SRINIVASAN COLLEGE OF ARTS & SCIENCE

(Affiliated to Bharathidasan University, Trichy)

PERAMBALUR – 621 212.

DEPARTMENT OF MICROBIOLOGY

Course : B.Sc Year: **II** Semester: **IV**

Course Material on:

INTRODUCTORY VIROLOGY

Sub. Code : 16SCCMB4

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Month & Year : APRIL – 2020

INTRODUCTORY VIROLOGY

OBJECTIVES

To make the student and learn about the structure, classification, morphology, pathological importance of viruses and viral diseases.

Unit I

History of virology, terminologies, origin of viruses, occurrence, morphology of viruses, helical, icosahedral and complex viruses. Viral envelope, nucleic acids, proteins, carbohydrates, classification of viruses- LHT and ICTV system of classification.

Unit II

Purification, Characterization, Separation and Assay. Cultivation and quantification of viruses, Separation and characterization of viral components.

Unit III

Bacteriophages- Life Cycle, Classification, Morphological groups, the virulent dsDNA phage, the ssDNA phage, phage lambda, Temperate and Transposable phage, Phage Mu the ssDNA phages, phage M13, Bacteriophage typing, Phage therapy (bacteriophage therapy), Cyanophages, Mycoviruses (Mycophages), Rhizobiophages.

Unit IV

General characteristics and multiplication of DNA containing viruses- Adenoviruses, Herpes viruses, Poxviruses. RNA containing viruses- Picorna virus, Rhabdo viruses, Orthomyxo viruses, Reoviridae, SARS and H1N1- Influenza A virus. Subviral agents - Viroids, Prions.

Unit V

History, Classification and nomenclature of plant viruses, Transmission, Multiplication, symptoms and control of plant viral diseases - DNA containing virus - Cauliflower mosaic virus, RNA containing virus - Tobacco mosaic virus - Poty virus, Tomato spotted wilt, Potato leaf roll virus, Rice tungro virus, Mosaic disease of sugarcane. Sub viral agents –Virusoids and Satellite virus.

UNIT 1

SOME MILESTONES IN THE HISTORY OF VIROLOGY

Date	Discoverer(s)	Discovery(ies)
1796	E. Jenner	Application of cowpox virus for vaccination against smallpox
1885	L. Pasteur	Development of rabies vaccine
1892	D. Ivanovsky, M. Beijerinck	Ultrafiltration of tobacco mosaic virus
1898	F. Loeffler, P. Frosch	Ultrafiltration of foot-and-mouth disease virus—clear proof of virus etiology of disease—discovery of the first virus
1898	G. Sanarelli	Discovery of myxoma virus
1900	W. Reed, J. Carroll, A. Agramonte, J. Lazear, C. Finlay	Discovery of yellow fever virus and its transmission by mosquitoes
1903	M. Remlinger, Riffat-Bay, A. di Vestea	Discovery of rabies virus
1907	P. Ashburn, C. Craig	Discovery of dengue viruses
1909	K. Landsteiner, E. Popper	Discovery of polioviruses
1911	P. Rous[a]	Discovery of the first tumor virus: Rous sarcoma virus
1911	J. Goldberger, J. Anderson	Discovery of measles virus
1915	F. Twort, F. d'Herelle	Discovery of bacterial viruses (bacteriophages)
1918		Beginning of global pandemic of influenza
1919	A. Löwenstein	Discovery of herpes simplex virus
1930	K. Meyer, C. Haring, B. Howitt	Discovery of Western equine encephalitis virus
1931	M. Theiler[a]	Attenuation of yellow fever virus—vaccine development
1933	C. Andrews, P. Laidlaw, W. Smith	Isolation of human influenza viruses in ferrets

Date	Discoverer(s)	Discovery(ies)
1933	R. Muckenfuss, C. Armstrong, H. McCordock, L. Webster, G. Fite	Discovery of St. Louis encephalitis virus
1934	C. Johnson, E. Goodpasture	Discovery of mumps virus
1934	M. Hayashi, S. Kasahara, R. Kawamura, T. Taniguchi	Discovery of Japanese encephalitis virus
1935	W. Stanley[a]	Purification/crystallization of tobacco mosaic virus
1936	C. Armstrong, T. Rivers, E. Traub	Discovery of lymphocytic choriomeningitis virus
1937	L. Zilber, M. Chumakov, N. Seitlenok, E. Levkovich	Discovery of tick-borne encephalitis virus (Russian spring summer encephalitis virus)
1938	B. von Borries, H. Ruska, E. Ruska	First electron micrograph of viruses (ectromelia, vaccinia viruses)
1939	E. Ellis, M. Delbrück	Development of one-step growth curve—bacteriophage
1940	K. Smithburn, T. Hughes, A. Burke, J. Paul	Discovery of West Nile virus
1941	G. Hirst	Discovery of agglutination of red blood cells by influenza virus
1945	M. Chumakov, G. Courtois, colleagues	Discovery of Crimean-Congo hemorrhagic fever virus
1948	G. Dalldorf, G. Sickles	Discovery of Coxsackieviruses
1949	J. Enders[a], T. Weller[a], F. Robbins[a]	Development of cell culture methodology for polio, measles, and other vaccines
1950	L. Florio, M. Miller, E. Mugrage	Discovery of Colorado tick fever virus
1952	R. Dulbecco, M. Vogt	Development of plaque assay for animal viruses—polioviruses, Western equine encephalitis virus
1953	W. Rowe	Discovery of human adenoviruses

Date	Discoverer(s)	Discovery(ies)
1954	J. Salk, J. Youngner, T. Francis	Development of inactivated polio vaccine
1958	J. Lederberg[a]	Discovery of genetic recombination and the organization of the genetic material of bacteria
1959	A. Sabin, H. Cox, H. Koprowski	Development of attenuated live-virus polio vaccine
1962	A. Lwoff, R. Horne, P. Tournier	Classification of the viruses based on virion characteristics
1964	M. Epstein, B. Achong, Y. Barr	Discovery of Epstein–Barr virus and its association with Burkitt's lymphoma
1965	D. Tyrrell, M. Bynoe, J. Almeida	Discovery of human coronaviruses (B814 and 229E)
1965	F. Jacob[a], A. Lwoff[a], J. Monod[a]	Discoveries of genetic control of enzymes and virus synthesis: the operon
1967	B. Blumberg[a], H. Alter, A. Prince	Discovery of Australia antigen and its link to hepatitis B
1969	M. Delbrück[a], A. Hershey[a], S. Luria[a]	Discoveries related to the replication mechanism and the genetic structure of viruses
1970	H. Temin[a], D. Baltimore[a], R. Dulbecco[a]	Discoveries related to the interaction between tumor viruses and the genetic material of the cell—reverse transcriptase
1972	A. Kapikian, colleagues	Discovery of Norwalk virus (norovirus)
1973	R. Bishop, G. Davidson, I. Holmes, T. Flewett, A. Kapikian	Discovery of human rotaviruses
1973	S. Feinstone, A. Kapikian, R. Purcell	Discovery of hepatitis A virus
1975	Y. Cossart, A. Field, A. Cant, D. Widdows	Discovery of parvovirus B-19 and its association with aplastic crisis in hemolytic anemia
1975	P. Sharp[a], L. Chow, R. Roberts[a], T. Broker	Discovery of RNA splicing and split genes (adenovirus)

Date	Discoverer(s)	Discovery(ies)
1976	D. C. Gajdusek[a]	Discovery of transmissible spongiform encephalopathies
1976	K. Johnson, P. Webb, J. Lange, F. Murphy, S. Pattyn, W. Jacob, G. Van der Groen, P. Piot, E. Bowen, G Platt, G. Lloyd, A. Baskerville, D. Simpson	Discovery of Ebola virus
1976	J. Bishop[a], H. Varmus[a]	Discovery of the cellular origin of retroviral oncogenes
1977	D. Henderson, F. Fenner, I. Arita, many others	Global eradication of smallpox
1978	D. Nathans[a], W. Arber[a], H. Smith[a]	Discovery of restriction enzymes and their application to problems of molecular genetics
1978	S. Harrison, M. Rossman, N. Olson, R. Kuhn, T. Baker, J. Hogle, M. Chow, R. Rueckert, J. Johnson	Atomic structure of viruses (tomato bushy stunt virus, polioviruses, rhinoviruses)
1980	P. Berg[a]	The development of recombinant-DNA technology
1980	R. Gallo, B. Poiesz, M. Yoshida, I. Miyoshi, Y. Hinuma	Discovery of human T lymphotropic viruses 1 and 2
1981	V. Racaniello, D. Baltimore	Development of an infectious recombinant clone of a virus (poliovirus)
1982	S. Prusiner[a]	Concept of the prion and their etiologic role in spongiform encephalopathies
1982	A. Klug[a]	Crystallographic electron microscopy and structural elucidation of biologically important nucleic acid–protein complexes
1983	F. Barré-Sinoussi[a], L. Montagnier[a], J. Chermann	Discovery of human immunodeficiency virus 1 (HIV1)

Date	Discoverer(s)	Discovery(ies)
1983	M. Balayan	Discovery of hepatitis E virus and its transmission
1985	F. Barin, F. Clavel, M. Essex, P. Kanki, F. Brun-Vézinet	Discovery of human immunodeficiency virus 2 (HIV2)
1988	G. Hitchings[a], G. Elion[a]	Discoveries of important principles for drug treatment—acyclovir
1989	M. Houghton, Q.-L. Choo, G. Kuo, D. Bradley, H. Alter	Discovery of hepatitis C virus
1993	S. Nichol, C. Peters, P. Rollin, T. Ksiazek	Discovery of Sin Nombre virus and its association with hantavirus cardiopulmonary syndrome
1994	Y. Chang, P. Moore	Discovery of human herpesvirus 8—Kaposi sarcoma herpesvirus
1995	K. Murray, P. Hooper, A. Hyatt	Discovery of Hendra virus and its reservoir host fruit bats
1996	P. Doherty[a], R. Zinkernagel[a]	Discovery of the genetic specificity of the cell-mediated immune response
1996	R. Will, J. Ironside, J. Collinge, colleagues	Discovery that bovine spongiform encephalopathy prion is the cause of variant Creutzfeldt–Jakob disease in humans
1999	K. Chua, S. Lam, W. Bellini, T. Ksiazek, B. Eaton, colleagues	Discovery of Nipah virus
1999	D. Asnis, M. Layton, W.I. Lipkin, R. Lanciotti	Extension of West Nile virus range to North America
2001	B. van den Hoogen, A. Osterhaus, colleagues	Discovery of human metapneumovirus
2003	C. Urbani, J. Peiris, S. Lai, L. Poon, G. Drosten, K. Stöhr, A. Osterhaus, T. Ksiazek, D. Erdman, C. Goldsmith, S. Zaki, J. DeRisi, others	Discovery of SARS coronavirus

Date	Discoverer(s)	Discovery(ies)
2003	B. La Scola, D. Raoult, others	Discovery of mimivirus, the largest virus known at the time
2005	J. Taubenberger, P. Palese, T. Tumpey, A. Garcia-Sastre, others	1918 influenza virus genome sequenced and the virus reconstructed
2005		Beginning of global pandemic of chikungunya
2005	E. Leroy, J. Towner, R. Swanepoel, others	Discovery that the reservoir hosts of Ebola/Marburg viruses are bats
2007	T. Allander, D. Wang, Y. Chang, others	Discovery of human polyomaviruses KI, WU, MC
2008	H. zur Hausen[a]	Discovery that human papilloma viruses cause cervical cancer
2008	B. La Scola, D. Raoult, others	Discovery of virophage, Sputnik
2010	W. Plowright and the FAO Global Rinderpest Eradication Programme	Global eradication of rinderpest
2011	B. Hoffmann, M. Beer, T. Mettenleiter, colleagues	Discovery of Schmallenberg virus
2012	A.M. Zaki, R. Fouchier, W.I. Lipkin	Discovery of MERS coronavirus
2014		Beginning of an Ebola hemorrhagic fever epidemic in West Africa, the largest ever
2015		Beginning of a global epidemic of Zika virus disease—discovery of microcephaly as consequence of *in utero* infection

Virus

Viruses are uniquely different from the many uni-cellular micro-organisms you have studied so far. Protozoa, yeasts, bacteria, mycoplasmas, rikettsiae and chlamydiae are all living organisms with the following features in common:

- They are all **cells**
- They store their **genetic information** as **DNA**
- Within their cell, they contain all the **organelles** necessary for producing energy and **synthesizing proteins, carbohydrates**, cell wall structures etc.
- Replicate by means of **binary fission**

- **Viruses do not share these properties**. They are **not cells**. They are very simple structures consisting essentially of a **nucleic acid genome**, protected by a shell of **protein**. They are metabolically inert and can only replicate once they are inside a host cell.
- The **genome** consists of only one type of nucleic acid: **either RNA or DNA**. Most DNA viruses are double stranded and most RNA viruses have a single stranded (ss) genome. A ssRNA genome may be either positive sense (this means that it can be used as mRNA to make proteins) or negative sense.
- Negative sense RNA is complimentary to mRNA, in other words, it has to be copied into mRNA. The viral genome codes only for the few proteins necessary for replication: some proteins are **non-structural**e.g. polymerase and some are **structural**, i.e. they form part of the virion structure.
- They have **no organelles**.
- They are very **small**, sizes range from 20 to 200 nm, with newly discovered viruses as large as 800nm. Most viruses are beyond the resolving power of the light microscope.

Terminology:

- **Abortive Infection**: An infection where a virus is present but is unproductive in spreading or maintaining the viral infection.
- **Acute Infection**: An active infection from a virus that may have severe symptoms or be occurring over a short period of time.
- **Arboviruses**: The type of viruses that are transmitted through vectors, such as mosquitoes, ticks, or spiders.
- **Assembly**: The gathering and replication of viruses within a cell by using the metabolism of the host organism.
- **Attachment**: The condition where the capsid proteins of the virus bind to certain receptors of the host organism.
- **Capsid**: The protein covering of a virus particle.
- **Chronic Infection**: An infection from a virus that lasts an extended period of time. Symptoms may vary in severity.
- **Complement Fixation** (CF): The combination of antigen and antibody binding to functional serum complement.
- **Envelope**: A lipid casing that surrounds the capsid that covers a virus. A viral envelope assists the virus in infiltrating the cells of the host organism.
- **Fusion Protein**: Two or more proteins that are produced by the attachment of two genes together.
- **Gene Expression**: An activity where information from a gene is made into functional gene material.
- **Genome Replication**: The reproduction of genetic material, particularly that in the structure of DNA.
- **Hemagglutination-Inhibition**: The suppression of a reaction to an antibody by red blood cells.
- **Latent Infection**: A viral infection that exists in dormancy and does not exhibit symptoms.
- **Matrix protein**: A type of protein that connects the components of the viral envelope to the nucleus of the virus.
- **Maturation**: The phase during replication at which a virus becomes infectious.
- **Molecular Epidemiology**: The study of the occurrence, spread, and control of various molecules, particularly in the areas of disease transmission.
- **mRNA**: A form of ribonucleic acid which carries copied genetic information from DNA to the cell ribosome.
- **Neutralization**: The process of rendering a specific virus ineffective by a particular viral antibody.

- **Neucleocapsid**: The composition of a virus that includes the DNA, RNA, and the capsid protein cover.
- **Penetration**: The process of the virus entering the cell of the host organism, causing infection.
- **Persistent Infection**: A situation where a virus continues to exist within a host. Symptoms may be manifested or in a state of remission, but the virus remains.
- **Peplomers =** proteins found in the envelope of the virion. They are usually glycosylated and are thus more commonly known as **glycoproteins.**
- **Polyprotein**: A protein that splits to form various polypeptides. Certain viruses produce polyproteins.
- **Receptor**: A specific type of molecule found on a cell membrane that a virus is able to attach to.
- **Release**: The process of the death of a host cell that discharges a virus.
- **Tropism**: The growth or movement of an organism in a specific direction that is provoked by an outside stimulant.
- **Uncoating**: A condition when the protein capsid of the virus is unsheathed due to enzymes of the cells of the host organism.
- **Vector**: Insects, such as mosquitoes or ticks, that carry disease from one organism to another.
- **Virions**: A virus particle, which invades the cells of a host organism, causing infection.
- **Virus Attachment Protein**: A specific protein found on a virus in charge of fixating to the receptor.
- ## Origin of Viruses:
    - Broadly speaking, only three general hypotheses of the origin of viruses are taken into consideration.

    - (i) The ancestors of viruses were at one time cellular organisms. As a result of parasitic existence in other cells, they gradually lost more and more of their own cellular machinery until they eventually became reduced to their present form.

    - (ii) The ancestors of viruses were once free-living pre-cellular forms of life, which managed to survive after the evolutionary emergence of cellular organisms only by becoming parasitic on them.

    - (iii) The viruses have not evolved from organisms, either pre-cellular or cellular, but have arisen from detached fragments of the genetic material of cellular organisms. These genetic fragments, as a result of detachment from the rest of the genetic system, acquired the ability to multiply more rapidly than the other constituents of the cell, and their unregulated growth caused disease and death of the cell.

    - Liberated after cell death, the genetic fragments were able to ensure their own perpetuation by entering adjacent healthy cells and again multiply there.

    - Originally passed from cell to cell in the form of nucleic acid, they eventually acquired the capacity to direct the simultaneous synthesis of the infected cell of a special protein, which served to enclose the nucleic acid fragments, and thus made their transfer from cell to cell a much less hazardous operation.

    - The above hypotheses have not yet been supported by factual information.

Properties of virus

- Some general properties of virus are;

1. Size:

- The size of virus ranges from (20-300) nm in diameter.
- Parvovirus is the smallest virus with size 20nm whereas Poxvirus is largest being 400nm.

2. Shape:

- The overall shape of virus varies in different groups of virus.
- Most of animal viruses are spherical shape, Pox virus is rectangular shape, TMV is rod shape, Poliovirus is bullet shape etc
- Some virus are irregular and pleomorphic in shape.

3. Symmetry:

- Morphological protein subunits of capsid are arranged together to from a symmetrical structure of the virus.
- Two basic symmetry are recognized in virus, they are helical symmetry and icosahedral symmetry.
- In some virus, symmetry is more complex, which is other than helical or icosahedral.

4. Structure and Chemical composition:

i. Genome:

- Viral genome or nucleic acid contains either DNA or RNA but not both.
- The genome can be either ds DNA or ss DNA or ds RNA or ss RNA
- The genome can exist as single piece or segmented. Eg, Influenza virus contains 8 segments of ss RNA genome.
- The genome may be linear or circular. Most virus possess linear genome except Papova virus which contains circular ss DNA.
- Genome helps replication of virus in host cell.

ii. Capsid:

- Capsid is the outer shell of a virus.
- It is chemically a viral protein.
- Capsid is composed of capsomere.
- Structure of capsid gives the symmetry of virus.
- Capsid protects the nuceic acid and also helps in attachments on host cell surface during infection.

iii. Envelope:

- Some virus contains phospholipid bilayer known as envelope.
- Virus lacking envelope is called naked virus.

- Envelope is a lipid bilayer which is acquired from host cell membrane

iv. Glycoprotein spike:

- Envelope of some virus contains viral coded spike projected outside the envelope called glycoprotein spike or peplomers.
- Glycoprotein spike are viral coded protein with carbohydrate head.
- Glycoprotein spikes is an important antigenic structure.
- Neuraminidase and Haemagglutinin are glycoprotein spikes which helps in virus attachment to cellular receptor on host cell to establish infection.

v. Enzymes:

- Some virus possess their own enzymes.
- Retrovirus possess reverse transcriptase

5. Viral replication:

- Virus only replicates inside host cell

6. Metabolism:

- Viruses are metabolically inert outside host cell.
- They are also called as obligate intracellular parasite

7. Resistance:

i. Temperature:

- Most viruses are heat labile.
- Viruses are inactivated by heating at 60°C for 30 minutes or 100°C for few seconds.

ii. Cold:

- Viruses are stable and resistant to cooling.
- Virus can be stored for long duration at -40°C to -70°C by lyophilization or freeze drying.

iii. Radiation:

- Both non-ionizing and ionizing radiation can kill virus.
- UV rays causes pyrimidine dimer formation while ionizing radiation eg, X-rays causes lethal break of viral genome.

iv. Organic solvent:

- Chloroform, ether and bile salt can destroy all viruses by lipid solubiliation.

v. Disinfectant:

- Most viruses are destroyed by oxidizing agents such as chlorine, H_2O_2, iodine etc.
- Many viruses are resistant to phenol and chlorination. The phenol and chlorine do not always inactivates enterovirus, particularly if they are present in faecal materials.

vi. Antibiotics:

- Viruses are resistant to antibiotics.
-

Virus: Structure and Symmetry

Size:

Variable. Most viruses are much smaller than bacteria. The size ranges in between 100A to 250 mu. Some viruses are larger than bacteria, for example the psittacos is a virus measuring 0.75 mu in diameter.

- Virus are very small infectious agents with size ranging from 20-300nm in diameter.
- Viruses are non-cellular entities so they are also called as particles.
- Virus lacks their own independent metabolism and cannot replicate outside the host cell. So they are also called as obligate intracellular parasites.
- Virus that infects bacteria are called bacteriophage or simply phage. Animal virus infects animals and similarly plant virus infects plants.

Structure of virus

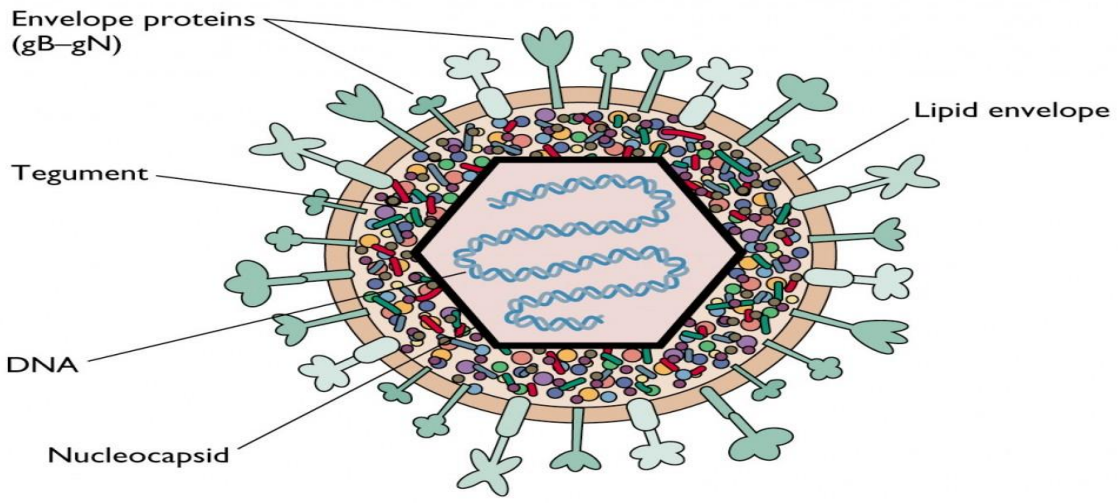


Figure: a diagrammatic sketch of an icosahedral virus

- A basic structure of virus is nucleic acid core (either DNA or RNA but not both) surrounded by protein coat.
- Central core of nucleic acid of a virus is called genome and the protein coat surrounding is called as capsid.
- In some virus, an envelope made up of glycoprotein and phospholipid bilayer is present outside the capsid.

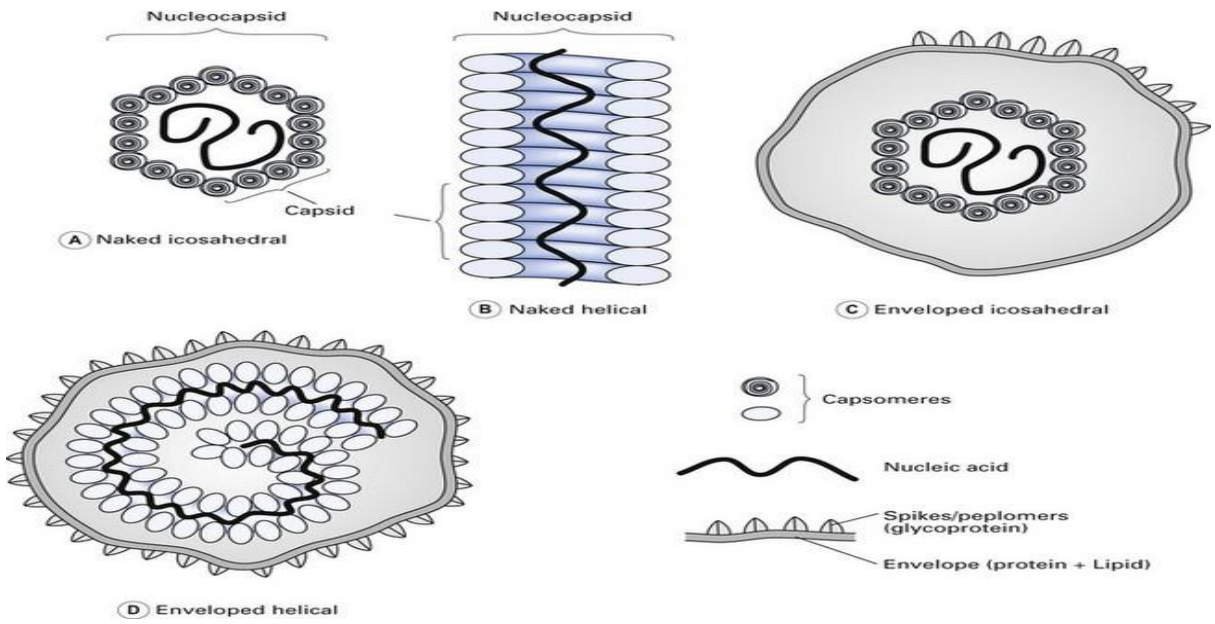
The basic structural components of a virus are;

1. Genome:
   - Virus contains either DNA or RNA as genetic material but not both. Virus which contains DNA as genetic material are called DNA virus and those containing RNA are called RNA virus.

- Unlike other living cell where ds DNA is always a genetic material, a viral genome may consists of linear or circular ds DNA, single stranded DNA, ss linear RNA or ds linear RNA.
- Examples; Reo virus is a RNA virus which contains ds RNA genome. Parvovirus contains ss DNA, Papovavirus contains ds circular DNA as genetic materials.

2. Capsid:
   - Capsid is the outer layer. Sometime it is refered as coat or shell.
   - Capsid serves as impenetrable shell around the nucleic acid core.
   - Capsid also helps to introduce viral genome into host cell during infection.
   - The protein coat or capsid is made up of number of morphological similar sub units called capsomere. Each capsomere is further composed of protomere.
   - Capsomere are arranged precisely and tightly together in a repetitive pattern to form complete capsid.
   - The number of capsomere in a capsid varies from virus to virus.
   - The complete complex of nucleic acid and protein coat of a virus particle is called as virus nucleo-capsid.
   - Structure of capsid give the symmetry to the virus. Virus particle may be either cubicl or helical or binal or complex symmetry.
3. Envelope:
   - Some virus contains envelope that surrounds nucleocapsid. The virus without envelope is called naked virus.
   - The envelope is a bilayer of lipoprotein and glycoprotein.
   - The envelope is acquired by the progeny virus from host cell during virus release by budding process.
   - In some virus the glycoprotein projects out in the form of spike called peplomere. Some of the peplomers or glycoprotein spike such as Haemaglutinin and Neuraminidase which are involved in binding of virus to host cell.
4. Enzymes:
   - Some virus contains enzymes which play central role during infection process. Eg. Some bacteriophage contains an enzyme lysozyme, which makes small hole in bacterial cell that allows viral nucleic acid to get in.
   - Some virus contains their own nucleic acid polymerase which transcribe the viral genome into mRNA during replication process. Eg. Retro virus are RNA virus that replicates inside host cell as DNA intermediate. These virus possess an RNA dependent DNA polymerase called reverse transcriptase.

Symmetry of virus



- Symmetry refers to the way in which capsomere units are arranged in viral capsid.
- Two kinds of symmetry are recognized in the viruses which corresponds to two primary shape ie. Rod and spherical shape of virus.
- Rod shaped virus have helical symmetry and spherical shaped virus have icosahedral symmetry.

i. Helical (spiral) symmetry:

- The capsomere and nucleic acid are wined together to form helical or spiral tube like structure.
- Most of the helical viruses are enveloped and all are RNA viruses.
- The typical virus with helical symmetry is tobacco mosaic virus (TMV), which is a RNA virus with 2130 identical capsomeres arranged in a helix.

ii. Icosahedral (cubical) symmetry:

- An icosahedral is a polygon with 12 vertices (corner), 20 facet (sides) and 30 edges.
- Each facet is an equilateral triange.
- Icosahedral capsid is the most stable and found in human pathogenic virus eg. Adenovirus, Picornavirus, Papovavirus, herpes virus etc.
- Icosahedral capsid are of two types;
- **Pentagon;** Pentagonal capsomere at the vertices
- **Hexagon;** Hexagonal capsomere at the vertices

iii. Complex symmetry:

- Some virus are more complex, being composed of several separate capsomere with separate shape and symmetry.
- They do not have either icosahedral or helical symmetry due to complexity of their capsid structure. Eg. Pox virus, Bacteriophage.

Binal symmetry: it is a type of complex symmerty

- Some viruses such as T-phage (T2,T4 etc) have compex symmetry including head and tail
- The most complicated virus in terms of structure are some bacteriophage which possess icosahedral head and helical tail. Such structure is called binal symmetry.

Classification of virus

A] Classification on the basis of nucleic acid

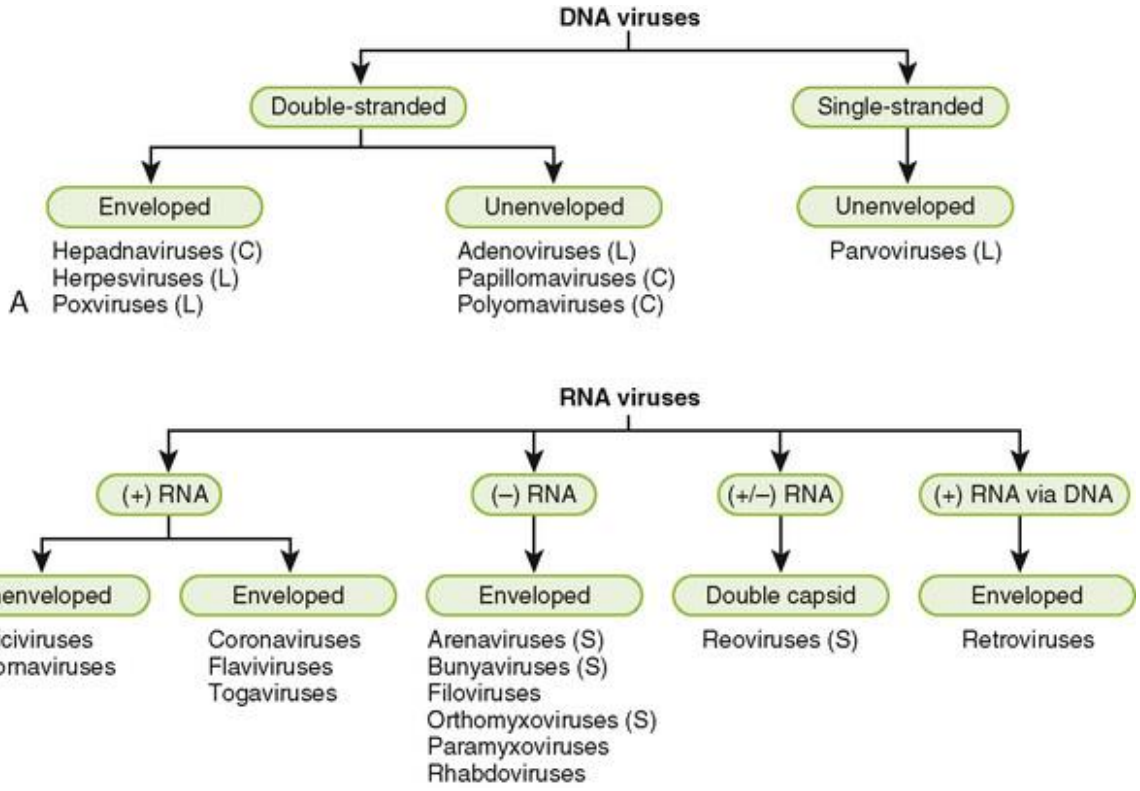
B] Classification on the basis of structure or symmetry

C] Classification on the basis of replication properties and site of replication

D] Classification on the basis of host range

E] Classification on the basis of mode of transmission

Classification of virus on the basis of nucleic acid



1. DNA virus:

- viral genome is DNA

i) Double stranded DNA virus: eg. Adenovirus, Herpesvirus

ii) Single stranded DNA virus: eg. Parvovirus, φ174 virus

2. RNA virus:

- genome is RNA

i) Double stranded RNA virus: eg. Reo virus

ii) Single stranded RNA virus: these are further classified into two groups

- Positive sense RNA (+RNA): Polio virus, Hepatitis A
- Negative sense RNA (-RNA): Rabies virus, Influenza virus

Some examples of DNA and RNA viruses:

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		

Classification of virus on the basis of structure

1. Cubical virus:

- they are also known as icosahedral symmetry virus
- Eg. Reo virus, Picorna virus

2. Spiral virus:

- they are also known as helical symmetry virus
- Eg. Paramyxovirus, orthomyxovirus

3. Radial symmetry virus:

- eg.Bacteriophage

4. Complex virus:

- eg. Pox virus

Classification of virus on the basis of replication properties and site of replication

1. Replication and assembly in cytoplasm of host:

- Eg. All RNA virus replicate and assemble in cytoplasm of host cell except Influenza virus

2. Replication in nucleus and assembly in cytoplasm of host:

- Eg. Influenza virus, Pox virus

3. Replication and assembly in nucleus of host:

- All DNA viruses replicate and assemble in nucleus of host cell except Pox virus.

4. Virus replication through ds DNA intermediate:

- Eg. All DNA virus, Retro virus and some tumor causing RNA virus replicates through ds DNA as intermediates.

5. Virus replication through ss RNA intermediate:

- Eg. All RNA virus except Reo virus and tumor causing RNA viruses.

Classification of virus on the basis of host range:

1. Bacteriophage:

- Phage are virus infecting bacteria. Eg, λ phage, T2, T4, φ174, MV-11

2. Plant virus:

- Those virus that infects plants. Eg. TMV, cauliflower mosaic virus

3. Animal virus:

- Those virus that infects animals. Eg. Polio virus, Retro virus, Herpes virus, Adeno virus

4. Insect virus:

- Virus that infects insects. Eg. Baculovirus, Sacbrood virus, Entomopox virus, Granulosis virus

Classification of virus on the basis of mode of transmission:

1. Virus transmitted through respiratory route:

- Eg, Swine flu, Rhino virus

2. Virus transmitted through faeco-oral route:

- Eg. Hepatitis A virus, Polio virus, Rota virus

3. Virus transmitted through sexual contacts:

- Eg. Retro virus

4. Virus transmitted through blood transfusion:

- Eg. Hepatitis B virus, HIV

5. Zoonotic virus:

- virus transmitted through biting of infected animals;
- Eg. Rabies virus, Alpha virus, Flavi virus

LHT System of Virus Classification

- Lwoff, Home and Tournier (1962)
- The LHT System of Virus Classification is based on chemical and physical characters like nucleic acid (DNA or RNA), symmetry (helical or icosahedral or complex), presence of envelope, diameter of capsid, number of capsomers.
- This classification was approved by the Provisional Committee on Nomenclature of Virus (PNVC) of the International Association of Microbiological Societies (1962). It is as follows:

- **Phylum Vira** (divided into 2 subphyla)
    - **Subphylum Deoxyvira** (DNA viruses)
    - **Class Deoxybinala** (dual symmetry)
    - **Order *Urovirales***
    - **Family *Phagoviridae***
        - **Class Deoxyhelica** (helical symmetry)
    - **Order *Chitovirales***
    - **Family *Poxviridae***
        - **Class Deoxycubica** (cubical symmetry)

- **Order *Peplovirales***

- **Family *Herpesviridae*** (162 capsomeres)

    - **Order *Haplovirales*** (no envelope)

- **Family *Iridoviridae*** (812 capsomeres)
- **Family *Adenoviridae*** (252 capsomeres)
- **Family *Papiloviridae*** (72 capsomeres)
- **Family *Paroviridae*** (32 capsomeres)
- **Family *Microviridae*** (12 capsomeres)

- **Subphylum Ribovira** (RNA viruses)

- **Class Ribocubica**

- **Order *Togovirales***

- **Family *Arboviridae***

    - **Order *Tymovirales***

- **Family *Napoviridae***
- **Family *Reoviridae***

    - **Class Ribohelica**

- **Order *Sagovirales***

- **Family *Stomataviridae***
- **Family *Paramyxoviridae***
- **Family *Myxoviridae***

    - **Order *Rhabdovirales***

- **Suborder *Flexiviridales***

- **Family *Mesoviridae***
- **Family *Peptoviridae***

    - **Suborder *Rigidovirales***

- **Family *Pachyviridae***
- **Family *Protoviridae***

Family *Polichoviridae*

ICTV classification

- The International Committee on Taxonomy of Viruses began to devise and implement rules for the naming and classification of viruses early in the 1970s, an effort that continues to the present.
- The ICTV is the only body charged by the International Union of Microbiological Societies with the task of developing, refining, and maintaining a universal virus taxonomy**.**
- The system shares many features with the classification system of cellular organisms, such as taxon structure. However, this system of nomenclature differs from other taxonomic codes on several points.
- A minor point is that names of orders and families are italicized, Unlike in the International Code of Nomenclature for algae, fungi, and plants and International Code of Zoological Nomenclature.
- Viral classification starts at the level of realm and continues as follows, with the taxon suffixes given in italics[]:

  Realm (-*viria*)

  Subrealm (-*vira*)

  Kingdom (-*viriae*)

  Subkingdom (-*virites*)

  Phylum (-*viricota*)

  Subphylum (-*viricotina*)

  Class (-*viricetes*)

  Subclass (-*viricetidae*)

  Order (-*virales*)

  Suborder (-*virineae*)

  Family (-*viridae*)

  Subfamily (-*virinae*)

  Genus (-*virus*)

  Subgenus (-*virus*)

  Species
- Species names often take the form of *[Disease] virus*, particularly for higher plants and animals. As of November 2018, only phylum, subphylum, class, order, suborder, family, subfamily, genus, and species are used.
- 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.
- As of 2018, one realm, four *incertae sedis* orders, 46 *incertae sedis* families, and three *incertae sedis* genera are accepted:

Realms: *Riboviria*

***Incertae sedis* orders**: *Caudovirales*, *Herpesvirales*, *Ligamenvirales*, *Ortervirales*

***Incertae sedis* families**: *Adenoviridae*, *Alphasatellitidae*, *Ampullaviridae*, *Anelloviridae*, *Ascoviridae*, *Asfarviridae*, *Bacilladnaviridae*, *Baculoviridae*, *Bicaudaviridae*, *Bidnaviridae*, *Circoviridae*, *Clavaviridae*, *Corticoviridae*, *Fuselloviridae*, *Geminiviridae*, *Genomoviridae*, *Globuloviridae*, *Guttaviridae*, *Hepadnaviridae*, *Hytrosaviridae*, *Inoviridae*, *Iridoviridae*, *Lavidaviridae*, *Marseilleviridae*, *Microviridae*, *Mimiviridae*, *Nanoviridae*, *Nimaviridae*, *Nudiviridae*, *Ovaliviridae*, *Papillomaviridae*, *Parvoviridae*, *Phycodnaviridae*, *Plasmaviridae*, *Pleolipoviridae*, *Polydnaviridae*, *Polyomaviridae*, *Portogloboviridae*, *Poxviridae*, *Smacoviridae*, *Sphaerolipoviridae*, *Spiraviridae*, *Tectiviridae*, *Tolecusatellitidae*, *Tristromaviridae*, *Turriviridae*

- ***Incertae sedis* genera**: *Dinodnavirus*, *Rhizidiovirus*, *Salterprovirus*
- Higher virus taxa span viruses with varying host ranges.
- The *Ortervirales* (Groups VI and VII), containing also retroviruses (infecting animals including humans e.g. HIV), retrotransposons (infecting invertebrate animals, plants and eukaryotic microorganisms) and caulimoviruses (infecting plants), are recent additions to the classification system orders.
- Other variations occur between the orders: *Nidovirales*, for example, are isolated for their differentiation in expressing structural and nonstructural proteins separately.

Virus replication:

- Virus are the obligate intra cellular particles, they replicate inside host cell only.
- For a specific virus to replicate within a specific host cell, certain condition must be fulfilled. Some of the criteria that are required to be fulfilled in order to viral replication are;
  - The host cell must be permissive and the virus must be compatible to host cell.
  - The host cell must not degrade the virus.
  - The viral genome must possess the information for multiplying utilizing the normal metabolism of host cell.
  - The virus must be able to use the metabolic capability of host cell to produce new progeny virus particles containing replicated copy of viral genome.
- A cell within which virus replicates is called host cell. Therefore the host may be permissive or non-permissive.
- Those host cell within which virus replicates is called permissive or compatible host cell and those within which virus cannot replicate is called non-permissive or non-compatible host cell.
- The host cell range of a virus is defined by the types of cells within which replication of that particular virus occurs.
- Some virus have broad host range and can replicates within several types of host cell whereas other virus have narrow host range.

Outcome of virus replication:

Virus replication of host cell can have three possible outcomes.

i. Productive infection:

- It occurs in permissive cell which results in viral replication within it producing progeny viruses that can infect other compatible host cells.
- The complete infectious virus produced in such infection is called virions.

ii. Abortive infection:

- It occurs in non-permissive host cell so that virus replication does not occurs or because virus replication produces viral progeny that are incapable of infecting other host cell.

iii. Restrictive infection:

- It occurs when host cell is transiently permissive so that infective viral progeny are sometime produce and other time the virus persists within cell without production of infective viral progeny.
- It results in occasional release of virus with no cell death.

Stages of virus replication:

- Although the specific detail of virus replication vary from one virus to another, general replication is same for most virus.
- The stages includes;
    - Attachment of virus to outer surface of suitable host cell; a process called Adsorption
    - Penetration of virus into host cell
    - Release of viral genome from capsid; a process called un-coating that sometime occurs simultaneously with penetration)
    - Synthesis of viral proteins
    - Synthesis of viral genome
    - Assembly of viral progeny (virion)
    - Release of progeny virus from host cell.

i. **Attachment (Adsorption):**

- This is the first step in virus infection in which interaction of virion with a specific receptor site on the surface of host cell occurs.
- The receptors sites are normal cell surface components of host cell such as protein, polysaccharides or lipoprotein-polysaccharide complex to which virus attach.
- For eg. HIV binds to CD4 cell receptor of T-lymphocytes
    - Rhinovirus binds to ICAM-1
    - Epstein Barr virus binds to C3 complement receptor.
- Each host cell contains upto 100,000 receptor sites for a given virus.
- In general viral receptor carryout normal function in cell.
- For eg. In some bacteriophage, receptor are pilli and flagella and in other virus receptor site may be transport binding protein etc.

- Receptor of influenza virus is glycoprotein found in RBC and on other cell of mucus membrane of susceptible host.

ii. **Penetration:**

After binding of virus, virus is taken up inside the cell which is referred as penetration or engulfment.

- The entry of virus into host cell may involves;
  - Transfer of only genome across cytoplasmic membrane
  - Transport of entire virus across cytoplasmic membrane by endocytosis
  - Fusion of viral envelope with cytoplasmic membrane of host cell.

iii, **Uncoating;**

- Shortly after penetration, uncoating of virus take place.
- Uncoating is defined as release of viral genome from capsid and is accessible to enzymes required to translate, transcribe and replicate it.
- The uncoating process vary from virus to virus.
- Transcription of viral genome is usually the next step in all virus except in those virus whose genome acts directly as mRNA (eg. Picorna virus).
- RNA viruses that carry minus(-) stranded RNA first transcribe their DNA to plus (+) stranded RNA that function as mRNA.
- The transcription is catalyzed by viral RNA polymerase released during uncoating.

iv. **Biosynthesis:**

- The biosynthesis process of virus replication can be divided into early event and late events.
- **Early event:**
  - In most virus, only part of nucleic acid is initially transcribed into mRNA.
  - The early mRNA codes for early proteins (enzymes) required for nucleic acid replication
  - After nucleic acid replication, many copy of progeny nucleic acids formed.
- **Late event:**
  - Late mRNA is transcribed from progeny genome.
  - Late mRNA codes for structural proteins by the process of translation. The translation process always occurs in cytoplasm of host cell, even if the mRNA synthesized in nucleus, it enter cytoplasm for translation.

v. **Assembly:**

- When critical number of various viral components have been synthesized, they assembled into mature virus.
- The assembly occurs in nucleus or cytoplasm of host cell depending upon types of virus.

- DNA virus assembled in nucleus except Poxvirus and RNA viruses assembled in cytoplasm except Influenza virus and Reo virus.

vi. **Release:**

- Release of mature virus from host cell is the final event in virus replication.
- The mechanism of virus release vary with types of virus.
- The naked viruses are generally released by cell lysis.
- The enveloped viruses are released by budding through special area of host cell membrane; during which virion acquire a portion of host cell membrane.
- In some animal and plant virus, host cells are not killed, the virus release through special channels.

For example: Replication of Herpes simplex virus

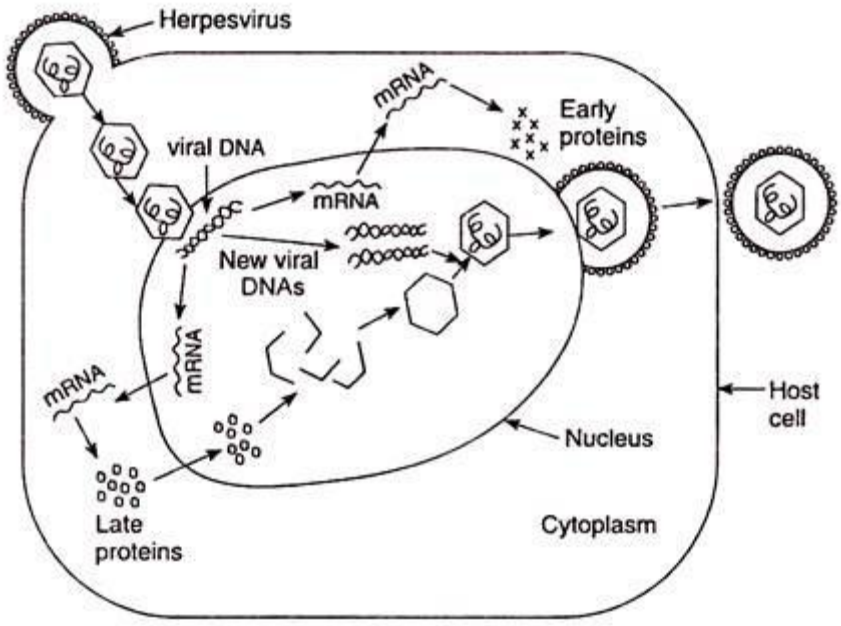


FIG. 14.6. Replication of herpesvirus genome and the formation of new viral particles.

UNIT 2

Cultivation of virus

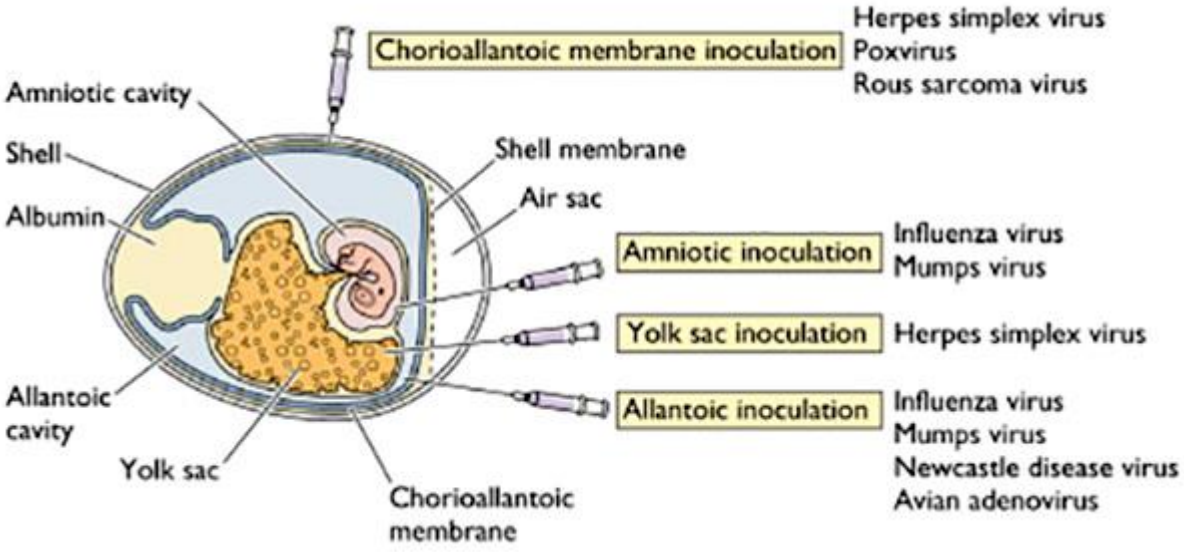
Virus lacks its independent metabolism and they can only replicates inside host cell, so viruses cannot be cultured in non-living medium as bacteria and fungi. Virus can only be cultured in embryonated egg, cell line culture and animal inoculation.

Techniques of virus cultivation

1. Animal inoculation

2. Embryonated egg culture
3. Cell culture

1. Animal inoculation:
   - Animal inoculation is one of the primary method for isolation of certain viruses and for study of pathogenesis of certain viral diseases.
   - Lab mice (white mice) particularly suckling one are animal of choice for virus cultivation. Suckling mice of age less than 48 hrs are used for culture of Toga virus and Coxsackie virus.
   - Other animals such as hamsters, Guinae pig, Chimpanzee etc are sometimes used as alternative for virus culture.
   - After inoculation of virus sample, the animals are observed for symptoms of disease till death. And finally virus is isolated from tissue of animal.
2. Embryonated egg culture:
   - For virus cultivation, an egg embryo of 7-12 days is used.
   - At first egg is kept in incubator for embryo development up to 7-12 days and then virus sample is inoculated into the egg.
   - Opening in egg should be shield with paraffin and it is incubated for sufficient time.
   - Virus can be cultured in different parts of embryonated egg, such as choriallontoic membrane, amniotic sac, allantoic cavty or yolk sac depending upon types of virus.



i. Chorioallontoic membrane (CAM):
   - Pox virus are cultured in choriallontoic membrane.
   - The growing virus produce grey-white lesions called Pocks.
   - Each Pock is developed by a single virus.
   - The number of pocks indicates the number of virus present in inoculated sample.

ii. Allantoic cavity:
   - Viruses such as Influenza virus, Mumps virus, Yellow fever virus and Rabies virus are cultivated in allantoic cavity.

- Allantoic cavity culture of virus is mainly done for vaccine preparation, to obtain large amount of virus load.

iii. Amniotic cavity:

- Influenza virus are cultured in amniotic cavity for isolation of virus from clinical sample.

iv. Yolk sac:

- Herpes virus is cultured by inoculating in yolk sac.
- This is also used for cultivation of some bacteria such as *Chlamydia* and *Rickettsia.*

3. Cell culture (tissue culture) technique:

- This technique is most commonly used technique for cultivation of virus.
- There are three types of cell culture technique.

i. Organ culture:

- Small bits of organ from human or animal is maintained in tissue culture media.
- This technique is used in specific purposes only. For eg, to culture Corona virus tracheal ring culture is done.

ii. Explant culture:

- In this small fragment of tissue is extracted from human or animal and used for virus culture.
- This technique is very rarely used.

iii. Cell line culture:

- This is the most commonly used technique.
- Cell line culture is routinely used in lab for virus culture, isolation and identification.
- In cell line culture, at first growth media is prepared by maintaining balanced salt concentration, all essential aminoacids, glucose, buffering agents, some antibiotic, serum etc.
- Some tissue fragment is obtained, it is trypsionised to dissociate cells.
- The dissociated cell are washed and suspended in culture media in a tube or petriplates and incubated for sufficient time.
- On incubation, cell divides and spread out on the glass surface to form a confluent mono-layer of cells, which is now used for virus culture.

On the basis of origin, chromosomal characteristics and number of generation through which cell culture can be maintained, cell line culture are of three types.

I. Primary cell line

II. Semi-continuous (diploid) cell line

III. Continuous cell line

I. Primary cell line:

- These are normal cells, obtained from fresh organ of animals or human and cultured.
- Once the cell attached to the surface of culture vessel, they divides by mitosis until confluent mono-layer of cells covers the surface.
- These cells are capable of limited growth for limited generation. They cannot be maintained in serial sub culture.

- These primary cell line culture is used for isolation of virus and for preparation of vaccines.
- Examples: Monkey kidney cell line, Human amnion cell line, etc

II. Semi-continuous cell line (Diploid cell):

- These cell are fibroblastic cell.
- They are diploid cell containing same number of chromosome as the parent cell.
- Fibroblastic cells are obtained from embryo tissue.
- These diploid cell can be sub cultured for limited generation.
- There is a rapid cell division and after 50 serial sub culture, they undergoes senescence.
- The diploid cell are susceptible for wide range of Human virus culture and also used for vaccine production.
- Examples: Rhesus embryo cell, human embryonic lung strain, etc

III. Continuous cell line:

- These are cells of single type capable of infinite growth in vitro.
- These are usually cancer cell derived from cancerous tissue. These cells grow faster and they are haploid cells.
- They are termed as continuous cell line as they can be serially sub culture for infinite generation without going senescence.
- Examples; **HeLa** cell is obtained from cervical cancer, **HEP-2** (Humman Epithelioma of larynx cell line), **Vero** (Vervet monkey) kidney cell lines, **BHK-21** (Baby Hamster Kidney cell line).
- Continuous cell line is maintained by serial sub culture or by deep freezing at -70C, so that these cell be reused when necessary.
- Continuous cell line is used for virus culture but it is not used for vaccine preparation because vaccine prepared by continuous cell culture are not considered safe to Human use.

DETECTION OF A VIRUS

Cytopathic effect:

- Many viruses can be detected and initially identified by observation of the morphological changes in the cultured cells in which they replicate.
- The CPE produced by different types of viruses are characteristic and help in the initial identification of virus isolates.
- Nuclear shrinking, vacuoles in the cytoplasm, syncytia formation, rounding up, and detachment are the examples ofalteration of morphology of the cells.
- Most CPEs can be demonstrated in unfixed and unstained monolayer of cells under low power of microscope.
- For example, adenoviruses produce large granular changes resembling bunches of grapes, SV-14 produces well-defined cytoplasmic vacuolation, measles virus produces syncytium formation, herpes virus produces discrete focal degeneration, and enteroviruses cause crenation of cells and degeneration of the entire cell sheet.

Hemadsorption:

- Hemadsorption is the process of adsorption of erythrocytes to the surfaces of infected cells which serves as an indirect measurement of viral protein synthesis.

- This property is made use of to detect infection with noncytocidal viruses as well as the early stage of cytocidal viruses.
- Viruses, such as influenza virus, parainfluenza virus, mumps virus, and togavirus, when infect cell lines code for the expression of red cell agglutinins, which are expressed on the infected cell membrane during infections.
- These hemagglutinins bind some erythrocytes to the infected cell surface.
- Sometimes, viruses can be detected by agglutination of erythrocytes in the culture medium.

Heterologous interference:

- This property is used to detect viruses that do not produce classic CPEs in the cell lines.
- In this method, the growth of non-CPE-producing virus in cell culture can be tested by subsequent challenge with a virus known to produce CPEs.
- The growth of the first virus will inhibit infection by the cytopathic challenge virus by interference.
- For example, rubella virus usually does not produce any CPE, but prevents the replication of picornaviruses, which is inoculated as a cytopathic challenge virus.

Transformation:

- Oncogenic viruses that are associated with formation of tumors induce cell transformation and loss of contact inhibition in the infected cell lines.
- This leads to surface growth that appears in a piled-up fashion producing microtumors.
- Examples of such oncogenic viruses that produce transformation in cell lines are some herpes viruses, adenoviruses, hepadanoviruses, papovavirus, and retroviruses.

Light microscopy:

- Viral antigens in infected cell cultures are demonstrated by staining virus-infected cells of tissue sections with specific viral antibody conjugated with horseradish peroxidase.
- This is followed by addition of hydrogen peroxide along with a benzidine derivative substance.
- In a positive reaction, a red insoluble precipitate is deposited on the cell line, which is demonstrated by examination under ordinary light microscope.

Immunofluorescence:

- Direct immunofluorescence using specific antibodies is frequently used to detect viral antigens in inoculated cell lines for identification of viruses.

Electron microscopy:

- The viruses can also be demonstrated in infected cell lines by EM.

PURIFICATION OF VIRUS IN PLANTS

The following points highlight the nine main steps involved in the purification of virus in plants. The steps are:

1. Virus Propagation in a Suitable Host Plant
2. Selection of Infected Part of the Plant
3. Factors
4. Extraction of Virus Using a Suitable Buffer
5. Infectivity Test
6. Criteria for Purity of Virus
7. Virus Yield
8. Storage of Purified Virus
9. Uses of Purified Virus.

Step # 1. Virus Propagation in a Suitable Host Plant:
A large amount of infected plant material is needed to purify the virus. It can be achieved by inoculation of a water or buffer extract of the infected plant to a number of suitable hosts or to the same healthy host. If a virus cannot be transmitted by mechanical means, it can also be transmitted by grafting or by suitable vector to a healthy host for multiplication only those suitable plants as hosts are selected which are free from the chemicals which inhibit infectivity of virus (Table 1) for e.g., cow pea, Petunia hybrida, Chenopodium and certain tobacco varieties.

Step # 2. Selection of Infected Part of the Plant:
It is also essential because mid rib and petioles contain low concentration of the virus and should be separated before use for virus extraction. Young leaves should be preferred to older leaves because inhibitory materials that adsorb or adhere to the virus are frequently low in concentration in young leaves. Plant parts to be used for virus isolation should not the contaminated by another virus or strain of the same virus. Roots of pea plant are best to extract clover yellow mosaic virus (C1YMV).

Step # 3. Factors:
Certain climatic factors like temperature, light intensity and its duration, host nutrition and time after inoculation affect the virus concentration in the host plant. Some virus attain peak concentration in about 12 days after inoculation, rapidly decreased in the next four days and reaches at very low concentration by 48 days.

So, the conditions for growth of the infected plants and time of harvest should be standardized for each virus to maximize the virus yield.

Step # 4. Extraction of Virus Using a Suitable Buffer:
An extraction medium must enable extraction of maximum amount of virus from the infected plant material, keep the virus in stable, infective, un-aggregation condition and minimize host contaminants. A buffer of suitable pH and molarity with additives to prevent oxidation and avoid co-precipitation other materials with the virus, yet retain virus infectivity is chosen by trial and error based on the knowledge of the methods used for other viruses.

Phosphate acetate and borate buffers are commonly used at different pH and molarity. Some virus need a cation like Ca^{2+}or Mg^{2+} to preserve their infectivity, besides the ionic strength of the buffer. Mortar pestle, food blenders, meat mincer and the electrically operated glass mortar and pestle are useful to homogenize the virus infected plant material in a suitable buffer.
All extractions of virus are done in cold conditions at 4°C or using salt-ice bath around mortar to prevent oxidation reactions. Cell components (Ribosomes, RuBP carboxylase protein from chloroplast, fragments of lower molecular weight compounds should be removed in the extraction process, leaving the infective virus in the solution.

It can be achieved by the following methods:
Heating:
Heating the extract for a few minutes at 50°-60° helps to coagulate the unwanted materials. It is very good method to extract stable virus TMV.

Centrifugation:

Centrifugation at different speeds separates virus particles and host components of different densities. Low speed 500-10,000 rpm centrifugation, is used in the initial stages of clarifying the crude infective plant extract to sediment the gross host material. High speed centrifugation at speeds of 30,000 rpm is used at later stages to get relatively pure virus, devoid at most of the host components.

Crystallization or Salt precipitation:
Ammonium sulphate to one third saturation of the crude extract is used to precipitate TMV, which can be suspended in suitable buffer. (Stanley, 1935).

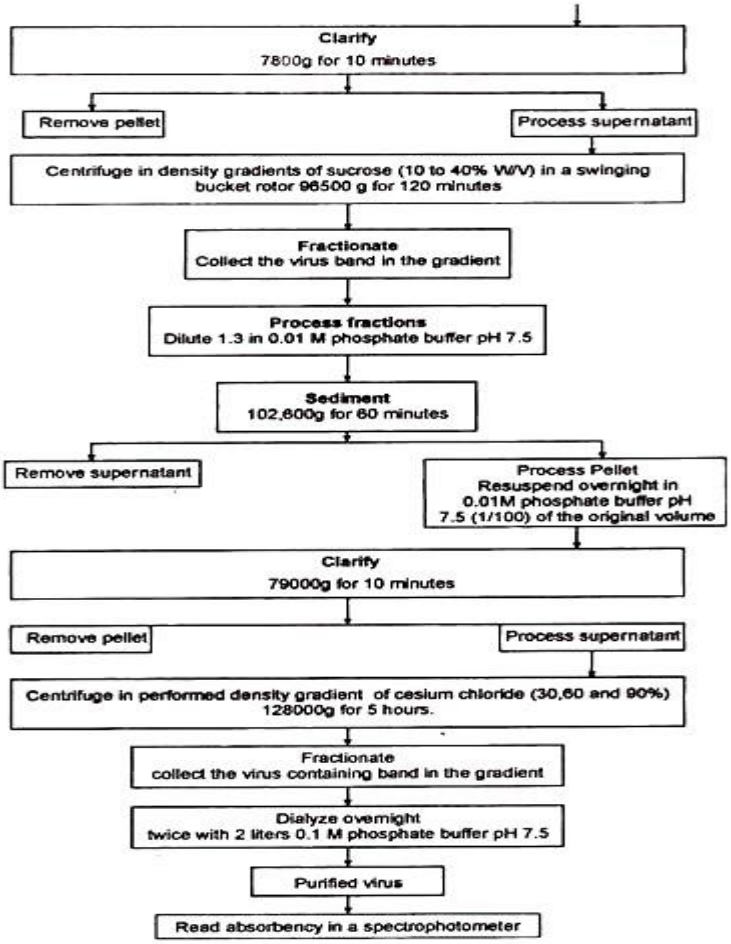
Precipitation at Isoelectric Point:
Virus which are nucleoproteins are precipitated at specific pH. The precipitate is collected by low speed centrifugation and re-suspended in suitable buffer.

Coacervation:
It involves separation of macromolecules in two liquid layers, one of which is rich in colloid. A virus separates into one of the two layers.

Gel Filtration:
Sephadex, agar and agarose remove smaller host components by adsorption.

Density Gradient Electrophoresis:
Virus components can be separated by layering the preparation on a suitable buffered density gradient formed in a U-tube and applying electrical charge.

Step # 5. Infectivity Test:
Infectivity of the purified mechanically transmissible virus can be checked by inoculating to a suitable host.



Step # 6. Criteria for Purity of Virus:

Criteria for purity of a virus as a single entity may be by specific characteristic of that virus in a specific test plant, serological tests, UV spectrophotometric absorption data, gel electrophoresis, PCR analysis and molecular characterization.

Step # 7. Virus Yield:

The total weight of purified virus is called virus yield. It varies with the virus and plant used. It ranges from 0.05 ug/g leaf with Barley yellow Dwarf-virus (BYDV) to 2000 µg TMV/g tobacco.

Step # 8. Storage of Purified Virus:

Extracted or purified virus is stored in small quantities in vials with equal volume of glycerol and 3 or 4 crystals of thymol or sodium azide (to prevent microbial growth) at 4°C or frozen.

Step # 9. Uses of Purified Virus:

Purified virus free from contaminants is required to determine its structure, biochemical and molecular composition in relation to function and development of probable practicable control or management measures for the disease by various methods, including manipulation of parts of its genome.

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.

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.

B. 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.

C. $CsCl_2$ 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:

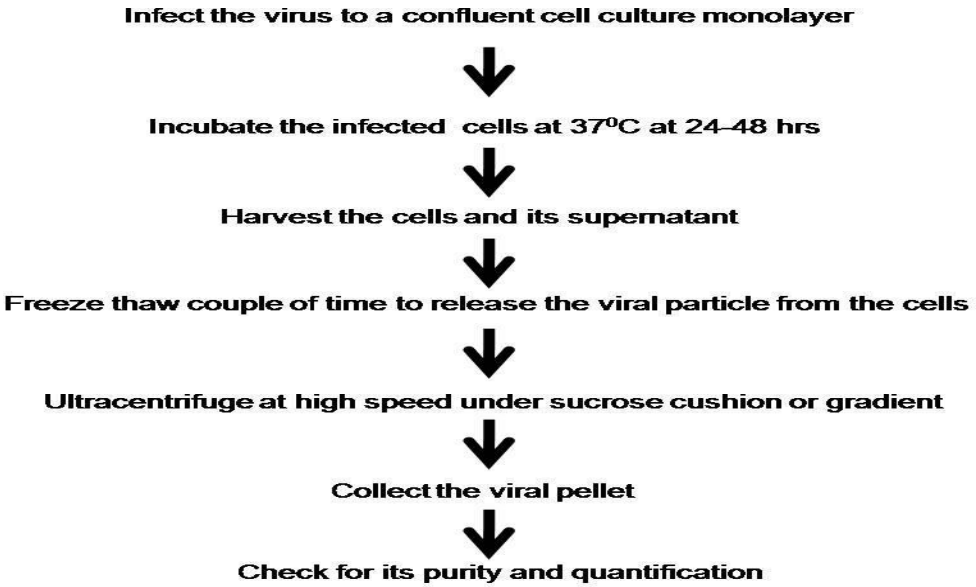
Viruses can also be separated by electrophoresis and column chromatography but these are not the preferred way to separate virus while sometimes they are used to separate viral nucleic acids or proteins. Both the methods separate the virus on the basis of charge and/or size. Virus contains a variety 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 6.2. A general approach for purifying a virus from tissue culture cells



UNIT 3

BACTERIOPHAGES

Introduction

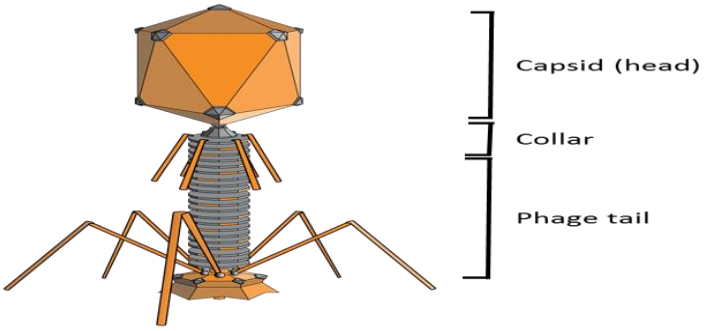
Even bacteria can get a virus! The viruses that infect bacteria are called **bacteriophages**, and certain bacteriophages have been studied in detail in the lab (making them some of the viruses we understand best).

two different cycles that bacteriophages may use to infect their bacterial hosts:

- The **lytic cycle**: The phage infects a bacterium, hijacks the bacterium to make lots of phages, and then kills the cell by making it explode (*lyse*).
- The **lysogenic cycle**: The phage infects a bacterium and inserts its DNA into the bacterial chromosome, allowing the phage DNA (now called a **prophage**) to be copied and passed on along with the cell's own DNA..

Phage structure

Bacteriophage structures are diverse, but the vast majority of characterised phage share some common characteristics. Many phage have an icosahedral, head structure made of repeat protein subunits known as the capsid. This head structure contains the viral genome. The primary difference in phage are the presence or absence of a 'tail' structure.



Above - a diagrammatic representation of the structure of phage λ (lambda) which was the first and phage discovered, and perhaps the best characterised in the modern day.

Bacteriophage infections

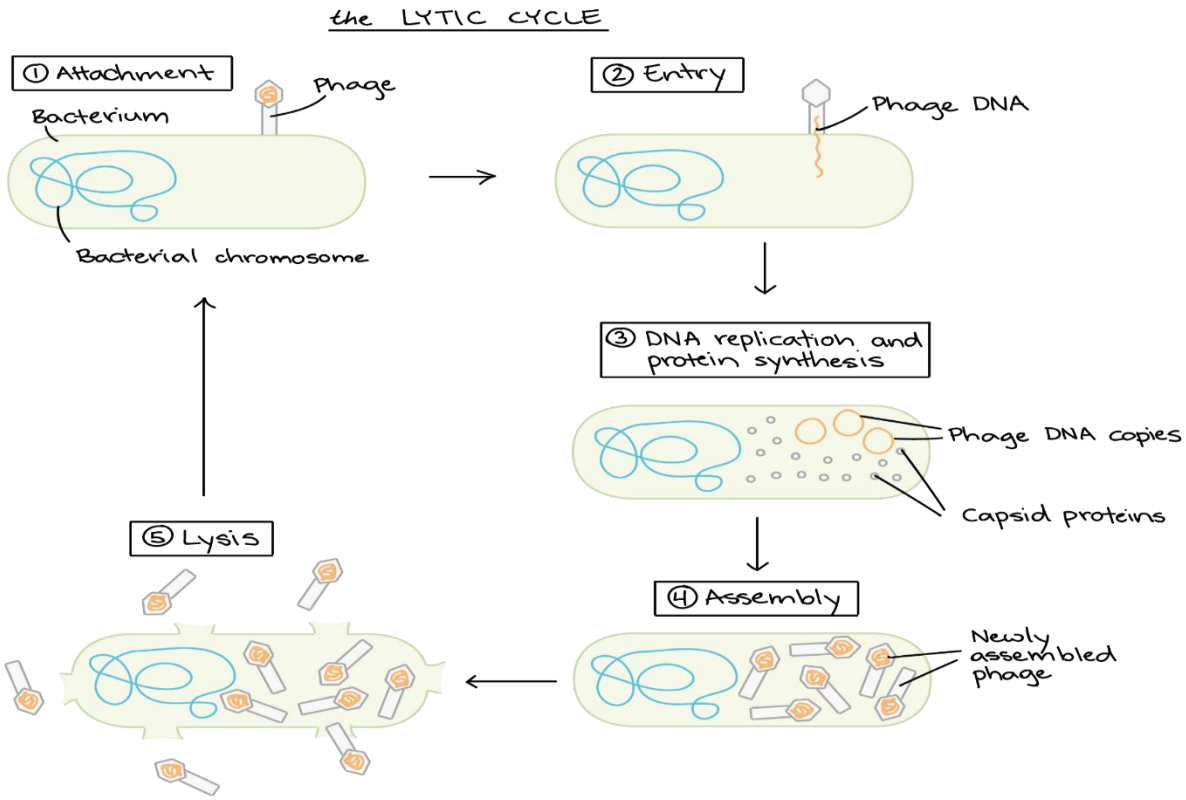
Bacteriophages, just like other viruses, must infect a host cell in order to reproduce. The steps that make up the infection process are collectively called the **lifecycle** of the phage.

Some phages can only reproduce via a lytic lifecycle, in which they burst and kill their host cells. Other phages can alternate between a lytic lifecycle and a lysogenic lifecycle, in which they don't kill the host cell (and are instead copied along with the host DNA each time the cell divides).

Let's take closer look at these two cycles. As an example, we'll use a phage called lambda (\lambdaλlambda), which infects *E. coli* bacteria and can switch between the lytic and lysogenic cycles.

Lytic cycle

In the **lytic cycle**, a phage acts like a typical virus: it hijacks its host cell and uses the cell's resources to make lots of new phages, causing the cell to **lyse**(burst) and die in the process.



1. **Attachment**: Proteins in the "tail" of the phage bind to a specific receptor

The stages of the lytic cycle are:

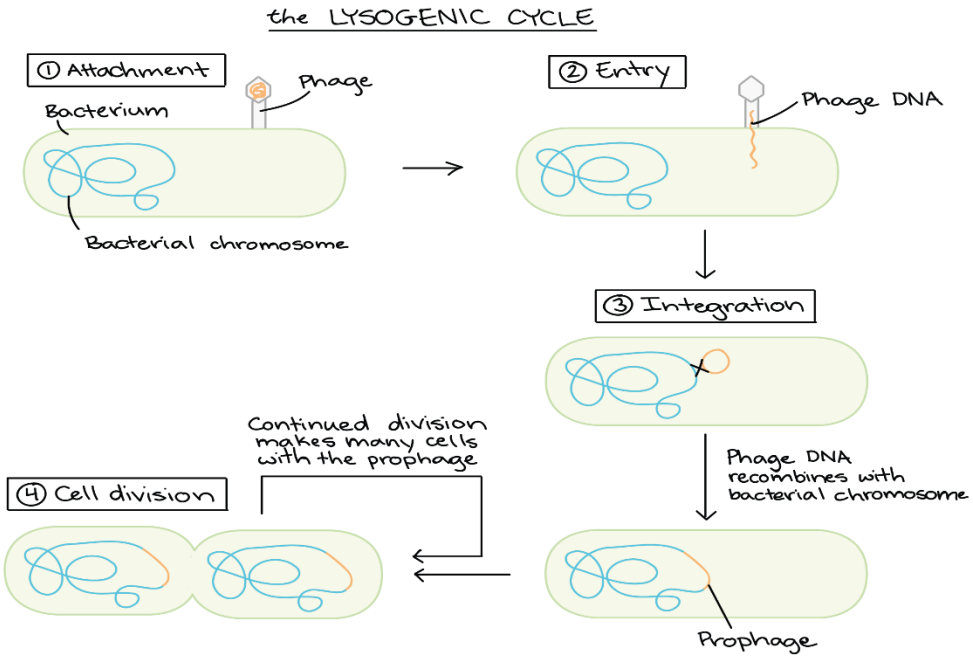
1. **Attachment**: Proteins in the "tail" of the phage bind to a specific receptor (in this case, a sugar transporter) on the surface of the bacterial cell.
2. **Entry**: The phage injects its double-stranded DNA genome into the cytoplasm of the bacterium.
3. **DNA copying and protein synthesis**: Phage DNA is copied, and phage genes are expressed to make proteins, such as capsid proteins.
4. **Assembly of new phage**: Capsids assemble from the capsid proteins and are stuffed with DNA to make lots of new phage particles.
5. **Lysis**: Late in the lytic cycle, the phage expresses genes for proteins that poke holes in the plasma membrane and cell wall. The holes let water flow in, making the cell expand and burst like an overfilled water balloon.
   Cell bursting, or **lysis**, releases hundreds of new phages, which can find and infect other host cells nearby. In this way, a few cycles of lytic infection can let the phage spread like wildfire through a bacterial population.

Lysogenic cycle

The **lysogenic cycle** allows a phage to reproduce without killing its host. Some phages can only use the lytic cycle, but the phage we are following, lambda (\lambdaλlambda), can switch between the two cycles.

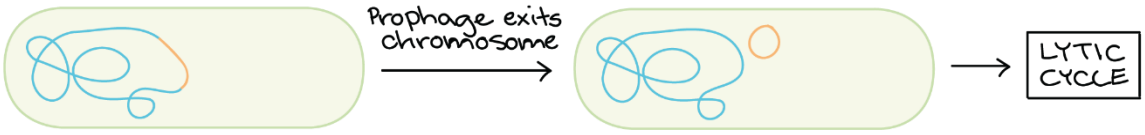
In the lysogenic cycle, the first two steps (attachment and DNA injection) occur just as they do for the lytic cycle. However, once the phage DNA is inside the cell, it is not immediately

copied or expressed to make proteins. Instead, it recombines with a particular region of the bacterial chromosome. This causes the phage DNA to be integrated into the chromosome.



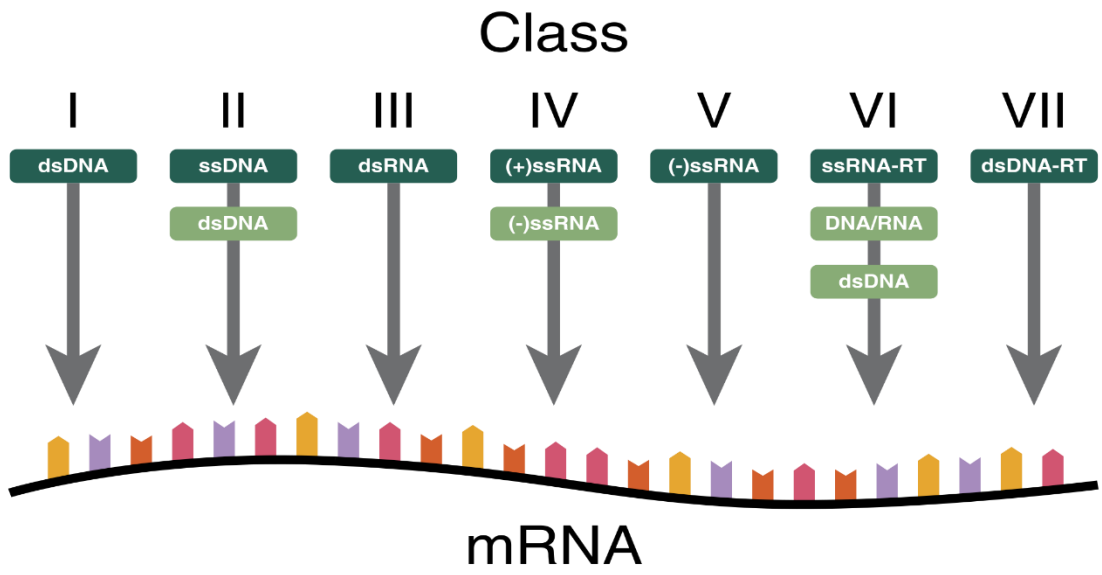
The integrated phage DNA, called a **prophage**, is not active: its genes aren't expressed, and it doesn't drive production of new phages. However, each time a host cell divides, the prophage is copied along with the host DNA, getting a free ride. The lysogenic cycle is less flashy (and less gory) than the lytic cycle, but at the end of the day, it's just another way for the phage to reproduce.

Under the right conditions, the prophage can become active and come back out of the bacterial chromosome, triggering the remaining steps of the lytic cycle (DNA copying and protein synthesis, phage assembly, and lysis).



1. Prophage exits chromosome and becomes its own circularized DNA molecule.

Classification of Bacteriophages:



The balitmore scheme for viral classification. Viruses within the Baltimore scheme are grouped based on the composition of their genomes, and their method for genome replication.

Bacteriophage are viruses that specifically infect bacterial cells. Bacteriophage, even within similar Baltimore taxa are extremely varied, with wide diversity in both their genomic and coat structures.

On the basis of presence of single or double strands of genetic material, the bacteriophages are categorized as under:

1. The ssDNA Bacteriophages:

(i) Icosahedral phages = φx 174, St-1, φR, BR2, 6SR U3 and G series, e.g., G4, G6, G13, G16. All are like φx 174.

(ii) Helical (filamentous)

(a) The Ft group: They are F specific phages and absorb to the tip of F type sex pilus e g E. coli phages (fd, fl, M13).

(b) If group: They are absorbed to I-type sex pilus specified by R factors, e.g.. If_1,IF_2, etc.

(c) The third group is specific to strains carrying RF_1 sex factor.

2. The dsDNA Phages:

Following are the examples of dsDNA phages:

(i) T-odd phage of E. coli, e.g., T1, T3, T5, T7.

(ii) T-even phage of E. coli, e.g., T2, T4, T6.

(iii) The other E. coli phages, e.g., P1, P2, Mu, φ80.

(iv) The phages of Bacillus subtilis, e.g., PBS 1, PBSX, SPO1, SPO2.

(v) The phage of Shigella a, e.g., P2.

(vi) The phage of Salmonella; e.g., PI, P22.

(vii) The phage of Haemophilus, e.g., HP1.

(viii) The phage of Pseudomonas, e.g., PM2.

3. The ssRNA phages.

Examples of the ssRNA bacteriophages are as below:

(i) Group I:

E. coli. phages such as f2, MS2, M12, R17, fr, etc.

(ii) Group II:

The Qβ phages.

4. The dsRNA phages.

Example:

The φ6 bacteriophage.

Morphological Groups of Bacteriophages:

On the basis of EM studies, Bradley (1967) has described the following six morphological types of bacteriophages.

Type A:

This type of virus has hexagonal head, a rigid tail with contractile sheath and tail fibers dsRNA, T-even (T2, T4, T6) phages.

Type B:

This type of phage contains a hexagonal head but lacks contractile sheath. Its tail is flexible and may or may have tail fiber, for example dsDNA phages, e.g., T1, T5 phages.

Type C:

Type C characterized by a hexagonal head and a tail shorter than head. Tail lacks contractile sheath and may or may not have tail fiber, for example dsDNA phages, e.g., T3, T7.

Type D:

Type D contains a head which is made up of capsomers but lacks tail, for example ssDNA phages (e.g., φX174).

Type E:

This type consists of a head made up of small capsomers but contains no tail, for example ssRNA phages (e.g., F2, MS2).

Type F:

Type F is a filamentous phage, for example ssDNA phages (e.g., fd, f1).

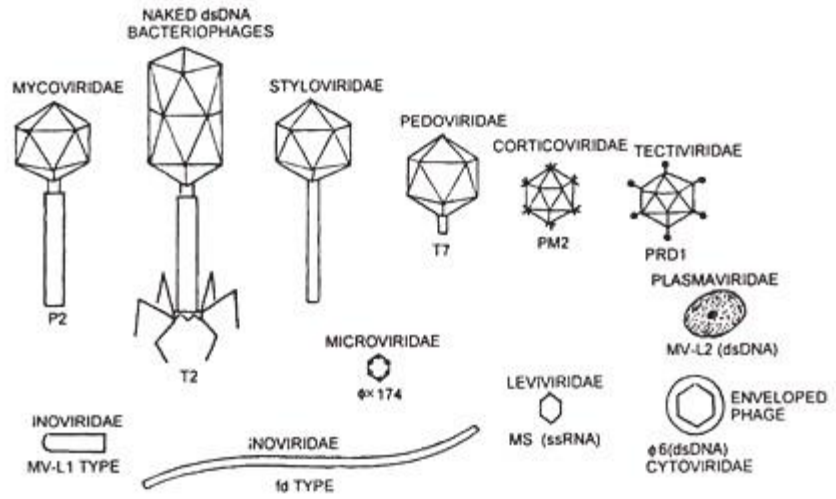


FIG. 13.1. Families of bactenophages (diagrammatic).

Further a group G is recently discovered which has a lipid containing envelope and has no detectable capsid, for example a dsRNA phage, MV-L2.

(i) T-Even Phages (dsRNA Virulent Phages):

The T-even phages (T_2, T_4, T_6) are homologous and much of our knowledge about bacteriophages is based on them, particularly T_4 phage.

1. Structure:

The T-even phage (Fig. 13.2) is characterized by the presence of a hexagonal head about 900 Å wide. It consists of dsDNA molecule protected by a protein coat made up of numerous facets. The DNA molecule, measuring about 52,000 Å in length, is coiled and packed inside the head. The head is attached with a cylindrical tail consisting of a hollow core surrounded by protein sheath.

The hollow central core measures about 80-100 Å in diameter and is considered continuous from the head to the end of the tail forming a channel through which the nucleic acid moves into invade the host cell being infected. The protein sheath is spirally coiled and is connected to a thin disc-like structure called ‘collar’ at the base of the head and to a hexagonal ‘end plate’ at the end of the tail.

The protein sheath of the tail is capable of contracting in the longitudinal direction. At the six corners of the hexagonal plate there are small ‘spikes’ to which very long fibers called ‘tail-fibres’ are connected. The tail fibres are the organs of attachment to the wall of the bacterial cell.

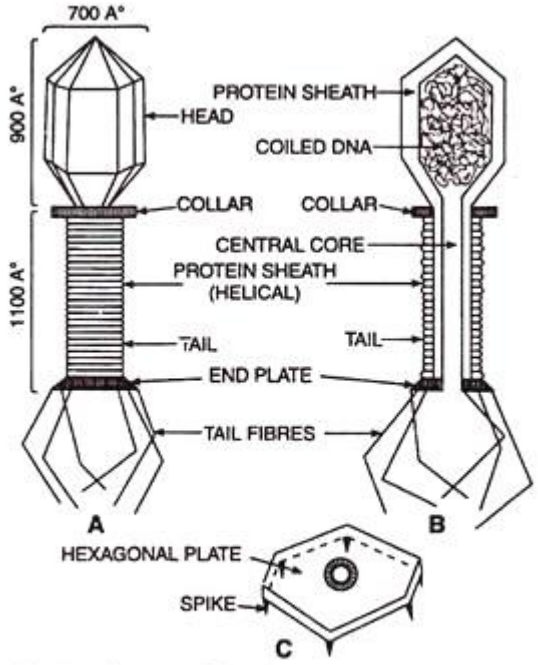


FIG. 13.2. Structure of T-even bacteriophage (diagrammatic). **A.** external structure, **B.** internal structure, and **C.** end plate (enlarged).

2. Life-Cycle (Multiplication or Infection Cycle):
The infection cycle (Fig. 13.3) of T-even bacteriophage lasts about 20 minutes, culminating in lysis (bursting-open) of the host cell, E. coli.

The whole process can be classified into:
(i) Adsorption or infection,

(ii) Penetration or injection,

(iii) The eclipse or the latent period

(iv) Maturation and

(v) Lysis or release.

(i) Adsorption or infection:
Attachment of the virus particle onto the surface of the host cell is adsorption or infection. The virus particles possess one or more proteins on the outside that interact with cell surface components called receptors; the receptors are normal surface components of the host (e.g., proteins, carbohydrates, glycoproteins, lipids, lipoproteins, etc.).

Infect, these are the receptors that determine which cells will be susceptible-to infection. In the absence of the receptor site, the virus cannot adsorb and hence cannot infect. If the receptor site is altered, the host may become resistant to virus infection.

(ii) Penetration or Injection:

This process is very interesting and has been studied and beautifully elucidated by B, Kellenberger. After the tail fibres get adsorbed, an ‘enzyme-system’ is supposed to make a pore’ or ‘hole’ in the cell wall of the host.

It is believed that the enzyme-system consists of a ‘phage- lysozyme’, which is synthesized during the multiplication of the parent phage inside the host cell and its molecules remain attached to the extreme tip of the tail-fibres of the new progeny phages.

This enzyme-system becomes active when the released phage particle infects the new host cell. However, the tail-fibres attached on the surface of the host cell bend to bring the end-plate in contact with the cell wall surface.

Now, the protein sheath of the tail longitudinally contracts pushing the central tubular core through the pore inside the wall of the host cell and the phage DNA molecule is released or injected into the cytoplasm. After the DNA is released, the empty protein coat becomes of no use.

(iii) The Eclipse or the Latent Period:
When the DNA molecule is released in the host cytoplasm, it is not degraded by the nuclease enzymes of the host cell. It has been studied, particularly in T_4 phage, that the phage DNA contains glucosylated hydroxymethyl cytosine instead of cytosine, which prevents the nucleases of the bacterium from degrading the phage DNA.
The phage DNA. first makes the host cell immune against infection by genetically similar phage particles.

Secondly, it immediately takes over the charge of the cell machinery and suppresses all cellular activities such as synthesis of cellular DNA, RNA, proteins, etc. This is the parasitism of a virus at the genetic level. This suppression is short lived and the cell machinery of protein synthesis starts functioning under the control of viral DNA in the place of cellular-DNA.

New messenger-RNA molecules are synthesized very rapidly and a series of new enzymes, namely, ‘early proteins’ is synthesized. Some of the early proteins are used as enzymes for the viral DNA synthesis.

The newly synthesized viral DNA molecules direct the formation of new type of proteins, namely, ‘late proteins’. Majority of the late proteins are viral coat proteins, whereas some are phage lysozyme. The viral coat proteins constitute the sheath of the phage and the phage-lysozyme later help in the injection process.

(iv) Maturation:
Assembly of the various components to constitute a new phage particle within the host cell is called ‘maturation’. Head and tail formation start separately, the protein components aggregate around the DNA and form the head of the phage.

End-plate is formed first followed by the formation of tubular core. 1 ail fibres are formed later. Hundreds (about 200) of new phage particles are produced from each bacterium by the time of lysis.

(v) Lysis or Release:

After the production of new bacteriophages, the host bacterial cell bursts open and the phage particles are released (Fig. 13.3(J)). Bursting open of the host bacterial cell is called 'lysis'.

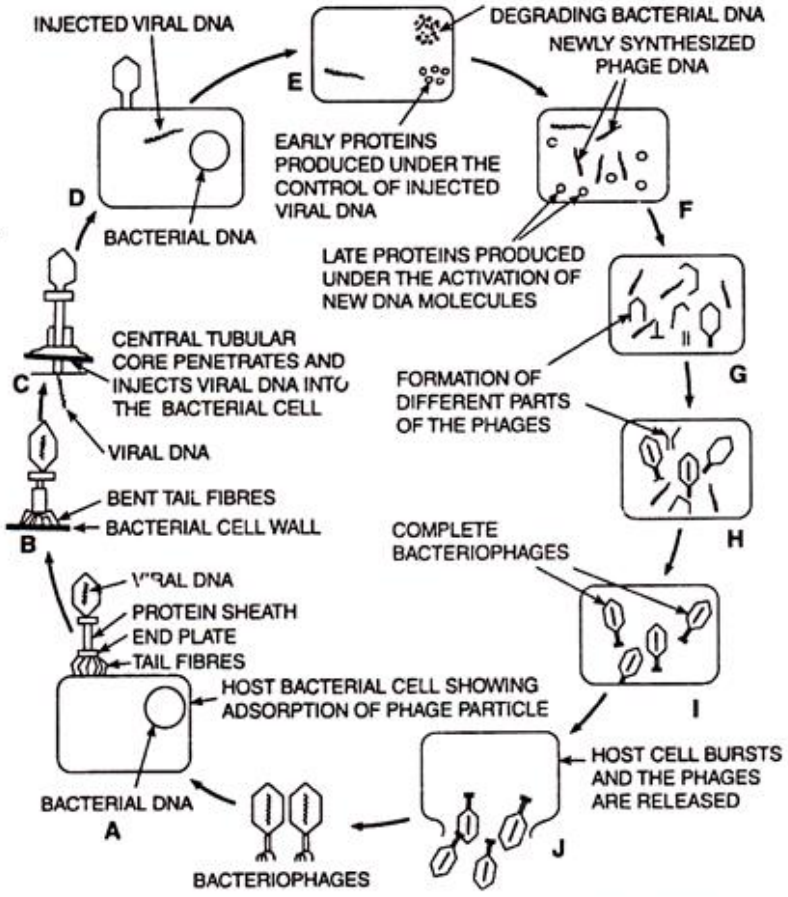


FIG. 13.3. Lytic life cycle of T-even bacteriophage. **A.** adsorption; **B, C, D.** penetration or infection; **E & F.** eclipse or latent stage; **G, H, I** maturation; **J.** lysis.

(ii) Lambda (λ) Phage (dsDNA Temperate Phages):

1. Structure:

Morphological structure of phage λ, which infects E. coli K12, is given in Fig 13.4. The phage λ contains double stranded (ds) circular DNA of about 17 µm in length packed in protein head of capsid. The head is icosahedral, 55 nm in diameter consisting of 300-600 capsomers (subunits) of 37,500 dalton. The capsomers are arranged in clusters of 5 and 6 subunits i.e., pentamers and hexamers.

The head is joined to a non-contractile 180 µm long tail by a connector. There is a hole in capsid through which passes this narrow neck portion expanding into a knob like structure inside. The tail possesses a thin tail fibre (25 nm long) at its end which recognises the hosts. Also, the tail consists of about 35 stacked discs or annuli. Unlike T-even phage, it is a simple structure devoid of the tail sheath.

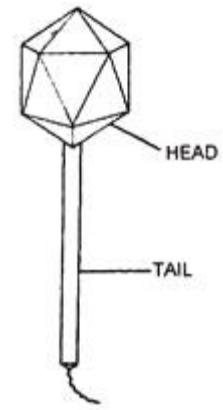


FIG. 13.4. Structure of bacteriophage lambda (λ).

2. Life-Cycle (Multiplication):

The bacteriophage is first adsorbed on the host wall surface and its DNA is injected into the bacterial cell cytoplasm (Fig. 13.5). The viral DNA, instead of starting lytic cycle, gets inserted into the bacterial DNA and travels through many generations by means of the successive divisions of the cell.

Under certain conditions, the inserted viral DNA may get dissociated from the bacterial DNA, and start functioning as virulent phage culminating in the lysis of the host cell. Such conversion of temperate phage (especially pro-phage) into the virulent phage is referred to as 'induction', which can be artificially achieved by treatment of the bacterial cells with ultraviolet radiation or with hydrogen peroxide.

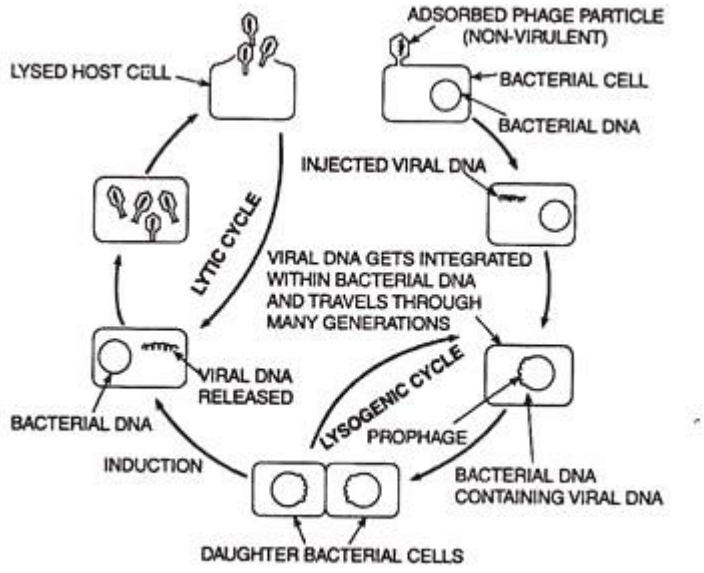


FIG. 13.5. Life cycle of lambda (λ) phage.

(iii) Bacteriophage Mu (Transposable dsDNA Phage):

Mu bacteriophage is temperate, like lambda, but is more interesting due to its unusual property of replicating as a transposible element (transposible elements are sequences of DNA which move from one location to another on their host genome). This bacteriophage is called Mu due to its property of inducing mutations in host genome into which it is integrated.

Its mutagenic property arises because the genome of the phage inserts into the middle of the host genes and these genes become inactive and hence the host behaves as a mutant. Mu is a useful phage as it can be used to generate a wide variety of bacterial mutants very easily. It is also used as vector in genetic engineering.

1. Structure:
Mu phage (Fig. 13.6) is a large dsDNA phage possessing an icosahedral head, a helical tail, and six tail fibres. The DNA molecule of the virion is approximately 39 kilobase pairs long but only 37.2 kilobase pairs make up the actual Mu genome. This is due to the fact that both ends of this DNA molecule contain DNA of the host.

These host DNA sequences are not unique and represent DNA adjacent to the location where Mu was inserted into the DNA of its previous host. Entry of Mu DNA into the head during new progeny formation is so variable that each virion arising from a single infected host cell will have a different amount of host DNA, and the host DNA base sequence in each virion from the same cell will be different.

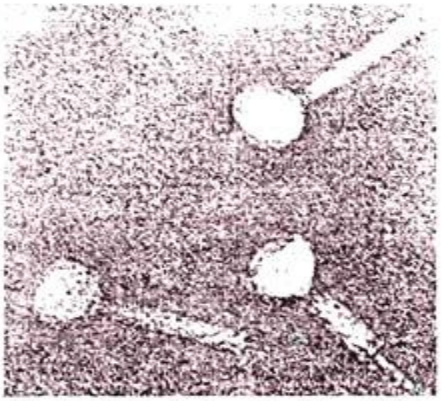


FIG. 13.6. Bacteriophage Mu virion (electron microscopic view).

2. Multiplication:
Genetic map (Fig. 13.7) of Mu genome shows a specific segment called G (distinct from G gene). This segment is invertible and determines the kind of tail fibres that are made for the phage.

Since phage adsorption on host cell is related to the specificity of the tail fibres, the host range of Mu is determined by which orientation of this invertible G segment is present in the virus. If the orientation is G^+, then the phage infects E. coli K12. If the orientation is G^-, the phage infects E. coli strain C or many other species of enteric bacteria.

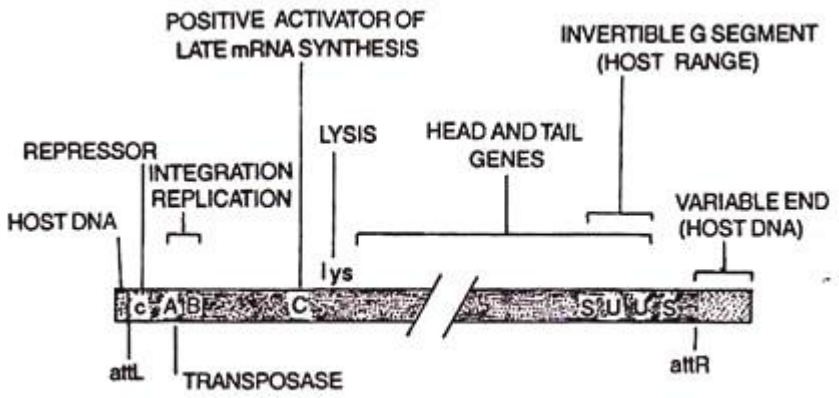


FIG. 13.7. Genetic map of Mu phage.

When the Mu phage adsorbs on its specific host, its DNA is injected into the cytoplasm and integrates into the host genome. Contrary to lambda (λ) phage, integration of Mu phage DNA into the host genome is essential for both lytic and lysogenic cycle of growth. At the site of host genome where the viral DNA becomes integrated, a five base-pair duplication of the host DNA takes place.

As shown in Fig. 13.8, this host DNA duplication arises because staggered cuts are made in the host DNA at the point where Mu DNA is inserted, and the resulting single-stranded segments are converted into double-stranded segments as part of the integration process. Mu phage can enter into the lytic cycle either on the beginning of the infection or by induction of a lysogen. The lytic cycle is initiated only if the formation of Mu repressor (the product of gene c) is suppressed. In either case, however, Mu DNA replication involves repeated transposition of Mu to multiple sites on the DNA of the host.

Transcription of only the early genes of Mu takes place in the beginning, but the synthesis of Mu head and tail proteins occurs when gene C protein (a positive activator of late RNA synthesis) is expressed. Eventually, expression of the lytic function occurs and mature progeny-virions are released from the host cell.

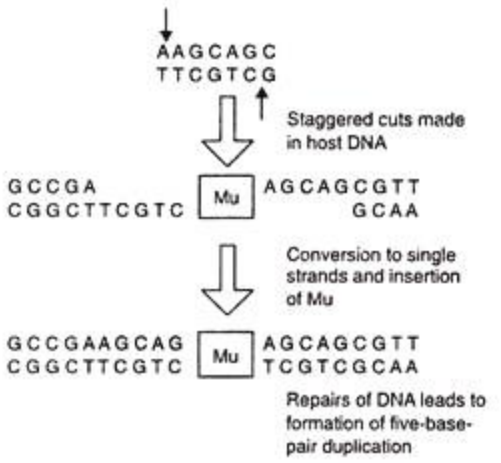


FIG. 13.8. Integration of Mu phage into the host DNA showing the generation of a five-base pair duplication of host DNA.

M13 infection and replication

M13 is a filamentous *bacteriophage* which infects *E. coli* host. The M13 genome has the following characteristics:

- Circular *single-stranded* DNA
- 6400 base pairs long
- The genome codes for a total of 10 genes (named using Roman numerals I through X)

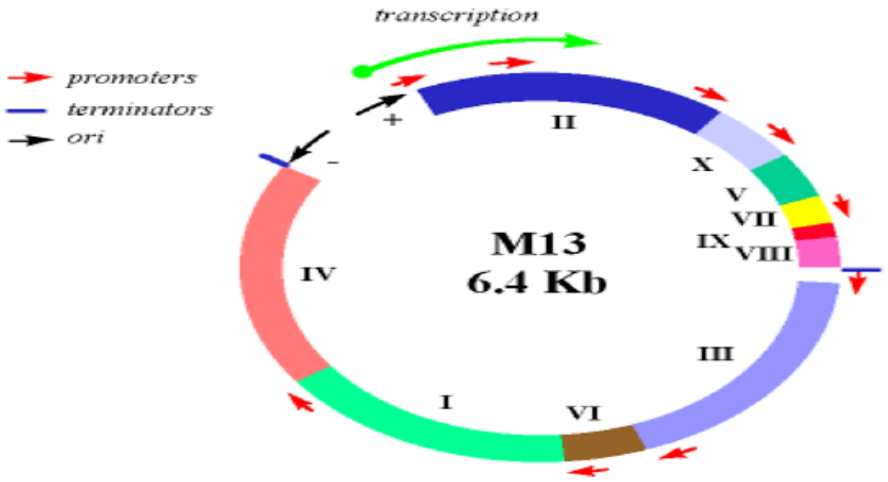
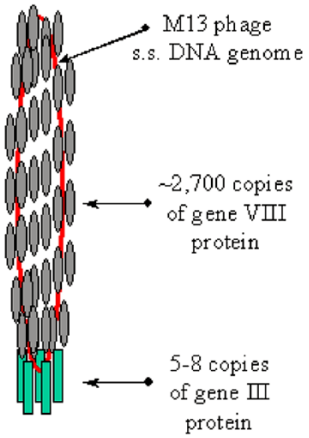


Figure 4.2.1: *M13 genome*

- *Gene VIII* codes for the major structural protein of the bacteriophage particles
- *Gene III* codes for the minor coat protein



Gene III and gene VIII

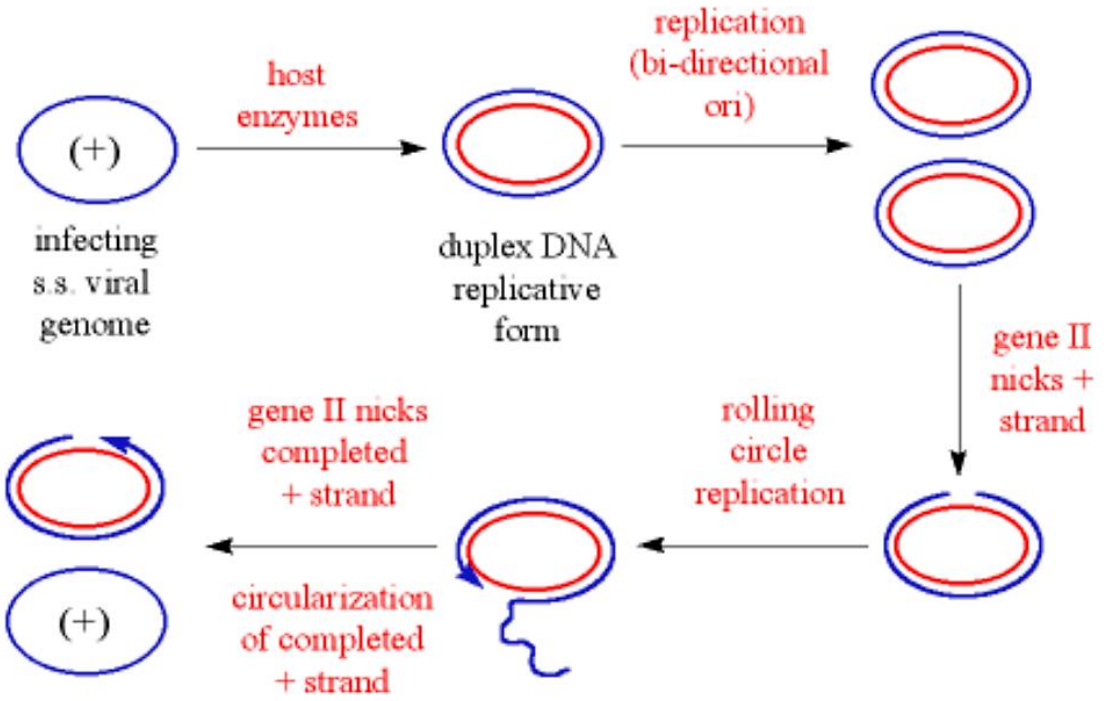
- The gene VIII protein forms a tubular array of approx. 2,700 identical subunits surrounding the viral genome
- Approximately five to eight copies of the gene III protein are located at the ends of the filamentous phage (i.e. genome plus gene VIII assembly)
- Allows binding to bacterial "sex" pilus
  - Pilus is a bacterial surface structure of *E. coli* which harbor the "F factor" extrachromosomal element

Infection

- Single strand genome (designated '+' strand) attached to pilus enters host cell
  - Major coat protein (gene VIII) stripped off
  - Minor coat protein (gene III) remains attached
- Host components convert single strand (+) genome to double stranded circular DNA (called the replicative or "RF" form)
- Transcription begins
  - Series of promoters
    - Provides a gradient of transcription such that gene nearest the two transcription terminators are transcribed the most
  - Two terminators
    - One at the end of gene VIII
    - One at the end of gene IV
  - Transcription of all 10 genes proceeds in same direction

Amplification of viral genome

- Gene II protein introduces 'nick' in (+) strand
- Pol I extends the (+) strand using *strand displacement* (and the '-' strand as template)
- After one trip around the genome the gene II protein nicks again to release a completed (linear) '+' genome
  - Linear (+) genome is circularized
- During first 15-20 minutes of DNA replication the progeny (+) strands are converted to double stranded (RF) form
  - These serve as additional templates for further transcription
- Gene V protein builds up
  - This is a *single stranded DNA binding protein*
  - Prevents conversion of single (+) strand to the RF form
- Now get a buildup of circular single stranded (+) DNA (M13 genome)



Amplification of genome

Phage packaging

- Major coat protein (Gene VIII) present in *E. coli* membrane
- M13 (+) genome, covered in ss binding protein - Gene V protein, move to cell membrane
- Gene V protein stripped off and the major coat protein (Gene VIII) covers phage DNA as it is extruded out
  - Packaging process is therefore *not linked to any size constraint* of the M13 genome
  - Length of the filamentous phage is determined by size of the DNA in the genome
  - Inserts of up 42 Kb have been introduced into M13 genome and packaged (7x genome size)
- ~8 copies of the Gene III protein are attached at the end of the extruded genome

Phage typing method: Principle, Procedure and Results

Bacteriophages, also known as phages, are viruses that attack bacteria. Depending on the method of replication phages can be broadly classified as virulent phages (replicate via lytic pathway) and temperate phage (replicate via both lytic and lysogenic pathway).

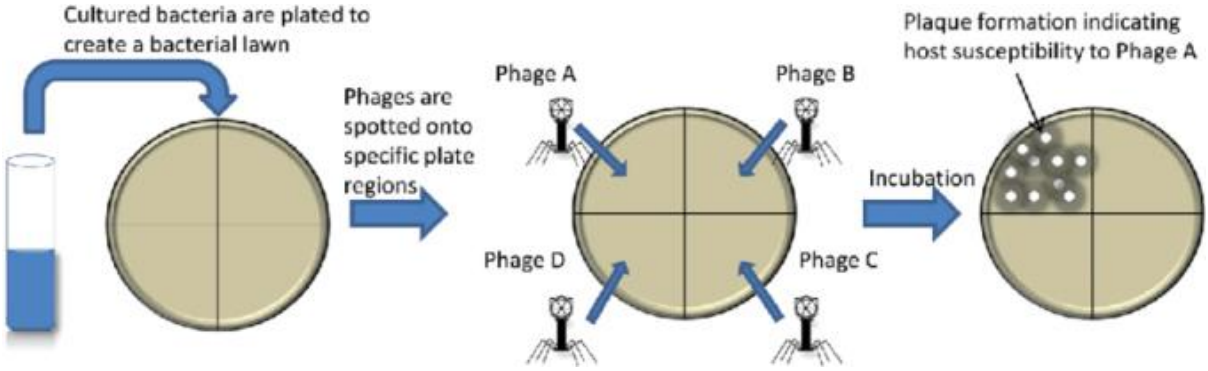
In phage typing, a panel of lytic phages is inoculated on a lawn inoculum of the bacteria under investigation. Phages which are able to set up a lytic infection in that isolate produce a clear zone. As the ability to be infected (and lysed) by different phages varies between different strains of bacteria, the pattern of lysis forms the basis of phage typing.

Microbiologists are using phage typing for several decades to determine the relatedness of species and also for various epidemiological purposes (surveillance, outbreak investigations etc.) This phenotypical method is being replaced by various molecular typing methods

Phage-typing methods are gradually being superseded by genotypic techniques such as clustered regularly interspaced short palindromic repeats (CRISPR) typing, whole-genome sequencing etc.

Principle:

Bacterial strains are grown on a suitable culture medium and then subjected to attack by a series of different known phages. Some phages will kill the bacteria and lyse their colony, which can be visualized and measured but others won't be able to kill a given bacteria. Depending on which groups of phages can lyse or fail to lyse bacterial strain, the bacteria are given a number, also called phage-type.



Bacteriophage typing method (Image source: Ref-2)

Phage typing has been used for decades for subtyping of *Salmonella* Typhimurium to determine the epidemiological relation among isolates. The system distinguishes more than 300 definitive phage types (DT) of *Salmonella* Typhimurium based on their patterns of lysis to a unique collection of Salmonella phages e.g., *S.* Typhimurium DT104. Phage typing is also done for other species of Salmonella e.g. *Salmonella* Enteritidis PT4. Similarly, Staphylococci are typed to determine whether the isolates belonged to the more virulent phage types so that the appropriate infection control method could be instituted.

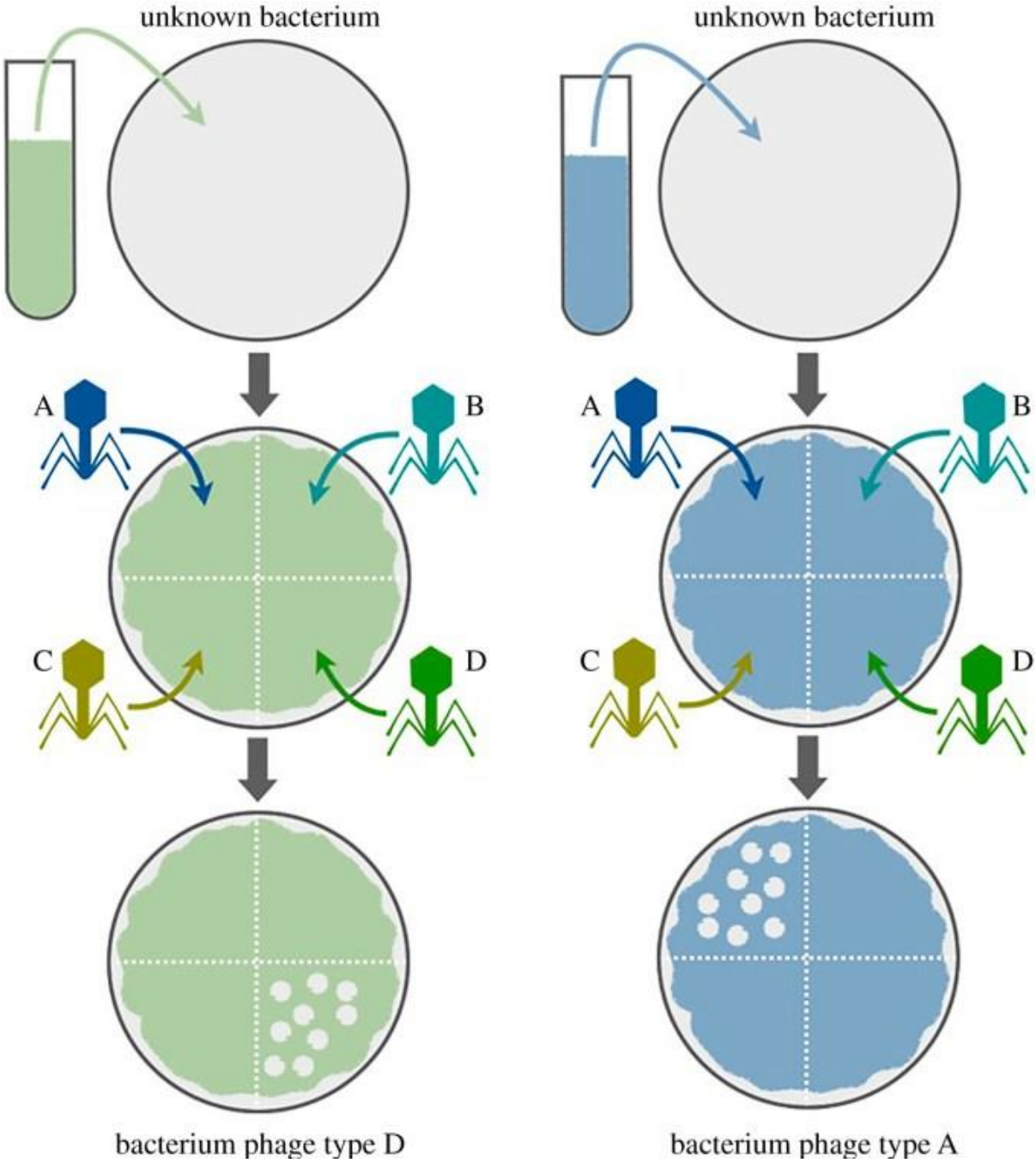
Salient features of phage typing methods

1. Phage typing is a rapid and low-cost approach for the epidemiological surveillance and outbreak investigation (identification of the source of infection).
2. Phage-typing is the most widely recognized typing method for *Staphylococcus aureus* and is also still used widely for sub-dividing serotypes of ***Pseudomonas aeruginosa*** and *Salmonella / Shigella* spp. Phage typing still remains as the gold standard method for epidemiological surveillance of *S.* Typhimurium.

Procedure

1. Label each plate with the name/number of test bacterium.
2. Place a sterile cotton swab in the bacterial suspension and remove the excess fluid by pressing and rotating the cotton against the inside of the tube above the fluid level.
3. Streak the swab in three directions over the surface of the agar medium to obtain uniform growth. A final sweep is made around the rim of the agar. This is done to make a lawn culture of bacterium.
4. Allow the plates to dry for five minutes.

5. Divide the plate in four quarters (using a pencil, by drawing a line in the backside of the plate) and name each quarter with the name of the bacteriophage which you are going to inoculate in that region.
6. One the agar media dried completely, spot-inoculate 10 µl phages (according to the labelling) by dropping just a tiny amount of the phage suspension from the pipette tip.
7. Repeat the above procedure with a fresh pipette tip and spot-inoculate this phage on its specifically labelled region.
8. Allow the phage inocula to dry completely.
9. Incubate at 37°C for 1-2 days (or 30°C if incubation is more than 2 days).



Phage Typing Procedure (Image source: Ref-3)

Reporting

Examine the plates for evidence of lysis (a giant plaque) in the area where phage was inoculated and tabulate the results. Record positive for lysis (= sensitivity of a bacterial strain to a particular phage).

Limitations

1. Phage typing requires different phages so phage typing is beyond the scope of local diagnostic laboratories. It is generally performed only at reference laboratories.
2. Phage typing requires substantial technical expertise to perform. Careful control of environmental conditions and other variables is technically demanding.
3. Maintenance of typing phages by the reference laboratory is time consuming and expensive approach.
4. phage-types can change following lysogenic conversion, loss of prophages, or gain or loss of R plasmids, and this variability is coupled with the continuous need to maintain the typing set of bacteriophages in a viable state by regular serial passage.

PHAGE THERAPY

- Phage therapy (PT) is also called bacteriophage therapy. It uses viruses to treat bacterial infections. Bacterial viruses are called phages or bacteriophages. They only attack bacteria; phages are harmless to people, animals, and plants.

- Bacteriophages are the natural enemies of bacteria. The word bacteriophage means "bacteria eater." They're found in soil, sewage, water, and other places bacteria live. These viruses help keep bacteria growth in check in nature.

- Phage therapy might sound new, but it has been used for 100Trusted Source years. However, the treatment isn't well known. More research is needed on bacteriophages. This therapy for disease-causing bacteria may be a useful alternative to antibiotics.

How phage therapy works

- Bacteriophages kill bacteria by making them burst or lyse. This happens when the virus binds to the bacteria. A virus infects the bacteria by injecting its genes (DNA or RNA).

- The phage virus copies itself (reproduces) inside the bacteria. This can make up to 1000Trusted Source new viruses in each bacterium. Finally, the virus breaks open the bacteria, releasing the new bacteriophages.

- Bacteriophages can only multiply and grow inside a bacterium. Once all the bacteria are lysed (dead), they'll stop multiplying. Like other viruses, phages can lay dormant (in hibernation) until more bacteria show up.

Phage therapy vs. antibiotics

- Antibiotics are also called anti-bacterials. They're the most common type of treatment for bacterial infections. Antibiotics are chemicals or drugs that destroy bacteria in your body.

- Antibiotics save lives and prevent disease from spreading. However, they can cause two main problems:

1. **Antibiotics attack more than one kind of bacteria**

- This means they can kill both bad and good bacteria in your body. Your body needs certain kinds of bacteria to help you digest food, make some nutrients, and keep you healthy.

- Good bacteria also help stop other bacterial, viral, and fungal infections from growing in your body. This is why antibiotics can cause side effects like:

- upset stomach
- nausea and vomiting
- cramping
- bloating and gassiness
- diarrhea
- yeast infections

- **Antibiotics can lead to "superbugs"**

- This means that instead of stopping, some bacteria become resistant or immune to antibiotic treatment. Resistance happens when bacteria evolve or change to become stronger than the antibiotics.

- They can even spread this "superpower" to other bacteria. This may trigger dangerous infections that cannot be treated. Untreatable bacteria can be deadly.

- Use antibiotics correctly to help prevent resistant bacteria. For example:

- Only use antibiotics for bacterial infections. Antibiotics will not treat viral infections like colds, flus, and bronchitis.
- Don't use antibiotics if you don't need them.
- Don't pressure your doctor to prescribe antibiotics for you or your child.
- Take all antibiotics exactly as prescribed.
- Complete the full dosage of antibiotics, even if you feel better.
- Don't take expired antibiotics.

- Throw away expired or unused antibiotics.

- **Phage therapy benefits**

- The benefits of phage therapy address the shortcomings of antibiotics.

- Just as there are many kinds of bacteria, there are several types of bacteriophages. But each kind of phage will only attack a certain bacterium. It won't infect other kinds of bacteria.

- This means that a phage can be used to directly target disease-causing bacteria. For example, a strep bacteriophage will only kill bacteria that cause strep throat infections.

A 2011 research reviewTrusted Source listed some pros of bacteriophages:

- Phages work against both treatable and antibiotic-resistant bacteria.
- They may be used alone or with antibiotics and other drugs.
- Phages multiply and increase in number by themselves during treatment (only one dose may be needed).
- They only slightly disturb normal "good" bacteria in the body.
- Phages are natural and easy to find.
- They are not harmful (toxic) to the body.
- They are not toxic to animals, plants, and the environment.

Phage therapy disadvantages

Bacteriophages are not yet widely used. This therapy needs more research to find out how well it works. It's not known if phages may harm people or animals in ways unrelated to direct toxicity.

Additionally, it's not known if phage therapy may trigger bacteria to become stronger than the bacteriophage, resulting in phage resistance.

Cons of phage therapy include the following:

- Phages are currently difficult to prepare for use in people and animals.
- It's not known what dose or amount of phages should be used.
- It's not known how long phage therapy may take to work.
- It may be difficult to find the exact phage needed to treat an infection.

- Phages may trigger the immune system to overreact or cause an imbalance.
- Some types of phages don't work as well as other kinds to treat bacterial infections.
- There may not be enough kinds of phages to treat all bacterial infections.
- Some phages may cause bacteria to become resistant.

Phage use in the United States

Phage therapy isn't yet approved for people in the United States or in Europe. There has been experimental phage use in a few rare cases only.

One reason for this is because antibiotics are more easily available and are considered to be safer to use. There is ongoing research on the best way to use bacteriophages in people and animals. The safety of phage therapy also needs more research.

In the food industry

Phage therapy is being used in the food industry, however. The U.S. Food and Drug Administration (FDA) has approved of some phage mixtures to help stop bacteria from growing in foods. Phage therapy in food prevents bacteria that can cause food poisoning, such as:

- *Salmonella*
- *Listeria*
- *E. coli*
- *Mycobacterium tuberculosis*
- *Campylobacter*
- *Pseudomonas*

The phages are added to some processed foods to help prevent bacterial growth.

Another use for phage therapy that is being tested involves adding bacteriophages to cleaning products to destroy bacteria on surfaces. This may be beneficial in hospitals, restaurants, and other places.

Conditions that may benefit from phage therapy

Phage therapy may be very important in treating infections that don't respond to antibiotics. For example, it may be used against a powerful *Staphylococcus*(staph) bacterial infection called MRSA.

There have been successful cases of phage therapy use. One such success storyinvolved a 68-year-old man in San Diego, California, who was treated for a resistant kind of bacteria called *Acinetobacter baumannii*.

After more than three months of trying antibiotics, his doctors were able to stop the infection with bacteriophages.

The takeaway

Phage therapy isn't new, but its use in people and animals also isn't well researched. Current studies and some successful cases may mean that it could become more common. As phage therapy is considered safe and approved for use in the food industry, this may be quite soon.

Phage therapy is nature's "antibiotics" and may be a good alternative treatment. It may also be beneficial for other uses such as a surgical and hospital disinfectant. More research is needed before its use is approved for people.

TABLE

Some of the major human phage therapy studies performed in Poland and the former Soviet Union

Reference(s)	**Infection(s)**	**Etiologic agent(s)**	**Comments**
Babalova et al. (7)	Bacterial dysentery	*Shigella*	*Shigella* phages were successfully used for prophylaxis of bacterial dysentery.
Bogovazova et al. (11)	Infections of skin and nasal mucosa	*K. ozaenae, K. rhinoscleromatis*, and *K. pneumoniae*	Adapted phages were reported to be effective in treating *Klebsiella* infections in all of the 109 patients.
Cislo et al. (17)	Suppurative skin infections	*Pseudomonas, Staphylococcus, Klebsiella, Proteus*, and *E. coli*	Thirty-one patients having chronically infected skin ulcers were treated orally

Reference(s)	Infection(s)	Etiologic agent(s)	Comments
			and locally with phages. The success rate was 74%.
Ioseliani et al. (22)	Lung and pleural infections	*Staphylococcus*, *Streptococcus*, *E. coli*, and *Proteus*	Phages were successfully used together with antibiotics to treat lung and pleural infections in 45 patients.
Kochetkova et al. (25)	Postoperative wound infections in cancer patients	*Staphylococcus* and *Pseudomonas*	A total of 131 cancer patients having postsurgical wound infections participated in the study. Of these, 65 patients received phages and the rest received antibiotics. Phage treatment was successful in 82% of the cases, and antibiotic treatment was successful in 61% of the cases.
Kucharewicz-Krukowska and Slopek (27)	Various infections	*Staphylococcus*, *Klebsiella*, *E. coli*, *Pseudomonas*, and *Proteus*	Immunogenicity of therapeutic phages was analyzed in 57 patients. The authors concluded that the phages' immunogenicity did not impede therapy.
Kwarcinski et al. (29)	Recurrent subphrenic abscess	*E. coli*	Recurrent subphrenic abscess (after stomach resection) caused by an antibiotic-resistant strain of *E. coli* was successfully treated with phages.

Reference(s)	Infection(s)	Etiologic agent(s)	Comments
Litvinova et al. (32)	Intestinal dysbacteriosis	*E. coli* and *Proteus*	Phages were successfully used together with bifidobacteria to treat antibiotic-associated dysbacteriosis in 500 low-birth-weight infants.
Meladze et al. (33)	Lung and pleural infections	*Staphylococcus*	Phages were used to treat 223 patients having lung and pleural infections, and the results were compared to 117 cases where antibiotics were used. Full recovery was observed in 82% of the patients in the phage-treated group, as opposed to 64% of the patients in the antibiotic-treated group.
Miliutina and Vorotyntseva (35)	Bacterial dysentery and salmonellosis	*Shigella* and *Salmonella*	The effectiveness of treating salmonellosis using phages and a combination of phages and antibiotics was examined. The combination of phages and antibiotics was reported to be effective in treating cases where antibiotics alone were ineffective.

Cyanophage

- **Cyanophages** are viruses that infect cyanobacteria, also known as Cyanophyta or blue-green algae.
- Cyanobacteria are a phylum of bacteria that obtain their energy through the process of photosynthesis

- Although cyanobacteria metabolize photoautotrophically like eukaryotic plants, they have prokaryotic cell structure.
- Cyanophages can be found in both freshwater and marine environments. Marine and freshwater cyanophages have icosahedral heads, which contain double-stranded DNA, attached to a tail by connector proteins.
- The size of the head and tail vary among species of cyanophages.

- **Nomenclature**

- The following three families of cyanophages have been recognized by the International Committee on Taxonomy of Viruses (ICTV): Myoviridae, Siphoviridae and Podoviridae; all contain double-stranded DNA.

- Initially, cyanophages were named after their hosts. However, the ability of cyanophages to infect multiple hosts and lack of a universal naming system can cause difficulties with their taxonomic classification**.**

- **Morphology**
- Like all other tailed bacteriophages cyanophages have a tail and a protein capsid surrounding genetic material.
- The double-stranded DNA is approximately 45 kbp long .
- The tail binds the virus to the host cell and transfers viral DNA to the host cell upon infection. Based on morphological characteristics, cyanophages are placed into the families
- Myoviridae, Podoviridaeand Siphoviridae, and although not formally recognized by the International Committee on Taxonomy of Viruses, historically cyanophages have been further classified into as a Cyanomyovirus, Cyanopodovirus or Cyanostylovirus based on which of the three families in which they are grouped.

- **Host**
- The host range of cyanophages is very complex and is thought to play an important role in controlling cyanobacterial populations.
- Freshwater cyanophages have been reported to infect hosts in more than one genus although this may also reflect problems in the taxonomic classification of their hosts.

- **Replication**

- **cyanophage replication has two dominant cycles: the lytic cycle and the lysogenic cycle.**

- ## Mycoviruses:
- The viruses associated with fungi are called mycoviruses, and also mycophages. They are often typically latent but some induce symptoms. They are wide spread in all taxonomic groups of fungi. Much less is known about the mycoviruses of the lower fungi.

- During 1950s, several disorders in fungi were described and some authors suspected for the involvement of viruses. For the first time Boilings (1962) gave the conclusive evidence of viruses that infected the cultivated mushrooms, Agaricus bisporus causing die back disease.

- . Subsequently mycoviruses from different taxonomic group of fungi were described. So far at least 5,000 fungal species are known to contain mycoviruses.

- It is interesting to note that most of species of Penicillium and Aspergillus have been found to be infected with viruses, whereas in other genera the viruses have not been found so frequent.

- ***Types of Mycoviruses:***
- So far very few mycoviruses have been fully characterized, and most are only the 'virus like particles' (VLPs) in electron micrograph of partially purified extracts from the fungus or sometimes from thin section studies. Several different morphological types of VLPs have been observed, some corresponding fairly close with well known viruses of the other host taxa. Some of the examples of mycoviruses are given in Table.

- **Table : Virus particles reported in fungi.**

Fungal Species	*Virion size (nm)*
Agaricus bisporus	25-50,19-50 (rods)
A.campestris	25-50,19x50 (rods)
Alternaria tenuis	30-40
Aspergillus foetidus	40-42
A.glaucus	25
A.niger	40-42
Endothia parasitica	300. club shaped
Gaeumannomyces tritici	29
Laccaria laccata	28
Pencillium brevicompactum	40
P.chrysogenum	35
P.funiculosum	25-30
P.notatum	25
P. stoloniferum	40-45
Peziza ostracoderma	17 × 350 (rods)
Stemphilium botryosum	25. VLPs
Saccharomyces cerevisiae	40

- Some of the isometric particles (105-110 nm diameter) i.e. viruses containing capsid roughly spherical polyhedron, superficially resemble iridoviruses, and some others (about 50 nm diameter) resemble caulimoviruses.

- Tailed DNA phage particles and paired 20 nm isometric particles of possibly Gemini virus type have also been reported. Most of the virus particles recorded in fungi have been isometric with a genome of several species.

- The mycoviruses have a heterogeneous properties with a diameter ranging from 25-50 nm and particle weight from 6-13 x 10^6 Dalton. They possess 1-8 segments of dsRNA with a total molecular weight of 2-8.5 x 10^6 Dalton.
- All the examined samples had only a single capsid protein but of varying molecular weight from 25 to 130 x 10^3Dalton in different viruses.

- However, the mycroviruses with more than one capsid polypeptide have also been reported in the purified preparations of many potyviruses, the other viruses of higher plants and in Aspergillus foetidus virus- S (Afv-s).
-
- ***Morphology of Mycoviruses****:*
- Mycoviruses recorded so far show morphologically variable forms, viz., bacilliform, rod- shaped, filamentous and herpes types. But majority of the known mycoviruses are typically isodiametric ranging usually from 25 and 50 nm in diameter and particle weight from 6-13 x 10^6dalton.
- The most outstanding feature common to mycoviruses is possession of double-stranded ribonucleic acid (dsRNA) usually segmented into 1-8 segments with a total molecular weight of 2 to 8.5 x 10^6 dalton.
- The dsRNA segments are separately enclosed into identical capsids. This feature of mycoviruses differentiates them from plant and animal dsRNA viruses in which the genetic material segments are, usually, all enclosed in a single virion.

- ***Replication of Mycoviruses:***
- Replication of mycoviruses inside the fungal cell has been reviewed by Buck (1979, 1980). He has reported some host enzymes capable of transcribing the ssRNA and dsRNA in laboratory conditions and probably dsRNA in vivo.

- Highly specific virus-coded RNA polymerases are necessary for effective in vivo transcription and replication of dsRNA. Such polymerase has been reported in some dsRNA mycoviruses. It is thought that the polymerases remain confined within the virion during the replicative cycle of mycoviruses.

- The mechanism of infection and transmission of mycoviruses is still obscure. They have been found in fungal spores and it is believed that they are transmitted through the spores. The presence of viral-RNA in the fungal cells does not appear to affect any cellular properties such as antibiotic production.

- For example Penicillium notation contains a dsRNA mycovirus, but penicillin production by the fungus is not affected at all. In recent years the dsRNA mycoviruses have attracted the attention of scientists since they have ability to induce interferon production in animal cells. Also, they do not appear to the animal cells be toxic unlike other chemicals that induce interferon production.

- RHIZOBIOPHAGES

- Bacteriophages that infect rhizobia (termed rhizobiophages) were first reported by Gerretsen et al. (1923) and isolated from root-nodule bacteria .

- Rhizobiophages have been implicated in the control of rhizobial populations in soil. so through their lytic activity, they can decrease N_2 fixation in legumes.

- mechanism underlying loss of symbiotic properties of rhizobiophages are still unknown .

UNIT 4

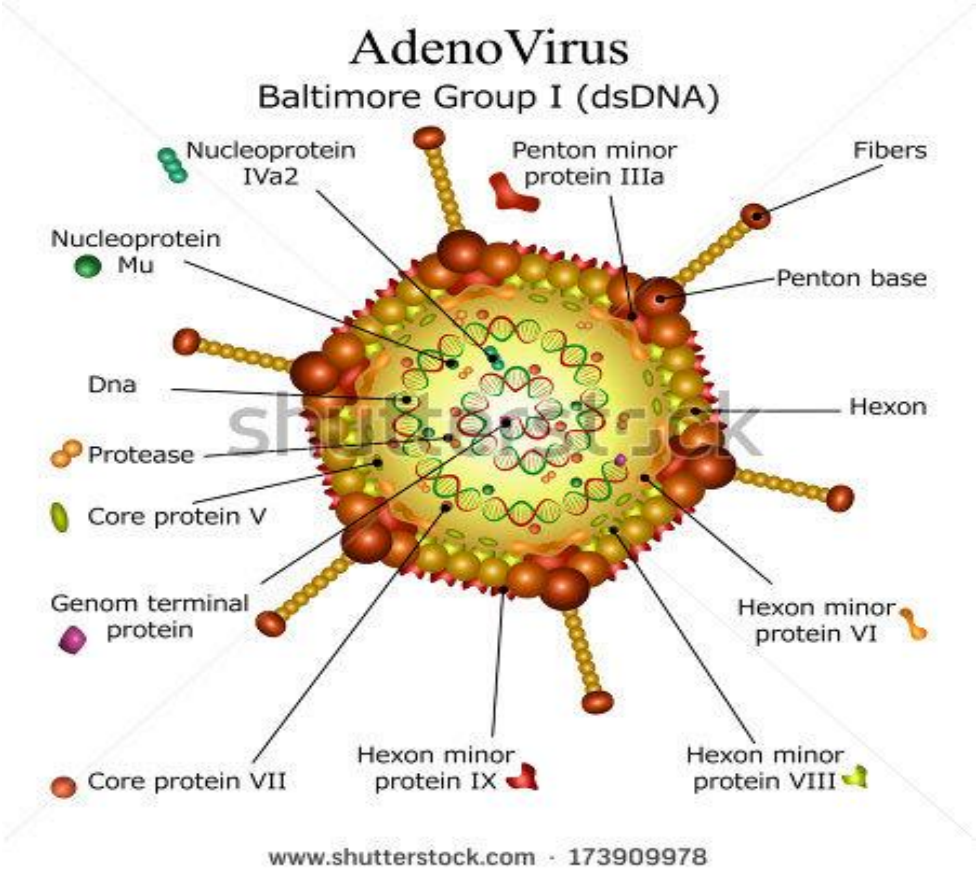
ADENOVIRUS

I. Structure

Adenoviruses are the group of medium sized, non enveloped ds DNA virus that share common complement fixing antigen.

Size: 70-90 nm in diameter

Shape: Icosahedral



Genome:

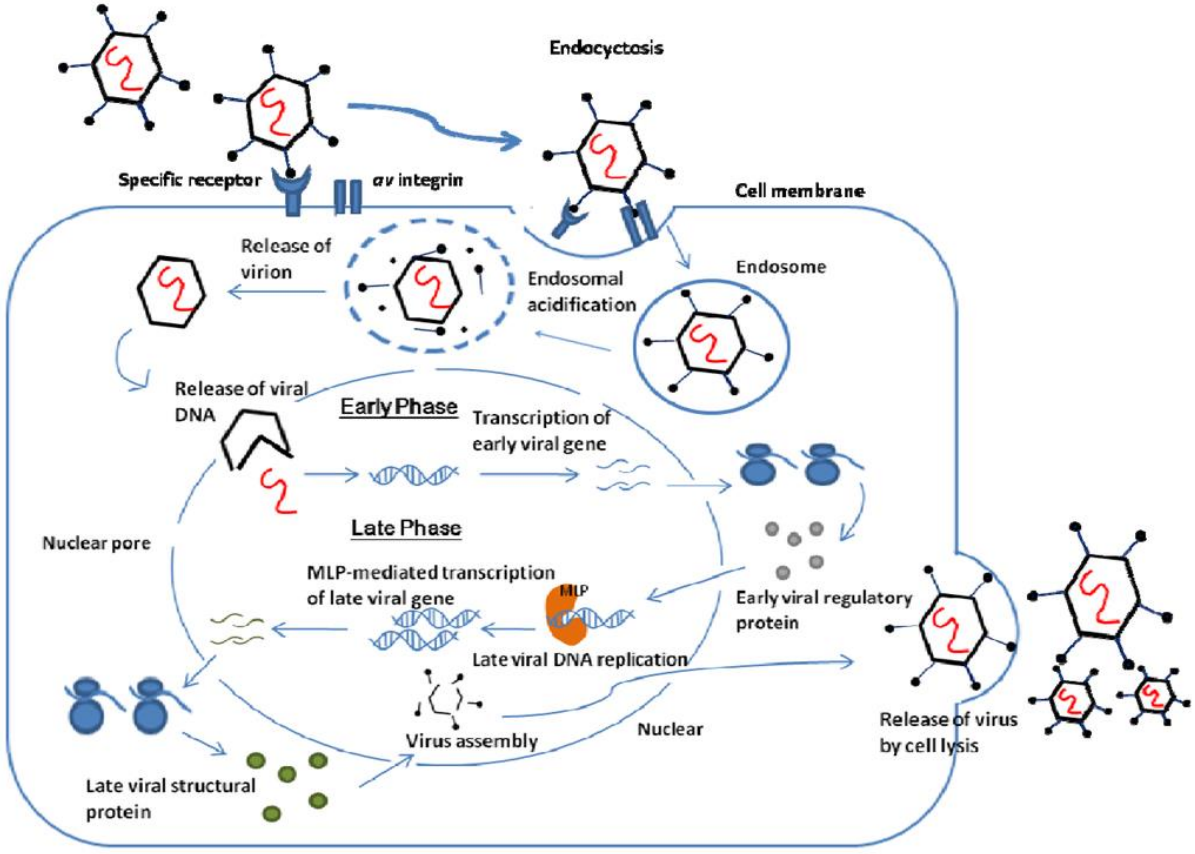
- Linear ds DNA molecule of 26-45 kbp long and DNA have inverted terminal repeats of approximately 100bp at both ends.
- Each DNA strand is covalently attached to virus encoded protein at 5’ end

Capsid:

- Capsid is Icosahedral in shape and is composed of 292 capsomeres with 20 triangular facets and 12 vertices.
- The capsid consists of 240 hexons and 12 pentons.

- Each pentons unit consists of a penton base anchored in capsid and a projection (fiber or knob) with a knot at distal end. Thus the virion looks like space vehicle.
- The projection or fiber helps to bind Adenovirus to host cell.
- The fiber contains viral attachment protein which acts as hemagglutinin.
- Penton base carries a toxin like activity that causes cytopathic effect (CPE) on host cell.

II. Replication of Adenovirus



Step I: Attachment and entry

- Adenovirus attaches to the host cell via its fiber structure to Coxsackie and Adenovirus receptor (CAR) receptor on host cell.
- The attachment of fiber to its receptor on host cell is followed by interaction of penton base with cellular integrin which promote receptor mediated internalization.

Step II: Uncoating

- The virus is internalized into clathrin coated endosome and the high pH of endosome helps in uncoating of virus
- Transport of viral DNA into nucleus
- The viral nucleocapsid is transported from cytosol to nucleus by the help of microtubules.

Step III: Early Transcription

- It is the early event in viral replication, and occurs before viral DNA synthesis begins.
- It is the preparatory phase in which transcription of viral DNA occurs to mRNA (early transcript).
- Early transcript undergoes translation to produce about 20 different early proteins. These early proteins induces host cell to enter into S-phase of cell cycle and to create condition favorable for viral replication.

Step IV: DNA replication

- Viral DNA replication takes place in nucleus.
- The viral encoded protein at 5' end of viral DNA strand acts as primer for initiation of viral DNA synthesis.
- Late event begins concomitantly with onset of viral DNA synthesis.

Step V: Late Transcription and translation

- A large single primary transcript is synthesized from virus DNA which spliced into 18 fragments and each fragment acts as mRNA and are transported to cytoplasm.
- In the cytoplasm, translation occurs and viral structural proteins are synthesized.

Step VI: Viral morphogenesis and release

- Morphogenesis of Adenovirus occurs inside nucleus.
- Viral DNA then gets packaged into preformed capsid forming mature virus particle.
- Mature virus particles are stable, infectious and resistant to nuclease enzyme of host cell.
- Adenovirus infection does not lyse the host cell.
- Mature virus is then release from host cell by budding.

III. Mode of transmission of Adenovirus

- Adenovirus infection transmits from person to person directly by-
- Aerosol droplets (respiratory route)
- Faeco-oral route
- Contaminated fingers to infects conjunctiva
- Contaminates fomites

IV. Pathogenesis

- Adenovirus can infects and replicates in epithelial cells of respiratory tracts, gastro-intestinal tracts, urinary bladder and eyes.
- After entry of Adenovirus inside human body, it can multiply in epithelial cells of conjunctiva, pharynx or small intestine according to mode of entry and then spreads to regional lymph nodes.
- Usually Adenovirus does not spread beyond regional lymph node.
- Adenovirus produces three types of infections:

1. Lytic infection

- Virus actively replicates inside host cell producing lytic effects causing cell death and releases progeny viruses.

- After local infection viruses may spread to visceral organs.

2. Latent infection:
   - Adenovirus has a property to become latent in lymphoid organs such as tonsil and payer's patches.
   - Latent infection can be reactivated in person with underlying immunity.
3. Oncogenic transformation:
   - Certain Adenovirus (group A and B) can transform host cell into cancerous cell by integrating viral DNA into host DNA.
   - Although the oncogenicity has not been seen in Human infection.

V. Adenovirus infection and diseases

- Adenovirus primarily infects children and accounts less in adults.
- Most Adenovirus infections are mild and self limiting.

1. Respiratory diseases:
   - **Pharyngitis:** Adenovirus is major causes of non-bacterial pharyngitis.
   - **Pneumonia:** Adenovirus 3 and 7 are associated with pneumonia.
   - **Acute respiratory disease**: fever, rhinorrhoea, cough , sore throat that lasts for 3-5 days.
   - **Pharyngoconjunctival fever**: Fever, red eyes, sore throat that occurs primarily in school going children.
2. Eye infection:
   - **Keratoconjunctivitis:** It is highly contagious and characterized by photophobia, tearing, pain and inflammation of conjunctiva.
   - **Gastro-intestinal infection**
   - **Intangible gastroenteritis:**I It is characterized by fever and watery diarrhoea.
3. Other infections:
   - **Pertussis** like symptoms in children
   - **Musculoskeletal disorder**
   - **Genital infection**
   - **Skin infection**

VI. Lab diagnosis

1. **Specimens:**
   - Depends upon nature of infections.
   - Throat swab, nasopharyngeal aspirates, conjunctival aspirates, conjunctival swab, urine, stool, blood, body fluids
2. **Microscopy:** Electron microscope
3. **Antigen detection**: ELISA, DFA test
4. **Serology** or antibody detection
5. **Molecular techniqu**e: PCR, DNA probe
6. **Virus culture**: cell line culture

VII. Prevention from Adenovirus infection

- Maintain personal hygiene
- Wash hands often with soap and water
- Avoid direct personal contact with diseased person
- Cover mouth and nose while coughing and sneezing
- Avoid touching eye, nose or mouth without washing hands
- Visit Hospital in case of any symptoms

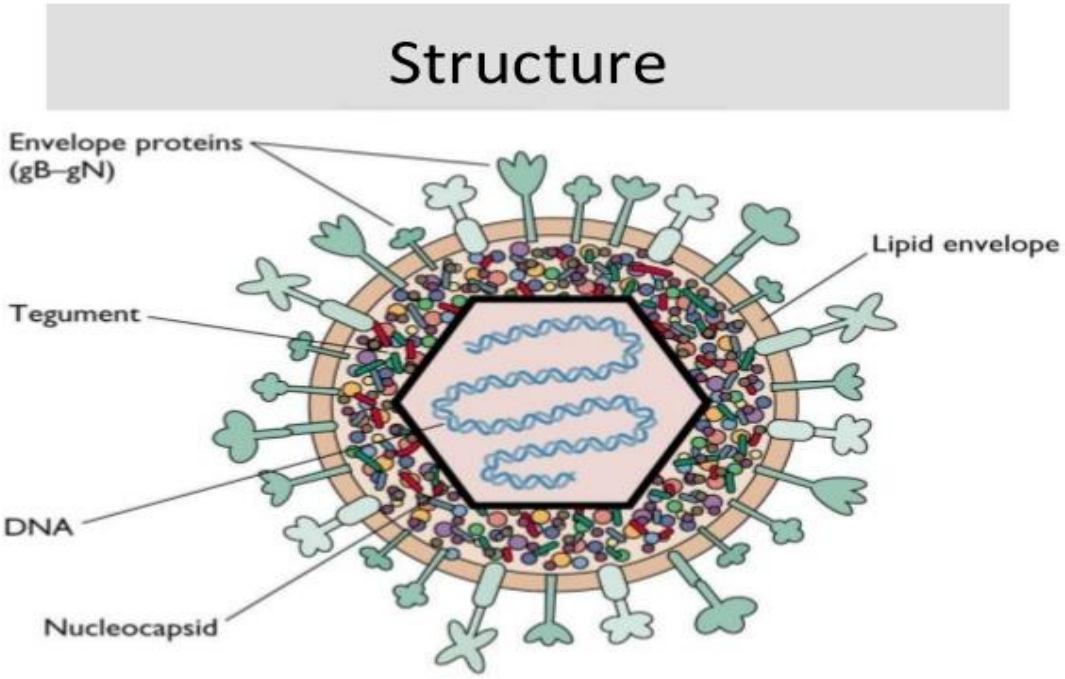
VIII. Treatments

- Not specific
- Some antiviral drugs such as Ganciclovir, Vidarabine, Ribavirin, Cidofovir

HERPES SIMPLEX VIRUS(HSV)

Structure and genome

- HSV is large enveloped icosahedral virus
- Human is only natural host for HSV. It is also known as Human Herpesvirus.
- HSV are of two types: HSV1 and HSV2. Two viruses are structurally and morphologically similar. However they are distinguished antigenically by using type specific monoclonal Ab, restriction endonuclease pattern of their genome, and site of lesions



- **Size:** 120-200nm in diameter

- **Genome:** linear ds DNA
- Enveloped
- Replication and assembly occur in nucleus of infected cell

Mode of transmission:

- Direct contact with lesion fluid or saliva
- Sexual transmission; genital herpes
- Perinatal route; child gets Herpes during birth from infected mother
- Congenital transmission; it is rare

Replication cycle:

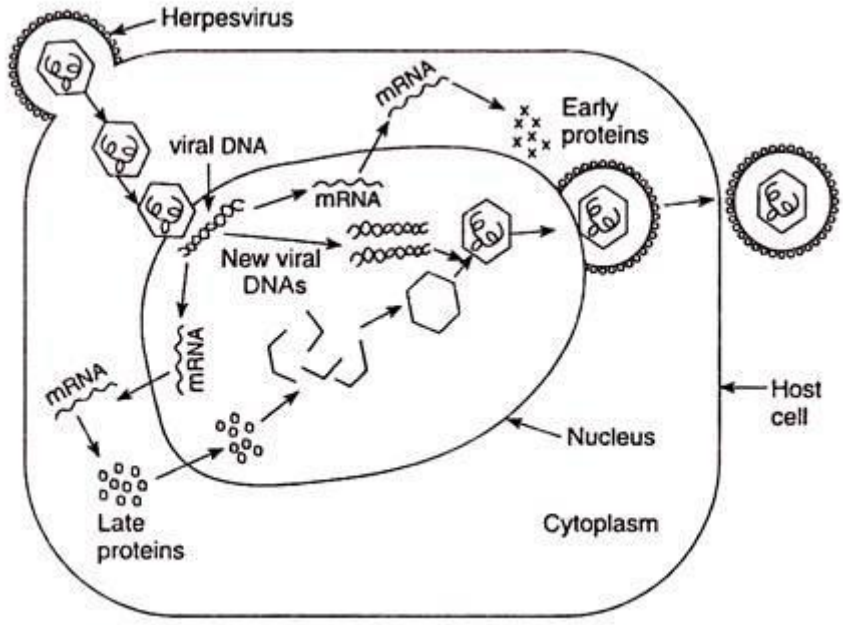
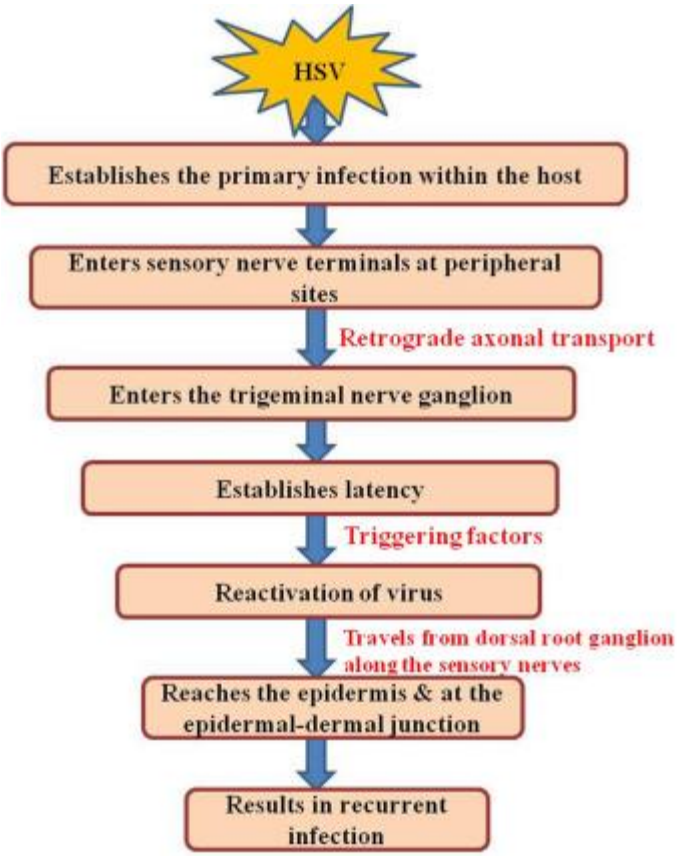


FIG. 14.6. Replication of herpesvirus genome and the formation of new viral particles.

Pathogenesis:

- Virus enter through skin and mucus membrane and multiply locally
- Virion interacts with specific cell surface receptor through its glycoprotein spike
- HSV1 and HSV2 have several cell surface receptor, they uses one of them for entry in host cell
- Binding of glycoprotein trigger fusion of viral envelope with host cell membrane and this fusion causes the release of nucleo-capsid into cytoplasm and is transported to nucleus.
- In the nucleus of host cell, virus replication occur immediately. At first Viral gene will be transcribed leading to synthesis of virus protein, replication of virus genome and assembly of progeny viruses.



1. Primary infection:
   - HSV1 infection are usually limited to oropharynx and transmitted by respiratory droplet or saliva whereas HSV2 infection usually transmits by genital route.
   - Primary infection of Herpes results in vesicle formation under the layer of keratinized squamous epithelium cell. The vesicle is filled with fluid which contains multinucleated giant cells and eosinophilic intranuclear inclusions bodies along with inflammatory cell and cellular debris
   - After primary infection, the virus invades local nerve ending and travel from retrograde intra axonal flow to sensory root ganglia where they further multiply.
   - The virus settle within neuron in sensory ganglia (trigeminal ganglia in case of HSV1 and sacral ganglia in case of HSV2) and remain latent.
2. Latent infection:
   - During latency no viral particles are produced. Also the latent infection does not causes any demonstrable damage in neuron.
   - This latency phase may be reactivated periodically in some individuals causing recurrent oral and genial lesion.
3. Recurrence herpes:
   - Various stimulus such as physical or emotional stress, trauma, fever, sunlight, certain food, menstrual cycle in female etc can induce recurrence of latent infection.
   - In recurrence the virus travels back from neuron and multiply in mucosal epithelial cell producing lesion at the same spot each time.

- The recurrent infection can also occurs in presence of specific antibodies. However recurrent infection are less severe, more localized and of shorter duration than that of primary infection due to presence of past immune response.
- As a general rule HSV-1 produce lesion above waist and HSV-2 below waist. But the rule is not absolute.
- The lesion of HSV are thin walled, umbilicated which break causing superficial ulcer and heal without scaring.

Clinical manifestation:

- Clinical manifestation and course of disease depends upon site of infection, age and immune status of host as well as antigenic type of Herpes virus.

1. Cutaneous or skin infection:

- It is characterized by fever blister or herpes febrilis on face, cheeks, chin, around mouth and forehead.
- It occurs by reactivation in febrile patients by certain stimulus such as stress, common cold, exposure to sun etc.
- Generalized eruption of lesion over body may occurs in children suffering from eczema.
- Herpetic whitlow is an occupational cutaneous herpes seen in doctors, dentists and nurses.

2. Mucosal or Oropharyngeal infection:

- Gingivostomatitis and pharyngitis are frequent.
- Acute Gingvostomatitis is the primary HSV-1 infection occurring in children between 6 month to 5 years old.
- It is characterized by vesicular lesion on oral mucosa, tongue and lips. The lesions subsequently rupture and coalesce together leaving behind ulcerated plaques.
- Pharyngitis and tonsillitis manifests with fever, malaise, headache and sore throat.

3. Ophthalmic or eye infection:

- Keratoconjunctivitis
- Follicular conjunctivitis
- Corneal blindness
- Chorioretinitis and acute necrotizing retinitis

4. Nervous system infection:

- Herpes encephalitis; caused by HSV-1
- Herpes meningitis; caused by HSV-2
- Autonomic dysfunction of nervous system
- Guillain-barre syndrome
- Bell’s palsy

5. Visceral herpes:

- Herpes esophagitis
- Tracheobronchitis
- Pneumonitis
- Hepatitis

6. Genital herpes:

- It is mostly caused by HSV-2
- Primary genital herpes is asymptomatic caused by both HSV-1 and HSV-2. Recurrent is more frequent in HSV-2
- In male lesions appears in glans or shaft and occasionally on urethra.
- In female lesions appears on valve, vagina, cervix, perianal area
- In both sexes, genital herpes is characterized by fever, pain, dysuria, mucoid urethral discharge with enlarged inguinal lymph node,

7. Congenital herpes:

- Transplacental transmission leads to congenital infection but it is rare.
- Neonatal herpes is caused by HSV-2, and it is manifested as infection of eye, mouth, skin and more commonly a disseminated infection with multiple organ involvement.
- Mortality rate is high and survivors may have neurological disabilities.

Laboratory diagnosis:

1. Specimens:

- Saliva, vesicle fluids, conjunctival fluids, corneal scrapping, skin swab and CSF.
- Depends upon site of infection

2. Microscopy:

- Tzanck smear preparation: Smear is prepared from lesion and stained with 1% aqueous solution of toluidine blue for 15 seconds. Multinucleated giant cells are visualized in positive smear.
- Giemsa stain can also be used to see inclusion bodies.

3. Electron microscope

4. Virus Culture:

- Primary human embryonic kidney **cell line culture**, Hela cell, Human amnion, Hep2
- Cytopathic effect should be visualized within 1-3 days

5. Serology:

- ELISA, neutralization test, Complement fixation test (CFT), Immunofluorescent test

7. Molecular diagnosis:

- **PCR**, DNA probe

Treatment:

- Acyclovir; orally or parenterally
- Ganciclovir, Viderabine, Famiciclovir; orally
- Idoxuvidine; topically in eye and skin infection

POXVIRUS (SMALLPOX)

Introduction:

- The largest and most complex viruses that occur in humans, birds, animals, and insects.
- Poxviruses (family Poxviridae) are large, brick-shaped or ovoid double-stranded DNA viruses of about 200–300 nm in diameter with a complex structure.

- Include a large group of DNA viruses that are morphologically similar and share a common nucleocapsid protein
- They cause primarily vesicular lesions in the host.
- They replicate in the cytoplasm of vertebrate or invertebrate cells
- These viruses are of special interest because of their unique biologic properties and impact on human health.
- Smallpox is a major disease among all. It is also known as variola virus.
- The primary reason for infection in humans is due to its ability to evade the host immune responses and avoid complement activation.
- The name smallpox is derived from the Latin word "spotted" and refers to the raised bumps that appear on the face and body of an infected person.
- The disease provides at least three 'firsts': the first vaccine, the first disease to be totally eradicated by immunization, and the first virus infection against which chemotherapy was clinically effective.

Classification:

- The family Poxviridae contains two subfamilies: the Chordopoxvirinae, with eight genera infecting a wide range of mammals and birds; and the Entomopoxvirinae, with three genera that affect insects only.
- The genera are distinguished on the basis of morphology, genome structure, growth characteristics, and serological reactions; there is a close serological relationship between the viruses within each genus and good cross-protection between genera.
- The poxviruses pathogenic for the man include Orthopoxvirus (smallpox, vaccinia, monkeypox and cowpox viruses), Parapoxvirus (or/ and pseudopoxviruses), Molluscipoxvirus and Yatapoxvirus.
- Most of the poxviruses that cause diseases in humans belong to the genera *Orthopoxvirus* and *Parapoxvirus*

Virus classification	
Group:	Group I (dsDNA)
Order:	*Megavirales*
Family:	*Poxviridae*
Subfamily:	*Chordopoxvirinae*
Genus:	*Orthopoxvirus*
Type species	
Vaccinia virus	
Species	
Variola virus	

Orthopoxvirus	Variola	Man	Smallpox
	Vaccinia	Man	Vesicular vaccination lesion
	Cowpox	Cattle, cats, rodents	Lesions on hands
	Monkeypox	Monkeys, squirrels	Resembles smallpox
Parapoxvirus	Pseudocowpox	Prairie dogs (USA), cattle	Localized nodular lesions ('milkers' nodes')
	Orf	Sheep, goats	Localized vesiculogranulomatc lesions
Yatapoxvirus	Tanapox	Monkeys	Vesicular skin lesions and febrile illness
Molluscipoxvirus	Molluscum	Man	Multiple small skin nodules

Morphology:

- Variola virus is a large, brick-shaped virus measuring 300×200×200nm, almost visible by light microscopy.

The poxviruses are neither icosahedral nor helical: their structure is referred to as complex.

- The outer membrane consists of a network of tubules and is sometimes surrounded by an envelope.
- Inside there is a dumbbell-shaped core structure and two accompanying lateral bodies, so named after their location in the virion.
- It has a protein-rich multilayered coat that makes it resistant to disinfectants and antiseptics
- Four major elements:

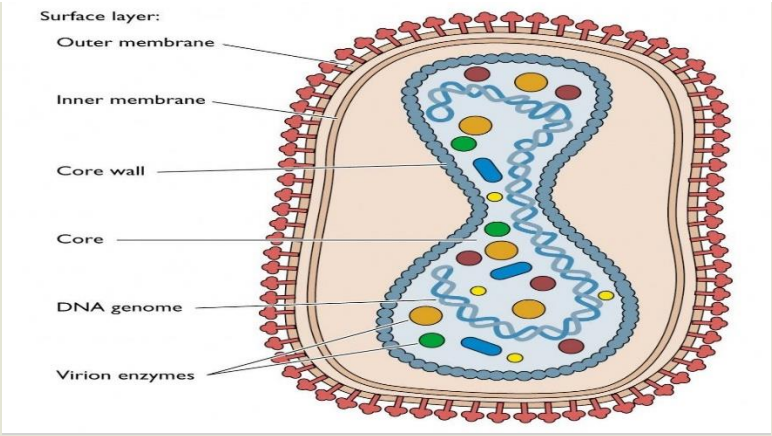
1. **core** (9 nm thick membrane, biconcave disk, a tightly compressed nucleoprotein)
2. **lateral bodies** (unknown function)
3. **outer membrane** (a protein shell 12nm thick, the surface consists of irregularly arranged tubules)
4. **envelope** (an inconstant element, proteins are glycosylated and acylated)

- Viral genome consists of a large double-stranded linear DNA that is fused at both ends.
- The DNA measures 130-375 kbp in size and has a terminal loop.
- The extracellular virion possess two envelopes while the intracellular virus has only one envelope.
- The outer envelope which encloses the extracellular virion consists of host cell lipids and several virus-specific proteins including hemagluttinins.
- These virions consist of a large number of a large number of proteins (more than 100), at least 10 of which show enzymatic activity needed for replication of the genome
- Poxvirus consists of many enzymes which facilitate in the synthesis, polyadenylation and methylation of messenger RNA (mRNA)
- They replicate in the cytoplasm of the host cell, therefore they must provide their own mRNA and DNA synthetic machinery (including DNA-dependent RNA polymerase)

- Virons are present in two infectious forms:

1. **EEV (Extracellular Enveloped Virus)-** released from cells spontaneously, by exocytoses, are enclosed within a lipoprotein envelope, which contains the haemagglutinin and other specific polypeptides – CEV (Cell Associated Enveloped Virus)

2. **IMV (Intracellular Mature Virus)** – released by cellular disruption, lacks envelope, "naked virus"

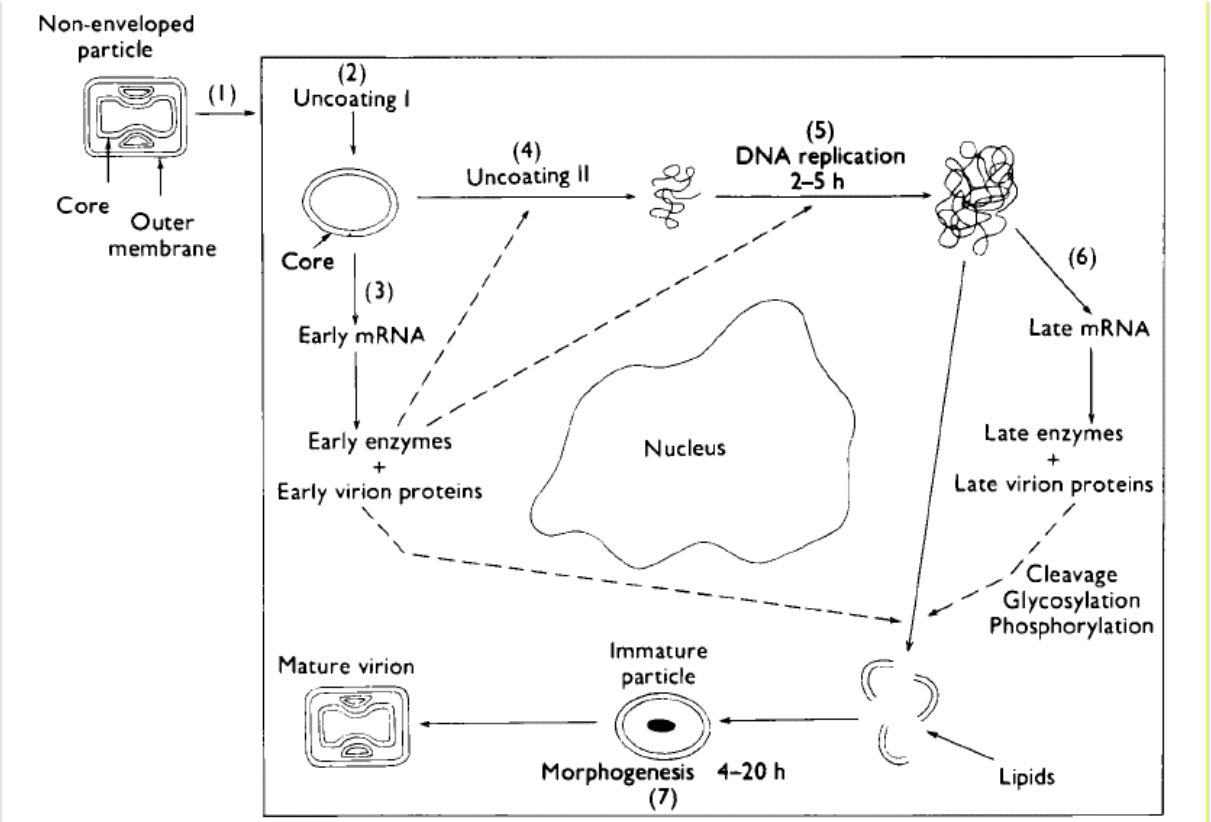


Genome:

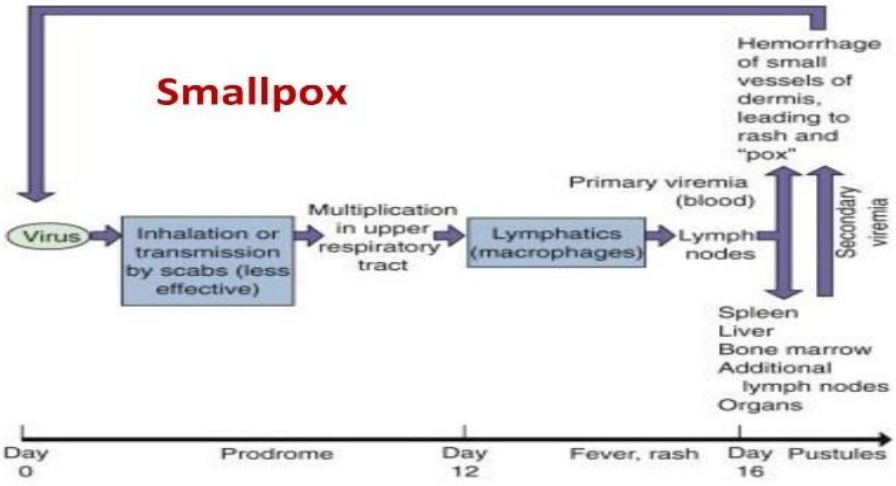
- The nucleic acid is dsDNA ranging in size from 186 kbp (variola) to 220 kbp (cowpox).
- This large genome codes for more than 100 polypeptides, including a DNA-dependent RNA polymerase and other enzymes.
- Centrally located genes are conserved and are essential for virus replication, whereas genes near the two termini affect host range and virulence.

Replication:

- Unlike most DNA viruses, the poxviruses replicate only in the cytoplasm and can replicate in cells without a nucleus.
- The virion enters the cell either by endocytosis or by a fusion event.
- The viral core enters the cytoplasm and acts as a scaffolding for subsequent replication events.
- Furthermore, the virus transports many essential enzymes such as viral transcriptase, transcription factors, capping and methylating enzymes and a poly(A) polymerase.
- Transcription of viral DNA is therefore initiated rapidly and approximately 100 early viral genes are activated, particularly genes coding for enzymes involved in viral DNA replication.
- Transcription of intermediate and late genes is initiated at the same time as DNA replication.
- Virus assembly takes place in particular areas of the cytoplasm and immature viruses can be visualized quite easily.
- During maturation, the virions move to the Golgi complex, where they are enveloped before being released by budding or by cell disruption.
- Some virions are enveloped and these may have some advantages, including speed of uptake by cells.



Pathogenesis & Immunity



Clinical manifestation:

- There are two forms of smallpox – variola major, which causes a mortality rate of 20–30% and variola minor, with a case fatality rate of 1%.
- The most characteristic feature of smallpox is the rash, which evolves over several days from macule to vesicle to a pustule.
- Finally, a scab forms, which falls off, leaving a pockmark.
- The rash first appears on the face, then on the arms, and later, on the lower limbs. It is usually more profuse on the face and limbs than on the trunk.

- The incubation period varies from 10 to 14 days
- The prodromal phase, which correlated with the phase of viremia, was the first to appear. The onset of disease was sudden. The condition manifested as sudden onset of fever, severe headache, nausea, pharyngitis, body malaise, and backache.
- An exanthematous rash would appear on the palate, tongue, and pharynx during the later part of the prodromal stage.
- The smallpox rash was characterized by skin lesions that are in the same stage of evolution, unlike those lesions were seen in chickenpox
- The skin lesions first appear on the face and extremities and then spread centrifugally to the trunk
- These lesions begin as macules and then develop into papules, vesicles, pustules, and finally crusts during a period of approximately 17 days
- The lesions heal with the formation of characteristic scarring
- Overwhelming toxemia was the usual cause of death in patients with smallpox
- There were two variants of smallpox: variola major and variola minor
- Variola major is the lethal strain, while variola minor is no lethal, but a mild strain, which is very similar to major but is only genetically different
- The variola major was associated with a fatality rate of 25–30%, while variola minor was associated with a low fatality rate of less than 1%
- In addition, flat smallpox and hemorrhagic variola were the unusual manifestations of smallpox in some patients and were usually fatal.

Laboratory diagnosis:

- **Specimens:** Skin lesions, such as vesicular fluid [characteristic smallpox lesions of the skin (deep, firm, round), which could be umbilicated or confluent; and skin lesions in the same stage of development on any part of the body]
- The differential diagnosis most often needed was between smallpox and chickenpox, which could on occasion resemble each other clinically..

Serodiagnosis:

- Useful to confirm the diagnosis of poxvirus infection
- Indirect immunofluorescent antibody test and HI, CF, and NT (neutralization test) tests are available for demonstration of serum antibodies that appear after the first week of infection.

Treatments:

- No specific antiviral agents are available against variola virus.
- Methisazone, a thiosemicarbazone, would prevent or modify an attack if given during the incubation period; but soon after the discovery of this, the first effective antiviral compound, it was made redundant by the success of the vaccination campaign.
- New antivirals are now being developed in case the virus re-emerges.
- Marboran, a thiosemicarbazone, was used to treat some of the last smallpox infections three decades ago; a more modern drug, cidofovir (HPMPC) shows antiviral effects in animal models.

PICORNAVIRUSES

- **Clinical Manifestations**
- Most infections are inapparent. Some picornaviruses cause mild illnesses; a few serotypes give rise to serious conditions of the central nervous system, heart, skeletal muscles, and liver. These varied manifestations are presented under each of the five genera.

- **Structure**
- The picornavirus virion is an icosahedral, nonenveloped, small (22 to 30 nm) particle. The capsid proteins encase a sense RNA strand consisting of approximately 7,500 nucleotides. The RNA carries a covalently bound noncapsid viral protein (VPg) at its 5′ end and a polyadenylated tail at its 3′ end.

- **Classification and Antigenic Types**
- Classification is based on morphology, physicochemical and biologic properties, antigenic structures, genomic sequence and mode of replication. The family PICORNAVIRIDAE comprises five genera, namely, enteroviruses, rhinoviruses, hepatoviruses, cardioviruses, and aphthoviruses.
- The enteroviruses are subdivided into human polioviruses (1–3); human coxsackieviruses A1–22, 24 (CA1–22 and CA24, CA23 = echovirus 9); human coxsackieviruses (B1–6 (CB1–6); human echoviruses 1–7, 9, 11–27, 29–33 (E1–7, 9, 11–27, 29–33; E8=E1; E10 = Reovirus; E28 = Rhinovirus 1A and E34 = CA24 prime strain); human enterovirus 68–71 (EV68–71); vilyuish virus; simian enteroviruses 1–18 (SEV1–18); porcine enteroviruses 1–11 (PEV1–11); bovine enteroviruses 1–2 (BEV1–2).

- **Multiplication**
- Picornaviruses multiply in the cytoplasm, and their RNA acts as a messenger to synthesize viral macromolecules. Viral RNA replicates in complexes associated with cytoplasmic membranes via two distinct, partially double-stranded RNAs - the “replicative intermediates.” One complex uses the sense RNA strand, and the other uses the antisense RNA strand as template.

- **Pathogenesis**
- Enterovirus can replicate in epithelium of the nasopharynx and regional lymphoid tissue, conjunctiva, intestines, mesenteric nodes, and the reticuloendothelial system. Viremia may cause virus transfer to the spinal cord, brain, meninges, heart, liver, and skin. Some chronic enterovirus infections result in postviral fatigue syndrome. Rhinoviruses infect and replicate mainly in nasopharyngeal epithelium and regional lymph nodes. Hepatitis A virus replicates in the intestinal epithelium, viremia transports the virus to the liver where secondary virus multiplication in the hepatocytes and Kupffer cells results in infectious hepatitis A.

- **Host Defenses**

- Interferon and virus-specific IgA, IgM, and IgG antibodies are important in host defense. Neutralizing antibody confers serotype-specific immunity.

- **Epidemiology**

- Picornaviruses are widely prevalent. Enteroviruses are transmitted by the fecal-oral route, via salivary and respiratory droplets, and in some cases via conjunctival secretions and skin lesion exudates. Cockroaches and flies may be vectors. Rhinoviruses are transmitted by saliva, respiratory discharge, and contaminated inanimate objects. Immunity is serotype specific.

- **Diagnosis**

- Viruses must be isolated and identified in the clinical laboratory. Serology is used to confirm the virus as the cause of infection and for the assessment of immune status.

- **Control**

- Poliomyelitis can be prevented by Salk-type (inactivated) and Sabin-type (live) attenuated poliovirus vaccines. Hepatitis A can be prevented by inactivated hepatitis A vaccine (Havrix). Control can be achieved via public education on transmission modes and personal hygiene. Adequate sewage disposal and uncontaminated water supplies are critical for prevention of enteroviral infections. There is no specific therapy.

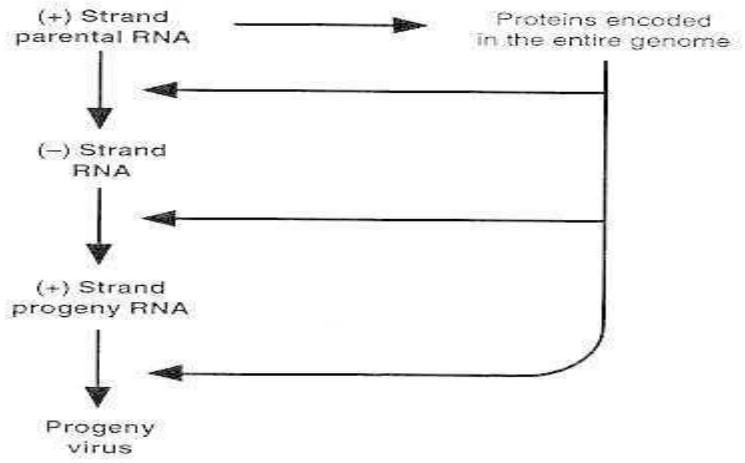
- **Enteroviruses**

- ***Poliovirus***

- Poliovirus has tropism for epithelial cells of the alimentary tract and cells of the central nervous system. Infection is asymptomatic or causes a mild, undifferentiated febrile illness. Spinal and bulbar poliomyelitis occasionally occurs. Paralytic poliomyelitis is not always preceded by minor illness. Paralysis is usually irreversible, and there is residual paralysis for life. All three poliovirus serotypes (1 to 3) can give rise to paralytic poliomyelitis.

- ***Coxsackieviruses***

- Most infections are inapparent or mild. Rashes and vesicular lesions are most commonly caused by group A coxsackieviruses and pleurodynia and viral pericarditis/myocarditis by group B coxsackieviruses. The coxsackievirus A24 variant causes epidemic and pandemic outbreaks of acute hemorrhagic conjunctivitis. Occasionally, coxsackieviruses are associated with paralytic and encephalitic diseases. Coxsackieviruses are characterized by their pathogenicity for suckling mice. They are classified by antibody neutralization tests as coxsackievirus group A (A1 to A24) and coxsackievirus group B (B1 to B6).



- ***Echoviruses***
- Echoviruses have been associated with febrile and respiratory illnesses, aseptic meningitis, rash, occasional conjunctivitis, and paralytic diseases.

- ***New Enteroviruses***
- Enterovirus types 68 and 69 cause respiratory illnesses; type 70 causes acute hemorrhagic conjunctivitis and occasionally polio-like radiculomyelitis; type 71 can cause meningitis, encephalitis and outbreaks of hand-foot-mouth disease with or without encephalitis.

- ***Rhinoviruses***
- Rhinoviruses cause mainly respiratory infections including the common cold. There are to date 115 serotypes. Immunity is type specific.

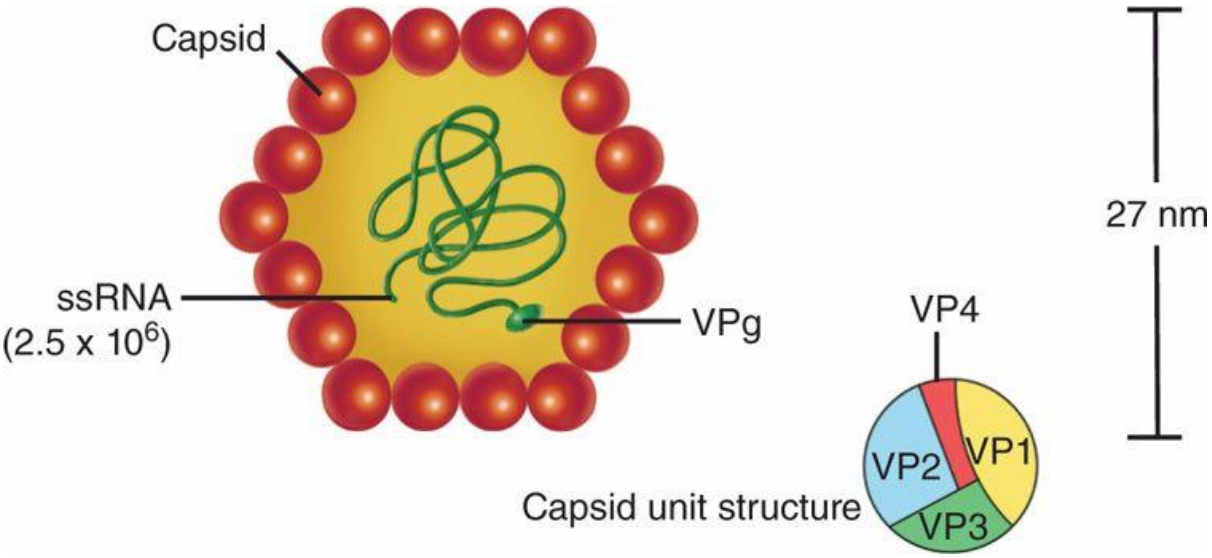
- **Hepatovirus**
- There is only one serotype of Hepatitis A virus. This virus causes gastroenteritis infections and hepatitis A

Hepatitis A virus (HAV

Properties and classification of HAV:

- Hepatitis A virus (HAV) ia a member of Family Picornaviridae. It was previously classified as **Enterovirus 72.** Now it is assigned to new genus **Heparnavirus** (Hepatovirus).
- HAV is a RNA virus.(7.48kb)
- **Genome:** the genome is single stranded positive sense RNA (+ss RNA). The 3′ end of RNA is polyadenylated and 5′ end consists of viral protein VPg. There are 4 viral proteins ie. VP1, VP2, VP3 and VP4. (* VP1 and VP3 are major antibody binding site)
- **Shape:** Small spherical
- **Capsid:** icosahedral

- **Size:** 27nm
- **Envelope:** absent (No envelope)



- **Replication:** In cytoplasm of Host cell
- **Resistance:** HAV is stable to treatment with 20% ether, acid (pH 1.0 for 2 hours), heat(60C for 1 hours) and temperature of 4C or below.
- **Susceptible:** HAV is destroyed by Autoclaving, boiling in water for 5 minutes, by dry heat (180 C for 1 hours), by UV light, formaldehyde (1: 4000 at 37C for 72 hours0 and chlorine (10-15 ppm for 30 minutes).
- Hepatitis A virus causes an acute highly contagious hepatitis, also known as infectious hepatitis in children and young adults.
- **Classification of Heparnavirus;** Hepatitis A virus is the only known species of the genus Hepatovius. There is only one serotype of HAV and 4 genotypes

Mode of transmission:

- HAV is mainly transmitted through Faeco-oral route by eating/drinking contaminated food and water.
- also transmitted by direct person to person contact
- virus is also transmitted by food handlers and children
- Rarely transmitted by blood products, blood transfusion or intravenous drug use.
- HAV is excreted in faeces during incubation period of 3-5 weeks

Pathogenesis of Hepatitis A virus:

- Human is the primary reservoir of Hepatitis A virus. The virus may survive outside the body for months.
- HAV infection is caused by ingestion of contaminated foods or water. The virus first multiplies in the intestinal epithelium and then enter the blood stream and then migrates to liver parenchymal cells.

- HAV attaches to liver cell through Ig-like cellular receptor on the host cell and enter inside the cell by receptor mediated endocytosis.
- Inside the endosome, virus releases VPg protein which creates pores on the endosomal membrane and releases viral genome in the host's cytoplasm and damage the hepatocytes.
- Virus multiplication occurs in hepatocytes and Kupffer's cells causing monuclear infiltrate, ballooning of hepatocytes, degeneration and acidophilic bodies in the hepatocytes.
- Liver damage is not due to HAV but rather due to immunological response by cytotoxic T cells.
- Newly synthesized viruses in liver cells sheds into bile ducts and excreted in faeces.
- HAV occasionally caused transient viremia.
- Rapid infection of virus is faster than development of immune response

Clinical features of Hepatitis A:

- The incubation period is between 2-1o weeks.
- After about 2 weeks of infection, virus is detectable in the liver blood and faeces.

clinical features includes-

I. Mild illness without Jundice with little liver cell damage

- Loss of appetite
- Nausea
- Gastrointestinal upset
- Enlarged and painful liver

II. Acute hepatitis with jaundice with severe liver cell damage

- More acute symptoms especially nausea
- Loss of appetite
- Fever
- Jaundice
- Headache
- Pain in muscle and joint
- Skin rashes

Laboratory diagnosis of Hepatitis A:

Specimen: faece, blood

1. Liver function test (LFT):
   - Increase in both Alanine aminotransferase (ALT) and Aspartate aminotransferase enzyme by 4-100 times in Hepatitis patient than normal.

- A sharp rise of ALT with short duration (4-20 days) is suggestive of HAV infection.
- Increase serum bilirubin level (5-20 mg/dl)
- Increase serum globulin level
- Decrease in serum albumin level

2. Serology:
   - Antibody detection: rapid kit for detection of anti-HAV IgM in serum
   - Antigen detection: detection of hemagglutination antigen or virus particle in faeces
3. Virus culture:
   - culture of HAV is difficult and takes upto 4 weeks to get result
   - In cell line culture, virus does not replicate well and produces variability till cytopathetic effects.
4. Molecular diagnosis:
   - Electron microscopy
   - RT-PCR.

POLIOVIRUS

Characteristics of Poliovirus

- **Family:** Picornaviridae
- **Genus:** Enterovirus
- Poliovirus are small, hexagonal, +ssRNA virus
- **Symmetry:** Icosahedral capsid composed of 60 capsomere
- Each capsomere consists of four viral protein (VP1- VP4)
- **Size:** 22-30nm
- **Shape:** spherical
- **Genome:** positive sense single stranded RNA (+ssRNA).
- The genome is polyadenylated at 3' end and
- Non-enveloped

Other characteristics of polio virus

- Poliovirus can survive for 4-6 months in cold water.
- It can be inactivated by pasteurization temperature, 0.3% formaldehyde, 0.1M HCL, residual cholorine of (0.3-0.5) ppm
- It is resistant to lipid soluble agents such as Ether, Chloroform, bile and proteolytic enzymes of intestine
- It can survive in faeces for months at 4° C and for years at -20°

Epidemiology:

- WHO recorded Polio cases have decreased by over 99% since 1988, from an estimated 350 000 cases in more than 125 endemic countries then, to 37 reported cases in 2016.
- Of the 3 strains of wild poliovirus (type 1, type 2, and type 3), wild poliovirus type 2 was eradicated in 1999 and no case of wild poliovirus type 3 has been found since the last reported case in Nigeria in November 2012.

- Pakistan , Afghanistan and Nigeria are endemic to poliovirus

Serotypes of Poliovirus:

On the basis of neutralization test poliovirus is divided into three serotypes.

- Poliovirus 1: it is most common and virulent type. It is frequent isolated from patients with poliomyelitis and causes epidemics
- poliovirus 2: it is usually associated with endemic infection
- poliovirus 3: it causes occasional endemic infection

Mode of transmission

- Human are only natural host for Poliovirus
- primarily by: Faeco-oral route by Ingestion of virus contaminated food and water
- Droplet infection, inhalation

Pathogenesis

1. Initial multiplication:
   - in oropharynx and intestinal epithelium
   - Incubation period: 9-12 days
   - Virus regularly present in throat and in stool of patient before clinical symptoms
2. Primary viremia:
   - Virus enter the lymphatic and blood from oropharynx and intestinal epithelium producing primary viremia
   - In most of the case, primary viremia is cleared by host defense. But in children who fail to control primary viremia develop Poliomyelitis.
3. Secondary viremia:
   - When primary viremia is not controlled then there is a secondary viremia
   - After multiplication in reticuloendothelial syetem, it envades the blood stream again causing major or secondary viremia.
   - During secondary viremia, virus crosses the blood-brain barrier and gain access to brain and spinal cord.
   - Virus multiply in nerve cell of CNS and damage anterior horn of spinal cord as well as nerve cell of medulla oblongata, pons etc. therefore patients suffer from neurological symptoms

Clinical manifestation:

Few suffer from minor illness, very few suffer from meningitis and less than 1% suffer from major paralytic disease

1. Asymptomatic illness:
   - In most of the infection is asymptomatic and self-limiting

2. Abortive poliomyelitis:
   - Non-specific symptoms such as headache, fever, sore throat, loss of appetite
   - Disease last for 5 days
3. Non paralytic poliomyelitis
   - Very few patients suffer from non-paralytic poliomyelitis
   - Stiffness of neck
   - Pain in back and neck
   - Disease last for 2-10 days
4. Paralytic Poliomyelitis:
   - Less than 1% patients suffer from major paralytic poliomyelitis
   - It damages the motor nerves causing oedema and muscle paralysis
   - Malaise
   - Anorexia
   - Nausea and vomiting
   - Sore throat
   - Constipation
   - Abdominal pain
   - Headache and fever
   - **Flaccid paralysis:** motor neuron damage
   - **Bulbar paralysis:** respiratory paralysis
5. Post poliomyelitis muscle atropy:
   - Muscle wasting
   - Loss of neuromuscular function
   - Physically Disabled

6. Death is rare. And if occur it is due to respiratory paralysis

Lab diagnosis:

Specimen: nasal secretion, faecal samples, throat swab, CSF

1. Electron Microscopy: virus detection
2. Virus isolation: culture on monkey kidney cell line, Human amnion, HeLA, Hep-2, Buffalo green monkey (BGM), MRC-5 cell line
3. Antibody detection: ELISA, complement fixation test
4. Antigen detection: neutralization test
5. Molecular diagnosis: PCR

Treatment: no antiviral drus

Prevention and control:

1. Vaccination

i. Salk's killed polio vaccine:

- Prepared by Jonas Salk in 1956
- Also known as Inactivated poliovirus vaccine (IPV)
- Prepared by formalin inactivation of poliovirus
- It is injected deep subcutaneous or intramuscular
- Given to child at age of 2 months, 4 months, at school entry age
- Effective against all serotype of poliovirus

ii. Sabin's vaccine: live attenuated vaccine

- Developed by Albert Sabin in 1962
- Contains live attenuated strain of all serotypes of poliovirus
- It is administered Orally at 2 months of age simultaneously with first DPT
- It is recommended for all children below 5 years
- In endemic countries monovalent oral poliovirus type I vaccine (MOPvI) is introduced to eliminate the last reservoir of poliovirus

2. proper sanitation
3. safe drinking water

- ## HUMAN RHINOVIRUSES

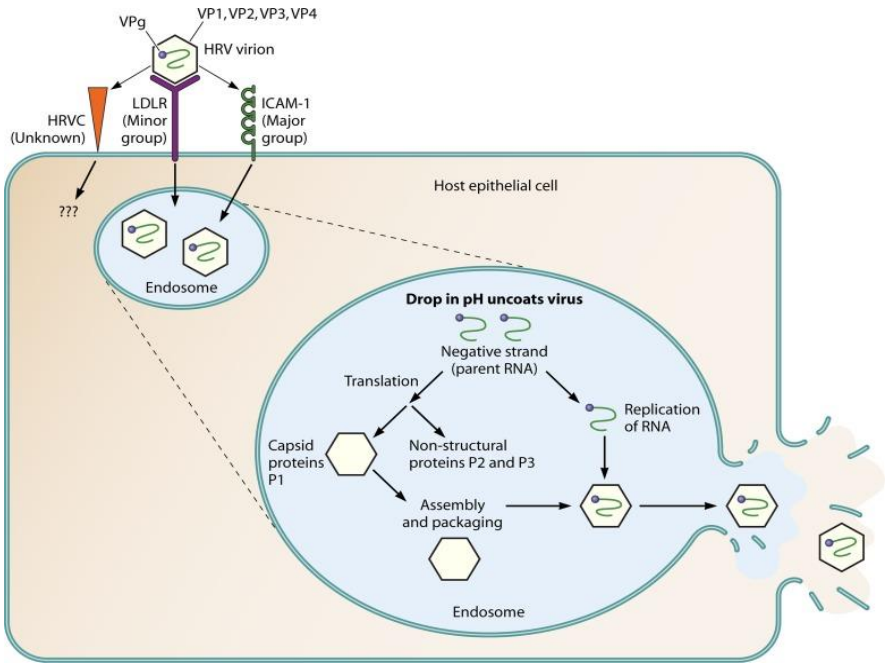
- Human rhinoviruses (HRVs) were first discovered in the 1950s in an effort to identify the etiology of the common cold. Nearly 60 years later, the search for a "cure" for the common cold virus is still ongoing.
- Efforts at vaccine development are hindered by the existence of more than 100 HRV serotypes with high-level sequence variability in the antigenic sites.

- **Virion Structure and Genomic Organization**

- HRVs, members of the family *Picornaviridae* and the genus *Enterovirus*, are positive-sense, single-stranded-RNA (ssRNA) viruses of approximately 7,200 bp. The viral genome consists of a single gene whose translated protein is cleaved by virally encoded proteases to produce 11 proteins ,

- Four proteins, VP1, VP2, VP3, and VP4, make up the viral capsid that encases the RNA genome, while the remaining nonstructural proteins are involved in viral genome replication and assembly

- **Viral Replication**

- 

- Viral replication in airway epithelial cells. Depending on the receptor type, virus uptake occurs via clathrin-dependent or -independent endocytosis or via macropinocytosis. A drop in the pH leads to viral uncoating. Negative-strand (parental) RNA is replicated as well as translated into structural (capsid) and nonstructural proteins. The virion is then assembled and packaged prior to cellular export via cell lysis (6, 7). LDLR, low-density-lipoprotein receptor; ICAM-1, intercellular adhesion molecule 1

- **Transmission**
- HRVs are transmitted from person to person via contact (either direct or through a fomite) or aerosol (small or large particle)
- HRV infection is efficiently initiated by intranasal and conjunctival inoculation but not by the oral route. In studies of natural and experimental HRV infection, the virus is regularly deposited onto the hands and introduced into the environment.
- ***Upper respiratory infections.***
  (i) Common cold.
- Studies using both molecular methods and viral culture demonstrated that HRV is the etiology of one-half to two-thirds of common colds
- ***ii) Acute otitis media.***
- In both experimental and natural settings, HRV is linked to otitis media (OM), which complicates approximately one-third of cold-like illnesses in early childhood
- ***iii) Rhinosinusitis.***
- Sinus abnormalities are frequently detected by computed tomography (CT) (109) and magnetic resonance imaging (MRI)
- **PREVENTION**

- Potential modes of person-to-person HRV transmission include small-particle aerosols, large-particle aerosols
- Maintaining hygienic practices
- **TREATMENT**
- Pirodavir
- Ruprintrivir

RHABDOVIRUSES: RABIES VIRUS

- **Clinical Manifestations**
- Rabies virus causes acute infection of the central nervous system. Five general stages are recognized in humans: incubation, prodrome, acute neurologic period, coma, and death. The incubation period is exceptionally variable, ranging from fewer than 10 days to longer than 2 years, but is usually 1–3 months.

- **Structure**
- Rabies virus is a rod- or bullet-shaped, single-stranded, negative-sense, unsegmented, enveloped RNA virus. The virus genome encodes five proteins.

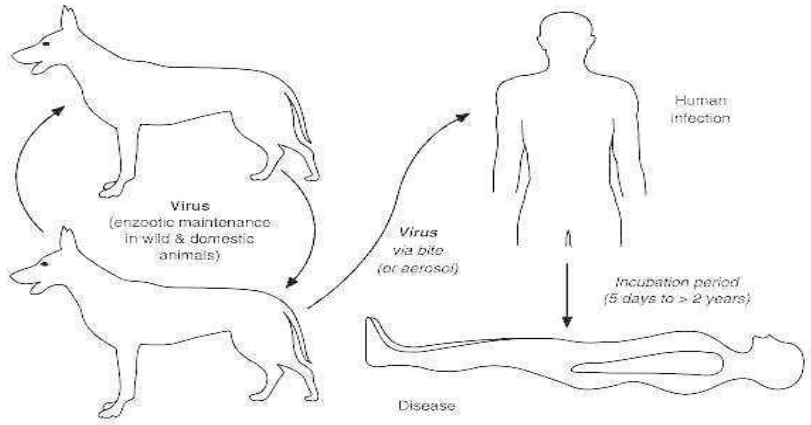
- **Classification and Antigenic Types**
- Placement within the family is based on the distinctive morphology of the virus particle. Cross- reactive nucleoprotein antigens or comparative genomic sequences determine inclusion in the genus *Lyssavirus,*which includes rabies virus and at least five other pathogenic rabies-like viruses.

- **Multiplication**
- The viral RNA uncoats in the cytoplasm of infected cells. The genome is transcribed by a virion-associated RNA-dependent RNA polymerase.
- Viral RNA is then translated into individual viral proteins. Replication occurs with synthesis of positive-stranded RNA templates for the production of progeny negative-stranded RNA.

- **Pathogenesis**
- After inoculation, rabies virus may enter the peripheral nervous system directly and migrates to the brain or may replicate in muscle tissue, remaining sequestered at or near the entry site during incubation, prior to central nervous system invasion and replication.
- It then spreads centrifugally to numerous other organs. The case:fatality ratio approaches unity, but exact pathogenic mechanisms are not fully understood.

- **Host Defenses**
- Susceptibility to lethal infection is related to the animal species, viral variant, inoculum concentration, location and severity of exposure, and host immune status. Both virus-neutralizing antibodies and cell-mediated immunity are important in host defense.

- **Epidemiology**
- Rabies occurs in nearly all countries. Disease in humans is almost always due to a bite by an infected mammal. Nonbite exposures (e.g., mucosal contact) rarely cause rabies in humans.



- **Diagnosis**
- Early diagnosis is difficult. Rabies should be suspected in human cases of unexplained viral encephalitis with a history of animal bite. Unvaccinated persons are often negative for virus-neutralizing antibodies until late in the course of disease. Virus isolation from saliva, positive immunofluorescent skin biopsies or virus neutralizing antibody (from cerebrospinal fluid, or serum of a non-vaccinated patient), establish a diagnosis.

- **Control**
- Vaccination of susceptible animal species, particularly dogs and cats, will control this zoonotic disease.

- ORTHOMYXOVIRUSES

- **Clinical Manifestations**
- Classic influenza is a febrile illness of the upper and lower respiratory tract, characterized by sudden onset of fever, cough, myalgia, malaise, and other symptoms. Many patients do not exhibit the full syndrome. Pneumonia is the most common serious complication.

- **Structure**
- Influenza viruses are spherical or filamentous enveloped particles 80 to 120 nm in diameter. The helically symmetric nucleocapsid consists of a nucleoprotein and a multipartite genome of single-stranded antisense RNA in seven or eight segments. The envelope carries a hemagglutinin attachment protein and a neuraminidase.

- **Classification and Antigenic Types**
- Influenza viruses are divided into types A, B, and C on the basis of variation in the nucleoprotein antigen. In types A and B the hemagglutinin and neuraminidase antigens undergo genetic variation, which is the basis for the emergence of new strains; type C is antigenically stable.

- **Multiplication**
- The virus binds to host cells via the hemagglutinin. Transcription and nucleocapsid assembly take place in the nucleus. Progeny virions are assembled in the cytoplasm and bud from the cell membrane, killing the cell. In cells infected simultaneously with more than one parent virion, the genome segments may undergo reassortment.

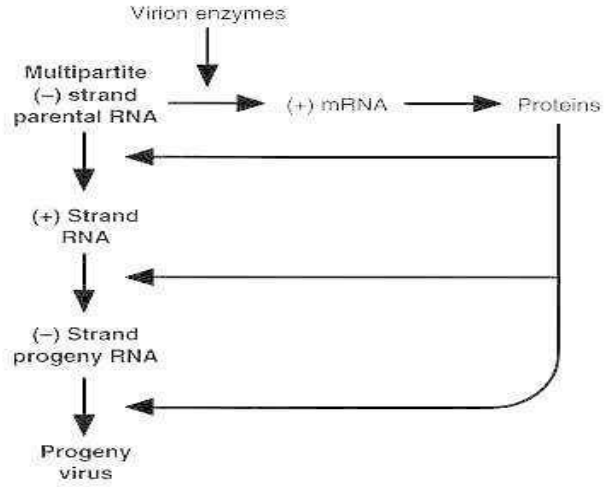
- **Pathogenesis**
- The virus is transmitted in aerosols of respiratory secretions. It multiplies in the respiratory mucosa, causing cellular destruction and inflammation.

- **Host Defenses**
- Both a cell-mediated response and antibody develop after infection. Antibody provides long-lasting immunity against the infecting strain.

- **Epidemiology**
- Influenza epidemics involving all age groups occur each winter; worldwide pandemics appear irregularly. Changes in the hemagglutinin and neuraminidase surface antigens are responsible for the appearance of antigenically novel strains that evade host immunity and cause reinfections.

- **Diagnosis**
- The diagnosis is suggested by the symptoms, particularly if an influenza epidemic is under way. Definitive diagnosis depends on detecting the virus or a rise in antibody titer.

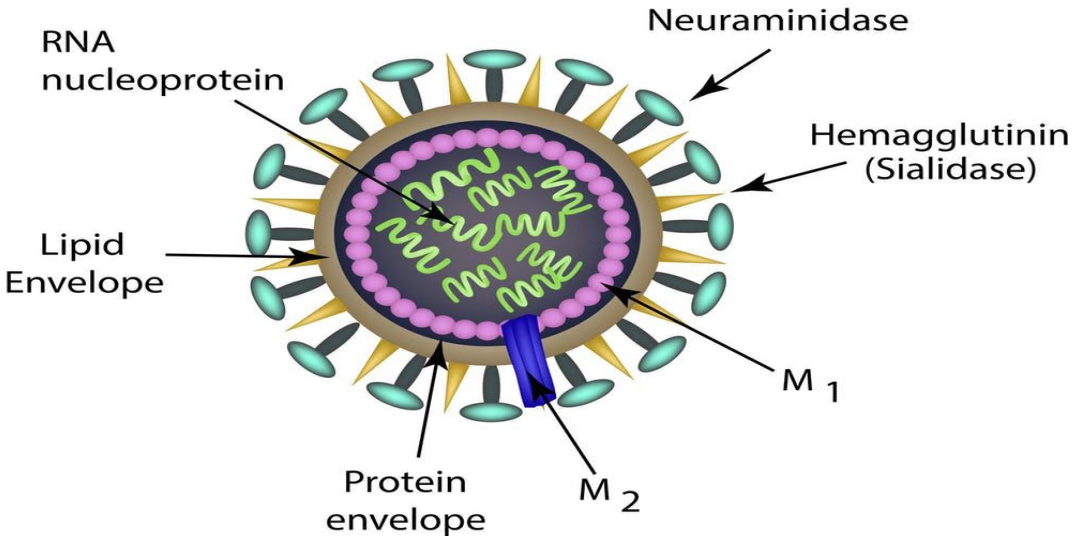
- **Control**
- An inactivated virus vaccine is developed each year against the strains most likely to cause disease the next winter. The drugs amantadine and rimantadine can be used for prophylaxis and treatment of influenza A infections.



INFLUENZA VIRUS

- The family Orthomyxoviridae contains a single genus Influenza virus with three types- A, B and C. Influenza viruses are classic respiratory viruses. They cause influenza, an acute infections disease of the respiratory tract that occurs in sporadic, epidemic and pandemic forms
- A unique feature of influenza virus is its ability to undergo antigenic variations. The surface glycoprotein (Haemagglutinin and Neuraminidase) show variations and are primarily responsible for antigenic variations. Antigenic variability is highest in influenza virus type A and less in type B, while it has not been demonstrated in types C.

Structure of Influenza virus



- **Shape**:
  - The influenza virus particle is typically spherical with a diameter of about 80-120 mm but pleomorphism is common.

- Filamentous forms upto several micrometers in length and readily visible under the dark ground microscope are frequent seen in freshly isolated strains.

- **Symmetry**:
  - The virus is core consists of ribonucleoprotien in helical symmetry.
  - The nucleoprotein (NP) associates with the viral RNA to form a ribonucleoprotein (RNP) which is a structure of 9 mm in diameter that assumes a helical configuration and forms the viral nucleocapsid.
- **Genome and protein**:
  - Influenza virus contains negative sense single stranded RNA (-ssRNA) genome which is segmented.
  - Type A and B influenza virus consist of 8 pieces of segmented RNA (while type C influenza virus contains 7 segments), which encode for 11 proteins (HA, NA, NP, M1, M2, NS1, NEP, PA, PB1, PB1, PB2).
  - Because of the segmented nature of the genome when a cell is infected by two different viruses of a given type, mixtures of parental gene segments, may be assembled in progeny visions. This phenomenon call genetic re-assortment, may result in sudden changes in viral surface antigens- a property that explains the epidemiological features of influenza and poses significant problems for vaccine development.
  - Also contains a viral RNA-dependent RNA polymerase that transcribes the negative polarity genome into mRNA.
  - Three large proteins (PB1, PB2, and PA) are bound to the viral Ribonucleoprotein and are responsible for RNA transcription and replications.
  - The matrix (M1) protein which forms a shell underneath the viral lipid envelope is important in particle morphogenesis and is a major competent of the virion.
- **Envelope and glycoprotein spikes:**
  - The nucleocappsid is surrounded by an envelope which has an inner membrane of protein known as matrix or M protein which is virus encoded and outer lipid layer derived from infected host cell membrane during the process of replication by budding.
  - Two virus encoded glycoproteins, the haemagglutinin (HA) and the neuraminidase (NA) are inserted into the envelope and are exposed as spikes about 10mm long on the surface of the surface of the particles.
  - These glycoproteins are triangular and mushroom shaped respectively. They are synthesized in the early period of replication cycle, and get attached to the plasma membrane at specialized patches where budding occurs.
  - These two surface glycoproteins are the important antigens that determine antigenic variation of influenza viruses and host immunity.
  - The HA represents about 25% of viral protein the NA about 5%.
  - The M2 ion channel and the NS2 protein are also present in the envelope but a few copies per particle.
- **Haemagglutinin (HA):**
  - Haemagglutin derives its name from its ability to agglutinate erythrocytes under certain conditions.

- Haemagglutinin is a glycoprotein composed of two polypeptides- HA 1 and HA2, responsible for hemadsorption and haemagglutination. These two polypeptides are joined together by disulfide bond.
- The Haemagglutinin consists of 500 spikes each measuring 12nm in length.
- The triangular shaped HA is inserted into the virus membrane by its tail end which is hydrophilic in nature. The distal end which contains five antigenic sites (HA1-HA5) is responsible for binding of vision to host cells.
- Haemagglutinin is one of the major antigen of influenza virus and is responsible for antigenic variation.
- HA enables the virus to absorb to muco-protein receptors on red cells as well as an respiratory epithelial cells.
- HA agglutinates certain RBCs which is inhibited by the neutralizing antibodies. This forms the basis of the **haemagglutination inhibition test** used in the sero-diagnosis of influenza.

- **Neurominidase (NA):**
  - Neuraminidase is a glycoprotein receptor and is important in determining the subtype of influenza virus isolates.
  - It consists of 100 mushroom-shaped spikes that is a tetramer, composed of four identical monomers. A slender stalk is tapped with a box shaped head.
  - NA functions at the end of the viral replication cycle.
  - Neuraminidase is a sialidase enzyme that removes sialic acid from glycol-conjugates. It causes hydrolysis of N-acetyl neuraminic acid or sialic acid residues present on the glycoprotein receptors on red cells, hence causes elution or detachment of cells absorbed to virion particles.
  - It facilitates release of the virus particles from infected cells surface during the budding processes and helps prevent self-aggregation of virions by removing sialic acid residues from viral glycoprotains.
  - It also degrades the mucus layer, thereby exposing the epithelial membrane of the respiratory tract for infection of the virus.

Types of Influenza viruses

On the basis of antigenic differences in nucleoprotein and the matrix protein (M) the influenza virus is divided into 3 types.

ROTAVIRUS

- Family: Reoviridae
- Genus: Rotavirus

<u>Classification of Rotavirus:</u>

- Classified into seven distinct groups (A to G) based on structural antigen VP6.
- Group A, B, and C Rotaviruses are found in Human infection as well as animal infection
- Group A Rotaviruses are most frequent Human pathogen

Structure, composition and properties of Rotavirus

1. Structure:

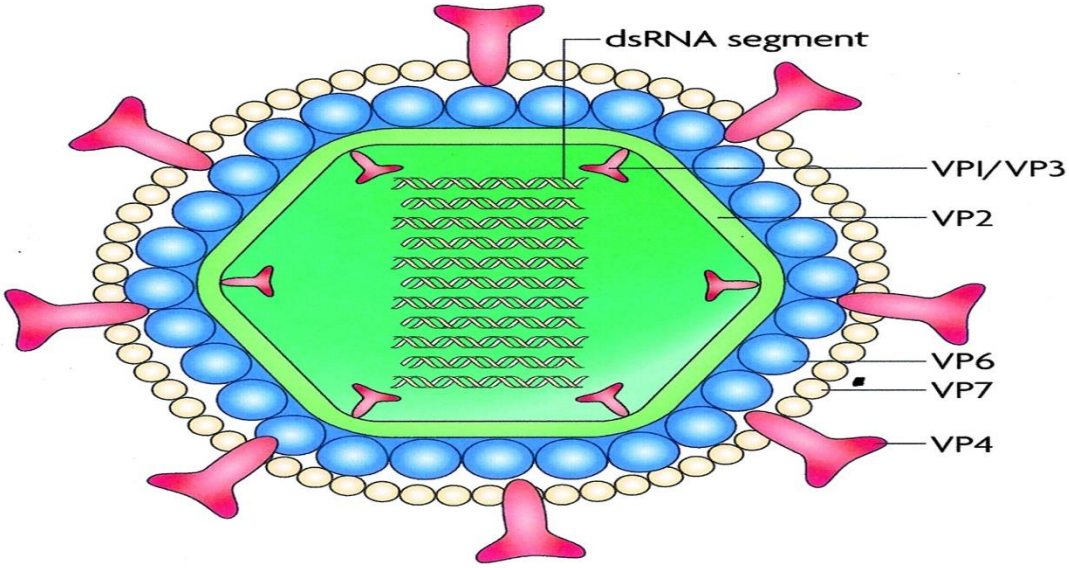


Figure: Structure of Rotavirus

- Characteristics **"wheel"** like appearance (Rota-means wheel)
- **Size:** 65nm-100nm in diameter
- **Shape:** Spherical shape
- **Symmetry:** Icosahedral

2. Genome composition:

- **Genome:** 11 segments of **double stranded RNA (ds RNA**)
- **Protein:** 6 structural protein (VP) and 6 Non-structural protein (NSP)
- **Envelope:** Absent
- Nucleic acid is surrounded by two layer of capsid- **inner capsid (VP6)** and **outer capsid (VP7)**
- **VP4** is the spike protein, it is a cell surface receptor

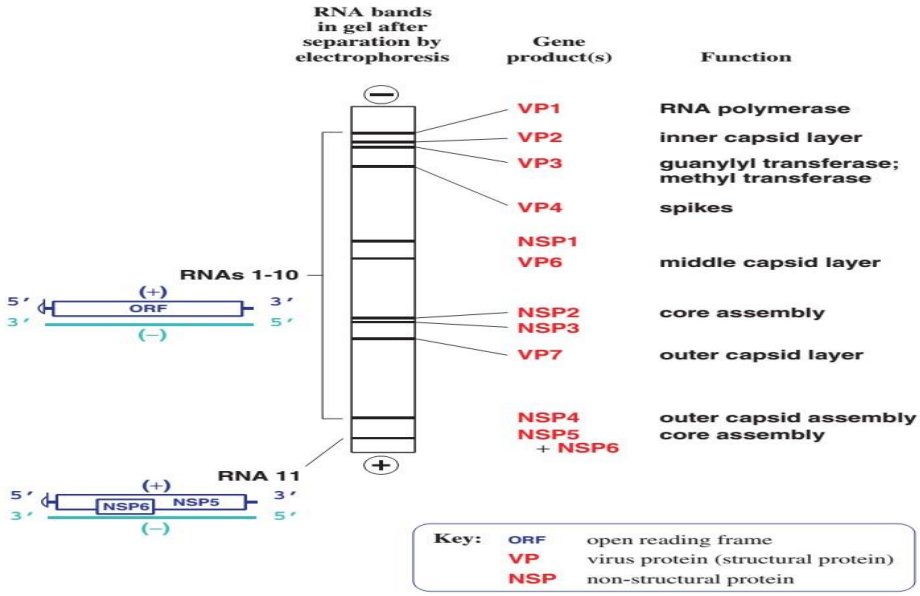


Figure: 11 segments of genome of Rotavirus

3. Other properties
   - **Replication:** Occurs in cytoplasm of infected cell.
   - Rota virus contain an **RNA-dependent RNA polymerase** and other enzymes capable of producing capped RNA transcripts
   - Rota virus do not grow in cell line culture

Rotaviruses are inactivated by-

- Heating to 100°C
- Treatment with acid (pH<3),
- Glutaraldehyde (3%),
- Phenol,
- Formalin,
- Chlorine
- Alcohol (70%),

Replication of Rota virus:

1. Attachment: by VP4 on cell surface receptor
2. Penetration: receptor mediated endocytosis
3. Un-coating in lysosome
4. Transcription is mediated by endogenous virus dependent RNA polymerase (transcriptase)
5. Translation to produce viral structural protein
6. Synthesis of full length transcript
7. Some of the full length transcript are encapsidate
8. Synthesis of –ve sense RNA strand with capsid to form ds RNA
9. Formation of inner capsid
10. Morphogenesis: budding of single shelled virus into RER acquiring pseudo envelope
11. Removal of pseudo envelope and replaced by outer capsid in RER
12. Maturation
13. Cell lysis and Release

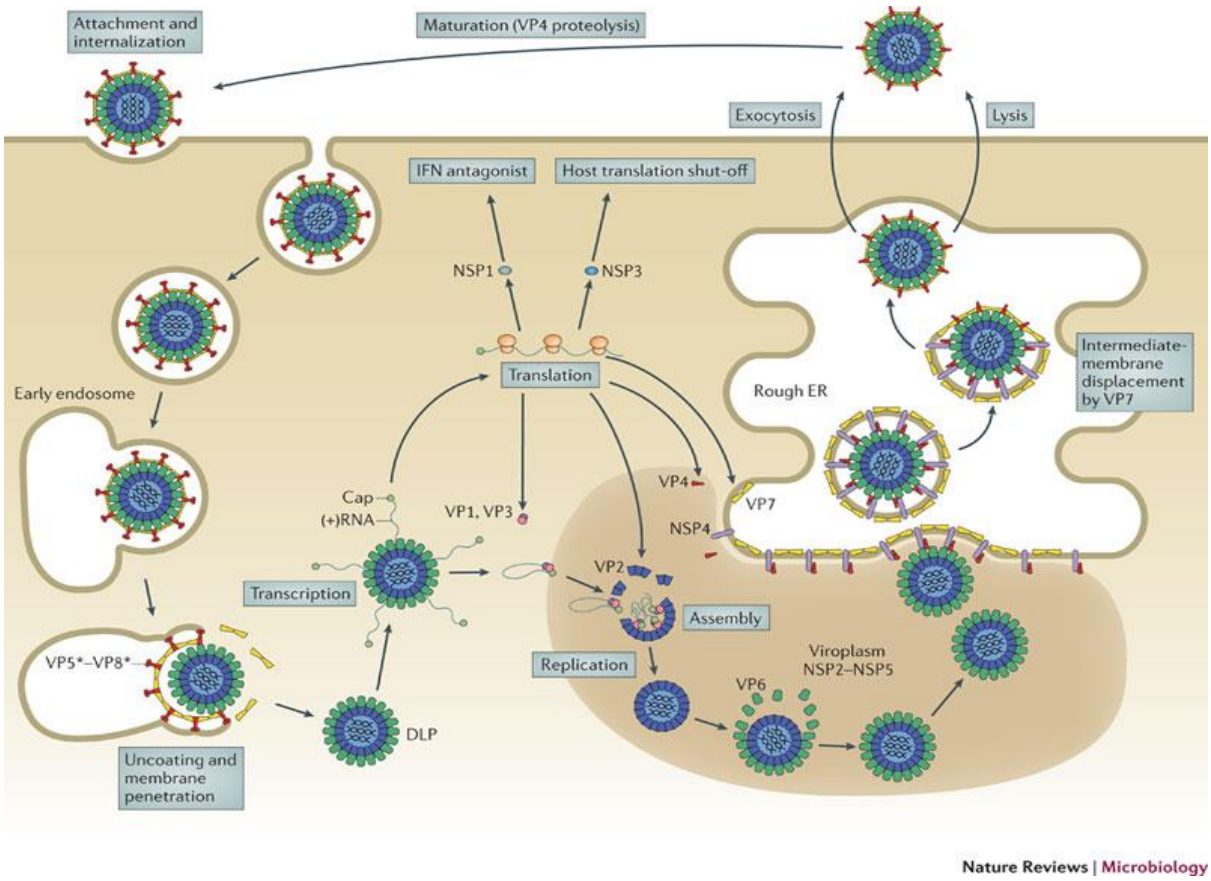


Figure: Replication cycle of Rotavirus

Mode of Transmission:

- Ingestion of contaminated food and water
- Directly from faeces contaminated fingers
- Occasionally by droplet infection
- Children below 5 years are mostly affected
- Adults are infected by contact with pediatric cases

Incubation period: 2-3 days

Pathogenesis:

- Rota virus replicates in enterocyte near the tip of villi destroying enterocytes
- Viral encoded toxin: early profuse, secretory diarrhea is caused by enterotoxin, NSP4.
- Disruption of intestinal epithelium due to virus replication
- Histologic changes of enterocytes that triggers enteric nervous system, intestinal secretion and immune response

- The acute infection and diarrhoea normally resolves within 7 days in immunocompetent hosts.

Clinical symptoms:

1. Local infection:

- Acute Gastroenteritis, severe in case of infants aged 6-24 months.
- Infected Infants are unable to digest milk due to lactase deficiency caused by destruction of enterocytes
- Diarrhoea, nausea and vomiting
- Malabsorption of Na+, water, and disaccharides
- **Symptoms of Dehydration:** decrease in urination, dry mouth and throat and feeling dizzy when standing up.

2. Systemic infection:
   - High grade Fever
   - Lymphocytosis and transient neutropenia.
3. Complication:
   - Febrile Convulsion in small children
   - Severe dehydration, hypotonia and shock

Laboratory diagnosis:

Specimen: faeces in early infection,

- Viral antigen detection: solid phase agglutination, ELISA
- Electron microscopy
- EIA (enzyme immune assay): it is sensitive to detect virus in stool
- Dip stick/ rapid test
- PCR: For genotyping of Rotavirus
- Virus culture: No cell line culture

Treatment:

- Oral rehydration
- Other supportive rehydration therapy to control loss of water and electrolytes
- Vaccine: Two Oral rotavirus vaccines are currently licensed for use in infants
    1. RotaTeq® (RV5) is given in 3 doses at ages 2 months, 4 months, and 6 months
    2. Rotarix® (RV1) is given in 2 doses at ages 2 months and 4 months

Prevention and control:

- Sanitation
- Waste water treatment

- Health professional should wash their hands with soap and water before and after patient contact.

SARS (SEVERE ACUTE RESPIRATORY SYNDROME)

- ***Cause***
- SARS coronavirus (SARS-CoV) – virus identified in 2003. SARS-CoV is thought to be an animal virus from an as-yet-uncertain animal reservoir, perhaps bats, that spread to other animals (civet cats) and first infected humans in the Guangdong province of southern China in 2002.
- ***Transmission***
- An epidemic of SARS affected 26 countries and resulted in more than 8000 cases in 2003. Since then, a small number of cases have occurred as a result of laboratory accidents or, possibly, through animal-to-human transmission (Guangdong, China).
- Transmission of SARS-CoV is primarily from person to person.
- It appears to have occurred mainly during the second week of illness, which corresponds to the peak of virus excretion in respiratory secretions and stool, and when cases with severe disease start to deteriorate clinically.
- Most cases of human-to-human transmission occurred in the health care setting, in the absence of adequate infection control precautions. Implementation of appropriate infection control practices brought the global outbreak to an end.

Nature of the disease

- Symptoms are influenza-like and include fever, malaise, myalgia, headache, diarrhoea, and shivering (rigors). No individual symptom or cluster of symptoms has proved to be specific for a diagnosis of SARS.
- Although fever is the most frequently reported symptom, it is sometimes absent on initial measurement, especially in elderly and immunosuppressed patients.
- Cough (initially dry), shortness of breath, and diarrhoea are present in the first and/or second week of illness. Severe cases often evolve rapidly, progressing to respiratory distress and requiring intensive care.

Geographical distribution

- The distribution is based on the 2002–2003 epidemic. The disease appeared in November 2002 in the Guangdong province of southern China.
- This area is considered as a potential zone of re-emergence of SARS-CoV.
- Other countries/areas in which chains of human-to-human transmission occurred after early importation of cases were Toronto in Canada, Hong Kong Special Administrative Region of China, Chinese Taipei, Singapore, and Hanoi in Viet Nam.

Risk for travellers

- Currently, no areas of the world are reporting transmission of SARS. Since the end of the global epidemic in July 2003, SARS has reappeared four times – three times from laboratory accidents (Singapore and Chinese Taipei), and once in southern China where the source of infection remains undetermined although there is circumstantial evidence of animal-to-human transmission.
- Should SARS re-emerge in epidemic form, WHO will provide guidance on the risk of travel to affected areas.

- Travellers should stay informed about current travel recommendations. However, even during the height of the 2003 epidemic, the overall risk of SARS-CoV transmission to travellers was low.

Prophylaxis

- None. Experimental vaccines are under development.
- ***Precautions***
- Follow any travel recommendations and health advice issued by WHO.

- ## H1N1 FLU VIRUS (SWINE FLU)

- H1N1 flu is also known as swine flu. It's called swine flu because in the past, the people who caught it had direct contact with pigs. That changed several years ago, when a new virus emerged that spread among people who hadn't been near pigs.
- In 2009, H1N1 was spreading fast around the world, so the World Health Organization called it a pandemic. Since then, people have continued to get sick from swine flu, but not as many.
- While swine flu isn't as scary as it seemed a few years ago, it's still important to protect yourself from getting it. Like seasonal flu, it can cause more serious health problems for some people. The best bet is to get a flu vaccine, or flu shot, every year. Swine flu is one of the viruses included in the vaccine.
- **How Do You Catch It?**
- The same way as the seasonal flu. When people who have it cough or sneeze, they spray tiny drops of the virus into the air. If you come in contact with these drops, touch a surface (like a doorknob or sink) where the drops landed, or touch something an infected person has recently touched, you can catch H1N1 swine flu.

What Is Age-Related Macular Degeneration?

Age-related macular degeneration is an eye disease that may get worse over time. It’s the leading cause of severe vision loss in people over age 60. Learn more about the symptoms,

Causes of Polycythemia Vera

You can have polycythemia vera for years without knowing it. Find out more about the symptoms of polycythemia vera, how it affects your body, and what causes it.

SWINE FLU SYMPTOMS

These, too, are pretty much the same as seasonal flu. They can include:

- Cough
- Fever
- Sore throat
- Stuffy or runny nose
- Body aches

- Headache
- Chills
- Fatigue

Like the regular flu, swine flu can lead to more serious problems including pneumonia, a lung infection, and other breathing problems. And it can make an illness like diabetes or asthma worse. If you have symptoms like shortness of breath, severe vomiting, pain in your belly or sides, dizziness, or confusion, call your doctor or 911 right away.

Are There Tests for Swine Flu?

Yes. Without one it's hard to tell whether you have swine flu or seasonal flu, because most symptoms are the same. If you have swine flu, you may be more likely to feel sick and your stomach and throw up than with regular flu. But a lab test is the only way to know. Even a rapid flu test you can get in your doctor's office won't tell you for sure.

To test for swine flu, your doctor runs a swab -- a bigger version of the ones in your bathroom -- up the inside of your nose around the back of your throat. But the test isn't as common or widespread as those for regular flu. So the only people who really need to be tested are those in the hospital or those at high risk for life-threatening problems from swine flu, such as:

- Children under 5 years old
- People 65 or older
- Children and teens (under age 18) who are getting long-term aspirin therapy and who might be at risk for Reye's syndrome after being infected with swine flu. Reye's syndrome is a life-threatening illness linked to aspirin use in children.
- Pregnant women
- Adults and children with chronic lung, heart, liver, blood, nervous system, neuromuscular, or metabolic problems
- Adults and children who have weakened immune systems (including those who take medications to suppress their immune systems or who have HIV)
- People i in nursing homes and other long-term care facilities

How Is It Treated?

Some of the same antiviral drugs that are used to treat seasonal flu also work against H1N1 swine flu. Oseltamivir (Tamiflu), peramivir (Rapivab), and zanamivir (Relenza) seem to work best, although some kinds of swine flu don't respond to oseltamivir.

These drugs can help you get well faster. They can also make you feel better. They work best when you take them within 48 hours of the first flu symptoms, but they can help even if you get them later on.

Antibiotics won't do anything for you. That's because flu is caused by a virus, not bacteria.

Over-the-counter pain remedies and cold and flumedications can help relieve aches, pains, and fever. Don't give aspirin to children under age 18 because of the risk of Reye's syndrome. Make sure that over-the-counter cold medications do not have aspirin before giving them to children.

SUB-VIRAL ENTITY # 1. VIROIDS:

Viroids are a novel class of sub-viral pathogens that are found to cause diseases on plants and are the smallest known infectious agents.

They are also known by the names ‘metaviruses’ or ‘pathogene’ and differ basically from viruses in at least following features:

(i) Virus-RNA is enclosed in a protein coat while the viroids lack any protein coat and apparently exist as free-RNA,

(ii) Viroid-RNA is of small size consisting of 246-375 nucleotides as compared to 4-20 kb of virus-RNA, and

(iii) Viroid-RNA consists of only one molecular species only, while many virus-RNA exist as more than one molecular species within the same capsid.

Discovery:

The first viroid was discovered by T.O. Diener in 1971 who found it to be the causative agent of Potato spindle tuber disease (Diener, 1979), the disease previously considered to be caused by Potato spindle tuber virus.

Since then, several other plant diseases are now known to be caused by viroids; some important ones are Chrysanthemum chlorotic mottle disease, Chrysanthemum stunt disease, Citurs excortis disease,Coconut cadang-cadang disease, Tomato bunchy top disease, Tomato apical stunt disease etc.

Morphology:

Viroids are small, circular, single-stranded RNA molecules ranging from 246 nucleotides (Coconut cadang-cadang viroid) to 375 nucleotides (Citrus excortis viroid) in size. Their molecular weight is low and ranges from 85,000 to 1,30,000 daltons. The extracellular form of viroid is naked-RNA, there is no oapsid of any kind.

Even more interestingly, the RNA molecule contains no protein encoding genes and, therefore, the viroid is totally dependent on host function for its replication. Although the viroid is a single- stranded circular RNA molecule, there is such considerable secondary structure possible that it resembles a short-stranded molecule with close ends (Fig. 11.10).

FIG. 11.10. Diagram of viroid ss-RNA showing how single-stranded circular RNA forms a seemingly double-stranded structure by intra-strand base pairing.

Replication:

Viroids seem to be associated with the cell nuclei, particularly the chromatin, and possibly with the endomembrane system of the host cell. There is evidence that viroids replicate by direct RNA copying in which all components required for viroid-replication including the RNA polymerase are provided by the host.

Branch et al. (1981), Owens and Diener (1982) and Branch and Robertson (1983) have proposed the following mode for viroid (Potato Spindle Tuber Viroid; PSTV) replication (Fig. 11.11).

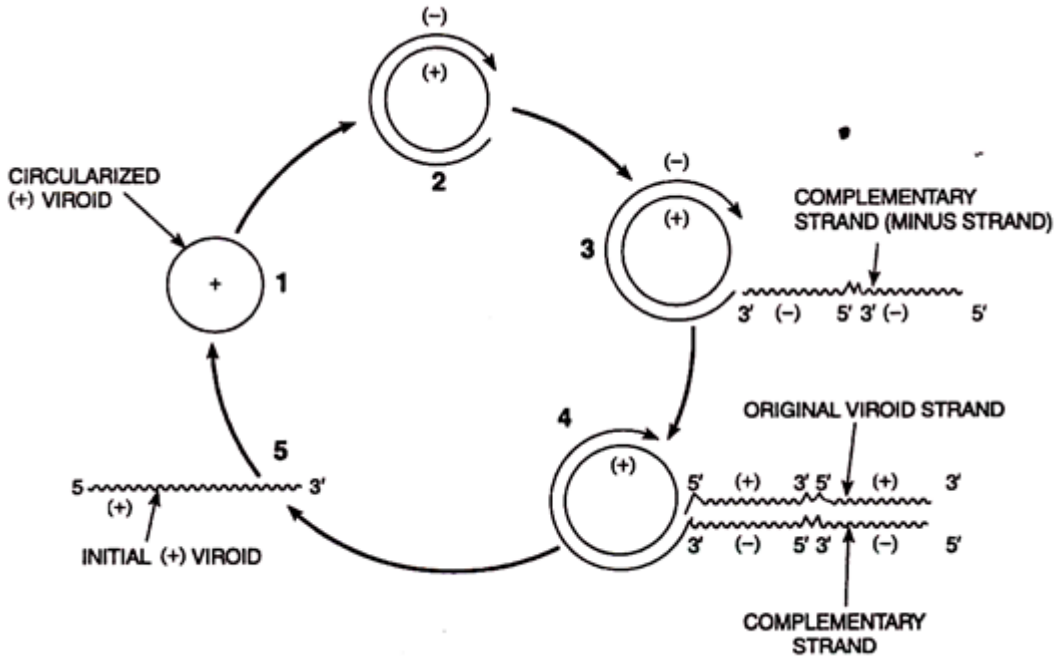


FIG. 11.11. Schematic representation of presumed viroid replication. 1-5 are the sequential steps of replications.

The infecting viroid strand (marked '+') enters a cell, moves into the nucleus and initiates the synthesis of minus (-) strand (i.e., the complementary strand) by a rolling circle mechanism proposed earlier by Brown and Martin (1965) for replication of certain viral RNAs.

The linear (-) strand of RNA then serves as a template (complementary) for replication of strand of (+) RNA. The (+) RNA is subsequently cleaved by enzymes that release linear, unit length viroid (+) RNAs, and these circularize and produce many copies of the original viroid RNA.

Transmission:

Viroids possibly cannot be transmitted as naked RNAs because of their susceptibility to nuclease enzyme. They, however, are protected from this enzyme-attack by being localized within the nuclei of infected cells (Sanger, 1979). Presumably, the viroids are transmitted in association with pieces of nuclei or chromatin and not as free RNA.

Their transmission from diseased to healthy plants takes place primarily by mechanical means, i.e., through sap carried on hands or tools during propagation or cultural practices, and by vegetative propagation. No specific insect or other vectors of viroids are known.

Origin of Viroids:

Origin of viroids is still speculative as there is no sufficient knowledge available in this regard.

However, there are different views to explain the origin of viroids, the most favoured views are as follows:

(i) The viroids are considered to have originated by circularization of spliced-out intervening sequences (introns) during RNA splicing. The introns are usually considered as nonsense

sequences that possibly rapidly degrade. If these excised sequences (introns) undergo extensive intramolecular base- pairing and become circularized, they may become stabilized, do not undergo degradation and give rise to viroids.

These 'viroids' may possess the relevant recognition sites and could be transcribed successfully by RNA dependent RNA polymerase enzyme. Interestingly, introns, including the ones of viroids size, have been observed to undergo circularization.

In the light of this view, the viroids may be considered 'escaped' introns and, like self-splicing introns, appear to be the remnants of an 'RNA World' evolved during the early stages of biological evolution.

(ii) Watson et al. (1987) emphasize the other view. According to them the viroids are supposed to be the 'primitive viruses' and must have originated from cellular RNAs. It has been found that RNA synthesis on RNA template takes place in most of the healthy plants. Viroids would have originated from this RNA as they did not induce the biosynthetic machinery of their host from their own replication.

SUB-VIRAL ENTITY # 2. VIRUSOIDS:

Similar to viroids, the virusoids are small, low molecular weight, circular RNAs; they are always associated with a larger RNA molecule of a virus. The virusoids were discovered by Randles (1981).

It is thought that some virusoids are necessary for the replication of RNA of the virus with which they are associated, and may form part of the viral genome. One virusoid has been found associated with Velvet tobacco mosaic virus.

Other virusoids have been found to be more like a satellite, i.e., extra RNA associated with virus capable only of replicating in cells infected by the virus. It has also been found that virusoids produce such structures in infected cell suggest that thereby that their replication cycles resemble those of the potato spindle tuber virusoid and other virusoids.

SUB-VIRAL ENTITY # 3. PRIONS (THE PUZZLING PROTEINS):

C. Gajdusek (1957) came across a mysterious disease in New Guinea tribals, which was later named as 'Kuru', and prepared neuropathological specimens from a person who died of Kuru. Williair Hadlow who was working on scrapic disease of sheeps and goats examined Gajdusek's neuropathological specimens and observed during 1957 remarkable similarities between the abnormalities found in brains of Kuru victim and the sheeps and goats dying of scrapie.

Similar observations were made by British investigators T. Alpher, D. Haig and M. Clark during 1966. In 1970s S.B. Prusiner, a biochemist at the University of California (USA), with his coworkers, initiated the isolation and identification of the infectious agent of scrapie.

After exhaustive research for a decade, he in 1982 discovered that the disease is caused by a proteinaceous infectious particle which he christened as 'prions' (derived from Proteinaceous and Infections). S.B. Prusiner has been awarded Nobel Prize in 1997 for the discovery of prions.

Prions represent the other extreme from viroids. They are considered to be devoid of their own genetic material (DNA or RNA) and consist of just a single, two, three, or more protein molecules i.e. a prion is merely an infectious protein. The discovery of prions has threatened the universally accepted concept that only the genetic material (DNA, in some cases RNA) is infectious.

If prions lack their own nucleic acids and are merely proteins, a very important question requires an answer. How can a protein enter a host cell and direct the process of replication? To answer this question a large number of hypotheses have been put forward.

An interesting hypothesis has been given by a group of scientists from the MRC Neuropathogenesis Unit at Edinburg. This hypothesis states that the existence of small piece of 'DNA gene' (also called PrP gene) is necessary to encode the amino acid sequence of prion protein at the time of its replication.

This 'DNA gene' is a component of the host genetic material (host DNA). The prion protein presumably serves as a promoter of 'DNA gene' expression.

Structure and Chemical Nature:

Prions are 100 times smaller than viruses, contain only protein, are heterogenous in size and density, and can exist in many molecular forms. Gel electrophoresis investigations have revealed that prions possess an apparent molecular weight between 27,000 and 30,000 daltons.

Electron microscopic studies have shown that a large number of prion molecules (~1000) aggregate together to form a composite structure called 'prion-seed'. The latter are typically 100 to 200 nm in length and 10-20 nm in diameter.

The chemical nature of the prions, as stated earlier, is considered to be proteinaceous and they have no nucleic acids of their own. This has been indicated by the various experimental evidences gathered so far. This aspect of prions has been investigated by treating them with nucleases (the enzymes that digest nucleic acids) and proteases (the enzymes that digest proteins).

It has been observed that the nucleases have no effect on prion infectivity, whereas proteases can drastically reduce a prion infectivity. In addition, prions show high resistance to ionizing and ultraviolet radiations, which act mainly on nucleic acids.

Prion Diseases:

Prion diseases, collectively called as transmissible spongiform encephalopathies (TSEs), are degenerative disorders of the central nervous system (neurodegenerative diseases) leading to motor dis function, dementia, and death. In all disorders now referred to as prion diseases, spongiform degeneration and astrocytic gliosis is found upon microscopic examination of the central nervous system.

Prion diseases may present as genetic, infectious, or sporadic disorders, all of which involve modification of the cellular prion protein (PrP^{c}), a constituent of normal mammalian cells, into infectious protein (PrP^{Sc}). However, some important prion diseases are listed in Table

TABLE 11.1. Some Important Prion Diseases

Disease	*Host*	*Remarks*
Kuru	Fore people of Papua-New Guinea	***Kuru*** was once prevalent prion disease found in Fore people (some cannibal trivals) of Papua-New Guinea who used to practice the trival ritual of eating the brains of dead Kinsmen (*i.e.* the prions were transmitted by ritual cannibalism). With the cessation of cannibalism at the urging of missionaries, Kuru began to decline long before and is now extinct.
CJD	Humans	***Creutzfeldt-Jakob disease (CJD)*** is the most common prion disease in humans and requires a period of 7-8 years for full development of symptoms and is usually fatal. CJD is of different subtypes, namely, iatrogenic CJD (iCJD), Variant CJD (vCJD), familial CJD (fCJD), and sporadic CJD (sCJD).
GSS	Humans	***Gerstmann-Straussler Sheinker disease*** (GSS) affects middle-aged persons. The affected persons loss control of coordinated movement (*ataxia*) and mental disorder.
FFI	Humans	***Fatal Familial Insomnia*** (FFI) appears in middle-aged persons. It is characterized by progressively severe insomnia, ataxia, and progressive degeneration of certain tissues of the thalamus of the brain leading to death.
Scrapie	Sheep	***Scrapie*** is an infection of sheep, usually transmitted from ewe to lamb. The incubation period is of upto 3 years, and once signs have appeared the disease progresses slowly but invariably to paralysis and death.
BSE	Cattle	***Bovine Spongiform Encephalopathy*** (BSE; commonly called ***'Mad cow disease'***) is similar to scrapie and appeared in Great Britain in 1990s. The disease entered into the cattle by feeding them with protein supplements of sheep containing bones, meat brains, spinal columns, etc. The peak of the mad cow disease in the British Islets reached during 1992-93 when thousand of cattle heads were afflicted. This heavily affected the meat industry of that country.
TME	Mink	***Transmissible Mink Encephalopathy*** (TME) is reported to appear due to infection with prions from sheep or cattle.
FSE	Cats	***Feline Spongiform Encephalopathy*** (FSE) is considered to appear due to infection with prion-contaminated bovine tissues or meat and bone meal.

Cellular PrP (PrP^c) Conversion into Prion (PrP^{sc}):

The host cell contains PrP gene that encodes PrP (for prion protein). Prions seem to be composed exclusively of a modified isomer of PrP designated PrP^{Sc} (Sc = scrapie-associated). No differences in the primary structure of PrP^c and PrP^{Sc} were detected, suggesting that they differ in their conformation.

The normal, cellular PrP, denoted Prp^c (C = cellular) is converted into PrP^{Sc} whereby a portion of its α-helical and coil structure is refolded into β-sheet. This structural transition is accompanied by profound alterations in the physicochemical properties of the PrP^c. Two models for the conformational conversion of PrP^C to PrP^{Sc} are proposed.

They are:

(i) "refolding" model and (ii) "nucleation" or "seedling" model. The refolding model (Fig. 11.12A) proposes that PrP^c unfolds to some extent and refolds under the influence of a PrP^{Sc} molecule and that the two states are separated by an activation energy barrier.

The nucleation or seedling model (Fig. 11.12B) postulates that PrP^C is in equilibrium with PrP^{Sc} (or a precursor thereof), that the equilibrium is largely in favour of $PrP^C$and the PrP^{Sc} is only stable when it forms a crystal-like aggregate of PrP^{Sc} (sown dark) called multimer (or seed).

Multimer (or seed) formation is rare. However, once a multimer (or seed) becomes present, monomer addition ensures rapidly. These multimers (or seeds) are continuously fragmented generating increasing surfaces for monomer addition.

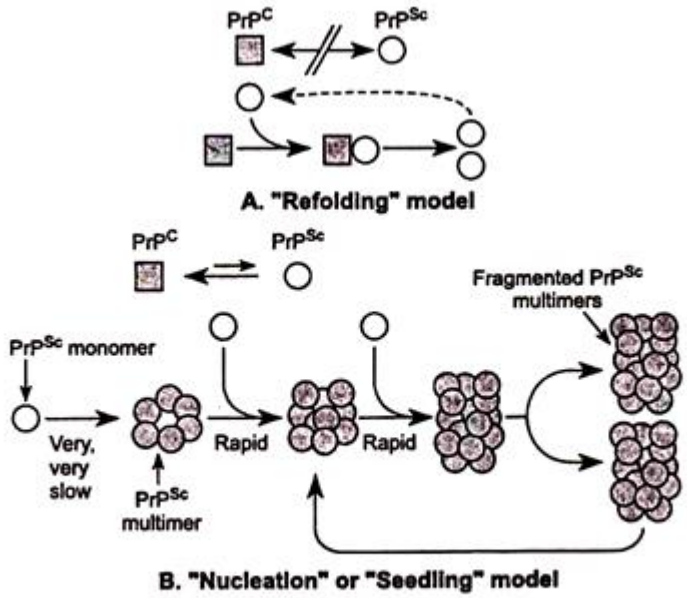


FIG. 11.12. Two models, "refolding" (A) and "nucleation" or "seeding" (B), for the cellular PrP (PrP^C) conversion into prion (PrP^{Sc}). See detail in text.

Prion Transmission:

Prion diseases are so far unique among conformational diseases in that they are transmissible, not only experimentally but also by natural routes. While prion diseases are not contagious (i.e. by direct contact), they are transmitted naturally perorally (predominantly by ingestion) and parenterally.

After oral uptake, the prion penetrates the mucosa through M cells of gastrointestinal tract and enter into the Peyer's patches as well as the enteric nervous system. Depending on the host, spleen and lymph nodes are sites in which prions replicate and accumulate.

It has been suggested by Huang and co-workers in 2002 that myeloid dendritic cells mediate transport within the lymphoreticular system (LRS). From the LRS and likely from other sites, prions proceed along the peripheral nervous system to finally reach the brain, either directly via the vagus nerve or via spinal cord (Fig. 11.13).

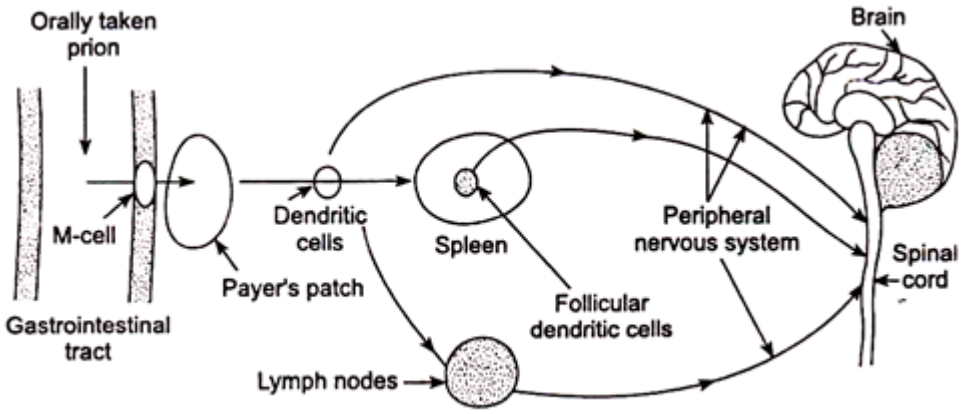


FIG. 11.13. Ingested prion transmission pathway.

UNIT 5

NOMENCLATURE AND CLASSIFICATION OF PLANT VIRUSES

- Traditionally, viruses are named after the most conspicuous symptom they produce on the first host. A virus causing mosaic on tobacco is called Tobacco mosaic virus, whereas the disease itself is called as tobacco mosaic.
- There have been frequent changes in the nomenclature and classification of viruses and generic names have been adopted.
- A genus is usually considered as a population of virus species that shares common characteristics and are different from other population of species.
- Currently 70 genera of plant viruses have been recognized.
- The genera are named either after the type species (Caulimovirus after Cauliflower mosaic virus) or are given a descriptive name often from a Greek or Italian word for a major feature of a genus, e.g., Closterovirus from the Greek word 'kloster' meaning 'a spindle or thread' – descriptive of virus particle shape; Geminivirus from the Latin word 'geminous' meaning twins to describe the particles.
- Secondly, genera are grouped together into family on common characteristics.
- There are 14 families recognized for plant viruses, such as Reoviridae and Rhabdoviridae, which are common with animal viruses. However, 22 genera have not yet been assigned any family and are called 'floating genera'.
- The family is either named after type member genus (e.g., Caulimoviridae named after Genus Caulimovirus) or given a descript to be named associated with genus for a major feature of family, e.g., Geminiviridae descriptive of virus particles.
- Only three orders have been accepted so far by International Committee for Taxonomy of Viruses (ICTV). The mononegavirales contains, among others the Rhabdoviridae in which there are two plant virus-families

Use of virus names

- The ICTV sets rules which are regularly revised on virus nomenclature and the orthography of taxonomic names.
- The last word of the species is 'virus'; and suffix word for a genus is 'virus', for a subfamily is 'virinae', for a family is 'viridae', for an order is 'virales'.
- In formal taxonomic usage, the virus order, family, genus and species names are printed in italics or underlined with first letter being capitalized.
- Other words in species names are not capitalized unless they are proper nouns or parts of proper noun.
- Also in formal usage, names of taxons should proceed the name being used e.g. Family Caulimoviridae, the Genus Closterovirus, the species Potato virus Y.
- However, in less formal instances which are widely used, the taxonomic unit is omitted.
- The plant viruses are classified on the basis of structure, physico-chemical properties, serological relationships, activities in the host plants and transmission.

Latest Classification

The plant viruses are classified in five major groups based on:

– Nature of the genome (RNA or DNA)
– Strandedness (single or double stranded)
– Method of replication

- Each group (not a recognized taxon) has orders, families, genera and species.
- The five groups are:

i) Single stranded positive sense RNA [(+) RNA] viruses
ii) Single stranded negative sense RNA [ss (-) RNA] viruses
iii) Double stranded RNA (ds RNA) viruses
iv) Double stranded DNA virus [ds DNA (RT)] viruses
v) Single stranded DNA [ss DNA] viruses

I. Single stranded positive sense RNA [(+) RNA] viruses:

- **Order:** Nidovirales

i) Family: Bromoviridae, e.g., Bromovirus (Brome mosaic virus-BMV), Alfamovirus (Alfalfa mosaic virus-AMV), Cucumovirus (Cucumber mosaic virus-CMV) and Ilarvirus (Tobacco streak virus-TSV).
ii) Family: Closteroviridae, e.g., Closterovirus (Beet yellows virus-BYV), Ampelovirus (Grapevine leaf roll associated virus GLRaV).
iii) Family: Comoviridae, e.g., Comovirus (Cowpea mosaic virus), Fabavirus (Broad bean wilt virus), Nepovirus (Nematode transmitted polyhedral virus, like Tobacco ring spot virus).
iv) Family: Flexiviridae, e.g., Potexvirus (Potato virus X), Carlavirus (Carnation latent virus).
v) Family: Luteoviridae, e.g., Luteovirus (Barley yellow dwarf virus-BYDV) and Polerovirus (Potato leaf roll virus- PLRV)
vi) Family: Potyviridae,

This family is largest single group of plant viruses and has been studied more extensively. Members of genus Potyvirus are one of the most successful plant viral pathogens. e.g., Potyvirus (Potato virus Y-PVY), Ipomovirus (Sweet potato mild mottle virus-SPMMV) and Bymovirus (Barley yellow mosaic virus).

vii) Family: Sequiviridae, e.g., Sequivirus (Parsnip yellow fleck virus-PYFV) and Waikavirus (Rice tungro spherical virus- RTSV).
viii) Family: Tombusviridae, e.g., Tombusvirus (Tomato bushy stunt virus- TBSV), Carmovirus (Carnation mottle virus), Necrovirus (Tobacco necrosis virus-TNV)
ix) Family: Tymoviridae, e.g., Tymovirus (Turnip yellow mosaic virus- TYMV).

- Some of the very important viruses like Tobamovirus (Tobacco mosaic virus- TMV), Tobravirus (Tobacco rattle virus-TRV), Potexvirus (Potato virus X-PVX) etc. have not been assigned any family yet.

II. Single stranded negative sense RNA [ss (-) RNA] viruses: Members of this group are only enveloped plant viruses.

Order: Mononegavirales

- **Family:** Rhabdoviridae, e.g., Cytorhabdovirus (Lettuce necrotic yellows virus-LNYV) and Nucleorhabdovirus (Potato yellow dwarf virus- PYDV).

- **Family:** Bunyaviridae, e.g., Tospovirus (Tomato spotted wilt virus-TSWV; Groundnut bud necrosis virus- GBNV)

III. Double stranded RNA (ds RNA) viruses : There is no order assigned.

- **Family:** Rheoviridae, e.g., Fijivirus (Fiji disease virus-FDV) and Phytorheovirus (Wound tomur virus- WTV).
- **Family:** Partiviridae, e.g., Alphacryptovirus (White clover crypto-virus 1) and Betacryptovirus (White clover crypto-virus 2)

IV. Double stranded DNA virus [ds DNA (RT) virus]: No order has been assigned.

- **Family:** Caulimoviridae, e.g., Caulimovirus (Cauliflower mosaic virus- CaMV)

V. Single stranded DNA [ss DNA] virus: No order has been assigned.

- **Family:** Geminiviridae, e.g., Mastrevirus (Maize streak virus- MSV), Curtovirus (Beet curly top virus-BCTV), Begomovirus (Bean golden mosaic virus- BGMV), Bhendi yellow vein mosaic virus- BYMV and Cassava latent virus- CLV.
- **Family:** Circoviridae, e.g., Nanovirus (Subterranean clover stunt virus; Banana bunchy top virus-BBTV).

Transmission of Plant Viruses

he following points highlight the eight chief methods used for the transmission of plant viruses. The methods are:

1. Seed Transmission of Virus
2. Transmission by Vegetative Propagation
3. Transmission by Mechanical Means
4. Transmission by Cuscuta
5. Soil Transmission
6. Insect Transmission
7. Transmission by Fungi
8. Some Soil Inhabiting Viruses have Nematode Vectors.

Method # 1. Seed Transmission of Virus:

Transmission through the seeds of the host plant was earlier considered to play a minor part in the spread of virus diseases. Recently Bennett (1969) listed 53 viruses which are transmitted by seeds of about 124 plant species.

The seeds are important in the spread of a few viruses of legumes, wild cucumber, tomatoes, and curly top virus of beet sugar. In the latter case the seeds carry a high percentage of the virus. The virus, however, does not enter the embryo. It is carried in a portion of the seed of the diseased plants.

Method # 2. Transmission by Vegetative Propagation:

It is one of the chief methods of transmission of virus diseases especially of Potato, Rose, Sugarcane, Raspberry, Strawberry, Turnips, Bulb plants, fruit trees and many ornamentals.

The vegetative parts, the infected plants such as the tubers, bulbs, roots, offshoots, buds and scions which are used for propagation, will contain the virus present in the parent. The new plants raised by the above-mentioned vegetative methods are nearly always infected.

Method # 3. Transmission by Mechanical Means:
Many mosaic viruses are transmitted mechanically from diseased plants to healthy ones by the following methods:
(i) By contact of infected and healthy leaves brought about by wind.

(ii) By rubbing the juice of the diseased plants over the surface of the leaves of healthy plants.

(iii) By grafting infected buds on to healthy plants.

(iv) Agricultural implements also play quite an important part. The knife used for cutting the seed pieces and the pruning shears will spread the disease.

(v) Some viruses spread below ground by contact between the roots of diseased and healthy plants.

(vi) Handling plants at planting time and in cultural operation will also help in the spread of viruses such as Sugar beet. Curly top virus and Cucumber mosaic virus.

EXAMPLE OF MECHANICAL MODE OF TRANSMISSION

Method # 4. Transmission by Cuscuta*:*
In many cases Dodder (Cuscuta) serves as a transmitting agent and an effective bridge between the infected host and the healthy plants by establishing intimate biological contact through its haustoria.

Method # 5. Soil Transmission*:*
Quite a number of viruses are transmitted through the soil. Common examples of soil borne viruses are Potato mosaic virus, Oat mosaic, Wheat mosaic, etc. In all these cases the disease is contracted from the soil.

EXAMPLES OF SOIL TRANSMISSION

Method # 6. Insect Transmission:

Some plant and animal viruses are spread and complete particles introduced into host cells by arthropod vectors and even by dog-bite as in rabies. Among the arthropods most important agents of spread of virus diseases are the insects.

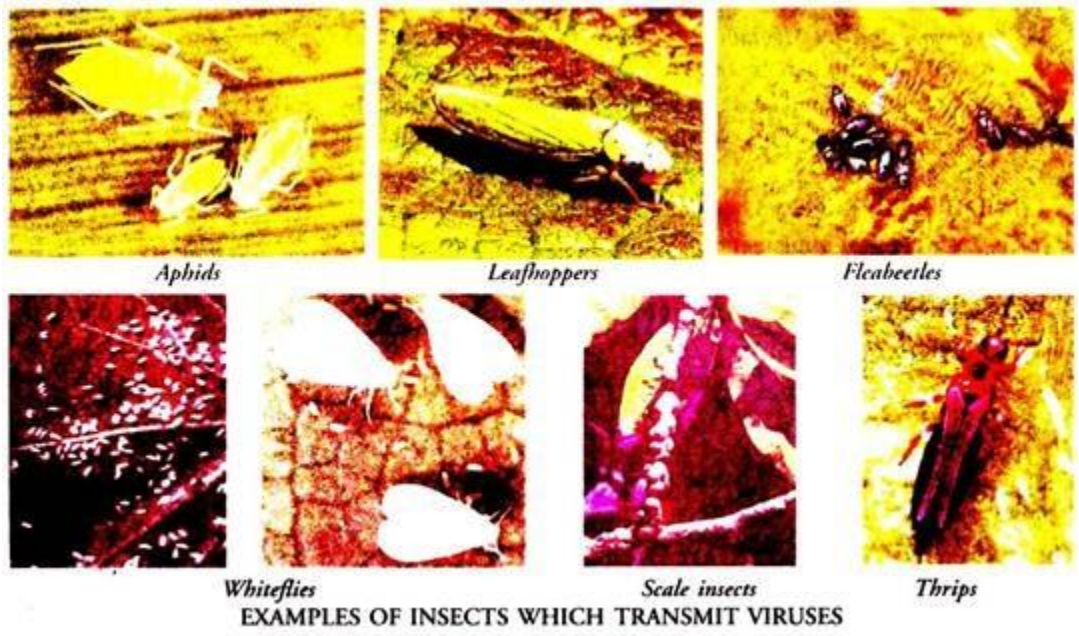
The insect which carries the disease is called a vector. The insect vectors which play a major role in the dissemination of plant viruses are the Aphids, Leafhoppers, Flee beetles, Scale insects, thirps and White flies.

Most of the insect vectors are sucking insects. Aphids transmit more plant viruses than any other insects. Leafhoppers come next in the list. About three hundred plant virus diseases are known to have insert vectors.

The insert obtains virus through its mouth parts at the time of feeding on the diseased plant. It is then inoculated in the healthy plant by means of the mouth part. Inoculation in many cases must be in a certain tissue or upon young leaves.

The virus may remain active in the body of the vector for many days. Instances are however, known when infectivity is soon lost. There are also cases where a vector cannot infect a healthy plant immediately after it has fed on a diseased plant.

There is delay in the development of infective power within the vector. This period of development of infectivity for the virus within the vector is called the incubation period. The duration of the incubation period varies with different viruses from a few hours to days



EXAMPLES OF INSECTS WHICH TRANSMIT VIRUSES

There also appears to be some relationship between the plant viruses and the insect vectors which transmit them. The precise nature of this relationship is still unknown. The virus disease of sugar beet known as curly leaf or curly top is spread by the leaf-hopper Circulifer tenellus.

Other sucking insects which feed on sugar beet are unable to transmit this virus. On the other hand peach aphid is the vector of Sugar beet mosaic virus. The leafhopper does not transmit this virus. Thirps transmit the spotted-wilt virus. All vectors of yellow group of viruses are leafhoppers and of mosaic group are aphids.

Method # 7. Transmission by Fungi*:*
The first proof of the fungus as a vector of plant viruses was found by Gorgon in 1958. Fie found that the diseased lettuce was invariably infected by a soil chytrid, Olpidium. Later he discovered that the fungus acts as a reservoir and vector of the big vein virus.

The virus acquired by the fungus remains in the oospore. The latter germinates and produces the zoospores which function as infective agents and penetrate lettuce roots. Similarly tobacco necrosis virus has been reported by Teakle (1960) to enter roots of its host by the zoospores of O. brassicae.

Method # 8. Some Soil Inhabiting Viruses have Nematode Vectors*:*
Animal viruses may gain access to the higher animals through the mouth and nose from dust or contaminated food. Besides infection from outside, virus may also be transmitted from cell to cell but the internal transmission need not be in the form of virus particles.

PLANT VIRAL DISEASE SIGNS:

- None – the viruses themselves can’t be seen

Plant Viral disease symptoms:

- Mosaic leaf pattern
- Crinkled leaves
- Yellowed leaves
- Plant stunting

Treatment for plant disease

There are no cures for viral diseases such as mosaic once a plant is infected. As a result, every effort should be made to prevent the disease from entering your garden.

1. Fungicides will *NOT* treat this viral disease.
2. Plant resistant varieties when available or purchase transplants from a reputable source.
3. Do *NOT* save seed from infected crops.
4. Spot treat with least-toxic, natural pest control products, such as Safer Soap, Bon-Neem and diatomaceous earth, to reduce the number of disease carrying insects.
5. Harvest-Guard® row cover will help keep insect pests off vulnerable crops/ transplants and should be installed until bloom.
6. Remove all perennial weeds, using least-toxic herbicides, within 100 yards of your garden plot.
7. The virus can be spread through human activity, tools and equipment. Frequently wash your hands and disinfect garden tools, stakes, ties, pots, greenhouse benches, etc. (one part bleach to 4 parts water) to reduce the risk of contamination.
8. Avoid working in the garden during damp conditions (viruses are easily spread when plants are wet).
9. Avoid using tobacco around susceptible plants. Cigarettes and other tobacco products may be infected and can spread the virus.
10. Remove and destroy all infected plants

CAULIFLOWER MOSAIC VIRUS (CAMV):

- Cauliflower mosaic virus (CaMV) is a type member of the caulimoviruses which falls under the family Caulimoviridae in the Group VII dsDNA-RT viruses. The CaMV are the only plant viruses that have dsDNA genome.

- Due to the presence of dsDNA, the caulimoviruses are exploited as vector in genetic engineering of plants. The CaMV consists of open circular dsDNA as genetic material with single strand discontinuity like hepadna Virus. The DNA is linear in situ but gets circularized after extraction. CaMV shows icosahedral symmetry of the capsid with a 50 nm diameter. It consists of more than one protein shell.

- ***Symptoms of CaMV:***
- From the cell walls of infected leaves, there arise finger like processes. It has also been observed that mitochondria and nuclei become abnormal in the infected cells of host leaves.

- ***Structure of CaMV:***
- Virions occur in the cytoplasm, and in some cases in the nucleus. Cytoplasmic virions are associated with electron-dense proteinaceous inclusion bodies (for caulimoviruses, the product of ORF6). The product of 0RF2 also forms inclusion bodies which are electron translucent.

- Inclusion bodies can be seen by light microscopy as well as by electron microscopy. The virion CaMV shows an icosahedral symmetry with a diameter of 52 nm which is made up of 420 capsid protein (CP) subunits arranged with a triangulation T = 7

- The protein subunits surround a solvent-filled central cavity. Each virion consists of a circular dsDNA molecule of about 8.0 kb which is interrupted by site-specific discontinuities.

It results from its replication by reverse transcription. The single stranded nicks in the viral DNA are repaired after entering the host. It results in formation of a supercoiled molecule that binds to histone proteins. Transcription of this DNA results into a full length 35S RNA and a sub-genomic 19S RNA molecule, which are terminally redundant.

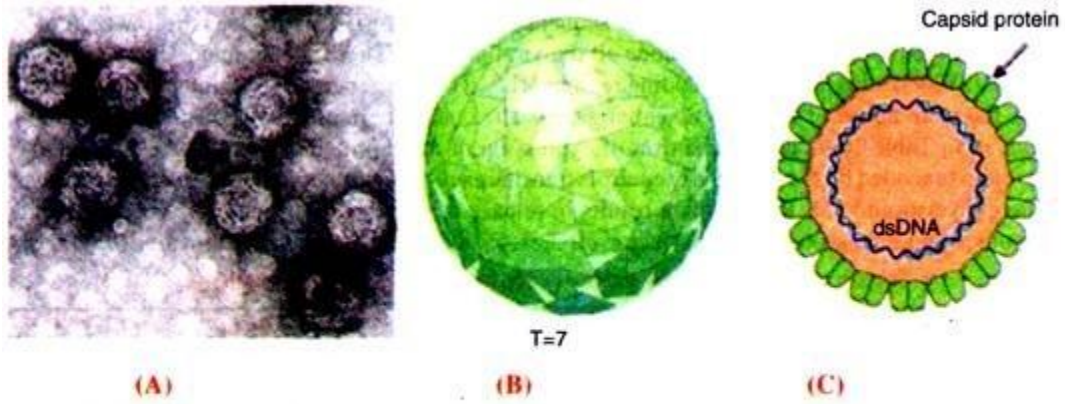


Fig. 16.14. Cauliflower mosaic virus (A); A- an electron micrograph of virions; B- icosahedral structure (T=7); C- structural organization of a virion showing viral genome.

Genome of CaMV:
The genome is a mono-partite, open circular dsDNA of about 8000 base pairs with one discontinuity in one strand, and one or more discontinuity in the other strand (Fig. 16.15).

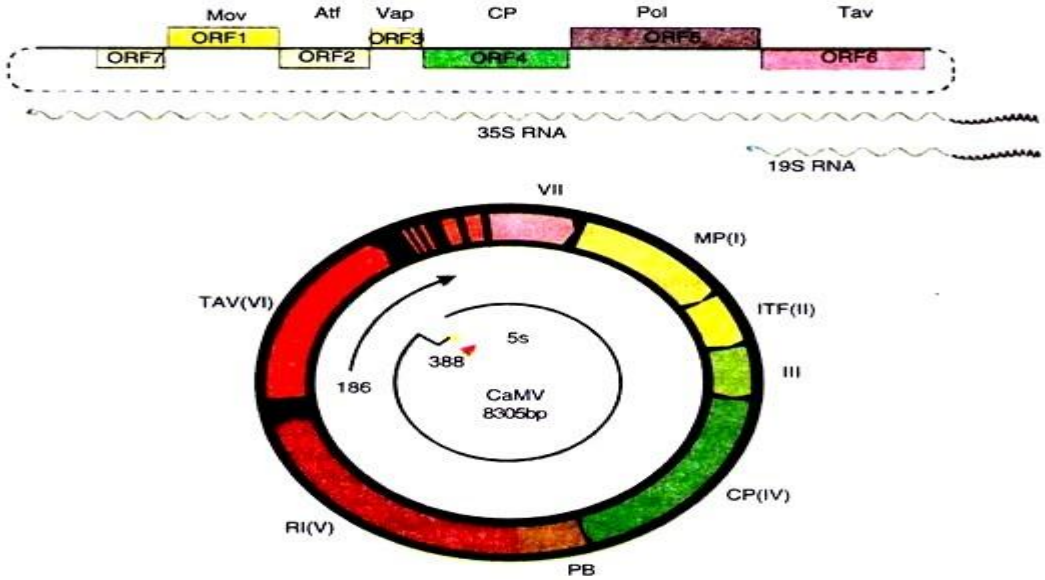


Fig. 16.15: Open circular, double stranded DNA showing seven ORFs. The genome contains discontinuities in both strands, one in the transcribed strand and 1 to 3 in the non-transcribed strand

- Strands displacement causes gaps in the genome, which are associated with the replication of the virus. The promoter of the 35S RNA is very strong. It is used in plant transformation. The 35S RNA is complex and contains one leader sequence (600 nt long). This leader is followed 6 to 8 tightly arranged longer ORFs that encode all the viral proteins.

- There are six major coding regions (I, III, IV, V, VI, VI11) and 2 minor coding regions (II. VII) in the genome. The minor coding regions act as the store house of the non- essential genes. The coding regions of ORFs and their function are given in Table 16.4. The mechanism of expression of these proteins is very special.

- The ORF VI protein (encoded by the 19S RNA) controls translation re-initiation of major open reading frames oh the polycistronic 35S RNA, which results in release of virions. After making association with polysomes and eukaryotic initiation factor eIF3, TAV starts its function.

Table 16.4 Different coding regions and their functions

Coding region (ORF)	Function
I	Codes for 38 kDa protein (MP) which helps in movement of virus from one cell to another
II	Codes for 19 kDa protein which attracts aphids
III	Codes for 15 kDa viral capsomeres
IV	Codes for 57 kDa coat protein (CP)
V	Codes for 79 kDa protease, reverse transcriptase (RT) and RNaseH
VI	Codes for 58 kDa proteips which is transactivator/viroplasmin (TAV) used as translational activator tor building of inclusion bodies
VII	Unknown functions; possibly codes for protein which targets newly formed virus to inclusion body

Replication of CaMV:

- CaMV replicates by reverse transcription in cytoplasm/nucleus. Virion of CaMV interacts with a cellular receptor of the host and enters inside the cell. The viral dsDNA genome is transported to the nucleus where the host RNA polymerase II carries out its transcription.

- Then translation of 35S RNA and 19S RNA takes place where viral proteins are produced thereafter, reverse transcription of 35S RNA occurs to produce new dsDNA genomes in cytoplasm. Capsid protein encapsulates the viral genome. The new viral particles get targeted to inclusion body and are released outside.

POTATO LEAF ROLL VIRUS (PLRV):

- Potato leaf roll virus (PLRV) belongs to the Genus Polerovirus, family Luteoviridae and Group IV (+) sense ssRNA virus according to the Baltimore classification.

- The family Luteoviridae consists of three genera viz., Polerovirus, Luteovirus and Enamovirus. Leaf curl of tomato has been reported from many parts of the world. It is most common is winter season in India. It has non-enveloped particles with isometric symmetry. It possesses one linear (+) ssRNA as genetic material.

Leaf rolling symptom on potato caused by *Polerovirus*.

The viral host range is restricted to members of family Solanacae. PLRV is transmitted via green peach aphids (Myzus persicae) in a circulative manner without any evidence for propagation in its invertebrate vector.

It is restricted to the phloem tissue of the infected plants:

Symptoms of PLRV:

The symptoms that develops in the infected plants are dwarfing, puckering, twisting and curling towards the dorsal side of leaves, mottling, vein clearing, excessive branching, shortening of whole plant, and partial or complete sterility in the plants.

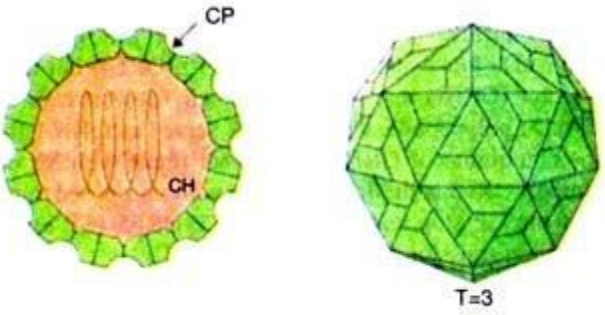


Fig. 16.16 Virion structure of Polerovirus.

Structure of PLRV:

PLRV particles are isometric icosahedral, non-enveloped virion (T=3 particles) of 25-30 nm diameter with 180 copies of the capsid protein (CP) (Fig. 16.16). It contains 30% of single stranded, positive-sense RNA with a molecular weight of -5.9 kb. The viral genome is covalently linked to a 7.2 kDa protein (VPg) at its 5′ start and not polyadenylated at the 3'end.

Genome Organization of PLRV:

Virion consists of a linear, (+) sense ssRNA genome of 5.3-5.7 kb in size (Fig. 16.19). Poleroviruses have a VPg bound at the 5′ end. There is no poly (A) tail or tRNA-like structure at the 3′ end. Different isolates of PLRV have been sequenced. The RNA codes for at least 8 open reading frames (ORFs) that are located in two clusters of genes and separated by 197 nucleotides (nt) of non-coding sequences.

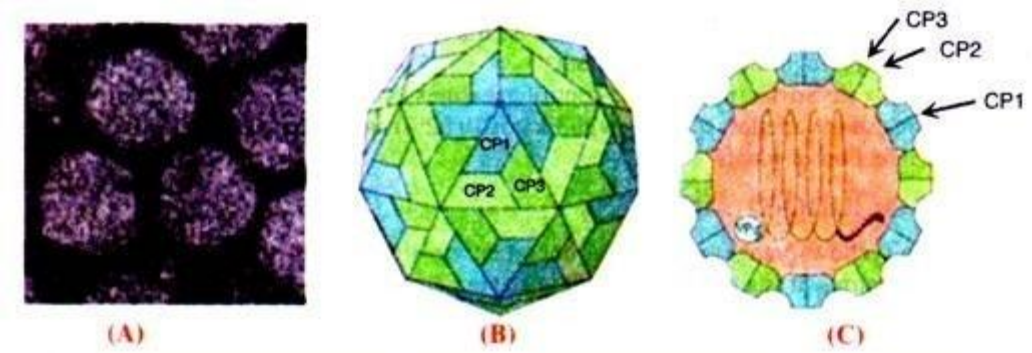


Fig. 16.19 A-Rice tungro spherical virus (RTSV) *(Waikavirus)*: B-icosahedral virion; C-organization of virion showing CP1, CP2 an CP3 proteins and a genome

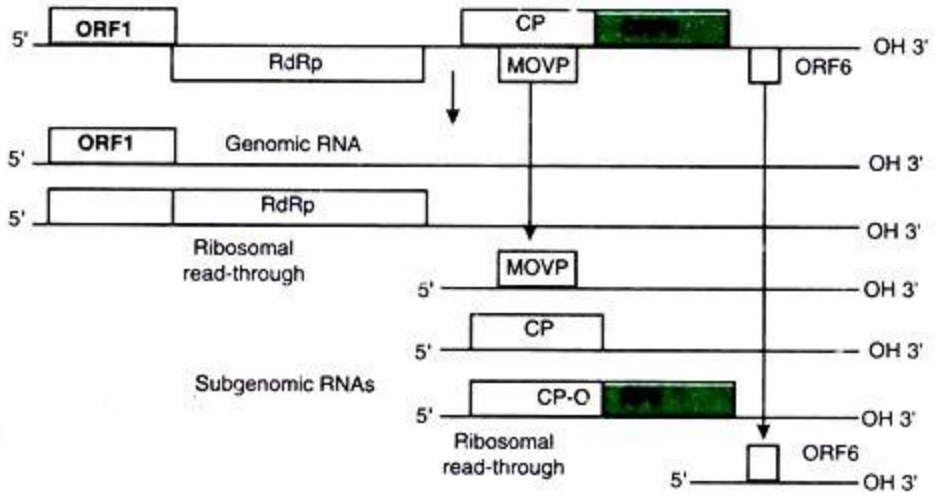
The first cluster is preceded by 1 74 nt of non-coding sequences, whereas the second cluster is followed by 141 nt of non-coding sequences. The first gene cluster is translated directly from genomic RNA to produce ORFO (with Mr of 28 kDa and responsible for the symptom development), ORF1 (with Mr of 70 kDa with a chymotrypsin-like serine protease domain), and the VPg (downstream of the protease domain).

ORFO and ORF1 are overlapped in different frames. ORF2 (with Mr of 118 kDa) is produced as a frame-shift from ORF1 and contains RNA dependent RNA polymerase (RdRp) motifs.

The only proteins translated directly from genomic RNA are the polymerase (ORF2) which is expressed as a fusion protein by – 1 translational frameshift at the end of ORF1, and ORF1 protein. All other ORFs are translated from sub-genomic RNAs (sgRNAs). The second cluster is translated from sub-genomic RNA1 (sgRNA1) and sgRNA2 with Mr of 2.3 kb and ~0.8 kb, respectively.

The sgRNA1 serves as an mRNA [for the coat protein (ORF3)] with MW of 23 kDa and completely overlaps the 17 kDa movement protein (MOVP) (ORF4) but in a different frame and the aphid transmission factor (ORF5) that is located directly downstream the ORF3 amber stop codon and encodes a 53-kDa polypeptide.

The sgRNA2 serves as mRNA for at least two ORFs. ORF6 present within the C terminus of ORFS but in other frame with Mr of 7.1 kDa and ORF7 representing the C terminus of ORFS with Mr of 14.1 kDa with a proposed transcriptional regulation function. Read-through of the CP stop codon produces CP-ORF5 (CP extended) i.e. the capsid subunit essential for aphid transmission (Fig. 16.17).



Genome organisation and gene expression of Polerovirus

Replication of PLRV:

- Polerovirus replicates inside the host's cytoplasm and it does not depend on a helper virus. The virion RNA is infectious and serves as both the genome and viral messenger RNA.

- Virus particle penetrates into the host cell, un-coats, and releases the genomic RNA into the cytoplasm. The viral RNA ORF1 and ORF2 are translated to produce the RdRp fusion protein. A negative-sense complementary ssRNA is synthesized by using the genomic RNA as a template.

- Thereafter, new genomic RNA is synthesized by using the negative-sense RNA as a template. The negative-sense complementary ssRNA also serves as template for the synthesis of 3′ co-terminal sgRNAs. Transduction of these sgRNAs yields the capsid (and extended CP) and movement proteins. Consequently formation of new virus particles takes place.

Transmission of PLRV:

The green peach aphid, Myzus persicae is also one of the most effective vectors. During winter, the virus remains within the infected plant and acts as the primary source of the PLRV inoculum. After infection the virus is largely contained to the phloem tissue and infects daughter tubers through the vascular system.

Control of PLRV:

So far no definite control measures have given fruitful result. However, developing resistant varieties, spraying with Ekatox (0.02%) and Regor (0.05%) at 10 days interval reduce the infection of plants.

MOSAIC DISEASE OF SUGARCANE:

- Sugarcane is one of the important crops of India which has been sown since the dawn of human civilisation. Besides the attack of several pathogens and pests, it is infected by many viruses which cause different types of diseases such as Fiji disease, chlorotic streak, mosaic, ratoon stunting, Australian dwarf, etc.

- In 1921, this disease was noticed first. But it is widespread in all sugarcane growing areas of India. It becomes a potential threat through development of pathogenic strains.

- Mosaic of sugarcane is caused by sugarcane mosaic virus (SCMV) or Saccharum virus 1 or sugarcane virus 1 that falls under the Group IV (+) sense ssRNA viruses. It is a member of family Potyviridae. There are six different strains of SCMV that cause mosaic disease.

- For the development of symptoms, SCMV must be inside the living tissues especially in phloem established bundles. Six weeks after planting there develop pale patches or blotches in the green tissues of the leaves.

- Moreover, the diseased leaves show mottling, chlorotic or light coloured, irregularly spread stripes or streaks. An area of proteolysis develops in one part of the cytoplasm in the cells of affected plants. This areas shows a vacuolated mass having X bodies. These can be heavily stained and observed under microscope.

- There are three principal modes of spread of SCMV: by aphid vectors, infected seed cane, and mechanical inoculation. Only aphid vectors and infected seed cane are important in the field.

- Mechanical transmission i important only in greenhouse and laboratory. There are at least 12 species of aphids that can transmit SCMV from diseased to healthy sugarcane. Perennial grasses also act as host and a source of reservoir of inoculum of SCMV.

Chlorotic symptom on sugarcane leaf caused by SCMV.

There are several methods which have been recommended for the control of mosaic disease of sugarcane such as:

(a) Use of healthy setts as seeding material,

(b) Systemic roguing of infected cane when infection is not severe,

(c) Elimination of perennial grasses acting as weeds and host,

(d) Avoiding rationing practices if plants are heavily infected,

(e) Using resistant varieties,

(f) Operating of good quarantine, etc..

TOBACCO MOSAIC VIRUS (TMV):

- TMV is the most serious pathogen causing mosaic on tobacco leaves. It is transmitted by artificial inoculation but not by insect vectors. TMV is the most resistant virus known so far of which the thermal death point is 90°C for 10 minutes.

- TMV is very stable and the preparations of unpreserved plant juice have been found to retain infectivity even after 50 years. But a temperature- sensitive, nitrous acid-induced mutant is much less stable.

They are very stable to heat. Some strains can retain infectivity after 10 minute exposures to more than 90°C. Dilutions of 10^6 of expressed tobacco sap can be infectious. Purified virus preparations may be preserved at 4°C for long periods by mixing a few drops of chloroform that inhibits the growth of microorganisms.

Symptoms of TMV:

TMV damages the solanaceous plants. However, it can infect the other plants too. Several strains of TMV has also been reported. After infection, it develops symptoms of lightening of leaf colour along the veins in early stages. Thereafter, it turns into light and dark green mosaic symptoms.

Along the veins green colour turns into dark green and the internal region turns into chlorotic. Sometimes dark green blisters appear in the leaf blade. If the plants are infected early in season they become stunted. However, symptoms vary with varieties of tobacco. The virus reduces the yield as well as quality of the products i.e. the nicotine content is decreased by 20-30 %.

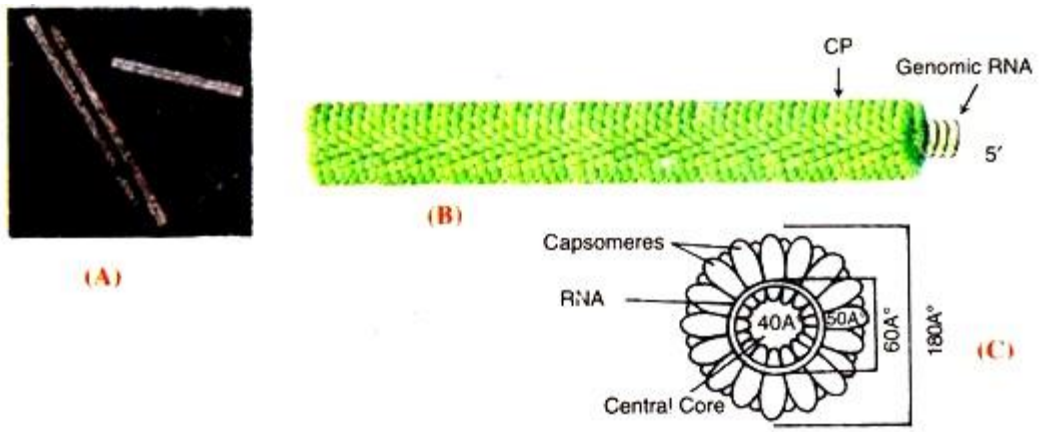


Fig. 16.3. Tobacco mosaic virus; A-an electron micrograph of virions; B-non-enveloped, rigid rod with a helical symmetry (virion about 18 nm in diameter and 300-310 nm in length); C-radial symmetry of the rigid rod showing the arrangement of capsomers, central core and RNA.

Virus Structure of TMV:

Fig. 16.3 shows an electron micrograph of TMV viron (A), a non- enveloped rigid rod with a helical symmetry (B) and a radical symmetry of the rigid rod showing the arrangement of capsomers, central core and RNA (C). Franklin (1957) have described the structure of TMV. It is rod-shaped helical virus measuring about 280 × 150 µm with a molecular weight of 39 × 10^6 Daltons. The virion is made up of 2,130 protein subunits of identical size.

The protein subunits are arranged around a central hole of 4 nm (40Å) (Fig. 16.4 A-B). Each protein subunit is made up of a single polypeptide chain which possesses 158 amino acids, the molecular weight of which is 17,500 Daltons. Inside the protein capsid there is a single stranded RNA molecule which is also spirally coiled to form helix.

Virus RNA consists of 6,500 nucleotides. In one turn the RNA contains 49 nucleotides. Total number of protein subunits counting in three turns is 49 i.e. 49/3 subunits per turn. Therefore a single protein subunit is linked with 3 nucleotides of RNA. Arrangement of capsomers on RNA is shown in Fig.16.4 B.

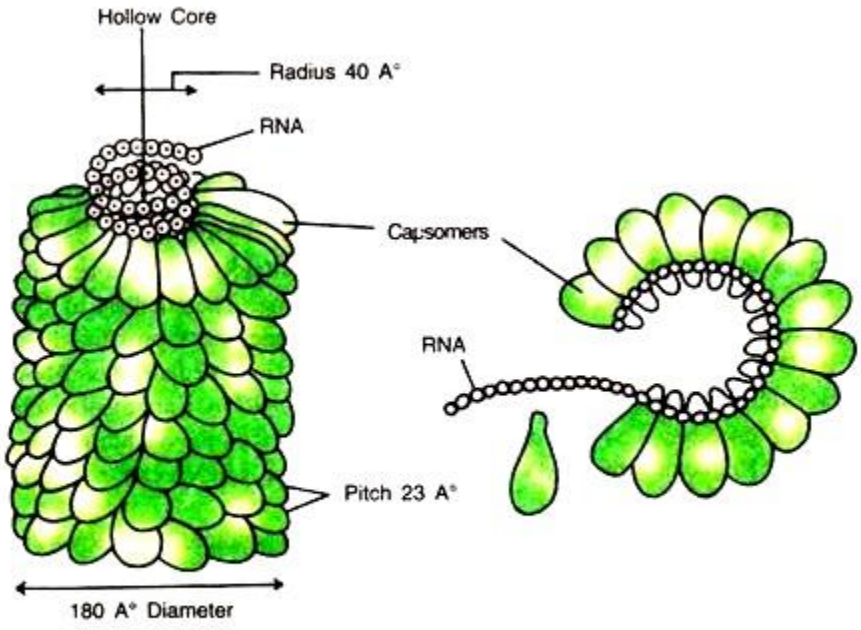


Fig. 16.4 : Structure of tobacco mosaic virus. A, helical model of TMV showing structure of RNA containing capsomers; B, arrangement or capsomers on RNA in one turn.

Genome of TMV:

Fig. 16.5 shows the functional domains of replication proteins of TMV genomic and sub-genomic RNA. The genome of TMV is a mono-partite, linear, (+) sense mRNA of 6.3-6.5 kb that produces five proteins during virus infection. The nucleotide sequence and genome organization of Tobamovirus were determined over 20 years ago. But there is still controversy concerning the number of proteins encoded by the TMV genome.

The RNA possesses a cap (m7G5'pppG) at the 5′-end and a tRNA-like structure charged with the amino acid histidine (His) at the 3′-end. Two RNA polymerase proteins are produced after direct translation of the genomic RNA. The 5′ proximal open reading frame (ORF1) encodes a 126 kDa protein involved in replication, which contains motifs characteristic of methyl transferase (MET, capping enzyme) and helicase (HEL) domains.

Read-through of the amber terminator of ORI 2 yields a 183 kDa protein which is considered to be the viral polymerase. Only the 126 and 183 kDa proteins are required for replication of the genomic RNA or various defective RNAs generated in vitro.

Another protein is produced due to ribosomes by-passing a leaky stop codon UAG. Besides, it also contains an RN A-dependent RNA polymerase (RdRp) domain. Two other proteins are

also produced from the viral RNA; these are called movement protein (MP) from ORF3 and capsid protein (CP) from ORF4.

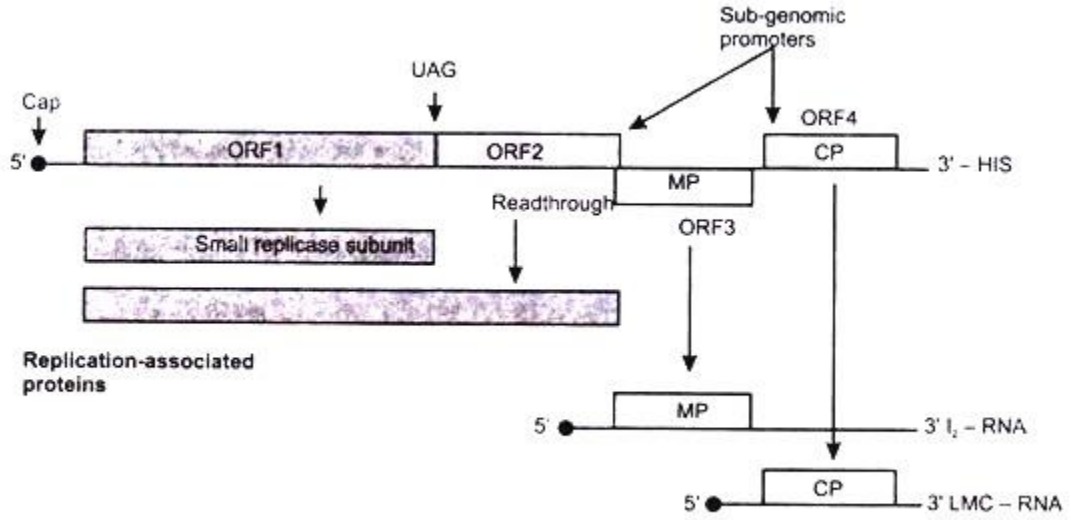


Fig. 16.5. TMV genomic RNA showing functional domains of replication proteins.

During the infection by TMV, three sub-genomic RNAs (sgRNAs) have been identified. All of which have been found in association with polyribosomes. The smallest of these sgRNAs, designated LMC-RNA (low molecular mass component), encodes the 3′ proximal 17-5 kDa capsid protein (CP) (0RF4).

The other largest sgRNA, designated I_2 (intermediate-class RNA 2), encodes the 30 kDa viral movement protein (MP) (0RF3). The I_1 sgRNA (intermediate-class RNA 1) contains an ORF encoding a 54 kDa protein, which coincides with the read-through portion of the 183 kDa protein. Unlike the other TMV-encoded proteins, the I_1sgRNA expressed 54 kDa protein has not been detected in vivo.
Sulzinski (1985) have characterized this third sub-genomic TMV RNA (i.e. 11-RNA. It is associated with polyribosomes; therefore, it is presumed that it acts as a messenger RNA in vivo. The 11-RNA was shown to be a subset of the TMV genome, representing the 3′-half of the molecule. The 11 -RNA was thus shown to be a distinct RNA species and not a class of heterogeneous molecules of approximately the same size.

The II-RNA 5′ terminus is residue 3405 in the genome. After an un-translated sequence of 90 bases, an AUG codon at residues 3495-3497 initiates a protein of 54 kDa terminating at residue 4915. Thus, the amino acid sequence of the 54 kDa protein is coincident with those residues of the carboxy terminus of the well-known 183 kDa TMV protein.

During the derivation of viral genome organizations from the sequences of their genomes, separate ORFs of less than 10 kDa are ignored, unless they occurred in several strains of the same virus or in different viral species in the same genus. An ORF which is referred to as ORF6 in the genomes of TMV and the tobamoviruses and Tobacco mild green mosaic virus have been described by Canto (2004) which encodes a protein of about 4-5 kDa.

Multiplication of TMV*:*
A virus needs to enter the vascular system of the plant for successful colonization of an entire plant. The process of cell-to-cell movement of TMV is relatively slow; it takes one too few hours to multiply in a cell and move to the next cell. Systemic transport of TMV normally

occurs through the phloem sieve elements where viruses move passively with the flow of photosynthates.

After rapid systemic spread of the virus in the phloem, it moves from the phloem to surrounding cells where it reproduces and spreads by cell-to-cell movement. The time between initial infection of one or few cells and systemic infection of the plant varies from few days to few weeks depending on the virus, host plant, and environmental conditions.

Thereafter, TMV moves into the neighboring cells. Depending on the virus, its genomes or the virions are transported into neighboring cells through small channels called plasmodesmata that form connections between cells. Many viruses produce movement proteins that modify the plasmodesmata channels and facilitate viral movement into neighboring cells.

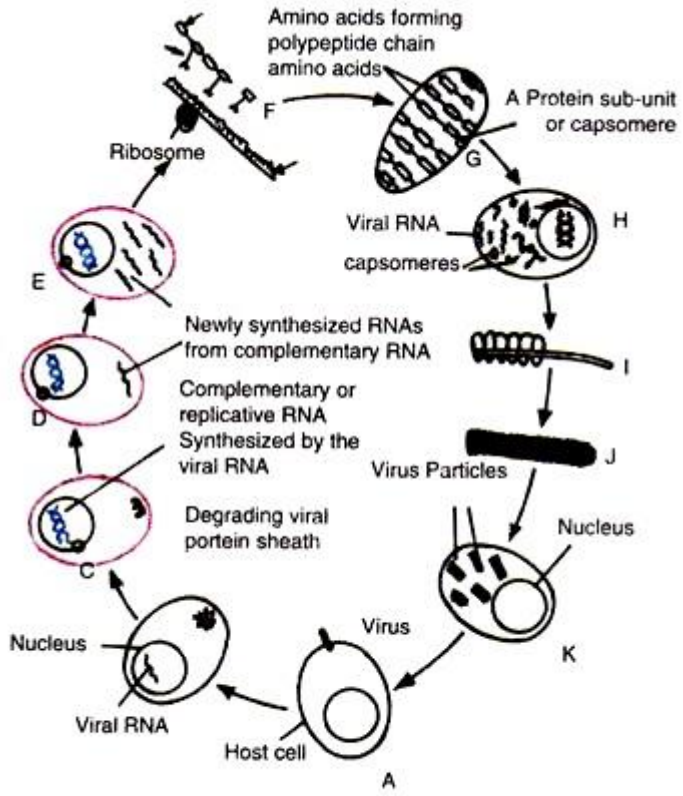


Fig. 16.6. Life cycle of TMV (diagrammatic).

a. Gene expression:

The virion RNA of TMV is infectious and serves as both the genome and viral messenger RNA. The genome encodes at least four proteins. The methyltransferase/ helicase (mt/hel) and RNA-dependent RNA polymerase (RdRp) involved in vims replication are in the 5′ half of the genome.

The first one is directly translated from genomic RNA, and the second one is produced by read-through of the termination codon of the first gene. The other two, a movement protein and the capsid protein, are expressed from separate sub-genomic mRNAs.

b. Replication:

After penetration, TMV enters the host cells (Fig. 16.6A), and replicates in cytoplasm of the infected cells. Inside the cell, the protein coat dissociates and viral nucleic acid becomes free in the cell cytoplasm.

The sites for different steps of the viral multiplication and formation of new viruses have not yet been completely determined. But the studies suggest that after becoming free in the cell cytoplasm the viral-RNA moves into the nucleus (possibly into the nucleolus) (B).

The viral-RNA first induces the formation of specific enzymes called 'RNA polymerases', in the presence of which the single-stranded viral-RNA synthesizes an additional RNA strand called 'replicative RNA' (C).

This RNA strand is complementary to the viral genome and serves as 'template' for producing new RNA single strands of which are the copies of the parental viral-RNA (D). The new viral RNAs are released from the nucleus into the cytoplasm and serve as mRNAs (E). In cooperation with ribosomes and tRNA of the host cell, each mRNA directs the synthesis of protein sub-units (F).

After the production of desired protein sub-units (capsomeres) (G-H), the new viral RNA is considered to organize the protein subunits around it resulting in the formation of complete virions (I-K). Unlike virulent bacteriophages, no lysis of the host cell takes place. The host cells remain alive and viruses move from one cell to the other causing systemic infection.

Protein Synthesis of TMV:
Takeba (1975) demonstrated the direct entry of TMV into the isolated protoplast from mesophyll cells of tobacco. After making entry, RNA rapidly starts uncoating by removing the subunits from the capsid by using host enzymes. The parental RNA is localized in nucleus but not in cytoplasm.

It performs two important functions:
(i) It acts as mRNA and directs and the synthesis of protein, and

(ii) Functions as template for synthesis of complementary strand.

The virus RNA utilizes the amino acids, ribosomes and tRNA of the host and synthesizes the complementary strand and proteins i.e. coat proteins of 17,500 Daltons and two other polypeptides (of molecular weight 160,000 and 140,000 Daltons).

The ratio of nucleic acid and protein differs with each virus. Nucleic acid is about 5-40% of the virus and protein 60-70%. Each protein subunit of TMV consists of 158 amino acids making a total number to about 17,531.

Transmission of TMV:
TMV is transmitted through the cell sap of host and enters a new host through wound incision. Wound is caused in plant due to various cultural operations such as clipping or topping the shoot. It is not seed transmitted but acts as seed contaminant. It is also transmitted by wind and water. Various control methods of the disease are regular roguing diseased plants and weeds, sanitation and use of resistant varieties.

Control of TMV:
TMV occurs in tobacco- growing areas, and is considered to be one of the most important tobacco viruses economically. Insects are not important in its spread. Control is by crop rotation, effective sanitation and use of resistant cultivars of tobacco. For the first time, TMV has been used to demonstrate for development of coat protein-mediated resistance, replicase-mediated resistance, and movement protein-mediated resistance.

Demonstration of TMV RNA as Genetic Material:
Gierer and Schramm (1956) and Fraenkel-Conrat and Singer (1957) have demonstrated that RNA is the genetic material in TMV by using viral strains. They selected two strains of TMV e.g. TMV A and TMV B, separated protein and RNA and reconstructed new TMV particles as given in Fig. 16.7.

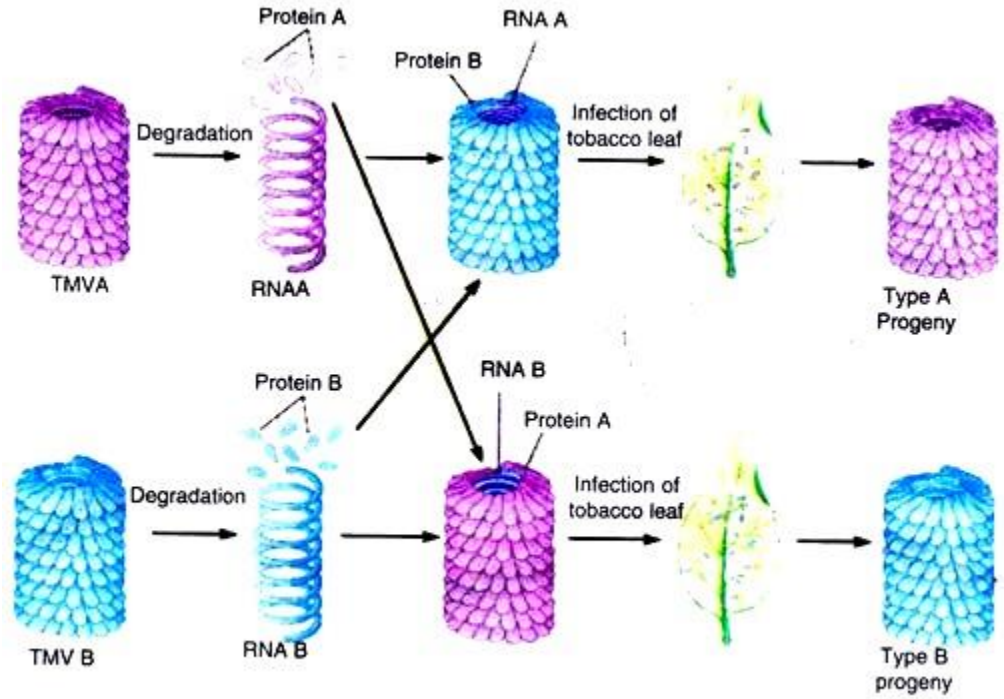


Fig. 16.7 : Demonstration of TMV RNA as genetic material.

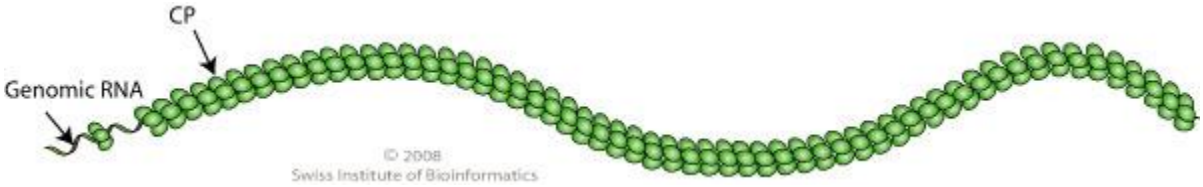
i. Type A particles contained RNA of TMV A + protein of TMV B

ii. Type B particles contained RNA of TMV B + protein of TMV A

These reconstructed particles were inoculated on healthy tobacco leaves to develop symptoms. Symptoms developed on both kinds of inoculated tobacco leaves according to types of viral RNAs, but not according to capsid proteins. This confirms that in TMV, RNA acts as genetic material, not the capsid protein.

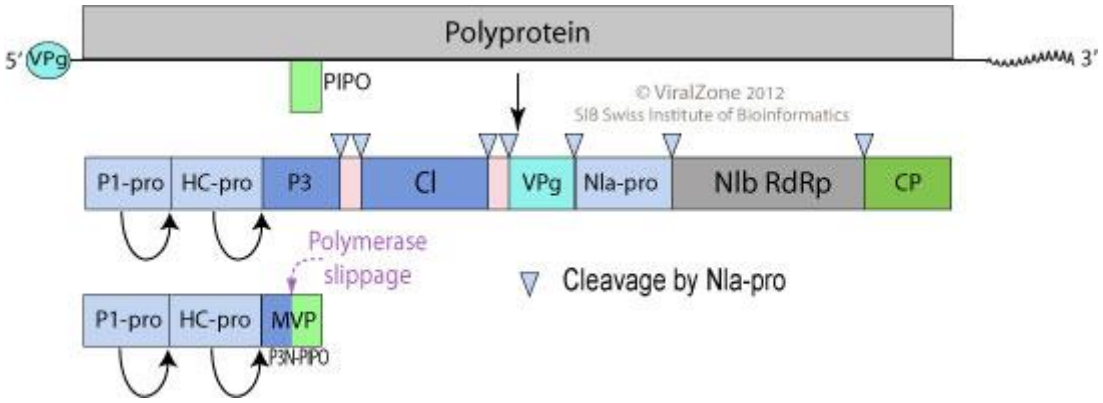
POTYVIRUS

VIRION



Non-enveloped, flexuous, filamentous, of 720-850 nm long and 12-15 nm in diameter. Symmetry helical. Presence of characteristic inclusion bodies within infected plant cells.

GENOME



Monopartite, linear, ssRNA(+) genome of 10 kb in size. 3' terminus has a poly (A) tract. 5' terminus has a genome-linked protein (VPg).

GENE EXPRESSION

The virion RNA is infectious and serves as both the genome and viral messenger RNA. The genomic RNA is translated into polyproteins which are subsequently processed by the action of three viral-encoded proteinases into functional products. P3N-PIPO is expressed by polymerase slippage mechanism from the P3 ORF and probably acts as a movement protein .

REPLICATION

CYTOPLASMIC

1. Virus penetrates into the host cell.
2. Uncoating, and release of the viral genomic RNA into the cytoplasm.
3. The viral RNA is translated to produce a polyprotein which is processed by viral proteases into the RdRp protein and structural proteins.
4. Replication takes place in cytoplasmic viral factories. A dsRNA genome is synthesized from the genomic ssRNA(+).
5. The dsRNA genome is transcribed/replicated thereby providing viral mRNAs/new ssRNA(+) genomes.
6. Virus assembly in the cytoplasm.

7. Viral movement protein P3N-PIPO probably mediates virion cell-to-cell transfer.

CONDITIONS FOR DISEASE DEVELOPMENT

All potyviruses are vectored in a non-persistent manner by several species of aphid. These viruses can also be mechanically transmitted by workers and equipment to a lesser extent. The host range for some of these viruses includes legumes and weeds, however, infected weeds may remain asymptomatic.

CONTROL

Grow resistant cultivars, control aphids and weeds and avoid planting near older cucurbit fields. Reflective mulches, equipment and worker sanitation, deep plowing of crop debris and destruction of cull piles may also help control these diseases.

TOMATO SPOTTED WILT VIRUS

Taxonomic Tree

- Domain: Virus
- Group: "Positive sense ssRNA viruses"
- Group: "RNA viruses"
- Order: Mononegavirales
- Family: Bunyaviridae
- Genus: Tospovirus
- Species: Tomato spotted wilt virus

Identification

Leaves:

- Yellow or brown spots or streaks on the foliage, leaf petioles and stems.
- Leaves develop a pale green color and become very distorted.
- Stems can develop purplish-brown streaking.
- Newer plant growth may be very stunted.

Fruit:

- Plants infected early in the season may produce few or now fruit.
- Green immature tomato fruit mottled, with faint concentric rings.
- Distinct red or yellowish-white rings on mature fruit.
- Blotches with sunken areas.
- Necrotic ringspots form on tomato fruits with *Tomato chlorotic spot virus*.

Pictures of symptoms caused by *Tomato chlorotic virus* (left; center) and *Tospovirus* (right).
Favorable Environmental Condtions
Dry and warm conditions (approximately 75ºF) are favorable for thrips reproduction.

Often Confused With

- Fusarium wilt
- Verticillium wilt
- *Pepino mosaic virus*

Scouting Notes

- Look for signs of thrips damage on the plants or keep sticky traps around the high tunnel to catch thrips.
- Remove any plants exhibiting viral symptoms.

TUNGRO DISEASE

Symptoms

Plants affected by tungro exhibit stunting and reduced tillering. Their leaves become yellow or orange-yellow, may also have rust-colored spots.
Discoloration begins from leaf tip and extends down to the blade or the lower leaf portion
Delayed flowering, - panicles small and not completely exerted
Most panicles sterile or partially filled grains
Tungro virus disease affects all growth stages of the rice plant specifically the vegetative stage.

Special detection technique

Collect leaf samples at 6 a.m.
The top 10 cm portion of the leaf is immersed in a solution containing 2 g of iodine and 6 g of potassium iodide in 100 ml of water for 15 minutes or 10 ml of tincture of iodine + 140 ml of water for one hour.Washed in water and when examined.
Tungro infected leaves develop dark blue streaks.

Identification of pathogen

Tungro virus disease is transmitted by leafhoppers, wherein the most efficient vector is the green leafhopper, *Nephotettix virescens* (Distant). The disease complex is associated with rice tungro baciliform virus (RTBV) and rice tungro spherical virus (RTSV). RTBV cannot be transmitted by leafhoppers unless RTSV is present.
Insects could acquire the virus from any part of the infected plant. After acquiring the virus, the vector can immediately transmit to the plants.
RTBV particles are rod-shaped and 100-300 nm in length and 30-35 nm in width. It contains DNA of 8.3 kb. RTSV particles are isometric and 30 nm in diameter. It has a polyadenylated single-stranded RNA of about 12 kb.

Factors favouring disease development

Presence of the virus sources.
Presence of the vector.
Age and susceptibility of host plants.
Synchronization of the three above factors.
All growth stages of the rice plant specifically the vegetative stage

Management Strategies

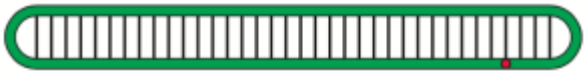
Trap methods

Light traps are to be set up to attract and control the leaf hopper vectors as well as to monitor the population.
In the early morning, the population of leafhopper alighting near the light trap should be killed by spraying/dusting the insecticides.
This should be practiced every day.

Cultural methods

Planting of resistant varieties against tungro virus disease is the most economical means of managing the disease.
Use Resistant varieties like **IR 36, IR 50 ,ADT 37, Ponmani,**Co 45, Co 48, Surekha, Vikramarya, Bharani, IR 36 and**white ponni .**
Among the cultural management practices, adjusting the date of planting is recommended.
Likewise, observing a fallow period of at least a month to eliminate hosts and viruses and vectors of the disease.
In epidemic areas follow rotation with pulses or oil seeds.
Apply neem cake @ 12.5 kg/20 cent nursery as basal dose.
plouging and harrowing the field to destroy stubbles right after harvest in order to eradicate other tungro hosts are also advisable.
Destruction of weed hosts on bunds.

SATELLITES – THE VIRAL KIND



- Satellites are subviral agents that differ from viroids because they depend on the presence of a helper virus for their propagation. **Satellite viruses** are particles that contain nucleic acid genomes encoding a structural protein that encapsidates the satellite genome.
- **Satellite RNAs** do not encode capsid protein, but are packaged by a protein encoded in the helper virus genome. Satellite genomes may be single-stranded RNA or DNA or circular RNA, and are replicated by enzymes provided by the helper virus.
- The origin of satellites remains obscure, but they are not derived from the helper virus.
- Satellite viruses may infect plants, animals, or bacteria. An example of a satellite virus is satellite tobacco necrosis virus, which encodes a capsid protein that forms an icosahedral capsid that packages only the 1,260 nucleotide satellite RNA. The helper virus, tobacco necrosis virus, encodes an RNA polymerase that replicates its genome and that of the satellite.

- Satellite RNAs do not encode a capsid protein and therefore require helper virus proteins for both genome encapsidation and replication. Satellite RNA genomes range in length from 220-1500 nucleotides, and have been placed into one of three classes.

- Class 1 satellite RNAs are 800-1500 nucleotide linear molecules with a single open reading frame encoding at least one non-structural protein. Class 2 satellite RNAs are linear, less than 700 nucleotides long and do not encode protein. Class 3 satellite RNAs are 350-400 nucleotide long circles without an open reading frame.

- In plants, satellites and satellite viruses may attenuate or exacerbate disease caused by the helper virus. Examples of disease include necrosis and systemic chlorosis, or reduced chlorophyll production leading to leaves that are pale, yellow, or yellow-white. The symptoms induced by satellite RNAs are thought to be a consequence of silencing of host genes. For example, the Y-satellite RNA of cucumber mosaic virus causes systemic chlorosis in tobacco. This syndrome is caused by production of a small RNA from the Y-satellite RNA that has homology to a gene needed for chlorophyll biosynthesis. Production of this small RNA leads to degradation of the corresponding mRNA, causing the bright yellow leaves.

- The giant DNA viruses including *Acanthamoeba polyophaga* mimivirus, *Cafeteria roenbergensis* virus, and others are associated with much smaller viruses (sputnik and mavirus, respectively) that depend upon the larger viruses for reproduction.
- For example, sputnik virus can only replicate in cells infected with mimivirus, and does so within viral factories. Whether these are satellite viruses or something new (they have been called virophages) has been a matter of controversy.
- Like satellite viruses, sputnik and others have similar relationships with their helper viruses: they require their helper for their propagation, but their genomes are not derived from the helper, and they negatively impact helper reproduction.

- Others argue that the definition of satellite viruses as sub-viral agents cannot apply to these very large viruses. For example, sputnik virophage contains a circular dsDNA genome of 18,343 bp encoding 21 proteins encased in a 75 nm t=27 icosahedral capsid. Sputnik is dependent upon mimivirus not for DNA polymerase – it encodes its own – but probably for the transcriptional machinery of the helper virus. Those who favor the name virophage argue that dependence upon the cellular transcriptional machinery is a property of many autonomous viruses – the only difference is that Sputnik depends upon the machinery provided by another virus. It seems likely that a redefinition of what constitutes a satellite virus will be required to solve this disagreement.

- Most known satellites are associated with plant viruses, but hepatitis delta satellite virus is associated with a human helper virus, hepatitis B virus. The genome (illustrated) is 1.7 kb – the smallest of any known animal virus – of circular single-stranded RNA that is 70% base paired and folds upon itself in a tight rod-like structure. The RNA molecule is replicated by cellular RNA polymerase II.

- These properties resemble those of viroid genomes. On the other hand, the genome encodes a protein (delta) that encapsidates the RNA, a property shared with satellite nucleic acids. The hepatitis delta satellite virus particle comprises the satellite nucleocapsid packaged within an envelope that contains the surface protein of the helper, hepatitis B virus.

Infection with hepatitis delta satellite virus only occurs in individuals infected with hepatitis B virus: it is globally distributed, present in about 5% of the 350 million carriers of hepatitis B virus. Acute co-infections of the two viruses can be more severe than infection with hepatitis B virus alone, leading to more cases of liver failure.

In chronic hepatitis B virus infections, hepatitis delta satellite virus aggravates pre-existing liver disease, and may lead to more rapid progression to cirrhosis and death than monoinfections. Why co-infection with both viruses leads to more serious outcomes is not known.