VIROLOGY

M.Sc. MICROBIOLOGY

SEMESTER-I, PAPER-I

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VIROLOGY

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FOREWORD

Since its establishment in 1976, Acharya Nagarjuna University has been forging ahead in the path of progress and dynamism, offering a variety of courses and research contributions. I am extremely happy that by gaining 'A+' grade from the NAAC in the year 2024, Acharya Nagarjuna University is offering educational opportunities at the UG, PG levels apart from research degrees to students from over 221 affiliated colleges spread over the two districts of Guntur and Prakasam.

The University has also started the Centre for Distance Education in 2003-04 with the aim of taking higher education to the door step of all the sectors of the society. The centre will be a great help to those who cannot join in colleges, those who cannot afford the exorbitant fees as regular students, and even to housewives desirous of pursuing higher studies. Acharya Nagarjuna University has started offering B.Sc., B.A., B.B.A., and B.Com courses at the Degree level and M.A., M.Com., M.Sc., M.B.A., and L.L.M., courses at the PG level from the academic year 2003-2004 onwards.

To facilitate easier understanding by students studying through the distance mode, these self-instruction materials have been prepared by eminent and experienced teachers. The lessons have been drafted with great care and expertise in the stipulated time by these teachers. Constructive ideas and scholarly suggestions are welcome from students and teachers involved respectively. Such ideas will be incorporated for the greater efficacy of this distance mode of education. For clarification of doubts and feedback, weekly classes and contact classes will be arranged at the UG and PG levels respectively.

It is my aim that students getting higher education through the Centre for Distance Education should improve their qualification, have better employment opportunities and in turn be part of country's progress. It is my fond desire that in the years to come, the Centre for Distance Education will go from strength to strength in the form of new courses and by catering to larger number of people. My congratulations to all the Directors, Academic Coordinators, Editors and Lesson-writers of the Centre who have helped in these endeavors.

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SEMESTER-I

M.SC. MICROBIOLOGY

1O1MB24 -VIROLOGY

UNIT-I

Brief outline of discovery of viruses; properties of viruses. Morphology of viruses- Structure, Capsid architecture; envelopes and peplomers. Chemistry of viruses- viral proteins, genome - structure and types. Study of sub-viral agents - Brief account of diseases caused by viroids - PSTV, Cadangcadang; Prions- Scrape, Cruetzfeldjakob; Satellite viruses, Satellite RNA's.

UNIT-II

General methods of cultivation of viruses-in embryonated eggs, experimental animals and cell cultures. monolayer cultures, cell lines.General methods of purification of viruses. Serological methods for detection of viruses- haemagglutination & HAI, immunoflourescense, ELISA, PCR and RIA. Infectivity assay - plaque method.

UNIT-III

Taxonomy of plant viruses, Symptoms of diseases caused by plant viruses (morphological, physiological and histological), Ultra structure and life cycles of TMV and CaMV, Transmission of plant viruses mechanical and biological (vector and nonvector), Basic control measures of plant diseases- vector and chemical control.

UNIT-IV

Taxonomy of human viruses. Ultra structure and brief account on life cycles of RNA viruses- polio" Influenza and HIV. Ultra structure and brief account on life cycles of DNA viruses- Vaccina, Adenovirus. SV4O.

UNIT-V

Ultra structure and life cycles of bacteriophages- Ml3, Mu T4 &lambda. General account of viruses of Cyanobacteria, algae and fungi. Viral vaccines- Types, preparation and production of vaccines. New generation vaccines- genetic recombinant vaccines. General account on interferons and antiviral drugs.

REFERENCE BOOKS

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2. Morag, C And Timbury M (1994). Medical Virology, Churchill Livingstone, CONRAT HF, KIMBALL PC and LEVIr JA. (1994). Virology -Ill Ed. Englewood cliff, New Jersey.
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CENTRE FOR DISTANCE EDUCATION

ACHARYA NAGARJUNA UNIVERSITY

Model Question Paper

M.Sc. Degree Examination

MICROBIOLOGY- I SEMESTER

VIROLOGY

Time: 3 hours **Maximum Marks: 70**

Answer ALL Questions **(5x14 = 70 marks)**

UNIT-I

1. a) Give an account on the discovery of viruses in chronological order.

OR

b) Explain the chemical nature of the viruses with suitable examples.

UNIT-II

2. a) Write an account on the general methods for cultivation of viruses.

OR

b) Describe the ELISA and RIA serological methods for detection of viruses.

UNIT-III

3. a) Give an account on the morphological, physiological and histological symptoms of plant viral diseases.

OR

b) Explain the ultrastructure and life cycle of TMV.

UNIT-IV

4. a) Write an account on ultrastructure and life cycle of HIV virus.

OR

b) Describe the ultrastructure and life cycle of Vaccinia virus.

UNIT-V

5. a) Give a general account on viruses of Cyanobacteria, algae and fungi.

OR

b) Write an account on genetic recombinant vaccines with suitable examples.

CONTENTS

LESSON - 1

DISCOVERY, PROPERTIES AND MORPHOLOGY OF VIRUSES

Objective of the lesson

Students will know about the discovery of viruses, their properties and morphological features.

Structure of the lesson

1.1 INTRODUCTION

Several viral diseases can be found in ancient records. The famous Greek poet Homer described the "Rabid dogs" before 1,000 BC. Egyptian Hieroglyphs depicted a man with a withered leg and the "drop foot" syndrome characteristic of poliomyelitis and pustular lesions characteristic of small pox. Small pox was endemic in the Ganges river basin during 5^{th} century BC and subsequently spread to the other parts of Asia and Europe. The other viral diseases such as mumps, measles, influenza, and yellow-fever are known from ancient times. Yellow fever disease has been described since the discovery of Africa by Europeans. Striping patterns of petals known as "colour breaking" in tulips were described in 1579 in Western Europe and were caused by a virus infection.

1.2 DISCOVERY OF VIRUSES

The first report of a pathogenic agent smaller than any known "bacterium" appeared in 1892. Adolf Mayer, a German scientist named "tobacco mosaic disease" after the dark and light spots on infected leaves in the year 1876 from Holland. This was the first experimental transmission of tobacco mosaic disease, but he failed to prove the Koch's postulates. Mayer concluded that the mosaic disease "is a bacterial, but that the infectious forms have not yet been isolated, not are their forms and mode of life known". In 1890 Dimitri Iwanowski, a Russian Scientist, passed the infected tobacco leaf sap through the Chamberland filter and reported that "the sap of leaves infected with tobacco mosaic disease retains its infectious properties even after filtration through Chamberland filter candles" on February 12, 1892. The term virus (Slimy liquid or poison in Latin) was applied to the causal agent of tobacco mosaic disease, and also for any infectious filterable agent.

Martinus Beijerinck, (1896), a Dutch soil microbiologist who collaborated with Adolf Mayer and showed that the sap of infected tobacco plants retained its infectivity after filtration and also he proved that the filtered sap regain its "strength" of infection after dilution. He explained that the pathogen is an organism smaller than bacteria, not observable in the light microscope and able to produce itself only in the living plant tissue, and hence, it cannot be cultured outside the host. Beijerinck called this filterable agent as a "Contagium vivum fluidum" (contagious living liquid). Loeffler and Frosch (1898) isolated and described the first filterable agent from animals, the foot-and-mouth disease virus (FMDV).

Walter Reed and his Co-workers (1901) recognized the first filterable agent from human, yellow fever virus. Lode and Gruber (1901) reported the virus causing plague in fowls and named it as Fowl Plague virus. In 1903 Remlinger and Riffat-Bay identified a causal agent infecting the dogs known as Rabies virus. Negri (1903) demonstrated that the nerve cells of rabies infected dogs contained prominent crystalline inclusion bodies, and they were later named as "Negri bodies". In 1911 Ellermann, Bang and Rous discovered and confirmed the cancer producing capacity of filterable agents in chicken and fowl. Peyton Rous first demonstrated a solid tumor virus of chicken, known as Rous Sarcoma virus, is a filterable agent.

In 1915, Frederick W. Twort noticed that some bacterial colonies underwent a visible change and become "Water looking" (more transparent). He called this phenomenon as glassy transformation and named the clear circular spots as '*taches vierges*' (plaques). d'Herelle developed the plaque assay in 1917 and named the agents infecting bacteria are "Bacteriophages". First successful cultivation of vaccinia virus in tissue culture was reported by Parker and Nye in the year 1925. Max Schleisinger (1932) purified the phages and reported that they were composed of protein and DNA in roughly equal proportions.

Emory Ellis and Max Delbruck (1939) designed one-step growth curve experiment, in which an infected bacterium releases hundreds of phages synchronously after 90 minutes latent or eclipse period. The first clear pictures of bacteriophages had been obtained by Tom Anderson and Delbruck (1942). The first mutants of bacteriophages were isolated and characterized by Delbruck in the year 1946, and he also reported that mixed phage infection leads to genetic recombination. Seymour Cohen (1947) examined the effects of phage infection on DNA and RNA levels in infected cells using a colorimetric analysis. The result of Monod and Wollman in 1947 made the clear point that a virus could redirect cellular macromolecular synthetic processes in infected cells.

In 1949, first successful cultivation of poliovirus in Human tissue culture was performed by John Enders. In 1949, Andre Lwoff studied the lysogenic phages of *Bacillus magaterium*. When lysogenic bacteria were lysed from without, no virus was detected. Hershey and Chase (1952) utilized labeled viral protein with ^{35}S and nucleic acids with ^{32}P to follow phase attachment to bacteria. They found that the viral DNA was the genetic material not the viral protein coat. In 1953, Lowoff and Wollman discovered the temperate phages. In 1956, Takahasi and Frankel–Conrat demonstrated the reconstitution of TMV. The closed circular and super helical nature of polyoma virus DNA was first elucidated by Dulbecco and Vogt (1963).

The crystallization of TMV in 1935 by Wendell Stanley brought this infectious agent into the

world of the chemists. Bawden and Pirie (1936) demonstrated that crystals of TMV contained 0.5% phosphorus and 5% RNA. Kaushe and his coworkers in 1939 had taken the first electron microscopic picture of TMV and it confirmed the rod shape of the virus particles. Jonas Salk (1955) successfully developed a killed vaccine for intravenous use against poliomyelitis, and Albert Sabin in1957 developed an attenuated virus vaccine for oral use against polio virus. Single stranded DNA was discovered in ϕX 174 bacteriophages by R.L. Sinsheimer (1959). The ultrastructure of T_2 bacteriophage was reported by S. Brenner, G. Strisinger, R.W. Horne and D. Crowther in 1959. In 1962, Caspar and Klug described the geometric principles of icosahedral structure of TMV. In 1962, Woff Home and Tournier formulated a unified system of classification of viruses. The viruses infecting cyanobacteria named as cyanophages were discovered by Shaflerman and Moris (1963). Reverse transcription *i.e.* DNA synthesis from RNA, a unique phenomenon observed in viruses alone, was reported by Howard Temin and David Baltimore in 964.

In 1967 Kornberg and Co-workers made attempts for artificial synthesis of viruses. Cancer causing virus was discovered by Schidolovski, Ahmad and Gallow in 1971. Sabin, Tam and Dress (1973) reported that the human cancer may also be caused by Herpes simplex virus. In 1976 Sanger made genetic mapping of the bacteriophage ϕX174. Galibert (1979) elucidated the nucleotide sequence of Hepatitis B virus (HBV) genome and Sninsky (1979) cloned HBV genome in *E. coli*. Robert Gallow (1984) identified AIDS as a viral disease.

1.3 PROPERTIES OF VIRUSES

Viruses exhibit the following characteristic properties –

1. **No independent metabolism** – Viruses cannot multiply outside a living cell. No virus has been cultivated in a cell-free medium. Viruses do not possess an independent metabolism for synthesis of proteins, carbohydrates and nucleic acids.
2. **Nucleocapsid** – Viruses have a very simple structure and are composed of a nucleic acid core surrounded by a protein coat known as nucleocapsid.
3. Absence of cellular structure – Viruses does not have any cytoplasm, limiting cell membrane and cytoplasmic organelles. The viruses utilize the ribosomes of the host cell for protein synthesis during reproduction.
4. **Nucleic acids** – Viruses have only one type of nucleic acid, either DNA or RNA. The Rous Sarcoma Virus (RSV) has both RNA and DNA and is therefore called RNA-DNA virus.
5. **Crystallization** – Many viruses can be crystallized, and thus behave like chemicals. The crystallized forms retain their infectivity.
6. **No growth and division** – The power of growth and division is absent in viruses. Various components of the virus are formed independently and assembled to form daughter viruses.
7. **Enzyme** – Lysozyme and transcriptase enzymes are present in viruses.
8. **Absence of respiration** – Viruses does not have any respiratory system.
9. **Transmission** – Viruses require transmission because it cannot pass from one living organism to another without transmission.
10. **Host specific** – Viruses are host-specific. Each virus can infect only a specific species and requires definite cells within host organisms for reproduction.
11. **Antibiotics have no effect on viruses** – Most of the antibiotics usually block a specific reaction in bacterial metabolism. The viruses have no metabolism of their own and utilize the metabolic machinery of the host cell for their reproduction. So,

the antibiotics have no effect on viruses.

12. **Mutation** – They show mutation like other living beings.

Biological status/nature of viruses

The biological status of viruses has always remained a debatable question. Biologists consider them as animate while the biochemists consider them simply inanimate chemical molecules. As they exhibit properties of both living as well as non-living objects, two theories have been proposed to explain their nature.

1. Organismal Theory: the supporters of this theory consider viruses as living or animate objects due to following characteristics –
    a) Viruses exhibit growth and reproduction, the chief distinctive features of all the living organisms.
    b) They possess genetic material in the form of nucleic acids (DNA or RNA).
    c) Some viruses are reported to possess their enzymes (e.g. HIV possesses reverse transcriptase protease and integrase).
    d) They are capable of causing infections in other living organisms.
    e) They exhibit high degree of host specificity.
    f) They exhibit antigenic properties.
    g) They undergo mutations to adapt to the environment through natural selection.

2. Molecular Theory: the supporters of this theory consider the viruses as non-living or inanimate objects because of the following features –
    a) They do not have cell structure, the basic unit of life.
    b) The viruses spontaneously assemble into viral particles inside the host cells, but do not exhibit cell division – a characteristic of all accepted forms of life.
    c) They do not exhibit respiration.
    d) They do not have independent metabolism but instead hijack the host's metabolic machinery.
    e) They can be crystallized as other chemical substances.
    f) They can exist in free state indefinitely like simple chemical substances.

1.4 MORPHOLOGY OF VIRUSES

Shapes of viruses: Viruses are of different shapes such as spheroid or cuboid (adenovirus), elongated (potatoviruses), flexous or coiled (beet yellow), bullet shaped (rabies virus), filamentous (bacteriophage M13), pleomorphic (alfalfa mosaic virus) etc.

Size of viruses: Viruses vary in their sizes (Fig. 1.1). Initially their sizes are estimated by passing them through membranes of known pore diameter. In recent years, their size is determined by ultracentrifugation and electron microscopy. In majority of the cases, they range from 10-300 nm in diameter. In Filo viruses, the length of the particle can be up to 1400 nm with a diameter of 80 nm. Small pox virus is of the same size as smallest bacterium and Lymphogranuloma virus is larger than the smallest bacteria. Recently, Mimi virus with a

diameter of 400 nm have been discovered, which possess the largest viral genome with 1000 genes, whereas some bacteria contain only around 500 genes.

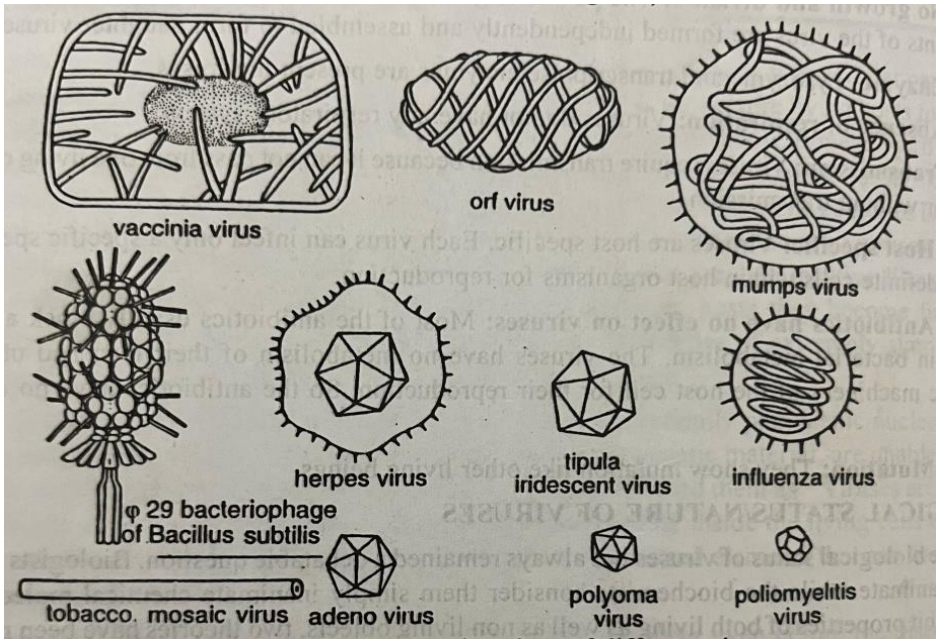

Figure- 1.1: Comparative sizes of different viruses

The broadest distinction is enveloped and non-enveloped viruses. Enveloped viruses contain a lipid-bilayer membrane and non-enveloped viruses do not contain the lipid-bilayer membrane. Further categorization of virus structure depends on their molecular organization. The progress made in understanding the viral architecture in atomic detail now allows studying the similarities across various families of viruses.

Electron microscopy is the most useful tool to determine the general morphology of a virus particle. The isolated and purified virus particles from the tissue gives more detailed images in electron microscopy. The traditional thin sections of infected cells are also used to examine the virus particles and their localization in the cells. Quantitative methods for image analysis, originally developed for studying negatively stained particles, have been applied effectively to such images. Cryoelectron microscopy is used to study the unstable or relatively impure preparations. Higher resolution picture can be obtained by X-ray diffraction method, if single crystals of the relevant structure can be prepared. In 1930, the simple plant virus such as Tomato bushy stunt virus (TBSV) was crystallized and studied. Crick and Watson (1956) proposed that virus shells would be highly symmetric objects. Identical subunits with specific interactions in general produce symmetric structures.

Morphologically, the viruses are classified as Helical, Polyhedral and Complex viruses (Fig. 1.2) based on electron microscopy and crystallography studies. The completely assembled virus particle is known as virion. A virion consists of a nucleic acid core surrounded by a protein coat or capsid. The complete set of virion is known as nucleocapsid. In turn the nucleocapsid may be naked or enveloped by loose covering. The capsid is composed of a large number of subunits known as capsomeres.

I) Helical (Cylindrical) Viruses:

(a) Naked capsid e.g. TMV and the bacteriophage M13, etc.
(b) Enveloped capsid e.g. Influenza virus, etc.

II) Polyhedral (Icosahedral) Viruses:

(a) Naked capsid e.g. Adenovirus, Polio virus, etc.
(b) Enveloped capsid e.g. Herpes simplex virus, etc.

III) Complex Viruses:

(a) Capsids not clearly identified e.g. Vaccinia virus, etc.
(b) Capsids with some attached structures e.g. some bacteriophages, etc.

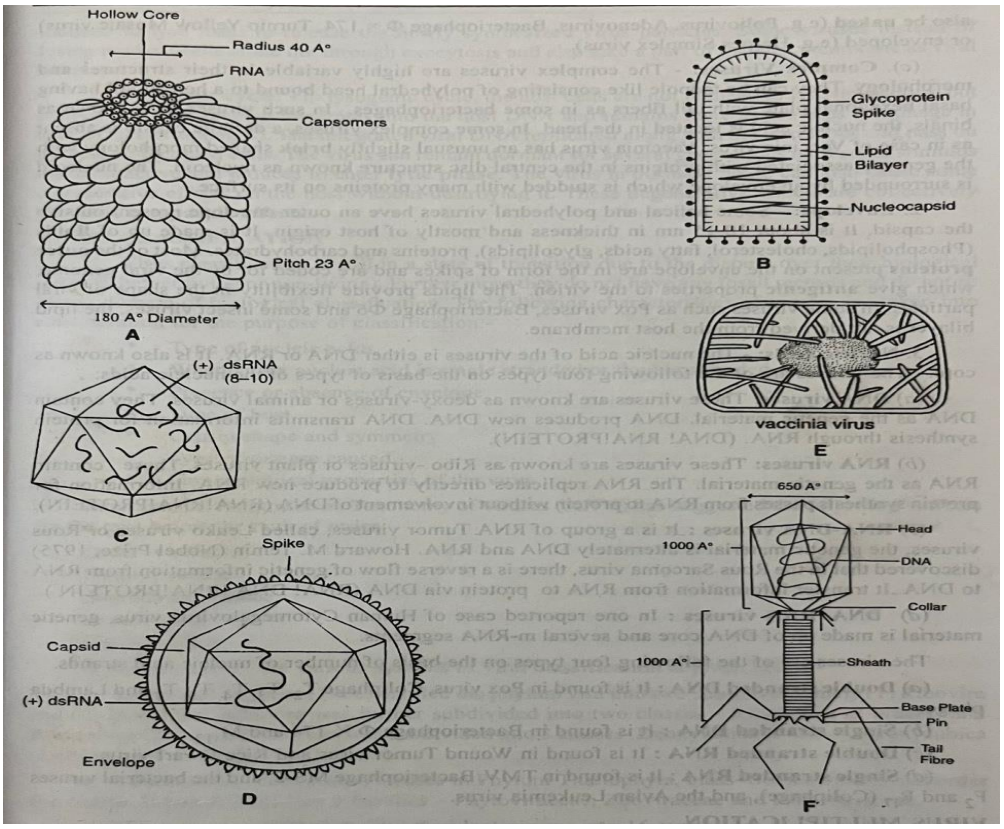

Figure-1.2: Types of viruses- A. Naked helical; B. Enveloped helical; C. Naked polyhedral; D. Enveloped polyhedral; E & F. Complex viruses
(Source: A.K. Thakur and S.K. Bassi)

Helical Viruses

The helical viruses are elongated, rod-shaped, rigid or flexible. Their capsid is hollow cylinder with a helical structure. Capsid consists of monomers arranged helically in a rotational axis. The helical capsids may be naked (e.g. TMV) or enveloped (e.g. Influenza virus). Helical symmetry is described by the number of structural units per turn of the helix. A characteristic feature of a helical structure is that any volume can be enclosed simply by varying the length of the helix, such a structure is said to be "open". In contrast, capsids with icosahedral symmetry are "closed" structures of fixed internal volume.

Naked viruses: One of the examples of naked viruses is the TMV (Fig. 1.3) that infects the tobacco plants and cause mosaic disease. This virus is rod-shaped measuring about 280 x 150-180 µm. It consists of a protein tube with a lumen of 20Å which encloses a single stranded (ss) helix of coiled RNA. Protein coat of the virus contains a number of identical subunits (monomers) which are arranged in helical manner. The capsid is composed of 2,130 subunits, and each subunit is made up of 150 amino acids forming a single polypeptide chain. The ssRNA of TMV has the molecular weight of 2.06 x 10^6 Daltons and consists of 6,500 nucleotides in total and coiled to form a helix. Each turn of the RNA helix consists of at least 49 nucleotides. The capsid protects the RNA molecule.

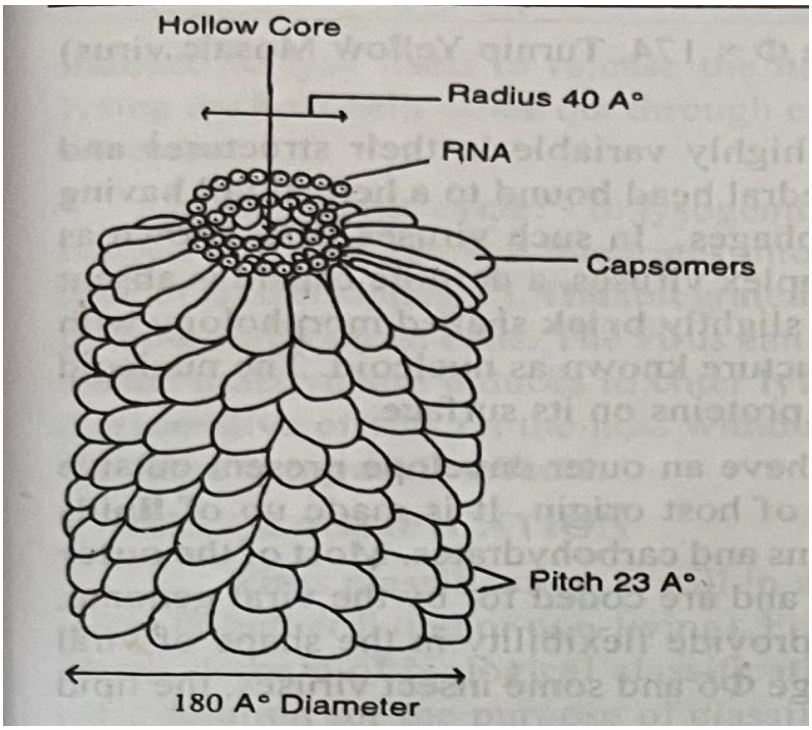

Figure-1.3: The naked helical structure of TMV

Enveloped viruses: When the helical viruses are enclosed within an envelope they are known as enveloped helical viruses, for example influenza virus. The envelope is composed of a viral protein and the host cell components i.e. lipid and carbohydrates. The envelope consists of numerous spikes. The helical capsid exists in folded form inside the envelope and sometimes may show pleomorphic appearance.

Polyhedral (Icosahedral) Viruses

There are several animal, plant and bacterial viruses which have either naked or enveloped icosahedral shape. Polyhedral structure has the three possible symmetries such as tetrahedral, octahedral and icosahedral. The viruses are more-or-less spherical. The icosahedral symmetry is the best one for packaging and bonding of subunits. Several inter molecular bonds of low free energy is formed. An icosahedron is a regular polyhedron with 20 triangular faces and 12 corners. The capsomers of each face form an equatorial triangles and 12 intersepting points or corners (Fig. 1.4).

Basically, there are two types of capsomers, the pentamers and hexamers. The pentamer is a cluster of 5 monomers and the hexamer is a cluster of 6 monomers. The monomers are linked

together by bonds. The capsomers are also linked together by bonds which are weaker than those of monomers. All subunits interact with their neighbors in an identical or equivalent manner. In icosahedral capsid, certain capsomers only in certain number may be present, but theoretically the minimum number is 12. In different viruses, the number of capsomers varies, for example 72 in polyomavirus, 92 in reovirus, 162 in herpesvirus, 252 in adenovirus.

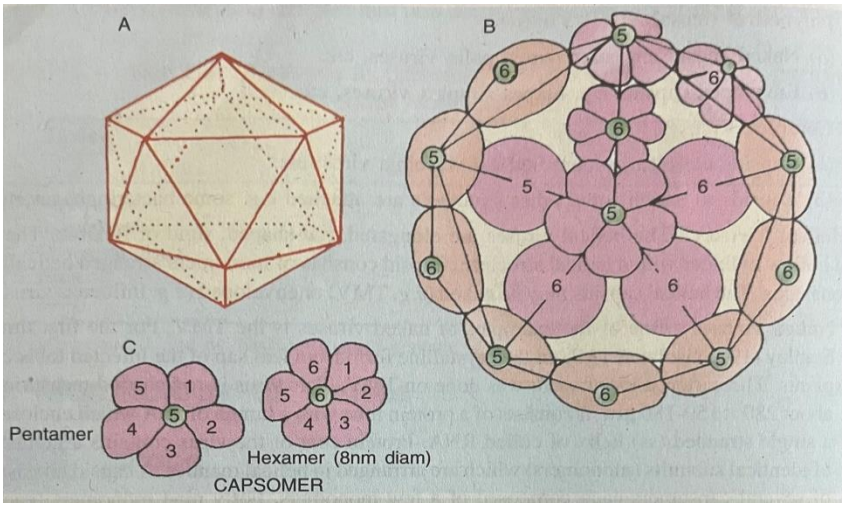

Figure-1.4: A. Icosahedral symmetry; B. Architecture; C. Subunits of capsomers

In 1962, Caspar and Klug developed a theory for the structural properties of larger particles with icosahedral symmetry. The theory proposes that when a capsid contains more than 60 subunits, each subunit occupies a quasi-equivalent position. The triangulation number was also proposed by Caspar and Klug, the description of the triangular faces of large icosahedral structure in terms of its subdivision into smaller triangles termed facets. The triangulation number and quasi-equivalent bonding among subunits describe the structural properties of many simple viruses with icosahedral symmetry.

Quasi-equivalent designs are exemplified by a number of animal and plant viruses such as Norwalk viruses and Tomato bushy stunt virus (TBSV). These consist of 180 genetically and chemically identical subunits that form the capsid. The shell domain (S domain) of these two viruses is about 200 residues and folded structure of the domain is again a Jelly – roll – barrel model. The contents of an icosahedral asymmetric unit can be described as A, B and C, which are chemically identical subunits with different conformations. The A and B conformations are nearly identical with discarded arms and similar hinge angles. C confirmation has an ordered arm and a different hinge angle from A and B.

An icosahedral symmetric structure is folded–up as a hexagonal net, 12 uniformly spaced six fold vertices are transformed into five-fold vertices. The intervening two fold, three fold and six fold symmetry axes of the flat net are transformed either into quasi-two fold, quasi-three fold and quasi- six fold axes of the icosahedral net. A number of the viral architecture designs predicted by Caspar and Klug among various viruses of plant, vertebrates and insect viruses. Herpes virus capsids which had T=16 structure with 12 pentamers and 150 hexamers of the major capsid protein assemble around a scaffold protein. Adenoviruses exhibit a combination of non-equivalent and quasi-equivalent interactions with T=25 icosahedral lattice. It had 12 pentons on the five-fold positions and 240 hexons on the six-fold positions. Double stranded RNA viruses (Blue tongue virus) exhibit both non-equivalent and quasi-equivalent interactions in separate protein shells consisting of 120 identical subunits with T=13.

Naked icosahedral viruses: Naked icosahedral viruses are turnip yellow mosaic virus (TYMV), poliovirus, adenovirus, and bacteriophage ϕx174, QB, etc. Adenovirus is icosahedral containing dsDNA. The capsid contains 12 pentamers and 32 hexamers. The capsomers are assembled to construct the capsid in a specific geometrical pattern where the pentamers form the corners of the icosahedron and the hexamers occupy the internal space. Some spike like structures arise from 12 points of the five-fold symmetry.

Enveloped icosahedral viruses: There are some enveloped icosahedral viruses, e.g. herpes simplex virus. In this virus, the capsid is enclosed inside an envelope of 30nm thickness which is made up of by glycoprotein-lipid complex. The envelope consist spikes on its surface. The capsid spherical enclosing a dense core of dsRNA molecule and contains 162 capsomers – 12 pentamers at apices and 150 hexamers at the faces.

Complex viruses

The viruses which have the unidentifiable capsids or have the capsids with additional structures are called complex viruses, e.g. Vaccinia virus and T-even bacteriophages. In addition, the other variations in the structure of complex viruses are also found such as (i) a definite capsid is absent (vaccinia virus), (ii) capsid present and consists of a tail. The second group consists of different types, for example, tadpole shaped viruses (with head and tail e.g. t-even phage), viruses with tail less head (lambda phage), brick-shaped virus devoid of flattened cylinder (pox virus), and bullet shaped capsid viruses. The best studied example for complex viruses is the binal structure of the T4 bacteriophage (member of T-even bacteriophages).

Binal structure

Binal structure is a complex one and bacteriophages are the best examples of binal viruses. They exhibit morphology of combination of two structures - the 'head' and 'tail'. The head is usually icosahedral and the tail is helical in symmetry (Fig. 1.5). The head shows all types of triangulation numbers as exhibited by normal icosahedral viruses. The head of lambda exhibit the highest triangulation number of T = 7 with 12 pentamers and 60 hexamers. Other morphological variations observed among phages include phages with much elongated heads - myoviridae, short tails - podoviridae and long tails - siphoviridae.

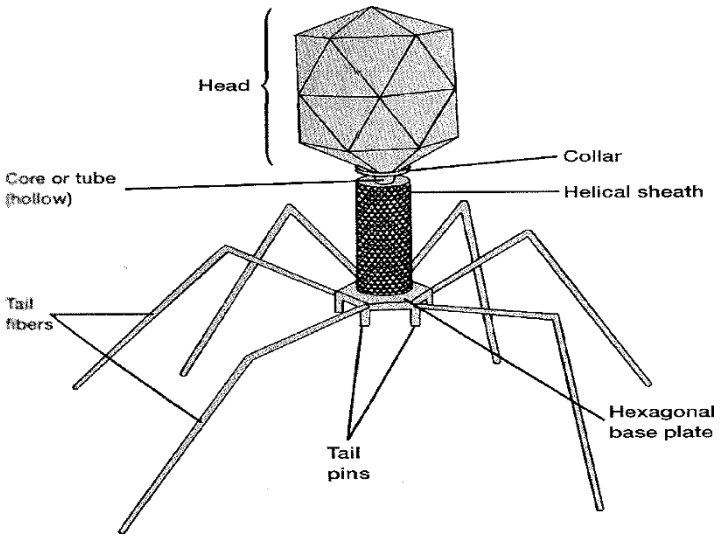

Figure-1.5: The binal structure of T4 bacteriophage.

Envelope of the viruses

Some helical and polyhedral viruses have an outer envelope present outside the capsid. It is about 10-15 nm in thickness and mostly of host origin. It is made up of lipids (phospholipids, cholesterol, fatty acids, glycolipids), proteins and carbohydrates. Most of the outer proteins present on the envelope are in the form of spikes and are coded for by the viral genome, which give antigenic properties to the virion. The lipids provide flexibility in the shape of viral particles. In some viruses such as pox viruses, bacteriophage ϕ6 and some insect viruses, the lipid bilayer is not derived from the host membrane.

Peplomers or Spike proteins

The term 'peplomer' refers to an individual spike from the viral surface. The term is derived from the Greek 'peplos' meaning 'a loose outer garment'. Early systems of viral taxonomy, such as Lwoff-Horne-Tournier system proposed in 1960s, used the appearance and morphology of the peplomers as an important characteristic feature for classification. Peplomers or spikes are usually rod or club shaped projections from the viral surface. These are membrane proteins, usually glycoproteins, with typically large external ectodomains, a single transmembrane domain that anchors the protein in the viral envelope, and a short tail in the interior of the virion. Peplomers are found in enveloped viruses such as orthomyxoviruses, paramyxoviruses, rhabdoviruses, filoviruses, coronaviruses, bunyaviruses, arenaviruses, and retroviruses. They play a role in viral entry into the host, by interacting with cell-surface receptors on the host cell. They may have hemagglutinizing activity or enzymatic activity. For example, influenza virus has two surface proteins having two functions namely hemagglutinin and neuraminidase activities. Many of these spike proteins are membrane fusion proteins. Some peplomer proteins are antigenic in nature being exposed on the virion surface.

1.5 SUMMARY

The discovery of viruses started with the studies on tobacco mosaic disease. The causal agent of the disease can pass through the bacterial filters. Hence, it was first described as "contagium vivum fluidum" and general term virus (meaning poison in Latin) was applied to causal agent of tobacco mosaic disease and causal agents of other diseases that can pass through bacterial filters. Stanley in 1935, crystallized the virus and its chemical nature was determined by a number of workers. The first electron microscopic picture of TMV was taken by Kaushe and his coworkers. Structurally the viruses are of three types viz. helical, icosahedral and binal. Chemically viruses are made of nucleic acids, proteins, carbohydrates and lipids. Viruses have a major role in medicine research and diagnosis. They are useful in vaccine production, gene therapy, cancer therapy, virus based diagnosis, research aspects, as biopesticides.

1.6 TECHNICAL TERMS

Virion, Bacteriophage, Helical structure, Icosahedral symmetry, Binal structure, capsomeres, Pentamers, Hexamers, Capsid.

1.7 SELF ASSESSMENT QUESTIONS

Q.1 Describe the properties and morphology of viruses with suitable examples.

Q.2 Discuss about the structural details of viruses.

Q.3 Give a brief account on the discovery of viruses.

Q.4 Discuss the best examples of helical and complex viruses.

1.8 SUGGESTED READINGS

1. Virology - Frankel- Conrat et al., 3rd Edition, 1994, Prentice Hall publications
2 Principles of Virology - S.J. Flint et al., 2000, ASM Press
3 Introduction to Modern virology – Dimmock et al., 5th edition, 2001, Blackwell Sci. Publications
4 Plant Virology - R. Hull, 4th edition, 2001, Academic Press
5 Fundamentals of Virology - D.M. Knipe and P.M. Howley, 4th edition, 2001, Lippincott.
6 Applied plant Virology - D.G.A. Walkey, 1985, Heinemann Publication.

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LESSON - 2

CHEMISTRY OF VIRUSES

Objective of the lesson

Students will gain knowledge on the chemical nature of different viruses and also understand the types of viruses based on their chemical nature.

Structure of lesson

2.1 INTRODUCTION

Max Schlesinger (1933) partially established the chemistry of viruses through the studies on bacteriophages and concluded as possibly nucleoproteins. The complete chemical identity was determined in TMV by Stanley (1935) through crystallization. Later, Bawden and Pirie (1937) established that the nucleoprotein nature of TMV strains as Ribonucleic acid. The field of "Chemical era of Virology" was launched by C.A. Knight (1974). Chemically, viruses are nucleoproteins and consist of two components, one is outer proteinaceous covering or sheath (capsid) and the other is internal component, which is a nucleic acid (genome) – either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). Some of the viruses contain third component made up of lipids and carbohydrates (envelope) in the case of enveloped viruses. The composition of proteins, lipids, and carbohydrates varies both qualitatively and quantitatively from virus to virus. The type or nature of nucleic acids are also varies from virus to virus – bacterial and animal viruses are mostly DNA viruses, whereas plant viruses are in most cases RNA viruses. More variety is found in the genomes of viruses than in those of prokaryotes and eukaryotes. They may be either single stranded or double stranded DNA or RNA. The nucleic acid strands can be linear, closed circle, or able to assume their shape. The genome is either non-segmented or segmented in viruses. The nucleic acid moiety is the active disease specific and host specific infective part of a virus. The nucleic acid content per particle varies from one virus to another virus. Generally, larger the size of the virus particle, larger is the contents of nucleic acid and vice-versa. The nucleic acid content of a virus can be roughly calculated from the molecular weight of a virus and its nucleic acid percentage.

2.2 VIRAL NUCLEIC ACIDS

Viruses are exceptionally flexible with respect to the nature of their genetic material. Infectiousness of the viruses depends on nucleic acid moiety only. The type and content of nucleic acid varies from virus to virus. Large size viruses consist of high quantity of nucleic acid. Greater amount of nucleic acid is necessary for the synthesis of complex viruses. The nucleic acid content of a virus can be calculated from the molecular weight of virus and its nucleic acid percentage.

Viruses employ all four possible nucleic acid types – single stranded DNA, double stranded DNA, single stranded RNA and double stranded RNA. All the four types are found in animal viruses. Plant viruses most often have single stranded RNA genomes. Although phages may have single stranded DNA or single stranded RNA, bacterial viruses usually contain double stranded DNA. Variations seen in viral nucleic acids are summarized in table 2.1. The size of viral genetic material also varies greatly. The smallest genomes are around $1x10^6$ (MS2 QB viruses), just large enough to code for three or four proteins. At the other extreme, T-even bacteriophages, herpes virus, and vaccinia virus have genomes of 1.0 to 1.6 $x10^8$ Daltons and may be able to direct the synthesis of over 100 proteins.

Table-2.1: Types of Viral Nucleic Acids

Nucleic Acid Type	Nucleic Acid Structure	Virus Examples
DNA		
Single-Stranded	Linear single strand	Parvoviruses
	Circular single strand	ϕX174, M13, fd phages
Double-Stranded	Linear double strand	Herpesviruses (herpes simplex viruses, cytomegalovirus, Epstein-Barr virus), adenoviruses, T coliphages, lambda phage, and other bacteriophages
	Linear double strand with single chain breaks	T5 coliphage
	Double strand with cross-linked ends	Vaccinia, smallpox
	Closed circular double strand	Papovaviruses (polyomavirus, human papilloma viruses, SV40), PM2 phage, cauliflower mosaic
RNA		
Single-Stranded	Linear, single stranded, positive strand	Picornaviruses (polio, rhinoviruses), togaviruses, RNA bacteriophages, TMV, and most plant viruses
	Linear, single stranded, negative strand	Rhabdoviruses (rabies), paramyxoviruses (mumps, measles)
	Linear, single stranded, segmented, positive strand	Brome mosaic virus (individual segments in separate virions)
	Linear, single stranded, segmented, diploid (two identical single strands), positive strand	Retroviruses (Rous sarcoma virus, human immunodeficiency virus)
	Linear, single stranded, segmented, negative strand	Paramyxoviruses, orthomyxoviruses (influenza)
Double-Stranded	Linear, double stranded, segmented	Reoviruses, wound-tumor virus of plants, cytoplasmic polyhedrosis virus of insects, phage ϕ6, many mycoviruses

Tiny DNA viruses like ϕX174 and M13 bacteriophages or the parvoviruses possess single stranded DNA genomes. Some of these viruses have linear pieces of DNA, whereas others have a single, closed circular DNA. Most DNA viruses have dsDNA as their genetic material. Linear dsDNA, variously modified, is found in many viruses and others have circular dsDNA. The lambda phage has linear dsDNA with cohesive ends – single stranded complementary segments of 12 nucleotides long – that enable it to cyclize when they base pair with each other. Besides the normal nucleotides, many virus DNAs contain unusual

bases. For example, the T-even phages of *E. coli* have 5-hydroxymethylcytosine instead of cytosine and glucose is usually attached to the hydroxymethyl group.

Most RNA viruses employ ssRNA as their genetic material. The RNA base sequence may be identical with that of viral mRNA, in which case the RNA strand is called the 'plus strand' or 'posistive strand'. However, the viral RNA genome may instead be complementary to viral mRNA, and then is called as 'minus strand' or 'negative strand'. Polio, tobacco mosaic, brome mosaic, and Rous sarcoma viruses are all positive strand RNA viruses. Whereas, rabies, mumps, measles, and influenza viruses are of negative strand RNA viruses. Many of these RNA genomes are segmented genomes – that is, they are divided into separate parts. It is assumed that each fragment or segment codes for one protein. Usually, all the segments are probably enclosed in the same capsid even though some virus genomes may be composed of as many as 10 to 12 segments. Plus strand viral RNA often resembles mRNA in more than the equivalence of its nucleotide sequence. Just as eukaryotic mRNA usually has a 5' cap of 7-methylguanosine, many plant and animal viral RNA genomes are capped. In addition, most or all plus strand RNA animal viruses also have a poly-A stretch at the 3' end of their genome, and thus closely resemble eukaryotic mRNA with respect to the structure of both ends. In fact, the plus strand RNAs can direct protein synthesis immediately after entering the cell. Strangely, a number of single stranded plant viral RNAs have 3' ends that resemble eukaryotic tRNA, and the genomes of tobacco mosaic virus will actually accept amino acids. Capping is not seen in the RNA bacteriophages. A few viruses have ds RNA genomes. All appear to be segmented; some such as reoviruses, have 10 to 12 segments. These dsRNA viruses are known to infect animals, plants, fungi, and even one bacterial species.

Genomic diversity is one of the most important characteristic features in viruses. Various types of viral nucleic acid structures viz., straight chain, cyclic, super coiled were recorded and reported by different scientists. Single stranded and double stranded nucleic acid types were identified. Segmented and non-segmented genomes of virus nucleic acid are the special feature for viruses. Recently the sense of the viruses was also noticed on the bases of polarity in single stranded genome (RNA) viruses.

Primary structure of viral nucleic acids relates to the proportion and arrangement of various nucleotides in a specific manner. The primary structure of viral nucleic acids can be determined into two ways – one is to determine the proportions of purines and pyrimidines and the second one is to determine the sequence arrangement of the nucleotides. In double stranded nucleic acid molecules, there is direct one to one correspondence between the purine and the pyrimidine bases. Base ratios are almost identical in similar viruses. Eg., Papilloma virus, Polyoma virus and SV40 virus. Sometimes dissimilar viruses may also have nearer identical base ratios. Eg., Coliphage, T4 and Iridescent virus. Higher proportion of uracil was estimated than other bases in influenza virus. The base ratio analyses do not allow a close insight into the primary structure of nucleic acid molecules. The nucleotide sequence analysis is the better approach to know about the arrangement of nucleotides in viral nucleic acids. The nucleic acid base ratios of some DNA viruses and RNA virues are given in tables 2.1 and 2.2 respectively.

The analysis of the molar base ratios of the viral nucleic acids revealed some significant information –

1) There is a great variation in the base ratios of nucleic acids, both RNA and DNA,

obtained from different viruses. This is clearly reflected in AT/GC or AU/GC ratios.

2) In double stranded nucleic acid molecules there is direct one to one correspondence between the purine and the pyrimidine bases. This is evident from the data obtained on nucleic acid from all the DNA containing viruses except ϕx174 which has single stranded DNA. Similarly, there is such correspondence in the cases of only two RNA containing viruses, wound tumor and reo, both of which are double stranded RNA. As a result, there is a lopsided proportion of certain bases. For instance, a higher proportion of uracil than other bases is found in influenza virus.
3) Similar viruses may have almost identical base ratios. For example, papilloma, polyoma and SV40 viruses, all of which are oncogenic and possess nucleic acids with similar base ratios.
4) Dissimilar viruses may also have almost identical base ratio, for instance Coliphage T4 and Tipula iridescent virus, both having similar GC content.

Table-2.1: Nucleic acid base ratios of some DNA viruses

Virus	Adenine	Guanine	Cytosine	Thymine	AT/GC
Adenovirus	21	29	29	21	0.73
Coliphage T4	24	25	19	32	1.92
Coliphage λ	26	24	24	26	1.04
Herpes Simplex	16	34	34	16	0.47
Polyoma	26	24	24	26	1.04
Papilloma	26	24	24	26	1.04
SV40	26	24	24	26	1.04
Tipula Iridescent	34	16	16	34	2.12

Table-2.2: Nucleic acid base ratios of some RNA viruses

Virus	Adenine	Guanine	Cytosine	Uracil	AU/GC
Broadbean mottle	27	25	19	29	1.27
Coliphage M12	23	26	26	25	0.93
Influenza	23	20	24	33	0.80
poliomyelitis	29	24	22	25	1.15
Tobacco Mosaic	28	24	22	26	1.15
Tobacco Necrosis	28	26	22	24	1.05
Tomato bushy stunt	25	28	21	26	1.04
Reo	28	22	22	28	1.27

2.3 VIRAL PROTEINS

Proteins found in viruses may be grouped into four categories- i) Envelope proteins; ii) Nucleocapsid (structural) proteins; iii) Core proteins; and iv) Viral enzymes.

i) Envelope proteins

Envelope of the viruses consists of proteins specified by both virus and host cell. In several viruses, its capsids are covered by a smooth and unbroken lipid layer. Such a coat is effectively inert and acts as a protective layer to prevent the desiccation or enzymatic damage of the particles. And sometimes, the receptor molecules on the host surface cannot be

recognized. Viruses can also change their lipid envelopes through the synthesis of several classes of proteins which get associated with the envelope in one of three ways—

Matrix proteins – these are the internal virion proteins that link the assembly of the internal nucleocapsid.

Glycoproteins – these are the proteins that contain oligosaccharide chains (glycans) covalently attached to their polypeptide side chains. Conjugate proteins such as glycoproteins make up the peplomers present on the surface of the viral envelope. A type of non-glycosylated protein is found to be present on the inside of the envelope in orthomyxo and pramyxo viruses. Basing on the functions, glycoproteins are divided into two types – External glycoproteins and Transport channels.

External glycoproteins – these are embedded in the envelope by a single transmembrane domain. Most of the proteins are present on the outside of the membrane. They have relatively a short tail. Individual monomers are mostly gets associated to form spikes on the surface of viral envelope. Such proteins act as the major antigens of enveloped viruses.

Transport channels – these proteins contains multiple hydrophobic transmembrane domains forming a protein-lined channel through the envelope. It helps the virus to change the permeability of the membrane.

ii) Nucleocapsid proteins

The viral capsids are made up of by proteins of identical subunits (protomers). The helical capsids contain single type of protein and icosahedral capsid contains several types of protein. For example, TMV contains single protein types, adenovirus contain 14 protein types, T4 bacteriophage contains 30 protein types, etc.

iii) Core proteins

Protein found in nucleic acid is known as core protein, for example nucleoproteins of influenza virus, and proteins V and VI of adenoviruses.

iv) Viral enzymes

In animal viruses especially in the enveloped viruses, many virion specific enzymes have been characterized, for example RNase and reverse transcriptase in retroviruses, protein kinase in herpes virus and adenovirus, and DNA dependent RNA polymerase in pox virus.

Proteins are the basic biochemical units of the viruses which are wrapped as outer component of the genome as a capsid or coat or sheath. The coat protein gives the characteristic shape to viruses. The protein coats of viruses are not in unitary structure and composed of varying number of identical subunits. Subunits present in the capsid are called as capsomeres. These capsomeres which are assembled and form a structural unit to the virus. These capsomeres are either homopolymers or heteropolymers. In complex viruses, along with coat protein, some of the non-coat proteins are also associated and act as functional enzymes as an internal proteinaceous entities, Eg., Neuraminidase (Influenza virus), lysozyme (Phages).

The primary structure is the basic form of a viral protein. The secondary, tertiary and quaternary structures of viral proteins are essentially dependent upon the primary features, which in turn derived from the composition of the amino acids and their sequential arrangements. Amino acid analysis is being used to understand the primary structure of viral proteins. In this analysis, the cleavage of a large polypeptide chain into smaller fragments, determination of sequence of these fragments and determination of the sequence of amino acids in the individual fragments are performed. In 1959, Woody and Knight successfully analysed the TMV coat protein by tryptic digestion method. Other TMV proteins were analysed by using various proteolytic enzymes like chemotrypsin, pepsin and subtilisin. The secondary structures and higher configuration of the viral proteins are being studied by using X-ray crystallography analyses. This method is useful to study the spatial arrangement of various subunits of the coat proteins and their alignment with central nucleic acid. List of some non-coat proteins associated with viruses are given in table 2.3.

Table-2.3: Non-coat proteins associated with viruses

Non-coat proteins	**Virus**
Viral Lysozyme	Bacteriophage T2
Influenzal neuraminidase	Influenza virus
RNA transcriptase	Reovirus
RNA polymerase	New Castle disease virus
RNA directed DNA polymerase	Murine Leukaemia virus

The amino acid composition data revealed that viral coat proteins are constituted of the common protein amino acids. Studies also indicated that all the amino acids are L-isomers, the form which is universally found in other living forms. The amino acid composition of some viral coat proteins is given in table 2.4.

Table-2.4: Amino acid composition of some viral coat proteins (relative no. per unit)

Virus Amino Acid	Tobacco Mosaic virus	Tomato Necrosis virus	Cucumber Mosaic virus	Coli- phage f_d	Coli phage Qβ
Alanine	14	13	17	9	15
Arginine	11	14	24	0	7
Asparagine	10	0	0	0	8
Aspartic Acid	8	18	30	3	7
Cysteine	1	2	0	0	2
Glutamine	9	0	0	1	8
Glutamic Acid	7	20	20	2	5
Glycine	6	8	16	4	7
Histidine	0	1	4	0	0
ISO-Leucine	9	11	16	4	4
Leucine	12	10	26	2	12
Lysine	2	12	18	5	7
Methionine	0	6	8	1	0
Phenylalanine	8	12	7	3	3
Proline	8	15	6	1	8
Serine	16	14	24	4	9
Threonine	16	16	10	3	12
Tryptophan	3	0	2	1	0
Tyrosin	4	11	4	2	4
Valine	14	14	7	4	13

2.4 VIRAL CARBOHYDRATES

Two types of carbohydrates are associated with viruses. The first type of carbohydrates is associated with viral nucleic acids namely the ribose and deoxyribose sugars, either of them is found in viruses. The second type of carbohydrates is mostly associated with capsid or nucleocapsid proteins (glycoproteins) and lipids (glycolipids). These are simple sugars which are linked with hydroxyl methyl cytosine residue. The analysis of glycoproteins and glycolipid components revealed that the carbohydrate component is mainly made of fructose, galactose, glucosamine and mannose. It has also been revealed that the protein and carbohydrate moieties in glycoproteins are linked by formation of bonds between the carbohydrate chain and asparagine, serine, threonine residues of proteins. A substantial amount of carbohydrate specified by either host cell (arbovirus) or viral genome (vaccinia virus) is found in viral envelope. The forms of carbohydrates found in glycoproteins and/or glycolipids are hexoses and hexamines. Influenza virus, parainfluenza virus, SV5 and sindbis virus contain galactose, mannose, glucose, fucose, glucosamine and galactosamine forms of carbohydrates.

2.5 VIRAL LIPIDS

Lipids are found in most of the enveloped viruses. Several kinds of lipids (phospholipids, cholesterol, fatty acids, and glycolipids) are associated with various animal viruses, plant viruses and bacteriophages. Only a few plant viruses have lipids as one of the chemical constituents (e.g. potato yellow dwarf virus, tomato spotted wilt virus). Phospholipids of different types are found associated with many of the viruses. Cholesterol and triglycerides are also very common. These lipids are located in the envelope of the viruses. Lipids play an important role at the time of virus maturation and budding. Recent studies revealed that there is a significant difference in lipid component both qualitatively and quantitatively, in the envelop of viruses. The proteins and polysaccharides are linked loosely and form a lipoprotein and glycolipid complex in the viruses.

Some of the viruses consist of special components in their structure. These are polyamines (T2, T4 phages). The polyamines are putrescine, spermidine and spermine. Polyamines are also reported in Herpes virus and Influenza virus. Traces of polyamines are identified in Turnip yellow mosaic virus and Broad bean mottle virus. Inorganic divalent metal ions are also found in certain viruses. Eg., Ca^{2+}, Mg^{2+} (TMV, Southern Bean Mosaic Virus). Some plant viruses such as Tobacco streak virus (TSV) have a zinc finger binding domain that specifically binds an atom of zinc in a protein involved in nucleic acid binding.

2.6 SUMMARY

Chemically, viruses consist of nucleic acids, proteins, carbohydrates, lipids and enzymes. Viral nucleic acids can be either single stranded or double stranded DNA or RNA. Most DNA viruses have double stranded DNA genomes that may be linear or closed circles. RNA viruses usually have ssRNA that may be either plus (positive) or minus (negative) when compared with mRNA (positive). Many RNA genomes are segmented. Although viruses lack true metabolism, some contain a few enzymes necessary for their reproduction. Phospholipids of different types are found associated with many of the viruses. Cholesterol and triglycerides are also very common. These lipids are located in the envelope of the viruses.

2.7 TECHNICAL TERMS

ssDNA, dsDNA, ssRNA, dsRNA, phospholipids, cholesterol, fatty acids, plus strain, minus strain, linear genome, circular genome, segmented genome, non-segmented genome.

2.8 SELF ASSESSMENT QUESTIONS

Q.1 Explain the nucleic acids nature of different viruses with suitable examples.

Q.2 Give an account on chemistry of viruses.

Q.3 Describe the different proteins, lipids and carbohydrates associated with viruses.

2.9 SUGGESTED READINGS

1. Virology - Frankel-Conrat et al., 3rd Edition, 1994, Prentice Hall publications
2. Principles of Virology - S.J. Flint et al., 2000, ASM Press
3. Introduction to Modern virology – Dimmock et al., 5th edition, 2001, Blackwell Sci. Publications
4. Plant Virology - R. Hull, 4th edition, 2001, Academic Press
5. Fundamentals of Virology - D.M. Knipe and P.M. Howley, 4th edition, 2001, Lippincott.
6. Applied plant Virology - D.G.A. Walkey, 1985, Heinemann Publication.

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LESSON – 3

VIROID AND PRION DISEASES AND SATELLITE VIRUSES AND SATELLITE RNAS

Objective of the lesson

Students will gain knowledge on the sub-viral agents like viroids and prions and also become aware of the most important diseases caused by them. Also know about the satellite viruses and satellite RNAs.

Structure of the lesson

3.1 INTRODUCTION

Infectious agents simpler than viruses also exist. Viroids are the short strands of infectious RNA responsible for several plant diseases. Prions or virinos are somewhat mysterious proteinaceous particles associated with certain degenerative neurological diseases in humans and livestock. Viroids are able to infect different crops and cause economic loss. Potato spindle tuber and coconut cadang-cadang are the prominent examples that causes severe yield losses. As viroids spread through transmission, uninfected tools and hands have to be used. Always the leaf contact or usage of same tools employed in infected crops should be avoided. Different chemical substances like 1-5% sodium hypochlorite, 6% hydrogen peroxide and 2% formaldehyde can be used as disinfectants to prevent viroid transmission by tools in field conditions. Prions are the proteinaceous infectious subviral agents that cause diseases in animals and humans. Prion diseases are often called as spongiform encephalopathies because of the post mortem appearance of the brain with large vacuoles in the cortex and cerebellum. Probably most mammalian species develop these diseases. Specific examples include – scrapie in sheep, transmissible mink encephalopathy (TME) in mink, chronic wasting disease (CWD) in muledeer, bovine spongiform encephalopathy (BSE – mad cow disease) in cows, Creutzfeldt-Jacob disease (CJD) in humans etc.

3.2 VIROIDS

Viroids, also referred as sub-viral agents, are the smallest infectious pathogens made up of a short RNA molecule without the protein coat. These pathogens affect only the plants and are therefore known as plant pathogens. These single stranded circular RNA molecules vary in length from 246 to 463 nucleotides and found only in plants. They do not encode proteins but replicate independently when introduced into host plants. So far, 30 viroids have been characterized, 28 infecting the dicotyledonous plants and the other 2 infecting the monocotyledonous plants. The crops that are infected by viroids result in crop failure due to huge losses to the agriculture industry.

The 30 known viroids belong to two families – Pospiviroidae and Avsunviroidae.
Pospiviroidae: Members of this family are named after the potato spindle tuber viroid. They have rod-like structure with a small single stranded region with a central conserved region. In plants that are infected, these viroid RNA is imported into the nucleus and replicated or copied by the plant DNA-dependent RNA polymerase II. The viroid is copied by a rolling circle mechanism which produces complementary linear and concatermeric RNAs. These are copied again to produce concatermeric linear molecules which are cut by the host enzyme RNA polymerase III. Their ends are joined by a host enzyme to form circles.

Avsunviroidae: This is named after avocado sun blotch viroid. This viroid also has rod-like branched regions. However, this viroid lacks a central conserved region and replicates in the chloroplast. RNA molecules are working ribozymes and this activity is crucial for replication. In the plants infected by the members of this family, viroid RNA is imported into the chloroplast and concatermeric RNAs are produced by chloroplast-dependent RNA polymerase.

Viroids are known to affect only plants and this affliction of the disease can cause economic loss and destroy important crops, fruits, and plants. Two clear examples of economically significant viroids are ‘coconut cadang-cadang viroid’ (cause deadly infection in coconut palm trees) and ‘apple scar skin viroid’ (cause disease in apple trees resulting in unappealing apple fruits). The other diseases caused by viroids are citrus exocortis, cucumber pale fruit, chrysanthemum stunt, etc. Some infections spread due to the propagation of seeds in plants by cutting, tubers, etc. The diseases can even spread by mishandling contaminated equipment and implements. Viroid progeny moves to adjacent cells through plasmodesmata and can travel systemically via phloem to infect other cells. They also enter into the pollen and ovule from where they are transmitted to the seed. When these seeds germinate, the newly emerging plants also become a victim of infection. Viroids can also spread among the plants by contaminated insects.

The symptoms are not very prominent unless the crop is infected. The most common symptom of the viroid infection is ‘retardation of plant growth and stunting’, which causes dwarfism in crops. **In** citrus trees, the symptom includes scaling of the bark below the graft union. In case of hop plants, tomato plants, and chrysanthemum plants, the main symptom is stunting of plants.

3.2.1 Potato Spindle Tuber Viroid

The potato spindle tuber viroid (PSTVd, *Pospiviroid fusituberis*) was the first viroid to be identified. It was discovered in 1971 by T.O. Diener, plant pathologist at the U.S. Department

of Agriculture's Research Centre in Beltsville, Maryland. PSTVd is a small ($1/80^{th}$ size of a virus), single stranded circular RNA molecule and closely related to the chrysanthemum stunt viroid. The natural hosts for this viroid are potatoes (*Solanum tuberosum*), and tomatoes (*Solanum lycopersicum*). Natural infections have also been seen in avacados and infections in other solanaceous crops have been induced in the laboratory. Pstv also causes 'Tomato bunchy top' and is seed transmitted. In potatoes, symptoms are quite prominent and the tubers are spindle shaped and smaller in size (Fig.3.1).

Figure-3.1: Potatoes infected by PSTVd

The PSTVd comprises 359 nucleotides and its putative secondary structure is given in figure 3.2. Determination of the complete nucleotide sequence of PSTVd made by Gross and his colleagues. In 1985, Keese and Symons used the information of Gross and proposed that PSTVd viroid contain five structural and functional domains which include i) a conserved central domain (C-domain) which is capable of forming two or more alternative structures that may regulate the replication cycle, ii) a domain associated with pathogenicity (P-domain), iii) a domain exhibiting high sequence variability (V-domain), iv) two terminal domains that are interchangeable between viroids (T_L and T_R). The Central Conserved Region (CCR) is located within the C domain and contains a UV-sensitive loop E motif with non-canonical base-pairs (denoted by circles). The T_L domain of posiviroids contains either a Terminal Conserved Region (TCR) or Terminal Conserved Hairpin.

Some of the nucleotides that are highlighted in the picture are also found in most other viroids.

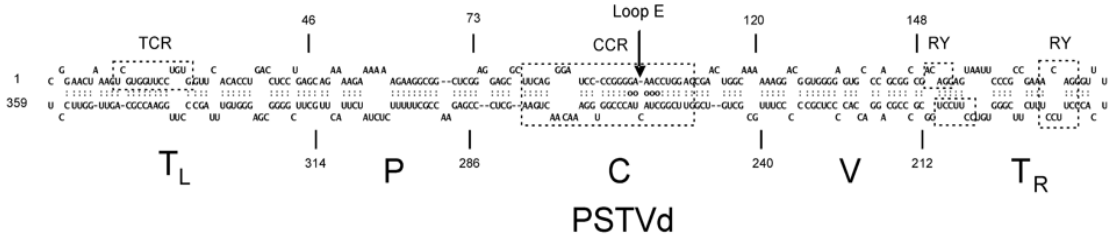

Figure-3.2: Putative secondary structure of PSTVd

Different strains of PSTVd exist and symptoms caused by them range from mild to severe. Mild strains produce no obvious symptoms. In case of severe stains, symptoms dependent on environmental conditions and are most severe in hot conditions. Symptoms may be mild in initial infections but become progressively worse in the following generations. The most common symptoms of severe infections include colour changes in the foliage, smaller leaves and spindle-like elongation. Sprouting also occurs at a slower rate than in unaffected potatoes. Infected tomatoes are slower to show symptoms which include stunted growth with a 'bunchy top' caused by shorted inter-nodes. Leaves become yellow or purple and often become curled and twisted. Eventually necrosis occurs in the veins of the bottom and middle leaves and the top leaves decrease in size. Fruit ripening is also affected leading to hard, small, and dark green tomatoes. Long distance spread of PSTVd usually occurs via infected seeds.

3.2.2 Cadang-cadang disease of coconut

Cadang-cadang is a disease caused by Coconut cadang-cadang viroid (CCCVd, Cocadviroid cadangi), a lethal viroid of several palms including coconut (*Cocos nucifera*), African oil palm (*Elaeis guineensis*), anahaw (*Saribus rotundifolius*), and buri (*Corypha utan*). The name cadang-cadang comes from the word gadang-gadang that means dying in Bicol. It was originally reported on San Miguel Island in the Phillippines in 1927. In 1962, an epidemic occurred in that island. The natural hosts of cadang-cadang are *Cocos nucifera*, *Corypha utan*, *Elaeis guineensis* and *Roystonea regia*. Whereas, the experimental hosts are *Adonidia merrillii*, *Areca catechu*, *Caryota cumingii*, *Dypsis lutescens*, *Saribus rotundifolius*, *Phoenix dactylifera*, *Ptychosperma macarthurii* and *Roystonea regia*.

CCCVd are small, circular or linear single stranded RNA with a basic size of 246 nucleotides. Of these 246 nucleotides, 44 are common with most viroids. CCCVd can have an addition of a cytosine residue in the 197 position and increase the size to 247 nucleotides. This 247 nucleotide form of CCCVd can cause severe symptoms. Their minimum size is 246 nucleotides, but they can produce different forms with 287 to 301 nucleotides. The sequence and structure prediction of CCCVd has been documented. There are four low-molecular weight RNA species (ccRNAs) found associated with cadang-cadang disease. Of the four, two of them are fast (ccRNA 1 fast and ccRNA 2 fast) and the other two are slow (ccRNA 1 slow and ccRNA 2 slow). The difference between fast and slow RNA is their mobilities in electrophoresis gel. In early stages of the disease, RNA 1 fast and RNA 2 fast appear, while RNA 1 slow and RNA 2 slow are appear in late stages of infection. Moreover, they share sequence homology with other viroids.

Symptoms of cadang-cadang develop slowly over 8 to 15 years making it difficult to diagnose at an early time. The first symptoms in the early stage develop within two to four years of infection, which include scarification of the coconuts which also become rounded. The leaves (fronds) display bright yellow spots. Two years later, at medium stage, inflorescences become stunted and eventually killed, and so no more coconuts are produced. The yellow spots becomes larger and in greater abundance to give the appearance of chlorosis. During the final stage, roughly 6 years after the first symptoms are recorded; the yellow/bronze fronds start to decrease in size and number. Finally, all the leaves coalesce, leaving just the trunk of the palm. Palms under 10 years of age are rarely affected by cadang-

cadang; the incidence of disease increases until about 40 years of age and then plateaus. No recovery has ever been observed and the disease is always fatal (Fig. 3.3).

CCCVd can spread through mechanical inoculation primarily through contaminated farm tools and due to improper sanitary conditions. The efficiency of the mechanical inoculation has been influenced by factors such as the age of the test plant and the mode of inoculation. The transmission through pollen and seed can occur but with relatively low rate. Detection of pathogen by molecular diagnostic method like 'Dot-blot hybridization' is the best one, and the viroid can be detected even six months before noticing the first symptom. Coconut cadang-cadang disease has not treatment. But the control strategies are elimination of reservoir species, vector control, mild strain protection and breeding for host resistance. Eradication of diseased plants is usually performed only to minimize the spread of the viroid.

Figure-3.3: Coconut cadang-cadang disease

3.3 PRIONS

The infectious agent different from both viruses and viroids that can cause disease in animals and humans is called as 'prions' (Proteinaceous infectious particles). Stanley B. Prusiner, an American neurologist and biochemist, discovered the prions. He won the Nobel Prize in Physiology or Medicine in 1997 for his work proposing an explanation for the cause of bovine spongiform encephalopathy (mad cow disease) and its human equivalent, Creutzfeldt-

Jacob disease. He coined the term prion which means '**pr**oteinaceous and **in**fectious **(-on** by analogy to viri**on**) that lacks the nucleic acid. Prion is self-reproducing pathogenic agent mainly composed of protein. And the specific protein that makes the prion is named as 'PrP' i.e. 'Protease resistant Protein'. The PrP seems to be a 33 to 35 kDa hydrophobic membrane protein encoded by *PrP* gene. This gene is present in many normal vertebrates and invertebrates, and the prion protein is bound to the surface of neurons. Presumably an altered PrP is at least partly responsible for the disease.

Prions are determined to be the infectious agents because transmission is difficult to prevent with heat, radiation and disinfectants, the agent does not evoke any detectable immune response, and it has a long incubation period between 18 months and 5 years. The agent is thought to be much smaller than the smallest known virus. Prions multiply by causing normally folded proteins of the same type to take on their abnormal shape, which then go on to do the same, in a kind of chain reaction. These abnormal proteins are gradually accumulated in the body, especially in nerve cells, which subsequently die. The prion gene that codes for the prion protein is highly conserved in most mammals, meaning the gene is similar and present in most species of mammals. Three locations on the prion protein gene have been identified as highly polymorphic and may have an effect on scrapie: codons 136, 154, and 171. Codon 154 has not shown any evidence of having a high effect on scrapie susceptibility, but is most likely having an effect on incubation time of the disease. Codons 136 and 171 are thought to control the incubation time as well as the susceptibility of the disease, and are the ones the USDA uses in its breeding standard. Codon 171 has been determined to be the major genetic factor in scrapie susceptibility.

3.3.1 Scrapie disease

Scrapie disease is a fatal, degenerative disease that affects the nervous systems of sheep and goats. It is one of several 'transmissible spongiform encephalopathies' (TSEs) and as such it is thought to be caused by a prion protein (PrP^{sc}). The scrapie has been known since at least 1732 and does not appear to be transmissible to humans. However, it has been found to be experimentally transmissible to transgenic mice and non-human primates. The name scrapie is derived from one of the clinical signs of the condition, wherein affected animals will compulsively scrape off their fleeces (wooly covering of the sheep or goat) against rocks, trees or fences. The disease apparently causes an itching sensation in the animals. Other clinical signs include excessive lip smacking, altered gaits, and convulsive collapse.

Scrapie is infectious and transmissible among conspecifics (members of the same species), so one of the most common ways to contain it is to quarantine and kill those affected as it is incurable. However, scrapie tends to persist in flocks and can also arise spontaneously in flocks that have not previously had cases of the disease. Recent studies suggest that prions may be spread through urine and persist in the environment for decades. Scrapie is usually affects the sheep around three to five years of age. The potential for transmission at birth and from contact with placental tissues is apparent. In scrapie disease, the primary mode of transmission of prion is from mother to lamb through ingestion of placental or allantoic fluids. The agent can also enter through cuts in the skin. The pathogenesis of scrapie involves the lymphatic system. Once the agent is absorbed through the intestines, misfolded prions first appear and accumulate in the lymph nodes, especially in Peyer's patches at the small intestine. Eventually, the infection invades the brain, often through the spinal cord or the medulla oblongata by creeping up the sympathetic and parasympathetic nervous system, respectively.

In the disease development, changes are mild at first; slight behavioral changes and an increase in chewing movements may occur. Ataxia and neurological signs then develop, and affected sheep struggle to keep up with the flock. The signs and effects of scrapie typically appear 2-5 years after the infection, but also may appear at later years. Signs of scrapie may vary among the infected individual animals. After the onset of clinical signs, sheep typically live for 1-6 months. In some cases, they may live longer, but death is an inevitable consequence of the condition. Due to the nerve cell damage caused by the condition, affected animals may exhibit behavioral changes, tremor, pruritus, and locomotor incoordination. Some sheep scratch excessively and show patches of wool loss and lesions on the skin. Scratching sheep over the rump area may lead to a nibbling reflex, which is characteristic for the condition. Signs of a chronic systemic disease appear later, with weight loss, anorexia, lethargy, and death (Fig. 3.4). Post mortem examination is important for the diagnosis of scrapie. Histology of tissues shows accumulation of prions in the central nervous system, and immunohistochemical staining and ELISA can also be used to demonstrate and detect the prion protein (PrP^{sc}).

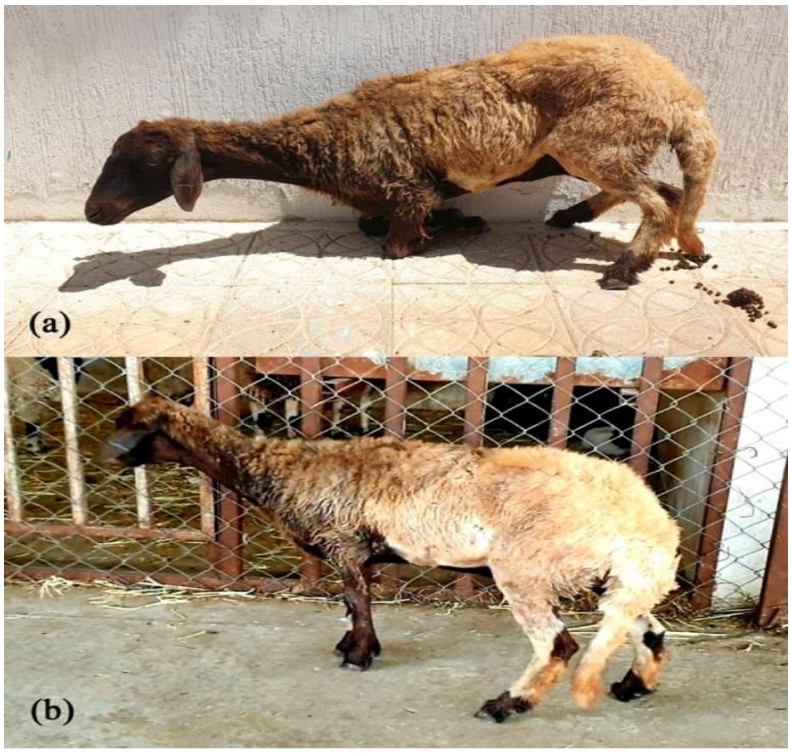

Figure-3.4: Scrapie affected sheep (a) Two-year-old female sheep showing ataxia, weakness and body weight loss (b) Two-year-old female sheep showing loss of weight, wool and incoordination of movement.

3.3.2 Creutzfeldt-Jacob disease

Creutzfeldt-Jacob disease (CJD) is an incurable, always fatal neurodegenerative disease belonging to the transmissible spongiform encephalopathy (TSE) group. The name ‘Creutzfeldt-Jacob disease’ was introduced by Walther Spielmeyer in 1922, after the German neurologists Hans Gerjard Creutzfeldt and Alfons Maria Jakob. CJD is caused by abnormal folding of prion protein. Infectious prions are misfolded proteins that can cause normally

folded proteins to also become misfolded. So, the CJD prion is dangerous as it promotes the refolding of the cellular prion protein into the diseased state. The number of misfolded protein molecules will increase exponentially and the process leads to a large quantity of insoluble protein in affected cells. This mass of misfolded proteins disrupts neuronal cell function and causes cell death. Mutations in the gene for the prion protein can cause a misfolding of the dominantly alpha helical regions into beta pleated sheets. This change in conformation disables the ability of the protein to undergo digestion. Once the prion is transmitted, the defective proteins invade the brain and induce other prion protein molecules to misfold in a self-sustaining feedback loop. The defective protein can be transmitted by contaminated harvested human brain products, corneal grafts, dural grafts etc.

The first symptom of CJD is usually rapidly progressive dementia, leading to memory loss, personality changes, and hallucinations. Myoclonus (jerky movements) typically occurs in 90% of cases, but may be absent at initial onset. Other frequently occurring features include anxiety, depression, obsessive-compulsive symptoms, and psychosis. This is accompanied by physical problems such as speech impairment, balance and coordination dysfunction (ataxia), changes in gait, and rigid posture. In most people with CJD, these symptoms are accompanied by involuntary movements. The duration of the disease varies greatly, but sporadic (non-inherited) CJD can be fatal within months or even weeks. Most affected people die in six months after initial symptoms appear, often of pneumonia due to impaired coughing reflexes. About 15% of people with CJD may survive for two or more years. The symptoms of CJD are caused by the progressive death of the brain's nerve cells. Many tiny holes occur where the nerve cells died and the affected parts of the brain may resemble a sponge appearance.

People can also develop CJD because they carry a mutation of the gene that codes for the prion protein (PRNP), located on chromose 202p12-pter. This occurs in only 10-15% of all CJD cases. In sporadic cases, the misfolding of the prion protein is a process that is hypothesized to occur as a result of the effects of aging on cellular machinery, explaining why the disease often appears later in life.

The different types of CJD include-
Sporadic CJD (sCJD): caused by spontaneous misfolding of prion-protein in an individual. This accounts for 85% cases of CJD.
Familial CJD (fCJD): caused by an inherited mutation in the prion-protein gene. This accounts for the majority of the other 15% of cases of CJD.
Acquired CJD: caused by contamination with tissue from an infected person, usually as the result of a medical procedure such as blood transfusion from infected person, use of human-derived pituitary growth hormones, gonadotropin hormone therapy etc. Variant CJD (vCJD) is a type of acquired CJD potentially acquired from BSE or caused by consuming food contaminated with prions.

Diagnosis of CJD is problematic, due to non-specific nature of the early symptoms and difficulty in safely obtaining brain tissue for conformation. The diagnosis may initially be suspected in a person with rapidly growing dementia along with some other medical signs and symptoms such as involuntary muscle jerking, difficulty with coordination/balance and waking, and visual disturbances. Brain MRI is the most useful imaging modality for changes related to CJD. Of the MRI sequences, diffuse-weighted imaging sequences are most sensitive. Through the image of MRI, the obvious precipitation of prion protein in the brain is visible. There is no cure or effective treatment for CJD. However, symptoms like twitching

can be managed. Psychiatric symptoms like anxiety and depression can be treated with sedatives and antidepressants. Myoclonic jerks can be handled with 'clonazepam or sodium valproate'.

3.4 SATELLITE VIRUSES AND SATELLITE RNAS

Satellites are the subviral agents which lack genes that could encode functions needed for replication. They depend for their multiplication on the co-infection of host cell with a helper virus. Satellite genomes have a substantial portion or all of their nucleotide sequences distinct from those of genomes of their helper virus. According to this definition, two major classes of satellites may be distinguished. **Satellite viruses** encode a structural protein that encapsidates their genome and so have nucleoprotein components distinct from those of their helper viruses. **Satellite nucleic acids** encode their non-structural proteins, or no proteins at all, and are encapsidated by the CP of helper viruses.

Satellites are classified into –
1) Satellite viruses
- Single stranded RNA satellite viruses
  -Subgroup 1: Chronic bee-paralysis satellite virus
  -Subgroup 2: Tobacco necrosis satellite virus

2) Satellite nucleic acids
- Single stranded satellite DNAs
- Double stranded satellite RNAs
- Single stranded satellite RNAs
  -Subgroup 1: Large satellite RNAs
  -Subgroup 2: Small linear satellite RNAs
  -Subgroup 3: Circular satellite RNAs (virusoids)

Satellites do not constitute a homogenous taxonomic group and are not formally classified into species and higher taxa by ICTV. Their arrangement is based largely on features of the genetic material of the satellites. The physicochemical and biological features of the helper virus and of the helper virus host are the important secondary characters. It also appears that no taxonomic correlation between the viruses that are associated with satellites. Satellites would appear to have arisen independently a number of times during virus evolution. A further complication is that some viruses are associated with more than one satellite and some satellites are supported by more than one species of helper virus. Satellites can even depend on both a second satellite and a helper virus for multiplication. The very first satellites characterized were mostly ssRNA satellites that use ssRNA plant viruses as helpers.

In 1981, J.W. Randles and coworkers discovered the virusoids. These are the small, circular RNAs which are similar to viroids but they are always linked with larger molecules of the viral RNA. Robertson et al. (1983) have reported that virusoids are essential for the replication of the large RNA and therefore form a part of the viral genome. Virusoids belong to a larger group of infectious agents called satellite RNAs. They are found in bacteria, plants, fungi, invertebrates and vertebrates. Some examples of virusoides are – barley yellow dwarf virus satellite RNA and a helper Luteovirus; tobacco ring spot virus satellite RNA and a helper Nepovirus.

Satellites are genetically distinct from their helper virus with a nucleotide sequence that is substantially different from that of their helper virus. However, the genomes of the most satellites have short sequences, often at the termini, which are identical to the genome of the helper virus. This is presumably linked to the dependence of nucleic acids of both satellite and helper virus on the same viral polymerase and host-encoded proteins for replication. Satellites are distinct from defective interfering (DI) RNAs or defective RNAs because such RNAs are derived from their helper virus genomes. Nevertheless, satellite viruses may from their own DI RNAs that specifically interfere with the satellite virus genomic RNA as shown for satellite panicum mosaic virus. Recombination can occur between satellites and their helper viruses. For example, chimeric molecules can be formed from a satellite RNA associated with turnip crinkle virus (genus *Carmovirus*) and parts of the helper virus genome.

3.5 SUMMARY

Infectious agents simpler and smaller than viruses exist in nature. For example, several plant diseases are caused by short strands of infectious RNA called as viroids. These single stranded circular RNA molecules vary in length from 246 to 463 nucleotides and found only in plants. They do not encode proteins but replicate independently when introduced into host plants. So far, 30 viroids have been characterized, 28 infecting the dicotyledonous plants and the other 2 infecting the monocotyledonous plants. The crops that are infected by viroids result in crop failure due to huge losses to the agriculture industry. Prions or virinos are the small agents associated with at least six degenerative nervous system disorders namely scrapie, bovine spongiform encephalopathy, kuru, fatal familial insomnia, the Gerstmann-Sraussler-Scheinker syndrome, and Creutzfeldt-Jakob disease. Satellites are the subviral agents which lack genes that could encode functions needed for replication. They depend for their multiplication on the co-infection of host cell with a helper virus. **Satellite viruses** encode a structural protein that encapsidates their genome and so have nucleoprotein components distinct from those of their helper viruses. **Satellite nucleic acids** encode their non-structural proteins or no proteins at all, and are encapsulated by the CP of helper viruses.

3.6 TECHNICAL TERMS

Subviral agents, Viroids, Prions, PrP, PSTV, Cadang-cadang, Scrapie, CJD, Kuru disease, Bovine spongiform encephalopathy, Satellite viruses, Satellite RNAs.

3.7 SELF ASSESSMENT QUESTIONS

Q.1 Write an account on viroids.

Q.2 Describe in detail about the prions and important diseases caused by them.

Q.3 Give an account on satellite viruses and satellite RNAs.

3.8 SUGGESTED READINGS

1. Microbiology – Prescott et. al.
2. A Text Book of Microbiology – R.C. Dubey and D.K. Maheswari.
3. Introduction to Modern Virology – N.J. Dimmock et. al.
4. Introduction to Plant viruses – C.L. Mandahar.

Prof. V. Umamaheswara Rao

LESSON - 4

GENERAL METHODS OF CULTIVATION OF VIRUSES

Objective of the lesson

Students will have the knowledge about the methods of culturing the plant, animal and bacterial viruses.

Structure of the lesson

4.1 INTRODUCTION

For the study of any virus it is essential to understand the nature and properties of individual virus. Cultivation and purification are the initial steps in the study of a virus. Cultivation of viruses can be done only on living cells because all are obligate pathogens, need a living cell for their survival. Different methods, including the whole organism, organ and cell cultures are used for animal viral cultivation and tissue culture and protoplast culture are used for plant viruses.

4.2 CULTIVATION OF ANIMAL VIRUSES

Animal Viruses can be isolated from an affected host by harvesting excreted or secreted material, blood, or tissue and testing for induction of the original symptoms in the identical host or for induction of some abnormal pathology in a substitute host or in cell culture. Historically, dogs, cats, rabbits, rats, guinea pigs, hamsters, mice and chickens have all been found to be useful in laboratory investigations although most animal methods have now been replaced by cell culture methods. Once the presence of a virus has been established, it is often desirable to prepare a genetically pure clone, either by limiting serial dilution or by plaque purification.

Laboratory animals and embryonated chicken eggs

Prior to the advent of cell culture, animal viruses could be propagated only on whole animals or embryonated chicken eggs. Whole animals could include the natural host- laboratory animals such as rabbits, mice, rats and hamsters. In the case of laboratory animals, newborn

or suckling rodents often provide the best hosts. Today, laboratory animals are rarely used for routine cultivation of virus but they still play an essential role in studies of viral pathogenesis.

The use of embryonated chicken eggs was introduced by Goodpasture *et.al.* in 1932 and developed subsequently by Beveridge and Burnet. The developing chick embryo, 10 to 14 days after fertilization, provides a variety of differentiated tissues, including the amnion, allantois, chorion, yolk sac, which serve as substrates for growth of a wide variety of viruses, including orthomyxoviruses, paramyxoviruses, rhabdoviruses, togaviruses, herpesviruses and poxviruses (Fig. 4.1). Several viruses from each of the groups cause discrete and characteristic foci – pocks, when introduced onto the chorioallantoic membrane (CAM) of embryonated eggs, thus providing a method for identification of virus types, or for quantifying virus stocks or assessing virus pathogenicity. Although the embryonated eggs have been almost wholly replaced by cell culture techniques, they are still the most convenient method for growing high tier stocks of some viruses and they thus continue to be used both in research laboratories and for vaccine production. In addition, pock formation on the CAM still provides a specialized method for assay of variants of poxviruses - wild type rabbit pox and cowpox viruses cause red hemorrhagic pocks on the CAM, whereas viruses deficient in specific virulence genes cause white pocks as a result of the infiltration of the lesions with inflammatory cells.

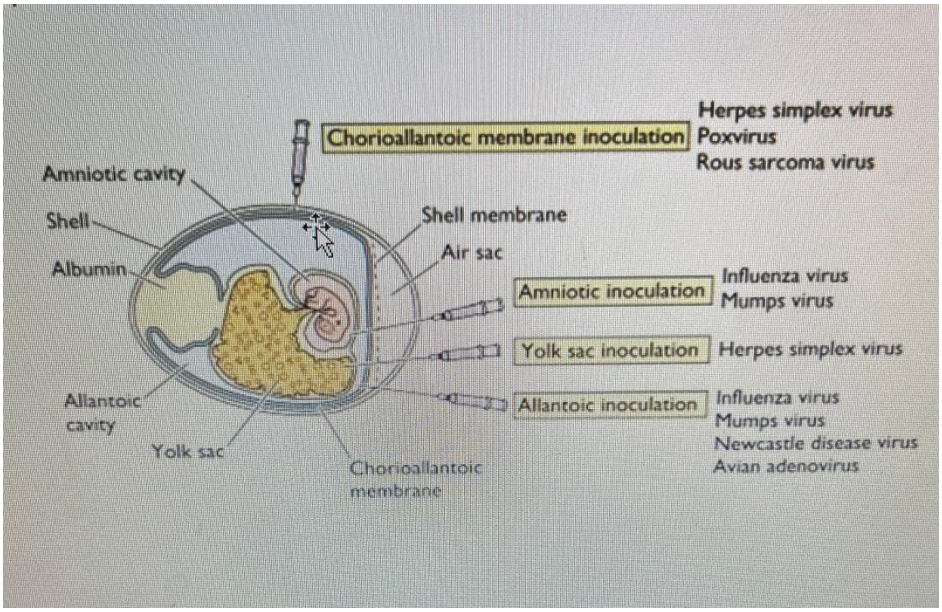

Figure-4.1: Cultivation of viruses in chicken embryonated egg.

Organ Cultures

Organ cultures use the whole organ for culturing which provides the natural conditions for the virus. They have the advantage of maintaining the differentiated state of the cell. However, there are technical difficulties in their large-scale use, and as a result they have not been widely used. Ciliated cells lining the trachea continue to beat in coordinated waves while the tissue remains healthy. Multiplication of some viruses causes the synchrony to be lost and eventually causes the ciliated cells to death. Virus is also released into fluids surrounding the tissue and can be measured if appropriate assays are available.

Cell Cultures

In the 1950-60 period more than 400 viruses were isolated and cultured (Golden Age) by the cell cultures. Two discoveries greatly enhanced the usefulness of cell culture for virologists. First, the discovery and use of antibiotics made it possible to prevent bacterial contamination. Second, biologists found that proteolytic enzymes, particularly trypsin, can free animal cells from surrounding tissues without injuring the free cells. Cells in culture are kept in an isotonic solution, consisting of a mixture of salts in their normal physiological proportions supplemented with serum (usually 5-10%), and in such a growth medium most cells rapidly adhere to the surface of suitable glass or plastic vessels. Serum is a complex mixture of proteins and other compounds, without which mitosis does not occur. After cell division the cells form a mono layer in the vessels. Synthetic substitutes are now available but these are expensive and employed mainly for specialized purposes. All components used in cell culture have to be sterile and handled under aseptic conditions to prevent the growth of bacteria and fungi. Antibiotics have been invaluable in establishing cells in culture, and routine cell culture dates from 1950s when they first appeared on the market.

Cultured cells are either diploid or heteroploid (having more than the diploid number of chromosomes but not simple multiple of it). Diploid cell lines undergo a finite number of divisions, from around 10 to 100 whereas the heteroploid cells will divide forever. The latter are known as continuous cell lines and they originate from naturally occurring tumors or from some spontaneous event that alter the control of division of a diploid cell. Diploid cell lines are most easily obtained from embryos by reducing lungs, kidneys or the whole body to a suspension of single cells. Cell cultures are of three basic types: Primary cell cultures, Cell strains and Cell lines, which may be derived from many animal species, and differ substantially in their characteristics.

Primary cell cultures (Fig. 4.2.)

A primary cell culture is defined as a culture of cells obtained from the original tissue that have been cultivated in vitro for the first time, and that have not been sub cultured. Primary cell cultures can be established from whole animal embryos or from selected tissues from embryos, newborn animals or adult animals of almost any species. The most commonly used cell culture in virology obtained from primates, including humans and monkeys, rodents including hamsters, rats and mice and birds most notably chickens. Cells to be cultured are obtained by mincing tissue and dispersing individual cells by treatment with proteases and/or collagenase to disrupt cell-cell interactions and interactions of cells with the extra-cellular matrix. With the exception of cells from the hemopoietic system, normal vertebrate cells will grow and divide only when attached to a solid surface. Dispersed cells are therefore placed in a plastic flask or dish, the surface of which has been treated to promote cell attachment. The cells are incubated in a buffered nutrient medium in the presence of blood serum, which contains a complex mixture of hormones and factors required for the growth of normal cells. The blood serum may come from a variety or sources, but bovine serum is most commonly used. Under these conditions, cells which attach to the surface of the dish will divide and migrate until the surface of the dish is covered with a single layer of cells, a mono layer, whereupon they will remain viable but cease to divide. If the cell mono layer is wounded by scraping cells from an isolated area, cells on the border of the wound will resume division and migration until the mono layer is reformed, whereupon cell division again ceases. Primary cultures may contain a mixture of cell types and they retain the closet resemblance to the tissue of origin.

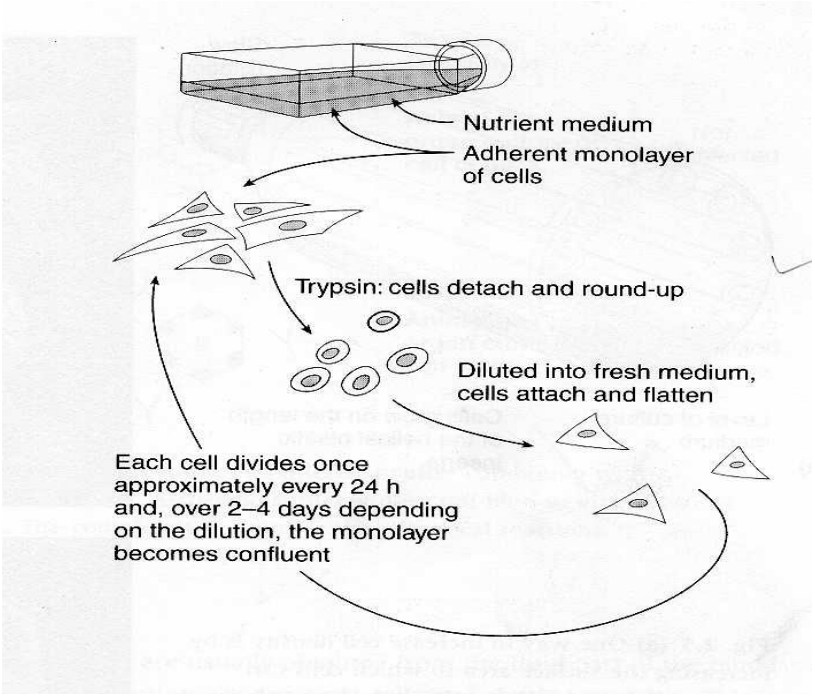

Figure-4.2: Cell cultures (Source: Dimmock *et al* 2001)

Cell strains

Normal vertebrate cells cannot be generated indefinitely in culture. Instead, after a limited number of cell generations, usually 20 to 100 depending on the age and species of the original animal, cultured normal cells cease to divide and they degenerate and die, a phenomenon called crisis or senescence. Primary cell cultures may contain a mixture of cell types but only a few cell types survive after sub culturing and by subsequent generations, after second and third, typically only one cell type remains in the cell strain.

Cell strains are usually composed of one of two basic cell types, fibroblast like or epithelial-like, cells. Fibroblasts have an elongated, spindle shape, whereas epithelial cells have a polygonal shape. Although after only a few generations, only one cell type may remain in a cell strain, continued generations may select for faster growing variants, such that the characteristic of a cell strain may change with increasing generation number. Despite the fact that normal cell strains experience senescence in culture, they may be maintained for many years by expanding the culture to a large number of cells.

Cell lines

At any time during the culture of a cell strain, cells in the culture may become transformed. Transformation is a complex phenomenon, in the context of cell culture. The most important characteristic of transformation is that the transformed cells become immortalized. Immortal cell cultures are called cell lines or sometimes continuous cell lines to distinguish them from primary cultures and cell strains. Immortalization can occur spontaneously during passage of a cell strain or it can be induced by treatment with chemical mutagens, infection with tumorogenic viruses or transfection with oncogenes. In addition, cells cultured from tumor tissue frequently readily establish immortal cell lines in culture. Spontaneous immortalization does not occur in cultured cells from all animal species. Thus immortalization occurs frequently during culture of rodent cells, for example in mouse and hamster cell strains and in monkey kidney cells, but it occurs rarely in chicken or human cells. Like cell strains,

cell lines are usually composed of cells that are either fibroblast-like or epithelial-like in morphology. These cell lines play a important role in the present day viral vaccine preparations.

Modern methods of cell culture

The methods described above are suitable for research and clinical or diagnostic laboratories, but it is difficult to scale up for commercial purposes which need increased cell densities. One of the earliest method to increase the cell density is to grow cells in suspension, and this has been refined, using hybridoma cells (which are immortalize antibody-synthesizing or B cells) that produces monoclonal antibodies (Mabs). As many cells grow only when anchored to a solid surface, the modified modern method is aimed to increase the surface area, by fitting the spiral inserts into the conventional culture bottles (Fig. 4.3.). The added advantage of this method is by rotating the bottle slowly (5 rev/h) only a small volume of culture medium is enough for culturing. Another method is to grow cells on 'micro carriers'- tiny particles, on which cells attach and divide. The surface area afforded by 1kg of micro carriers is about 2.5 m^2 and the space take up (a prime consideration in commercial practice) is very economical. This method combines the ease of handling cell suspensions with matrix for the cell to grow on.

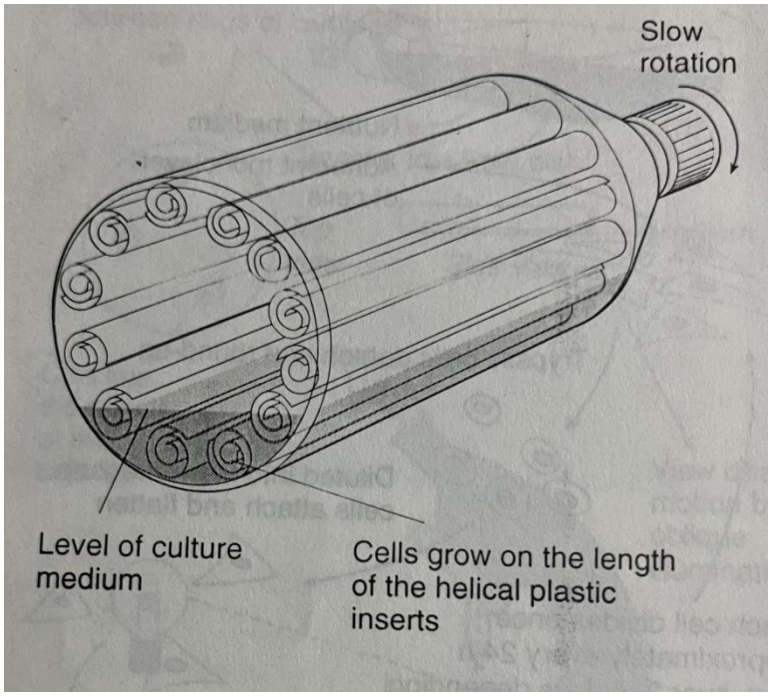

Figure-4.3: Culture bottle lined with spiral plastic coils. (Source: Dimmock *et al* 2001)

The plaque assay

The plaque assay is the most quantitative and the most useful biologic assay for viruses. Developed originally for the study of bacteriophage by d'Herelle in the early 1990s, the plaque assay was adapted to animal viruses by Dulbecco and Vogt in 1953. This assay was relatively simple and permits a qualitative assay for individual virus variants that differ in growth properties or cytopathology.

The plaque assay is based simply on the ability of a single infectious virus particle to give rise to a macroscopic area of cytopathic effect on a normal monolayer of cultured cells.

Specifically, if a single cell in a monolayer is infected with a single virus particle; new viruses resulting from the initial infection can infect surrounding cells, which in turn produce viruses that infect additional surrounding cells. Over a period of days, the exact length of time depends on the particular virus; the initial infection thus gives rise through multiple rounds of infection to an area of infection called a plaque.

The plaque assay can be used to quantify virus in the following manner. A sample of virus of unknown concentration is serially diluted in an appropriate medium, and measured aliquots of each dilution are seeded on to mono layers of cultured cells. Infected cells are overlaid with a semisolid nutrient medium usually consisting of growth medium and agar. The semisolid medium prevents formation of secondary plaques through diffusion of virus from the original site of infection to new sites, ensuring that each plaque that develops in the assay originated from a single infectious particle in the starting inoculum. After an appropriate period of incubation to allow development of plaques, the mono layer is stained so that the plaques can be visualized. The staining technique depends on the cyto-pathology, but vital dyes such as neutral red are common. Neutral red is taken up by living cells but not by dead cells, so that plaques become visible as clear areas on a red mono layer of cells. In cases where the virus cytopathology results in cell lysis or detachment of cells from the dish, plaques exist literally as holder in the monolayer, and a permanent record of the assay can be made by staining the monolayer with a general stain such as crystal violet, prepared in a fixative such as ethanol.

The aim of this assay is to identify a dilution of virus that yields 20 to 100 plaques on a single dish that is, a number large enough to be statistically significant. Usually a series of four to six 10-fold dilutions are tested. Dishes inoculated with low dilutions of virus will contain only dead cells or too many plaques to count. Whereas dishes inoculated with high dilutions of virus will contain very few plagues. Dishes containing an appropriate number of plaques are counted, and the concentration of infectious virus in the original sample can then be calculated by taking into account the serial dilution. The resulting value is called a titer, and it is expressed in plaque-forming units per milliliter or pfu/ml, to emphasize specifically that only viruses that are capable of forming plaques have been quantified. In this method an error of up to 100% is always possible because it mainly involves the multiple serial pipetting steps. However, a critical benefit of the plaque assay is that it measures infectivity, but it is important to understand that infectivity does not necessarily correspond exactly to the number of virus particles in a preparation.

4.3 CULTIVATION OF PLANT VIRUSES

Although viruses cannot be grown in a synthetic medium, the cell, in which they live, can be propagated. This procedure is called tissue culture. Plant viruses can be cultivated either by tissue cultures or by protoplast culture method. In tissue culture method, the ex-plants pieces of tissues are used, while in protoplast culture the cells from the host plants are used. In tissue culture various plant parts- roots, endosperm, pollen, and pieces of stem are commonly used.

Plant Tissue Culture

White (1934) was the first to examine the possibilities of growing plant viruses in tissue-culture. He investigated the multiplication of tobacco and cucumber mosaic viruses in growing excised tomato root tips. A tomato plant already systematically infected with the viruses was used and the stem was cut up into segments, these were thoroughly washed

and were suspended by threads in 3-litre conical flasks containing a little water. The pieces of stem were kept out of contract with the water on the sides of the flask, the flasks were then plugged with cotton – wool and allowed to stand till roots developed. After 11 days, the root tips were removed and placed in 125 ml conical flasks, each flask contain 50 ml of nutrient medium as follows

$Ca(NO_3)_2$	0.60 millimols
$MgSO_4$	0.30 millimols
KNO_3	0.80 millimols
KCl	0.87 millimols
KH_2PO_4	0.09 millimols
$Fe_2(SO_4)_3$	0.006 millimols
Sucrose	2 % by weight
Yeast extract	0.01 %

At the end of a week, the surviving cultures were cut into pieces of about 10 mm long. After further subculture, a single root tip was selected as parent stock for all subsequent subcultures. It was found that the two viruses continued to multiply actively in growing isolated root tips for at least 25 to 30 weeks. Using the above technique, tobacco mosaic (TMV) and tobacco necrosis (TNV) viruses can be cultured in root tips of tobacco plants also. In addition to root tip cultures, plant viruses can also be cultured in callus tissues. In general, the tissues grew on a wide range of concentrations of the salts tested, but best growth was apparent when concentrations were increased over those of the basal medium (given above). Further it was found that increased phosphate concentrations increase the growth of tobacco-callus tissue. After callus development, infecting the callus with virus is also a difficult task in this method.

Since it is difficult to infect tissue cultures with viruses *de novo*, it is better to start the culture with tissues from systemically infected plants. However it is not always possible to get such type of plants, then it is better to employ alternative methods. One possible way is to use the natural vector of a virus to infect cultured tissue. In case of tobacco necrosis, the virus was inoculated to tobacco callus tissues by zoospores of the fungus *Olpidium brassicae*. Two strains of tobacco necrosis virus and three isolates of *O. brassicae* were used. One day before inoculation, the callus tissues were transferred to small filter-paper cups pushed into vials containing 5 ml Hoaglands solution (1:20 dilution). The solution just touched the bottom of the paper cup, which was used to prevent the callus cells being lost in the liquid. The method of inoculation was to add to each vial 1ml of Hoagland's solution containing zoospores and 0.5 ml containing purified virus. Four or 5 days after inoculation, the virus in the tissues and in the fluid beneath them was assayed by infectivity tests on French beans, the test plant for TNV. All three isolates of *Olpidium* transmitted both strains of TNV to the tobacco callus tissue.

Similarly it is also possible to propagate the virus in tissue cultures of insect vectors. Tissue cultures derived from the vector insect *Agallia constricta* (Van Duzee) were infected with wound-tumor virus and the infection was detected by staining with fluorescent-conjugated antibody, and by infectivity tests. These experiments demonstrate multiplication of the wound- tumor virus in the inoculated tissues of the leaf-hopper. Many viruses - chilli mosaic virus, sun hemp mosaic virus and ring spot strain of potato virus X and a type strain of TMV have also been successfully cultivated in normal callus tissue obtained from virus-affected White Burley tobacco plants.

Protoplast culture

Use of protoplasts and isolated cells in the study of viruses has many advantages over the inoculated leaves. In the latter case only a few cells are initially infected and the virus replication must be studied against an overwhelming background of uninfected cells. Moreover the cells in the inoculated leaf are in varying stages of virus synthesis ranging from uninfected cells to cells in which virus synthesis is completed. Replication of virus within protoplast may be demonstrated in various ways, staining with fluorescent antibodies, infectivity assays, electron microscopy, incorporation of radioactive precursors into viruses and serology.

Certain conditions must be satisfied in order to achieve successful protoplast infection.

1. Protoplasts should be freshly washed with 0.7 M mannitol immediately before adding the inoculum.
2. Poly-l-Ornithine should be used to the inoculum
3. pH and osmolarity during inoculation should be within acceptable limits.
4. After infection takes place the protoplasts should be washed to remove excess virus and inoculation medium and then re-suspended in the incubation medium.

It is necessary to use very large numbers of virus particles to establish infection in inoculated protoplasts. Infection of protoplasts can be 10 times more efficient than is infection in a leaf probably because of the easier accessibility of the protoplast. A simplified method of obtaining tobacco protoplasts for infection with tobacco mosaic virus is - incubating the tobacco leaf tissue, from which the lower epidermis was peeled, overnight with 0.3-0.4 percent Macerozyme and 0.6-1.2 percent cellulase, depending on leaf condition, produced a good yield of protoplasts that were susceptible to infection by TMV. Highest concentration of virus can be attained, when the protoplasts were inoculated as soon as they were washed free from the enzymes. Protoplast culture of viruses is an important tool in plant virus study and offers much scope for progress. The time course of virus replication and its kinetics can be measured, and with protoplasts infected *in vitro* it is possible to get a picture of the generation time of plant viruses.

Abrasive method

This is one of the simplest methods for cultivation of plant viruses. The viruses that are sap transmitted are generally cultivated by this method. In this method carborundum was used an abrasive to make injury on the leaf, through which virus can make entry. First, a fine homogenate of the infected leaf was prepared by using K_2HPO_4/Na_2SO_3 solution in the ratio of 1.5 ml solution to 1 g leaf, in a pre-cooled mortar. In a healthy plant, the leaf to be inoculated is marked and dusted with carborundum. Then by using the folded square of muslin cloth, the filtered homogenate of the infected leaf was applied on the surface of the leaf which was dusted with carborundum. The square of muslin dipped in the sap was firmly stroked on the upper surface of the leaf until complete leaf was moistened. These treated plants were kept in a green house at 22- 25^oC. Then plants were observed for the appearance of symptoms from 4 to 5 days after inoculation. By this method viruses like *Cucumber mosaic virus* can be cultured in *Chaenopodium quinoa*, the local lesion host.

4.4 CULTIVATION OF BACTERIOPHAGES

Bacterial viruses are easily isolated and cultivated in young, actively growing cultures of bacteria in broth or on agar plates. In liquid cultures, lysing of the bacteria may cause a

cloudy culture to become clear, whereas in agar-plate cultures, clear zones, or plaques, become visible to the unaided eye. The principal requirement for the isolation and cultivation of phages that optimal conditions for growth of the host organisms be provided. The best and most usual source of bacteriophages is the host habitat. For example, coliphages or other phages pathogenic for other bacteria found in the intestinal tract can be best isolated from sewage or manure. This can be done by centrifugation or filtration of the source material and addition of chloroform to kill the bacterial cells. A small amount (0.1 ml) of this preparation is mixed with the host organism and spread on an agar medium. Growth of phage is indicated by the appearance of plaques in the otherwise opaque growth of the host bacterium occurs (Fig. 4.4).

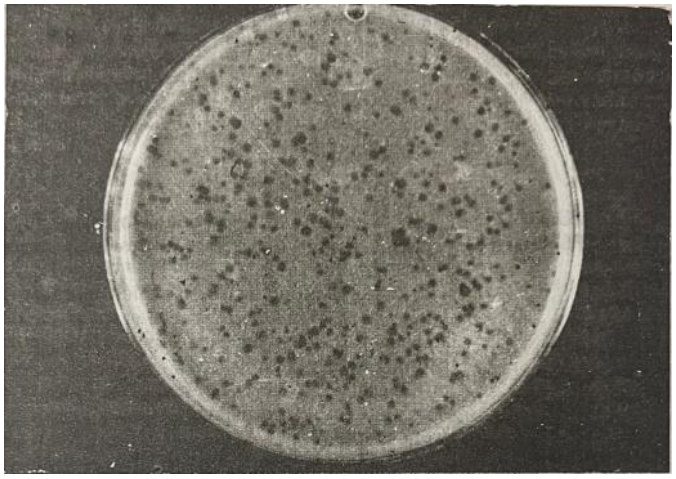

Figure-4.4: Plaque assay of bacteriophages

4.5 SUMMARY

Cultivation of viruses can be done different methods depending on the nature of the virus. In the past whole organism was used as media for cultivation. Later, the organ and now it is the cell that is used a s media for cultivation. Obviously virus needs a living system for its multiplication. Animal viruses are mostly cultivated in mono cell culture consisting of either epithelial or fibroblast cells, though the embryonated chicken egg is the method of choice for many viruses. Plant viruses are usually cultivated by tissue culture methods and protoplast cultures. Protoplast cultures are advantageous over tissue cultures is that with protoplasts infected in vitro it is possible to get a picture of the generation time of plant viruses and course of virus replication and its kinetics can be measured.

4.6 TECHNICAL TERMS

Organ culture, Cell lines, Plaque assay, Abrasion, Embryonated chicken egg, Allontoic cavity, Amniotic cavity, Chorioallontoic membrane, Yolk sac.

4.7 SELF ASSESSMENT QUESTIONS

Q.1 Discuss the cultivation methods of plant viruses.
Q.2 Give an account on cultivation of animal viruses.
Q.3 Describe the general methods used for the cultivation of viruses.

4.8 SUGGESTED READINGS

1. Virology - Cornal, F.H and Kimball P.C. 1988 2nd Edition, Prentice Hall, New Jersey.
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Prof. V. Umamaheswara Rao

LESSON - 5

GENERAL METHODS OF PURIFICATION OF VIRUSES

Objective of the lesson

Students will have the knowledge and know how the different viruses can be purified.

Structure of the lesson

5.1 INTRODUCTION

Purification is defined as separation of virus particles from host components and their concentration in vitro. Crude extracts of viruses prepared from natural or propagated hosts are not qualify for further investigations as they contain variety of cell constituents with similar properties and with same broad size range. For the study of any virus it is essential to understand the nature and properties of individual virus. Cultivation and purification are the initial steps in the study of a virus. To know the properties of individual viruses, it is essential to purify the viruses by different methods including precipitation, filtration and centrifugation. Any of the processes should not disrupt the physical, chemical, biological and infectivity properties of the viruses. The crucial factors to consider during isolation and purification of viruses are temperature, pH and salt concentration.

5.2 PURIFICATION OF VIRUSES

Purification is essential for the study of structure, replication and other biological aspects of viruses. These procedures are mainly aimed at removal of all contaminants without the loss of viruses. Viruses are basically proteins which are often more stable than normal cell components. Because of these characteristics, many techniques useful for the isolation of proteins and organelles can be employed in virus isolation and purification. Many viruses are purified quite satisfactorily by differential centrifugation or by repeated precipitation. However, more selective separation techniques are necessary, where the contaminant material

have similar properties to those of the virus. Preferred procedures for the isolation and subsequent purification of viruses, are – Differential centrifugation; Density gradient centrifugation (Rate zonal and isopycnic density gradient centrifugation), Precipitation with ammonium sulfate or polyethylene glycol and filtration. Clarification of extract is the first step, which is designed to remove much of the macromolecular host material leaving viruses in solution. Extract is subjected to some treatments like heating to 50-60o C for few minutes, freezing, thawing, and acidification to a pH less than 5.0, addition of K2HPO4 etc., to coagulate much of the host material. But viruses should not be damaged during the process. Then centrifugation at low speed (5000-10,000g for 10-20 minutes) causes the sedimentation of cell debris and coagulated host material. Butanol-chloroform system of centrifugation separates the viruses in aqueous phase and denatured protein at interface. However, organic solvents should not be used for enveloped viruses.

5.2.1 Precipitation

Many viruses can be precipitated simply by lowering the pH (hydrogen ion concentration) of the extracts until the virus is precipitated at its isoelectric point. The pH at which viruses can be precipitated is usually in the range of 3.4 –5.5. Proteins are ampholytes i.e. they possess two types of surface charges, positive and negative on them. Proteins remain in the solution till the charges are unequal in number and till they are neutralized. Once that happens, they precipitate out. For a particular type of protein only one pH level is capable of precipitating that type of protein. This pH is called the 'isoelectric point'. The pH can be adjusted by adding more cation (Mg^{2+}, Ca^{2+}, H^{+} etc.) and anions (Cl^{-}, PO4) in the form of salt solution. These ions neutralize the negative and positive charges, respectively and the isoelectric point is reached. Thus proteins are precipitated out. Since the viruses have an outer protein coat, they can also be precipitated in this manner. Alfalfa mosaic virus is one of the first viruses to be precipitated by this method. This is the simplest method for precipitating the viruses, however some viruses are inactivated during this procedure.

Another simplest method is precipitation by salts - ammonium sulfate. Only salt tolerant viruses can be precipitated by this method. Most of the viruses are precipitated at saturated ammonium sulfate concentrations between 20% and 40%, however some viruses require about 80% saturation as for broad bean mottle virus. The suspension or extract containing virus particles is initially purified using the techniques of ultrafiltration and ultracentrifugation. The purified preparation containing the virus particles is then precipitated. Some chemical agents like ethanol or ammonium sulfate has been found to be effective concentration for precipitating viruses. The precipitates obtained could be re-dissolved in some suitable buffer solution and precipitated. Then, precipitate is treated with gradually increasing amounts of water saturated with ammonium sulfate. When a precipitate appears, it is separated by low-speed centrifugation and more ammonium sulfate is added slowly to the supernatant until another precipitate forms, which is centrifuged off. Usually the salt, as saturated solution or as crystals, is added slowly to sap and left for several hours or overnight for better precipitation. Methanol, protamine etc., have been found to be the effective precipitants of animal viruses.

The same principle is used in stepwise precipitation with polyethylene glycol, which can be used, in contrast to ammonium sulfate, with salt-sensitive viruses. Precipitation with PEG is also commonly used as an early purification step to concentrate animal viruses or bacteriophage from large volumes of culture media into which the viruses were released from infected cells. Some plant viruses could be preferentially precipitated in a single-phase

polyethylene glycol (PEG) system, although some host DNA may also be precipitated. Since that time, precipitation with PEG has become one of the commonest procedures used in virus isolation. In case of viruses which are stable in organic solvents, precipitation by adding solvents is preferable. Ethanol and methanol have been used to precipitate plant and animal viruses. However, these alcohols are known to denature some viruses especially when the precipitation was performed at unfavorable temperatures. The only advantage in this method is – it minimizes the time and cost, particularly when large samples are processed.

5.2.2 Ultrafiltration

This technique is based on the fact that virus particles have a smaller size dimension than any other microorganism. Therefore, to separate viruses from amongst a mixture of variously sized particles, the simple way is to use a filtration device capable of retaining the larger particles while allowing the smaller virus particles to pass through. In this process, a viral suspension having impurities like bacteria or any other materials is allowed to pass through a suitable filter paper (Seitz type filters or Porcelain G-5 filters) capable of allowing only the virus particles to pass through, while keeping back the larger particles. The clear filtrate obtained should contain only viruses. In order to retain these virus particles, the viral suspension may further be passed through some ultra-filters made of cellulose acetate or collodion membranes. The very small pore size of these filters enables them to retain the comparatively larger particles of the virus. Such membrane filters with graded porosity, like Millipore filters (manufactured by Millipore Filters Corporation, USA) have accurately determined pore size. Therefore, these can be used to segregate particles having different sizes. Particles larger than a particular pore size get wedged in the small pores of these filters. Thus the smaller ones get through but may again be retained by filters having still smaller pore size. The filtered particles of a particular size can be brought in a fluid suspension by washing the filters in reverse direction. In recent years, diatomaceous earth filters are increasingly used as a filtering aid. However, this technique is not of much use, no matter how small the pore size, when the viral suspension is contaminated with Mycoplasmas and Rickettsia which have the same size range of viruses.

5.2.3 Differential centrifugation

Differential centrifugation consists of alternating cycles of high and low speed centrifugation at 20,000 to 50,000 rpm (e.g. a force of 1,00,000 g) for 1 to 3 hr sediments viruses together with other particulate material and leaves soluble components of the extract of less than 106 molecular weight in the supernatant. The sediment is then dispersed in water or in a suitable buffer; this must often be done by allowing the pellet to soften rather than by vigorous stirring. The extract is then again centrifuged at 8000 to 10,000 g to remove membranous and fibrous cell components, denatured proteins, and so on. Subsequent centrifugation of the supernatant solution at the higher speed again brings down viruses, and two or three repetitions of this procedure, discarding low-speed sediments and high-speed supernatants, yields many plant viruses including TMV in comparatively pure form.

Density Gradient Centrifugation

One of the most useful procedures for purification, particularly for less stable viruses is density gradient centrifugation (Fig. 2.1). Sucrose is the most commonly used material for making the gradient. It is a relatively mild procedure, although there may be a loss of infectivity with some viruses. It can give some indication of the purity of the preparation. It allows a correlation between particles and infectivity to be made and it frequently

reveals the presence of non-infective virus like multi-particle viruses. Gradient centrifugation can be done in two different way, one by considering the size and density of the particles together (rate zonal centrifugation) and the second by considering only the density (isopycnic centrifugation).

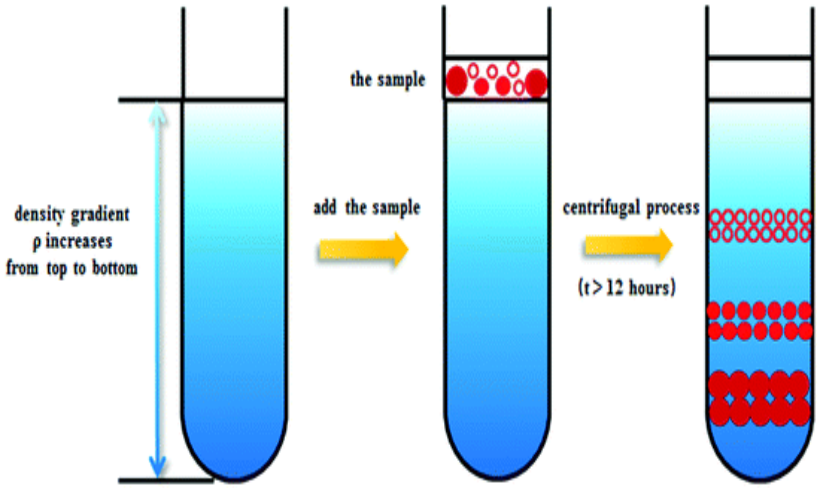

Figure-5.1: Density gradient centrifugation

Rate zonal density gradient centrifugation

This method utilizes centrifugation through increasing concentrations of sucrose, glycerol, cesium chloride and so on. Sucrose gradients (e.g. 25 to 5 percent) are mostly preferred and easily prepared, and they afford good separations of viruses and other particles and molecules of various sizes and densities. The gradient was prepared by pouring the sucrose solution in a test tube such that its concentration smoothly and linearly increase between the top and the bottom of the tube, the highest density of which does not exceed the densest viral particle to be separated. The virus preparation is layered on top of the gradient and centrifuged. The particles are separated based on differences in their sedimentation rates i.e., based on both size and density. The virus was clearly visible in the test tube as a light scattered band, when a light beam is allowed to pass through the test tube. From the test tube the virus is then recovered either by piercing the test tube at the scattered light region or by introducing the syringe from top of the test tube up to the zone. Pure virus preparation can be obtained by this method. Salt sensitive and low-density viruses are preferentially purified by this method.

Isopycnic gradient centrifugation

A different use of density gradient centrifugation is isopycnic equilibrium centrifugation. This method relies strictly on the different buoyant densities of viruses, proteins, nucleic acids and so on. Each type of molecule will move in an increasing density gradient to the level at which its density equals that of the gradient and stop there. Most commonly, salts of heavy metals (Cesium or Rubidium) at high density (above 1-2 g/ml) are used since these form gradients automatically under the g forces of the ultracentrifuge. Thus, centrifugation of viruses in such a salt solution of appropriate concentration will result in location of the virus as a sharp band at the particular level of the tube where the solution density equals to the buoyant density of virus. This centrifugation requires a long time (36-48h) for equilibrium to be reached and can be used for salt-stable viruses only. Isopycnic density gradient centrifugation is particularly useful in the separation and

characterization of nucleic acids, since these have higher density than viruses and other cell components and are not dissociated by salts. Very slight differences in the density of nucleic acids due to different ratios of G-C as compared with A-T base pairs, or the presence of heavy elements (5-bromo-uracil-instead of uracil) or isotopes can easily be detected by this method.

5.2.4 Adsorption Chromatography

One of the effective physico-chemical methods is the adsorption chromatography (Fig.5.2). This method is dependent on the surface properties of the protein coats of the virus particles. In this, the virus can get associated with or adsorbed on the surface of solid but porous substances having the similar properties (charge etc.) on them. Chromatographic substances like calcium or aluminium phosphate are generally used. These are packed in the column of glass tube designed specially to hold them. The packing of chromatographic material has to be done under special conditions. The viral suspension along with the impurities is them poured on the column. The liquid is allowed to drain off by keeping the outlet stopcock of the column open. Virus particles get adsorbed onto the surface of the material. Water flows out along with some of the impurities. The adsorbed virus particles can be reclaimed by washing the column with a large volume of a specific medium such as $MgCl_2$ solution of an ionic concentration higher than that of the viral suspension. The virus particles come out of the column material as they have greater affinity towards the washing medium ($MgCl_2$ solution). The reclaimed virus is now purer than it was earlier. The presence of virus could be subsequently detected in the outflowing fluid (eluate) which is collected in small volumes. Ion exchange resin like, Dowex I have also been successfully used. Separation on columns of neutral substance like silica gel and magnesium pyrophosphate have also been reported.

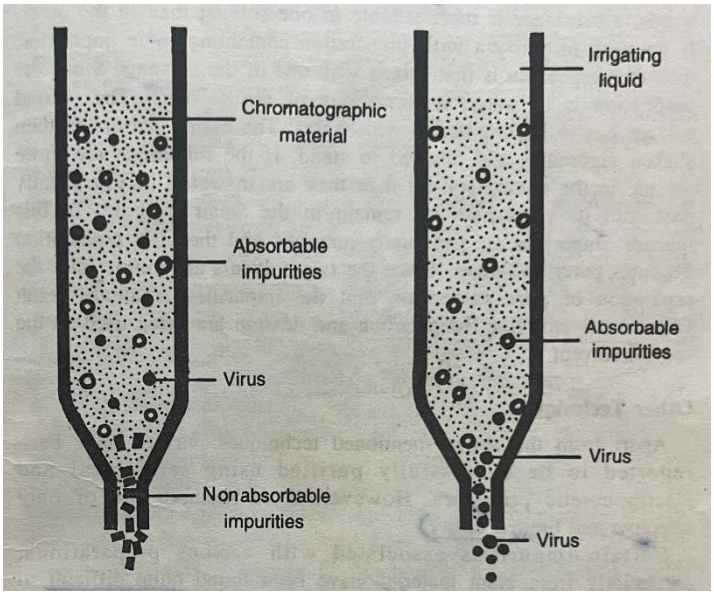

Figure- 5.2: Purification of viruses by Adsorption Chromatography

5.2.5 Solvent Extraction

Purification of viral preparations may also be achieved by what is known as partition between solvents. It is well known that a substance could be soluble in more than one solvent. Usually, the solubility of the material in these solvents also differs. In other words, a

substance is more soluble in one solvent than in the other. To purify a virus preparation containing some impurities, then the suspension is first mixed with one of the solvents. The second solvent has to be immiscible with water. The entire mixture is then shaken vigorously and allowed to stand. If the impurities are more soluble in the second solvent than they are in water, they gradually pass into it. Virus particles remain in the water medium. In this manner, impurities are eventually removed and the viral preparation becomes purer and purer. Since the two solvents are immiscible, the separation of pure suspension and the impurities is almost total. Organic solvents like fluorocarbon and dextran are often used as the second solvent.

5.3 SUMMARY

Purification of the viruses can be achieved by different methods which include mainly protein purification. Depending on the tolerance towards the precipitating agent, viruses are precipitated using either by ammonium sulfate, polyethylene glycol or solvents. Sometimes they can be isolated by filtration through filter papers with Celite pads. Differential centrifugation is the first option for purification of majority of viruses. In Gradient centrifugation low density and high density viruses are conveniently purified by using sucrose and cesium chloride gradients, respectively.

5.4 TECHNICAL TERMS

Centrifugation, Isopycnic, Adsorption chromatography, Precipitation, Equilibrium, Iso-electric point.

5.5 SELF ASSESSMENT QUESTIONS

Q.1 Give an account on the methods for purification of viruses.
Q.2 Explain the differential centrifugation method for the purification of viruses.
Q.3 Describe the ultrafiltration and adsorption chromatography techniques for purification of viruses.

5.6 SUGGESTED READINGS

1. Virology - Cornal, F.H and Kimball P.C. 1988 2nd Edition, Prentice Hall, New Jersey.
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Dr. J. Madhavi

LESSON – 6

SEROLOGICAL METHODS FOR DETECTION OF VIRUSES

Objective of the lesson

The students gain the knowledge on how to detect the viruses by using different serological based methods.

Structure of the lesson

6.1 INTRODUCTION

The union of antigen and antibody in vitro produces either a visible reaction or one that can be made visible in a variety of ways. These techniques can be used to identify viruses, microorganisms, to quantitate and identify antigens and antibodies etc. The older classic tests are named according to what happens to the antigen – agglutination, complement fixation, precipitation, and neutralization. More recent tests are named according to the technique used: – enzyme-linked immunosorbent assay, immunodiffusion, immunoelectrophoresis, immunofluorescence, immunoprecipitation, and radioimmunoassay. Several serological techniques are also in extensive, though restrictive, use in the study of viruses. With these techniques, it is possible to detect the presence of a virus or viral product with rapidity and high degree of specificity. These methods are based on the direct interaction between the virion or viral antigen present *in situ* in tissues or in secretions and specific antibodies which are pre labeled or tagged in some way as to permit the ready recognition of the interaction. Of the techniques, ELISA has been found wide acceptance and usage for its high degree of sensitivity and operational simplicity.

6.2 HAEMAGGLUTINATION

The haemagglutination assay (HA) and the haemagglutination inhibition assay (HAI) are developed in 1941-42 by American virologist, George Hirst as the methods for quantifying the relative concentration of viruses, bacteria, or antibodies. HA and HAI apply the process of haemagglutination, in which sialic acid receptors on the surface of red blood cells (RBCs) bind to the haemagglutinin glycoprotein found on the surfaces of some viruses (e.g. influenza virus) and create a network or lattice structure of interconnected RBCs and virus particles. The agglutinated lattice maintains the RBCs in a suspended distribution, typically viewed as a diffuse reddish solution. The formation of the lattice depends on the concentrations of the virus and RBCs, and when the relative virus concentration is too low, the RBCs are not constrained by the lattice and settle to the bottom of the well. Haemagglutination is observed in the presence of staphylococci, vibrios, and other bacterial species, similar to the mechanism viruses use to cause agglutination of erythrocytes. The RBCs used in HA and HAI assays are typically of chickens, turkeys, horses, guinea pigs, or humans depending on the selectivity of the targeted virus or bacterium and the associated surface receptors on the RBCs.

In the general procedure for HA, a serial dilution of virus is prepared across the rows in a U or V-bottom shaped 96-well microtiter plate. The most concentrated sample in the first well is often diluted to be 1/5x of the stock, and subsequent wells are typically two-fold dilutions (1/10, 1/20, 1/40, etc.). The final well serves as a negative control with no virus. Each row of the plate typically has a different virus and the same pattern of dilutions. After serial dilutions, a standardized concentration of RBCs is added to each well and mixed gently. The plate is incubated for 30 minutes at room temperature. After incubation, the assay can be analyzed to distinguish between agglutinated and non-agglutinated wells. The images across a row will typically progress from agglutinated wells with high virus concentration and diffuse reddish appearance to a series of wells with low virus concentrations containing dark red pellet, or button, in the center of the well. The low concentration wells appear nearly identical to the no-virus negative control well. The button appearance occurs because the RBCs are not held in the agglutinated lattice structure and settle into the low point of the U or V-bottom well. The transition from agglutinated to non-agglutinated wells occurs distinctively, within 1 to 2 wells. The relative concentration, or titre, of the virus sample is based on the well with the last agglutinated appearance, immediately before a pellet is observed. Relative to the initial viral stock concentration, the virus concentration in this well will be some dilution of the stock, for example, 1/40 fold. The titer value of that sample is the inverse of the dilution i.e., 40 (Fig. 6.1).

HAI is closely related to the HA assay, but includes anti-viral antibodies as ‘inhibitors’ to interfere with the virus-RBC interaction. The goal is to characterize the concentration of antibodies in the antiserum or other samples containing antibodies. The HAI assay is generally performed by creating a dilution series of antiserum across the rows of a 96-well microtiter plate (Fig. 6.2). Each row would usually be a different sample. A standardized amount of virus of bacteria is added to each well, and the mixture is allowed to incubate at room temperature for 30 minutes. The last well in each row would be negative control with no virus added. During the incubation, antibodies bind to the viral particles, and if the concentration and binding affinity of the antibodies are high enough, the viral particles are effectively blocked from causing haemagglutination. Next, a standardized amount of RBCs is added to each well and allowed to incubate at room temperature for an additional 30 minutes. The resulting HAI plate images usually progress from non-agglutinated, ‘button’ wells with

high antibody concentration to agglutinated, red diffuse wells with low antibody concentration. The HAI titre value is the inverse of the last dilution of serum that completely inhibited haemagglutination.

The described HA and HAI processes are generalized, and specific details can vary depending on the operator and laboratory. For example, serial dilutions across the rows are described, but some laboratories use an alternate orientation and perform dilutions down the columns instead. Similarly, the starting dilution, serial dilution factor, incubation times, and choice of U or V-bottom plate can be depend on the specific laboratory. On the advantage point, HA and HAI assays are simple, relatively inexpensive and provide results within a few hours.

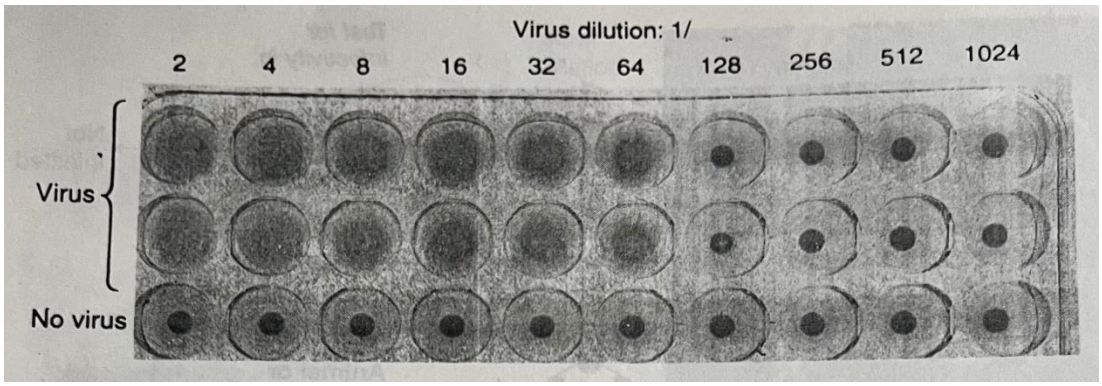

Figure-6.1: Haemagglutination titration

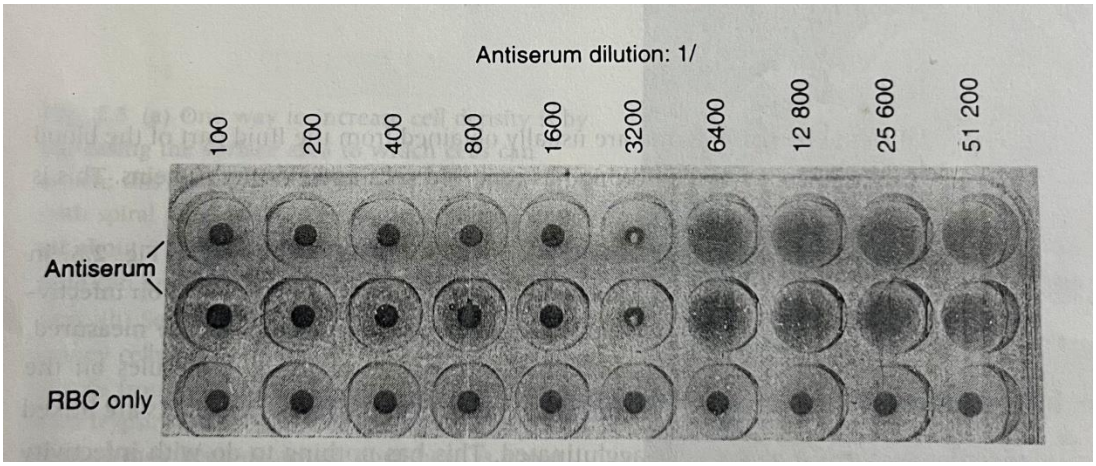

Figure-6.2: Haemagglutination-inhibition test

6.3 IMMUNOFLUORESCENCE

Albert Coons, in 1944, showed that antibodies could be labeled with molecules that have the property of fluorescence. Fluorescent molecules absorb light of one wavelength and emit light of another wavelength. If antibody molecules are tagged with a fluorescent dye, or fluorochrome, immune complexes containing these fluorescently labeled antibodies (FA) can be detected by colored light emission when excited by light of the appropriate wavelength.

Antibody molecules bound to antigens in cells or tissue sections can similarly be visualized. The emitted light can be viewed with a fluorescence microscope, which is equipped with a UV light source. In this technique, known as 'Immunofluorescence', the most commonly used fluorescent dyes are fluorescein and rhodamine, but other highly fluorescent substances, such as phycoerythrin and phycobiliproteins have also come into use. The dyes can be conjugated to the Fc region of an antibody molecule without affecting the specificity of the antibody. Each of these dyes absorbs light at one wavelength and emits light at a longer wave length.

- **Fluorescein:** an organic dye that is the most widely used label for immunofluorescence procedures, absorbs blue light (490 nm) and emits an intense yellow-green fluorescence (517 nm).

- **Rhodamine:** another organic dye, absorbs in the yellow-green range (515 nm) and emits a deep red fluorescence (546 nm). Because it emits fluorescence at a longer wavelength than fluorescein, it can be used in two-colour immunofluorescence assays. An antibody specific to one determinant is labeled with fluorescein, and an antibody recognizing a different antigen is labeled with rhodamine. The location of the fluorescein-tagged antibody will be visible by its yellow-green colour, easy to distinguish from the red colour emitted where the rhodamine-tagged antibody has bound. By conjugating fluorescein to one antibody and rhodamine to another antibody, one can visualize simultaneously two different cell membrane antigens on the same cell, for example.

- **Phycoerythrin:** Phycoerythrin and other phycobiliproteins are light-gathering proteins that play important roles in photosynthesis in some species of algae. Because they are efficient absorbers of light (30-fold greater than fluorescein) and brilliant emitters of red fluorescence, these proteins have become widely used labels for immunofluorescence.

Fluorescent-antibody staining of cell membrane molecules or tissue sections can be direct or indirect (Fig.6.3). In 'direct staining', the specific antibody (the primary antibody) is directly conjugated with fluorescein; in 'indirect staining', the primary antibody is unlabeled and is detected with an additional fluorochrome-labeled reagent. A number of reagents have been developed for indirect staining. The most common is a secondary antibody, a fluorochrome-labeled anti-isotype antibody, such as fluorescein-labeled goat anti-mouse immunoglobulin. Another reagent is fluorochrome-labeled protein A from *Staphylococcus aureus*, which binds with high affinity to the Fc region of IgG antibody molecules. A third indirect approach uses a secondary biotin-conjugated anti-isotype antibody followed by fluorochrome-conjugated avidin, a protein that binds to biotin with extremely high affinity.

Indirect immunofluorescence staining has two advantages over direct staining. First, the primary antibody does not need to be conjugated with a fluorochrome. Because the supply of primary antibody is often a limiting factor, indirect methods avoid the loss of antibody that usually occurs during the conjugation reaction. Second, indirect methods increase the sensitivity of staining because multiple molecules of the fluorochrome reagent bind to each primary antibody molecule, increasing the amount of light emitted at the location of each primary antibody molecule.

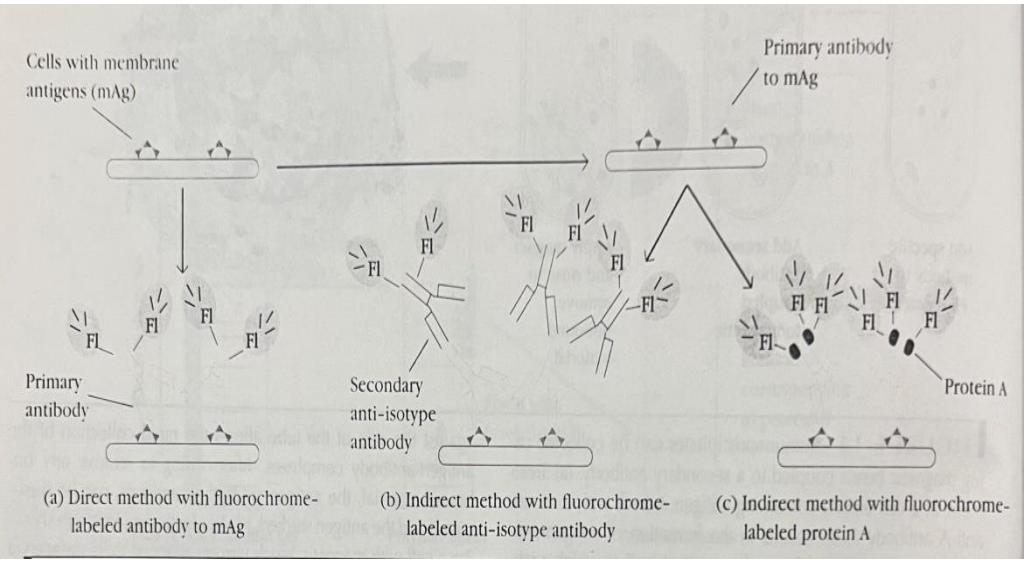

Figure-6.3: Direct and indirect immunofluorescence staining of membrane antigen.

Applications of IFA

Diagnosing diseases – IFA can be used to detect the presence of specific antigens or antibodies in patient samples, helping to diagnose diseases like autoimmune disorders and infectious diseases.

Research – IFA is **a** powerful tool for studying cell biology and identifying the location and expression of specific proteins in cells and tissues.

Quality control – IFA can be used to ensure the quality of reagents and materials used in other biological experiments.

Advantages

IFA offers several advantages over other techniques, including –

- Sensitivity – IFA can detect very low concentrations of antigens or antibodies.
- Specificity – The technique can be very specific for detecting target antigens, minimizing the risk of false positives.
- Visualization – IFA allows for the visualization of the location and distribution of specific proteins in cells and tissues.

6.4 ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

Enzyme-linked immunosorbent assay is commonly known as ELISA or EIA. The principle of ELISA is similar to RIA but depends on an enzyme rather than a radioactive label. An enzyme conjugated with an antibody reacts with a colourless substrate to generate a coloured reaction product. Such a substrate is called as chromogenic substrate. ELISA is a valuable tool for virus detection, particularly for identifying antibodies in blood samples that indicate a past or current infection. It works by detecting the presence of specific antigens or antibodies related to a virus, using a combination of antigens, antibodies, and enzymes to generate a detectable signal. A number of enzymes have been employed for ELISA, including alkaline

phosphatase, horseradish peroxidase, and β-galactosidase. These assays approach the sensitivity of RIAs and have the advantage of being safer and less costly.

A number of variations of ELISA have been developed, allowing qualitative detection or quantitative measurement of either antigen or antibody. Each type of ELISA can be used qualitatively to detect the presence of antibody or antigen. Alternatively, a standard curve based on known concentrations of antibody or antigen is prepared, from which the unknown concentration of a sample can be determined.

Indirect ELISA

Antibody can be detected or quantitatively determined with an indirect ELISA (Fig.6.4A). Serum or some other sample containing primary antibody (AB_1) is added to an antigen-coated microtiter well and allowed to react with the antigen attached to the well. After any free Ab_1 is washed away, the presence of antibody bound to the antigen is detected by adding an enzyme-conjugated secondary anti-isotype antibody (Ab_2), which binds to the primary antibody. Any free AB_2 then is washed away, and a substrate for the enzyme is added. The amount of colored reaction product that forms is measured by specialized spectrophotometric plate readers, which can measure the absorbance of all the wells of a 96-well plate in less than a few seconds. Indirect ELISA is the method of choice to detect the presence of serum antibodies against human immunodeficiency virus (HIV), the causative agent of AIDS. In this assay, recombinant envelope and core proteins of HIV are adsorbed as solid-phase antigens to microtiter wells. Individuals infected with HIV will produce serum antibodies to epitopes on these viral proteins. Generally, serum antibodies to HIV can be detected by indirect ELISA within 6 weeks of infection.

Sandwich ELISA

Antigen can be detected or measured by a sandwich ELISA (Fig.6.4 B). In this technique, the antibody (rather than the antigen) is immobilized on a microtiter well. A sample containing antigen is added and allowed to react with the immobilized antibody. After the well is washed, a second enzyme-linked antibody specific for a different epitope on the antigen is added and allowed to react with bound antigen. After any free second antibody is removed by washing, substrate is added, and the colored reaction product is measured.

Competitive ELISA

Another variation for measuring amounts of antigen is competitive ELISA (Fig.6.4 C). In this technique, antibody is first incubated in solution with a sample containing antigen. The antigen-antibody mixture is then added to an antigen-coated microtiter well. The more antigen present in the sample, the less free antibody will be available to bind to the antigen-coated well. Addition of an enzyme-conjugated secondary antibody (Ab_2) specific for the isotype of the primary antibody can be used to determine the amount of primary antibody bound to the well as in an indirect ELISA. In the competitive assay, however, the higher the concentration of antigen in the original sample, the lower the absorbance.

Chemiluminescence

Measurement of light produced by chemiluminescence during certain chemical reactions provides a convenient and highly sensitive alternative to absorbance measurements in ELISA assays. In versions of the ELISA using chemiluminescence, a luxogenic (light-generating) substrate takes the place of the chromogenic substrate in conventional ELISA reactions.

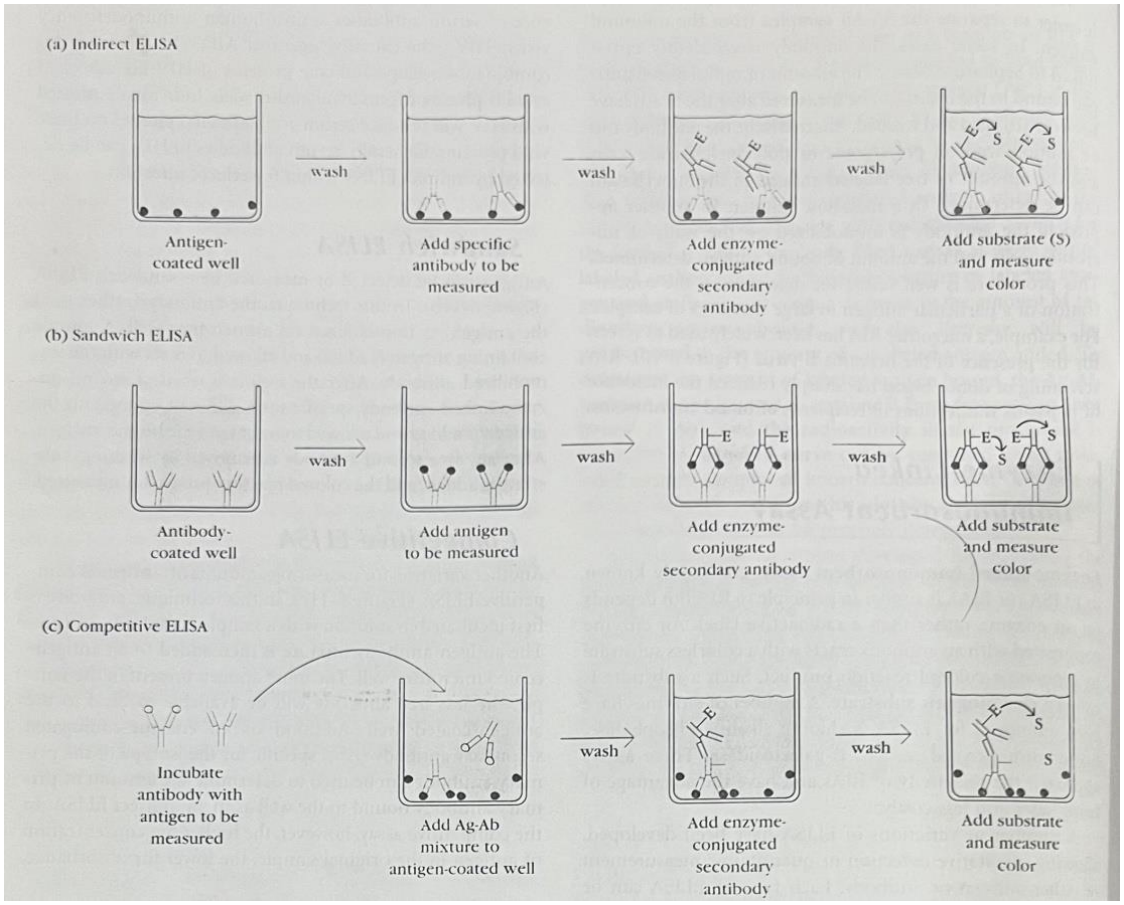

Figure-6.4: ELISA technique

6.5 POLYMERASE CHAIN REACTION

Polymerase chain reaction (PCR) is a powerful molecular technique used to detect and amplify specific DNA or RNA sequences, including those of viruses. This method allows for the detection of even minute amounts of viral genetic material, making it highly sensitive and reliable for diagnosing and monitoring viral infections. Kary Mullis, between 1983 and 1985, developed the PCR technique that made it possible to synthesize large quantities of a DNA fragment without cloning it. So, the PCR technique is a powerful technique for amplifying specific DNA sequences even when they are present at extremely low levels in a complex mixture.

The first step in this is to synthesize fragments with sequences identical to those flanking the targeted sequence. This is readily accomplished with a DNA synthesizer machine. These synthetic oligonucleotides are usually about 20 nucleotides long and serve as primers for DNA synthesis. The PCR cycle itself takes place in 3 steps. First, the target DNA containing the sequence to be amplified is heat denatured to separate its complementary strands (step 1). Normally the target DNA is between 100 and 5,000 base pairs in length. Next, primers are added in excess and the temperature is lowered so that they can hydrogen bond or anneal to the DNA on both sides of the target sequence (step 2). Because the primers are present in excess, the targeted DNA strands will almost always anneal to the primers rather than to each other. Finally, nucleoside triphosphates and a DNA polymerase are added to the mixture. The DNA polymerase extends the primers and synthesizes copies of the target DNA sequence

(step 3). Only polymerases able to function at the high temperatures are employed in the PCR technique can be used. Two popular enzymes are the 'Taq polymerase' from the thermophilic bacterium *Thermus aquaticus* and the 'Vent polymerase' from *Thermococcus litoralis*. At the end of one cycle, the targeted sequences on both strands have been copied. When the three-step cycle is repeated (Fig.6.5), the four strands from the first cycle are copied to produce eight fragments. The third cycle yields 16 products. Theoretically, 20 cycles will produce about one million copies of the target DNA sequence; 30 cycles yield around one billion copies. Pieces ranging in size from less than 100 base pairs to several thousand base pairs in length can be amplified, and only 10 to 100 pico moles of primer are required. PCR technology is improving continually, and now RNA can also be efficiently used in PCR procedures. Cellular RNAs and RNA viruses may be studied even when the RNA is present in very small amounts. PCR can also quantitate the DNA products without the use of isotopes. Quantitative PCR is quite valuable in virology and gene expression studies.

Advantages

1. Sensitivity – PCR can detect very small amounts of viral material, even when the viral load is low.
2. Applications – PCR is used for diagnosing viral infections, monitoring viral load, and understanding disease progression.
3. Types – Real-time PCR (qPCR) is a common type, which allows for quantification of viral load during amplification.
4. Clinical significance – PCR-based detection methods have provided a rapid and reliable way to detect viral nucleic acids in the clinical setting, facilitating timely diagnosis and management of viral infections.

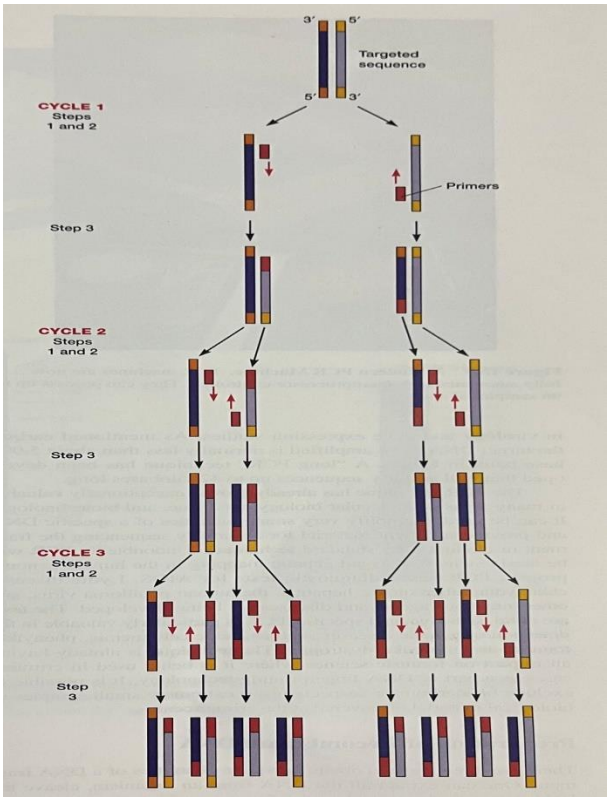

Figure-6.5: Polymerase Chain Reaction technique

6.6 RADIOIMMUNOASSAY (RIA)

Radioimmunoassay is a method used to detect and measure the concentration of specific viral antigens or antibodies in the sample. It involves using a radioactive isotope, often 1251, to label a portion of the target antigen or antibody. This labeled target is them mixed with a sample, and any matching antigen or antibody in the sample will compete for binding to the antibody. The amount of radioactivity in the unbound fraction is them measured, which is directly proportional to the concentration of the target in the sample.

To perform this, a known amount of the target antigen or antibody is labeled with a radioactive isotope, typically 1251. The labeled target is mixed with the sample that is suspected to contain the virus or viral component. If the sample contains the target antigen or antibody, it will compete with the labeled target for binding to an antibody that recognizes it. The mixture is then processed to separate the labeled target that is bound to the antibody from the unbound labeled target and any target in the sample that has also bound to the antibody. The radioactivity in the unbound fraction is measured, and this value is directly proportional to the concentration of the target in the sample.

Applications
Detecting viral antigens – RIA can be used to detect the presence of specific viral antigens in a sample, such as influenza A and B viruses in nasopharyngeal specimens.
Detecting viral antibodies – RIA can also be used to detect the presence of antibodies against specific viruses, such as mumps-specific IgA or measles-specific IgA in saliva samples.

Advantages
High sensitivity – RIA is a highly sensitive technique, allowing for the detection of viral antigens or antibodies at very low concentrations.
Specificity – RIA is a specific technique, allowing for the identification of specific viral antigens or antibodies.

Disadvantages
1. RIA involves the use of radioactive isotopes, which requires special handling and disposal procedures.
2. RIA can be a complex and time-consuming procedure.

6.7 PLAQUE ASSAY

One of the methods for enumerating the viruses is the ‘plaque assay or method’. In this method, a suspension culture of appropriate host cells is prepared initially and later cultured in Petri plates containing suitable agar based media. Such cultures are then inoculated with aliquots of the virus titer to be enumerated. On proper incubation, virus particles start infecting the host cells. One susceptible host cell is infected by a single virion. After some time, disintegration of the host cells occurs resulting in the liberation of more virus particles. These particles, in their turn, infect the host cells in the immediate vicinity and destroy them as well. In the meanwhile, the cells that are not infected continue to grow and divide normally. Thus, soon transparent, visible, circular areas appear on the beneficial lawn in the plate. These areas are called as ‘Plaques’.

Since each virion of the original suspension causes one plaque, the number of virus particles in it can be determined with ease. For example, if 1 ml of the original suspension was diluted

10^4 times before being distributed with the host cell culture; and 42 plaques are formed. So, there are 42 x 10^4 particles per ml or precisely 42 x 10^4 'plaque forming units (PFUs) per ml are present in the stock. In this method, virus suspensions are often mixed with the host cells prior to incubation. Plaque assay has been most effective in the counting of bacterial viruses.

Plaque assay is also extensively used for quantitative evaluation of animal viruses. Dulbecco, in 1952 introduced a modified bacteriophage plaque assay which has now become a standard procedure for enumeration of animal viruses. In this method, a series of 10 fold dilutions of a viral suspension is first inoculated into monolayers of cell cultures for an hour to allow the virions to adsorb onto the cells. The infected cells are then overlaid with agar or nitrocellulose gel based medium to trap the infected cells. Spread of virus progenies gest restricted to the immediate vicinity of the originally infected cell. Thus, after a few days, a focus of infected cells become large enough to be visible to the naked eye, and represents a plaque. To make the plaques more visible, the cell monolayers are usually stained with neutral red or crystal violet. The non-infected, living cells take up the stain and the plaques appear as distinct clear zones against a coloured background. In this case, also the number of virions or the infectivity titer is expressed as PFUs. Herpes virus and pox viruses can form plaques even when monocultures are maintained in liquid medium, because the progeny of these viruses remain associated with the infected cell. Therefore, they do not spread out and can infect only adjoining cells.

6.8 SUMMARY

Haemagglutination is a reaction that causes clumping of red blood cells in presence of some enveloped viruses such as influenza virus. A glycoprotein on the vial surface, namely haemagglutinin, interacts with red blood cells, leading to the clumping of red blood cells and the formation of a lattice.

In essence, Immunofluorescence assay is a versatile and powerful technique that combines the specificity of antibody-antigen binding with the visualization power of fluorescence microscopy, making it a valuable tool for both research and clinical applications. It is a process in which certain dyes called fluorochromes are irradiated with UV, violet, or blue light to make them fluoresce. These dyes can be coupled to antibody. There are two main kinds of fluorescent antibody assays: direct and indirect.

The enzyme-linked immunosorbent assay involves the linking of various enzymes to either antigens or antibodies. Two basic methods are involve – the double antibody sandwich method and the indirect immunosorbent assay. The first method detects antigens and the latter, antibodies.

Radioimmunoassay use a purified antigen that is radioisotope-labeled and used to compete with unlabeled standard or antigen in experimental samples for a specific antibody.

PCR detection of viruses is a highly sensitive and rapid method used to identify viral nucleic acids (DNA or RNA) in clinical samples. This technique amplifies the specific DNA sequences from a viral sample, making them detectable even in minute amounts. PCR is widely used for diagnosing and monitoring viral infections.

Plaque assay is a method used to quantify the number of infectious viral particles, also known as plaque forming units. It relies on the principle that a single infectious virus particle can

initiate infection and replication in a host cell, eventually leading to the death of that cell and its neighbors, creating a visible clear area, or plaque, in a cell culture.

6.9 TECHNICAL TERMS

HA, HAI, ELISA, RIA, Fluorochrome, Antigen, Antibody, PCR, Microtiter plate, Titre, Taq polymerase, Plaque assay, Virion.

6.10 SELF ASSESSMENT QUESTIONS

Q.1 Write an account on Haemagglutination and Haemagglutination-inhibition assays to detect the viruses.
Q.2 Explain the Enzyme-linked immunosorbent assay the detection of viral antigens.
Q.3 Describe in detail about Plaque assay.
Q.4 Give an account on Immunofluorescence and Radioimmunoassay.

6.11 SUGGESTED READINGS

1. Virology - Cornal, F.H and Kimball P.C. 1988 2nd Edition, Prentice Hall, New Jersey.
2. Introduction to Modern virology - Dimmock, N.J; Easton, A.J. and Leppord, K.N. 2001. 5th Edition, Blackwell Sciences.
3. Fundamental Virology - Knipe, D.M, Hoeley M.P. 2001. 4th Edition, Lippincott, Williams Publ.
4. Plant Virology - Mathews, R.C.F. 1981, Academy Press.
5. Plant Viruses - Smith, K.M. 1990. 6th Edition. UBS publ.
6. Applied Plant Virology - Walkey, D.G.A 1988. Heinemann; London.

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LESSON - 7

TAXONOMY OF PLANT VIRUSES

Objective of the lesson

Students will have a clear knowledge on the taxonomy of plant viruses and how they are classified.

Structure of the lesson

7.1 INTRODUCTION

Viruses are autonomous infectious particles that differ widely from other microorganisms in a number of characteristics: they have no cellular structure, consisting only of proteins and nucleic acid (DNA or RNA). They have no metabolic systems of their own, but rather depend on the synthetic mechanism of a living host cell, whereby the viruses exploit normal cellular metabolism by delivering their own genetic information, i.e., nucleic acid, into the host cell. The host cell accepts the nucleic acid and proceeds to produce the components of new viruses in accordance with the genetic information it contains. One thus might call viruses "vagabond genes". Plant viruses, like other viruses, contain a core of either DNA or RNA. As plant viruses have a cell wall to protect their cells, their viruses do not use receptor-mediated endocytosis to enter host cells as is seen with animal viruses. For many plant viruses to be transferred from plant to plant, damage to some of the plants' cells must occur to allow the virus to enter a new host. This damage is often caused by weather, insects, animals, fire, or human activities such as farming or landscaping. Additionally, plant offspring may inherit viral diseases from parent plants.

Adolf Mayer(1886) coined the term 'Mosaic'. First to prove the transmissible plant virus i.e. TMV. He thought that the causal agent of tobacco mosaic disease was bacteria. D. Ivanovsky (1892) proved that the causal agent of tobacco mosaic disease could pass through bacterial proof

filters, and thus, must be smaller than bacteria. He described them to be 'crystalline' and amorphous (X-bodies) in TMV. Beijernick (1898) - called the liquid material of virus to be "*Contagium vivumfluidum"* (Latin – contagious living fluid) which cause the infection of Tobacco Mosaic virus. Considered to be the "Founder and Father of Virology". George Clinton (1908) was the first to demonstrate cross inoculation of TMV. Twort (1915) and FD Herelle (1917) individually discovered "bacteriophages". Helen Alice Purdy Beale (1928) reported that virus infected plants contain a specific antigen and she prepared an antiserum to neutralize its effects. Diener and Raymer (1971) discovered that potato spindle tuber was caused by small naked ssRNA which he called viroid. Imperial et al (1981) confirmed that the causative agent for the Cadang- Cadang disease in coconut as viroid.

7.2 TAXONOMY OF PLANT VIRUSES

At the International Congress for Microbiology held in Moscow in 1966, the International Committee on Nomenclature of Viruses (ICNV) was established by an international group of 43 virologists. An international organization was set up with the aim of developing a unique world-wide recognized taxonomy and nomenclature system for all viruses. The name of the ICNV was changed in 1974 to a more appropriate one: the International Committee on Taxonomy of Viruses (ICTV), which **is** active today. The ICTV is now considered the official body for all matters related to taxonomy and nomenclature of viruses. At the first meeting of the ICNV in Mexico City (1970), two families with a corresponding two genera and 24 floating genera were accepted to begin the grouping of vertebrate, invertebrate and bacterial viruses. In addition, 16 plant virus groups were designated.

The Fifth ICTV Report describes one order, 40 families, nine subfamilies, 102 genera, two floating genera and two subgenera for vertebrate, invertebrate, bacterial and fungal viruses and 32 groups and seven subgroups for plant viruses. While most virologists shifted to the grouping of viruses in families and genera, plant virologists have persisted in clustering plant viruses in 'groups' until very recently. It is only in 1993 that the ICTV will propose a uniform system for all viruses with two orders, 50 families, 9 subfamilies, 126 genera, 23 floating genera and 4 subgenera encompassing 2644 assigned virus species.

Table I. List of orders, families and groups of viruses[a]

Nature of the presentation criteria		Order	Family or group	Morphology	Genome configuration	Genome size (kbp)	Virus host	Number of species		
								Members	Tentative	Total
dsDNA	Enveloped		*Baculoviridae*	Bacilliform	1 circular supercoiled	90–230	Invertebrate	14		14
			Hepadnaviridae	Isometric	1 circular	3	Vertebrate	5		5
			Herpesviridae	Isometric	1 linear	120–220	Vertebrate	19	4	23
			Lipothrixviridae	Rod	1 linear	16	Bacteria	2		2
			Plasmaviridae	Pleomorphic	1 circular	12	Bacteria	2	5	7
			Polydnaviridae	Rod, fusiform	1 circular supercoiled	2–28	Invertebrate	2		2
			Poxviridae	Ovioid	1 linear	130–375	Vertebrate, invertebrate	61	16	77
			SSV-1 group	Lemon-shape	1 circular supercoiled	15	Bacteria	3		3
dsDNA	Nonenveloped	(*Caudovirales*)	*Myoviridae*	Tailed phage	1 linear	336	Bacteria	83		83
			Podoviridae	Tailed phage	1 linear	40	Bacteria	51		51
			Siphoviridae	Tailed phage	1 linear	53	Bacteria	111		111
			Adenoviridae	Isometric	1 linear	32–48	Vertebrate	111		111
			Caulimovirus	Isometric	1 circular	8	Plant	11	6	17
			Commelina yellow mottle virus group	Bacilliform	1 circular	8	Plant	4	11	15
			Corticoviridae	Isometric	1 circular supercoiled	10	Bacteria	1	1	2
			Iridoviridae	Isometric	1 linear	160–400	Vertebrate, invertebrate	70	2	72
			Papovaviridae	Isometric	1 circular	5–8	Vertebrate	28		28
			Phycodnaviridae	Isometric	1 linear	250–350	Algae	47		47
			Rhizidiovirus	Isometric	1 linear	27	Fungus	1		1
			Tectiviridae	Isometric	1 linear	16	Bacteria	8		8
ssDNA	Nonenveloped		*Geminivirus*	Isometric	1 or 2 circular	3–6	Plant	35	13	48
			Inoviridae	Rod	1 circular	7–20	Bacteria, mycoplasmas	32		32
			Microviridae	Isometric	1 circular	6	Bacteria	28		28
			Parvoviridae	Isometric	1 – strand	6–8	Vertebrate, invertebrate	4	11	15
dsRNA	Enveloped		*Cystoviridae*	Isometric	3 segments	17	Bacteria	1		1
dsRNA	Nonenveloped		*Birnaviridae*	Isometric	2 segments	6	Vertebrate, invertebrate	5		5
			Cryptovirus	Isometric	2 segments	3–5	Plant	20	10	30
			Partitiviridae	Isometric	2 segments	4–10	Fungus	9	5	14
			Reoviridae	Isometric	10–12 segments	19–62	Vertebrate, invertebrate, plant	136	33	169
			Totiviridae	Isometric	1 segment	5–7	Fungus	4	8	12

Nature of the presentation criteria		Order	Family or group	Morphology	Genome configuration	Genome size (kb)	Virus host	Number of species		
								Members	Tentative	Total
ssRNA	Enveloped; no DNA step; positive sense genome		*Coronaviridae*	Pleomorphic	1+segment	28–33	Vertebrate	11	3	14
			Flaviviridae	Isometric	1+segment	10–22	Vertebrate, invertebrate	35	19	54
			Togaviridae	Isometric	1+segment	10–13	Vertebrate, invertebrate	29	2	31
ssRNA	Enveloped; no DNA step; negative non-segmented genome	*Mononegavirales*	*Filoviridae*	Bacilliform	1 – segment	13	Vertebrate	2		2
			Paramyxoviridae	Helical	1 – segment	15–16	Vertebrate	32	4	36
			Rhabdoviridae	Bacilliform	1 – segment	10–13	Vertebrate, invertebrate, plant	75	100	175
ssRNA	Enveloped; no DNA step;		*Arenaviridae*	Spherical	2 – segments	11	Vertebrate	15		15
			Bunyaviridae	Spherical	3 – segments	12–23	Vertebrate invertebrate, plant	253	45	298
	negative segmented genome		*Orthomyxoviridae*	Helical	8 – segments	13–14	Vertebrate	3	2	5
ssRNA	Enveloped; DNA step		*Retroviridae*	Spherical	dimer 1+segment	7–10	Vertebrate	32		32
ssRNA	Nonenveloped; monopartite genome; Isometric particles		*Caliciviridae*	Isometric	1+segment	8	Vertebrate	4	1	5
			Carmovirus	Isometric	1+segment	4	Plant	8	9	17
			Leviviridae	Isometric	1+segment	3–4	Bacteria	43		43
			Luteovirus	Isometric	1+segment	6	Plant	14	12	26
			Maize chlorotic dwarf virus group	Isometric	1+segment	9	Plant	1	2	3
			Marafivirus	Isometric	1+segment	6–7	Plant	3		3
			Necrovirus	Isometric	1+segment	4–5	Plant	2	2	4
			Parsnip yellow fleck virus group	Isometric	1+segment	10	Plant	2	1	3
			Picornaviridae	Isometric	1+segment	7–8	Vertebrate, invertebrate	215	13	228
			Sobemovirus	Isometric	1+segment	4	Plant	10	6	16
			Tetraviridae	Isometric	1+segment	5	Invertebrate	1	14	15
			Tombusvirus	Isometric	1+segment	5	Plant	12		12
			Tymovirus	Isometric	1+segment	6	Plant	18	1	19
ssRNA	Nonenveloped; monopartite genome; rod-shaped particles		*Capillovirus*	Rod	1+segment	7	Plant	2	2	4
			Carlavirus	Rod	1+segment	7–8	Plant	27	29	56
			Closterovirus	Rod	1+segment	7–18	Plant	10	12	22
			Potexvirus	Rod	1+segment	6	Plant	18	21	39
			Potyvirus	Rod	1+segment	8–10	Plant	73	84	157
			Tobamovirus	Rod	1+segment	6	Plant	12	2	14

Table I. Continued

Nature of the presentation criteria	Order	Family or group	Morphology	Genome configuration	Genome size (kb)	Virus host	Number of species		
							Members	Tentative	Total
ssRNA	Nonenveloped; bipartite genome; isometric particles	*Comovirus*	Isometric	2+segments	9	Plant	14		14
		Dianthovirus	Isometric	2+segments	4	Plant	3		3
		Fabavirus	Isometric	2+segments	10	Plant	3		3
		Nepovirus	Isometric	2+segments	12	Plant	28	8	36
		Nodaviridae	Isometric	2+segments	5	Invertebrate	6		6
		Pea enation mosaic virus group	Isometric	2+segments	9	Plant	1		1
ssRNA	Nonenveloped; bipartite genome; rod-shaped particles	*Furovirus*	Rod	2+segments	9–11	Plant	5	6	11
		Tobravirus	Rod	2+segments	9–11	Plant	3		3
ssRNA	Nonenveloped; tripartite genome; bacilliform particles	Alfalfa mosaic virus group	Bacilliform	3+segments	8	Plant	1		1
ssRNA	Nonenveloped; tripartite genome; isometric particles	*Bromovirus*	Isometric	3+segments	8	Plant	6		6
		Cucumovirus	Isometric	3+segments	9	Plant	3	1	4
		Ilarvirus	Isometric	3+segments	8	Plant	20		20
ssRNA	Nonenveloped; tripartite genome; rod-shaped particles	*Hordeivirus*	Rod	3+segments	10	Plant	4		4
ssRNA	Nonenveloped; tetrapartite genome	*Tenuivirus*	Rod	4–? segments	19	Plant	3	4	7
Total no. species							1970	530	2500

[a] The taxa are listed according the Fifth ICTV Report with the following criteria: nature and strandedness of the nucleic acid, presence or absence of a lipoprotein envelope, the single-stranded (ss)RNA enveloped viruses are arranged on the basis of genome strategy and the ssRNA nonenveloped viruses are arranged on the basis of the number of segments of their genome and their particle morphology. For each family or group of viruses, also indicated are the morphology of the virions, the genome configuration, the genome size in kb, the virus host, the number of species and tentative members in the taxa, and the total number of species listed in 1990.

7.2.1 System for Virus Classification

There are two systems for classifying organisms: the Linnean and the Adansonian systems. The Linnean system is the monothetic hierarchical classification applied by Linnaeus to plants and animals while the Adansonian system is a polythetic hierarchical system proposed by Adanson in 1763. Since a taxonomic structure above the level of family or group (with the exception of the order **Mononegavirales** and the pending order **Caudovirales)** has not been developed extensively, any listing must be arbitrary. The order of presentation is generally the same as in the Fifth ICTV Report. The order of presentation of virus families and groups follows three criteria: (1) the nature of the viral nucleic acid, (2) the strandedness of the nucleic acid and (3) the presence or absence of a lipoprotein envelope. There are no known single-stranded (ss)DNA viruses with envelopes, so these three criteria give rise to seven clusters comprising the 73 families and groups of viruses (comprising one floating genus). Within two of these clusters, the ssRNA enveloped and nonenveloped viruses, the families have been arranged as follows: the ssRNA enveloped viruses are arranged on the basis of genome strategy, i.e. DNA/no DNA step in the replication cycle, positive or negative sense genome and monopartite/multipartite genome. The ssRNA non-enveloped viruses are arranged on the basis of number of segments of RNA of their genome, i.e. mono-/bi-/tri-/tetrapartite genome and their virion morphology: isometric/bacilliform/rod-shaped particles.

7.2.2 Nomenclature of Virus Taxa

However, none of these type species has received an official name and only English vernacular names are indicated. Use of Latinized binomial names for virus names was supported by animal and human virologists of ICTV for many years, but has never been implemented. This suggestion was in fact withdrawn from ICTV nomenclature rules in 1990 and consequently such names as ***Herpesvirus varicella*** or ***Polyomavirus hominis*** should not be used. For several years, plant virologists have set up a different nomenclature, using the vernacular name of a virus but replacing the word ‘virus’ by the group (genus) name; for example, cucumber mosaic cucumovirus and tobacco mosaic to bamovirus. Though this usage is favored by many scientists and examples of such practice can be found for human, animal and insect viruses (e.g. human rhinovirus, canine calicivirus, ***Acheta*** densovirus), it has not been adopted by the ICTV.

The ICTV has a set of rules for virus nomenclature and orthography of taxonomic names. The international genus names universally end in ‘-virus’, the international subfamily names end in ‘-virinae’, the international family names end in ‘-viridae’ and the international order names end in ‘-virales’. In formal taxonomic usage, the virus order, family, subfamily and genus names are printed in italics (or underlined) and the first letter is capitalized. Species names, which are used in English vernacular form, are not capitalized or italicized (or underlined). In formal usage, the name of the taxon precedes the name of the taxonomic unit; for example, ‘the family

Picornaviridae' or 'the genus ***Rhinovirus'.*** In informal vernacular usage, virus order, family, subfamily, genus and species names are written in lower case Roman script; they are not capitalized or italicized (or underlined). Additionally, in informal usage, the name of the taxon should not include the formal suf**fix,** and it should follow the term for the taxonomic unit; for example, 'the mononegavirales order', 'the adenovirus family', 'the avihepadnavirus genus' or'the tobamovirus group'. To avoid ambiguous identifications, it has been recommended to journal editors to follow ICTV guidelines for proper virus identification and nomenclature, and to cite viruses with their full taxonomic terminology when they are first cited in an article, as in the following examples. Order ***Mononegavirales,*** Family ***Paramyxoviridae,*** Subfamily ***Paramyxovirinae,*** genus ***Paramyxovirus,*** avian paramyxovirus 1. Order ***Mononegavirales,*** Family ***Rhabdoviridae,*** Plant rhabdovirus group, Plant rhabdovirus subgroup **A,** lettuce necrotic yellows virus. Family ***Iridoviridae,*** genus ***Iridovim, Chilo*** iridescent virus. Family ***Podoviridae,*** genus T7 phage group, coliphage T7.

Universal Classification System

The present universal system of virus taxonomy is set arbitrarily at hierarchical levels of order, family (in some cases subfamily), genus and species. Lower hierarchical levels, such as subspecies, strain, variant, pathotype and isolate, are established by international specialty groups or/and by culture collections, but not by the ICTV.

Virus species

The species taxon is always regarded as the most important taxonomic level in classification but it has proved to be the most difficult to apply for viruses. The ICTV definition of a virus species was long considered to be 'a concept that will normally be represented by a cluster of strains from a variety of sources, or a population of strains from a particular source, which have in common a set or pattern of correlating stable properties that separates the cluster from other clusters of strains'. This was a general definition which was in fact not very precise for delineating species in a particular family or in all families. Furthermore, this definition directly addressed the definition of a virus strain, which had never been attempted in the history of virus taxonomy.

Members of a polythetic class are defined by more than one property and no single property is absolutely essential and necessary. Thus in each family it might be possible to determine the set of properties of the taxonomic level 'species' and to check if the family members are species of this family or if they belong to a lower taxonomic level. The ICTV is currently conducting this exercise throughout all virus families. This should ultimately result in an excellent evaluation of a precise definition of each virus species in the entire classification. Several practical matters are related to the definition of a virus species with the goal of a better usage of a virus classification. These include: (1) homogeneity of the different taxa; (2) diagnostic related matters; (3) virus collections; (4) evolution studies; (5) biotechnology; (6) sequence database projects;
and (7) virus database projects.

7.2.3 Virus families, genera and groups

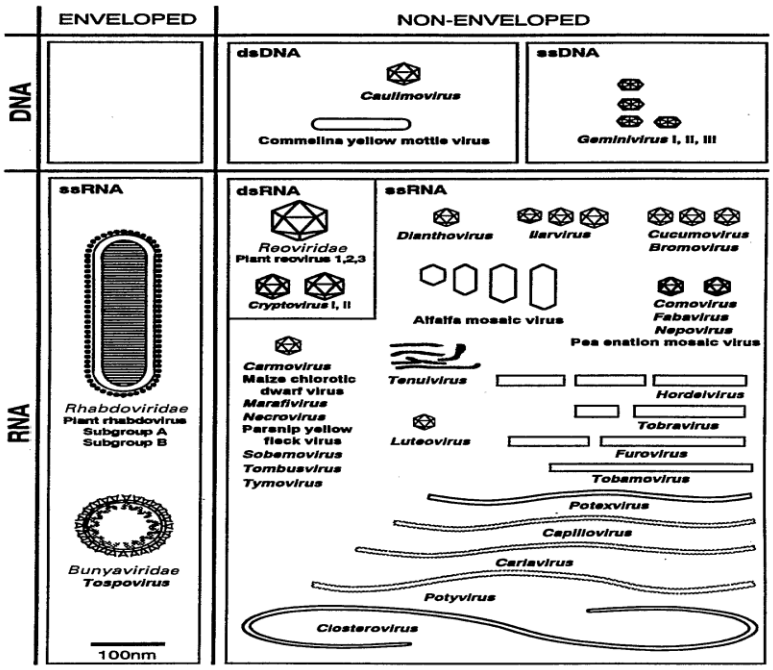
There is no formal definition for a genus, but it is commonly considered as: 'a population of virus species that share common characteristics and are different from other populations of species'. Although this definition is somewhat elusive, this level of classification seems stable and useful; some genera have been moved from one family to another but the composition and description of these genera have remained stable over the years. The characteristics defining a genus are different from one family to another and there is a tendency to create genera with fewer differences between them. Upon examination, there is more and more evidence that the members of a genus have a common evolutionary origin. The use of subgenera is very limited in current virus classification (see Table 1); only one subgenus classification exists in the entire family *Bamloviridae* and there are three other examples in plant virus groups. However, these may disappear when plant virus groups are reorganized into families and genera (see below). Since the creation of the ICTV, plant virologists have always kept the classification of plant viruses in 'groups' and strongly refused to place them in genera and families.

However, due to obvious similitude, plant reoviruses and rhabdoviruses have been integrated into the families Reoviridae and Rhabdoviridae (Table 1). This position was mostly due to the refusal of plant virologists to accept binomial nomenclature. Since this form of nomenclature has been withdrawn from the ICTV rules, they have subsequently accepted classification of plant viruses into genera and families. The current classification still presents plant viruses in groups but the next report will only have families and genera for all 'virus kingdoms'. Five plant virus families and 39 genera have been proposed for the next ICTV Report. Nevertheless, it has been stated several times that the creation of orders could be considered on a case-by-case basis. The first virus order Mononegavirales was established in 1990. This order comprises the non-segmented ssRNA negative-sense viruses, namely the families Filoviridae, Paramyxoviridae and Rhabdoviridae. This decision has been taken because of the great similitude between these families at many points of view including the replication strategy of these viruses. A second order is under consideration; it is named Caudovirales, and it includes all the families of dsDNA phages having a tail, including Myoviridae, Podoviridae and Siphoviridae. Many members of the ICTV advocate the creation of many more orders, but it has been decided to proceed cautiously to avoid creation of short-life orders. The creation of formal taxa higher than orders, for example, kingdoms, classes and subclasses, has not been considered by ICTV.

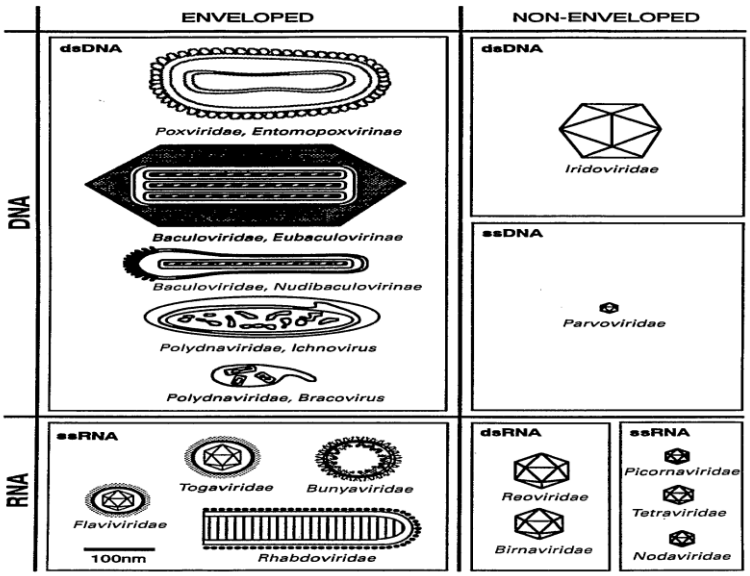
Virus Taxa Descriptions

Virus classification continues to evolve with the technologies available for describing viruses. The first wave of descriptions, before 1940, mostly took into account the visual symptoms of the diseases caused by viruses and their modes of transmission. A second wave, between 1940 and 1970, brought an enormous amount of information from studies of virion morphology (electron microscopy, structural data), biology (serology and virus properties) and physicochemical properties of viruses (nature and size of genome, number and size of viral proteins). Since 1970, the third wave of virus descriptions has included genome and replicative information (sequence of genes, sequence of proteins), as well as molecular relationships with virus hosts. There has been a correlative modification of the list of virus descriptors and Table 3 lists the family and genera Report. The impact of descriptions on virus classification has been particularly influenced

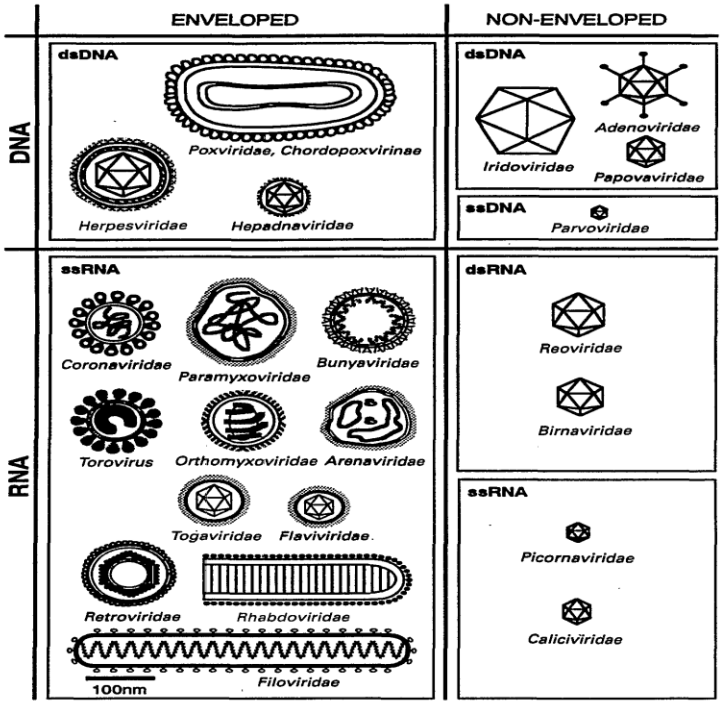
by electron microscopy and the negative staining technique for virions. This technique had an immediate effect on diagnostics and classification of viruses. These techniques in conjunction with the determination of the nature of the genome provided a major source of information for the system of virus classification established in the 1980s.



Diagrammatic representation of the families of viruses infecting plants, grouped according to the nature and strandedness of their genome and the presence or absence of an envelope.



Diagrammatic representation of the families of viruses infecting plants, grouped according to the nature and strandedness of their genome and the presence or absence of an envelope



Diagrammatic representation of the families of viruses infecting plants, grouped according to the nature and strandedness of their genome and the presence or absence of an envelope

7.3 SUMMARY

Virus classification continues to evolve with the technologies available for describing viruses. The first wave of descriptions, before 1940, mostly took into account the visual symptoms of the diseases caused by viruses and their modes of transmission. A second wave, between 1940 and 1970, brought an enormous amount of information from studies of virion morphology (electron microscopy, structural data), biology (serology and virus properties) and physicochemical properties of viruses (nature and size of genome, number and size of viral proteins).

7.4 TECHNICAL TERMS

International Committee on Nomenclature of Viruses (ICNV), Linnean system, Adansonian system, Filoviridae, Paramyxoviridae and Rhabdoviridae, Sequence database projects, Virus database projects

7.5 SELF ASSESSMENT QUESTIONS

Q.1 Write an account on Taxonomy of plant viruses.
Q.2 Explain about System for virus classification.
Q.3 Explain in detail about Nomenclature of virus taxa.
Q.4 Discuss about virus families, genera and groups.

7.6 SUGGESTED READINGS

1. Microbial Genetics - Maloy S.R, Cronan JR, JE. Freifelder, D;. Jones and Barlette publishers. 1994.
2. Molecular Cell Biology - Lodish H, Baltimore O, Berk A, Zipursky SL, MAtsudaira P, Darnell, J.; Scientific American Books. 1995.
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Prof. A. Amruthavalli

LESSON - 8
SYMPTOMS CAUSED BY PLANT VIRUSES

Objective of the lesson

The students will have a concept and general information on the different symptoms caused by plant viruses.

Structure of the lesson

8.1 INTRODUCTION

Plant viruses cause a variety of symptoms, often involving changes in leaf color, shape, and overall plant health. The first plant virus, the Tobacco mosaic virus (TMV), was discovered in 1886 by Adolf Mayer. Plant viruses are viruses that affect plants. Like all other viruses, plant viruses are obligate intracellular parasites that do not have the molecular machinery to replicate without a host. Plant viruses can cause major crop losses and greatly reduce quality and storage or products (vegetable/ornamentals and grains).

Viruses can remain dormant and express when plants are unhealthy or stressed. They can join with other pathogens and plant viruses to form disease complexes that can decimate crops. They mutate very fast and new strains emerge all the time. By identifying the viruses infecting your plants you can look closely at the life cycle / the method of transmission/vector/host plants and level of damage that they can cause. Then you can make an informed, fact driven, scientifically based strategy to control it. Propagation is a common form of virus transmission – it is important to screen plants, cuttings, seeds, starter material for viruses to ensure that the material is clean and virus free at the onset of any project, especially projects in new areas.

8.2 GENERAL CONCEPTS AND CLASSIFICATION OF PLANT DISEASES

- **Disease:** According to Horsfall and Diamond (1959), disease may be defined as a malfunctioning process that is caused by continuous irritation by a pathogen and/or environmental factor resulting in some suffering producing symptoms.
- **Disorder:** The diseases caused by the deficiency of nutrients or unfavourable environmental are sometimes termed as disorders or physiological disorders.

- **Pathogen:** It is the agent responsible for inciting pathos 'i.e. ailment or damage.
- **Parasite:** These are the organisms which derive the food materials needed for their growth from other living organism (the host). All the pathogens are parasites but all the parasites are not pathogens. As some of the parasites live on their hosts without causing any damage to them as symbiotic relationships, e.g., Rhizobium bacterium in legume roots, mycorrhizae and lichens.
- **Biotrophs** are the organisms which regardless of the ease with which they can be cultivated on artificial media obtain their food from living tissues only in nature in which they complete their life cycle). They were earlier also called **obligate parasites**, e.g., rusts, smuts, powdery mildews etc.
- **Saprophytes/saprobes** are the organisms which derive their nutrition from the dead organic matter. Some parasites and saprophytes may have the faculty or (ability) to change their mode of nutrition.
- **Facultative saprophytes** are ordinarily parasites which can grow and reproduce on dead organic matter under certain circumstances. They are also called **hemibiotrophs** which attack the living tissues in such a way as biotrophs but continue to grow and reproduce after the tissues is dead.
- A parasite is called **necrotroph** when it kills the host tissue in advance of penetration and then lives saprophytically, e.g. *Sclerotium rolfsii* and *Pythium* species. Similar to **necrotrophs** are **facultative parasites** which live as saprophytes but under favourable conditions they can attack living plants and become parasites. The necrotrophs are also known as **perthotrophs** or **perthophytes**.
- **Pathogenicity** is the ability of a pathogen to cause disease under a given set of environmental conditions. Whereas, **pathogenesis** is the chain of events that leads to development of a disease in the host.
- **Parasitism** is a phenomenon by which a plant parasite becomes intimately associated with the plant; it draws nutrition and multiplies and grows at the expense of the plant host.
- **Virulence** is a measure or degree of pathogenicity of an isolate or race of the pathogen. The term **aggressiveness** is often used to describe the capacity of a pathogen to invade and grow in the host plant and to reproduce on or in it. This term like virulence is used as measure of pathogenicity.
- **Immunity** of a plant against a disease is absolute quality. It denotes the freedom of plant from disease, when the pathogen cannot establish parasitic relationship with the host. High resistance and low susceptibility approach immunity.
- **Disease resistance** is the ability of an organism to overcome completely or in some degree the effect of a pathogen or other damaging factor; whereas susceptibility in the inability of the plant to resist the effect of the pathogen or other damaging factor.
- **Hypersensitivity** is the extreme degree of susceptibility in which there is rapid death of the cells in the vicinity of the invading pathogen. It halts the further progress of the pathogen. Thus, hypersensitivity is a sign of very high resistance approaching immunity.
- **Infection** is the establishment of the parasitic relationship between the pathogen and host following entry or penetration.
- **Incubation period** is the time elapsing between penetration and completion of infection i.e. development of the disease symptoms.
- **Invasion and colonization** is the growth and multiplication of the pathogen through the tissue of the host varying extent.

8.3 SYMPTOMS OF PLANT DISEASES

- **Symptoms**- External expression or the evidence of the abnormalities in the appearance of the diseased plants brought about by the pathogens after host-pathogen interaction.
- **Sign**- When the pathogen itself becomes visible on the host surface in the form of its organs or structures. eg. Sclerotia, mycelium etc.
- **Disease syndrome**- A sum total of variety of symptoms produced by the disease.

8.3.1 Morphological and Physiological Symptoms of Plant Diseases

i. Mildews

Mildews consist of white, grey, brownish or purplish pathogen growth on the host surface. Downy mildew is characterized by a tangled cottony or downy growth mostly on the lower surface of the leaves or other plant parts. Powdery mildew consists enormous number of spores are formed on superficial growth of the fungus giving a dusty or powdery appearance on the host surface. Black minute fruiting bodies may also develop in the powdery mass.

Grapevine downy mildew

Pea powdery mildew

ii. Rust

Rust appears as relatively small pustules of the spores, usually breaking through the host epidermis. Pustule is a small blister-like elevation of the epidermis, often opening to expose spores. The pustules may be dusty or compact, and red, brown, yellow or black in colour.

Pea rust

iii. Smut

Smut means a sooty or charcoal like powder. The affected parts of the plants show black or purplish black dusty areas. Symptoms usually appear on floral organs, particularly the ovulary areas. The pustules on the leaves and stems are usually larger than those of rusts.

iv. White Blister

White blister-like pustules appear on the leaves and other parts of cruciferous plants which break open the epidermis and expose powdery masses of spores. Such symptoms are called white rust, although there is nothing common with them and the rusts.

White blisters on crucifer

v. Blotch

It consists of superficial growth giving the affected plant parts i.e., fruits and leaves smoky (blotched) appearance, e.g. sooty blotch of apple.

Sooty blotch of apple

vi. Sclerotia

A **sclerotium** is a compact, often hard mass of dormant fungus mycelium. **Sclerotia** are mostly dark in colour and are found mixed with the healthy grains as in the case of ergot of wheat and rye.

vii. Exudation

Mass of bacterial cells ooze out on the surface of the affected organs where they may be seen as a drop or smear in several bacterial diseases such as bacterial blight of paddy, gummosis of stone fruits and fire blight of apple and pear. They form crusts after drying.

viii. Mycelial growth

Appearance of white cottony, mycelial growth of the fungi like *Dematophora necatrix* on affected roots of apple is an important diagnostic feature of white root rot in the field.

Sclerotium rolfsii sclerotia

White rot of apple

Mango gummosis

8.3.2 Histological Symptoms

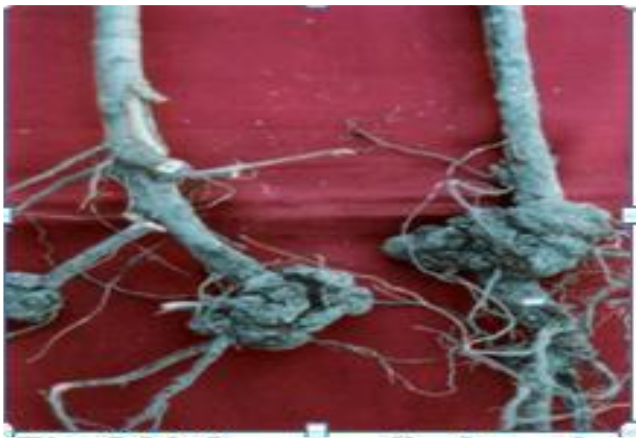
i. Colour change

- **Discolouration** is change of colour from normal. It is one of the most common symptoms of plant diseases. The green pigment of leaves disappears entirely and is replaced by yellow pigments.
- **Etiolation** is yellowing due to the lack of light.
- **Chlorosis** is yellowing due to low temperature, lack of iron, excess of the lime or alkali in soil and infection by viruses, fungi and bacteria.
- **Albilinism** is the phenomenon in which the leaves become devoid of any pigment and look bleached or white.
- **Chromosis** is change of colour to red, purple or orange.

Mosaic symptom on cucurbit leaf

ii. Overgrowths or hypertrophy

Hypertrophy is the abnormal increase in the size of the plant organs due to increase in the size of the cells of a particular tissue, whereas **Hyperplasia** is the abnormal increase in the size of the plant organs due to increase in the number of cells of which the tissue or organ is composed, owing to increased cell division. The overgrowths cause galls, curl, pockets or bladders, hairy root, witches‘ broom, intumescence etc.



Crown gall of peach

iii. Atrophy or Hypoplasia or Dwarfing

Atrophy is inhibition of growth and thereby showing stunting and dwarfing effect on the plants. The whole plant may be dwarfed or only certain organs are affected. e.g. rice dwarf, phony peach etc.

iv. Necrosis

Death of the cells, tissues and organs occurs as a result of parasitic activity. The characteristic appearance of the dead areas differs with different hosts, host organs and with different parasites. Necrotic symptoms include spots, streaks or stripes, canker, blight, damping off, burn, scald or scorch and rot.

Colocasia blight

Brown rot of pear

v. Wilt

Characterized by drying of the entire plant. Leaves and other green or succulent parts loose their turgidity, become flaccid and droop down. Usually seen first in some of the leaves. Later, the young growing tip or the whole plant may dry up. May be caused by injury to the

host system or the conducting vessels. Wilting due to disease is different from the physiological wilting where the plant recovers as soon as the supply of water is retained.

Fusarium wilt of pea

Bacterial wilt of capsicum

vi. Die-back or Wither Tip

Symptoms are characterized by drying of plant organs, especially stems or branches, from the tip backwards. It is also a form of necrosis caused directly by the pathogen or its toxins.

Die-back symptom on mango

8.4 SUMMARY

External expression or the evidence of the abnormalities in the appearance of the diseased plants brought about by the pathogens after host-pathogen interaction. When the pathogen itself becomes visible on the host surface in the form of its organs or structures. eg. sclerotia, mycelium etc. A sum total of variety of symptoms produced by the disease

8.5 TECHNICAL TERMS

Discolouration, Etiolation, Chlorosis, Albilinism Chromosis, Wilt, Necrosis etc.

8.6 SELF ASSESSMENT QUESTIONS

Q.1 Explain the general concepts and classification of plant diseases.
Q.2 Discuss about morphological and physiological Symptoms caused by plant viruses.
Q.3 Explain in detail about histological symptoms caused by plant viruses.

8.7 SUGGESTED READINGS

1. Microbial Genetics - Maloy S.R, Cronan JR, JE. Freifelder, D;. Jones and Barlette publishers. 1994.
2. Molecular Cell Biology - Lodish H, Baltimore O, Berk A, Zipursky SL, MAtsudaira P, Darnell, J.; Scientific American Books. 1995.
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Prof. A. Amruthavalli

LESSON - 9

ULTRA STRUCTURE AND LIFE CYCLE OF TOBACCO MOSAIC VIRUS (TMV) AND CAULIFLOWER MOSAIC VIRUS (CAMV)

Objective of the lesson

Students will gain the knowledge on ultra structure and life cycles of Tobacco mosaic virus (TMV) and Cauliflower mosaic virus (CaMV).

Structure of the lesson

9.1 INTRODUCTION

Adolf Mayer, a German chemist first started studying the diseases in tobacco plants in 1879. By 1886 he was able to describe how leaf mottling disease was able to transfer from infected plants to healthy plants by rubbing infected plant juice over the healthy one, similar to bacterial infection. Dmitri Ivanowsky, a Russian microbiologist, started investigating the tobacco mosaic disease between 1887 and 1890. He came to the conclusion that the causal organism could pass through the porcelain filter that was fine enough to hold the bacterial organisms and thus he believed that it was a non-bacterial infectious agency. Later, Martinus Beijerinck replicated Ivanowsky's work independently and showed that the causative agent was able to replicate and multiply in the tobacco plant host cell. Thus he coined the term 'virus' to show the non-bacterial nature of the tobacco mosaic disease.

9.2 ULTRA STRUCTURE OF TOBACCO MOSAIC VIRUS (TMV)

TMV is a simple rod-shaped helical virus (Fig. 9.1) consisting of centrally located single-stranded RNA (5.6%) enveloped by a protein coat (94.4%). The rod is considered to be 3,000 Å in length and about 180 Å in diameter. The protein coat is technically called 'capsid'. R. Franklin

estimated 2,130 sub-units, namely, capsomeres in a complete helical rod and 49 capsomeres on every three turns of the helix; thus, there would be about 130 turns per rod of TMV. The genetic material is a single-stranded RNA molecule. The diameter of RNA helix is about 80 Å and the RNA molecule lies about 50 Å inward from the outer-most surface of the rod. The central core of the rod is about 40 Å in diameter. Each capsomere is a grape like structure containing about 158 amino acids and having a molecular weight of 17,000 daltons. The RNA is found in a coiled manner inside the capsid coat and is made up of approximately 6395 nucleotides. It has structural chirality and inherent symmetry in the structure which gives the organism an easy way for chemical or genetic modifications.

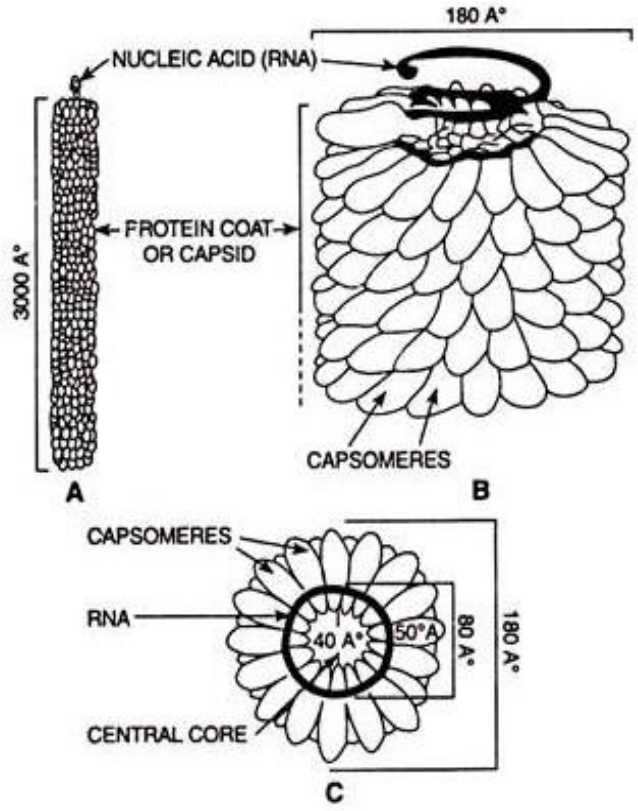


Figure-9.1: Tobacco mosaic virus (TMV) A. Surface view B. An enlarged portion showing RNA-capsomere arrangement C. View in section

9.3 LIFE-CYCLE OF TOBACCO MOSAIC VIRUS (TMV)

Plant viruses like TMV penetrate and enter the host cells in toto and their replication completes within such infected host cells (Fig. 9.2). Inside the host cell, the protein coat dissociates and viral nucleic acid becomes free in the cell cytoplasm. Although the sites for different steps of the viral multiplication and formation of new viruses have not yet been determined with absolute certainty, the studies suggest that after becoming free in the cell cytoplasm the viral-RNA moves into the nucleus (possibly into the nucleolus). The viral-RNA first induces the formation of specific enzymes called 'RNA polymerases' the single-stranded viral-RNA synthesizes an additional RNA strand called replicative RNA.

This RNA strand is complementary to the viral genome and serves as 'template' for producing new RNA single strands which is the copies of the parental viral-RNA. The new viral-RNAs are released from the nucleus into die cytoplasm and serve as messenger-RNAs (mRNAs). Each mRNA, in cooperation with ribosomes and t-RNA of the host cell directs the synthesis of protein subunits. After the desired protein sub-units (capsomeres) have been produced, the new viral nucleic acid is considered to organize the protein subunit around it resulting in the formation of complete virus particle, the virion. No 'lysis' of the host cell, as seen in case of virulent bacteriophages, takes place. The host cells remain alive and viruses move from one cell to the other causing systemic infection. When transmitted by some means the viruses infect other healthy plants.

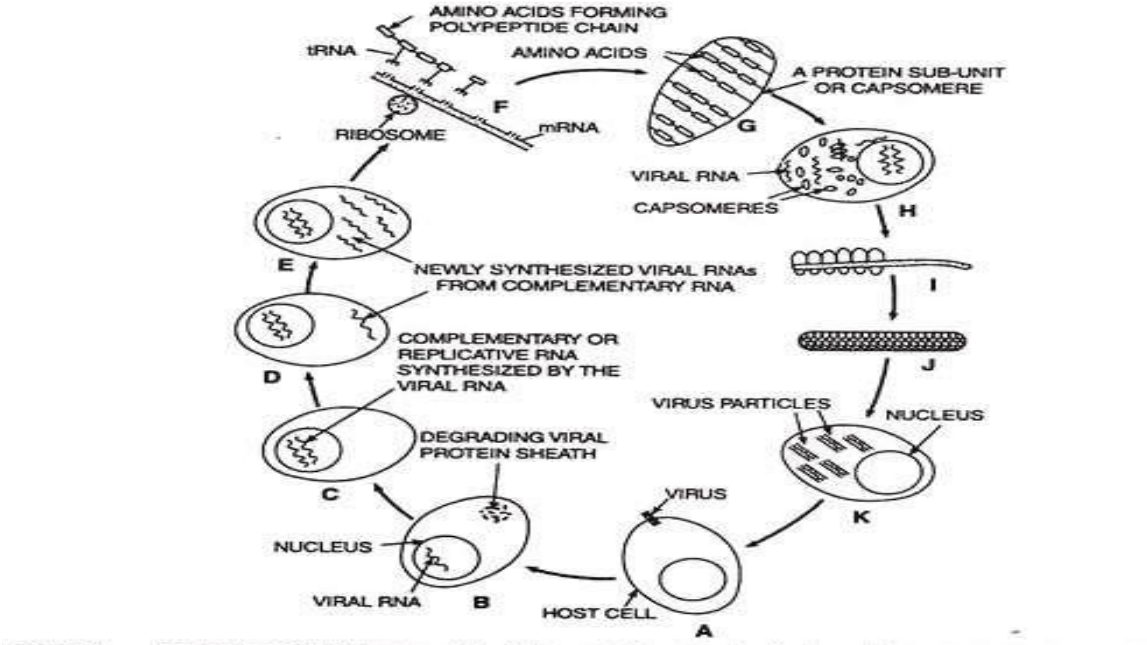


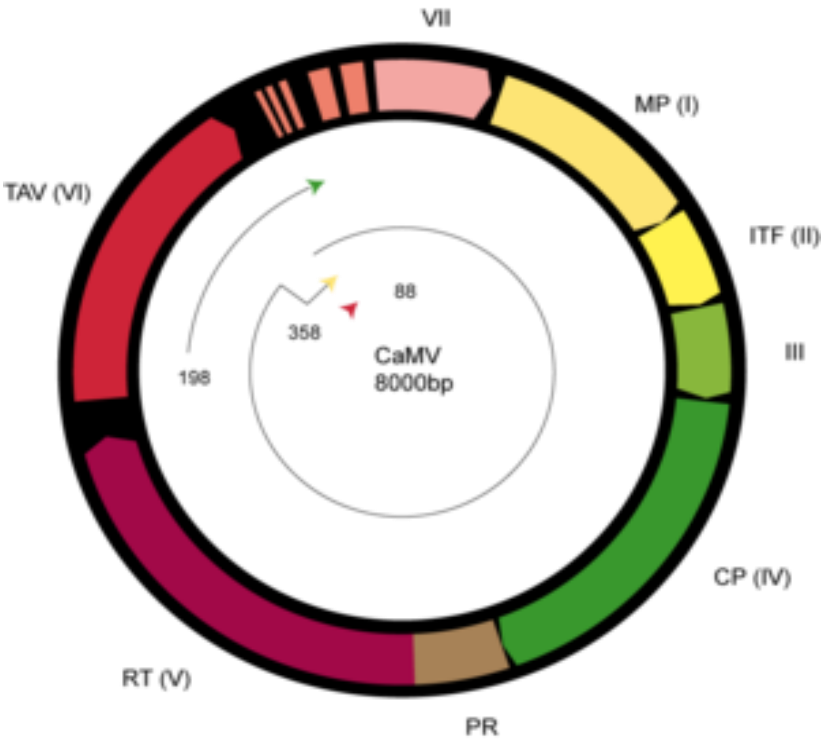
Figure-9.2: Replication of TMV

9.4 ULTRA STRUCTURE OF CAULIFLOWER MOSAIC VIRUS (CAMV)

CaMV belongs to the Caulimo viridae family of circular, ds-DNA viruses. It predominantly infects members of the Brassicaceae family, including radish, turnip, canola, mustard, cauliflower, broccoli, and cabbage. Some CaMV strains (D4 and W260) are also able to infect Solanaceae species, such as devil's trumpets (genus *Datura*) and tobacco plants (genus *Nicotiana*). CaMV is a wide spread virus in temperate regions and can cause significant loss. The CaMV particle is an icosahedron with a diameter of 52 nm built from 420 capsid protein (CP) subunits arranged with a triangulation T = 7, which surrounds a solvent-filled central cavity.

CaMV contains a circular double-stranded DNA molecule of about 8.0 kilo bases, interrupted by nicks that result from the actions of RNase H during reverse transcription. These nicks come from the Met-tRNA, and two RNA primers used in reverse transcription. After entering the host

cell, these single stranded "nicks" in the viral DNA are repaired, forming a supercoiled molecule that binds to histones. This DNA is transcribed into a full length, terminally redundant, 35S RNA and a sub-genomic 19S RNA. CaMV genetic variants have been described in different host species with different symptoms, virulence, and transmission rates, and recent studies have identified a high diversity of CaMV genomic sequences. The CaMV genome consists of approximately 8,000 base-pairs of circular, double- stranded DNA. The genome encodes seven genes (gene I to gene VII), also called P1 to P7 for encoded proteins 1-7. CaMV replicates by reverse transcription, and its genes are transcribed from two promoters, the 19S and 35S promoters.



The promoter of the 35S RNA is a very strong constitutive promoter responsible for the transcription of the whole CaMV genome. It is well known for its use in plant transformation. It causes high levels of gene expression in dicot plants. However, it is less effective in monocots, especially in cereals. The differences in behavior are probably due to differences in quality and/or quantity of regulatory factors. Recent study has indicated that the CaMV 35S promoter is also functional in some animal cells, although the promoter elements used are different from those in plants. While this promoter had low activity compared to canonical animal promoters, levels of reporter products were significant. This observation suggests that the 35S promoter may have potential for use in animals.

The promoter was named CaMV 35S promoter ("35S promoter") because the coefficient of sedimentation of the viral transcript, whose expression is naturally driven by this promoter, is 35S. It is one of the most widely used, general-purpose constitutive promoters. It was discovered at the beginning of the 1980s, by Chua and collaborators at The Rockefeller University. The 35S RNA is particularly complex, containing a highly structured 600 nucleotide long leader sequence with six to eight short open reading frames (ORFs).

Genomic map of CaMV

This leader is followed by seven tightly arranged, longer ORFs that encode all the viral proteins. The mechanism of expression of these proteins is unique, in that the ORF VI protein (encoded by the 19S RNA) controls translation reinitiation of major open reading frames on the polycistronic 35S RNA, a process that normally only happens on bacterial mRNAs. TAV function depends on its association with polysomes and eukaryotic initiation factor eIF3.

- ORF I – P1: movement protein
- ORF II – P2: aphid/insect transmission factor
- ORF III – P3: virion-associated protein (VAP): structural protein, DNA-binding capabilities
- ORF IV – P4: capsid protein (CP)
- ORF V – P5: pro-pol (P0): protease, bifunctional reverse transcriptase and RNaseH
- ORF VI – P6: transactivator/viroplasmin : inclusion body Formation/trafficking; possibly other functions (see text)
- ORF VII/VIII – unknown (appears note to be required for infection,
- Contains a tRNA-Met binding site

9.5 LIFE-CYCLE OF CAULIFLOWER MOSAIC VIRUS (CAMV)

CaMV is transmitted between host plants by more than 27 aphid species in a non-persistent and non-circulative manner, meaning that after an aphid acquires the virus from an infected plant, the virus does not circulate or replicate within the insect. The virus is retained for a short period (a few hours) in the aphid stylets (mouth parts), where CaMV receptor candidates have recently been identified (Protein P2 required for aphid transmission). The virus can then be released to initiate a new infection during aphid feeding on healthy plants. There are no known cases of CaMV transmission via seeds. The infection cycle starts with virus particles (VPs) being delivered into the cytoplasm of a plant cell after it has been punctured by the stylets of anaphid vector. VPs dock at the nuclear envelope and disassemble to allow the naked viral DNA to enter the nucleus, where viral DNA associates with host histones, forming a mini chromosome. There, the viral genome is transcribed to produce two mRNAs, the 19S mRNA that encodes protein P6 and the 35SmRNA that encodes the other six proteins.

P6 belongs to the early proteins that are translated in the cytoplasm. Within the cytoplasm, P6 accumulates in foci that will give rise to the virus factories [here is exemplified one (VF)] with P6 forming the matrix protein, where all viral synthesis occurs and most progeny VPs are stored. Viral synthesis in the VFs involves many coordinated events including the P6-mediated translation transactivation required for the translation of all viral proteins from the polycistronic 35S RNA. The translation products include P1 or MP, the movement protein that associates with the plasmodesmata and is required for cell-to-cell and systemic movement of the virus. P2 or ATF, the aphid transmission factor that binds the virus particles to the aphid vector mouth parts during plant-to-plant transmission. P3orVAP, the virus-associated protein, P4 or CP (capsid protein), and P5 or RT, the reverse transcriptase generating progeny DNA genomes from the 35S RNA. P7 function is unknown (appears note to be required for infection).

P6 or TAV (trans activator-viroplasmin) is, besides a trans activator and VF matrix protein, an RNA silencing suppressor that interferes with specific anti-viral defense pathways. While the

pre-genomic 35S RNA is responsible for genome replication by reverse transcriptase, it also contains a non-coding 600 base pair leader sequence that serves as an important mRNA for the production of factors involved in viral counter-defense. A number of hosts of CaMV possess small RNA-based viral silencing mechanisms that serve to limit viral infection. The products of the afore mentioned 600-bp sequence are viral small RNAs (vsRNA) of 21, 22, and 24 nucleotides in length that serve as decoys, binding and inactivating effectors of host silencing machinery. Besides VFs, a second type of viral inclusions, the transmission bodies (TBs), forms during infection. TBs contain P2, P3 and some VPs and are entirely dedicated to aphid transmission. The life cycle of CaMV is given in figure 9.3.

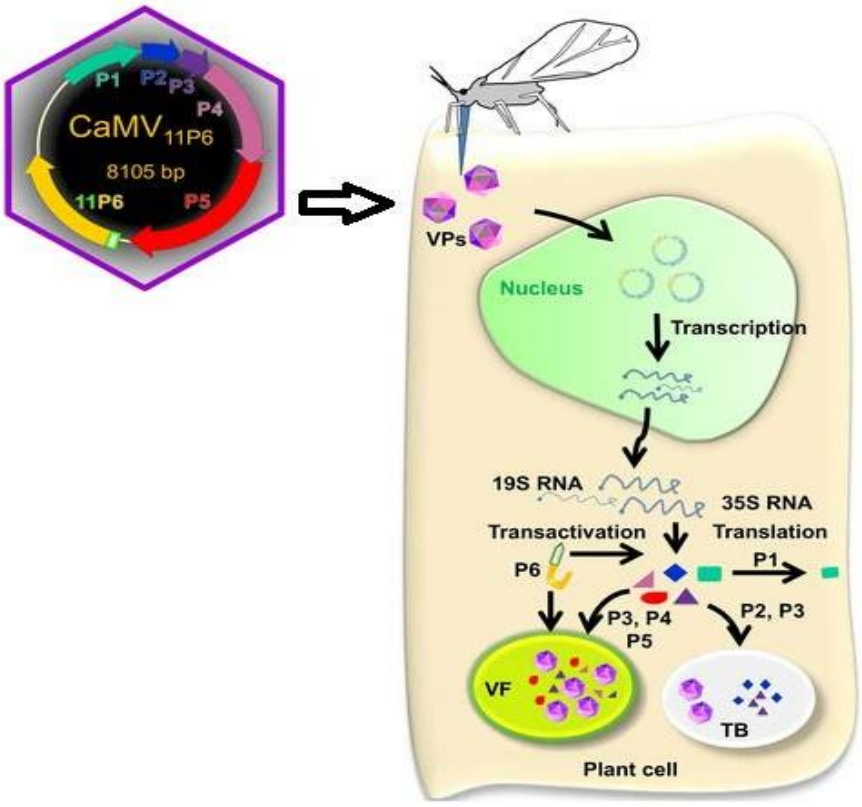


Figure-9.3: Life cycle of CaMV

9.6 SUMMARY

Dmitri Ivanowsky, a Russian microbiologist, started investigating the tobacco mosaic disease between 1887 and 1890. CaMV belongs to the Caulimo viridae family of circular, ds-DNA viruses. It predominantly infects members of the Brassicaceae family, including radish, turnip, canola, mustard, cauliflower, broccoli, and cabbage. Some CaMV strains (D4 and W260) are also able to infect Solanaceae species, such as devil'strumpets (genus *Datura*) and tobacco plants (genus *Nicotiana*). CaMV is a wide spread virus in temperate regions and can cause significant loss.

9.7 TECHNICAL TERMS

Tobacco Mosaic Virus (TMV), Capsomeres, Cauliflower mosaic Virus (CaMV), Trans activator-viroplasmin, Open reading frames (ORFs).

9.8 SELF ASSESSMENT QUESTIONS

Q.1 Explain about the Ultra Structure of Tobacco Mosaic Virus (TMV).
Q.2 Discuss about Life cycle of Tobacco Mosaic Virus (TMV).
Q.3 Explain in detail about the Ultra Structure of Cauliflower mosaic Virus (CaMV).
Q.4 Discuss about Life cycle of Cauliflower mosaic Virus (CaMV).

9.9 SUGGESTED READINGS

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LESSON - 10

TRANSMISSION OF PLANT VIRUSES AND CONTROL MEASURES OF PLANT DISEASES

Objective of the lesson

Students will know the various modes of transmission of plant viruses and different types of symptoms caused by plant viruses.

Structure of the lesson

10.1 INTRODUCTION

Viruses must be brought in contact with the contents of living host cells. They achieve this quite effectively by transmission from an infected plant to a healthy plant in a number of ways. A variety of chemicals are available that have been designed to control plant diseases by inhibiting the growth of or by killing the disease-causing pathogens. Chemicals used to control bacteria (bactericides), fungi (fungicides), and nematodes (nematicides) may be applied to seeds, foliage, flowers, fruit, or soil. They prevent or reduce infections by utilizing various principles of disease control. Eradicants are designed to kill a pathogen that may be present in the soil, on the seeds, or on vegetative propagative organs, such as bulbs, corms, and tubers. Protectants place a chemical barrier between the plant and the pathogen. Therapeutic chemicals are applied to combat an infection in progress. Soil treatments are designed to kill soil-inhabiting nematodes, fungi, and bacteria. This eradication can be accomplished using steam or chemical fumigants. Soil borne nematodes can be killed by applying granular or liquid nematicides. Most soil is treated well before planting; however, certain fungicides can be mixed with the soil at planting time.

10.2 TRANSMISSION OF PLANT VIRUSES

(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 thseed 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

Through vegetative propagation

The viruses are transmitted from the infected plant parts to the healthy ones of the same generation and it results in only primary infection and occurs in monocyclic diseases. 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 mentioned vegetative methods are nearly always infected. 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.

Mechanical transmission through sap

Some viruses can transmit from diseased to healthy plants through the mechanical transmission of the infected sap by touch. Virus infected sap adhering to tools, implements, insect mouth parts, and body, hands, clothes of man etc. can transmit viruses to the wounded plant cells. This type of mechanical transmission is wide spread in Tobacco mosaic virus (TMV), Potato virus X, etc.

Seed transmission

Majority of seed transmitted viruses are carried internally. Virus may be carried to the seed from the infected ovule or the pollen. Examples are muskmelon mosaic virus in watermelon, barley stripe mosaic virus, tobacco ring spot virus in soybean, common bean mosaic virus. 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.

Pollen transmission

Pollen transmission of virus occurs in sour cherry infected with Prunus necrotic ring spot virus.

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.

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

Biological Transmission

Insect transmission

Aphids, leaf hoppers, white flies, mealy bugs and scale insects constitute the insect vectors. However, aphids and leaf hoppers transmit a larger number of viral diseases and are most important. The viruses can be classified as:

a) **Stylet borne viruses** (mostly aphid transmitted): which do not go into the system of insects and remain near the tip of the stylet and are lost after one or two visits. They are also called non-persistent viruses, e.g., Cucumber mosaic virus, Papaya ring spot virus, etc.
b) **Circulative viruses**: Mostly vectored by leaf hoppers, which take them into their system and after circulation, they are returned to the stylet, mixed with the saliva and are transmitted to healthy plants they visit, e.g., Maize streak virus Some viruses of circulative nature may multiply inside the insect body and are called as propagative viruses‘. Leaf hoppers transmitted viruses are mostly circulative and propagative in nature, e.g., Tomato spotted wilt virus
c) **Persistent viruses**: which are acquired by leaf hoppers and are incubated for 1-2 weeks and become viruliferous. Once they start transmitting viruses, they remain infective for long periods or even for rest of their life, e.g., Banana bunchy top virus.
d) **Transovarial transmission**: in which the viruses once acquired are transferred to their following generations.

Examples of other Insects transmitting viruses:

- Green peach aphid transmits potato virus Y (Potyvirus) and potato leaf roll virus (PLRV)

- Leaf hoppers transmit rice stunt virus, aster yellow virus etc.
- White flies can transmit Papaya leaf curl virus, Yellow vein mosaic virus, Tomato leaf curl virus
- Mealy bug transmits Swollen shoot of cocoa
- Thrips transmit Tomato spotted wilt virus
- Beetles transmit Squash mosaic virus, Cowpea mosaic virus and Turnip yellow mosaic virus
- Grass hopper transmits Tobacco mosaic virus (TMV), Potato virus X (PVX) and Tobacco ring spot virus.

EXAMPLES OF INSECTS WHICH TRANSMIT VIRUSES

Mite transmission: Mites transmitting viruses have piercing and sucking mouth-parts. Examples are Wheat streak mosaic virus, Peach mosaic virus and viruses of sterility disease of pigeon pea.

Fungus transmission:
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*.

Three major classes of fungi can transmit viruses.

(a) Chytridiomycetes- Olpidium brassicae transmits Lettuce big vein virus; and *Synchytrium endobioticum* transmits Potato virus X and Potato mop top virus.
(b) Plasmodiophoromycetes- *Polymyxa graminis* transmits Wheat soil borne mosaic virus, *Spongospora subterranea* transmits Potato mop top virus.
(c) Oomycetes- Pythium ultimum transmits Pea false leaf roll virus.

Nematode transmission or 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.

10.3 CONTROL MEASURES OF PLANT DISEASES

A variety of chemicals are available that have been designed to control plant diseases by inhibiting the growth of or by killing the disease-causing pathogens. Chemicals used to control bacteria (bactericides), fungi (fungicides), and nematodes (nematicides) may be applied to seeds, foliage, flowers, fruit, or soil. They prevent or reduce infections by utilizing various principles of disease control. Eradicants are designed to kill a pathogen that may be present in the soil, on the seeds, or on vegetative propagative organs, such as bulbs, corms, and tubers. Protectants place a chemical barrier between the plant and the pathogen. Therapeutic chemicals are applied to combat an infection in progress. Soil treatments are designed to kill soil-inhabiting nematodes, fungi, and bacteria. This eradication can be accomplished using steam or chemical fumigants. Soil borne nematodes can be killed by applying granular or liquid nematicides. Most soil is treated well before planting; however, certain fungicides can be mixed with the soil at planting time.

Seeds, bulbs, corms, and tubers are frequently treated with chemicals to eradicate pathogenic bacteria, fungi, and nematodes and to protect the seeds against organisms in the soil—mainly fungi—that cause decay and damping-off. Seeds are often treated with systemic fungicides, which are absorbed and provide protection for the growing seedling. Protective sprays and dusts applied to the foliage and fruit of crops and ornamentals include a wide range of organic chemicals designed to prevent infection. Protectants are not absorbed by or translocated through the plant; thus they protect only those parts of the plant treated before invasion by the pathogen. A second application is often necessary because the chemical may be removed by wind, rain, or irrigation or may be broken down by sunlight. New, untreated growth also is susceptible to infection. New chemicals are constantly being developed. Most serious diseases of crop plants appear on a few plants in an area year after year, spread rapidly, and are difficult to cure after they have begun to develop. Therefore, almost all control methods are aimed at protecting plants from becoming diseased rather than at curing them after they have become diseased. Few infectious plant diseases can be controlled satisfactorily in the field by therapeutic means.

Depending on the nature of the agents employed, various control methods can be classified as:

1. Biological control methods
2. Chemical methods of control.

1. Biological methods

Biological control of pathogens, i.e., the total or partial destruction of pathogen populations by other organisms, occurs routinely in nature. There are, for example, several diseases in which the pathogen cannot develop in certain areas either because the soil, called **suppressive soil**, contains microorganisms antagonistic to the pathogen or because the plant that is attacked by a pathogen has also been inoculated naturally with antagonistic microorganisms before or after the pathogen attack. Sometimes, the antagonistic microorganisms may consist of avirulent strains of the same pathogen that destroy or inhibit the development of the pathogen, as happens in **hypovirulence** and **cross protection**. Insome cases, even higher plants reduce the amount of inoculum either by trapping available pathogens (trap plants) or by releasing into the soil substances toxic to the pathogen. Agriculturalists have increased their efforts to take advantage of such natural biological antagonisms and to develop strategies by which biological control can be used effectively against several plant diseases. Biological antagonisms, although subject to numerous ecological limitations, are expected to become an important part of the control measures employed against many more diseases.

Suppressive Soils: Several soilborne pathogens, such as *Fusarium oxysporum* (the cause of vascular wilts), *Gaeumannomyces graminis* (the cause of take-all of wheat), *Phytophthora cinnamomi* (the cause of root rots of many fruit and forest trees), *Pythium* spp.(a cause of damping-off), and *Heteroderaavenae* (the oat cyst nematode), develop well and cause severe diseases in some soils, known as **conducive soils**, whereas they develop much less and cause much milder diseases in other soils, known as **suppressive soils**. The mechanisms by which soils are suppressive to different pathogens are not always clear but may involve biotic and / or Abiotic factors and may vary with the pathogen. In most cases, however, it appears that they operate primarily by the presence in such soils of one or several microorganisms antagonistic to the pathogen. Such antagonists, through the antibiotics they produce, through lytic enzymes, through competition for food, or through direct parasitizing of the pathogen, do not allow the pathogen to reach high enough populations to cause severe disease. Numerous kinds of antagonistic microorganisms have been found to increase in suppressive soils; most commonly, however, pathogen and diseases uppression has been shown to be caused by fungi, such as *trichoderma*, *Penicillium*, and *sporidesmium*, or by bacteria of the genera *Pseudomonas*, *Bacillus*, and *streptomyces*.

However, in several diseases, continuous cultivation (monoculture) of the same crop in a conducive soil, after some years of severe disease, eventually leads to reduction in disease through increased populations of microorganisms antagonistic to the pathogen. For example, continuous cultivation of wheat or cucumber leads to reduction of take - all of wheat and of *Rhizoctonia* damping-off of cucumber, respectively. Similarly, continuous cropping of the water melon variety ‘crimson sweet’ allows the buildup of antagonistic species of *Fusarium* related to that causing *Fusarium* wilt of watermelon with the result that *Fusarium* wilt is reduced rather than increased. Such soils are suppressive to future disease development. That suppressiveness is due to antagonistic micro flora canbe shown by pasteurization of the soil at 60°C for 30 minutes, which completely eliminates the suppressiveness.

Reducing amount of pathogen inocula through antagonistic microorganisms

Soil borne Pathogens: Among the most common mycoparasitic fungi are *Trichoderma* sp., mainly *T. Harzianum*. The latter fungus has been shown to parasitize mycelia of *Rhizoctonia*

and *sclerotium*, to inhibit the growth of many oomycetes such as *Pythium*, *Phytophthora*, and other fungi, e.g., *Fusarium* and *Heterobasidion* (*Fomes*), and to reduce the diseases caused by most of these pathogens. Also, some *Pythium* species parasitize species of *Phytophthora* and other species of *Pythium*. Several yeasts, e.g., *Pichia gulliermondii*, also parasitize and inhibit the growth of plant pathogenic fungi such as *Botrytis* and *Penicillium*. In addition to fungi, bacteria of the genera *Bacillus, Enterobacter, Pseudomonas,* and *Pantoea* have been shown to parasitize and/or inhibit the pathogenic oomycetes *Phytophthora* sp., *Pythium* sp, and the fungi *Fusarium*, *Sclerotium ceptivorum*, and *Gaeumanno mycestritici*; the mycophagous nematode *Aphelenchus avenae* parasitizes *Rhizoctonia* and *Fusarium*; and the Amoeba *Vampyrella* parasitizes the pathogenic fungi *Cochliobolus sativus* and *Gaeumannomyces graminis.*

Mechanisms of Action: The mechanisms by which antagonistic microorganisms affect pathogen populations are not always clear, but they are generally attributed to one of four effects: (1) direct parasitism or lysis and death of the pathogen (2) competition with the pathogen for food, (3) direct toxic effects on the pathogen by antibiotic substances released by the antagonist, and (4) indirect toxic effects on the pathogen by volatile substances, such as ethylene, released by the metabolic activities of the antagonist. Many of the antagonistic microorganisms mentioned earlier are naturally present in crop soils and exert a certain degree of biological control over one or many plant pathogens regardless of human activities. New microorganisms added to the soil of a field cannot compete with the existing micro flora and cannot maintain themselves for very long. Also, soil amendments, so far, have not been selective enough to support and build up only the populations of the introduced or existing antagonists. Thus, their potential for eventual disease control is quite limited. There are several cases of successful biological control of plant pathogens when the antagonistic microorganism is used for direct protection of the plant from infection by the pathogen.

Control through Trap Plants: If a few rows of rye, corn, or other tall plants are planted around a field of beans, peppers, or squash, many of the incoming aphids carrying viruses that attack the beans, peppers, and squash will first stop and feed on the peripheral taller rows of rye or corn. Because most of the aphid-borne viruses are non persistent in the aphid, many of the aphids lose the bean, pepper, or squash infecting viruses by the time they move onto these crops. In this way, trap crops reduce the amount of inoculum that reaches a crop. Trap plants are also used against nematodes, although in a different way. Some plants that are not actually susceptible to certain sedentary plant-parasitic nematodes produce exudates that stimulate eggs of these nematodes to hatch. The juveniles enter these plants but are unable to develop into adults and eventually they die. Such plants are also called **trap crops**. By using trap crops in a crop rotation program, growers can reduce the nematode population in the soil. For example, *Crotalaria* plants trap the juveniles of the root-knot nematode *Meloidogyne* sp. And black night shade plants (*Solanum nigrum*) reduce the populations of the golden nematode *Heterodera rostochiensis*. Similar results can be obtained by planting highly susceptible plants, which after infection by the nematodes are destroyed (plowed under) before the nematodes reach maturity and begin to reproduce.

Control through Antagonistic Plants: A few kinds of plants, e.g., *Asparagus* and marigolds, are antagonistic nematodes because they release sub stances in the soil that are Toxic to several plant parasitic nematodes. When inter planted with nematode susceptible crops, antagonistic plants decrease the number of nematodes in the soil and in the roots of the susceptible crops. Antagonistic plants, however, are not used on a large scale for the practical control of nematode diseases of plants for the same reasons that trap plants are not used.

Chemical control measures: Chemical pesticides are generally used to protect plant surfaces from infection or to eradicate a pathogen that has already infected a plant. A few chemical treatments, however, are aimed at eradicating or greatly reducing the inoculum before it comes in contact with the plant. They include soil treatments (such as fumigation), disinfestations of ware houses, sanitation of handling equipment, and control of insect vectors of pathogens.

Soil Treatment with Chemicals: Soil to be planted with vegetables, strawberries, ornamentals, trees, or other high-value crops, such as tobacco, is frequently treated with chemicals for control primarily of nematodes but occasionally also of soil borne fungi, such as *Fusarium* and *Verticillium*, weeds, and bacteria. Certain fungicides are applied to the soil as dusts, liquid drenches, or granules to control damping-off, seedling blights, crown and root rots, and other diseases. In fields where irrigation is possible, the fungicide is sometimes applied with the irrigation water, particularly in sprinkler irrigation. Fungicides used for soil treatments include metalaxyl, diazoben, pentachloronitrobenzene (PCNB), captan, and chloroneb, although the last two are used primarily as seed treatments. Most soil treatments, however, are aimed at controlling nematodes, and the materials used are volatile gases or produce volatile gases (fumigants) that penetrate the soil throughout (fumigate). Some nematicides, however, are not volatile but, instead, dissolve in soil water and are then distributed through the soil.

Fumigation: The most promising method of controlling nematodes and certain other soil borne pathogens and pests in the field has been through the use of chemicals usually called fumigants. Some of them, including chloropicrin, methyl bromide, dazomet, and metamsodium, either volatilize as they are applied to the soil or decompose into gases in the soil. These materials are general purpose pre plant fumigants; they are effective against a wide range of soil microorganisms, including nematodes, many fungi, insects, certain bacteria, and weeds. Contact nematicides, such as fensulfothion, carbofuran, ethoprop, and aldicarb, are of Low volatility, are effective against nematodes and insects, and can be applied before and after planting of many crops that are tolerant to these chemicals.

Nematicides used as soil fumigant sare available as liquids under pressure, liquids, emulsify able concentrates, and granules. These materials are applied to the soil eitherby spreading the chemical evenly over the entire field (broadcast) or by applying it only to the rows to be planted with the crop (row treatment). In both cases the fumigant is applied through delivery tubes attached at the back of tractor-mounted chisel-tooth injection shanks or disks spaced at variable widths and usually reaching six inches below the soil surface.

Highly volatile nematicides are covered immediately with polyethylene sheeting, which should be left in place for at least 48 hours. When small areas are to be fumigated, the most convenient method is through injection of the chemical with a hand applicator under a tarp that has been placed over the area. The edges of the tarp are covered with soil prior to injection of the chemical. Applications may also be made by placement of small amounts of granules in holes or furrows six inches deep, 6 to 12 inches apart, which should be covered immediately with soil. In all cases of pre plant soil fumigation with phyto toxic nematicides, several days to two weeks must elapse from the time of treatment to seeding or planting in the field to avoid plant injury. In the above mentioned types of nematicide application, only a small portion of the soil and its microorganisms immediately come in contact with the chemical. The effectiveness of the fumigants, however, is based on their diffusion in a

gaseous state through the pores of the soil throughout the area in which nematode and other pest control is desired. The distance the vapors move is influenced by the size and continuity of soil pores, by the soil temperature (the best range is between 10 and 20°C), by soil moisture (best at about 80% of field capacity), by the type of soil (more material is required for soils rich in colloidal or organic matter), and by the properties of the chemical itself. Nematicides with low volatility, such as carbofuran, do not diffuse through the soil to any great extent and must be mixed with the soil mechanically or by irrigation water or rainfall. Except for the highly volatile methyl bromide and chloropicrin, most nematicides can be applied in irrigation water when it is provided as trickle soaks or drenches, but only low-volatility nematicides can be applied through over head sprinkler systems. In practice, chemical nematode control in the field is generally obtained by pre plant soil fumigation with one of the nematicides applied only before planting. These chemicals are nonspecific, i.e., they control all types of nematodes, although some nematodes are harder to control than others no matter what the nematicide. Chloropicrin, methyl bromide, dazomet, and metam sodium are expensive, broad-spectrum nematicides that must be covered on application either with tarps(the first two) or with water or through soil (the others). All nematicides are extremely toxic to humans and animals and should be handled with great caution.

Disinfestation of Ware houses: Stored products can be protected from becoming infected by pathogens left over in the warehouse from previous years by first cleaning thoroughly the storage room sand by removing and burning the debris. The walls and floors are washed with bleach, a copper sulfate solution (1pound in 5 gallons of water), or some other sanitizing agent. Warehouses that can be closed airtight and in which the relative humidity can be kept at nearly 100% while the temperature is between 25 and 30°C can be fumigated effectively with chloropicrin (tear gas) used at 1 pound per each 1,000 cubic feet. In all cases the fumigants should be allowed to act for at least 24 hours before the warehouse doors are opened for aeration.

10.4 SUMMARY

The mechanisms by which antagonistic microorganisms affect pathogen populations are not always clear, but they are generally attributed to one of four effects: (1) direct parasitism or lysis and death of the pathogen (2) competition with the pathogen for food, (3) direct toxic effects on the pathogen by antibiotic substances released by the antagonist, and (4) indirect toxic effects on the pathogen by volatile substances, such as ethylene, released by the metabolic activities of the antagonist. Many of the antagonistic microorganisms mentioned earlier are naturally present in crop soils and exert a certain degree of biological control over one or many plant pathogens regardless of human activities. New microorganisms added to the soil of a field cannot compete with the existing micro flora and cannot maintain themselves for very long.

10.5 TECHNICAL TERMS

Vegetative propagation, Mechanical transmission, Seed transmission, Pollen transmission, Transmission by *Cuscuta*, Hypovirulence, Suppressive Soils, Conducive soils, *Trichoderma*, *Penicillium*, *Sporidesmium*, *Pseudomonas*, *Bacillus*, S*treptomyces*, *Rhizoctonia*, *Fusarium.*

10.6 SELF ASSESSMENT QUESTIONS

Q.1 Explain in detail about the mechanical transmission of plant viruses.
Q.2 Discuss about biological transmission of plant viruses.
Q.3 Explain the biological control measures of plant diseases.
Q.4 Describe the chemical control measures of plant diseases.

10.7 SUGGESTED READINGS

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Prof. A. Amruthavalli

Lesson - 11

TAXONOMY OF HUMAN VIRUSES

Objective of the lesson

The students will understand how the of human viruses are taxonomically categorized into hierarchical groups based on their genetic material, structure, replication strategies, and other characteristics based on the current classification system.

Structure of the Lesson

11.1 INTRODUCTION

Viruses are non-cellular, microscopic infectious agents that can only replicate inside a host cell. From a biological perspective, viruses cannot be classified either a living organism or non-living. A virus can be an infectious agent which only replicates within a host organism. This is due to the fact that they possess certain defining characteristic features of living organisms and non-living entities. For instance, a virus cannot replicate itself outside the host cell. This is because viruses lack the required cellular machinery. Therefore, it enters and attaches itself to a specific host cell, injects its genetic material, reproduces by using the host genetic material and finally the host cell splits open, releasing the new viruses.

Viruses mainly depend on a host to deliver the complex metabolic machinery of prokaryotic or eukaryotic cells for propagation. The main task of the virus is to carry its DNA or RNA genome to the host cell, which then can be transcribed by the host cell. The viral genome structure is packed in a capsulated symmetric protein. The protein associated with nucleic acid (also known as nucleoprotein) produces the nucleocapsid with the genome. Virus derived from the Latin word virus meaning poison. Viruses are intermediates to supramolecular complexes and very simple biological entities and exist in two states. When not in contact with suitable host cell, they remain entirely dormant. During this time there are no internal biological activities occurring within the virus. During this simple, clearly non-living state, viruses are referred to as virions. Virions can remain in the dormant state for extended periods of time waiting in patience for a suitable host, when the virions come into contact with an appropriate host; it becomes active and is then referred to as a virus. It now displays the properties characteristic of living organism, such as reacting to environment and directing efforts toward self- replication.

11.2 CLASSIFICATION OF VIRUSES

Viruses can be classified primarily on their phenotypic characteristics, core content, chemical composition, capsid structure, size, shape, modes of replication and other viral genome structures. Using systems like the Baltimore classification and the International Committee on Taxonomy of Viruses (ICTV).
ICTV (International Committee on Taxonomy of Viruses)

- The ICTV is the authority responsible for developing and maintaining a universal system for virus taxonomy.
- They classify viruses based on a hierarchical system: order, family, subfamily, genus, and species.
- The names of orders end with "-virales," families with "-viridae," subfamilies with "-virinae," and genera with "-virus".
- ICTV considers various factors, including genome structure, capsid structure, and vira proteins, to classify viruses.

The Baltimore classification is the most commonly used for studying the system of human and animal virus classification. This system was developed by an American biologist David Baltimore in the 1970s, for which he was awarded the Nobel Prize. In addition to the differences in morphology and genetics mentioned above, the Baltimore classification scheme groups viruses according to how the mRNA is produced during the replicative cycle of the virus.

- **Class I:** Double-stranded DNA (dsDNA) viruses (e.g., Adenoviruses, Herpesviruses, Poxviruses)
- **Class II:** Single-stranded DNA (ssDNA) viruses (e.g., Parvoviruses)
- **Class III:** Double-stranded RNA (dsRNA) viruses (e.g., Reoviruses)
- **Class IV:** Positive-sense single-stranded RNA (+ssRNA) viruses (e.g., Coronaviruses, Picornaviruses, Togaviruses)
- **Class V:** Negative-sense single-stranded RNA (-ssRNA) viruses (e.g., Orthomyxoviruses, Rhabdoviruses)
- **Class VI:** Single-stranded RNA viruses with a DNA intermediate in their life cycle (e.g., Retroviruses)
- **Class VII:** Double-stranded DNA viruses with a ssRNA intermediate (e.g., Hepadnaviruses)

11.3 CLASSIFICATION BASED ON GENETIC MATERIAL

DNA Viruses
The virus has DNA as its genetic material. There are two different types of DNA virus
Single-stranded (ss) DNA virus: e.g. Picornaviruses, Parvovirus, etc.
Double-stranded (ds) DNA virus: e.g. Adenovirus, Herpes virus, etc

- **Adenoviridae:** Adenoviridae belongs to Non-enveloped, icosahedral virions containing linear ds DNA genomes of 25-48kb. Includes viruses causing respiratory infections (e.g., Adenovirus), conjunctivitis, and gastroenteritis.
- **Herpesviridae:** Herpes viruses have a unique 4-layered structure, a core containing the large, ds DNA genome is enclosed by an icosapentahedral capsid which is composed of capsomers. The capsid is surrounded by an amorphous protein coat called the tegument.

Herpes simplex viruses (HSV-1, HSV-2), Varicella-zoster virus (VZV), Epstein-Barr virus (EBV), Cytomegalovirus (CMV), and others causing a range of diseases from cold sores to mononucleosis.

- **Papillomaviridae:** The genetic material of papillomaviruses is double stranded DNA. The genome is circular and ranges from 5,748 to 8,607 base pairs in size. Human papillomaviruses (HPV), are responsible for warts and some cancers like cervical cancer.
- **Poxviridae:** The poxviruses share a conserved genetic structure including linear, double-stranded DNA genomes in which the two strands are joined by palindromic hairpin termini. Poxvirus genomes range widely in size, varying from 135 kb to greater than 300kb. Smallpox virus (Variola virus), now eradicated, and other poxviruses causing diseases like cowpox and molluscum contagiosum.

RNA Viruses

The virus has RNA as its genetic material. There are two different types of RNA virus
Double-stranded (ds) RNA virus: e.g. Reovirus, etc.

Single-stranded (ss) RNA virus. It is further classified into two Positive sense RNA (+RNA) and negative sense RNA (-RNA). Poliovirus, Hepatitis A, Rabies virus and Influenza virus are examples of single-stranded RNA viruses.

- **Picornaviridae:** The genetic material of Picornaviridae is a single-stranded RNA molecule that is positive-sense. This means that the RNA is read from 5' to 3' in the same direction as mammalian mRNA. Enteroviruses (e.g., Poliovirus, Coxsackievirus), Rhinoviruses (common cold), Hepatitis A virus.
- **Flaviviridae:** The genetic material of Flaviviridae is a single-stranded, positive-sense RNA molecule. The genome is linear and ranges in size from 9 to 13 kilobases (kb). Hepatitis C virus (HCV), Dengue virus, Zika virus, Yellow fever virus.
- **Paramyxoviridae:** The genetic material of Paramyxoviridae is a single-stranded, negative-sense RNA genome. The genome is linear and has a size of about 18–20 kb. Measles virus, Mumps virus, Respiratory syncytial virus (RSV).
- **Orthomyxoviridae:** Viruses of the family Orthomyxoviridae contain six to eight segments of linear negative-sense single-stranded RNA. They have a total genome length that is 10,000–14,600 nucleotides (nt). The influenza A genome, for instance, has eight pieces of segmented negative-sense RNA (13.5 kilobases total). Influenza viruses (Influenza A, B, C).
- **Retroviridae:** The genetic material of retroviruses is RNA, but they are able to replicate using DNA. This is because retroviruses carry an enzyme called reverse transcriptase that converts their RNA into DNA. The DNA is then integrated into the DNA of the host cell. Human Immunodeficiency Virus (HIV), Human T-lymphotropic virus (HTLV).
- **Coronaviridae:** The genetic material of a coronavirus is a single-stranded RNA (ribonucleic acid) molecule. This RNA is similar to DNA, and it acts as a blueprint for producing proteins that help the virus replicate. Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV), Middle East Respiratory Syndrome Coronavirus (MERS-CoV), and the more recent SARS-CoV-2 (responsible for COVID-19).

11.4 CLASSIFICATION BASED ON STRUCTURE AND CHARACTERISTICS

Enveloped Viruses

- These viruses have an outer lipid envelope derived from the host cell membrane. Examples include Herpesviruses, Influenza viruses, and Coronaviruses.
- A viral envelope is the outermost layer of many types of viruses. It protects the genetic material in their life cycle when traveling between host cells. Not all viruses have envelopes. A viral envelope protein or E protein is a protein in the envelope, which may be acquired by the capsid from an infected host cell.

Non-enveloped Viruses

- These viruses lack an outer lipid envelope and are composed primarily of a protein capsid. Examples include Adenoviruses, Papillomaviruses, and Picornaviruses.
- Non-enveloped viruses, which lack a lipid envelope, display higher resistance to disinfectants, soaps and sanitizers compared to enveloped viruses. The capsids of these viruses are highly stable and symmetric protein shells that resist inactivation by commonly employed virucidal agents.

11.5 CLINICAL AND EPIDEMIOLOGICAL CLASSIFICATION

Respiratory Viruses

- Viruses causing respiratory infections such as Influenza viruses, Respiratory syncytial virus (RSV), Coronaviruses, and Rhinoviruses.
- A respiratory virus is a virus that infects the respiratory system, including the nose, throat, and lungs. Respiratory viruses are a common cause of illness and can lead to serious infections.

Enteric Viruses

- Viruses primarily affecting the gastrointestinal tract, including Rotaviruses, Noroviruses, and Enteroviruses.
- Enteric viruses are viruses that infect the gastrointestinal tract and are primarily transmitted through the fecal-oral route.

Hepatitis Viruses

- Viruses causing hepatitis (inflammation of the liver), including Hepatitis A, B, C, D, and E viruses.
- Hepatitis A is an acute infection and people usually improve without treatment. Hepatitis B and hepatitis C can cause a chronic, persistent infection, which can lead to chronic liver disease. There is a vaccine to prevent hepatitis A and B, however there is not one for hepatitis C.

Sexually Transmitted Viruses

- Viruses transmitted through sexual contact, such as Herpes simplex viruses (HSV-1 and HSV-2), Human papillomaviruses (HPV), and Human Immunodeficiency Virus (HIV).
- **Human papillomavirus (HPV)**
  A viral STI that can cause cervical cancer. There is a vaccine to prevent HPV.
- **Herpes simplex virus (HSV)**
  A viral STI that can affect the mouth, genital area, and anal area. HSV-1 usually affects the mouth, while HSV-2 usually affects the genital area.

- **Human immunodeficiency virus (HIV)**
  A viral STI that causes AIDS. There is no cure for HIV, but there are treatments that can suppress the virus.

11.6 EVOLUTIONARY AND PHYLOGENETIC CLASSIFICATION

Viruses are also classified based on their evolutionary relationships and phylogenetic analysis, which helps in understanding their origins, spread, and adaptation.

Phylogenetic analysis

- Uses complex rules to separate virus families into study groups.
- Uses phylogenetic clustering to group viruses into high-rank taxa.
- Helps establish families like the Marnaviridae and Dicistroviridae.
- Helps determine if a virus fits into a family based on its genome characteristics.

Virus evolution

- Viruses evolve through genome mutation and selection.
- Viruses with RNA genomes tend to have high mutation rates.
- Viruses may have originated from mobile genetic elements or from previously free-living organisms.
- Viruses may have played a role in the evolution of cellular life.

Challenges in classifying viruses

- It's difficult to create a single viral phylogenetic tree because viruses don't have genes that are common to all species
- Viruses may be polyphyletic in origin

11.7 SUMMARY

Viruses are non-cellular organisms, which is enclosed in a protective envelope. They can be classified primarily on their phenotypic characteristics, core content, chemical composition, capsid structure, size, shape, modes of replication and other viral genome structures. The Baltimore classification is the most commonly used for studying the system of virus classification, divided in to seven groups.

11.8 TECHNICAL TERMS

Naked viruses, Enveloped viruses, non-enveloped viruses, ssDNA, dsDNA, DNA viruses, RNA viruses, ICTV.

11.9 SELF ASSESSMENT QUESTIONS

Q.1 Write about the classification of viruses based on their genetic material.
Q.2 Classify the viruses based on their morphological characteristics.
Q.3 Give a detailed account on viral taxonomical classification.

11.10. SUGGESTED READINGS

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Dr. J. Madhavi

LESSON-12

ULTRA STRUCTURE AND LIFE CYCLES OF RNA VIRUSES – POLIO, INFLUENZA AND HIV

Objective of the lesson

Students will gain knowledge on the ultra structure and life cycles of Polio, Influenza and HIV viruses and the notable human diseases caused by them.

Structure of the lesson

12.1 INTRODUCTION

RNA virus is the virus that has single-stranded as well as double-stranded RNA as its genetic material. Noticeable diseases caused by RNA viruses are polio, influenza and AIDS. Poliovirus is the causative agent of polio disease or poliomyelitis. Poliovirus is serotype of the species Enterovirus C, in the family of Picornaviridae. There are three poliovirus serotypes, numbered 1, 2, and 3. Influenza viruses cause influenza infectious disease also called as flu. Influenza may progress to pneumonia from the virus or a subsequent bacterial infection. There are four types of influenza virus – types A, B, C, and D. Influenza types B and C primarily infect humans, and type D found in cattle and pigs. The human immunodeficiency viruses (HIV) are two species of Lentivirus that infect humans. HIV is a virus that attacks cells which help the body to fight against infection, making a person more vulnerable to other infections and diseases. Over time, they cause acquired immunodeficiency syndrome, a condition in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive.

12.2 ULTRASTRUCTURE AND LIFE CYCLE OF POLIOVIRUS

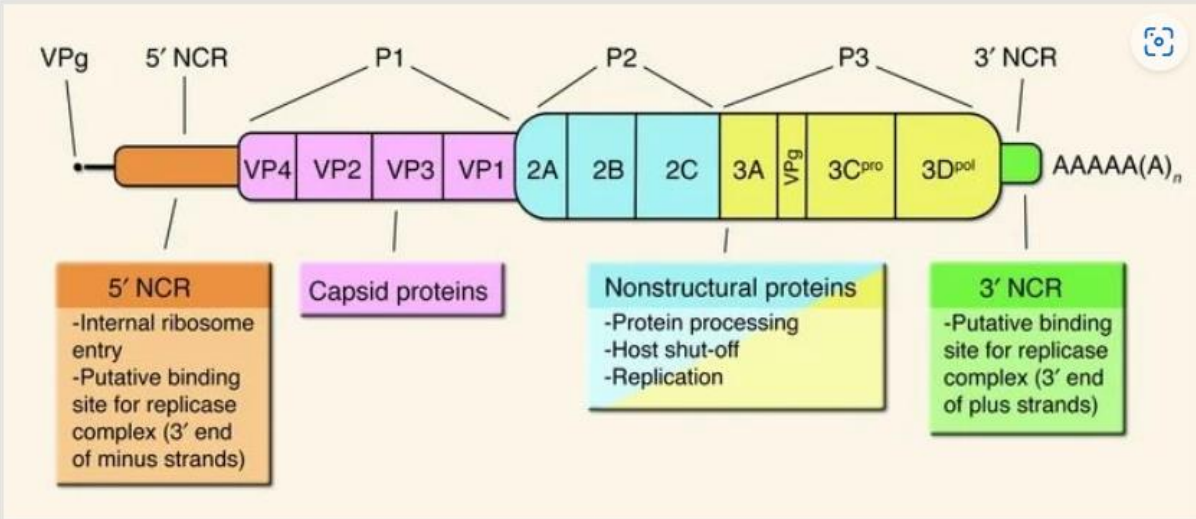
Poliovirus is a member of a family of viruses called the *Picornaviridae*. Virions are spherical in shape with a diameter of about 27nm.The particles are simple in that they are composed of a protein shell surrounding the naked RNA genome. The genome is a monopartite, linear ssRNA (+) genome of 7.2-8.5 kb, polyadenylated, composed of a single ORF encoding a polyprotein. The capsids are composed of four structural proteins: VP1, VP2, VP3, and VP4. The basic building block of the picornavirus capsid is the protomer, which contains one copy each of VP1, VP2, VP3, and VP4. The shell is formed by VP1 to VP3, and VP4 lies on

its inner surface. The virus particles lack a lipid envelope, and their infectivity is insensitive to organic solvents.

Genome of Polio Virus

Polio virus genome can be divided into three parts

- a 5′ noncoding region (NCR) that comprises approximately 10% of the genome, is uncapped and is covalently linked at the 5′ terminus to viral protein VPg.
- a single open reading frame that appears to encode all of the viral proteins, with regions designated as P1 for capsid proteins and P2 and P3 for non-structural proteins.
- a short 3′ NCR terminating in a polyA tail.



Source: https://microbenotes.com/polio-virus/

The genomes vary in length from 7,209 to 8,450 bases. The 5′-noncoding region contains the internal ribosome entry site (IRES), an element that directs the translation of the mRNA by internal ribosome binding. The region P1 contains four segments for structural proteins which make up the capsid protein; 1A-VP4, 1B- VP2, 1C-VP3, 1D-VP1. P2 comprises of three non-structural proteins; 2A, 2B, 2C which play a role in viral replication. P3 makes up four non-structural proteins.

i) 3A- anchors the replication complex to the cell membrane.
ii) 3B- it is VPg protein.
iii) 3C- it is a cysteine protease that cleaves the protein from polypeptides.
iv) 3D- it is RNA-dependent RNA Polymerase.

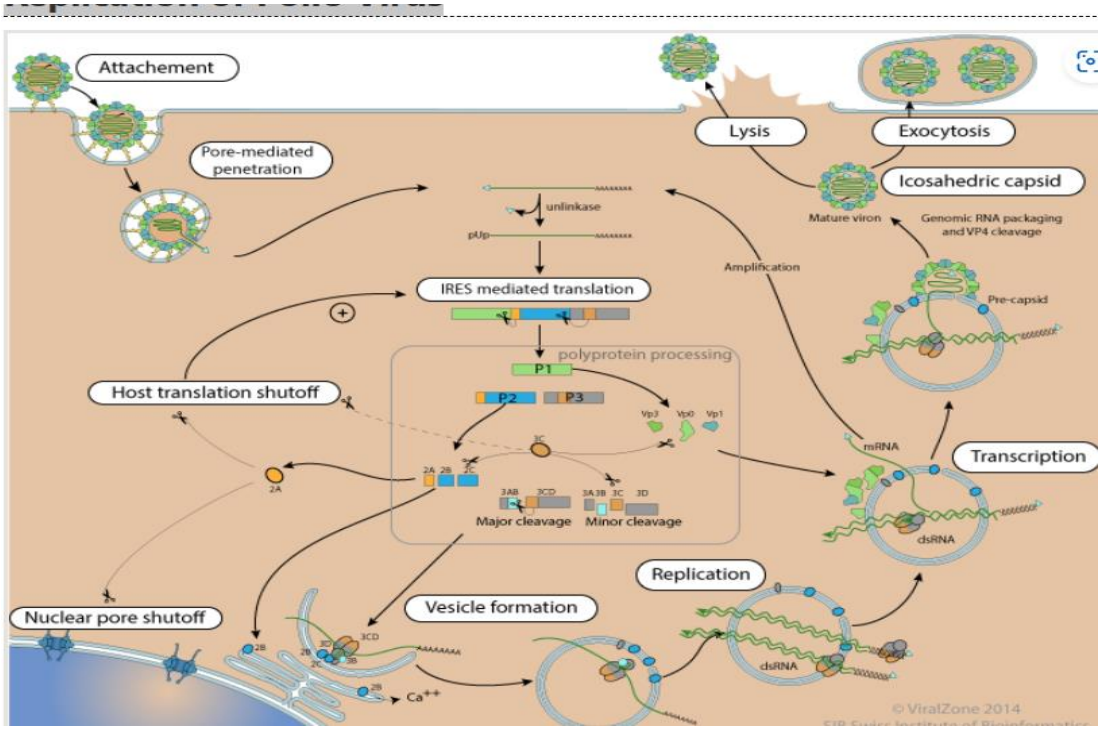
Epidemiology of Polio Virus

Poliomyelitis has had three epidemiologic phases: endemic, epidemic, and the vaccine era. Before global eradication efforts began, poliomyelitis occurred worldwide—year-round in the tropics and during summer and fall in the temperate zones. Winter outbreaks were rare. The disease occurs in all age groups, but children are usually more susceptible than adults because of the acquired immunity of the adult population. In developing areas, where living conditions favour the wide dissemination of virus, poliomyelitis is a disease of infancy and early childhood (“infantile paralysis”). In developed countries, before the advent of vaccination, the age distribution shifted so that most patients were older than age 5 years, and 25% were older than age 15 years. The case fatality rate is variable and is highest in the

oldest patients and may reach from 5% to 10%. Before the beginning of vaccination campaigns in the United States, there were about 21,000 cases of paralytic poliomyelitis per year. Humans are the only known reservoir of infection. In temperate zones with high levels of hygiene, epidemics have been followed by periods of little spread of virus until sufficient numbers of susceptible children have grown up to provide a pool for transmission in the area.

Replication of Polio Virus

The virus binds to a cellular receptor and the genome is uncoated. VPg is removed from the viral RNA, which is then translated. The polyprotein is cleaved nascent to produce individual viral proteins. RNA synthesis occurs on membrane vesicles. Viral (+) strand RNA is copied by the viral RNA polymerase to form full-length (–) strand RNAs, which are then copied to produce additional (+) strand RNAs. Early in infection, newly synthesized (+) strand RNA is translated to produce additional viral proteins. Later in infection, the (+) strands enter the morphogenetic pathway. Newly synthesized virus particles are released from the cell by lysis.



Source: https://microbenotes.com/polio-virus/

Pathogenesis

The mouth is the portal of entry for the virus, transmitted by faecal oral route on ingestion of contaminated water. Virus initially multiplies in the oropharynx and gastrointestinal mucosa. The virus is regularly present in the throat and the stools before the onset of illness. Virions are resistant to the acidity of the stomach and to lytic activities of the protease and other enzymes of the intestinal tract and bile. On entering the body, the virus infects and multiplies in the tonsils and Peyer's patch of ileum. The incubation period is 9-12 days. The virus then spreads to regional lymph nodes and enters the blood causing primary viremia. Antibodies to the virus appear early in the disease, usually before paralysis occurs. The antibodies are produced to prevent infection from spreading. On continued infection and multiplication of virus in the Reticulo-Endothelial System (RES), it invades the blood stream causing

secondary viremia. During this period of viremia, the poliovirus crosses the blood brain barrier and gain access to the brain. The virus shows tissue tropism by specifically combining with neural cells. The virus recognizes the receptor present on the anterior horn of spinal cord, dorsal root ganglia and motor neurons. The destruction of motor neurons leads to paralysis. The virus also infects brain stem causing bulbar poliomyelitis.

Clinical Manifestations of Polio Virus

The earliest features associated with phase of viremia consist of fever, malaise, headache, drowsiness, constipation, and sore throat and lasts for 1 to 5 days. Incubation period is usually 10 days but may vary from 4 days to 4 weeks.

Asymptomatic illness

- It is caused as a result of viral infection confined to the oropharynx and the intestine.

Abortive poliomyelitis

- It is minor illness occurring in approximately 5% of infected people.
- It is febrile illness characterized by fever, headache, sore throat, loss of appetite, vomiting, and abdominal pain.
- Neurological symptoms are typically absent.

Non paralytic poliomyelitis

- Some people who develop symptoms from the poliovirus contract a type of polio that doesn't lead to paralysis (abortive polio).
- This usually causes the same mild, flu-like signs and symptoms typical of other viral illnesses.
- Signs and symptoms, which can last up to 10 days, include: Fever, sore throat, headache, vomiting, fatigue, back pain or stiffness, neck pain or stiffness, pain or stiffness in the arms or legs and muscle weakness or tenderness.

Paralytic poliomyelitis

- Initial signs and symptoms of paralytic polio, such as fever and headache, often mimic those of non-paralytic polio.
- Within a week, however, other signs and symptoms appear, including: Loss of reflexes, severe muscle aches or weakness and loose and floppy limbs (flaccid paralysis)

Post poliomyelitis syndrome

- Post-polio syndrome is a cluster of disabling signs and symptoms that affect some people years after having polio.
- Common signs and symptoms include: Progressive muscle or joint weakness and pain, fatigue, muscle wasting (atrophy), breathing or swallowing problems, sleep-related breathing disorder; such as sleep apnea, and decreased tolerance of cold temperatures.

Bulbar poliomyelitis

- This is caused due to involvement of the cranial nerves, most commonly 9^{th}, 10^{th}, and 12^{th}.
- This condition tends to be more severe with involvement of the muscles of the pharynx, vocal cords and respiration.
- The condition may cause death in 75% of the patient.

Laboratory Diagnosis of Polio Virus
Specimen: stool, rectal swab, throat swab, CSF (rare)

Microscopy
Virus can be detected in stool specimens by direct electron microscopy or also by immune electron microscopy. Although virus is rarely demonstrated in CSF, microscopy of CSF demonstrates predominantly lymphocytic pleocytosis.

Virus isolation
Virus may be recovered from pharyangeal aspirations and feces. Virus isolation from feces and throat swab is carried out by cultivation on monkey kidney, human amnion, HeLa cells, Hep-2, Buffalo green monkey (BGM), MRC-5 and other cell cultures. Cytopathogenic effects appear in 3–6 days. Cytopathic effects include cell retraction, increased refractivity, cytoplasmic granularity, and nuclear picnosis. An isolated virus is identified and typed by neutralization with specific antiserum.

Serodiagnosis
Demonstration of fourfold increase of antibody titre in the serum sample collected at the time of acute illness and time of convalescence. Neutralization test and complement fixation test is carried out to demonstrate antibodies presence.

Molecular diagnosis
Virus can also be identified more rapidly by polymerase chain reaction (PCR) assays.
Treatment of Polio Virus
No antiviral treatments are available for the treatment of poliomyelitis.

Prevention and Control of Polio Virus
- Provision of clean water, improved hygienic practices and sanitation are important for reducing the risk of transmission in endemic countries.
- Immunization is the cornerstone of polio eradication and both live-virus and killed-virus vaccines are available.
- Formalin-inactivated vaccine (Salk) is prepared from virus grown in monkey kidney cultures.
- Killed-virus vaccine induces humoral antibodies but does not induce local intestinal immunity so that virus is still able to multiply in the gut.
- Live attenuated vaccine (Sabin) is grown in primary monkey or human diploid cell cultures and delivered orally.
- The live polio vaccine infects, multiplies, and immunizes the host against virulent strains.
- The vaccine produces not only immunoglobulin M (IgM) and IgG antibodies in the blood but also secretory IgA antibodies in the intestine, enabling mucosal immunity.
- Both killed-virus and live-virus vaccines induce antibodies and protect the CNS from subsequent invasion by wild virus.
- Oral polio vaccine has been the vaccine used predominantly in the past in global campaigns and is still used in endemic areas.
- It has the advantages of inducing both humoral and intestinal immunity and of being cheap and easy to administer.

- However, the gut develops a far greater degree of resistance after administration of live-virus vaccine indicating it as a potential limiting factor of interference for oral vaccine.
- The disadvantage is the small risk of vaccine associated paralytic poliomyelitis (VAPP), which occurs in about 4 out of every 1,000,000 vaccinated children and unvaccinated contacts.
- Inactivated poliovirus vaccine is injected intramuscularly and does not carry any risk of VAPP.
- The disadvantage of inactivated vaccine is that it does not confer intestinal immunity and is not effective for outbreak control and is more expensive and requires better trained staff for deliverance.
- European countries have gradually shifted from OPV to IPV over the last decades and today all EU Member States use IPV in their childhood immunization programmes.

12.3 ULTRASTRUCTURE AND LIFE CYCLE OF INFLUENZA VIRUS

Influenza is caused by any of several closely related viruses in the family orthomyxoviridae (a group of RNA viruses). Influenza viruses are categorized as types A, B, C, and D. These major types generally produce similar symptoms but are completely unrelated antigenically, so that infection with one type confers no immunity against the others. The A viruses cause the great influenza epidemics, and the B viruses cause smaller localized outbreaks. The C viruses cause only mild respiratory illness in humans. Influenza D viruses are not known to infect humans and have been observed only in pigs and cattle.

Influenza A viruses are classified into subtypes, and both influenza B and subtypes of influenza A are further divided into strains. Subtypes of influenza A are differentiated mainly by two surface antigens (foreign proteins)—hemagglutinin (H) and neuraminidase (N). Examples of influenza A subtypes include H1N1, H5N1, and H3N2. Influenza B viruses are subdivided into two major lineages, B/Yamagata and B/Victoria. Strains of influenza B and strains of influenza A subtypes are further distinguished by variations in genetic sequence. Influenza is caused by three types of RNA viruses called influenza types A, B and C (considered different genera), which all belong to the family *Orthomyxoviridae*. The disease, colloquially called "flu" in humans, is generally caused by the viruses A and B, which are transmitted by aerosols from infected individuals or via close contact with infected animals.

Subtypes of influenza A and B viruses can be further characterized into strains. There is a plethora of different strains of influenza B viruses and of influenza A subtypes, and new strains of influenza viruses can appear and replace older strains. Such newly emerging viruses contain antigenic variations known as antigenic drift and shift. The clinical outcome of the disease depends on both the influenza virus and ensuing host defence.

Influenza A

Influenza type A viruses are known to infect people, birds, pigs, horses, whales, seals and other animals, but wild birds represent the natural hosts for these viruses. The enveloped influenza A virions contain three membrane proteins (HA, NA, M2), a matrix protein (M1) just below the lipid bilayer, a ribonucleoprotein core (consisting of 8 viral RNA segments and three proteins – PA, PB1, PB2), as well as the NEP protein. Influenza type A viruses can be further divided into subtypes based on two membrane proteins on the surface of the virus. These proteins are called hemagglutinin (HA) and neuraminidase (NA). There are 18

different HA and 11 different NA subtypes (HA1 through HA18 and NA1 through NA11, respectively).

Although many different combinations of HA and NA proteins are possible, only a fraction of influenza A subtypes (*i.e.* H1N1, H1N2 and H3N2) are currently in general circulation among people. Other subtypes are found in other animal species. For example, H7N7 causes illness in horses, and H3N8 has been shown to elicit the disease in dogs and seals. Subtypes of influenza A virus are named in accordance with their HA and NA surface proteins. For example, an "H3N8 virus" designates influenza A subtype that has an HA 3 protein and an NA 8 protein. Likewise, an "H5N1" virus has an HA 5 protein and an NA 1 protein. An internationally accepted naming convention for influenza viruses also exists, accepted by WHO in 1979 and published in February 1980 in the Bulletin of the World Health Organization. This approach uses the components such as the antigenic type (A, B or C), the host of origin, geographical origin, strain number, year of isolation and, for influenza A viruses, the hemagglutinin and neuraminidase antigen description in parentheses.

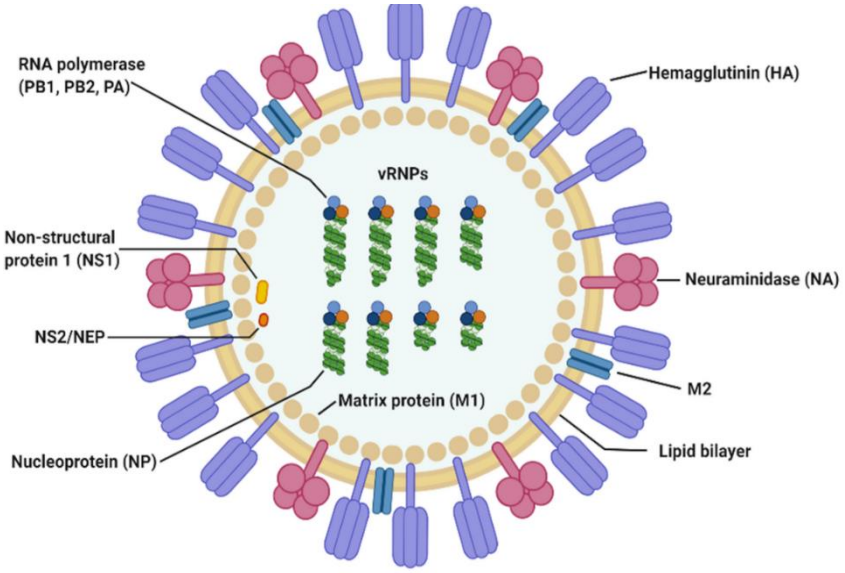
Influenza B

Influenza B viruses are responsible the same spectrum of disease as influenza A; however, influenza B viruses do not cause pandemics. Such a property may be a consequence of the limited host range of the virus (only humans and seals), which limits the occurrence of new strains by reassortment. In addition, they are not divided into subtypes, although can be broken down into lineages and strains. Currently circulating influenza B viruses belong to one of the two lineages: B/Victoria and B/Yamagata.

Influenza B virions contain four envelope proteins: HA, NA, NB, and BM2. The BM2 protein is a proton channel that is essential for the uncoating process (akin to the M2 protein of influenza A virus). The NB protein is thought to be an ion channel, not required for viral replication in cell culture. This virus is responsible for significant morbidity. For example, in the US in 2008, approximately one-third of all laboratory-confirmed cases of influenza were caused by influenza B. That is the reason why the seasonal trivalent influenza vaccine contains an influenza B virus component as its integral component.

Influenza C

Influenza C viruses are different in comparison to influenza A and B. The enveloped virions have hexagonal structures on the surface and form stretched cordlike structures (approximately 500 microns in length) as they bud from the cell. Analogous to the influenza A and B viruses, the core of influenza C viruses is composed of a ribonucleoprotein made up of viral RNA and four proteins. The M1 protein lies just beneath the membrane, similar to influenza A and B virions. A minor viral envelope protein is CM2, which has a function of an ion channel. This virus does not contain separate HA and NA glycoproteins, yet its function is consolidated in one glycoprotein called HEF (hemagglutinin-esterase-fusion). Therefore, the influenza virion contains 7 RNA segments, and not 8. Influenza type C infections cause a mild respiratory illness (comparable to other common respiratory viruses) and are not thought to cause epidemics. According to the seroprevalence studies, nearly all adults have been infected with the influenza C virus. Lower respiratory tract complications are rare, and vaccine against it is not available.



Source: https://www.mdpi.com/1999-4915/12/5/504

Transmission and symptoms

The flu may affect individuals of all ages, though the highest incidence of the disease is among children and young adults. Influenza is generally more frequent during the colder months of the year. Infection is transmitted from person to person through the respiratory tract, by such means as inhalation of infected droplets resulting from coughing and sneezing. As the virus particles gain entrance to the body, they selectively attack and destroy the ciliated epithelial cells that line the upper respiratory tract, bronchial tubes, and trachea. The incubation period of the disease is one to two days, after which the onset of symptoms is abrupt, with sudden and distinct chills, fatigue, and muscle aches. The temperature rises rapidly to 38–40 °C (101–104 °F). A diffuse headache and severe muscular aches throughout the body are experienced, often accompanied by irritation or a sense of rawness in the throat. In three to four days the temperature begins to fall, and the person begins to recover. Symptoms associated with respiratory tract infection, such as coughing and nasal discharge, become more prominent and may be accompanied by lingering feelings of weakness. Death may occur, usually among older people already weakened by other debilitating disorders, and is caused in most of cases by complications such as pneumonia or bronchitis.

Treatment and prevention

The antiviral drugs amantadine and rimantadine have beneficial effects on cases of influenza involving the type A virus. However, viral resistance to these agents has been observed, thereby reducing their effectiveness. A newer category of drugs, the neuraminidase inhibitors, which includes oseltamivir (Tamiflu) and zanamivir (Relenza), was introduced in the late 1990s; these drugs inhibit both the influenza A and B viruses. Other than this, the standard treatment remains bed rest, ingestion of fluids, and the use of analgesics to control fever. It is recommended that children and teenagers with the flu not be given aspirin, as treatment of viral infections with aspirin is associated with Reye syndrome, a very serious illness. Individual protection against the flu may be bolstered by injection of a vaccine containing two or more circulating influenza viruses. These viruses are produced in chick embryos and rendered noninfective; standard commercial preparations ordinarily include the type B influenza virus and several of the A subtypes. Protection from one vaccination seldom lasts more than a year, and yearly vaccination may be recommended, particularly for those

individuals who are unusually susceptible to influenza or whose weak condition could lead to serious complications in case of infection. However, routine immunization in healthy people is also recommended. Advances in scientific understanding of influenza and vaccine technologies enabled the development of a so-called universal influenza vaccine, capable of protecting individuals against a broad range of different influenza subtypes.

The pathogenesis of influenza virus involves several key steps that describe how the virus infects and causes disease in humans:

1. **Virus Entry**: Influenza viruses primarily enter the body through the respiratory route. This can occur when an infected person coughs or sneezes, releasing virus-containing droplets that can be inhaled by others.
2. **Attachment and Entry into Cells**: The influenza virus attaches to cells lining the respiratory tract, specifically targeting epithelial cells that line the nose, throat, and lungs. The viral attachment is mediated by the interaction between viral surface glycoproteins (hemagglutinin) and host cell receptors (sialic acid residues on cell surface glycoproteins).
3. **Replication**: Once attached, the virus enters the host cell by endocytosis. Inside the host cell, the virus releases its genetic material (negative-sense RNA) into the cell's cytoplasm. The viral RNA is then used as a template for transcription and replication by the viral RNA-dependent RNA polymerase. New viral RNA and proteins are synthesized in the host cell.
4. **Assembly and Release**: Viral RNA and proteins are assembled into new virus particles (virions) in the host cell. These virions then bud from the cell surface, acquiring an envelope derived from the host cell membrane that contains viral glycoproteins (including hemagglutinin and neuraminidase). The mature virions are then released and can infect neighboring cells or be expelled from the respiratory tract through coughing and sneezing.
5. **Host Response and Inflammation**: Infection with influenza virus triggers an immune response in the host. This includes the production of cytokines and chemokines by infected cells and immune cells, leading to inflammation in the respiratory tract. This inflammatory response contributes to the symptoms of influenza, such as fever, cough, sore throat, and muscle aches.
6. **Complications**: In some cases, particularly in individuals with weakened immune systems or underlying health conditions, influenza infection can lead to more severe complications such as pneumonia, acute respiratory distress syndrome (ARDS), and exacerbation of pre-existing conditions.
7. **Transmission**: The virus is transmitted from person to person primarily through respiratory droplets produced when an infected person talks, coughs, or sneezes. It can also spread by touching surfaces contaminated with influenza virus and then touching the mouth, nose, or eyes.

12.4 ULTRASTRUCTURE AND LIFE CYCLE OF HIV

HIV is an RNA retrovirus that infects specific white blood cells with the CD4 receptor on their surface (CD4+ cells). CD4 is hijacked by HIV which uses it to gain access to the cell. The family Retroviridae is so named for its possession of a reverse transcriptase (latin, retro = backwards) Of the 7 genera it is recognized that only 2 genera cause disease in humans - HIV -1 and HIV-2. Once inside the cell, the virus's genetic material, RNA, is converted to DNA in a process called reverse transcription. The viral DNA is then inserted into the host's DNA

where it remains for the lifetime of the cell. The host cell synthesizes viral RNA and proteins, new HIV particles are assembled, which escape and infect other CD4+ cells. As the virus leaves the cell it disrupts the cell membrane leading to host cell death. HIV is so destructive because it infects and destroys the white cells that are responsible for regulating other immune cells. This causes the individual to become severely immunocompromised, which leads to acquired immune deficiency syndrome (AIDS).

Transmission

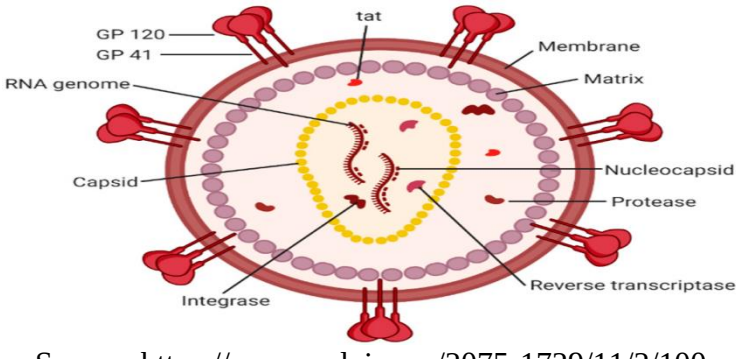
The three main routes for HIV transmission are -

- Contaminated blood (for example between injecting drug users).
- Sex: vaginal, anal (and very rarely oral).
- From mother to child (either in pregnancy, during birth or via breast milk).

Worldwide, approximately 60% of new HIV infections are contracted through sex between men and women. The other cases are usually due to - Babies who acquire the virus from their mothers (10%); Drug users sharing needles (10%); and Sex between men (5–10%).

AIDS: HIV the causative agent of a life threatening disease Acquired immune deficiency syndrome (AIDS). It is documented first in 1981.Amajor proportion of the infection world-wide is caused by the HIV -1 virus, which is identified in 1983. The type – 2 (HIV -2) virus was first detected in West Africa. AIDS occurs when the virus has destroyed the immune system, leaving the patient highly susceptible to other life-threatening infections. It is defined by the occurrence of any of more than 20 opportunistic infections or HIV-related cancers. Tuberculosis (TB) is the most common infection and is the number one cause of death among HIV-infected people in Africa. TB kills nearly a quarter of a million people living with HIV each year. Other opportunistic infections commonly associated with AIDS include candidiasis (or 'thrush'), Kaposi's sarcoma, fungal pneumonia, toxoplasmosis, infection by cytomegalovirus or herpes simplex virus. Further complications arise if patients are co-infected with other viruses such as hepatitis B and C.

Morphology of HIV: HIV is a spherical enveloped virus, about 90-120 nm in diameter. It has a unique three layered structure. The innermost is the genome-nucleocapsid complex. It is enclosed within a cone shaped capsid which in turn is surrounded by a matrix protein followed by a host cell membrane derived lipid bilayer envelope that contain glycoprotein spikes of 72 peplomers. The genome is diploid composed of two identical copies of (+) ssRNA molecules of 9.2 kb each which are associated with reverse transcriptase enzyme. The genome of HIV contain three major genes, viz gag (group specific antigen) gene encodes the core capsid and matrix proteins, the pol gene encodes the reverse transcriptase and the env gene encodes the virion envelope peplomer protein and transmembrane protein and antigens of HIV-1 and -2.

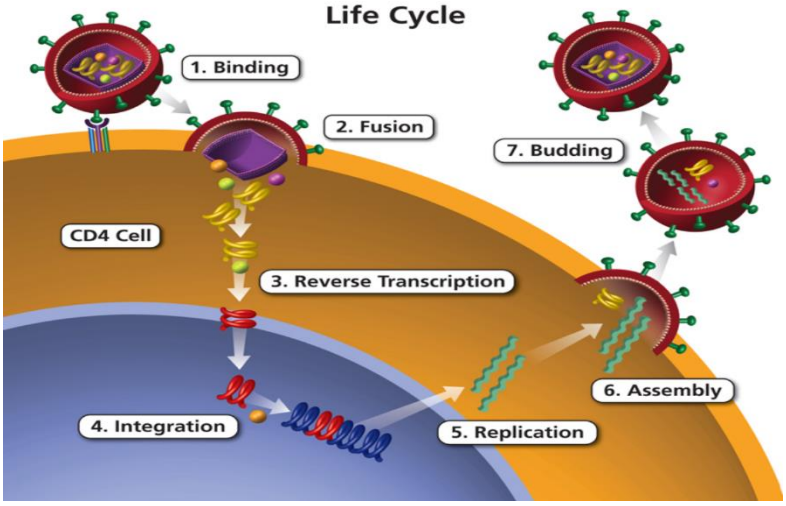


Source: https://www.mdpi.com/2075-1729/11/2/100.

Life cycle of the Human immunodeficiency virus

Like all viruses, human immunodeficiency virus (HIV) reproduces (replicates) using the genetic machinery of the cell it infects, usually a cd4+ lymphocyte.

1. HIV first attaches to and penetrates its target cell.
2. HIV releases RNA, the genetic code of the virus, into the cell. For the virus to replicate, its RNA must be converted to DNA. The RNA is converted by an enzyme called reverse transcriptase (produced by HIV). HIV mutates easily at this point because reverse transcriptase is prone to errors during the conversion of viral RNA to DNA.
3. The viral DNA enters the cell's nucleus.
4. With the help of an enzyme called integrase (also produced by HIV), the viral DNA becomes integrated with the cell's DNA.
5. The DNA of the infected cell now produces viral RNA as well as proteins that are needed to assemble a new HIV.
6. A new virus is assembled from RNA and short pieces of protein.
7. The virus pushes (buds) through the membrane of the cell, wrapping itself in a fragment of the cell membrane and pinching off from the infected cell.
8. To be able to infect other cells, the budded virus must mature. It becomes mature when another HIV enzyme (HIV protease) cuts structural proteins in the virus, causing them to rearrange.
9. When HIV infection destroys cd4+ lymphocytes, it weakens the body's immune system, which protects against many infections and cancers. This weakening is part of the reason that the body is unable to eliminate HIV infection once it has started. However, the immune system is able to mount some response. Within a month or two after infection, the body produces lymphocytes and antibodies that help lower the amount of HIV in the blood and keep the infection under control. For this reason, untreated HIV infection may cause no symptoms or only a few mild symptoms for an average of about 10 years (ranging from 2 to more than 15 years).



Source: https://clinicalinfo.hiv.gov/en/glossary/life-cycle

HIV Tests

Most labs use combination assays that detect the presence of host-generated antibodies against HIV as well as the virus itself. HIV proteins (antigens) are present in the blood before antibodies are produced (up to 3 months after infection), allowing infection to be detected sooner. Combination assays give a more conclusive result sooner after infection than traditional HIV tests which detect antibodies only.

Vaccines

There are many possible types of experimental HIV vaccines, although none have successfully passed a phase three clinical trial.

Treating HIV

There is currently no cure for HIV infection, or a vaccine to prevent it. Treatment consists of a combination of three or more anti-retroviral drugs (ARVs). This combination therapy [also known as Highly Active Anti-Retroviral Therapy (HAART)] slows down the progression of HIV, by inhibiting the 3 enzymes (reverse transcriptase, integrase, and protease) that the virus uses to replicate or to attach to and enter cell and prolonging the patient's life. There are currently 24 ARVs licensed for use. As per 2010 guidelines from the World Health Organization (WHO) promote earlier treatment for all patients, when their CD4+ cell count falls to 350 cells/mm3 or less, regardless of symptoms.

12.5 SUMMARY

An RNA virus is a virus characterized by a ribonucleic acid (RNA) based genome. The genome can be single stranded RNA (ssRNA) or double-stranded (dsRNA). Notable human diseases caused by RNA viruses include the Polio Virus, Influenza Virus & HIV. The viruses are transmitted by different ways. Polio virus transmitted through the mouth by contaminated water and multiplies in the tonsils and causes the disease polio. Influenza virus spread through flu and Influenza is caused by three types of RNA viruses called influenza types A, B and C. AIDS is caused by HIV and it is transmitted by various ways like human contact and blood transfusion. And the three diseases are eradicating through vaccination and medication.

12.6 TECHNICAL TERMS

Polio virus, Influenza virus, HIV, AIDS, ss RNA, CD4 cells, Poliomyelitis.

12.7 SELF ASSESSMENT QUESTIONS

Q.1 Give a detailed account on life cycle of polio virus.
Q.2 Discuss in detail about the types and lifecycle of Influenza.
Q.3 Explain the structure and lifecycle of HIV in causing AIDS in humans.

12.8 SUGGESTED READINGS

1. Introduction to Modern Virology - Dimmock NJ, Primrose SB (1994). IV Edition, Blackwell Scientific publications. Oxford.
2. Medical Virology - Morag, C and Timbury M (1994), Churchill Livingstone.
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4. Functional of plant Virology - Mathews, RE (1992), Academic Press, San Diego.
5. Text book on Principles of Bacteriology, virology and immunology - Topley and Williams (1995), Edward Arnold, London
6. The genetics of bacteria and their viruses - William Hayes (1985), Black well scientific publishers, London.
7. Applied Plant Virology - David GA Walkey (1985). William Heinemann Ltd, London.

Dr. J. Madhavi

LESSON – 13

ULTRA STRUCTURE AND LIFE CYCLES OF DNA VIRUSES – VACCINIA, ADENO AND SV40

Objective of the lesson

Students will understand the ultra structure and life cycle pattern of Vaccinia virus, Adenovirus and SV40 virus which are the examples of DNA viruses.

Structure of the lesson

13.1 INTRODUCTION

A DNA virus is a virus that has a DNA genome and replicates using a DNA polymerase. DNA viruses can be single-stranded DNA (ssDNA) or double-stranded DNA (dsDNA). They are more stable than RNA viruses and are found in many environments, including the marine ecosystem.

13.2 ULTRA STRUCTURE AND LIFE CYCLE OF VACCINIA VIRUS

The vaccinia virus is a large, complex, enveloped virus belonging to the poxvirus family. It has a linear, double-stranded DNA genome approximately 190 kbp in length, which encodes approximately 250 genes. The dimensions of the virion are roughly 360 × 270 × 250 nm, with a mass of approximately 5–10 fg. The vaccinia virus is the source of the modern smallpox vaccine, which the World Health Organization (WHO) used to eradicate smallpox in a global vaccination campaign in 1958–1977. Although smallpox is no longer exists in the wild, vaccinia virus is still studied widely by scientists as a tool for gene therapy and genetic engineering.

Smallpox had been an endemic human disease that had a 30% fatality rate. In 1796, the British doctor Edward Jenner proved that an infection with the relatively mild cowpox virus would also confer immunity to the deadly smallpox. Jenner referred to cowpox as *variolae vaccinae* (smallpox of the cow). However, the origins of the smallpox vaccine became murky over time, especially after Louis Pasteur developed laboratory techniques for creating

vaccines in the 19th century. Allan Watt Downie demonstrated in 1939 that the modern smallpox vaccine was serologically distinct from cowpox, and *vaccinia* was subsequently recognized as a separate viral species. Whole-genome sequencing has revealed that *vaccinia* is most closely related to horsepox, and the cowpox strains found in Great Britain are the *least* closely related to *vaccinia*.

Vaccinia virus is closely related to the virus that causes cowpox; historically the two were often considered to be one and the same. The precise origin of vaccinia virus is unknown due to the lack of record-keeping, as the virus was repeatedly cultivated and passaged in research laboratories for many decades. The most common notion is that vaccinia virus, cowpox virus, and variola virus (the causative agent of smallpox) were all derived from a common ancestral virus. There is also speculation that vaccinia virus was originally isolated from horses, and analysis of DNA from an early (1902) sample of smallpox vaccine showed that it was 99.7% similar to horsepox virus.

Classification of vaccinia infections

In addition to the morbidity of uncomplicated primary vaccination, transfer of infection to other sites by scratching, and post-vaccinial encephalitis, other complications of vaccinia infections may be divided into the following types:

- Generalized vaccinia
- Eczema vaccinatum
- Progressive vaccinia (vaccinia gangrenosum, vaccinia necrosum)
- Roseola vaccinia

Structure of Vaccinia Virus

Virion Structure

Envelope: Vaccinia virus is enveloped, meaning it has a lipid membrane derived from the host cell.

Core: Inside the envelope, the virus contains a complex structure called the core, which houses the viral genome and essential proteins.

Genome: The genome of Vaccinia virus is large (~190 kbp) and consists of double-stranded DNA. It encodes approximately 250 genes. The genome is linear but forms a covalently closed circular molecule inside the virion.

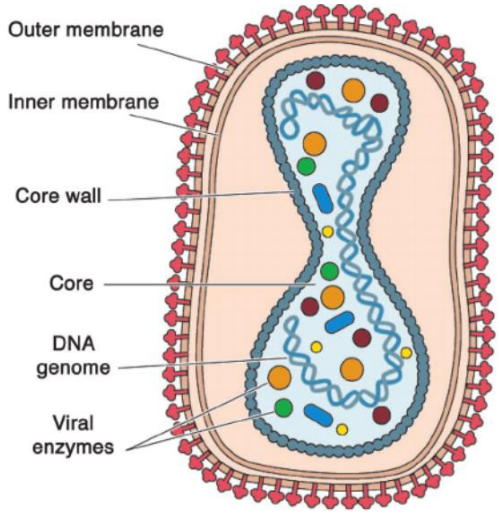


Image source Poxyviridae- Poxyvirus Flashcards - Quizlet

Replication Cycle of Vaccinia Virus

Attachment and Entry - Vaccinia virus attaches to host cells via interactions with cell surface receptors, although the specific receptors can vary depending on the host cell type. Entry into the cell involves fusion of the viral envelope with the host cell membrane, releasing the core into the cytoplasm.

Early and Late Gene Expression - Once inside the cytoplasm, the virus begins transcription and translation of its genes. Early genes are transcribed first, encoding proteins involved in viral DNA replication and modification of host cell functions to favour virus replication. Late genes encode structural proteins and are expressed after viral DNA replication has begun.

DNA Replication - Vaccinia virus replicates its DNA in the cytoplasm of infected cells using viral enzymes and factors. Viral DNA synthesis is independent of the host cell nucleus, which is unusual for DNA viruses.

Assembly and Maturation - Newly synthesized viral DNA and proteins assemble to form mature virions. Viral assembly occurs in cytoplasmic viral factories, specialized structures where replication and assembly take place. Maturation involves packaging of viral DNA into capsids, acquisition of an envelope from the host cell membrane, and incorporation of viral glycoproteins.

Release - Mature virions are released from infected cells by cell lysis or by budding through the host cell membrane. The released virions can then infect neighbouring cells or be transmitted to new hosts.

Immunological Properties

a) Smallpox Vaccine

- Vaccinia virus was used as the basis for the smallpox vaccine, which led to the eradication of smallpox globally.
- The smallpox vaccine consists of live, attenuated Vaccinia virus strains that induce immunity against variola virus (the causative agent of smallpox) without causing smallpox disease itself.

b) Host Range

- Vaccinia virus has a broad host range and can infect various mammalian cells in culture.
- It does not naturally infect humans but can replicate efficiently in human cells, making it a suitable vaccine vector for delivering antigens from other pathogens.

Treatments

Dissemination of vaccinia infection is rare due widespread immunization. Immuno compromised patients may be at risk of developing severe infection. The only current FDA-approved treatment is serotherapy (intravenous infusion of anti-anti-vaccinia immunoglobulin

Used as a vaccine

Vaccinia virus infection is typically very mild and often does not cause symptoms in healthy individuals, although it may cause rash and fever. Immune responses generated from a vaccinia virus infection protect the person against a lethal smallpox infection. For this reason, vaccinia virus was, and still is, being used as a live-virus vaccine against smallpox. Unlike vaccines that use weakened forms of the virus being vaccinated against, the vaccinia virus

vaccine cannot cause a smallpox infection because it does not contain the smallpox virus. However, certain complications and/or vaccine adverse effects occasionally arise. The chance of this happening is significantly increased in people who are immunocompromised. Approximately 1 to 2 people out of every 1 million people vaccinated could die as a result of life-threatening reactions to the vaccination. The rate of myopericarditis with ACAM2000 is 5.7 per 1,000 of primary vaccinees.

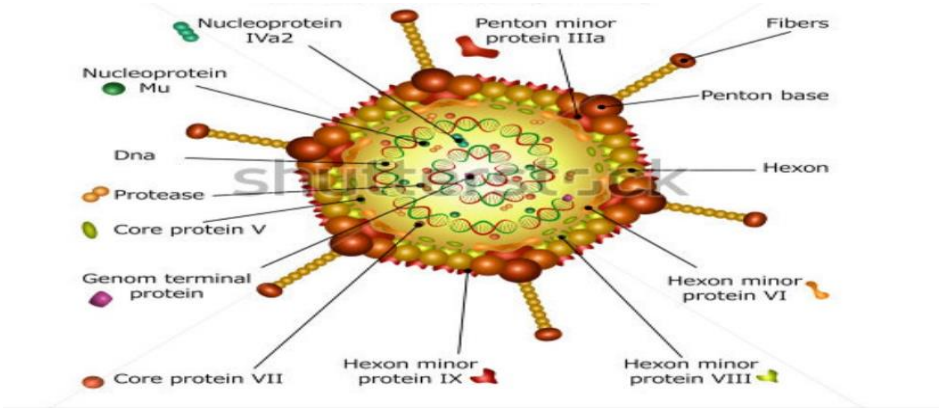
On September 1, 2007, the U.S. Food and Drug Administration (FDA) licensed a new vaccine ACAM2000 against smallpox which can be produced quickly upon need. Manufactured by Sanofi Pasteur, the U.S. Centres for Disease Control and Prevention stockpiled 192.5 million doses of the new vaccine. A smallpox vaccine, Imvanex, which is based on the Modified vaccinia Ankara strain, was approved by the European Medicines Agency (EMA) in 2013. This strain has been used in vaccines during the 2022 monkey pox outbreak. *Vaccinia* is also used in recombinant vaccines, as a vector for expression of foreign genes within a host, in order to generate an immune response. Other poxviruses are also used as live recombinant vaccines

13.3 ULTRASTRUCTURE AND LIFE CYCLE OF ADENOVIRUS

Adenoviruses cause acute respiratory disease (usually), pneumonia (occasionally), acute follicular conjunctivitis, epidemic kerato conjunctivitis, cystitis, and gastroenteritis (occasionally). In infants, pharyngitis and pharyngeal-conjunctival fever are common. The adenoviruses are common pathogens of humans and animals. Moreover, several strains have been the subject of intensive research and are used as tools in mammalian molecular biology. More than 100 serologically distinct types of adenovirus have been identified, including 49 types that infect humans. The family Adenoviridae is divided into two Genera, the mammalian adenoviruses (mastadenoviruses) and the avian adenoviruses (aviadenoviruses). The adenoviruses are named after the human adenoids, from which they were first isolated. Several adenoviruses can cause respiratory and conjunctival diseases. In addition, a few types of human adenoviruses induce undifferentiated sarcomas in newborn hamsters and other rodents and can transform certain rodent and human cell cultures. There is currently no evidence that adenoviruses are oncogenic in humans, but the possibility remains of interest.

Structure

The icosahedral capsid (70 to 100 nm) is made up of 252 capsomeres, 240 hexons forming the faces and 12 pentons at the vertices. Each penton bears a slender fibre. The double-stranded linear DNA is associated with two major core proteins and carries a 55-kDa protein covalently attached to its 5′ end.



Adenoviruses are the group of medium sized, non-enveloped ds DNA virus that share common complement fixing antigen. Virus is Icosahedral in shape with a size range of 70-90 nm in diameter. 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 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 penton unit consists of a penton base anchored in capsid and a projection (fibre or knob) with a knot at distal end. Thus the virion looks like space vehicle. The projection or fibre helps to bind Adenovirus to host cell. The fibre contains viral attachment protein which acts as hemagglutinin. Penton base carries a toxin like activity that causes cytopathic effect (CPE) on host cell.

Classification and Antigenic Types

More than 100 antigenic types of adenoviruses have been identified that infect mammals (mastadenoviruses) and birds (aviadenoviruses); 47 human adenovirus types are classified, 5 more candidate types are presently studied.

Multiplication

Infection may be productive, abortive, or latent. In productive infections, the viral genome is transcribed in the nucleus, mRNA is translated in the cytoplasm, and virions self-assemble in the nucleus. In latent infections and in transformed and tumor cells, viral DNA is integrated into the host genome. Virus-host DNA recombinants are also found in productive infections.

Life Cycle

Step I: Attachment and entry

- Adenovirus attaches to the host cell via its fibre structure to Coxsackie and Adenovirus receptor (CAR) receptor on host cell.
- The attachment of fibre 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 favourable 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 the 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.

Mode of transmission of Adenovirus

Adenovirus infection transmits from person to person directly by -

- Aerosol droplets (respiratory route)
- Faeco-oral route.
- Contaminated fingers to infect conjunctiva.
- Contaminates fomites.

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.

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.
- **Pharyngo conjunctival 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:** It is characterized by fever and watery diarrhoea.

3. Other infections:

- **Pertussis** like symptoms in children
- **Musculoskeletal disorder**
- **Genital infection**
- **Skin infection**

Lab diagnosis

Specimens: Depends upon nature of infections. Throat swab, nasopharyngeal aspirates, conjunctival aspirates, conjunctival swab, urine, stool, blood, body fluids
Microscopy: Electron microscope
Antigen detection: ELISA, DFA test
Serology or antibody detection
Molecular technique: PCR, DNA probe
Virus culture: cell line culture

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.

Treatments

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

13.4 ULTRASTRUCTURE AND LIFE CYCLE OF SV40 (SIMIAN VIRUS 40)

SV40 virus is an abbreviation for simian vacuolating virus 40 or simian virus 40, a polyomavirus that is found in both monkeys and humans. Like other polyomaviruses, SV40 is a DNA virus that is found to cause tumours in humans and animals, but most often persists as a latent infection. SV40 has been widely studied as a model eukaryotic virus, leading to many early discoveries in eukaryotic DNA replication and transcription. Following contamination of polio vaccine batches in the 1950s and 1960s, SV40 came under suspicion as a possible cancer risk, but no subsequent increased cancer rate was observed, making such a risk unlikely. Nevertheless SV40 has become a *cause célèbre* for anti-vaccination activists, who have blamed it for multiple ills, including cancer and HIV/AIDS. Simian virus 40

(SV40) belongs to polyomavirus family, the family of viruses that induce tumours in animals; indeed, the suffix oma means tumour. Simian virus 40 was first isolated from monkeys hence so named. SV40 was one of the first genetic elements to be studied by genetic engineering techniques and has been used extensively as a vector for transferring genes into eukaryotic cells. It contains small DNA virus originally discovered in 1960 as a contaminant in poliovirus vaccines prepared using monkey kidney cells.

Multiplicity reactivation

SV40 is capable of multiplicity reactivation (MR). MR is the process by which two or more virus genomes containing otherwise lethal damage interact within an infected cell to form a viable virus genome. Yamamato and Shimojo observed MR when SV40 virions were irradiated with UV light and allowed to undergo multiple infection of host cells. Hall studied MR when SV 40 virions were exposed to the DNA crosslinking agent 4, 5', 8-trimethylpsoralen. Under conditions in which only a single virus particle entered each host cell, approximately one DNA cross-link was lethal to the virus and could not be repaired. In contrast, when multiple viral genomes infected a host cell, psoralen-induced DNA cross-links were repaired; that is, MR occurred. Hall suggested that the virions with cross-linked DNA were repaired by recombinational repair. Michod et al. reviewed numerous examples of MR in different viruses and suggested that MR is a common form of sexual interaction that provides the advantage of recombinational repair of genome damages.

History

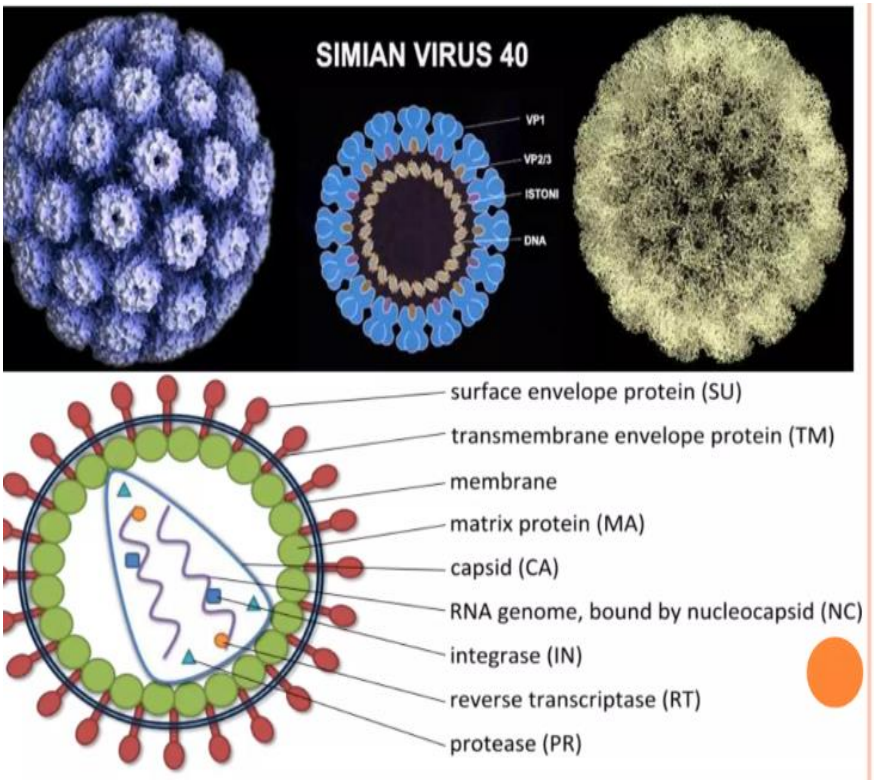
SV40 was first identified by Ben Sweet and Maurice Hilleman in 1960 when they found that between 10 and 30% of polio vaccines in the US were contaminated with SV40. In 1962, Bernice Eddy described the SV40 oncogenic function inducing sarcoma and ependymomas in hamsters inoculated with monkeys cells infected with SV40. The complete viral genome was sequenced by Weissman at Yale University (US) in 1978 and also by Fiers and his team at the University of Ghent (Belgium).

Other animals

SV40 is dormant and is asymptomatic in rhesus monkeys. The virus has been found in many macaque populations in the wild, where it rarely causes disease. However, in monkeys that are immunodeficient—due to, for example, infection with *simian immunodeficiency virus*—SV40 acts much like the human JC and BK polyomaviruses, producing kidney disease and sometimes a demyelinating disease similar to progressive multifocal leukoencephalopathy. In other species, particularly hamsters, SV40 causes a variety of tumours, generally sarcomas. In rats, the oncogenic SV40 large T antigen was used to establish a brain tumour model for primitive neuroectodermal tumour and medulloblastoma. The molecular mechanisms by which the virus reproduces and alters cell function were previously unknown, and research into SV40 vastly increased biologists' understanding of gene expression and the regulation of cell growth.

Structure and Genome of SV40

Structure: SV40 is a small, non-enveloped virus with an icosahedral capsid. The capsid encloses a circular, double-stranded DNA genome of approximately 5.2 kilobases (kb). Its capsid contains 72 protein subunits. SV40 does not have enzymes in it unlike RNA viruses.



Genome: The SV40 genome consists of early and late coding regions, divided by a non-coding control region. Early genes include those encoding regulatory proteins like large T antigen (Tag), small t antigen (tag), and others involved in viral DNA replication and transcriptional regulation. Late genes encode structural proteins (VP1, VP2, VP3) necessary for virion assembly. The genome of SV40 is a single molecule of dsDNA of 5243 base pairs and is very small. ds DNA of this virus is circular and exists in supercoiled configuration having the sedimentation coefficient of 21S. Total G+C content of the nucleic acid is 41%. A linear form of 14S sedimentation coefficient is formed after double-stranded break in the supercoil. Complete base sequence of SV40 has been determined.

Replication of Simian Virus 40 (SV40)

When the virus enters the host cell, its genome migrates to the nucleus. SV40 genome replicates inside the nucleus of the cell, but the capsid protein are synthesized in the cytoplasm and migrate inside the nucleus where, finally, the assembly of the virus take place. The replication of SV40 genome can be divided into two distinct stages: early and late.

Replication Cycle:

a) **Attachment and Entry**: SV40 attaches to host cell receptors, typically via interactions with sialic acid-containing glycoproteins on the cell surface. Entry into the host cell is mediated by endocytosis, where the virus is taken up into endosomes.

b) **Uncoating and Genome Release**: The virus undergoes uncoating, releasing the viral genome into the cytoplasm. The viral genome is transported to the nucleus, where transcription and replication occur.

c) Transcription and Replication:

(i) Early stage: The early region of the viral genome is transcribed to begin early protein synthesis during this stage within 12 hour of infection and before start of genome replication. A single RNA molecule, called primary transcript, is synthesized by the RNA polymerase of

the host ceil and is processed into two mRNAs, a large one and a small one. These mRNAs are capped in the cytoplasm and translated to yield two proteins, small-T and large-T. The large-T (the T-antigen) binds to the site on (origin of replication) on the parental DNA; this initiates the synthesis of viral genome.

(ii) Late stage: Replication of viral genome starts in a bidirectional fashion from a single origin of replication. Late mRNA molecules are synthesized using the strand complementary to that used for early mRNA synthesis. Transcription starts at a promoter near the origin of replication. This late RNA is then processed by splicing, capping, and polyadenylation to yield mRNA corresponding to the three coat proteins, namely, VP1, VP2, and VP3 (VP = virion protein). Viral coat protein mRNAs are then transported to the cytoplasm and translated into the viral coat proteins, which are then transported back into the nucleus where the assembly of progeny viruses takes place. Release of progeny virus particles occurs by the lysis of the host cell.

d) Assembly and Release: Late viral genes are transcribed and translated, leading to the assembly of new virions. Virion assembly occurs in the nucleus, where capsid proteins encapsidate newly synthesized viral genomes. Mature virions are released from the infected cell by cell lysis, allowing them to infect new cells.

Biological Properties

Transformation and Tumorigenicity: SV40 has the ability to transform infected cells in culture, inducing cellular proliferation and forming foci of transformed cells. In laboratory settings, SV40-transformed cells are used as a model to study cellular transformation and oncogenesis.

Tumorigenic Potential: SV40 has been associated with the induction of tumours, particularly in experimental animals. In studies, SV40 has been shown to induce tumours like mesotheliomas in hamsters and tumours in rodents at injection sites.

13.5 SUMMARY

A DNA virus is a virus characterized by a ribonucleic acid (DNA) based genome. The genome can be single stranded DNA (ssDNA) or double-stranded (dsDNA). Notable human diseases caused by DNA viruses include the Adeno Virus, Vaccinia Virus and SV40 Virus. The adenoviruses are common pathogens of humans and animals. The vaccinia virus is the source of the modern smallpox vaccine. Simian virus 40 (SV40) belongs to polyomavirus family, the family of viruses that induce tumours in animals.

13.6 TECHNICAL TERMS

Vaccinia virus, Adenovirus, SV40, Simian virus, ssDNA, dsDNA, Vaccine, Oncogenic, Tumours.

13.7 SELF ASSESSMENT QUESTIONS

Q.1 Explain the structure, life cycle, pathogenesis and diagnosis of Adenovirus.
Q.2 Write structure, classification and lifecycle of vaccina virus.
Q.3 Give a detailed account of genome, replication and transmission of simian virus40.

13.8 SUGGESTED READINGS

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Dr. J. Madhavi

LESSON - 14

ULTRA STRUCTURE AND LIFE CYCLES OF M13, MU, T4 AND LAMBDA BACTERIOPHAGES

Objective of the lesson

The students will gain the knowledge on the Ultra structure and life cycles of Bacteriophages such as M13, Mu, T4 and Lambda.

Structure of the lesson

14.1 INTRODUCTION

Viruses are ultramicroscopic intracellular parasites, capable of autonomous replication inside a host cell. Viruses were established as distinct from bacteria by the work of Frederick Twort and Felix D'Herelle during 1910-1917. D'He'relle called viruses, destroying bacteria as bacteriophages. Researchers began using bacteriophages as experimental organisms in 1930s to understand more about the genetic fine structure. The general stages to a viral life cycle are: infection, replication of the viral genome, assembly of new viral particles and then release of the progeny particles from the host. The ultra-structure of Mu phage is made up of a head, neck, tail, and base plate. The head is icosahedral in shape, and the tail is contractile.

14.2 ULTRA STRUCTURE OF M13

The M13 bacteriophage is a filamentous virus with a cylindrical shape and a genome packaged in five capsid proteins (Fig.14.1). The M13 phage structure is made up of a protein sheath that encapsulates a single-stranded DNA (ssDNA). M13 phage is a type of non-lytic filamentous bacteriophage, with a diameter of ~ 6 nm and a length of ~ 880 nm. Its well-

defined genome was packaged in five capsid proteins (pIII, pVI, pVII, pVIII, pIX) and determines the length of M13 phage. The phage's body is made up of 2,700 copies of the major capsid protein, pVIII. The phage's ends have five to seven copies each of the minor capsid proteins, pIII, pVI, pVII, and pIX. The phage coat is primarily assembled from a 50 amino acid protein called pVIII (or p8), which is encoded by gene VIII (or g8) in the phage genome. For a wild type M13 particle, it takes about approximately 2700 copies of p8 to make the coat about 900nm long. The coat's dimensions are flexible though and the number of p8 copies adjusts to accommodate the size of the single stranded genome it packages. Viable phages appear to be limited at approximately twice the natural DNA content.

There are four other proteins on the phage surface, two of which have been extensively studied. At one end of the filament are five copies of the surface exposed pIX (p9) and a more buried companion protein, pVII (p7). If p8 forms the shaft of the phage, p9 and p7 form the "blunt" end that is seen in the micrographs. These proteins are very small, containing only 33 and 32 amino acids respectively, though some additional residues can be added to the N-terminal portion of each which are then presented on the outside of the coat. At the other end of the phage particle are five copies of the surface exposed pIII (p3) and its less exposed accessory protein, pVI (p6). These form the rounded tip of the phage and are the first proteins to interact with the *E. coli* host during infection. p3 is also the last point of contact with the host as new phage bud from the bacterial surface.

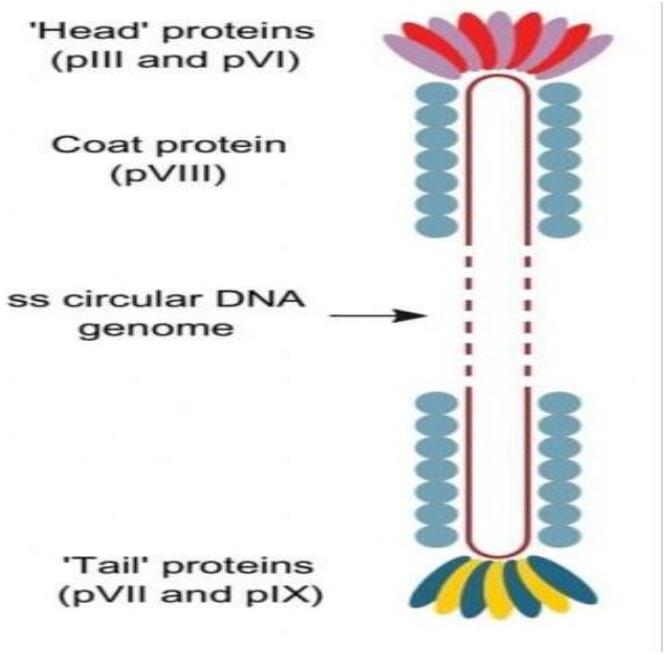

Figure-14.1: Structure of bacteriophage M13

14.2.1 Life cycle of M13

The general stages to a viral life cycle are: infection, replication of the viral genome, assembly of new viral particles and then release of the progeny particles from the host (Fig.14. 2). Filamentous phage use a bacterial structure known as the F pilus to infect *E. coli*, with the M13 p3 tip contacting the TolA protein on the bacterial pilus. The phage genome is then transferred to the cytoplasm of the bacterial cell where resident proteins convert the

single stranded DNA genome to a double stranded replicative form ("RF"). This DNA then serves as a template for expression of the phage genes.

Two phage gene products play critical roles in the next stage of the phage life cycle, namely amplification of the genome. pII (aka p2) nicks the double stranded form of the genome to initiate replication of the + strand. Without p2, no replication of the phage genome can occur. Host enzymes copy the replicated + strand, resulting in more copies of double stranded phage DNA. pV (aka p5) competes with double stranded DNA formation by sequestering copies of the + stranded DNA into a protein/DNA complex destined for packaging into new phage particles. Interestingly there is one additional phage-encoded protein, pX (p10), that is important for regulating the number of double stranded genomes in the bacterial host. Without p10 no + strands can accumulate. What's particularly interesting about p10 is that it's identical to the C-terminal portion of p2 since the gene for p10 is within the gene for p2 and the protein arises from transcription initiation within gene 2. This makes the manipulation of p10 inextricably linked to manipulation of p2 (an engineering headache) but it also makes for a compact and efficient phage in nature.

Phage maturation requires the phage-encoded proteins pIV (p4), pI (p1) and its translational restart product pXI (p11). Multiple copies (on the order of 12 or 14) of p4 assemble in the outer membrane into a stable, i.e. detergent resistant, barrel-shaped structure. Similarly a handful of the p1 and p11 proteins (5 or 6 copies of each) assemble in the bacterial inner membrane, and genetic evidence suggests C-terminal portions of p1 and p11 interact with the N-terminal portion of p4 in the periplasm. Together the p1, p11, p4 complex forms channels through which mature phage are secreted from the bacterial host.

To initiate phage secretion, two of the minor phage coat proteins, p9 and p7, are thought to interact with the p5-single stranded DNA complex at a region of the DNA called the packaging sequence (aka PS). The p5 proteins covering the single stranded DNA are then replaced by p8 proteins that are embedded in the bacterial membrane and the growing phage filament is threaded through the p1, p11, p4 channel. This replacement of p5 by p8 explains the microphage data presented earlier indicate how the size of the phage particle is determined by the number of bases the phage packages. The bacterial host can continue to grow and divide, allowing this process to continue indefinitely.

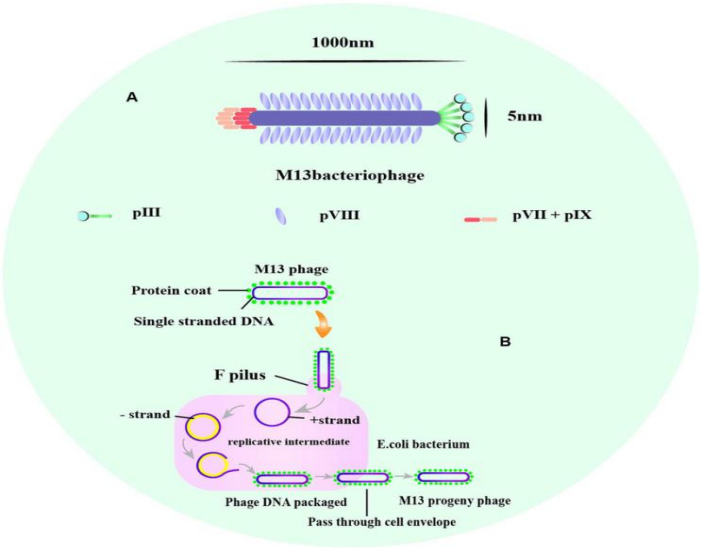

Figure-14.2: Life cycle of M13 Phage

14.3 ULTRA STRUCTURE OF Mu

Phage Mu is non-enveloped, with a head and a tail. The head has an icosahedral structure of about 54 nm in width. The neck is knob-like, and the tail is contractile with a base plate and six short terminal fibers. The genome has been fully sequenced and consists of 36,717 nucleotides, coding for 55 proteins. The ultrastructure of Mu phage is made up of a head, neck, tail, and base plate. The head is icosahedral in shape, and the tail is contractile (Fig. 14.3). The head of Mu phage is icosahedral in shape, with a width of about 54 nanometers. The head is filled with double-stranded DNA. The neck of Mu phage is knob-like. The tail of Mu phage is contractile and has a base plate and six short terminal fibers. The base plate is located at the end of the tail. The genome of Mu phage is a single, linear DNA molecule with about 37,000 base pairs. The genome codes for 55 proteins. The transpososome of Mu phage resembles a pair of scissors, with the phage end DNAs forming the handles and the target DNA forming the blades. MuB is a protein that helps to maintain the Mu prophage as a separate and stable chromosomal domain of E. coli.

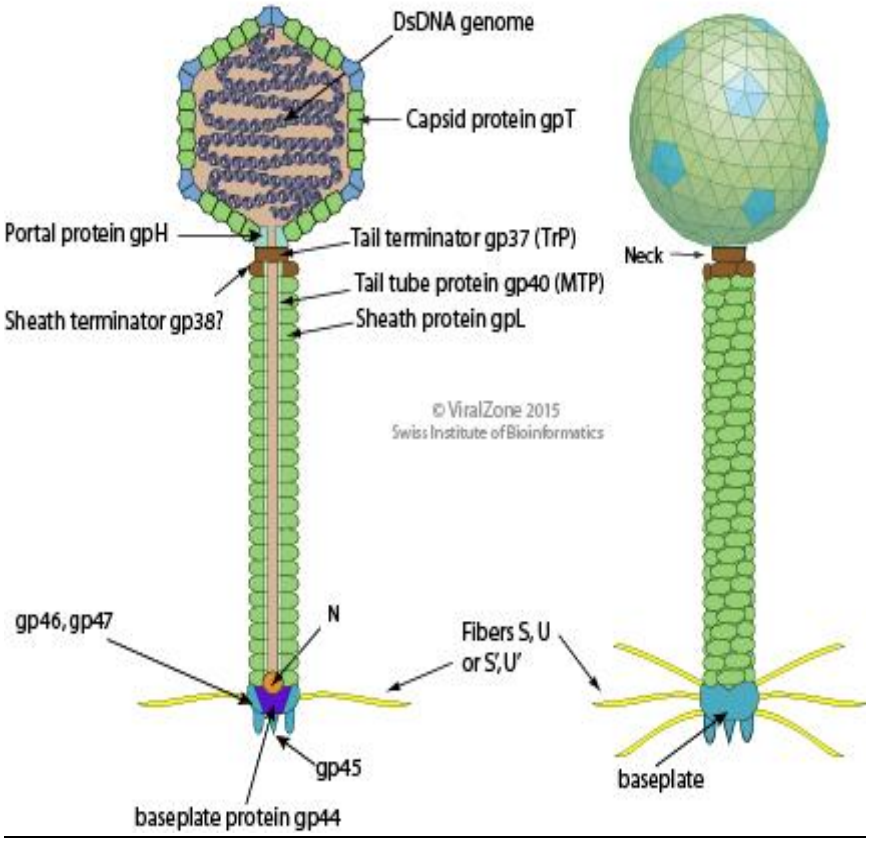

Figure-14.3: Structure of bacteriophage Mu

14.3.1 Life cycle of Mu

The virion fibers attach to the host cell surface lipopolysaccharides (LPS) thereby initiating infection. Upon binding to the host cell surface, the baseplate changes its conformation and triggers sheath contraction, driving the rigid internal tail tube through the cell envelope leading to viral genome entry. The genome of Mu phage dsDNA is linear, about 40Kb in length and encodes about 56 genes. 50-150 bp and about 2 Kb of bacterial DNA are respectively covalently linked at the left and the right end of Mu genome (Fig.14.4). The presence of these pieces of bacterial DNA is due to the headful packaging mechanism. The N

protein, which is present in the virion, is ejected with, and binds to the viral DNA in order to circularize it. The DNA ends are thus protected from host nucleases.

There must be some early transcription giving rise to at least the Repc and Ner repressors and to the DDE recombinase A (MuA) which performs the integration. Flanking bacterial sequences are cut away from the viral genome prior to integration. The ratio of Repc and Ner repressors determines if the phage enters latency or lytic cycle (replication). Repc represses the early promoter thereby establishing latency. Ner represses Repc expression thereby promoting early genes expression leading to the onset of viral replication. When the repressor Repc is inactivated, both the DDE recombinase A (MuA) and the target DNA activator B (MuB) are expressed. MuA is part of the transpososome complex which performs recombination between the viral genome ends and the host DNA. This viral-host DNA structure is resolved by target-primed replication leading to two copies of the viral genome. This process is called replicative transposition.

The selection of the transposition sites is performed by the MuB. Successive rounds of replicative transposition can lead up to about 100 copies of the viral genome. Late transcription allows the expression of the adenine modification enzyme which modifies the adenines in the viral DNA in order to make it resistant to the host restriction enzymes. The new viral particles that will be formed will thus be protected. Structural genes are also expressed in the late phase leading to the assembly of empty capsids, fibers and tails. The bacterial DNA is cut 50-150 bp on the left of the integrated Mu genome to initiate packaging and a second cut occurs once the phage head has been filled. Since there is a bit more space in the procapsid than required for the viral genome, some bacterial DNA on the right side of the Mu genome is also taken and packaged with the viral genome. Since each Mu genome is packaged from a different site in the bacterial genome, the host DNA on Mu ends is unique in every different phage head. The newly synthesized virions are released by lysis.

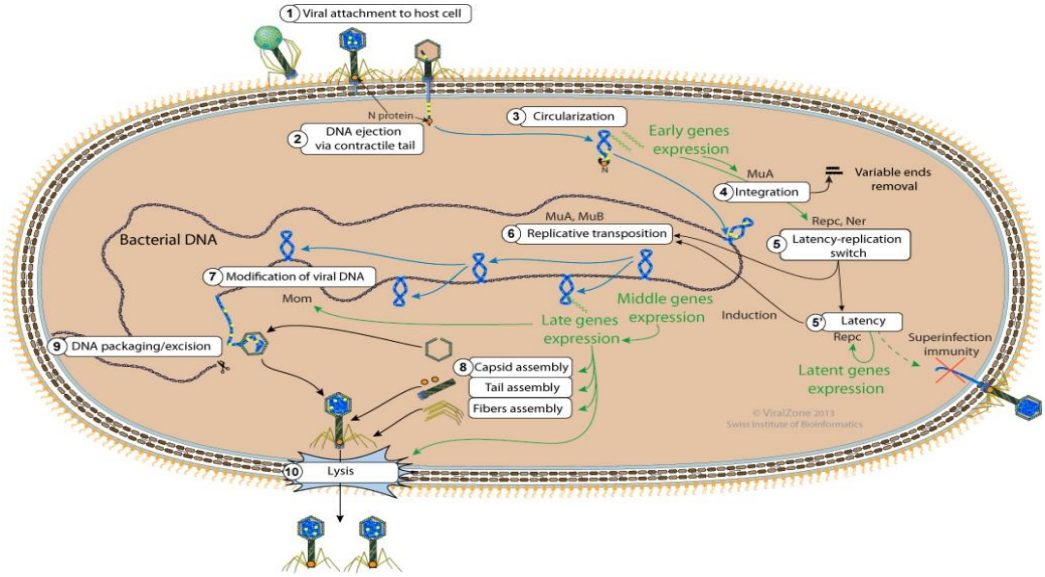

Figure-14.4: Mu Phage Life Cycle

14.4 ULTRA STRUCTURE OF T4

Viruses are ultramicroscopic intracellular parasites, capable of autonomous replication inside a host cell. Viruses were established as distinct from bacteria by the work of

Frederick Twort and Felix D'Herelle during 1910-1917. D'Herelle called viruses, destroying bacteria as bacteriophages. Researchers began using bacteriophages as experimental organisms in 1930s to understand more about the genetic fine structure. T phages, TI through T7 of *E. coli* are among the most intensely studied bacterial viruses. Though some structural variation occurs between T-phages, they are all tadpole-shaped. The head is composed of several proteins, a polyhedron. Inside the head is a single molecule of double-stranded DNA. Attached to the head is a tail, composed of hollow tubes. The tail fibres uses to find a bacteria to infect. The tail pins anchor to virus to the host cell during infection.

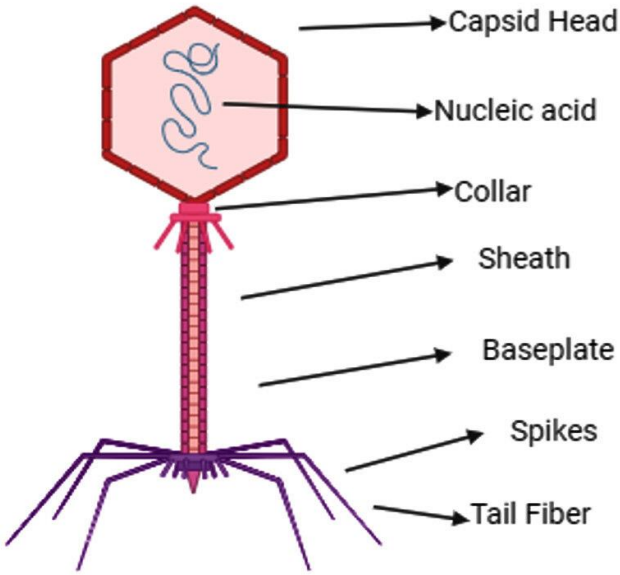

Figure-14.5: Structure of bacteriophage T4

The structure of bacteriophage T4 is given (Fig.14.5). When phage has absorbed to the bacterial cell surface, phage genetic material enters the bacterial cell. During the infection, the cells genetic material is destroyed, while the genetic material of the virus is replicated many times. New virus particles are assembled within the host cell, which bursts open releasing several hundred viral particles. After DNA bacteriophages have reproduced within the host cell, many of them are released when the cell is destroyed by lysis. A phage life cycle that culminates with the host cell bursting and releasing virions is called a lytic cycle, and viruses that reproduce solely in this way are called virulent viruses. The events taking place during the lytic cycle are reviewed in this section, with the primary focus on the T-even phages of *E. coli*, which are some of the most complex viruses known. T-even phages are double-stranded DNA bacteriophages with complex contractile tails. They are placed in the family *Myoviridae.* Certain phages are capable of replicating through different life-cycle stages lytic and lysogenic cycles.

14.4.1 Life cycle of T4

Lytic cycle: They replicate in the host cytoplasm and cause destruction of the host cell and liberate phage particles.

Lysogenic cycle: These phages are capable of integrating into the host chromosome. Some temperate bacteriophages such as phage lambda, when infects *E. coli*, it may either cause

lysis or become lysogenic depending on the prevailing conditions. The integrated phage is termed a prophage (Fig.14.6). Majority of research on lysogeny has been done on phage λ.

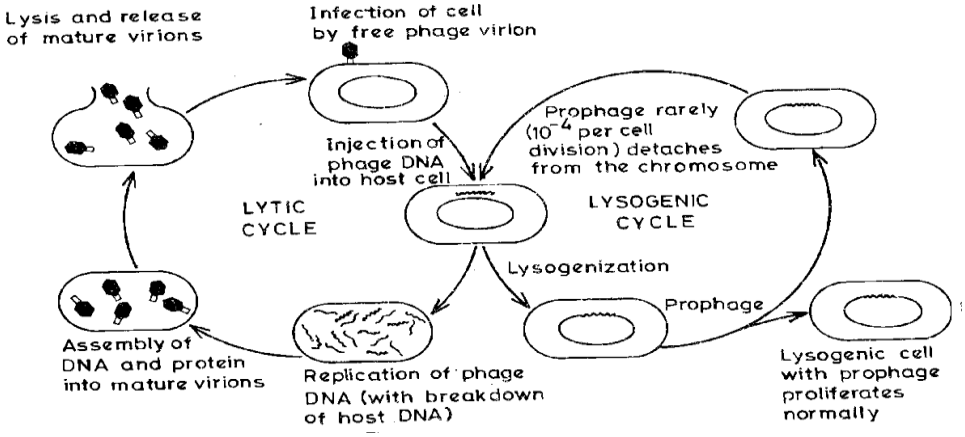

Figure-14.6: Life cycle of T4 bacteriophage

Adsorption and Penetration

Like all viruses, bacteriophages do not randomly attach to the surface of a host cell; rather, they fasten to specific surface structures called receptors. The nature of these receptors varies with the phage; cell wall lipopolysaccharides and proteins, teichoic acids, flagella, and pili can serve as receptors. The T-even phages of *E. coli* use cell wall lipopolysaccharides or proteins as receptors. The baseplate contains the protein gp5, which has lysozyme activity. This aids in the penetration of the tube through the peptidoglycan layer. Finally, the linear DNA is extruded from the head, through the tail tube, and into the host cell. The tube may interact with the plasma membrane to form a pore through which DNA passes. Penetration of the membrane tube is made possible partly by two enzymes, both of which break the same glycosidic bond that is attacked by lysozyme.

Synthesis of Phage Nucleic Acids and Proteins

With the exception of the *Hepadnaviridae*, all double-stranded DNA viruses follow a similar route for synthesis of viral nucleic acids and proteins. The DNA genome serves as the template for mRNA synthesis, and the mRNA molecules made are translated to yield viral proteins. Sometime after the onset of mRNA synthesis, DNA replication ensues and more viral genomes are made. The details of nucleic acid and protein synthesis vary from virus to virus, but all are designed to manipulate the host cell to the advantage of the virus. Within 2 minutes after injection of T4 DNA into a host *E. coli* cell, the *E. coli* RNA polymerase starts synthesizing T4 mRNA. This mRNA is called early mRNA because it is made before viral DNA is made. DNA replication is initiated from several origins of replication and it proceeds bidirectionally from each. Viral DNA replication is followed by the synthesis of late mRNAs, which are important in later stages of the infection.

The T4 genome is linear dsDNA and shows what is called terminal redundancy—that is, a base sequence is repeated at each end of the molecule. These two characteristics contribute to the formation of long DNA molecules called concatemers, which are composed of several genome units linked together in the same orientation. T4 does not have telomerase activity. The T4 genome is said to be circularly permuted, and the genetic map of T4 is drawn as circular molecule.

Assembly of Phage Particles

The assembly of T4 phage is an exceptionally complex self-assembly process that involves special virus proteins and some host cell factors. Late mRNA directs the synthesis of three kinds of proteins: (1) phage structural proteins, (2) proteins that help with phage assembly without becoming part of the virion structure, and (3) proteins involved in cell lysis and phage release.

Release of Phage Particles

Many phages lyse their host cells at the end of the intracellular phase. The lysis of *E. coli* by T4 takes places after about 150 virus particles have accumulated in the host cell. Two proteins are involved. One directs the synthesis of an enzyme that attacks peptidoglycan in the host's cell wall. It is sometimes called T4 lysozyme. Another T4 protein called holin creates holes in the *E. coli* plasma membrane, enabling T4 lysozyme to move from the cytoplasm to the peptidoglycan.

14.5 ULTRA STRUCTURE OF PHAGE λ

The lambda phage, also known as bacteriophage lambda, is a virus with a complex structure that includes a head, tail, and tail fibers. The phage λ contains double stranded DNA of about 17 µm in length packed in protein head of capsid. The head is 55 nm in diameter consisting of 300-600 capsomers (subunits) of 37,500 Daltons. The capsomers are arranged in clusters of 5 and 6 subunits i.e. pentamers and hexamers (Fig.14.7). The phage's DNA is housed in the head, and the tail injects the DNA into the host cell.

The head is also known as the capsid, It is made of proteins, It contains the phage's double-stranded DNA genome. The head is icosahedral in shape. The head is made up of capsomers, which are subunits arranged in clusters of five or six. The tail is long, flexible, and non-contractile. It is helical in shape. The tail has a baseplate that connects the head and tail. The tail tip complex (TTC) is located at the bottom of the tail tube. The tail injects the phage's DNA into the host cell's cytoplasm. The tail fibers are part of the phage particle. The phage binds to the host cell, *E. coli*. The phage's DNA is ejected into the host cell's cytoplasm. The phage's DNA is replicated, producing new phage particles. The cell lyses, releasing the new phage particles into the environment.

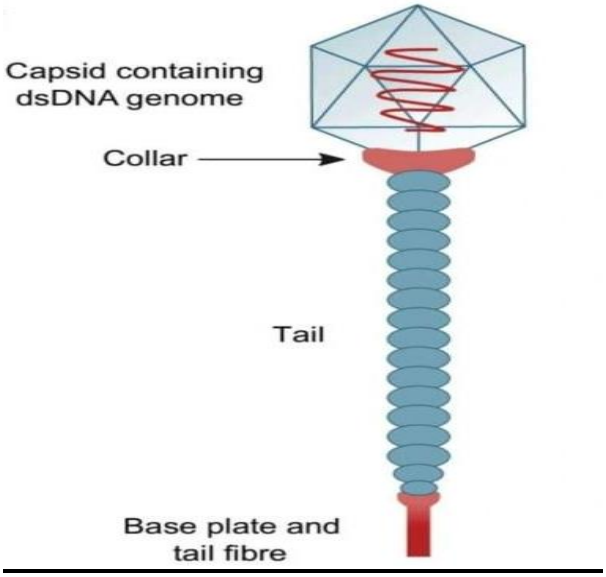

Figure-14.7: Structure of Lambda phage

14.5.1 Life cycle of lambda phage

Like most bacteriophages, lambda attaches to its host and then injects its genome into the cytoplasm, leaving the capsid outside. Once inside the cell, the linear genome is circularized when the two cohesive ends base pair with each other; the breaks in the strands are sealed by the host cell's DNA ligase. The lambda genome has been carefully mapped, and over 40 genes have been located. Most genes are clustered according to their function, with separate groups involved in head synthesis, tail synthesis, lysogeny, DNA replication, and cell lysis. This organization is important because once the genome is circularized, a cascade of regulatory events occurs that determine if the phage pursues a lytic cycle or establishes lysogeny. Regulation of appropriate genes is facilitated by clustering and coordinated transcription from the same promoter.

The cascade of events leading to either lysogeny or the lytic cycle involves a number of regulatory proteins that function as repressors or activators or both. Two regulatory proteins are of particular importance: the lambda repressor (product of the *cI* gene) and the Cro protein (product of the *cro* gene). The lambda repressor promotes lysogeny, and the Cro protein promotes the lytic cycle. In essence, the decision to pursue lysogeny or to pursue a lytic cycle is the result of a race between the production of these two proteins. If lambda repressor prevails, the production of Cro protein is inhibited and lysogeny occurs; if the Cro protein prevails, the production of lambda repressor is inhibited and the lytic cycle occurs. This is because the lambda repressor prevents transcription of viral genes, while Cro does just the opposite: it ensures viral gene expression.

The lambda repressor is 236 amino acids long and folds into a dumbbell shape with globular domains at each end. One domain binds DNA while the other binds another lambda repressor molecule to form a dimer. The dimer is the most active form of the repressor. Lambda repressor binds two operator sites,*OL* and *OR*, thereby blocking transcription of most viral genes. When bound at *OL*, it represses transcription from the promoter *PL* (promoter leftward). Likewise, when bound at *OR* it represses transcription in the rightward direction from *PR* (promoter rightward). However, it also activates transcription in the leftward direction from the *cI* promoter *PRM* (RM stands for *r*epressor *m*aintenance). Recall that *cI* encodes the lambda repressor.

Thus lambda repressor controls its own synthesis. As noted earlier, if the lambda repressor wins the race with the Cro protein, lysogeny is established and the lambda genome is integrated into the host chromosome. Integration is catalyzed by the enzyme integrase, the product of the *int* gene, and takes place at a site in the host chromosome called the attachment site (*att*). A homologous site is found on the phage genome, so the phage and bacterial *att* sites can base pair with each other. The bacterial site is located between the galactose (*gal*) and biotin (*bio*) operons, and as a result of integration, the circular lambda genome becomes a linear stretch of DNA located between these two host operons. The prophage can remain integrated indefinitely, being replicated as the bacterial genome is replicated.

14.6 SUMMARY

Researchers began using bacteriophages as experimental organisms in 1930s to understand more about the genetic fine structure. The general stages to a viral life cycle are: infection, replication of the viral genome, assembly of new viral particles and then release of the progeny particles from the host. T phages, TI through T7 of *E. coli* are among the most intensely studied bacterial viruses. Though some structural variation occurs between T-phages, they are all tadpole-shaped. The head is composed of several proteins, a

polyhedron. Inside the head is a single molecule of double-stranded DNA. Attached to the head is a tail, composed of hollow tubes. The tail fibres uses to find a bacteria to infect. The tail pins anchor to virus to the host cell during infection. The lambda genome has been carefully mapped, and over 40 genes have been located. Most genes are clustered according to their function, with separate groups involved in head synthesis, tail synthesis, lysogeny, DNA replication, and cell lysis. This organization is important because once the genome is circularized, a cascade of regulatory events occurs that determine if the phage pursues a lytic cycle or establishes lysogeny. Regulation of appropriate genes is facilitated by clustering and coordinated transcription from the same promoter.

14.7 TECHNICAL TERMS

Bacteriophages, M13, Mu, T4, Lambda, Prophage, Lysogeny, Lytic cycle.

14.8 SELF ASSESSMENT QUESTIONS

Q.1 Write a detail account on Ultrastructure and life cycle of M13 Bacteriophage.
Q.2 Explain the Ultrastructure and life cycle of Mu Bacteriophage.
Q.3 Give a detail account on Ultrastructure and life cycle of T4 Bacteriophage.
Q.4 Discuss about the Ultrastructure and life cycle Lambda phage.

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Dr. K. Nagaraju

LESSON – 15

GENERAL ACCOUNT OF VIRUSES OF CYANOBACTERIA, ALGAE AND FUNGI

Objective of the lesson

Students have an understanding about the details of the characteristics of viruses that infect cyanobacteria, algae and fungi.

Structure of the lesson

15.1 INTRODUCTION

Cyanobacteria are a phylum of bacteria that obtain their energy through the process of photosynthesis. The first cyanophage studied by Safferman and Morris was the cyanophage attacking *Lyngbya*, *Plectonema* and *Phormidium*. Algae have been observed in freshwater, marine, and terrestrial systems, in unicellular, colonial, or multi-cellular forms, and in disparate taxonomic lineages. Phycophages are the viruses that infect algae or seaweed. They are also known as algal viruses. In 1948, a disease-causing dieback of the commercially produced mushroom, *Agaricus bisporus*, was reported and named the 'La France' disease. It was not until nearly a decade later that mycoviruses were identified as the causal agents of this economically important disease. This quickly led to the development of a new field of study, mycovirology.

15.2 CYANOPHAGES

Cyanophages are the 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. The first cyanophage studied by Safferman and Morris was the cyanophage attacking *Lyngbya*, *Plectonema* and *Phormidium*. They named the virus as LPP-1 using the first letter of the three genera. Thereafter, several serological strains of LPP were isolated from different parts of world and named LPP-1, LPP-2, LPP-3, LPP-4 and LPP-5. Besides LPP groups of cyanophages, a large number of other cyanophages such as SM-1, AS-1, N-1, C-1, AR-1, A-1, etc., have also been reported.

Morphology and Replication of Cyanophages

LPP group of cyanophages resemble T_3 and T_7 bacteriophages as they possess icosahedral head (580 Å diam.) and short (20 x 15 nm) tail. N-1 cyanophages resemble T_2 and T_4 phage because their head (550 Å dia.) is icosahedral but the tail is long (110 x 10 nm). SM-1 cyanophages have tailless icosahedral head (880 Å diam.) whereas As-1 viruses posses hexagonal head (900 Å diam.) and long tail (243 x 22 nm) (Fig.14.1). Like R-even phages, the tail may be contractile or non-contractile. AS-1 group has the largest cyanophages.

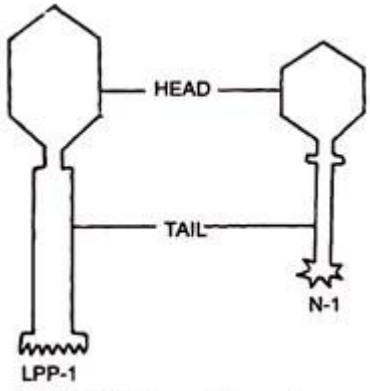

Figure-14.1: Structure of Cyanophage

Cyanophages resemble T-even bacteriophages in their growth cycle. However, cyanophage LPP-1 is much more studied and our discussion on growth cycle is based on this virus. After the LPP-1 is adsorbed on the host cyanobacterium, the viral-DNA is injected into the host cytoplasm; the injection mechanism is still obscure.

15.3 PHYCOPHAGES

Algae have been observed in freshwater, marine, and terrestrial systems, in unicellular, colonial, or multi-cellular forms, and in disparate taxonomic lineages. Phycophages are the viruses that infect algae or seaweed. They are also known as algal viruses. Viruses infecting eukaryotic algae are extremely diverse. They have been reported with DNA or RNA genomes in various architectures (linear, circular, double-stranded, single-stranded, segmented) and sizes (4.4 to 638kb)**.** It is anticipated that any single algal host can be permissive to many closely related virus variants, whereby phylogenetic comparisons of their core genes will reveal distinct clades (e.g., *Micromonas pusilla* and *Chlorella variabilis* viruses) with differences in latent phases, burst sizes, and genome size. In closely related viruses this is best resolved using concatenated alignments of marker protein sequences. At the same time, the origin of some of these genes is often attributed to gene transfer events.

Chloroviruses are the only viruses characterized thus far that infect freshwater algae. The hosts of chloroviruses are zoochlorellae, which are endosymbiotic green algae commonly associated with hosts *Paramecium bursaria*, coelenterate *Hydra viridis*, or the heliozoan *Acanthocystis turfacea*. In the ciliate *Paramecium bursaria*, for example, the algae lives within the cells of the host, providing nutrients via photosynthesis. Living inside the cells of the ciliate offers protection for the algae, and a mode of transportation. Zoochlorellae are resistant to infection in their symbiotic state. When the relationship between the algae and host is disrupted, for example, through grazing by copepods, infection by chloroviruses is permitted.

The only ssDNA alga-infecting viruses that have been isolated are those which infect diatoms (Bacillariophyceae). In total, diatoms are a collective of an estimated 12,000–30,000 species, representing one of the most abundant phytoplankton groups in freshwater and marine environments. Most diatom-virus systems currently in culture are those infecting the cosmopolitan genus *Chaetoceros*. These isometric virus particles are ~35 nm in diameter and house circular, ssDNA genomes ranging from ~5.5–6.0 kb. The genomes generally encode four open reading frames consisting of an endonuclease (Rep), a major capsid protein, and two ORFs with unknown function. The capsid and replication initiating endonuclease are used in phylogenetic analyses.

Algae-infecting viruses with single (ss) and double-stranded (ds) RNA genomes have also been isolated and characterized, although most attention has been focused on the ssRNA isolates. Both virus groups encode an RNA-dependent RNA polymerase (RdRP), as well as proteases and helicases that can be used to infer distant evolutionary relationships. Most information on dsRNA algal viruses has been derived from the original isolation papers describing the evolutionary relationships of the isolates. MpRV, a dsRNA virus of *Micromonas pusilla*, forms its own genus within the family *Reoviridae* (unassigned order) and has been proposed to be the ancestral line of the *Reoviridae* based on its placement between clades that demonstrate turreted or non-turreted virions. ssRNA viruses have received considerably more attention since their hosts are common marine phytoplankton with some species capable of forming harmful blooms. Most of the alga-infecting ssRNA viruses are members of the order Picornavirales, with a few contradictions that are awaiting a taxonomic re-evaluation based on molecular data. The viruses infecting *Heterocapsa* and *Heterosigma* are the sole members of the families *Alvernaviridae* and *Marnavirdiae* (order Picornavirales), respectively, while the genus *Bacillarnavirus* (order Picornavirales) includes formal members *Chaetoceros socialis forma radians* RNA virus, *Chaetoceros tenuissimus* RNA virus 01, and *Rhizosolenia setigera* RNA virus 01.

15.4 MYCOPHAGES

In 1948, a disease-causing dieback of the commercially produced mushroom, *Agaricus bisporus*, was reported and named the ‘La France’ disease. It was not until nearly a decade later that mycoviruses were identified as the causal agents of this economically important disease. This quickly led to the development of a new field of study: mycovirology. Not long after, mycoviruses were also identified in the ascomycete *Penicillium stoloniferum*, and were determined to cause interferon stimulation in mammals. It was not until the 1970s, however, that a significant breakthrough was made in the field of mycovirology. A mycovirus of the chestnut blight pathogen, *Cryphonectria parasitica*, was observed to reduce the virulence of its host and had potential as a biocontrol agent against this plant pathogenic fungus. This led to increased interest in the discovery of mycoviruses in plant pathogenic fungi, as they could serve as prospective biocontrol agents of such fungi.

Mycoviruses that elicit hypovirulence have been identified in both human and plant pathogenic fungi. These viruses frequently cause several adverse effects in their fungal hosts, such as decreased virulence, irregular growth, abnormal pigmentation, and defects in sexual development. The recent discovery of hypovirulence inducing mycoviruses in human pathogenic fungi presents an opportunity for the development of therapeutic interventions against fungal infections in humans. The majority of mycoviral research, however, has been concerned with hypovirulence-inducing mycoviruses of plant pathogenic fungi. These mycoviruses have the potential to be used as biocontrol agents against their fungal hosts,

thereby reducing the losses in agriculture and forestry due to fungal infections. Despite their potential, the use of mycoviruses as biocontrol agents has a number of challenges. Mycoviruses lack the extracellular transmission mechanisms of plant and animal viruses. Instead, viral transmission occurs intercellularly, through hyphal anastomosis (fusion of fungal hyphae), cell division, and sporulation. Hyphal anastomosis impedes the successful transmission of mycoviruses between different fungal strains since they need to be vegetatively compatible with hyphal fusion. Nevertheless, research is ongoing to find solutions to this problem, and mycovirus-based biocontrol has already been used successfully. Advances in high throughput sequencing have led to a surge in mycovirus discoveries and new insights into their origins, diversity, and impact on fungal hosts.

Mycoviruses (viruses of fungi) are ubiquitous throughout the fungal kingdom. Mycoviruses can have double-stranded RNA (dsRNA), single-stranded RNA (+ssRNA), or single-stranded DNA (ssDNA) genomes. Mycoviruses are transmitted intercellularly through cell division, sporulation, and cell fusion. Mycoviruses lack an extracellular route for infection. Mycoviruses lack a movement protein, which is essential for animal and plant viruses. They are known to associate with most of the major fungal taxonomic groups, including Ascomycota, Basidiomycota, Chytridiomycota, Zygomycota, and Neocallimastigomycota. The majority of mycoviruses have double-stranded RNA (dsRNA) or positive sense single-stranded RNA (+ssRNA) genomes. Mycoviruses with dsRNA genomes are classified into the viral families Chrysoviridae, Amalgaviridae, Megabirnaviridae, Quadriviridae, Partitiviridae, Polymycoviridae, Reoviridae, Totiviridae and the genus *Botybirnavirus*. Those with +ssRNA genomes are grouped into the families Endornaviridae, Alphaflexiviridae, Barnaviridae,Deltaflexiviridae, Gammaflexivirid ae, Hypoviridae, Narnaviridae, Mitoviridae, Hadakaviridae, Yadokariviridae, and the reverse transcribing (RT) families Metaviridae and Pseudoviridae. Mycoviruses with negative-sense single-stranded RNA (−ssRNA) genomes have also been discovered, and belong to the family Mymonaviridae. Recently a number of ssDNA mycoviruses have also been found; however, only two belong to a recognized mycoviral family, namely *Sclerotinia sclerotiorum* hypovirulence-associated DNA virus 1 and *Fusarium graminearum* gemytripvirus 1. Both belong to the family Genomoviridae.

15.5 SUMMARY

The first cyanophage studied by Safferman and Morris was the cyanophage attacking Lyngbya, Plectonema and Phormidium. Algae have been observed in freshwater, marine, and terrestrial systems, in unicellular, colonial, or multi-cellular forms, and in disparate taxonomic lineages. Phycophages are viruses that infect algae or seaweed. They are also known as algal viruses. Phycophages are viruses that infect algae or seaweed. They are also known as algal viruses. Viruses infecting eukaryotic algae are extremely diverse. Mycoviruses (viruses of fungi) are ubiquitous throughout the fungal kingdom. Mycoviruses lack an extracellular route for infection. Mycoviruses lack a movement protein, which is essential for animal and plant viruses. They are known to associate with most of the major fungal taxonomic groups, including Ascomycota, Basidiomycota, Chytridiomycota, Zygomycota, and Neocallimastigomycota.

15.6 TECHNICAL TERMS

Cyanophages, Phycophages, Mycoviruses, Ascomycota, Basidiomycota, Chytridiomycota, Zygomycota and Neocallimastigomycota.

15.7 SELF ASSESSMENT QUESTIONS

Q.1 Write an essay on Cyanophages.
Q.2 Discuss in detail about Phycophages.
Q.3 Give an account on Mycophages.

15.8 SUGGESTED READINGS

1. Essential Genetics - P.J. Russell, 1987, Blackwell Publishers.
2. Modern Genetics - F.J.Ayala & J.A. Kiger, Jr. 1980..The Benjamin/Cummings Publ. Com.
3. Genetics - M.W. Strickberger.– Prentice Hall of India, New Delhi.
4. Tamarin. **Principles of Genetics**.
5. Principles of Genetics - Snustad, D.P., M.J..Simmons, 2003. John Wiley & Sons.

Dr. K. Nagaraju

LESSON - 16

VIRAL VACCINES, INTERFERONS AND ANTIVIRAL DRUGS

Objective of the lesson

Students will understand the types and preparation of different vaccines, also have knowledge on different interferons and antiviral drugs.

16.1 INTRODUCTION

A vaccine is a medical preparation given to provide immunity from a disease. Vaccines use a variety of different substances ranging from dead microorganisms to genetically engineered antigens to defend the body against potentially harmful microorganisms. Effective vaccines change the immune system by promoting the development of antibodies that can quickly and effectively attack disease-causing microorganisms when it enters the body, preventing disease development. A vaccine may contain live-attenuated or killed microorganisms or parts or products from them capable of stimulating a specific immune response comprised of protective antibodies and T cell immunity. The viral vaccines should also be able to stimulate high titers of neutralizing antibodies. Injection of a vaccine into a non-immune subject induces active immunity against the modified pathogens. Vaccination is immunization against infectious disease through the administration of vaccines for the production of active (protective) immunity in humans or other animals.

16.2 TYPES OF VACCINES

Vaccines have proved to have a strong defense against some of the most fatal diseases and if they were still unavailable, the survival of individuals would be based on their immune defenses which could either resolve the infection or lead to death from the infection.

Therefore, the use of vaccines means, the vaccine will mimic the pathogen and cause an immune response that is similar to that that can be activated by the pathogen. Historically, these vaccines have eliminated fatal infections such as smallpox, almost eliminated polio, and saved many individuals from typhus, tetanus, hepatitis A and B, measles, rotavirus diseases, etc.
Vaccine types can broadly be classified into three groups:

1. Whole-organism Vaccines
3 Inactivated (Killed) Vaccine
4 Live-attenuated vaccines
5 Chimeric vaccine

2. Subunit Vaccines
- Polysaccharide Vaccine
- Conjugated Vaccines
- Toxoid Vaccines
- Recombinant Protein Vaccines
- Nanoparticle vaccines

3. Nucleic Acid Vaccines
- DNA plasmid vaccines
- mRNA vaccines
- Recombinant vector vaccine

16.2.1 Whole organism vaccines

Many vaccines that were developed early consist of an entire pathogen that is either killed (inactivated) or weakened (attenuated) so that they cannot cause disease. They are known as the whole-organism vaccines. These vaccines elicit strong protective immune responses and many vaccines used today are prepared in this manner, but not all disease-causing microbes can be effectively targeted with a whole-organism vaccine.

1. Inactivated (Killed) Vaccines
These were produced by killing the pathogen (bacteria, virus, or other pathogens) with chemicals or heat, or radiation. The killed pathogen cannot cause disease, and this means that they do not replicate in the host's body. Examples of inactivated vaccines include poliomyelitis (salk vaccine), rabies, typhoid, cholera, pertussis, pneumococcal, rabies, hepatitis B, and influenza vaccines.

2. Live-attenuated vaccines
These vaccines were developed in the 1950s when advances in tissue culture techniques were developed. These vaccines are prepared from a whole organism, by weakening their pathogenicity so that they cannot cause disease but can induce an immune response, hence the term attenuation**.** These vaccines elicit strong immune responses because they are similar to the actual disease pathogen and hence they confer a life-long immunity after only one or two doses, therefore they are very effective. They are also relatively easy to create for certain viruses, but difficult to produce for more complex pathogens like bacteria and parasites. Examples include Measles/Mumps/Rubella **(**MMR**)** and Influenza Vaccine Live, Intranasal, Polio (Sabin vaccine), Rotavirus, Tuberculosis, Varicella, Yellow fever. The attenuated strain of *Mycobacterium bovis* called Bacillus Calmette- Guérin (BCG) was developed by

growing *M. bovis* on a medium containing increasing concentrations of bile. After 13 years, this strain had adapted to growth in strong bile and had become sufficiently attenuated that it was suitable as a vaccine for tuberculosis.

3. Chimeric vaccines
The evolution of modern genetic engineering techniques has enabled the creation of chimeric viruses, which contain genetic information from one viral particle and display the biological properties of different parent viruses. Whole-organism vaccines, whether alive or dead, have another big drawback. Considering that they are composed of complete pathogens, they retain molecules that are not involved in evoking immunity, including unavoidable byproducts of the manufacturing process such as contaminants that can trigger allergic or immune disruptive reactions.

16.2.2 Subunit Vaccines

Some of the subunit vaccines produced to prevent bacterial infections are based on the polysaccharides or sugars that form the outer coating of many bacteria. Therefore, there are subtypes of subunit vaccines as follows:

1. Polysaccharide Vaccines:
Some microbes contain a polysaccharide (sugar) capsule which they use for protection and evading the human immune defenses, especially in infants and young children. Therefore, these are vaccines that are prepared using the sugar molecules, and polysaccharides from the outer layer of a bacteria or virus. They create a response against the molecules in the pathogen's capsule. Normally these molecules are small hence they are not immunogenic (cannot induce an immune response on their own). Hence, they tend to be ineffective in infants and young children between 18-24 months, and they induce a short-term immunity associated with slow immune responses, and slow activation, and it does not increase antibody levels and it does not create an immune memory. Therefore, these sugar molecules are chemically linked to carrier proteins and work similarly to conjugate vaccines. Examples of polysaccharide vaccines include Meningococcal disease caused by *Neisseria meningitidis* groups A, C, W135, and Y, as well as Pneumococcal disease.

2. Conjugated Vaccines
These vaccines are prepared by linking the polysaccharides or sugar molecules on the outer layer of the bacteria to a carrier protein antigen or toxoid from the same microbe. The polysaccharide coating disguises a bacterium's antigens so that the immature immune systems of infants and younger children cannot recognize or respond to them. Conjugate vaccines get around this problem through the linkage of polysaccharides with a protein. This formulation greatly increased the ability of the immune systems of young children to recognize the polysaccharide and develop immunity. The vaccine that protects against *Haemophilus influenzae* type B (Hib) is a conjugate vaccine. Today, conjugate vaccines are also available to protect against pneumococcal and meningococcal infections.

3. Toxoid Vaccines
These vaccines are prepared from inactivated toxins, by treating the toxins with formalin, a solution of formaldehyde, and sterilized water. This process of inactivation of toxins is known as detoxification and the resultant inactive toxin is known as a toxoid. Detoxification makes the toxins safe to use. The toxins used for the preparation of toxoids are obtained from the bacteria that secrete the illness-causing toxins. This means that when the host body

receives the harmless toxoid. the immune system adapts by learning how to fight off the natural bacterial toxin responsible for causing illness, by producing antibodies that lock onto and block the toxin. Examples of toxoid vaccines include diphtheria and tetanus toxoid vaccines.

4. Recombinant Protein Vaccines

After the start of the generic engineering era, recombinant DNA technology also evolved. This is where DNA from two or more sources is combined. This technology harnessed the development of recombinant protein vaccines. For recombinant vaccines to induce immunity against a pathogen, they have to be administered along with an adjuvant or expresses by a plasmid or harmless bacterial or viral vectors. Production of these recombinant protein vaccines involves the insertion of DNA encoding an antigen such as a bacterial surface protein, which stimulates an immune response into bacterial or mammalian cells, expressing the antigen in these cells, and then the antigen is purified from them. The classical example of a recombinant protein vaccine currently in use in humans is the vaccine against hepatitis B. The vaccine antigen is a hepatitis B virus protein produced by yeast cells into which the genetic code for the viral protein has been inserted. Vaccines that are also used to prevent human papillomavirus (HPV) infections are also based on the recombinant protein antigens, by preparing from the proteins of the outer shell of HPV, which form particles that almost resemble the virus.

5. Nano-particle vaccines

This vaccine development was based on a strategy to present protein subunit antigens into the immune system. The NIAID has also designed a universal flu vaccine, an experimental vaccine with protein ferritin which can self-assemble into microscopic pieces known as nanoparticles that display a protein antigen. A nanoparticle-based influenza experimental vaccine is also being evaluated in human trials (early stages). This new technology of vaccine delivery is also being evaluated and assessed for the development of vaccines against MERS coronavirus, respiratory syncytial virus (RSV), and Epstein-Barr virus.

16.2.3 Nucleic Acid Vaccines

These are vaccines designed to aim at introducing the genetic materials that code the antigen or the antigen that is aimed at inducing an immune response, enabling the host cells to use the genetic materials to produce the antigens. The advantages of the nucleic acid vaccine approach include: stimulating a broad long-term immune response, excellent vaccine stability, ease of large-scale vaccine manufacture, rapid production, reduces potential risks of working with the live pathogen, encoding only the key antigen without including other proteins, The advantage of the ease of production is a potential game-changer for targeting epidemic or emerging diseases where rapidly designing, constructing, and manufacturing the vaccine are crucial

Some of the know nucleic acid vaccines models include:

1. DNA plasmid vaccines

These are vaccines that are composed of a small circular piece of DNA known as a plasmid. The plasmid carries genes that encode proteins from the pathogen of interest. Experimental DNA plasmid vaccines have been designed by the National Institute of Allergy and Infection Disease (NIAID) to address some viral disease threats including SARS coronavirus (SARS-

CoV) in 2003, H5N1 avian influenza in 2005, H1N1 pandemic influenza in 2009, and Zika virus in 2016.

2. mRNA vaccines

mRNA is an intermediary between DNA and protein. Recent technological advances have developed mRNA vaccines overcoming the instability issues of mRNA and its delivery into the cells, with encouraging results. Some experimental mRNA vaccines have been designed to protect mice and monkeys against Zika virus infection, and administered in a single dose.

3. Recombinant vector vaccines

These are vaccines designed as vectors or carriers using harmless viruses or bacterium and they introduce the genetic material into cells. Majorly these vaccines are designed and approved for use to protect animals from infectious diseases, including rabies and distemper, but some have been developed to protect humans from viruses such as HIV, Zika virus, and Ebola virus.

16.3 PREPARATION AND PRODUCTION OF VACCINES

Steps involved in the production of vaccines: 1) Manufacturing of vaccines begins with the small number of specific pathogens. The pathogens should pass through the tests of sterility. The pathogen should be free from impurities and other bacteria, ideal conditions are maintained usually frozen –so that it does not become weaker and do not further divide. 2) Selecting strains: -The strain of pathogen to cause pathogenicity is selected. Restriction fragment length polymorphism (RFLP) is a method for identifying bacterial strains using unique fingerprints which relies on the presence of variations (polymorphisms) in homologous DNA sequences. 3) The strain is cultured in a Petridish with the appropriate media which act like stock culture. 4) Bulk production: - This is carried in fermenters with controlled pH, temperature, aeration by providing appropriate media. The cells are harvested till it reaches 10 8 /cells. 5) Cells are separated from the media by using different techniques like centrifugation, chromatography and filtration. 6) An adjuvant improves the immune response to the vaccine, sometimes by keeping the vaccine at the injection site for a little longer or by stimulating local immune cells. Eg: Aluminium phosphate, aluminium hydroxide or potassium aluminium sulphate. The adjuvant may be a tiny amount of aluminium salts (like aluminium phosphate, aluminium hydroxide or potassium aluminium sulphate). Aluminium has been shown not to cause any long-term health problems, and humans ingest aluminium regularly through eating and drinking. 7) Surfactants keep all the ingredients in the vaccine blended together. They prevent settling and clumping of elements that are in the liquid form of the vaccine. 8) Stabilizers prevent chemical reactions from occurring within the vaccine and keep the vaccine components from sticking to the vaccine vial. Stabilizers can be sugars (lactose, sucrose), amino acids (glycine), gelatine, and proteins (recombinant human albumin, derived from yeast). 9) Lyophilizer:- is a low temperature dehydration process that involves freezing the product, lowering pressure, then removing the ice by sublimation. This is in contrast to dehydration by most conventional methods that evaporate water using heat. Because of the low temperature used in processing, the quality of the rehydrated product is excellent.

New Generation Vaccines - Genetic Recombination Vaccines

Vaccine produced by using recombinant DNA technology (i.e. mixing of two DNA from different sources) is called recombinant vaccine. This involves inserting the DNA encoding antigen (such as bacterial surface protein) that stimulates an immune response into bacterial

or mammalian cells, expressing the antigen in these cells and then purifying it from them. Recombinant vaccines are prepared with the help of expression system, such as bacteria, yeast, and mammalian cells in which the DNA encoding the genetic determinant can be inserted and expressed. However many factors must be checked before choosing the system for antigen expression. The level of expression we get by using each expression vectors and promoter(Initiator), the selection marker of choice, the presence or absence of post-translational modifications by recombinant vector ,besides other are important characteristics that hinders in quality production of recombinant antigens as vaccines (Fig.16.1).

Bacterial expression system are most common in use because they are easy to handle and their ability for high level expression. On the other hand, for antigens in which post-translational modifications (e.g. glycosylation) are necessary, the use of mammalian or insect cells should be preferred.

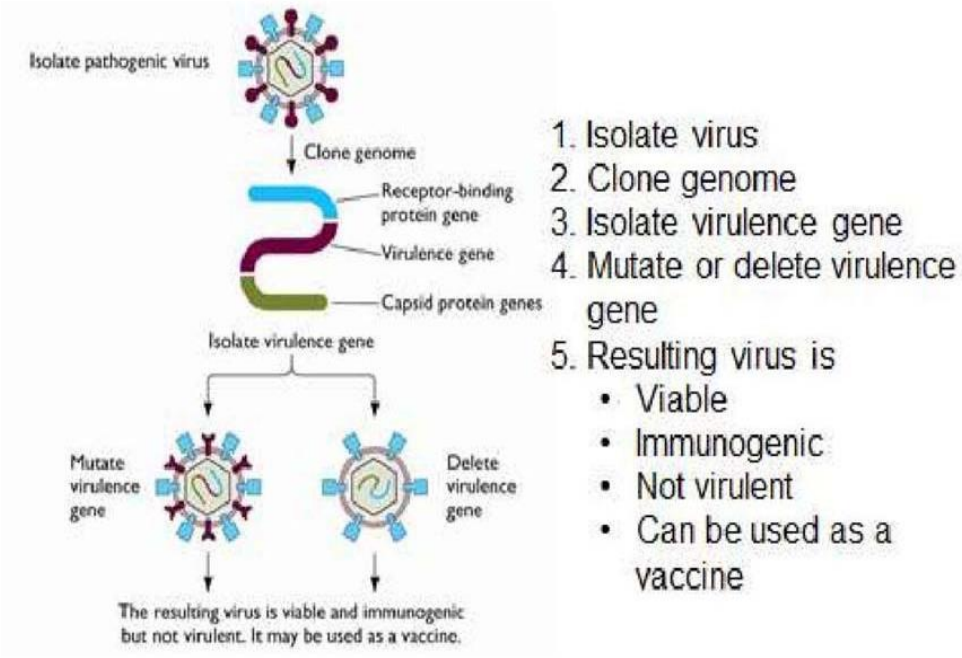

Figure-16.1: Construction of recombinant attenuated virus

Types of Recombinant vaccines

The recombinant vaccines can be broadly classified into three groups:

1. Subunit recombinant vaccines: These are the components of the pathogenic organisms. Sub unit vaccines are proteins, peptides and DNA.
2. Attenuated recombinant vaccines: In this method, genetically modified organisms (bacteria or viruses) that are made non-pathogenic are used as vaccines.
3. Vector recombinant vaccines: These are the genetically modified viral vectors that can be used as vaccines to protect from several pathogens.

Recombinant hepatitis B vaccine

Hepatitis B is a common viral disease in man. It basically affects liver causing chronic hepatitis and liver cancer. It contains a core having a viral genome (DNA) surrounded by phospholipids envelop carrying surface antigens. Scientists has identified the gene encoding for hepatitis B surface antigen (HBsAg) as a subunit and produced by cloning (growing) HbsAg gene in yeast cells. *Saccharomyces cerevisiae,* a harmless baking and brewing yeast, is used in this purpose. The HBsAg assembles into virus like particles (VLPs), which are

highly immunogenic, making the HBV vaccine, a very good vaccine. After expression in yeast system, it is purified. Hepatitis B vaccine is safe to use, very accurate and produces no allergic reactions (Fig.16.2).

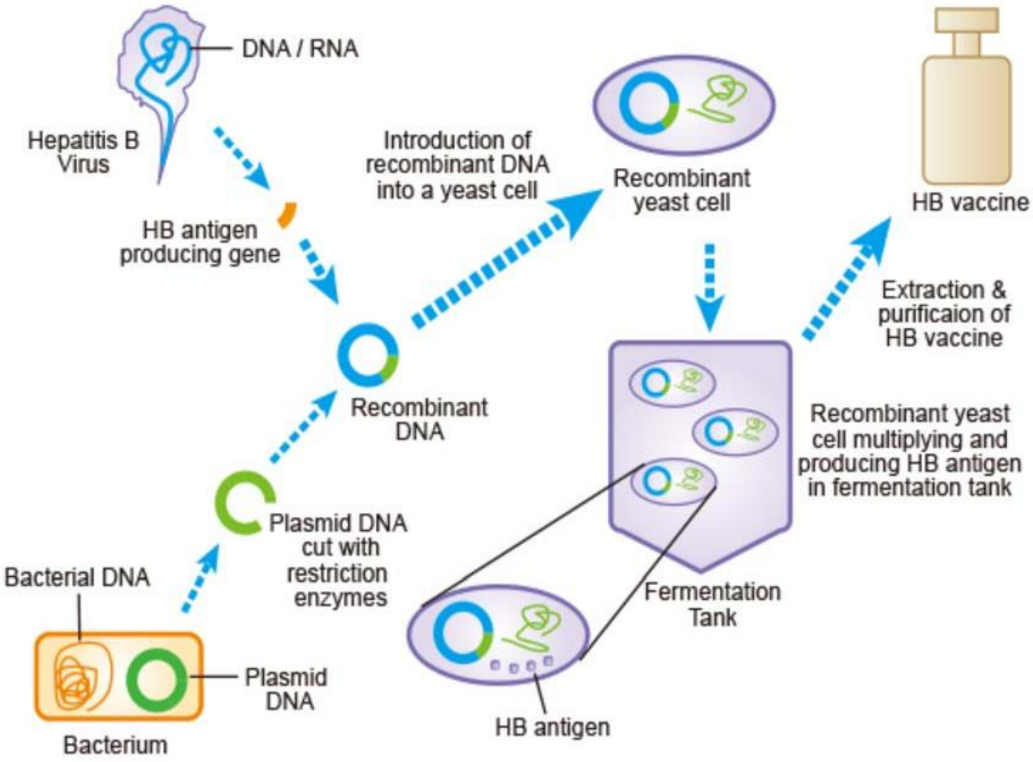

Figure-16.2: Hepatitis B vaccine production

DNA vaccines

The sequence of a pathogenic protein antigen is cloned in plasmid. The constructed plasmids are then subsequently grown in bacteria like *E. coli* and highly purified via chromatographic methods. LPS contamination of plasmids has to be prevented because of the immunotoxic properties of natural LPS. After purification the circular double-stranded DNA plasmids are ready for vaccination. The unique advantage of DNA vaccines is their ability to mimic the effects of live attenuated vaccines without the risk associated with the administration of infectious attenuated material. DNA vaccines are able to stimulate a complete, humoral and cellular immune response. Plasmid DNA is very stable also beyond a cold chain. Therefore, the storage, transportation, and distribution of DNA vaccines are more practical and also cheaper.

Mostly all plasmid DNA constructs used for vaccination share five main characteristics:

- Strong promoter/enhancer sequence for driving the incorporated foreign gene
- Convenient cloning site for insertion of foreign genes
- Origin of replication for initiation of plasmid replication
- Polyadenylation/termination sequence for production of mature mRNA
- Resistance/antibiotic marker for selection.

Present status of DNA Vaccines

After 1990, many groups of workers World-wide have been trying to develop DNA vaccines against several diseases. Genetic immunization has been done against a number of pathogenic organisms. These include influenza A virus, rabies virus, hepatitis B virus, herpes virus, HIV and plasmodium species (malarial parasite). It must be noted that DNA vaccines have not

been tried in humans for unknown risks of these foreign DNAs.

Recombinant vaccines for COVID-19

SARS-CoV-2, the causative agent of COVID-19, has imposed a major public health threat, which needs effective therapeutics and vaccination strategies. Several potential candidate vaccines being rapidly developed are in clinical evaluation. Considering the crucial role of SARS-CoV-2 spike (S) glycoprotein in virus attachment, entry, and induction of neutralizing antibodies, S protein is being widely used as a target for vaccine development. Based on advances in techniques for vaccine design, in activated, live-vectored, nucleic acid, and recombinant COVID-19 vaccines are being developed and tested for their efficacy.

16.4 INTERFERONS

Interferons (IFNs) are proteins made and released by host cells in response to the presence of pathogens such as viruses, bacteria, parasites, or tumor cells. IFNs belong to the large class of glycoproteins known as cytokines. Interferons are named after their ability to "interfere" with viral replication within host cells. IFNs have other functions: they activate immune cells, such as natural killer cells and macrophages, they increase recognition of infection or tumor cells by up-regulating antigen presentation to T lymphocytes, and they increase the ability of uninfected host cells to resist new infection by virus. Certain symptoms, such as aching muscles and fever, are related to the production of IFNs during infection (Fig.16.3).

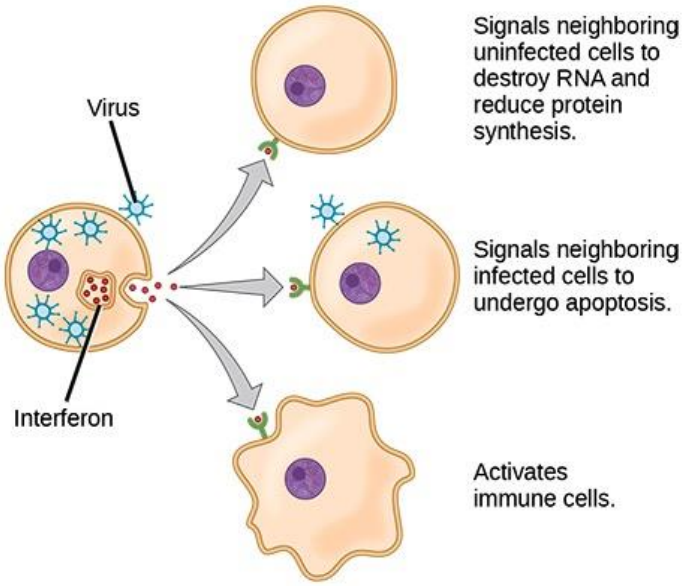

Figure-16.3: Mechanism of Interferon

About ten distinct IFNs have been identified in mammals; seven of these have been described for humans. They are typically divided among three IFN classes: type I IFN, type II IFN, and type III IFN. IFNs belonging to all IFN classes are very important for fighting viral infections.

Based on the type of receptor through which they signal, human interferons have been classified into three major types:

- **Interferon type I:** All type I IFNs bind to a specific cell surface receptor complex, known as the IFN-α receptor (IFNAR) that consists of IFNAR1 and IFNAR2 chains. The type I interferons present in humans are IFN-α, IFN-β and IFN-ω.

- **Interferon type II:** These bind to IFNGR that consist of IFNGR1 and IFNGR2 chains. In humans this is IFN-γ.
- **Interferon type III:** These signal through a receptor complex consisting of IL10R2 (also called CRF2-4) and IFNLR1 (also called CRF2-12). Acceptance of this classification is less universal than that of type I and type II, and unlike the other two, it is not currently included in *Medical Subject Headings*.

Effects of Interferons

All interferons share several common effects; they are antiviral agents and can fight tumors. As an infected cell dies from a cytolytic virus, viral particles are released that can infect nearby cells. In addition, interferons induce production of hundreds of other proteins—known collectively as interferon-stimulated genes (ISGs)—that have roles in combating viruses. They also limit viral spread by increasing p53 activity, which kills virus-infected cells by promoting apoptosis. The effect of IFN on p53 is also linked to its protective role against certain cancers. Another function of interferons is to upregulate major histocompatibility complex molecules, MHC I and MHC II, and increase immunoproteasome activity. Interferons, such as interferon gamma, directly activate other immune cells, such as macrophages and natural killer cells. Interferons can inflame the tongue and cause dysfunction in taste bud cells, restructuring or killing taste buds entirely.

By interacting with their specific receptors, IFNs activate signal transducer and activator of transcription (STAT) complexes. STATs are a family of transcription factors that regulate the expression of certain immune system genes. Some STATs are activated by both type I and type II IFNs. However, each IFN type can also activate unique STATs. STAT activation initiates the most well-defined cell signaling pathway for all IFNs, the classical Janus kinase-STAT (JAK-STAT) signaling pathway.

Key Points

- Interferons are named after their ability to "interfere" with viral replication within host cells.
- IFNs are divided into three classes: type I IFN, type II IFN, and type III IFNs.
- IFNs activate immune cells (natural killer cells and macrophages), increase recognition of infection and tumor cells by up-regulating antigen presentation to T lymphocytes, and increase the ability of uninfected host cells to resist new infection by virus.

16.5 ANTIVIRAL DRUGS

For many years the possibility of treating viral infections with drugs appeared remote because viruses enter host cells and make use of host cell enzymes and constituents. A drug that would block virus reproduction also was thought to be toxic for the host. Inhibitors of virus-specific enzymes and life cycle processes have now been discovered, and several drugs are used therapeutically. Some important examples are shown in figure 16.4.

Most antiviral drugs disrupt either critical stage in the virus life cycle or the synthesis of virus-specific nucleic acids. Amantadine and rimantadine can be used to prevent influenza A infections. When given early in the infection (in the first 48 hours), they reduce the incidence of influenza by 50 to 70% in an exposed population. Amantadine blocks the penetration and un-coating of influenza virus particles. Adenine arabinoside or vidarabine disrupts the activity of DNA polymerase and several other enzymes involved in DNA and RNA synthesis and function. It is given intravenously or applied as an ointment to treat herpes infections. A

third drug, acyclovir, is also used in the treatment of herpes infections. Upon phosphorylation, acyclovir resembles deoxy- GTP and inhibits the virus DNA polymerase. Unfortunately acyclovir- resistant strains of herpes are already developing. Effective acyclovir derivatives and relatives are now available. Valacyclovir is an orally administered pro-drug form of acyclovir. Pro-drugs are inactive until metabolized. Ganciclovir, penciclovir, and its oral form famciclovir are effective in treatment of herpesviruses. Another kind of drug, foscarnet, inhibits the virus DNA polymerase in a different way. Foscarnet is an organic analog of pyrophosphate that binds to the polymerase active site and blocks the cleavage of pyrophosphate from nucleoside triphosphate substrates. It is used in treating herpes and cytomegalovirus infections.

Several broad-spectrum anti-DNA virus drugs have been developed. A good example is the drug HPMPC or cidofovir. It is effective against papovaviruses, adenoviruses, herpesviruses, iridoviruses, and poxviruses. The drug acts on the viral DNA polymerase as a competitive inhibitor and alternative substrate of dCTP. It has been used primarily against cytomegalovirus but also against herpes simplex and human papillomavirus infections.

Research on anti-HIV drugs has been particularly active. Many of the first drugs to be developed were reverse transcriptase inhibitors such as azidothymidine (AZT) or zidovudine, lamivudine (3TC), didanosine (ddI), zalcitabine (ddC), and stavudine (d4T). These interfere with reverse transcriptase activity and therefore block HIV reproduction. More recently HIV protease inhibitors have also been developed. Three of the most used are saquinvir, indinavir, and ritonavir. Protease inhibitors are effective because HIV, like many viruses, translates multiple proteins as a single polypeptide. This polypeptide must then be cleaved into individual proteins required for virus replication. Protease inhibitors mimic the peptide bond that is normally attacked by the protease. The most successful treatment regimen involves a cocktail of agents given at high dosages to prevent the development of drug resistance. For example, the combination of AZT, 3TC, and ritonavir is very effective in reducing HIV plasma concentrations almost to zero.

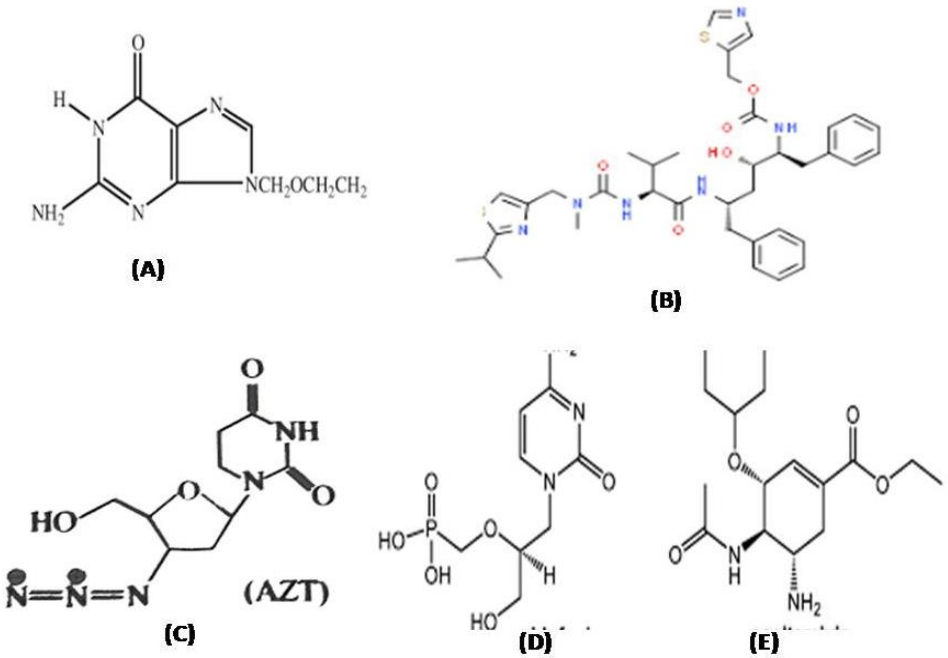

Figure-16.4: Representative Antiviral drugs: A. Acyclovir, B. Ritonavir, C. Azidothymidine, D. Cidofovir, E. Oseltamivir.

16.6 SUMMARY

Vaccination is immunization against infectious disease through the administration of vaccines for the production of active (protective) immunity in humans or other animals. Effective vaccines change the immune system by promoting the development of antibodies that can quickly and effectively attack disease-causing microorganisms when it enters the body, preventing disease development. A vaccine may contain live-attenuated or killed microorganisms or parts or products from them capable of stimulating a specific immune response comprised of protective antibodies and T cell immunity. The viral vaccines should also be able to stimulate high titers of neutralizing antibodies. Injection of a vaccine into a non-immune subject induces active immunity against the modified pathogens.

Interferon is a type of cytokine that activates specific proteins in host cells, leading to the inhibition of viral replication by inducing an antiviral state. Interferons (IFNs) are proteins made and released by host cells in response to the presence of pathogens such as viruses, bacteria, parasites, or tumor cells. IFNs belong to the large class of glycoproteins known as cytokines. Interferons are named after their ability to "interfere" with viral replication within host cells. For many years the possibility of treating viral infections with drugs appeared remote because viruses enter host cells and make use of host cell enzymes and constituents. A drug that would block virus reproduction also was thought to be toxic for the host. Inhibitors of virus-specific enzymes and life cycle processes have now been discovered, and several drugs are used therapeutically.

16.7 TECHNICAL TERMS

Live-attenuated vaccines, Chimeric vaccine, Polysaccharide Vaccines, Conjugated Vaccines, Toxoid Vaccines, Recombinant Protein Vaccines, Nano-particle vaccines, DNA plasmid vaccines, mRNA vaccines, Recombinant vector vaccine, Interferons, Acyclovir, Ritonavir, Azidothymidine, Cidofovir, Oseltamivir.

16.8 SELF ASSESSMENT QUESTIONS

Q.1 Define Vaccination and explain different types of Vaccines.
Q.2 Discuss about whole-organism Vaccines.
Q.3 Explain about Subunit Vaccines and Nucleic Acid Vaccines.
Q.4 Explain in detailed about Preparation and Production of Vaccines.
Q.5 Give an account on New generation vaccines - genetic recombination vaccines
Q.6 Write in detail about Interferons.
Q.7 Explain about different types of antiviral drugs?

16.9 SUGGESTED READINGS

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