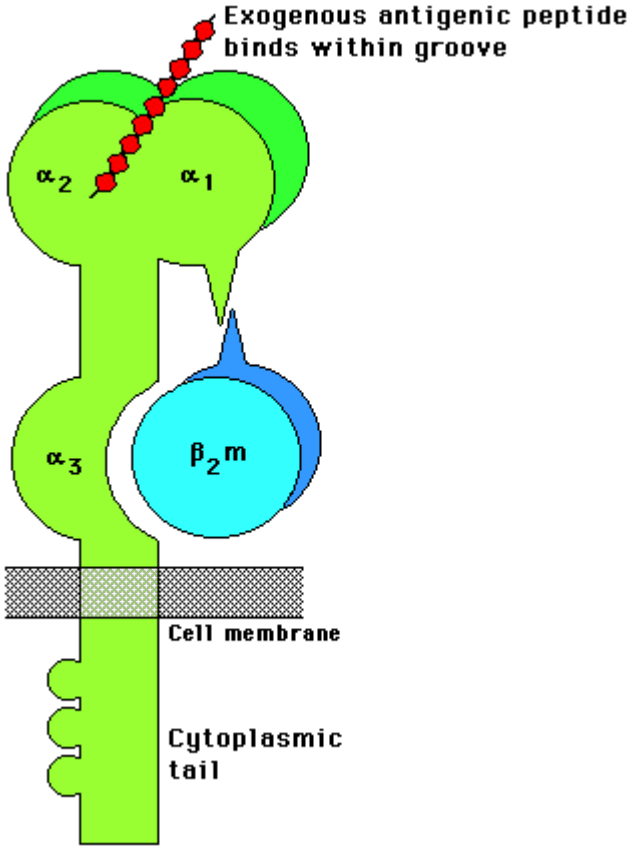
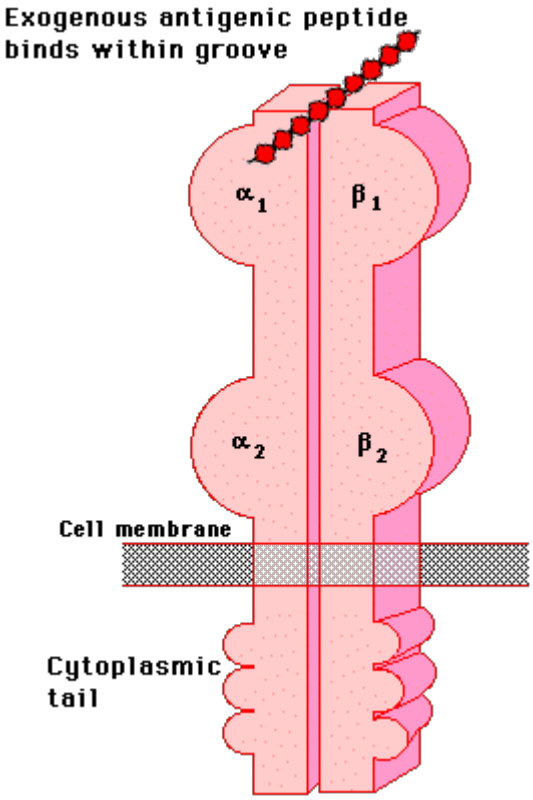

Exogenous antigenic peptide
binds within groove
α2
α1
α3
β2m
Cell membrane
Cytoplasmic
tail


Exogenous antigenic peptide
binds within groove
α1
β1
α2
β2
Cell membrane
Cytoplasmic
tail

Last Updated: Saturday, October 4, 1945624.

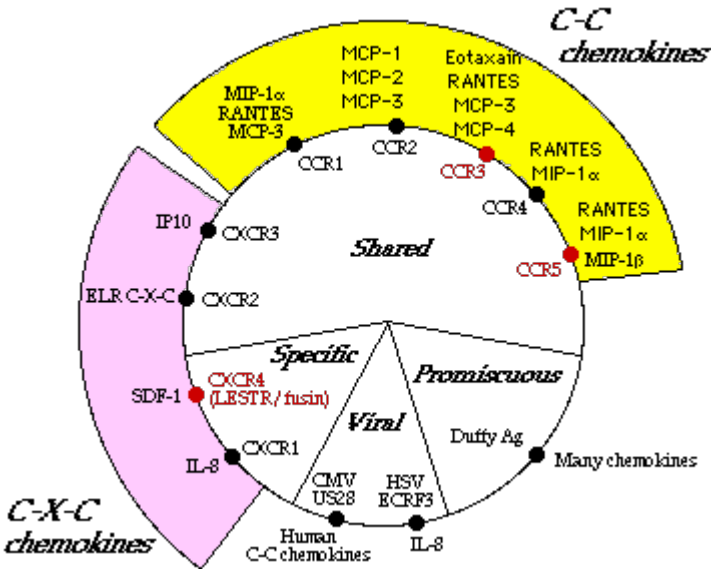
Who gets AIDS ?

Recent scientific discoveries have revealed new and important information on how the HIV virus is able to infect different types of human cell. This is already resulting in new ideas which will inform future thinking about possible therapeutic intervention in HIV-infected individuals. In future, this might well involve a two-pronged approach incorporating both an attack on the virus itself and therapeutic immunomodulation to prevent the decline of the immune system.

Chemokines:

Immune cell trafficking (above) is controlled by 2 components:

1. adhesion molecules
2. chemoattractant cytokines (**chemokines**) and their receptors:



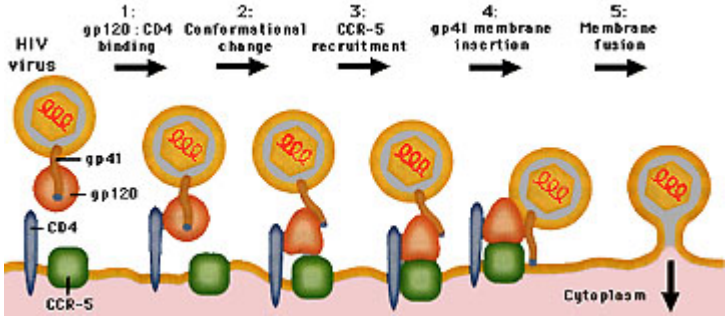
- 3 classes: C (1), C-C (beta), C-X-C (alpha)
- characteristic cysteine (C-C) bridge or C-X-C amino acid repeat
- 68-120 amino acid G-protein-associated glycoproteins
- 7 trans-membrane domains
- at least 50 members

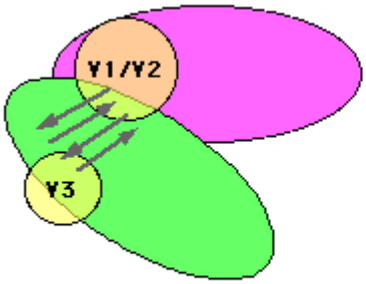
New information about the identity of the HIV receptor has been revealed by recent studies:

- The fact that CD4 is a component of the HIV receptor has been known since 1984, also that CD4 on its own is not sufficient to allow HIV to infect cells.
- Cocchi et al (Science 270: 1811,1995): the CC chemokines RANTES, MIP1-alpha and MIP-1beta block infection of lymphocytes with macrophage-tropic HIV isolates by interacting with the CCR5 receptor.
- Feng et al (Science 272: 872, 1996): CXCR4 ligand blocks infection with lymphocyte-tropic HIV isolates.

- (N.B. Some HIV isolates use CCR-3; some HIV isolates can use more than 1 CCR; pattern of use does not fall into neat categories).
- The number of co-receptors continues to grow (e.g. BOB, BONZO, etc - see Clapham & Weiss, Nature 388: 230, 1997).
- The binding site for HIV in the amino terminus of CCR5 has now been identified (Dragic T, et al. J.Virol. 72: 279-285, 1998).

The role of the second receptor components is believed to be as shown in the following diagrams:





Read:
Moore J.P. & Binley J. "Envelope's letters boxed into shape" Nature 393: 630 (1998).

Long term survivors:

Allikmets et al (Science 273: 1857, 1996) studied 1,955 men either infected with HIV or at high risk for HIV infection:

- Most people have two normal alleles for the CCR5 gene.
- 1:7 have one mutant allele (32bp deletion "Delta32") and one normal allele (heterozygous).
- 1:100 have two mutant alleles.
- Mutant allele has a frequency of 11% among Caucasians, 1.7% among African Americans, less than 1% in Asian populations.
- 17/612 (less than 3%) of people at high risk for HIV infection had two copies of the mutant CCR5 gene.
- **NONE** of 1,343 HIV-infected people had the mutation.
- Heterozygous genotype occurred in 15% of HIV-infected subjects, 14% of subjects not infected with HIV - one mutant copy of the gene does not protect from HIV infection
- **BUT** the average time to AIDS for all HIV-infected men in the study was 10 years, heterozygous men progressed to AIDS in an average of 13 years.

The observation that CCR5 mutations can protect against HIV infection **SUGGESTS** (but does not prove) that CCR5 plays a critical role early in HIV infection since infection can be blocked if this receptor is not present. At later stages of infection, other co-receptors such as CXCR4 may take over this role as the properties of the virus population in a patient changes. However, this is only a theory at present and more work is needed to confirm this idea.

It has recently been suggested (Berger E.A. et al, Nature 391:240, 1998) that co-receptor use should be used as the basis for classification of HIV isolates:

- Isolates that use CCR5 but not CXCR4 should be termed **R5** viruses
- Isolates that use CXCR4 but not CCR5 should be termed **X4** viruses

- Isolates able to use CCR5 and CXCR4 should be termed **R5X4** viruses

Under this scheme, R5 viruses are the most common sexually-transmitted isolates. In an infected individual, these eventually evolve into R5X4 and/or X4 viruses.

New Drugs - chemokines as receptor antagonists:

- Concentrations of chemokines used to block HIV replication in vitro are 10-100 greater than the levels at which they are produced in the body.
- Chemokines induce potent inflammation, hence derivatives will need to be used (Nature 383: 400, 1996).

- **Medscape Article:** Host Genes and HIV: The Role of the Chemokine Receptor Gene CCR5 and Its Allele (delta-32 CCR5).
- "How HIV Fools the Immune System"
- References: Click here

There is a LOT of infomation available online on this topic:

- Just to get you started, here's an example of the sort of relevant information available.

- Search **MEDLINE** for the latest publications on HIV/AIDS:

Click Here

Books about HIV & AIDS: click here

- Other online searches

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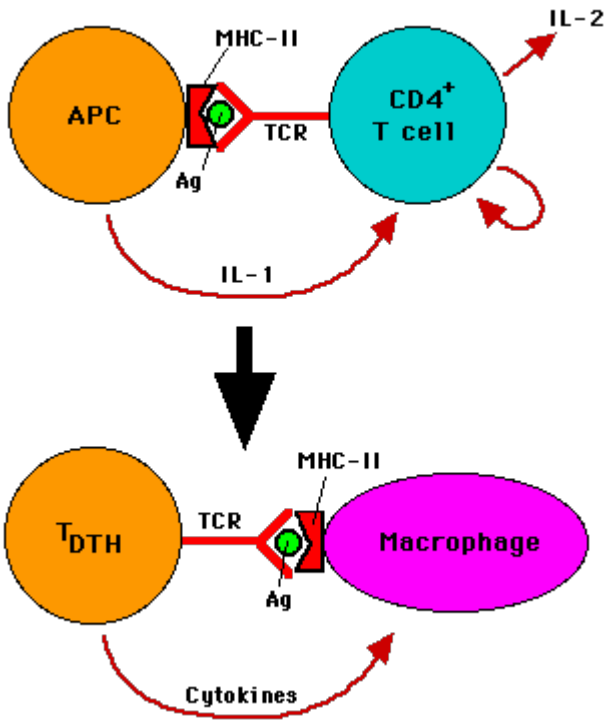
MHC-II
IL-2
APC
CD4+
T cell
TCR
Ag
IL-1
MHC-II
TDTH
TCR
Macrophage
Ag
Cytokines

Azidothymidine (Zidovudine/AZT):

You need the free CHIME plugin to view this molecule

Didanosine (ddI, Videx):

You need the free CHIME plugin to view this molecule

Zalcitabine (ddC, Hivid):

You need the free CHIME plugin to view this molecule

Stavudine (d4T, Zerit):

You need the free CHIME plugin to view this molecule

Lamivudine (3TC, Epivir):

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Nevirapine (Viramune):

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Delavirdine (Rescriptor):

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Saquinavir (Invirase):

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Ritonavir (Norvir):

You need the free CHIME plugin to view this molecule

This image © Chris Betts 1998.

Emerging & Re-emerging Viruses: An Essay

By:

Alison Jacobson

Department of Microbiology
University of Cape Town

- Introduction
- Theoretical Considerations
- A Historical Perspective
- Examples of Emerging & Potentially Emergent Viruses
- Arbovirus Diseases
- Aetiology
- Epidemiology
- Transmission
- Immunity
- Active Immunity
- Passive Immunity
- General Clinical Features
- Ecological Factors

CASES IN POINT:

- HANTAVIRUSES:
- Hantaan virus
- Seoul virus
- Four Corners virus
- Ebola virus
- Influenza virus: A model for emergence
- Mutation Frequency
- Selective Pressures & Constraints

- Conclusion

- References

INTRODUCTION

Within the last decade there has been an ever increasing awareness of the Darwinian struggle with which the human species is engaged. Microbial and viral predators abound, in no less abundance than before, and still present a constant threat to individual survival as well as to the success of the population at large. Following decades of general complacency in the antibiotic era, a startling turning point has been reached, spurred in large part by the global ravages of the human immunodeficiency virus (HIV) - one of the simplest of viral constructs - which still evades cure or even true understanding. The last two years have been accented by several striking episodes of disease emergence, such as multi-drug resistant tuberculosis, acute coccal infections, the rodent-borne pneumonic hantavirus in the United States, food- and waterborne outbreaks of *Salmonella* infections, cholera and illnesses caused by the *Shigella*-like *Escherichia coli* O157. Against these newer threats is a perpetual backdrop of a multiplicity of infections, which cycle throughout their ecological niches and are encountering opportunities in our modern world to spread with a frightening vigour. Currently, we are experiencing the epidemic potential of HIV, but the next epidemic of human disease may be entirely different. A few examples of general emerging infectious diseases are shown below:

Examples of Emerging Infectious Diseases:

USA:

- *E. coli* O157:H7 disease
- Cryptosporidiosis
- Hantavirus pulmonary syndrome
- Coccidioidiomycosis
- Vancomycin-resistant enterococci and multiply-resistant pneumococci

International:

- Vibrio cholerae O139 in Asia
- Vibrio cholerae O1 in Latin America
- Yellow fever virus in Kenya
- Rift Valley fever virus in Egypt *E coli*O157:H7 disease

To quote Donald A.Henderson of the U.S. Office of Science and Technology Policy:
"The recent emergence of AIDS and Dengue hemorrhagic infections, among others, are serving usefully to disturb our ill-founded complacency about infectious diseases. Such complacency has prevailed in this country (USA) throughout much of my career... It is evident now, as it should have been then, that mutation and change are facts of nature, that the world is increasingly interdependent, and that human health and survival will be challenged, *ad infinitum*, by new mutant microbes, with unpredictable pathophysiological manifestations...How are we to detect these at an early date so as to be able to devise appropriate preventative and therapeutic modalities? What do we look for? What types of surveillance and reporting systems can one devise?" (Morse 1993).

The Centers for Disease Control and Prevention (CDC), in an attempt to ensure that its prevention and control programs keep pace with the numerous and changing health problems that threaten all segments of our diverse society, has identified four priority areas:

1. strengthen core public health functions;
2. develop, maintain, and enrich the capacity to respond to urgent threats to health;
3. develop a nationwide prevention network and program; and
4. promote women's health.

Leading the list of urgent threats to health are new and emerging infections. It must be recognized that the health of the developed world's people is inextricably linked to the health of people in other nations, infectious diseases can and do disseminate rapidly around the globe, and global surveillance for emerging infections is vital to public health. While it is vital for us to recognize and promote this awareness, we too have an integral role to play in surveillance and basic research.

There seems to be two stages in public health crises. The first is, "I don't believe you." And the second is "Why didn't you tell me?" (Dowdle 1994). Everyone tends to think in very short terms. Although officials need to recognize and respond to the critical `disease of the year', it is also necessary to maintain the infrastructure needed to identify emerging diseases as well as to develop effective ways for combating them. The problem can be made more difficult when resources are set aside for the spotlighted problem without ensuring that the less glamorous infrastructure needs to continue to be met. In this essay I will attempt to address these issues, as well as to highlight appropriate examples of emergent disease, particularly those of viral origin.

THEORETICAL CONSIDERATIONS

Joshua Lederburg has commented that viruses are humanity's only real competitors for dominion of the planet, serving as both parasites and genetic elements in their hosts (Lederburg 1988). Not only do they have considerable plasticity, enabling them to evolve in new directions, but their genetic and metabolic entanglements with cells uniquely positions them to mediate subtle, cumulative evolutionary changes in their hosts as well. In contrast, they are also able to decimate entire populations. The fact that long term natural selection favours mutualism offers only limited encouragement to our species, with millions of people suffering before an equilibrium can be reached.

Coevolution of viruses and host can follow several possible lines estimated by modelling techniques, and pathogens may not always evolve towards lower virulence. For example, a virus strain that kills much faster will not be favoured over a less virulent strain if it has a modest transmission advantage, but it will prevail if it is much more readily transmitted than the less-virulent strain. In the trade-off between transmissibility and virulence, many viruses evolve toward a middle ground, favouring transmissibility but allowing them to retain some virulence. Viruses that are transmitted over a long time, for example HIV, have a selective advantage even when their effective rates of transmission are relatively low (Anderson and May 1987).

However, although mathematical approaches offer useful insights, they are often inadequate in predicting outcomes because viral emergence and host interactions are so complex, being dependent on both the genetics of the host and external conditions. These predictive attempts, a true test for chaos theorists, have even more variables than say, equations dealing with predictions of meteorological patterns, because such climatic conditions make up just one part of the ecological picture within which biological evolution maneuvers. Furthermore, stock market predictions, another favourite of chaos theorists, can also be seen to form just one piece of the puzzle that is infectious disease, as many of our responses to the latter problem are politically and financially interwoven.

A HISTORICAL PERSPECTIVE

New patterns of human movement, leading to new contacts across what had once been geographic boundaries, have been seen to give rise to a variety of emergent infections. Examples are the introduction of smallpox into the Americas and of syphilis into Europe. Yellow fever probably emerged in the New World as a result of the African slave trade, which brought *Aedes aegypti* in water containers of ships. Similarly, the rise of Dengue hemorrhagic fever in Southeast Asia in the late 1940s is attributed to rapid migration to cities with open water storage, which favoured proliferation of the mosquito or other suitable vectors. Of current concern in the USA is the fact that *Aedes albopictus*, an aggressive and competent dengue virus vector, was brought to Houston in used Asian tires and has established itself in at least 17 American states (Morse and Schluederberg 1990).

RECENT EXAMPLES OF EMERGING AND POTENTIALLY EMERGENT VIRUSES

Most emergent viruses are zoonotic, with natural animal reservoirs a more frequent source of new viruses than is the spontaneous evolution of a new entity. The most frequent factor in emergence is human behaviour that increases the probability of transfer of viruses from their endogenous animal hosts to man. Rodents and arthropods are most commonly involved in direct transfer, and changes in agricultural practices or urban conditions that promote rodent or vector multiplication favour increased incidence of human disease. Other animals, especially primates, are important reservoirs for transfer by arthropods. Because arthropod transmission plays a very large part in infectious animal disease, specifically potential emergent virus epidemics, I will dedicate the next part of this essay to a discussion of them.

ARBOVIRUS DISEASES

Approximately 100 of the more than 520 known arthropod-borne viruses (arboviruses) cause human disease. At least 20 of these might fulfill the criteria for emerging viruses, appearing in epidemic form at generally unpredictable intervals (Morse and Schluederberg 1990). These viruses are usually spread by the bites of arthropods, but some can also be transmitted by other means, for example through milk, excreta or aerosols. The arbovirus infections are maintained in nature principally, or to an important extent, through biological transmission between susceptible vertebrate hosts by blood-sucking insects; they multiply to produce viraemia in the vertebrates, multiply in the tissues of the insects and are passed on to new vertebrates by the bites of insects after a period of extrinsic incubation. The names by which these viruses are known are often place names such as West Nile or Rift Valley, or are based on clinical characteristics like yellow fever.

AETIOLOGY

Most arboviruses are spherical, measuring 17-150 nm or more, a few are rod-shaped, measuring 70 x 200 nm. All are RNA viruses. Many circulate in a natural environment and do not infect man. Some infect man only occasionally or cause only a mild illness; others are of great clinical importance causing large epidemics and many deaths. Specifically, these belong to the Togaviridae, the alphaviruses, flaviviruses, the Bunyaviridae, nairoviruses, phleboviruses and other subgroups.

EPIDEMIOLOGY

Vertebrate hosts

Maintenance, incidental, link and amplifier hosts are categorized according to Stickland Hunter's "Tropical Medicine" (1991) as the following:

Maintenance hosts are essential for the continued existence of the virus, usually living in symbiosis with the viruses, without actual disease, but they do develop antibodies. These include birds such as the prairie chicken, pigeon and wood thrush which transmit Eastern and Western equine encephalitis; heron and egrets transmitting Japanese encephalitis and migrating birds which travel over long distances carrying these and other similar viruses; rodents and insectivores such as rats, hedgehogs, lemmings and chipmunks are known to carry louping ill and Colorado tick fever; primates such as monkeys which carry Dengue fever; Leporidae (rabbits and hares) which carry Californian encephalitis; Ungulates (cattle and deer) which are implicated in the transmission of European tick-borne encephalitis; bats which carry Rio Brava virus; and marsupials, reptiles and amphibia such as kangaroos and snakes which also harbour encephalitis-causing viruses.

Incidental hosts become infected, but transmission from them does not occur with sufficient regularity for stable maintenance. Man is usually an incidental host, often, but not always, being a dead end in the chain. These hosts may or may not show symptoms. Link hosts bridge a gap between maintenance hosts and man, for example, between small mammals and man by goats (via milk) in tick-borne encephalitis. Amplifier hosts increase the weight of infection, as is the case with pigs which act between wild birds and man in Japanese encephalitis.

The populations and characters of the vertebrate hosts and their threshold levels of viraemia are important. Small rodents multiply rapidly and have short lives, thus providing a constant supply of susceptible individuals. In contrast, monkeys and pigs multiply slowly, and once they have recovered from an infection, remain immune for life. African monkeys are relatively resistant to Yellow Fever, but Asian and American monkeys are susceptible, probably because, unlike the African monkeys, they have not been exposed continuously for centuries to the infection. Also, possibly related arboviruses may offer partial immunization.

Invertebrate hosts

Mosquitoes, sandflies and ticks may imbibe virus from a vertebrate in a state of viraemia, after which the virus undergoes an incubation period within the arthropod, known as the extrinsic incubation period. In mosquitoes this period is short: 10 days at 30o C ambient temperature and longer at lower temperatures. Mosquitoes remain infective for life without any apparent ill-effects. In fact, their infectivity appears to increase with time after infection and their effectiveness as transmitters depends upon the frequency with which they bite. It is also possible that arthropods, whose mouth parts are contaminated by virus in the act of feeding, could transmit the virus mechanically if they feed soon afterwards on another animal. For instance, chikungunya virus can be transmitted mechanically by *A. aegypti* for 8 hours after infection. In general, mosquito-borne viruses may not use ticks as vectors nor can tick-borne viruses reside in mosquitoes.

TRANSMISSION

Arthropod transmission involves several stages:

1. Ingestion by the arthropods of virus in the blood (usually) or tissue fluids of the vertebrate hosts
2. Penetration of the viruses into the tissue of the arthropods, in the gut wall, or elsewhere after passing through the gut barrier
3. Multiplication of the viruses in the arthropod cells, including those of the salivary glands.

Stage 2 and part of stage 3 represent the extrinsic incubation period of the disease (Hunter 1991). The quantity of blood, and therefore the amount of virus ingested, seems to be important as each arthropod species must ingest a minimum quantity of a given virus before multiplication can take place. The same mosquito species can have two different thresholds for two different viruses and if one species has a low threshold, other species may have high thresholds or may be completely resistant. This threshold phenomenon is extremely important in determining the efficiency of a vector and may also vitally affect the course of an epidemic. Viruses reportedly persist in overwintering mosquitoes, while transovarian passage of virus has been seen in some tick species. For mosquitoes the availability of suitable breeding places (and therefore rainfall) is a major factor. An efficient vector may have a wide range of animals on which to feed, but if the arthropod species is abundant, and even if it bites man only infrequently in the presence of other (and preferred) animals, the large numbers enable it to maintain transmission to man. For example, *Culex tritaeniorhynchus*, which mostly bites birds, Bovidae, dogs and especially porcines, and only to a limited extent man, can maintain transmission of Japanese encephalitis from pigs to man by sheer numbers.

Although transmission of arboviruses usually takes place through the bites of arthropods, Lassa virus, for example, may be transmitted through contact with excreta of infective rodents, and others via urine or faeces infecting the nasopharynx, some through aerosol from a patient or others by one bird pecking another.

IMMUNITY

After a vertebrate has been infected, the arbovirus probably multiplies first in the regional lymph glands where the earliest formation of antibodies also probably takes place. Some do not produce high titres of antibodies in man and some antibodies are short-lived or appear late. In diagnosis, haemagglutination-inhibiting and complement-fixing antibodies are important, but the only protective antibody is of the neutralizing type, which is also the most specific.

Arboviruses are grouped according to antigenic characters, but after inoculation of one virus into a fresh animal, not only the homologous antibodies, but also heterologous antibodies reacting with other viruses of the same group tend to appear. Recovery from an infection by a member of one group of arboviruses may provide some degree of resistance to a subsequent infection by another member of the same group. For example, infection with West Nile virus may have modified the Ethiopian epidemic of Yellow Fever in 1962. Again, the effect of prior infection with Zika, Uganda S and other related viruses in the forest belt of Nigeria, leading to a high incidence of related antibodies, is suggested as the explanation of the absence of epidemic Yellow Fever in man in that area. These related infections probably modify the disease rather than prevent infection.

ACTIVE IMMUNITY

With Yellow Fever, neutralizing antibodies can be found as early as a few days after the beginning of the disease and are found constantly for many years in the sera. The persistence of immunity does not depend on exogenous reinfection. It is probable that a mosquito infected with Yellow Fever is not harmed by it, but continues to excrete the virus throughout life. This means a continuous supply and release of virus, probably from the epithelial cells of the salivary glands. The virus enters man (or other animals) and gains the liver and other epithelia, provoking the early antibodies in the blood, which neutralize circulating viruses. But, as suggested by Hunter (1991), antibodies which can be detected for so many years in man must stem from a continuing stimulus, and the sensitive cells and their progeny probably have a prophase equivalent of the virus incorporated into their genome, with occasional reversion to productive development which provides the stimulus for further antibody formation. A degree of immunity of this kind may possibly be provided when a related virus invades epithelial cells.

PASSIVE IMMUNITY

Infant rhesus monkeys and human infants born of mothers immune to Yellow Fever have transient protective antibodies in their sera at birth which persist for several months. They are probably placentally transferred, rather than coming from the mother's milk, because antibodies may disappear from infant sera while they are still suckling. Passive immunity induced by injection of homologous immune serum, has been used for protection against tick-borne encephalitis in cases of special risk and similar sera could be used against other infections, particularly after laboratory or hospital accidents.

GENERAL CLINICAL FEATURES

Most arbovirus infections are inapparent, that is they produce no symptoms or often only mild ones (fever and occasional rash). For example, in an epidemic of Japanese encephalitis it was estimated that for each case of apparent disease there were 500-1000 inapparent infections. If clinical manifestations arise after infection they do so after an intrinsic incubation period lasting from a few days to a week or more. Some arboviruses damage the endothelial lining of the capillaries increasing permeability which allows the virus to pass the blood brain barrier causing meningoencephalitis. Others damage the parenchymatous organs by direct damage to the cells in which they are situated, while with others damage is caused by the immune system of the host from the formation of antigen- antibody complexes and disordered complement formation which damage the renal tubules and alter the coagulation and fibrinolytic systems of the body causing haemorrhage (viral hemorrhagic fevers). There is a general pattern of biphasic illness, the first phase associated with viraemia ending when antibodies appear in the blood and the second phase when the virus is located in organs, such as the liver or brain.

The onset of clinical manifestations is usually abrupt, generally occurring after the onset of viraemia. Fever is usual and is sometimes the only sign. In many cases the clinical manifestations last only while the virus is disseminated, but in other cases there is remission, short or long. If long, the disease is biphasic. After this, fever returns with signs indicating localization of the virus in certain organs. If the period of viraemia has been symptomless and the virus becomes localized in the central nervous system, encephalitis appears. In hemorrhagic cases there is a special risk of shock which can rapidly become irreversible unless promptly treated (Hunter 1991).

ECOLOGICAL FACTORS

Microorganisms and viruses are adapted to extremely diverse econiches. One of the most complex sets of adaptive characteristics concern arthropod transmission of viruses. The arthropod-borne viruses are spectacular examples of emergence and re-emergence resulting from innocent environmental manipulation or natural environmental change. Deforestation, amateur irrigation and the introduction of new species (usually livestock) gives rise to many virus disease threats of humans and animals. Important aspects of ecological change and their relation to arbovirus life cycles are:

1. Population movements and the intrusion of humans and domestic animals into new arthropod habitats, particularly tropical forests
2. Deforestation, with development of new forest-farmland margins and exposure of farmers and domestic animals to new arthropods
3. Irrigation, especially primitive irrigation systems, which are oblivious to arthropod control
4. Uncontrolled urbanization, with vector populations breeding in accumulations of water (tin cans, old tires etc.) and sewage
5. Increased long distance air travel, with potential for transport of arthropod vectors
6. Increased long-distance livestock transportations, with potential for carriage of viruses and arthropods (especially ticks)
7. New routing of long-distance bird migration brought about by new man-made water impoundments (Murphy 1994).

To illustrate the effect ecological change can have on the emergence of a new disease and the course of it afterwards one can look to dengue, one of the most rapidly expanding diseases in tropical parts of the world, with millions of cases occurring each year. For example, Puerto Rico had five dengue epidemics in the first 75 years of this century, but has had six epidemics in the past 11 years, at an estimated cost of over $150 million. Simultaneously, Brazil, Nicaragua and Cuba have had their first major dengue epidemic in over 50 years, involving multiple virus types. At the lethal end of the dengue spectrum is dengue haemorrhagic fever, first occurring in the Americas in 1981. Since then, 11 countries have reported cases, and since 1990 over 3000 cases have been reported annually. Figure 3 illustrates the extent of dengue occurrence globally.

The primary reason that dengue is emerging and re-emerging is vector control. National priority lists are political in nature and tend to emphasize daily problems, not episodic ones. Expensive mosquito control tends to fall off the bottom of the list. Meanwhile, as older cheaper chemicals lose effectiveness or are banned, new and expensive chemicals replace them. Before 1970, *A. aegypti*, the vector of dengue and Yellow Fever, was targeted for regional or even global eradication through the use of DDT (dichlorodiphenyltrichloroethane) (Murphy 1993). Obviously,

this solution is no longer applicable, but nothing has effectively supplanted it.

CASES IN POINT

HANTAAN VIRUS

The hantavirus (mentioned above) is also the focus of much international attention. During the Korean War of 1950-1952, thousands of United Nations troops developed a mysterious disease marked by fever, headache, hemorrhage and acute renal failure; the mortality rate was 5-10%. Despite much research, the agent of this disease remained unknown for 28 years, when a new virus, named Hantaan virus, was isolated in Korea from field mice. Recently, related viruses have been found in many parts of the world in association with different rodents and as the cause of human diseases with a variety of little-known local names. Epidemic haemorrhagic fever, one of the most important diseases in China, causes more than 100000 cases per year. Transmission to humans is primarily by inhalation of aerosolized excreta. In May 1993 a cluster of deaths in the southwestern United States set in motion a multiagency local, state and federal investigation that led to the discovery of a highly pathogenic hantavirus and to the definition of a new clinical syndrome (Peters 1994).

SEOUL VIRUS

Another virus of current interest in the USA, Seoul virus, was identified about 10 years ago in Korea as a Hantaan-like virus whose natural host is the urban rat. Serologic surveys detect it worldwide, including seroprevalence rates of 12% in urban rats in Philadelphia and about 64% in Baltimore rats (Le Duc 1986). Although acute hemorrhagic fever was not identified in inner-city Baltimore, 1.3% of 1148 local residents were antibody-positive and the possibility of viral association with chronic renal disease is under study.

"FOUR CORNERS VIRUS"

The disease hantavirus pulmonary syndrome (HPS), is characterized by an initial fever followed by the abrupt onset of acute pulmonary edema and shock. After recognition of the initial cases by observant clinicians in the Southwest, investigations were swiftly mounted by local university and public health workers but, in spite of efficient and competent studies, failed to find the cause. By the time the CDC became involved, a number of possible causative agents had been ruled out, leading most of the investigators to believe they were dealing with a new entity. This observation led to a broadly based approach to the field epidemiology and the laboratory study of the disease. Samples from the field investigations were distributed among many different laboratories of the National Center for Infectious Disease (NCID) for analysis by the most sensitive classic and modern molecular biological tests for a wide range spectrum of infectious agents.

Somewhat surprisingly, successful results were obtained after only a few days of straightforward serologic tests for hantaviruses. The hemoconcentration, thrombocytopenia and shock observed in some of the patients had raised speculation about the involvement of these viral agents; however they had been previously known as associated with renal syndromes only. The serologic results came from established techniques such as indirect fluorescent-antibody assays and enzyme-linked immunosorbent assays. The next steps utilized reverse transcription and PCR amplification of RNA in postmortem tissue samples (60% of confirmed cases to date have been fatal), using consensus primers based on known hantavirus RNA sequences. These yielded products with sequences typical of hantavirus but clearly different from any known member of the genus. This provided additional evidence for the hantavirus etiology and linked the new hantavirus closely to the human disease by its presence in the tissues of people dying of the infection. Using the genomic sequences from human tissues, investigators were subsequently able to implicate the deer mouse as the principle reservoir of the virus.

Hantaviruses have traditionally been difficult to propagate, and this one was no exception. Thus a full-length cDNA clone of the small RNA segment of the virus was synthesized. This technique provided a diagnostic reagent of increased sensitivity that could be made widely available. Eventually, full length RNA sequences were developed for the medium segment and a partial sequence was determined for the large segment, permitting the definitive determination that the new virus, isolated weeks later and registered as Muerto Canyon virus, was not a reassortant of any known hantavirus.

Immunohistochemical identification of hantavirus antigens and *in situ* hybridization with genomic sequences also confirmed the hantavirus etiology of the syndrome. The extensive presence of antigen in pulmonary capillaries provided an explanation for the pathophysiology and target organ specificity differing from that of other known disease-causing hantaviruses. This method, when applied to paraffin- imbedded tissues, has also served as a retrospective diagnostic tool, firmly identifying fatal cases from 10 to 15 years ago.

The rapid recognition of the hantavirus etiology of this disease was important in that it alleviated heightened fear among the general American population, and saved lives by focusing public health recommendations on the avoidance of contact with potentially infected rodents. Different hantaviruses have been isolated in Louisiana, Florida and also Brazil, indicating the uncommon, yet widespread nature of this disease. Recently (Diglisic 1994), isolation of a hantavirus from *Mus musculus* captured in Yugoslavia was reported.

As stated by C.J. Peters, chief of the Special Pathogens Branch of the Division of Viral and Rickettsial Diseases at NCID, the crucial role of modern techniques in virology was possible only in a context of past hantavirus research, and as part of efforts of a multidisciplinary team of clinicians, epidemiologists, field ecologists and classic microbiologists. The need for basic research is highlighted by the applied practical success which resulted from it, as was the case in identifying a new strain of hantavirus. Future research will need to investigate the molecular mechanisms for induction of pulmonary edema and an appropriate blocking therapy. The evolutionary relationships of the hantaviruses and their rodent host specificity must be understood to predict the future course of transmission, and finally the basis for the different tropisms of the viruses must be examined at a molecular level.

To view a negatively-stained electron micrograph of the four corners virus, click here.

EBOLA VIRUS

Ebola virus, a member of the Filoviridae, burst from obscurity with spectacular outbreaks of severe, haemorrhagic fever. It was first associated with an outbreak of 318 cases and a case-fatality rate of 90% in Zaire and caused 150 deaths among 250 cases in Sudan. Smaller outbreaks continue to appear periodically, particularly in East, Central and southern Africa. In 1989, a haemorrhagic disease was recognized among cynomolgus macaques imported into the United States from the Philippines. Strains of Ebola virus were isolated from these monkeys. Serologic studies in the Philippines and elsewhere in Southeast Asia indicated that Ebola virus is a prevalent cause of infection among macaques (Manson 1989).

These threadlike polymorphic viruses are highly variable in length apparently owing to concatemerization. However, the average length of an infectious virion appears to be 920 nm. The virions are 80 nm in diameter with a helical nucleocapsid, a membrane made of 10 nm projections, and host cell membrane. They contain a unique single-stranded molecule of noninfectious (negative sense) RNA. The virus is composed of 7 polypeptides, a nucleoprotein, a glycoprotein, a polymerase and 4 other undesignated proteins. Proteins are produced from polyadenylated monocistronic mRNA species transcribed from virus RNA. The replication in and destruction of the host cell is rapid and produces a large number of viruses budding from the cell membrane.

Epidemics have resulted from person to person transmission, nosocomial spread or laboratory infections. The mode of primary infection and the natural ecology of these viruses are unknown. Association with bats has been implicated directly in at least 2 episodes when individuals entered the same bat-filled cave in Eastern Kenya. Ebola infections in Sudan in 1976 and 1979 occurred in workers of a cotton factory containing thousands of bats in the roof. However, in all instances, study of antibody in bats failed to detect evidence of infection, and no virus was isolated form bat tissue.

The index case in 1976 was never identified, but this large outbreak resulted in 280 deaths of 318 infections. The outbreak was primarily the result of person to person spread and transmission by contaminated needles in outpatient and inpatient departments of a hospital and subsequent person to person spread in surrounding villages. In serosurveys in Zaire, antibody prevalence to Ebola virus has been 3 to 7%. The incubation period for needle-transmitted Ebola virus is 5 to 7 days and that for person to person transmitted disease is 6 to 12 days.

The virus spreads through the blood and is replicated in many organs. The histopathologic change is focal necrosis in these organs, including the liver, lymphatic organs, kidneys, ovaries and testes. The central lesions appear to be

those affecting the vascular endothelium and the platelets. The resulting manifestations are bleeding, especially in the mucosa, abdomen, pericardium and vagina. Capillary leakage appears to lead to loss of intravascular volume, bleeding, shock and the acute respiratory disorder seen in fatal cases. Patients die of intractable shock. Those with severe illness often have sustained high fevers and are delirious, combative and difficult to control.

EBOLA SEROLOGY

The serologic method used in the discovery of Ebola was the direct immunofluorescent assay. The test is performed on a monolayer of infected and uninfected cells fixed on a microscopic slide. IgG- or IgM-specific immunoglobulin assays are performed. These tests may then be confirmed by using western blot or radioimmunoprecipitation. Virus isolation is also a highly useful diagnostic method, and is performed on suitably preserved serum, blood or tissue specimens stored at -70oC or freshly collected.

TREATMENT OF EBOLA

No specific antiviral therapy presently exists against Ebola virus, nor does interferon have any effect. Past recommendations for isolation of the patient in a plastic isolator have given way to the more moderate recommendation of strict barrier isolation with body fluid precautions. This presents no excess risk to the hospital personnel and allows substantially better patient care, as shown in Table 2. The major factor in nosocomial transmission is the combination of the unawareness of the possibility of the disease by a worker who is also inattentive to the requirements of effective barrier nursing. after diagnosis, the risk of nosocomial transmission is small.

PREVENTION AND CONTROL OF EBOLA

The basic method of prevention and control is the interruption of person to person spread of the virus. However, in rural areas, this may be difficult because families are often reluctant to admit members to the hospital because of limited resources and the culturally unacceptable separation of sick or dying patients from the care of their family. Experience with human disease and primate infection suggests that a vaccine inducing a strong cell- mediated response will be necessary for virus clearance and adequate protection. Neutralizing antibodies are not observed in convalescent patients nor do they occur in primates inoculated with killed vaccine. A vaccine expressing the glycoprotein in vaccinia is being prepared for laboratory evaluation.

INFLUENZA VIRUS: A MODEL FOR VIRAL EMERGENCE

Influenza, both human and avian, would be very high on the list of disease with epidemic potential. Up to 20% of the population has been seen to become ill during a single epidemic, with 50000 deaths per year in the United States (Murphy 1994). In the 1918 pandemic more than 500000 people throughout the world died.

The probability of interspecies transfer can be increased not only by increased contact between humans and an animal reservoir, but also by increased opportunity for viral genetic reassortment or recombination within animal or insect hosts. Because influenza virus has an eight-segmented genome, it has considerable freedom for such a genetic reassortment. While small epidemics may arise from mutation (antigenic drift), all known human pandemic strains have been the result of reassortment, mostly involving the hemagglutinin (H) gene. Kida *et al.*(1988) and also Scholtissek and Naylor (1988) state that influenza virus maintained in shore and migrating birds infect ducks raised on farms and reassort in pigs, from which new strains emerge to infect humans.

Virulent strains of influenza virus can also arise from a single mutation, even if pandemic strains have not generally arisen this way. For example, in 1983 a single mutation in a relatively avirulent strain gave rise to an H5N2 strain that caused a fatal epidemic in chickens in Pennsylvania. The point mutation in the H gene changed threonine to lysine, exposing a previously glycosylated site. Similarly, and remarkably, if pigs are infected experimentally with an avirulent mutant, the swine virulent parental phenotype emerges within a few days, indicating rapid evolution and emergence *in vivo* of the virulent form (Kilbourne *et al.* 1988).

For viruses with non-segmented genomes, recombination provides another genetic avenue for emergent diseases. For instance, viral genetic sequence analysis revealed that Western equine encephalomyelitis virus, an alphavirus,

arose from a recombination event that seems to have involved a Sindbis-like virus and Eastern equine encephalomyelitis virus, probably occurring some 100-200 years ago (Hahn *et al.* 1988). Genetic recombination also seems to have occurred between the envelope protein genes of human T lymphotropic virus (HTLV)-I and HTLV-II (Doolittle *et al.* 1989).

MUTATION FREQUENCY

The mutation rate of any genome is inversely proportional to its size. However, RNA viruses usually have higher mutation rates than do DNA viruses of the same genome size. This is generally ascribed to the lack of error-correcting mechanisms in RNA synthesis. High mutation rates have been reported in influenza genes. The changes occurred in the non-structural protein (NS) gene of influenza A virus during a single cycle of replication in tissue culture. A mutation rate of approximately 10-5 changes per nucleotide site per replication cycle was observed (Parvin *et al.* 1986). Similar tissue culture experiments revealed mutation rates of about 10-fold lower for poliovirus and 10-fold higher for Rous sarcoma virus. Also, the evolution rate of influenza virus has been considered, this is in contrast to the mutation rate as it takes place when the viruses are passaged into humans. The evolution rate of the influenza A virus NS gene is 1.95 x 10-3 changes/site/year, several orders of magnitude greater than that of eukaryotic genes.

Doolittle *et al.* (1989) looked at the evolutionary rates of change of ten genes from retroviruses. Overall, the reverse transcriptase showed the slowest rate of change and the outer portion of the envelope protein the most rapid, evolving three times faster. The core portion of the gag protein changed about 1.6 times as fast as the transcriptase, the proteinase 1.8 times as fast, and the 140 amino acids at the amino terminal of gag 2.5 times as fast. The viral proteinase is pepsin-like, and that from HIV is as similar to that of visna virus as human pepsin is to its fungal homologue. The proteinases of HIV and HTLV-I differ from one another even more than human pepsin does from the fungal proteinase. In other words, the retroviruses are changing extraordinarily rapidly. Most species of RNA viruses actually consist of a population of genomes showing considerable variation around a master sequence (Domingo *et al.* 1988). The population concept is important, as in experimental systems, defective members of the genomic population can play a significant role in viral expression.

SELECTIVE PRESSURES AND CONSTRAINTS

It is of interest to determine, what, if any, limits are placed on virus variation. Despite high mutation rates and opportunities for genetic reassortment, many factors act to minimize emergence of new influenza A epidemics (Morse and Schluederberg 1988). even though avian and human influenza viruses are widespread (in humans an estimated 100 million infections yearly), pandemic influenza viruses emerge infrequently (every 10-40 years). Powerful constraints appear to exist since pandemic human influenza strains vary in their H gene, whereas the neuraminidase and most other genes are conserved.

These constraints on viral evolution are not surprising when one considers the selective pressures imposed by the host at each stage of the virus life cycle. Tissue tropism determinants, include site of entry, viral attachment proteins, host cell receptors, tissue- specific genetic elements (for example promoters), host cell enzymes (like proteinase), host transcription factors, and host resistance factors such as age, nutrition and immunity. Host factors contribute significantly: sequences such as hormonally responsive promoter elements and transcriptional regulatory factors can link viral expression to cell state.

The interaction of virus and host is thus complex but highly ordered, and can be altered by changing a variety of conditions. Unlike bacterial virulence, which is largely mediated by bacterial toxins and virulence factors, viral virulence often depends on host factors, such as cellular enzymes that cleave key viral molecules. Because virulence is multigenic, defects in almost any viral gene may attenuate a virus. For example, some reassortments of avian influenza viruses are less virulent in primates than are either parental strain, indicating that virulence is multigenic (Treanor and Murphy 1990).

Viral and host populations can exist in equilibrium until changes in environmental conditions shift the equilibrium and favour rapid evolution (Steinhauer and Holland 1987). It seems reasonable to expect that new viruses will emerge occasionally, but the stochastic and multifactorial nature of viral evolution makes it difficult to predict such events. According to Doolittle, retrovirus evolution is sporadic, with retroviruses evolving at different rates in different situations. For instance, the human endogenous retroviral element is shared with chimpanzees, indicating

no change in over 8 million years, whereas strains of HIV have diverged in mere decades. Endogenous retroviruses carried in the germline evolve slowly compared with infective retroviruses. Generation of new viral pathogens is rare, and often possible only because of high mutation rates that permit many neutral mutations to accumulate before selective pressure forces a change. The seeming unpredictability of these events ensure that recognition of new pathogens must await their emergence.

CONCLUSION

The proposed American fiscal budget for 1995 allows allocations for the CDC which remain basically the same as those for past years and the $11.5 billion budget for the National Institutes of Health includes only a modest increase for non-AIDS infectious and immunological diseases research (Cassell 1994). In view of the magnitude of the problem, this budget is unacceptable. Currently, infectious diseases remain the leading cause of death worldwide. In the United States infectious diseases directly account for 3 and indirectly account for 5 of the 10 leading causes of death, AIDS is the ninth leading cause. Infectious diseases account for 25% of all visits to physicians in the United States. In total, the annual cost of AIDS and other infectious diseases reached $120 billion in 1992, about 15% of the nation's total health-care expenditure. The expanding pool of immunodeficient patients due to the AIDS epidemic, cancer treatment, transplant recipients, and hemodialysis has caused an explosion of opportunistic infections due to a number of fungal, parasitic, viral and bacterial agents.

According to the Gail H. Cassel, president of the American Society of Microbiology, the public health system is not prepared to meet the challenges of new and re-emerging infections. Perhaps the most obvious defect is inadequate disease surveillance and reporting. In America, only one-quarter of the states have a professional position dedicated to surveillance of food-borne and waterborne diseases. In 1992, only $55000 was spent on federal, state and local levels tracking drug-resistant bacterial and viral infections. In addition, the public health laboratories are eroding. Overall, CDC's budget for infectious diseases unrelated to AIDS has declined approximately 20% in the last decade. This is the case in the developed world of the United States, and we in developing South Africa are certainly no better off in terms of disease surveillance and concomitant protection. It should be clear that a mixture of basic and applied research related to infectious disease is needed. Coupled with this, better diagnostic techniques, prevention strategies and risk factor analysis is needed. Finally, enhanced communication among health care professionals and the public is integral in coming to terms and dealing with this issue. The American National Institute of Allergy and Infectious Diseases (NIAID) plans to develop a research and training infrastructure to elucidate the mechanisms of molecular evolution and drug resistance and to learn more about actual disease transmission through molecular and environmental studies and to continue their emphasis on vaccine development. For example, NIAID-funded research has already led to the creation of a new Haemophilus influenzae type B vaccine which is expected to save nearly $400 million in health-care costs each year. Similarly, the NIH spent less than $27 million dollars to find the connection between *Helicobacter pylori* and chronic peptic ulcers, yet using antibacterial therapy for the disease will save $760 million dollars in health care costs annually.

Given the diverse nature of threats from infectious diseases, it is not adequate merely to face each crisis as it emerges, as this may provide a strategy which proves to be too little and too late. Instead, a more holistic approach is required. This must include a global perspective as well as the need to address the issue of infectious disease within the context of shared environmental responsibility. Improved health care derived from socioeconomic betterment is crucial, as are long term policies involving systems thinking as opposed to the limiting nature of long term over-specialization.

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Hantaviruses, with emphasis on Four Corners Hantavirus

Synonyms: Sin Nombre virus, Convict Creek virus, Muerto Canyon virus

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Summary:

Hantaviruses have been implicated as etiologic agents for two acute diseases: hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). Both diseases are carried by rodent vectors. HFRS viruses are carried by Old World rodents, and HPS viruses by New World rodents. The true number and disease potentials of hantaviruses is doubtlessly much higher than currently appreciated. Hantaviruses are classic "emerging viruses" because of their tendency to appear, sometimes explosively, in new populations in which they are unexpected.

Introduction

Hantaviruses are serologically-related members of the family Bunyaviridae (Elliott, 1991).

They are enveloped viruses with a tripartite negative-sense RNA genome. The three genome segments are called L, M and S; they encode the viral transcriptase, envelope glycoproteins, and nucleocapsid protein, respectively.

The prototype hantavirus is Hantaan virus (HTN), which infects the Asian striped field mouse *Apodemus agrarius* (Lee, 1978, Yanagihara, 1987a). In the rodent host HTN causes a chronic infection that does not appear to result in disease. When the virus is transmitted from mice to humans, an acute disease known as hemorrhagic fever with renal syndrome (HFRS, Korean hemorrhagic fever) results. Similar syndromes, although generally less severe, are caused by the related Puumala virus (PUU), Seoul virus (SEO), and Belgrade (Dobrava, DOB) virus. Mortality rates for HTN and DOB HFRS are the highest among the hantavirus-associated diseases, at approximately 5-20%. It is estimated that more than 100,000 people in China contract HFRS annually (Yanagihara, 1987a). All of the hosts for viruses associated with HFRS are associated with rodent hosts indigenous to the Old World (table). SEO is somewhat exceptional in that the worldwide spread of its host rodent, *Rattus norvegicus*, has resulted in the worldwide spread of SEO.

HFRS is an acute febrile illness that was first recognized in the western world after a 1951 outbreak among U.S. troops stationed in Korea. Fever and myalgia (muscle pain) develops days or weeks after exposure to rodents. The disease progresses to hemorrhage (gastrointestinal, subconjunctival, etc) and hemodynamic instability, occasionally progressing to shock. There is acute renal failure, but the precise pathophysiologic lesion producing renal failure is uncertain. Mortality is usually from shock or hemorrhage.

The prototypic New World hantavirus is Prospect Hill virus (PHV) of the meadow vole *Microtus pennsylvanicus*. It has not been associated with human disease, but is widespread among voles in the United States and Canada (Lee, 1982; Yanagihara, 1987b). Serologic evidence for hantavirus infection in deer mice (*Peromyscus maniculatus*) has been recognized for several years (Tsai, 1985, Yanagihara, 1987b) but was generally ascribed to "spillover" of PHV from *M. pennsylvanicus*. Isolation attempts had been unsuccessful.

Despite extensive study, no arthropod vector has been implicated in the transmission of any hantavirus. The route by which the virus is transmitted to humans therefore is believed to be via aerosols of infected excreta of rodents (Zu, 1985; Tsai, 1987). Infected rodents appear to be persistently infected and viruric (have virus in their urine). HTN seroprevalence among mice in endemic areas, as assessed by seroreactivity to culture-adapted Hantaan virus, fluctuates in a biphasic manner: a small peak of seroprevalence in the spring season usually is followed by a decline over the summer months; then a higher peak occurs in the autumn. The incidence of HFRS among humans follows a similar trend, but the curve slightly trails the seroprevalence curve among *A. agrarius* populations. Those individuals whose occupations (eg, farmer) place them at greater exposure to dust or aerosols containing *A. agrarius* excreta are the most commonly affected.

Hantaviruses in murid and cricetid rodents.

A. agrarius and *Rattus norvegicus* are members of the family Muridae. The majority of hantavirus isolates have come from these rodents. Most older classifications of rodents recognize the families Muridae and Cricetidae. All of the New World rodents that have been identified as vectors of HPS are confined within the family Cricetidae. The family Cricetidae includes voles, hamsters, gerbils, deer mice, and harvest mice, and has members that are hosts to PUU, Four Corners virus (FCV), harvest mouse hantavirus (HMV-1), Black Creek Canal virus (BCCV), and Prospect Hill virus (PHV). Members of the PUU/FCV/HMV-1/BCCV/PHV group are contained within a clade (phylogenetic grouping) that is distinct from the HTN/SEO group, as judged by parsimony analyses of nucleic acid sequence (Xiao 1994). The serologic relatedness of the various hantaviruses parallels their relatedness based upon nucleic acid sequence comparisons (Lee, 1985; Chu, 1994; Xiao, 1994). Less is known about the extent of hantavirus infection in the cricetid rodents, as compared with the murid rodents, in part because the cricetid genera have not been subjected to sufficient study with tests of sufficient sensitivity.

Table 1. Hantaviruses and rodent hosts

Hantavirus pulmonary syndrome in the Americas.

Interest in New World hantaviruses accelerated with the recognition of the HPS outbreak in 1993. A cluster of cases of an illness characterized by fever, chills, and severe myalgia was noted in rural residents of the Four Corners region of the United States, where the states of Colorado, New Mexico, Utah, and Arizona share a common border. The disease inevitably progressed to variably severe respiratory compromise and hemodynamic instability. Thrombocytopenia was common, and hemoconcentration and other hematologic abnormalities occur commonly in severe cases. Mortality is slightly more than 50% in the 94 U.S. patients known as of early October, 1994. Four cases have occurred in Canada (British Columbia and Alberta), and one in Sao Paulo, Brazil.

The Four Corners hantavirus (FCV) was shown to be the etiologic agent for cases occurring in the western United States. P. maniculatus was implicated as the host for FCV in New Mexico, Colorado, and Arizona (CDC, 1993a; Nichol, 1993; Childs, 1994; Nerurkar, 1993; Nerurkar, 1994). Of the 79 cases of HPS known as of July 1, 1994, nearly all have been linked to FCV, and isolates (Sin Nombre virus; Convict Creek virus) were made from New Mexico and California deer mice (CDC, 1994a; Hjelle, 1994b; Schmaljohn, 1995). In most cases, that linkage was made possible through the use of reverse transcription-PCR (RT-PCR) analyses of the viral genetic material, using human autopsy tissue (Nichol, 1993) or peripheral blood mononuclear cells (Hjelle, 1994d) as template. For a few cases, RT-PCR studies were not conducted because (a) frozen, unfixed autopsy tissues were not available and immunohistochemical staining was used to reveal hantaviral antigenic material in the fixed lung tissue, or (b) the patient recovered and cleared the virus, and infection was documented retrospectively on clinical and serologic grounds (Jenison, 1994; CDC, 1994a; Zaki, 1993; Wilson, 1994). In two cases, the serologic response to distant hantavirus infections in New Mexico case-patients included antibodies to the FCV G1 glycoprotein, which appears to be distinctive for FCV or BCCV infection (Wilson, 1994; Jenison, 1994; Hjelle, 1994c; Jenison S and Hjelle B, unpublished). For a small, unknown number of western HPS case-patients, RT-PCR or serologic studies have not formally proven that FCV was the etiologic agent.

A few exceptional cases have greatly expanded the definition of potential agents of HPS. A man in Florida contracted HPS at a drug rehabilitation center. He recovered before the illness was recognized as HPS, and viral genetic material could not be amplified from his blood (CDC, 1994b). A novel hantavirus, BCCV, was later isolated from seropositive cotton rats (Sigmodon hispidus) trapped at the rehabilitation center, and persuasive evidence was

presented that BCCV was responsible for the man's illness (CDC, 1994b; CDC, 1994c; Hjelle, 1994c). A young man succumbed to HPS after exposure in Rhode Island or New York in January, 1994. The etiologic agent proved to have 93% nucleocapsid protein sequence identity to a New Mexico FCV, but nucleotide sequence similarity was much lower (83%) (Hjelle, 1995b; CDC, 1994c). A patient from Monroe, Louisiana, died of HPS in 1993. A hantavirus M segment cDNA was amplified from autopsy lung tissue, and shown to be phylogenetically distinct from FCV (CDC, 1994b). In these 3 cases, and in at least one additional case (eastern Texas), HPS was apparently contracted outside of the range of P. maniculatus (CDC, 1994a; CDC, 1994c).

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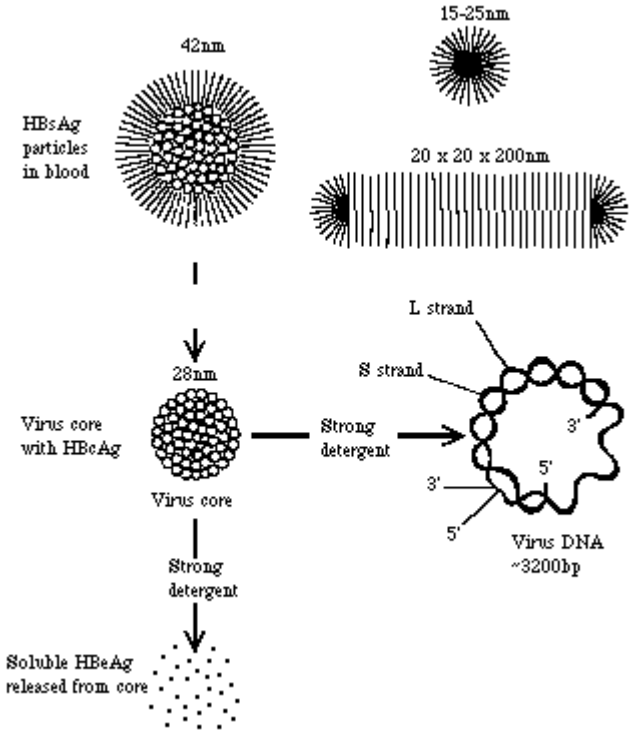
Hepatitis B Virus (HBV):

'Serum hepatitis' was distinguished clinically from 'infectious hepatitis' in the 1930's indicating that at least at least 2 different infectious agents were responsible for hepatitis. Infection often results from inoculation with human serum - blood transfusions, transplants or passive immunization (common among IVDAs). However, the virus is also transmitted sexually, by ingestion and from mother to child (transplacenta and breast milk) - accounting for familial clusters. All blood/organ/tissue donations in developed countries are now tested for HBV and risk of transmission is extremely low. Incubation period 45-120 days. HBV does not grow in tissue culture and this has hindered investigations.

- Blumberg, 1963: Was searching for novel antigens useful for blood/tissue typing. Looked at U.S. haemophiliacs (multiply transfused, each transfusion pooled from 00's donors) - found Abs which recognised antigen in the serum of an Australian aborigine **'Australian antigen - Au'**.
- 1967: It was recognised that Au was a viral antigen = **HBsAg** (surface antigen).
- Dane, 1970: Discovered **42nm 'Dane particles'** in blood of hepatitis patients = **HBcAg** (core antigen).
- 1973: **HBeAg** discovered (endogenous antigen = a truncated version of HBcAg).



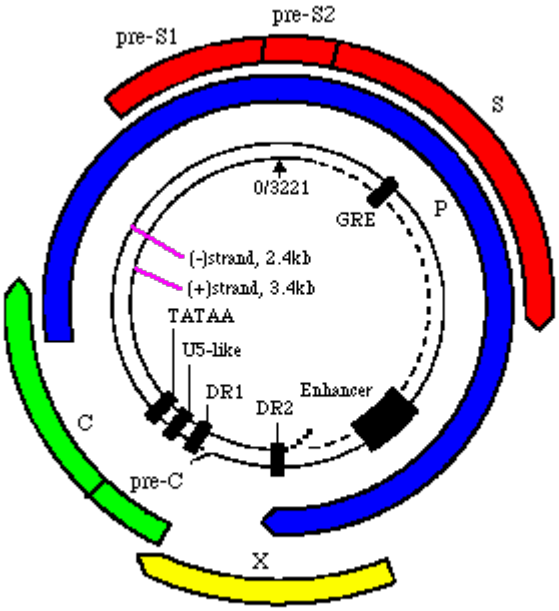
HBV is the prototype member of the family Hepadnaviridae.

Features:

Spherical, enveloped (? lipid-containing, detergent disrupted ?) particles 42-47nm diameter containing partially d/s DNA plus an RNA-dependent DNA polymerase (i.e. reverse transcriptase)
(c.f. retroviruses - package RNA + RT).

Hepadnaviruses have the among the smallest genomes of all known viruses, consisting of two uneven strands of DNA:

(-)sense strand, 3.0 - 3.3kb (size varies between different Hepadnaviruses)
(+)sense strand, 1.7 - 2.8kb (size varies between different particles)



Replication:

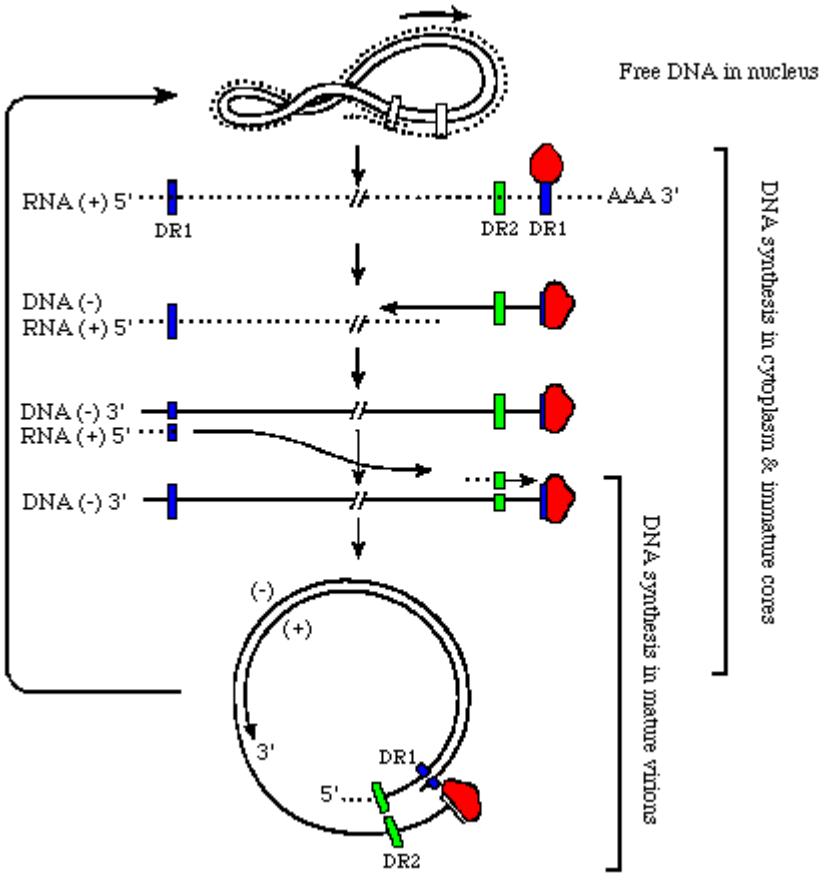
HBsAg is composed of 3 polypeptides (below) and is presumably responsible for receptor binding (not known), which in turn presumably determines the tropism of the virus for hepatocytes (not known). 3 major genome transcripts are produced: 3.5kb, 2.4kb, 2.1kb. All have same polarity, same 3' ends but different 5' ends (i.e. initiation sites). These transcripts are somewhat heterogeneous in size and it is not completely clear which proteins each transcript encodes, but there are 4 known genes in the virus:

- **C** - the core protein
- **P** - the polymerase
- **S** - the 3 polypeptides of the surface antigen (preS1, preS2 and S - produced from alternative translation start sites.
- ***X*** - a transactivator of viral transcription (and cellular genes?)

Closed circular DNA is found soon after infection in the nucleus of the cell and therefore is probably the source of

these transcripts. This DNA is produced by repair of the gapped virion DNA as follows:

1. completion of the (+)sense strand
2. removal of a protein primer from the (-)sense strand and an oligoribonucleotide primer from the (+)sense strand
3. elimination of terminal redundancy at the ends of the (-)sense strand
4. ligation of the ends of the two strands



It is not known how or by which proteins (viral/cellular) these events are carried out. The 3.5kb RNA transcript, core antigen and polymerase form core particles in the cytoplasm. The polymerase converts the RNA to DNA in these particles **INSIDE THE PARTICLE** in the cytoplasm c.f. retroviruses, where RNA is packaged then converted to DNA as the first stage of genome replication:

Features:	Hepadnaviruses:	Retroviruses:
Genome:	DNA	RNA
Primer for (-)strand synthesis:	Protein	tRNA
Terminal Repeats (LTRs):	No	Yes
Specific integration site in virus genome:	No	Yes

The surface antigen and lipid envelope may be acquired at any stage after encapsidation of the RNA, apparently from intracellular membranes.

Assembly/release appear to be rather disordered events for Hepadnaviruses - some extracellular particles contain DNA-RNA hybrids or (-)sense DNA strands without (+)sense strands (intermediates in reverse transcription). Assembly of the particle is initiated by packaging of the RNA pregenome and the viral reverse transcriptase-DNA polymerase into a nucleocapsid. The pregenome is then reverse transcribed into single-stranded minus-polarity DNA, which is subsequently replicated to double-stranded DNA. All replicative intermediates are observable in

capsids within infected liver, but only relatively mature nucleocapsids containing partially double stranded DNA are found in secreted virions. This observation suggests that maturation of the genome within the capsid is required for envelopment and secretion.

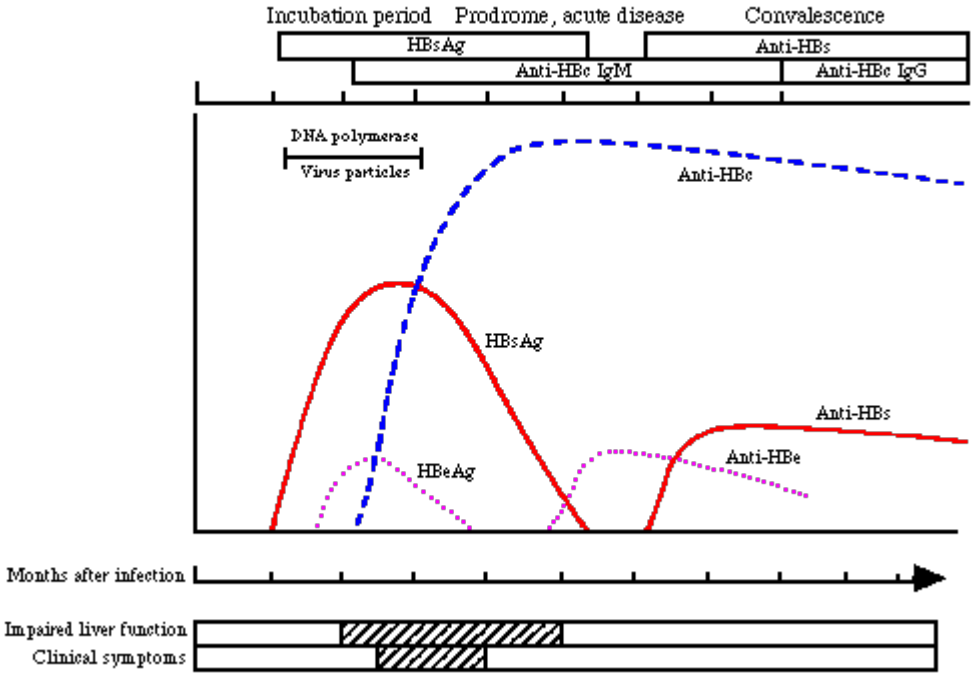
Release of mature particles occurs by secretory mechanisms - "reverse endocytosis", without cell lysis.

Pathogenesis:

HBV infection has 3 possible outcomes:

1. **Acute** course with complete recovery and immunity from reinfection (>90%).
2. **Fulminant** hepatitis with liver failure and mortality ~90% (~1% cases).
3. **Chronic** infection - carrier state with virus persistence (~10% cases).

There are >200m HBV carriers worldwide (total population of world ~4bn. = 5%).



Primary Hepatocellular Carcinoma (PHC): A rare tumour in the west (<2% fatal cancers) and most cases are alcohol-related. In S.E. Asia and China PHC is the most common fatal cancer, ~$5x10^5$ deaths p.a.
Relationship between HBV infection and PHC is not clear cut:

- Cirrhosis appears to be a prerequisite + chronic liver damage/repair results in malignant transformation.
- Co-factors such as aflatoxins and nitrosamines can induce PHC-like disease in experimental animals.

The key factor is then what determines the development of chronic vs. acute infection:

- Age (chronic infections decrease with increasing age)
- Sex:

Syndrome:	Males : Females
Chronic Infection:	1.5 : 1
Cirrhosis:	3 : 1
PHC:	6 : 1

- Route of infection (oral/sexual infections give rise to less chronic cases than serum infection)

Prevention/Therapy:

Purified (!) HBsAg from the blood of chronic carriers was originally used as a vaccine - risky! A recombinant HBsAg vaccine produced in yeast is now used - safe and reasonably effective - one of the few recombinant vaccines to date. Effective vaccination campaigns could:

1. save ~1m lives p.a.
2. eradicate the virus (no animal reservoir).

Alpha-IFN is used for therapy of chronic HBV infection. 30-40% chronic carriers respond to this (expensive) treatment, c.f. 10-20% spontaneous loss of virus markers in untreated control groups.
Lamivudine (3TC - 2'deoxy, 3'thiacytidine - a reverse transcriptase inhibitor) is currently being investigated for therapy of chronic HBV infection. Early results suggest this drug may be effective in patients who have previously failed to clear the virus with alpha-IFN.
A number of other drugs and a therapeutic vaccine are also in clinical trials. *Read: Terrault, N.A. "Treatment of chronic hepatitis B and chronic hepatitis C" Rev.Med.Virol. 6:215-228 (1996).*

- References: Click here
- **Medscape Article:** Ask the Experts: Hepatitis B
- Search for more information on this topic
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HAV | HCV | HDV | HEV | GBV-C/HGV | TTV

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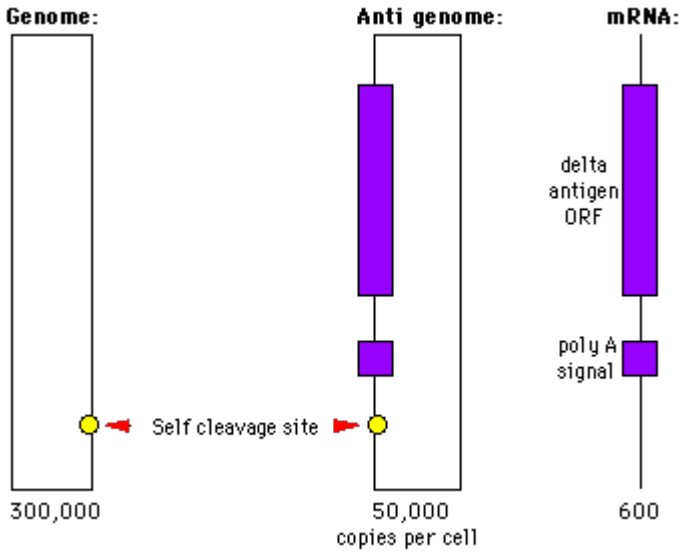
Last Updated: Saturday, October 4, 1945624.

Hepatitis D (delta):

In 1977, a novel nuclear antigen was discovered in patients with chronic HBV infection - called delta antigen (after the 3 known HBV antigens). By 1980, transmission experiments in chimpanzees showed that HDV was a defective transmissible pathogen dependent on HBV for replication - similar to plant virus satellite RNAs. Delta antigen is found wherever HBV infection occurs and seems to potentiate the pathogenic effects of HBV infection. More then 10 million people are believed to be infected with HDV. Fulminant hepatitis (mortality ~80%) is 10 times more common than with HBV infection alone. Patients with dual infection with hepatitis B virus (HBV) and delta virus (HDV) respond poorly to interferon (IFN) therapy. Liver transplantation for HDV cirrhosis seems to be more successful than in any other viral hepatitis, with comparatively few reinfections of the grafted liver.

The HDV genome is a unique chimaeric molecule with some of the properties of a satellite virus and some of a viroid - a covalently closed circular RNA molecule 1679-1683 bases long with a branched or rod-like configuration:



This molecule is thought to be replicated by host cell RNA polymerase II using a rolling circle mechanism. This produces linear concatemers which must be cleaved for infetcivity. The cleavage is carried out by a ribozyme domain present in the HDV RNA, the only known example of a ribozyme in an animal virus genome ("Crystal structure of a hepatitis delta virus ribozyme" Nature 395: 567-574, 1998).

However, unlike all other viroids, HDV encodes a protein, the delta antigen, which is a nuclear phosphoprotein. Post-transcriptional RNA editing results in the production of two slightly different forms of the protein, **S-HDAg** (195 amino acids) which is necessary for HDV replication and **L-HDAg** (214 amino acids) which is necessary for the assembly and release of HDV-containing particles. Preparations from HDV-infected animals contain heterologous particles distinct from HBV but with ill-defined structure. L-HDAg and small-form hepatitis B surface antigen (HBsAg) of the helper hepatitis B virus have previously been shown to be the minimum components for the assembly of these particles.

Replication of HDV is hard to study since it requires the presence of a helper virus (HBV) which itself cannot be cultured! HDV can be controlled by controlling HBV infection. The worldwide distributions of HDV infection is currently changing as HBV vaccination programmes begin to take effect, for example in Italy: in a study of HBsAg chronic carriers, HDV prevalence reduced from 23% in 1987 to 14% in 1992.

- References: Click here

- **Medscape Article:** Treatment of Chronic Hepatitis D
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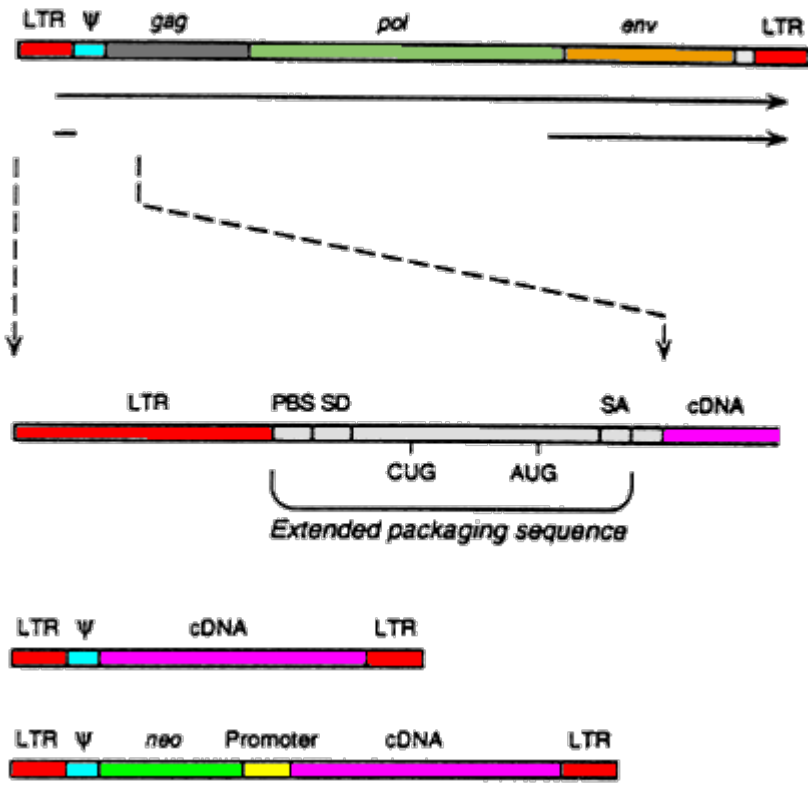
Retroviral Vectors

Retroviruses are a class of enveloped viruses containing a single stranded RNA molecule as the genome. Following infection, the viral genome is reverse transcribed into double stranded DNA, which integrates into the host genome & is expressed as proteins. The viral genome is approximately 10kb, containing at least three genes: gag (coding for core proteins), pol (coding for reverse transcriptase) & env (coding for the viral envelope protein). At each end of the genome are long terminal repeats (LTRs) which include promoter/enhancer regions & sequences involved with integration. In addition there are sequences required for packaging the viral DNA (psi) & RNA splice sites in the env gene.



Some retroviruses contain protooncogenes, which when mutated can cause cancers, however, in the production of vectors these are removed. Retroviruses can also transform cells by integrating near to a cellular protooncogene & driving inappropriate expression from the LTR, or by disrupting a tumour suppresser gene. This event, termed insertional mutagenesis, though extremely rare could still occur when retroviruses are used as vectors.

Retroviral vectors are most frequently based upon the Moloney murine leukaemia virus (Mo-MLV), which is an amphotrophic virus, capable of infecting both mouse cells, enabling vector development in mouse models, & human cells, enabling human treatment. The viral genes (gag, pol & env) are replaced with the transgene of interest & expressed on plasmids in the packaging cell line. Because the non-essential genes lack the packaging sequence (psi) they are not included in the virion particle. To prevent recombination resulting in replication competent retroviruses, all regions of homology with the vector backbone should be removed & the non-essential genes should be expressed by at least two transcriptional units (Makowitz et al, 1988). Even so, replication competent retroviruses do occur at a low frequency.

The essential regions include the 5' & 3' LTRs & the packaging sequence lying downstream of the 5' LTR. Transgene expression can either be driven by the promoter/enhancer region in the 5' LTR, or by alternative viral (e.g. cytomegalovirus, Rous sarcoma virus) or cellular (e.g. beta actin, tyrosine) promoters. Mutational analysis has shown that up to the entire gag coding sequence & the immediate upstream region can be removed without effecting viral packaging or transgene expression (Kim et al, 1998). However the exact positioning of the transgene start codon & small alterations of the 5' LTR influence transgene expression (Rivire et al, 1995). To aid identification of transformed cells selectable markers, such as neomycin & beta galactosidase, can be included & transgenes expression can be improved with the addition of internal ribosome sites (Saleh, 1997). The available carrying capacity for retroviral vectors is approximately 7.5 kb (Verma & Somia, 1997), which is too small for some genes even if the cDNA is used.

The retroviral envelope interacts with a specific cellular protein to determine the target cell range. Altering the env gene or its product has proved a successful means of manipulating the cell range. Approaches have included direct modifications of the binding site between the envelope protein & the cellular receptor, however these approaches tend to interfere with subsequent internalisation of the viral particle (Harris & Lemoine, 1996). By replacing a portion of the env gene with 150 codons from the erythropoietin protein (EPO), Kasahara et al (1994) were able to target EPO receptor bearing cells with high affinity. Coupling an antibody to the viral particle with affinity for a second cell specific antibody via a streptovadin bridge, improves viral uptake, but internalisation tends to lead to viral degradation (Roux et al, 1989). Neda et al (1991) treated viral particles with lactose which resulted in uptake by cells, principally hepatocytes, expressing asiaglycoprotein receptors. Subsequently there was efficient viral transgene expression, possibly due to acidification of the endosome allowing fusion of the viral envelope with the endosome membrane.

Viruses differ with respect to their tropisms, therefore by replacing the env gene with that of another virus, the host range can be extended, in a technique known as pseudotyping. Vesicular stomatitis virus G protein has been included in Mo-MLV derived vectors (Burns et al, 1994), which are also more stable when purified by ultracentrifugation. Recently, Qing et al (1997) improved transduction into numerous cell lines by first treating the recipient cells with an adeno-associated vector (discussed below) expressing the cellular receptor for retroviral envelope protein.

A requirement for retroviral integration & expression of viral genes is that the target cells should be dividing. This limits gene therapy to proliferating cells in vivo or ex vivo, whereby cells are removed from the body, treated to stimulate replication & then transduced with the retroviral vector, before being returned to the patient. When treating cancers in vivo, tumour cells are preferentially targeted (Roth et al, 1996, Tait et al, 1997). However, ex vivo cells can be more efficiently transduced, due to exposure to higher virus titres & growth factors (Glimm et al, 1997). Furthermore ex vivo treated tumour cells will associate with the tumour mass & can direct tumouricidal effects (Oldfield & Ram, 1995, Abdel-Wahab et al, 1997).

Though transgene expression is usually adequate in vitro & initially in vivo, prolonged expression is difficult to attain. Retroviruses are inactivated by c1 complement protein & an anti-alpha galactosyl epitope antibody, both present in human sera (Rother et al, 1995, Rollins et al, 1996). Transgene expression is also reduced by inflammatory interferons, specifically IFN-alpha & IFN-gamma acting on viral LTRs (Ghazizadeh et al, 1997). As the retroviral genome integrates into the host genome it is most likely that the viral LTR promoters are being inactivated, therefore one approach has been to use promoters for host cell genes, such as tyrosine (Diaz et al, 1998). Clearly this is an area where continued research is needed.

Lentivirus Vectors:

Lentiviruses are a subclass of retroviruses which are able to infect both proliferating & non-proliferating cells. They are considerably more complicated than simple retroviruses, containing an additional six proteins, tat, rev, vpr, vpu, nef & vif. Current packaging cell lines have separate plasmids for a pseudotype env gene, a transgene construct, & a packaging construct supplying the structural & regulatory genes in trans (Naldini et al, 1996). Early results using marker genes have been promising, showing prolonged in vivo expression in muscle, liver & neuronal tissue (Blmer et al, 1997, Miyoshi et al, 1997, Kafri et al, 1997). Interestingly the transgenes are driven by an internally engineered cytomegalovirus promoter, which unlike when in MoMLV vectors, is not inactivated. This may be due to the limited inflammatory response to the vector injection, which was equal in magnitude to the saline control (Blmer et al, 1997).

The lentiviral vectors used are derived from the human immunodeficiency virus (HIV) & are being evaluated for safety, with a view to removing some of the non-essential regulatory genes. Mutants of vpr & vif are able to infect neurones with reduced efficiency, but not muscle or liver cells (Blmer et al, 1997, Kafri et al, 1997).

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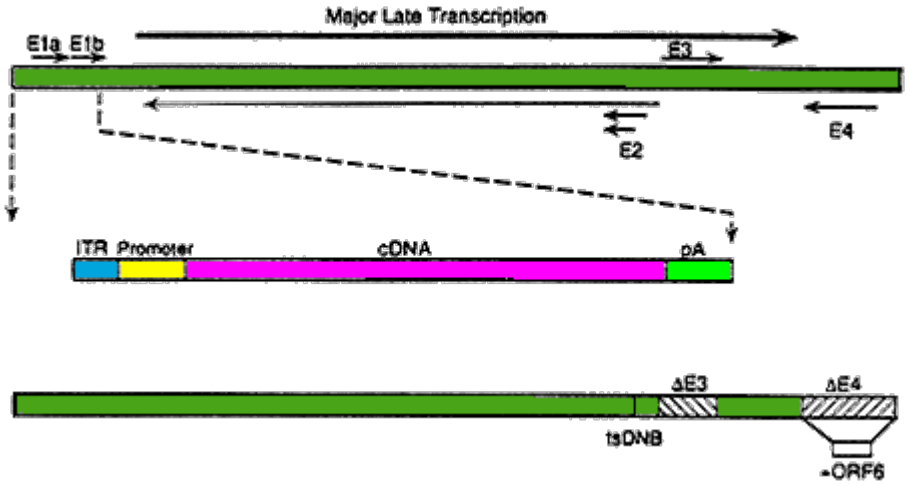
Adenovirus Vectors

Adenoviruses are non-enveloped viruses containing a linear double stranded DNA genome. While there are over 40 serotype strains of adenovirus, most of which cause benign respiratory tract infections in humans, subgroup C serotypes 2 or 5 are predominantly used as vectors. The life cycle does not normally involve integration into the host genome, rather they replicate as episomal elements in the nucleus of the host cell & consequently there is no risk of insertional mutagenesis.

The wild type adenovirus genome is approximately 35 kb of which up to 30 kb can be replaced with foreign DNA (Smith, 1995, Verma & Somia, 1997). There are four early transcriptional units (E1, E2, E3 & E4), which have regulatory functions, & a late transcript, which codes for structural proteins. Progenitor vectors have either the E1 or E3 gene inactivated, with the missing gene being supplied in trans either by a helper virus, plasmid or integrated into a helper cell genome (human fetal kidney cells, line 293, Graham et al, 1977). Second generation vectors additionally use an E2a temperature sensitive mutant (Engelhardt et al, 1994) or an E4 deletion (Armentano et al, 1997). The most recent "gutless" vectors contain only the inverted terminal repeats (ITRs) & a packaging sequence around the transgene, all the necessary viral genes being provided in trans by a helper virus (Chen et al, 1997).



Adenoviral vestors are very efficient at transducing target cells in vitro & vivo, & can be produced at high titres (>10^{11}/ml). With the exception of Geddes et al (1997), who showed prolonged transgene expression in rat brains using an E1 deletion vector, transgene expression in vivo from progenitor vectors tends to be transient (Verma & Somia, 1997). Following intravenous injection, 90% of the administered vector is degraded in the liver by a non-immune mediated mechanism (Worgall et al, 1997). Thereafter, an MHC class I restricted immune response occurs, using CD8+ CTLs to eliminate virus infected cells & CD4+ cells to secrete IFN-alpha which results in anti-adenoviral antibody (Yang & Wilson, 1995). Alteration of the adenoviral vector can remove some CTL epitopes, however the epitopes recognised differ with the host MHC haplotype (Sparer et al, 1997, Jooss et al, 1998). The remaining vectors, in those cells that are not destroyed, have their promoter inactivated (Armentano et al, 1997) & persisting antibody prevents subsequent administration of the vector.

Approaches to avoid the immune response involving transient immunosupressive therapies have been successful in prolonging transgene expression & achieving secondary gene transfer (Jooss et al, 1996; Kay et al, 1997). A less interventionist method has been to induce oral tolerance by feeding the host UV inactivated vector (Kagami et al, 1998). However, it is desirable to manipulate the vector rather than the host. Although only replication deficient vectors are used, viral proteins are expressed at a very low level which are presented to the immune system. The development of vectors containing fewer genes, culminating in the "gutless" vectors which contain no viral coding sequences, has resulted in prolonged in vivo transgene expression in liver tissue (Schieder et al, 1998). The initial delivery of large amounts of DNA packaged within adenovirus proteins, the majority of which will be degraded & presented to the immune system may still cause problems for clinical trials. Moreover the human population is heterogeneous with respect to MHC haplotype & a proportion of the population will have been already exposed to the adenoviral strain (Gahry-Sgard et al, 1997)

Until recently, the mechanism by which the adenovirus targeted the host cell was poorly understood. Tissue specific expression was therefore only possible by using cellular promoter/enhancers e.g. the myosin light chain 1 promoter

(Shi et al, 1997) & the smooth muscle cell SM22a promoter (Kim et al, 1997), or by direct delivery to a local area (Rome et al, 1994). Uptake of the adenovirus particle has been shown to be a two stage process involving an initial interaction of a fibre coat protein in the adenovirus with a cellular receptor or receptors, which include the MHC class I molecule (Hong et al, 1997) & the coxsackievirus-adenovirus receptor (Bergelson et al, 1997). The penton base protein of the adenovirus particle then binds to the integrin family of cell surface heterodimers (Wickham et al, 1993) allowing internalisation via receptor mediated endocytosis. Most cells express primary receptors for the adenovirus fibre coat protein, however internalisation is more selective (Harris & Lemoine, 1996). Methods of increasing viral uptake include stimulating the target cells to express an appropriate integrin (Davison et al, 1997) & conjugating an antibody with specificity for the target cell type to the adenovirus (Wickham et al, 1997b, Goldman et al, 1997). The use of antibodies though increases the production difficulties of the vector & the potential risk of activating the complement system. By incorporating receptor binding motifs into the fibre coat protein, Wickham et al (1997a) were able to redirect the virus to bind the integrin expressed by damaged endothelial or smooth muscle cells, or heparin sulphate receptors which is expressed by numerous cells types.

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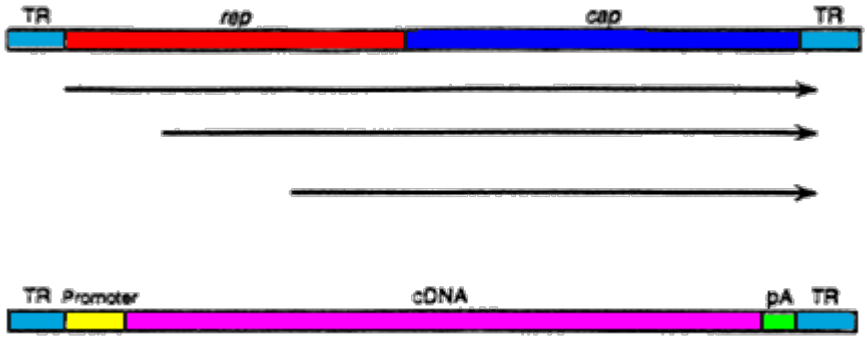
Adeno-Associated Viruses

Adeno-associated viruses (AAV) are non-pathogenic human parvoviruses, dependant on a helper virus, usually adenovirus, to proliferate. They are capable of infecting both dividing & non dividing cells, & in the absence of a helper virus integrate into a specific point of the host genome (19q 13-qter) at a high frequency (Kotin et al, 1990). The wild type genome is a single stranded DNA molecule, consisting of two genes; rep, coding for proteins which control viral replication, structural gene expression & integration into the host genome, & cap, which codes for capsid structural proteins. At either end of the genome is a 145 bp terminal repeat (TR), containing a promoter:



When used as a vector, the rep & cap genes are replaced by the transgene & its associated regulatory sequences. The total length of the insert cannot greatly exceed 4.7 kb, the length of the wild type genome (Smith, 1995). Production of the recombinant vector requires that rep & cap are provided in trans, along with helper virus gene products (E1a, E1b, E2a, E4 & VA RNA from the adenovirus genome). The conventional method is to cotransfect two plasmids, one for the vector & another for rep & cap, into 293 cells infected with adenovirus (Samulski et al, 1989). This method, however, is cumbersome, low yielding ($<10^4$ particles/ml) & prone to contamination with adenovirus & wild type AAV. One of the reasons for the low yield is the inhibitory effect of the rep gene product on adenovirus replication (Vincent et al, 1997). More recent protocols remove all adenoviral structural genes & use rep resistant plasmids (Xiao et al, 1998) or conjugate a rep expression plasmid to the mature virus prior to infection (Fisher et al, 1996).

In the absence of rep, the AAV vector will only integrate at random, as a single provirus or head to tail concatamers, once the terminal repeats have been slightly degraded (Rutledge & Russell, 1997). Interest in AAV vectors has been due to their integration into the host genome allowing prolonged transgene expression. Gene transfer into vascular epithelial cells (Maeda et al, 1997), striated muscle (Fisher et al, 1997, Herzog et al, 1997) & hepatic cells (Snyder et al, 1997) has been reported, with prolonged expression when the transgene is not derived from a different species. Neutralising antibody to the AAV capsid may be detectable, but does not prevent readministration of the vector or shut down promoter activity. It is possibly due to the simplicity of the viral capsid, that the immune response is so muted. As AAV antibodies will be present in the human population this will require further investigation. There has been no attempt to target particular cell types other than by localised vector delivery.

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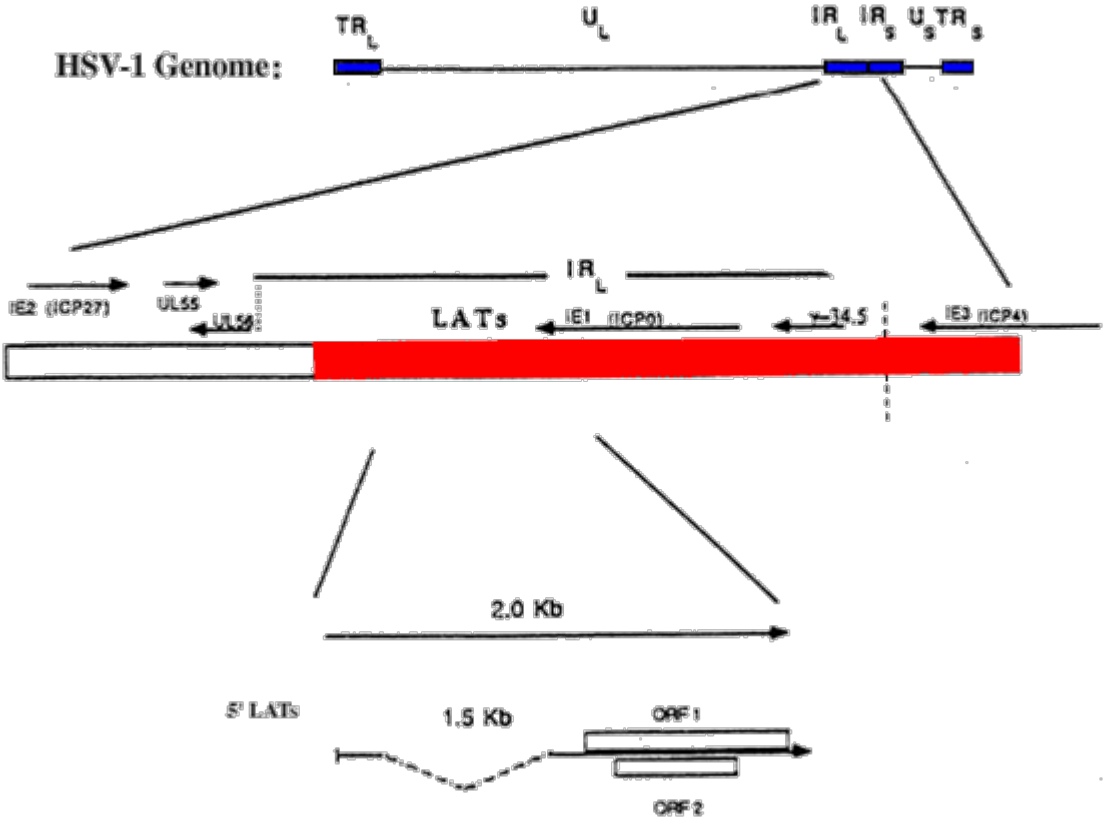
Herpes Simplex Virus

Herpes simplex virus type 1 (HSV-1) is a human neurotropic virus, consequently interest has largely focused on using HSV-1 as a vector for gene transfer to the nervous system. The wild type HSV-1 virus is able to infect neurones & either proceed into a lytic life cycle or persist as an intranuclear episome in a latent state. Latently infected neurones function normally & are not rejected by the immune system. Though the latent virus is transcriptionally almost silent, it does possess neurone specific promoters that are capable of functioning during latency. Antibodies to HSV-1 are common in the human population, however complications due to herpes infection, such as encephalitis, are very rare.

The viral genome is a linear double stranded DNA molecule of 152 kb. There are two unique regions, long & short (termed UL & US) which are linked in either orientation by internal repeat sequences (IRL & IRS). At the non-linker end of the unique regions are terminal repeats (TRL & TRS). There are up to 81 genes (Marconi et al, 1996), of which about half are not essential for growth in cell culture. Once these non essential genes have been deleted, 40-50 kb of foreign DNA can be accommodated within the virus (Glorioso et al, 1995). Three main classes of HSV-1 genes have been identified, namely the immediate-early (IE or alpha) genes, early (E or beta) genes & late (L or gamma) genes.

Following infection in susceptible cells, lytic replication is regulated by a temporally co-ordinated sequence of gene transcription. Vmw65 (a tegument structural protein) activates the immediate early genes (IP0, ICP4, ICP22, ICP27 & ICP477), which are transactivating factors allowing the production of early genes. The early genes encode genes for nucleotide metabolism & DNA replication. Late genes are activated by the early genes & code for structural proteins. The entire cycle takes less than 10h & invariably results in cell death.



The molecular events leading to the establishment of latency have not been fully determined. Gene expression during latency is driven by the latency associated transcripts (LATs) located in the IRL region of the genome. Two LATs (2.0 & 1.5kb) are transcribed in the opposite direction to the IE gene ICP0. LATs have a role in HSV-1 reactivation from latency (Steiner et al, 1989) & the establishment of latency (Sawtell & Thompson, 1992). Two latency active promoters which drive expression of the LATs have been identified (Marconi et al, 1996) & may prove useful for vector transgene expression.

Two basic approaches have been used for production of HSV-1 vectors, namely amplicons & recombinant HSV-1

viruses. Amplicons are bacterially produced plasmids containing col E1 ori (an Escherishia coli origin of replication), OriS (the HSV-1 origin of replication), HSV-1 packaging sequence, the transgene under control of an immediate-early promoter & a selectable marker (Federoff et al, 1992). The amplicon is transfected into a cell line containing a helper virus (a temperature sensitive mutant) which provides all the missing structural & regulatory genes in trans. Both the helper & amplicon containing viral particles are delivered to the recipient. More recent amplicons include an Epstein-Barr virus derived sequence for plasmid episomal maintenance (Wang & Vos, 1996).

Recombinant viruses are made replication deficient by deletion of one the immediate-early genes e.g. ICP4, which is provided in trans. Though they are less pathogenic & can direct transgene expression in brain tissue, they are toxic to neurones in culture (Marconi et al, 1996). Deletion of a number of immediate-early genes substantially reduces cytotoxicity & also allows expression from promoters that would be silenced in the wild type latent virus. These promoters may be of use in directing long term gene expression.

Replication-conditional mutants are only able to replicate in certain cell lines. Permissive cell lines are all proliferating & supply a cellular enzyme to complement for a viral deficiency. Mutants include thymidine kinase (During et al, 1994), ribonuclease reductase (Kramm et al, 1997), UTPase, or the neurovirulence factor g34.5 (Kesari et al, 1995). These mutants are particularly useful for the treatment of cancers, killing the neoplastic cells which proliferate faster than other cell types (Andreansky et al, 1996, 1997).

A number of neurological diseases could be amenable to gene therapy by HSV-1 vectors (Kennedy, 1997). Though most attention has focused on cancers, there has been some success in Parkinsons disease by expressing tyrosine hydroxylase in striatal cells (Geller et al, 1995, During et al, 1994), thus replacing the supply of L-dopa. Federoff et al (1992) induced nerve repair following axotomy of the superior cervical ganglion, by injection of a vector expression nerve growth factor. This resulted in restored levels of tyrosine hydroxylase.

However, Wood et al (1994) observed strong inflammatory responses to HSV-1 amplicon vectors, both at the primary site of the injection & at secondary sites supplied by nerve fibres originating from area of the injection. In addition up to 20% of experimental animals may die shortly after injection with HSV-1 vector (Kucharizuk et al, 1997), though the reason is unknown. A viral protein, ICP47, has been identified, which reduces viral antigen presentation & may be employed in future HSV-1 vectors to reduce cytotoxicity (York et al, 1994).

Because of its tropism for neuronal tissue issues of cellular targeting have been largely overlooked. However, wild type HSV-1 can infect & lyse other non-neuronal cell types, such as skin (Al-Saadi et al, 1983), & it would be advantageous to target a specific subset of neurones. As HSV-1 will travel down the length of nerve efficient cellular targeting would improve its safety profile when used as a vector. Indeed a replication-restricted HSV-1 vector has been used to treat human malignant mesothelioma (Kucharizuk et al, 1997).

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Non-Viral Methods of DNA Transfer

Viral vectors all induce an immunological response to some degree & may have safety risks (such as insertional mutagenesis & toxicity problems). Furthermore their capacity is limited & large scale production may be difficult to achieve. Non-viral methods of DNA transfer require only a small number of proteins, have a virtually infinite capacity, have no infectious or mutagenic capability & large scale production is possible using pharmaceutical techniques. There are three methods of non-viral DNA transfer. namely:

- naked DNA
- liposomes
- molecular conjugates

Naked DNA (in the form of a plasmid) can be directly injected into muscle cells (Wolff et al, 1990) or attached to gold particles that are bombarded into the tissue (Cheng et al, 1993). Though not very efficient, this can result in prolonged low level expression in vivo. The simplicity of this method, & sustained expression has led to the development of DNA vaccines. Compared to conventional attenuated & protein based vaccines, they are unaffected by pre-existing immunity e.g. due to maternal antibodies, relatively cheap, & can deliver a number of pathogen antigens on a single plasmid (Manickan et al, 1997). DNA vaccines are being developed for those pathogens where there is no existing vaccine e.g. HIV (Lekutis et al, 1997) or the current vaccine not fully effective e.g. influenza (Macklin et al, 1998). By using a highly conserved gene Ulmer et al (1993) were able to immunise mice against two serologically distinct influenza virus strains. In most cases however, DNA vaccines have not been shown to be better than the existing vaccines (Macklin et al, 1998). The actual type of immune response can be controlled by cotransformation of a gene coding for the appropriate cytokine (Xiang & Ertl, 1995) & this method may prove useful in redirecting inappropriate immune responses (Manickan et al, 1997). Other uses for naked DNA include cancer immunopotentiation (discussed below, Corr et al, 1997), repair of pancreatic insulin function (Goldfine et al, 1997), & stimulation of collateral blood vessel development (Takeshita et al, 1997). Expression of the gene product in muscle tissue can be improved by the coadministration of collagenase, papaverine & ischaemic conditions (Budker et al, 1998). The use of a muscle specific promoter (Skarli et al, 1998) & other intragene regulatory sequences, such as the poly A & transcription termination sequence (Hartikka et al, 1996) will also improve transgene expression.

Liposomes are lipid bilayers entrapping a fraction of aqueous fluid. DNA will spontaneously associate to the external surface of cationic liposomes (by virtue of its charge) & these liposomes will interact with the cell membrane (Felgner et al, 1994). In vitro up to 90% of certain cell lines may be transfected. By including a small amount of an anionic lipid in an otherwise cationic liposome the DNA can be incorporated into the internal surface of the liposome, thus protecting it from enzymatic degradation. (Crespo et al, 1996, cited Alio, 1997). To facilitate uptake into the cell as endosomes, targeting proteins have been included in liposomes, e.g. anti-MHC antibody (Wang & Huang, 1987) transferrin (Stavridis et al, 1986), & the Sendai virus or its F protein (Dzau et al, 1996). The Sendai virus additionally allows the plasmid DNA to escape from the endosome into the cytoplasm, thus avoiding degradation. The inclusion of a DNA binding protein (28 kDa high mobility group 1 protein) enhances transcription by bringing the plasmid into the nucleus (Dzau et al, 1997). Further proposed improvements include incorporating the Epstein-Barr virus Ori p & EBNA1 genes in the plasmid (as described above for HSV-1 amplicons) to maintain the plasmid as an episomal element (Alio, 1997).

Molecular conjugates consist of protein or synthetic ligands to which a DNA binding agent has been attached. Delivery to the cell can be improved by using similar techniques to those for liposomes. Targeting proteins include asialoglycoprotein (Wagner et al, 1991), transferrin (Wu et al, 1989), polymeric IgA (Ferkol et al, 1993) & adenovirus (Madon & Blum, 1996). Transgene expression tends to be transient & is limited by endosome/lysosomal degradation.

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Comparison of Vectors

Objective studies comparing the relative merits of different delivery systems are rarely carried out, making comparisons difficult. Lozier et al (1997), compared the merits of retroviruses, adenoviruses & liposomes for transducing gut epithelial cells & found that all three methods efficiently expressed the transgene. The ideal vector combines features of all the delivery systems described above. Preparation of this ideal vector would be relatively simple (e.g. not involving multiple steps such as attachment of a ligand targeting a particular cell type) & result in high vector concentrations (>10^8 particles/ml). To allow subsequent readministration & avoid undesired host reactions there would be no significant immune response to any component of the vector. The lack of an immune response may allow transgene expression to be sufficiently prolonged from episomal systems, such that readministration is not necessary. Alternatively, integration into the host genome, preferably in a site specific location, would ensure that the transgene is not lost during the lifetime of the cell. Whatever the means of delivery, transcriptional control as exerted by the host cell or by the physician, would enable greater tissue specificity & regulation of the transgene expression. As yet no one vector has all these requirements, but improvements are being made to all systems, e.g. immunological problems of adenoviruses, production problems of AAV, the short duration of retroviruses, etc. The genomes of viral vectors are being progressively reduced to contain the transgene & a minimum of maintenance genes, whilst developments to molecular conjugates & liposomes are making them more like viruses.

It is likely that all delivery systems will find a niche, either as dictated by biological needs, e.g. HSV-1 for neuronal applications, or because the expense of developing a system to replace an existing functional method would be prohibitive. A very large number of research groups have invested considerable effort in developing a given system & are unlikely to abandon it before a therapeutic application has been found.

Promoter Control:

A recurring theme throughout this review has been the value of tissue specific promoters, allowing a second level of control over transgene expression, in addition to that of selective transduction by the vector. The most frequently used promoters are viral in origin, often derived from a different virus than the vector backbone, for example cytomegalovirus promoters have been used in all vector systems. Viral promoters have the advantages of being smaller, stronger & better understood than most human promoter sequences. One of the drawbacks of viral promoters is their tendency to be silenced when there is an immunological response. Qin et al (1997) showed that a range of commonly used viral promoters were silenced by IFN-gamma & TNF-alpha at the level of mRNA stability. By contrast a constitutive human promoter, beta actin, was less effected.

A range of cellular promoters have been developed for specific tissues including the liver (albumin; Miyatake et al, 1997), muscle (myosin light chain 1; Shi et al, 1997) & endothelial cells (von Willebrandt; Ozaki et al, 1996 & smooth muscle 22a; Kim et al, 1997). Tumour specific promoters are also being used in developing cancer therapies, including tyrosine kinase for B16 melanoma (Diaz et al, 1998), DF3/MUC1 for certain breast cancers (Wen et al, 1993) & afetoprotein for hepatomas (Chen et al, 1995). The temporal expression of the transgene construct can be controlled by drug inducible promoters, for example by including cAMP response element enhancers in a promoter, cAMP modulating drugs can be used (Suzuki et al, 1996). Alternatively repressor elements can prevent transcription in the presence of the drug (Hu et al, 1997). Spatial control of expression has been developed by using ionising radiation (radiotherapy) in conjunction with the erg1 gene promoter (Hallaham et al, 1995).

Despite the plethora of different promoters that have been identified, their behaviour in the context of a vector can be very difficult to predict (Harris & Lemoine, 1996). Regulatory elements may be distant from the promoter sequence & when included within a smaller distance may fail to operate. With greater understanding of the human genome, consensus sequences can be constructed to improve the strength of cellular promoters (Suzuki et al, 1996).

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Strategies for Gene Therapy

Gene therapy can either be applied ex vivo or in vivo. Ex vivo methods are technically simpler with regard to vector transfer & gene expression, but surgery is required to obtain & replace the cells. To enhance in vivo delivery the target organ may be stimulated, for example a partial hepatectomy will improve retroviral transduction to the liver (Ferry et al, 1991), however minimal manipulation of the patient would be desirable.

For some diseases the pathology affects the function of a particular organ which must be directly treated, e.g. cystic fibrosis in the lungs (Wheeler et al l996), inflammatory bowel disease (Jobin et al, 1998), Parkinsons disease in the corpus striatum region of the brain (During et al, 1994) & retinitis pigmentosa (Miyoshi et al, 1997). Other diseases are systemic in their effects, e.g. haemophilia & metabolic diseases such as adenosine deaminase deficiency. For these diseases the common sites for therapy are the liver (Numes & Rapier, 1996), gut (Lozier et al, 1997) & muscle (Herzog et al, 1997). These sites are chosen for their ease of access, bulk & metabolic activity. Monogenic recessive diseases only require the functional gene to be expressed, often therapeutically useful levels being much lower than that those found in normal individuals. Monogenic dominant diseases require that the aberrant gene is silenced, usually by means of an anti-sense DNA which is complementary to the aberrant gene (Gerad & Collen, 1997).

Gene Therapy for Cancer:

Many gene therapy protocols to date have concentrated upon treatments for cancer. Though many cancers have a genetic predisposition, they all involve acquired mutations, & as they progress their cells become less differentiated & more heterogeneous with respect to the mutations they carry. In general cancers have at least one mutation to a protooncogene (yielding an oncogene) & at least one to a tumour suppressor gene, allowing the cancer to proliferate. The range of different cancers encountered & the mutations they carry, have led to a variety of strategies for gene therapy namely; immunopotentiation, oncogene inactivation, tumour suppressor gene replacement, molecular chemotherapy & drug resistance genes.

The aim of immunopotentiation is to enhance the response of the immune system to cancers, thereby leading to their destruction. Passive immunotherapy aims to increase the pre-existing immune response to the cancer whilst active immunotherapy initiates an immune response against an unrecognised or poorly antigenic tumour (Barnes et al l997). Passive immunotherapy usually involves harvesting tumour infiltrating lymphocytes & treating them to express increased cytokines e.g. IL-2 & TNF-alpha (Yanelli et al, 1993). The cell population is then expanded in vitro & returned to the patient. Tumour cells are used for active immunotherapy, genetically modifying them to increase expression of antigen presenting molecules/costimulatory molecules (e.g. B7; Chen et al, 1992), local concentrations of cytokines (e.g. IL-2; Lemig et al, 1996) or tumour antigens (erbB2 oncoprotein; Disis et al, 1994). The cells are then irradiated prior to being returned to the patient, preventing the reintroduction of replication competent tumour cells. These approaches have been termed cancer vaccines.

Oncogene inactivation uses the same techniques employed for dominantly inherited monogenic diseases. The oncogene may be targeted at the level of the DNA, RNA transcription or protein product. Oligodeoxynucleotides are short single stranded pyridimine rich DNA sequences that form a triple helix with purine rich double stranded DNA sequence. Oligodeoxynucleotides are designed in a sequence specific manner to target the promoter regions of oncogenes, (e.g. erb2; Ebbinghouse et al, 1993). At the RNA level antisene techniques prevent transport & translation of the oncogene mRNA by providing a complementary RNA molecule (e.g. c-myc; Collins et al, 1992). Ribozymes, antisense oligoribonucleotides with a cleavage action, will also reduced the stability of oncogene mRNA (e.g. brc, abl; Snyder et al, 1993). Transport of the oncogene product to the cell surface can be prevented by a single chain antibody with specificity for the oncogene product & a localisation signal for the endoplasmic reticulum.(e.g. erb2; Beerli et al, 1994).

Despite multiple genetic abnormalities, restoration of the tumour suppressor gene, such as p53, can be sufficient to cause cellular apoptosis & arrest tumour growth. Moreover, expression of p53 is synergistic with chemotherapeutic drugs such as cisplastin (Ogawa et al, 1997) & adjacent tumour cells that have not been transduced are killed, in what is termed the bystander effect (Roth et al, 1996). The p53 gene has been identified as important in a range of cancers & though less elegant than oncogene inactivation techniques, this method has proved robust enough to be included in a number of clinical trials. Other tumour suppressor genes may also prove useful e.g. BRCA1sv (Tait et al, 1997).

An alternative means of killing a tumour cell is to transduce a gene coding for a toxic product, known as molecular chemotherapy. The gene of choice is usually herpes simplex virus thymidine kinase (HSV/TK) which converts the prodrug ganciclovir into toxic metabolites. The effected cell is supported via the gap junctions of adjacent cells (Wygoden et al, 1997), until the toxin burden is too great killing both the affected cell & its neighbours. An advantage to this system is that all the transduced cells will be killed, allowing allogenic tumour cells to be prepared in advance (Paillard, 1998, Oldfield & Ram, 1995). HSV/TK is also used in other gene therapy protocols, allowing the treatment to be aborted at any time (Contassot et al, 1998).

Many cancer chemotherapy regimes are limited by their low therapeutic index, as determined by heamopoetic cells. By harvesting precursor bone marrow cells & transducing them with the gene MDR1, coding for the drug efflux protein p170, a population of resistant cells can be cultured. When returned to the patient, much higher doses of chemotherapy may be used (Champlin et al, 1994).

Clinical Trials:

Numerous systems have been demonstrated in vitro & with animal models. Phase 1 human trials are primarily designed to demonstrate safety & efficacy & will not provide complete cures. The patients chosen have advanced metastatic cancer & the early results are comparable to single agent chemotherapy (Roth & Cristiano, 1997).

Clinically, viral gene transfer to cancer cells has proved more efficient than expected from normal organ gene transfer & viral vectors can spread through three dimensional cell matrices (Fujiwara et al, 1994). Perhaps due to the heterogeneous nature of many malignant tumours, the most successful approaches do not rely on manipulating the immune system, rather act as adjuncts to conventional therapies (drug resistance genes) or use established agents (molecular chemotherapy). The morbidity associated with cancer gene therapies is significantly lower than of conventional treatments, therefore more aggressive application of gene therapies may be beneficial (Roth et al, 1996). Development of therapies for non malignant diseases have been hampered by the short duration of transgene expression. Recent advances however, make it likely that monogenic recessive diseases, such as haemophilia, will be treatable by administering a single dose of the gene vector

Current objectives include improvement of cell targeting through vector & promoter specificity (discussed above) & reducing the immune response to the current vectors. As the understanding of the molecular biology, immunology & virology underlying vector technology & oncology improves, so will our ability to treat cancer. Many of the developments reviewed above are present only in isolated systems, therefore consolidation of existing technology may yield significant improvements. Public expectation, though understandable & used by scientists to generate funds, should be tempered as gene therapy will not be the cure all in the near future. However, in conjunction with existing therapies, gene therapy will substantially reduced the burden of morbidity experienced by an ageing population.

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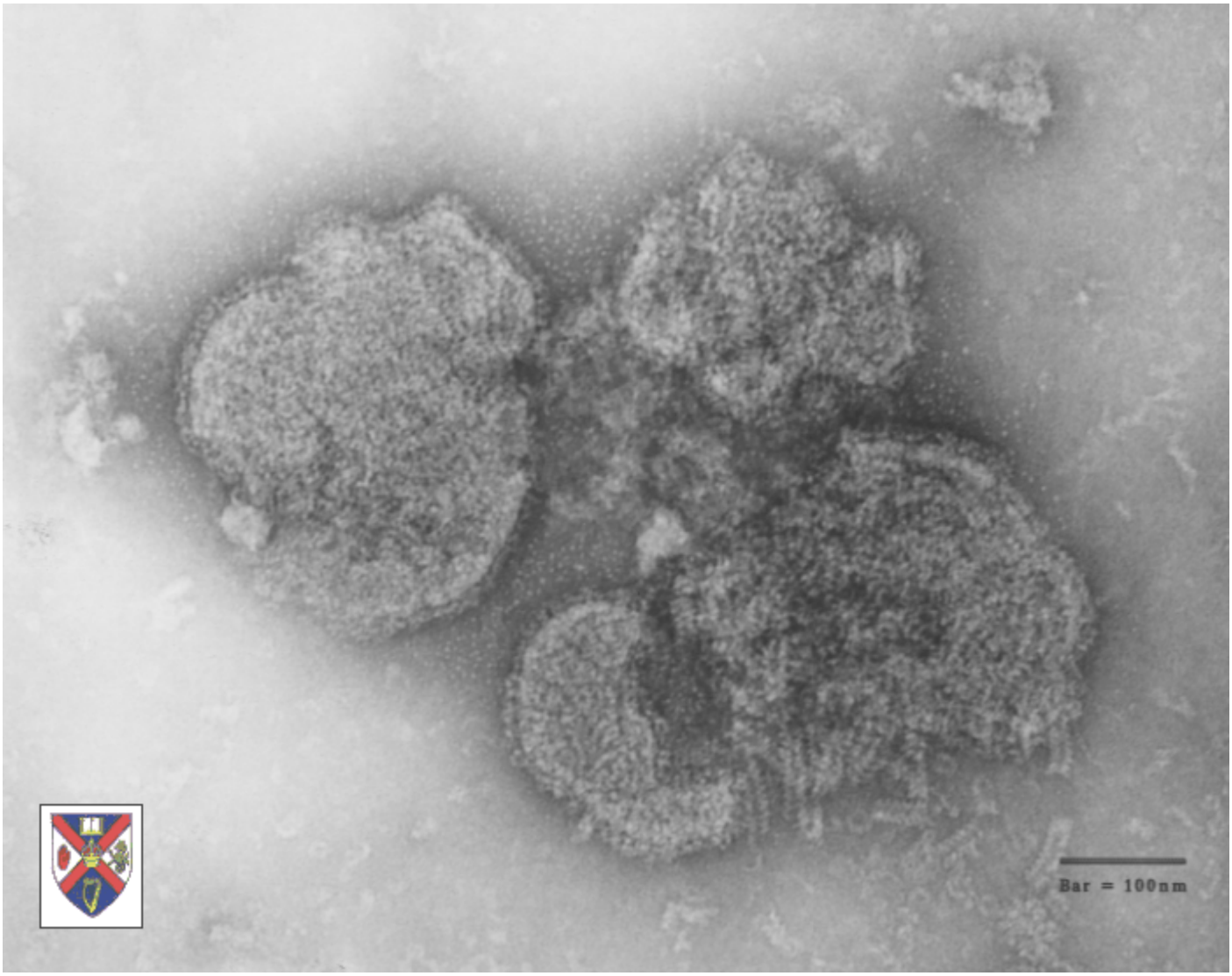
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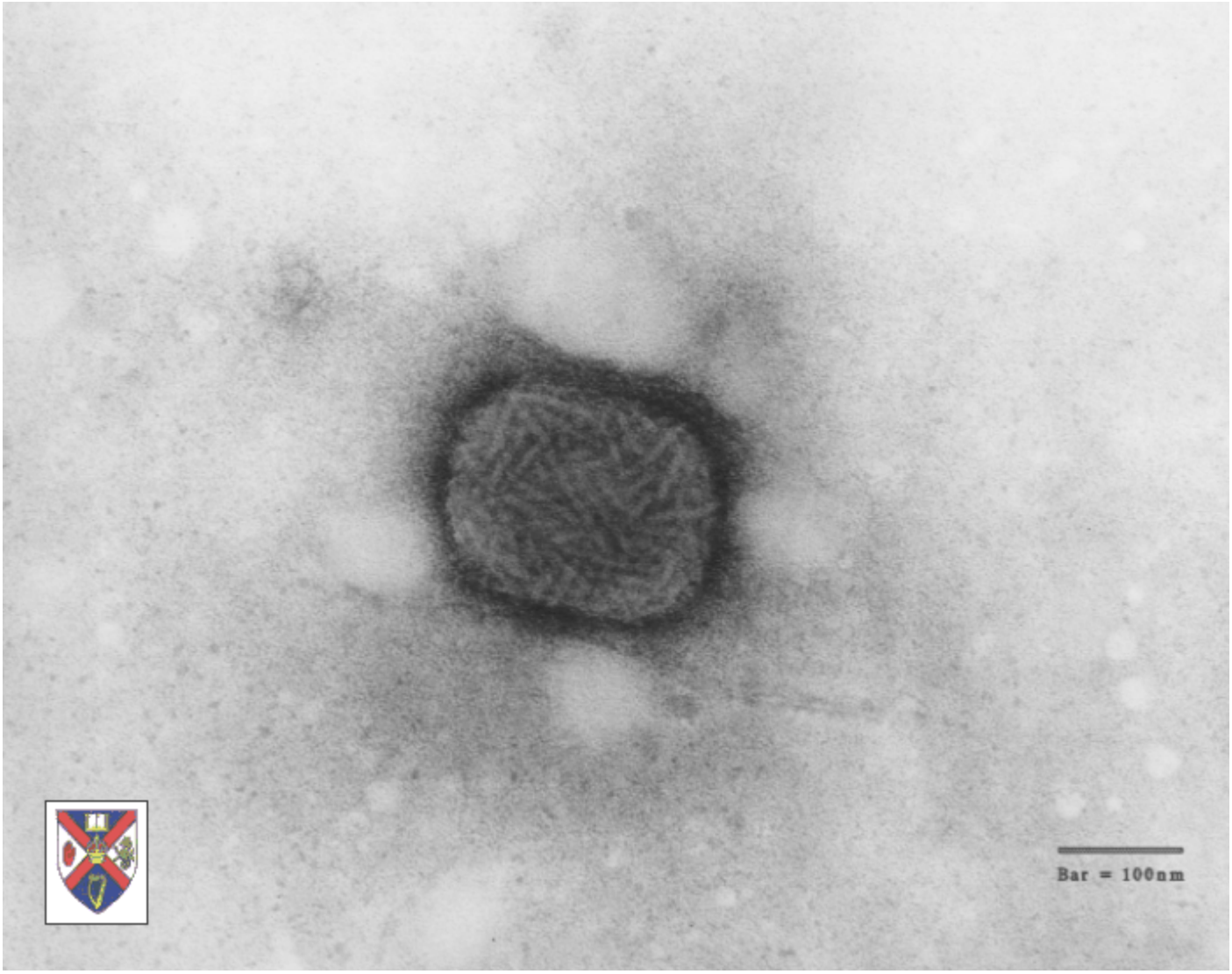

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Smallpox:

has been known for many centuries. The characteristic "pocks" produced by variola (smallpox) virus gave their name to all forms of infectious disease: "a dose of the pox".

Smallpox:

first appeared in China and the Far East at least 2000 years ago.
The Pharaoh Ramses V died of smallpox in 1157 B.C.

Smallpox:

reached Europe in 710 A.D. and was transferred to America by Hernando Cortez in 1520.
3,500,000 Aztecs died in the next 2 years.

Smallpox:

reached plague proportions in the cities of 18th century Europe and was a highly feared scourge.

Smallpox:

killed five reigning European monarchs during the 18th century.

Smallpox:

has now been eradicated.
The last naturally occurring outbreak was in Somalia on 26th October 1977.

How did they do that ?

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² ²Ztion. Virus isolation is also a Pox1.htmltmll 12umentslantesses TEXTR*ch 8²*“µ î m, blood or tissue specimens stored at -70oC or freshly collected.

TREATMENT OF EBOLA

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Monkeypox also produces a disease clinically identical to smallpox (e.g. pustular rash, fever & respiratory symptoms) but is not usually fatal. Is human monkeypox infection emergent disease or has it always been there but masked by smallpox?
Monkeypox is an orthopoxvirus with enzootic circulation in rainforests of central and western Africa. From February to August 1996, a total of 71 clinical cases of monkeypox, including six deaths, occurred in 13 villages in Zaire. Preliminary investigation of this outbreak suggests that person-to-person transmission accounted for most monkeypox cases investigated in 1996 and 1997 in contrast to previous years when sporadic cases resulted from animal-to-human transmission.
If you want to see the latest research findings on monkeypox, click here!

Source: World Health Organization (WHO); **Date:** 14 Apr 1997:

Monkeypox changes its pattern of human infection

Following reports of ongoing cases of human monkeypox in Zaire representing a new pattern of the disease, the Ministry of Health in Zaire and the World Health Organization (WHO) organized an investigation in February 1997. Monkeypox, a disease which closely resembles smallpox, is preventable by the vaccination against smallpox. WHO is concerned that the ending of vaccination programs for smallpox may have led to the seriousness of the latest outbreak.

The study revealed that this outbreak represents the largest cluster of monkeypox cases ever reported, and the proportion of patients that were 15 years of age or older (27%) was higher than previously reported (8%). Young children have been most affected in previous outbreaks. These findings contradict previously accepted findings on the nature of the disease.

The investigation

Studies over a twenty year period have shown that the rate of transmission of monkeypox within households was low, suggesting that the disease had a low potential for transmission from person-to-person and that an outbreak could not last long after the first patients recovered. However, the recent study has shown that active cases in February 1997 were linked to cases that occurred during the preceding 12 month period. The study also revealed the following:

The rate of transmission from person-to-person (73%) was higher than previously reported (30%). This was associated with the clustering of cases in household compounds and prolonged chains of transmission from person-to-person.

The proportion of deaths (3%) was lower than previously reported (10%).

The disease

Monkeypox disease closely resembles ordinary forms of smallpox. The viruses causing the two diseases are related and vaccination against smallpox also protects against monkeypox. The emergence of monkeypox does not mean that smallpox, which has been eradicated, is re-emerging.

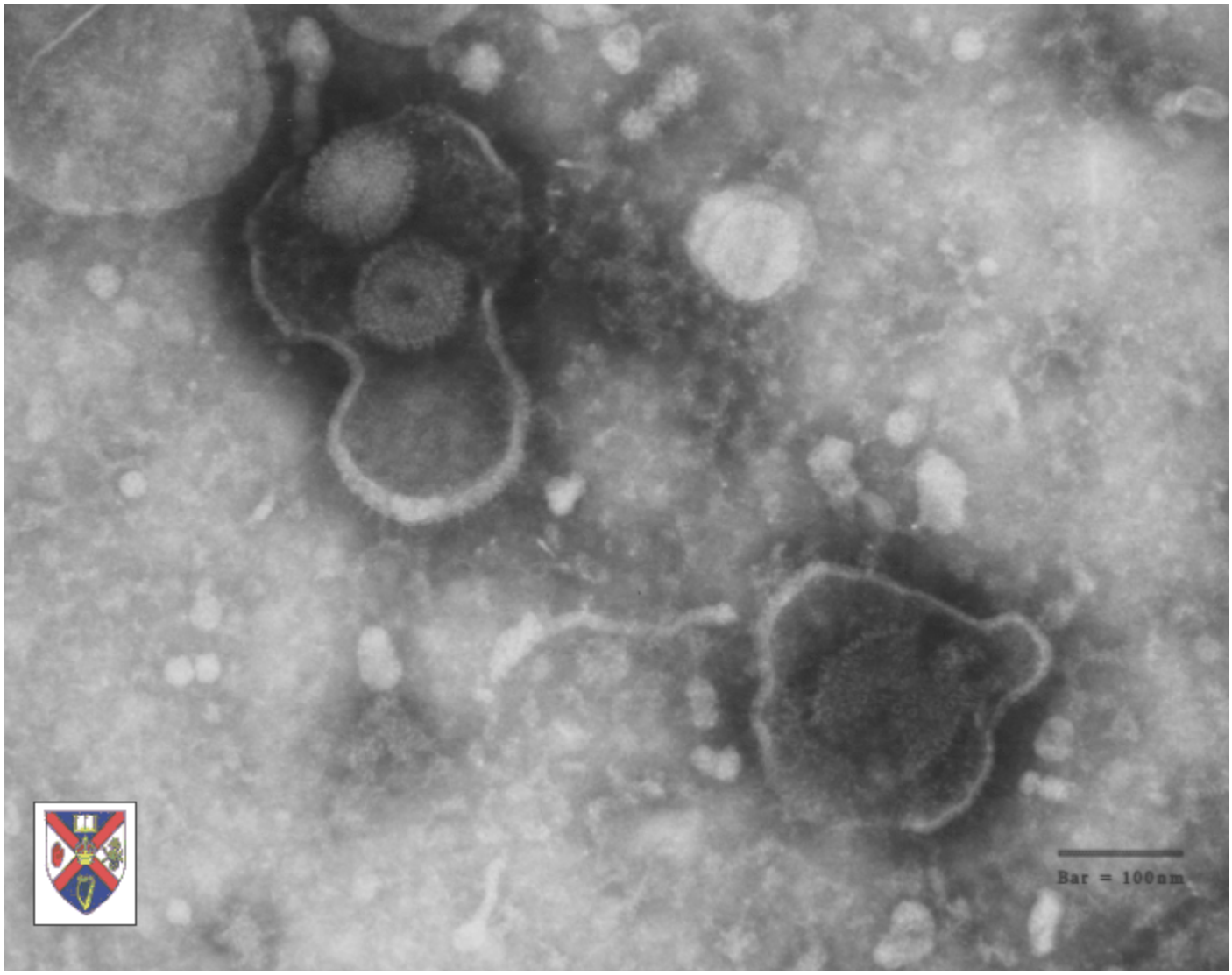
All age groups are affected, but children who have not been vaccinated are at the highest risk of death, about a 10% case fatality rate. Most cases occur in remote villages of Central and West Africa close to tropical rainforests where there is frequent contact with infected animals. Monkeypox is usually transmitted to humans from squirrels and primates.

In the past, an outbreak of monkeypox would not go very far in the village or last long because it did not spread extensively after the first patients recovered. However, what concerns WHO is that the present study indicates that monkeypox disease is changing its pattern of infection in humans. This outbreak has a much higher rate of person-to-person transmission than seen before, and spread through many generations of transmission, thus maintaining the outbreak for more than a year.

Concern for WHO

The ending of vaccination programmes against smallpox in the late 1970's has probably led to an increase in susceptibility to monkeypox and could explain the larger size of the outbreak, the higher proportion of patients aged 15 and over, and the spread through many generations of transmission.

The changing nature of monkeypox disease is of concern to WHO. In the past, before the eradication of smallpox in 1980, smallpox vaccination was widespread and protected against both smallpox and monkeypox. Since the eradication of smallpox, vaccination programmes have been discontinued as recommended by WHO. Though monekypox has been shown to not be a threat to smallpox eradication by studies done throughout the 1980s, WHO is concerned that monkeypox could pose a localized public health problem. WHO will therefore continue to maintain vigilance by strengthening detection systems for monkeypox and completely investigating future outbreaks so that the best strategy for prevention and control may be developed.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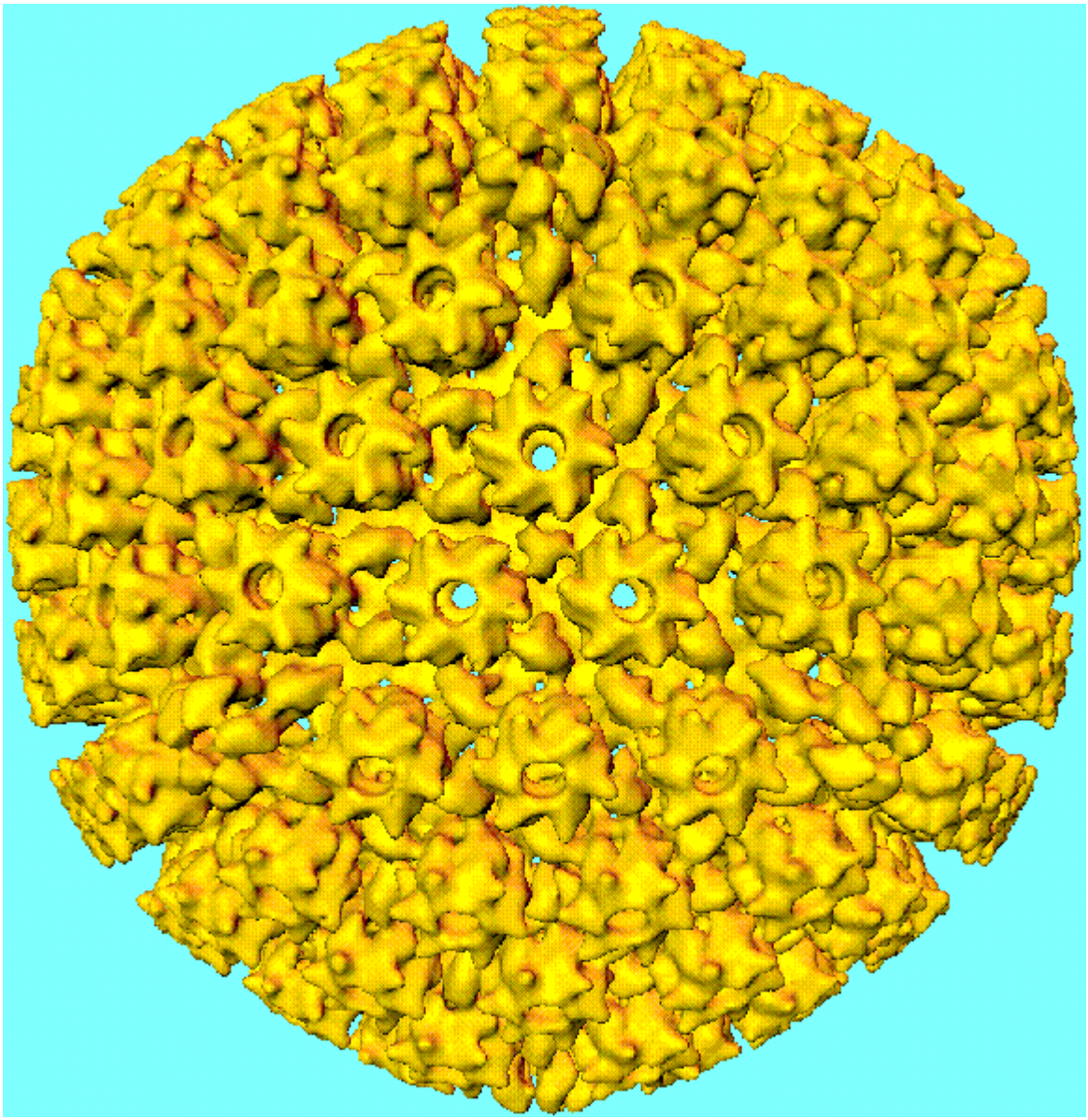

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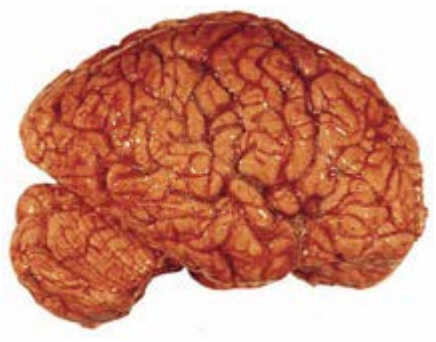
Together with comparatively better understood diseases such as AIDS and Ebola virus, Alzheimer's disease is one of the three plagues of the late twentieth century.



Alzheimer's disease is characterized by the deposition of plaques and 'tangles' composed of beta-amyloid protein in the cells of the brain. Amyloid deposits appear to be inherently poisonous to cells. Although the molecular mechanisms involved in cell death are unclear, it is this toxic effect which gives the **'spongiform encephalopathies'** their name, due to the characteristic holes seen in thin sections of affected brain tissue under a microscope. The holes are caused by the death of both neurons and their supporting framework of glial cells, which actually make up the structure of the brain. When this loss of cells affects the cerebral cortex, the brain shrinks perceptibly and higher cognitive abilities are progressively lost.

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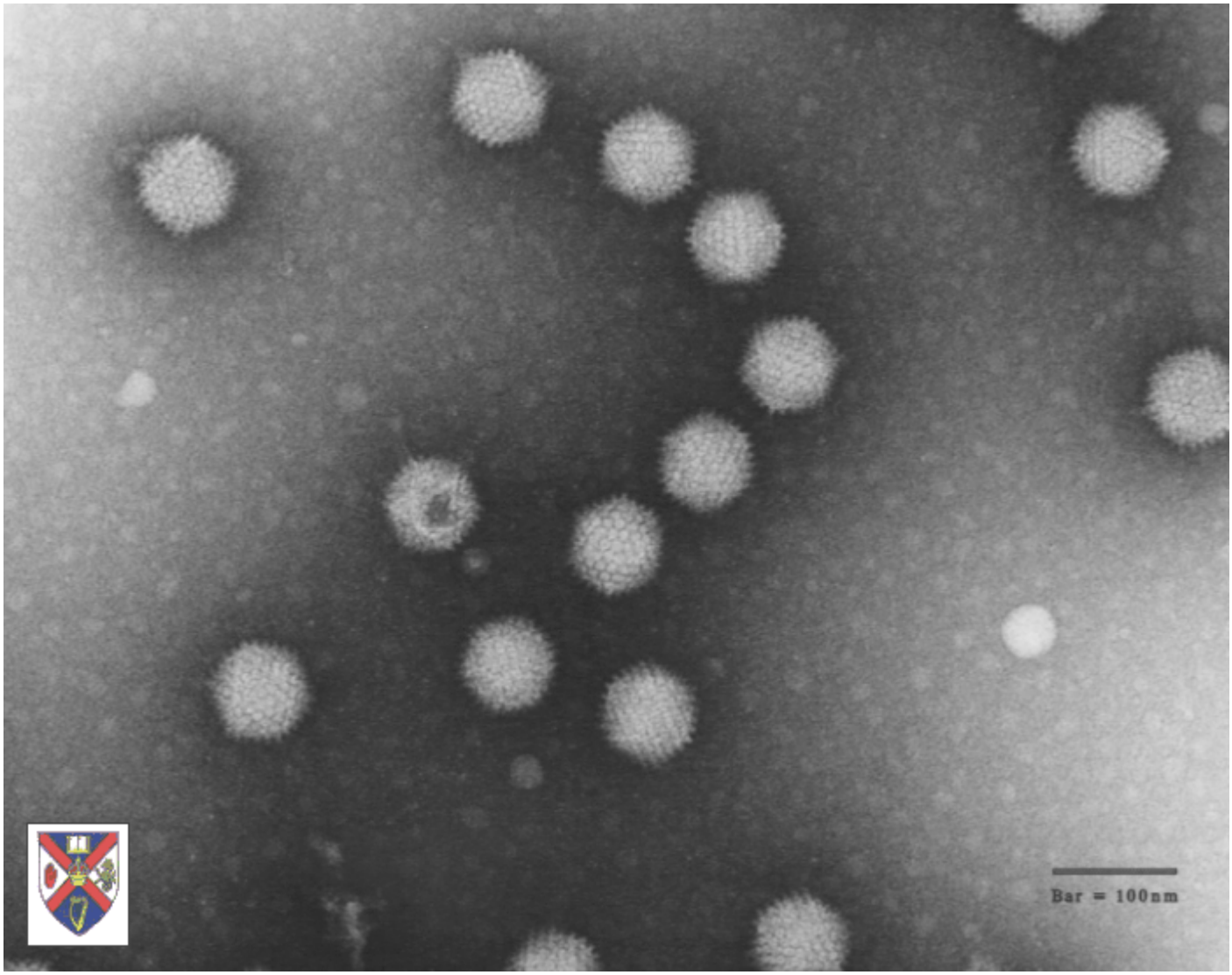

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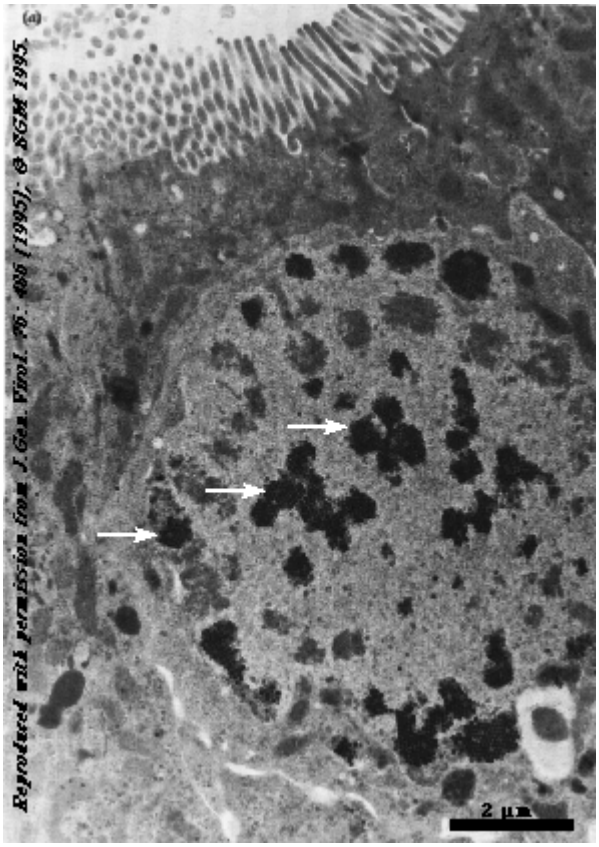

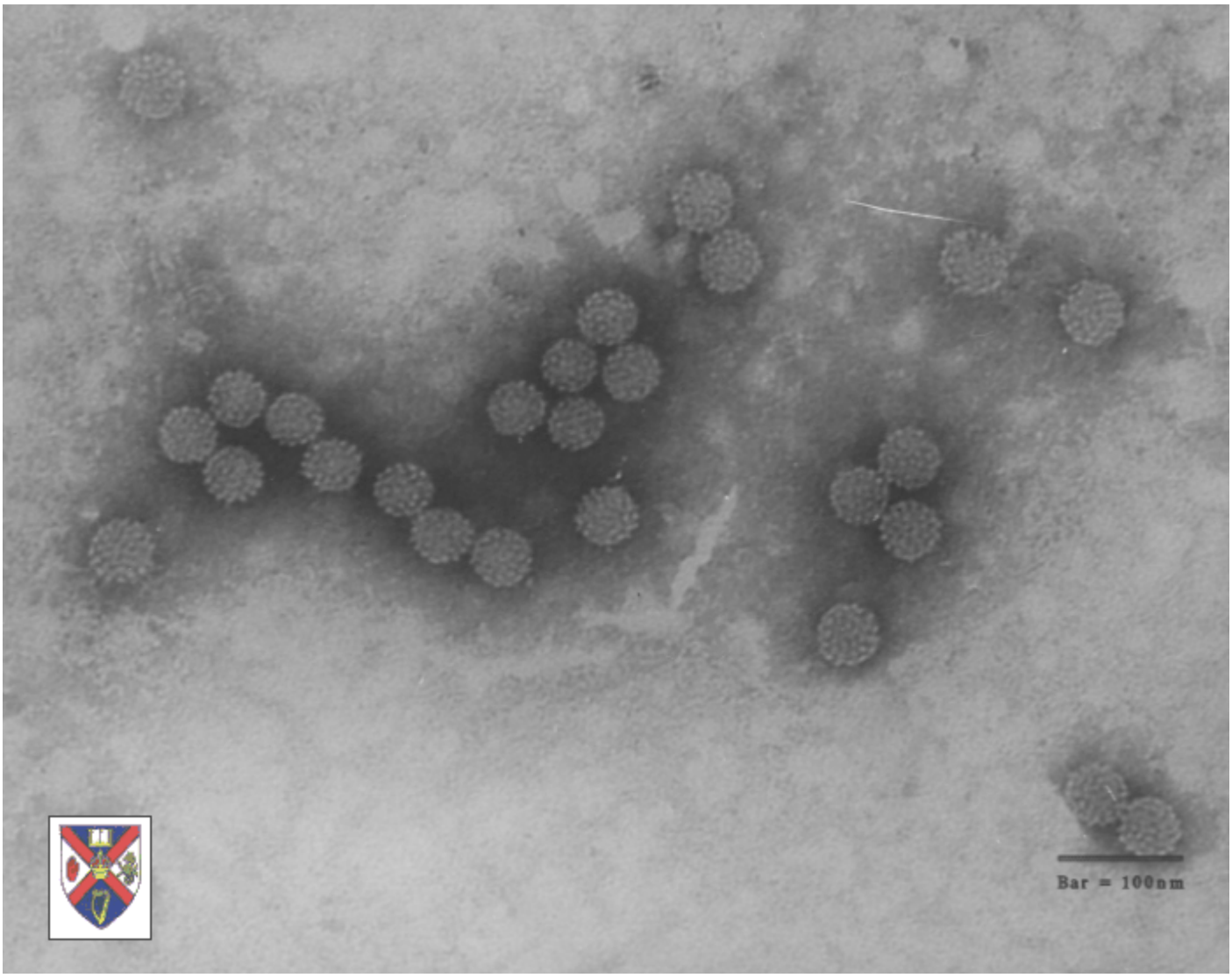
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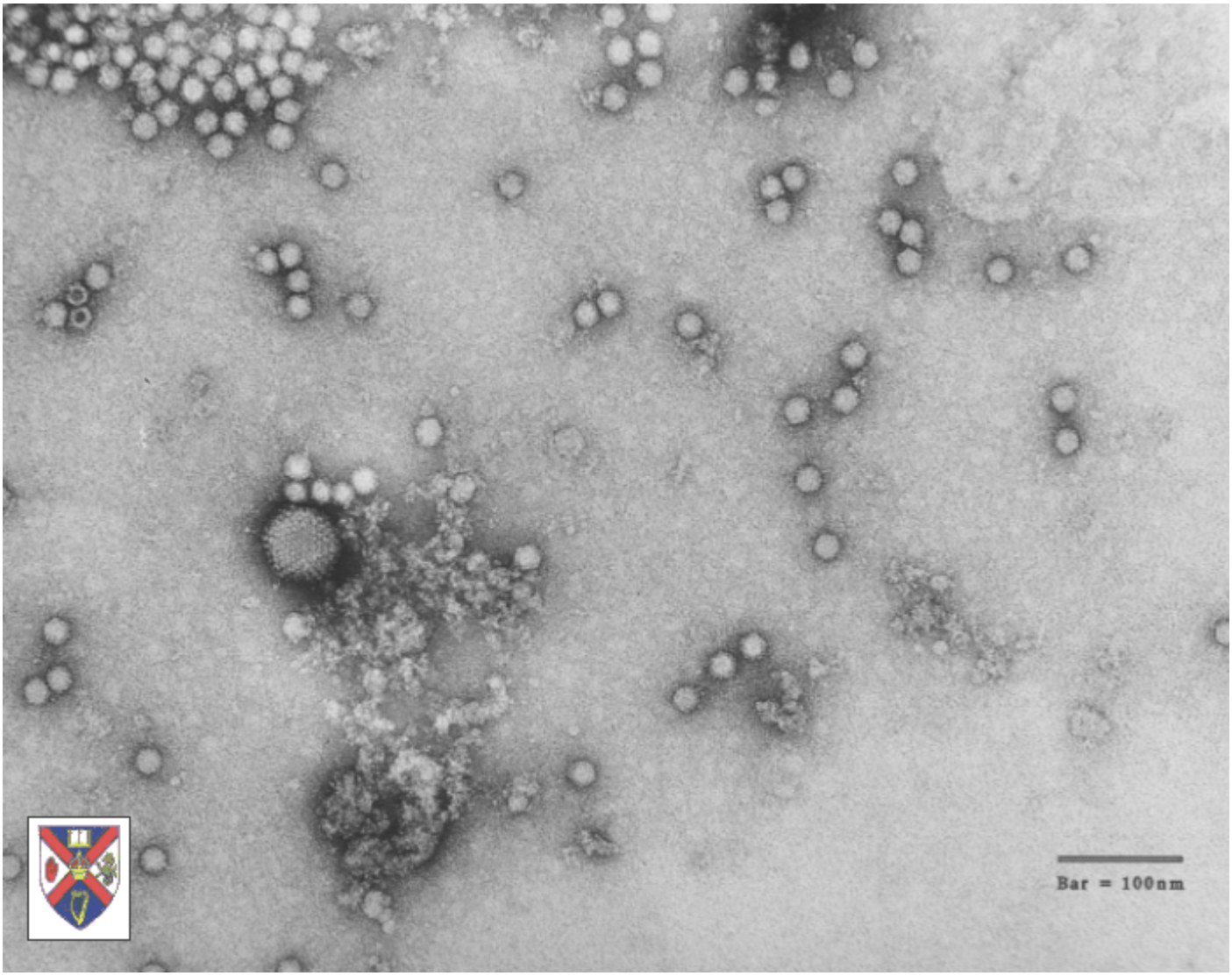

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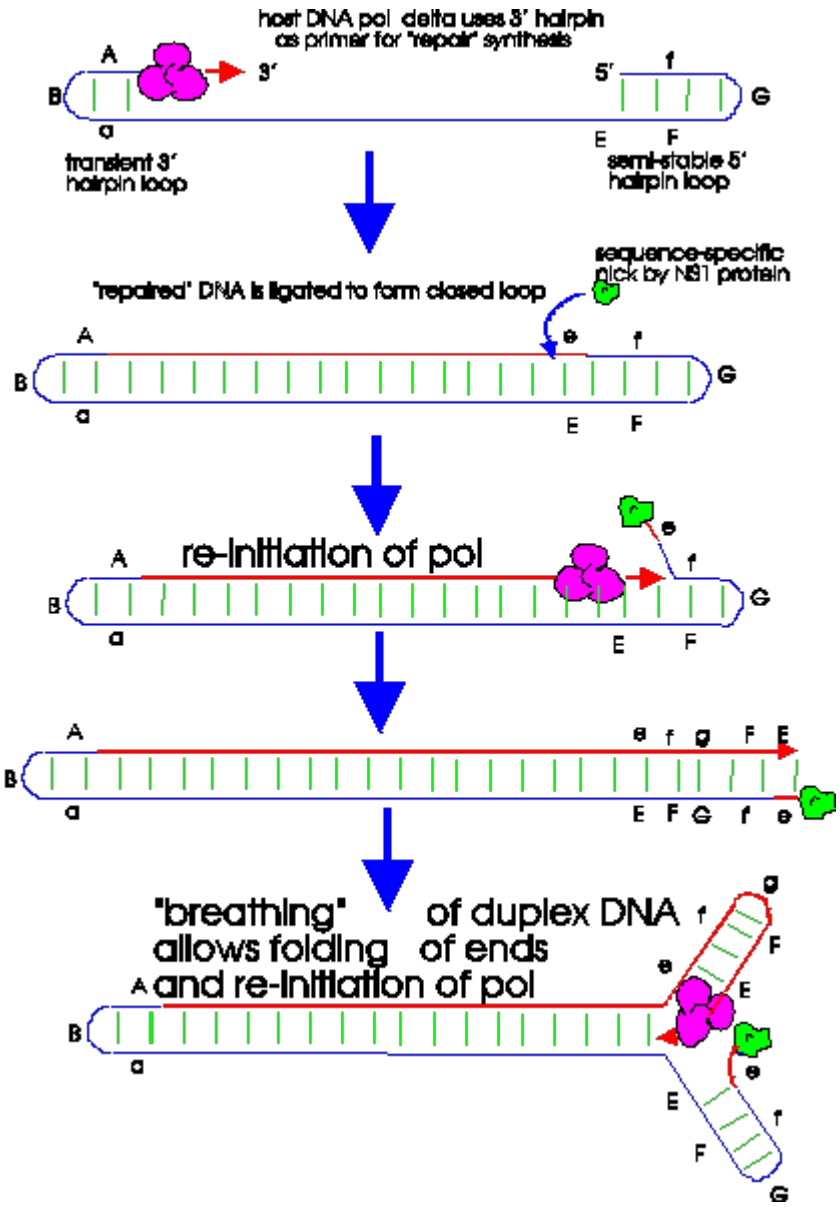

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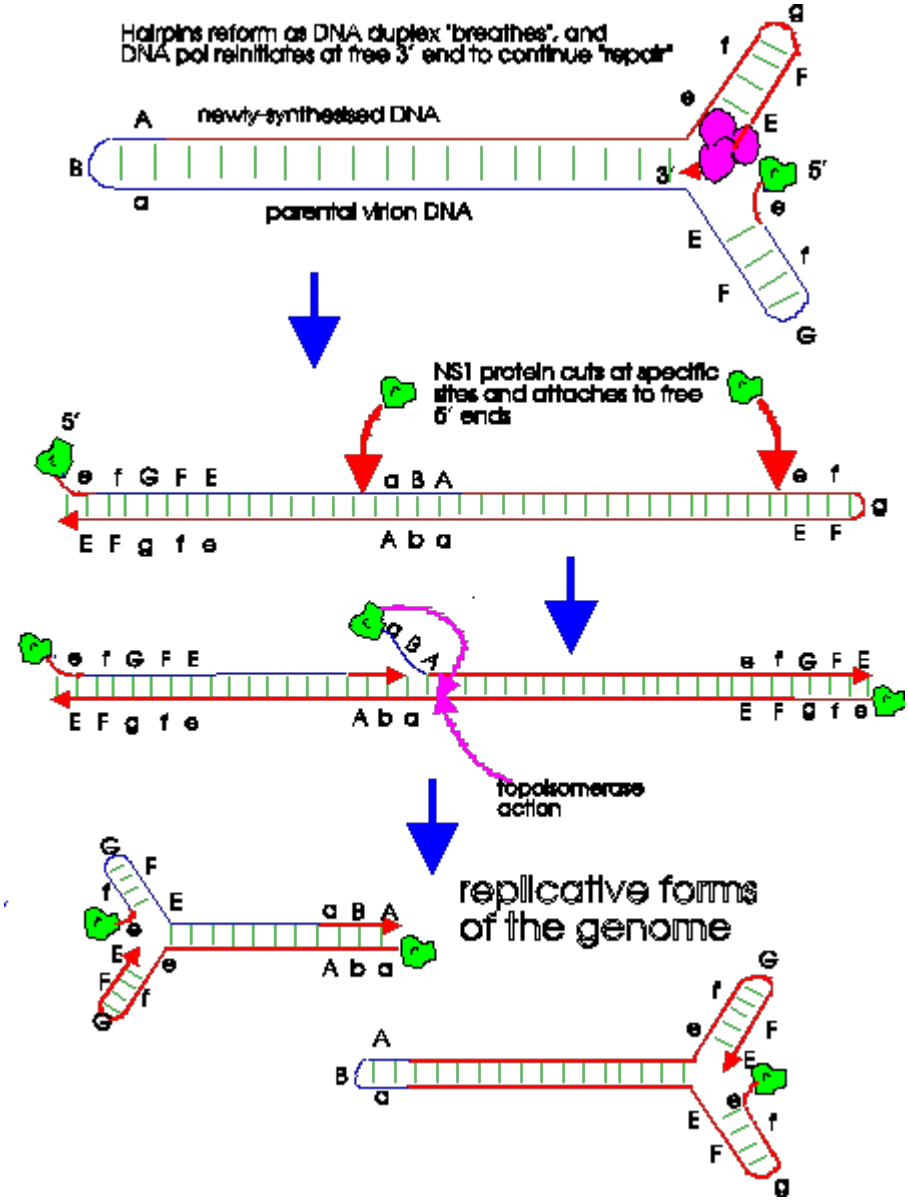

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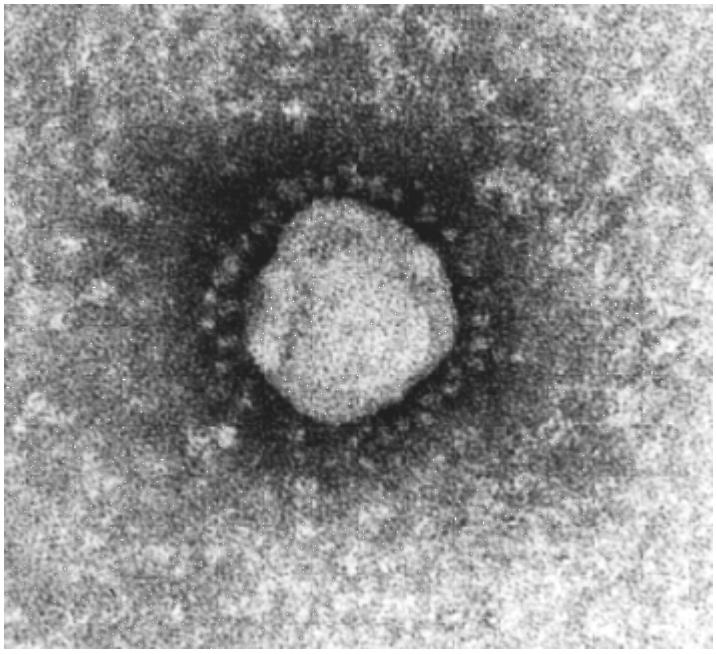

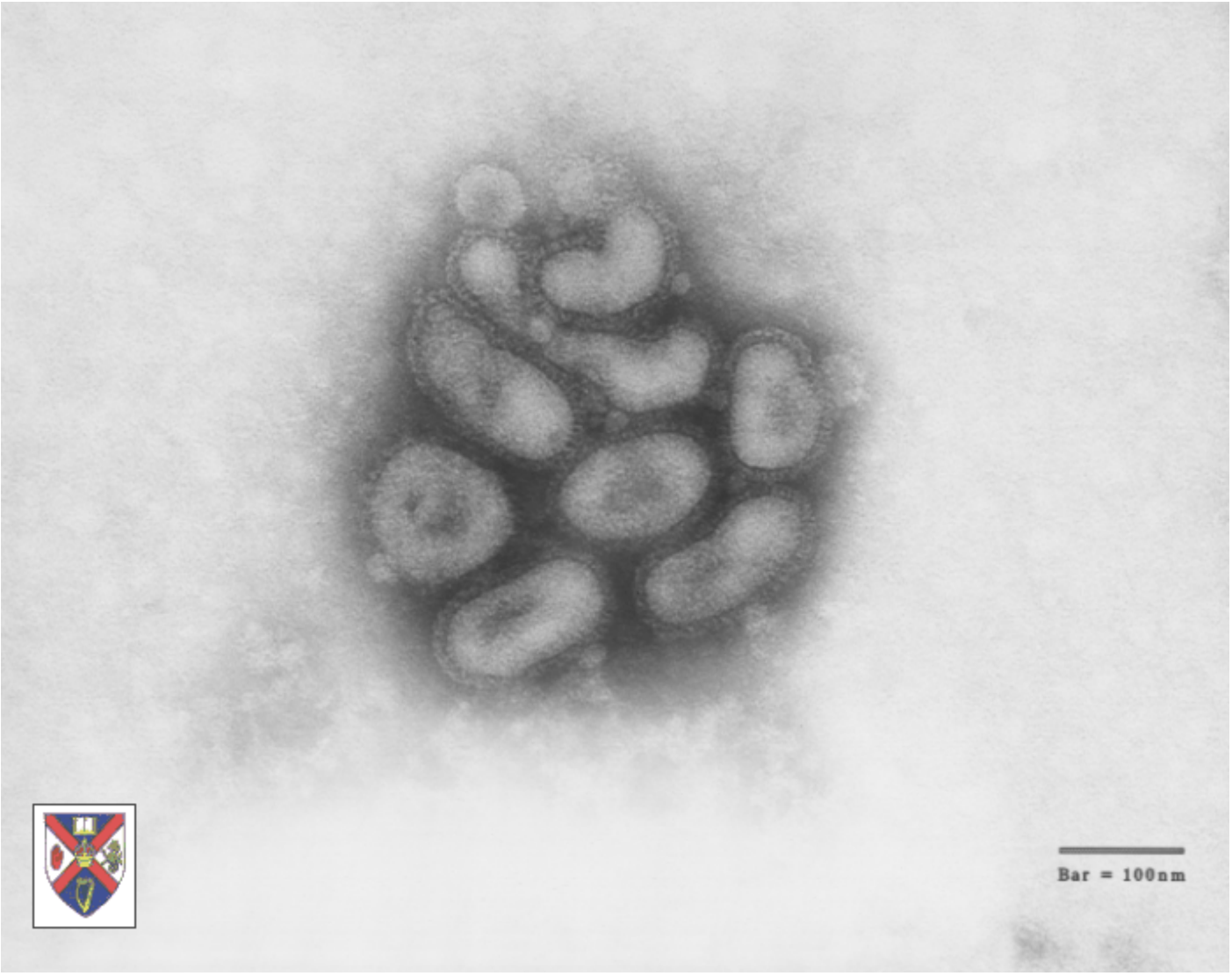
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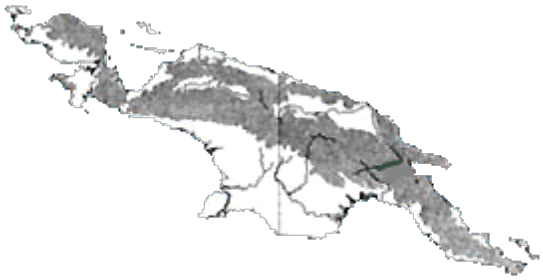

New Guinea, 1949:

Ibuso pushed the twig up through the small hole at the base of the skull, then moved it in a circular motion around the inside of the man's cranium. A thin grey paste began to drip from the skull into the bowl. It had been easy to remove the Shaman's head from his neck using the broad steel knife. The last time Ibuso had performed the funeral rights had been on her own husband, just under a year ago. Then, she had still used the stone tools copied from those which her family had used for centuries. It was good to use the old tools, which had honoured so many Ancestors, but the task was much easier with the knife, especially since Ibuso no longer possessed the strength she once had.

She paused for a second to suck the liquid from her fingers so that she could keep a firm grip on the skull. It would dishonour the Shaman's spirit and might anger it if she dropped the skull in the dust. Ibuso looked across to the men's hut and saw Kiguda sitting beside the doorway. It was Kiguda who had brought the knife back the village after a raid on a distant village to the North. Normally, the men would not have gone further than the adjacent villages a few miles away, but Kiguda had persuaded the elders that they were stronger than the distant tribe. Three men had died in the raid - the other village had put up much stronger resistance to the surprise attack than expected. Because of the distance, it was not possible to bring back any prisoners from the raid, although Ibuso suspected Kiguda had become scared and run away from the fight after stealing the white man's metal knife. Even the powerful spirit in the knife had not been enough to change the course of the skirmish, which was soon lost. Kiguda led the survivors back to their own village. He walked into the centre of the village shouting about his brave deeds during the raid, and held up the knife so that everyone could see it. Some of the elders were angry with Kiguda for bringing the white man's magic into the village, but it was generally agreed that the raid had been a great success. Ibuso had left the feast early that night when she could not listen to Kiguda's boasting any more. She knew he was a coward who should not be allowed to sleep in the men's hut.

The loss of the three men had greatly weakened the village, and then the Shaman had been possessed by evil spirits. The Shaman had been powerful in his time and good for the village, which had prospered under his guidance. His magic had been powerful and the men's hunting trips were successful. Attacks on the village by neighbouring tribes had been repulsed because he marshalled the spirits of the Ancestors which guided village life. But after the raid he had changed. He became listless and sat in his hut for much of the time, shivering. He began to forget key elements of the rituals he performed whenever the men went hunting, or when a child was born. He had stumbled and dropped the skull of the most powerful chieftain the village had ever had. Ibuso was afraid when this happened, for she knew the Ancestors were angry at this sacrilege. After a few months, the Shaman did not emerge from his hut any more. The women were not allowed into his hut because the magic there was too powerful, but the men took him food the women had prepared. Yesterday he had been found dead in his hut, sitting propped up against the wall, his hand still gripping the talisman he always carried.

Ibuso had nearly finished carefully scooping the liquefied brain from the Shaman's skull. The other women watched her in silence, afraid to talk and pensive about the powerful spirit of the Shaman which might be angered if the funeral rights were not performed with due deference. When she was satisfied that she could not extract any more of the brain, she set the skull down carefully beside her on the mat, careful that it did not touch the ground. The bowl was half full of a thick grey liquid, in which some intact pea-sized lumps of jelly-like brain tissue could still be seen. She began to add the herbs that the Shaman himself had gathered from secret sites in the valley and stirred the mixture. After a few minutes, it became smooth and she began to pour it into hollow bamboo tubes. She sealed these by tying leaves around the open end, then the other women placed them in the embers of the cooking fire.

The women sat in the hut while the brain was warmed by the fire. At 7,000 feet above sea level, the heat from the embers warmed the greyish soup to a temperature of 94°C. Because of the low atmospheric pressure, it began to bubble gently, but did not get any hotter. After an hour, Ibuso stood up and called the children. They came slowly towards the hut, aware that the women were pensive and uncertain of themselves at the liberation of such a powerful spirit. The children had always been afraid of the Shaman when he was alive and gave him a wide berth. Now, with the women, they would honour his spirit by consuming his brain. The eldest boy was eleven years old. Ibuso's youngest son was a few months older and had gone to live in the men's hut after his father had died, so could not participate in the funeral rites any longer.

The bamboo tubes were passed around the group, who received them and drank in silence. For the infants and babies in arms, the mothers poured a little liquid into their hands and fed them. They also smeared the liquid on their faces in the approved manner. After half an hour, the funeral was over and the mood lightened a little. Nothing untoward had happened and the Shaman's spirit had been honoured. The children ran off to play and women began to drift away to other tasks. Ibuso began to clean to Shaman's skull so that it could take its place on the Ancestors shelf in the men's hut. Kiguda's eldest son sat close by, watching Ibuso. He seemed to be quiet these days and no longer ran through the village with the other children. Well, perhaps he was just getting tired of children's games. He was, after all, nearly ready to go and live in the men's hut. But Ibuso had seen him stumble as he walked. Maybe he was going to get **kuru** - the shivering.

(click on the arrow)

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Glossary:

(You can search this document using the Find command from the Edit menu)

ACDP:	UK government Advisory Committee on Dangerous Pathogens which regularly produces information regarding the handling of dangerous microorganisms.
ADAS:	Agricultural Development and Advisory Service. UK government organisation that providing veterinary and agricultural advice to farmers. Greatly reduced in size during the Thatcher Government.
Agent:	An element able to transmit a disease.
Alzheimer's disease:	The most common dementing illness of the elderly in industrialized countries.
Amyloid:	A chemical structure which can accumulate in brain or other tissues in an amorphous way as a result of a variety of different diseases. In CJD the amyloid is made-up of the prion protein.
Amyloidosis:	The build up of amyloid which causes damage to the tissues in the body.
Astrocyte:	Common cell type in the brain that supports and assists the function of the nervous tissue (neurons). Sometimes called an 'accessory cell'.
Astrocytosis:	The proliferation of astrocytes in brain tissue due to the death of neurons (nerve cells). A common finding in CJD but it not specific to the disease.
Ataxia:	Loss of muscular coordination resulting in jerky, uncontrolled movement.
BSE:	Bovine spongiform encephalopathy (mad cow disease, la vache folle, etc).
Chronic wasting disease:	TSE of deer and elk.
CJD:	Creutzfeldt-Jakob disease. The most common human spongiform encephalopathy (SE). Identified in the 1920s through the work of Creutzfeldt and Jakob.
CNS:	Central nervous system, i.e. the brain and the spinal cord.
CSF:	Cerebrospinal fluid. A clear fluid that surrounds the brain and spinal cord.
CVL:	UK government Central Veterinary Laboratory, which performed much of the early work on BSE.
DNA:	Deoxyribonuclic acid. The chemical that carries the genes in the chromosomes of animals, plants and some viruses.
DoH:	The UK Department of Health.
Downer cows:	A term used in the US to describe cattle that do not have the ability to stand.
EEG:	Electroencephalogram. The trace of electrical activity in the brain. In patients with CJD this may show characteristic signs.
Epidemiology:	Study of the distribution of cases of a disease.
EU:	European Union.
FFI:	Fatal Familial Insomnia. A genetic disease in humans caused by an inherited mutation of the prion protein gene.
	Feline spongiform encephalopathy. SE in domestic cats, believed to be related to the

FSE:	consumption of BSE material in cat food.
Gene:	Unit of DNA and forming part of chromosome which is responsible for particular functions within a cell function.
Genome:	The total make-up of all the genes of an organism.
Genotype:	The genetic make-up of an individual.
GSS:	Gerstmann-Straussler-Scheinker Disease. A familial SE of humans and associated with inherited mutations of the PrP gene.
Horizontal transmission:	Lateral transmission of a disease from one animal to another (c.f. vertical transmission).
Iatrogenic:	A disorder caused by medical treatment, e.g. CJD cases resulting from injections of growth hormone or transplants of tissue from an infected donor.
Immunohistochemistry:	Chemical methods used to locate deposits of particular chemicals or proteins in tissues. Often used to identify the prion protein in brain tissue.
Kuru:	TSE found only in the Fore tribe in New Guinea, related to ritualistic cannibalism carried out among members of the tribe.
MAFF:	UK Ministry of Agriculture Fisheries and Food.
Maternal transmission:	The transmission of disease from the mother to the offspring.
MBM:	Meat and bone meal.
MRM:	Mechanically recovered meat, stripped from the carcasses of animals & used in processed foods, e.g. hamburgers.
Mutation:	A chemical change in a gene of a cell which alters or destroys its normal function.
Myoclonus:	Involuntary muscular contractions.
Neuroanatomy:	Anatomy of the brain, spinal cord and peripheral nervous system.
Passage:	The inoculation of an infectious agent into an animal.
Pathogenesis:	The production of damage (pathology) in a tissue or animal.
Pathology:	Study of disease-affected tissues.
Plaques:	Accumulation of protein that may build up in brain tissue as a result of infection.
Prion:	PROteinaceous INfectious agent. Term coined by Stanley Prusiner in 198?. The prion theory suggests that the infectious agent of CJD and the other TSEs is composed only of a single protein and does not contain a nucleic acid genome which would be necessary if the agent was a conventional virus.
PrP:	Prion protein. A normally occurring protein found on the surface of particular cell types - PrPC. The abnormal form PrPCJD (or PrPSc in scrapie) accumulates in the brain.
SAF:	Scrapie associated fibrils. Filamentous structures, visible only in an electron microscope found only in brains from TSE.
SBO:	Specified bovine offals. Offal of cows thought to represent a risk of BSE transmission. A ban on the use of these organs (thymus, brain, spinal cord, gut (below duodenum), spleen, tonsils) in the food chain was introduced (but not properly enforced) in 1989.
SBM:	Specified bovine materials. Additional parts of slaughtered cows considered not acceptable for human consumption from December 1995.

Scrapie:	TSE of sheep or goats.
SE:	Spongiform encephalopathy.
SEAC:	Spongiform Encephalopathy Advisory Committee. UK government committee consisting of 'experts' in the field of spongiform encephalopathy.
Species barrier:	A naturally occurring barrier between different species of animal which makes transfer of TSE from one to the other difficult.
Sporadic:	Cases of CJD that occur at random throughout the world are said to be sporadic if they are not associated with a genetic mutation or any known iatrogenic cause.
Strains:	Different forms of the scrapie have been isolated by transmission to mice. These strains show distinct variations in the incubation period and pattern of pathology observed in the infected mice.
Thirty Month Rule:	Regulation introduced in April 1996 that cattle over 30 months of age should not be used for human food.
Transgenic mice:	Mice carrying a specific gene from another animal. There may be more than one copy of the gene (for instance, in many transgenic mice containing the human PrP gene there may be over 20 copies) and may actually replace the gene that is there normally. The gene is unlikely to be at the same site as the normal gene in the animal chromosome. To insert the gene requires the DNA to be inserted into the fertilised ovum of the animal and hence is very difficult indeed, with many failures.
TME:	Transmissible mink encephalopathy. TSE of mink found on mink farms in the USA, first reported in the 1960s.
TSE:	Transmissible spongiform encephalopathy. A disease that can be transmitted from one animal to another which causes microscopic holes in the brain that similar to a sponge.
Vertical transmission:	Transmission of an illness from a parent to its offspring.
Virino:	Alternative hypothesis to explain TSEs. Proposed to contain protein and nucleic acid, but distinct from conventional viruses.
Virus:	An extremely small infectious agent with a specific structure and nucleic acid genome, able to cause its own multiplication after infection of cells.

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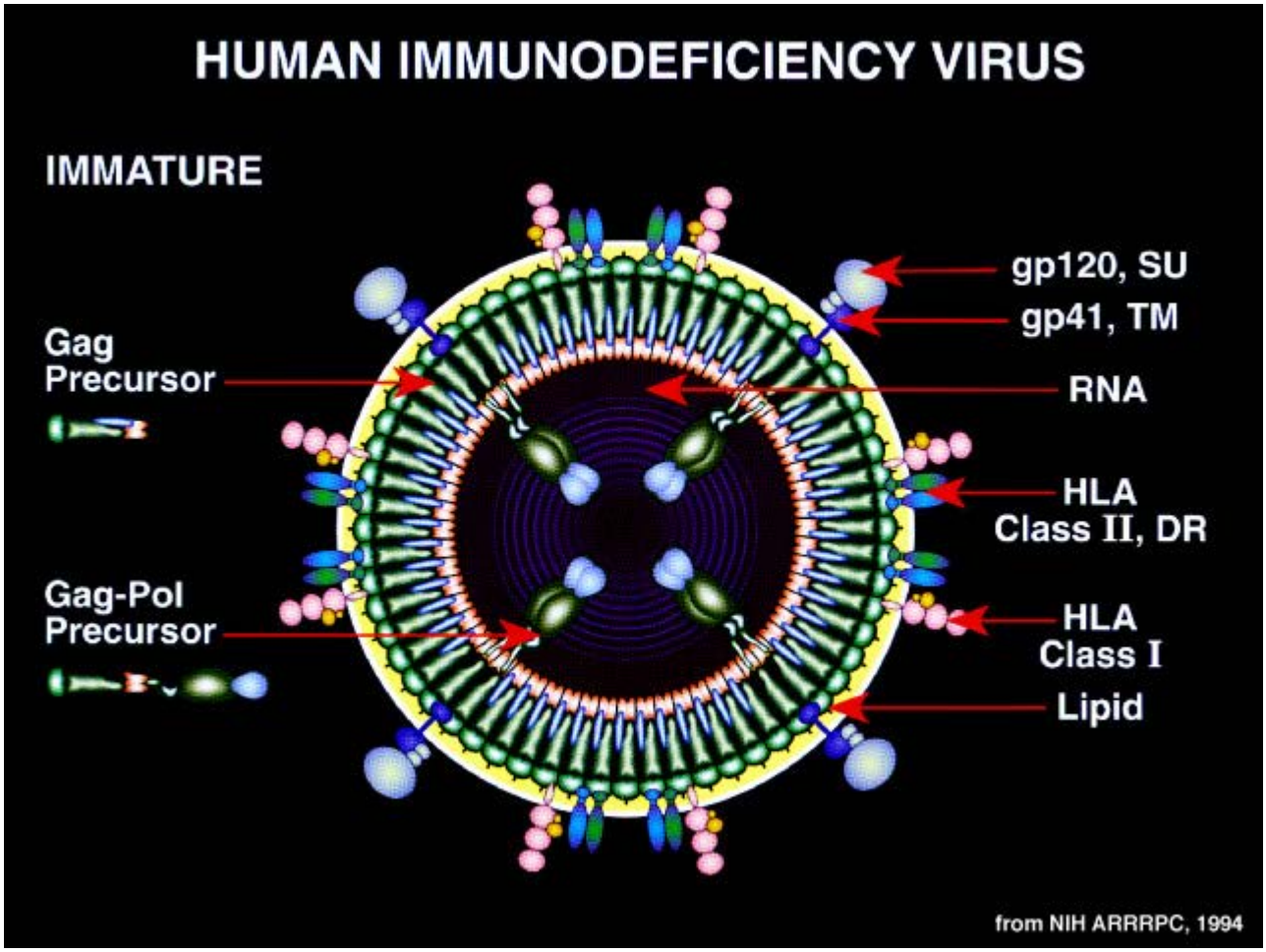
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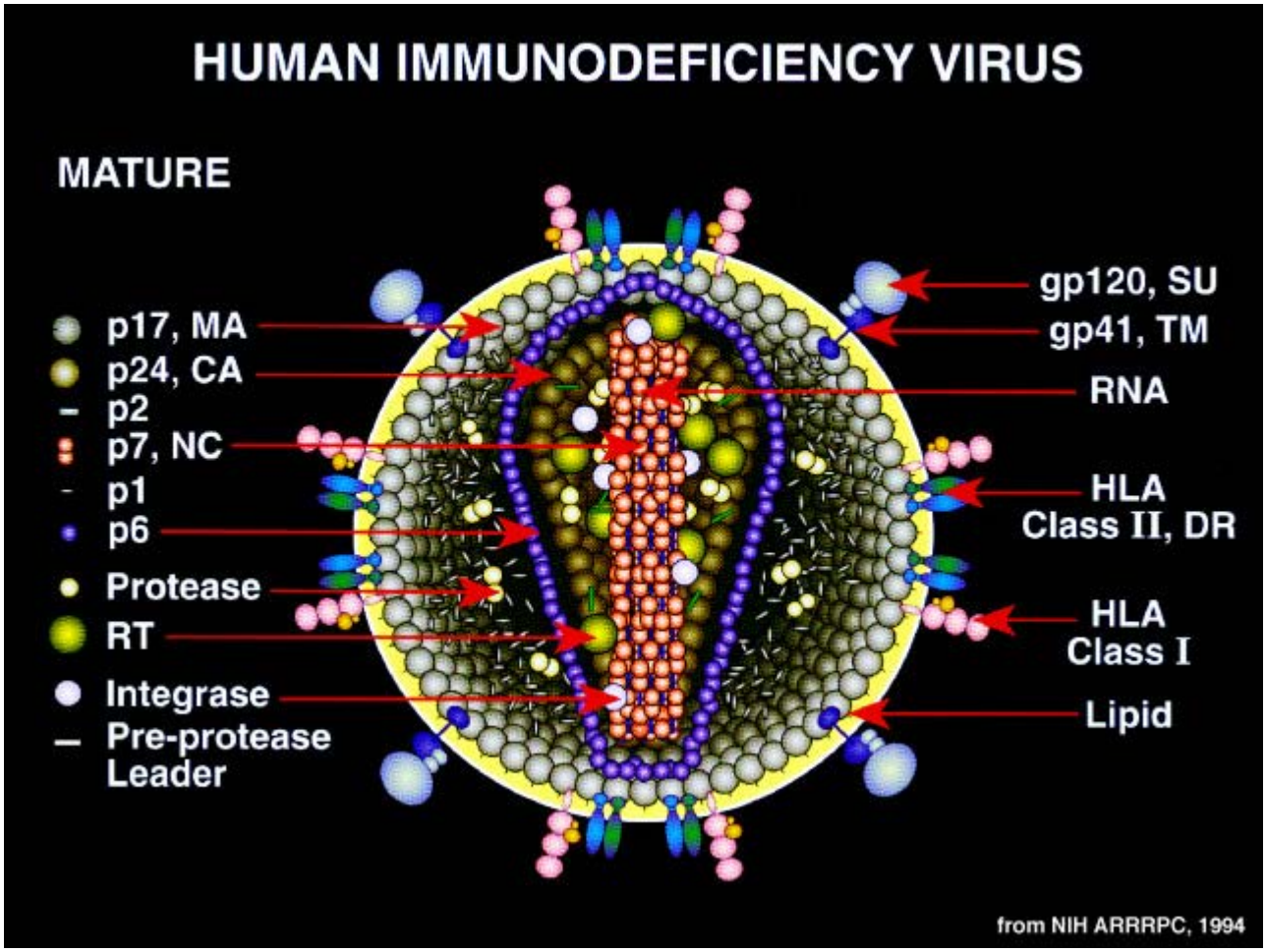
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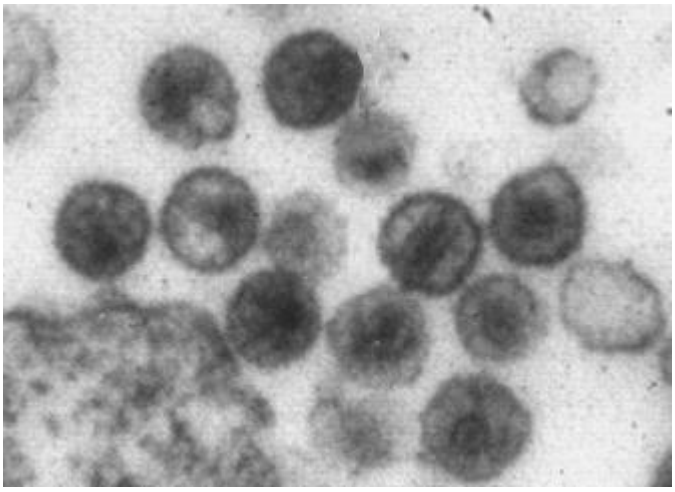
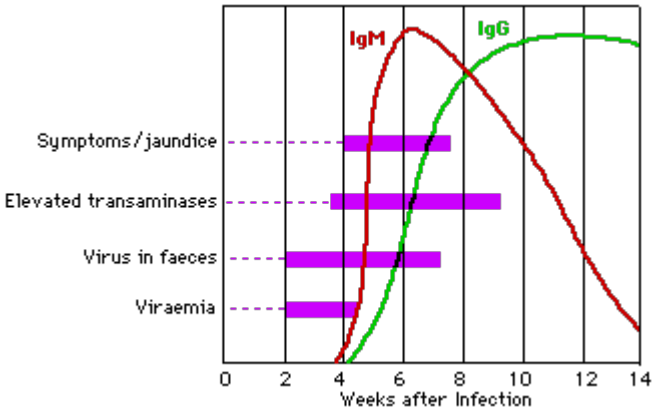


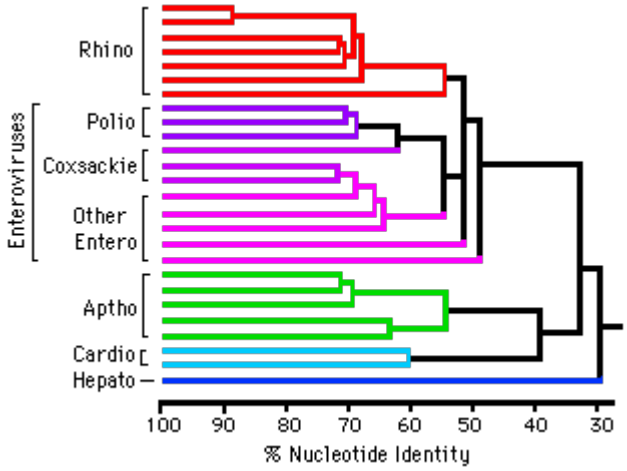
Last Updated: Saturday, October 4, 1945624.

Hepatitis A Virus (HAV)

HAV causes '**infectious/epidemic hepatitis**'. Known for centuries & (wrongly) believed to be spread by aerosols. Spread by faecal-oral route - outbreaks frequently associated with consumption of shellfish.
Clinically, very variable; >90% childhood infections asymptomatic, 25-50% adult infections (as usual, the older you get, the worse it is). Incubation period from 10-50 days, fever, jaundice are main symptoms. 99% cases recover completely, a few cases experience permanent liver damage, fatalities ~0.1%.



The virus was first isolated by Purcell in 1973. In vitro, grows in a variety of cell lines, but rather poorly. HAV is a Picornavirus, formerly classified in the genus *Enterovirus*. Genome studies (sequence homology) showed that it did not belong in this genus and it has been reclassified in a genus of its own: *Hepatovirus*.



Both inactivated and attenuated **vaccines** are available, the inactivated form being more widely used. The availability of assays for and vaccines against HAV means that the incidence is likely to decrease in future.

- References: Click here
- Search for more information on this topic

  Read: The Bad Bug Book - Foodborne Pathogenic Microorganisms.
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HBV | HCV | HDV | HEV | GBV-C/HGV | TTV

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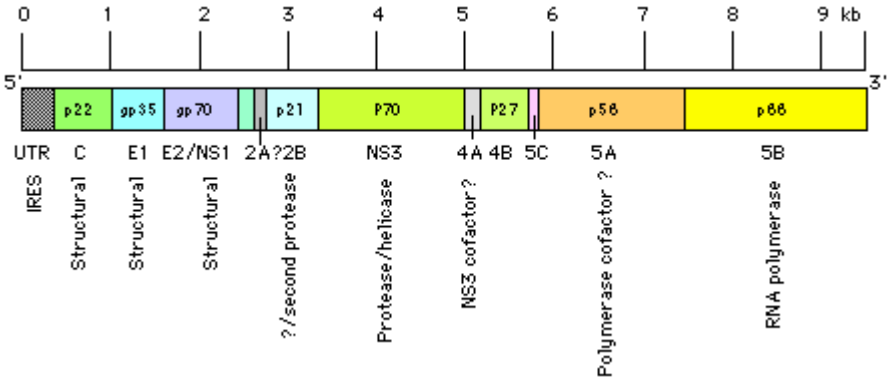
Hepatitis C Virus (HCV):

Originally one source of NANBH (~90%?), hepatitis C virus (HCV) was first definitively identified by molecular cloning of the virus genome in 1989 (Choo et al, Science 244: 359-62, 1989). The virus cannot be cultured in vitro & this has hampered investigations. The HCV genome consists of a positive-sense RNA molecule approximately 9.5kb in length:



In terms of genome organization, HCV is a member of the Flaviviridae and has now been placed in a new monotypic genus in this family. The genome contains a single long open reading frame which encodes a polyprotein of about 3000 amino acids. There is a non-coding region (NCR) of 324-341 nucleotides at the 5' end & a 3' NCR of variable length including a poly(U) tract. The 5' NCR contains an IRES apparently similar in function (but not structure) to that of picornaviruses.

The nucleotide sequence of HCV is highly variable, the most divergent isolates sharing only 60% nucleotide sequence homology. Isolates from all over the world have now been grouped into 6 main types, each with several subtypes, based on sequence data (Simmonds et al, Hepatology 21: 570-83, 1995). Types 1-3 account for almost all infections in Europe, type 4 is prevalent in Egypt & Zaire, type 5 in South Africa & type 6 in Hong Kong. It is not yet clear whether immunity to one type prevents subsequent infection with another, but there is some evidence that various genome types differ in their biological properties.

HCV infection occurs in approximately 0.1% of the UK population (inferred from the level of infection seen in UK blood donors), although rates of infection in other parts of the world are much higher. Since autumn 1991 the BTS has screened all donated blood for HCV. Several diagnostic tests have been developed, serological (used for screening blood donations) - using recombinant antigens, and molecular (PCR - being used to determine the extent of virus variation).

The parenteral route of infection seems to be most prevalent, with high rates of infection seen in intravenous drug abusers, haemophiliacs and recipients of unscreened blood transfusions. The possibility of sexual transmission cannot be eliminated, but if it occurs, the risk seems to be very low. Vertical transmission of HCV in utero & perinatally has also been reported, but again, appears to be rare.

Pathogenesis:

In the majority of cases HCV infection gives rise to an acute illness; **80% of such cases develop into chronic hepatitis**. Almost all patients develop a vigorous antibody and cell-mediated immune response which fails to clear the infection but may contribute towards liver damage. Most flavivirus infections are cytopathic, but this has not been directly tested in the case of HCV since the virus cannot be cultured.
Spontaneous resolution of chronic liver disease is very rare (<2%) and patients with chronic disease are at risk of developing hepatocellular carcinoma (HCC). AS with HBV, chronic HCV infection is a major risk factor for HCC

(30% of UK HCC patients?). Time from HCV transmission to development of cancer ranges from 10 to 50 years (median 30 years). There is a strong association between chronic HCV infection, cirrhosis, and hepatocarcinogenesis (Haydon et al Gut 40: 128-132, 1997). The duration from the onset of acute hepatitis until the time of diagnosis of cirrhosis of the liver and of HCC is about 20 and 30 years, respectively. The acute phase lasts from the onset of disease until 2-3 years thereafter, and the silent phase which follows lasts for 10-15 years. Since so little is known about the biology of HCV, it is presently unclear how this RNA virus establishes a persistent infection.

Therapy:

Recurrent HCV infection occurs in >90% infected liver transplant recipients, although not all patients develop subsequent liver disease. At the present time, the only therapy with any demonstrated efficacy against HCV-induced liver disease involves the use of alpha-IFN, but this approach is not entirely successful. Even in the best trials reported, only 40-50% of chronic suffers respond and 50% of these individuals relapse when treatment is stopped and there is evidence that alpha-IFN treatment is much less effective against some HCV genotypes than others. The development of vaccines, although proceeding, has been slowed by the extreme antigenic variability of the virus. Even when available, vaccines will not alleviate the problems faced by millions of chronic HCV carriers worldwide. No conventional drugs active against HCV infection (i.e. other than IFN) have yet been developed.

IFN therapy is expensive due to the high cost of recombinant human interferons, the large doses & lengthy course of administration necessary to achieve maximum response rates in recipients. Typical therapeutic regimes may be 3 million units IFN administered by injection three times a week for six months. However, recent studies suggest that 3-6 million units IFN three times a week for one year or even longer may be necessary to achieve maximum response rates (Chemello et al. Hepatology 22: 700-706, 1995; Hakozaki et al. Am.J.Gastroenterol., 90: 1246-1249, 1995). The cost of the treatment effectively rations the number of patients able to receive this therapy. There are data from early studies which suggest that sustained response rates are enhanced if ribavirin, a broad spectrum antiviral agent, is administered in combination with IFN.
Read: Terrault, N.A. "Treatment of chronic hepatitis B and chronic hepatitis C" Rev.Med.Virol. 6:215-228 (1996).

Most antibodies to HCV do not play a major role in clearance of infection, although neutralising antibodies do exist. However, these tend to be strain-specific & are ineffective against emerging strains, allowing infection to persist. Formidable obstacles remain to be overcome before a generally useful HCV vaccine is available. Thus there is clearly a desperate need for effective antiviral therapy to complement future vaccination programmes when these become available and to mitigate the effects of long-term HCV infection. However, our inability to propagate HCV in any tissue-culture system and due to the lack of any other animal model than the chimpanzee has confounded the development of antiviral compounds active against this agent.

- References: Click here
- Search for more information on this topic
- Hepatitis C: Therapy
- Managing Hepatitis C (Medical Sciences Bulletin)
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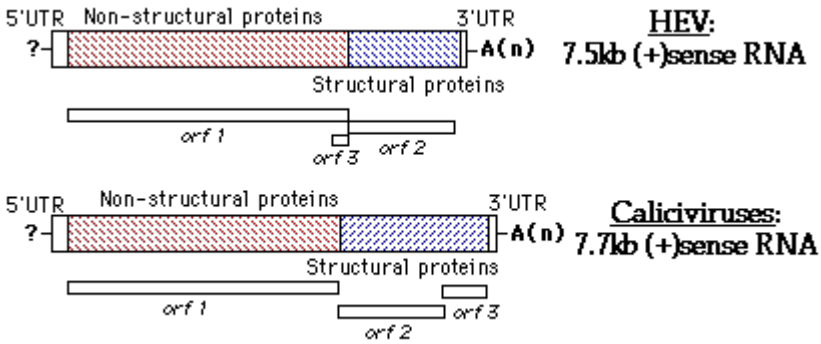
Hepatitis E Virus (HEV):

HEV has now been cloned and sequenced. The virion is a 30-32nm non-enveloped particle containing a s/s (+)sense RNA genome of ~7.5Kb. Genetic organization similar (not identical) to Caliciviruses:



Phylogenetic analysis of the genus Calicivirus in the family Caliciviridae based on the nucleotide and amino acid sequences of human and animal calicivirus 3D RNA-dependent RNA polymerase (approximately 470nt) and capsid hypervariable regions (approximately 1,200nt) to generate phylogenetic trees indicates that there are five separate genogroups:

- small round-structured viruses (SRSV)
- Sapporo-like human calicivirusers (HuCVs)
- **hepatitis E virus (HEV)-like HuCVs**
- rabbit-like animal aclicivirus (AnCVs)
- vesicular exanthema of swine virus (VESV)-like AnCVs

Primate studies indicate faecal-oral (waterborne) transmission of the virus. HEV is endemic in S.E. Asia, ex 'USSR', India, Mid-East, Africa and C. America. ~1% of US blood donors have anti-HEV antibodies. Large epidemics with person to person spread have been known to occur. Normal course of infection seems to be an acute but relatively benign illness (c.f. HAV), except during pregnancy - 15-30% mortality. Recombinant vaccines are currently being prepared.

A closely related virus, swine hepatitis E virus (swine HEV), was recently identified in pigs. Swine HEV crossreacts with antibody to the human HEV capsid antigen & is a ubiquitous agent in pigs. The putative capsid gene (ORF2) of swine HEV shares about 80% sequence identity at the nucleotide level and 90-92% identity at the amino acid level with human HEV strains. The possible medical significance of this finding remains unclear at present.

- References: Click here
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HGV

History:

- In 1967, Deinhard et al. inoculated tamarin monkeys with serum from GB, a surgeon suffering from acute hepatitis. The monkeys also showed biochemical and histologic evidence of acute viral hepatitis. Both GB and the tamarins recovered from their hepatitis: this form of the disease is self-limiting.
- 1970s-early 80s: multiple passages in tamarins and marmosets using the serum from the tamarins originally infected with GB serum were performed. The "GB agent" was characterized biophysically (filtration, etc) and shown to be of viral origin.
- Almeida et al (Nature, 261: 608-609, 1976) perform the 11th passage study of the "GB agent". This material was deposited at the ATCC in the USA (also known as: "H205 GB pass 11").
- Researchers at Abbott Laboratories used GB passage 11 serum for tamarin inoculations. Serum from tamarins with hepatitis were used for molecular biology experiments designed to identify the viral "GB agent". Methods used included representational difference analysis (RDA) and cDNA library immunoscreening.
- RDA identified cDNA fragments isolated from infectious tamarin serum with sequence similarity to other flaviviruses, but most similar to HCV. The genomes of two viruses were eventually cloned and sequenced in their entirety from the infectious tamarin serum produced at Abbott Laboratories. These viruses were designated GB viruses A and B (GBV-A, GBV-B) (Simons et al, PNAS, 92:3401-3405, 1995).
- Serologic studies identified humans with antibodies against nonstructral proteins from GBV-A and B, but PCR studies failed to identify humans infected with GBV-A or B even though they were antibody positive.
- Consensus (i.e. degenerate) PCR primers were designed based upon alignments of nucleotide sequences from the helicase genes of GBV-A, GBV-B, and HCV.
- These primers identified a PCR positive individual from West Africa. The sequence of the PCR product was similar to but distinct from GBV-A and GBV-B and believed to be a portion of the genome of a third virus, designated GB virus C. (Simons et al Nature Medicine, 1: 564-569, 1995).
- The complete genome sequence of GBV-C was determined (Leary et al, J.Med.Virol, 48: 60-67, 1996).
- Linnen et al (Science, 271: 505-508, 1996) described the isolation of a new virus, designated HGV, from a patient diagnosed with non-ABC hepatitis.
- Both GBV-C and HGV are positive, single-stranded RNA viruses containing approximately 9400 nucleotides, have a genomic organization resembling that of the Flaviviridae, and are distantly related to HCV. They have 86 percent nucleotide sequence similarity and 96 percent predicted amino acid sequence similarity. Thus they are considered to be different isolates of the same virus, which is now generally known as **HGV**.

I am grateful to A.Scott Muerhoff (Research Investigator, Virus Discovery Group, Abbott Laboratories) for the above account of the discovery of GBV-C/HGV.

HGV infection was originally suggested to be connected with fulminant hepatitis, but recent studies have failed to prove a connection between HGV and clinical illness. Some studies have suggested that in contrast to HCV, the liver is **not** the primary replication site for HGV. Where does HGV grow? Virus circulating in the bloodstream is difficult precipitate with antibody to immunoglobulins, but can precipitated with antibody to apolipoproteins. Since no hypervariable regions have yet been identified in the envelope proteins of HGV, the lipoprotein coat may help the virus evade immune surveillance and contribute to its persistence.

In many countries, 1-2% of blood donors test positive for HGV RNA & the prevalence of HGV infection is up to 10-15% in West African children. How this high prevalence is maintained is unknown, but this does suggest that sub-clinical infection is common. Antibodies to E2, an envelope protein of HGV, can be detected in >50% IVDAs who are HGV RNA negative, but in relatively few IVDAs who have HGV RNA. Therefore, HGV infection is probably much more frequent than studies of the prevalence of HGV RNA suggest.

The virus is transmitted by the same routes as HCV & co-infection is common; however, this may represent a common source of infection rather than any clinical similarity between the two viruses. The clinical significance of HGV infection and HGV-HCV co-infection remains to be fully elucidated, but at present does not seem to be a major disease-causing factor. The majority of patients infected with HGV by blood transfusion do not develop chronic hepatitis, but viremia frequently persists without biochemical evidence of hepatitis. Given the high prevalence of HGV worldwide and the association with mild or no clinical illness, is HGV merely an **accidental tourist** that occasionally travels with other hepatitis viruses?

- References: Click here
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HAV | HBV | HCV | HDV | HEV | TTV

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Last Updated: Saturday, October 4, 1945624.

TTV

History:

In 1997, a novel DNA virus was identified in serum of a Japanese patient (TT) with post-transfusion hepatitis (Nishizawa et al, BBRC 241:92-97, 1997). The virus has little sequence similarity with any known virus, thus it is presently not clear to which family it belongs.

Distribution & Pathogenesis:

Since it's discovery, it has become clear that TTV infection is present worldwide among blood donors and is common in patients with liver disease, including cryptogenic cirrhosis and fulminant hepatic failure. In some studies, TTV DNA has been found more frequently in patients with liver cirrhosis and hepatocellular carcinoma than in those with chronic hepatitis. However, virus DNA is not integrated in tumour cells, which may suggest that the virus is a passenger rather that causative of the tumour. Further studies are required to determine the role of TTV in the pathogenicity of acute and/or chronic liver disease. Therefore, the significance of TTV infection in liver disease is, at present, analagous to that of HGV.

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The Panama Puzzle

The name's Reed.

Walter Reed: Major, US Army Medical Corps.

I need your help. It's June 25th, 1900, and I've just been posted to Cuba after the end of the Spanish-American war. We lost more men to disease than bullets in that campaign. One disease in particular. In Panama, our boys have started digging a canal so that our warships can get from the Atlantic to the Pacific. Some canal! We've got big trouble - hundreds of our men have died so far from that damn disease. We call it "yellow jack" *(yellow fever)*. They get a high fever and jaundice. Up to half of those who show symptoms die. It's my responsibility to find out what's causing it and put a stop to it. Like I said, I need your help.

The surgeon general has put three assistant surgeons under my command: James Carroll, Jesse W. Lazear and Aristides Agaramonte. We've got a few ideas so far about what could be causing the disease:

- The hot, humid air the men have to breathe
- A disease spread by rats
- A disease spread by mosquitos
- A disease spread by drinking water
- A disease caused by germs *(bacteria)*

What do you think?

Click on one of the choices above.

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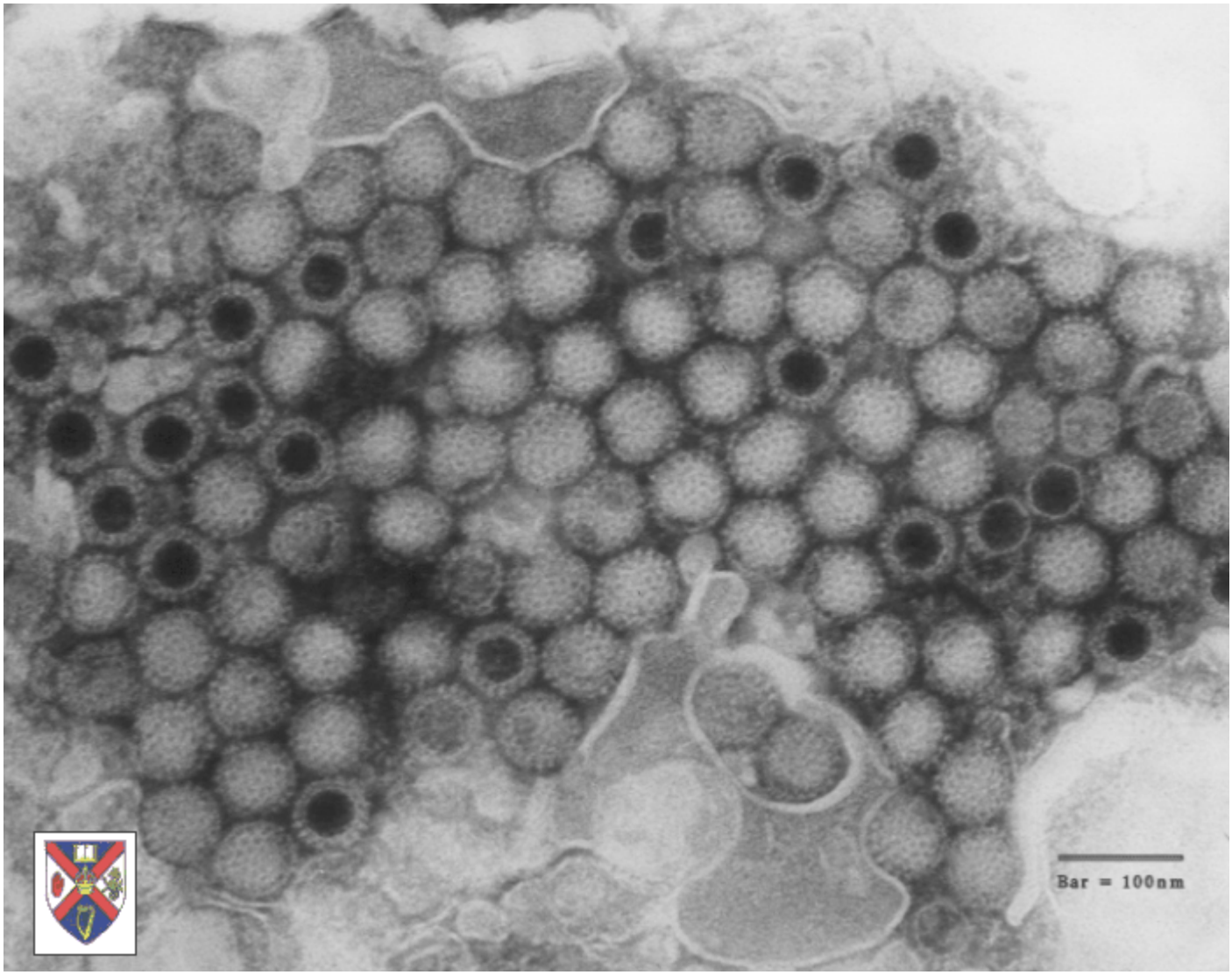

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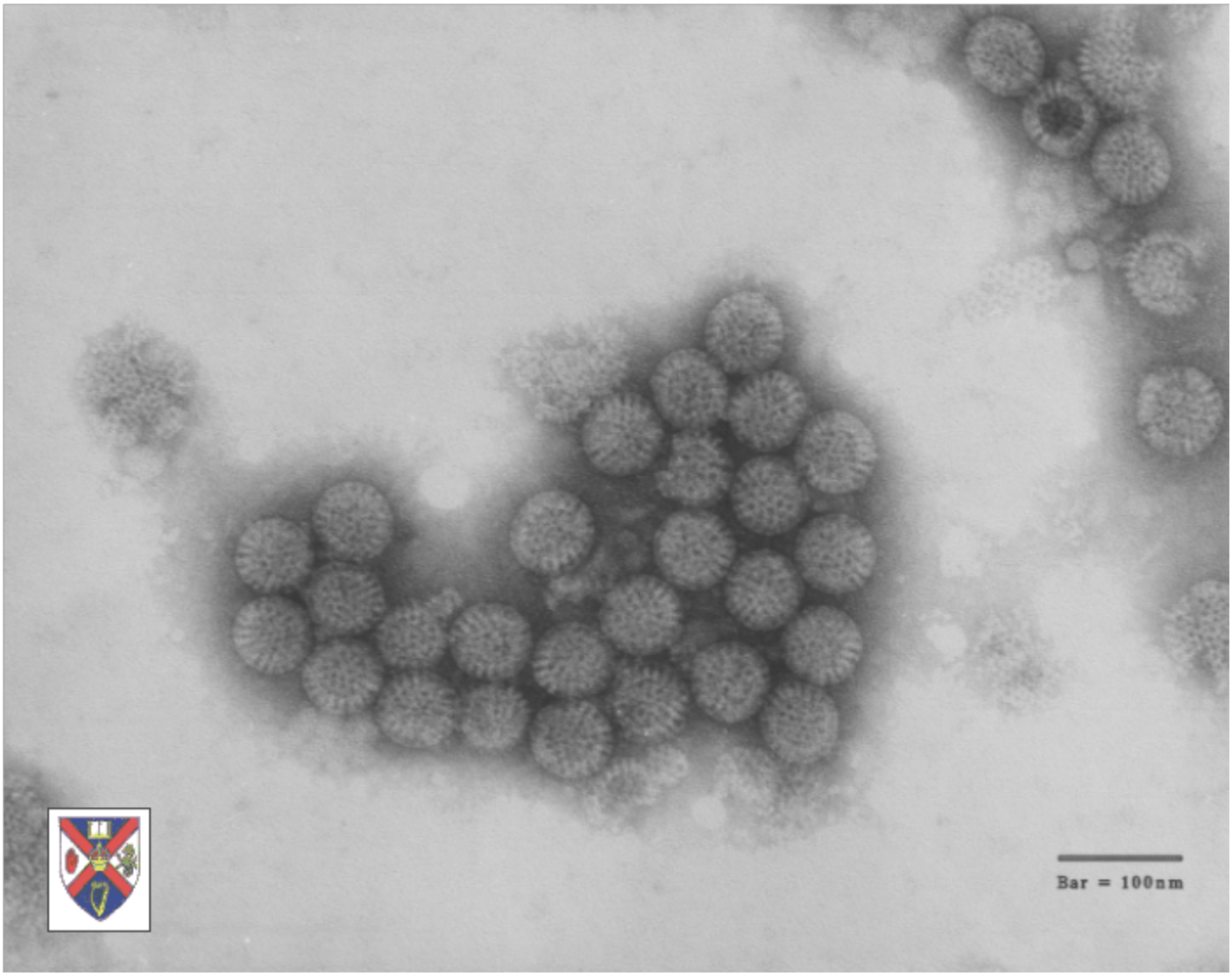

How HIV Fools the Immune System.

Professor Andrew McMichael

Institute of Molecular Medicine
Oxford University.

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We still do not fully understand how some viruses persist and cause chronic disease, including cancer. For instance, while we know that AIDS results from the HIV virus overcoming part of the immune system, we don't know exactly how it does this, while avoiding being destroyed itself. But we do know that the relationship between the infecting virus and the immune system is central to the story. If we could find out why the immune response fails to see off invaders such as HIV, we should be able to develop more effective treatments. Work in my laboratory at Oxford, supported by the MRC, is focusing on one key player: a type of immune or white blood cell known as the cytotoxic or 'killer' T-cell.

When the immune system encounters a virus for the first time, every component of the system goes on red alert. At first, as viruses infect susceptible cells, they multiply unconstrained by any immune response. But after a variable length of time - four or five days for influenza but two to three weeks for HIV - the first immune response becomes detectable. This is the T-cell response, made up of both 'killer' and 'helper' cells. The killer cells destroy virus-infected cells before they have time to release large quantities of new virus particles. The helper cells secrete special compounds, called cytokines, some of which have antiviral activity - interferon is a well known example. Other cytokines are involved in controlling inflammation, while yet others drive the B-cells of the immune system to make antibodies to help combat the invaders.

In some infections the action of these T-cells is visible to the naked eye: they cause a rash as they promote inflammation around a focus of infected cells in the skin. Measles, for example, appears to get worse as the rash appears but then recovery is rapid. In another example, as the virus which causes hepatitis A multiplies quietly in liver cells, the T cells move into the scene. Killer cells destroy virus-infected cells, but in so doing damage the liver, causing jaundice. But once the patient is through that stage, recovery is usually rapid and complete.

The production of antibodies, which lags behind the T-cell response, may help in recovery but is not essential. Antibodies' real role in life is to prevent further viral attacks. In this they are incredibly successful. For example, it is extremely rare for anyone to have more than one attack of the childhood viral infections such as mumps, measles or chicken pox. Furthermore, pregnant mothers pass on antibodies to their babies, providing protection against infection during their first six months of life. Thus the baby benefits from the mother's previous infections, even if the viral 'challenge' happened more than thirty years earlier! Some viruses, however, can evade anti body immunity by changing their outer coats; the classic example is the rapidly evolving influenza virus which changes its surface

coating from year to year. Unlike measles or mumps, humans suffer flu infections every few years throughout life.

Viruses that are major health threats:

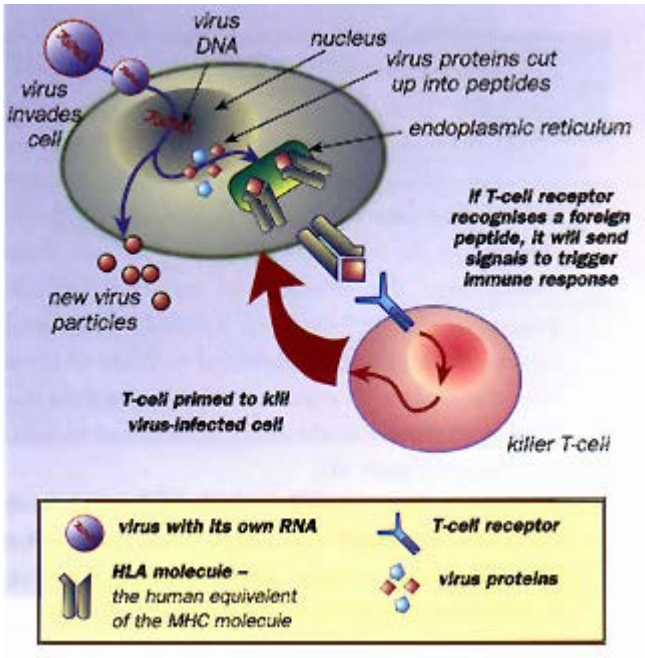
Virus:	Disease:	Vaccine?
HIV	AIDS	No
RSV	Respiratory Infection	No
Hepatitis B	Liver Cancer	Yes
Hepatitis C	Cirrhosis/Cancer	No
Epstein Barr virus	Lymphomas, NPC	No
HPV	Cervical cancer	No
Measles	Pneumonia (infants)	Yes
Influenza	Pneumonia	Yes

How does HIV avoid detection?

The role of killer T cells in viral infections has been of major interest to my laboratory at the Institute of Molecular Medicine in Oxford for some time now. We have built on the work of many MRC scientists, notably Brigitte Askonas of the National Institute for Medical Research, London. She showed the importance of killer T cells in influenza and respiratory syncytial virus infections, and in the 1970s and 1980s trained many of those involved in the field today, including Alain Townsend, Charles Bangham, Peter Openshaw, Helen Bodmer and myself.

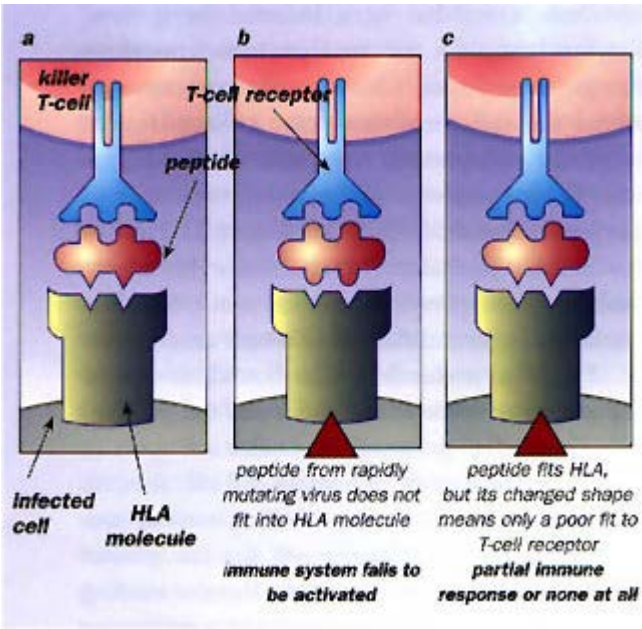
In our laboratory we have focused on the immune response to HIV in an attempt to understand how the virus escapes from control by killer cells - this is probably one important reason why it can go on to cause AIDS. As in other viral infections, the initial burst of viral proliferation is at first brought under control by the killer T-cell response. Then antibodies against the virus appear which can neutralise some of the released virus particles. For most people with HIV, the infection is kept at bay for some years and the patient feels healthy. We now know, however, that, despite an immune system which is still active, the virus somehow continues to replicate at quite high levels during this period. This results in the infected cells, the helper T-cells and macrophages of the immune system, being slowly but relentlessly destroyed. Eventually the whole immune response fails.

Once a cell has been infected by a virus, enzymes chop up the viral proteins into short strings, or peptides, which are then transported across the cell membrane into a compartment called the endoplasmic reticulum. Here the peptides fit into a groove on the top of molecules of a cell protein called HLA. The HLA molecule and its bound bit of viral peptide then travels to the surface of the infected cell. There, if all goes well, the killer cell spots it, recognises the peptide as foreign and kills the cell. In effect, the infected cell is labelling itself as can non fodder - sacrificing itself for the greater good of the organism as a whole. But the binding of peptide to the HLA molecules, a very precise process, is a vital step in the immune response. If something goes wrong with this process, the immune response can be fatally undermined.



We each possess a unique array of HLA genes, which define and produce molecules that vary in structure from one individual to another. Thanks to our distinctive HLA molecules, each of us reacts to viruses in a distinctive way: our HLA molecules react to and bind different peptides from distinct viruses. This may have major consequences for our susceptibility to persistent viruses.

Unfortunately, viruses that are highly variable, like HIV, have found a way to interfere with the binding process. In particular, small genetic changes in the virus - mutations - alter the structure of the virus peptide, destroying the 'fit' between it and the HLA molecules. As a result, the altered peptide may no longer stimulate an immune response, and so may be able to persist in the body undetected by killer T cells. Often the mutated peptide still binds with HLA but the combined shape is so altered that the killer cell simply does not recognise it. Sometimes the mutated duo no longer fit into the appropriate docking molecule or receptor on the T cell. Alternatively, the mutated peptide may dock only half-heartedly, so that the receptor delivers a weakened signal to the T cell, effectively putting the T-cell out of action:



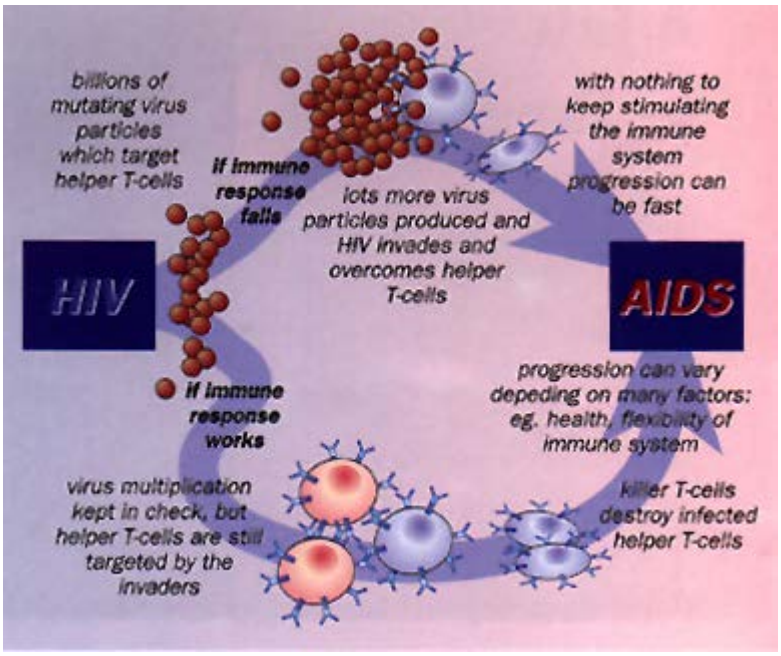
The key question is: why can't killer T-cells control or eliminate this virus? Clues come from other persisting viruses; it is becoming clear that these viruses have evolved strategies to evade killer cells. Epstein Barr virus, for instance, causes glandular fever but then persists harmlessly in more than 85% of infected individuals. It persists because it stops the cells it infects from generating, fragments of viral proteins, or peptides, that normally signal its presence and stimulate killer cell responses.

As a result of this interference, the virus goes by undetected. Herpes simplex virus, which causes recurrent cold sores, also interferes with the processing of the viral proteins into the immunologically stimulating peptide fragments, but in a different way. Adenoviruses, like the ones which cause colds, make themselves non immunogenic by interrupting the same process in another way. HIV however, does not have to use this kind of strategy: it can probably subvert the killer T-cell response simply by having such a variable molecule.

The problems with large numbers.

Lamentably for our immune system, the potential for mutations in HIV is enormous: over a billion virus particles are made every day by an infected patient, even in the early stages of infection, and on average each contains one mutation. This gives the virus the power to escape from both the killer cells and antibodies. Some patients are more lucky than others, however. The virus cannot mutate certain parts of its genes without damaging its ability to survive and replicate. Some people infected with HIV may be able to generate a more effective immune response to the virus if their particular HLA molecules happen to pick peptides from these 'conserved' regions.

In the absence of a killer response HIV invades and kills the helper cells and multiplies rapidly so that progression to AIDS is fast. When the killer cells are able to recognise and kill HIV infected cells, the virus is controlled but only at cost: for inevitably, the helper cells infected with HIV are also killed. Sadly, the maintenance of the killer response depends on the helper cells, so the killer cells eventually cut off their own lifeline:



This may be one reason why killer cells eventually fail to control HIV. The rate of this failure depends on various factors including the intrinsic aggression of the infecting strain of HIV, the overall health of the individual, their ability to replenish stocks of helper cells and the vigour of the killer cell response. The strength of the killer cells in turn depends largely on ever-mutating virus. If by good fortune an individual's HLA molecules zero in on the less variable, 'conserved' parts of the virus, the killer cell response will be more stable. The specificity of the killer response is itself determined by the HLA molecules, and ultimately, by the HLA genes an individual happens to possess.

This brief summary of HIV immunity illustrates another point about killer T-cells: they can sometimes contribute to disease. Some viruses infect cells but do not cause them much damage and do not themselves kill the cell, at least in the short term.

A good example is hepatitis B virus. If a killer response is stimulated, the T cells kill the infected liver cells, damaging the liver. If in doing so they eliminate the virus, this may be a price worth paying, but sometimes they can cause more damage than the virus ever would. But although hepatitis B virus may itself cause little direct damage, the infection is strongly associated with liver cancer - one of the world's commonest cancers, and so total elimination of the virus is desirable in the long term.

An understanding of the killer response and its role in immunity is beginning to lead to better vaccines and new

kinds of treatment, known as immunotherapy. Traditional vaccines have been aimed at stimulating an effective antibody response against viruses such as influenza, hepatitis B, measles, mumps and polio. Yet researchers have long realised that live virus vaccines - where the virus is attenuated or made less vicious are more effective, probably because they are better at stimulating the T-cell responses. Unfortunately, sometimes it is not feasible or is too dangerous to attenuate a virus - and this is the case with HIV. So researchers in both industrial and academic laboratories are now trying to develop safe and simple vaccines that stimulate strong killer responses. Such vaccines should offer better protection against infection with viruses, including HIV. There is an attenuated version of the monkey variant of HIV, known as SIV, which protects macaques from infection with SIV and the development of AIDS. This work was done by Ron Desrosiers in Boston and in the UK by Jim Stott, Martin Cranage and colleagues funded by the MRC. This research has led some scientists to ask whether an attenuated version of HIV could be developed. The problem is that such a vaccine would infect permanently and might perhaps cause disease in the long run. It would be much better to mimic these immune responses by vaccines with less potential risk.

It would be better if we could control or eliminate established viral infections by directly manipulating the immune responses in infected patients. Redirecting immune responses to the most vulnerable parts of a virus-such as the conserved regions of HIV might be an effective approach. Altering immune responses to a variety of infections by injecting antibodies, cytokines or even cells is also under investigation. One of the most encouraging examples is the remission of a cancer linked to Epstein-Barr virus, called EBV associated lymphoma, in people receiving bone marrow transplants, whose immune systems are suppressed. Their cancers receded after they were given infusions of EBV-specific killer cells grown in culture. It might also be possible to modify such cells by gene therapy before treatment.

The killer T-cell response is a key part of the immune response to a viral infection, complementing the antibody response. Killer cells are usually good at inhibiting the infection, but they may fail, allowing the virus to persist. Unravelling how and why this happens is central to a better understanding of viral infections. Furthermore, developing vaccines that stimulate killer cells is an important priority for many viruses where vaccines are either not available or unsatisfactory.

Professor Andrew McMichael is a MRC Clinical Research Professor of Immunology and heads the Molecular Immunology Group at the Institute of Molecular Medicine in Oxford. His research focuses on the immune response to virus infections, in particular HIV. He is a member of the MRC's Molecular and Cell Medicine Board, and also sits on the AIDS Research Coordinating Committee.

Further Reading:

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Meier U-C et al, (1995) Cytotoxic T lymphocyte lysis inhibited by viable HIV mutants, Science, 270: 1360-1362.

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Emerging & Re-Emerging Viral Diseases: A Challenge For Biotechnology

Ed Rybicki, Dept. Microbiology, University of Cape Town

Recent years have seen an increasing, rather than decreasing, incidence of viral diseases that had been confidently supposed to be under control. Chief among these is yellow fever - the disease which almost stopped the building of the Panama Canal - which is still endemic throughout the tropics, despite there having been extensive eradication and vaccination campaigns over several decades. Now it is threatening to spread to more temperate zones, including the continental US, largely as a result of inadvertent importation of vector mosquitoes in water in used tyres transported from Asia to the US. Another factor in its recent epidemiology has been the settling of cleared forest areas in the Amazon and elsewhere, which results in disturbance of the natural mosquito-small mammal virus transmission cycle. Although there has been a vaccine for the virus for many years, it is apparently not particularly effective in terms of conferring lasting immunity, and there have been complications resulting from its use: these factors and the recent spread of the virus combine in making it essential to engineer new and more efficient (and cheap!) vaccines for combatting its spread.

Although it is not an emerging virus, hepatitis C virus was only finally identified (after years as non-A-non-B-hepatitis virus) as recently as five years ago. This was as a result of cDNA cloning of a short viral sequence from the nucleic acid in patient serum followed by sequencing, synthesis of PCR primers, and "walking" along the hardly-detectable genome, till the entire genome had been cloned and sequenced. Although the sequences of at least five distinct strains of HCV are now known - thanks to cDNA PCR - and the true incidence of the virus is finally understood, HCV has not yet ever been isolated as RNA or even as particles from patient serum. That we know so much the virus despite the fact that it never either been purified or cultured, is due entirely to modern molecular biology techniques, such as diagnosis of HCV in serum samples by PCR. It is also possible to do molecular epidemiological studies on HCV by using PCR products for molecular hybridisation and sequencing.

The recent spate of publicity surrounding what was called the "Four Corners virus" - and now known to be a hantavirus, sometimes called Muerto Canyon hantavirus - has brought home with a vengeance the realisation that even First World countries such as the USA are not immune to the sudden emergence of an indigenous disease with life-threatening potential. Muerto Canyon virus is related to the Hantaan virus which caused great problems among GIs in the Korean War. Both are members of the genus Hantavirus, family Bunyaviridae, have three segments of negative-sense single-strand RNA, and have small rodents (deer mice in the case or MC virus) as natural hosts. Humans are exposed to the virus when rodent infestation of dwellings is high, when rodent urine and faeces serves as inoculum. MC virus became prominent because of its high fatality rate - 60% of confirmed cases have been fatal - and because it was first recognised in the Navajo country of the US south-west, which sparked fears among potential tourists across the country.

Identification of the virus as a novel hantavirus had occurred within a month of the first alarm over the disease in May 1993: first attempts to grow the virus failed, but serological screening of patient samples indicated a hantavirus. Subsequent progress depended on degenerate primer PCR, which allowed amplification via cDNA of a conserved sequence which could be used as a molecular probe, and could be sequenced. Use as a probe allowed detection of virus in deer mice, and post-mortem tissue samples. Sequencing allowed molecular cloning of most of the virus genome, and further sequence proved that the virus was novel. It has also been found all across the US, indicating a wide distribution. Subsequent molecular probing of paraffin-embedded tissues proved also that the disease must have been around as long as 15 yrs. PCR amplification using newly designed degenerate primers has shown that other novel hantaviruses are present in other rodents elsewhere in the US. Within a year of the initial identification of a cluster of suspected hantavirus pulmonary syndrome has come not only a confirmation of the disease, but molecular characterisation and epidemiological studies that have done much to allow prevention of outbreaks of the same and similar viruses. This progress is thanks to the rapid application of sophisticated biotechnology after an initial serological diagnosis.

As stated earlier, this outbreak is warning that severe and previously unidentified diseases could strike at any time in any society: Joshua Lederberg warns (ASM News, 60: 233) that we may well be in line for another influenza epidemic on the scale of the 1918 Spanish 'Flu, which would spread far faster and potentially reach more people, thanks to modern transport systems and porous borders. Although we partially understand the mechanisms which give rise to epidemic 'flu strains - genetic reassortment of human, avian and porcine strains in Chinese intensive farming schemes - there is the distinct possibility that other, more lethal diseases may well be about to burst out of some ecology that we do not understand, but have somehow upset to the point that hitherto secluded/sequestered virus reservoirs, or novel vectors, are coming into contact with humans.

As an example, although monkeypoxvirus causes smallpox-like symptoms in humans, it is not nearly as contagious. However, we do not know what might make it more so - and as it gets into humans living in tropical trainforests in Africa due to contact with arboreal rodents, and pressure on rainforests is becoming more acute, it may become a matter of necessity that we find out just why it has not yet caused an epidemic. Like the HIV work that is being done here and elsewhere, the monkeypox work focussed on looking for changes on viral genomes in the human and animal populations which could explain differences in biology. In the case of monkeypox, important differences were found relative to the smallpox genome which could explain differences in human disease potential. In the case of HIV - which, thanks to molecular epidemiology and molecular phylogenetic investigations has almost certainly been shown to be an escape from monkeys - the virus exhibits far more variability in humans due to rapid mutation, than in monkeys, in which hosts it is most often temperate i its effects. However, knowledge of the variability of the virus has allowed the formulation of test kits that more accurately detect virus in African patients (in whom the variability of strains is greatest).

Although the emerging virus problem in the human sphere is more important in social terms, emerging virus diseases in crop plants should not be overlooked in terms of their potential impact on the human population. For example, a speaker at the last International Congress of Virology in Glasgow in 1993 cited HIVs, yellow fever, hantaviruses, and tomato spotted wilt and whitefly-transmitted geminiviruses as beng the most important of the emerging or re-emerging virus diseases affecting humankind.

TSWV is - coincidentally - another of the Bunyaviridae, has a very wide plant host range, causes a great deal of damge to infected plants, and is cutting a swathe through agriculture from Asia, through the Americas, in Europe, and in Africa. Its spread has been due to diseemination of its thrips insect vector, and of diseased plant material. Again, molecular biological techniques such as PCR have allowed its rapid identification and characterisation of strains and distinct related viruses. Molecular epidemiology of the virus is a logical corollary, and is proceeding by means of large-scale internation collaborations.

WT- geminiviruses have caused a great deal of crop damage in plants such as tomatoes, beans, squash, cassava and cotton, and their spread may be directly linked to the inadvertent world-wide dissemination of a particular (so-called silverleaf) biotype of the whitefly *Bemisia tabaci*. This happens to be a particularly indiscriminate feeder, and a very good vector, allowing rapid and efficient spread of viruses from indigenous weed species to neighbouring crops. The detection of these viruses in plants and vector has again been hugely aided by the advent of PCR and molecular hybridisation. To illustrate how rapid is the advance in our understanding of these viruses thanks to modern techniques, there are currently over 40 complete and hundreds of partial viral genomic DNA sequences known, compared to only 1 complete sequence in 1983. PCR and sequencing and restriction mapping has similarly allowed the characterisation of a wide variety of strains of the extremely agriculturally important maize streak virus, and to show that viruses infecting wheat and grasses and sugarcane are distinct from those infecting maize, meaning traditional ideas on virus spread will have to be re-examined.

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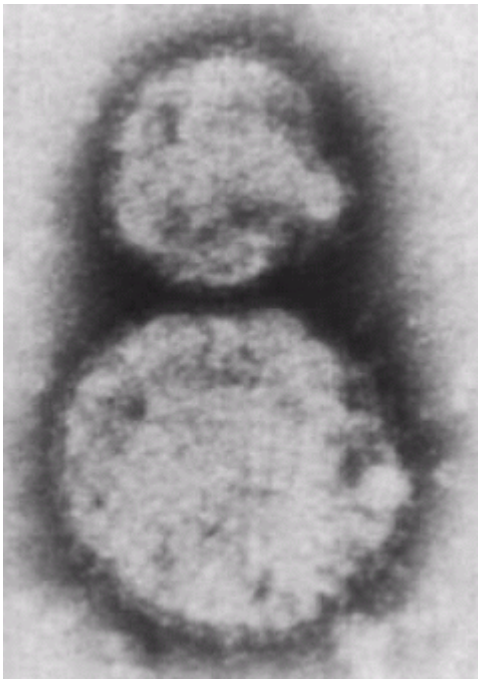

Virus	Host	Distribution (host)	Disease
Hantaan (HTN) (Lee,1978)	Apodemus agrarius (striped field mouse)	China, southern Siberia, central Asia, Korea, Europe	HFRS
Dobrava/Belgrade (DOB) (Gligic, 1992)	(yellow-necked field mouse) Apodemus flavicollis	Most of Europe, Asia Minor	HFRS
Seoul (SEO) (Lee, 1983)	Rattus norvegicus (Norway rat)	Worldwide, according to distribution of commensal rats	HFRS
Thailand (THAI)(Xiao, 1994)	Bandicota indica (bandicoot rat)	India, southeastern China, northern Malay Peninsula	unknown
Puumala (PUU)	Clethrionomys spp.(bank voles)	Russia, Europe, Asia Minor, Hokkaido, Korea	milder HFRS (nephropathia epidemica)
Prospect Hill (PHV)(Lee, 1982)	Microtus pennsylvanicus (meadow vole)	United States, Canada	unknown
Four Corners (FCV) (Nichol, 1993)	Peromyscus spp. (deermice)	United States, Mexico, Canada	HPS
Harvest Mouse(HMV-1) (Hjelle,1994e)	Reithrodontomys megalotis	United States, Mexico, Canada	unknown
Black Creek Canal (BCCV) (CDC, 1994c)	Sigmodon hispidus (cotton rat)	United States to Venezuela, Peru	HPS
Monroe, Louisiana (CDC, 1993b)	unknown	unknown	HPS
Rhode Island/New York (CDC, 1994c)	unknown	unknown	HPS

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Edward Jenner was the first person to try to prevent smallpox.

- True?

or

- False?

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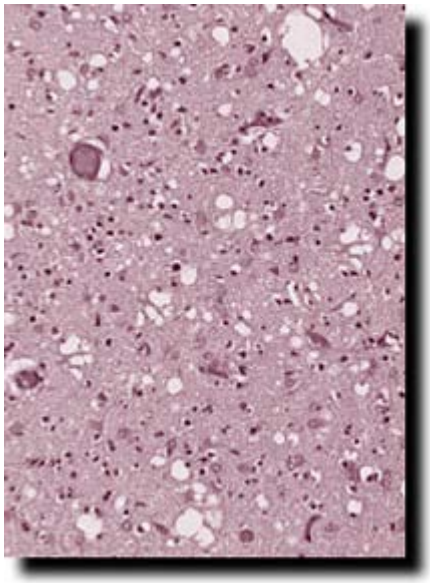
In 1920, two Austrian doctors called H.G.Creutzfeldt and A.Jakob first described a disease which in many respects is similar to Alzheimer's disease:



Looking at the victims brains under the microscope, they saw that many of the normal brain cells had died, causing the development of many tiny holes, too small to be seen by the naked eye, and a mesh of fine fibres. The appearance of the brain tissue resembled a microscopic sponge, and the expression "spongiform encephalopathy" was used to describe the disease. At first, suffers of Creutzfeldt-Jakob disease (**CJD**) are usually unaware that anything is wrong. Often there are subtle symptoms such as lapses of memory for daily events, and sometimes mood changes. Increasing apathy and loss of interest in normal daily and social activities is common. At this point, straightforward tasks that were previously simple become progressively more difficult for the sufferer. Early in the disease, the illness is frequently disregarded by friends, relatives and the sufferers themselves as the results of stress, being 'run down' or as mild depression.

Within weeks of the onset of the disease, other less easily discounted symptoms begin to appear. Neurological signs such as loss of balance, hesitancy in walking, deteriorating vision (sometimes including terrifying hallucinations), slurring and slowing of speech are common. After this, sufferers decline rapidly, becoming incontinent and experiencing involuntary, jerky movements of the limbs (known as myoclonus). Most patients slip into a comatose state without the ability to speak or move. Fortunately, the very apathy and loss of mental functions which CJD causes seems to protect victims from awareness of their fate. People affected by CJD usually die within 6 months of the onset of the disease. Only in 10% of cases does the disease run a more prolonged course of, for example, 2-5 years.

There is no known cure for CJD nor is there any treatment which can halt the progress of the disease.

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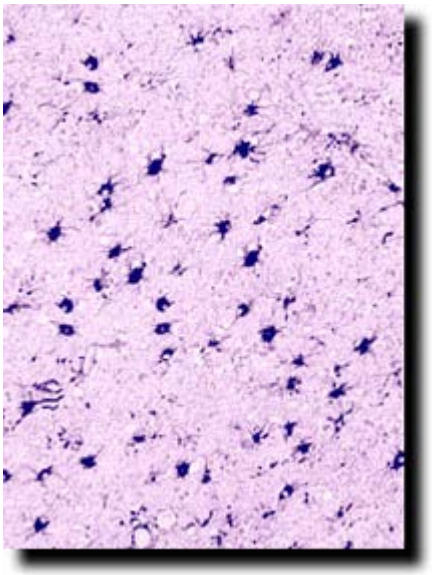
Industrialized societies are in the middle of an epidemic. The killers of youth are being replaced by the killers of old age:



Among the most disturbing of these 'new' killers are a set of degenerative brain diseases known as the amyloidoses. A number of different diseases are characterized by the deposition of strange protein deposits in various organs of the body, e.g. the kidney, spleen, liver or brain. These abnormal or **'amyloid'** deposits consist of accumulations of many different proteins in the form of spherical plaques or thin fibrils, depending on their origin. Diverse and varied medical conditions may show deposition of amyloid as part of disease changes. These include diverse, unrelated conditions which can strike at any age, such as tuberculosis, leprosy and some tumours.

Scientists and doctors do not understand exactly how and why proteins become locked up in these deposits, except that the normal mechanisms which keep vigorous, healthy cells free of this accumulated junk must become damaged or blocked. During various diseases, amyloid deposits can occur in many different tissues throughout the body. In some cases, sufferers recover when their underlying diseases improve or are cured. However, there is disease where there can be no recovery and where the formation of these protein deposits can only lead to a long and lingering decline which inevitably ends in death.

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This was a popular theory, but Reed and his colleagues were always sceptical about this.

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This was a possibility.

However, Reed and his colleagues noted that the disease tended to strike in houses which were close to water but not those on the tops of hills. It also affected soldiers who had been into the forest. Neither of these observations was consistent with the idea of rats spreading the disease.

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††ZITLE>The Panama Puzzle < Panama2.htmltafsumentslontmlsss TEXTR*ch P ¬Œ í àlay, a local physician in Havana, insists that mosquitos spread this disease. But how can we prove it? *(Remember, it's 1898):*

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The Panama Puzzle

Since the 1860s, Carlos J. Finlay, a local physician in Havana (that's him opposite), has insisted that mosquitos spread this disease. But how can we prove it?

- Try to show that the disease is **not** spread by personal possessions/clothing of victims
- Try to show that the disease is transmitted in the blood of victims ?
- Try to show that mosquitos could spread the disease from victims to human volunteers (!)

What do you think we should try first?

Click on one of the choices above.

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This didn't fit the facts.

Why should some people be affected and others spared when they had all used the same water supply?

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Reed and his colleagues initially tried to isolate bacteria from the corpses of those who had died. They were unsucessful in finding any unknown strains which could be causing this disease and soon abandoned this approach.

They eventually showed that the disease was caused by "a filterable agent" i.e. a virus.

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Wrong.

That's the answer you get from most textbooks.

But Jenner was not the first to try to prevent smallpox.

If you want to know who was, click here.

Click on the link above.

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False.

In spite of what you may read in textbooks, that answer is false.
Or at least, misleading.

Great guy, Eddie Jenner, (we'll get back to him in a while), but not the first to try to prevent smallpox.

If you want to know who was, click here.

Click on the link above.

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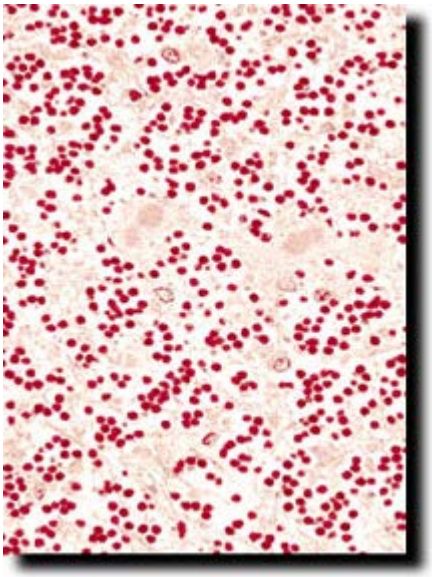
Across the world, there is approximately one death per million people from CJD annually:



This figure does not vary significantly from one country to another, although the number of cases reported by different countries are influenced by the stage of development of medical services in different countries and by varying medical priorities. The average age of death is 67 and death rates are highest for people in their 70s.
Equal numbers of men and women are affected and 98% of deaths occur in people over 45 years old. Similar to Alzheimer's disease, approximately 10% of CJD cases are familial, i.e. inherited. A number of mutations in the human **PrP gene** are known to give rise to inherited forms of CJD - two of these are known as familial fatal insomnia (FFI) and Gerstmann-Straussler-Scheinker disease (GSS). These diseases are relatively easy to recognise since many members of the same family are affected.

In the early stages, it is relatively easy to confuse CJD for a wide variety of other conditions which affect the brain. A number of medical tests are commonly used to try to confirm a diagnosis of CJD. A small amount of the fluid which surrounds the brain and spinal cord may be taken by lumbar puncture and tested to ensure there is no infection of the brain which may be causing the symptoms. CT or "CAT" scans are a specialised form of X-rays used to examine the shape of the brain, although they cannot see the "spongy" changes characteristic of CJD directly. MRI scans (magnetic resonance imaging) are an alternative way of looking at the brain without using X-rays. Electroencephalograms (EEG) record the electrical activity of the brain - in most cases of CJD there are characteristic disturbances of the brainwave pattern which can help to confirm the diagnosis. In spite of all of this, the only certain way in which a diagnosis of CJD can be confirmed is to examine the brain microscopically after death.

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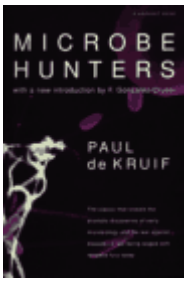
Reed and his colleagues conducted a gruesome series of experiments.

Some volunteers slept for weeks in the blood-soaked, vomit-stained faecal-contaminated nightgowns of recently deceased disease victims.
Others allowed themselves to be bitten mercilessly by (uninfected) mosquitoes.
I asked two volunteers, Private John R. Kissinger and clerk John J. Moran, why they were agreeing to such life-threatening experiments. They said "We volunteer solely for the cause of humanity and in the interest of science".
None of these subjects came down with yellow fever.



"Those three men opened the tightly-nailed, suspicious-looking boxes. They opened those boxes inside that house, in air already too sticky for proper breathing. Phew! There were cursings, there were holdings of noses. But they went on opening those boxes, and out of them Cooke and Folk and Jernegan took pillows, soiled with the black vomit of men dead of yellow fever; out of them they took sheets and blankets, dirty with the discharges of dying men past helping themselves. They beat those pillows and shook those sheets and blankets - "you must see the yellow fever poison is well spread around that room!" Walter Reed had told them. Then Cooke and Folk and Jernegan made up their little army cots with those pillows and blankets and sheets. They undressed. They lay down on those filthy beds. They tried to sleep - in that room fouler than the dankest of mediaeval dungeons! And Walter Reed and James Carroll guarded that little house, tenderly, to see no mosquito got into it."

FROM "Microbe Hunters" by Paul De Kruif

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Reed and his colleagues injected volunteers with with blood from yellow fever victims.

I asked two volunteers, Private John R. Kissinger and clerk John J. Moran, why they were agreeing to such life-threatening experiments. They said "We volunteer solely for the cause of humanity and in the interest of science".

None of these subjects came down with yellow fever.

To understand why this was, continue with the tutorial.

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The Panama Puzzle

In August, I was called back to Washington. In my absence, Carroll, Lazear, and Agaramonte pushed forward and bred mosquitoes from eggs given to them by Finlay. Without my knowledge, they allowed the mosquitoes to feed on patients with established cases of yellow fever, and then applied the insects to the skin of volunteers, including themselves.

The results they obtained were confusing: some volunteers got yellow fever, but others did not. Neither Carroll, Lazear nor Agaramonte became infected.

We had still not proved that mosquitos could spread the disease.

What should we do?

- Give up?
- Keep trying?
- Hope for a lucky break?

Click on one of the choices above.

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This was the first person to try to prevent smallpox.
His name was ... err, well, we don't know what his name was, but he lived in China ...

More than a thousand years ago, there are records of Chinese medicine men carrying out a practice known as **variolation** (named after smallpox, or variola, virus). This involved taking the dried scabs from smallpox victims, grinding them to a powder and blowing them up the victim's ... err, sorry ... patient's nose.

Did it work ?

Yes ... and no. Smallpox strains can be divided into **variola major** (bad news, 25-30% death rate) and **variola minor** (same symptoms but 1% or lower death rate). Talented medical men probably tried to select variola minor patients to prepare the material for inoculation. Cut price doctors, however ...

So how long did this carry on?

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There is one other human disease which closely resembles CJD:

Kuru was the first human spongiform encephalopathy to be investigated in detail and is one of the most fascinating and horrifying stories to have emerged from any epidemiological investigation. This disease occurred in 169 villages occupied by the Fore tribes in the highlands of New Guinea.

The first cases were recorded in the 1950s and involved symptoms very similar to those of CJD. The Fore people practised ritual cannibalism as a rite of mourning for their dead. Women and children participated in these ceremonies but adult men did not take part, explaining the age/sex distribution of kuru - it was never seen in very young children, rarely in adult men and was most common in adolescents and in adult women. This was the first clue that kuru was not caused by a conventional infectious agent, e.g. a virus. The incubation period for kuru can be longer than thirty years, but is usually shorter than this, e.g. fifteen years. Ritual cannibalism was stopped in the late 1950s and the occurrence of kuru has declined dramatically over the last few decades. Kuru is now very rare and only occurs in those who participated in cannibalistic feasts over thirty years ago.

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We were in the Army - we had our orders!

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The Panama Puzzle

Well, we got "lucky", if that's what you call it.

Click on me!

James Carroll decided feed an "old" mosquito some of his own blood. Three days later, Carroll was ill with the yellow fever. Jesse Lazear's notebook had noted that the insect had fed twelve days before on a yellow fever patient, who was then in his second day of disease.

Then Lazear was working at the bedside of a yellow fever patient when he was bitten by a stray mosquito. Five days later, he developed yellow fever; on the seventh day of his illness, Jesse died.

The reason we could not interpret our earlier experiments was that we had not allowed for the incubation period of the disease. If we followed this precise course of events, we could transmit yellow fever by mosquitos every time.

We had our proof that mosquitos spread the disease.

What happened next?

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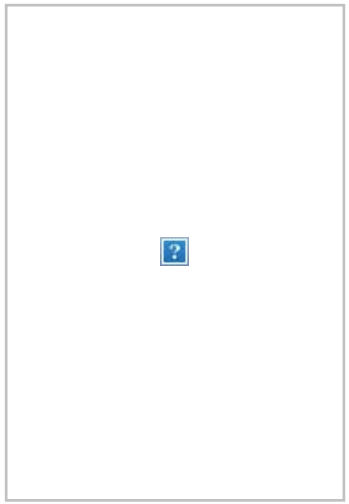
Variolation spread from China and was widely practised in the middle east for many centuries, often by inoculating the fluid from smallpox vesicles into the recipient's arm by making a small scratch with a needle.

In the 17th century, physicians in Europe became aware of this.

Lady Mary Wortley Montagu was a prominent member of society, noted beauty and the wife of the British Ambassador to the Ottoman Empire. In 1715, she contracted smallpox & suffered severe facial scarring and loss of her eyelashes (!)
In 1718, while living in Turkey, she had her 6 year old son variolated, despite the violent opposition of the British Embassy staff in Constantinople. This act did much to publicize the practice of variolation after her return to Britian.
In 1721, a smallpox epidemic broke out in Britian and Lady Mary was instrumental in getting several children of the aristocracy variolated, which came to the attention of the College of Physicians.

At the same time in colonial America, trials of variolation were being carried out in Boston by the Reverend Cotton Mather.

However, as in Britian, these experiments met with disapproval from the heirachy of the medical profession, but nevertheless continued.



So what has all this got to do with Jenner?

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There is one other human disease which closely resembles CJD:

In 1965, an American doctor called Carleton Gajdusek, who had been working on kuru in New Guinea for many years, injected some extract from the brain of a kuru victim into the brains of chimpanzees. One and a half years after the inoculation, the chimpanzees developed kuru. The discovery that the kuru could be transmitted from one species to another came as a huge surprise, but more surprises were to follow. In 1968 Gajdusek was also able to transmit Creutzfeldt-Jakob disease to chimpanzees and other monkeys by inoculation into the brain.

These experiments have now been repeated many times and the conclusion - *that under some conditions, CJD behaves as an infectious disease* - was inescapable. There are many reasons why chimpanzees are rarely used for such experiments today, the major one being that they are now an endangered species. However, kuru and CJD can also be transmitted to mice and other rodents. Distasteful as animal experiments are, there are few alternative ways of studying these diseases. Since the 1960's it has become apparent that not all cases of CJD behave as infectious agents - in fact only about 1% of sporadic CJD cases can be transmitted to mice.

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Oops. Due to an incident involving some old dilithium crystals and a dodgy antimatter containment field generator I got cheap, there appears to have been a small explosion. This has blown you through a wormhole in the space-time continum.

The year is now 1937 and you're in Max Theiler's lab in South Africa.

Max Theiler (for it is he):

"Since Reed's original work, we've found out a lot more about yellow fever. For one thing, we now know that the disease is caused by a virus, something which followed from Reed's eventual demonstration that this was a filterable agent.

Also, we discovered that we could transmit the virus to a variety of animals and animal cells in culture. We've been growing a strain of the virus we call **17D** in chick embryo cells for a number of years. We think that this could be the basis of a useful vaccine to prevent yellow fever.

Care to guess how we made the vaccine?

- By growing the virus in chick cells and inactivating it with formalin
- By attenuating the 17D strain after many sequential passages in chick cells
- By cloning the virus and expressing it in *E. coli*

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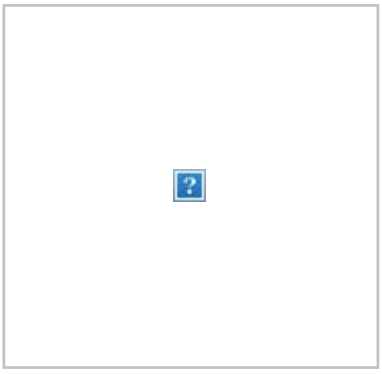
Edward Jenner was born on 17th May 1749, the youngest son of the vicar of Berkley, Gloucestershire:



Field Trip

Jenner's parent's died when he was five and he was educated in local schools.

Jenner himself underwent variolation in 1756 and nearly died, an experience he did not forget.

At the age of 13, he was apprenticed to a surgeon for 7 years, then worked for several more years in London.

So, Jenner was a famous London doctor and important member of society then?

True? or False?

Click on one of the choices above.

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Creutzfeldt-Jakob disease does not normally spread from person to person:

In the conventional sense, it is not an infectious disease like influenza, herpes or AIDS. In the 1970's, a few cases of CJD occurred in people who contracted the disease after receiving corneal or other transplants from a donor with the disease. Similarly, three cases occurred in people who had undergone brain surgery where someone with CJD had been operated on earlier in the same day with the same surgical instruments. It is now standard practice for all instruments used during an operation on patients with CJD to be destroyed, since it is impossible to be sure that any sterilization method has removed all traces of infection. (There is no evidence that blood transfusions present a risk of CJD transmission).

In 1985 the first case of CJD was reported in a recipient of contaminated human growth hormone (HGH). Since the 1960's, this hormone had been given to children who were unable to manufacture it themselves and who would have otherwise experienced dwarfism. The original source of the material was from the pituitary glands of human cadavers - corpses donated for medical research. Most of the donors were elderly and the fact that batches of the hormone were made from pooled tissue from many donors increased the chances of contamination. Tragically, over 60 HGH-related cases have been discovered world-wide in addition to 4 cases associated with another human-derived hormone, gonadotrophin. HGH manufactured from the cloned human gene became available in the late 1980's. This is entirely free from the risk of infection and the use of pituitary-derived hormone was stopped. However, the incubation period for the development of CJD from this source was 13 years and so the last cases have only recently been diagnosed.

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Possible... but wrong.

Try another choice.

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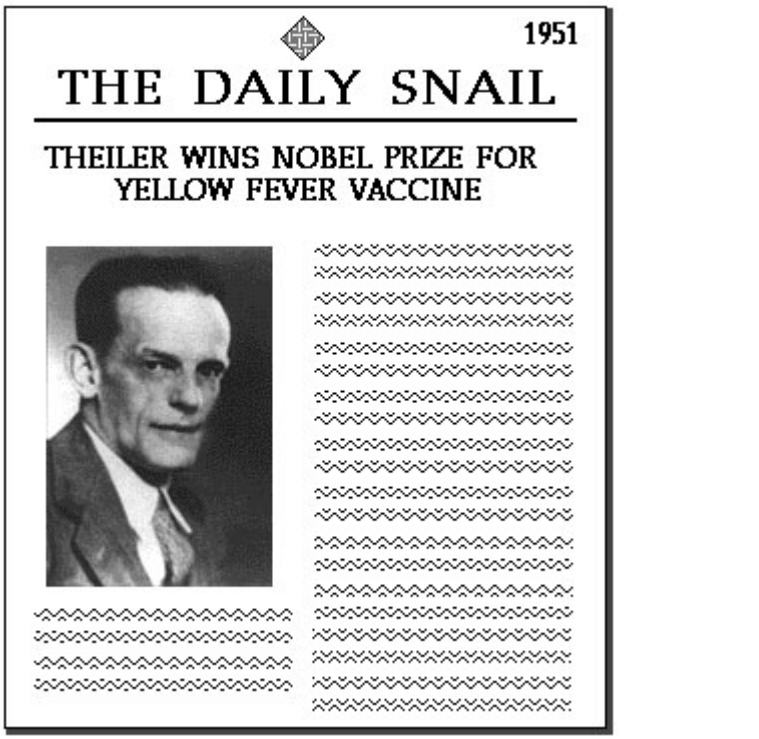
1951

THE DAILY SNAIL

THEILER WINS NOBEL PRIZE FOR YELLOW FEVER VACCINE

The 17D yellow fever vaccine is still in use today and millions of doses are administered every year.

What happened next ?

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You're clearly still dazed from the explosion.

Cloning in 1937?

Try another choice.

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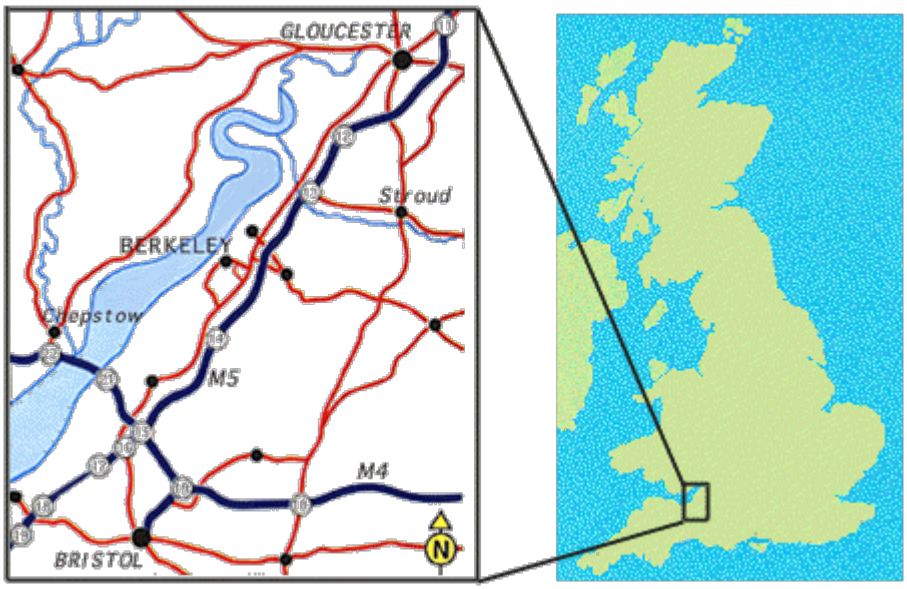
Field Trip:

Visit the Jenner Museum:



The Jenner Museum
High Street
Berkeley
Gloucestershire GL13 9BH
UK.
Tel. +44 (0)453 810631.

- Learn about the life and interests of Edward Jenner
- Discover how he developed the smallpox vaccine and how smallpox is the only disease to have been eradicated from the world
- Admire Jenner's fine Georgian house, its oldest part dating from the 14th century
- See his study as it was 200 years ago
- Visit his rustic hut with its ingenious fireplace and curious decoration and wander in the tranquil gardens
- Wonder at the thriving vines; two were from Hampton Court and probably tended by Jenner himself
- Visit the Museum shop for gifts, postcards, specialised and general books for adults and children and, in season, vine plants and grapes from the Jenner vine
- Use the fully-equipped Conference Centre in the grounds for meetings or social gatherings

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Wrong!

Try again.

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66Zotential for carriage of viruses Pox8.htmleDatafsumentslontmlsssTEXTR*ch*²<▯ • rd migration brought about by new man-made water impoundments (Murphy 1994).

To illustrate the effect ecological change can haÊ : €€€@ A €€€Y _ €€ €l , €€€“ H Monaco0ÿÈ- d š F,¸ Ú~$ F Fm` , * ,, * ,²‹* R*ch , HH Ø (ÿáÿâ ù F G (ü HH Ø (d ' h Timeso Helvetica Confidential €€€€ x 6 6Z Ú…ì $ Z styl MPSR&BBST2 èÿÿ íÿÿÎ €ÿÿ Ú~¨

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After 2 years (aged 23), Jenner resigned his post in London and moved back to Berkley.

There he was mostly interested in natural history and published several monographs about hedgehogs (like our friend here) and cuckoos: *"Observations on the Natural Histroy of the Cuckoo" Phil. Trans. Roy. Soc. 78: 218-237, 1788.*

So that's how he thought up the ideas of vaccination then...

- True?

  or

- False?

Click on one of the choices above.

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BSE was first recognised in dairy cattle the UK in 1986:

Affected cattle showed altered behaviour and a staggering gait, giving the disease its name in the press - **'mad cow disease'**. On microscopic examination, the brains of affected cattle showed extensive spongiform degeneration.

The initial explanation for the emergence of BSE in the UK was as follows:

Since **scrapie** (a spongiform encepalopathy of sheep) is endemic in Britain, it was assumed that this was the source of the infectious agent in the feed. It was concluded that BSE is due to the use of contaminated foodstuffs.

But cows don't eat sheep - or do they?

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We learned a lot more about yellow fever virus and other flaviviruses:

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ID   FLYF17DG   standard; RNA; VRL; 10862 BP.
AC   X03700; K02749;
DT   18-NOV-1986 (Rel. 10, Created)
DT   12-SEP-1993 (Rel. 36, Last updated, Version 3)
DE   Yellow fever virus genome (17D vaccine strain)
KW   capsid protein; envelope glycoprotein; genome; glycoprotein;
KW   polyprotein; replicase.
OS   Yellow fever virus
OC   Viridae; ss-RNA enveloped viruses; Positive strand RNA viruses;
OC   Flaviviridae; Flavivirus (arbovirus group B).
RN   [1]
RP   1-10862
RX   MEDLINE; 85272570.
RA   Rice C.M., Lenches E.M., Eddy S.R., Shin S.J., Sheets R.L.,
RA   Strauss J.H.;
RT   "Nucleotide sequence of yellow fever virus: implications for
RT   flavivirus gene expression and evolution";
RL   Science 229:726-733(1985).
DR   SWISS-PROT; P03314; POLG_YEFV1.
FH   Key             Location/Qualifiers
FT   source          1..10862
FT                   /organism="Yellow fever virus"
FT                   /strain="Flavivirus (mosquito-borne)"
FT   mRNA            1..10862
FT                   /note="PP mRNA"
FT   virion          1..10862
FT                   /note="virion RNA"
FT   CDS             119..10354
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FT                   /note="pid:g59339"
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FT                   /product="capsid protein"
FT   mat_peptide     482..973
FT                   /product="M protein precursor"
FT   mat_peptide     749..973
FT                   /product="M protein"
FT   mat_peptide     974..2452
FT                   /product="envelope protein"
FT   mat_peptide     2453..3679
FT                   /product="NS1 (soluble complement-fixing antigen)"
FT   mat_peptide     3680..4180
FT                   /product="non-structural protein ns2a (putative)"
FT   mat_peptide     4181..4569
FT                   /product="non-structural protein ns2b (putative)"
FT   mat_peptide     4570..6439
FT                   /product="NS3 (put. replicase component)"
FT   mat_peptide     6440..7300
FT                   /product="non-structural protein ns4a (putative)"
FT   mat_peptide     7301..7636
FT                   /product="non-structural protein ns4b (putative)"
FT   mat_peptide     7637..>7639
FT                   /partial
FT                   /product="NS5 (put. replicase component)"
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     acacatttgg attaatttta atcgttcgtt gagcgattag cagagaactg accagaacat       120
     gtctggtcgt aaagctcagg gaaaaaccct gggcgtcaat atggtacgac gaggagttcg       180
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To continue this tutorial, click here.

If you'd like to learn more about flaviviruses, click here.

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Jenner had been told about the idea that infection with cowpox protected against smallpox by a milkmaid who visited the surgeon he was apprenticed to many years before.

In addition, in 1774, a farmer named **Benjamin Jesty** had vaccinated his wife and two sons with cowpox taken from the udder of an infected cow and had written about his experience.
It is not clear if Jenner knew of this event...

So when did Jenner first try vaccination?

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To obtain higher milk yields and growth rates, the nutritional value of feed for farmed animals was routinely boosted by the addition of protein derived from waste meat products and bonemeal (MBM) prepared from animal carcasses, including sheep and cows.

This practice was not unique to the UK but was widely followed in most developed countries.
By March 1996, a total of 161,663 cases of BSE had been reported in the UK and 412 cases elsewhere (including Switzerland, Ireland, Portugal, France, Germany, Italy, Oman, Canada, Denmark and the Falklands).

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So that's it then? End of problem?

Nope.

End of tutorial, problem continues.

If you'd like to know more about :

- Books about the history of early studies into yellow fever: Click Here
- Emerging Infections Information Network: Thomas P.C. Monath, MD "The Resurgence of Yellow Fever"
- Yahoo:Health:Diseases and Conditions:Yellow Fever

Back to the Tutorials Page

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On **14th May 1796**, Jenner vaccinated an 8 year old boy, James Phipps, with material from a cowpox lesion on the hand of a milkmaid, Sarah Nelmes.
James, who had never had smallpox, developed a small lesion at the site of vaccination which healed in 2 weeks.

On 1st July 1796, Jenner challenged the boy by deliberately inoculating him with material from a real case of smallpox **!!!**

He did not become infected !!!

So that's it then, vaccination was accepted?

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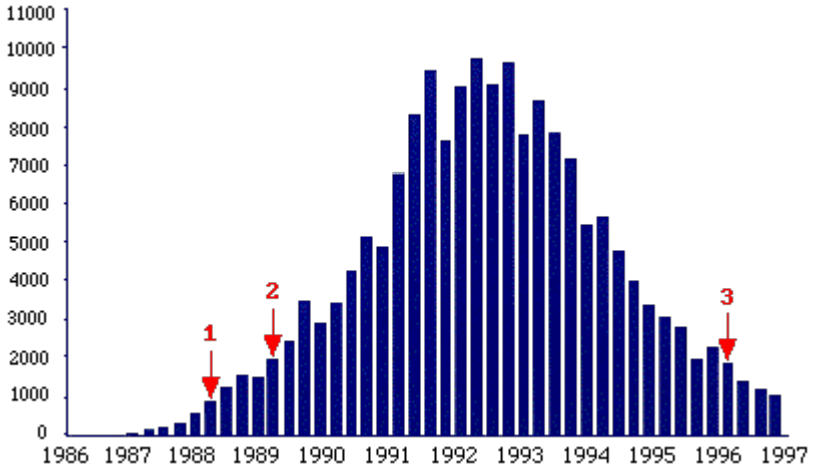
Traditionally, MBM was prepared by a rendering process involving steam treatment and hydrocarbon extraction.

- a protein-rich fraction called 'greaves' containing about 1% fat from which MBM was produced
- a fat-rich fraction called 'tallow' which was put to a variety of industrial uses.

In the late 1970s, the price of tallow fell and the use of expensive hydrocarbons in the rendering process was discontinued, producing an MBM product containing about 14% fat in which the infectious material may not have been inactivated. As a result, a ban on the use of ruminant protein in cattle feed was introduced in July 1988 (1):



In November 1989, human consumption of specified bovine offals (SBO) thought most likely to transmit the infection (brain, spleen, thymus, tonsil and gut) was prohibited (2). A similar ban on consumption of offals from sheep, goats and deer was finally announced in July 1996 to counter concerns about transmission of BSE to sheep (3). The available evidence suggests that milk and dairy products do not contain detectable amounts of the infectious agent.

The total number of BSE cases continued to rise, as would be expected from the long incubation period of the disease, and the peak incidence was reached in the last quarter of 1992, since when the number of new cases has started to fall.

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Nope. Lots of people were very upset.

- **James Gilray,** the famous cartoonist for one:
- **Sir Joseph Banks**, the president of the Royal Society for another. He refused to accept Jenner's manuscript for publication.
- And the group of doctors who brought **Benjamin Jesty** to London to claim that Jenner was a fraud.

Cartoon

So how did vaccination catch on?

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Unfortunately, there are a few problems with the accepted wisdom concerning BSE:

- It is now known that none of the rendering processes used, before or after the 1980s, completely inactivates the infectivity of prions. Therefore cattle would have been exposed to scrapie prions in all countries worldwide where scrapie was present and MBM was used, not just in the UK in the 1980s. For example, the incidence of scrapie in the USA is hard to determine but in the 8 years after the level of compensation for slaughter of infected sheep was raised to $300 in 1977, the reported number of cases went up 10 fold to a peak of about 50 affected flocks a year.

BSE is not scrapie:

- The biological properties of the scrapie and BSE agents are distinct, e.g. transmissibility to different animal species and pattern of lesions produced in infected animals (Bruce ME. et al. Transmissions to mice indicate the 'new variant' CJD is caused by the BSE agent. Nature 389: 498-501, 1997). There is no evidence to support the assumption that BSE is scrapie in cows. The only feasible interpretation based on present knowledge is that BSE originated as an endogenous bovine (cow) prion which was amplified by the feeding of cattle-derived protein in MBM back to cows.

  Thus the emergence of BSE in the UK appears to have been due to 'bad luck' compounded by poor husbandry practices, i.e. use of MBM in ruminant feed.

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Some people would say that left to Jenner, it would never have happened (e.g. see Nature 381: 18, 1996).

However, vaccination eventually became widely practiced for two reasons:

- Because the benefits were obvious.
- Because Jenner spent the rest of his life promoting the benefits of vaccination worldwide.

Jenners friends built him this rustic hut in the garden of his house called "The Temple of Vaccinia" where he vaccinated the local poor, free of charge.

By the time Jenner died on 25th January 1823, vaccination was accepted and widely practiced throughout much of the world, including the United States and the British Empire.

What happened next?

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The reported international distribution of BSE is at odds with established facts.

Approximately 40,000 tonnes of MBM were exported from the UK between 1985 and 1988; France alone imported at least 17,000 tonnes during this period and yet has only reported 20 cases of BSE compared with nearly 100,000 in the UK during the same period. During 1985-1990 the UK exported 57,900 cattle. These animals would have resulted in 1,668 cases of BSE had they remained in Great Britain, but only a small fraction of these cases have been reported by the recipient countries.
It has been suggested that only one in six probable cases of BSE within the EC has been reported.

It couldn't happen here...

The United States Department of Agriculture (USDA) maintains that no cases of BSE have been confirmed in the USA, but transmissible mink encephalopathy (TME) in the USA has been found in mink fed on 'downer cows' and never fed on sheep which therefore could not have been exposed to scrapie (McKenzie et al. Seminars in Virology, 7: 201-206, 1996). Recently (August 1997), 5 cases of CJD were reported in Kentucky in patients reported to have a history of eating squirrel brains (don't ask...)

Mad Cow U.S.A.: Could the Nightmare Happen Here?

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The number of cases of smallpox in countries practising vaccination fell rapidly.

However, smallpox was not eliminated from Europe and the United States until the late 1940s.
The last fatal case of smallpox in the USA occurred in **1949**.

So the problem was solved then?

Not entirely. Smallpox continued to be imported into these regions from other parts of the world:

In **1972**, an outbreak occurred in Yugoslavia, started by an immigrant from Iran, which resulted in 175 cases and 35 deaths.

So how was smallpox eradicated?

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Can BSE Infect Humans?

Dr S. Heaphy.

"The spontaneous occurrence of a novel prion strain that is the same in 14 individuals in the UK over the last two years seems extraordinarily unlikely as an explanation for NV-CJD. The alternative conclusion is that these cases have arisen from a common source of exposure to a new prion strain, the lack of any history of common iatrogenic exposure indicates that this is a new animal strain." Collinge et al, Nature 383: 685-690,1996.

In this paper they described the first direct evidence that BSE is the cause of NV-CJD. They found that PrPsc isolated from 10 cases of NV-CJD all had an identical 'fingerprint' on SDS polyacrylamide gels. This fingerprint was quite distinct from that seen in cases of familial, sporadic and iatrogenic CJD. However it was identical to the fingerprint found in BSE strains isolated from several animal species. Strain typing experiments currently in progress will be necessary to confirm this result but I think we can now answer the above question with a **Yes**.

Towards a Risk Assessment for the UK Population:

The figures used in this very simplistic assessment are taken from a variety of sources, some unpublished. Therefore there accuracy is more than unusually certain. If anyone wishes to comment on them please do so.

1. How much infected beef did we eat, 1983-1995 ?

Pre-1989 (before any offal controls), 446,000 BSE-infected animals were eaten
Between 1989 and 1995 when there was only a 50% compliance with the specified offals ban, a further 283,000 BSE-infected animals were eaten.
It has been estimated that this represents 3-5% of the animals entering the human food chain.

2. What meat was infected?

In the main: brain, spinal cord, eye and parts of the gut. Infectivity has not been detected in lymphoid tissues, other organs, or milk. However that does not mean that these tissues are safe. Brain and nervous tissue represents 0.1% by wt. of edible cow, 1% of offal.

Conservative(?) assumption 1 beef meal per week for 12 years with a 3% chance of the beef being infected = 500 x 0.03 = **18 independent exposures**.

Whatever the risk of infection, it is additive.

3. Infectious dose:

1 LD_{50} unit by definition is the amount of agent required to kill 50% of the population exposed to it. This dosage varies depending on the route of exposure e.g intracerebral inoculation (i.c.) or via the diet.

Some claim 10^{10} LD_{50}/g wet tissue by the intracerebral (i.c.) route. Most agree 10^7 by the i.c. route in scrapie. Unpublished reports suggest 10^7 in BSE. Most of the infected animals will have been incubating the disease and have had a titre lower than 10^7. But some will have had full blown BSE and titres approaching 10^7.

Assuming that of the 18 exposures 1 was major, (beefburger, pate, your favourite meat pie, pastrami, cows brain**!**) cooking will have done little to reduce the titre. 10g@ 10^7 = 10^8 ic LD_{50} eaten.

1 gastric LD_{50} = 100,000 i.c. LD_{50} (measured by comparing BSE titres in mice) i.e. 10^3 or 1,000 gastric LD_{50}.

Unless these assumptions are badly flawed, it seems reasonable to assume that most of us have been exposed to an enormous infectious BSE prion dose. In which case we have to thank the powers that be for the species barrier

4. The species barrier is impossible to predict a priori.

It reflects the fact that interspecies transmission is less efficient than within the species. BSE titres in cows of 10^7 are reported to be about 10^4 in mice, i.e. there is a species barrier of about 1,000. A similar value has been reported for sheep scrapie in mice. CJD has not been transmitted to mice, there is a very large species barrier. A species barrier of 10^7 is considered large. It seems reasonable but not necessarily correct to assume the species barrier between cow and human is greater than 1,000 (Collinge transgenic mouse experiments.)
The table below gives some idea of the fatalities that might be expected depending upon the size of the species barrier assuming an exposure of 1000 gastric LD_{50} to a population of 50 million:

Species Barrier:	Effective LD_{50}:	Deaths:
10^4	10^{-1}	2.5 million
10^5	10^{-2}	250,000
10^6	10^{-3}	25,000
10^7	10^{-4}	2,500
10^8	10^{-5}	250

Other risk factors include the PrP 129 polymorphism. For the Caucasian population:

- 12%V/V
- 37% M/M
- 51%M/V

The relative Sporadic CJD Risk for these genotypes is 11 : 4 : 1.
For growth hormone iatrogenic CJD it is 50% V/V, 31%M/M and 19% M/V.
So far 100% of NV-CJD patients have had a M/M genotype.

Final words:

- BSE is almost certainly the cause of 15 confirmed NV-CJD cases.
-
- Is beef safe ? As the chief medical officer states, it depends on what you mean by safe.
-
- The major risk of infection from beef was pre-1995 .
- The nature of the epidemic is not yet fully predictable but is now completely beyond our control.
-
- The size of any epidemic will depend on the magnitude of the species barrier. Experimentation may yet give us an idea of what this is.
-
- Kuru had a 5-30 years incubation period (10 years is a reasonable guess for the average incubation). Likely that BSE would take longer than 10 years to manifest in humans because of the species barrier.

Worst case scenario:

Current infections are fast responders to early 1980s infections when even retrospectively we can't see BSE in the national heard. By 1992, cattle infections were increased by more than 1000 fold. This would imply that we will see at least tens of thousands of human cases.

Best case scenario:

Current cases are the sensitive responders to the peak of the BSE epidemic in 1989-93. This would imply only a few hundred deaths.

For an alternative view, read: Cousens SN. et al.
Predicting the CJD epidemic in humans. Nature 385: 197-8 (1997).

© Dr Shaun Heaphy, 1996.

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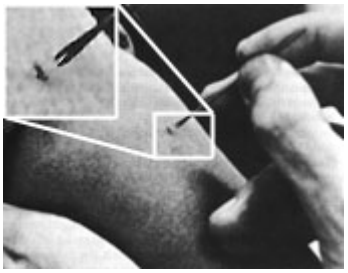
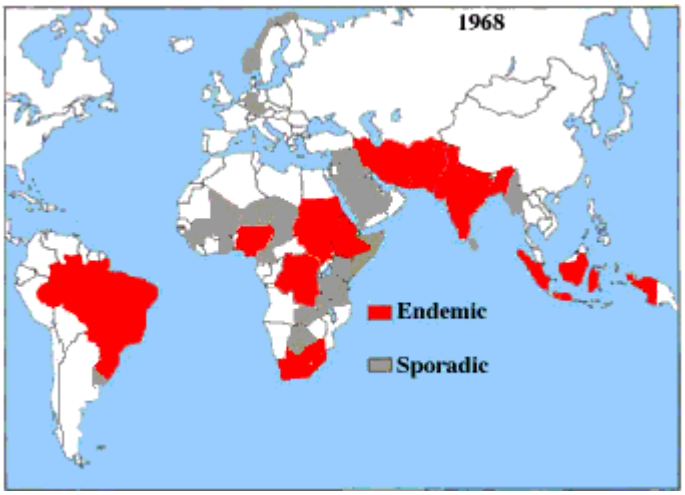
Eradication:

Jenner had first proposed the idea of worldwide eradication of smallpox by vaccination in 1801.
In 1950, the World Health Organization proposed a programme to eradicate smallpox from the Americas. This was acheived in 8 years.
In 1958, the W.H.O. proposed a worldwide eradication campaign, but this did not really get under way until 1965.
Between 200 and 300 million doses of a standardized vaccine were produced and administered annually.



Smallpox vaccination was greatly helped by the development of the "bifurcated needle" in 1968, making administration of the vaccine simpler and more effective.

Even in the best organized campaigns, it was not possible to vaccinate 100% of the population, so a new strategy was adopted in addition to blanket vaccination campaigns - early detection and vigorous containment of outbreaks. This proved to be highly effective.



The last naturally occurring case of smallpox occurred on 26th October, 1977.

On 9th December 1979, the W.H.O. officially declared smallpox to be completely eradicated

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So now I understand. Vaccinia virus is really cowpox virus, and this gives protection against infection with smallpox. Right?

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32 cases of nvCJD have now been confirmed.

(as of November 1998: most recent figures).

The official line is that BSE will eventually die out in the British beef herd, it's demise hastened by a massively expensive culling programme.

However, important unanswered questions remain concerning BSE. Many of these are raised by the large number of infected cattle (27,000) born after the 1988 feed ban. It is now generally acknowledged that the feed ban was initially improperly enforced and moreover, only applied to cattle feed. The same mills that were producing cattle feed were also producing sheep, pig and poultry food containing MBM, allowing many opportunities for contamination. As a result, in March 1996 the use of all mammalian MBM in animal feed was prohibited in the UK.
Up to 10% of calves born to cows which die of BSE will themselves die of BSE caught from their mothers. Similarly, there is a possibility of environmental transmission similar to that known to occur with scrapie. This could potentially sustain the epidemic and make it very difficult to eliminate completely.

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نحن أعضاء اللجنة العالمية للإشهاد الرسمى باستئصال الجدرى نشهد بأنه قد تم إستئصال الجدرى من العالم.

WE, THE MEMBERS OF THE GLOBAL COMMISSION FOR THE CERTIFICATION OF SMALLPOX ERADICATION, CERTIFY THAT SMALLPOX HAS BEEN ERADICATED FROM THE WORLD.

NOUS, MEMBRES DE LA COMMISSION MONDIALE POUR LA CERTIFICATION DE L'ÉRADICATION DE LA VARIOLE, CERTIFIONS QUE L'ÉRADICATION DE LA VARIOLE A ÉTÉ RÉALISÉE DANS LE MONDE ENTIER.

我们，全球扑灭天花证实委员会委员，证实扑灭天花已经在全世界实现。

МЫ, ЧЛЕНЫ ГЛОБАЛЬНОЙ КОМИССИИ ПО СЕРТИФИКАЦИИ ЛИКВИДАЦИИ ОСПЫ, НАСТОЯЩИМ ПОДТВЕРЖДАЕМ, ЧТО ОСПЫ В МИРЕ БОЛЬШЕ НЕТ.

NOSOTROS, MIEMBROS DE LA COMISION MUNDIAL PARA LA CERTIFICACION DE LA ERRADICACION DE LA VIRUELA, CERTIFICAMOS QUE LA VIRUELA HA SIDO ERRADICADA EN TODO EL MUNDO.

Frank Fenner
Keith Dumbell
Robert Kelle
Donald A. Henderson
Michal Rodzy
Minister
R. N. Baan
C. Маренникова

Genève, le 9 décembre 1979

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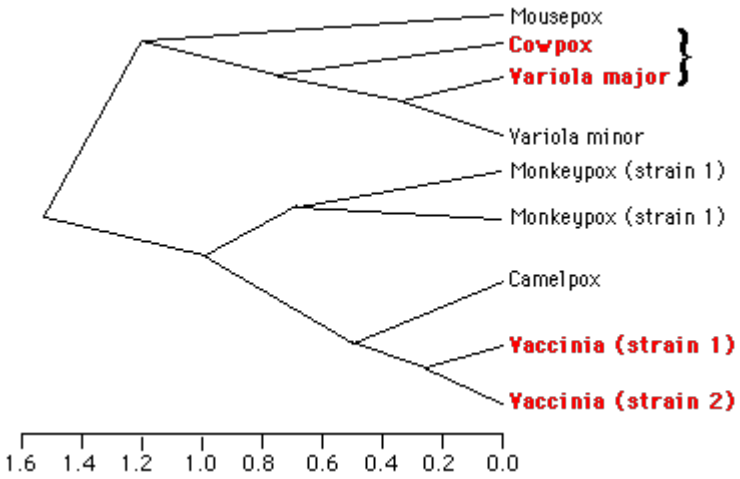
Aah, err, well... um,

No.

There's a slight problem. Although we administered more than a **billion** doses, and although it works (it eradicated smallpox, didn't it?) ... it's a bit embarrassing, this ... we don't actually know what vaccinia virus is, or where it came from.

What are you talking about?

It was always assumed that vaccinia was a variant of cowpox, but genome sequencing studies of poxviruses during the last few years have shown that it's not:



Originally, "vaccinia" was propagated from one human arm to the next, keeping the strain going.

In 1845, it was shown that the virus could be propagated in cows and the vaccine was then made by scarifying the entire flank of the cow which allowed the production of a large quantity of vaccine. (Remember, this was during the 19th Century - more recently, vaccina was produced in cell culture). Even this century in India, cows and oxen treated in this way were led from door to door in order to deliver the vaccine 'fresh' to the inhabitants.

Jenner's original vaccine was certainly cowpox. It is not clear what happened between 1796 and the present day, but vaccinia's closest relative appears to be camelpox!

So what does the future hold?

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Are we all going to die ?

Books about:

Mad Cow Disease | BSE (technical)

The Trembling Mountain: A Personal Account of Kuru, Cannibals, and Mad Cow Disease
by Robert Klitzman.

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Smallpox as a disease has been eradicated.

The entire genomes of variola (smallpox) and vaccinia viruses have now been sequenced.

It has been proposed that the last remaining stocks of smallpox virus should be destroyed, to prevent any accidential escapes. However, this suggestion has been intensely debated, since some people feel:

- the virus should be kept in secure facilities for future study should the need arise - there is a limit to the amount of information which can be derived from a nucleotide sequence.
- we have no right to destroy any form of "life" (the dodo, whales, viruses ???)

Currently, it is intended that the last remaining laboratory stocks of variola virus will be destroyed on 30th June 1999:

Continue

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