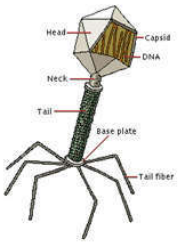
What are viruses?



- **Obligate intracellular parasites**
- **they cannot be observed using a light microscope**
- **they have no internal cellular structure**
- **they contain either DNA or RNA, but not both.**
- **they are incapable of replication unless occupying an appropriate living host cell**
- **they are incapable of metabolism individuals show no increase in size.**

A particular virus has a limited host range

Typically, **bacteria** range from **1 to 10 micrometers** (mM) in length, whereas a **virus** would fall in the range of **0.03 to 0.1 mM** in length [or 30-90 nanometers). Of course there are always some exceptions: Some viruses, such as **poxviruses**, can be **200 to 400 nanometers (nm)** in length, and **Filoviruses** (such as **Ebola**) can be up to**1000 nm**

5.1 DISCOVERY OF VIRUSES

Understanding of the viruses evolved in the late 1800 from the results of experiments done by a number of scientists. Therefore, no one person deserves exclusive credit for their discovery. It was not until the invention of porcelain bacterial filters in 1884 by Charles Chamberland, one of Pasteur's co-worker and inventor of the autoclave, that the field of virology really began to develop. In 1886, A Mayer working in Holland demonstrated that a mosaic disease of tobacco was transmissible through crude sap taken from the diseased plants.

In 1892, Russian scientist Dimitrii Ivanowsky observed that the causative agent of tobacco mosaic disease was not retained by the porcelain filters as the disease could be transmitted with sap filtrates from plant to plant. Therefore, it became apparent that the microbial world contained even smaller members than had been previously recognised. Six years later, Martinus Beijerinck (1898), a Dutch microbiologist, made the same observation independently in Holland. Beijerinck proposed that tobacco mosaic disease was caused by an entity different from bacteria and called it **contagium vivum fluidum**—a living infectious fluid, a filterable virus that would multiply only in living plant cells, but could survive for long periods in a dried state.

In the same year (1898), two German scientists, Friedrich Loeffler and Paul Frosch, both former students of Robert Koch, observed that the causative agent of foot and mouth disease of cattle was also filterable and dependent on host organism for replication. All these infectious agents that passed through bacterial filters came to be called as ultrafilterable viruses. In 1900, Walter Reed and co-workers discovered that yellow fever disease of man that was widespread in tropical countries since the fifteenth century was due to a filterable virus that was transmitted by mosquitoes. Rapid introduction of mosquito control by William Gorgas dramatically reduced the incidence of yellow fever disease by 1902, and even today mosquito control remains to be the most effective method for control of yellow fever. Subsequent discoveries by Vilhelm Ellerman and Olaf Bang in 1908 and by Peyton Rous in 1911 revealed that leukemias or solid tumours in chickens could be transmitted between chickens by cell-free filtrates and was probably caused by a virus. The study of viruses associated with cancers in chickens, particularly the Rous sarcoma virus, eventually led to an understanding of the molecular basis of neoplastic disease.

It was soon discovered that viruses could infect bacteria also. This discovery was made independently by Frederick W Twort in England in 1915 and by Felix d' Herelle at the Pasteur Institute in Pairs in 1917. Twort observed that bacterial colonies on the surface of agar plates sometimes underwent lysis (dissolved and disappeared) and that this lytic effect could be transmitted from colony to colony. Even high dilutions of a lysed colony that had been passed through a bacterial filter could transmit the lytic effect but heating the filtrate destroyed its lytic property. From these observations, Twort suggested that the lytic agent might be a virus. Felix d' Herelle rediscovered this phenomenon in 1917 and demonstrated that these viruses could reproduce only in live bacteria. He coined the word **bacteriophage** (*phage* is a Greek word for eating) for these viruses because they were parasitic for bacteria (bacteria eater). Later, investigations on bacteriophages provided the foundations for the field of molecular biology as well as insight into interactions of viruses with their host cells.

In 1935, Wendell M Stanley, a biochemist of Rockefeller Institute, purified and crystallised tobacco mosaic virus (TMV), making it possible for the first time to carry out chemical and structural studies on a purified virus. Stanley found TMV to be largely protein and was rewarded with the 1946 Nobel Prize in Chemistry. Two years later, in 1937, Frederick C Bawden and Norman W Pirie were able to separate the TMV particles into protein and a small but constant fraction of RNA. Thus, it became known that viruses were complexes of nucleic acids and proteins (**nucleoproteins**) that were able to reproduce only in living cells. Chemical studies of other viruses revealed that some contain DNA in addition to protein, but no virus contains both DNA and RNA. In addition to nucleic acid, some viruses contain lipid and small amounts of carbohydrate conjugated to their protein components.

TMV is the first virus to be crystallised.

Two more significant developments of the 1940s revolutionised virology. One was the invention of the electron microscope, which permitted scientists to visualise the virus particles and to morphologically distinguish one type from another. This opened the way for the first rational classification of viruses. The other was the successful cultivation of viruses using tissue (cell) culture techniques, which facilitated the study of these organisms and made vaccines possible for controlling certain viral diseases. Tissue culture technology established that viruses are **molecular parasites** as they reproduce by exploiting their host cell biosynthetic machinery for synthesis of their components (nucleic acid and protein) from which they are made. Few other developments that extended our understanding about viruses included the discovery of myco-

Tissue culture is the technology of maintenance or culture of isolated tissues and of plant or animal cell lines *in vitro*.

5.2 EVOLUTIONARY ORIGIN OF VIRUSES

The origin and subsequent evolution of viruses is an intriguing question and has been the subject of considerable speculation in the scientific community. As viruses differ greatly from one another, it is likely that they have originated independently many times during the course of evolution. Probably some viruses have evolved from other viruses just as cellular organisms have arisen from their predecessors.

There are two major theories that have been advanced by virologists for the origin of viruses. It is possible that viruses have arisen by means of both the mechanisms.

Retrograde evolution Viruses are thought to have originated by retrogressive evolution from free-living or parasitic microorganisms. This theory is based on the concept that a parasite evolves downwards or retrogressively because it takes the ready-made metabolites from the host instead of synthesising them itself. If the parasite continues to evolve retrogressively, then in order to save labour and energy, it would slowly shed some of its physiological, morphological and even unused genetic information. Genes for biosynthesis of intermediates supplied by the host could be lost even by mutation without harm to the invader. Eventually, this prokaryote may have evolved to nothing more than a group of genes now termed as virus.

Escaped gene theory Evidence accumulated so far strongly supports the proposal that viruses originated from "escaped" host nucleic acid. According to this theory, viruses represent pieces of host cell RNA or DNA that have managed to escape the cell and have achieved independence from cellular control. Possibly, a few mutations could also convert nucleic acids into infectious nucleic acids whose replication could not be controlled.

The origin of viruses may have been with plasmids or transposons, circular DNA molecules that replicate in cytoplasm, and can be integrated into or excised from various sites in the host chromosome. Plasmids can move from cell to cell carrying information, whereas transposons are bits of DNA present in both prokaryotic and eukaryotic cells that can move from one site in a chromosome to another site carrying genetic information. There is one type of transposon known that carries a gene for synthesis of the reverse transcriptase, and transposon elements with such properties have some similarity to retroviruses. Analysis of nucleotide sequences in the small, infectious RNAs called as viroids have indicated that they have base sequences complementary to transposons, introns and portions of host DNA. This has prompted speculation that they have arisen from introns or transposons.

General Properties of Viruses

Virology

Virology is the bioscience for study of viral nature,and the relationship between viruses and hosts

Definition of Virus

Viruses may be defined as acellular organisms whose genomes consist of nucleic acid, and which obligately replicate inside host cells using host metabolic machinery and ribosomes to form a pool of components which assemble into particles called VIRIONS

Properties of Viruses

- Obligate intracellular parasites of bacteria, protozoa, fungi, algae, plants, and animals
- Ultramicroscopic size, ranging from 20 nm up to 450 nm (diameter)
- Not cellular in nature; structure is very compact and economical.
- Do not independently fulfill the characteristics of life
- Inactive macromolecules outside the host cell and active only inside host cells
- Basic structure consists of protein shell (capsid) surrounding nucleic acid core.
- Nucleic acid can be either DNA or RNA but not both.
- Nucleic acid can be double-stranded DNA, single-stranded DNA, single-stranded RNA, or double-stranded RNA.
- Molecules on virus surface impart high specificity for attachment to host cell.
- Multiply by taking control of host cell's genetic material and regulating the synthesis and assembly of new viruses
- Lack enzymes for most metabolic processes
- Lack machinery for synthesizing proteins

Properties of bacteria and viruses

	Cellular organisation	Growth on inanimate media	Binary fission	Both DNA and RNA	Ribosome	Sensitivity to antibiotics	Sensitivity to Interferons
Bacteria	+	+	+	+	+	+	-
Mycoplasma	+	+	+	+	+	+	-
Rickettsiae	+	-	+	+	+	+	-
Chlamydiae	+	-	+	+	+	+	+
Viruses	-	-	-	-	-	-	+

Morphology

Size

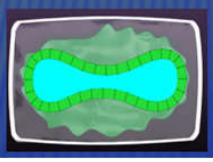
Methods of analysis

1. Passing through collodion membrane filters of graded porosity (gradocol membranes)
2. Ultracentrifuge
3. Electron microscope
4. X-ray crystallography

Size of Viruses

A small virus has a diameter of about 20 nm.
Example: Parvovirus

A large virus have a diameter of up to 400 nm.
Example: Poxviruses



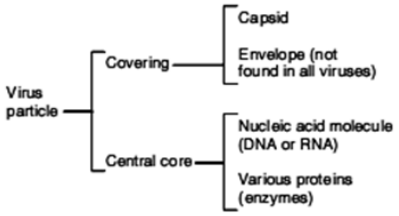
Host Range and Specificity of Viruses

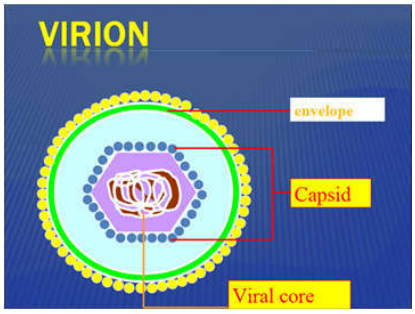
The host range of a virus refers to the spectrum of organisms that a particular virus can infect. On this basis, viruses are grouped as animal viruses, plant viruses, bacterial viruses, mycoviruses, etc. Another important property of viruses is their specificity. A particular virus may infect only a single species or sometimes only certain members of a species or only specific cells and tissues of the species. For example, polioviruses can be grown in the laboratory in monkey kidney cells but have never been observed to cause a natural infection in any animal excepting humans. In contrast, rabies virus can affect the central nervous system of many warm-blooded animals. Therefore, the host range of rabies virus is much more wide than that of polioviruses. Similarly, certain papillomaviruses (causing warts) are so specific that they can infect and replicate only in skin cells. The particular host range of a virus is determined largely by:

1. The presence of specific receptor sites on the host cell surface.
2. Availability within the potential host of cellular factors required for viral multiplication.
3. Whether replicated viruses can be released from the cell to spread the infection to other cells.

Viral Structures: CAPSIDS, CAPSOMERES AND ENVELOPES

Virion: A single complete infective viral particle





Capsid: The protein coat that surrounts the core nucleic acid or nucleopretein assembly

Capsomere: Component protein subunits of capsids are called capsomeres.

Protomers: Some capsomeres are mulimeric each subunit is referred to as protomers

Envelop: For some **viruses**, the capsid is surrounded by lipid bilayer that contains **viral** proteins, usually including the proteins that enable the**virus** to bind to the host cells. This lipid and protein structure is called the **virus envelope**, and is derived from the host cell membranes.

Viral proteins (70-90 %):

- **structural** (capside, envelope, matrix, core, associated with nucleic acid)
- **non-structural**

- **Structural proteins** are in virion in its extracellular state.

Functions:

- ✓ protection of nucleic acid,
- ✓ interaction with the membrane of susceptible cell
- ✓ provide viral penetration into the cell,
- ✓ have RNA- and DNA-polymerase activity etc.

- **Non-structural proteins** are absent in virion in its extracellular state, but they are formed during viral reproduction

Functions :

- ✓ provide regulation of viral genome expression,
- ✓ are viral precursor proteins and can inhibit cell biosynthesis.

Lipids (15-35 %) are in enveloped viruses in their envelope

Functions :

- Stabilization of viral shell,
- Protection of inner virion shells and nucleic acid,
- Deproteinization of virions

Carbohydrates molecules are in glycoproteins and glycolipids (3,5-9 %).

They protect these molecules from cell proteases action

Capsid functions

- ☎Packaging or condensation of nucleic acid
- ☎Protection of nucleic acid
- ☎Transport nucleic acid from cell to cell
- ☎Provides specificity for attachment

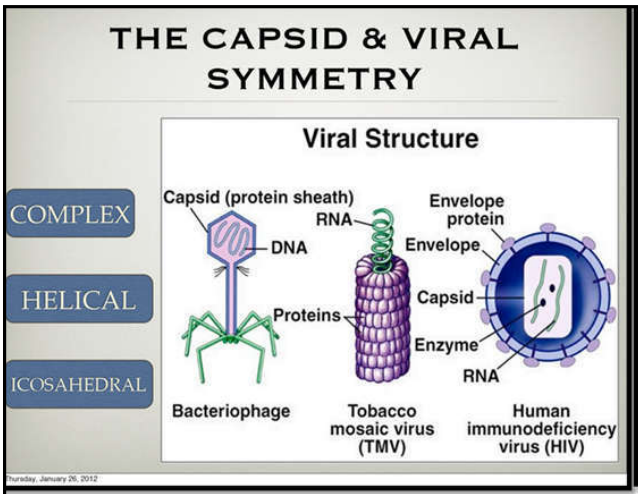
Functions of envelope

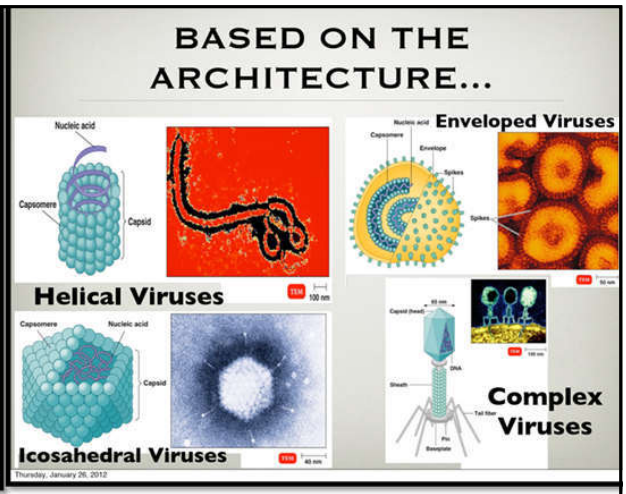
- u Antigenicity
  some viruses possess neuraminidase
- u Infectivity
- u Resistance

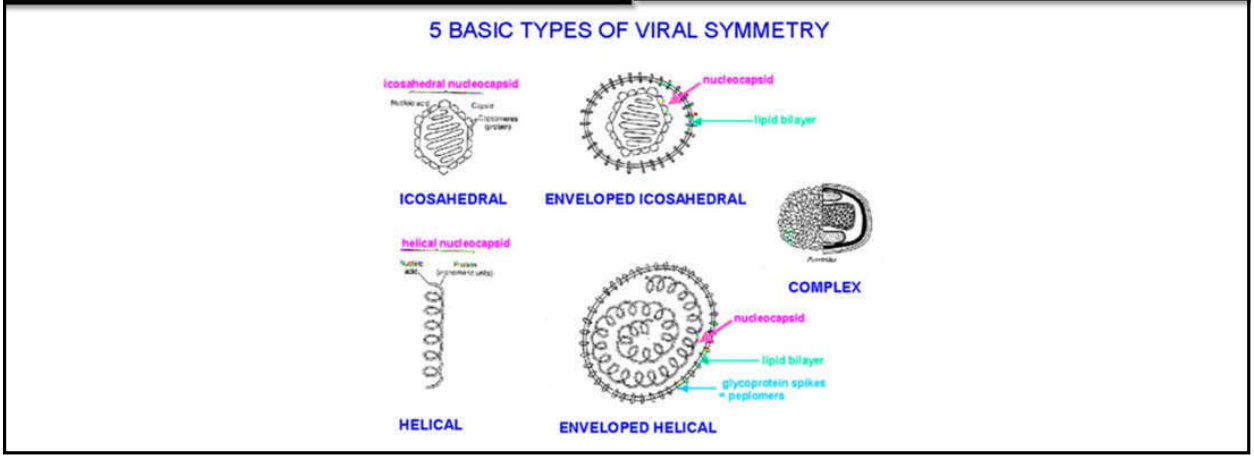
How Viruses are classified

Main criteria presently used are:

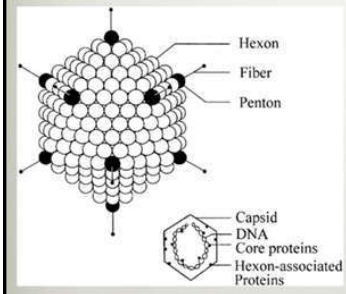
- Acid type.
- Size and morphology, including type of symmetry, number of capsomeres, and presence of membranes.
- Presence of specific enzymes, particularly RNA and DNA polymerases, and neuraminidase
- Susceptibility to physical and chemical agents, especially ether.
- Immunologic properties.
- Natural methods of transmission.
- Host, tissue, and cell tropisms.
- Pathology; inclusion body formation.
- Symptomatology.



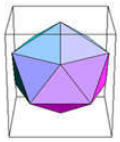




ICOSAHEDRAL CAPSID

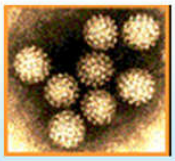


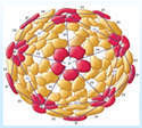
- Icosahedral morphology is characteristic of the nucleocapsids of many *"spherical"* viruses
- The icosahedral capsid structure of adenovirus is made up of three proteins, ***hexon, penton base, and fiber***
- Some proteins are associated with viral DNA, whereas others are associated with hexon and are involved in the formation of the capsid

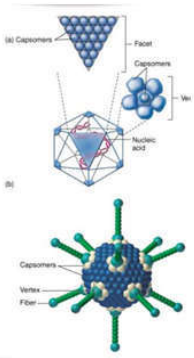


Icosahedral Capsid

- A polyhedron with 20 equilateral faces and 12 vertices
- **capsomers**
  - ring or knob-shaped units made of 5 or 6 protomers
  - **pentamers (pentons)** – 5 subunit capsomers
  - **hexamers (hexons)** – 6 subunit capsomers







Icosahedral

- Adeno-associated Virus (AAV)
- Adenovirus
- B19
- Coxsackievirus - A
- Coxsackievirus - B
- Cytomegalovirus (CMV)
- Eastern Equine Encephalitis Virus (EEEV)
- Echovirus
- Epstein-Barr Virus (EBV)
- Hepatitis A Virus (HAV)
- Hepatitis B Virus (HBV)
- Hepatitis C Virus (HCV)
- Hepatitis Delta Virus (HDV)
- Hepatitis E Virus (HEV)
- Herpes Simplex Virus 1 (HHV1)
- Herpes Simplex Virus 2 (HHV2)
- Human Immunodeficiency Virus (HIV)
- Human T-lymphotrophic Virus (HTLV)
- Norwalk Virus
- Papilloma Virus (HPV)
- Polio virus
- Rhinovirus
- Rubella Virus
- Saint Louis Encephalitis Virus
- Varicella-Zoster Virus (HHV3)
- Western Equine Encephalitis Virus (WEEV)
- Yellow Fever Virus

Calculating the number of subunits in a virus particle

The way in which each triangular face of the icosahedron can be subdivided into smaller, identical equilateral triangles is governed by the law of solid geometry. This can be calculated from the expression:

$$T = Pf^2,$$

where T, the *triangulation number*, is the number of smaller, identical equilateral triangles, P is given by the expression $h^2 + hk + k^2$. In this expression, h and k are any pair of integers without common factors, i.e. h and k cannot be divided by any whole number to give the same values.

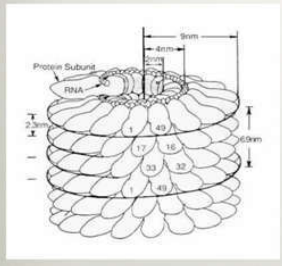
$$f = 1, 2, 3, 4, \text{ etc.}$$

For viruses examined so far, the values of P are 1 ($h=1$, $k=0$), 3 ($h=1$, $k=1$) and 7 ($h=1$, $k=2$). Representative values of T are shown in Table 2.1. Once the number of triangular subdivisions is known, the total number of subunits can easily be determined since it is equal to $60T$.

Values of capsid parameters in a number of icosahedral viruses. The value of T was obtained from examination of electron micrographs, thus enabling the values of P and f to be calculated.

P	f	$T (= Pf^2)$	No of subunits (60T)	Example
1	1	1	60	Tobacco necrosis satellite virus
3	1	3	180	Tomato bushy stunt virus, picornaviruses*
1	2	4	240	Sindbis virus
1	4	16	960	Herpesviruses
1	5	25	1500	Adenoviruses**

HELICAL CAPSID



- The icosahedral capsid structure of adenovirus is made up of three proteins, ***hexon, penton base, and fiber***
- Helical morphology is seen in nucleocapsids of many filamentous and ***pleomorphic*** viruses
- Helical nucleocapsids are characterized by length, width, pitch of the helix, and number of protomers per helical turn

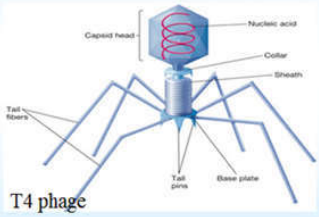
Thursday, January 26, 2012

Helical Symmetry

California Encephalitis Virus
Coronavirus
Hantavirus
Influenza Virus (Flu Virus)
Measles Virus (Rubeola)
Mumps Virus
Parainfluenza Virus
Rabies Virus
Respiratory Syncytial Virus(RSV)

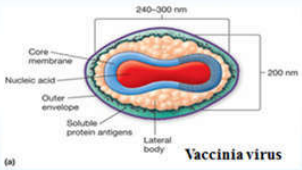
Complex Symmetry

- many viruses do not fit into helical or icosahedral symmetry
- Examples: poxviruses and large bacteriophages



T4 phage

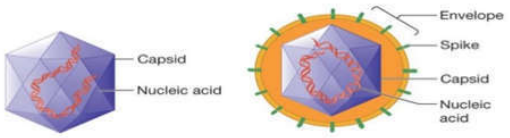
Binal symetry: head icosahedron, tail helical. Tail fibers and sheath used for binding and pins for injecting genome



Vaccinia virus

200x400x250 nm, enveloped virus DNA With double membrane envelope.

Viral Structure: Envelope

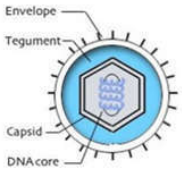


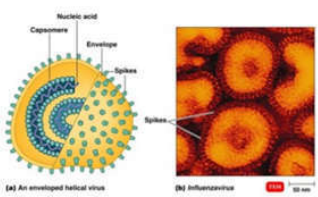
(a) Naked virus (b) Enveloped virus

a) Non-enveloped viruses/ Naked Viruses = viruses whose capsids are not covered by an envelope
b) Sometimes, Capsid covered with envelope
- **SPIKES** = carbohydrate-protein complexes (glycoproteins) that project from the envelope
  - Can be used to attach to host cell

Viral Envelope

- Presence
  - Enveloped
  - Naked (non-enveloped)
- Location
  - Surrounds capsid
- Source
  - Host plasma membrane
  - Nuclear membrane
  - Endoplasmic reticulum
- Components
  - Phospholipid
  - Proteins
  - Glycoprotein spikes (+/-)
- Examples
  - Influenza
  - Rabies
  - Herpes
  - HIV



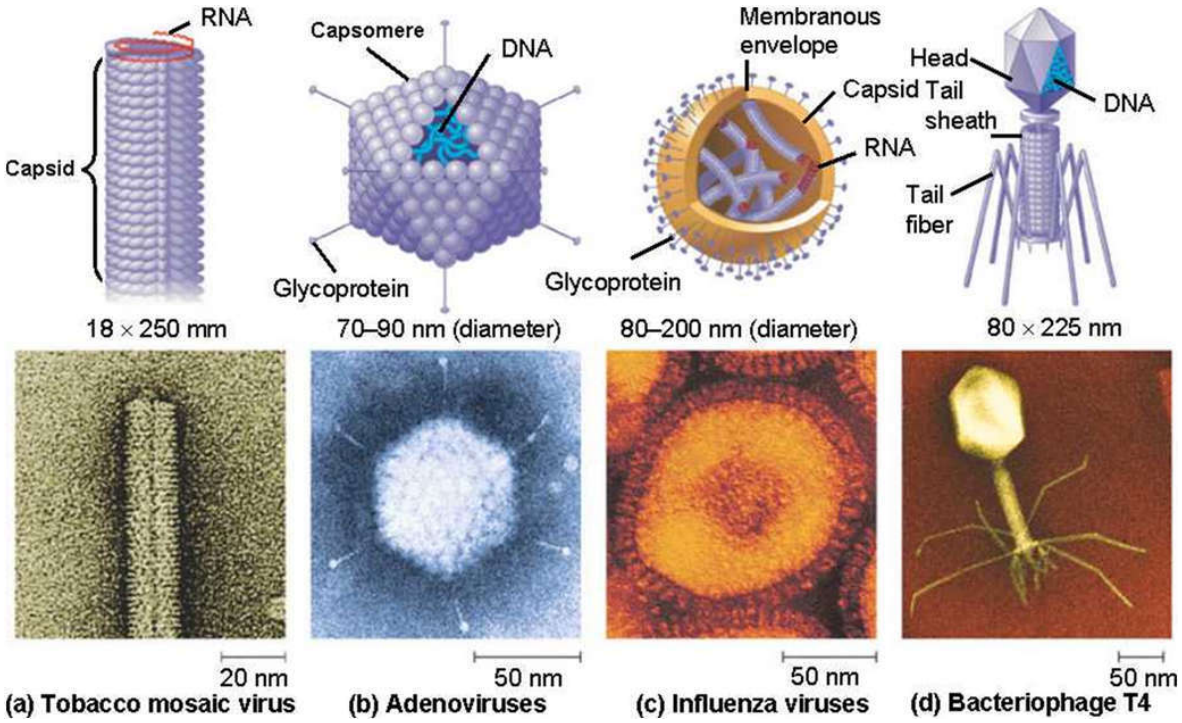


Viral envelope

- When **enveloped viruses** (mostly animal) are released from the host cell, they take with them a bit of its membrane system in the form of an envelope.
  - Some viruses bud off the cell membrane; others leave via the nuclear envelope or the endoplasmic reticulum.
- Some proteins form a binding layer between the envelope and capsid of the virus, and glycoproteins (proteins bound to a carbohydrate) remain exposed on the outside of the envelope.
  - These protruding molecules, called **spikes** or peplomers, are essential for the attachment of viruses to the next host cell.

Functions of Capsid/Envelope

- The outermost covering of a virus is indispensable to viral function
  - it protects the nucleic acid from the effects of various enzymes and chemicals when the virus is outside the host cell.
- Capsids and envelopes are also responsible for helping to introduce the viral DNA or RNA into a suitable host cell,
  - by binding to the cell surface
  - by assisting in penetration of the viral nucleic acid



Classification by Symptomatology

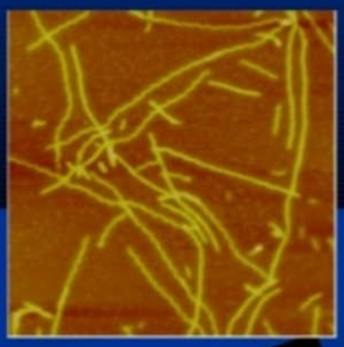
- **Dermatotropic** - lesions of skin and mucous membranes
  - cold sores, shingles, warts
- **Pneumotropic**
  - flue, parainfluenza, respiratory syncytial viral pneumonia,
- **Neurotropic** - CNS
  - encephalitis
- **Viscerotropic** - organs
  - hepatitis, infectious parotitis
- **Generalized**

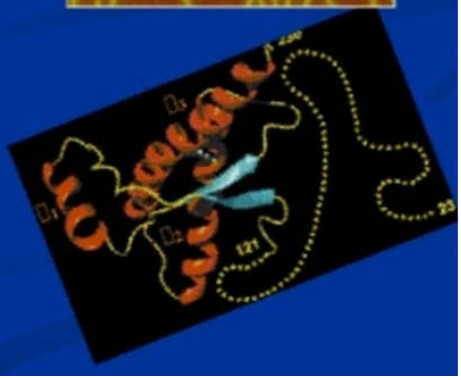
Classification According to Routes of Transmission

- **Respiratory transmission**
  - ✓ Influenza A virus
- **Faecal-oral transmission**
  - ✓ Enterovirus
- **Blood-borne transmission**
  - ✓ Hepatitis B virus
- **Sexual Transmission**
  - ✓ HIV
- **Animal or insect vectors**
  - ✓ Rabies virus, Western equine encephalitis, yellow fever, West Nile fever, dengue fever

Prions

- Prions are "infectious proteins"
- They are normal body proteins that get converted into an alternate configuration by contact with other prion proteins
- They have no DNA or RNA
- The main protein involved in human and mammalian prion diseases is called "PrP"(neurons)
- Prions don't simply subvert host enzymes in the cell but convert a normal protein into a self propagating conformational state.

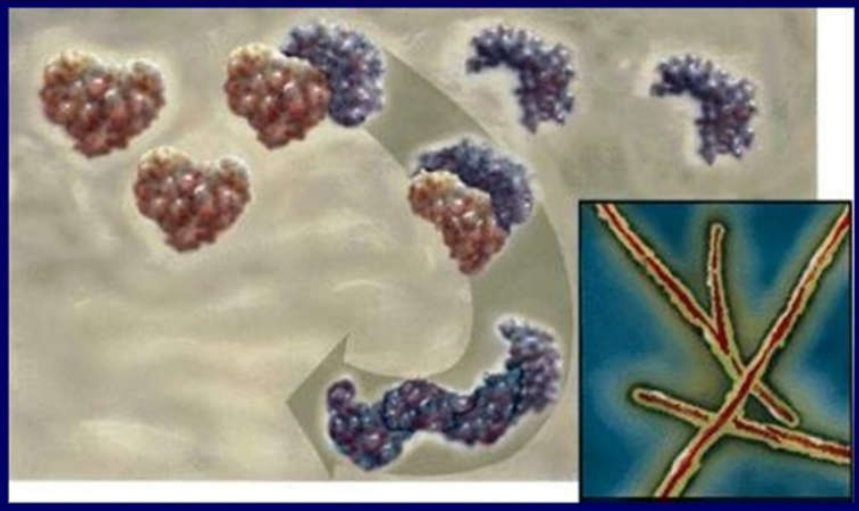




Effect of prions on neural tissue

- Convert PrPc into PrSc
- PrSc has identical primary structure but different beta structures leading to resistance of protease cleavage.
- Brain tissue collects PrSc causing too much protein accumulation.
- Distinguished by nerve cell death causing large vacuoles and plaques in brain tissue

How do prions function?



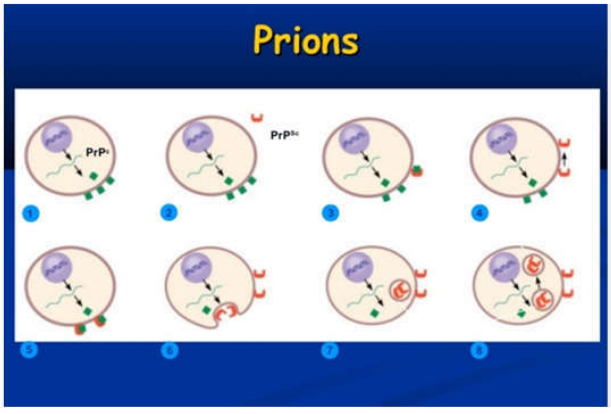
PrP^{c}

PrP^{Sc}

The prion protein exists in two forms. The normal, innocuous protein (PrPc) can change its shape to a harmful, disease-causing form (PrPSc). The conversion from PrPc to PrPSc then proceeds via a chain-reaction. When enough PrPSc proteins have been made they form long filamentous aggregates that gradually damage neuronal tissue. The harmful PrPSc form is very resistant to high temperatures, UV-irradiation and strong degradative enzymes.

The normal form of the PrP protein is called PrP^{c} (where "C" refers to "cellular" PrP), and it is found on the cell membrane of many mammalian cells, especially brain cells. PrP^{c} is produced initially as a precursor protein containing 253 amino acid residues. Its role has been shown to bind copper ions and thereby, help the brain cells to resist the toxic effects of highly reactive oxygen radicals. The enzyme, superoxide dismutase, which protects cell from oxygen radicals, requires copper for its activity. In absence of PrP^{c}, the cells cannot bind copper and the enzyme remains inactive. This leads to death of the cells due to oxygen toxicity.

The PrP that forms prions has a different structure and is resistant to proteases, the enzymes in the body that can normally break down proteins. The infectious forms of PrP proteins are called **PrP^{Sc}** (in the case of scrapie) or **PrP^{CJD}** (in the case of Creutzfeld–Jakob disease). They have been shown to possess the same amino acid sequence as the normal one, but they differ in conformation (Fig. 8.17). As a result of such conformational change, they are thought to stick together inside cells, forming small fibers, or fibrils. Because the fibrils cannot be organised in the cell membrane correctly, such aggregations possibly block molecular traffic in the infected cells and eventually kill the cell.



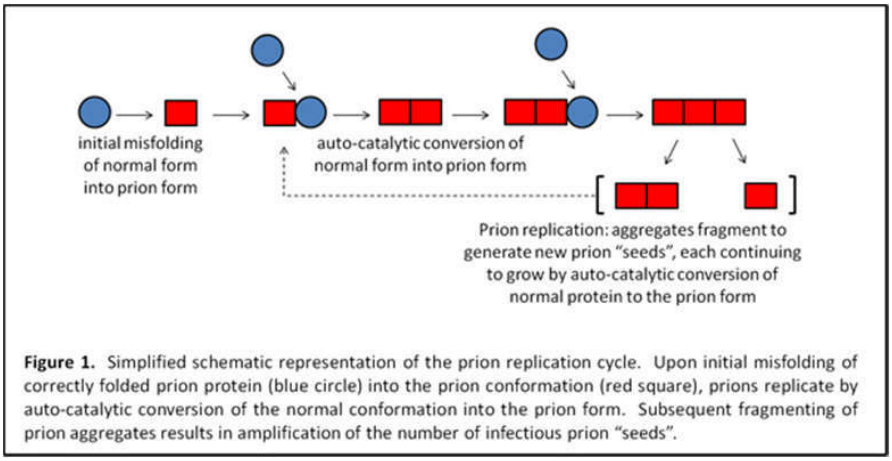
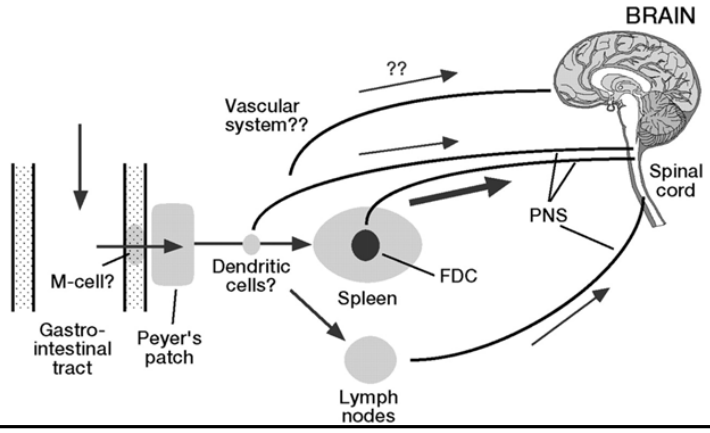


Figure 1. Simplified schematic representation of the prion replication cycle. Upon initial misfolding of correctly folded prion protein (blue circle) into the prion conformation (red square), prions replicate by auto-catalytic conversion of the normal conformation into the prion form. Subsequent fragmenting of prion aggregates results in amplification of the number of infectious prion "seeds".



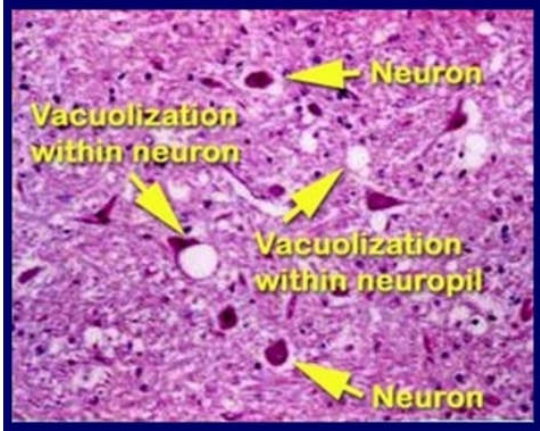
Examples of Human Prion Diseases

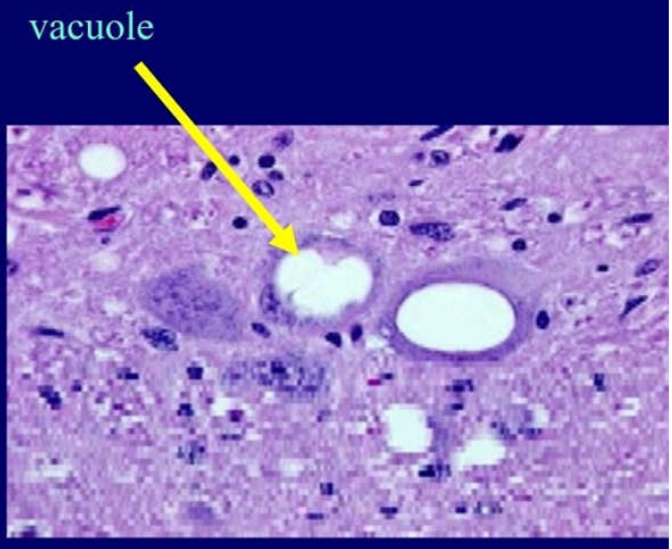
Disease	Transmission	Clinical Signs and Symptoms	Treatment
Creutzfeldt-Jacob disease (CJD)	Spontaneous transformation of normal prion proteins; 10%–15% inherited (autosomal dominant)	Dementia Early neurologic signs: Periodic waves on the electroencephalogram (EEG); presence of "florid plaques" on neuropathology; presence of agent in lymphoid tissue	None known; rapidly progressive and always fatal
Variant Creutzfeldt-Jacob disease (vCJD)	"Mad cow" disease: Contracted when exposed to food contaminated with bovine spongiform encephalopathy (BSE)	Prominent psychiatric/behavioral symptoms; painful dyesthesiasis (distortion of the sense of touch); delayed neurological signs; large amounts of "florid plaques" on neuropathology	Supportive: No specific treatment; fatal
Gerstmann-Sträussler-Scheinker syndrome	Inherited	Various levels of ataxia; dysarthria (slurring of speech), dementia, nystagmus, spasticity, visual disturbances	No cure, no known treatment, only supportive
Kuru	Cannibalism	Unsteady gait, tremors, slurred speech	No cure, no treatment, only supportive

Prions

- First identified with “Spongiform encephalopathies”
- Characteristics of infection:
  - Loss of motor control
  - Dementia
  - Paralysis
  - Encephalitis
  - Widespread neuronal loss
- Ways of infection:
  - Infectious (including diet, after surgical procedures, corneal transplants etc.)
  - Hereditary (autosomal and dominant)

Brain Damage from Spongiform Encephalopathy





VIROIDS: *The Plant Invaders…*

A **VIROID** is a…

VIR(virus)OID(like) particle.

Viroids are "sub-viruses" composed exclusively of a single circular strand of nucleic acid (RNA) that codes for a single protein.

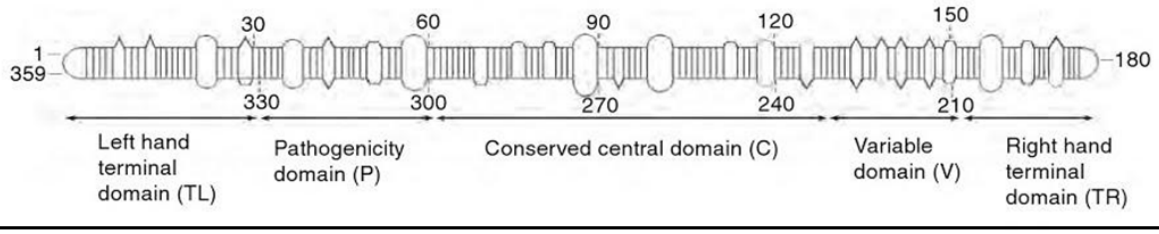
OR

Small, circular RNA molecules without a protein coat

Structure of Viroids

Plant viroids are the smallest known infectious agents. They differ from viruses as they do not possess a protein coat and exist only as short, infectious molecules of RNA having a molecular weight of 1,30,000 daltons, which is at least ten times smaller than a typical RNA-virus genome. Viroid genome encodes no gene product and they do not require a helper for infectivity. Some known viroids are single-stranded, covalently closed, circular RNA molecules that contain 246–375 nucleotides. Under electron microscope, the viroid RNA measures about 40 nm in length and it has thickness of double-stranded RNA. All viroids have some degree of overall sequence similarity. Most of the viroids have a relatively high G+C content (53–60%), but Avocado sunblotch viroid is rich in A+U (62%).

The single-stranded RNA molecules of the viroids have extensive intra-strand base-pairing with intermittent loops produced by unpaired bases (Fig. 8.15). This probably contributes to the stability of the RNA as it lacks a protein coat. In many viroids, the folded RNA molecule consists of five structural regions (domains): a left and right terminal region, a pathogenicity region, a conserved central region, and a variable region (Fig. 8.15). The left and right terminal domains are involved in replication. The pathogenicity domain located near the left terminal domain, determines the pathogenicity of a viroid, that is, its ability to infect and multiply, and also the severity of the symptoms that will develop on the host plants. However, the severity of symptoms can be altered by changes in one or two bases of the pathogenicity domain. The central portion contains the conserved sequence, while the base sequences are variable in the variable domain. This correlation of structure and function is observed in most of the viroids.

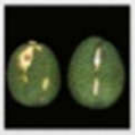


Genus *Pospiviroids:*
PSTVd (potato spindle tuber)

Genus *Coleviroids:*
CbVd 1 (coleus blumei 1)

Genus *Hostuviroids:*
HSVd (hop stunt)

Genus *Avsunviroids:*
ASBVd (avocado sunblotch)



Genus Cocadviroids:
CCCVd (coconut cadang-cadang)

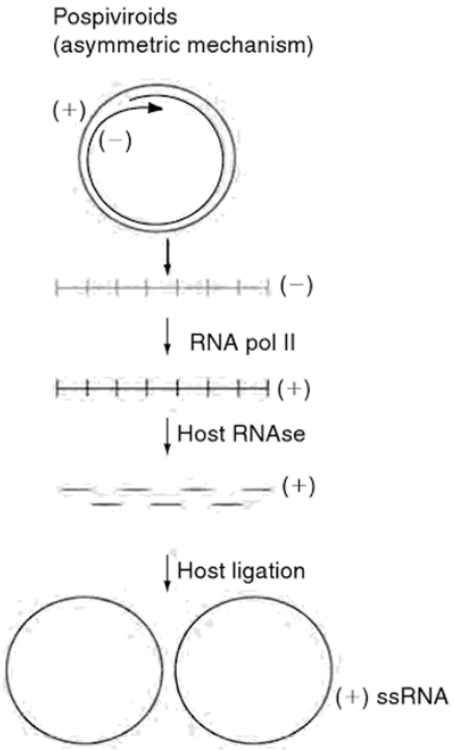
Genus *Pelamoviroids:*
PLMVd (peach latent mosaic)

Genus *Apscaviroids:*
ASSVd (apple scar skin)

Family (Subfamily)	Genus	Species	Hosts
Pospiviroidae	*Pospiviroid*	Potato spindle tuber viroid	Plants
	Hostuviroid	Hop stunt viroid	
	Cocadviroid	Coconut cadang-cadang viroid	
	Apscaviroid	Apple scar skin viroid	
	Coleviroid	Coleus blumei viroid 1	
Avsunviroidae	*Avsunviroid*	Avocado sunblotch viroid	Plants
	Pelamonviroid	Peach latent mosaic viroid	

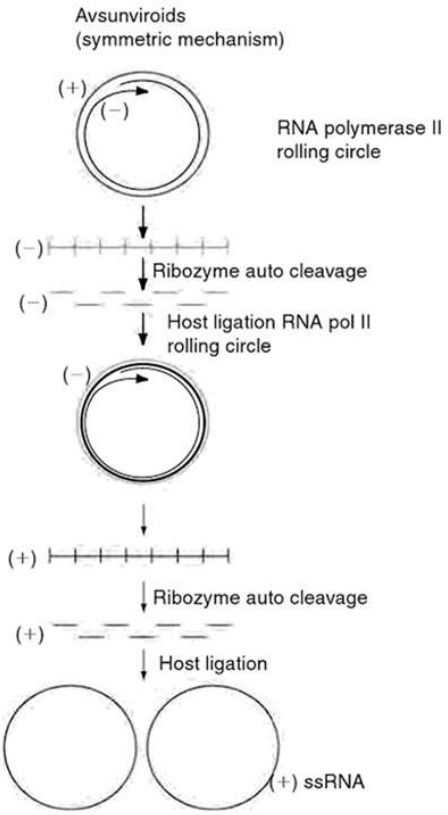
The RNAs of Pospiviroidae members are found in the nucleus and the available evidence is that this family of viroids replicates in the nucleus and nucleolus. The enzyme most likely to be responsible for replicating the genome RNA is the host cell DNA-dependent RNA polymerase. It is not known how this enzyme functions on an RNA template, but this may be, at least in part, the result of the extensive base-pairing structure of the genome RNA.

In case of Pospiviroids, replication begins at a precise point on the positive infecting circular genome with the help of RNA polymerase II, but the nature of initiation event is not known. This results in the formation of a large linear multimeric negative strand. The Pospiviroids with an asymmetric replication pathway then make positive strand RNA from this long linear molecule. A host RNAase activity cleaves the positive strand into unit viroid lengths. These molecules then ligate to form many copies of the original circular viroid RNA



On the other hand, members of the Avsunviroidae are associated with chloroplasts, and it is speculated that they may replicate in the chloroplasts. The replication of viroid RNA begins by adopting a rolling circle mechanism. Avsunviroids probably replicate via symmetric rolling circle mechanism, whereas Pospiviroids probably use an asymmetric mechanism.

Avsunviroid group are different as they replicate in the chloroplasts, and there is evidence for a chloroplast polymerase being involved in their replication. The replication process generates linear concatameric RNA, of opposite sense to the genome, from which genome length RNA molecules must be excised. The excision depends on the action of an unusual RNA sequence within the newly synthesised molecule, called a ribozyme. These ribozyme sequences autocatalytically cleave the RNA at a specific site, generating genome length, linear, (—) and ssRNA molecules. These molecules are then converted into circles. The circular antigenome RNA is then used as a template to produce more genomes by the rolling circle



Satellite Virus

➢A **satellite** is a subviral agent composed of nucleic acid that depends on the co-infection of a host cell with a helper or master **virus** for its replication. When a **satellite** encodes the coat protein in which its nucleic acid is encapsidated it is referred to as a **satellite virus**.

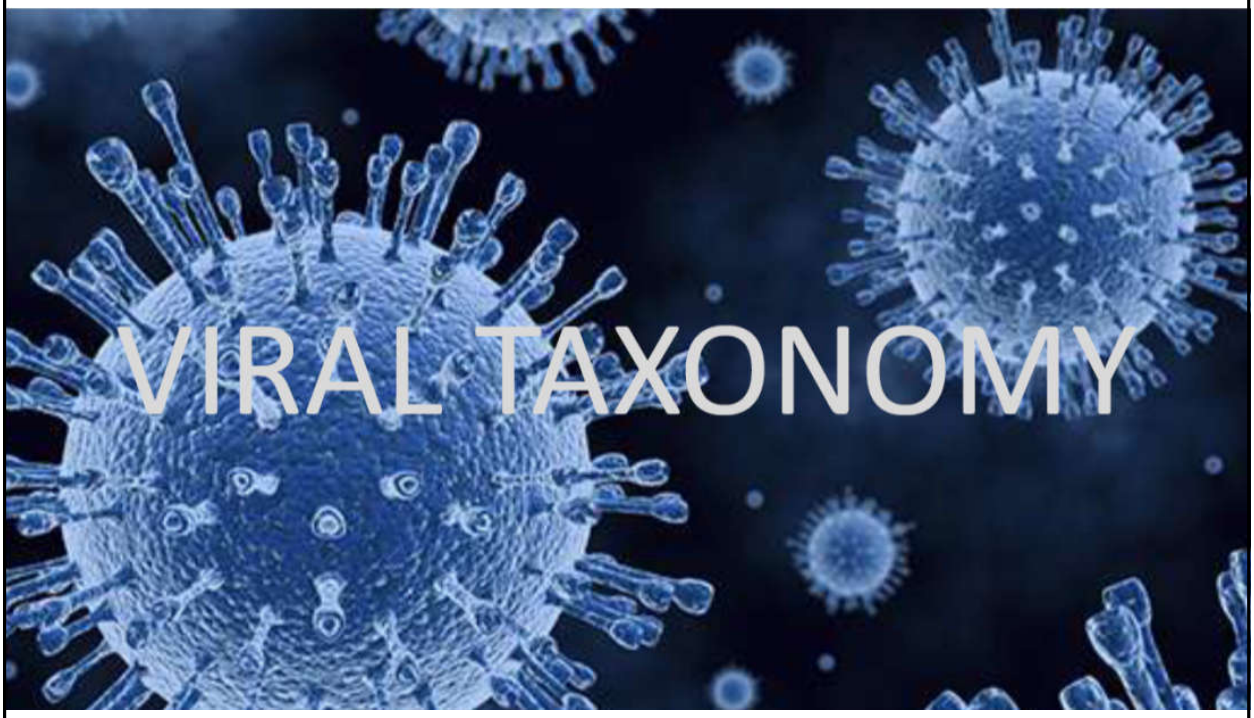
➢They lack genes that would encode the enzymes needed for their replication. And hence depend on a co-infecting helper virus to provide essential functions.

➢The satellite genome does not have any significant homology with that of the helper virus.

➢Representative genera: Adeno Associated Virus

Virusoids (Satellites)

Virusoids or satellites RNAs have genomes from 220 to 388 nucleotides long, their genomes are circular and single stranded, and they have ribozyme activity. Virusoids replicate in the cytoplasm of their host, using an RNA-dependent RNA polymerase. Although the RNA replication is similar to that of viroids, virusoids require that the host cell be infected with a specific helper virus. Virusoids are generally associated with plant infections, but there are similar agents that infect animals. One of these agents infecting humans is the hepatitis delta virus (HDV). For hepatitis D virusoid to function, a cell must be simultaneously infected with the hepatitis B virus. However, there is a significant difference between viroids and HDV: viroids cannot produce proteins whereas HDV produces two proteins named small and large delta antigens.



INTRODUCTION

- **Virus classification** is the process of naming viruses and placing them into a taxonomic system.
- Viruses are mainly classified by phenotypic characteristics, such as morphology, nucleic acid type, mode of replication, host organisms, and the type of disease they cause.
- Currently there are two main schemes used for the classification of viruses:

1. The International Committee on Taxonomy of Viruses (ICTV) system and
2. Baltimore classification system,

which places viruses into one of seven groups. Accompanying this broad method of classification are specific naming conventions and further classification guidelines set out by the ICTV.

International Committee on Taxonomy of Viruses (ICTV)

- The ICTV had adopted the principle that A VIRUS SPECIES IS A POLYTHETIC* CLASS OF VIRUSES THAT CONSTITUTES A REPLICATING LINEAGE AND OCCUPIES A PARTICULAR ECOLOGICAL NICHE.
- The formal definition of a polythetic class is "a class whose members always have several properties in common although no single common attribute is present in all of its members"
- The qualification of a replicating lineage implies that members of a species experience evolution over time with consequent variation, but that members share a common ancestor.
- The qualification of occupation of an ecologic niche acknowledges that the biology of a virus, including such properties as host range, pathogenesis, transmission, and habitat, are fundamental components of the characterization of a virus.

International Committee on Taxonomy of Viruses (ICTV)

The official objectives of the ICTV are:

- To develop an internationally agreed taxonomy for viruses
- To develop internationally agreed names for virus taxa, including species and sub-viral agents
- To communicate taxonomic decisions to all users of virus names, in particular the international community of virologists, by publications and via the Internet
- To maintain an index of virus names
- To maintain an ICTV database on the Internet, that records the data that characterize each named viral taxon, together with the common names of each taxon in all major languages

International Committee on Taxonomy of Viruses (ICTV)

- In July 2013, the ICTV definition of species changed to state: "A species is a monophyletic group of viruses whose properties can be distinguished from those of other species by multiple criteria."

Viral classification starts at the level of order and continues as follows, with the taxon suffixes given in italics:

- Order (*-virales*)
- Family (*-viridae*)
- Subfamily (*-virinae*)
- Genus (*-virus*)
- Species

Species names generally take the form of *[Disease] virus*.

**Of, pertaining to, or affecting a single phylum (or other taxon) of organisms.

International Committee on Taxonomy of Viruses (ICTV)

- The establishment of an order is based on the inference that the virus families it contains have most likely evolved from a common ancestor.
- The majority of virus families remain unplaced.
- Currently (2012), 7 orders, 96 families, 22 subfamilies, 420 genera, and 2,618 species (2,619 acc. to ICTV master species list) of viruses have been defined by the ICTV.
- The orders are the *Caudovirales, Herpesvirales, Ligamenvirales, Mononegavirales, Nidovirales, Picornavirales,* and *Tymovirales*.
- These orders span viruses with varying host ranges. The *Ligamenvirales*, infecting archaea, are the most recent addition to the classification system.

International Committee on Taxonomy of Viruses (ICTV)

As an example, the full formal written description of human respiratory syncytial virus is

- Order: *Mononegavirales*
- Family: *Paramyxoviridae*
- Subfamily: *Pneumovirinae*
- Genus: *Pneumovirus*
- Species: *Human respiratory syncytial virus.*

International Committee on Taxonomy of Viruses (ICTV)

- *Caudovirales* are tailed dsDNA (group I) bacteriophages.
- *Herpesvirales* contain large eukaryotic dsDNA viruses.
- *Ligamenvirales* contains linear, dsDNA (group I) archaean viruses.
- *Mononegavirales* include nonsegmented (-) strand ssRNA (Group V) plant and animal viruses.
- *Nidovirales* are composed of (+) strand ssRNA (Group IV) viruses with vertebrate hosts.
- *Picornavirales* contains small (+) strand ssRNA viruses that infect a variety of plant, insect and animal hosts.
- *Tymovirales* contain monopartite (+) ssRNA viruses that infect plants.
- Other variations occur between the orders: *Nidovirales*, for example, are isolated for their differentiation in expressing structural and nonstructural proteins separately.

Baltimore Classification of viruses

The division of the viruses into classes based on genome type and mode of replication and transcription

Suggested by David Baltimore – Seven Baltimore classes.

Major groups of viruses are distinguished first by their nucleic acid content as either DNA or RNA

RNA and DNA viruses can be single-stranded (ssRNA, ssDNA) or double-stranded (dsRNA, ssDNA)

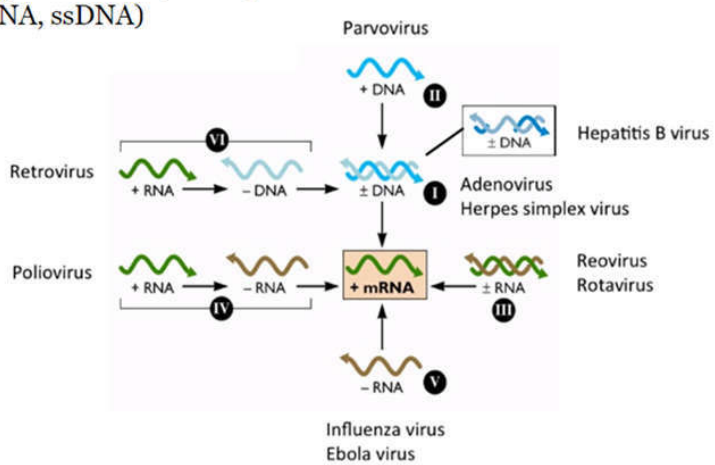
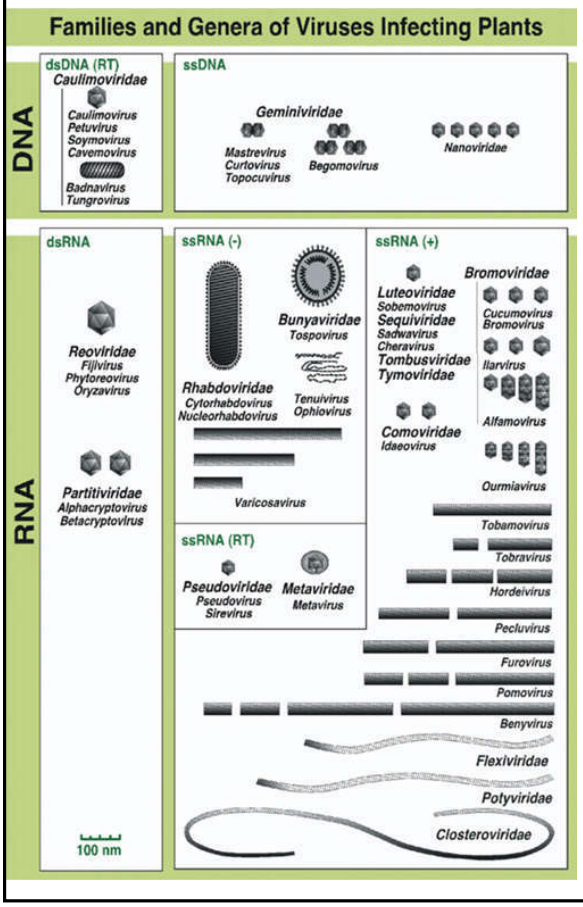


Table 17.1 : The animals viruses.

Family	Virions	Genome (kb)	Example
1. dsDNA viruses			
Adenoviruses	Naked, icosahedral	35-40	Human and Animal adenoviruses
Herpes virus	Enveloped, icosahedral	120-200	Herpes simplex, chicken-pox, Epstein-Bar virus
Papovavirus	Naked, icosahedral	5-8	SV40, polyoma, papilomaviruses
Poxvirus	Enveloped, complex	120-300	Small pox, vaccinia
2. ssDNA viruses			
Parvovirus	Naked, icosahedral	4-5	Adeno-associated virus, Human parvovirus
3. (+) ssRNA viruses			
Coronavirus	Enveloped, helical	16-21	Human common cold like diseases, mouse hepatitis virus
Picorna virus	Naked, icosahedral	7	Polio, common cold virus, foot and mouth disease virus
Togavirus	Enveloped, icosahedral	17	Encephalitis virus
4. (-) ssRNA virus			
Paramyxoviruses	Enveloped, helical	15	Measles, mumps
Rhabdovirus	Enveloped, helical (Bullet shaped)	12-15	Rabies virus
Orthomyxovirus	Enveloped, helical	14	Influenza
5. dsRNA viruses			
Reovirus	Naked, icosahedral	18-30	Reovirus, human and animal diarrhoea virus
6. (+)ssRNA-RT viruses			
Retrovirus	Enveloped, icosahedral (diploid)	7-10	Rous sarcoma, Avian mouse, Mammary tumour virus
7. dsDNA-RT viruses			
Hepadnavirus	Enveloped, icosahedral	13	Hepatitis-B virus, humans, rodents and birds



Nature of Nucleic Acid	Type of Nucleic Acid	Representative Genera	Disease Caused
DNA	ds (Class I)	**Badnavirus**	**Commelina yellow mottle disease**
	ss (Class II)	**Geminivirus**	**Maize Streak Disease**
RNA	ds (Class III)	**Oryzavirus**	**Rice Ragged Stunt Disease**
	(+) sense ss (Class IV)	**Tobamovirus**	**Tobacco Mosaic Disease**
	(-) sense (Class V)	**Cytorhabdovirus**	**Barley yellow striate mosaic disease**
	(+) sense ss retro-virus (Class VI)	**Pseudovirus**	**Infects different plants**
	ds reverse virus (Class VII)	**Caulimovirus**	**Cauliflower mosaic virus**

Techniques in cultivating and identifying animal viruses

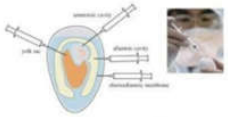
- viruses require living cells as their "medium".
  - In vivo – laboratory-bred animals and embryonic bird tissues.
  - In vitro - cell or tissue culture methods.

The primary purpose of virus cultivation is:

- To isolate and identify viruses in clinical samples.
- To do research on viral structure, replication, genetics and effects on host cell.
- To prepare viruses for vaccine production.

Methods for Cultivation of Virus

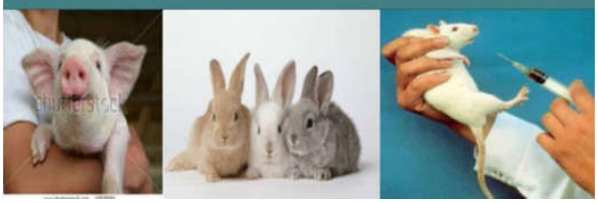
- Generally three methods are employed for the virus cultivation
  1. Inoculation of virus into animals
  2. Inoculation of virus into embryonated eggs
  3. Tissue culture



1. Animal Inoculation

- Viruses which are not cultivated in embryonated egg and tissue culture are cultivated in laboratory animals such as mice, guinea pig, hamster and rabbits are used.
- The selected animals should be healthy and free from any communicable diseases.
- Mice(less than 48 hours old) are most commonly used.
- Mice are susceptible to togavirus and coxsackie virues, which are inoculated by intracerebral and intranasal route.

- After inoculation, virus multiply in host and develops disease. The animals are observed for symptoms of disease and death.
- Then the virus is isolated and purified from the tissue of these animals.
- Live inoculation was first used on human volunteers for the study of yellow fever virus.



Advantages of Animal Inoculation

- Diagnosis, Pathogenesis and clinical symptoms are determined.
- Production of antibodies can be identified.
- Primary isolation of certain viruses.
- Mice provide a reliable model for studying viral replication.
- Used for the study of immune responses, epidemiology and oncogenesis .

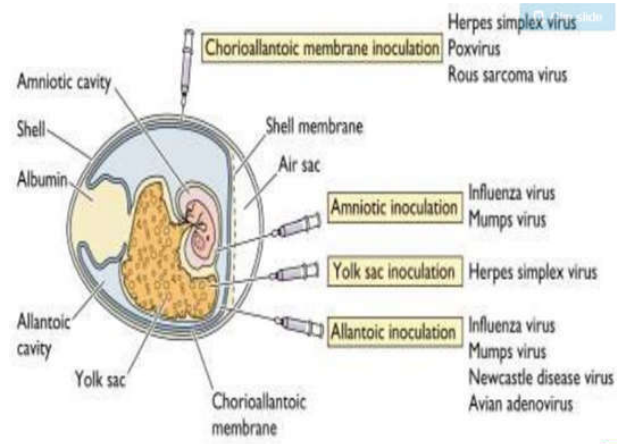
Disadvantages of Animal Inoculation

- Expensive and difficulties in maintenance of animals.
- Difficulty in choosing of animals for particular virus
- Some human viruses cannot be grown in animals , or can be grown but do not cause disease.
- Mice do not provide models for vaccine development.
- Issues related to animal welfare systems.

2. Inoculation into embryonated egg

- Good pasture in 1931 first used the embryonated hen's egg for the cultivation of virus.
- The process of cultivation of viruses in embryonated eggs depends on the type of egg which is used.
- Viruses are inoculated into chick embryo of 7-12 days old.
- For inoculation, eggs are first prepared for cultivation, the shell surface is first disinfected with iodine and penetrated with a small sterile drill.

- After inoculation, the opening is sealed with gelatin or paraffin and incubated at 36°c for 2-3 days.
- After incubation, the egg is broken and virus is isolated from tissue of egg.
- Viral growth and multiplication in the egg embryo is indicated by the death of the embryo, by embryo cell damage, or by the formation of typical pocks or lesions on the egg membranes
- Viruses can be cultivated in various parts of egg like chorioallantoic membrane, allantoic cavity, amniotic sac and yolk sac.



Allantoic cavity:

- Inoculation is mainly done for production of vaccine of influenza virus, yellow fever, rabies.
- Most of avian viruses can be isolated using this method.

Amniotic sac:

- Inoculation is mainly done for primary isolation of influenza virus and the mumps virus.
- Growth and replication of virus in egg embryo can be detected by haemagglutination assay.

Chorioallantoic Membrane (CAM):

- Inoculation is mainly for growing poxvirus.
- After incubation and , visible lesions called pocks are observed, which is grey white area in transparent CAM.
- Herpes simplex virus is also grown.
- Single virus gives single pocks
- This method is suitable for plaque studies.

Yolk sac inoculation

- It is also a simplest method for growth and multiplication of virus.
- It is inoculated for cultivation of some viruses and some bacteria (Chlamydia, Rickettsiae)
- Immune interference mechanism can be detected in most of avian viruses.

Advantages of Inoculation into embryonated egg

- Widely used method for the isolation of virus and growth.
- Ideal substrate for the viral growth and replication.
- Isolation and cultivation of many avian and few mammalian viruses.
- Cost effective and maintenance is much easier.
- Less labor is needed.
- The embryonated eggs are readily available.
- They are free from contaminating bacteria and many viruses.
- Widely used method to grow virus for some vaccine production.

Disadvantages of Inoculation into embryonated egg

- The site of inoculation for varies with different virus. That is, each virus have different sites for their growth and replication.

3. Tissue Culture

- Cultivation of tissue or organ for the growth of viruses
- Three types of tissue culture.
- **Organ cultures** - are mainly done for highly specialized parasites of certain organs e.g. tracheal ring culture is done for isolation of coronavirus.
- **Explant culture** – Fragments of tissue can be grown as explant in plasma clot. This method is rarely used.
- **Cell culture** – it is mostly used for cultivation of viruses.

- **Cell culture** is mostly used for identification and cultivation of viruses.
  - ❖Cell culture is the process by which cells are grown under controlled conditions.
  - ❖Cells are grown in vitro on glass or a treated plastic surface in a suitable growth medium.
  - ❖At first growth medium, usually balanced salt solution containing 13 amino acids, sugar, proteins, salts, calf serum, buffer, antibiotics and phenol red are taken and the host tissue or cell is inoculated.
  - ❖On incubation the cell divide and spread out on the glass surface to form a confluent monolayer.

Types of cell culture

Based on the origin and the chromosome property the tissue culture are classified into 3 types.

1. Primary cell culture:

- ❖ These are normal cells freshly taken from animal or human body.
- ❖ They are able to grow only for limited time and cannot be maintained in serial culture.
- ❖ They are used for the primary isolation of viruses and production of vaccine.
- ❖ Examples: Monkey kidney, cell culture, Human embryonic kidney, chick embryo cell culture.

2. Diploid cell culture (Semi-continuous cell lines)

- They are diploid and contain the same number of chromosomes as the parent cells.
- They can be sub-cultured up to 50 times by serial transfer following senescence and the cell strain is lost.
- They are used for the isolation of some fastidious viruses and production of viral vaccines.
- Examples: Human embryonic lung strain, Rhesus embryo cell strain.

3. Continuous cell lines

- They are derived from cancer cells.
- They can be serially cultured so named as continuous cell lines
- They can be maintained either by serial subculture or by storing in deep freeze at -70°c.
- Due to derivation from cancer cells they are not useful for vaccine production.
- Examples: HeLa (Human Carcinoma of cervix cell line), HEP-2 (Humman Epithelioma of larynx cell line), BHK-21 (Baby Hamster Kidney cell line).

Advantages of cell culture

- Relative ease, broad spectrum, cheaper and sensitivity.

Disadvantage of cell culture

- The process requires trained technicians with experience in working on a full time basis.
- State health laboratories and hospital laboratories do not isolate and identify viruses in clinical work.
- Tissue or serum for analysis is sent to central laboratories to identify virus.

Detection of virus growth in cell culture

- **Cytopathic effects** – Many viruses causes morphological changes in cultured cells in which they grow. These changes can be observed microscopically and are known as cytopathic effects.

  The cytopathic effect are characteristic for a particular group of viruses and helps in the presumptive identification of viruses.

  ex- entroviruses causes orientation of cells.

- **Metabollic inhibition** – In normal cell cultures the medium turns acidic due to cellular metabolism. Growth of viruses inhibits metabolism and hence no acidic production. This can be made out by the colour of the indicator (phenol red).

- **Haemadsorption** - When Haemogglutinating viruses (influenza and Para influenza) grow in cell culture. They adsorb Guinea pig RBCs onto the surface of cell cultures.

Cultivation of Plant Virus

Plant viruses cause many diseases of economically important crops, such as tomatoes (tomato spotted wilt virus), corn and sugarcane (wound tumor virus), potatoes (potato yellow dwarf virus), and lettuce (lettuce necrotic yellows). The effects of viral infection on some plants are shown in FIGURE 16.8. As with bacterial and animal viruses, it is important for microbiologists to be able to cultivate and study plant viruses in a controlled laboratory setting.

Plant viruses can be cultivated by inoculating a plant with a virus suspension through a hypodermic needle or by scratching plant leaves with virus material. Scratching is aided with an abrasive such as carborundum. This can lead to formation of local lesions as well as general infection. Some plant viruses can replicate to large numbers in infected plants. For example, a single hair cell of an infected tobacco plant may contain over 107 (10 million) tobacco mosaic virions. Indeed, as much as 10 percent of the dry weight of infected leaves may be tobacco mosaic virus.

Transfer of infection from cell to cell occurs by passage of virions through bridges that connect the cells of plant tissue. Also, in most plant diseases, infected cells can continue to manufacture virus without either disintegrating or dying. Diseased plant material can be crushed to release plant juices containing the virions, which can then be purified, sometimes to crystals. Using this method in the 1930s, Wendell M. Stanley obtained purified tobacco mosaic virus in crystal form, the first crystallization of any virus. He was awarded the 1946 Nobel Prize in chemistry for this research.

In recent years some progress has been made in the preparation of plant cells which have had their walls removed. Such wall-less cells are bounded only by the cytoplasmic membrane and are called *protoplasts.* For instance, protoplasts made from specific tobacco plant cells can be infected directly with tobacco mosaic virus. Progress has also been made in the development of mono-layer cultures of susceptible cells from the insects that transmit some viral plant diseases. For example, rhabdovirus has been cultivated in leafhopper cell culture, with a yield of over 10,000 virions per cell.

(A) (B) (C)

Illustrations of plant infections caused by viruses. [A] Leaf of *Grammatophyllum scriptum* (orchid) exhibiting necrotic ringspots caused by bacilliform particles (virions). [B] *Nicotiana tabacum* (tobacco) leaf exhibiting necrotic ringspot resulting from infection by tomato ringspot virus. [C] Leaf of *N. glutinosa* (tobacco) exhibiting local lesions resulting from mechanical inoculation with tobacco mosaic virus.

Bacteriophages:

- Bacteriophages are viruses that can infect and destroy bacteria.
- They have been referred to as bacterial parasites, with each phage type depending on a single strain of bacteria to act as host.

Like most viruses, bacteriophages typically carry only the genetic information needed for replication of their nucleic acid and synthesis of their protein coats.. They require precursors, energy generation and ribosomes supplied by their bacterial host cell.

- At present, over 5000 bacteriophages have been studied by electron microscopy and can be divided into 13 virus families.
- Bacteriophages make up a diverse group of viruses, some of which have complex structures, including double-stranded DNA.
- Also known simply as a phage; a virus that attacks and infects bacteria. The infection may or may not lead to the death of the bacterium, depending on the phage and sometimes on conditions. Each bacteriophage is specific to one form of bacteria.

Viral Multiplication – general phases in the life cycle of viruses

Multiplication/Replication cycles involving 5 steps:

1. **Adsorption:** the attachment of viruses to host cells
2. **Penetration:** entry of virions (or their genome) into host cells
3. **Synthesis:** new nucleic acids, capsid proteins, and other viral components- *transcription, translation and genome replication*
4. **Maturation:** assembly of newly synthesized viral components into complete virions
5. **Release:** departure (pemergian) of new virions from host cells

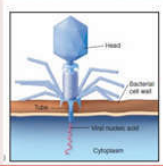
Adsorption

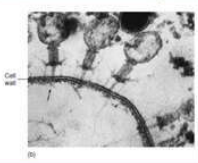
- Bacteriophages have specialized structures for attaching to bacterial cell walls – adsorption involve attachment of specific tail fibers to bacteria's cell wall
- They adsorb to host bacteria using specific receptors on the bacterial surface

Penetration

- Bacteriophages have a mechanism for injecting their nucleic acid into host cells (nucleic acid and capsid usually penetrate animal cells)
- This eliminates the need for uncoating.

Only nucleic acid penetrate into host cell.





Penetration of a bacterial cell by a T-even bacteriophage.
(a)After adsorption, the phage plate becomes embedded in the cell wall, and the sheath contracts, pushing the tube through the cell wall and membrane and releasing the nucleic acid into the interior of the cell.
(b)Section through *E. coli* with attached phages. Note that these phages have injected their nucleic acid through the cell wall and now have empty heads.

Synthesis

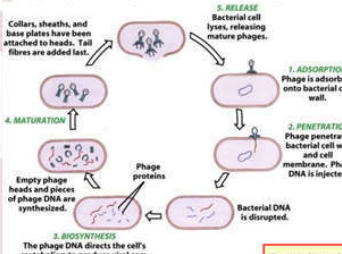
- Entry of the nucleic acid causes the cessation of host cell DNA replication and protein synthesis. Soon the host cell machinery is used for viral replication and synthesis of viral proteins.

Maturation

- As the host cell produces new phage parts, the parts spontaneously assemble into bacteriophages.
- An average-sized *Escherichia coli* cell can contain up to 200 new phage units at the end of this period.

Release

- Eventually, the host cell becomes so packed with viruses that it lyses (splits open) - releasing the mature virions.
- The process is hastened by viral enzymes produced late in the infection cycle that digest the cell envelope, thereby weakening it. Upon release, the virulent phages can spread to other susceptible bacterial cells and begin a new infection.



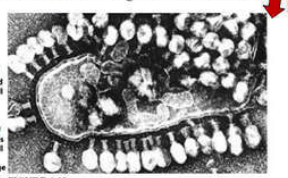


FIGURE 6.19
A weakened bacterial cell, crowded with viruses. The cell has ruptured and released numerous virions that can then attack nearby susceptible host cells. Note the empty heads of "spent" phages lined up around the ruptured wall.

Involve lysozyme to break the host cell wall

Replication of a virulent bacteriophage: A virulent phage undergoes a lytic cycle to produce new phage particles within a bacterial cell. Cell lysis releases new phage particles that can infect more bacteria

T4 virulent (lytic) phages

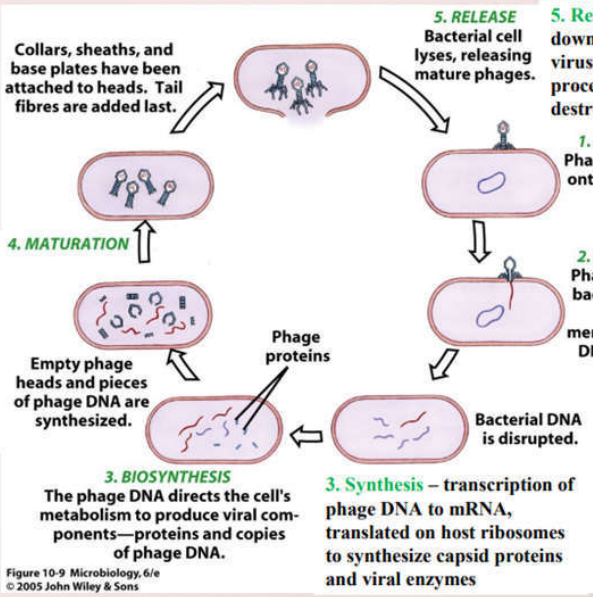


Figure 10-9 Microbiology, 6/e
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5. Release – Lysozyme breaks down the cell wall, allowing viruses to escape – in the process the host cell is lysed ∴ destroy the host

1. Adsorption – chemical attraction, specific protein recognition factors found in tail fibers that bind to specific receptor sites on the host cells.

2. Penetration – lysozyme, weakens the bacterial cell walls – for T4 phages DNA are introduce into the periplasmic space

3. Synthesis – transcription of phage DNA to mRNA, translated on host ribosomes to synthesize capsid proteins and viral enzymes

4. Maturation – T4 head is assembled in host cell cytoplasm from new capsid protein, phage tails from new formed base plates, sheaths and collars. After heads and tails are attached

Replication of a temperate bacteriophage: Following adsorption and penetration, the virus undergoes prophage formation

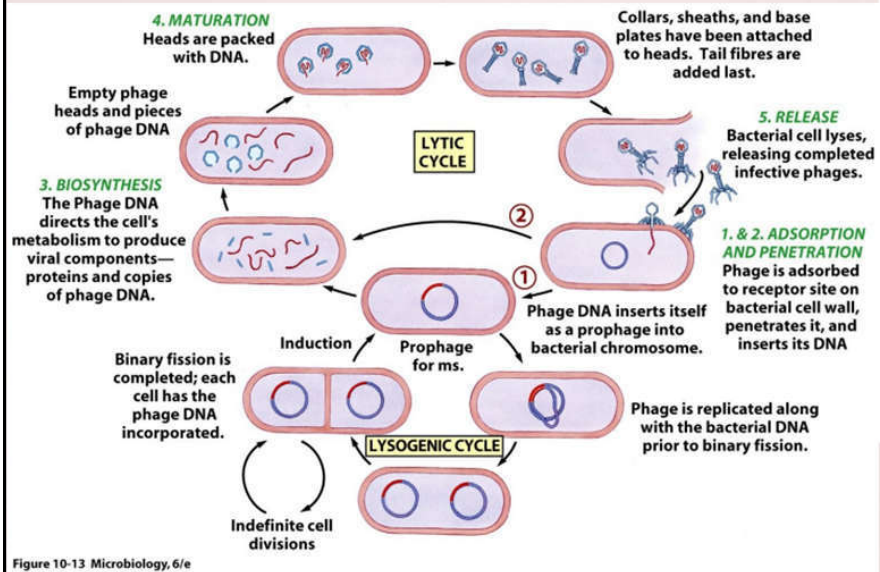

Figure 10-13 Microbiology, 6/e
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Temperate phages have alternative replication cycle
i)A productive lytic cycle
ii)A reductive infection, in which the phage remain latent in the host establishing lysogeny

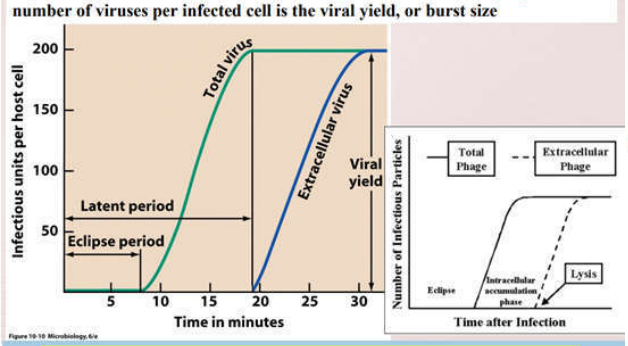
- Lysogeny – occurs when environmental conditions are poor. Allowing survival as a prophage in the host. - lysogen

Induction: Due to lack of nutrients for bacterial growth or the presence of chemical toxic to lysogen

Lysogenic phages - λ phage of *E. coli.*

Phage Growth

Growth curve for a bacteriophage: The eclipse phage represents the time after penetration through the biosynthesis of mature phages. The latent period represents the time after penetration through release of mature phages. The number of viruses per infected cell is the viral yield, or burst size



Cultivation and identification of phages

1. **Obtaining bacteriophage from sample**
2. **Amplification/multiplication of phages**

Solution (sample) into liquid media (eg. NB, TSB)

Addition of host – sewage: enteric bacteria, faec *E.coli*

Incubation: 37° C, 24 hrs

increase the numbers of phages (in the sewage sample) by allowing them to infect and reproduce within fresh host.

1. **Isolation of multiplied phages**

Separate the remaining host cell/cell debris via centrifugation and filtration (0.2µm filter)

Preparation of pure phage suspension

1. **Plaque assay**

Detection, identification , phage isolation for storage and future research

Estimation of Phage Numbers

- Plaque assay:

1.Serial dilutions of suspension of phages

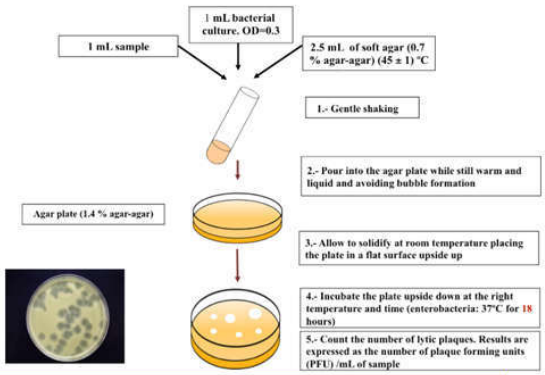
2.Each dilution is inoculated onto a plate containing bacterial lawn

3.As a result of infection, new phages will lyse the bacteria

4.After several round of lysis, the bacterial lawn shows clear areas called plaques.

5.Plaque-forming units (pfu) – counting the no. of plaques X dilution factor = the no. of phages in ml of suspension.

Bacteriophage enumeration by double agar layer method



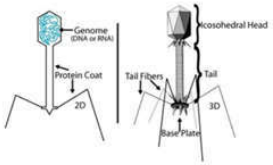
Identification of phages – Plaque assay technique

➢ **Plaque ?** Zone of cell death/ a clear area in a bacterial lawn culture where viruses have lysed host cells

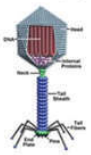
HOW TO IDENTIFY TEMPERATE PHAGE? - Cloudy plaque

Structure of T4 Bacteriophage

- Size
  - Approximately 200 nm long and 80-100nm wide
- Head or Capsid
  - Icosahedral
  - Protective covering for the nucleic acid
- Tail
  - A hollow tube through which the nucleic acid passes during infection.
  - Surrounded by a contractile sheath.
  - Base Plates
  - Tail fibers



T4 bacteriophage



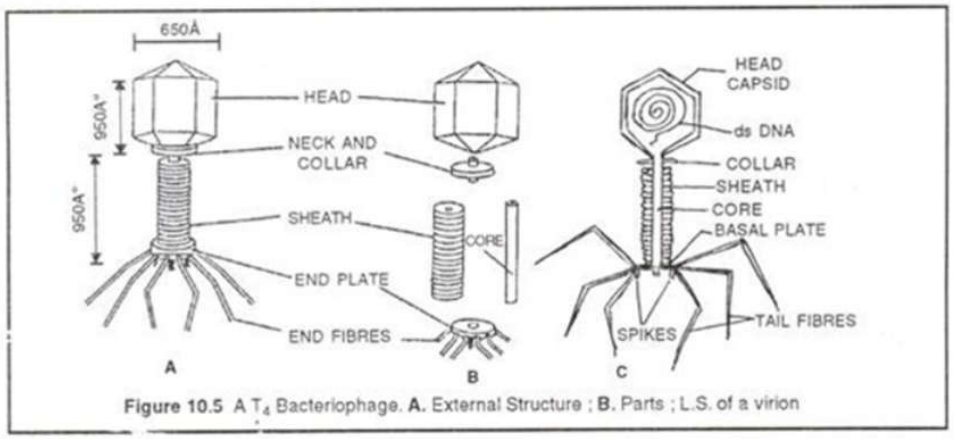
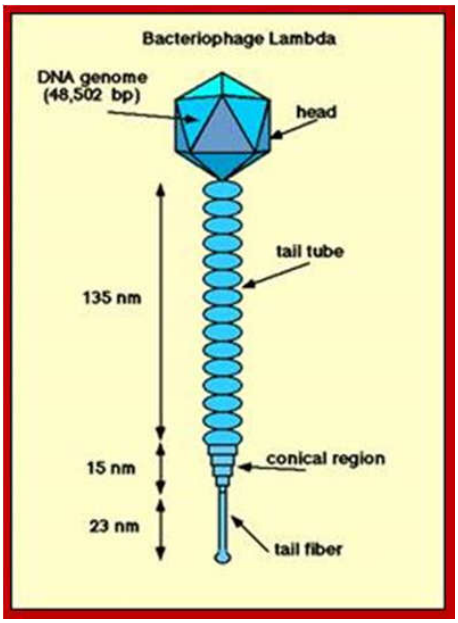
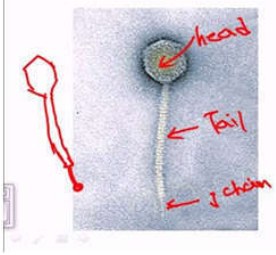


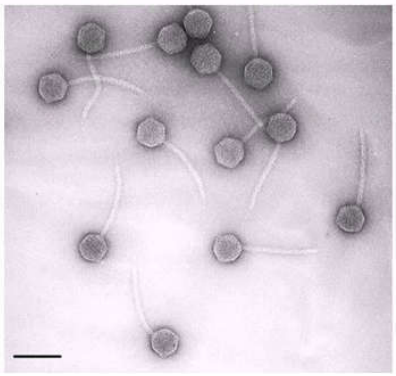
Figure 10.5 A T_4 Bacteriophage. **A.** External Structure ; **B.** Parts ; L.S. of a virion



Lambda (λ)

Family – *Siphoviridae.*
Host – K12 strain of Escherichia coli.
Structure – icosahedral head of 55 nm in diameter and a 150nm long non-contractile tail with a thin tail fiber at its end.





INTRODUCTION

- An **oncovirus** is a virus that can cause cancer. This term originated from studies of acutely transforming retroviruses in the 1950–60s, often called oncornaviruses to denote their RNA virus origin.
- Now refers to any virus with a DNA or RNA genome causing cancer and is synonymous with "tumor virus" or "cancer virus".
- The vast majority of human and animal viruses do not cause cancer, probably because of long-standing coevolution between the virus and its host.

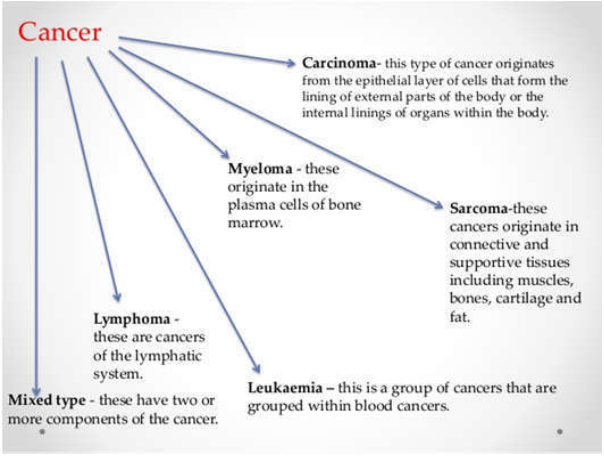
Oncogenesis & Oncogenes

- **Oncogene**
  - A gene that has the potential to convert a normal cell to a cancerous or transformed cell
- **Viral oncogene (v-*onc*)**
  - A viral gene responsible for the oncogenicity of the virus
    - Retroviruses may carry altered cellular genes that are tumor promoters
- **Proto-oncogene**
  - Cellular genes that promote the normal growth and division of cells.

Oncogenes

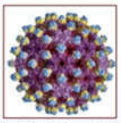
Oncogenes : Originally identified as the transforming genes of viruses
: The dorminantly acting transforming genes
: Altered forms of normal cellular genes called proto-oncogenes
Tumor suppressor genes : function normally to constrain cell growth
Proto-oncogenes and their products

- Highly conserved in evolution
- Important regulators of normal cell growth and differentiation.
- Maintain the ordered progression of the cell cycle, cell division, and differentiation
- In the cancer cell, the ordered progression is partially lost
- Mutation that alter the structure, levels, or sites of expression of the gene products activates oncogenic potential
- Extracellular cytokines, Growth factors, Transmembrane growth factor receptors, Cytoplasmic proteins that act to transmit the signal to the nucleus, Nuclear proteins that include transcription factors and proteins involved in the control of DNA replication



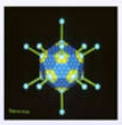
Major human Oncogenic Viruses

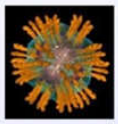
- At least 6 viruses are thought to contribute to cancers (15 – 20%):
- DNA Viruses
  - Small DNA tumor viruses
    - Human Papilloma virus (HPV)
    - SV40
    - Adenovirus
  - Herpesviruses (large)
    - Epstein Barr virus (EBV)
    - Kaposi's Sarcoma Herpesvirus (KSHV)
  - Other
    - Hepatitis virus B


Hepatitis B virus HBV


Human Papillomavirus

- RNA Viruses
  - Human T-cell Leukemia Virus 1 (HTLV1)
  - Hepatitis virus C


Adeno virus


Hepatitis virus C

- 80% of viral-associated cancers
  - Cervical cancer (HPV)
  - Liver cancer (HBV and HCV)

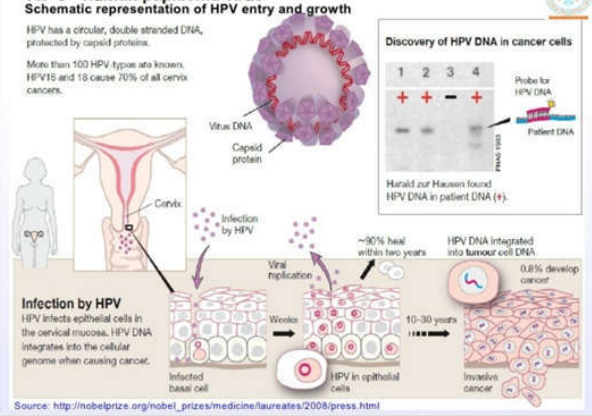
How do Viruses contribute to cancer?

- **Integrations** that cause activation or inactivation of oncogenes or tumor suppressors (e.g. RNA viruses)
- **Expression of genes** that alter key signal transduction pathways - this is our focus
- Chronic activation of **inflammatory**

Can You Guess What are these?

HPV– human papilloma virus

Schematic representation of HPV entry and growth

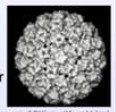


Source: http://nobelprize.org/nobel_prizes/medicine/laureates/2008/press.html

Human Papilloma Virus (HPV)

DNA TUMOR VIRUS

- Small (52-55 nm in diameter)
- Non-enveloped
- Icosahedral-shaped
- Circular dsDNA genome (~8000 bp in length)
- 100 types identified - most common are types 6 and 11
- HPV infections most common among sexually active adults and adolescents.
- There are over 100 different types of HPV's (low, medium and high-risk).
- Low-risk types cause warts or papillomas (e.g. genital warts).
- High-risk types cause cervical, vulva, vagina, anus and penis cancers (e.g. types 16 and 18).
- 10% of human cancers may be HPV-linked
- 16% of all female cancers linked to HPV
- Papilloma viruses are found in 91% of women with cervical cancer

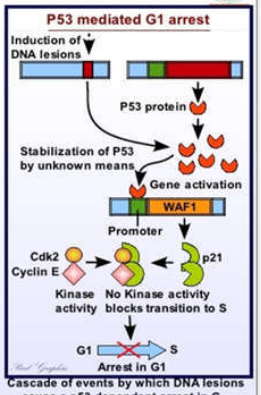


Human Papilloma virus – mechanism

- Composed of eight genes - E1, E2, E4, E5, E6, E7, L1 and L2, expressed at different times during the HPV life cycle - categorized as early or late
- HPV virion infects epithelial tissues through micro-abrasions and enters the basal epithelial cells, genome exists as 20-100 copy episome
- Viral genome then enters the cell nucleus and now the early E1 and E2 genes replicate the virus genome. E1 and E2 then build messenger RNA molecules
- As the host epithelial cells divide and differentiate, a transcription cascade takes place
- E4 and E5 genes, respectively, assist in the virus genome production
- E7 interfere with the work of the human genes Rb gene that regulate normal cell division
- The function of Rb is to accumulate important proteins like the E2F protein that are essential for cell division; if enough proteins are not accumulated, Rb prevents cell division from taking place
- E6 binds & stops p53 from repairing cell damage or causing the damaged cell to die
- Leading to continued cell division of damaged cells, and can end up causing cancer or tumors.
- The L1 and L2 genes help create viral capsid proteins required to build new viruses.
- The entire HPV life cycle depends strictly on the process of epithelial cell differentiation.

The *p53* Tumor Suppressor Gene

- **Most cancers result from mutations in several genes. A gene mutated in about 1/2 of human cancers is p53. Tumors arise when the second p53 allele is mutated, so the trait is inherited as an autosomal dominant. The p53 tumor suppressor protein (393 amino acids) is involved in many processes, including: a). Transcription b). Cell cycle control c). DNA repair and d). Apoptosis (programmed cell death).**
- **Damage to cellular DNA causes p53 to initiate the cascade of events leading to G_1 arrest.**
- **At least 17 cellular and viral proteins interact with p53. Virus proteins typically inactivate p53, allowing products needed for replication to be expressed.**



Cascade of events by which DNA lesions cause a p53 dependent arrest in G_1

BURKITT'S LYMPHOMA

- Burkitt's lymphoma (or Burkitt Lymphoma) is an uncommon type of Non-Hodgkin Lymphoma (NHL).
- Burkitt's lymphoma **commonly affects children.**
- It is a highly aggressive type of B-cell lymphoma that often **starts and involves body parts other than lymph nodes**

BURKITT LYMPHOMA

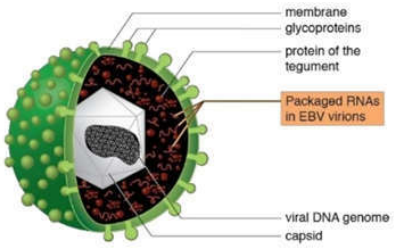
- highly aggressive NHL that has the highest cell proliferation rate among human neoplasms.

- occurs predominantly in the first decades of life
- mostly in males
- with significant affinity for gnathic bones – 50 – 70% cases
- maxilla: mandible – 2:1
- Posterior segments of the jaw
- Sometimes – 4 quadrant involvement

Jaw involvement seems to be age related:

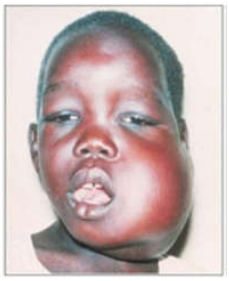
- 90% - 3 yrs old pt
- 25% - pts older than 15 yrs

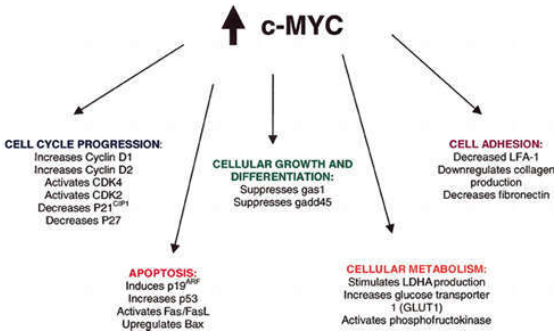
HHV 4 Ebstein Barr virus - EBV



Epstein-Barr Virus (EBV)

- 30-50 day incubation
- most cases asymptomatic
- **Burkitt's lymphoma**
  - associated with chronic co-infections with malaria
- nasopharyngeal carcinoma in Chinese & African men





AETIOLOGY OF BL

- Early EBV infection causes massive proliferation of B-cells because these cells have C3-d –like receptors for the virus.
- Anti-EBV antibodies are commonly elevated in pre-BL children.
- *P. falciparum* promotes further enhancement of B-cell proliferation by suppressing T-cells.
- T-cells are needed to eliminate EBV-infected cells.

EBV - Pathogenicity

39

Virus enters the Pharyngeal epithelial cells

Through CR 2 or CD 21 receptors present on B lymphocytes

Multiplies locally | Invades blood stream

Infects B lymphocytes

A. Latent infection – Cells transformed & Immortalised – Indefinite growth in vitro

B. Lytic infection – Cell death and release of progeny virions

Polyclonal activation – produce many types of Immunoglobulin

Ex: Heterophile sheep erythrocyte agglutination

12/17/2015

AETIOLOGY OF BL

- Chromosome 8 contains C-myc gene, an oncogene which regulates cellular proliferation.
- When the C-myc gene is translocated to Chromosomes 2, 14 or 22, it loses its capacity to regulate cellular proliferation.
- The final result is uncontrolled excessive proliferation of B-cells.

Human T-Lymphotropic Viruses (HTLV) type I and type II:

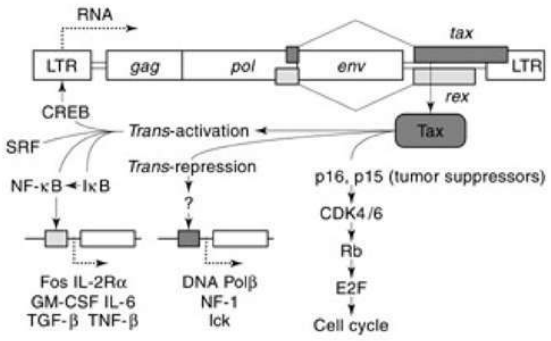
HTLV is a human RNA *Deltaretrovirus* that is known to cause **adult T-cell leukemia** and lymphoma.

The Viral genes, particularly the *tax* gene, are activating a variety of host cell genes; interleukin-2 (IL-2) and its receptors (IL-2Rα) genes.

The lymphokine activity places the infected cell in an uncontrolled autocrine mode of growth.

Virology:

- Single stranded RNA enveloped virus.
- Three major genes: **gag** (structural protein), **pol** (reverse transcripatse), and **env** (envelope glycoproteins).
- Classification: Retrovirusvirus; subfamily: *Deltaretrovirus.*



ATL cells have clonally integrated HTLV-1 proviruses. However, no common site of integration was observed among ATL patients. Therefore, the role of HTLV-1 in leukemogenesis is independent of its site of integration. In this feature, HTLV-1 differs from other chronic retroviruses of animals, in which integration was commonly adjacent to a proto-oncogene (*cis*-acting effect). As a consequence, a *trans*-acting function of HTLV-1 is postulated in leukemogenesis, and unique genes in the pX sequence have been the focus of interest.

The pX region is able to code for three proteins, $p40^{tax}$, $p27^{rex}$ and $p21^{x}$. Among these protein products, Tax was shown to immortalize human CD4-positive T cells, transform rodent fibroblasts in cooperation with *ras* oncogene, and induce mesenchymal tumors in its transgenic mice. These properties, together with the *trans*-acting functions of Tax protein, suggest that Tax may play critical roles in the development of ATL.

Tax

Tax was originally identified as a *trans*-activator of viral gene transcription as described above, and then also identified as a *trans*-activator of specific cellular genes. It was then identified as a *trans*-repressor of transcription of another set of cellular genes, and also as an inhibitor of tumor suppressor proteins and cell cycle regulators (**Fig. 4**).

Trans-activation of transcription by Tax protein requires a specific enhancer in the target gene; however, Tax itself does not bind to the enhancer directly. The mechanism includes Tax binding to enhancer binding proteins, CREB and CREM, that bind to the 21 bp enhancer of HTLV-1, NF-κB family proteins which bind to NF-κB binding site, and SRF which binds to serum responsive element (SRE). Through the binding to these enhancer binding proteins, Tax *trans*-activates transcription of the viral genome, interleukin (IL)-2Rα, IL-6, GM-CSF, TNF-α, PTHrP, major histocompatability complex (MHC) class I, c-Fos, c-Egr, and others. Abnormal expression of some of these genes obviously enhances abnormal proliferation of infected cells. Activation of IL-2Rα and PTHrP genes by Tax is a reasonable explanation for the abnormal expression of IL-2Rα chain on ATL cells and the high incidence of hypercalcemia among ATL patients, respectively. *Trans*-activation of NF-κB-directed transcription is also achieved by suppression of the inhibitors, IκBα, β and γ, in the cytoplasm. Tax binds to IκBα and induces its rapid degradation, which is normally induced by stimuli for cell growth or differentiation. Consequently, NF-κB regulation is activated by Tax at two distinct steps; one is inactivation of the inhibitor IκB in the cytoplasm and the other is activation of NF-κB protein in the nucleus.

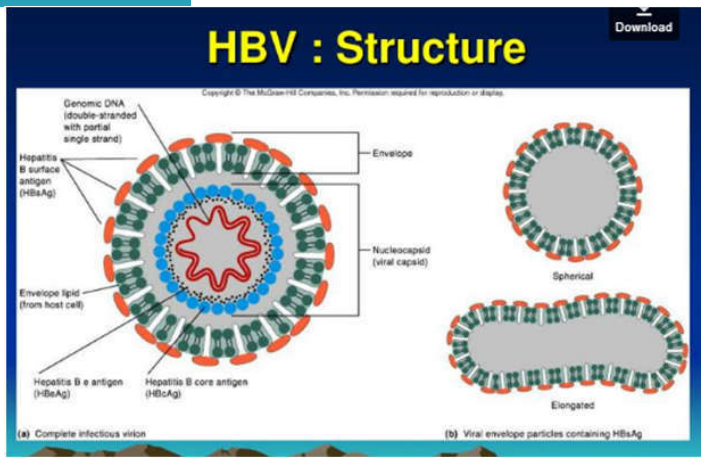
Trans-repression of transcription has been described for DNA polymerase β, NF-1 and lck. More cases will be found on other genes. The mechanism for *trans*-repression is not well characterized; however, the *trans*-repression of DNA polymerase β suggests inefficient DNA repair resulting in a high frequency of mutation. NF-1 gene is a tumor suppressor gene; thus, its suppression might contribute to induction of abnormal cell proliferation.

Inactivation of cell cycle inhibitors is also achieved by Tax; Tax binds to $p16^{ink4A}$ and $p15^{ink4B}$, which are also known to be tumor suppressor proteins, and inhibits their function. These INK family proteins inhibit CDK4 and CDK6, which phosphorylate and inactivate the negative function of Rb protein, and thus, arrest cells at the G1 phase of the cell cycle. Inactivation of these INK family proteins by binding with Tax releases cells from G1 arrest, promoting abnormal cell proliferation. Inactivation by Tax of cell cycle checkpoint protein is also reported at the G2/M phase.

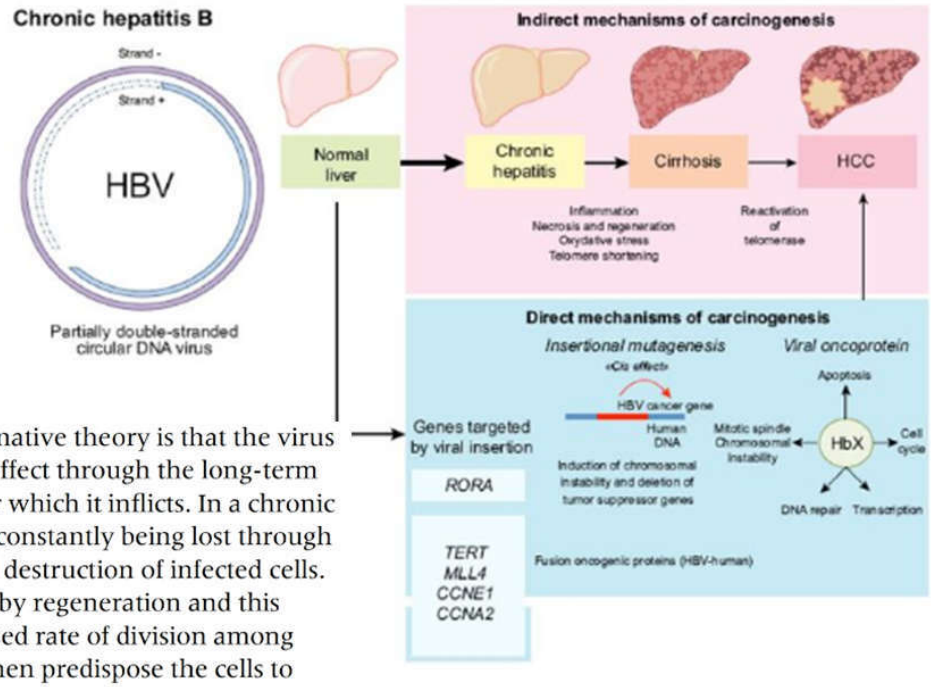
1、Properties of HBV

- a member of the hepadnavirus group
- Circular partially double-stranded DNA viruses
- Replication involves a reverse transcriptase.
- endemic in the human population and hyperendemic in many parts of the world.
- a number of variants
- It has not yet been possible to propogate the virus in cell culture

HBV : Structure



Pathophysiology of HCC in hepatitis B



Therefore, an alternative theory is that the virus exerts an indirect effect through the long-term damage to the liver which it inflicts. In a chronic infection, cells are constantly being lost through immune-mediated destruction of infected cells. The liver responds by regeneration and this persistently increased rate of division among hepatocytes may then predispose the cells to accumulate mutations, ultimately leading by chance to the emergence of a cell with a set of mutations which gives it cancerous properties.