Viruses

1. Virus classification

There is no definition of viruses, but viruses **are not** bacteria and it is important to considering their biological properties in evolutionary studies.

The Baltimore system for virus classification complements the ICTV classification system. It is especially useful for understanding viral replication strategies and will be discussed later. Classical virus classification schemes have been based on the consideration of four major properties of viruses:

1. The type of nucleic acid in the virion (RNA or DNA)
2. The symmetry and shape of the capsid
3. The presence or absence of an envelope
4. The size of the virus particle

More recent classification systems adopted by the International Committee on Viral Taxonomy (ICTV) have emphasized the viral genome as the primary determinant for viral taxonomy. Furthermore, there is a drift towards the use of genomics for virus classification – that is, sequence analysis of the viral genome, and comparison to other known viral sequences.

Latinized virus family names start with capital letters and end with the suffix –viridae (e.g., Herpesviridae). These formal names are often used interchangeably with the common names for viruses (e.g., herpesviruses).

i. Genetic Content of Viruses

DNA viruses: Almost all DNA viruses that infect animals contain double-stranded DNA. Exceptions include the Parvoviridae (e.g., parvovirus B19, adeno-associated virus) and the Circoviridae (these include the recently discovered TT virus, which may be related to the development of some cases of hepatitis). Here we also have some of the most commonly used phages, such as T7 and ϕX174.

RNA viruses: Almost all RNA viruses contain single-stranded RNA. Exceptions include the Reoviridae (e.g., rotaviruses) which contain double-stranded RNA. Other RNA viruses can be broadly subdivided as follows:

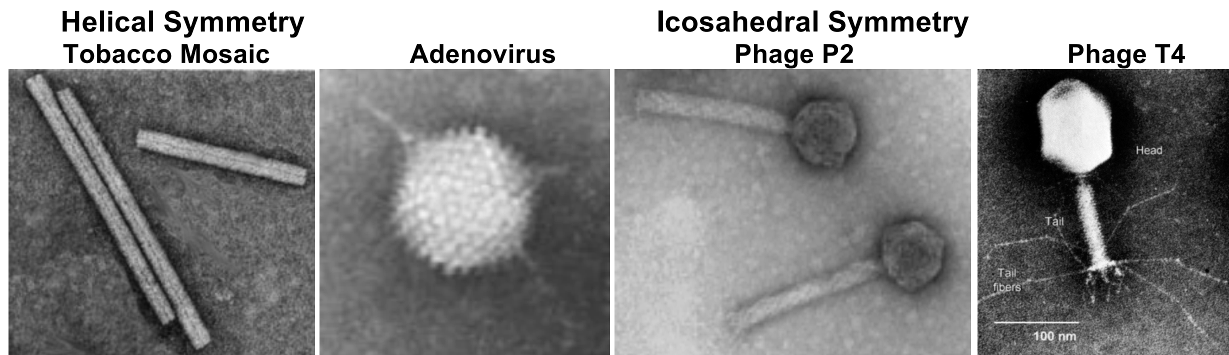
Viruses with positive strand (+) RNA genomes – i.e., genomes of the same polarity as mRNA. Viruses in this category include picornaviruses, astroviruses, togaviruses, nidoviruses, flaviviruses and caliciviruses. In addition, retroviruses contain two copies of (+) RNA, although they replicate by a unique mechanism.

Viruses with negative strand (-) RNA genomes – i.e., genomes with a polarity complementary to mRNA. Viruses in this category all have helical capsids. Three members of the class are sufficiently closely related to comprise a distinct taxonomic order – the Mononegavirales (rhadboviruses, paramyxoviruses and filoviruses). The other (-) strand DNA viruses have segmented genomes (orthomyxoviruses).

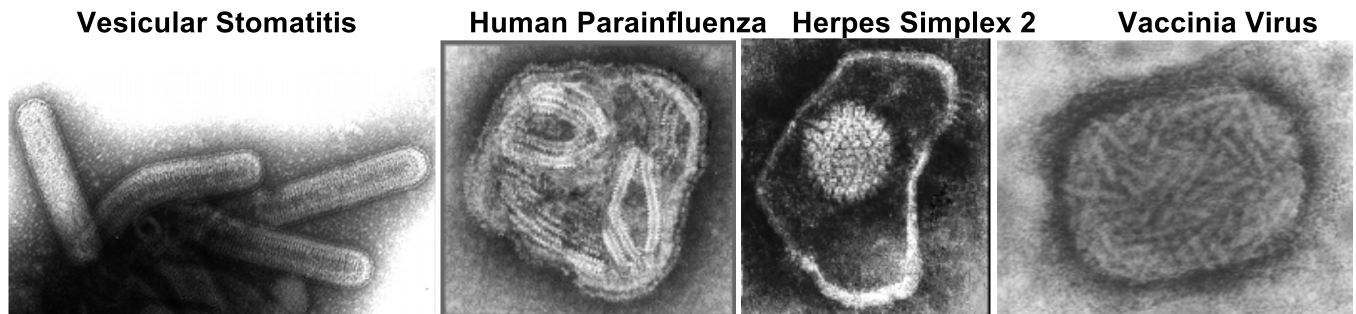
Ambisense viruses – i.e., genomes that contain positive sense genes and negative sense genes. This group includes arenaviruses and bunyaviruses, all with segmented genomes, and they generally follow the same replication strategy as negative strand viruses. Here we have phage ϕ6.

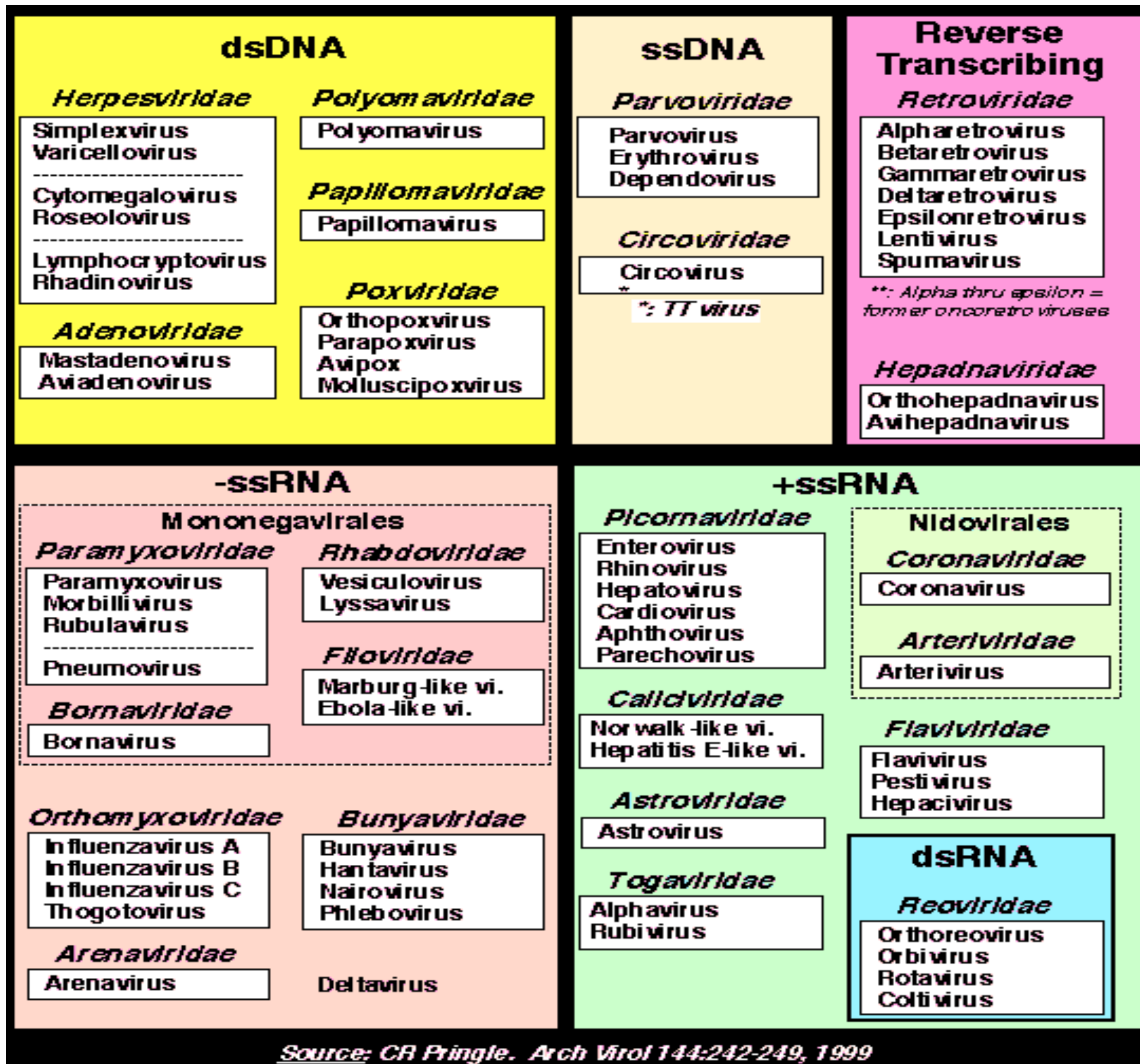
ii. Physico-chemical Properties

Capsid (sometimes referred to as nucleocapsid): This is the protective protein shell surrounding the viral genome. Capsids are typically formed from a small number of protein subunits, which are assembled into repeating, symmetrical structures. The major classes of capsid symmetry are (1) helical (rod-like) and (2) icosahedral (sphere-like). The size of the capsid will, in large measure, dictate the amount of genetic material that can be packaged into the virus particle.



Envelope: Many animal viruses are surrounded by a lipid bilayer that is derived from the host cell membrane during the process of virus budding. These viral envelopes also contain virally-encoded proteins, which are often glycoproteins. These envelope proteins and glycoproteins often play a role in the processes of virus attachment and entry/uptake. Note that envelopes are not present on all viruses, and that viruses which contain envelopes are usually less stable those that do not, e.g. herpes-viruses, which do and polio and human papillomaviruses (wart viruses), which do not.





2. Viral replication

2.1. One-step growth curves

Our understanding of the life cycle of viruses has been developed in large part by studying virus infections under conditions where cells become infected synchronously, in such a way that only a single cycle of infection can occur. Synchronous infection can be achieved by infecting cultures with a high amount of virus, such that all cells within the culture become infected rapidly. To do this, one typically infects cells at a multiplicity of infection (m.o.i.) of 5 to 10.

One-step growth analysis begins with the addition of virus to cells. After a period of adsorption, the virus inoculum is washed away, and cells are cultured. Aliquots of the cells and the cell-culture fluid are then collected at various time points and analyzed for the presence of virus. A typical one-step growth analysis can be divided into several phases:

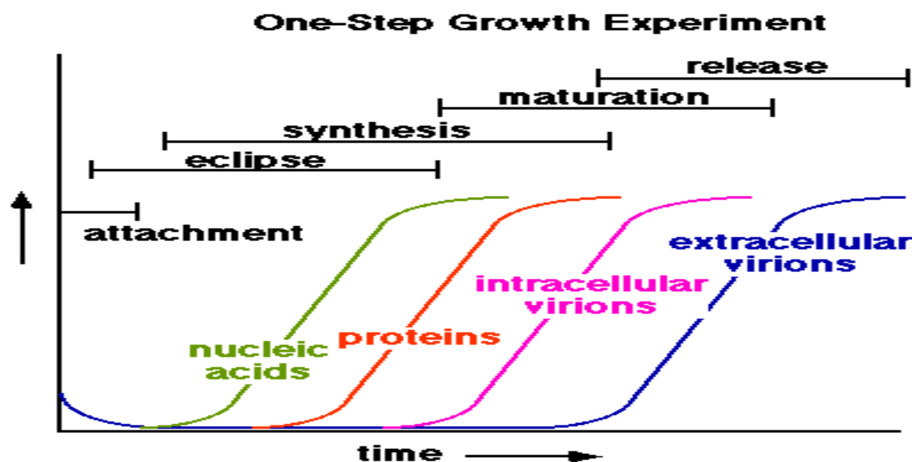
1) Attachment of virus. Viral proteins interact specifically with the receptor.

2) Eclipse phase. It corresponds to the period between the entry of incoming virus and the release of viral progeny. As a result, no infectious virus can detected during this time (any infectious virus detected is simply virus that is still stuck on the cell membrane). The eclipse phase is not necessarily the same as the latent phase, which ends when the first viral particles are completed.

3) Synthetic phase. This corresponds to the time during which new virus components are synthesized. Viruses need to make new nucleic genomes of the correct polarity and new proteins.

4) Maturation period. Viral particles are assembled

5) Release (productive) period. Virus is detected in the extracellular medium. In enveloped viruses steps 4 and 5 are combined.



The virus burst size (amount of infectious virus produced, per infected cell) can be calculated on the basis of the results from a one-step growth experiment. Burst sizes for viruses typically vary between 10 and 10,000. Viruses vary considerably in terms of the kinetics of their replication, and the specifics of their one-step growth curves. The viral titer is defined by quantity of virus per ml of medium.

2.2. Steps in the replication cycle

2.2.a. Attachment

Viruses typically attach to cells via specific cell surface receptors. Very often, virus receptors are molecules projecting some distance away from the cell surface, allowing them to be contacted more easily by viruses. Receptors are usually (but not always) proteins. Often one virus can recognize more than one receptor, or one virus may require a correceptor (an additional molecule).

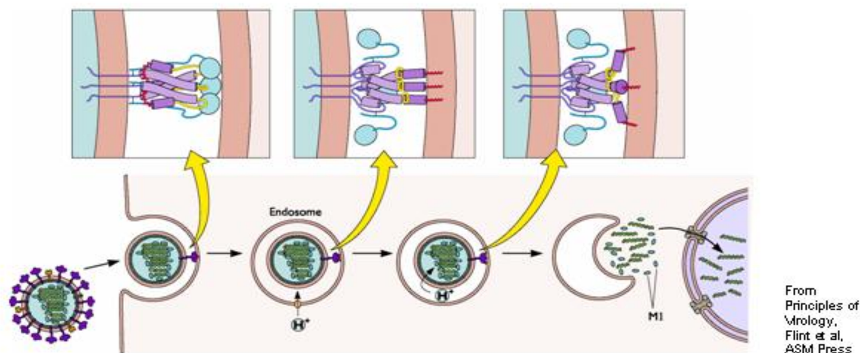
2.2.b. Entry and uncoating

Following attachment, viruses must enter cells without killing them. Once inside, it must disassemble in such a way that its genome and any associated enzymes remain intact and are directed to the appropriate cellular compartment. The process of entry in animal viruses can proceed via several pathways:

Direct fusion. In the case of the Paramyxoviridae, the virus is able to fuse directly with the host cell membrane at neutral pH, via the action of the viral fusion protein (F). This results is uncoating of the viral genome at the cell membrane.

Receptor-mediated endocytosis with endosome fusion. Other viruses do not fuse directly with the host cell membrane, but instead undergo uptake into endocytic vesicles. One example of this is the HA (hemagglutinin) protein of influenza virus.

Influenza entry and uncoating



- The low pH has a second very important role for influenza entry - the virus contains an ***ion channel*** in its envelope (M2).

- The presence of M2 allows acidification of the virus interior, and promotes uncoating of the M1/vRNPs

- Drugs that block M2 block infection - ***amantadine***. This is highly specific for the viral M2 ion channel, with no effect on the cellular H+/vATPase

HA undergoes a low-pH induced shape change, such that the protein enters a fusion-competent state. This allows the virus to fuse with the host cell membrane, thereby bringing the virus particle into the cell. Influenza also contains a second protein that is activated under conditions of low pH. This is the M2 protein, which has ion channel activity at low pH, thereby allowing protons to enter the virus. The entry of protons in turn disrupts electrostatic interactions between the viral ribonucleoprotein (RNP) and the major virion envelope protein, M1. This leads to virion disassembly while the virus is still in the endosome, and it is an essential part of the viral life cycle (so much so, that antiviral drugs such as amantadine target this step). A similar pathway is followed by enveloped viruses like rabies. In this case acidification triggers conformatinal changes in the virus G protein that becomes fusogenic. Upon fusion of the viral and endosomal membranes the nucleocapsid is released into the cytoplasm.

Endosome lysis. Non-enveloped viruses cannot enter cells via a simple process of membrane fusion between the virus envelope and the host cell membrane. One strategy employed by non-enveloped viruses is exemplified by adenovirus. This virus attaches initially to CAR, an immunoglobulin-like molecule, and it then binds to cellular integrins. Following binding, the integrin and virus are internalized, and the virus then begins to disassemble in the mildly acidic environment of the early endosome (~pH 6). As the endosome acidifies further, the penton base protein is

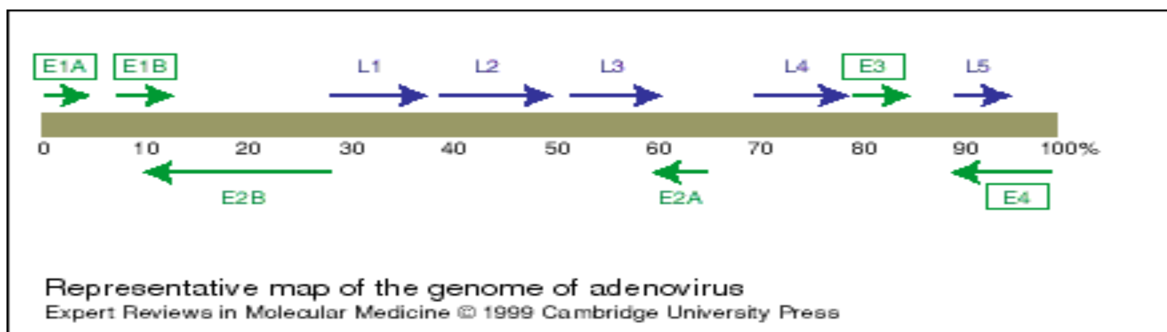
released and this is thought to trigger endosome lysis, thereby mediating the escape of the partially disassembled core particle into the cytosol.

Pore formation. Some non-enveloped viruses, such as picornaviruses, form pores in the cell membrane. One example is poliovirus, which after binding to its receptor (PVr) undergoes a conformational change that exposes a hydrophobic domain on the VP1 virion protein. This forms a pore in the cell membrane, through which the viral RNA is then released into the cytosol.

2.2.c. Biosynthesis (transcription, translation, replication)

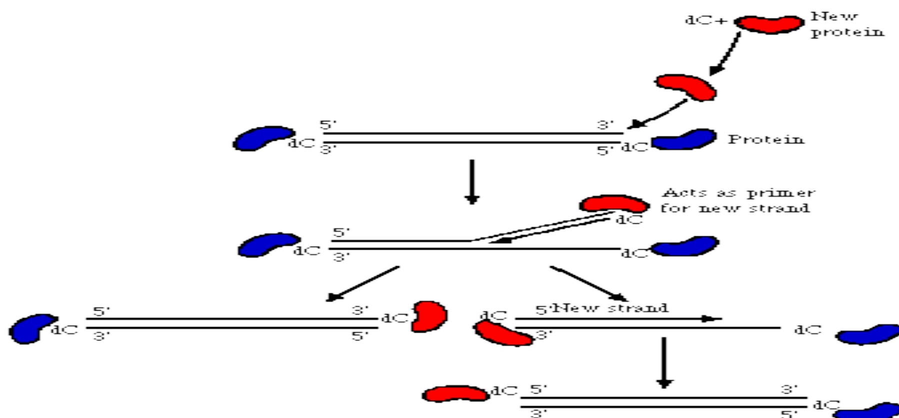
A DNA virus: Adenovirus

Adenoviruses are an important cause of colds, but they also cause a wide range of other illnesses, often with involvement of the eye. Virions are non-enveloped, icosahedral particles. The shell is made of viral protein, hexons and pentons, and there are projections at the vertices called fibers. Inside the particle there is 36-38 Kb. of linear, double-stranded DNA associated with other viral proteins forming the core. After entry, the core is transported to the nucleus, where replication occurs. There is transcription of both strands and mRNA splicing to yield early (E) and late (L) proteins. The early proteins are involved in replication, the late proteins have structural functions.



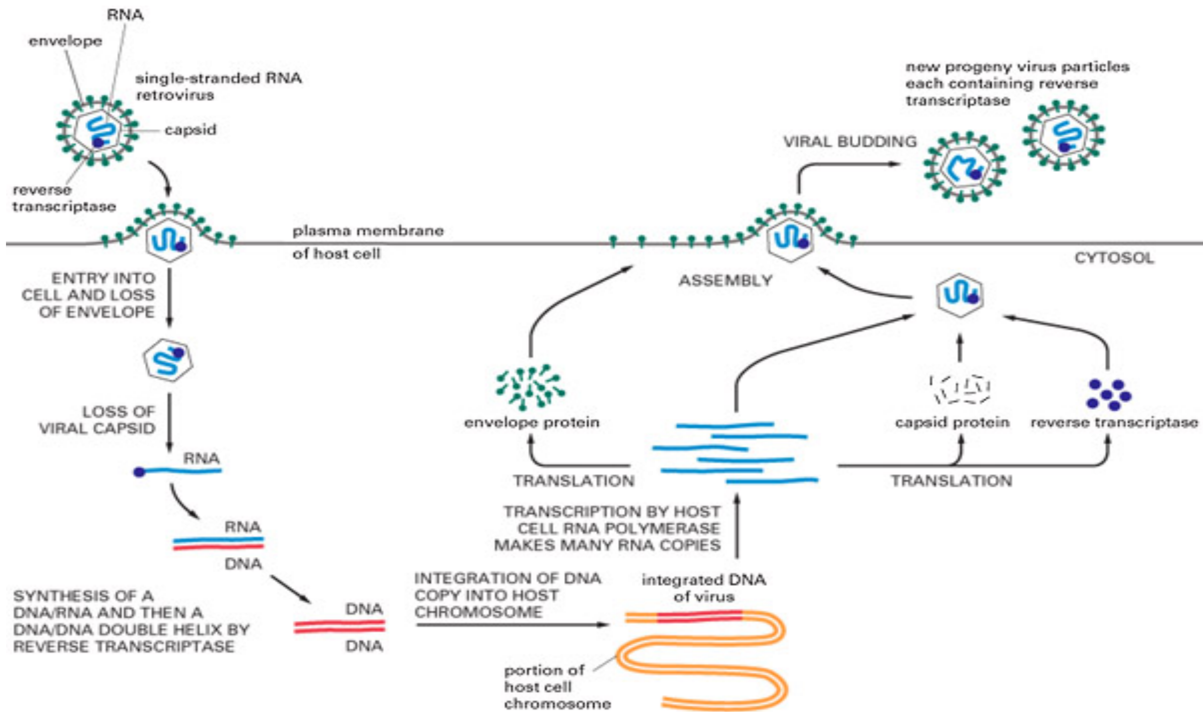
Representative map of the genome of adenovirus
Expert Reviews in Molecular Medicine © 1999 Cambridge University Press

DNA is copied starting at both ends by strand displacement. The polymerase, together with the Terminal Protein (Tp) bind the termini and serve as a primer to initiate the incorporation of nucleotides to form the progeny genome.



An RNA virus with DNA replication: HIV-1

Many of the viruses belonging to this group target cells of the immune system and are usually involved in different types of immunodeficiencies. Retroviruses' virions carry two copies of RNA genomes of positive polarity. Replication includes copying RNA into DNA, which is incorporated into the cellular genome. DNA synthesis is mediated by virally-encoded RNA-dependent-DNA polymerases (reverse transcriptases.)



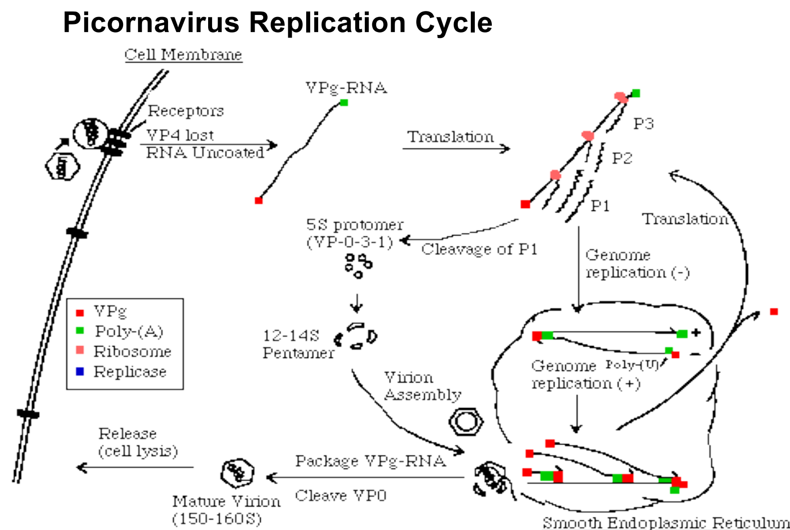
An RNA (+) stranded virus: poliovirus.

The Picornaviridae is a large family of icosahedral viruses that includes many important human pathogens. More than 230 picornaviruses have been described, and these are divided into five genera, Enterovirus, Rhinovirus, Heparnavirus, Aphthovirus and Cardiovirus. The enteroviruses include poliovirus. They replicate in the gastrointestinal tract and are transmitted by the fecal-oral route. Although enteroviruses replicate in the gastrointestinal tract, they do not cause severe enteric disease. More serious disease results when the virus spreads to the central nervous system or other sites. As a result of such spread, enteroviruses are able to cause a wide variety of human illnesses including paralytic disease and encephalitis. Rhinoviruses replicate in the nose and upper respiratory tract; they are the most important cause of the common cold. Hepatitis A virus is the only Heparnavirus. Hepatitis A virus replicates in the gastrointestinal tract, is transmitted by the fecal oral route, and causes infectious hepatitis in humans. The Aphthoviruses and Cardioviruses cause disease in animals, but not in humans. Foot-and-mouth disease virus of cattle, for example, is an Aphthovirus.

Picornavirus genomes are single stranded RNA of positive polarity and 8 Kbs in length. All picornavirus genomes are organized in the same way with capsid proteins encoded near the 5' end of the RNA and RNA polymerase plus proteases near the 3' end. The virus RNA serves as an mRNA immediately after it enters a host cell, so it has some properties of an mRNA including a poly-A tract at the 3' end (but no cap!). A small protein (VPg) involved as a primer in RNA replication is bound

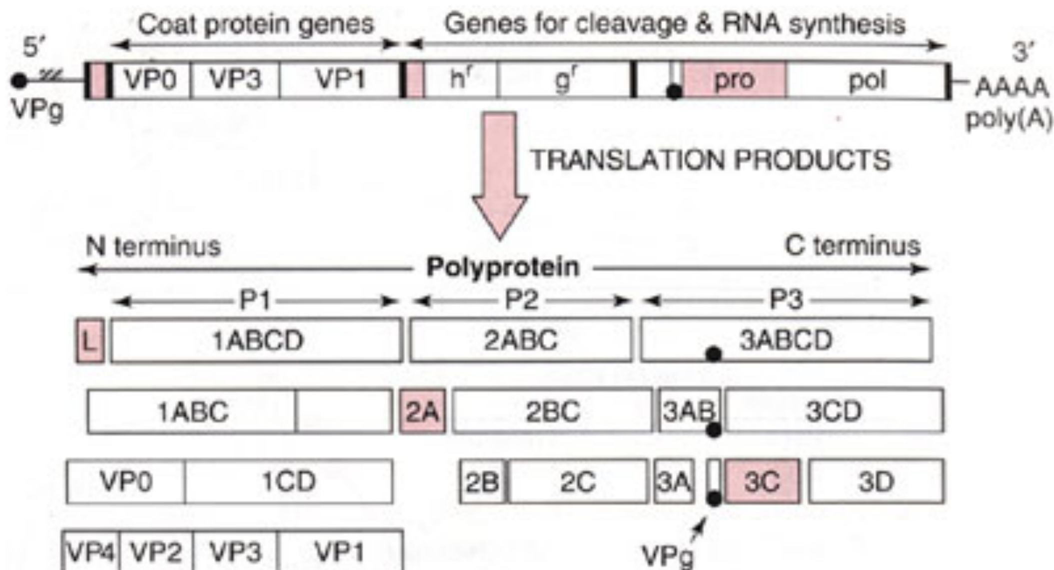
covalently at the 5' end of picornavirus RNAs. Since picornavirus RNAs can serve directly as mRNA, the virion RNA is infectious if it is introduced into an appropriate host cell. Poliovirus replicates entirely in the cytoplasm. Once it enters the cells the RNA is translated to produce a single polypeptide chain, the polyprotein. The polyprotein is greater than 2300 amino acids in length and it corresponds to all the virus genes. Once it is synthesized, the polyprotein undergoes self-cleavage to create individual virus proteins including the RNA dependent RNA polymerase (RdRP). Using the parental virus RNA as a template, the RdRP then makes a minus strand copy. Using the minus strand as a template, it then makes progeny plus strands. Progeny plus strands are used for three purposes: (1) as templates for further minus strand synthesis that can lead to more plus strands; (2) translation to produce additional polyprotein molecules which are cleaved to produce virus proteins; and (3) encapsidation by capsid proteins to form progeny virus.

Picornavirus Replication Cycle



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.

Polyprotein Processing



An RNA (-) stranded virus: Vesicular stomatitis virus

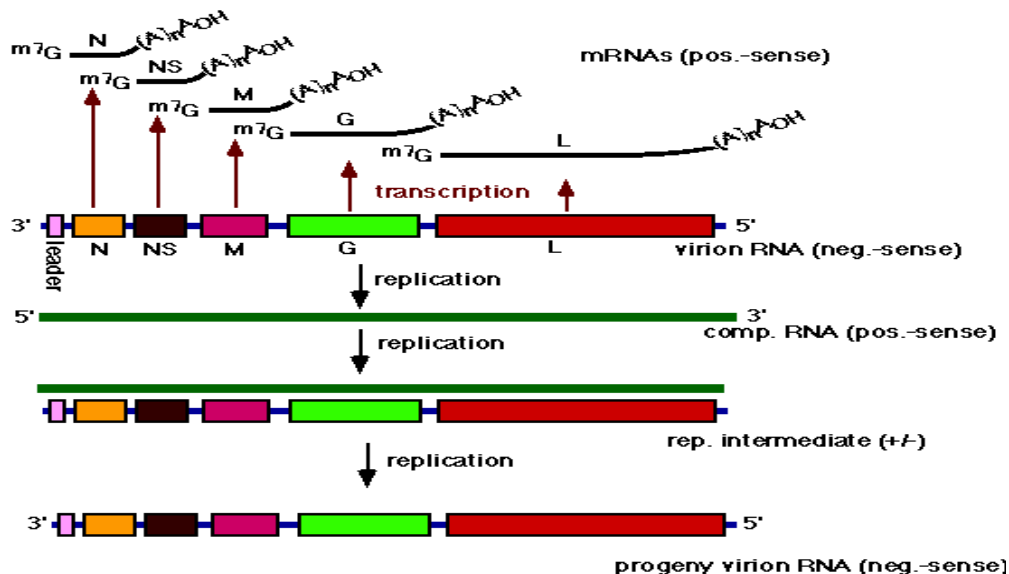
Vesicular stomatitis virus (VSV) belongs to the Rhabdoviridae family. The use of negative sense (-) RNA genomes means, by definition, that the viral genome is complementary to mRNA. Thus, the viral genome cannot be used to make proteins until it has first been transcribed to produce mRNAs. This has the following implications: (i) purified virion RNA is not infectious (as noted above, it cannot be translated into proteins); and (ii) the viruses must bring their own RNA polymerase into the cell in order to make mRNA (i.e., the viral polymerase must be incorporated into the viral particle, or virion)

The other key feature of these viruses is that they make monocistronic mRNAs (i.e., each mRNA corresponds to a single ORF). This is achieved by the use of transcriptional stop and start signals, which are located at the boundaries of all of the viral genes (intergenic regions). Stop/start transcription has two major results:

1. Since there is only a single promoter, located at the 3' end of the viral genome, the polymerase can only load onto its RNA template at one site. As it moves along the viral RNA, the polymerase encounters stop/start signals at intergenic regions. This results in pausing of the enzyme, which often falls off the template. The result is that more mRNA is made from genes that are located close to the promoter, and less mRNA is made from genes located far from the promoter. This means that there is a polarity of transcription (see Figure below). The viruses use this to regulate the expression of their genes, since highly expressed proteins are encoded close to the promoter (e.g., structural proteins such as the nucleocapsid protein, N), while proteins that are needed in only small amounts (e.g., enzymes such as the RNA polymerase, L) are encoded far away from the promoter.

2. The other major consequence of stop/start transcription is that the complete viral RNA genome can be copied is if the transcriptional stop/start signals can be ignored. If the stop/start signals here are always obeyed, then only subgenomic mRNAs will be produced. However, if the stop/start signal here is ignored a complete copy of the viral genome can be made. The switch from transcription to replication is triggered by binding of the N protein to the nascent RNA and resulting changes in the host-cell proteins associated to the polymerase.

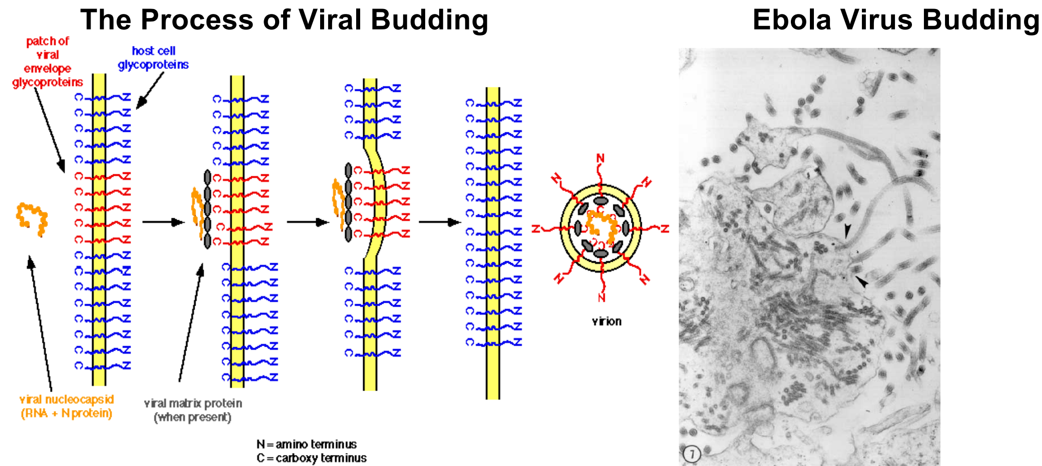
Genomic Organization of Vesicular Stomatitis Virus



2.2.d. Assembly and Release

Virus particles are released from cells either by lysis of cells, or budding from cell membranes, usually the cytoplasmic membrane. Both processes lead to changes in cell membranes, which can often be observed in vitro, as part of the virally-induced cytopathic effect. Non-enveloped viruses assemble spontaneously inside the cells, which burst to release the progeny. Enveloped viruses need the intact cell to acquire their membranes.

The Process of Viral Budding **Ebola Virus Budding**



Budding of Eggplant Mottled Dwarf Virus

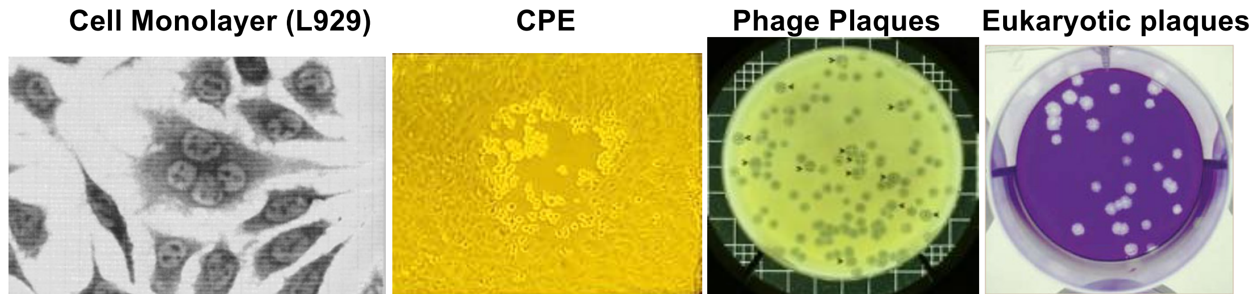
3. Methods in virology

3.1. Plaque assays.

To study and quantitate virus infectivity in the laboratory one very widely used technique is the plaque assay. This method relies upon (1) the use of confluent monolayers of eukaryotic cells or lawns of bacteria which are susceptible to the virus, (2) the induction of a visible cytopathic effect by the virus (i.e., cell killing or cell damage) and (3) the use of a semisolid overlay (usually agar) which prevents virus diffusion from one infected cell to other nearby cells. As a result, small round plaques (clear areas) form in the cell monolayer as the virus replicates. These plaques can be visualized by staining the cells with a vital dye like crystal violet or neutral red. Currently there are no plaque assay methods for plant viruses.

Growth is inhibited when a cell contacts other cells and thus the cultured hosts form a true **mono**layer. The number of susceptible cells determines the number of generations that occur during

replication which, given the constancy of cell size then is a function of flask size and, of course, virus inoculum.



3.2. Virus-inducible reporter genes

A related method is the use of indicator cells with a virus-inducible reporter gene. Examples of such systems include the Multinuclear Activation of Galactosidase Indicator (MAGI) cell system for detection of HIV infection. In this case, HIV-susceptible monolayer cells were genetically engineered to contain a reporter gene cassette comprising the viral long terminal repeat (LTR), placed upstream of the gene encoding *E. coli* b-galactosidase. Expression of the lacZ gene is therefore dependent upon the transcriptional activity of the HIV LTR. Under normal circumstances this activity is weak and little b-galactosidase is produced. However, if the cell becomes infected by HIV-1, the virus will produce its transcriptional activator protein, Tat, and this will upregulate the activity of the viral LTR. As a result, expression of lacZ is greatly enhanced and one can now detect b-galactosidase using a chromogenic substrate such as X-gal.

Sources of genetic variation

1.1. Mutation

Different organisms have different mutation rates. Eukaryotes replicate their genomes with high fidelity (10^{-9} substitutions/nt/round of replication), thanks to the presence of a variety of DNA repair mechanisms that correct most nucleotides misincorporated during replication or modified by damage. However, because their large genomic sizes (many megabase pairs) they average of 1 mutation/genome, which may be hidden from selection due to diploidy and redundancy.

Bacteria also replicate through DNA and follow the rules of DNA replication. In contrast to eukaryotes, bacterial genomes are relatively small, and that results in an average of 0.003 mutation/genome. DNA viruses also behave similarly, but there are not very many studies.

Unlike in the DNA world, the RNA world is subjected to high error rates, in the order of 10^{-4} substitutions/nt/round of replication. This very high mutation rate is largely the result of lack of proofreading and repair mechanisms. Mutations can be the result of difference processes:

Polymerase error.

Generally there is a good correlation between genomic size and error rate. RNA viruses, and viruses that undergo a step of RNA replication (hepadnaviruses, retroviruses,) are copied by RNA-dependent polymerases. These enzymes lack correcting mechanisms, so all misincorporations produced during replication remain and lead to very high mutations rates, in the order of 10^{-4} substitutions/nt/round of replication. Because of their very small genomic size, the genomic mutation

rate is also 1 mutation/genome. Bacterial replication is much more accurate, resulting in ~0.03 mutations/genome.

Even in the absence of correcting mechanisms RNA polymerases can differ in fidelity. Typically, retroviral polymerases are more accurate than polymerases from riboviruses. Within the riboviruses, it is possible to isolate mutants whose polymerases differ in their fidelity several-fold. In poliovirus, increased fidelity correlates with lower pathogenicity and is due to a slight pause at the time when the incoming nt pairs with its template. Influenza fidelity mutants have also been isolated, but the bases for changes in fidelity are unknown.

Editing.

Cellular enzymes and some environmental determinants (Mn^{2+}, unbalanced dNTP pools) can lead to modification of properly incorporated nucleotides with high frequency, a process known as hypermutagenesis, which results in extensively mutated viral genomes.

Measles virus infection of the CNS can lead to a deadly subacute sclerosing panencephalitis (SSPE). Virus isolated from infected patients is characterized by extensive deletions, particularly in the M and F genes, and multiple A to G substitutions throughout the genome. These mutations are the result of cellular adenosine deaminase activity, which deaminates A to produce inosine (I), and then I pairs with C instead of U.

HIV-1 *nef* mutants are less virulent than those with wt versions of the gene. This gene inhibits the activity of cellular APOBEC enzymes, which edit G residues and, in the absence of *nef*, produce hypermutation of the HIV genomes. APOBEC and other editing enzymes are essential for proper editing of mRNA in eukaryotic cells. Widespread hypermutation of the retroviral genome results in loss of meaningful genetic information and, thus, inhibits viral replication.

Polymerase stuttering

Polymerase stuttering is a common process in the RNA world of viruses and eukaryotes. Polymerase stuttering consists of the repeated copying of template sequences, and mediates the generation of polyA tails in mRNA. As during mRNA synthesis, stuttering is favored by the presence of repeats in the template, wither single-nucleotide repeats or repeats of a few nts in length. The extent and significance of this type of recombination in the biology of viruses is unknown.

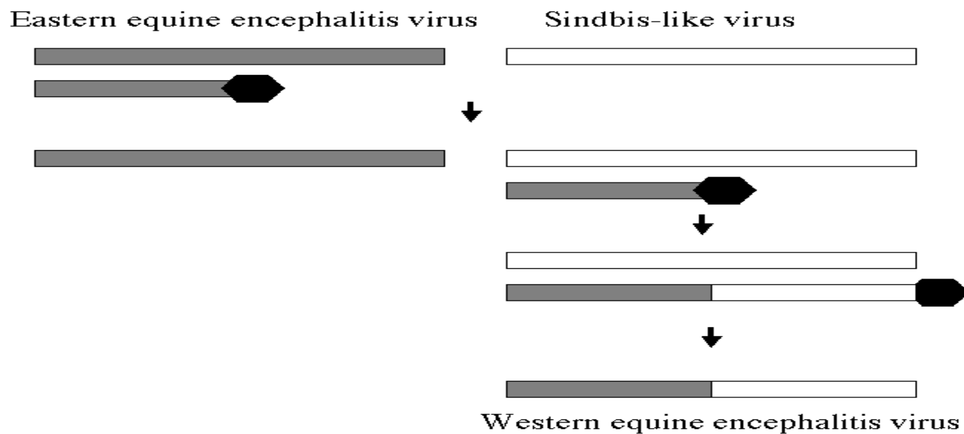
1.2. Recombination.

In eukaryotes recombination results from cut-and-paste of the DNA from sister chromatids. In bacteria recombination occurs by three mechanisms: transformation (DNA is naturally taken up and incorporated), transduction (a phage mediates DNA interchange) and conjugation (plasmid DNA is transferred from one bacteria to another by special bacterial structures). In general, in the DNA world recombination is the result of physical exchange of genes or gene fragments. In RNA viruses, recombination may take place in different ways:

Recombination by copy choice.

Recombination is widespread in positive strand viruses (poliovirus) and retroviruses (HIV), but is virtually absent in negative-stranded viruses. The most common mechanism is copy-choice with template switching. It involves release of a subgenomic RNA copy together with the polymerase complex. Synthesis is resumed after a new template interacts with the subgenomic RNA+polymerase

complex to generate a new strand with information from two different strains. An example of a natural virus recombinant is Western equine encephalitis virus, which originated during a recombination event between Eastern equine encephalitis virus and a Sindbis-like progenitor.



Replicative recombination without detachment

There is a second mechanism of homologous replicative recombination: copying with a supporting molecule. The main difference between this case and template-switching is that the two parental molecules are in close proximity to allow a single round of copying.

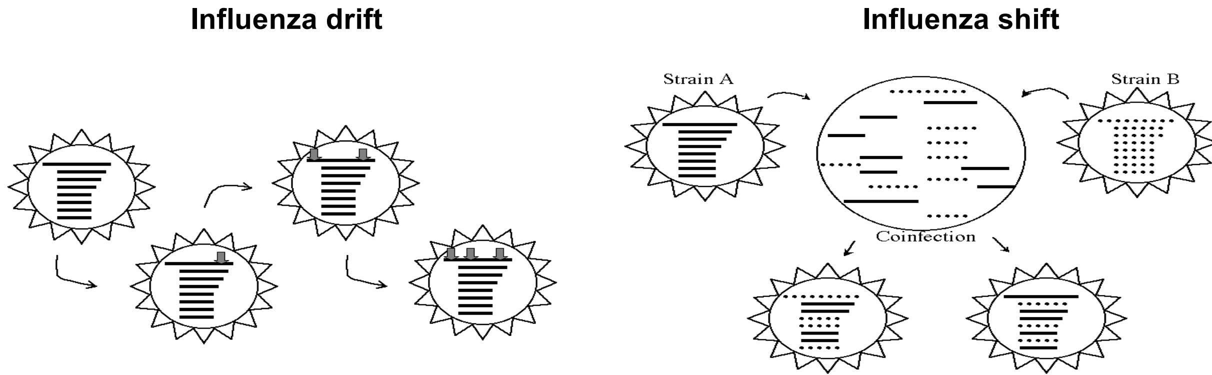
Non-replicative recombination.

While template switching and copy choice require an active polymerase, recombination can also take place in the absence of replication. In this case, the reaction consists of a ligation between two RNA molecules. The origin of these ligated molecules is unclear, but it is possible that they are the result of subgenomic RNA synthesis, ribozyme activity and/or release of nascent templates. Non-replicative recombination can also be homologous or non-homologous.

Reassortment.

This process occurs in segmented viruses, such as influenza. It occurs when RNA segments from different strains are packed together. Influenza pandemics are often the result of avian and human viruses reassorting in pigs. Influenza virus has two modes of evolution: antigenic drift (sigh!) and antigenic shift. Antigenic drift describes the gradual accumulation of mutations in response to the human immune system. Antigenic shift refers to sudden evolutionary changes in the virus precipitated by the acquisition of whole new genomic segments during reassortment.

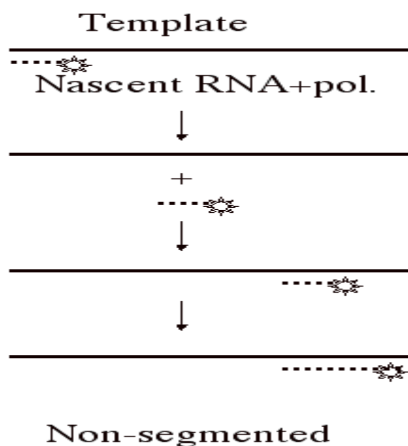
Influenza drift **Influenza shift**



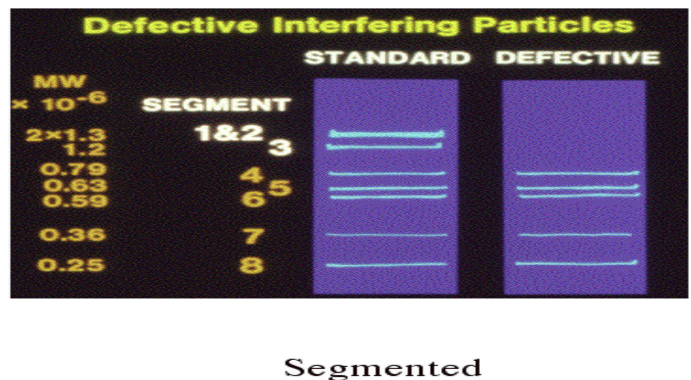
Non-homologous recombination

The same processes that results in full-length progeny genomes when template-switching takes place at a specific site may occur a a non-homologous site. This type of recombination may lead to the generation of viruses with extended length or with deleted genes. In single-stranded viruses, such as rabies, DIPs are generated during synthesis of positive- or negative-sense RNA molecules, by two mechanisms: (i) the polymerase stops somewhere in an internal region of the genome and turns around to copy the daughter RNA strains; and (ii) the polymerase with a nascent strands is released from the template, reattaches to a region much downstream and finishes the remainder of the copying. DIP can also result from packaging of an incomplete set of segments in segmented viruses.

DIPs lack components of the replication machinery, and they cannot replicate by themselves. However, they can replicate if the missing products are provided by a full-length helper virus during coinfection. When DIPs use proteins from a helper virus, the helper virus does not have as many of those proteins for its own use, and cannot replicate properly or as effectively. This inhibition is called interference. DIPs occur during normal infection, and they may play a role in limiting host damage as well as during persistent infections and chronic diseases. The presence of DIPs may also alter the experimental conditions in ways that, at best, are difficult to predict. Thus, experimental design must consider the potential accumulation of DIPs.



Non-segmented



Segmented

1.3. Acquisition of cellular genes and gene fragments

Acquisition of host-cell genes has been reported in positive strand viruses (including retroviruses). It occurs by both homologous non-homologous recombination. In positive-strand riboviruses it is a rare event and its role in evolutionary processes is unclear. In retrovirus it is a very common event, and there are multiple crossovers during replication, but the genetic material that originates from the host is typically only a few nts in length.

The exact mechanism of replication determines the possibilities of new gene acquisition. The shape and size of particles are also important: The icosahedral viruses are limited by the volume inside the virion, while helical viruses do not have such constraint.

Appendix Glossary of Virology

Abortive Infection: When a virus infects a cell (or host), but cannot complete the full replication cycle, i.e. a non-productive infection.

Acute Infection: Relatively brief infections, i.e. a few days to a few weeks, following which the virus is usually eliminated completely from the body by the immune system.

Antigenome: A full-length viral sequence complementary to that of the sequence present in the virion.

Antirreceptor: The region of the attachment protein(s) that interacts with the receptor.

Arboviruses: A large a diverse group of viruses, taxonomically unrelated which are classically transmitted by arthropod vectors, e.g. mosquitoes, ticks, etc.

Assembly: The stage of replication during which all the structural components come together at one site in the cell and the basic structure of the virus particle is formed.

Attachment: The binding of a virus particle to a specific receptor on the surface of a host cell.

Attenuated virus. Virus showing decreased virulence, often used as vaccines.

Burst size: Number of infectious virus produce by a single cell.

Capsid: A protein shell comprising the main structural unit of a virus particle.

Cytopathic effect (cpe). Abnormal morphology typically observed during viral infection.

CTL: Cytotoxic T lymphocyte (a type of white blood cell that kills other cells)

Editing: Post-transcriptional modification of RNA, typically the insertion of a non-template nucleotide or the mutation of a template nucleotide.

Envelope: A lipid membrane around a virus particle.

Fusion Protein: The protein(s) on the surface of a virus particle responsible for fusion of the virus envelope with cellular membranes.

Haemagglutination-inhibition: An assay used for certain types of viruses that are able to agglutinate red blood cells. Haemagglutination-inhibition records blocking of this process by antibodies, and thus, the presence of antibodies against the virus.

Helper virus: Virus that provides a function to another virus that lacks them.

Host range: Diversity of organisms or cell that can support viral replication.

Latent Infection: Infection in which an initially acute phase is followed by alternation of quiescent and boost periods.

Lytic virus. Virus that kills the cells.

LTR: Long terminal repeats.

MAGI: Multinuclear activation of galactosidase indicator.

Matrix Protein: A structural protein of a virus particle that underlies the envelope and links it to the core.

Maturation: The stage of viral replication at which a virus particle becomes infectious.

Monolayer: A sheet of cells, one cell deep.

Multiplicity of infection (MOI): Number of virus particles per cell.

Neutralization: Blocking of virus infection by antibodies; also, an assay which measures this.

Nucleocapsid: The core of a virus particle consisting of the genome plus a complex of proteins.

Pathogenicity: The ability to cause disease.

Penetration: The stage of viral replication at which the virus genome enters the cell.

Persistent Infection: Infections in which ongoing virus replication occurs, but the virus adjusts its replication so as to avoid killing host.

Phage (bacteriophage): A virus that infects a bacterium.

Permissive cell: Cell that the virus can use to produce infectious progeny.

Plaque forming unit (PFU). Infectious virion.

Polyprotein: A long polypeptide encoding several individual proteins that are subsequently released by protease cleavage.

Portal of entry: The organ through which the virus enters a body.

Productive infection. Infections in which viable virus is produced

Prophage (provirus): The phage (virus) DNA inserted into a cell chromosome.

Protomer: Minimal building unit of an icosahedral capsid

Provirus: Virus genome integrated in the cell chromosome.

RdRP: RNA-dependent RNA polymerase.

Receptor: A specific molecule on the surface of a cell that is used by a virus for attachment.

Release: The stage of viral replication at which virus particles escape the infected cell.

Replication complex: The minimal combination of element physically in contact that allows replication.

Retroelements: Genetic units for which there is evidence of movement from one locus to another by retrotransposition. We humans are some 25% retroelements.

Endogenous retrovirus. They have direct repeats, LTRs and gag, pol and env genes (10^2copies)

Retrotransposons. As above, minus env (10^4 copies)

Retroposons (LINEs). As above, minus ORFs, plus polyA (10^5 copies)

Retrosequences (SINES). As above, but smaller (10^6 copies)

Processed pseudogenes. As above, minus direct repeats (10^2 copies)

Restrictive cell: Cell in which a virus cannot produce infectious progeny.

Serotype: Group of closely related viruses that share recognition by specific antibodies.

Susceptible cell: Cell that the virus can enter.

Superinfection: Infection of a cell that already carries a virus.

Syncytia: Multinucleated cells originated from the fusion of many infected cells. They are one of the hallmarks of cytopathic effect.

Target cell: Last cell type infected in the organism, where the virus typically does most of the replication.

Terminal repeats: Repetitive sequences often found at the termini of viral genomes. They often carry cis-acting signals for transcription and replication.

Titer: Concentration of a virus (normally in PFU/ml).

Titration. Determination of the titer.

Tropism: The predilection of a virus to infect specific cell or tissue types.

Uncoating: The stage of viral replication at which structural proteins are lost and the virus genome is exposed to the replication machinery.

UTR: Untranslated terminal repeat.

Vector: An organism responsible for transmitting a pathogen from one host to another, e.g. a mosquito. (In molecular biology, a molecule used to clone nucleic acid sequences).

Viremia: The presence of virus in the bloodstream.

Virions: Structurally mature, extracellular virus particles.

Viroceptors. Homologues of cytokine receptors that compete with cellular receptors and thus inhibit signaling. They are thought to be originated from cellular genes that were incorporated in the genome of viruses.

Virulence: Degree of damage caused by a pathogen.

Virus attachment protein: The protein on the surface of a virus particle responsible for binding the receptor.

Zoonosis: Infection shared by humans and other animals.