VIRUS

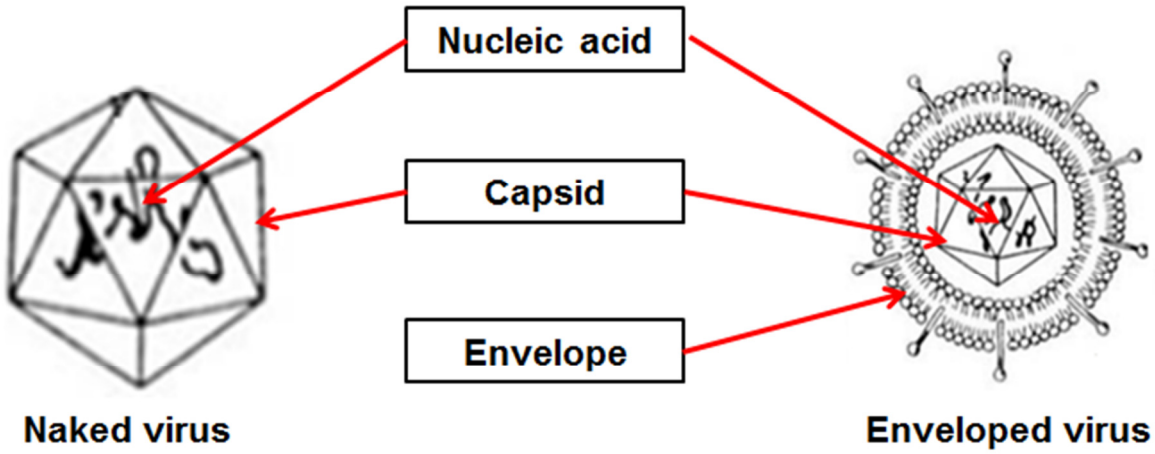
A **virus** is a tiny, acellular, non-living, infectious particle composed mainly of protein coats that harbors nucleic acid either in the form DNA or RNA in the core. As it is acellular, it is completely depends on host cells for its reproduction by hijacking metabolic pathways of host cells. Therefore, it is also a type of obligatory parasite. However, it differs from bacteria in having smaller size and acellular nature compared to unicellular nature of bacteria that does not depend on host cells for their reproduction but it derives nutrition from the host cells. It has only similarity with living organism in having a nucleic acid genomes based on the same genetic code that's used in cells of all living organisms, and therefore viruses also have genetic variations like all other organisms.

Viruses are studied under the specialized branch of physiology called **Virology**, which focuses the study of the structure, classification and evolution of viruses, their mechanism of infecting and utilizing host cells for reproduction, interaction with host's defiance mechanism, the disease they cause, techniques of their isolation and culture and further how to combat their infection through various therapeutic measures.

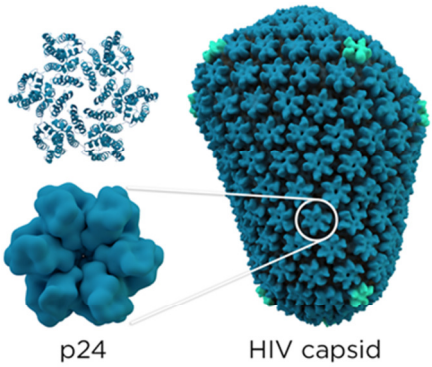
Viruses are tiny particle diameter of which ranges from 20 to 300 nm (1 nm = 10^{-9} meter). The largest virus is said to be *Pithovirus sibericum* that measures 1500 nm in length and 500 nm in diameter while *Escherichia coli* bacterium has roughly 100 nm diameter. But in terms of size of genome, *Megavirus chilensis* having a genome length of 12,59,197 bp with around 1120 predicted proteins is the largest virus. The smallest virus is thought to be Porcine Circovirus with an average size of only 17 nm; Parvovirus is a second in size with an average capsid size of 18 – 25 nm. However, in terms of size of genome, the hepadnavirus such as Hepatitis B is smallest having only 3200 bp of genetic material with a average capsid size of 42 nm. Parvovirus is second in the list having slightly larger genetic material of 5000 bp but smaller capsid size of 18 – 26 nm.

Structure of viruses

There are very large number of viruses on the earth which greatly differs in terms of shape, size, constituent, and structure. Roughly, viruses are composed of three structures on average namely a nucleic acid genome that may be of DNA or RNA, a protective protein shell or capsid composed of capsomere as unit, and a membranous envelope that is taken off from host cells. However, all viruses do not have membranous envelope and therefore known as **naked** or **simple virus** or **non-envelope virus**.



But, those viruses which have envelope are known as **enveloped virus** or **complex viruses**. Envelopes are bilayer structure found mainly in those viruses that infects animal cells. The envelope is composed of phospholipid bilayer where glycoproteins that forms spikes needed for attachment and fusion to the host cells, matrix proteins needed for the attachment to the nucleocapsid, and enzymes and proteins needed for virus life cycle are studded. Nucleic acid in a protein core may be of double stranded or single stranded and possess either DNA or RNA genetic material rarely both.



Arrangement of proteins oligomers to form capsomeres which arranged precisely to form a capsid that are unique to a virus.

Capsid is the protein coat that surrounds the viral genetic material. It is consists of several oligomeric structural subunits of proteins called as **protomers**, 5 to 6 of which congregates to form an individual protein subunits known as **capsomeres**. Capsomeres join each other via **intercapsomeric triplexes** comprised of two copies of one protein and one copy of other protein. Furthermore, each virus has a finite number of capsomeres as for example enterovirus has a capsid of icosahedral shape containing only 60 capsomeres, herpesvirus has 162 capsomeres in their protein coat of icosahedral shape, hepatitis B virus has an icosahedral capsid containing 180 capsomeres, naked and non-enveloped adenovirus has a capsid containing 252 capsomeres in their protein coat.

The capsomeres organize in a precise and repetitively around the nucleic acid to provide an outer morphology to the virus particles called as capsid. Therefore, a single virion is composed of large number of capsomeres arranged differently in different viruses that is unique to a particular virus. Besides harboring genetic material, it has immense role in specificity, viral infectivity and transferring between hosts due to the presence of glycoprotein protrusion, called spikes that interacts with certain receptors on host the host cells. Moreover, they protect genetic material of viruses from physical, chemical and enzymatic damages.

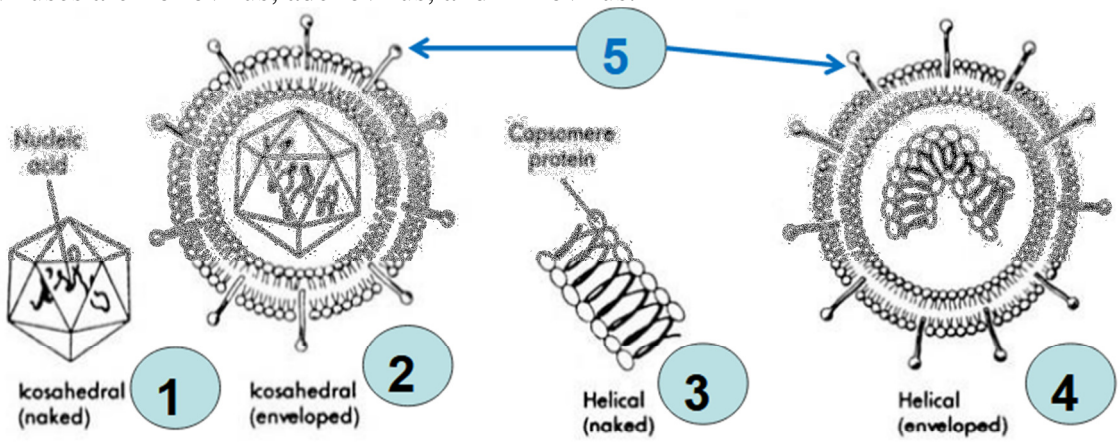
Classification of viruses

Viruses are morphologically different from each other. As already stated earlier, viruses may be of naked or enveloped type on the basis of presence or absence of outer bilayer. Viruses are generally classified on the basis of structure of the capsid, genetic material, chemical composition and mode of replication. Commonly, they are classified on the basis of their morphology.

I. On the basis of morphology

1. **Helical:** Viruses that have helical structure, their protein coat is composed of identical set of capsomeres arranged typically around a hollow central structure or cavity. They are also called as rod-shaped or filamentous virus when they appear thread-like. The length generally ranges from 300 – 500 nm and width 15 – 19 nm and depend mainly on the length of the genetic material. *Tobacco Mosaic Virus* or TMV, a plant RNA virus, is a classic example of a helical virus, while *Orthomyxoviridae* genera that comprises influenza viruses is helical viruses with single-stranded RNA in animals.
2. **Icosahedral:** Icosahedral structure is formed by the fusion of numerous equilateral triangles spherically, and thus geometrically it has 12 corners or vertices, 20 sides or

equilateral triangles and 30 edges. Icosahedral capsids can be further structurally identified as hexagonal or pentagonal at the corners. The classic examples of icosahedral viruses are Poliovirus, adenovirus, and rhinovirus.



The most common viral morphology
Left to right. (1) A naked virus, (2) an enveloped icosahedral virus, (3) a naked helical virus, and (4) an enveloped helical virus. (5) Some human viruses also contain an envelope, which possess unique viral proteins drwan in figure as "spikes".

3. **Prolate:** It is basically elongated icosahedral shapes that are mostly present in bacteriophages infecting bacteria.
4. **Head or Tail:** This structure is said to be a hybrid between icosahedral and helical or filamentous morphology consisting basically of an icosahedral capsid attached to a filamentous tail e.g. some bacteriophages.
5. **Complex or asymmetrical:** This group falls under types of viruses whose capsid is neither helical nor icosahedral and is asymmetric. It is because of having added structure on the outer wall or having extra proteins. The poxvirus is an example of a complex virus due to its unique capsid and outer layer.

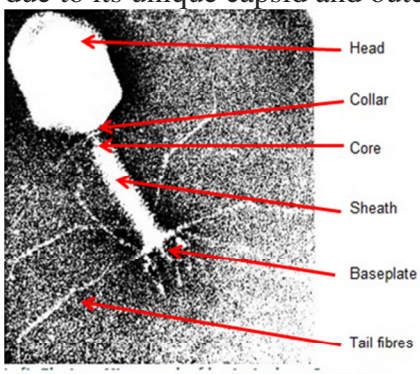


Image credit: Kenneth Todar/extbookofbacteriology.net/

Head and Tail morphology (Bacteriophage)

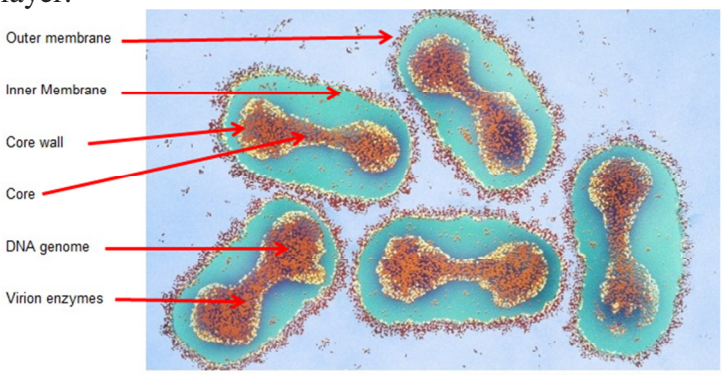


Image credit: Chris Bjornberg/npr.org

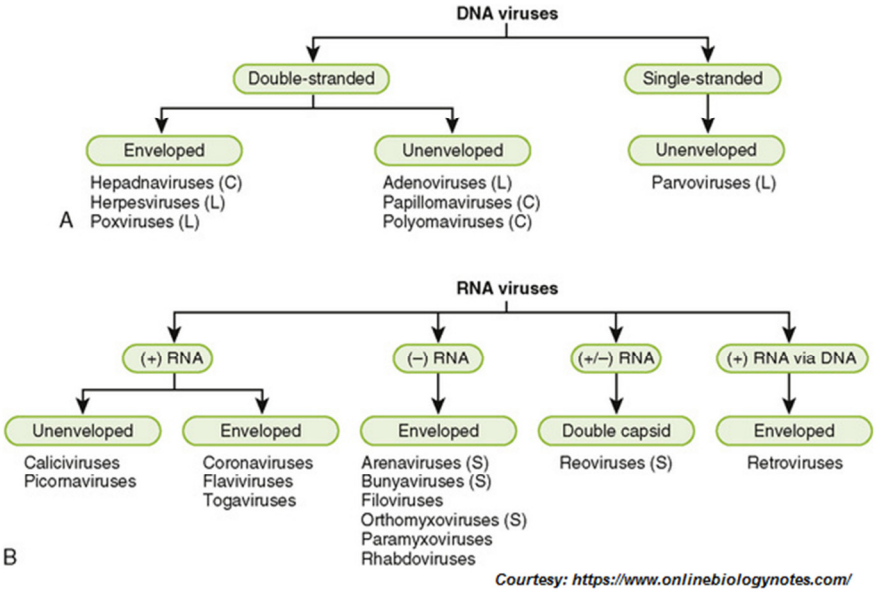
Complex or asymmetric morphology (Pox virus)

II. On the basis of genetic material

1. **DNA viruses:** Those viruses which have DNA as genetic material in their core, are called as DNA virus. They can affect both humans and animals and cause simple benign symptoms to serious health hazards. The common examples of DNA viruses are parvovirus, papillomavirus and herpesvirus. DNA viruses may be single stranded (ssDNA) or double stranded (dsDNA) viruses.

Difference between dsDNA and ssDNA	
dsDNA	**ssDNA**
dsDNAs are linear or filamentous.	ssDNA is star-shaped or stellate.
Total number of adenine is equal to total number of thymine similarly total of guanine is equal to total number of cytosine.	No such relationship in ssDNA.
A/T ratio is 1.	A/T ratio is 0.77.
G/C ratio is 1.	G/C ration is 1.3.
More stable	Less stable
Resistant to formaldehyde.	Highly susceptible, amino group readily reacts with the formaldehyde.
Behaves like rigid rod like structure.	Behaves as random coiled structure.
Present in almost all organisms.	Present in few viruses such as Bacteriophage ϕX174.
Follow Chargaff's rule.	Don't follow Chargaff's rule.

2. **RNA viruses:** Viruses that posses RNA as genetic material are called RNA virus. Examples include rotavirus, poliovirus, yellow fever virus, dengue virus, hepatitis C virus, measles virus, rabies virus, influenza virus, Ebola virus, AIDS virus, Nipah virus, Zika virus, and of course Corona virus. RNA virus may also be double stranded RNA (dsRNA) virus such as Bacteriophage ϕ6 that infects *Pseudomonas* bacteria or single stranded RNA (ssRNA) virus.
3. **DNA-**RNA viruses: Exceptionally, a few viruses do contain both DNA and RNA as their genetic material. Examples include Leukoviruses and Rous's viruses that cause cancer. These are a type of tumor viruses.



Classification of viruses on the basis of presence of genetic material

III. On the basis of host range

1. **Bacteriophage:** These are vruses which infect bacteria such as ʎ phage, T2, T4, ϕ174, ϕ6, MV-11, etc.
2. **Plant viruses:** Infect only plants e.g. tobacco mosaic virus (TMV), cauliflower mosaic virus, etc.
3. **Insect viruses:** Infect insects such as Baculovirus, Sachbrood virus, Entomopox virus, etc.
4. **Animal viruses:** Those viruses that infect animals as for example Polio virus, Retro viruses, etc.

IV. On the basis of mode of transmission

1. **Virus transmitted through respiratory route:** E.g. Swine flu, Rhino virus, adeno virus, influenze virus.
2. **Virus transmitted through faeco-oral route:** E.g. Hepatitis A virus, Polio virus, Rota virus, etc.
3. **Virus transmitted through sexual contacts:** E.g. Retro virus.
4. **Virus transmitted through blood transfusion:** E.g. Hepatitis B virus, Hepatitis C virus, HIV.
5. **Zoonotic viruses:** Viruses that are transmitted through bitin g of infected animals e.g. Rabies virus, Alpha virus, Flavi virus and recently Corona virus (it is considered that this virus descended from Bats to human beings).

V. On the basis of replication properties and site of replication

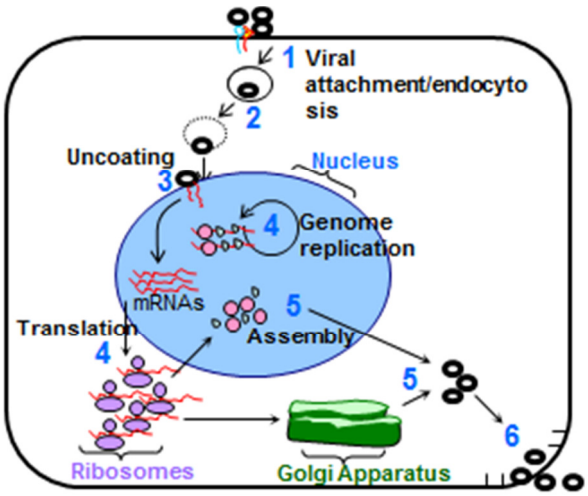
1. **Replication and assembly in cytoplasm of host:** E.g. All RNA virus replicates and assembles in the cytoplasm of host cells except influenza virus.
2. **Replication in nucleus and assembly in cytoplasm of host:** E.g. Influenza virus, Pox virus, etc.
3. **Replication and assembly in nucleus of host:** All DNA viruses replicate and assembvle in nucleus of host cell except Pox virus.
4. **Replication through dsDNA intermediate:** E.g. All DNA viruses, Retro virus and some tumor causing RNA virus replicate through dsDNA as intermediates.
5. **Replication through ssRNA intermediate:** All RNA viruses except Reo virus and tumor causing RNA viruses.

Viral replication:

Viruses are obligate intracellular pathogens therefore they cannot replicate without the utilization of machinery and metabolism of a host cells. Though, the replicative life cycle of viruses differs greatly between species and category of virus, there are six basic stages that are essential for viral replication namely attachment, penetration, uncoating, replication, assembly, and release.

1. **Adsorption or attachment:** Viral proteins on the capsid or phospholipid envelope interact with specific receptors on the host cellular surface leading to attachment to the surface of host cells. Some accessory protein molecules may present on the surface of host cells that intensify the process. This specificity determines the host range (**tropism**) of a virus.
2. **Penetration:** Attachment to the host cell surface through receptor results in conformational changes in the capsid of viruses or lipid envelope leading to the fusion of viral and cellular membranes. However, some DNA viruses can also enter the host cell through receptor-mediated endocytosis.

3. **Uncoating:** In the process of fusion to the host cells membrane, viral capsid is removed and degraded by viral enzymes or host enzymes releasing the viral genomic nucleic acid to the host cell cytoplasm or directly to nucleus in some cases.



4. **Replication:** After uncoating of genetic material of virus, transcription and translation of genetic material are started. This process is very different in DNA and RNA viruses and further amongst the genetic materials having different polarity. This process results in the de novo synthesis of viral proteins and genome.

DNA Viruses: In animal DNA viruses, transcription and translation are not coupled except for poxviruses, transcription occurs in the nucleus and translation in the cytoplasm. Generally, the primary transcripts, generated by host's RNA polymerase II, are larger than the mRNAs found on ribosomes, and therefore in some cases, as much as 30% of the transcribed RNA remains untranslated in the nucleus. In most DNA viruses, only a small fraction of genome is transcribed as early messengers, which leads to translation of early viral proteins that is key to viral DNA replication. The viral messengers are monocistronic. After DNA synthesis, the remainder of the genome is transcribed into late messengers[1] and finally into most of the viral proteins.

Regulation is carried out by proteins present in the virions, or specified by viral or cellular genes, interacting with regulatory sequences at the 5' end of the genes. These sequences may respond in *trans* to products produced by other genes and act in *cis* on the associated genes. Different classes of genes may be transcribed from different DNA strands and therefore in opposite directions e.g. polyomaviruses. The transcripts may undergo post- transcriptional processing so that nonessential intervening sequences are removed. The mode of replication is semiconservative but the nature of the replicative intermediates depends on the manner of replication. Several methods of replication can be recognized.

A. Adenoviruses - Adenoviruses show asymmetric replication, which initiates at the 3' end of one of the strands using a protein primer. The growing strand displaces the preexisting strand of the same polarity and builds a complete duplex molecule. The displaced strand in turn replicates in a similar manner after generating a panhandle structure by pairing the inverted terminal repetitions.

B. Herpesviruses - Herpesviruses have linear genomes with terminal repeats. On reaching the nucleus, the terminal ends undergo limited exonucleotic digestion and then pair to form circles. Replication is thought to take place via a rolling circle mechanism,

[1] The complex viruses have immediate early genes, which are expressed in the presence of inhibitors of protein synthesis, and delayed early genes, which require protein synthesis for expression.

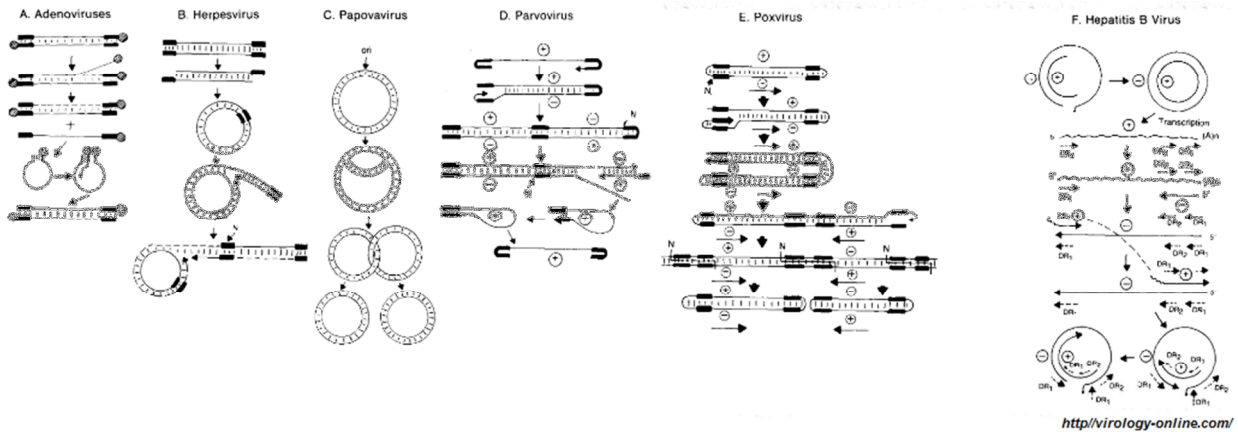
where concatemers are formed. During maturation, unit-length molecules are cut from the concatemers.

C. Papovaviruses - The DNA of papovaviruses are circular and the replication is bidirectional and symmetrical, via cyclic intermediates.

D. Parvoviruses - The replication of single stranded parvoviruses is initiated when +ve and -ve stranded DNA from different parvovirus particles come together to form a double stranded DNA molecule from which transcription and replication takes place.

E. Poxviruses - The striking feature of poxvirus DNA is that the two complementary strands are joined. The replicative intermediates, present in the cytoplasm, are special concatemers containing pairs of genomes connected either head to head or tail to tail.

F. Hepadnaviruses - Hepatitis B virus employs reverse transcription for replication. The genome consists of a partially double-stranded circular DNA with a complete negative strand and an incomplete positive strand. Upon entering the cell, the positive strand is completed and transcribed. RNA transcripts are in turn reverse-transcribed into DNA by a viral enzyme in several steps, following closely the model of retroviruses, including a jump of the nascent positive strand from one direct repeat (DR) to another.



RNA viruses: Replication in RNA virus is dictated by the absence of multiple translation units within the same messenger. To overcome this difficulty, viruses developed three strategies which are as follows:

1. The viral mRNA acts directly as the messenger and is translated monocistronically, followed by cleavage to form different proteins.
2. The virion RNA is transcribed to yield various monocistronic mRNAs by initiating transcription at various places.
3. The genome itself is a collection of separate RNA fragments that are transcribed into monocistronic mRNAs.

RNA viruses can be placed into 7 classes, according to the nature of the viral RNA and its relation to the messenger.

Class I: The genome, having +ve polarity, itself act as the messenger, specifying information for the synthesis of both structural and nonstructural proteins. The same RNA molecules also initiate replication that requires the expression of proteins first. This

format allows little control over replication e.g. Poliovirus has no independent mechanism of controlling the numbers of structural proteins made. Examples are picornaviruses, and flaviviruses.

Class II: Many +stranded RNA viruses have subgenomic RNA as part of their cycle. This would allow a certain amount of control. The subgenomic mRNA cannot be recognized by the RNA polymerase. It can be used solely for the synthesis of structural proteins etc. A second way to get round the problem is to make a nested set of RNAs. The nested set of RNA is the most efficient form of control. They can control which part of their genome to express. E.g. coronaviruses, togaviruses and picronavirus.

Viruses	Subgenomic	PTC	Nested	Splicing
Picornaviridae	N	Y	N	N
Togaviridae	Y	Y	N	N
Coronaviridae	Y	N	Y	N

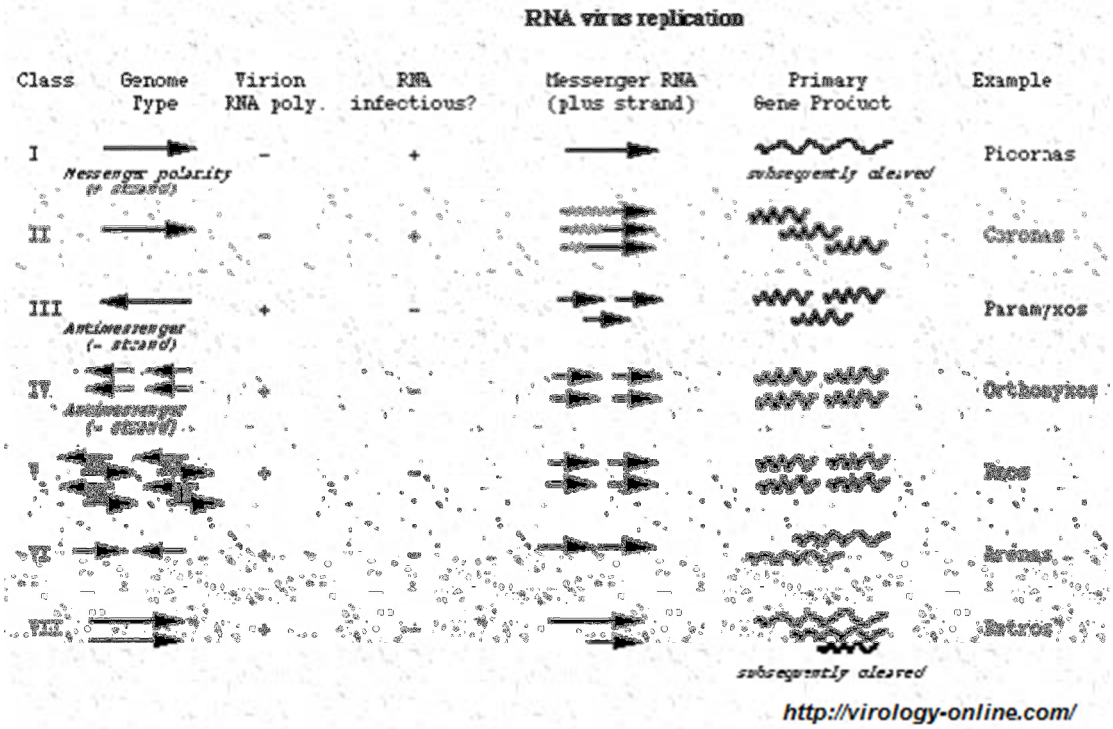
Class III: The genome is of -ve polarity to the messenger. A virion RNA-dependent RNA transcriptase first transcribes the genomes into separate monocistronic messengers initiating at a single promoter. The transcriptase stops and restarts at each juncture between different genes. E.g. paramyxoviruses and rhabdoviruses.

Class IV: The -ve genome is in several distinct nonoverlapping pieces of ssRNA. The virion transcriptase generate a messenger from each piece. With orthomyxoviruses, most genomic segments contain a single gene but 2 fragments contain 2 overlapping genes: one is expressed by a full-length messenger, the other by a shorter messenger obtained from the former by splicing. The replication of orthomyxoviruses is unusual amongst RNA viruses in that it takes place within the nucleus. The nuclear function it requires is the 5' cap of cellular messengers, which it "pinches" after endonucleotic cleavage of the host messengers. The 5' cap is then used as primers in the synthesis of viral messengers. E.g. orthomyxoviruses and most bunyaviruses.

Class V: Arenaviruses have an ambisense genome in that half the genome is of -ve polarity and is transcribed into a messenger by a virion transcriptase, but the other half, which is of +ve polarity is transcribed twice: first a complete transcript of the genome is made, then the mRNA is transcribed form this transcript. This strategy is seen in the S (small) segment of the genome of phleboviruses. Ambisense genomes are unusual for RNA viruses but not for dsDNA viruses.

Class VI: This class of RNA viruses contain distinct nonoverlapping segments of dsRNA, each is transcribed into an independent mRNA by the virion transcriptase. Most messengers are monocistronic, but one is bicistronic and expresses a second protein by initiating at an internal AUG in a different reading frame. Each segment of reovirus RNA is replicated independently. A nascent mRNA strand is first generated by the virion transcriptase, which then serves as the template for the replicase to make the negative strand. The two strands remain associated in a dsRNA molecule that ends up in a virion. This replication is asymmetric and conservative because (1) the -ve strand of the virion RNA servers as the initial template and (2) the parental RNA does not end up in the progeny. Example is Reoviruses.

Class VII[2]: This class includes retroviruses which are unique in that their genomes are transcribed into DNA and not RNA. They contain two identical ssRNA of +ve polarity, with a poly A tail at the 3' end and a cap at the 5' end. Each is transcribed into DNA by reverse transcriptase that then integrates into the cellular DNA as provirus. Transcription of the provirus by the cellular transcriptase yields the viral molecules that end up in virions.



5. **Assembly:** After *de novo* synthesis and post-transcriptional modification of viral genome and proteins, viral proteins are packaged with newly replicated viral genome into new virions that are ready for release from the host cell. This process can also be referred to as maturation that varies in different type of viruses, which are as follows:

   Naked icosahedral viruses: Naked icosahedral viruses are released from infected cells in different ways. Poliovirus is rapidly released, with death and lysis of infected cells. In contrast, the virions of DNA viruses that tend to mature in the nucleus tend to accumulate within infected cells over a long period and are released when the cell undergoes autolysis, and in some cases, may be extruded without lysis.

   Enveloped Viruses - Viral proteins are first associated with the nucleic acid to form the nucleocapsid, which is then surrounded by an envelope. In nucleocapsid formation, the proteins are all synthesized on cytoplasmic polysomes and are rapidly assembled into capsid components whereas in envelope assembly, virus-specified envelope proteins[3] go

[2] RNA viruses of class III to VII require a virion transcriptase for synthesizing a messenger, their purified viral RNA s are not infectious. Only those of class I and II are infectious.

[3] Envelope glycoprotein synthesis: Polypeptide backbone is first formed on polysomes bound to the rough endoplasmic reticulum, which then moves via transport vesicles to the Golgi apparatus where it attains full glycosylation and fatty acid acylation. However, the matrix proteins are usually not glycosylated and stick to the cytoplasmic side of the plasma membrane through hydrophobic domains.

directly to the appropriate cell membrane (the plasma membrane, the ER, the Golgi apparatus), displacing host proteins. The viral envelope has the lipid constitution of the membrane where its assembly takes place. (E.g. the plasma membrane for orthomyxoviruses and paramyxoviruses, and the nuclear membrane for herpesviruses) that determines their physical, biological, and antigenic properties. Envelopes are formed around the nucleocapsids by budding of cellular membranes.

The selection of viral glycoproteins is efficient but not exclusive (rhabdovirus contain 10 – 15% of non-viral glycoproteins. They may also contain glycoproteins specified by another virus infecting the same cell that results in cross-reactivity in antibody test.

Complex viruses: Maturation of the highly organized complex virus such as poxviruses takes place in cytoplasmic foci. In contrast to simple viruses, the poxvirus membrane contains newly synthesized lipids that differ in composition to the cellular lipids. The maturation of poxviruses after the precursors have been enclosed within the primitive membranes suggests that poxviruses may be transitional forms towards a cellular organization.

*In the course of replication of viruses, interference may occur because of generation of **D**etective **I**nterference particles (**DI Particles**), in particular during infection by RNA viruses such as rhabdoviruses, togaviruses, orthomyxoviruses, paramyxoviruses, coronaviruses and some DNA viruses (herpesviruses). These particles are usually smaller than regular particles with comparatively shorter genome that requires the helper functions of a normal virus co-infecting the same cell.*

They deprive the regular virus of its replicase by binding to it more effectively. They do not make a replicase of their own because they are always defective in their replicase gene. The formation of DI genomes of RNA viruses is the consequence of high variability of these genomes. The DI genomes are formed by a copy choice mechanism when the replicase, having replicated part of the template, skips to another part of the same or another template. With VSV and other negatively stranded RNA viruses, 4 types of defective genomes are seen;

1. ***Deletions** - the polymerase jumps to a site beyond on the same template, skipping a fragment.*
2. ***Snapbacks** - this occurs when the replicase, having transcribed part of the + strand, switches to the just-made - strand as template. The resultant RNA contain half+ and half- and can produce a hairpin on annealing.*
3. ***Panhandle** - this is formed by a similar mechanism, when the polymerase carrying a partially made - strand switches back to transcribing the extreme 5' of it, so that on annealing, the strand forms a panhandle.*
4. ***Compounds** - these genomes are made by a combination of deletions and snapbacks.*

The competition of DI genomes with competent genomes depends not only on the structure of the DI genome but on that of the normal competent genome. Different DI genomes may interfere to very different degrees with the same competent genome that may acquire mutations making them resistant to the existing DI genomes. Subsequently, this is overcome by the new types of DI genomes. During viral multiplication, many types of DI genomes are continuously made and they are very heterogeneous.

6. **Release:** As stated, after packaging of newly synthesized viral genomes into the core of newly synthesized viral capsid, the viruses are released from the host cells. There are two methods of viral release: **lysis (Lytic cycle)** or **budding**. Lysis results in the death of an infected host cell, these types of viruses are referred to as **cytolytic viruses** such as smallpox virus (*Variola major*).

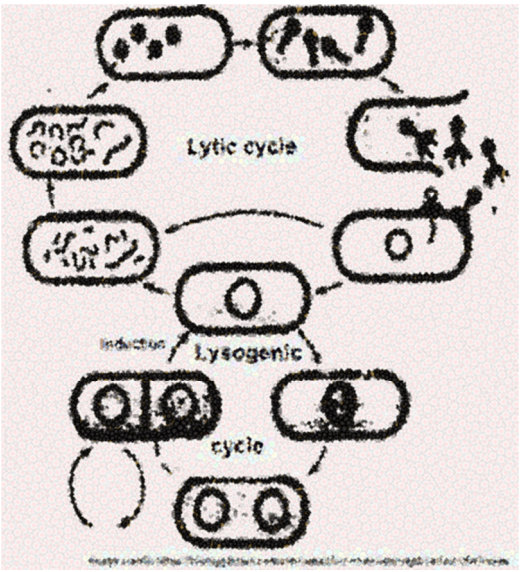
Adapted from http://www.virology-online.com/general/Replication.htm

Enveloped viruses, such as influenza A virus, are typically released from the host cell by budding which results in acquisition of the viral phospholipid envelope. These types of virus do not usually kill the infected cell and are termed **cytopathic viruses**.

Lytic vs. Lysogenic cycle of viruses

Viruses are pathogens that cannot multiply on their own and therefore, they are solely dependent on host cells for their multiplication that is commonly termed as **virus replication**, by utilizing host cellular machinery and metabolic pathways. There are two different replication patterns of virus namely **lytic cycle** or **lysogenic cycle** which are interchangeable. Some viruses are capable of showing both these patterns of replication by replicating first through lysogenic cycle and then by switching to lytic cycle.

Lytic cycle: It is the principal pattern of virus replication. In the lytic cycle, viruses first enter the host cell, inject its nucleic acid, replicate by taking over the metabolic activities of the host, direct host cell to produce more viral genes and proteins, assemble to new virion and then cause host cell to burst, releasing new viruses. Therefore, as the name implies, host cell lysis ocurrs. Hence, the viruses that show lytic cycles are virulent than the viruses that undergo lysogenic cycle. It is estimated that virus replication in lytic cycle is fast taking only about 30 minutes to come out from the host cell.



Lysogenic cycle: In lysogenic cycle, virus is in dormant stage comparable to viruses of lytic cycle which are very active. In this cycle, viruses first enter the host cell, inject its nucleic acid, integrate it with the nucleic acid of the host cell, and make it replicated as the host cell multiplies. The new set of genome that contains viral genome is now called as "**prophage**". These types of viruses rest inside the host cell to very long time resulting in alteration in functional characteristics of the host cell but, they do not die due to virus infection unless the virus replication switches to lytic cycle.

Difference between lytic and lysogenic cycle		
Attributes	Lytic cycle	Lysogenic cycle
Definition	Lytic cycle is type of virus replication in which host cell bursts during the release of viruses.	Lysogenic cycle is another type of virus replication in which viral DNA is integrated into host cell DNA.
Time taken	Short	Long
Virulence	Highly virulent	Less virulent
Lysis of the host cell	occurs	Does not occur
Integration of viral genome into host genome	Integration does not occur	Integration occurs
Prophage formation	Prophages are not formed.	Prophages are formed.
Formation of many viruses	Many viruses form.	Viruses do not form.

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