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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – I – MEDICAL VIROLOGY – SMB3102

GENERAL CONCEPTS

VIRUS HISTORY

The history of virology goes back to the late 19th century, when German anatomist Dr Jacob Henle (discoverer of Henle's loop) hypothesized the existence of infectious agent that were too small to be observed under light microscope. This idea fails to be accepted by the present scientific community in the absence of any direct evidence. At the same time three landmark discoveries came together that formed the founding stone of what we call today as medical science. The first discovery came from Louis Pasture (1822-1895) who gave the spontaneous generation theory from his famous swan-neck flask experiment. The second discovery came from Robert Koch (1843-1910), a student of Jacob Henle, who showed for first time that the anthrax and tuberculosis is caused by a bacillus, and finally Joseph Lister (1827-1912) gave the concept of sterility during the surgery and isolation of new organism.

The history of viruses and the field of virology are broadly divided into three phases, namely discovery, early and modern.

The discovery phase (1886-1913)

In 1879, Adolf Mayer, a German scientist first observed the dark and light spot on infected leaves of tobacco plant and named it tobacco mosaic disease. Although he failed to describe the disease, he showed the infectious nature of the disease after inoculating the juice extract of diseased plant to a healthy one. The next step was taken by a Russian scientist Dimitri Ivanovsky in 1890, who demonstrated that sap of the leaves infected with tobacco mosaic disease retains its infectious property even after its filtration through a Chamberland filter. The third scientist who plays an important role in the development of the concept of viruses was Martinus Beijerinck (1851-1931), he extended the study done by Adolf Mayer and Dimitri Ivanofsky and showed that filterable agent form the infectious sap could be diluted and further regains its strength after replicating in the living host; he called it as *"contagium vivum fluidum"*. Loeffler and Frosch discovered the first animal virus, the foot and mouth disease virus in 1898 and subsequently Walter Reed and his team discovered the yellow fever virus, the first human virus from Cuba in1901. Poliovirus was discovered by Landsteiner and Popper in 1909 and two years later Rous discovered the solid tumor virus which he called Rous sarcoma virus.

The early phase (1915-1955)

In 1915, Frederick W. Twort discovered the phenomenon of transformation while working with the variants of vaccinia viruses, simultaneously Felix d'Herelle discovered bacteriophage and developed the assay to titrate the viruses by plaques. Wendell

Stanley (1935) first crystallized the TMV and the first electron micrograph of the tobacco mosaic virus (TMV) was taken in 1939. In 1933 Shope described the first papillomavirus in rabbits. The vaccine against yellow fever was made in 1938 by Thieler and after 45 years of its discovery, polio virus vaccine was made by Salk in 1954.

The modern phase (1960-present)

During this phase scientists began to use viruses to understand the basic question of biology. The superhelical nature of polyoma virus DNA was first described by Weil and Vinograd while Dulbecco and Vogt showed its closed circular nature in 1963. In the same year Blumberg discovered the hepatitis B virus. Temin and Baltimore discovered the retroviral reverse transcriptase in 1970 while the first human immunodeficiency virus (HIV) was reported in 1983 by Gallo and Montagnier. The phenomenon of RNA splicing was discovered in Adenoviruses by Roberts, Sharp, Chow and Broker. In the year 2005 the complete genome sequence of 1918 influenza virus was done and in the same year hepatitis C virus was successfully propagated into the tissue culture.

Many discoveries are done using viruses as a model. The transcription factor that binds to the promoter during the transcription was first discovered in SV40. The phenomenon of polyadenylation during the mRNA synthesis was first described in poxviruses while its presence was first reported in SV40. Many of our current understanding regarding the translational regulation has been studied in poliovirus. The oncogenes were first reported in Rous sarcoma virus. The p53, a tumor suppressor gene was first reported in SV40.

Important discoveries

Date	Discovery
1796	Cowpox virus used to vaccinate against smallpox by Jenner.
1892	Description of filterable infectious agent (TMV) by Ivanovsky.
1898	Concept of the virus as a contagious living form by Beijerinck.
1901	First description of a yellow fever virus by Dr Reed and his team.
1909	Identification of poliovirus by Landsteiner and Popper.
1911	Discovery of Rous sarcoma virus.
1931	Virus propagation in embryonated chicken eggs by Woodruff and Goodpasture.
1933	Identification of rabbit papillomavirus.
1936	Induction of carcinomas in other species by rabbit papillomavirus by Rous and Beard.
1948	Poliovirus replication in cell culture by Enders, Weller, and Robbins.
1952	Transduction by Zinder and Lederberg.
1954	Polio vaccine development by Salk.
1958	Bacteriophage lambda regulation paradigm by Pardee, Jacob, and Monod.
1963	Discovery of hepatitis B virus by Blumberg.
1970	Discovery of reverse transcriptase by Temin and Baltimore.
1976	Retroviral oncogenes discovered by Bishop and Varmus.
1977	RNA splicing discovered in adenovirus.
1983	Description of human immunodeficiency virus (HIV) as causative agent of acquired immunodeficiency syndrome (AIDS) by Montagnier, Gallo)
1997	HAART treatment for AIDS.
2003	Severe acute respiratory syndrome (SARS) is caused by a novel coronavirus.
2005	Hepatitis C virus propagation in tissue culture by Chisari, Rice, and Wakita.
2005	1918 influenza virus genome sequencing.

VIRUS DIVERSITY

Viruses are minute, non-living entities that copy themselves once inside the living host cells. All living organisms (animals, plants, fungi, and bacteria) have viruses that infect them. Typically viruses are made up of coat (or capsid) that protects its information molecule (RNA or DNA); these information molecules contain the blue prints for making more virus. The viruses are highly diverse in their shape, size, genetic information, and infectivity. Viruses are all around us, on an average a human body encounters billion virus particles every day. Our intestinal, respiratory, and urogenital tract are reservoirs for many different kinds of viruses, it is astonishing that with such constant exposure, there is little or no impact of these organisms in human health. The host defense mechanism is quite strong to remove all these in normal condition, while they cause many nasty diseases only when the person is immune-compromised. Although viruses have a limited host range but sometimes they may jump the species barrier and causes fatal disease, recent spread of swine influenza is an ideal example of such kind of spread.

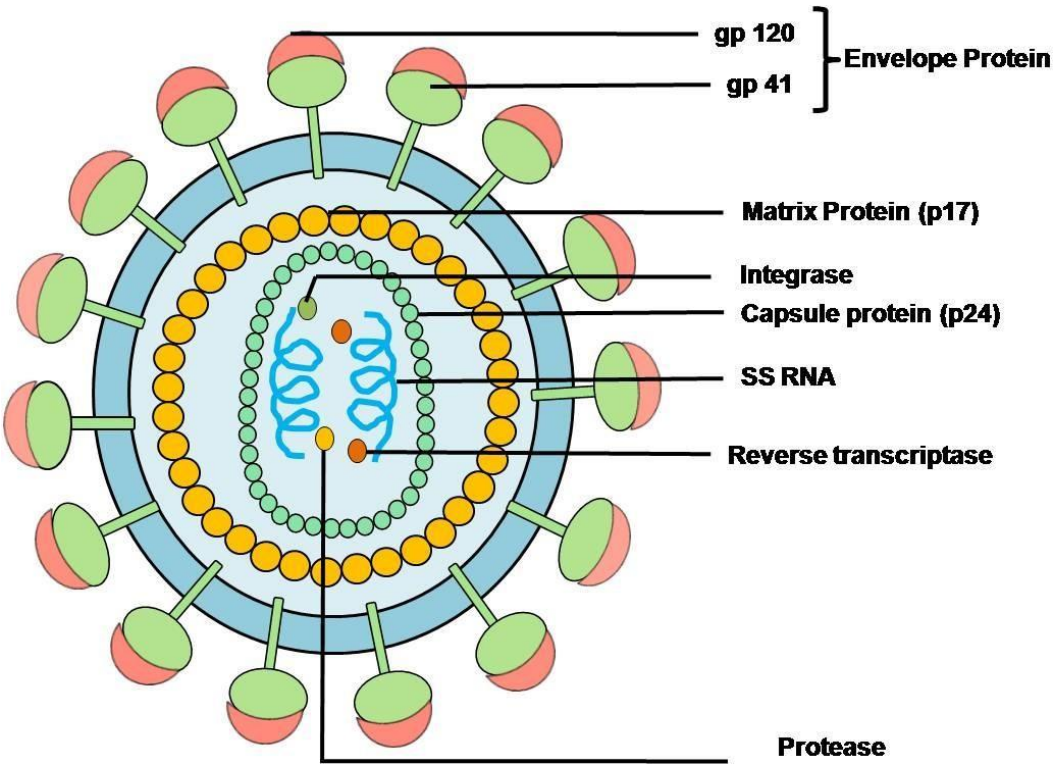
The epidemic viruses, such as influenza and severe acute respiratory syndrome (SARS), cause diseases that rapidly spread to a large human population within no time, and seem to attract more scientific and public attention than do endemic viruses, which are continually present in a particular population.

Virology as a discipline is merely 100 years old and the way it expanded in this small period of time is rampant. To group the new emerging viruses in a specific group by specifying certain parameters was initiated in 1966 when international committee on the taxonomy of viruses (ICTV) was formed with the aim to classify the viruses. The ICTV has adopted a norm for the description of the viruses. Name for genera, subfamilies, families, and orders must all be a single word, ending with the suffixes -virus, -virinae, - viridae, and -virales respectively. In written usage, the name should be capitalized and italicized.

Viruses are obligate parasite which means their absolute dependence on living host system. This property of virus made it a valuable tool to study cell functions and its biology. Adenovirus is an example of DNA virus that enters the host nucleus but remains separated from the host genome and at the same time use host cell machinery for

its replication. On the other hand influenza is a RNA virus that carries its own enzyme to replicate its genome while the viral proteins are synthesized by using the host cell machinery. Human immunodeficiency virus (HIV) is a retrovirus; it contains RNA as a genetic material but it converts into DNA after entering the host cell by an enzyme called reverse transcriptase. It also contains enzymes in its virion namely, integrase and viral protease which helps HIV during maturation process inside the infected cells. Outer surface of HIV virion contains two surface glycoproteins called as gp120 and gp41 which helps in the attachment of virus to the cell surface.

Figure 1.1. Schematic diagram of HIV



VIRUS SHAPES

Early study with tobacco mosaic virus (TMV) strongly suggested that viruses were composed of repeating subunits of protein which was later supported by crystallization of TMV. A major advancement in determining the morphology of virus was the development of negative stain electron microscopy. Another modification of classical electron microscopy is cryo-electron microscopy where the virus containing samples were rapidly frozen and examined at a very low temperature; this allows us to preserve the native structure of the viruses.

A virion is a complete virus particle that is surrounded by the capsid protein and encapsidates the viral genome (DNA or RNA). Sometime structure without nucleic acid can be visible under the electron microscope those structures are called as empty capsids. In some of the viruses like paramyxoviruses the nucleic acid is surrounded by the capsid proteins and the composite structures are referred as nucleocapsid. Some of the viruses contain the lipid envelope which surrounds the nucleocapsids. The envelopes are derived from the host cell membrane during the budding process. As the envelopes are derived from host cell membrane they contain many of the surface proteins present in the host cells.

There are two kinds of symmetry found among the viruses: icosahedral and helical. In theory the icosahedral symmetry may sometime referred as spherical based on the external morphology. Icosahedral symmetry has 12 vertices, 30 edges, and 20 faces. They also have two, three, or five fold symmetry based on the rotation through axes passing through their edges, faces, and vertices respectively (Figure 3.1). The viruses of this kind look spherical in shape. In helical symmetry the genomic RNA forms a spiral within the core of the nucleocapsids (Figure 3.2). The viruses of this kind look rodlike or filamentous. The viruses which contain large DNA genomes are more complex in structure, for example- poxviruses and herpesviruses.

Figure.1.2. An icosahedral virion structure showing two, three, and fivefold symmetry

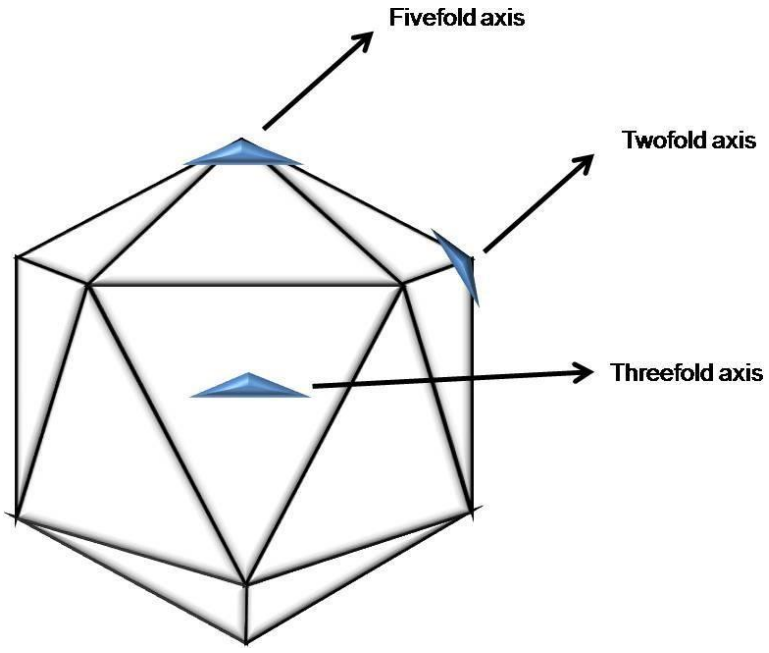


Figure 1.3. Virus structure with helical symmetry

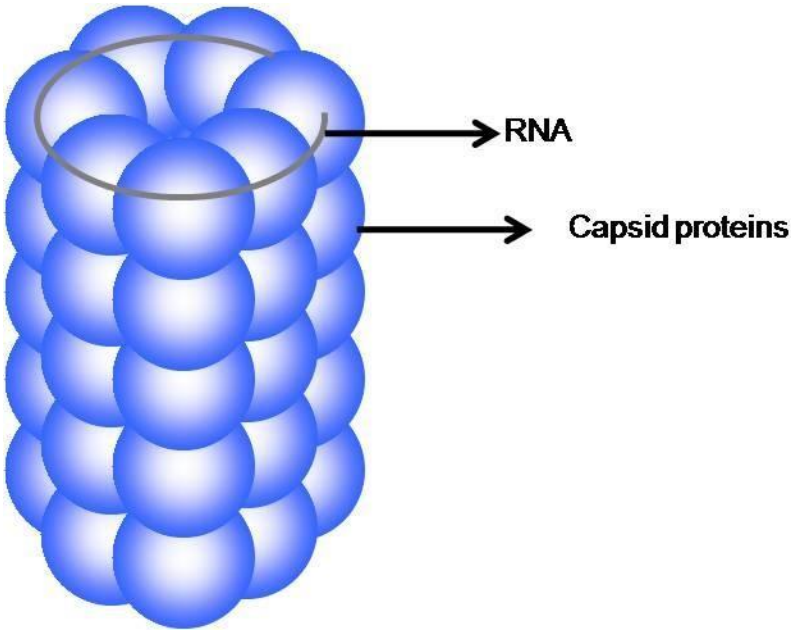


Table 1.1. Shape of viruses belonging to different families

Family	Shape
Poxviridae	Pleomorphic
Iridoviridae	Icosahedral
Asfarviridae	Spherical
Herpesviridae	Icosahedral
Adenoviridae	Icosahedral
Polyomaviridae	Icosahedral
Papillomaviridae	Icosahedral
Hepadnaviridae	Spherical
Circoviridae	Icosahedral
Parvoviridae	Icosahedral
Retroviridae	Spherical
Reoviridae	Icosahedral
Birnaviridae	Icosahedral
Paramyxoviridae	Pleomorphic
Rhabdoviridae	Bullet shaped
Filoviridae	Filamentous
Bornaviridae	Spherical
Orthomyxoviridae	Pleomorphic
Bunyaviridae	Spherical
Arenaviridae	Spherical
Coronaviridae	Spherical

Arteriviridae	Spherical
Picornaviridae	Icosahedral
Caliciviridae	Icosahedral
Astroviridae	Icosahedral
Togaviridae	Spherical
Flaviviridae	Spherical

VIRUS SIZE

Viruses are generally much smaller than the bacteria and its average size varies from 25- 300 nm in diameter. They are visible under electron microscope and only the largest and complex viruses are seen under light microscope with high resolution. Among all, the smallest viruses belong to the families *Circoviridae*, *Parvoviridae* and *Picornaviridae* which measure about 20 - 30 nm in diameter while the largest one belongs to *Poxviridae* that measures around 250-300 nm in diameter. Recently, scientists isolated a new form of virus that infects amoeba and grouped it under a separate family *Mimiviridae*. The members of the family *Mimiviridae* range from 400-800 nm in diameter.

On an average a bacterial cell is about 1400 nm in diameter while an average epithelial cell is about 20,000 nm. Considering both viruses and bacteria to be nearly spherical a bacterial cell has a volume about 30,000 times greater than a virus while an epithelial cell is about 60 million times larger.

Table 1.2. Size of viruses belonging to different families

Family	Size (nm)
Poxviridae	300
Iridoviridae	135-300
Asfarviridae	170-220
Herpesviridae	150
Adenoviridae	80-100
Polyomaviridae	40-50
Papillomaviridae	55
Hepadnaviridae	50
Circoviridae	12-27
Parvoviridae	15-25
Retroviridae	80-100
Reoviridae	60-80
Birnaviridae	60
Paramyxoviridae	150-250
Rhabdoviridae	100
Filoviridae	80
Bornaviridae	80-100
Orthomyxoviridae	80-120
Bunyaviridae	80-120
Arenaviridae	50-280
Coronaviridae	120-150
Arteriviridae	60-70
Picornaviridae	30

Caliciviridae	30-40
Astroviridae	30
Togaviridae	70
Flaviviridae	40-60

COMPONENTS OF GENOMES

In general the viruses are made up of nucleic acids (genome), proteins (capsid), and lipids (envelope). Viral genomes can be either DNA or RNA, when once inside a host cell it directs synthesis of new viral proteins, and replication of new viral genomes. Capsid is a protein covering that surrounds and protects the viral genome. It is made up of many small subunits called as capsomeres which determine the shape of the virus. The arrangement and composition of the capsomeres varies among the virus families. Envelopes are the lipid bilayer membranes that are derived from the host cell membrane when virus "buds" out from the plasma membrane or passes through a membrane-bound organelle (such as the Golgi body or endoplasmic reticulum). The envelope contains sometimes glycoprotein (protein with carbohydrate) in the form of spikes which helps them in the attachment during the time of infection to the host cell surface (gp120 in HIV). In non-enveloped viruses, grooves present in the capsid and specific capsid proteins may bind to the cell surface receptor.

The most important and characteristic feature of a living organism is replication of its genetic information. The mechanism of genome replication is done with greater economy and simplicity among different viruses. Different families of viruses have their genome made of either double stranded (ds) DNA or single stranded (ss) DNA or RNA. The viruses that contain RNA genome may have either positive, negative, or mixed (ambisense) polarity. In addition, they either have single or multiple segments in their genome with linear or circular topology. Each of the above parameters have their consequences for the pathways of viral genome replication, viral gene expression, and virion assembly.

Among the families of viruses that infect animals and human, those containing RNA genome outnumber those containing DNA genome. This disparity is even more in case of plant viruses (no double stranded DNA virus that infect plant is known).

5.1. Viruses encode enzymes and follow unique pathways:

Almost all viruses encodes unique proteins and enzymes, moreover they follow unique pathways to transfer their genetic information. This phenomenon is more pronounced in case of RNA viruses, they either use RNA dependent RNA polymerase or in case of retrovirus (HIV) RNA dependent DNA polymerase to complete their replication cycle. Both of these processes requires unique enzymes that are encoded by the virus following infection to the host cells and are generally absent elsewhere.

The RNA dependent RNA polymerase and reverse transcriptase have minimal proofreading ability, as a result their error rate is very high (1 in 10,000) as compared to the DNA replication. This means that an RNA virus particle will contain 1 or more mutation from its parental wild type virus. Presence of many different subspecies of virus particle in a population is also called as quasispecies nature of RNA viruses. The error prone activity of

RNA virus polymerase restricts the upper size limit of the genome above which they cannot survive. As a result of this phenomenon most of the RNA virus have their genome size in the range of 5-15kb (coronavirus 30kb). The opposite is true in case of DNA viruses where proofreading and error repair activity ensures accurate replication of the viral DNA as big as 800 kb. The fact that DNA is more stable chemically than RNA likely explains us why all thermophilic hosts contain viruses that have dsDNA as their genetic material.

Table 1.3.. Nature of genome of viruses belonging to different families

Family	Nature of Genome
Poxviridae	dsDNA
Iridoviridae	dsDNA
Asfarviridae	dsDNA
Herpesviridae	dsDNA
Adenoviridae	dsDNA
Polyomaviridae	dsDNA
Papillomaviridae	dsDNA
Hepadnaviridae	dsDNA-RT
Circoviridae	ssDNA
Parvoviridae	ssDNA
Retroviridae	ssRNA-RT
Reoviridae	dsRNA
Birnaviridae	dsRNA
Paramyxoviridae	NssRNA
Rhabdoviridae	NssRNA
Filoviridae	NssRNA
Bornaviridae	NssRNA
Orthomyxoviridae	NssRNA
Bunyaviridae	NssRNA
Arenaviridae	NssRNA
Coronaviridae	ssRNA
Arteriviridae	ssRNA
Picornaviridae	ssRNA
Caliciviridae	ssRNA
Astroviridae	ssRNA
Togaviridae	ssRNA
Flaviviridae	ssRNA

dsDNA= double stranded DNA
ssDNA= single stranded DNA
dsRNA= double stranded RNA
ssRNA= single stranded RNA
NssRNA= single stranded RNA with negative polarity

Figure 1.4. Diversity among the viruses belonging to different groups

ISOLATION AND PURIFICATION OF VIRUSES AND COMPONENTS

Virus Isolation

Viruses are obligate intracellular parasites that require living cells in order to replicate. Generally cell culture, embryonated eggs and small laboratory animals are used for the isolation of viruses. Embryonated eggs are very useful for the isolation of influenza and paramyxoviruses. Although laboratory animals are useful in isolating different kind of viruses, cell culture is still a preferred way for virus isolation in many of the laboratories.

For primary cell cultures, tissue fragments are first dissociated into small pieces with the help of scissors and addition of trypsin. The cell suspension is then washed couple of times with minimal essential media and seeded into a flat-bottomed glass or plastic container bottle after resuspending it with a suitable liquid medium and fetal calf serum. The cells are kept in incubator at 37^0C for 24 to 48hrs depending on the cell type. This allows the cells to attach the surface of the container and its division following the normal cell cycle.

Cell cultures are generally of 3 types:-

1. Primary culture – These are prepared directly from animal or human tissues and can be subcultured only once or twice e.g. chicken embryo fibroblast.
2. Diploid cell culture – They are derived from neonatal tissues and can be subcultured 5-10 times. e.g. human diploid fibroblasts cells.
3. Continuous cells – They are derived from tumor tissues and can be subcultured more than 10 times. e.g. Vero, Hep2, Hela.

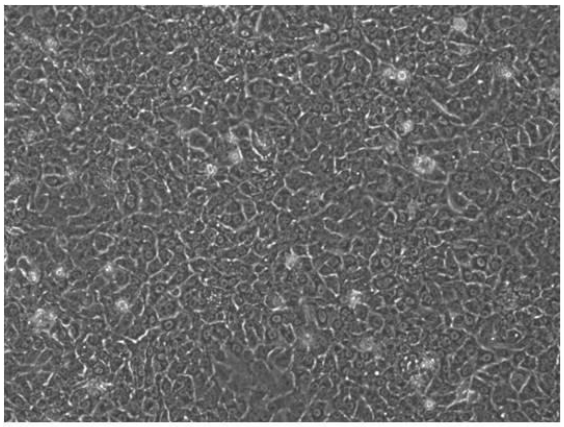
Specimens containing virus should be transported to the laboratory as soon as possible upon being taken. Oral or cloacal swabs should be collected in vials containing virus transport medium. Body fluids and tissues should be collected in a sterile container and sealed properly. If possible all the samples should be maintained and transported in a cold condition for higher recovery rates.

Upon receipt, the samples should be inoculated into cell culture depending on the history and symptoms of the disease. The infected cell culture flask should be observed every day for any presence of cytopathic effect (CPE). Certain kind of samples, such as faeces and urine are toxic to the cell cultures and may produce a CPE-like effect. When virus specific CPE is evident, it is advised to passage the infected culture fluid into a fresh cell culture. For cell-associated viruses such as cytomegaloviruses, it is required to trypsinize and passage the intact infected cells. Viruses such as adenovirus can be subcultured after couple of time freezing and thawing of the infected cells.

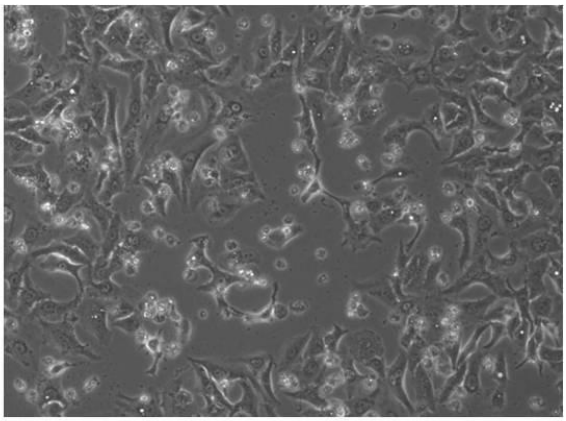
Susceptible cell lines:

Influenza virus- MDCK cells, Vero cells.
Paramyxoviruses- DF-1 cells, Vero cells.
Adenoviruses- HEK cells, HuH7 cells.
Herpesviruses- LMH cells.
Respiratory syncytia virus- Hep2 cells, Vero cells.

Figure 6.1. Virus induced CPE in cell culture



Normal human epithelial cells



Adenovirus infected human epithelial cells

Purification of virus and components:

Ultracentrifugation:

The viruses are usually purified with the help of ultracentrifugation. The machine is capable of rotating the samples at 20,000-100,000 rpm under the density gradient of CsCl2 or sucrose. Density at which viruses neither sink nor float when suspended in a density gradient is called as buoyant density. The rate at which viral particles sediment under a defined gravitational force is called as sedimentation coefficient. The basic unit is the Svedberg (S) which is 10^{-13} sec. The S value of a virus is used to estimate its molecular weight.

Types of sedimentation medium:

A. Sucrose cushions or gradient - A fixed concentration or a linear gradient of sucrose is used. Increasing the density and viscosity of the medium decreases the rate at which virus sediments through them. In general a "cushion" of sucrose is prepared at the bottom of the centrifuge tube and the sample containing virus is overlaid over the cushion. Since most viruses have greater densities than sucrose, separation is based on S values. This

method can be used to separate molecules with relatively close S values. Sometime glycerol is also used in place of sucrose.

B. $CsCl_2$ gradient centrifugation - A linear gradient of $CsCl_2$ in buffer is prepared in the ultracentrifuge tube. As the concentration of the $CsCl_2$ is increased the density of the medium increases in the tube so that density is low at the top and high at the bottom. Viral particle centrifuged through this medium will form a band at a position equal to their buoyant density. These are useful to separate viruses of different densities. Limitation of this method is that $CsCl_2$ can permanently inactivate some viruses.

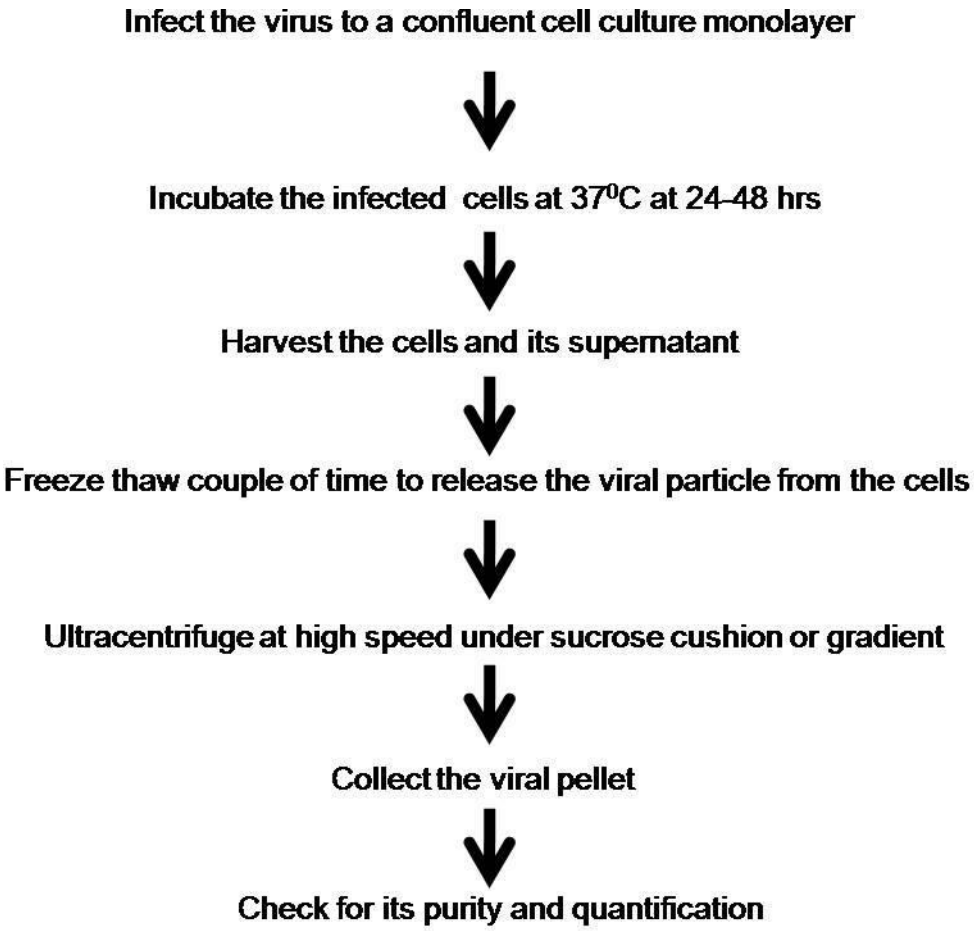
Other techniques for separation:

Viruses can also be separated by electrophoresis and column chromatography but these are not the preferred way to separate virus while sometimes they are used to separate viral nucleic acids or proteins. Both the methods separate the virus on the basis of charge and/or size. Virus contains a variety of charged macromolecule on its surface which contributes to its electrophoretic mobility or ion-exchange characteristics. Viruses are sometimes ligated with the charged group to be separated by ion exchange chromatography. Molecular sieve chromatography can also be used to purify the viruses where large pores are formed with the help of special agarose through which virus particles can enter.

Purity of viruses:

Many methods are used to assess the purity of virus. The ratio of UV absorption at 260 and 280 nm during a spectrophotometric analysis (260/280) is a characteristic feature to measure the purity of a virus sample and is dependent on the amount of nucleic acid and protein present in the virion. Serological methods such as enzyme-linked immunosorbent assay (ELISA), radioimmuno precipitation assay (RIPA), western blot, virus neutralization test (VNT), and complement fixation are also used to check the puirity of a virus sample. These methods require antibodies specific to viral proteins that may be monoclonal (single type of antibody specific to a single viral protein) or polyclonal (several different antibodies that may recognize several viral proteins or epitopes). Plaque assay is also performed in order to isolate the single colony from a pool of quasispecies viruses.

Figure 1.5. A general approach for purifying a virus from tissue culture cells



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VIRUS HOST INTERACTION

CONSEQUENCES OF VIRUS INFECTION TO ANIMALS AND HUMANS

Virus contains its genetic material in the form of nucleic acid (DNA/ RNA) surrounded by a protein coat called as capsid. Viruses are the obligatory intracellular parasites of cells. This means that the viruses can only replicate within a living host cell. The virus does this by subverting the biosynthetic pathways and protein synthesizing capacity of the cell. This helps the virus to replicate its viral nucleic acid, make viral proteins, and facilitate its escape from the parasitized cell.

In order to know the outcome of virus infection on the animal cells two factors play an important role -- **virulence** of the virus and the **susceptibility** of the host.

Virulence – It may be defined as the ability of the virus to cause disease or in other words it gives the relative degree of pathogenicity of the infecting virus. Viral virulence differs greatly among the strains depending on the pathogenic nature of the virus. Virus may be categorized as pathogenic or non- pathogenic. The pathogenicity of the virus range from mild to severe depending on the virulence of the viral strains. The term virulence is used as a quantitative measure of its pathogenicity. The degree of virulence is usually related with the ability of the pathogen to multiply within the host and depends on other factors such as host environment and its immune status.

Terms describing infections of an organism

Lytic infection- When virus enters the cell and hijacks its cellular machinery to rapidly multiply and in the process kills the cell is termed as lytic infection (many influenza viruses).

Lysogenic infection- It is the process characterized by the incorporation of viral DNA to the cellular DNA. Once incorporated, the viral DNA replicates along with the host DNA. The incorporated viral DNA permits the host cell to undergo normal cell cycle.

Acute infection- It is a rapid onset of disease symptoms resulting in severe illness or death of the infected animal (influenza, viral hemorrhagic fever).

Chronic Infection- It is a prolonged infection in which the organism is not immediately killed and may carry the virus for long period of time (hepatitis, HIV).

Terms describing virus transmission

Horizontal transmission is defined as the transmission of virus or other pathogen to host at any age after birth while **vertical** transmission is the passage of a virus from mother to

the new born child.

Zoonosis is defined as the disease which is naturally transmitted between animals and man (Rabies, H1N1 influenza virus, Rift valley fever virus).

Sometimes the virus can be transmitted through an insect vector (arboviruses). Viruses present in the saliva of the infected insect are transmitted during feeding of blood meal to the susceptible host.

Persistent infection is a condition where the virus remains associated with the cell without actively multiplying or killing it. This often occurs when the viral genome gets integrated into the host genome (retroviruses) and sometime without integration (Herpesvirus).
Persistence can be categorized into three types
(1) Virus genome persists within the cell without actual release of the virus, eg. Some retroviruses.
(2) Virus released sporadically but remains in a state of "latent" for most of the time (herpes simplex).
(3) Virus released continuously without lysis of the host cell, eg. hepatitis B virus.

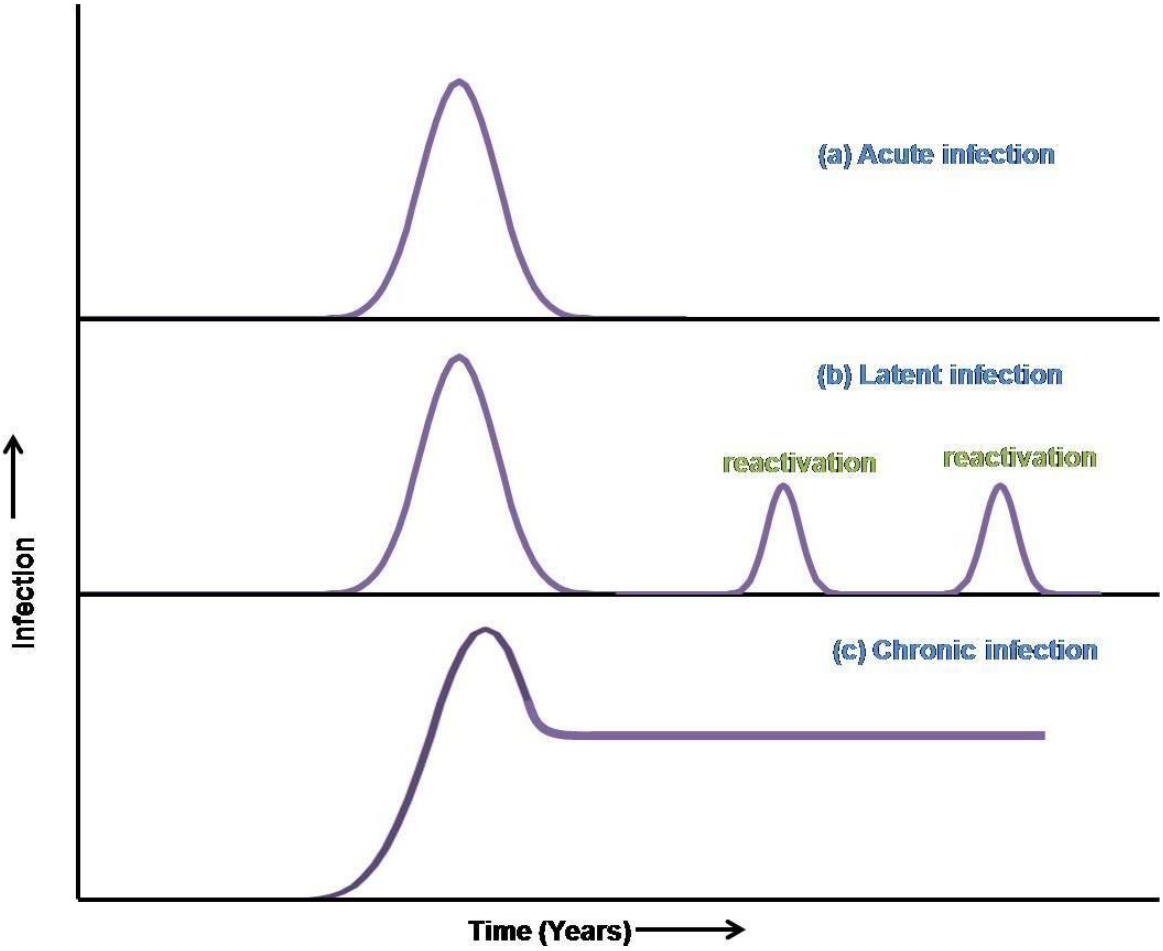
Multiplicity of Infection (m.o.i.)- This is the ratio of total virus infected to the number of target cells in an infection condition. This is usually used to describe the infection of a cell type grown *invitro* in a culture system.

Infectious dose50 (ID50)- The dose required to infect 50% of the inoculated animals.

Lethal dose50 (LD50)- The dose required to kill 50% of the inoculated animals.

Incubation period- The time between the initial infection to the actual onset of disease symptoms. This period can range from a few days (cold viruses) to years (HIV).

Figure 2.1 Schematic diagrams showing the patterns of viral infection.



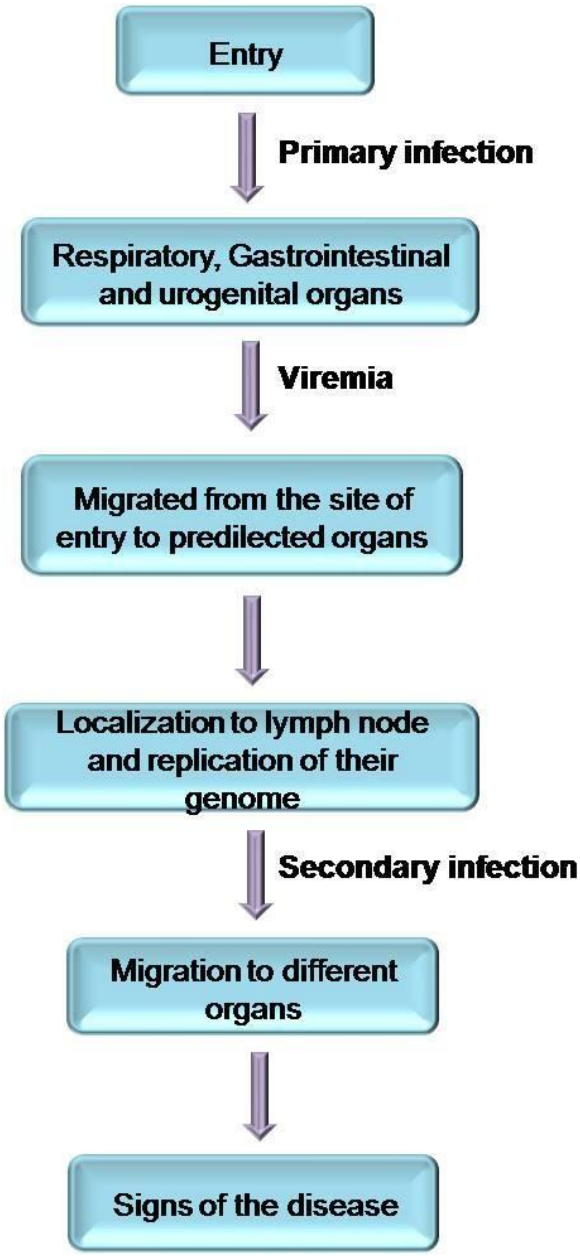
Virus entry to the host

The viruses generally enter the body through the epithelial surface of respiratory tract (influenza), alimentary tract (rotavirus), and reproductive tract (HIV). Sometimes they gain entry through small wounds in skin like insect bites (yellow fever virus) or through large wounds after animal bites (rabies). Herpesviruses (cold sores) and Epstein-Barr virus (EBV) are transmitted mostly by the oral secretions, while the HIV and herpes simplex virus are known to transmit vertically through mother to offspring. The disease caused by a virus is more generalized if it enters through the epithelial lining of the body (mumps, smallpox, measles etc).

7.1 Stages of viral infection

Primary infection occurs when virus enters the body through different portals. The viruses then enter into the blood streams and targeted to different organs, the stage is known as **viremia**. After entry into the redirected site they start their replication and transmitted to different organ and may shed outside through body secretions, the condition is referred as **secondary infection** (infection of brain tissue by encephalitis virus and liver by the hepatitis virus).

Figure 2.2 Schematic representation of viral infection from entry to the signs of the disease.



CONSEQUENCES OF VIRUS INFECTION TO ANIMALS AND HUMAN

Respiratory tract disease

Virus induced respiratory infections kill about millions of humans and children worldwide each year. Most viruses that infect only the upper and lower respiratory tract do not induce a strong immune response and therefore chances of reinfection with the similar or same strain is very common. On an average children get about 6 colds a year and adults 2-3.

Rhinitis (Common Cold) – Common signs and symptoms include nasal discharge and obstruction, sneezing, coughing, and mild sore throat.

Pharyngitis (mostly viral)- Common signs are sore throat, malaise, fever, and cough. RSV and adenovirus are the predominant causes in young children while Herpes viruses in young adults.

Laryngotracheobronchitis (Croup)- Common symptoms include fever, cough, respiratory distress, sometimes laryngeal obstruction. Most common causes are influenza and parainfluenza virus.

Bronchiolitis- Common signs and symptoms includes rapid and labored breathing, persistent cough, wheezing, cyanosis, atelectasis (Lung collapse), and emphysema. Major causes are Influenza, parainfluenza, and RSV.

Pneumonia- usually develops following upper respiratory tract infection. Symptoms include fever, cough, and difficulty in breathing. RSV, Influenza, parainfluenza, and adenoviruses are the major causes. It is a major cause of death to older people and young children. RSV is the major cause of death in young ones.

Gastrointestinal tract disease

It involves inflammation of the stomach and intestines leading to watery diarrhea. Fever and vomiting are common with some viral gastroenteritis. Diarrheal diseases kill 2 million children each year mostly in developing countries. Rotaviruses are the main cause of deaths. Astroviruses and Caliciviruses (Norwalk virus) can also cause diarrhea.

Central Nervous system diseases

Some viral infections can cause pathogenicity in the brain and spinal cord (central nervous system [CNS]). Viruses may be neuroinvasive (able to enter the CNS after crossing the blood brain barrier) and/or neurovirulent (can cause damage to the nerve cells). Mumps virus is highly neuroinvasive but not very neurovirulent while herpesviruses are more neurovirulent. Viruses can cause disease in a variety of ways including infection of a specific area of the brain or infect systemically to the CNS. Sometime their infection causes lysis of the neurons while other type of infections can cause demyelination of axons.

Meningitis- Virus infects the meningeal cells of the CNS. Symptoms include headache, fever, and neck stiffness with/or without vomiting. Mumps and Enteroviruses are most common agents.

Poliomyelitis- The disease involves demyelination of nerve cells and is most common in the countries where polio virus has not been eradicated.

Encephalitis- Symptoms include fever, headache, stiffness of the neck muscles, vomiting, and deviations from the normal state of consciousness. Patients are often lethargic and show signs of seizures. Sometimes paralysis may develop before coma and death. Recovered patience may show mental retardation, epilepsy, paralysis, deafness, and blindness. Many Arboviruses and Herpesviruses are associated with the severe form of encephalitis. **Guillain-Barre syndrome** is a condition caused by Epstein - Barr virus (EBV) infection. Similar kind of condition seen in the patients infected with influenza or chickenpox which are under aspirin treatment and are characterized by cerebral edema. The condition is often lethal and known as **Reye's syndrome**.

Urogenital system diseases

Herpes simplex virus and papillomaviruses are the major viruses infecting the genital area. Sexual transmission is the main way of acquiring these agents. Herpesvirus infection manifests as painful itching and ulcerated vesicular lesions occasionally accompanied by fever and malaise especially in woman. Recurrences are common although generally less severe than the initial infection. Certain types of HPV may progress over several years through stages of cervical neoplasia to invasive squamous cell carcinoma.

VIRAL DISEASE AFFECTING OTHER ORGANS AND SYSTEMS

Eye diseases- Many infants viral diseases can involve conjunctivitis (Inflammation of the conjunctiva which is the transparent membrane covering the sclera). It leads to redness, discomfort and discharges from the eye and is commonly termed as **pink eye** condition. Sometime it is also associated with cornea (kerato-conjunctivitis). Herpes simplex virus is the most common form of virus associated with this condition. Many other kind of eye disorder including cataract and glaucoma are associated with rubella virus and cytomegalovirus infection.

Viral Hepatitis- Inflammation of the liver accompanying damage of the hepatocytes (liver cells) is called as hepatitis. Besides Hepatitis viruses A, B, C, D, E, and G which infect the liver as the primary organ other viruses can also cause hepatitis such as herpes and hemorrhagic fever viruses. Symptoms include jaundice (yellowing of the skin caused by accumulation of bilirubin in the blood), and flu-like symptoms. The disease may become chronic depending on the infectious agent and terminally leads to cirrhosis (fibrosis of the liver tissue).

Viral arthritis- Characterized by stiffness in the joint accompanied by pain, fever, and myositis (inflammation of muscle tissue). The major causative agents are flaviviruses, togaviruses, and bunyaviruses.

Hemorrhagic fever- Symptoms include widespread hemorrhages from the epithelial tissue including eyes, ears, nose, and gastrointestinal tract. Ebola, yellow fever virus, Hantavirus, Lassa fever virus, and Marburg virus are the common cause of viral hemorrhagic fever. Severe damage of the internal organs is often associated with viral hemorrhagic fever. Ebola and Yellow fever virus can cause severe damage to the hepatocytes.

Chronic fatigue syndrome- There is no such evidence of any virus to be associated with this condition. The disease is characterized by extreme fatigue and is most common following the infection of CMV, EBV, enteroviruses, and HTLV.

Viral carditis-myocarditis- Characterized by inflammation of the heart muscles. The disease is often associated with certain enteroviruses (a family of picornaviruses) such as coxsackie B virus. The infections usually reoccurs leading to permanent myocardial damage, enlargement of the heart, or congestive heart failure.

Viral infection: Affect on host macromolecules

A cell is said to be **permissive** when it supports the virus multiplication. Viruses infecting the permissive cells are usually **cytocidal** (kill the host cell) while infection to non- permissive cells do not produce any effect upon infection hence called **abortive.** When the virus replication gets completed, no more viral mRNA or protein are produced in the infected cells and is referred as **restricted**. In some cases viral DNA or RNA may sequester indefinitely inside a host cell and this condition is called as **persistent infection**.

Cytolytic infections

Cytolytic infections can be clearly visualized under a light microscope. The characteristic of CPE effect is an important parameter for a virologist to identify the virus species. In some viral infections inclusion bodies which are formed upon viral infection are identified after specific staining methods and are used as a tool for identifying the virus. Seller's stain is used to visualize the Negri bodies in the cells infected with Rabies virus. Inclusion bodies are the remnants of viral structural and non-structural proteins. Alternatively, inclusion bodies may be formed by a host cell macromolecule upon virus infection. For example, Cytomegalovirus infection to a cell changes the cytoskeleton of infected cell which are then visible as inclusion bodies. Viral infection to a permissive cell is often associated with changes in cellular biosynthetic pathways, its morphology, and cell physiology.

Table 2.1 Viral inclusion bodies in some human diseases

Virus	Location in Cell	
	Nucleus	Cytoplasm
Adenoviruses	Cowdry type A	
Herpesviruses	do	Present (cytomegalovirus)
Rabies virus		Negri bodies
Reovirus		Present
Vaccinia		Guarnieri bodies

Effects on biosynthetic pathways

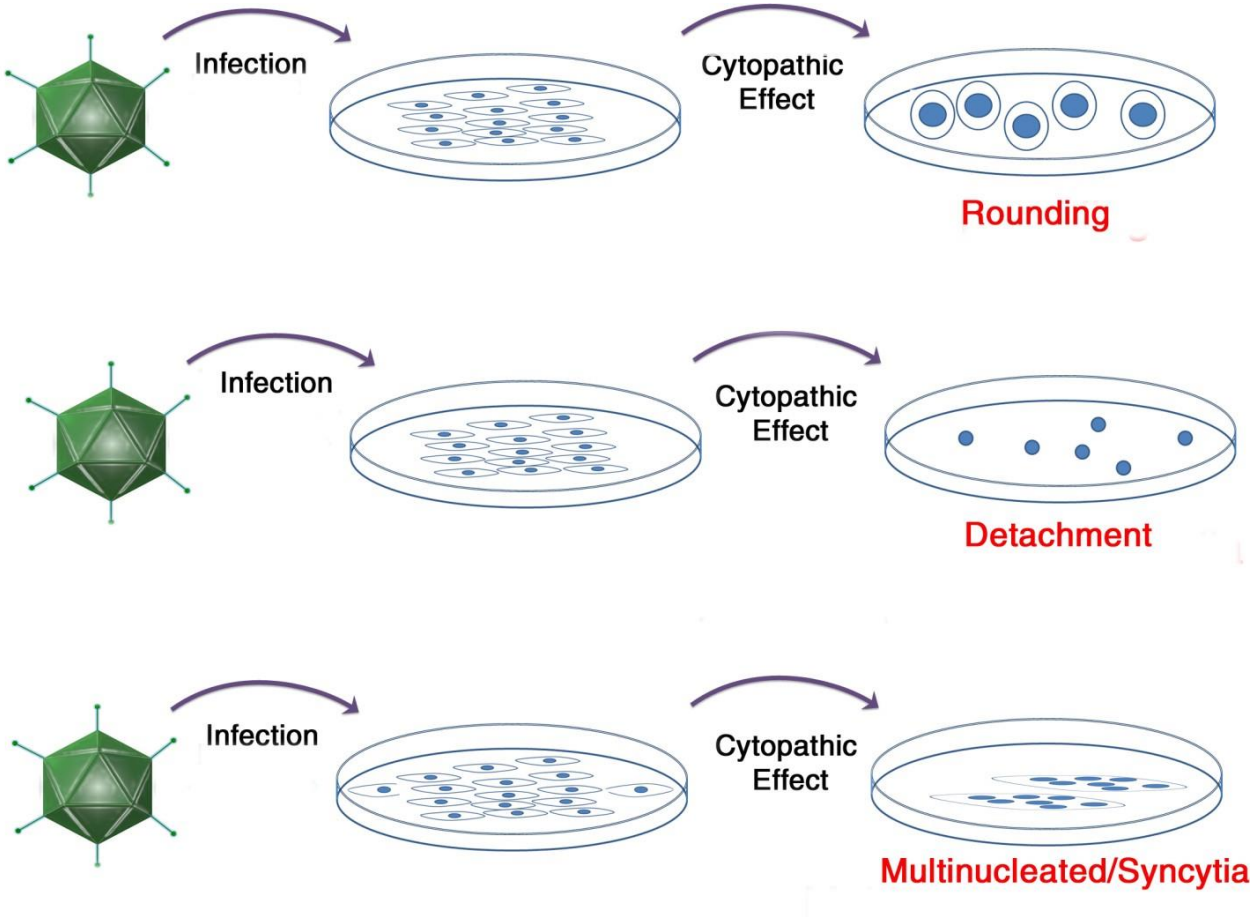
Virus infection to a host cell inhibits its DNA and/or RNA, and its protein synthesis. Sometimes it also causes breakage and fragmentation of host chromosome. Moreover it also changes the growth characteristics, shape, and surface protein expression of the infected host cell. Viruses often subvert the host biosynthetic pathway for their own benefits at the cost of cellular macromolecules.

Virus infection to a cell forms many early proteins that mediate the changes in cellular biochemical pathways. Viral nucleic acid contains specific signal sequences that help in migration of nucleic acids to different cellular locations. In addition, it also contains some motifs that bind to regulators of cellular transcriptional machinery. Therefore many viral early proteins contain binding sites for a wide range of cellular transcriptional factor. These interactions and bindings are very important for the activation of virus protein synthesis and production of progeny viruses. The biochemical events sometimes include glycosylation and phosphorylation of viral proteins. These modifications are often associated with the increase or decrease of the pathogenicity and virulence of the viruses. Generally virus alters the cascades that are involved in the synthesis of protein kinases and secondary messengers (cyclic AMP,cyclic GMP, etc). Occasionally virus triggers the cells to overproduce regulatory proteins that changes the cellular biochemical pathways. These regulatory proteins may be transforming growth factors, interleukins, cytokines, NF-kβ or TNFα and TNFβ (HIVand Herpesviruses). In some viral infections cellular mRNA get degraded (Influenza virus). Alternatively herpesviruses and reoviruses inhibit the cellular DNA synthesis. Interestingly Pox virus degrades the host DNA with the help of virus associated DNase.

Effects on cell morphology

Changes evident in a cell following the virus infection are called cytopathic effects (CPE). There are various kinds of CPE depending on type of infection. For example, detachment of cells from monolayer, rounding of cells, formation of syncytia (multinucleated cells formed after fusion of nuclei) and nuclear or cytoplasmic inclusion bodies formation.

Figure 2.3. Cytopathic effects in the cells infected with viruses



Effects on cell Physiology

Virus infection to a cell changes many of the physiological events, including changes in cellular metabolism, alteration in the ATP synthetic pathways, and deviation in the ion channel system. Physiological condition of a viable cell has a great effect on the outcome of a virus infection because the host cell provides the cellular machinery, regulatory proteins, and source for the viral nucleic acid, and protein synthesis. Attachment of virion with the receptors present on cell membrane leads to a series of events that are associated with the changes in morphological, physiological and biochemical characteristics of the cell. The receptor present on the cell surface determines the host range as well as tissue tropism of a viral species. Influenza virus infects the cell after binding to the sialic acid

receptor present on the cell membrane. Similarly HIV infects the T-cells upon binding to the chemokine receptors of the cell. Usually virus infection alters the intracellular ion concentration that affects the cell membrane permeability (For example picornaviruses).

Table .2.2. .Proposed cell membrane receptors for some viruses

Viruses	Representative cell type	Receptors
DNA viruses		
Adenovirus	Respiratory epithelium	Integrins
Epstein-Barr virus	B lymphocytes	CD21
Hepatitis B virus	Liver	IgA
Herpes simplex virus	Oral and genital epithelium	Heparin sulfate
Vaccinia virus	Oropharyngeal epithelium	Epidermal growth factor receptor
RNA viruses		
Echovirus	Alimentary epithelium	Integrins
Influenza	Respiratory epithelium	Sialic acid
HIV	T lymphocytes	CD4
Measles virus	Respiratory epithelium	CD46
Paramyxovirus	Respiratory epithelium	Sialic acid
Poliovirus	Oropharyngeal cells	Polio virus receptor
Rabies virus	Neurons	Acetylcholine receptor
Reovirus	Neurons, lymphocytes	Adrenergic receptor
Rhinoviruses	Nasal epithelium	Intercellular adhesion molecules

Effect on host chromosome

Virus infection to a cell directly or indirectly leads to the damage of the host cell chromosome that may be lethal to the cell. If the cell does not die, viral genome may persist within the cell causing instability of cellular genome and alteration in the expression of proteins.

Viral infection: Effect on host macromolecules

Persistent infection

In persistent infection virus is not eliminated from the cell. Persistent infection may be chronic, latent, and transforming. In chronic infection the spread of virus is checked by host immune system while in latent infection only few cells express the viral protein and virus replication is largely restricted. In transforming persistent infection cell undergoes genetic changes that results in malignancy.

Persistent infection may sometime cause autoimmune disease condition in the host cell. Newly viruses bud out from the cell membrane following the virus infection, this leads to change in the antigenicity of the host cell. Immune system recognizes it as a nonself and produces an immune response which eventually causes death of the cell. The immune response also causes formation of viral antigen-antibody (Ag-Ab) complexes which may get deposited into vital organs like brain and kidney. Deposition of Ag-Ab complex elicits inflammatory condition in those organs (nephritis and encephalitis)

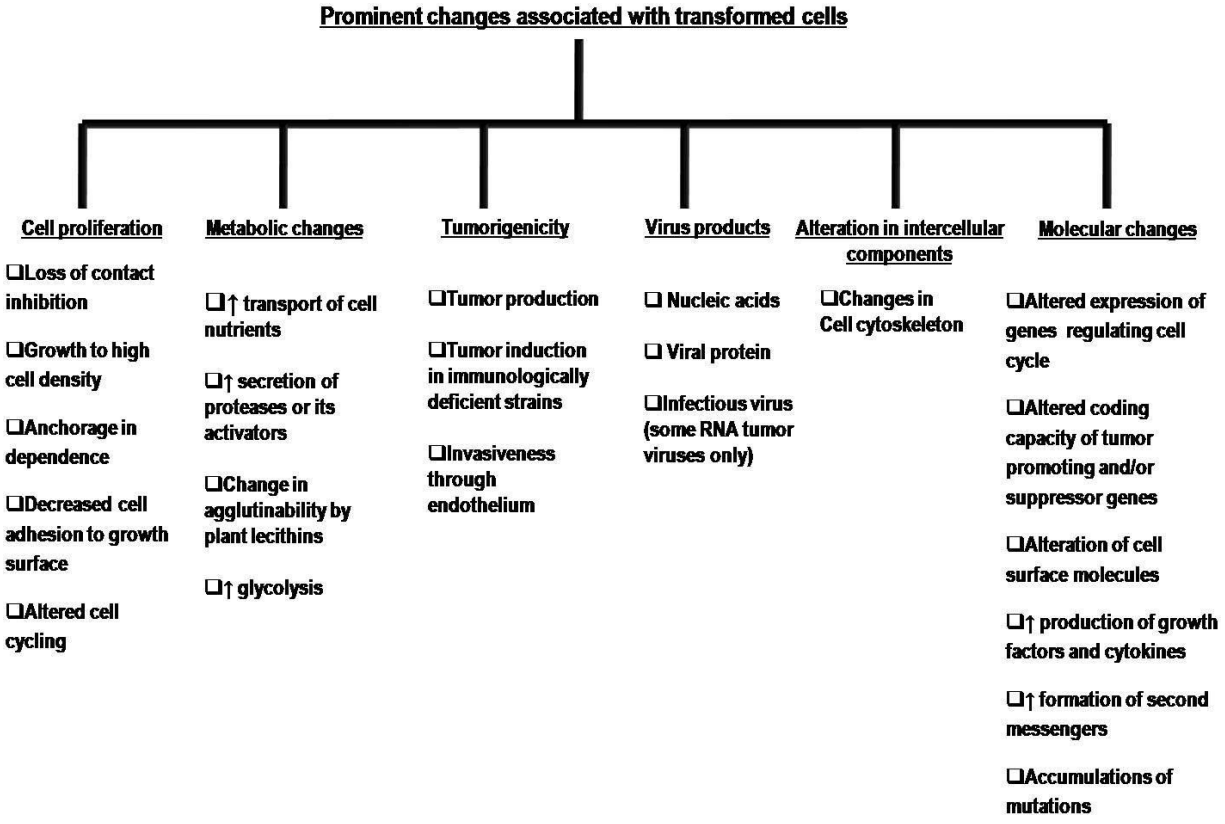
Transforming infection

Transformation refers to the ability of cells to multiply indefinitely that leads to cancerous condition. Mostly DNA viruses like Epstein Barr virus and polyoma virus can cause transformation in permissive cells. Transformation is essentially orchestrated by the viral proteins that may inactivate tumor suppressor proteins (Retinoblastoma proteins and p53) of the host cell.

Cellular transformation usually involves two stages namely

1) Immortalization and
2) Tumor production

Figure 2.4. List of changes associated with transformed cells:



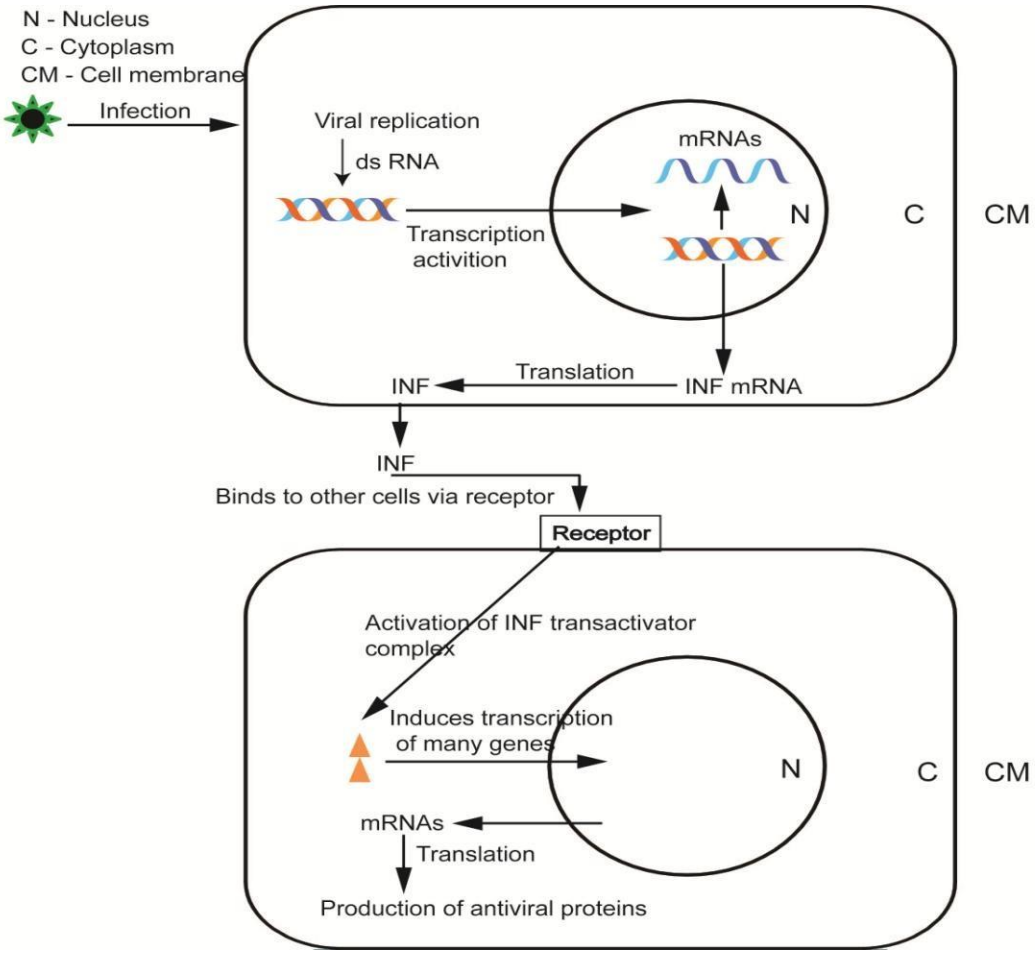
Viral infection: establishment of the antiviral state

Host immune response towards virus infection includes antibody mediated as well as cell mediated immune responses. Moreover macrophages, neutrophils and complement proteins also play an important role in clearing the virus infection from the body. Interestingly sometimes host cells sacrifice their life in order to protect other cells and restrict virus spread by a phenomenon called as **APOPTOSIS** (programmed cell death). Another very important player of host immune system that fights against viral invasion is **INTERFERON**.

INTERFERON:

Interferons are naturally occurring proteins secreted by cells in response to virus infections. When a cell is infected with a virus, it releases interferon which diffuses to the surrounding cells. After binding to the receptors present on the adjacent or surrounding cells; interferon stimulates the production of antiviral proteins in the cells.

Figure 2.5. Activation of interferon following virus infection:



Interferons are of two types

Type I (interferon α and β) and **type II** (interferon γ). Interferon α is produced by lymphocyte, β by fibroblast, and γ by T lymphocytes upon viral infection. The type of interferon are less or more potent against the class of virus species, for example, interferon α and β inhibits the vesicular stomatitis and encephalomyocarditis viruses better than interferon γ while interferon γ works better in case of vaccinia and reovirus infection.

How are interferons produced?

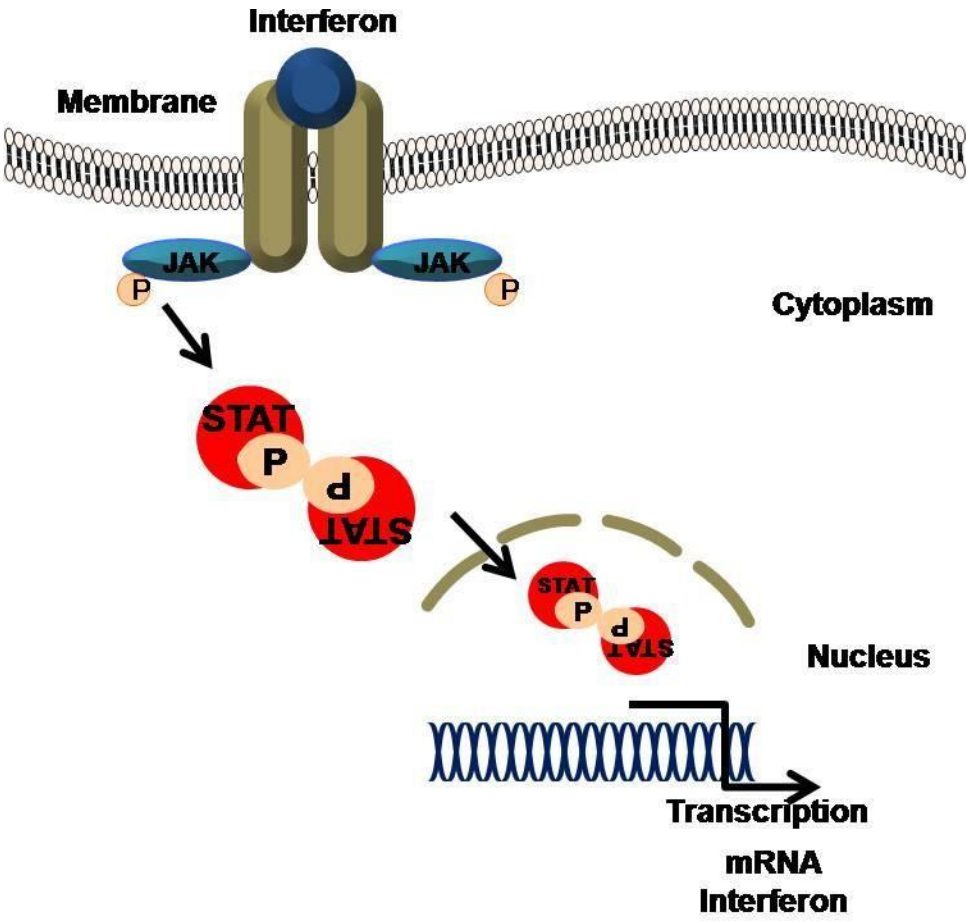
Generally all viruses can induce type-1 interferon production. Production of type-1 interferon is more pronounced in case of RNA viruses as compared to DNA viruses. In addition bacterial lipopolysaccharide and synthetic dsRNA analogs are known inducers of interferons. As a matter of fact dsRNA is a potent activator of interferon. dsRNA induce intereferon production by JAK/STAT signaling pathway. **Toll-like-receptor** 3 (TLR3) in presence of dsRNA can induce interferons by an alternate pathway. TLR7, TLR8, and TLR9 also induce interferon production through **interferon regulatory factor** 5 and 7 (IRF-5 and -7). Retinoic acid inducible gene 1 (RIG-1) activates interferon production by activating IRF- 3 and -7.

Interferons trigger **signal transducer and activator of transcription** (STAT) complexes by coordinating with their specific receptors. STATs belong to the family of transcription factors that control the expression of many immune system genes. Certain STATs are triggered by both type I and type II Interferons despite this each Interferon type can also activate unique STATs.

The classical **Janus kinase** – STAT (JAK-STAT) signaling pathway is the most explicit cell signaling pathway for all interferons which is also triggered by STAT activation.

The pathway involves coordination between JAKs and interferon receptors and phosphorylation of STAT1 and STAT2. Consequently this leads to the formation of a complex called as an **Interferon-stimulated gene factor** 3 (ISGF3). This complex comprises of STAT1, STAT2 and a third transcription factor called IRF9. After its formation the complex moves inside the cell nucleus where it binds to specific nucleotide sequences known as **interferon stimulated response elements** (ISREs) in the promoters of some specific genes called as **interferon stimulated genes** ISGs. Finally, coming together of ISGF3 and other transcriptional complexes triggered by interferon signaling initiates the transcription of genes responsible for secretion of interferons.

Figure 2.6. Interferon signaling pathway:



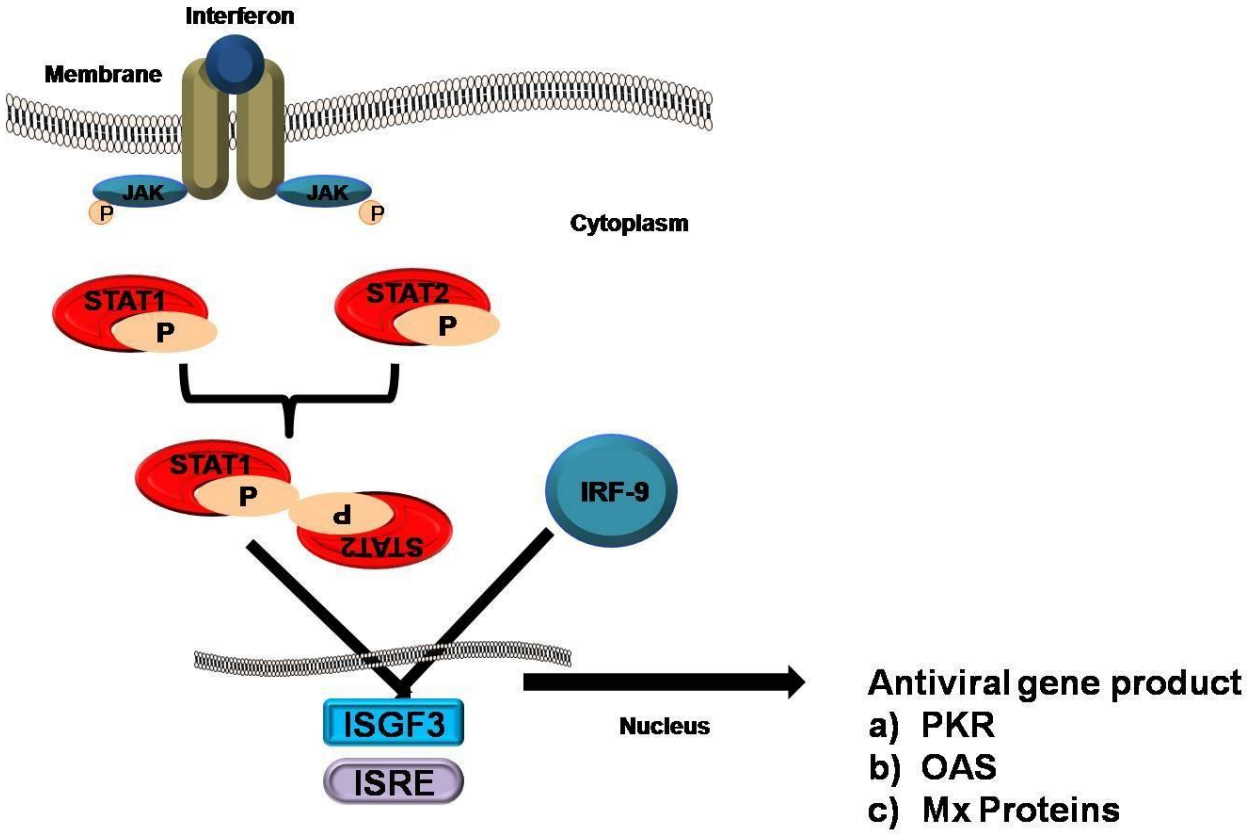
Viral infection: establishment of the antiviral state

Double stranded RNA activated antiviral state

Many genes are transcriptionally regulated by interferons following virus infection. Among all, three members have been studied extensively for their antiviral activities.

1) dsRNA activated protein kinase (PKR)
2) 2',5'- oligoadenylate synthetase (OAS)
3) Mx proteins

Figure 2.7.Schematic representation of interferon signaling for the activation of antiviral gene



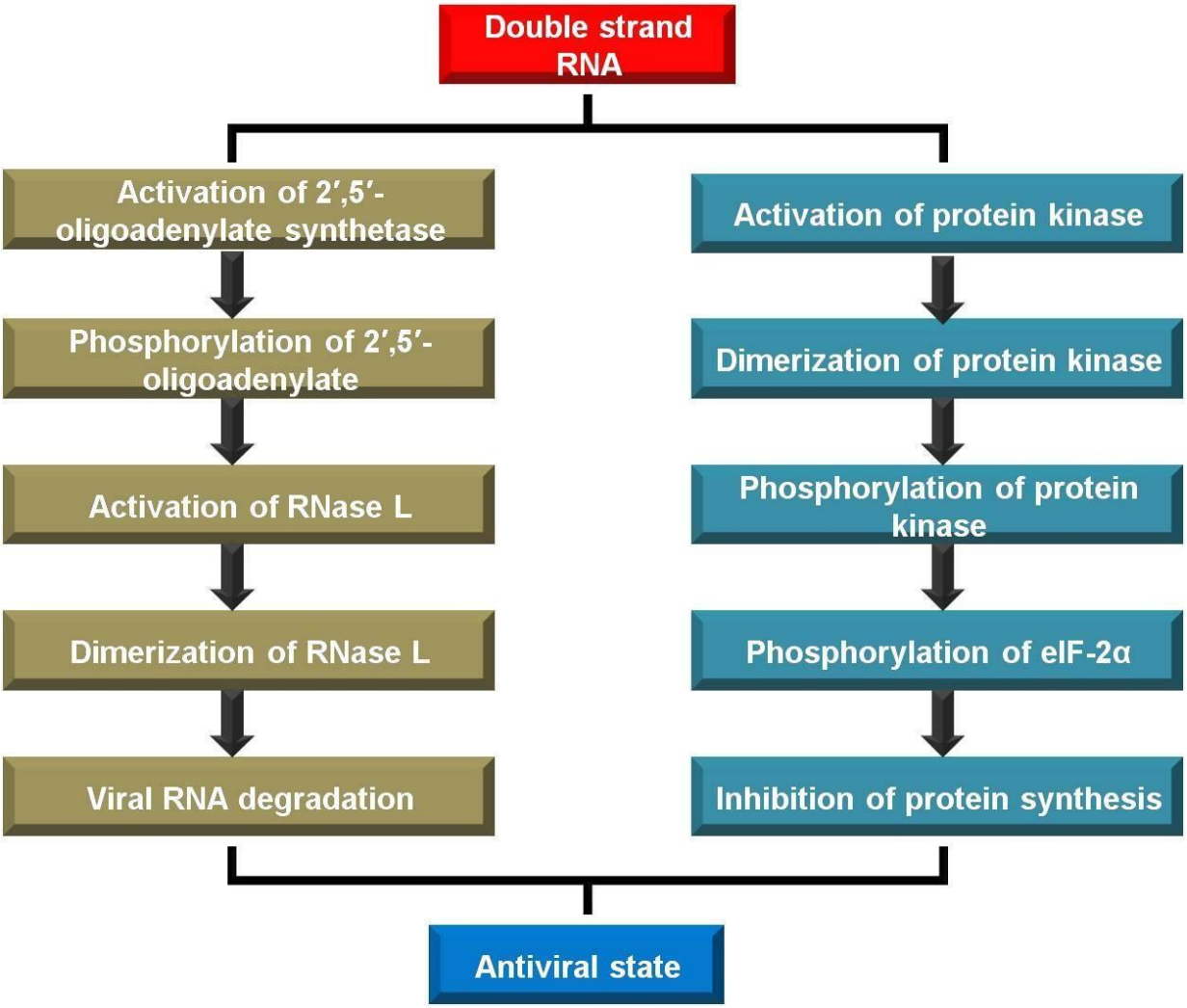
1) dsRNA activated protein kinase (PKR) – During dsRNA virus infection PKR forms the dimer, and is activated following phosphorylation. Eukaryotic translation initiation factor (EIF-2α) is the most important substrate phosphorylated by PKR. EIF-2α gets inactivated following its

phosphorylation leading to inhibition of viral protein synthesis. This way PKR exhibits antiviral activity.

2) 2', 5'- oligoadenylate synthetase (OAS) - Interferon inducible OAS is also activated by dsRNA formed during viral infection. It binds to RNase L triggering its dimerization and activation. Activated RNase L degrades the mRNA leading to inhibition of protein synthesis
3) Mx proteins - Interferon induced Mx protein have antiviral activity against several RNA viruses. Mice expressing Mx proteins are more resistant to many virus infections e.g. influenza.

   Mice deficient in PKR, RNase L and Mx proteins have been proved to be more sensitive to viral infection.

Figure 2.7. Schematic representation of dsRNA activated antiviral state



Apoptosis

Apoptosis (programmed cell death) is an interesting way explored by the host cell in order to prevent virus infection. In apoptosis cell must die before virus starts its replication. During apoptosis, cellular DNA undergo fragmentation and apoptotic bodies are formed which are then engulfed by macrophages and other cells of the immune system. Activation of apoptotic cycle involves release of "**cytochrome C**" from mitochondria and downstream activation of caspases (**c**ysteine-**as**partic **p**rote**ases**) cascade.

Role of the immune system against virus attack

Natural killer cells and cytotoxic T cells are the major type of immune cells involved against virus infection in the host. Natural killer cells are activated immediately upon virus infection and produce the cytokines such as **tumor necrosis factor** (TNF) and interferon γ. They also cause the direct cytotoxicity of the virus infected cells. In later stages of virus infection, the virus surface antigens are presented over the major histocompatibility antigens class I (MHC-I) molecules and activates the **cytotoxic T lymphocytes** (CTLs). CTLs exert antiviral state by secreting cytokines and apoptosis. Sometime the level of MHC-I gets downregulated in the virus infected cells, in that condition natural killer cells comes at the site of rescue to take over the task and kills the virus infected cells.

Viruses counter attack mechanisms

Viral strategies to escape host immune response

Adenoviruses upon entering into a permissive cell produces large amount of small RNA molecules called as **VA-1**. This VA-1 mimics the dsRNA and competitively binds to protein kinases to inhibit interferon production. Reoviruses and vaccinia virus produces dsRNA binding protein that inhibits the activity of dsRNA induced cascade of interferon production. Herpes virus produces 2'-5' oligoadenylate analogs that binds to RNase L and inhibits downstream activation pathway of interferon production. Some paramyxoviruses produce V protein which is known to inhibit interferon production by interfering STAT signaling pathway. Poxviruses produce soluble receptors known as decoy cytokine receptors which blocks the cell surface receptor and further inhibits the activation of cell antiviral response. Moreover many viruses inhibit the apoptotic pathways for their prolonged survival (herpesvirus, adenovirus, and poxvirus).

Table 2.3. Mechanism developed by virus to inhibit effects of interferon:

Virus	Mechanism
Adenovirus	Block interferon signaling
Vaccinia	Binds to dsRNA
Hepatitis B virus	Block interferon signaling
HIV	Degrades PKR
Reovirus	Binds to dsRNA
Epstein-Barr virus	Block PKR activation
Herpesvirus	Block RNaseL activation

APOBEC is an apolipoprotein B mRNA-editing enzyme that can change C to U in a DNA strand. This mutation inhibits RNA polymerase to synthesize viral RNA because it cannot read U in the DNA strand. APOBEC is a very important enzyme encoded by host

cell against HIV infection. HIV encodes a protein called viral infectivity factor (**Vif**) that degrades the APOBEC for its better survival inside host cell.

Major Histocompatability class I antigen (MHC-I) present on the macrophages are required to present the viral antigens to the immune cells. Viral antigen presentation by MHC-I activates the cells of immune system, which eventually helps to clear the virus from the infected cells (HIV, and paramyxoviruses).

Viral RNA polymerase encoded by many RNA viruses is highly error prone. Viruses often escape immune system by gradual incorporation of mutation into their genome. The variation in the antigenicity because of mutation is called as **ANTIGENIC DRIFT** (HIV). Viruses which contain segmented genome often exchange their genome segments between different viruses of same species to evolve as a new virus; the phenomenon is called as **ANTIGENIC SHIFT** (Influenza). Both antigenic drift and shift is a major way adopted by many viruses to escape the host immune system.

Evasion of interferon system by viruses

Interferons are well studied and established defense system against virus infection. Nevertheless, cohabitation between the host and viruses resulted in the procurement of mechanism to inhibit interferon system by most of the viruses. Viruses inhibit the interferon activation by blocking the different steps involved in the interferon signaling cascade. Some of the unique strategies used by the viruses to decoy the interferon system are enlisted below

Inhibition of protein synthesis

Many viruses hijack the host protein synthesis machinery for their own benefits. This leads to inhibition of cellular protein synthesis and upregulation of viral protein synthesis. As the interferons are also proteins, viral mediated inhibition of host protein synthesis can assist to the inhibition of interferons. Translation inhibition by phosphorylation of eIF2α is a host mediated antiviral mechanism, many viruses evolved in a way to carryout eIF2α independent translation in order to escape the immune surveillance.

Inhibition of interferon production

Type-I interferon production is activated by dsRNA formed during virus infections. Many viruses encode dsRNA-binding proteins that inhibit the enzymes protein kinases and 2'-5' oligoadenylate synthetase. The sigma protein of reoviruses, and the non

structural protein of rotavirus and influenza viruses are some examples of dsRNA-binding proteins.

Inhibition of interferon signaling

Herpes virus and papillomavirus blocks the interferon production by inhibiting the downstream signaling pathway. Adenoviruses, measles virus, and hepatitis viruses were also shown to inhibit the interferon production. All the essential components of interferon signaling pathways, i.e. interferon receptors, JAK/STAT and IRFs have been shown to be involved in virus mediated inhibition.

Despite the identification of the various strategies by which virus interfere with interferon action, little is known on the precise mechanism that exists between viruses and the interferon pathways, and its possible implications on viral pathogenicity, clearance, and viral immunity.

Viruses counter attack mechanisms

Virus response against apoptosis

Virus inhibits the apoptosis by interrupting the various stages of transcription and translation. Herpes and poxviruses are evolved in a way to modulate the apoptosis by blocking the activation of caspases. SV40 T antigen and E1 protein of adenovirus are known to bind with p53 and target it for proteasomal degradation. Although many viruses prevent apoptosis, herpes virus can selectively cause apoptosis in the lymphocytes in order to delay their removal from the host cell.

Virus response against host immune system

Many viruses come up with a system to reduce the expression of MHC-I molecules over the virus infected host cell surface. This explains the important role of MHC-I towards viral invasion into the susceptible host cells.

HIV, adenovirus, and herpesvirus inhibits the translocation of peptide within the endoplasmic reticulum, which is a necessary step for the loading and trafficking of the peptide over the MHC-I molecules. Cytomegalovirus produces a homologues of MHC-I molecule to decoy the host immune system.

Herpes simplex virus express a "glycoprotein E" that binds to the immunoglobulin molecules and prevents the activation of antibody mediated immune response.

Table2.4. Inhibition of viral antigen presentation by MHC-I:

Virus	Function
Epstein-Barr virus	**Inhibition of transporter associated with antigen processing during MHC maturation**
HIV	**Enhance the endocytosis of MHC-I**
Adenovirus	**Modulate the trafficking of MHC-I**
Cytomegalovirus	**Degrades the MHC-I**
Herpes simplex virus	**Inhibition of transporter associated with antigen processing during MHC maturation**

14.1 Virus response against different host factors

RNA interference is an antiviral mechanism in animals and many other living species. Many viruses encode "**suppressors of RNA interference"** that function against the host RNA interference machinery. Influenza virus and adenoviruses were demonstrated to have this activity.

Some viruses encode specific proteins called as **VIROCEPTORS,** which mimic the cellular receptors and acts as a trap for chemokines and interferon. Poxvirus and cytomegalovirus are known to encode viroceptors.

Table 2.5 Inhibition of chemokines by viruses:

Virus	Function
Epstein-Barr virus	**Homologues of interleukin -10**
Vaccinia virus	**Homologues of interferon γ**
Cytomegalovirus	**Homologues of chemokine receptor**
Poxvirus	**Inhibition of interleukin -2**
Myxoma virus	**Inhibition of interleukin -1β**

Some Paramyxoviruses like Newcastle disease virus encodes V protein which is formed following infection in the cells. These V proteins bind to helicase protein **Melanoma Differentiation-Associated protein 5** (MDA5) in the cells, which is a sensor for dsRNA molecules. Thus MDA5 acts as an interferon antagonist.

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DEPARTMENT OF BIOTECHNOLGOY

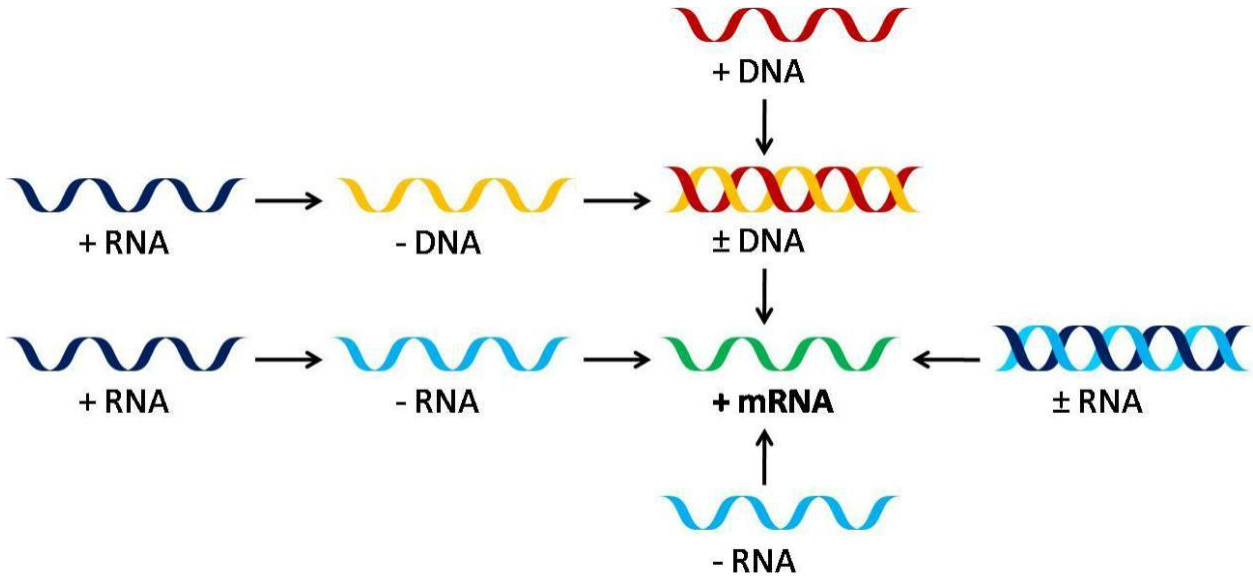
UNIT – III – MEDICAL VIROLOGY – SMB3102

POSITIVE STRAND RNA VIRUS
Classification of viruses and nomenclatures

Historically, all the viruses were grouped according to the illness they caused (for eg-hepatitis, encephalitis etc.). It was quite common to name the virus on the disease with which it is associated (foot and mouth disease virus) or the geographical location from which it is isolated (Rift valley fever virus). This kind of nomenclature changed with the advent of molecular biology and more advanced biochemical and biophysical techniques.

The most comprehensive and widely used classification was first given by Dr David Baltimore in which seven groups were proposed based on the genetic contents and replication strategies of the viruses. This classification is focused on the relationship between the viral genome and its mRNA and describes the formation of mRNA by the viruses with either DNA or RNA genome

Figure 3.1. Baltimore classification based on mRNA production by all viruses following infection



Group 1, dsDNA viruses – Replicating through DNA

Group 2, ss DNA viruses- Replicating through DNA

Group 3, ds RNA viruses- Replicating through RNA

Group 4, ssRNA viruses (+) polarity, (sense to mRNAs) - Replicating through RNA

Group 5, ssRNA viruses (-) polarity, (antisense to mRNAs) - Replicating through RNA

Group 6, RNA-retroid genomes (RNA -> DNA -> RNA) - Replicating using reverse transcriptase having dsDNA as an intermediate.

Group 7, DNA-retroid genomes (DNA -> RNA -> DNA) - Replicating using reverse transcriptase having ssRNA as an intermediate.

Current virus classification is based mainly on the morphology, nucleic acid type, host organism it infects, replication mode, and the disease type caused. International committee on taxonomy of viruses (ICTV) was established in 1966 in order to establish a universal system for virus classification. In the eighth report of ICTV which was published in 2005, three orders, 73 families, 9 subfamilies, 287 generas and more than 5000 viruses were approved. It is absolutely impossible to be up to date on the numbers that were approved by the ICTV as everyday new viruses are added to the database. The

most current information is available in the ICTV webpage (http://www.ictvonline.org/index.asp).

Family- It is defined as a group of genera with common characteristics. It is written as capitalized, Italicized, and ends in -*viridae*. Examples- *Paramyxoviridae*, *Poxviridae* (poxvirus family).

Subfamily- These are groups of viruses within some large families. They are written as capitalized, Italicized, and end with -*virinae.* Examples- *Paramyxovirinae, Parvovirinae, Alphaherpesvirinae.*

Genus- It is defined as a group of virus species sharing common characteristics. They are written as capitalized, Italicized, and end with -*virus*. Examples- *Parvovirus*, *Flavivirus, Coronavirus.*

Species- It is defined as a population of strains from one particular source, all of which have a common property that separates them from other strains. While writing the name of the species it is neither capitalized nor italicized. Eg. vaccinia virus, human immunodeficiency virus, influenza A virus.

Some specification not approved by the ICTV:

Strain- These are different lines of isolates of the same virus. Eg. Influenza viruses those were isolated from different geographical locations.

Type- They show different reactivity towards a positive serum sample, sometime called as serotypes (different antigenic specificity) of the same virus. Eg. Paramyxovirus type 1-9. There may also be subtypes within a particular type.

Group- These are divisions often based on nucleotide sequence similarities or origin.
HIV group M (Main), N (Neither M or O), or O (Outlier). There may also be subgroups.
(also called clade) within a particular group (M group HIV has A-J subgroups).

Variant- These are viruses whose phenotype differs from original wild type strain.

Origins of some viral names

Picorna: small having size in the scale of 10^{-12} RNA segment
Birna: two RNA segment

Toga: wearing a robe
Rota: Wheel like
Arbo- Arthopod borne
Papilloma: infections result in warts
Adeno: infections of glands
Hepadna: hepatitis + DNA
Herpes: produce scaly lesions
Pox: produce pox lesions
Corona: crown like

Satellite viruses and Defective Interfering particles:

Consider viruses to be a part of ecological habitat where organisms tend to share the relationships with one another: mutualism, commensalism, symbiosis, and parasitism. Viruses also act similarly.

Satellite viruses - Viruses with separate genomes that are encapsidated inside viral particles that are produced by a "helper" virus. They also require helper virus replicative machinery to replicate their genomes.

Defective Interfering particles (DI particles) - Their genomes are derived from a helper virus. They are deletion mutants which have lost their ability to encode proteins, but retain their ability to replicate with the help of a replication machinery of other helper virus. They called defective interfering particles because they are defective in their ability to produce proteins, and tend to interfere with the replication of helper virus by competing with the resources.

Classification of viruses and nomenclatures (Part II)

1 Principles of virus nomenclature

Followings are the principles of virus nomenclature set by ICTV

a) It aims to provide stability, avoid confusion, and to avoid creation of unnecessary names.

b) It is independent of any other biological nomenclature.

c) The basic unit of classification is **TAXON**.

d) A taxon should be officially approved by the ICTV committee.

The universal virus nomenclature should follow a hierarchical level of Order, Family, Subfamily, Genus, and Species.

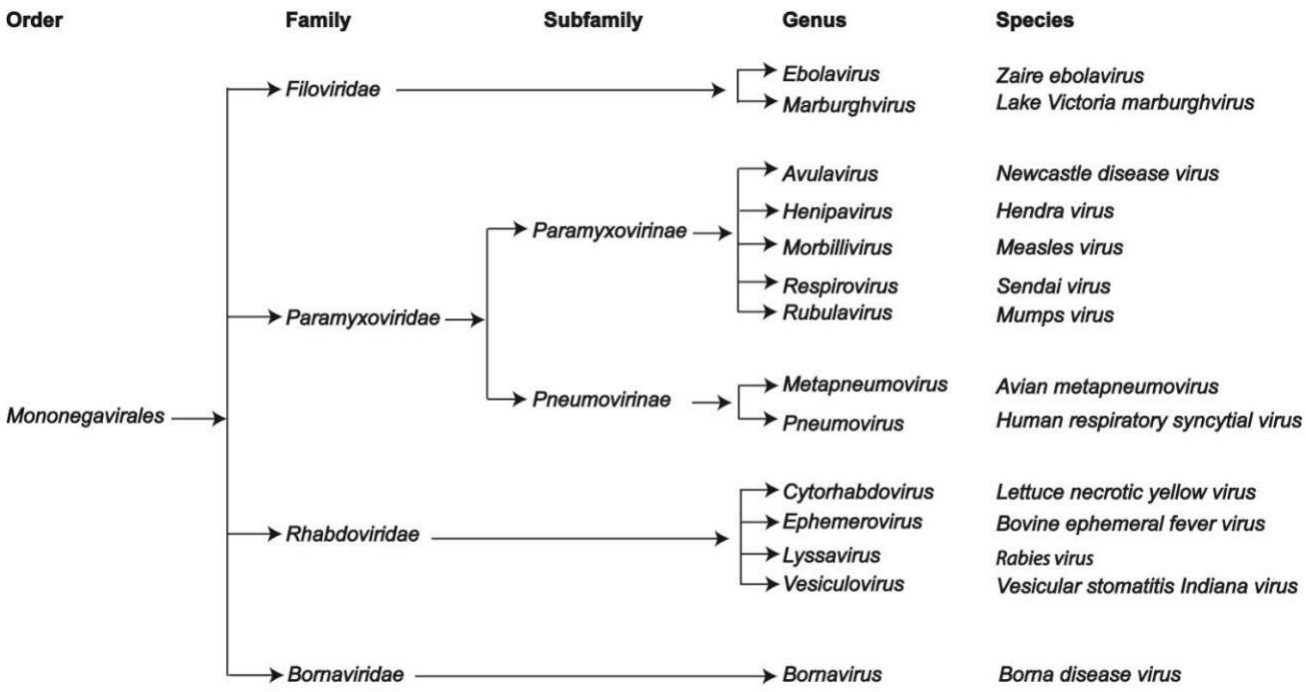
Table 3.1 Current status of virus taxonomy:

Order	Numbers of Families
Caudovirales	3
Herpesvirales	3
Mononegavirales	4
Nidovirales	3
Picornavirales	5
Tymovirales	4
Not assigned any order	72

Table 3.2 Classification of viruses based on the type of nucleic acid:

dsDNA Viruses	**ssDNA viruses**	**dsRNA viruses**	**ssRNA (+) viruses**	**ssRNA (-) viruses**	**RNA and DNA (RT) viruses**
Poxviridae	*Circoviridae*	*Reoviridae*	*Picornaviridae*	*Bornaviridae*	*Retroviridae* (RNA)
Asfaviridae	*Anellovirus*	*Birnaviridae*	*Caliciviridae*	*Rhabdoviridae*	*Hepadnaviridae*
Iridoviridae	*Parvoviridae*		*Hepevirus*	*Filoviridae*	(DNA)
Herpesviridae			*Astroviridae*	*Paramyxoviridae*	
Adenoviridae			*Nodaviridae*	*Orthomyxoviridae*	
Polyomaviridae			*Coronaviridae*	*Bunyaviridae*	
Papillomaviridae			*Arteriviridae*	*Arenaviridae*	
			Flaviviridae	*Deltavirus*	
			Togaviridae		

Figure 3.2 Classification of order *Mononegavirales*:



POSITIVE STRAND RNA VIRUSES

The viruses which contain positive strand RNA genome act directly as mRNA upon infection to a host cell. Most viruses in this category have icosahedral symmetry and vary approximately 50-150 nm in diameter. The members contain positive sense RNA genome containing either lipid envelope (*Togaviridae*, *Flaviviridae*, and *Coronaviridae*) or devoid of envelope (*Picornaviridae*). They are important because they can cause serious life-threatening diseases including hemorrhagic fever and encephalitis (dengue, yellow fever). Members of genus Alphavirus (Sindbis virus, Semiliki Forest virus, and Equine encephalitis) are transmitted to animal by mosquito bite. Among all Alphaviruses sindbis virus is well studied and understood.

Table 3.3 Examples of positive strand RNA viruses:

	Family	**Virus example(s)**
Animal viruses	*Flaviviridae*	West Nile virus Yellow fever virus Dengue virus bovine viral diarrhea virus
		classical swine fever Hepatitis C virus
	Coronaviridae	Severe acute respiratory syndrome (SARS) virus Avian infectious bronchitis virus (IBV)
	Togaviridae	Rubella virus Chikungunya virus [redacted] Semliki Forest virus Sindbis virus

		[redacted] Eastern equine encephalitis virus Western equine encephalitis virus Venezuelan equine encephalitis virus Ross River virus [redacted]
	Picornaviridae	Foot and mouth disease virus Human rhinovirus Encephalomyocarditis virus Polio virus Hepatitis A virus
Plant viruses	*Potyviridae*	Potato virus Y
	Flexiviridae	Potato virus X
	Comoviridae	Cowpea mosaic virus

Virion properties

Virions are spherical in shape and are having uniform appearance. The diameter of virus varies between 70-100 nm. The enveloped virion particle encircles the icosahedral capsid. Envelope contains spikes of viral glycoprotein which are major antigenic determinants of the virus. The spike glycoproteins are highly variable among strains and also between different serotypes.

Structure of positive strand RNA genome

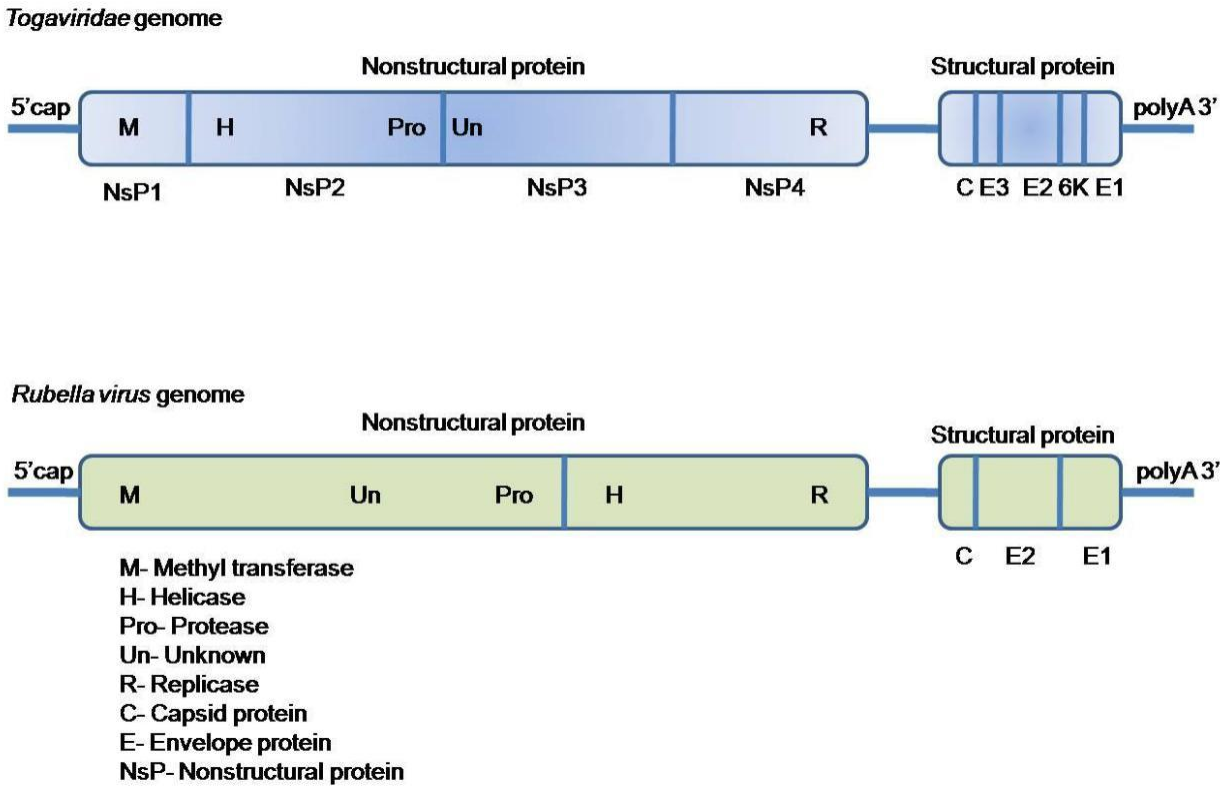
They are positive sense, single-stranded RNA, vary between 9-12 kb in size, with the exception of coronaviruses. Terminal 5' end of the genome is capped while 3' end is polyadenylated. Viruses with single stranded RNA genome do not require secondary or tertiary fold in their capsid to

accommodate its genome. That means they are highly organized and tightly packed. Generally a dimer of coat protein interacts with the 3' end of the RNA, which is essential for the virus replication. This interaction is also needed for the packaging of the genomic RNA inside the virion. The viral genomic RNA is arranged in the icosahedral capsid in various ways in order to neutralize the negative charge of the nucleic acids.

Replication of positive strand RNA

The positive strand RNA virus transfers its genome directly to the ribosome and starts translation for the synthesis of viral proteins. Infectious cycle begins with the entry of virus into the cell through endocytosis. The genomic RNA uncoats after getting into the cytoplasm of the infected cells. The RNA is then translated into the viral polyprotein precursors which are later cleaved by proteolysis to form the structural and non structural viral proteins. The structural proteins are involved in the maturation and assembly of the virion while nonstructural proteins act as RNA replicating enzyme for genomic RNA synthesis. Some of the viruses in this class form the **subgenomic RNA** during replication process (Coronavirus, Caliciviruses, and Togaviruses).

Figure 3.3 Schematic representations of togavirus and rubella virus genome:



1. Picornaviruses

Family *Picornaviridae* contains viruses that infect many species of animal as well as humans. Poliovirus was the first virus that was propagated in the cell culture and purified using plaque assay. Most of the picornaviruses grow in a variety of cell lines making them a useful tool to understand the biology of positive strand RNA viruses.

Important picornaviruses

1.1. Poliovirus

In general poliovirus cause mild disease condition in the oro-phrayngeal cavity and gut epithelium. The disease becomes serious when virus migrates to central nervous system and cause systemic viremia. Poliovirus is a disease associated with poor hygienic and sanitary conditions. Poliovirus infection to central nervous system can cause encephalitis and paralysis of limbs and respiratory muscles. Polio vaccine is very effective in controlling the outcome of the disease. Polio has been eradicated from most of the countries.

1.2. Hepatitis A virus

Hepatitis A virus is a problem of young children in the developing countries where hygienic condition is not good. Infection in the childrens is often mild and once infected the immunity lasts for life long. The infection to adults can cause severe jaundice which may prove sometimes fatal also.

1.3. Coxsackievirus

The first coxsackievirus was isolated from mice showing symptoms of inflammation in skeleton muscles. The virus can cause inflammation of central nervous system and heart muscles with rashes over the body.

1.4. Foot and mouth disease virus

Foot and mouth disease (FMD) virus is a highly contagious disease of cloven footed animals. The disease has a major impact on the trade and economy of the countries dependent on agriculture and animal products. The disease causes severe drop in milk production of cattle. Symptoms of the disease include lesions in hoof and mucosal surface of oral cavity. The condition is febrile and animal may die if not treated. Vaccines are available against FMD for cattle, sheep, goats and pigs.

1.5. Rhinovirus

Rhinovirus is very common cause of upper respiratory tract infection in humans. Most of the children get infected with rhinovirus by the age of 3 years. Most of the rhinovirus can survive and replicate in the lower temperature (33^0C) of respiratory epithelium.

PICORNAVIRUS VIRION

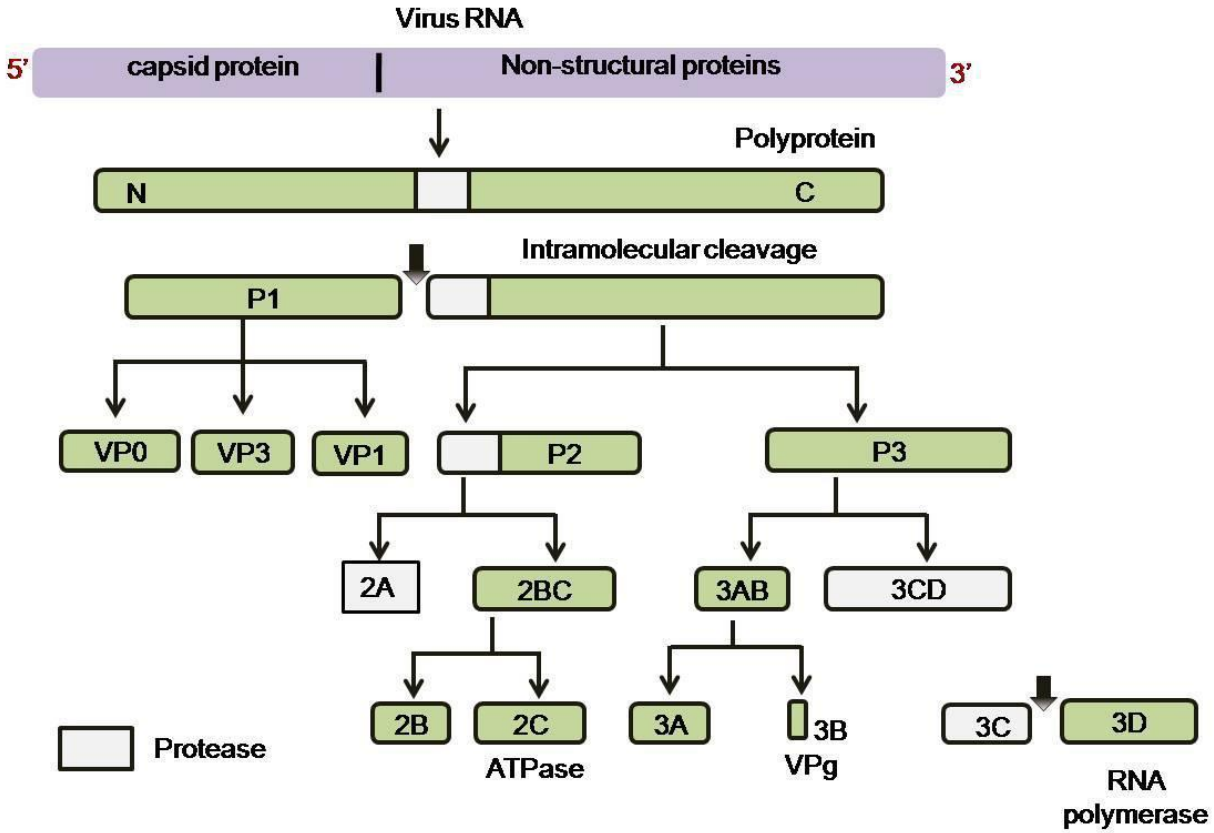
Picornaviruses are small RNA viruses having diameter of 25-30 nm. The virus has icosahedral symmetry and capsids are made up of four proteins (VP1-4). Some of the picornaviruses contain **CANYONS** over the vertices of the icosahedron which act as virus attachment site on those viruses (Poliovirus and rhinovirus). Many serotypes have been evolved because of the variation in the capsid proteins which creates a challenge to make a vaccine against the picornaviruses. The genome is composed of ssRNA of 7-8 kb in size. The 5' end of the RNA is covalently attached with a protein called as VPg (genome linked viral protein) while its 3' end is polyadenylated. Interestingly, 5' end of the genome contains many secondary structures called as **internal ribosome entry site** (IRES).

Picornavirus replication

Some picornaviruses enter cell through a receptor called as **CD155** (Poliovirus receptor). CD155 is a member of immunoglobulin super-family and are widely expressed in many cell types. Many Enteroviruses enter the cell after binding to **CD55** or decay accelerating factor, a member of complement system. Once inside the cell the viral encoded VPg gets detached from the 5' end of the RNA. The viral RNA acts as an mRNA and binds to the ribosome with the help of IRES present at the 5' end of the

genome. Virus encodes a single polyprotein which is cleaved by virus coded proteases into single structural and nonstructural proteins. The polyprotein is first cleaved into P1, P2, and P3. P1 get cleaved into VP0, VP1 and VP3 and myristylated at its N terminus. VP0 further cleaved into VP2 and VP4, other cleavage products include 2C (ATPase), 3B (VPg) and 3D (RNA polymerase). Virus replication takes place in the replication complexes with the help of RNA polymerase. Usually five copies each of VP0, VP3 and VP1 assembles to form a procapsid which encapsidates the viral genome. Lysis of the infected cells releases the progeny virions.

Figure 3.4. Schematic representation of picornaviruses genome:



Recombination in Picornavirus

The process of recombination is mostly relevant in cases of segmented viral genome. When a cell is infected simultaneously with two or more strains, the progeny virion may contain the genome derived from both the strains. A recombinant virus is one which contains part of its genome from one virus and remaining from the other. Polio virus vaccine contains the mixture of all three strains responsible for the disease in oral formulation.

2. FLAVIVIRUSES- WEST NILE VIRUS

The family *Falviviridae* contains around 70 pathogens of both human as well as animals. These 70 members are distributed among three genera, namely *Flavivirus, Pestivirus,* and *Hepacivirus*. Out of these 70 members, 30 were arthropod borne human pathogens. Yellow fever virus is the prototype of the genus flavivirus which was the major cause of human illness during the 18^{th} and 19^{th} centuries. Members such as West Nile virus, dengue virus, and Japanese encephalitis viruses are now considered as most important human pathogens. Genus *Pestivirus* contains many important pathogens of veterinary importance such as **hog cholera** (classical swine fever), **bovine viral diarrhoea**, and **border disease**. Genus *Hepacivirus* contains ***hepatitis C virus***, an important human pathogen which causes viral hepatitis.

Properties of Flaviviruses

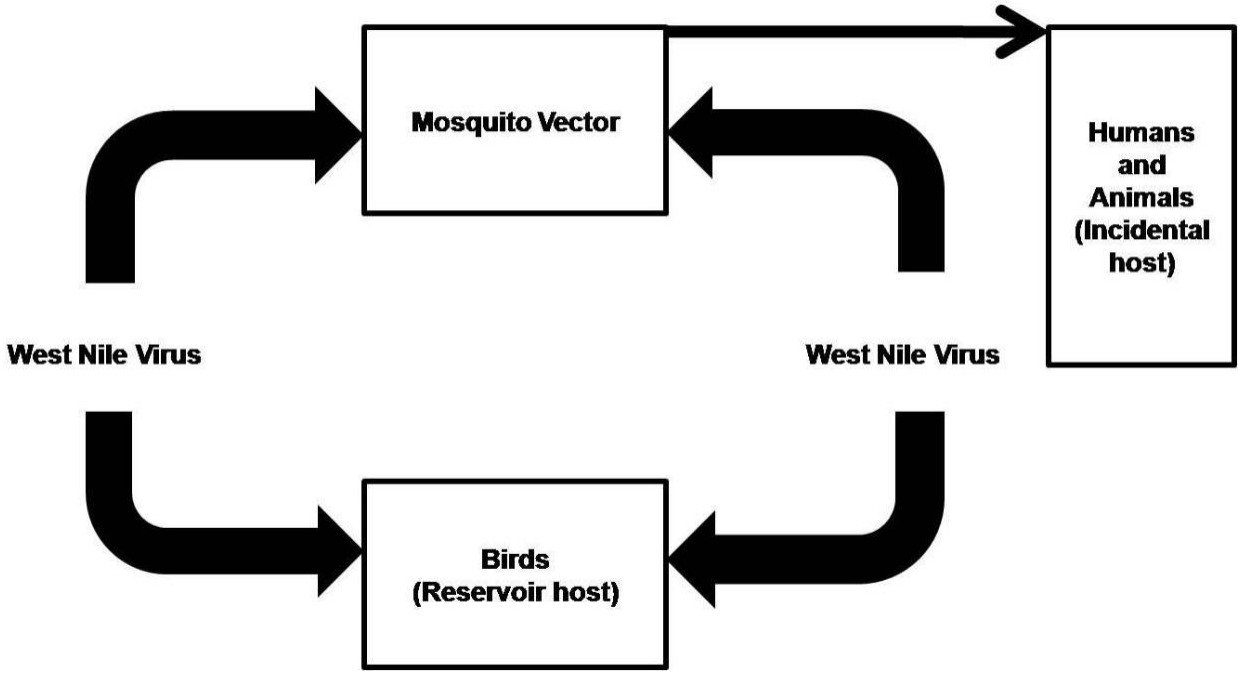
Virions are spherical and 40-60nm in diameter. They contain a lipid derived envelope with spikes of glycoprotein embedded on it. The genome consists of a positive sense single-stranded RNA of approximately 9.6 to 12.3 kbp. 5' cap is present only in the members of genus *Flavivirus*. The viral genome codes for both structural (3-5 in numbers) as well as non-structural (7-8 in numbers) proteins. The viruses are easily inactivated by common disinfectants and heat. Flaviviruses infect a variety of cells including Vero (African green monkey), BHK-21 (baby hamster kidney) and chicken embryo fibroblasts. Infection of flavivirus is lethal to new born mice. Viruses enter the cells through receptor mediated endocytosis and replication of the viral genome takes place in the cytoplasm. Replication involves synthesis of negative sense RNA from the positive sense genomic RNA, which then serves as a template for mRNA synthesis. Translation of viral mRNA leads to formation of a single polyprotein which is then cleaved into structural and non-structural proteins. The maturation of the virion takes

place in endoplasmic reticulum and are released following lysis of the infected cells.

2.1. West Nile Virus

West Nile virus was first identified in 1937 in Africa as a cause of mild febrile infection. Later on it was identified as a causative agent of fatal encephalitis in humans and horses. The virus is transmitted to the human being by the bite of Culex mosquitoes, while birds serve as a reservoir host. The animal and humans are considered as an incidental host when virus gets transmitted following insect bite harboring the virus in large quantity.

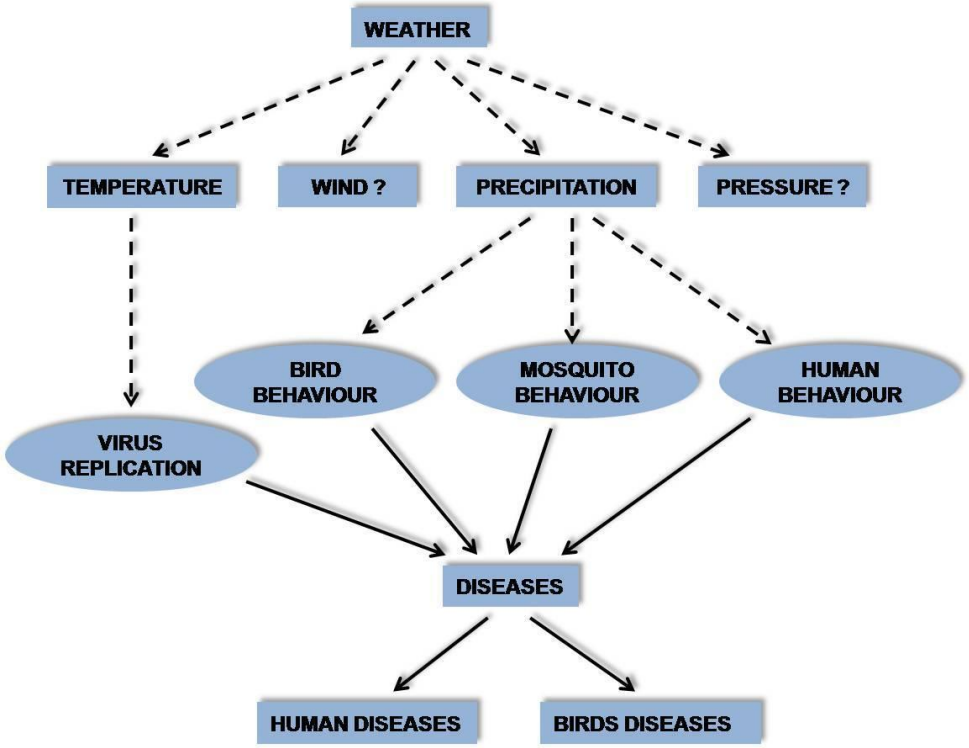
Figure 3.5 West Nile Virus transmission cycle



Ecology of West Nile virus transmission:

Weather plays an important role in the transmission of West Nile virus to humans and animals. Change in temperature and dryness is an ideal condition for the breeding of mosquitoes. Also during the rainy season the behavioral patterns of birds, mosquitoes, and humans changes. These lead to a hypothesis of having high incidence rate of West Nile virus during initial precipitation and dryness in the air. The role of wind velocity and pressure difference has also been suggested for the outcomes of West Nile virus incidences, but their actual impact is an open area of discussion.

Figure 3.6.Ecology of West Nile virus transmission:



Clinical Features:

Birds often contains virus and do not show any disease symptoms while humans and horses are dead-end hosts. Clinical manifestation of the disease is only evident in case of horses and humans. Infected animals show neurological signs, depression, muscle weakness, and fever. Death occurs in 40% of infected cases based on the immune status of the animal and the strain of virus. Milder form of disease in humans shows fever, abdominal pain, diarrhea and restlessness. These symptoms last for a week. More severe form that can be life threatening is called as West Nile encephalitis.

Pathogenesis:

Infection of West Nile virus causes high-titer viremia, necrosis, hemorrhage and inflammation in many vital organs. These include heart, brain, liver,

kidney, intestine, and nervous system. Lesions are evident on brain and spinal cord following West Nile virus infection.

Diagnosis:

Diagnosis of this disease is done mainly on the basis of serology for virus specific immunoglobulin in the serum of infected patient. Traditional techniques such as virus neutralization assay, *in–situ* hybridization, and immuno-histochemistry are regularly used to diagnose the disease. Modern molecular biological techniques such as reverse transcriptase polymerase chain reaction (RT-PCR) are also used to detect the virus infection from tissue samples.

Immunity, Prevention and Control:

Animal previously infected with West Nile virus are resistant to reinfection and many vaccines are available in the market against West Nile disease. The possible way to control the disease is by controlling mosquito population.

FLAVIVIRUSES

2.2. Dengue virus

Introduction

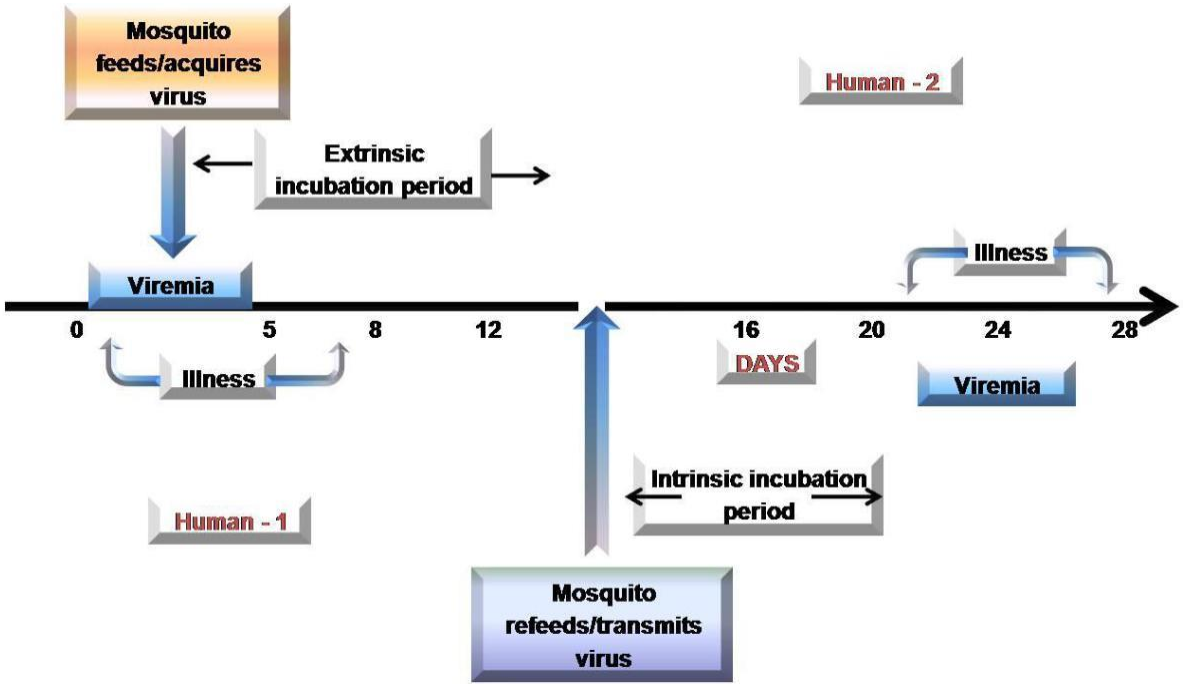
Dengue virus infection is a leading cause of illness and death in many tropical countries. It becomes the most important mosquito transmitted disease in many parts of the world. Nearly 100 million people are infected annually while millions are under risk. Dengue fever can be caused by 4 different serotypes of the dengue virus. Infection by one virus does not protect against the other virus because of different serological reactivity among viral serotypes. Dengue virus is transmitted among people by the bite of mosquitoes *Aedes aegypti* and *Aedes albopictus. They are approximately 5mm in size and bites during early morning or late afternoon. Only female mosquitoes can transmit the virus not the males. In some part of the world dengue is endemic, which means the disease occurs every year when the mosquito population is at its peak. The virus first originated from African monkeys, which was suggested as natural reservoir for dengue virus.*

Transmission and spread

Virus enters the human body through the mosquito saliva and localizes to various target organs including lymph nodes and liver and starts replication. The virus is then released from target organs and reaches to other lymphatic tissues through blood circulation. Finally virus starts circulating in blood following its release from the lymphatic tissues.

In general the cycle begins with a dengue-infected person. When an uninfected female *Aedes* bites to dengue-infected person during his viremic stage, the virus gets transmitted into the gut of the mosquito. The virus replicates during certain period of time within the mosquito which is called as extrinsic incubation period. The infected mosquito then bites to a susceptible person and transmits the virus, as well as to every other person for its entire life. The virus replicates in the second person and start producing symptoms, this is called as intrinsic incubation period (Inside human body).

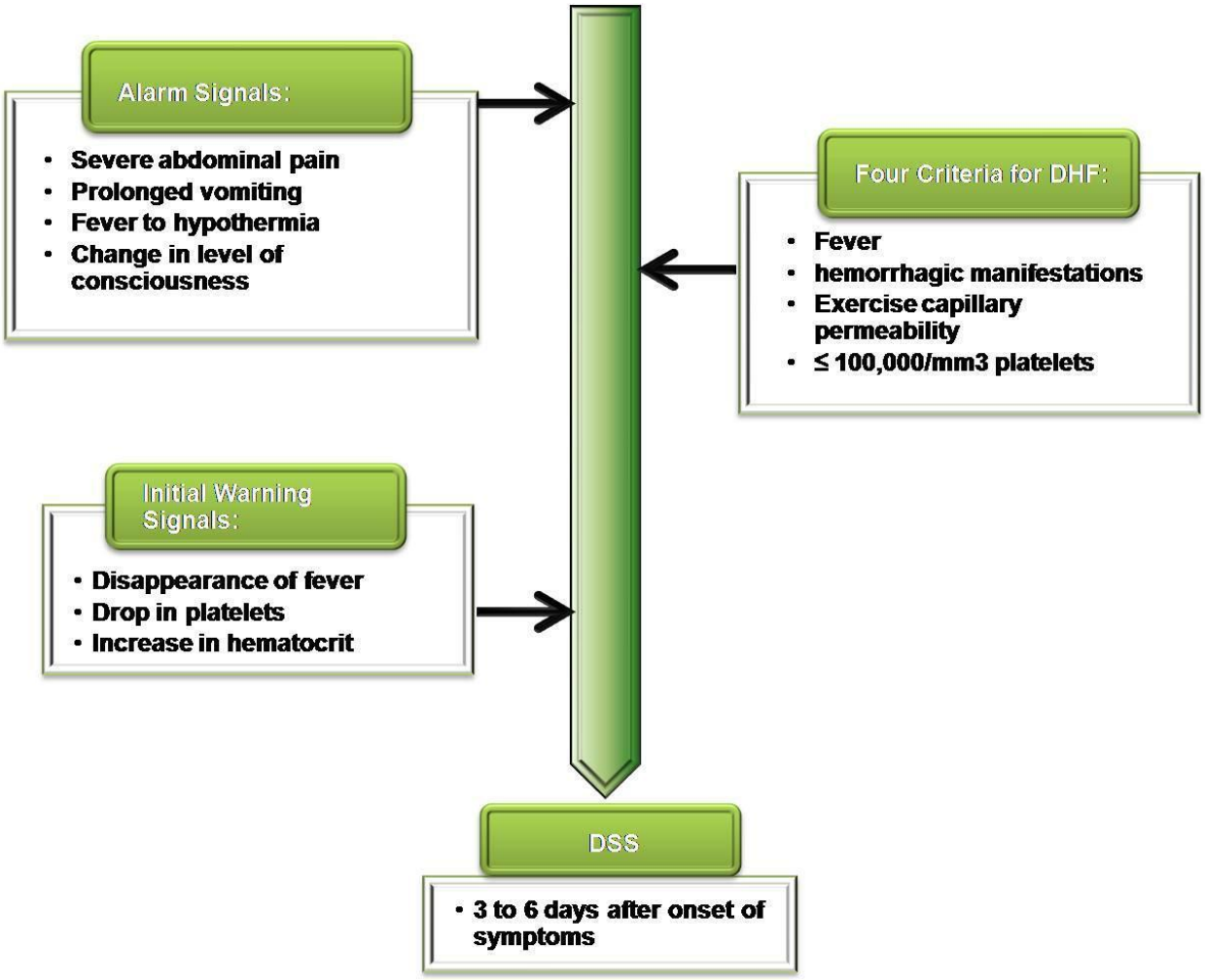
Figure 3.7 Dengue virus transmission cycle between human and mosquitoes



Clinical Features

The dengue virus infection causes fever, headache, joint pain, muscle pain, vomiting, and sometime hemorrhages. Patients may suffer from severe depression after acute form of the disease. Sequential infection with different serotypes of dengue virus put patient into greater risk of developing dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). DSS is the severe form of DHF. DSS is characterized by week and rapid pulse, low blood pressure, and altered mental status.

Figure 3.8 Clinical feature of Dengue shock syndrome



Control

Disease can be controlled by eliminating the female *Aedes* mosquito population. Educating the people to carry out vector control program in their homes and neighborhoods can also help to some extent.

3. Coronaviruses

Family *Coronaviridae* contains two genera, **coronavirus** and **torovirus**. Coronaviruses contain pathogens that mainly infect mammals and birds causing respiratory, gastroenteritis, reproductive and generalized infections. The toroviruses are so far reported in horses and cattle and are associated with diarrhea.

Important members of genus coronavirus

3.1. Transmissible gastro-enteritis virus

It is a highly contagious disease of swine. Clinical signs of this disease include vomiting, diarrhea, weight loss and dehydration. Disease is more severe in case of young piglets. The virus infects and destroys the enterocytes and intestinal villi resulting in loss of mucosal surface. The measure pathology is restricted to gastro intestinal tract.

3.2.Feline enteric coronavirus

It is systemic and fatal disease of cats. Clinical signs include fever, anorexia (Loss of appetite), and weight loss. Sometimes ocular and neurological signs are also visible. Virus replicates mostly in monocyte and macrophages.

3.3.Avian Infectious bronchitis virus

It is the respiratory disease of poultry characterized by cough, gasping, respiratory rales and dysponea. Clinical signs include visceral gout, nephritis and cheesy exudate in the trachea. Birds may develop secondary bacterial infection.

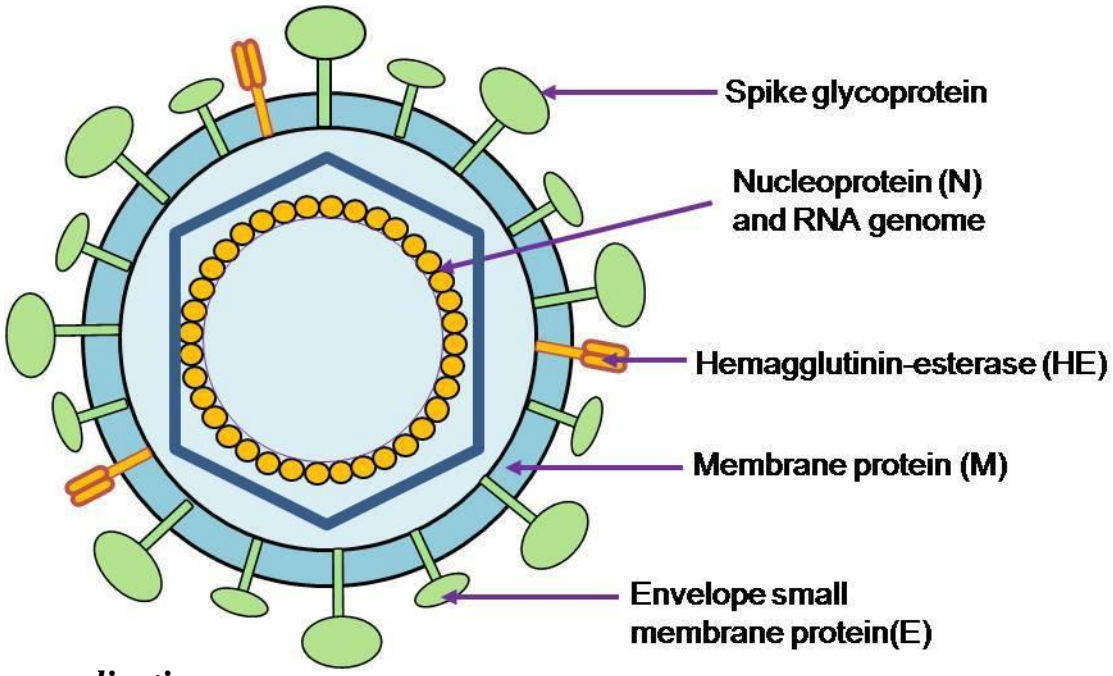
3.4.Mouse hepatitis virus

Mouse hepatitis virus is a causative agent of enteric infection in infant mice. It is a highly contagious virus of mice and mortality may approach to 100% in virulent outbreak cases. Symptoms include dehydration and rapid weight loss in infants. Lesions of the disease are visible mainly in small intestine and proximal part of large intestine.

Virion characteristics

Coronaviruses are enveloped, approximately 80-250 nm in diameter, and are pleomorphic in shape. The viruses contain large club shaped spike protrusion from the surface of the icosahedral internal core. The core contains helical nucleocapsids which is linear single stranded RNA of 25-31 kb in size. The 5' end of the genome is capped and 3' of the genome is polyadenylated similar to that of picornaviruses. The major structural protein of the coronavirus includes nucleoprotein (N), spike protein (S), membrane protein (M), envelope protein (E), and hemagglutinin esterase protein (HE). The S protein is a major antigenic determinant of the virus and contains the conformational epitopes towards their N terminal. Host immune responses are directed mainly against N and S proteins which are the main targets for the vaccine designing strategy.

Figure 3.9. Schematic representation of mouse hepatitis virus:

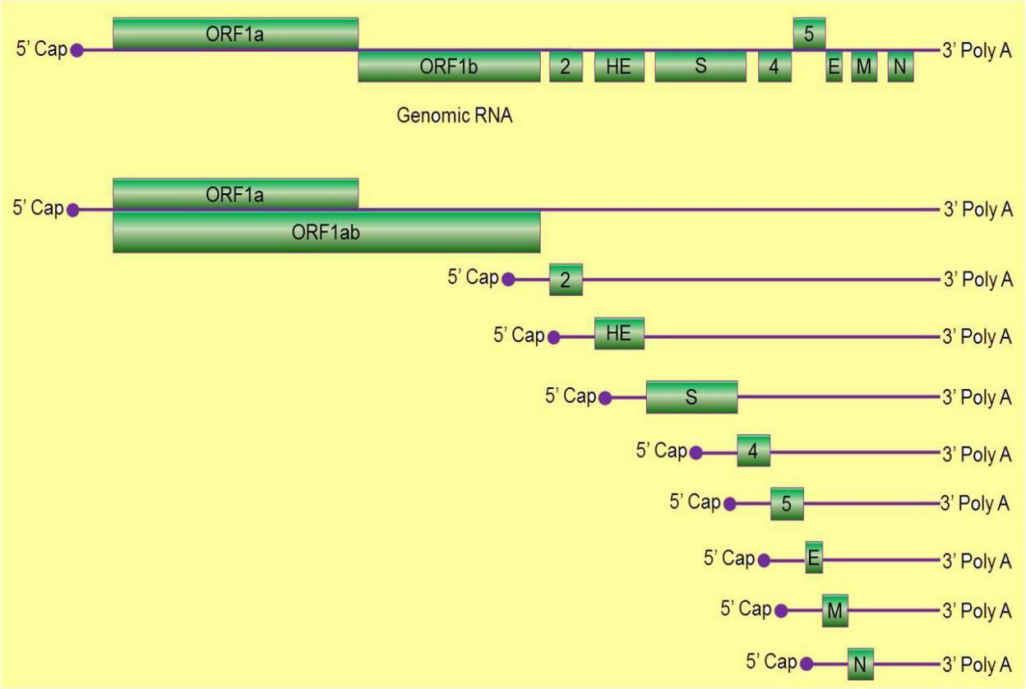


Virus replication

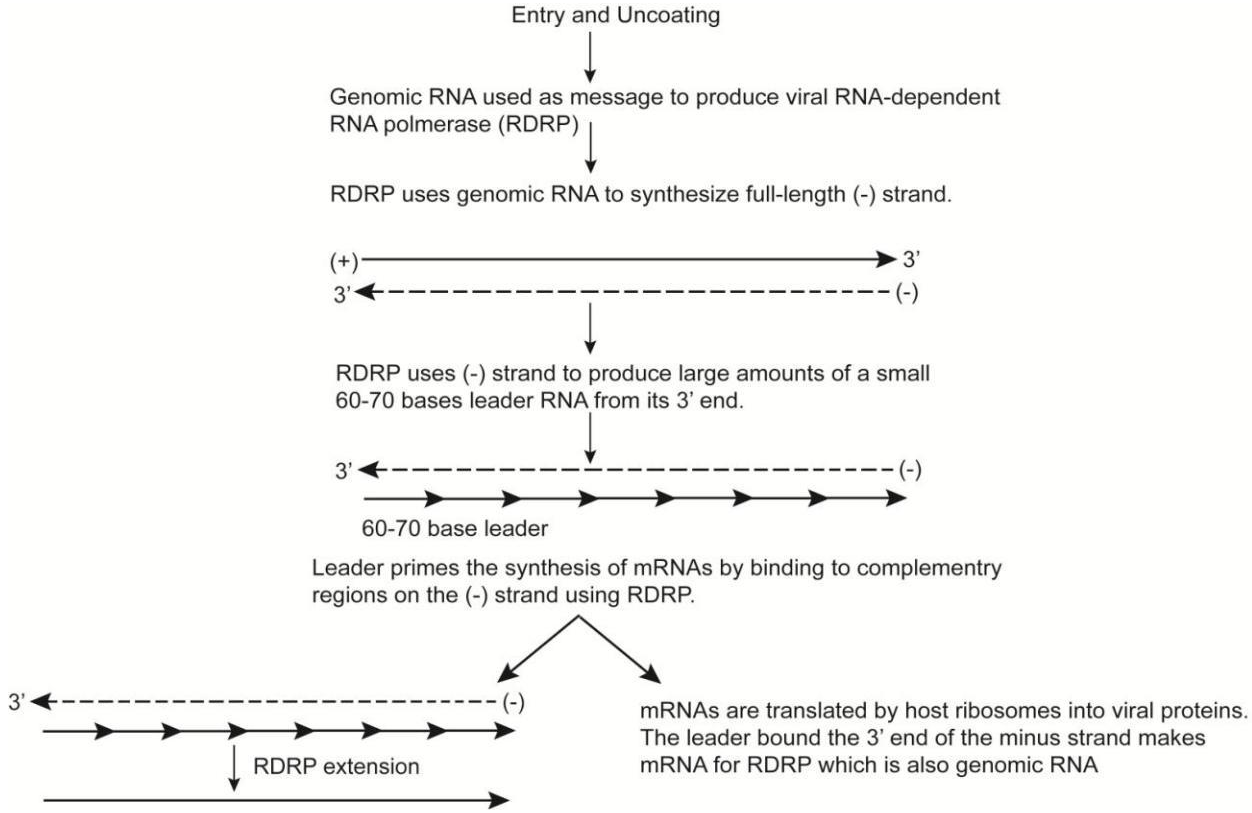
Coronaviruses are reported to utilize many receptors for their entry inside the cells. Severe acute respiratory syndrome (SARS) coronavirus uses angiotensin-converting enzyme 2 (**ACE-2**), whereas mice hepatitis virus uses carcinoembryonic antigen related cell adhesion molecule 1 (**CEACAM-1**) while others use **sialic acid** as a receptor.

Viral RNA serves as an mRNA for the synthesis of RNA dependent RNA polymerase (**RDRP**). Two large open reading frames are translated as a single polyprotein which are later cleaved into separate viral protein. The RDRP then forms the full length complementary genome which serves as the template for the formation of subgenomic RNA. All the mRNAs are then translated into various viral proteins. Many of the viral proteins undergo posttranslational modifications in endoplasmic reticulum and golgi body and therefore the progeny virions buds out from these location and not from cell membrane.

Figure 3.10. Schematic representation of replication scheme in mouse hepatitis virus:



igure 3.11. Overall view of coronavirus infectious cycle:



1. SEVERE ACUTE RESPIRATORY SYNDROME (SARS):

Pathogenesis

Causative agent

Severe acute respiratory syndrome (SARS) is a highly infectious viral disease caused by members of family *Coronaviridae*. The first case was recognized in 2003 with signs of atypical pneumonia. SARS is caused by a novel coronavirus (CoV) which can infect a wide range of animal species. Many SARS-CoVs has been found in bats suggesting it to be the reservoir of the virus. The first outbreak of SARS was notified in Southern China where the bats and humans lived in the close proximity with each other. It mainly affects the adult human beings. Transmission is through direct contact with infected patient or through infected body fluids. Incubation period varies

from 5 days to 2 weeks. Symptoms include cough, shortness of breath, and difficulty in breathing with mild fever.

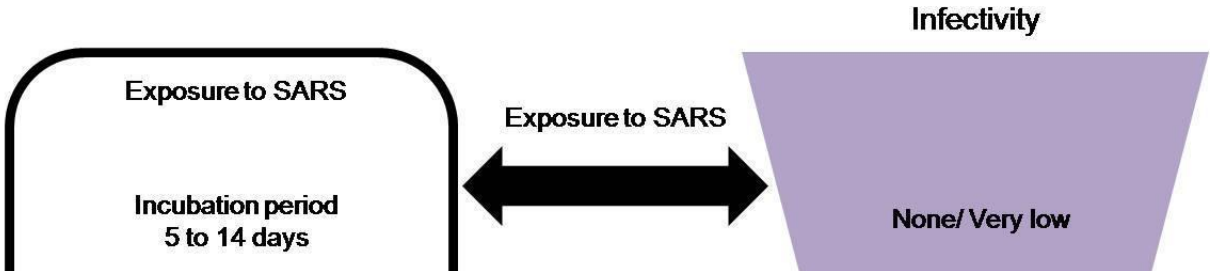
Virion properties

The viral genome of SARS-CoV contains ssRNA of 29.7 kb in size, one of the largest among RNA viruses group. The virion is spherical in shape around 80-100 nm in diameter. Various projections from the surface of the virion protrude to form a crown like structure (hence named coronavirus). 5' end of the virus encodes for an open reading frame which is translated into a large polyprotein that is cleaved by viral proteases to form many non-structural proteins. The non structural proteins include RNA dependent RNA polymerase, ATPase and helicase. The non structural proteins helps in replicating viral genome as well as viral transcripts and subgenomic mRNA's which are used to synthesize viral proteins. The viral membrane protein includes spike protein, and a membrane protein which completes its maturation in endoplasmic reticulum and golgi apparatus. The spike (S) protein present on the surface of the virion is the major antigenic determinant of the virus. Spike protein is responsible for tissue tropism as well as gradient of pathogenesis of SARS-CoV. The virus infects the cells of the respiratory epithelium by binding to its receptor, angiotensin-converting enzyme 2 (ACE-2).

Pathophysiology

Respiratory and gastrointestinal tract are the only organs reported to support SARS-CoV replication. The virus invades the respiratory epithelium and cause lytic effect on the infected cells. The effects are more pronounced in the pulmonary tissues where wide spread alveolar damages are visible. Although the morbidity and mortality is higher among old age group (65 years), intensive care treatment and mechanical ventilation can improve the life expectancy of the patients.

Figure 3.12 Clinical picture of SARS:



Diagnosis

Diagnosis is based on

I. PCR for identifying SARS-CoV genome sequence.

II. Enzyme-linked immunosorbent assay (ELISA) from serum samples collected from patient.

III. *Immunofluorescence* test (IFT) from serum samples collected from patient.

IV. Virus isolation from infected tissue samples using cell culture techniques.

Treatment

Normal treatment is mostly symptomatic and includes followings:-

I. Antibiotics to treat secondary bacterial infections

II. Antiviral drugs

III. Corticosteroids to reduce pathology of lungs

IV. Ventilators and artificial breathing

Prevention and control

a) Reduce the contact with SARS infected individuals

b) Hand washing and personal hygiene

c) Covering mouth and nose while sneezing to avoid droplet infection

d) Avoid sharing food and drinks from an infected person

e) Disinfection of the working space and surroundings.

Negative strand RNA viruses

Negative strand RNA viruses belong to order *Mononegavirales*. The viruses in this group have similar genome organization and replication strategies and are probably diverged from a common ancestor (*Filoviridae*, *Paramyxoviridae, Bornaviridae* and *Rhabdoviridae*). They are often associated with emerging infection and havoc to human population (Ebola, Marburg, Nipah and Hendra). Virus contains a negative sense RNA genome which means the polarity of the genome is opposite to that of an mRNA. The negative sense RNA cannot use its genome to synthesize proteins and hence its **RNA is not infectious** (absence of protein synthesis). Because of the above stated property viruses in this group encode their own polymerase (**RNA dependent RNA polymerase [RDRP]**). Another unique property about these viruses is about its transcription, first a leader RNA is synthesized, which is followed by sequential transcription of the genes in the 3' to 5' order to yield individual mRNAs by a stop-start mechanism guided by the conserved gene-start and gene-end signals.

Genome features

e) Linear non-segmented negative sense RNA genome

II. Organization of genome- 3'-Leader-Virion core- Surface proteins-Polymerase-Trailer 5'.

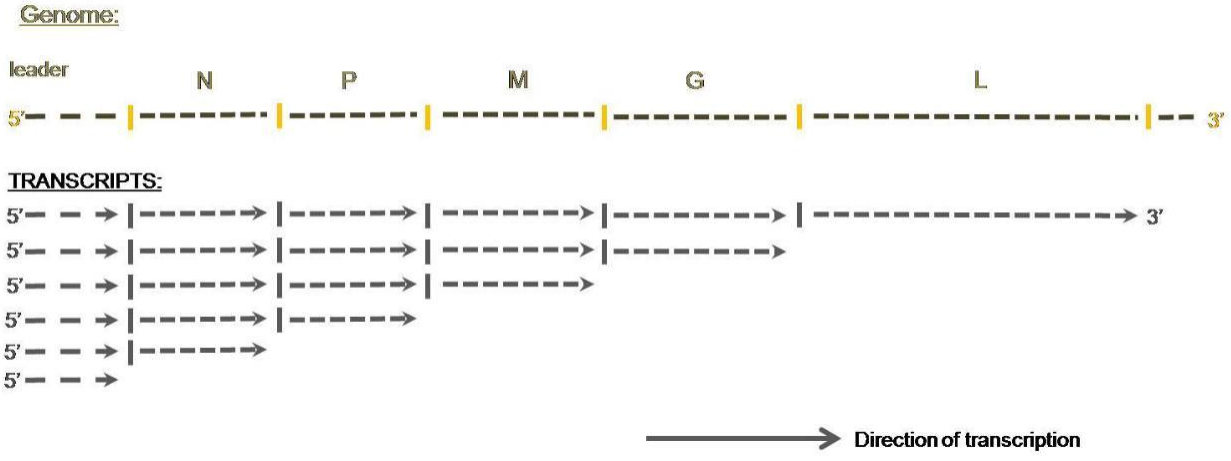
J. Helical nucleocapsid contains the RNA dependent RNA polymerase.

IV. The leader RNA is neither capped nor polyadenylated and is not functional as mRNA.

JJ. Replication occurs when the polymerase complex ignores the transcription stop signals at the 3' end of each gene and a full-length positive-sense antigenome is synthesized.

VI. Transcription at the gene-start site is not perfect, which leads to a gradient of mRNA abundance that decreases according to the distance from the 3' end of the genome.

Figure: 3.13. Gradient of mRNA abundance from 3' end towards 5' end:



N= Nucleocapsid protein

P= Phosphoprotein

M= Matrix protein

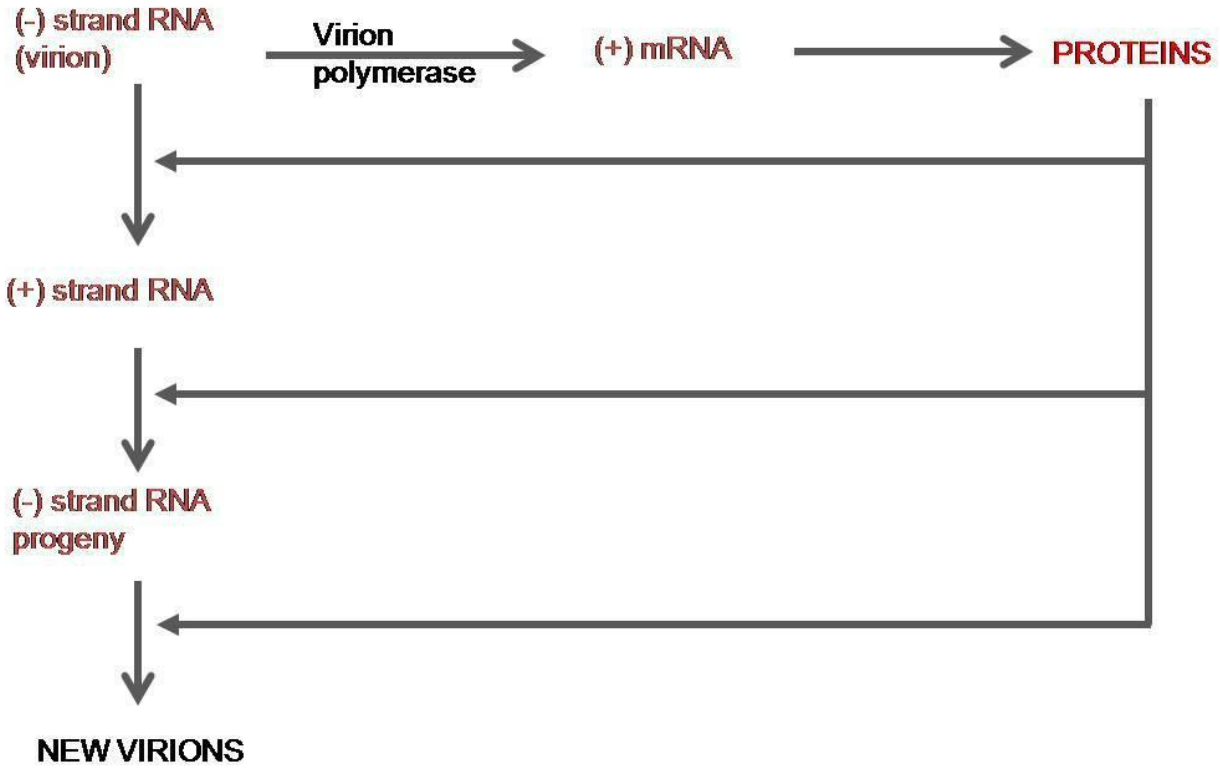
G= Membrane glycoproteins

L= Large polymerase protein

2 Genome replication

Virus enters the cell by receptor mediated endocytosis. The initial step after entering inside the cell is to transcribe viral mRNA from genomic RNA with the help of RDRP. The mRNA is further translated to form the viral proteins. The viral replication begins at 3' end and forms a complete positive sense RNA using negative sense genomic template. The viral transcription and replication occurs within a nucleocapsid-polymerase complex that consists of N, P, and L proteins. The switch from transcription to replication occurs when sufficient amount of N protein accumulates in the cytoplasm; it binds to the P protein to form a soluble complex, which is used for replication of the progeny RNA for the genome.

Figure 3.14. Replication strategy in negative sense RNA viruses:



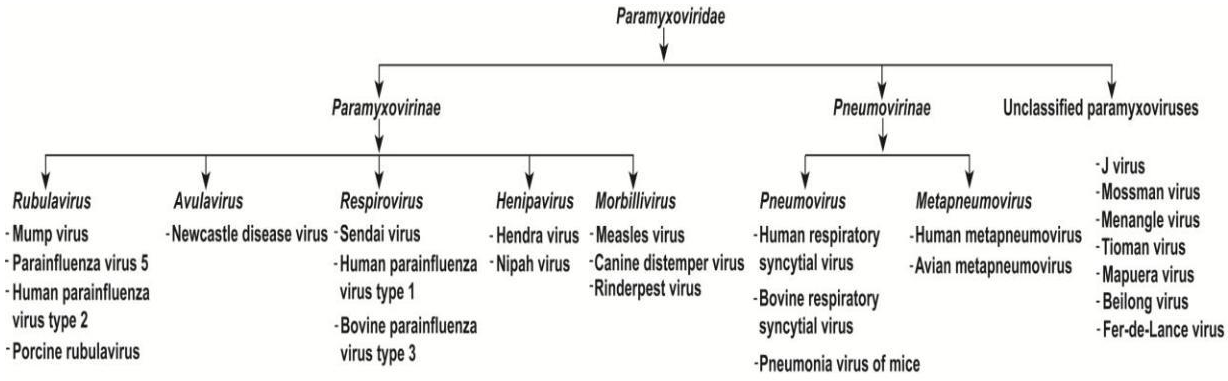
Paramyxoviruses

Paramyxoviruses are included under the family *Paramyxoviridae*, order *Mononegavirales* along with *Rhabdoviridae*, *Fliovіridae*, and *Bornaviridae*. All the viruses in this group are enveloped and contain surface glycoproteins over it. The genome of the virus is single stranded RNA of negative polarity. Many life threatening diseases are caused by members of the family *Paramyxoviridae*. The impact of the disease caused by these viruses has been reduced dramatically through the use of vaccine.

Classification of Paramyxoviruses:

The family is divided into two subfamilies which are further divided into different genera.

Figure:3.15. Classification of Paramyxoviruses



Virion properties

Paramyxoviruses are pleomorphic and about 150- 350 nm in diameter. Virions are enveloped and covered by surface glycoproteins. The genome consists of ssRNA of negative polarity and 13-19 kb in size. The RNA at 3’

end is not polyadenylated and 5' end of the RNA is not capped. With the exception of family *Pneumovirinae*, the genome size of the viruses are the even multiples (also called as rule of six) of six. The viral N protein binds effectively with the six nucleotide of the genomic RNA for its effective replication.

General Concepts

JJJ. The Paramyxoviruses are the leading cause of respiratory disease in children; general illnesses include croup and inflammation of respiratory tract.

J. Paramyxoviruses share similar features; they contain a bilayer envelope containing spikes, have a helical symmetry and contain a negative stranded ssRNA genome. An RNA-dependent RNA polymerase (RDRP) is carried by the virus particle in order to perform the replication of the RNA genome.

JJ. Replication of the virus takes place in the cytoplasm and are released by the budding process.

IV. Virus antigens are confined in the lipid envelopes (spikes) and within the nucleocapsid core.

JJJ. The viruses have a wide variety of host range that includes humans and primates.

VI. Viruses produce syncytia upon infection to susceptible cells by fusion and later cell lysis.

Different Paramyxoviruses:

Parainfluenza:

Nearly 25-35% of acute respiratory infections in infants and children are caused by this group of viruses. Disease starts with mild flu like symptoms which may progress to life-threatening (Croup, bronchiolitis and pneumonia) condition in the untreated cases. Parainfluenza viruses are the most common cause of **croup.** The viruses were divided into 4 distinct serotypes (numbered 1-4). These serotypes usually produce local inflammation in the upper and lower respiratory tract causing denudation of the ciliated epithelium (nose and throat). The virus generally sheds over 5-12 days following infection. Serotype 1 and 2 are attributed for the severe forms of the disease in young children.

Mumps:

This is another common disease of children. Acute infection of Mumps virus produces inflammation of salivary glands leading to its enlargement. Only one serotype is available for this group of virus. The common target tissues include glandular and nervous tissue. The virus enters through the pharynx or conjunctiva, systemic infection of the virus can cause viremia. Secondary dissemination of the virus occurs to salivary glands, gonads, pancreas, and central nervous system after their multiplication in the lymphoid tissues. Incubation period of the disease may vary between 18-24 days while in

many cases it is asymptomatic. The most characteristic feature of the disease is painful swelling of the parotid glands. Sometimes disease may lead to deafness and severe inflammation in the male reproductive system.

Measles:

It is also an acute disease of infants and children. The virus commonly causes a rash over the body with a high fever, occasionally conjunctivitis and pneumonia. In severe form of the disease virus may cause inflammation and pathological condition in the brain. Like Mumps virus only one serotype exists for Measles virus. Measles is also a systemic infection spread by dissemination of the virus through blood. Acute disease affects the lymphatic and respiratory systems while persistence of the virus in children leads to subacute sclerosing *panencephalitis*. Virus enters the body via the oropharyngeal route, multiplies locally within the lymphatic system, and spreads to the mucosal surface of respiratory, gastrointestinal and central nervous system. Clinically, respiratory symptoms and fever are evident during the early stages which on later changes to a rash during the eruptive phase. Rash over the body and head are sometimes called as **Koplik's spots**. They are ulcerated mucosal lesions characterized by necrosis and infiltration of neutrophils, and are the pathognomonic (hallmark and unique to measles) signs of the measles. Secondary infection by bacteria may sometimes complicate the situation and even turn worse in untreated condition.

Respiratory Syncytial virus:

Respiratory Syncytial virus is one of the leading causes of bronchiolitis and pneumonia in infants under one year of age. The viruses produce a characteristic syncytia formation in the respiratory epithelium cells; hence the name is given as respiratory syncytial virus. The virus starts its infection in the upper or lower respiratory tract infecting ciliated epithelium. Spread of the virus proceeds by cell fusion. Severe form of the disease may cause bronchiolitis, pneumonia, or croup in infants.

f) Croup is sometimes called as barking cough and is characterized by swelling around

the vocal cords. It usually associated with the inflammation of larynx, trachea, and bronchioles.

Orthomyxoviruses

The name originates from the Greek word "ortho" which means **correct** while "myxo" stand for **mucus**. The word essentially stands for virus that infects epithelial cells in the right way, exactly opposite to that of paramyxoviruses. The family *Orthomyxoviridae* contains viruses of single stranded segmented RNA genome (6-8 segments). Out of all, influenza viruses are the most important members of this family which includes influenza virus A, B, and C. The name originates during 18^{th} century from a disease that was thought to "**influence"** by stars. The pathogenic viruses are included in the genus influenza virus A, whereas other two genera (B and C) circulate constantly in human subjects. Several devastating pandemics caused because of influenza virus in past include famous Spanish Flu (1918), Asian Flu (1956), and Hong Kong Flu (1967) which killed millions of people.

Distinct Characters

I. The viruses cause an acute respiratory disease with prominent systemic symptoms with its major manifestation on the respiratory system.

II. Influenza virus type A is responsible for periodic epidemics worldwide; while virus types A and B cause regional epidemics during the cold weather.

III. **Antigenic drift** (minor changes in the viral surface proteins) and **Antigenic Shift** (major changes in the viral genome due to rearrangement of the virus segments or reassortments) are responsible for both epidemics and pandemics of influenza viruses.

Table 3.4 Differences between influenza virus types:

	Influenza virus type A	Influenza virus type B	Influenza virus type C
Animal Reservoir	Present	Absent	Absent
Spread	Pandemic/ Epidemic	Epidemic	Sporadic
Severity	High	Moderate	Mild
Variations	Antigenic shift/drift	Antigenic drift	Antigenic drift

Virus Classification

The family *Orthomyxoviridae* contains the genera Influenza virus A, Influenza virus B, Influenza virus C, Thogotovirus, and Isavirus.

Table 3.5. Different members of family *Orthomyxoviridae*:

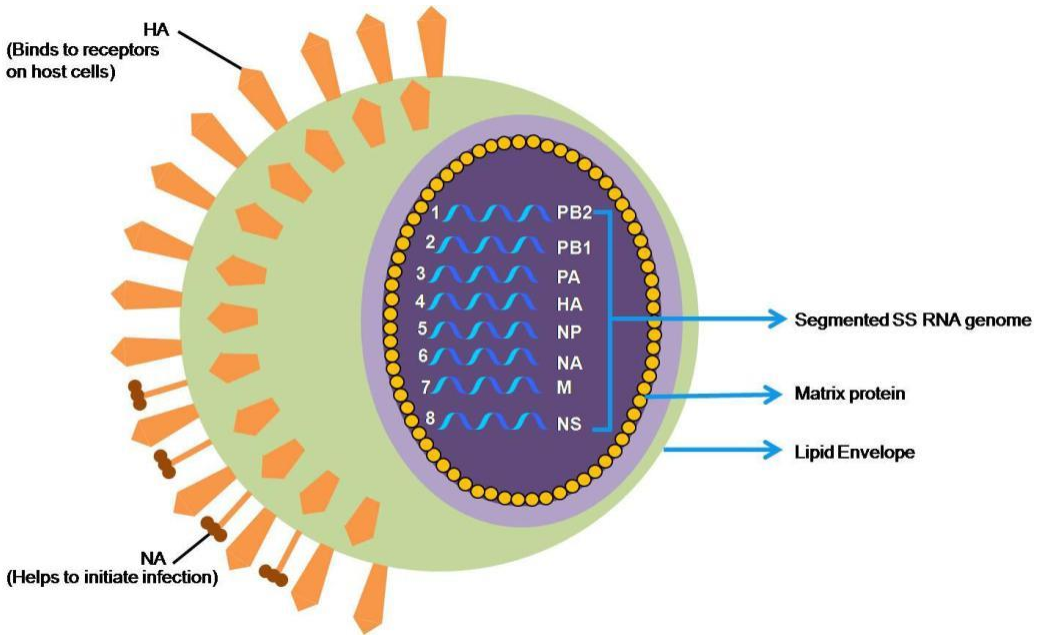
Genus	Virus type
Influenzavirus A	Influenza A virus
Influenzavirus B	Influenza B virus
Influenzavirus C	Influenza C virus
Thogotovirus	Thogotovirus Dhori virus Batken virus
Isavirus	Infectious salmon anemia virus

Virion properties

Orthomyxovirus virions are pleomorphic in shape and around 80–120 nm in diameter. The nucleocapsids are helical in symmetry and contains eight (influenza virus A, B, and isavirus), seven (influenza virus C), or six (Thogotovirus) RNA segments. Genome is single stranded negative sense RNA of 10 – 14Kb in size. The viruses also contain surface glycoproteins over the lipid envelope. The two envelope glycoproteins are hemagglutinin protein (H) and neuraminidase protein (N). There are **15** different subtypes of "H" and **9** different subtypes of "N". This provides a total of **135** (15 x 9) possible combinations. Hemagglutinin is the most important protein of the virus and determinant of its virulence while neuraminidase helps in the budding and release of the progeny virions. Both hemagglutinin and neuraminidase frequently undergo genetic modifications

decreasing the effectiveness of the host immune response. Influenza virus "C" lacks the "N" protein. Envelope is lined by Matrix protein "M1" and an ion channel matrix protein "M2". Three proteins namely PB1, PB2 and PA form the viral RNA polymerase complex which is associated with genomic RNA and nucleoprotein.

Figure: 3.16. Schematic representation of an influenza virus:



Virus Replication

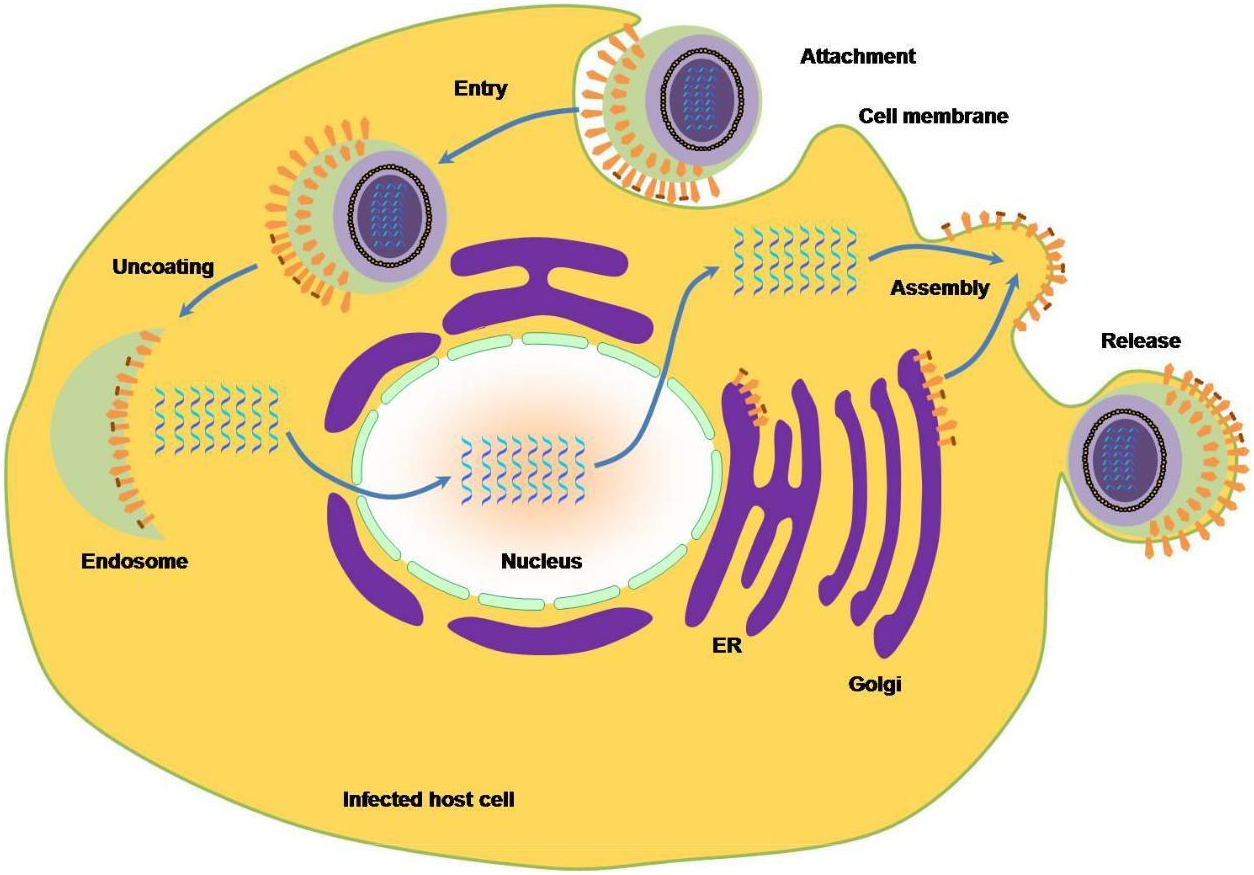
Influenza virus enters the cell after binding to sialic acid receptor present on the cell membrane. Different cells of the body contain different sialic acid receptor types which largely determine the host range of these viruses. The

respiratory tract epithelium of humans contains α 2-6 linkage sialic acid receptor while birds contain α 2-3 sialic acid receptor. The types of sialic acid receptor in the epithelium determines the tropism of human as well as avian influenza viruses. A single amino acid change in the hemagglutinin protein [E (Glutamic acid) 190D (Aspartic acid)] changed the binding efficiency of influenza virus from α2,3 to α2,6 which caused the outbreak of 1918 influenza virus (Spanish flu). Virus enters the cell by receptor mediated endocytosis and uncoats under the low pH condition of endosome. RNA synthesis of influenza virus takes place in the nucleus of the cell. Nucleoprotein of influenza virus contains nuclear localization signals (NLS) that help in transportation of ribonucleoprotein complex into the nucleus. Negative sense RNA genome of influenza viruses serve as a template for the synthesis of positive sense RNA. Positive sense replicative intermediate RNA acts as a template for progeny RNA genome. Interestingly, viral endonuclease activity of PB2

protein cleaves the 5' cap and 10-13 nucleotides from a cellular mRNA in order to transcribe the viral RNA. The phenomenon is called as **cap snatching**. All orthomyxoviruses undergo **splicing** phenomena to produce two proteins from one gene such as influenza virus A uses gene segment 7 to produce M1 and M2 protein. Similarly 8^{th} segment of influenza virus produces NS1 and NS2 protein after undergoing splicing. In certain influenza viruses, frame shift mutation leads to formation of PB1-F2 protein. Viral protein synthesis occurs in the cytoplasm and its maturation takes place in endoplasmic reticulum and Golgi apparatus. Viral nucleoproteins are required for replication of genomic RNA which then enters the nucleus

along with polymerase protein for transcription. Progeny virus is released by budding through the plasma membrane.

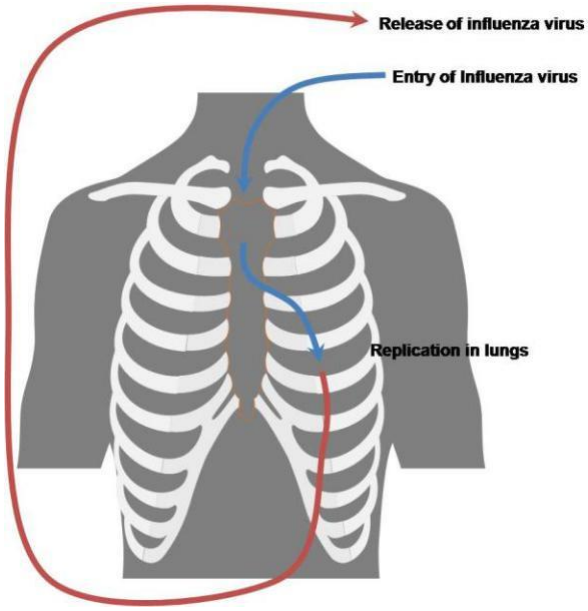
Figure:3.17. Schematic representation of an influenza virus replication cycle:



Influenza pathogenesis and bird flu

When influenza virus (commonly referred as flu) enters the host via aerosol it replicates in the epithelial cells of upper and lower respiratory tract. The infection causes destruction of the ciliated cells along the lining of the respiratory tract that leads to inflammation and formation of exudates. The signs include tracheitis, bronchitis, and pneumonitis. Sometime collapse of adjacent air sacs and emphysema is also evident. Microscopically, there is complete denudation of the epithelial lining and infiltration of inflammatory cells.

Figure:3.18. Mode of entry and exit of influenza virus in humans:



Molecular determinants

The hemagglutinin (HA) protein of influenza virus is a key in determining the virulence and pathogenicity of influenza virus. HA protein of influenza

virus has to cleave in order to start the infectious cycle. The highly pathogenic influenza virus has many basic amino acid (**arginine**) residues at the cleavage site of the HA protein, while the less pathogenic viruses contains less basic amino acid residues at the cleavage site. The virus containing less basic amino acids are restricted to multiply in certain selected tissues as the protease required for the cleavage site is present only in certain specific tissues. In humans and
birds respiratory and gastrointestinal tract contains the proteases to cleave the HA protein. In contrast, the viruses containing more basic amino acid residues at the cleavage site increases the range of cells capable of producing infectious virions. This property of the virus is important in determining its tissue tropism.

Non structural (NS1) protein in influenza virus is another key factor in determining the virulence of the viral strains. NS1 protein binds to dsRNA and blocks the interferon activation (refer lecture 11 and12). The influenza virus containing mutation in the NS1 proteins are attenuated as the mutated form of the protein does not bind with the dsRNA which causes increased level of interferon in the infected cells.

Polymerase protein PB2 is another important component that determines the virulence of influenza viruses. PB2 helps in RNA transcription and replication of the virus. The PB2 containing mutation in certain amino acid sequences may increase or decrease the virulence of the virus. Another protein such as PB1 is also suggested as a determinant of influenza virus virulence.

Clinical features

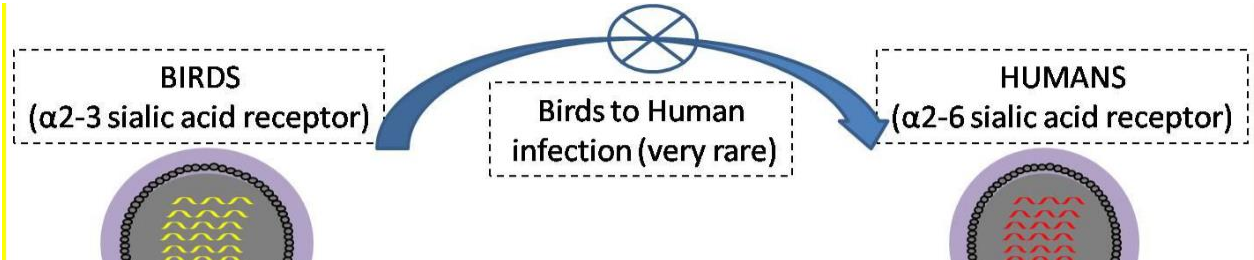
The incubation period of the virus in human is from 2 to 4 days, may extend up to 8 days based on the immune status of the patient. Initial symptoms of the disease include fever and lower respiratory tract illness. Diarrhea, abdominal pain, headache, and vomiting are other common symptoms observed during the early phase of infection. The later phase of the disease shows the symptoms of more advanced respiratory distress such as acute respiratory distress syndrome (ARDS). Multi-organ failure involving kidney and heart are also associated during the later part of the disease.

Influenza virus reassortment

Reassortment is another very interesting phenomenon and occurs in viruses having segmented genome. Reassortment stands for mixing of viral genetic information in order to evolve them as a new species having potential to cause a greater pandemic. If a cell or animal is infected with two different influenza viruses, the RNA segments of both viruses

are copied in the nucleus. When new virus particles are assembled at the cell membrane, each of the 8 RNA segments may originate from any one of them or a mixture of two infecting viruses. The progeny virion that contains RNA segments from both the parents is called as reassortant. This process is illustrated in the diagram below for involution of swine influenza virus

Figure:3.19.Reassortment of avian and human influenza virus in swine:



Bird Flu

Bird flu is also known as avian influenza (flu infection in birds). Historically the disease was known as **fowl plague**. The virus caused havoc in the human population when a bird infecting virus mutated to infect humans. The first case was reported in Hong Kong in 1997 and that was known as H5N1

(avian influenza A virus). Human cases of H5N1 were reported in many parts of the world. Major risk group includes farmers and others who are in contact with poultry, visiting the countries affected with bird flu, and people consuming undercooked poultry meat from infected birds.

Symptoms

In human subjects symptom include sore throat, nasal discharge, headache, fever, diarrhea, breathing difficulty, and cough. If birds survive following infection, they show complete loss of egg laying capacity, respiratory distress, diarrhea and nervous signs (tremors and paralysis).

Pathogenesis

The virus cause damages in the respiratory epithelium and alveoli. The alveolar spaces are filled with fibrinous exudates and inflammatory cells. Vascular congestions and proliferation of fibroblast is also evident in many cases. Varied degree of hepatic damage is also seen in many cases. In birds picture is quite different than humans, as virus replicates in the intestine also. Infected birds show the signs of viremia and necrosis of different visceral organs, and often succumb to death following few days of infection.

Treatments

Antiviral drugs such as oseltamivir (Tamiflu) or Zanamivir (Relenza) reduces the severity of infection if taken within 48 hrs after the onset of symptoms. In later stages of infection patient may need respirator for breathing.

Rhabdoviruses

Rhabdo in Greek means 'rod-shaped'. The first description of rabies dates from the 23rd century BC in Mesopotamia. In Latin word "*rabere"* means to rage (In sanskrit word *Rabhas* means to do violence). During early 1881, French chemist **Louis Pasteur** and his assistant, **Emile Roux**, begin their research on rabies and successfully vaccinated **Joseph Meister who was bitten by a rabid dog**. Rabies is a zoonotic viral disease most often transmitted after the bite of rabies infected animal. Large cases of the rabies are reported each year from different parts of the world. The members of the family *Rhabdoviridae* belong to order *Mononegavirales* and contain six genera. The Rhabdoviruses contain negative strand RNA genome ranging from 11 to 15 Kb in size. Those Rhabdoviruses that infect plants are rod shaped with rounded ends eg Potato yellow dwarf virus while those infecting animals are bullet shaped eg Rabies virus (Lyssa virus).

Rhabdoviridae is divided into six genera

1) Novirhabdovirus
2) Vesiculovirus
3) Lyssa virus
4) Ephemerovirus

↕ Animal viruses

1) Cytorhabdovirus

↕

2) Nucleorhabdovirus — Plant viruses

Virus	Virus type	Host species
Vesiculovirus	Vesicular stomatitis Indiana virus	Vertebrates
Lyssavirus	Rabies virus	Vertebrates
Ephemerovirus	Bovine ephemeral fever virus	Vertebrates
Novirhabdovirus	Infectious haematopoetic necrosis virus	Vertebrates
Cytorhabdovirus	Lettuce necrotic yellows virus	Plant
Nucleorhabdovirus	Potato yellow dwarf virus	Plant

Virion properties

Rhabdoviruses contain a linear, single stranded, negative sense RNA genome. Virions are 45- 100 nm in diameter and 100- 430 nm long. The virion has a cylindrical nucleocapsid surrounded by an envelope with large glycoprotein spikes. The virions are sensitive to heat and UV rays but stable towards the changes in pH. The virus is bullet shaped which is due to its lipid envelope. The genome is 11.9 kb in size which encodes for 5 genes in the following order.

3' –N-P-M-G-L- 5'

N- Nucleocapsid protein

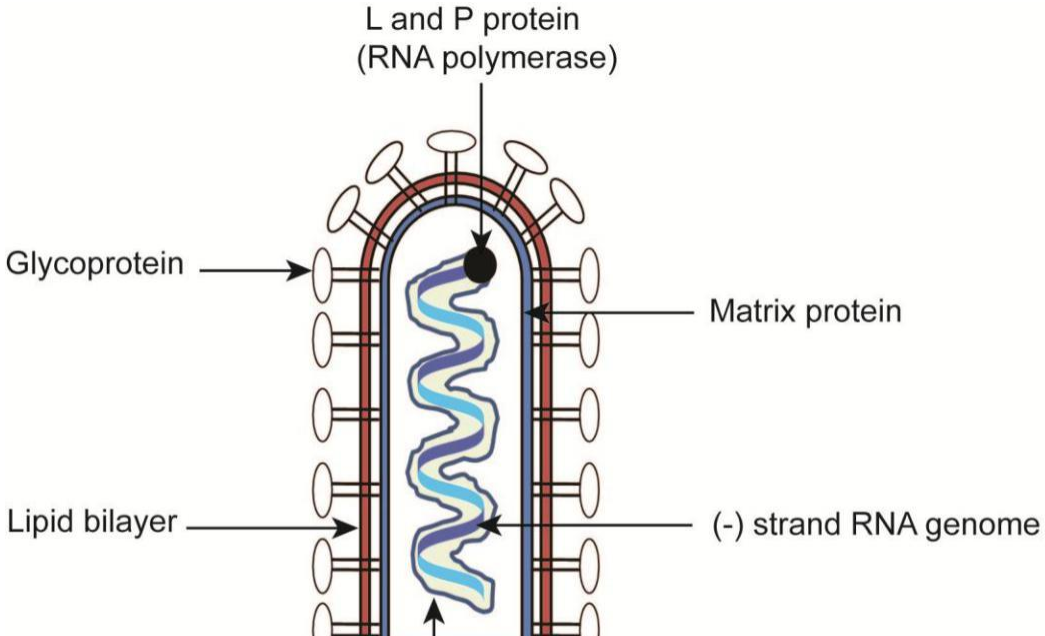
P- Phosphoprotein- cofactor of the viral polymerase

M- Inner virion protein/ helps in budding of the virion.

G- Glycoprotein that assists in making virion spikes

L- Large protein that represents RNA dependent RNA polymerase and helps in transcription and replication.

Figure 3.20. Schematic representation of rhabdovirus virion:



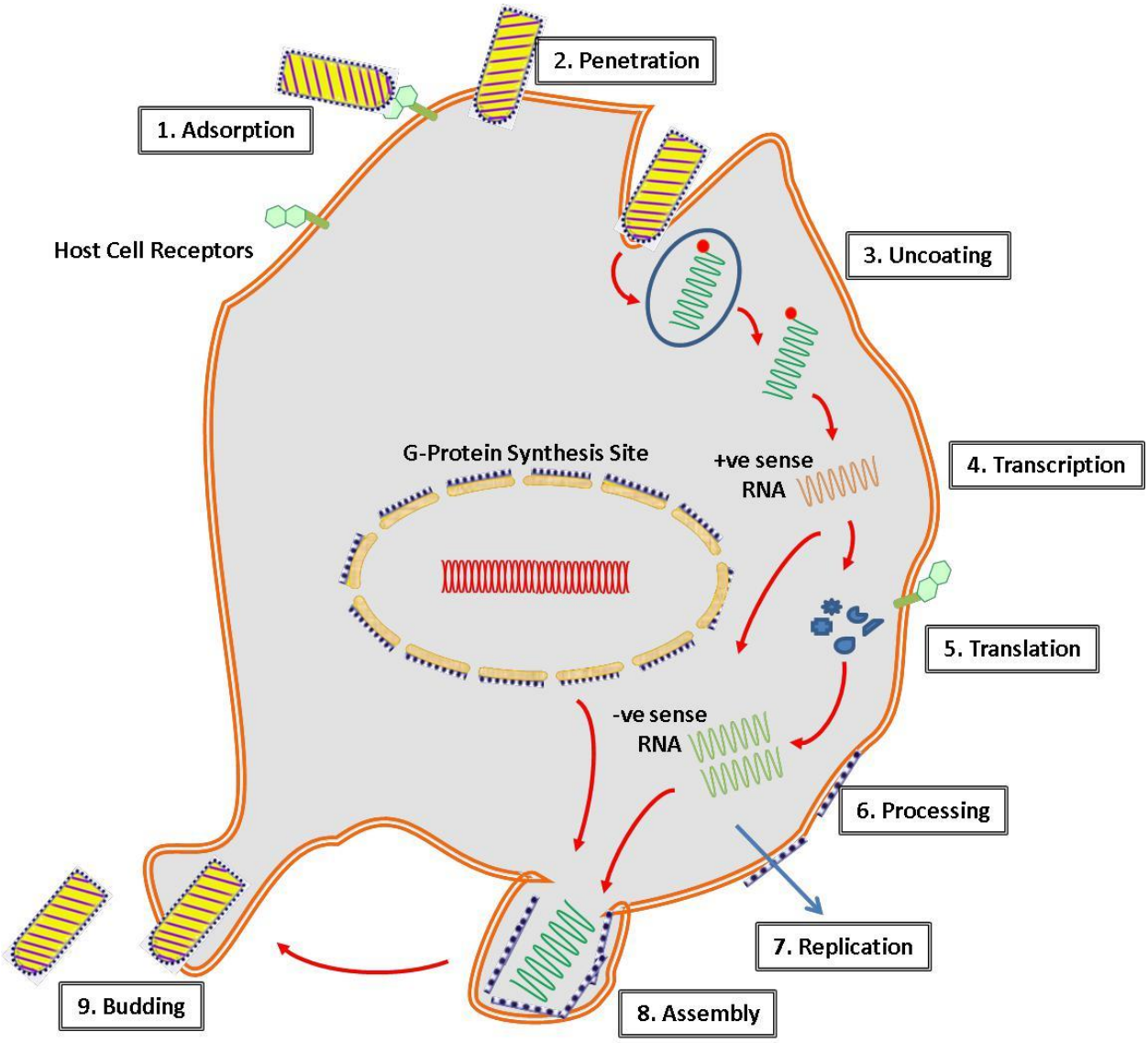
Virus Replication

In animals, virus replication takes place in the cytoplasm while in plants it may occur in nucleus. The virus entry into the host cell occurs by receptor mediated endocytosis followed by pH dependent fusion of virus envelope with endosomal membrane. As a result of fusion, the nucleocapsid is released into the cytoplasm. The first step of replication involves mRNA transcription from genomic RNA using RNA dependent RNA polymerase. For successful replication a large amount of nucleoprotein (N) and phosphoprotein (P) should be expressed. Switching of transcription to positive sense antigenome occurs after a threshold amount of N and P, which are then further used as a template for synthesis of negative stranded genomic RNA. There is a single promoter site at the 3' end of the viral genome where the polymerase attaches to the genomic RNA template and moves along the viral RNA. While moving it hits with start – stop signals at both the ends of the viral genes. Due to this only a small fraction undergoes continuous

transcription process and hence this phenomenon is also known as **attenuated transcription**. Consequently more mRNA is produced towards the genes that are located at the 3' end and hence producing a gradient of

mRNA in the order of N>P>M>G>L. As a result of the mRNA gradient, large amount of structural protein such as nucleocapsid protein is produced as compared to L protein.

Figure 3.21. Schematic representation of rhabdovirus replication cycle:



Important Rhabdoviruses

Vesicular stomatitis virus

Vesicle means blister and stomatitis means inflammation of oral mucous membrane. Vesicular stomatitis virus (VSV) is a disease of various animal species including cattle, horse, sheep and pig. Animals develop the lesions in the feet and mouth similar to that of foot and mouth disease. The VSV can replicate in a variety of cell lines. Most of our understanding regarding the rhabdovirus replication and transcription came from the study of VSV.

Bovine Ephemeral fever virus

It is an arthropod transmitted disease of cattle and buffalo characterized by biphasic or

polyphasic fever, depression, diarrhea, and loss of appetite. The disease is also called as

3-day stiff-sickness. The virus causes inflammation and injury to the inner lining of the

endothelial blood vessels.

Infectious hematopoietic necrosis virus

It is a disease of salmonid fishes. The virus is associated with significant economic losses to the countries producing salmon in large quantity. Infection is characterized by darkened body colour, pale gills, distension of abdomen due to accumulation of fluid, and hemorrhages around fins.

Rabies

WORLD RABIES DAY is being celebrated every year on **SEPTEMBER 28**.

Rabies is a dreaded disease and is of zoonotic importance. Disease is generally caused by the bite of a rabid dog/ animal. Although rabies in humans is 100% preventable by appropriate medical care, more than 55,000 people in Africa and Asia, die from rabies every year. Children are often at greatest risk as they are more likely to be bitten by dogs.

Clinical features and epidemiology

Rabies is a zoonotic disease and it may infect any warm blooded animal including humans. Virus is usually present in the saliva of the infected animal. The disease usually occurs in two clinical forms, furious and dumb (paralytic). The furious form is characterized by insomnia, confusion, agitation and often leading to delirium. It is also called as hydrophobia as the infected person cannot gulp water because of pharyngeal paralysis. Sometime profuse salivation and encephalitis are also evident. Progressive encephalitis leads to dumb form of the disease. Terminal stages include convulsive seizures, coma and respiratory arrest.

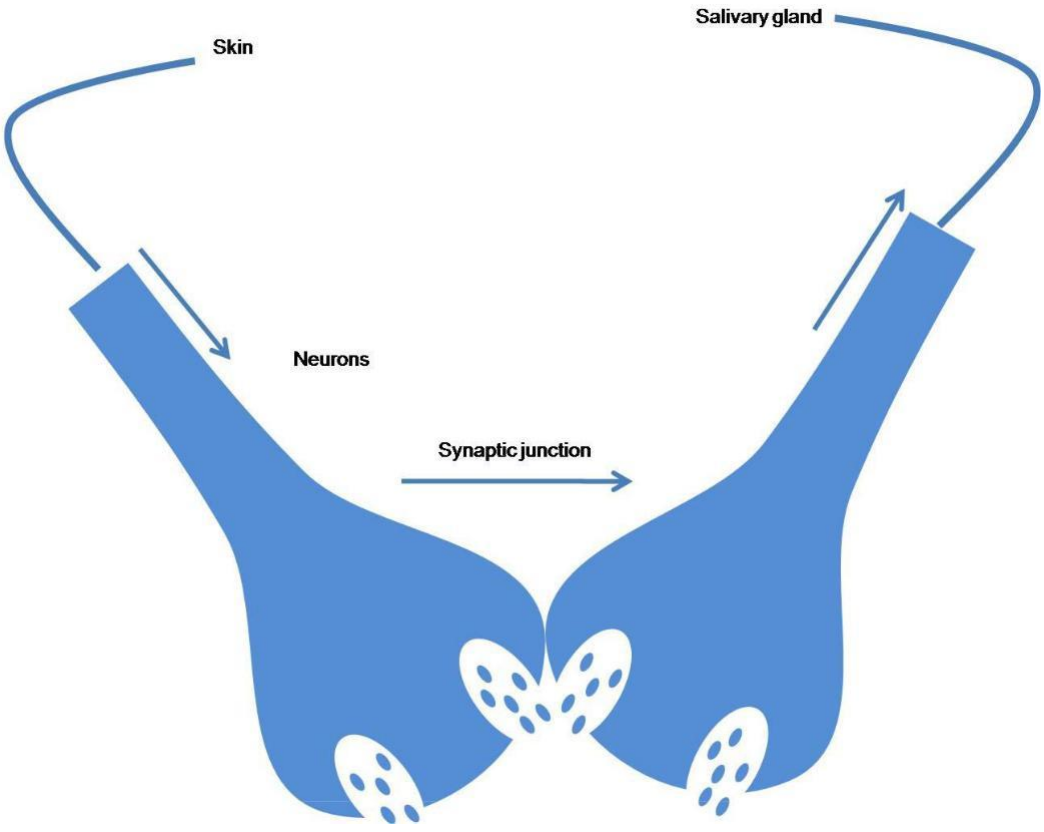
Pathogenesis and Pathology

In rabies the prognosis of the disease is mainly decided by the location and severity of the site of bite and the species of animals involved. Rabies has three stages. The first stage is characterized by behavioral changes also known as **prodromal** stage. In the second stage called as **excitative** stage the animal exhibits disease in furious forms and in the third stage the animal manifests the **paralytic** form of the disease. After entry the virus first affects the peripheral nervous system and replicates in the

brain. Dumb and paralytic forms of the disease appear as it progresses towards the central nervous system. In the nervous system the virus is formed by budding in various membranes and glands. Neurons accumulate ribonucleoprotein as intracytoplasmic inclusion bodies often termed as **Negri bodies** and are pathognomonic for rabies. Salivary glands help the virus to bud

on plasma membrane and release it in very high concentrations through the saliva. Death usually occurs due to respiratory arrest.

Figure : 3.22.progression of rabies virus through neurons in body:



Diagnosis

Any patient having a history of animal bite should be suspected for rabies. Differential diagnosis with West Nile virus, herpesvirus, enterovirus is required. History and clinical symptoms like encephalitis, salivation, etc provide concrete support for rabies. Besides this, **Negri** bodies in the neurons of affected animals can be visualized using Seller's stain. RT-PCR assay can be conducted to test for the presence of viral RNA in the brain of suspected animal. RT-PCR of saliva can also be carried out. Immuno-histochemical staining can also be done from frozen sections of brain tissue. Fluorescent antibody test can also be done for the detection of viral antigens from the tissue samples.

Immunity, prevention and control

Rabies is a highly immunogenic disease and many vaccines are available for the same. In early stages of infection, infectious rabies virus is susceptible to antibody mediated neutralization and this efficacy has been proved in exposed humans of the classical Pasteurian post- exposure vaccination and it provides even better results when administered along with hyper-immune globulin.

Blood brain barrier shows promising results when its permeability is increased artificially as it helps in viral clearance and does not allow most immune cells across. Thus altering the permeability of blood brain barrier to contribute to viral clearance can also be exploited in case of Rabies.

Besides this, prophylactic measures should be taken like scheduled vaccination, washing the wounds with soap for 5 to 10 times while treating the infected cases and regular spaying of the animals can prevent the disease.

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DEPARTMENT OF BIOTECHNOLGOY

UNIT – IV – MEDICAL VIROLOGY – SMB3102

RNA VIRUSES

DOUBLE STRANDED RNA (DSRNA) VIRUSES

Double stranded RNA (dsRNA) genome containing viruses infect wide range of animal as well as plants. The majority of the viruses in this group contain icosahedral capsid and similar strategies for the replication of genomic RNA. Despite similarities in the replication strategy, structure, and cognate proteins, the amino acid and nucleotide sequence identity between different genera is generally low. The diversity of proteins and genomic sequence might be the reason for the wide host spectrum of these viruses.

Table 4.1 Important members of viruses containing dsRNA genome:

Family	Host	Genome/ Segments	Genera	Structure
Reoviridae	Mammals, Plants, Fungi, Fish, Insects	10-12	*Rotavirus, Coltivirus, Orbivirus Aquareovirus Seadornavirus*	Icosahedral around 100 nm in diameter
Birnaviridae	Birds, Fish, insects	2	*Avibirnavirus Aquabirnavirus Entomobirnavirus*	Icosahedral, 75nm in diameter
Chrysoviridae	Fungi	4	*Chrysovirus*	Icosahedral, 30-50 nm in diameter
Hypoviridae	Fungi	1	*Hypovirus*	Pleomorphic, 60-100 nm in diameter
Totiviridae	Plants	2	*Varicosavirus*	Rod Shaped

Total of 8 families of dsRNA viruses are currently recognized by ICTV. Two most important family members are Reoviruses and Birnaviruses. These two groups contain viruses of medical as well as of veterinary importance. Rotavirus is a major cause of infant diarrhea while blue tongue virus is a big issue for cattle and sheep. Infectious bursal disease virus is another important member of family *Birnaviridae* which causes an

immunosuppressive disease in poultry and are of great economic importance. Another member of family *Birnaviridae*, Infectious pancreatic necrosis virus causes significant losses to the fisheries industry around the world.

Expression of viral proteins

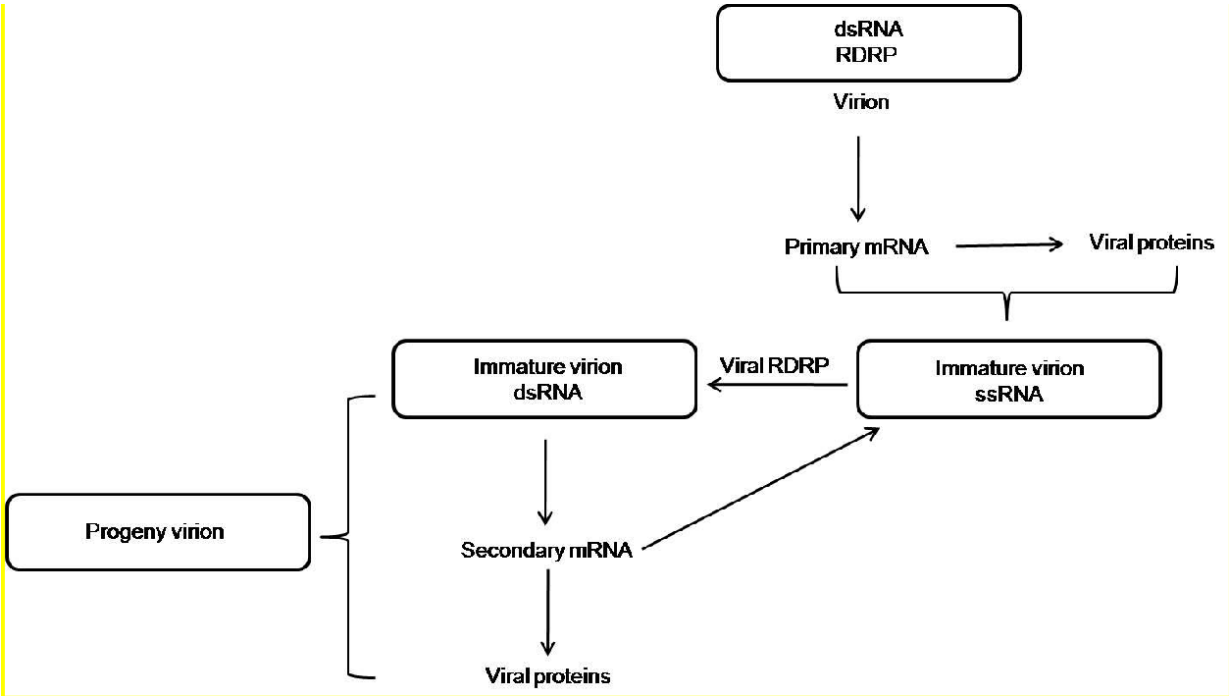
The size of dsRNA genome of the viruses is limited by the icosahedral capsid which can accommodate the segmented genome and at the same time actively allow the transcription of the viral messages. The viruses evolve in such a way to conveniently dissociate the total translation products from the genome into several distinct proteins. Sometimes individual genome segments can also be exchanged in the presence of a suitable donor or acceptor which increases the genetic diversity in virus population. The dsRNA transcribes separately to full-length +ve sense RNA. The full-length +ve sense RNA acts as mRNA for viral protein synthesis as well as template for the synthesis of genomic RNA for the progeny virions.

Replication of the genome

The replication of the genome is divided into several steps as follows

1. In the first step primary transcription of the viral genome takes place inside viral core in the cytoplasm using viral RNA dependent RNA polymerase (RDRP)
2. Positive sense RNA is then transported into the cytoplasm
3. Positive sense RNA is translated to form viral proteins
4. Positive sense RNA and viral proteins are assembled to form immature virions
5. Positive sense RNA is then transcribed to form dsRNA in virions by viral RDRP
6. dsRNA undergoes secondary transcription
7. Final assembly and maturation of virions

Figure 4.1 Replication strategy of dsRNA virus:



Reoviruses

The family Reoviridae contains viruses which are most complex in nature. The term Reo stands for "respiratory enteric orphan," which was named because the first member was identified in the respiratory and the enteric tract of animals and humans and was not associated with any type of disease. They are generally spherical in shape and have icosahedral symmetry. They do not contain any envelope. The different viruses belonging to this family have their names which are indicative of their unique morphological features. For example in case of rotaviruses, rota stands for wheel like capsid with spikes, similarly in orbiviruses, orbi stands for ring shaped capsid. Some of the reoviruses are transmitted through the bite of female culicoides (blue tongue virus) or through tick bite (Colorado Tick fever).

The family *Reoviridae* contains six genera based on group specific antigen present on VP6 capsid protein

1) Orthoreovirus
2) Orbivirus
3) Coltivirus
4) Rotavirus
5) Seadornavirus
6) Aquareovirus

Table 4.2. Diseases caused by Reoviruses:

VIRUS	DISEASE
Orthoreovirus	Respiratory and enteric diseases
Orbivirus	Blue tongue fever in cattle and Sheep African Horse Sickness disease
Coltivirus	Colorado Tick fever
Rotavirus	Gastroenteritis and Diarrhoea
Aquareovirus	Diseases of Fish

Virion Property

Reovirus particles are non-enveloped, spherical having a diameter of approximately 85 nm. Genome contains a linear dsRNA divided into 10-12 segments. The overall genome size varies from 16 to 29 Kbp. They contain a cap at their 5' end while poly A tails are absent from 3' end.

Virus Replication

Virus replication takes place in the cytoplasm of the cell. Because of the segmented RNA genome chances of reassortment of genomic segments between different strains is very high. This results in genetic drift and shift leading to diversity among viruses which is reflected by numerous serotypes within each genus.

Virus enters the cell by receptor mediated endocytosis. The coated vesicle uncoats and fuses to the lysosomes under low pH condition. Virions are disrupted and viral inner core is released into the cytoplasm. Virus associated RNA polymerase utilizes the negative strand of each dsRNA segment as template to transcribe viral mRNA. These viral mRNA's are translated to form viral structural proteins that eventually assemble to form the infectious virion. Progeny viruses remain cell associated but are often released by lysis of the infected cell. Genomic RNA replication takes place within sub-viral particles in the cytoplasm of infected cells.

Important Reoviruses

Rotavirus

Rotaviruses are the most important human pathogens which lead to life threatening diarrhoea in young ones. It was first isolated from a children hospital in Australia. Rotavirus infection leads to destruction of intestinal villi. Transmission of the virus mainly takes place by fecal-oral route. Destruction of enterocyte (intestinal cells) causes mal-digestion and poor absorption of food. Intestinal epithelium of young ones has a slow turnover rate and that is the reason that rotavirus infection is more severe in case of infants. Rotavirus infection is characterized by severe diarrhoea, dehydration, weight loss

and fatigue. Virus can be isolated in faeces in high amount. Enzyme immunoassay is commonly used to detect viral infection in infected individuals. Genetic reassortment vaccines are available for rotavirus infection.

African Horse Sickness

African horse sickness is caused by a member of genus orbivirus. It is a pantropic and fatal disease of horses, predominantly infecting endothelial cells and myocardium. Acute form of the disease is characterized by pneumonia**,** interlobular pulmonary oedema, pericarditis, haemorrhages and oedema of the visceral organs. The death can occur within 5 days in highly acute form of the disease. A more prolonged form of the sickness involves the **cardiac vascular system**. Mortality can be more than 80% depending on the immune status of the animal and virulence of the isolate. **Subclinical disease** can occur in donkeys and vaccinated horses. Diagnosis is usually carried out by using complement fixation tests and haemagglutination inhibition tests.

Bluetongue Virus

It is another important disease of livestock caused by a member of genus orbivirus. It causes high mortality in sheep and decrease in productivity of other farm animals. The virus is transmitted to the animals by the bite of Culicoides mosquitoes. The chances of outbreaks are more during the breeding season of Culicoides. Major signs of the disease include high fever, swelling of the face, hyperemia around the coronary band and **cyanosis of the tongue** (blue colour of the tongue). The disease can be restricted by controlling mosquitoes while polyvalent live vaccines are available for the animals.

Colorado Tick fever

Colorado Tick fever is a disease caused by bite of tick (*Dermacentor andersoni*) infected with Coltivirus. Transmission of this disease is reported to be through blood transfusion from an infected individual. The virus usually infects and replicates in the erythrocyte. The symptoms of the disease include fever, headache, vomiting, abdominal pain, and encephalitis.

Retroviruses: structure, classification, life cycle

Retroviruses are single stranded RNA viruses that undergo DNA phase during their replication. All the retroviruses contain reverse transcriptase enzyme and are capable of integrating with the host genome. Currently retroviruses are divided into 7 classes based on their biological and molecular properties. In general they are classified into three categories –

I. Oncoviruses
II. Lentiviruses
III. Spumaviruses

I. Oncoviruses

These viruses are associated with one or other form of cancer in different animal species. Some viruses carry the oncogenes as part of their genome e.g Rous sarcoma virus, Human T lymphotrophic virus (HTLV).

II. Lentiviruses

These viruses have long incubation period and are associated with immune deficiencies. Occasionally encephalopathy and arthritis may also be evident eg human immuno deficiency virus (HIV), feline immuno deficiency virus (FIV), and simian immuno deficiency virus (SIV).

III. Spumaviruses

Spuma stands for foamy. The virus causes vacuolation and froth like appearance in infected cells.eg simian foamy virus (SFV).

General Concept

a. Out of three subfamilies of Retroviruses two infect humans namely oncornaviruses (Human T Cell Lymphotrophic Viruses (HTLV) I, II, and V) and lentiviruses (HIV 1, HIV 2).

HTLV-I causes cutaneous T-cell lymphomas,

HTLV-II causes hairy T-cell leukemias,

HTLV-V causes T-cell lymphomas and leukemias.

HIV-1 causes Acquired Immunodeficiency Syndrome (AIDS),

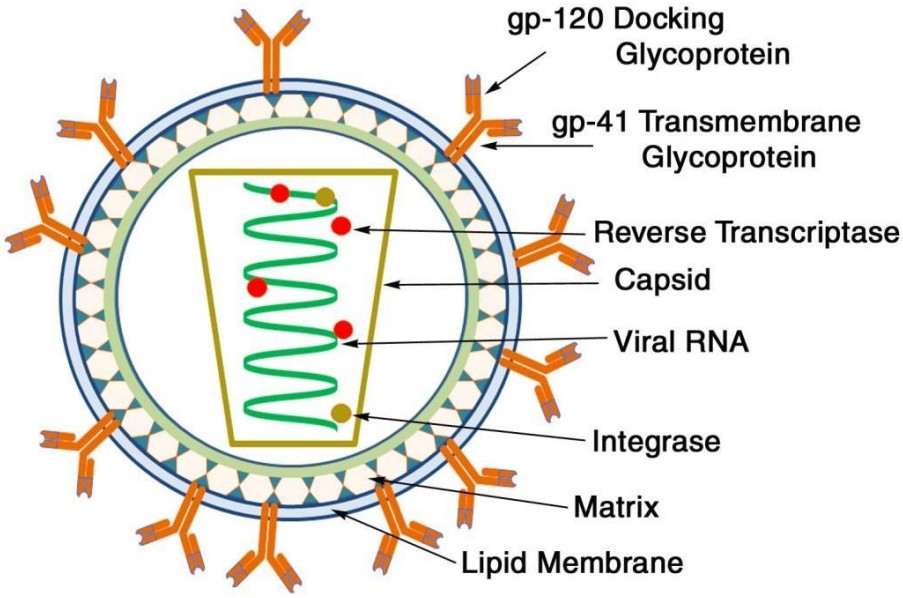
HIV-2 causes AIDS related syndrome in Africa.

b. Retroviruses contain RNA genome and an enzyme called reverse transcriptase, which makes circular DNA by using RNA as a template. The viral DNA integrates with the host cell chromosomes.
c. HIV is an enveloped virus which contains glycoprotein 120 (gp120) which binds to CD4 receptor present over the T helper cell.
d. Genome of HIV composed of two positive strands RNA which are capped at 5'end and polyadenylated at 3'end.
e. The tRNA acts as a primer for the synthesis of DNA using RNA as a template.
f. Long terminal repeats (LTR) present towards both the ends help in the integration of viral genome to host genome. It also serves as promoter and enhancer for the viral genome.
g. Group specific antigen (**gag**) helps in the formation of capsid protein. Polymerase (**pol**) encodes message for the formation of reverse transcriptase. Envelope (**env**) gene is associated with the formation of gp120 and gp41. **tax/rex** are the regions which encodes the factors involved in transactivation and other regulatory functions.

Morphology

Retroviruses are spherical and approximately 100 nm in size. The virion contains cone shaped nucleocapsid that encapsidates two copies of single stranded RNA about 10 Kb in size. In addition, capsid also contains three enzymes namely reverse transcriptase, proteases, and integrase. The nucleocapsid is covered by matrix protein which in turn is covered by an envelope containing two transmembrane glycoprotein called gp120 and gp 41.

Figure.4.2 Schematic representation of human immuno deficiency virus (HIV):



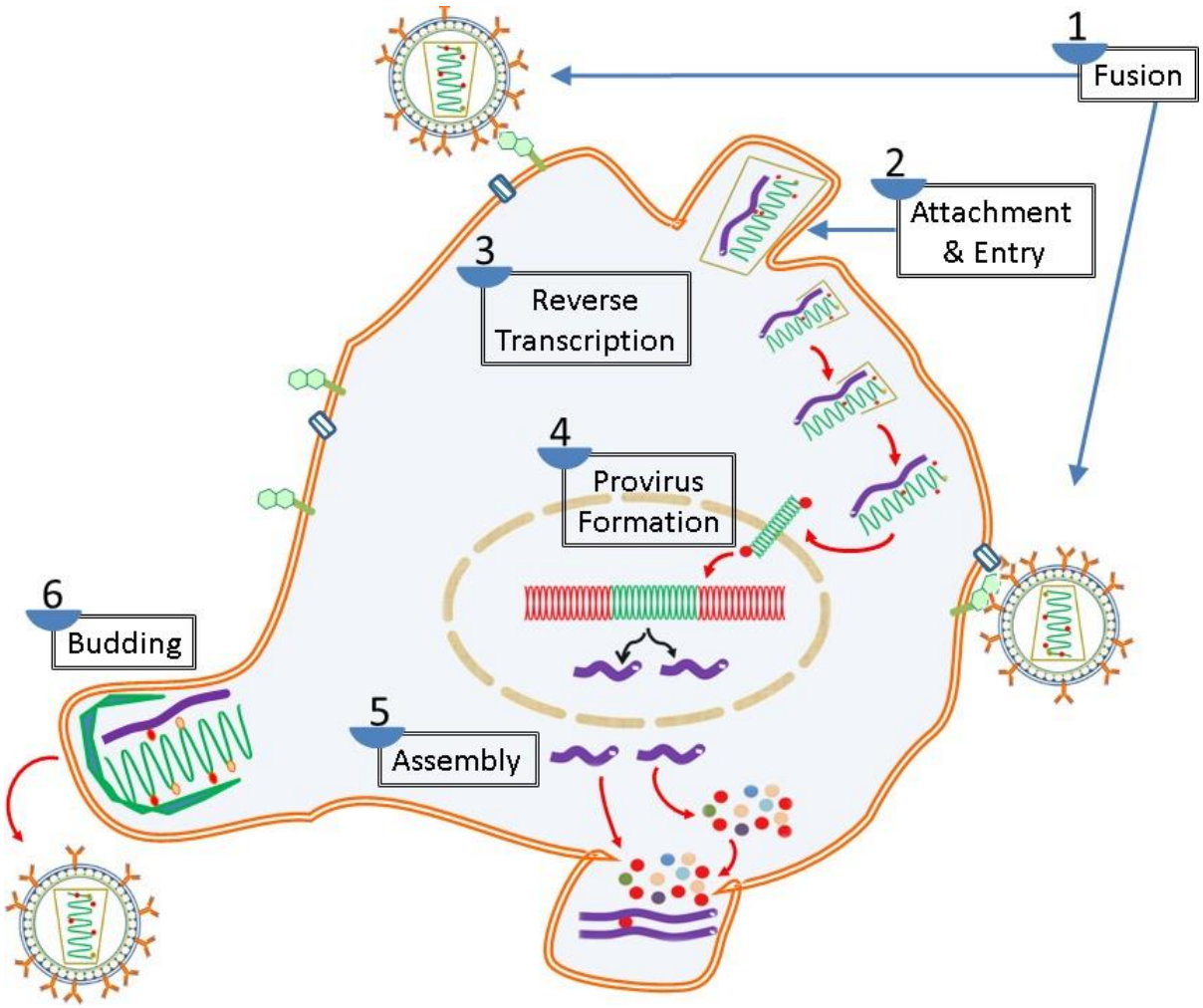
Virus entry

HIV enters the host cell by infecting the T- helper cells and macrophages which contain CD4 receptor. The viral gp120 binds to CD4 receptor to initiate the entry process. Coreceptors such as chemokine receptors (CCR5 and CXCR4) also assist in entry process. Different HIV serotypes use different coreceptors. Following the binding, the virus and cell membrane fuses releasing the nucleocapsid inside the cell.

Virus replication

Many of our present days understanding about the retrovirus comes from the work of HIV. Upon entry to the cells, viral RNA produces a polyprotein which is cleaved by virus encoded proteases to form individual protein. Virus uses the tRNA as a primer to synthesize DNA using reverse transcriptase enzyme. RNA from the hetroduplex form of RNA and DNA is then cleaved by RNase H. The remaining part of DNA is synthesized as double stranded and integrates into the host cell genome with the help of enzyme integrase. The viral genome remains integrated into the host genome and keeps on transcribing the viral RNA for the progeny virions. The progeny virions are assembled in the cytoplasm and exit by the process of budding taking the covering of the host cell membrane.

Figure .4.3. Schematic representation of HIV replication:



31.2 Endogenous Retroviruses

Genome of many vertebrate animals contains retroviral sequences. These sequences are mostly inactive or defective. On an average a normal human cell contains approximately 1, 00, 000 retroviral sequences. It is likely that the retroviruses can infect the germ lines early in the life, giving rise to these sequences in the human genome.

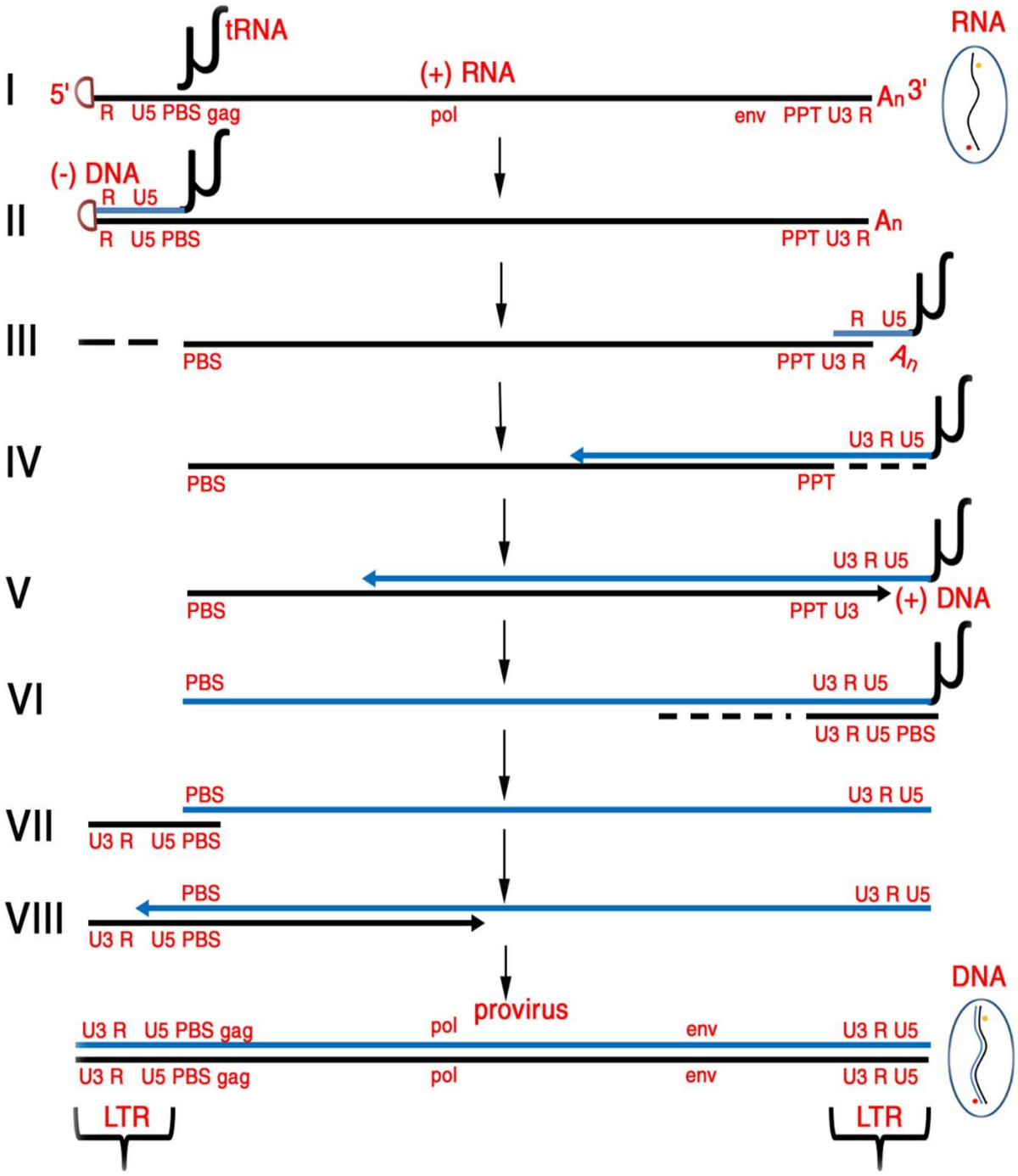
Reverse transcription

The virus binds to the cell surface with the help of surface glycoproteins present over the envelope. This interaction leads to change in the configuration of the proteins which allows the fusion of virions to the host cells. The virus releases its content into the cytoplasm and a reverse transcription complex is formed in order to complete the genome replication.

Reverse transcription

The phenomenon of reverse transcription takes place in the reverse transcription complex. **Cellular tRNA** acts as a primer which binds to the 5' end of the RNA to form negative sense DNA. **Polypurine tract (PPT)** present on the RNA acts as a primer for the synthesis of positive sense DNA. **RNase H** enzymatic property of the reverse transcriptase helps in the cleavage of RNA from a RNA-DNA hetroduplex. The DNA formed because of the reverse transcription is called as **PROVIRUS**. The DNA of the provirus is longer than the parental genomic RNA. The 3' end of the genomic RNA contains unique region called as U3, similarly, 5' end also contains U5 as unique region. The provirus contains an extra U5 along with U3 and similarly extra U3 with U5 (reason for longer length of provirus). The provirus contains U3- Repeat (R)-U5 sequence at both the ends which is also called as long terminal repeats (LTR).

Figure4.4. Schematic representation of the complete reverse transcription process:



Step1- A cellular tRNA binds to the primer binding site (PBS) in the RNA genome.

Step2- Negative sense DNA is synthesized towards 3' end with the help of virus reverse transcriptase.

Step3- RNase H digests the RNA from RNA-DNA hetroduplex. First jumps of the negative strand DNA occurs towards 5' end.

Step4- Negative strand DNA continues to elongate and RNase H digest the RNA- DNA hetroduplex till PPT.

Step5- Synthesis of positive strand DNA begins from PPT towards 5' direction.

Step6- All the remaining RNA degraded by RNase H.

Step7- Second jump occurs were positive sense DNA move from 5' end and binds to the 3' end of the negative sense DNA.

Step8- Synthesis of remaining strand of the DNA completed.

Integration of the provirus

Newly formed provirus associated with viral integrase is then migrated to the nucleus. The migration of the provirus usually occurs at the time of mitosis because of the fragility of the nuclear membrane at the time of cell division. The provirus with integrase is referred as pre-integration complex. Once inside nucleus, the viral integrase cuts the host chromosome and ligates the provirus into the gap. The provirus may express immediately or may be inactive in case of latent infection. When cell starts dividing the provirus is also copied into the daughter cells along with the host chromosome.

Transcription

The transcription of the provirus starts from the LTR sequence with the help of cellular RNA polymerase II. The transcription begins at U3-R junction and terminates at the R-U5 junction. All the transcripts are capped and polyadenylated. Some of these act as mRNA and some as a progeny genome (Fig 32.2). LTR present on the viral genome act as the promoter and other cellular transcription factors help RNA polymerase II to transcribe the RNA. Transcription produces mRNA's similar to that of genomic RNA that are spliced at different locations to form different mRNA subspecies. Without spliced mRNA forms gag and pol, one time spliced to env mRNA, and two times to TAT, REV and NEF mRNA's. During early phase of transcription only double spliced

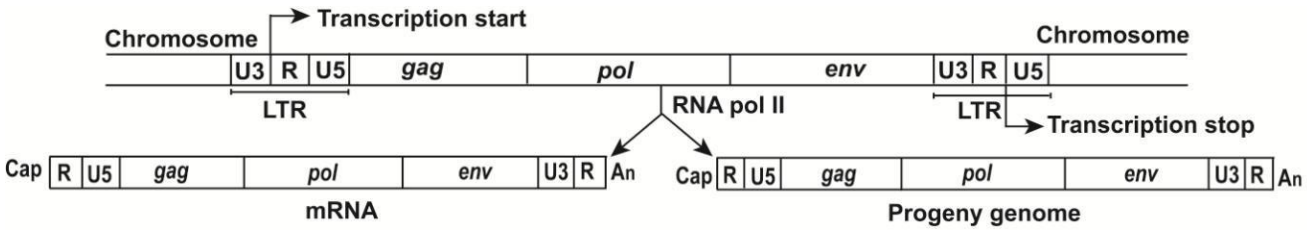
mRNA's are formed which increases the level of TAT, REV and NEF in the cell. TAT acts as transcription enhancer and REV helps in the export of spliced mRNA.

TAT stands for "Trans-Activator of transcription".

REV stands for "Regulator of Virion Expression".

NEF stands for "Negative Regulatory Factor".

Figure 4.5. Transcription process in the integrated provirus:



Translation, Assembly and Release

The envelope proteins are synthesized by the "env" gene following splicing. The proteins (gp 120 and gp41) become glycosylated in the endopolasmic reticulum and transported through Golgi apparatus to the surface of plasma membrane. Gag and Pol are produced as polyprotein which later on are cleaved by viral proteases to form individual protein. The virus usually needs more of Gag protein as compared to Pol, hence, the synthesis of Gag protein is always towards the higher side. The envelope proteins in retroviruses are glycosylated while the Gag and Pol proteins are myristylated. Virus assembly takes place at the plasma membrane. Viral protein also migrates to the membrane where genomic RNA interacts with viral protein and gets assembled. Many viral polyproteins undergo cleavage and rearrange after budding through plasma membrane.

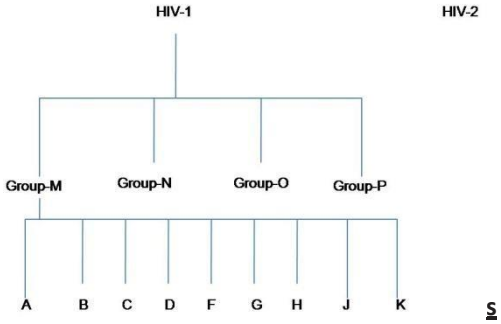
HIV- viral pathogenesis

Human immunodeficiency virus (HIV) -1 and -2 was thought to evolve from simian immunodeficiency virus (SIV). Both of these viruses emerged during 18^{th} century and prevalence of HIV-1 infection is far greater than HIV-2. The virion has a general features of other retroviruses (Please refer lecture 31 and 32). HIV-1 contains on an average 14 surface glycoprotein (gp120 and gp41) over its virion. In contrast, the glycoproteins in HIV-2 are composed of gp130 and gp38 and other internal proteins similar to HIV-1. The genome of HIV is around 9.6kb in size and contains all the essential genes for the expression of viral proteins (Please refer lecture 31 and 32). HIV infects the CD4+ T helper cells and macrophages. Apart from binding with cell receptor (CD4), the HIV also requires co-receptors such as chemokine receptors **CXCR4 (α)** and **CCR5 (β)**. HIV strains which uses CXCR4 are known as **X4** while those using CCR5 are called **R5**. The use of chemokine receptor by HIV largely determines the tropism of the HIV. T-cell tropic strain of HIV uses CXCR4 co-receptor while macrophage tropic strain of HIV uses CCR5 as a co-receptor.

HIV-1 and -2 clades or subtypes

HIV-1 is divided into four groups M, N, O and P. Group M is divided into nine different clades based on the sequence alignment of gag and env gene. Clades A, C, D, and E are responsible for high incidence rate of HIV.

Figure 4.6. groups and clades of HIV-1:



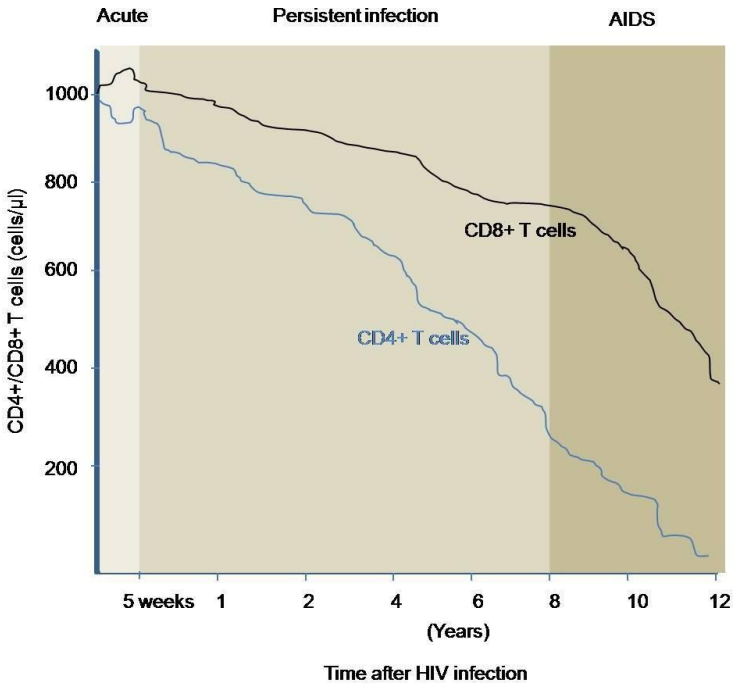
S

Primary HIV infection

Viruses infect the macrophages during the early phase of infection through binding with CCR5. The virus is then transferred to the dendritic cell with the help of DC-SIGN (dendritic cell specific ICAM-3 grabbing nonintegrins) receptor present over the surface of dendritic cells. Dendritic cells then deliver the virus to the draining lymph node where

it replicates and causes viremia after their dissemination into the blood circulation. As the disease progresses the body produces an immune response which reduces the viral load in the body. The virus set point is reached after 6 months of initial infection and persists till many years. 50-90% of the infections are symptomatic which occurs after 5-30 days post exposure.

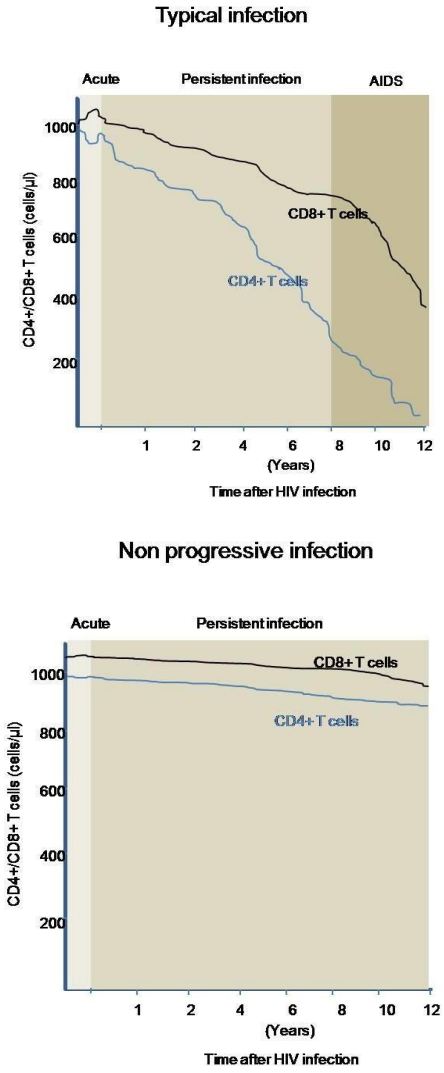
Figure 4.7. Graphical representation of HIV infection and its progression:

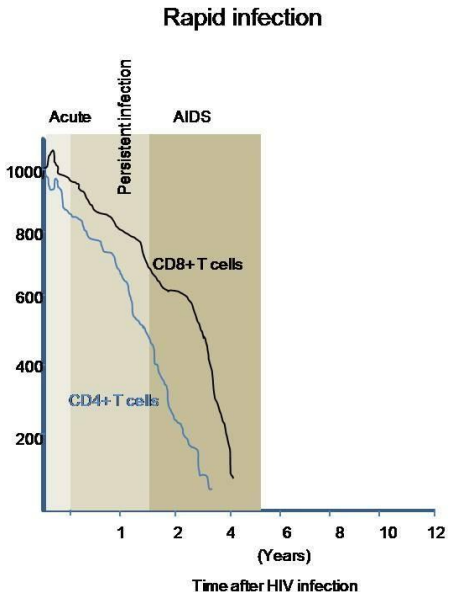


Established HIV infection

Viruses actively replicate throughout the course of the disease. The virus replicates outside the CD4+ cells in gut associated lymphoid tissues, central nervous system, and genital tracts. Around 10^{10} virus particles are made and destroyed everyday during the course of the disease. The half life of a mature HIV virion is around 30 min to 6 hrs in the plasma of infected patient.

Figure4.7. Type of HIV infection towards host susceptibility:





HIV and Cancer

HIV-1 infection leads to a variety of cancers in the infected individuals. This may occur because of immune dysfunction and absence of proper immune cells for surveillance inside the body. Generally this causes the replication of many oncogenic viruses. In addition, unregulated level of cytokines can cause proliferation of the cells and angiogenesis.

Acquired immuno deficiency syndrome (AIDS)

World AIDS day is celebrated every year on 1st of December.

Approximately 35 million people are affected with HIV worldwide. In India around 2.5 million people are suffering from HIV while in US more than one million people are suffering. There is decline in HIV infectivity in India as well as other parts of the world during the past 10 years. About 80 percent of the AIDS cases are reported in men between the age group of 20 to 44. In general males, percentage varies from 78 to 80 percent and the female around 20 to 22 percent. Nearly 1.8 percent of the total AIDS cases are children born to HIV infected mothers. In HIV infection an infectious doze means presence of ten thousand or more particles in the body. The level of HIV particle in different body fluids varies.

"AIDS is defined as HIV positive patient with CD4+ count less than 200cell/mm^3 or CD4+ count less than 14% of total lymphocyte population".

Transmission of HIV –

There are three major ways of HIV transmission

1) Sexual interaction
2) Blood transfusion
3) Perinatal infection

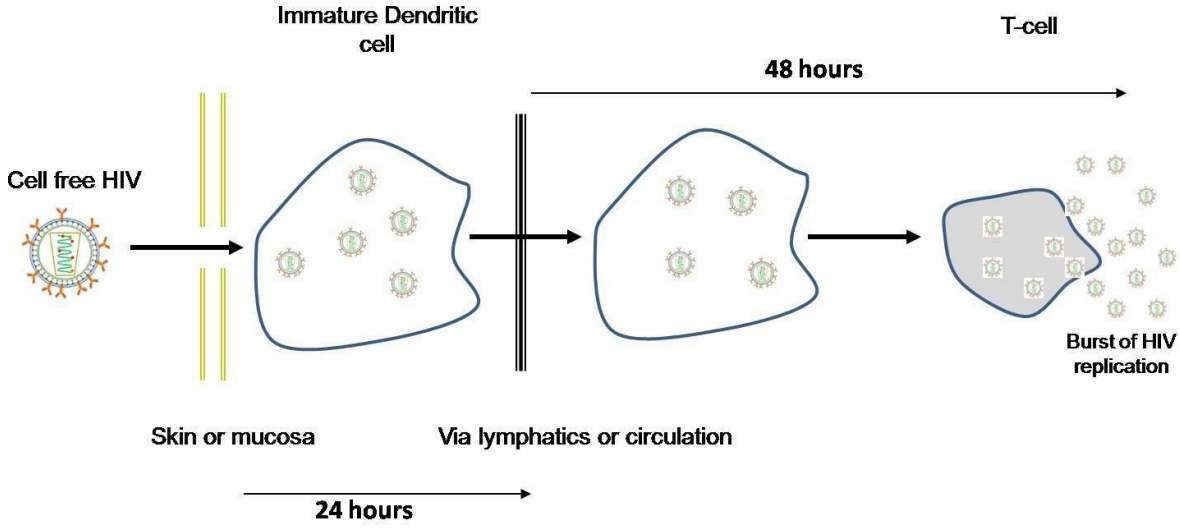
1) Sexual interaction - Risk of transmission of HIV is higher if person is infected with other sexually transmitted disease (STD) eg. herpes, syphyllis, Gonorrhoea etc. Passive partner is always in the higher risk side. This is more established way of HIV transmission.
2) Blood transfusion – HIV transmission is also possible by the way of infected blood, syringes, needles and other body fluids including saliva. Since now blood donors are screened first for HIV, the number of HIV cases decreased to a greater extent.

3) Perinatal infection – Young born children get infected if the mother is infected with HIV. There are several theories established regarding transmission of HIV through placenta or during delivery. Many studies have suggested involvement of breast milk for the transmission of HIV.

Early Phase of HIV Infection

Decrease in the number of circulating CD4+ lymphocyte is the hallmark of AIDS. CD4+ T helper cell and the macrophages are the major reservoir of HIV. Replication of HIV in macrophages results in budding of progeny virions through the membranes of endoplasmic reticulum (ER) which means that it acquires its envelope from ER similar to that of Coronaviruses. After entry into the body the virus travels to circulating lymph nodes and starts its replication. Initial infection of about 1 to 3 weeks results in fever, high virus titer in blood and high depletion of CD4+ T helper cell. After one month of infection the virus titer gets reduced in the blood circulation because of cytotoxic T cells, natural killer cells and antibody dependent cellular cytotoxicity

Figure 4.8. Mucosal entry of HIV and its path of circulation:



HIV-infected person during the latent period?

Cells are called latently infected if they are infected by HIV and are not active in producing virus but can be activated by various signals during the course of infection. Since they are not producing the virus they would not be detected by the immune system and acts as a perfect reservoir of the virus in the body. Only a small amount of cells are latently infected with HIV (around 1%). During the asymptomatic stage, the virus appears in low titers in the blood and it is counter balanced by the host immune system.

AIDS associated disease conditions

Several specific syndromes are associated with infection by HIV. These include:

1. **Fever and Lymphadenopathy** It is characterized by loss of weight and malaise.
2. **Opportunistic infections:** HIV infected patients have lower immune response because of depletion of CD4+ cells. Several diseases that rarely affect normal individuals may occur in HIV positive patient. The organisms are: *Pneumocystis carinii,* a causative agent of pneumonia, tuberculosis, fungal infection such as candidiasis, herpesvirus, *Salmonella*, *Shigella* and *Campylobacter*.
3. **Cancer:** Kaposi's sarcoma is a rare type of cancer that occurs in HIV-infected persons. These normally benign lesions become malignant and disseminate to involve visceral organs.
4. **Wasting disease:** Disease is characterized by hide bound condition.
5. **AIDS dementia:** Sometime HIV infection of the brain leads to condition that mimics Alzheimer's disease.

Antiviral drug against AIDS and some facts

a) Around 95% reduction of virus within 14 days after treatment with essentially any one of the following drug (nucleoside RT inhibitors, nonnucleoside RT inhibitiors, protease inhibitors).

b) Average half life of an HIV infected cell is about 24-48 hrs.

c) Approximately 5% of total CD4+ T lymphocytes are productively infected in an infected individual at any given time during the latent period.

d) The CD4+ T lymphocytes that are dying each day are being replaced nearly as fast as they die. This means that bone marrow, spleen and other reservoirs of T lymphocytes must be producing

the cells at an exponential rate.

e) Nearly within 7 -25 days after treatment with any one drug there is emergence of resistant virus.

f) The rapid emergence of mutant virus suggests that the resistant virus was already present in the population at the time drug treatment was started.

g) Resistant viruses do not grow quite as well as the wild-type viruses.

h) Upon removal of the anti HIV drug, the wild type virus once again becomes predominant over the course of infection.

i) One potential factor in the development of AIDS may be excessive stress on the immune system due to rapid turnover of T lymphocytes.

Control

I. The use of condoms during sexual intercourse can reduce the chance of infection.

II. Avoiding intravenous drugs (*Needle sharing*).

III. Monitoring the blood for HIV before transfusion.

IV. Educating people regarding the cause, severity, and preventive measure of HIV.

V. Anti HIV drugs: These drugs are often given in combination of two or three. Nucleoside and Non-nucleoside analogues are called as reverse transcriptase inhibitors.

VI. Protease Inhibitors- saquinavir

VII. Nucleoside analogues- Zidovudine and AZT

VIII. Fusion inhibitors- Enfuvirtide

IX. Non- nucleoside analogues- Nevirapine

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DEPARTMENT OF BIOTECHNOLGOY

UNIT – IV – MEDICAL VIROLOGY – SMB3102

DNA viruses

Small DNA viruses: Parvo and Polyomaviruses

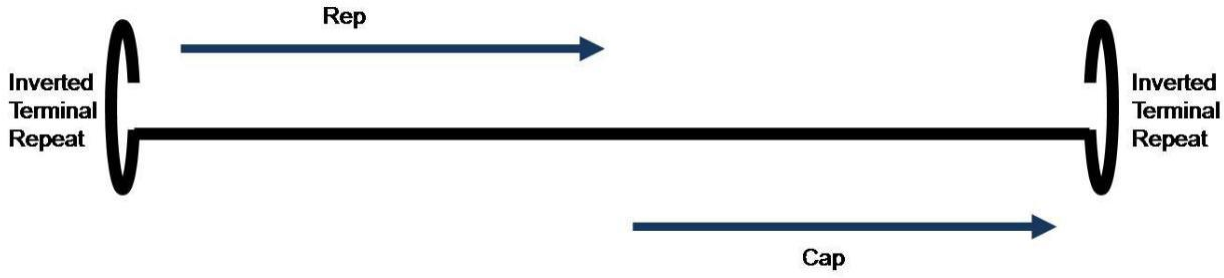
Cellular DNA synthesis occurs only during the S phase of the cell cycle, so the viruses which depend on host cell DNA polymerase must either wait for cells to enter S phase or express some protein early during infection to regulate the cell cycle (many small DNA viruses).

Parvoviruses

Parvo in latin stands for small. The virion is icosahedral, non-enveloped, and around 25 nm in diameter. These are the smallest of all animal viruses. They do not contain any viral or host enzyme and virion is made up of 80% protein and 20% DNA by weight.

The genome of parvovirus is linear, ssDNA which contains approximately 4500 to 5500 nucleotides. All parvoviruses contain terminal palindromic sequences at their 5' and 3' ends which allow the formation of Y or T shaped structures. In addition, they also contain inverted repeats at 3' and 5' ends which allow the circularization of the genome. **Rep** gene is required for replication of DNA while **cap** gene forms the capsid. The virion also contains 3 coat proteins namely, VP1, VP2, and VP3. VP3 is the most abundant among all three and it is made by the proteolytic cleavage of VP2. VP2 constitutes about 80% of capsid protein.

Figure 35.1 Genome map of parvoviruses:



There are 3 subfamilies of Parvoviridae

a) The family is divided into three subfamilies: *Parvovirinae*, which infect vertebrates and human, *Densovirinae*, which infect invertebrates, and *Hamaparvovirinae*.

Viruses infecting humans

Humans can be infected by viruses from five of the eight genera in the subfamily *Parvovirinae*:
i) *Bocaparvovirus* (human bocavirus 1–4, HboV1–4),

ii) *Dependoparvovirus* (adeno-associated virus 1–5, AAV1–5),
iii) *Erythroparvovirus* (parvovirus B19, B19V),

iv) *Protoparvovirus* (bufavirus 1–3, BuV1–3; cutavirus, CuV)

v) *Tetraparvovirus* (human parvovirus 4 G1–3, PARV4 G1–3).

As of 2018, no known human viruses were in the remaining three recognized genera:
vi) *Amdoparvovirus* (e.g. Aleutian mink disease virus),

vii) *Aveparvovirus* (e.g. chicken parvovirus), and

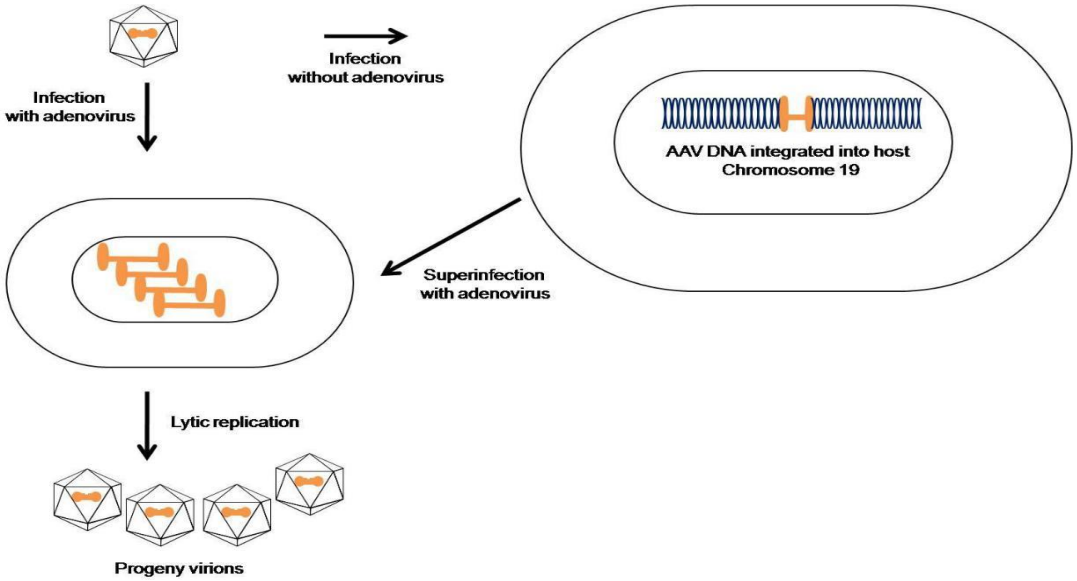
viii) *Copiparvovirus* (e.g. bovine parvovirus)

II. **Parvoviruses** – They only replicate in actively dividing cells. These viruses are highly resistant to heat, nucleases, detergents, proteases, and mild acid. They generally spread through body secretions. The most common strain of parvovirus that infects humans is B19.

I. **Dependovirus** – They are also called as adeno associated virus (AAV). They require adenoviruses to replicate. Upon infection in absence of helper virus they can establish latent infection by integrating into the host genome. They are one of the very important tools for targeted gene therapy viral vector.

3) **Densovirus** – They infect only invertebrates.

Figure 35.2 Helper dependent replication strategy of adeno-associated viruses:



Replication

The receptor for parvovirus has not been identified yet. It replicates with the help of host DNA polymerase and its assembly occurs in nucleus. Palindromic sequences at the termini are the initiation sites for DNA replication. Following replication, transcription is carried out by host cell RNA polymerase II. The genome of parvovirus codes for two non- structural proteins NS1 and NS2 apart from 3 coat proteins VP1, VP2 and VP3. Translation of the mRNA occurs in the cytoplasm and protein enters into the nucleus where assembly occurs. The virion exits the cell by lysis and the whole process is completed in 24hrs.

Papovaviruses

The name is derived from **papillomas** (warts), **polyomas** (multifocal tumors), and **vacuoles** in infected cells. These viruses contain dsDNA and are non- enveloped and spherical. They replicate and assemble in the nucleus of the infected cell and are released out following the lysis of the cell.

There are two genera

II. Papilloma

JJ. Polyoma

Papilloma - Genome is around 8000 bp long. They depend upon the replication machinery of the host cell. They often infect basal cell layers of the skin and hence are associated with warts. Papilloma viruses do not grow in tissue culture.

Polyoma - Genome is around 6000 bp long. They are associated with leukoencephalopathy and immunosuppression in humans. The most common virus SV40 that has been used to study mammalian replication belongs to this group. SV 40 makes large and small T-antigens which are required for viral DNA replication as it binds to origin of replication and is known to possess helicase and ATPase activity.

Replication

III. Adsorption of virions to the cell surface and entry by endocytosis.

II. Transport to the cell nucleus and uncoating

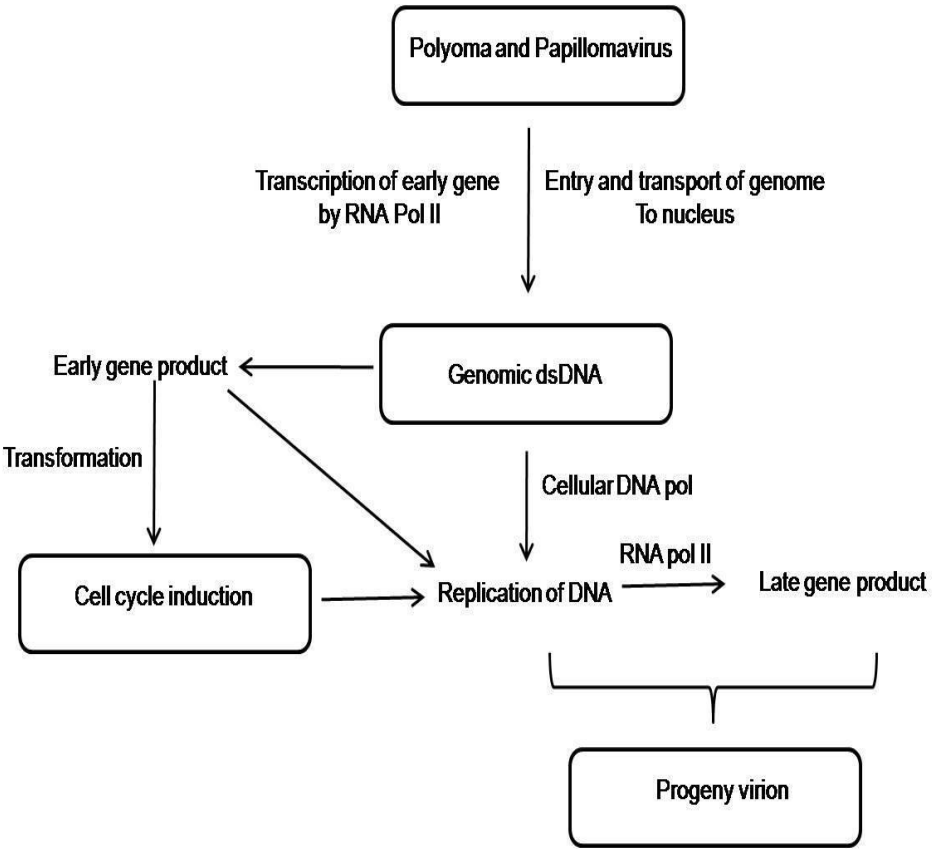
III. Transcription to produce early gene mRNAs and translation to produce early proteins (T antigens).

IV. Viral DNA replication and transcription of late gene mRNAs.

I. Translation to produce late proteins (capsid proteins) and assembly of progeny virions in the nucleus.

VI. Release of virions from the cell

Figure 35.3 General replication strategies of polyoma and papillomaviruses:

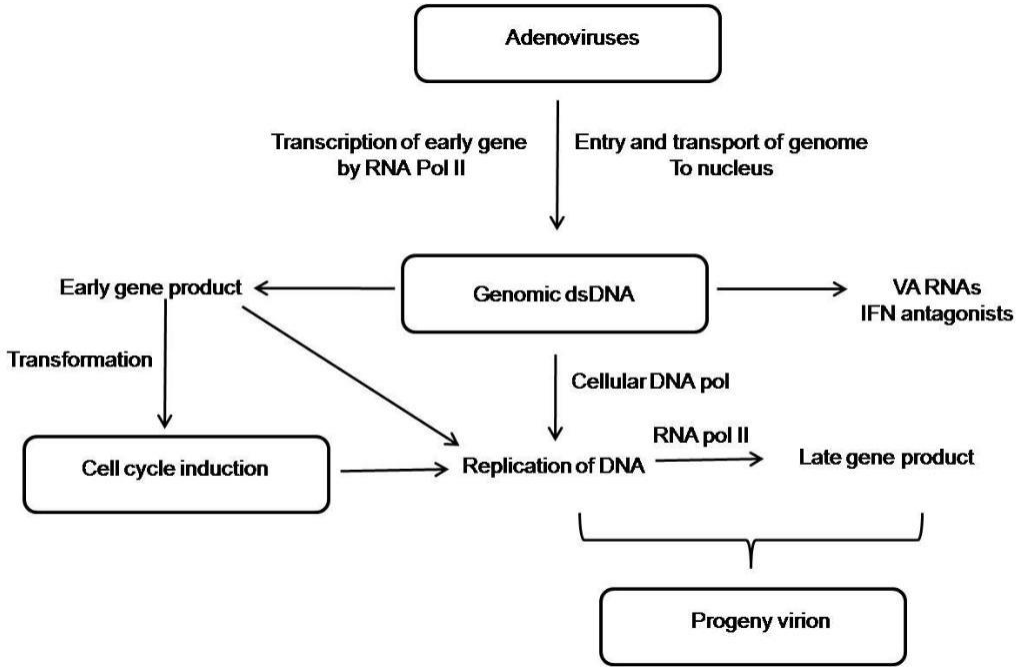


Large DNA viruses

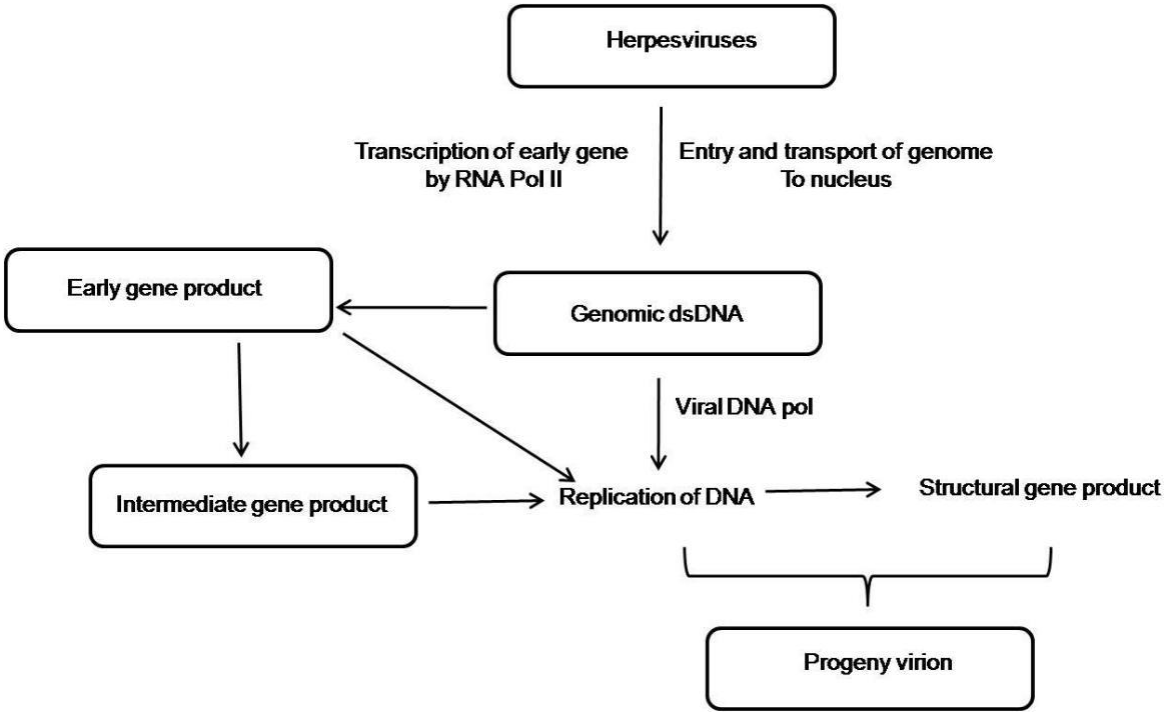
The viruses with large DNA have different strategies for their genome replication. The virus having intermediate size DNA (Adenoviruses) carry their own DNA replication machinery including DNA polymerase and other regulatory proteins. However, they still depend on the host cell RNA pol-II for the transcription of the viral RNA. The virus having large genome size like pox and herpesviruses have their own machinery to fulfill the requirement of RNA transcription and genomic DNA replication.

Virus type	Genome	Replication	Example
Adenovirus	dsDNA, 35 kb	Nucleus with DNA polymerase	Many serotypes infecting humans as well as animals
Herpesvirus	dsDNA, 120-230 kbp	Nucleus with DNA polymerase	HSV-1 and -2, Varicella/Zoster, HHV-5, 6, &7, Epstein-Barr
Poxvirus	dsDNA, 200 kbp	cytoplasm with DNA polymerase	Smallpox, Sheeppox, ORF, cowpox, vaccinia

Figure 36.1 General replication strategies of Adenoviruses:



igure 36.2 General replication strategies of herpesviruses:



Oncogenic DNA viruses

Epstein-Barr virus, *Hepatitis B* virus, papillomaviruses and Human Herpesvirus type 8 (HHV-8) or Kaposi's sarcoma are associated with one or other type of cancer in humans. In addition, HIV induces severe immunosuppression and facilitates development of cancer by other persisting infections, especially by HHV-8, Epstein-Barr virus and human papillomaviruses. Thus these agents contribute indirectly to human cancer. The mechanism about how these DNA viruses induce cancer is quite well known now. E6 and E7 gene of human papillomavirus modulate large array of cellular gene making cells vulnerable to undergo uncontrolled multiplication. Similarly, Epstein-Barr virus nuclear antigen 2 (EBNA-2) results in the induction of viral oncogenes that modulate many cell proteins. Many liver associated malignancies are caused by Hepatitis B virus. Bovine papillomaviruses can induce tumors (sarcoids) in horses and donkeys.

Herpesviruses

The herpes name is derived from the Greek word *herpein*, meaning to creep. Many of the herpesviruses were isolated from different species of animal and at least eight from human. One of the important characteristics of this virus is to cause latency in the infected individuals.

Herpesvirus virion

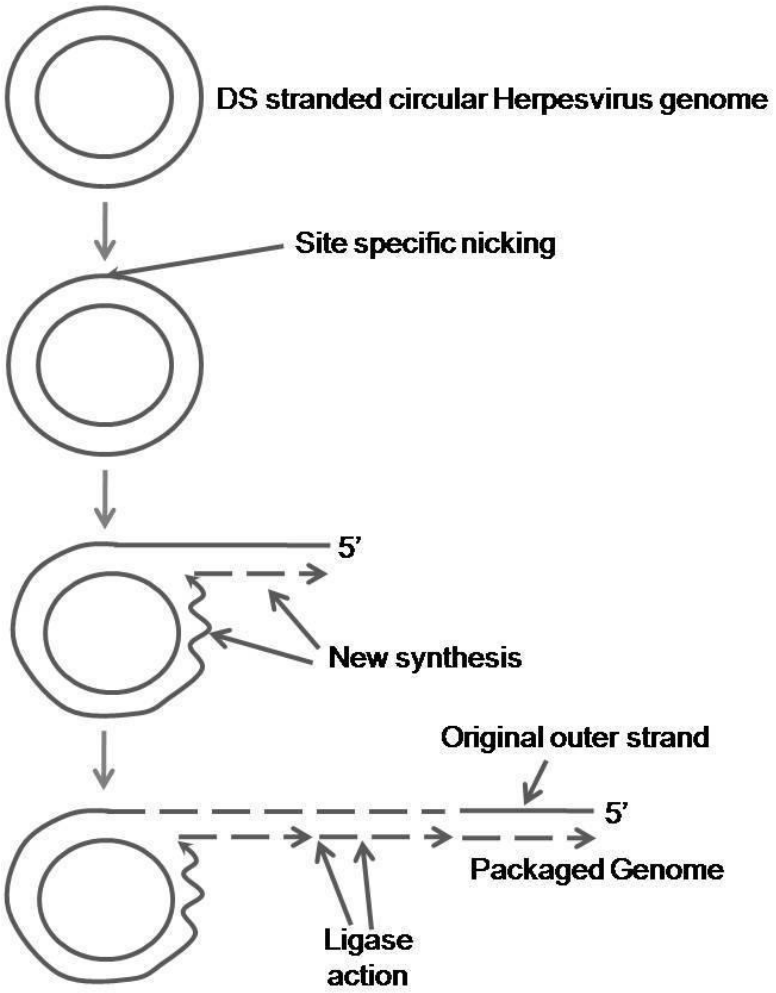
Herpesviruses have a complex structure because of its large size, multiple proteins, and tegument. The viral genome is a linear dsDNA of about 125-250 Kbp. The DNA is encapsidated inside an icosahedral capsid which is surrounded by a tegument. The tegument of herpesvirus contains many proteins while envelope is composed of 10 or more glycoproteins. The structural proteins of the herpesviruses are called as viral protein (VP) and VP5 is the most abundant protein present in the capsid. The envelope glycoproteins such as gB, gC and gD are the antigenic determinants and are involved in mounting the host immune response. In addition, the virion also contains the hexon and penton fibers. Genome of the herpesvirus contains two unique sequences; large and small and both are flanked by the repeat sequences. The genome encodes more than 75 proteins and many mRNA subspecies. Both strands of the DNA are used for the coding purpose. As some genes are present in the inverted repeats so the genome contains a pair of those genes in each strand.

Herpesvirus replication

Herpesvirus largely infects humans, many animals and lab animal in research laboratories. The virus binds first to **heparan sulfate** and then to cell adhesion molecules such as **nectins**. The virus then fuses with the cell membrane and enters the cell following endocytosis. The nucleocapsid and the tegument are released into the cytoplasm. The replication of the genome occurs in the nucleus and therefore nucleocapsid is first transported into the nucleus. The linear DNA molecule is converted into closed and circular in the nucleus of the host cells. The closed circular DNA then binds with the histones. The major tegument protein VP16 helps in modulating the viral gene expression and is transported along with the viral DNA to the nucleus. The herpesvirus genes express as immediate early (IE), early (E), and late (L). VP16 acts as a

transcription factor to recruit RNA polymerase II to activate the immediate early genes. Early proteins have their role in viral DNA replication and late proteins are formed during the assembly of the virion. Most of the late proteins are structural proteins. DNA replication in herpesvirus starts with a θ mode and later switches to rolling circle mode. Rolling circle mode of replication is the major form observed in the herpesviruses.

Figure 37.1 Rolling circle replication in herpesviruses:



Latency during herpesvirus infections

II. After entry virus releases many factors that initiate infection. The factors include **virion host shut-off** (VHS), **α-trans inducing factor** (αTIF) and many others.

α-TIF- helps in the synthesis of 5α mRNA.

VHS- favors to shut off host protein synthesis by degrading cellular mRNA.

III. The viral genome and αTIF migrates to the nucleus where viral gene expression begins.

a) New viruses bud out from the cell and infect other neighboring epithelial cells.

IV. Some newly formed virions cross the synapses and travel downwards the axon to the nerve cells towards peripheral ganglion.

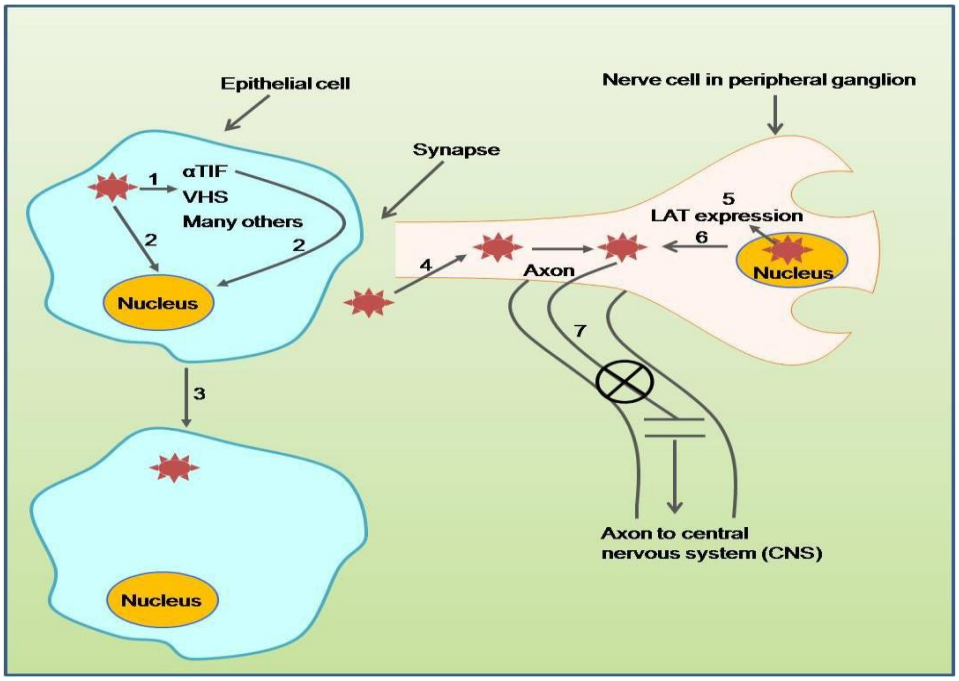
V. The virions become latent inside neurons with the expression of **Latency associated transcript** (LAT).

LAT- It is an mRNA made during latency by viruses.

VI. During the process of reactivation new viruses are made in the nerve cell. They travel back downwards the axon to infect the epithelial cells again.

VII. Nerve cells in the ganglion are well connected to the brain but virus rarely goes in that direction.

Figure 37.2 Latency in herpesviruses:



Important herpesviruses

Herpes simplex viruses 1 and 2

Herpes simplex viruses 1 and 2 (HSV-1 and HSV-2) infect epithelial cells of the buccal cavity, genital mucosa membrane, skin and cornea. Generally the virus migrates to central nervous system via neurons and initiates a latent infection. HSV-1 is mostly transmitted by lips and nasal contacts mostly to the young ones (1-2 years). HSV-2 is mostly transmitted by sexual contact and hence called as **genital herpes**.

Varicella-zoster virus

Varicella-zoster virus leads to a condition commonly called as **chicken pox** (varicella) where virus spreads to the skin and produces rashes. The rashes are mostly towards the face and trunk area. It may spread to CNS to produce a latent infection and reactivates during stress or administration of corticosteroids leading to a condition called as **shingles**. The symptoms include rashes in different body parts, fever, headache, joint pain, and swollen lymph nodes.

Epstein-Barr virus

Epstein-Barr virus is generally transmitted by saliva from an infected individual and spread in the body by its multiplication in the B cells. The virus infects the young ones with asymptomatic infection which activates during adolescence. The virus leads to a condition called as **infectious mononucleosis** or glandular fever. Epstein-Barr virus is also associated with different kind of cancers in humans. In medical science the infection is referred as **kissing disease** since it is transmitted by kissing through saliva.

Human cytomegalovirus

Human cytomegalovirus is transmitted vertically from mother to foetus. The infection at birth can cause reduced brain size and enlargement of the liver and spleen. During the later phase of life virus can cause hearing loss and mental retardation. HIV positive patients who are immunocompromised can easily be infected by cytomegalovirus which terminates into life threatening pneumonia or hepatitis.

ADENOVIRUSES

Adenoviruses are one of the major causative agents of upper respiratory tract or common cold infection in humans. In addition, they also cause conjunctivitis (eye inflammation), tonsillitis (inflammation of tonsils), gastroenteritis (inflammation of intestine), urinary tract infections, and infection to brain.

There are four genera of adenoviruses

I. Aviadenovirus- Infecting to avian species
J. Mastadenovirus- Infecting to mammals
K. Atadenovirus- Infecting to avian and humans
L. Siadenovirus- Infecting to avian, mammals, and reptiles.

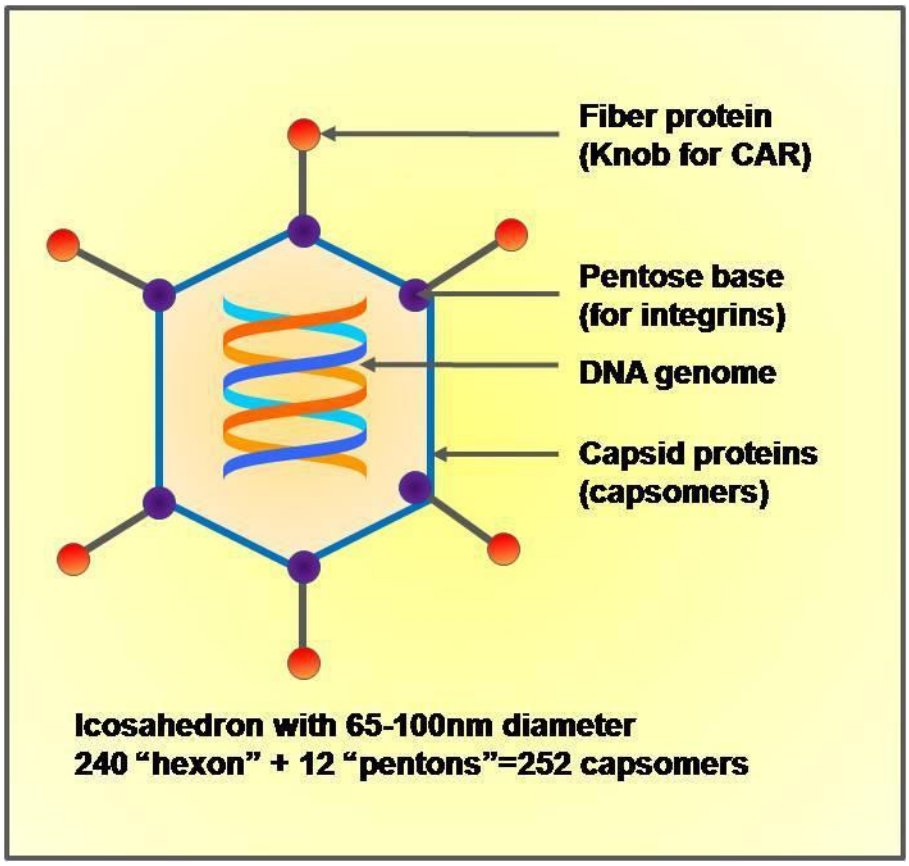
Adenovirus structure and genome organization

Adenoviruses are non-enveloped and icosahedral particles. They are 60-90 nm in diameter and contain 252 capsomers (240 hexons and 12 pentons) in the vertices of the icosahedrons.

The **hexons** on the virions are involved in the stabilization and assembly of the viral particle.

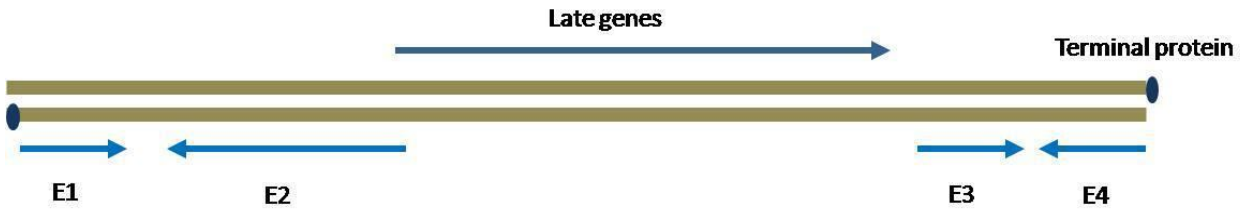
They contain a **penton** fiber that projects from each apex from the virion surface. The penton fiber consists of a shaft and a globular head. They are involved in the attachment of the virus to the surface of the host cell. They are very fragile and usually detached during preparation for electron microscopy.

Figure 38.1 Schematic representation of adenovirus virion:



Genome of adenovirus contains linear dsDNA of about 35 kb which encodes approximately 40 different proteins. The genome has inverted terminal repeats which are required during the replication process. The adenovirus DNA contains terminal protein at its 5' end. The early genes (E1-E4) are present towards either of the ends and are required to control the transcription and viral DNA replication. The late genes are generally associated with the viral structural proteins.

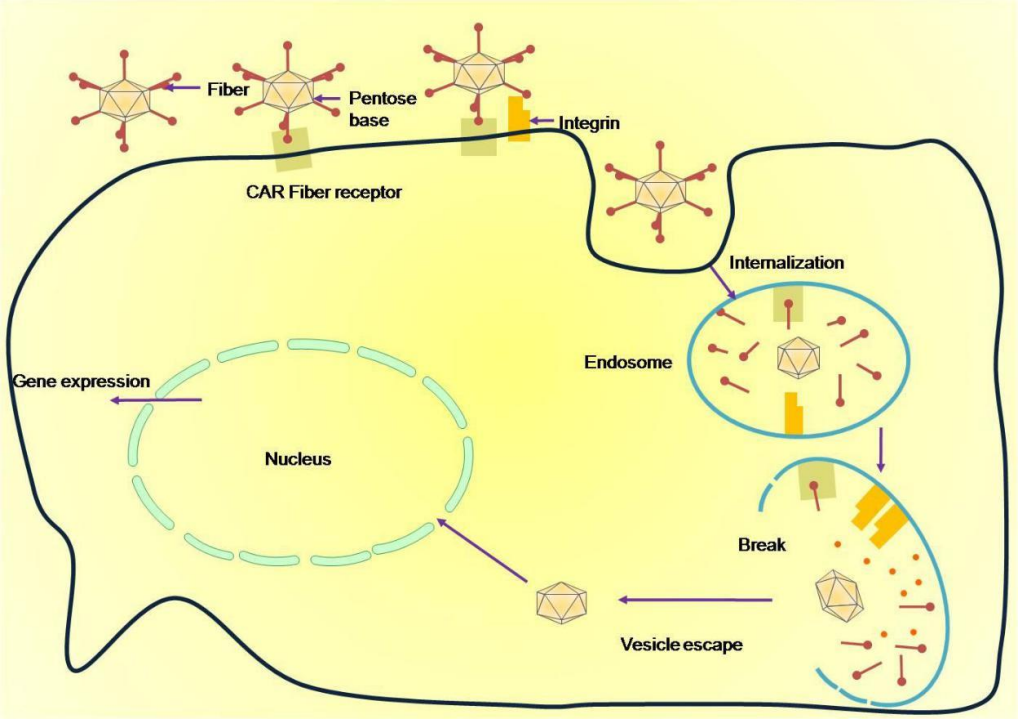
Figure 38.2 Adenovirus genome:



Adenovirus replication

To enter the cells they use a receptor present in the host cell called as **CAR** (coxsackie and adenovirus receptor). The internalization of the virus particles occur through receptor mediated endocytosis. After entry the endosome containing the virus particle migrates to nucleus and the genetic information of the virus is released into the nucleus. Transcription of the first gene is done by the terminal protein attached with the viral DNA. Viral mRNA is then transported to the cytoplasm and translated into the viral proteins. Virus assembly takes place in the cytoplasm and the mature viral particles get released from the infected cells after killing them by accumulated adenoviral death proteins.

Figure 38.3 Adenovirus life cycle:



Adenovirus associated diseases

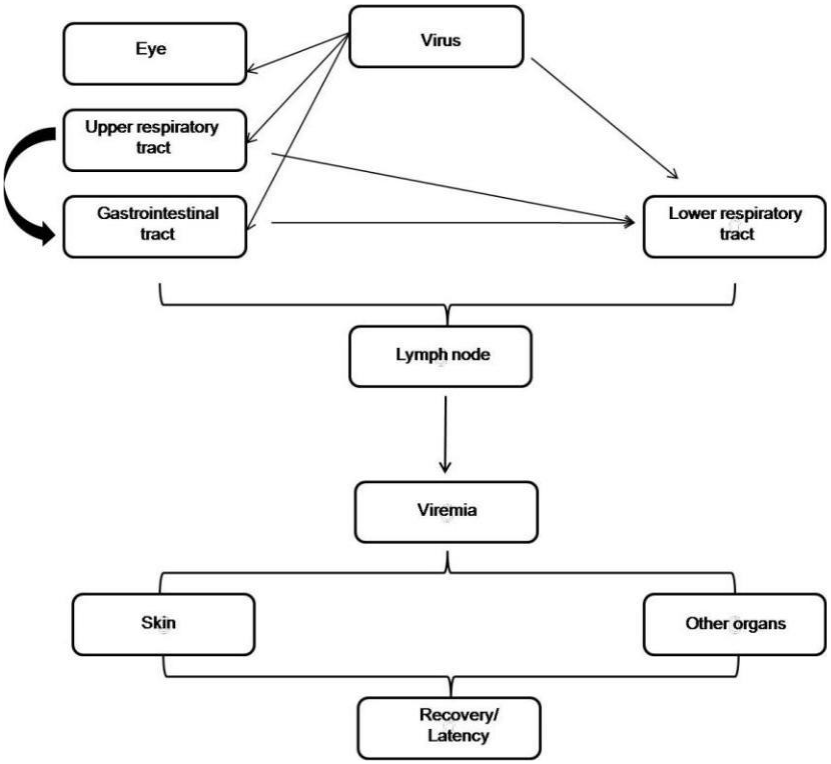
Respiratory diseases

In young children and infants it causes an acute febrile upper respiratory tract infection. It may progress to pneumonia and pharyngeal infection in untreated cases and in immuno-compromised individuals. In adults the symptoms include fever with pneumonia and pharyngitis.

Other diseases

In children the virus can cause acute gastroenteritis and hemorrhagic cystitis (inflammation of urinary bladder). Occasionally they may cause condition like meningoencephalitis in immuno-compromised patients. Sometimes they infect liver and eye leading to hepatitis and keratoconjunctivitis, respectively.

Adenovirus pathogenesis



Prevention and control

Currently no vaccine is available to protect against adenoviruses.

Good hygienic practices can prevent the infection.

Hand washing is still the best way to avoid adenovirus infection.

Wear protective clothing.

Heat and bleach will kill adenoviruses.

Adenoviruses are unusually stable to chemicals, physical agents, and adverse pH, causing them to survive longer in environment.

Poxviruses

Poxviruses belong to family *Poxviridae* and are among the complex viruses in the field of virology. The disease has a great historical impact; the first case of the poxvirus was reported about 2000 years ago in China. The virus produces a characteristic pock like lesions in the body (small pox). Last naturally occurring outbreak was reported in Somalia in 1977.

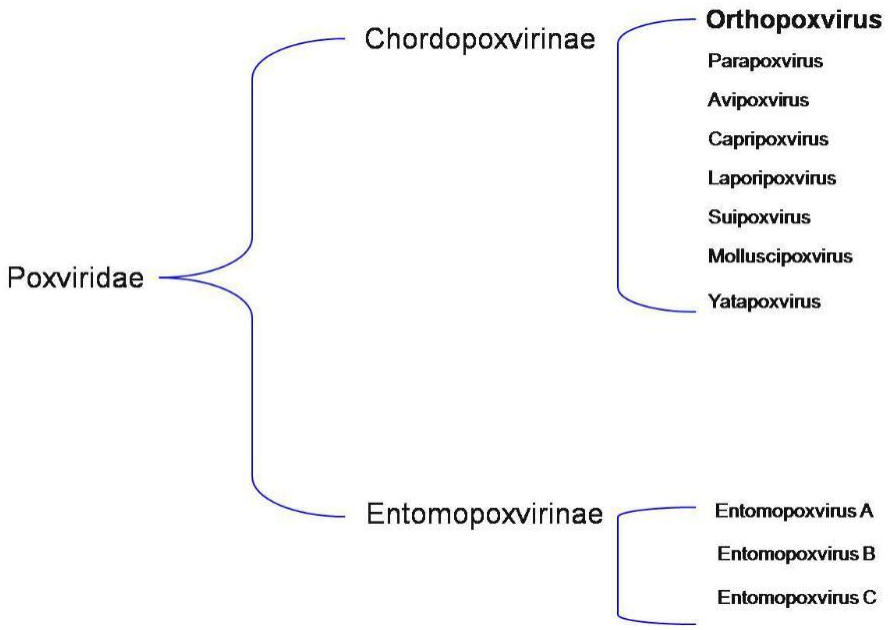
What characteristics of small pox made its eradication possible?

- Short incubation period
- No animal reservoir
- High morbidity and mortality
- Clinically apparent disease
- Mode of transmission
- An effective vaccine
- Social and economic factors

Classification

All human pox viruses are in the Chordopoxovirinae subfamily, and most of them belong to either the Orthopoxvirus (variola, vaccinia, cow pox) or the Parapoxvirus (Orf virus) genus.

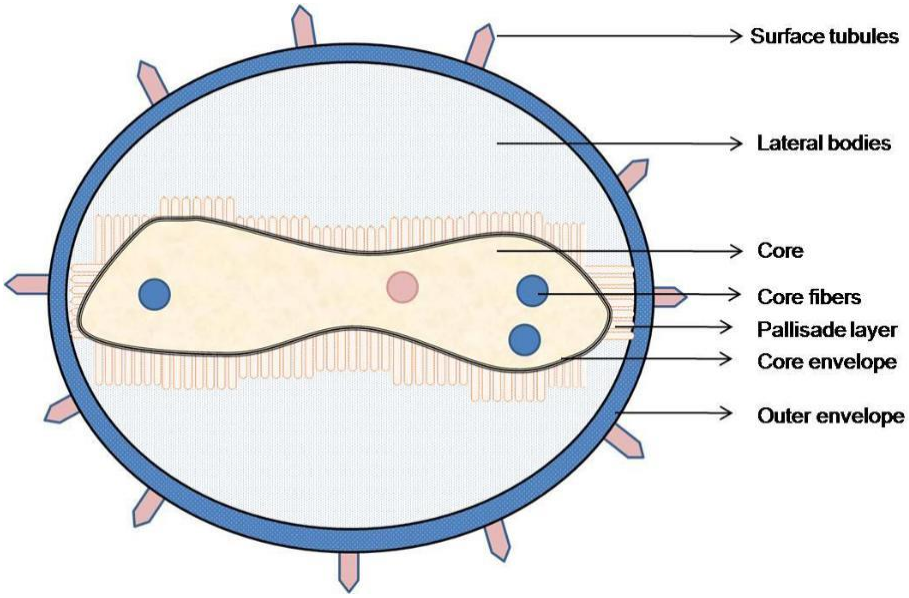
Figure 39.1 Classification of poxviruses:



Morphology

Virus is brick or oval shaped and around 300-400 nm in diameter. The viruses contain many proteins and are highly complex. The virus contains a lipid envelope that surrounds the core which is dumbbell shaped or biconcave. The virion may be beaded or smooth based on the presence or absence of surface tubules. Beaded form is converted into smooth form by the treatment of non-ionic detergent. The virus is present in both extracellular and intracellular form. The intracellular form contains a single envelope and is called as intracellular envelope virion (IEV) while the extracellular form has two envelopes and is called as extracellular envelope virion (EEV). Either side of the core (dumbbell shape) contains lateral bodies. The core is compactly packed with the genomic DNA. Antigenically, poxviruses are complex and produce a strong antibody response together with a long lasting memory. The genome of the virus contains dsDNA of about 130-300kbp. The terminal end of the viral genome contains inverted terminal repeats. More than 200 genes have been identified for the poxviruses; many of the essential genes are located in the center of the genome while non-essentials lie towards the ends.

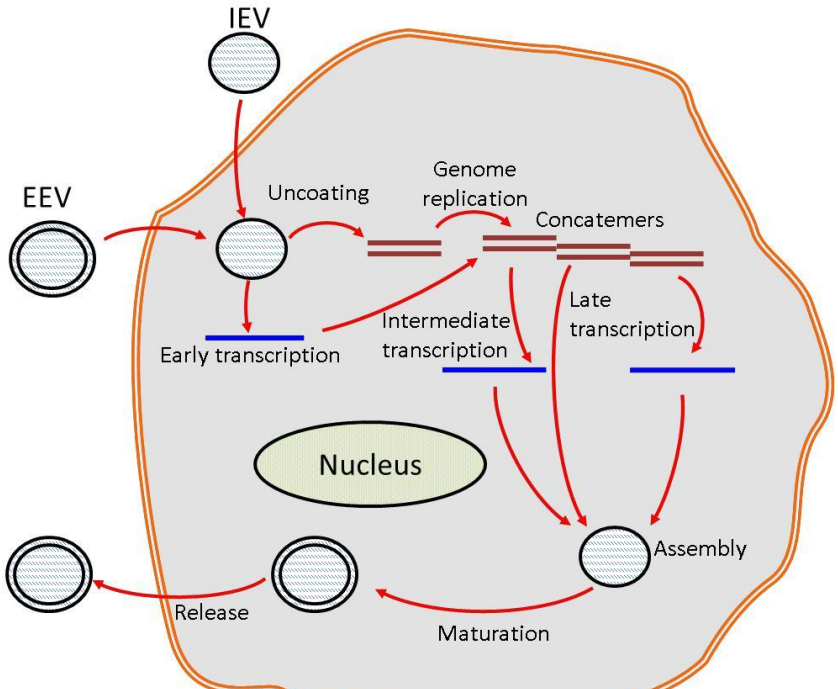
Figure 39.2 Poxvirus virion:



Replication

The replication of the genomes occurs in the cytoplasm. Many of the poxviruses attach to the cells with the help of **epidermal growth factor** as a receptor. Uncoating of the outer membrane occurs in the cytoplasm and genomic DNA is released into the cytoplasm. The virus contains both early and late gene based on its transcription preference. More than 50% of the early genes are transcribed before the DNA replication while late genes are transcribed after the completion of DNA replication. Many virus encoded enzymes help in the replication of DNA, concatemers are formed during the replication that later on cleave to form viral genome.

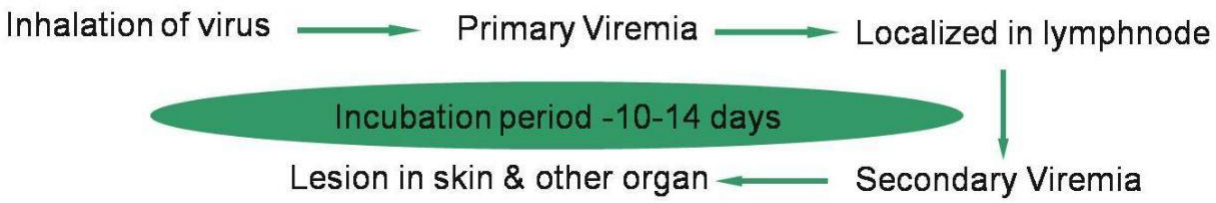
Figure 39.3 Life cycle of poxviruses in infected cell:



Transmission

In poxviruses, transmission is through direct contact. In case of small pox, the virus is found in lesions in the upper respiratory tract, which can be transmitted to others in droplet secretions, and in skin lesions. Route of transmission makes its spread relatively slow. The mechanical transmission of the virus by flies is also reported.

Pathogenicity



Important poxviruses

Vaccinia virus:

The virus causes a wide spread infection in animal and humans. The causative agent is an Orthopoxvirus. Symptoms of the disease includes pustular lesion in the teat and udder of the dairy cattle. Outbreaks in human produce lesions in hands and face of milkers who are not protected from smallpox.

Monkeypox virus

Monkey pox virus is a zoonotic agent with a wide host range. The virus was first reported in Democratic Republic of Congo. The signs of the disease include pustular rashes in the body, high fever and enlargement of lymph nodes.

Miscellaneous viruses

Infectious diseases have played a significant role throughout the history of mankind. Investigation of diseases dates back to ancient times and the query to understand it through science has lead to the discovery of viruses and bacteria as the causative agents of various types of infection and illness. Pathogenicity of viruses and susceptibility of host to infectious agents have constantly appeared through the emergence of new diseases and reappearance of pre-existing diseases. **Emerging infectious viral** diseases are those that have recently appeared in a population as a result of a new virus or the recognition of a previously undetected virus and are often zoonotic. Emergence of an infectious viral disease may occur due to the extension of the geographic or host range of the virus. Recently, bats have been implicated as an important reservoir and source of many emerging viruses. As new technology for detection of viruses becomes increasingly available, more viruses are likely to be detected. Enhanced molecular biology techniques will allow faster and more complete characterization of new and miscellaneous viruses.

Bat paramyxoviruses

Bats have been shown to be the reservoir hosts of a variety of viruses responsible for severe disease outbreaks in humans and animals, including filoviruses, coronaviruses and paramyxoviruses. Recently Hendra and Nipah viruses were also isolated from bats.

Hendra and Nipah viruses are zoonotic viruses of the genus *Henipavirus* under the family *Paramyxoviridae.* The natural reservoirs for both the viruses are fruit bats or flying foxes of the genus *Pteropus.* Hendra virus was first isolated from an acute febrile illness in horses and subsequently in

humans with a sign of fatal encephalitis. The first known human infections with Nipah virus were detected during an outbreak of severe febrile encephalitis in peninsular Malaysia and Singapore.

Menangle virus was isolated during an outbreak of reproductive disease in pigs in New South Wales, Australia in 1997. Symptoms of the disease included malaise, chills, fever, sweating, headache, weight loss and decrease in farrowing rate (birth giving process in pigs).

Tioman virus was isolated from the urine of fruit bats (*Pteropus hypomelanus*). Tioman virus is lethal in suckling mice 8-12 days post intracerebral inoculation. There role in human and animal infection is still under debate.

Mapuera virus was isolated from the salivary glands of an asymptomatic fruit bat (Sturnira lilium) in 1979 in Brazil.

Canine Distemper Virus

Canine distemper virus is an important pathogen which naturally infects a broad range of terrestrial and marine carnivores. Canine distemper virus is a member of genus *Morbillivirus* of the family *Paramyxoviridae*. The disease is characterized by skin rash, fever, gastrointestinal and respiratory signs, and a profound immune-suppression as well as by frequent neurological complications.

Rift Valley Fever Virus

Rift Valley Fever Virus (RVFV) is a member of the genus *Phlebovirus* (family *Bunyaviridae*). The RVFV is transmitted by the bite of mosquitoes. The disease was first reported in sheep in Kenya in 1918. Infection of RVFV is characterized by febrile illness with hemorrhages and inflammation of brain.

Hantavirus

Hantavirus belongs to the family *Bunyaviridae* (negative-sense, single-stranded RNA viruses). Hantavirus is transmitted by rodents (deer mice) via their urine and feces. Hantavirus is a cause of hemorrhagic fever with a renal (Kidney) syndrome. The early symptoms of the disease are similar to flu and include fever, chills, cough and muscle ache. The disease can progress to Hantavirus pulmonary syndrome.

Ebola Virus

Ebola virus belongs to family of RNA viruses called the *Filoviridae*. The virus leads to fatal hemorrhagic disease in humans and nonhuman primates. The transmission of the virus occurs by direct contact with the blood and/or secretions of an infected person. Sudden onset of illness is characterized by fever, sore throat, headache, joint and muscle

pain, and weakness, followed by diarrhea, vomiting, and abdominal pain. In highly fatal cases internal and external bleeding may be seen in the patients.

Arenaviruses

The family *Arenaviridae* contains the viruses which are usually associated with rodent-transmitted disease in humans. The virus particles are spherical with a diameter of around 110-130 nm. The virus contains negative strand RNA as a genetic material. Infection of Arenaviruses leads to hemorrhagic disease in humans that are often fatal.

Table 40.1 Different Arenavirus diseases:

Virus	Disease
Junin virus	Argentine hemorrhagic fever
Lassa virus	Lassa fever
Guanarito virus	Venezuelan hemorrhagic fever
Machupo virus	Bolivian hemorrhagic fever

Prions

Prions are the infectious agents made up of only proteins (No DNA or RNA) and were discovered by **Stanley Prusiner.** Prions are propagated by transmitting the misfolded form of the protein. Prion diseases or transmissible spongiform encephalopathies (TSEs) are a family of rare progressive neurodegenerative disorders of humans and animals. They are characterized by long incubation periods, spongiform changes in brain, and a failure to induce inflammatory response.

Table 40.2 Different prion diseases in human and animals:

Human Prion disease	Animal Prion disease
Creutzfeldt- Jakob Disease (CJD)	Scrapie
Kuru	Mad Cow Disease (Bovine Spongiform

	Encephalopathy)
Gerstmann- Straussler- Scheinker Syndrome	Chronic Wasting Disease
	Transmissible mink encephalopathy

Viroids

Viroids are plant pathogen that contains circular single stranded RNA as a genetic material. They are discovered by **Theodor Diener** in 1971. Viroids contain small RNA of around 250 to 500 nt and do not encode any proteins. The **Potato spindle tuber viroid** was the first viroid to be identified.