SHREE H. N. SHUKLACOLLEGE OF SCIENCE

(AFFILIATED TO SAURASHTRA UNIVERSITY)

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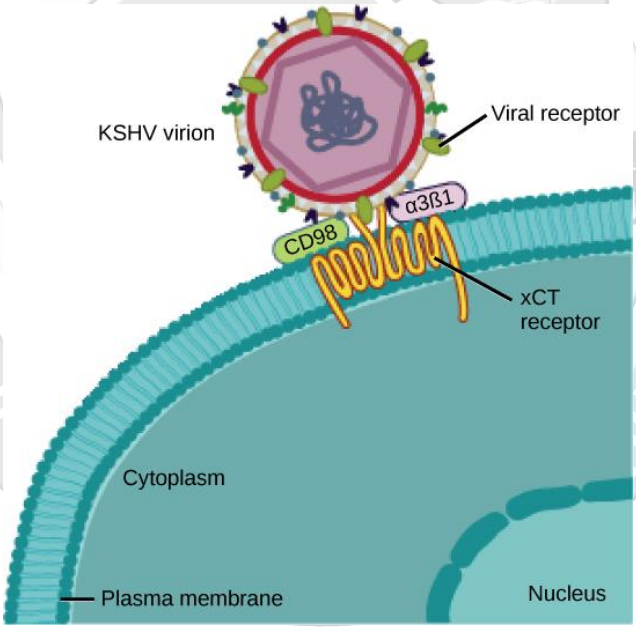
S.Y. B.Sc. (Sem. III) (CBCS)

MICROBIOLOGY

[301]: MICROBIAL DIVERSITY

Unit 5

UNIT 5: AKARYOTIC DIVERSITY



Prepared By

Jinesh Kaneriya

" Sky is the Limit"
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CONTENT

- **Introduction, General Characteristics and Classification (overview of different classifications)**
- **Cultivation of Viruses**
- **Bacterial Viruses: general structure (T4 phage), Lytic life cycle (T4 phage), lysogenic life cycle with genetics (Lambda phage)**
- **Introduction to Animal Viruses: Structure (HIV), Cytocidal effects, Viruses and Cancer, Prions**
- **Introduction to Plant Viruses: Structure of TMV, Economic importance, Viroids**

Title explanation

- In this unit we are going to learn and discuss about different unusual morphology of bacteria like gliding motility, sheathed bacteria, mycoplasma which has no cell wall, and budding bacteria
- Study of rickettsia and chlamydia and which disease caused by them cause in human being.
- Study about archaeabacteria which are extremophiles bacteria they are living in extreme conditions such as temperature, pH, salt concentration, pressure.
- Study of methanogens which can produce methane in strict anaerobic condition.
- Discuss about importance of prokaryotic organisms.

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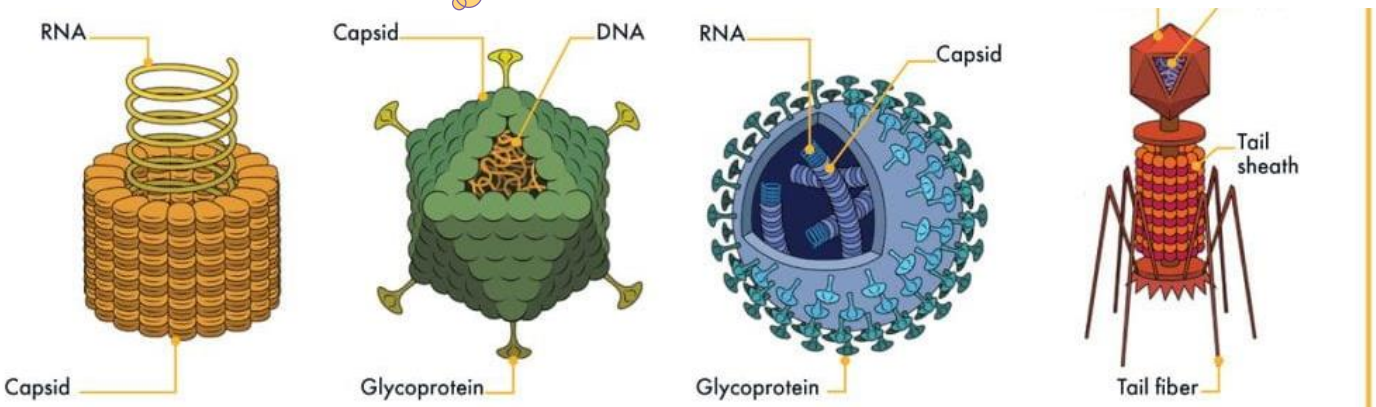
LEARNING OUTCOME



Helical viruses, like the Tobacco Mosaic Virus, which infects a number of different types of plants, have a slinky-shaped capsid that twists around and encloses its genetic material.

Polyhedral viruses, like adenoviruses, which are known to cause a range of illnesses from pink eye to pneumonia, are composed of genetic material surrounded by a many-sided capsid, usually with 20 triangular faces.

Spherical viruses, like the infamous Coronavirus, are essentially helical viruses enclosed in a membrane known as an envelope, which is spiked with sugary proteins that assist in sticking to and entering host cells.

Complex viruses, like bacteriophages, which infect and kill bacteria, resemble a lunar lander, and are composed of a polyhedral "head" and a helical body (or "tail sheath"), and legs (or "tail fibers") that attach to a cell membrane so that it can transfer its genetic material.

Viruses are microscopic organisms that can infect hosts, like humans, plants or animals. They're a small piece of genetic information (DNA or RNA) inside of a protective shell (capsid). Some viruses also have an envelope. Viruses can't reproduce without a host. Some common diseases caused by viruses include the flu, the common cold and COVID-19.

❖ What is a virus?

Viruses are small germs (pathogens) that can infect you and make you sick. They can infect humans, plants, animals, bacteria and fungi. Each one infects only specific types of hosts.

Viral infections in humans can cause no symptoms or make you extremely ill. Types of diseases they can cause include:

- Respiratory illnesses.
- Diarrhea and vomiting.
- Sexually transmitted infections (STIs).
- Skin conditions.

A virus is a small piece of genetic information in a "carrying case" — a protective coating called a capsid. Viruses aren't made up of cells, so they don't have all the equipment that cells do to make more copies of themselves. Instead, they carry instructions with them and use a host cell's equipment to make more copies of the virus.

It's like someone breaking into your house to use your kitchen. The virus brought its own recipe, but it needs to use your dishes, measuring cups, mixer and oven to make it. (Unfortunately, they usually leave a big mess when you finally kick them out.) Viruses are also sometimes called "virions."

Virus features

→ Viruses share some common features. Viruses:

→ Are made up of genetic material (RNA or DNA) and a protective protein coating (capsid).

→ Sometimes have another layer called an envelope around the capsid. Viruses without an envelope are called "naked viruses."

→ Are similar to parasites — they need a host to reproduce. They'll survive outside of a host until their capsid breaks down over time.

→ Are 100 to 1,000 times smaller than the cells in your body.

Classification of virus

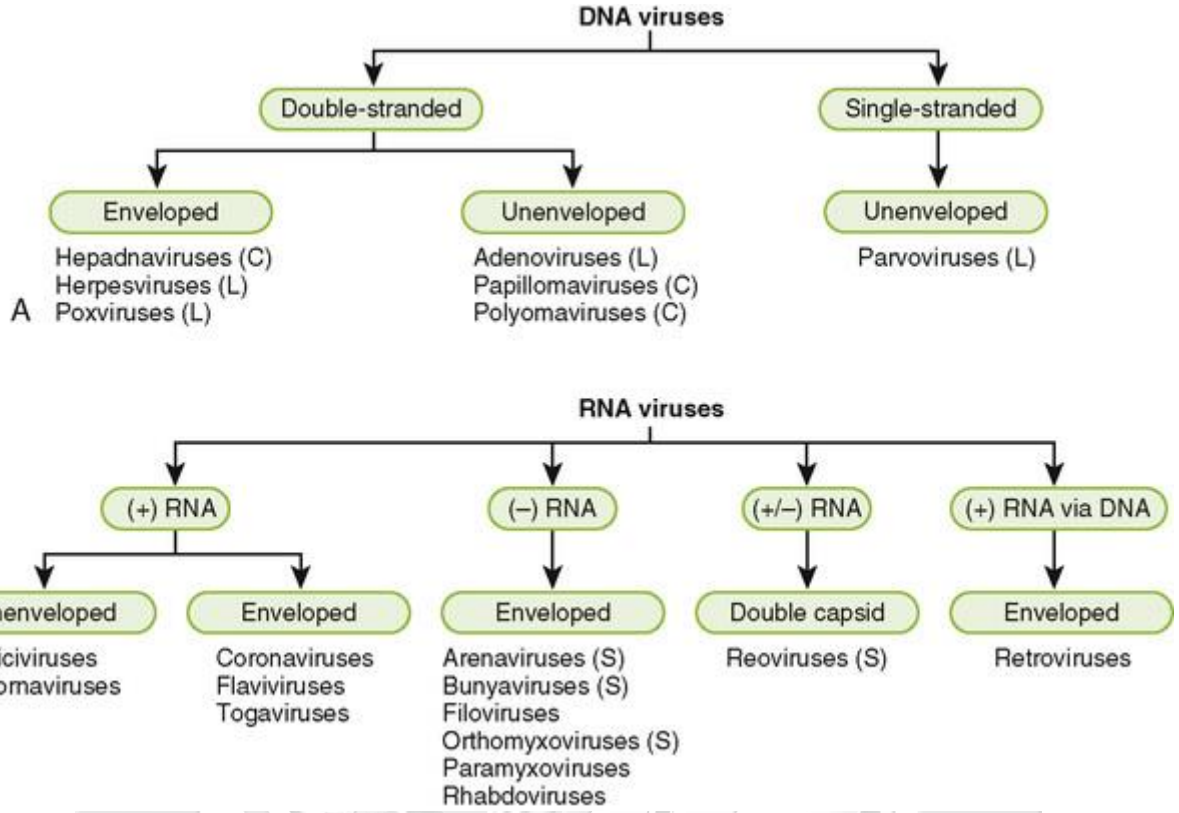
A] Classification on the basis of nucleic acid

B] Classification on the basis of structure or symmetry

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

D] Classification on the basis of host range

E] Classification on the basis of mode of transmission



1. DNA virus: viral genome is DNA

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

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

2. RNA virus: genome is RNA

i) Double stranded RNA virus: eg. Reo virus

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

Positive sense RNA (+RNA): Polio virus, Hepatitis A

Negative sense RNA (-RNA): Rabies virus, Influenza virus

Techniques of Virus Cultivation

Viruses are obligate intracellular parasites so they depend on host for their survival. They cannot be grown in non-living culture media or on agar plates alone, they must require living cells to support their replication. The primary purpose of virus cultivation is:

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

Cultivation of viruses can be discussed under following headings:

1. Animal Inoculation
2. Inoculation into embryonated egg
3. Cell Culture

1. Animal Inoculation

- Viruses which are not cultivated in embryonated egg and tissue culture are cultivated in laboratory animals such as mice, guinea pig, hamster, rabbits and primates are used.
- The selected animals should be healthy and free from any communicable diseases.
- Suckling mice (less than 48 hours old) are most commonly used.
- Suckling mice are susceptible to togavirus and coxsackie virues, which are inoculated by intracerebral and intranasal route.
- Viruses can also be inoculated by intraperitoneal and subcutaneous route.
- After inoculation, virus multiply in host and develops disease. The animals are observed for symptoms of disease and death.
- Then the virus is isolated and purified from the tissue of these animals.
- Live inoculation was first used on human volunteers for the study of yellow fever virus.

Advantages of Animal Inoculation

1. Diagnosis, Pathogenesis and clinical symptoms are determined.
2. Production of antibodies can be identified.
3. Primary isolation of certain viruses.

4. Mice provide a reliable model for studying viral replication.
5. Used for the study of immune responses, epidemiology and oncogenesis.

Disadvantages of Animal Inoculation

1. Expensive and difficulties in maintenance of animals.
2. Difficulty in choosing of animals for particular virus
3. Some human viruses cannot be grown in animals,or can be grown but do not cause disease.
4. Mice do not provide models for vaccine development.
5. It will lead to generation of escape mutants
6. Issues related to animal welfare systems.

Inoculation into embryonated egg

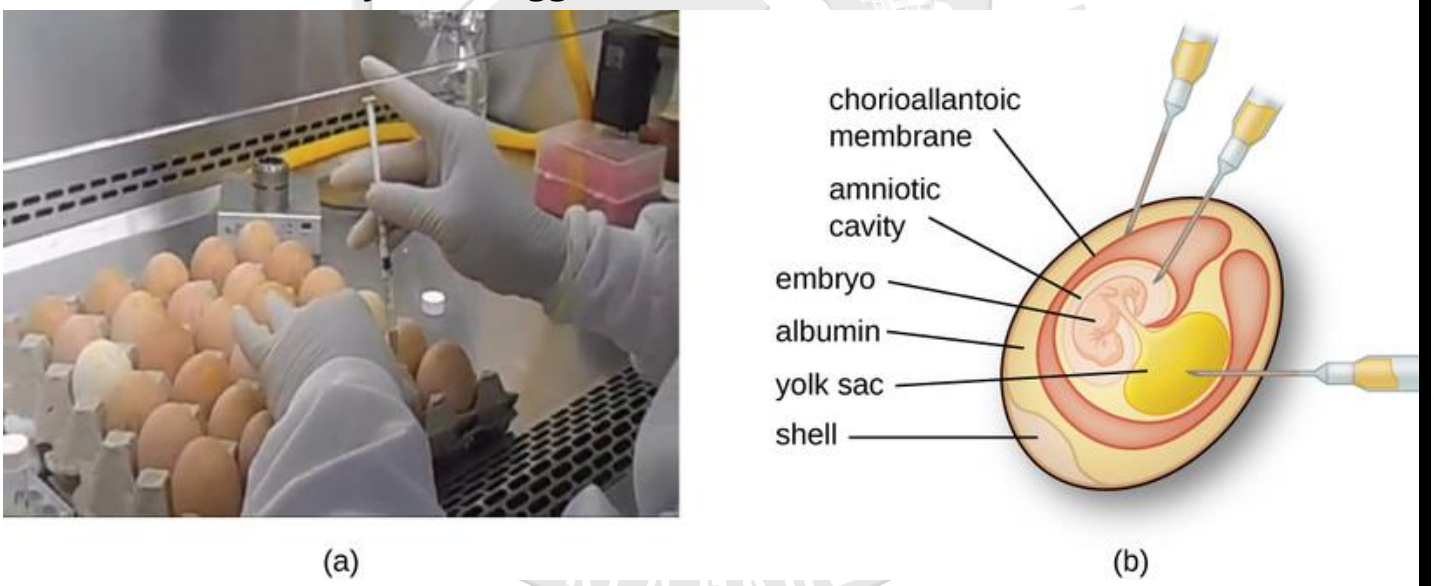


Figure: (a) The cells within chicken eggs are used to culture different types of viruses. (b) Viruses can be replicated in various locations within the egg, including the chorioallantoic membrane, the amniotic cavity, and the yolk sac.

- Good pasture in 1931 first used the embryonated hen's egg for the cultivation of virus.
- The process of cultivation of viruses in embryonated eggs depends on the type of egg which is used.
- Viruses are inoculated into chick embryo of 7-12 days old.

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

1. Chorioallantoic Membrane (CAM):

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

2. Allantoic cavity:

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

3. Amniotic sac:

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

4. Yolk sac inoculation:

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

Advantages of Inoculation into embryonated egg

1. Widely used method for the isolation of virus and growth.
2. Ideal substrate for the viral growth and replication.
3. Isolation and cultivation of many avian and few mammalian viruses.

4. Cost effective and maintenance is much easier.
5. Less labor is needed.
6. The embryonated eggs are readily available.
7. Sterile and wide range of tissues and fluids
8. They are free from contaminating bacteria and many latent viruses.
9. Specific and non specific factors of defense are not involved in embryonated eggs.
10. Widely used method to grow virus for some vaccine production.

Disadvantages of Inoculation into embryonated egg

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

Cell Culture (Tissue Culture)

There are three types of tissue culture; organ culture, explant culture and cell culture.

Organ cultures are mainly done for highly specialized parasites of certain organs e.g. tracheal ring culture is done for isolation of coronavirus.

Explant culture is rarely done.

Cell culture is mostly used for identification and cultivation of viruses.

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

Types of cell culture

1. Primary cell culture:

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

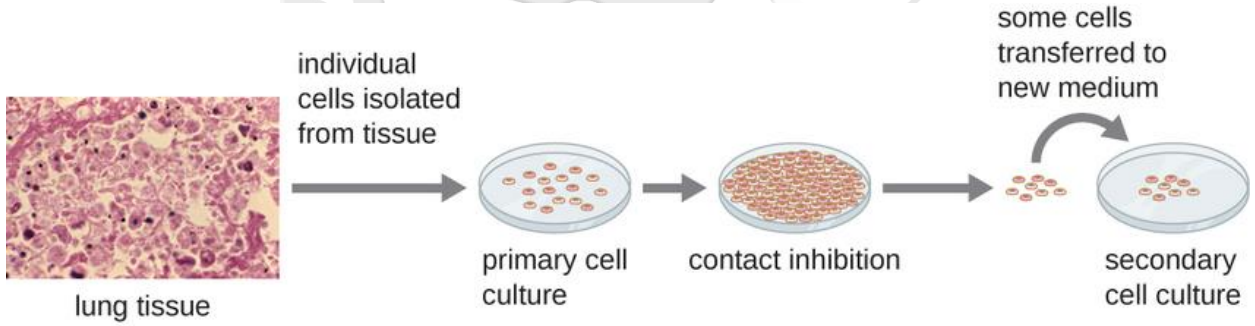
2. Diploid cell culture (Semi-continuous cell lines):

- They are diploid and contain the same number of chromosomes as the parent cells.

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

3. Heteroploid cultures (Continuous cell lines):

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



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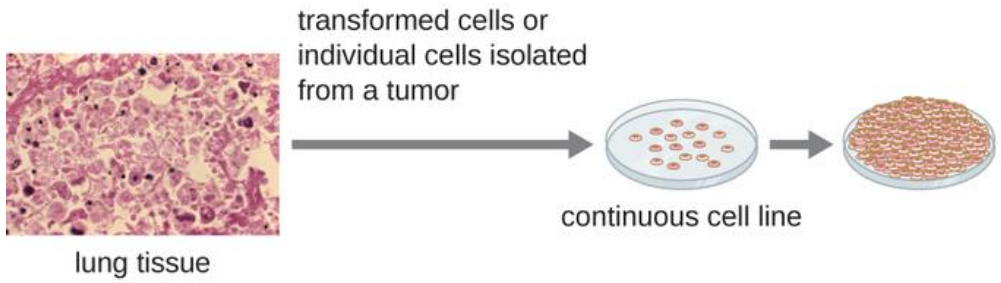

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Advantages of cell culture

1. Relative ease, broad spectrum, cheaper and sensitivity

Disadvantage of cell culture

1. The process requires trained technicians with experience in working on a full time basis.
2. State health laboratories and hospital laboratories do not isolate and identify viruses in clinical work.

Bacterial Viruses: general structure (T4 phage)

- The T4 virus is a bacteriophage that infects the members of the bacterial species Escherichia coli and thus, is also known as Escherichia virus T4.
- The virus is one of the seven Escherichia coliphages (name T1-T7), which were discovered by Delbruck and coworkers in 1944 as models to study different mechanisms of the phage community.
- The bacteriophage T4 belongs to the Caudovirales order of the Myoviridae family of bacteriophages based on the presence of a non-enveloped head and contractile tail.
- The structure of bacteriophage T4 consists of a protein capsid, called, head which consists of a linear double-stranded DNA molecule.
- At the end of the tail is a 925 Å long and 520 Å diameter contractile tail attached to a special portal at the base of the head.
- There are six short tail fibers emerging from the baseplate that can recognize receptor molecules on the host surface.
- Bacteriophage T-even viruses are among the most commonly studied and researched group of bacteriophages that also are similar to one another in various factors.
- These are also one of the largest and most complicated groups of bacterial viruses as their genetic makeup is made up of about 300 different genes.

Life Cycles of Bacteriophage

Viruses enter the host cell to reproduce during which the virus results in different forms of infections to the host cell. The overall process of the entry of the virus, its replication, and exit from the host cell comprises the lifecycle of viruses. Bacteriophages, like all other viruses, follow a similar trajectory where the virus enters the bacterial host cell in order to replicate. There are two types of lifecycles that differ in the mechanism of DNA replication where, in one, the viral DNA is incorporated into the host DNA, but in the other, the DNA replicates separately from the host DNA. These lifecycles might occur independently or alternatively in different types of bacteriophages.

1. Lytic Cycle

- The lytic cycle is one of the two lifecycles of bacteriophages where the viral DNA remains as a free-floating molecule and replicates separately from the bacterial DNA.
- The lytic cycle usually occurs in virulent phages as the phages result in the destruction of the infected cell membrane during the release of the viral particles.
- The lytic cycle is a virulent infection as it results in the destruction of a cell.

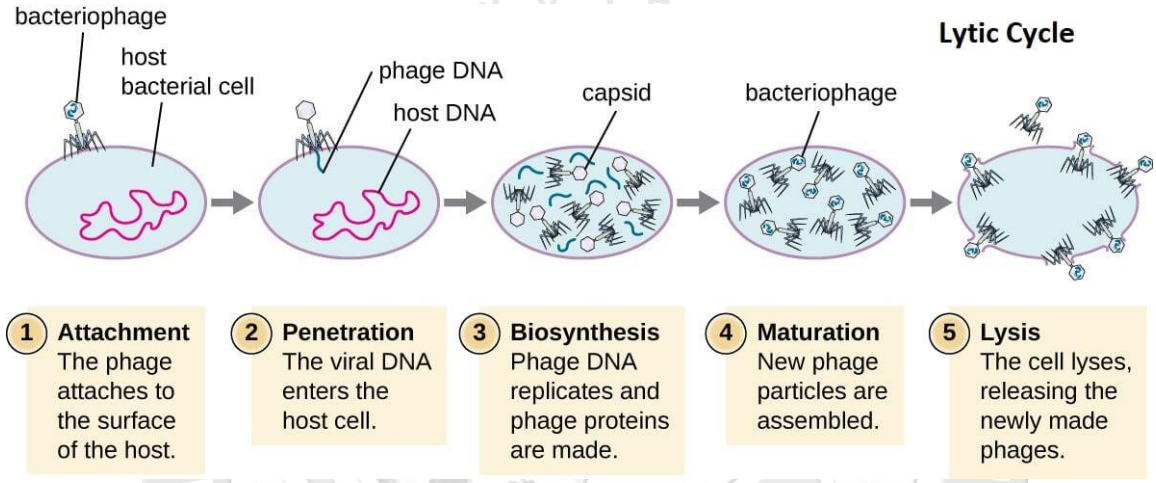


Figure: Lytic Cycle of Bacteriophage. Image Source: Openstax Microbiology.

a. Attachment and Penetration

- The first step in the lifecycle of a bacteriophage is attachment, where the ligands on specific molecules on the surface of the viral particles bind to the receptor molecules on the plasma membrane of the host cell.
- The receptors depend on the type of viruses as most orthomyxoviruses use receptors like terminal sialic acid on an oligosaccharide side chain of a cellular glycoprotein.
- The ligand, however, is an aperture at the distal end of each monomer of the trimeric viral hemagglutinin glycoprotein.
- Even though there is a high degree of specificity between the receptors and the ligands, a number of viruses might use the same receptors.
- Besides, some bacteriophages might use other membrane glycoproteins as their receptors.

- Once attached, the virus injects its nuclear material into the cytoplasm of the bacterial cell.
- The viral genome (either DNA or RNA) remains in the cytoplasm, and in some cases becomes circular and resemble the bacterial plasmid.

b. Biosynthesis and Transcription

- Once in the cytoplasm, the viral genome hijacks the host cellular mechanism and utilizes it to produce more viruses.
- In the case of DNA viruses, the DNA undergoes transcription to produce messenger RNA that then directs the ribosome of the host cell.
- In the case of the lytic cycle, the mRNA encodes for various polypeptides, the first of which destroy the host's DNA.
- In the case of RNA viruses, an enzyme called reverse transcriptase is involved which transcribes the viral RNA into DNA.
- The DNA is then transcribed back to mRNA, which then directs the destruction of host DNA.
- The viral DNA then takes control of the host cell and produces different proteins required for the assembly of new viruses.
- The viral DNA also undergoes replication to produce more genetic material for new viral particles.
- The process of biosynthesis and DNA replication is mediated by different genes and enzymes.

c. Assembly and Lysis

- As biosynthesis and replication continue, a large number of viral proteins and genomes are formed.
- Once enough viral particles are formed and matured, these particles under assembly during which the genetic material of the virus is incorporated into the viral protein, capsid.
- The newly assembled bacteriophages release the enzyme, lysin, into the cytoplasm. The enzyme causes the lysis of the bacterial cell wall, resulting in the release of newly formed phage particles.
- Thus, at the end of the lytic lifecycle, the infected bacterial cell and cell membrane are destroyed.

2. Lysogenic Cycle

- Lysogenic is one of the two lifecycles of bacteriophages defined by the incorporation of the bacteriophage genome into the host genome.
- During the lysogenic lifecycle, the host bacteria continue to live and reproduce normally after the replication of bacteriophages.
- The genetic material of bacteriophage incorporated in the bacterial DNA during the lysogenic lifecycle is called a prophage which can be transmitted to daughter cells during the bacterial cell division.
- The lysogenic cycle is a temperate and non-virulent infection as the bacteriophage doesn't kill the host cell.

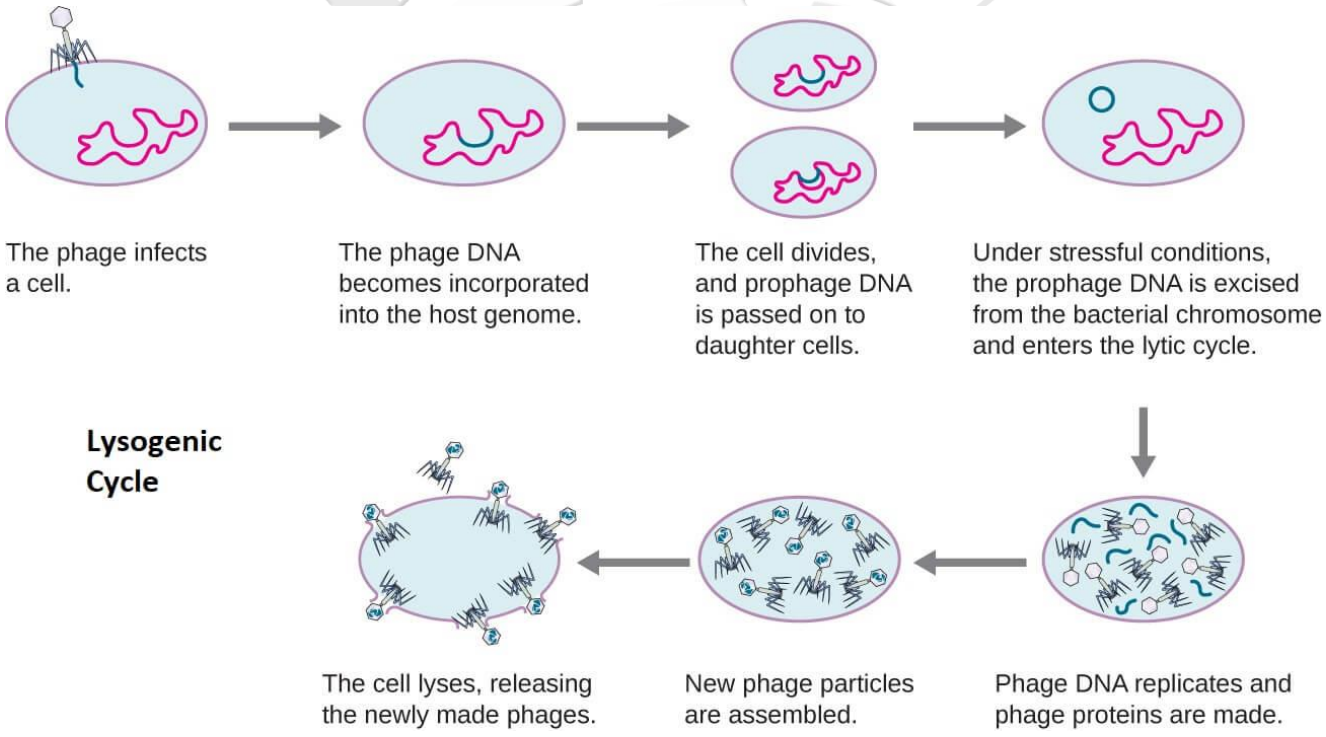


Figure: Lysogenic Cycle of Bacteriophage. Image Source: Openstax Microbiology.

The process of lysogenic lifecycle occurs in the following steps;

a. Attachment and Penetration

- The first step of the lysogenic lifecycle is identical to the first step of the lytic lifecycle.
- The bacteriophage ligands attach to the receptors on the surface of the bacterial cell wall.
- The attachment is highly specific as it is determined by the interaction between the ligands and the receptors present on the surface of the bacterial cell wall.
- After attachment, the viral genome is injected into the cytoplasm of the host cell.
- The infective viral DNA or prophage is then incorporated into the host chromosome, which converts the infective prophage into a non-infective prophage.

b. Replication

- The viral DNA then uses the host machinery to replicate as it continues to replicate with the host chromosomes during cell division.
- In some cases, the prophage might be ejected from the host chromosome, and the viral DNA might enter the lytic cycle.
- Unlike the lytic cycle, the bacterial cellular mechanism is not hijacked by the viral particles, and no biosynthesis of viral proteins takes place.
- The prophage, however, can be transferred to the daughter cells during the bacterial cell division.
- The process of replication continues until there are some stressors which can either be physical stressors like UV radiation, low nutrient condition or chemical, which might result in the transition of the lysogenic cycle into the lytic cycle.
- Once converted into the lytic cycle, the viral DNA undergoes transcription to produce viral proteins. The proteins and viral genome are then assembled to form complete viral particles which then are released from the host cell by lysis.

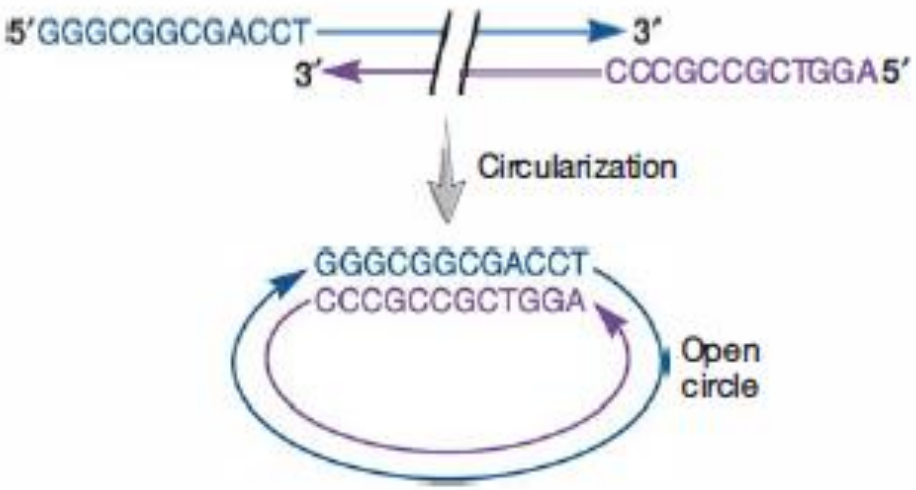
Genetics (Lambda Phage)

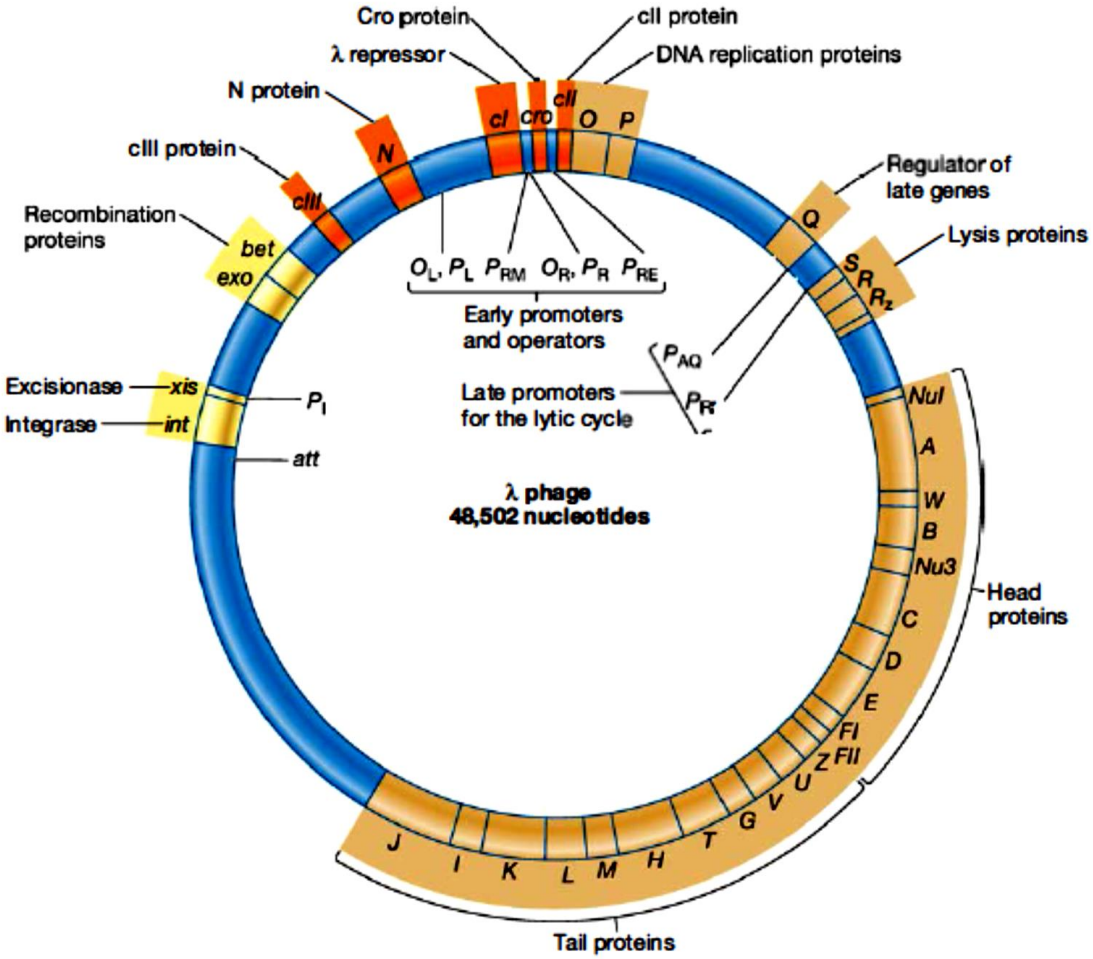
Unlike bacteriophage T4, temperate bacteriophages, such as phage lambda (family Siphoviridae, species Enterobacteria phage lambda), can enter either the lytic or lysogenic cycle upon infecting a host

cell. If a temperate virus enters the lysogenic cycle, its dsDNA genome often is integrated into the host's chromosome, where it resides as a prophage until conditions for induction occur Upon induction, the viral genome is excised from the host genome and the lytic cycle is initiated Like most bacteriophages, 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 X. genome has been carefully mapped, and over 40 genes have been located (figure 27.11). 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 promoters.





The complete sequence of the HIV-1 genome, extracted from infectious virions, has been solved to single-nucleotide resolution. The HIV genome encodes a small number of viral proteins, invariably establishing cooperative associations among HIV proteins and between HIV and host proteins, to invade host cells and hijack their internal machineries. HIV is different in structure from other retroviruses. The HIV virion is ~100 nm in diameter. Its innermost region consists of a cone-shaped core that includes two copies of the (positive sense) ssRNA genome, the enzymes reverse transcriptase, integrase and protease, some minor proteins, and the major core protein. The genome of human immunodeficiency virus (HIV) encodes 8 viral proteins playing essential roles during the HIV life cycle.

HIV-1 is composed of two copies of noncovalently linked, unspliced, positive-sense single-stranded RNA enclosed by a conical capsid composed of the viral protein p24, typical of lentiviruses. The two copies of RNA strands are vital in contributing to HIV-1 recombination, which occurs during reverse transcription of viral replication. The containment of two copies of single-stranded RNA within a virion but the production of only a single DNA provirus is called pseudodiploidy. The RNA component is 9749 nucleotides long[12][13] and bears a 5' cap (Gppp), a 3' poly(A) tail, and many open reading frames (ORFs). Viral structural proteins are encoded by long ORFs, whereas smaller ORFs encode regulators of the viral life cycle: attachment, membrane fusion, replication, and assembly.

The single-strand RNA is tightly bound to p7 nucleocapsid proteins, late assembly protein p6, and enzymes essential to the development of the virion, such as reverse transcriptase and integrase. Lysine tRNA is the primer of the magnesium-dependent reverse transcriptase. The nucleocapsid associates with the genomic RNA (one molecule per hexamer) and protects the RNA from digestion by nucleases. Also enclosed within the virion particle are Vif, Vpr, Nef, and viral protease. The envelope of the virion is formed by a plasma membrane of host cell origin, which is supported by a matrix composed of the viral p17 protein, ensuring the integrity of the virion particle. At the surface of the virion can be found a limited number of the envelope glycoprotein (Env) of HIV, a trimer formed by heterodimers of gp120 and gp41. Env is responsible for binding to its primary host receptor, CD4, and its co-receptor (mainly CCR5 or CXCR4), leading to viral entry into its target cell.

Prions

A prion is a type of protein that can cause disease in animals and humans by triggering normally healthy proteins in the brain to fold abnormally. The prion mode of action is very different to bacteria and viruses as they are simply proteins, devoid of any genetic material. Once a misfolded prion enters a healthy person – potentially by eating infected food – it converts correctly-folded proteins into the disease-associated form. To date, nobody knows quite how this happens.

Prions in "mad cow" brain. Coloured transmission electron micrograph (TEM) of prion fibrils in the brain of a cow infected with BSE (Bovine Spongiform Encephalopathy) or "mad cow" disease. Prions are virus-like organisms made up of a prion protein. These elongated fibrils (green) are believed to be

aggregations of the protein that makes up the infectious prion. Prions attack nerve cells producing neurodegenerative brain disease. "Mad cow" symptoms include glazed eyes and uncontrollable body tremor. Prions cause BSE in cattle; scrapie in sheep and goats; and Creutzfeldt-Jakob disease in humans.

Structure of Prions

Prions are found all over the body but the ones that cause diseases are structurally different. Few of them are even resistant to proteases. The two isoforms of prions are:

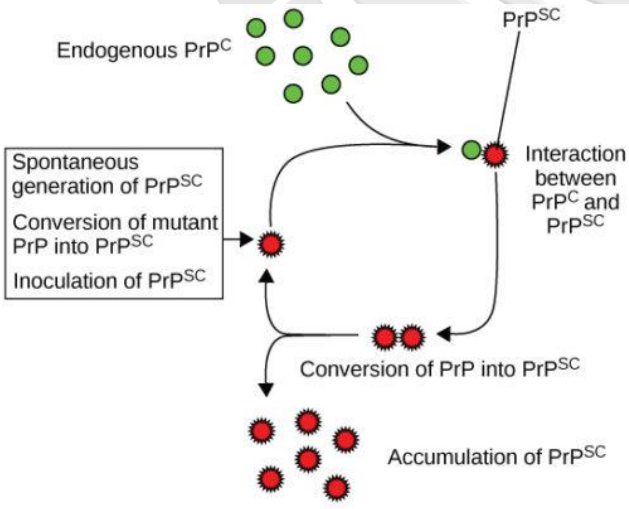
PrPc

These prion proteins are found on the cell membrane and play an important role in cell signalling and cell adhesion. More research is being carried out to discover its functions.

PrPsc

This is the disease-causing prion and is resistant to proteases. It affects the confirmation of PrPc and changes it. They are believed to have more beta sheets than the alpha helices.

It also forms highly structured amyloid fibres. The other free proteins also attach to the end of these fibres. Similar prions with similar amino acids can only bind. However, cross-species binding is also possible, but is very rare.



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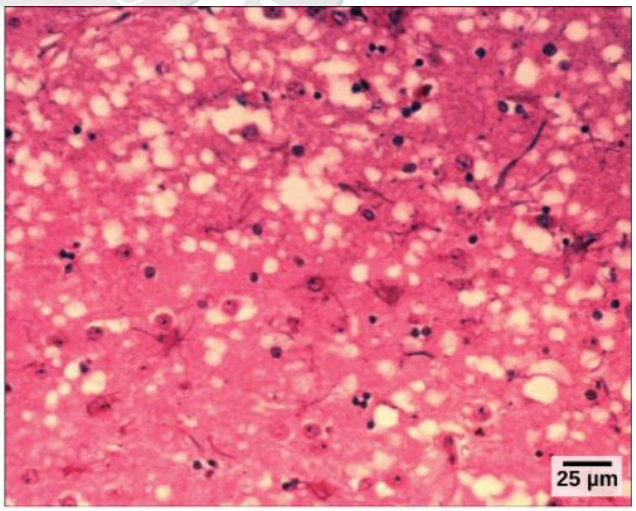


(b)

Figure: (PrP^{sc}) when it encounters this variant form of the protein. PrP^{sc} may arise spontaneously in brain tissue, especially if a mutant form of the protein is present, or it may occur via the spread of misfolded prions consumed in food into brain tissue. (b) This prion-infected brain tissue, visualized using light microscopy, shows the vacuoles that give it a spongy texture, typical of transmissible spongiform encephalopathies

Diseases Caused by Prions

Prions are quite rare and difficult to transmit. But they are progressive neurodegenerative diseases with no cure or treatment. These diseases develop gradually. These proteins affect many other animals in addition to humans. For eg., scrapie in sheep, mad cow disease in cows, chronic wasting disease in deer. The prion diseases in humans are Creutzfeldt-Jakob disease, Fatal Familial Insomnia, kuru disease, etc. Prions can spread in a person's brain for years without any symptoms. The prions start killing neurons and the symptoms strike the brain in no time. Soon the person's health starts declining. All the prion diseases are fatal, some last a few months, and some might last for years. A few experimental pieces of evidence show that the prions are not ordinary infectious materials. It is believed to be a "self-replicating protein".

Types of Prion Diseases

Prion diseases can be of three types- acquired, sporadic, or genetic.

Acquired Prion Disease

The acquired prion diseases occur when a person is exposed to the infectious protein. Though scary, these prions are rarely caught by the people. For eg., in kuru diseases, the prions were transmitted to people by cannibalism. Its main source was New Guinea pig.

Genetic Prion Disease

The familial prion diseases are caused as a result of genetic transmissions. However, it is not necessarily inherited from the ancestors. It may be caused due to the mutation in some DNA.

Sporadic Prion Disease

Prion diseases are also believed to be sporadic. This means that its cause is not confirmed. This form of prion disease is most common to date.

Causes of Prion Diseases

The main cause of prion diseases is the abnormal folding and clumping of prions in the brain causing brain damage. This leads to memory impairment, changes in the personality, difficulties in moving. Prions are by far the most dangerous infections caused by the agents already present within the body and are usually fatal. However, a lot has not been discovered about prion diseases.

Viroids

The photo shows shriveled, cracked potatoes.

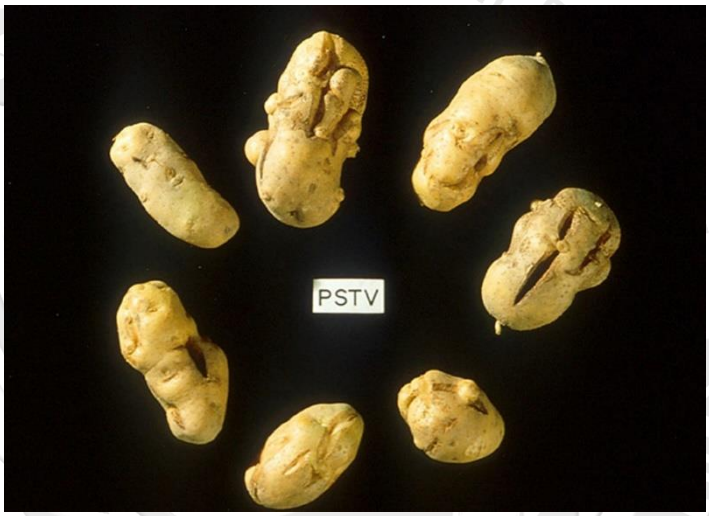


Figure: These potatoes have been infected by the potato spindle tuber viroid. (credit: Pamela Roberts, University of Florida Institute of Food and Agricultural Sciences, USDA ARS)

Viroids are plant pathogens: small, single-stranded, circular RNA particles that are much simpler than a virus. They do not have a capsid or outer envelope, but like viruses can reproduce only within a host cell. Viroids do not, however, manufacture any proteins, and they only produce a single, specific RNA molecule. Human diseases caused by viroids have yet to be identified.

Viroids are known to infect plants and are responsible for crop failures and the loss of millions of dollars in agricultural revenue each year. Some of the plants they infect include potatoes, cucumbers,

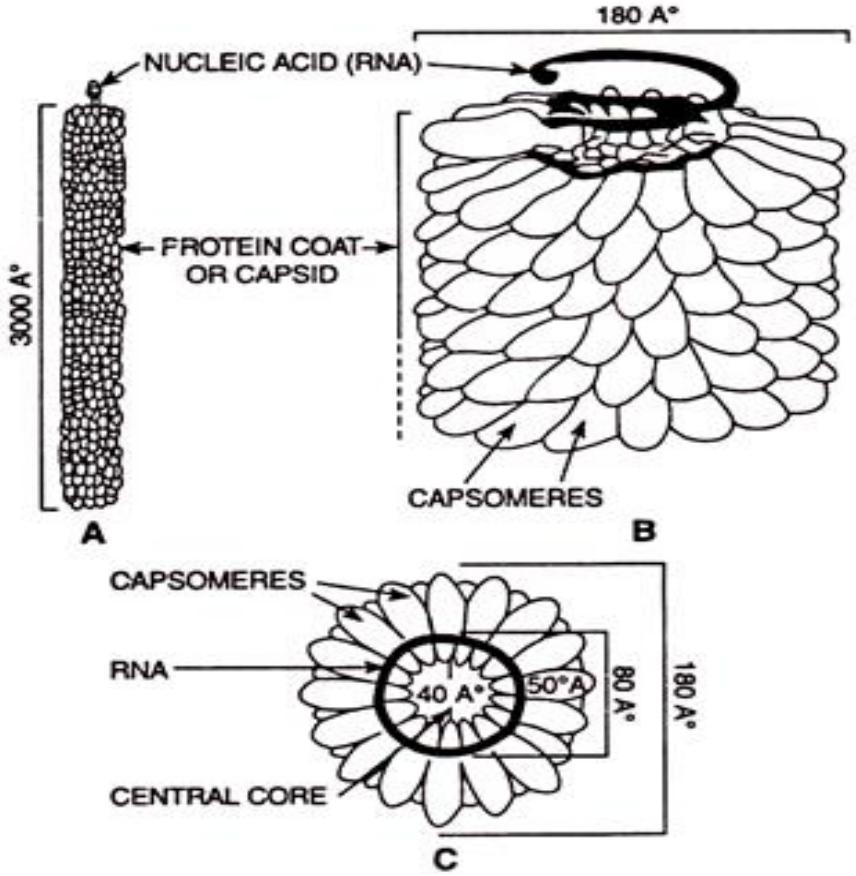
tomatoes, chrysanthemums, avocados, and coconut palms. For example, the potato spindle tuber viroid (PSTVd), which typically spreads when infected knives cut healthy potatoes in preparation for planting, can affect potatoes and tomatoes.

Tobacco Mosaic Virus (TMV): Structure and Replication

Structure of Tobacco Mosaic Virus (TMV):

TMV is a simple rod-shaped helical virus (Fig. 13.20) 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 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 dalton as determined by Knight. Tobacco mosaic virus The ssRNA is little more in length (about 3300 Å) slightly protruding from one end of the rod. The RNA molecule consists of about 7300 nucleotides; the molecular weight of the RNA molecule being about 25,000 dalton.



Life-Cycle (Replication) 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. 13.21). 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 ha alter 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.

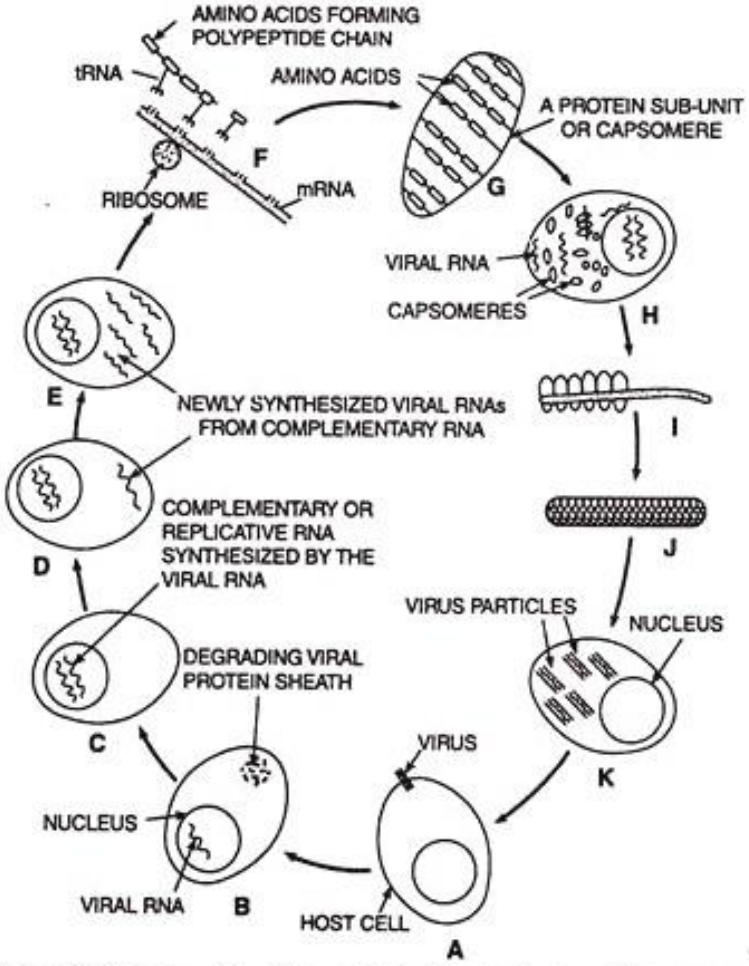


FIG. 13.21. Replication of TMV (diagrammatic). **A.** Virus particle entering inside the cell of the host plant; **B. & C.** Viral RNA enters inside the nucleus and synthesizes its complementary copy; **D. & E.** Complementary RNA synthesizes new viral RNA that comes in the cytoplasm; **F.** Polypeptide chain synthesis; **G., H. & I.** Arrangement of capsomeres around viral-RNA; **J.** Complete virus particle; **K.** Host cell containing many virus particles.

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 ells 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.