Government College (Autonomous) Rajamahendravaram

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Department of Microbiology

II Semester
Question bank for the course:
BACTERIOLOGY AND VIROLOGY

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Question bank- Essay type Questions:

1.Give a brief account on Biological systems of classification and branches of taxonomy

Introduction: Biological classification is a scientific method that involves grouping organisms into a hierarchical series of groups and subgroups based on their similarities and differences. Three types of systems of classification have been recognized-(1) Artificial (2) Natural (3) Phylogenetic

(1) Artificial system of classification: In an artificial system of classification gross, superficial and morphological characteristics are taken into account for classification.Vegetative (habit, number ,shape and colour of the leaf) and sexual characters (structure of androecium) give equal importance which is not acceptable as vegetative characters are more easily affected by the environment.

Example:

a.Aristotle:
i. He used simple morphological characters to classify plants into herbs, shrubs and trees.
ii. He classified animals into Anaima and Enaima, on the basis of absence and presence of RBCs   respectively.
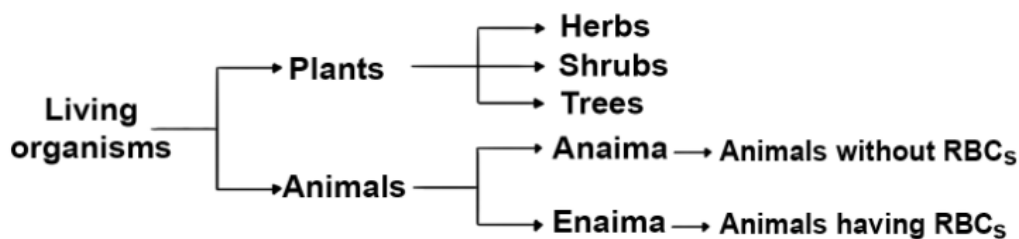
(2) The natural system of classification

- It is based on the affinities between organisms

- It takes into account not only external but internal features like ultrastructure, anatomy, embryology, and phytochemistry for classification.

- George Bentham and Joseph Dalton Hooker, both of whom were closely involved with the Royal Botanic Garden at Kew, England, proposed a natural system of classification of angiosperms that was published in 'Genera Plantarum' in 3 volumes.

- They classified the plant kingdom into two subkingdoms–Cryptogamia and Phanerogamia. The phanerogamia are classified into three classes–Dicotyledons, Gymnosperms and Monocotyledons.

Phylogenetic Classification (Cladistics):
- Evolutionary history of the organism is called Phylogeny.

  - These systems are based on Phylogenetic relationships of organisms.

  - Phylogenetic systems are also called Cladistics (Systematic classification based on evolutionary relationships of organisms in order of their assumed divergence from ancestral forms) and the graphic representation of evolutionary relationships is called family tree or Cladogram.

  - Engler and Prantl proposed the phylogenetic classification and published it in their book "Die Natürlichen Pflanzenfamilien" in 23 volumes.

  - Engler and Prantl divided plant kingdom into two sub kingdom (1) Cryptogamia: invisible sex organs e.g. Thallophyta, Bryophyta and Pteridophyta.; (2) Phanerogamia: visible sex organs e.g. Gymnosperm and Angiosperm.

  - Later on, well-developed phylogenetic systems of classification were created by Hutchinson, Tippo, Takhtajan, Robert Whitaker, Robert Thorne and Cronquist.

  - Oswald Tippo proposed the biggest phylogenetic classification of the plant kingdom and it is most accepted for books and study.

Fossil records are the most important evidence in systematics but if fossil records are not available then other branches of taxonomy like cytotaxonomy, numerical, chemotaxonomy etc. play important roles to find out phylogeny.

Branches of Taxonomy-

Introduction:Characterization, identification, classification and nomenclature are the processes that are basic to taxonomy.Based on characteristics, all living organisms can be classified into different taxa. This process of classification is taxonomy. External (Morphology) and internal structure (Anatomy), along with the structure of cells (Cytology), development process and ecological information of organisms are essential and form the basis of modern taxonomic studies.

1. Cytotaxonomy:

- It is a classification based on comparative cytological structure, the number of chromosomes, and the shape and meiotic behavior of chromosomes.
- In order to understand the interrelationship between different organisms the most important parameter is the chromosomal configuration.
- It has wide application in classification of plants as one of the lead parameters, e.g., The chromosomal number is a reliable trait for identifying grasses.

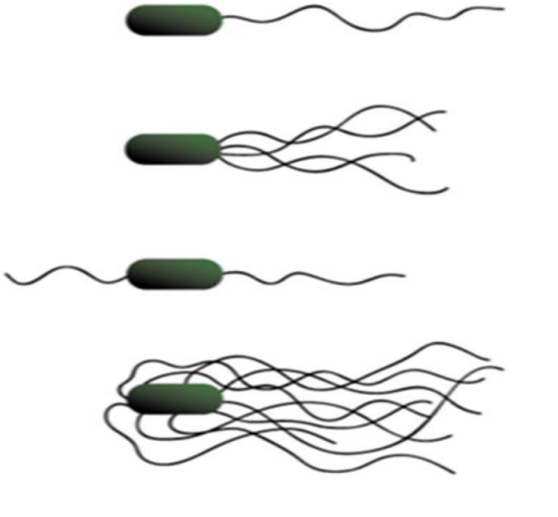## 2. Chemotaxonomy (Biochemical Systematics)

- It is a classification method based on amino acids, proteins, DNA sequences, alkaloids, crystals, betacyanins, and other chemical elements of organisms. Plant chemical components are often distinct and stable.

- They are not easily swayed. Plants were identified by their scent, flavor, and other chemical qualities in ancient times.

- Only 35 families of calcium oxalate crystals, such as raphides, exist. Similarly, some alkaloids are only found in a few closely related families, such as the benzylisoquinoline alkaloid found in the Papaveraceae, Berberidaceae, and Ranunculaceae families.

3. Numerical Taxonomy/Phenetics

- It uses statistical methods to assess resemblances and differences, as well as primitiveness and advancement, based on a huge number of features gathered from several areas of biology.

- In the 1950s, phenetics, often known as numerical taxonomy, was introduced.

- Phenetics is a branch of biology that aims to classify species into higher taxa based on overall resemblance, usually in appearance or other visible qualities, rather than phylogeny or evolutionary relationships. It was developed by Adanson which is based on all observable characteristics and each character is given equal importance. Hundreds of characters can be considered at the same time. Numbers and codes are assigned to all characters and data and then processed using a computer or statistical method.

- Sneath and Sokal (1973) gave various examples of numerical taxonomy being used in several angiosperm taxa, including Apocynum, Crotalaria, Cucurbita, Chenopodium, Oenothera, Salix, Zinnia, Barley cultivars, Maize cultivars, Wheat cultivars, and so on.

2. Explain the Ultrastructure of Bacterial Flagella and it functions

A. Flagella: The motile bacteria are provided with flagella. Bacterial flagella are about 100 to 200 A thick and show varied length.

1.Monotrichous Single polar flagellum Example: Vibrio cholerae 2. Lophotrichous Tufts of flagella at one or both sides Example: Spirillum 3. Amphitrichous Single flagellum on both sides Example: Alkaligens faecalis 4. Peritrichous Numerous falgella all over the bacterial body Example: Salmonella Typhi	

- Bacterial flagella are long (upto 15-20 µm) and thin (about 20 nm) appendages free at one end and attached to the cell at other end.
- They are the organs of locomotion and are so thin that a single flagellum can only be observed with a light microscope after staining with special flagella-stains that increase their diameter.
- Flagella can easily be seen with the electron microscope.
- Bacterial flagella arise from the plasma membrane and pass out through the cell wall.
- A bacterial flagellum is not straight but helical and consists of three distinct parts

THE FILAMENT, THE HOOK AND THE BASAL BODY
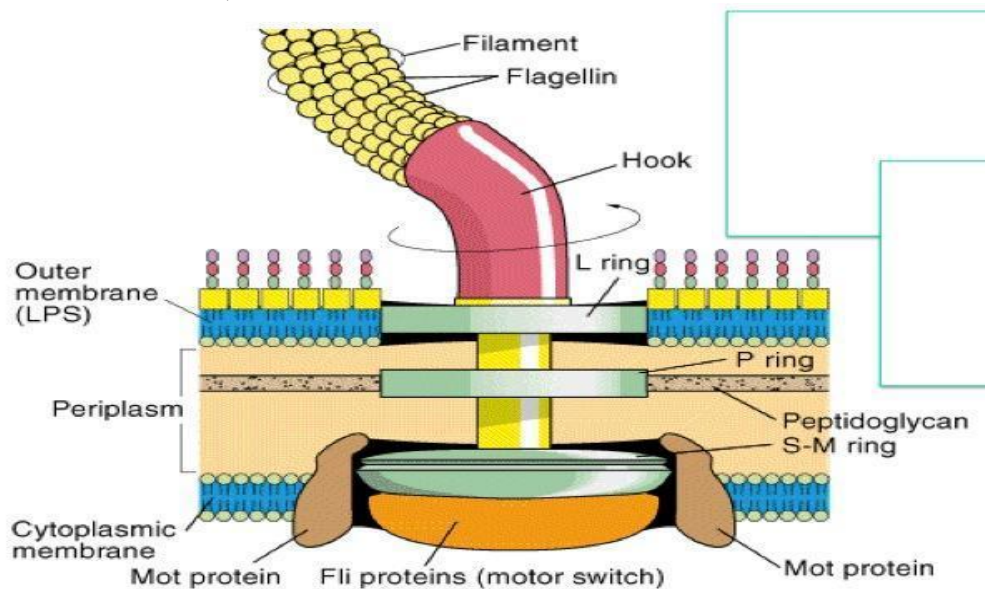
1. Filament:

☐   The filament is a fine, cylindrical, helical hollow structure, about 120-200 Å in diameter.

- It is composed of a fibrin protein called 'flagellin' structurally similar to keratin and myosin proteins and ranging in mol. Wt. from 30,000 to 60,000 dalton.
- A cross section of the filament reveals that there are eight flagellin molecules surrounding a central hollow cylinder.
- Actually, the 8 flagellin molecules seen in the cross section arc the parts of flagellin molecule-chain eight in number and running longitudinally around the central hollow cylinder.
- Each chain contains approximately 1,000 spherical, smaller flagellin molecules each of 40 Å diameter. In this way, the bacterial flagellum fundamentally differs from the flagellum of an eukaryotic cell, which has 9 + 2 type of arrangement in its filament.
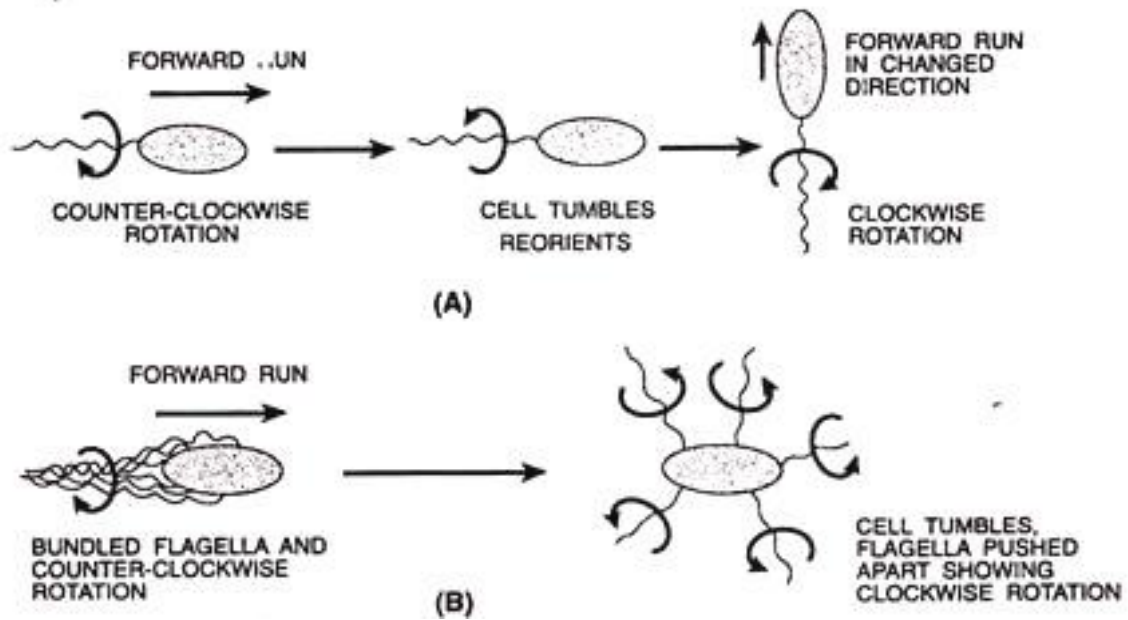
2. Hook:

- As said earlier, hook is a somewhat broader and thicker basal region of a flagellum and passes out through the cell wall. It is made up of a single type of protein and functions to connect the filament to the basal body of the flagellum.

3. Basal Body (The Motor Portion of the Flagellum):

- Basal body, the motor portion of the flagellum is the most complex part of a flagellum. In gram-negative bacteria, the basal body has five rings (L, P, S, M, and C) connected to a central rod.
- The outer L (lipopolysaccharide) and P (peptidoglycan) rings remain embedded in the lipopolysaccharide and the peptidoglycan layers, respectively; the inner S (supermembrane) and M (membrane) rings are located within the plasma membrane, and the innermost C (cytoplasmic) ring is located in the cytoplasm.
- In gram-positive bacteria, which lack the outer lipopolysaccharide layer, only S, M and C rings are present. There are a pair of proteins called Mot A and Mot B that surround the inner ring and are associated to the plasma membrane.
- The portion of the basal body that rotates (the motor) is consisted of the rod, the M-ring, and the Mot and Fli proteins.
- The Mot proteins drive the motor causing a torque that rotates the filament, whereas the Fli proteins function as the 'motor switch' reversing rotation of the flagella in response to signals send by the bacterial cell.

Flagellar Movement

- Bacterial (prokaryotic) flagella operate in different manner when compared to eukaryotic flagella.
- Each individual flagellum is a semi-rigid helix and moves by rotation like propellers on a boat
- Monotrichous and lophotrichous polar flagella rotate counter-clockwise and make the bacterial cell spring around and run forward from place to place with the flagellum trailing behind.
- These bacteria stop and tumble randomly by reversing the direction of flagellar rotation.
- The flagella of peritrichous bacteria rotate counter clockwise, like montrichous and lophotrichous ones, to move forward.
- The flagella bend at their hooks to form a rotating bundle that propels them forward.
- Clockwise rotation of the flagella disrupts the bundle and the cell tumbles.
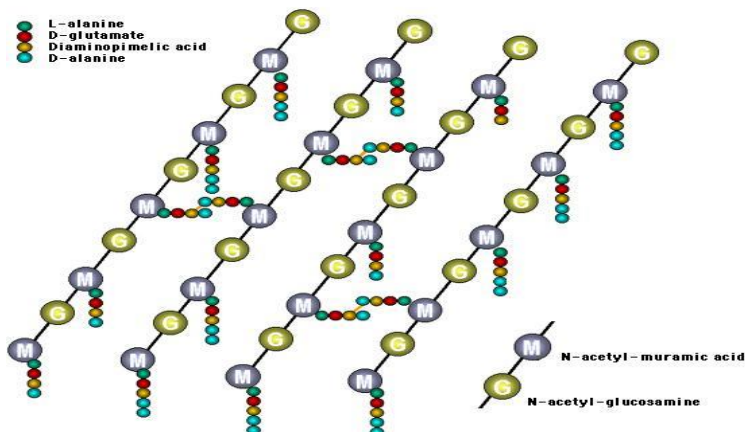
FORWARD ..UN

COUNTER-CLOCKWISE ROTATION

CELL TUMBLES REORIENTS

FORWARD RUN IN CHANGED DIRECTION

CLOCKWISE ROTATION

(A)

FORWARD RUN

BUNDLED FLAGELLA AND COUNTER-CLOCKWISE ROTATION

CELL TUMBLES, FLAGELLA PUSHED APART SHOWING CLOCKWISE ROTATION

(B)

4. Explain the Ultrastructure of bacterial cell wall with reference to gram staining
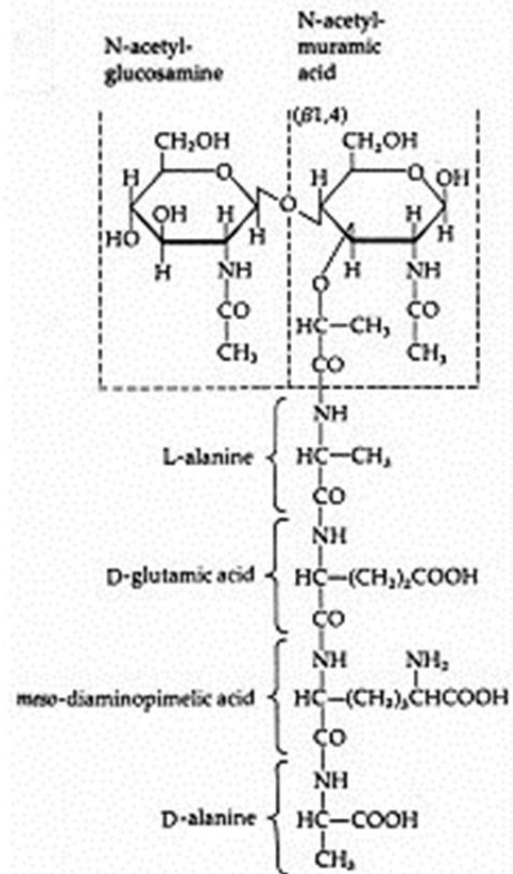
Cell Wall
- They are essential structures in bacteria.
- The primary function is to prevent rupture or osmotic lysis of the cell protoplast
- They are made of chemical components found nowhere else in nature.
- They may cause symptoms of disease in animals.
- They are the site of action of some of our most important antibiotics.
- Cell wall is a structure that completely surrounds the cell protoplast.All bacteria have a cell wall.

Arrangement of NAM and NAG in Peptidoglycan

L-alanine
D-glutamate
Diaminopimelic acid
D-alanine

M N-acetyl-muramic acid

G N-acetyl-glucosamine

Chemical nature of bacterial cell walls

7

- Bacterial cell walls always contain murein, which is a type of peptidoglycan

- Chemical nature of murein accounts for the function of the cell wall

- Murein is only found in the cell walls of bacteria

- Peptidoglycan (Murein) is made up of 2 amino sugars
  N-acetyl-glucosamine = NAG
 N- acetylmuramic acid = NAM

Gram Positive Cell walls

The cell walls of gram positive bacteria are composed predominantly of peptidoglycan. In fact, peptidoglycan can represent up to 90% of the cell wall, with layer after layer forming around the cell membrane. The NAM tetrapeptides are typically cross-linked with a peptide interbridge and complete cross-linking is common. All of this combines together to create an incredibly strong cell wall.

The additional component in a gram positive cell wall is **teichoic acid**, a glycopolymer, which is embedded within the peptidoglycan layers. Teichoic acid is believed to play several important roles for the cell, such as generation of the net negative charge of the cell, which is essential for development of a proton motive force. Teichoic acid contributes to the overall rigidity of the cell wall, which is important for the maintenance of the cell shape, particularly in rod shaped bacteria.

Teichoic acids appear to play a role in resistance to adverse conditions such as high temperatures and high salt concentrations, as well as to β-lactam antibiotics. Teichoic acids can either be covalently linked to peptidoglycan (**wall teichoic acids or WTA**) or connected to the cell membrane via a lipid anchor, in which case it is referred to as **lipoteichoic acid**.

| Gram positive	Gram negative
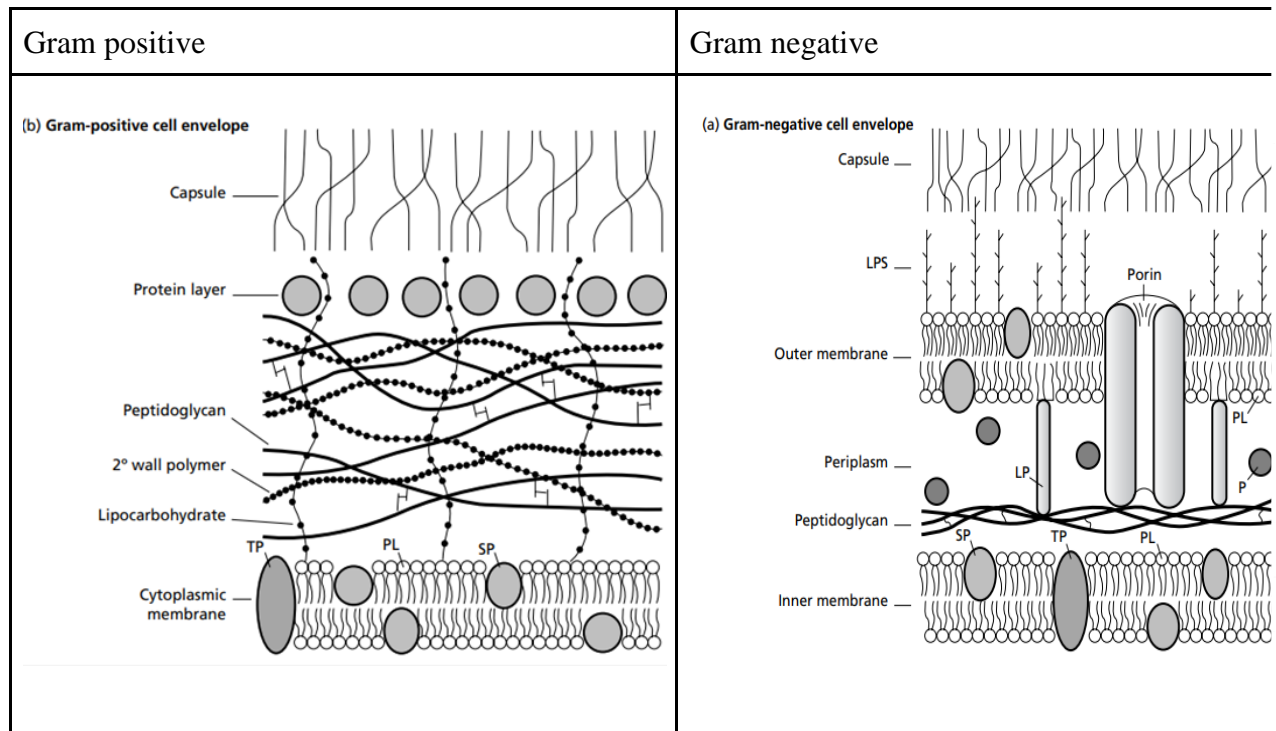
Gram Negative Cell Walls

The cell walls of gram negative bacteria are more complex than that of gram positive bacteria, with more ingredients overall. They do contain peptidoglycan as well, although only a couple of layers, representing 5-10% of the total cell wall. What is most notable about the gram negative cell wall is the presence of a plasma membrane located outside of the peptidoglycan layers, known as the **outer membrane**. This makes up the bulk of the gram negative cell wall. The outer membrane is composed of a lipid bilayer, very similar in composition to the cell membrane with polar heads, fatty acid tails, and integral proteins. It differs from the cell membrane by the presence of large molecules known as **lipopolysaccharide (LPS)**, which are anchored into the outer membrane and project from the cell into the environment. LPS is made up of three different components: 1) the **O-antigen or O-polysaccharide**, which represents the outermost part of the structure , 2) the **core polysaccharide**, and 3) **lipid A**, which anchors the LPS into the outer membrane. LPS is known to serve many different functions for the cell, such as contributing to the net negative charge for the cell, helping to stabilize the outer membrane, and providing protection from certain chemical substances by physically blocking access to other parts.

The peptidoglycan layers are linked to the outer membrane by the use of a lipoprotein known as **Braun's lipoprotein** (good ol' Dr. Braun). At one end the lipoprotein is covalently bound to the peptidoglycan while the other end is embedded into the outer membrane via its polar head. This linkage between the two layers provides additional structural integrity and strength of the cell wall.

The gram stain consists of these steps:

- **Crystal violet** - stains both gram negative and positive bacteria

- Gram's iodine - fixes the stain in gram positive bacteria

- Ethanol or acetone - washes the stain from gram negative bacteria

- **Safranin** - counterstain, will re-stain gram negative bacteria while not interfering with the previous stain in gram positive bacteria

GRAM + Fixation GRAM -
Crystal Violet
Iodine treatment
Decolorization
Counter stain (safranin)
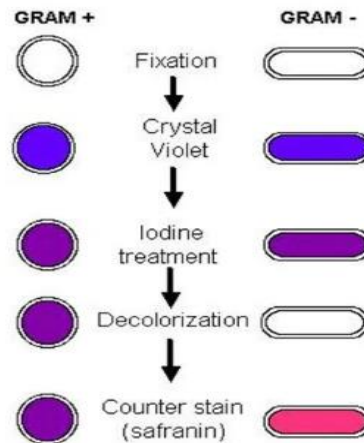
4. Write a brief note on Photosynthetic bacteria - Purple bacteria, Green bacteria and Anabaena

Photosynthetic bacteria

Classification of Photosynthetic Bacteria:

The photosynthetic bacteria are divided into two broad groups, Anoxygenic photosynthetic bacteria and oxygenic photosynthetic bacteria.

Anoxygenic Photosynthetic Bacteria: The anoxygenic photosynthesis depends on e– donors such as reduced sulphur compounds, molecular hydrogen or organic compounds. The ammonium salts are generally used as nitrogen source. Nitrogen fixation has been reported in some bacterial species.

Some can grow chemo-auto-trophically under aerobic/micro-aerobic condition. Fatty acids, ethanol and organic acids serve as carbon sources. They are found in fresh water, brackish water, and marine and hyper-saline water.

Table 13.2 : Characteristics and classification of anoxygenic bacteria.

Category	Name of organism	Family	Characteristics
Purple-Sulphur	Chromatium, Ectothiorhodospira Thioapsa, Thiospirillum	Chromatiaceae	Photolithotrophs
Purple non-Sulphur	Rhodomicrobium Rhodopseudomonas Rhodospirillum	Rhodospirillaceae	Photoorganotrophs
Green-Sulphur	Chlorobium, Pelodictyon,	Chlorobiaceae	Photolithotrophs
Green non-Sulphur	Chloroflexus, Heliothrix, Oscillochloris	Chloroflexaceae	Chemoorganotrophs and phototrophs

(i) Purple Bacteria (Proteobacteria):

The anoxygenic phototrophs grow under anaerobic conditions in the presence of light and do not use water as e-donor as in higher plants. The pigment synthesis is repressed by O_2.

They grow auto-trophically with CO_2 (C source) and hydrogen or reduced sulphur compounds act as e– donor. Photo-heterotrophy (i.e. light as energy source and an organic compound as a carbon source) also supports growth. Some purple bacteria also show chemo-organotrophy i.e. can grow in dark under similar conditions.

Ex:Rhodospirillum, Rhodopseudomonas, Rhodobacter, Rhodomicrobium.
Purple bacteria are of two types:

a. purple-sulphur bacteria and

b. purple non-sulphur bacteria.

(a) Purple sulphur bacteria (family; Chromatiaceae):

They are Gram-negative bacteria which contain BChl a and BChl b and grow chemolithotrophically in dark with thiosulphate as an e– donor. They are also chemoorganotrophs; utilise acetate, pyruvate and a few other compounds. The mole % of G+C varies from 46-70.

The cells of purple-sulphur bacteria are larger than green bacteria and packed with intracellular sulfide deposition. They are found in anoxic zone of lakes and sulphur springs (obligate anaerobe).e.g. Ectothiorhodospira, Chromatium, Thiocapsa, Thiospirillum, Thiodictyon, Thiopedia etc.

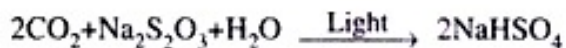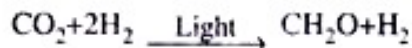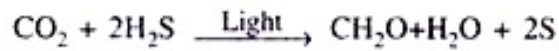**b)Purple non-sulphur bacteria (family:** Ectothiorhodospiraceae old name Rhodospirillaceae):

They also contain BChl a and b and use low concentration of sulphide. The concentration of sulphide utilized by purple-sulphur bacteria proved toxic to this category of bacteria. Earlier, scientists thought that these bacteria are unable to use sulphide as an e– donor for the reduction of CO_2 to cell material, hence named them non-sulphur.

They deposit sulphur extracellularly. Some non-sulphur bacteria grow anaerobically in the dark using fermentative metabolism, while the others can grow anaerobically in dark by respiration in which e– donor .

ii) **Green Bacteria:**Instead of green in colour, these are brown due to the presence of carotenoids components. They are Gram-negative. Hence, colour is not a suitable basis for these bacteria. They contain BChl c, BChl d or BChl e plus small amount of Bchl a.

The photosynthetic apparatus is chlorosomes which consist of a series of cylindrical structures underlying and/or attached to cytoplasmic membrane and are quite different with lamellae. These

may be an organic compound/inorganic compound as H2.vesicles are enclosed within a thin membrane devoid of bilayer but consist of transporter proteins located in the cytoplasmic membrane. They do not require vitamins for their growth.

$$CO_2 + 2H_2S \xrightarrow{\text{Light}} CH_2O + H_2O + 2S$$

$$CO_2 + 2H_2 \xrightarrow{\text{Light}} CH_2O + H_2$$

$$2CO_2 + Na_2S_2O_3 + H_2O \xrightarrow{\text{Light}} 2NaHSO_4$$

Green bacteria are of two types:

Green sulphur bacteria (family: Chlorobiaceae)

They are non-motile, rods, spiral and cocci. Some have appendages i.e. prosthecae. Chlorosomes are present in the cell. They do not possess gas vesicles (Chlorobium) except in Pelodictyon. They are strictly anaerobic and obligate phototroph.

Green non-sulphur bacteria(family: Chloroflexaceae):The green non-sulphur bacteria are filamentous, gliding bacteria, thermophilic in nature. The pigments are Bchl a, Bchl c, β- and Ɣ- carotenes. The chlorosomes are present when grown anaerobically. They are photoheterotrophic and photoautotrophic and show gliding movement. They do not deposit sulphur.

Oxygenic Photosynthetic Bacteria (Anabaena)

The oxygenic photosynthetic bacteria are unicellular or multicellular and possess bacterio-chlorophyll a and carry out oxygenic photosynthesis. They contain phycobilins. One group, prochlorophytes lack phycobilins, but contain both bacteriochlorophyll a and b. They are mostly represented by Gram-negative cyanobacteria having only membrane.

The light harvesting pigments are phycobilin proteins, phycoerythrin, phycocyanin, bacteriochlorophyll a and carotenoids but sheath capsule may contain yellow pigment called scytonemin or red-blue pigment gloeocapsin which may mask cellular pigmentation.

Phycobilins form phyobilisomes on both surfaces on double unit internal membrane called thylakoids. Bacteriochlorophyll a and carotenoids are part of it. Photosynthesis is oxygenic and autotrophic but chemoautotrophy also occurs.e.g. Cylindrospermum, Anabaena, Nodularia, Calothrix, Nostoc.

6. **Write about general characters and importance of Mycoplasma, Rickettsia, Chlamydia**

Mycoplasma:

Mycoplasma (plural mycoplasmas or mycoplasmata) is a genus of bacteria that lack a cell wall around their cell membranes.

They can be parasitic or saprotrophic. Several species are pathogenic in humans, including M. pneumoniae, which is an important cause of "walking" pneumonia and other respiratory disorders, and M. genitalium, which is believed to be involved in pelvic inflammatory diseases. The term mycoplasma is derived from the Greek word mykes (fungus) and plasma (formed).

They were the first discovered by Pasteur in 1843 when he was studying the causal organisms of pleuropneumonia in cattles.
These are first isolated by the two French bacteriologist E. Nocard and E.R. Roux in 1898 from pleuro fluids of cattles affected with pleuropneumonia.
These organisms were named as mycoplasma in 1929 by Nowak.

 Due to the absence of cell wall these organisms can change their shape and are pleomorphic. Lack of nucleus and other membrane-bound organelles. Genetic material is a single DNA duplex and is naked. ribosomes are 70S type.
Mollicute genomes are among the smallest found among bacteria, ranging from 0.7 to 1.7 Mb. The genomes of the human pathogens Mycoplasma genitalium, M. pneumoniae, and Ureaplasma urealyticum have fewer than 1,000 genes, suggesting a minimal genome size.

 Based on nutritional requirement, mycoplasmas are divided into three genera: Mycoplasma, Acholeplasma and Thermoplasma.
 Since mycoplasmas pass through many filters and grow on media without living tissue, these are considered to the intermediate between viruses and bacteria.
 They can grow in cell free media forming typical "fried egg shaped" colony.

They are highly plueromorphic (variable in shape) showing small coccoid bodies, rings forms and five filamentous forms which may be branched.
In Mycoplasmas rigid cell wall is absent. Cells are surrounded by a triple layered lipoproteinaceous unit membrane. It is about 10 nm thick. Unit membrane encloses the cytoplasm
Mycoplasmas reproduce by budding and/or binary fission.
 Mycoplasma like organisms (MLO) or phytoplasmas are usually present in phloem of the host plants.

Mycoplasmas cause different serious diseases, these are: in plants (Little leaf disease of brinjal, Bunchy top of papaya, Big bud of tomato, Witches broom of legumes, Yellow dwarf of tobacco, Clover dwarf etc)
 In animals (Mycoplasma agalactia causes agalactia of goat and sheep, Mycoplasma mycoides causes pleuropneumonia of cattle, M. bovigenitalium causes inflammation of genitals of different animals etc).
 In humans (Primary atypical pneumonia (PAP) by mycoplasma pneumoniae, Mycoplasma hominis causes pleuropneumonia, prostatitis, inflammations of genitals, Mycoplasma fermentants causes infertility in man etc).
The genome of mycoplsma are considered as the smallest genome.
 Mycoplasma are highly resistant to penicillium but inhibited by tetracyclines.

Mycoplasma

NO CELL WALL

Cytoplasm

Soluble RNA

Ribosome

Three layered cellular membrane

Soluble protein

DNA
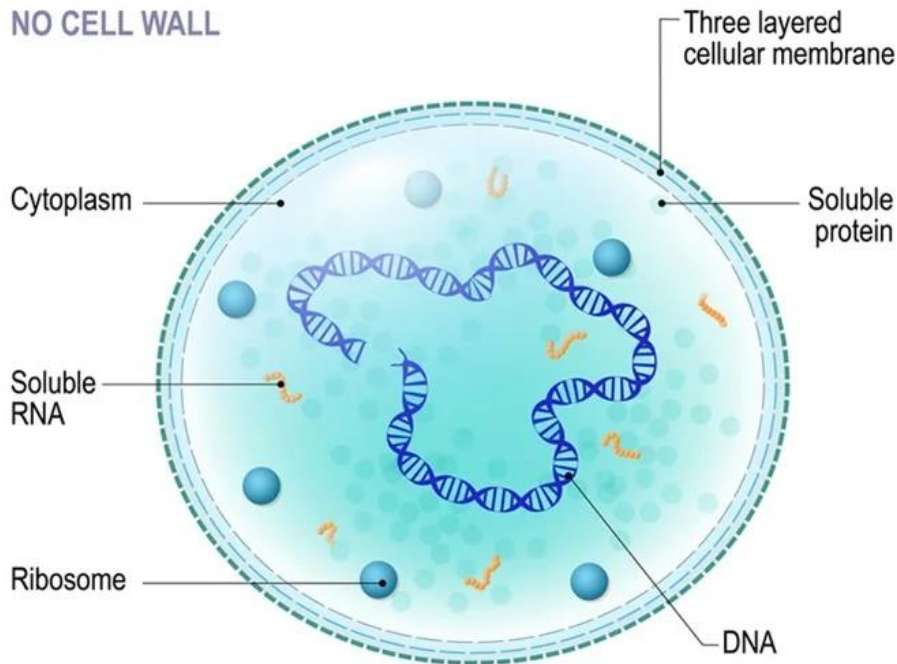
Rickettsia

General Features

The rickettsia are bacteria which are obligate intracellular parasites. They are considered a separate group of bacteria because they have the common feature of being spread by arthropod vectors (lice, fleas, mites and ticks). The cells are extremely small (0.25 u in diameter) rod-shaped, coccoid and often pleomorphic microorganisms which have typical bacterial cell walls, no flagella, are gram-negative and multiply via binary fission only inside host cells. They occur singly, in pairs, or in strands. Most species are found only in the cytoplasm of host cells, but those which cause spotted fevers multiply in nuclei as well as in cytoplasm. In the laboratory, they may be cultivated in living tissues such as embryonated chicken eggs or vertebrate cell cultures.

The family Rickettsiaceae is taxonomically divided into three genera:

1. *Rickettsia* (11 species)--obligate intracellular parasites which do not multiply within vacuoles and do not parasitize white blood cells.
2. *Ehrlichia* (2 species) - obligate intracellular parasites which do not multiply within vacuoles but do parasitize white blood cells.
3. *Coxiella* (1 species)--obligate intracellular parasite which grows preferentially in vacuoles of host cells.
4. *Baartonella* (3 species)--intracellular parasite which attacks the red blood cell.Structure

The structure of the typical rickettsia is very similar to that of Gram-negative bacteria. The typical envelope consists of three major layers: an innermost cytoplasmic membrane, a thin electron dense rigid cell wall and an outer layer. The outer layer resembles typical membranes

in its chemical composition and its trilaminar appearance. The cell wall is chemically similar to that of Gram-negative bacteria in that it contains diaminopimelic acid and lacks teichoic acid. Intracytoplasmic invaginations of the plasma membrane (mesosomes) and ribosomes are also seen. There are no discrete nuclear structures.

Metabolism

In dilute buffered salt solutions, isolated rickettsia are unstable, losing both metabolic activity and infectivity for animal cells. If, however, the medium is enriched with potassium, serum albumin and sucrose, the isolated organisms can survive for many hours. If ATP is added to the solution, the organisms metabolize and consume oxygen. The basis for the obligate parasitism of these cells is that they require the rich cytoplasm to stabilize an unusually permeable cell membrane.

The rickettsia have many of the metabolic capabilities of bacteria, but require an exogenous supply of cofactors to express these capabilities. The response to exogenous cofactors implies an unusually permeable cytoplasmic membrane.

Growth and Multiplication

Rickettsia normally multiply by transverse binary fission. Under poor nutritional conditions, the rickettsia cease dividing and grow into long filamentous forms, which subsequently undergo rapid and multiple division into the typical short rod forms when fresh nutrient is added. Immediately after division, the rickettsia engage in extensive movements through the cytoplasm of the cell. *C. burnetii* differs from other rickettsia in that it is enclosed in a persistent vacuole during growth and division. Six to ten daughter cells will form within a host cell before the cell ruptures and releases them.

Diseases

The rickettsial diseases of man are usually broken down according to the arthropod vector as seen in table below.

Disease	Causative agent	Animal reservoir
Rocky Mountain Spotted fever	*Rickettsia rickettsii*	Dogs, rodents
North Asian tick typhus	*Rickettsia siberica*	Wild rodents
Q- fever	*Coxiella burnetii*	Dogs, rodents
European epidemic typhus	*Rickettsia prowazekii*	—

Chlamydia

General Characteristics

The chlamydia, which are incorrectly called the PLT viruses or Bedsonia or basophilic viruses, are bacteria which are obligate intracellular parasites of higher animals (mammals and birds). The members of this group share a unique development cycle, a common morphology and a common family antigen. They are not transmitted by arthropods. These organisms are termed basophilic because they take up the Giemsa stain (i.e., they stain blue). They are gram-negative, non-motile and multiply in the cytoplasm of the host cell. These organisms generally parasitize epithelial cells. The clinical features, pathogenesis, pathology and epidemiology of chlamydial infections are similar to those of viral infections.

Taxonomy

The chlamydia fall into two main ecological groups. In the first group, are the agents causing trachoma, inclusion conjunctivitis, and lymphogranuloma venereum, which seem to infect man only. In the second group, are those agents transmitted to man as zoonotic infections. About 100 species of birds are naturally infected with chlamydia. This includes 71 species of parrots as well as finches, pigeons, chickens, ducks, turkeys and seabirds.

Morphology and Structure

The chlamydial cell is roughly spherical and measures between 0.3 and 1.0 u in diameter, according to the stage of development. Both the small and the large cell types contain complete cell walls which are similar to the cell walls of gram-negative bacteria.

Under the cell wall lies a separate cytoplasmic membrane made up of large amounts of lipid. The DNA occurs as an irregular mass in the cytoplasm. There is no nuclear membrane. Ribosomes can be seen throughout the cytoplasm. The cells contain no capsule or flagella.

Metabolism

There are no detectable flavoproteins or cytochromes. It appears that the basis of the obligate intracellular parasitism is due to a lack of ATP-generating ability and the need to obtain ATP from the host cell. The cells can synthesize DNA, RNA and protein.

Growth and multiplication

Chlamydia pass through a series of developmental forms while multiplying by binary fission. This is termed the "developmental cycle." Two morphologically different developmental forms with a continuous gradation of intermediates between them can be recognized. One is a small cell about 0.3 u in diameter, with an electron-dense nucleoid. The other is a large cell, 0.5 to 1.0 u in diameter without a dense centre.

There appears to be no significant difference in morphology or developmental cycle among the various chlamydia, and a single generalized description applies to all. The development cycle may be regarded as an orderly alternation of the small and large cell type. It is initiated by the highly infectious small cell which is taken into the host cell by phagocytosis. The engulfed small cell retains its morphological integrity in vacuoles bound by membrane derived from the surface of the host cell, and there is no eclipse (period in which the parasite loses the infectious ability). Instead, without loss of individuality, the small cell is reorganized into a large cell which is the vegetative multiplying form of these organisms. Then, still within the membrane-

bound vacuole, the large cell grows in size and multiplies by repeated binary fission. The developmental cycle is completed by the reorganization of most of the large cells into small ones which are then available for infection of new host cells. The time required for completion of a cycle varies from 24-48 hours, depending on the particular host/parasite system involved.
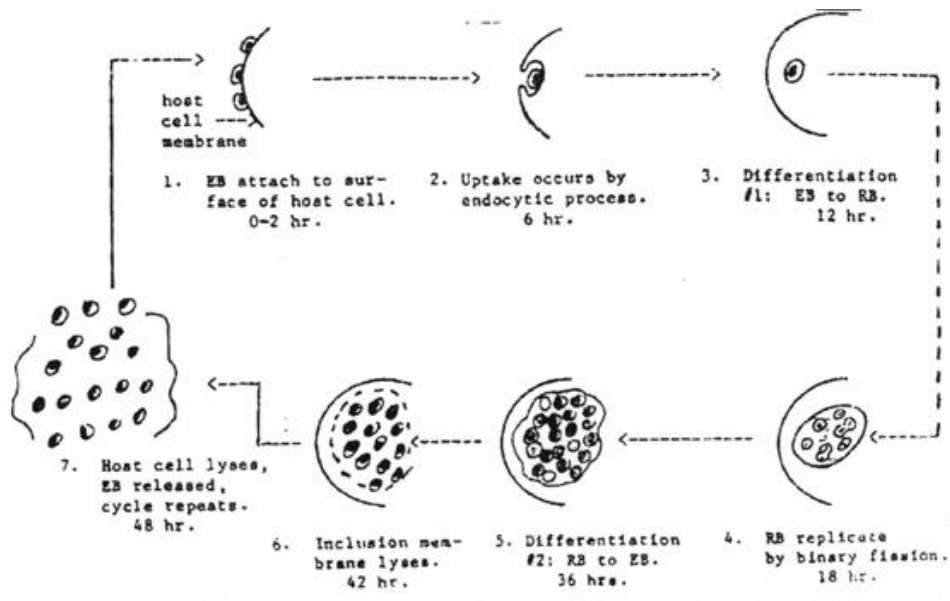
The   chlamydial   diseases   include:

DISEASE	CAUSUAL AGENT	HOST
Subgroup A (person-to-person transmition)		
Trachoma	*Chlamydia trachomatis*	Man
Inclusion conjunctivitis	*Chlamydia trachomatis*	Fowl, Man
Urethritis	*Chlamydia trachomatis*	Man
Cervicitis	*Chlamydia trachomatis*	Man
Ophthalmia neonatorum	*Chlamydia trachomatis*	Man
Myocarditis	*Chlamydia trachomatis*	Man

Lymphogranuloma venereum	*Chlamydia trachomatis*	Man
Atherosclerosis	*Chlamydia trachomatis*	Man
Neonatal Pneumonia	*Chlamydia trachomatis*	Man

**Q.6. Salient features of Extremophiles- Methanogens and halobacteria.**

Methanogen

Microbes capable of producing methane are called methanogens. They have been identified only from the domain Archaea – a group that is phylogenetically distinct from eukaryotes and bacteria – though many live in close association with anaerobic bacteria. The production of methane is an important and widespread form of microbial metabolism, and in most environments, it is the final step in the decomposition of biomass.

A methanogen pertains to any of the various archaeabacteria that are capable of Methanogens should not be confused with methanotrophs, which are prokaryotes that metabolize methane for nutrition and growth.

Methanogens produce methane as a metabolic byproduct. These microbes thrive in anoxic conditions and are common in wetlands. They are the ones responsible for the production of marsh gas. Some of them are also found in the digestive tracts of animals, e.g. ruminants and humans. These microbes that live in the digestive tract are responsible for the presence of methane in ruminants' belching and humans' flatulence. Others live as extremophiles in hot springs and hydrothermal vents.

Methanogens are strictly anaerobic. Most of them are sensitive to oxygen and are susceptible to oxygen stress especially for longer periods of time. Some of the methanogens are as follows: Methanobrevibacter gottschalkii, Methanobrevibacter smithii, Methanococcus jannaschii, Methanococcus maripaludis, Methanogenium frigidum, Methanopyrus kandleri, Methanosaeta concilii, Methanosarcina acetivorans, Methanosarcina barkeri, and Methanosphaera stadtmanae. Methanogenesis, or biomethanation, is a form of anaerobic respiration that uses carbon as the terminal electron acceptor, resulting in the production of methane. The carbon is sourced from a small number of low molecular weight organic compounds, such as carbon dioxide, acetic acid, formic acid (formate), methanol, methylamines, dimethyl sulfide, and methanethiol. The two bestdescribed pathways of methanogenesis use carbon dioxide or acetic acid as the terminal electron acceptor.

$$CO_2 + 4 H_2 \rightarrow CH_4 + 2H_2O$$

$$CH_3COOH \rightarrow CH_4 + CO_2$$
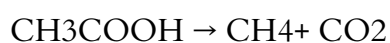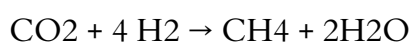
The biochemistry of methanogenesis is relatively complex. It involves the coenzymes and cofactors F420, coenzyme B, coenzyme M, methanofuran, and methanopterin.
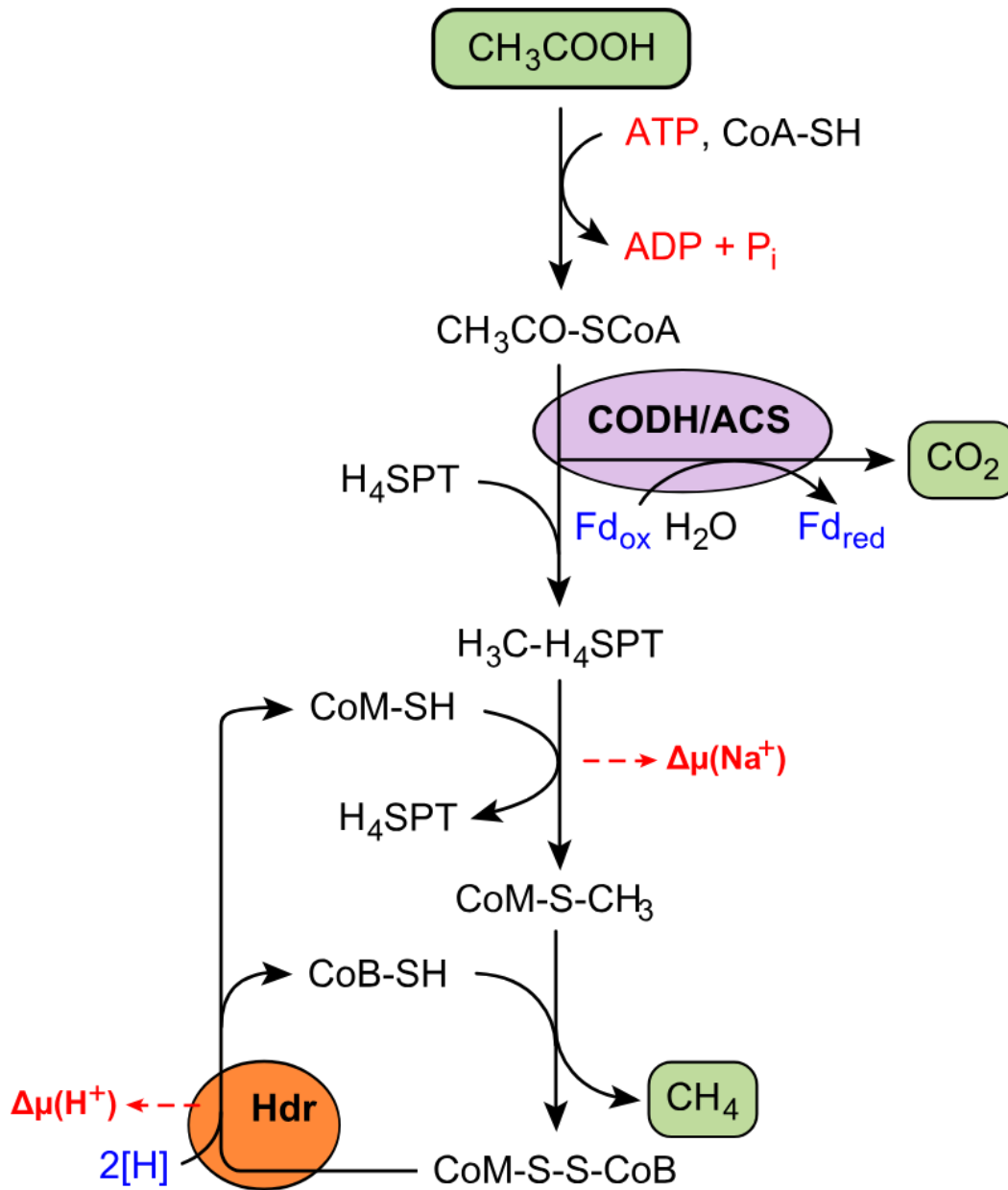
Figure: Methanogenesis of acetate: Acetate is broken down to methane by methanogenesis, a type of anaerobic respiration.

Halophile Definition and Characteristics

- Halophiles are a group of extremophiles that require high salt concentrations for their survival and growth.
- Halophiles are of two types; obligate halophiles that require NaCl concentration of 3% or more and halotolerant that survive at both average salt concentrations and higher.
- Halophilic microorganisms constitute the natural microbial communities of hypersaline
- etc.Alicyclobacillus acidoterrestris, A. acidocaidarius, etc.
- ecosystems, which are widely distributed around the world.

- The general features of halophilic microorganisms are low nutritional requirements and resistance to high concentrations of salt with the capacity to balance the osmotic pressure of the environment.
- The salt requirement in halophiles is classified into three groups; low (1-3%), moderate (3-15%), and extreme (15-30%).
- Salt requirement depends on factors like temperature, pH, and growth medium.
- They are physiologically diverse; mostly aerobic and as well anaerobic, heterotrophic, phototrophic, and chemoautotrophic.
- Ecologically, the halophilic microorganisms inhabit different ecosystems characterized by a salinity higher than seawater that range from hypersaline soils, springs, salt lakes, sabkhas to marine sediments.
- These organisms are found in all three domains of life, i.e., Archaea, Bacteria, and Eukaryota.
- Halophilic bacteria are more abundant in specific phylogenetic subgroups, most of which belong to Halomonadaceae, a family of Proteobacteria.

Halophile Mode of adaptation

In order to avoid excessive water loss under high salt conditions, halophiles have employed two distinct strategies to increase the osmotic activity of their cytoplasm with the external environment, either producing compatible organic solutes or accumulating large salt concentrations in their cytoplasm to reach an equilibrium state in which the overall salt concentration within cells correlates that of the environment.

a. High salt-in strategy

The high-salt-in strategy is another adaptation technique that protects halophiles from a saline environment in which they accumulate inorganic ions intracellularly to balance the salt concentration in their environment.

- This process involves Cl– pumps that are found only in halophiles that transport Cl– from the environment into the cytoplasm.
- Arginines and lysines are positioned at both ends of the channel to facilitate Cl– uptake, and release.
- Extreme halophiles maintain their osmotic balance by concentrating the K+ ions inside the cells.
- This is achieved by the combined action of the membrane-bound proton-pump bacteriorhodopsin, ATP synthase, and the Na+ antiporter that results in an electrical potential that drives the uptake of K+ into cells.

b. Organic salt-in strategy

- The high-salt-in strategy might be incompatible for the survival of moderate halophiles that thrive in habitats of fluctuating salinity.
- The solute-in strategy includes the evolution of inert, compatible organic solutes (osmolytes) in the halophiles.
- These osmolytes protect the microbial proteins from denaturation in the water of low salt concentrations while enhancing their tolerance to drastic fluctuations in the external saline environment.

c. Enzymes

- A high-salt environment substantially impacts protein solubility and stability and consequently, its function.
- The unfavorable interactions that disrupt internal microbial proteins caused by dehydration may be averted by modulating their net charge.
- Proteins and enzymes of halophiles have a larger proportion of glutamate and aspartate on their surfaces that result in a substantial number of protein charges and increased hydrophobicity.
- Both of these mechanisms work as a form of molecular halo adaptation of halophilic enzymes.
- Halophilic enzymes are more stable than their non-halophilic counterparts owing to their polyextremophilic characteristics.
- These enzymes remain active in high-salt environments, thermotolerant, and alkaliphilic.

Halophile Examples

Some common examples of halophilic organisms in terms of their salt requirement are:

1. **Slightly halophilic:** *Erwinia, Bacillus hunanensis, Halomonas zhaodongensis, Alkalibacterium thalassium*, etc.
2. **Moderately halophilic:** *Spiribacter salinus, Halobacillus sediminis, Halobacillus salicampi, Marinobacter piscensis, Idiomarina aquatica*, etc.
3. **Extreme halophile:** *Halococcus salifodinae, Halobacterium salinarum, Limimonas halophilia, Lentibacillus kimchii, Sporohalobacter salinus*, etc.

Q.7.write about general characters and structure of viruses

Viruses (*virus* means toxin or poison) are sub microscopic, acellular infectious agents. They are un able to grow or reproduce outside a host cell. The first known virus was TMV (tobacco mosaic virus). In 1892 Dimitri Ivanowski studied about tobacco mosaic disease and observed a filterable agent which was responsible for the infection. In 1899 Martinus Beijerink, repeated the experiments and became convinced that this was a new infectious agent.He called it as *contagium vivum fluidum* and introduced the word *virus.*In early 20th century Frederick Twort and Felix D' herily individually observed bacteriophages which were infecting agents of bacteria.The study of virus was known as virology.

Properties of viruses

- Viruses are acellular organisms.
- They are ultramicroscopic.
- They are obligate intracellular parasites.
- Viruses are filterable (Can pass through bacterial filters).
- Viruses are made up of nucleoproteins.
- The genetic material in viruses is either DNA or RNA, but never both.
- They can be crystalized. Viruses remain as inert particles outside the host.
- Viruses do not show cell division. They multiply within the host cell and give rise to the same genetic types.
- They can be transmitted from one host to another host either directly or through vectors.

● Viruses can be cultured only on living media (cells of chick embryo, tissue culture cells).

Structure and chemical composition:

Viruses are acellular as they do not have cell wall, membrane and cytoplasm. They display wide diversity of shapes and sizes. That may be rod shaped(TMV), round (Mumps, influenza virus, Tadpole shape (T- phages) bullet shaped (rabies), brick shaped (pox virus) filamentous (M13 phase) and pleomorphic.

Most viruses are 10-300 nanometers in size. Some viruses like F2 phase and foot and mouth disease virus are small and have a diameter of about 2-10nm. Viruses like pox virus are about 250 to 300nm.

Structure

A complete virus particle is known as virion. A virion consists of nucleic acid core surrounded by a protective protein coat called capsid they together called nucleo capsid. The nucleo capsid may be naked or surrounded by an additional lipid envelop derived from the host membrane.

Capsid: the capsid protects the viral genome and helps in the transfer of genetic material between host cells. Capsid is made up of identical protein subunits called capsomeres. The Capsomers are encoded by viral genome. Virus capsid mainly exhibit three kinds of symmetry.

They are 1. Helical

2. Icosahedral

3. Complex symmetry.

Helical or cylindrical symmetry : Viruses like TMV , Influenza virus, Rabies etc, exhibit helical symmetry. Helical capsids are composed of a single type of capsomears stacked around a central axis to form a helical structure which may have a central cavity, or hollow tube. This arrangement results in rod shaped or filamentous virion. They may be rigid or flexible. They may be naked (TMV), or enveloped (Influenza virus). The genetic material is generally single stranded RNA or ssDNA.
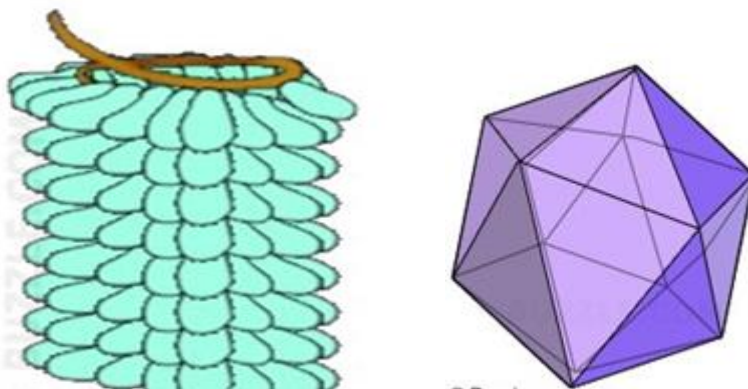
Icosahedral symmetry. Most of the viruses like polio virus, adeno virus, herpes virus, turnip yellow mosaic virus are cubical or near spherical in shape and have icosahedral symmetry. An Icosahedran is a regular polyhedron with 20 faces formed by equilateral triangles, and 12 intersecting corners. Capsomers at the apices are surrounded by five other Capsomers and are called pentons. Capsomers on the triangular faces are surrounded by six other s and are called hexons. The icosahedron capsid may be surrounded by envelop or naked.
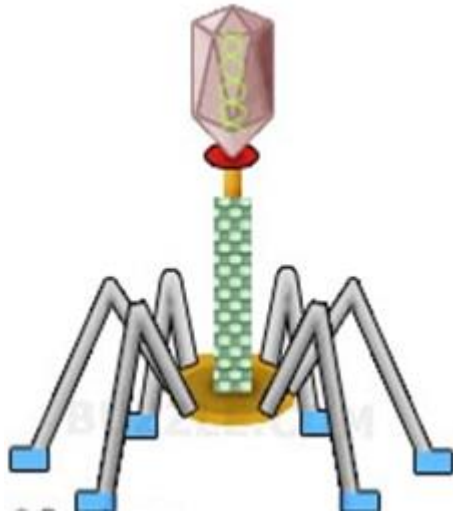
Complex symmetry: Some viruses possesses a capsid which was neither purely helical nor icosahedral. Such virus are called complex. They may be possess extra structures such as protein tails (Bacteriophage) or a complex outer wall(pox virus). The capsid of T bacteriophage s has two regions, head and tail. The head capsid is an icosahedron and is bound to a helical tail. Thus the bacteriophage has combined symmetry. The tail is connected with the head with a collar. At the free end the tail has a hexagonal base plate with protruding protein tail fibres.

The pox viruses have an unusual morphology. The viral genome is associated with proteins within a central disk-like structure known as nucleoid. The nucleoid is surrounded by a membrane and two lateral bodies of unknown function. The virus has outer envelope with a thick layer of protein. The whole particle is slightly pleomorphic, look like ovoid to brick shaped.

Genetic Material:

A wide variety of genomic diversity was observed with viruses. A virus has either DNA or RNA genes and is called DNA viruses and RNA viruses respectively. Plant viruses tend to have ssRNA and Bacteriophages tend to have ds DNA. [table genetic diversity.]

Viral genomes are circular as in polyomaviruses, or linear as in adenivirus. In RNA viruses the genome is often segmented and each segment codes for each protein. A viral genome is either single stranded or double stranded. Single stranded genome is un paired nuclic acid and double stranded genome consists of complimentary base pairing. Viruses like hepadna have partially double stranded and partially single stranded.

The viruses with ssRNA , the strand may be positive sence (+ strand), or negative sence (-Strand). Positive sense viral RNA is identical to viral mRNA. So, it immediately translated in the host cell. Negativesense viral RNA is complimentary to the mRNA and thus must be converted to positive sense RNA by RNA polymerases.

Envelop:

Most of the viruses were surrounded by a thin membranous envelop. This is about 10-15µm thick. It is made up of protein,lipid and carbohydrates which are combinedto form lipoproteins and glycoproteins. Protein component of the envelop is of viral origin and lipid and carbohydrates were of host origin. The viruses replicate in the host cytoplasm and release by budding through plasma membrane.

During release they are enveloped by a part of membrane of host cell.
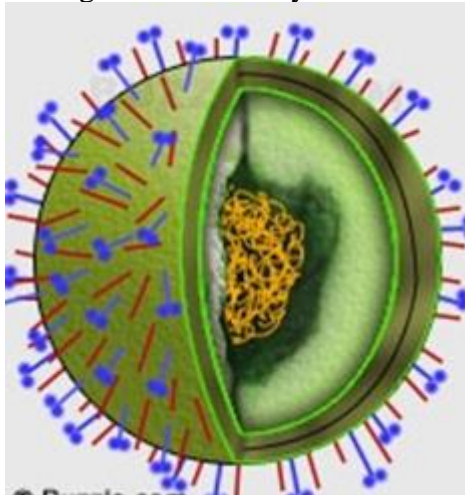
Lipids provide flexibility to the virion and therefore viruses are of variable size and shapes. Envelop of virus have two types of proteins they are matrix proteins and surface proteins. Matrix proteins are non-glycosylated proteins and are present in space between capsid and envelop. They provide strength to the envelop. Surface proteins are glycosylated proteins and are projected from envelop surface as spikes or peplomers.

Viral proteins:

- Proteins present in viruses are of four kinds. They are
- Envelop proteins: Proteins in envelop like glycoproteins are specific for virus and host cell.Eg: neuraminidase and haemagglutinin in influenza.
- Nucleocapsid proteins: Viral capsids are made up of identical protein subunits. Viruses with helical capsid (TMV) are made up of single type of protein whereas viruses with icosahedral capsid contain different kinds of proteins.
- Core protein: Protein found in nucleic acid are called core protein.
- Viral enzymes: In animal viruses many virion specific enzymes were found ex: RNase, reverse transcriptase in retrovirus, DNA dependent RNA polymerase in pox virus.
- Lipids: the lipids found in viral envelop fall under four classes. They are Phospholipids, cholesterols, fatty acids, and glycolipids.

- Carbohydrates: Carbohydrates in the envelop of viruses was specified by host cell or virion. They are hexoses (galactose, mannose, glucose)orhexamines (glucosamine, galacosamine) present in the form of glycoproteins or glycol lipids.

Q.8. Outline of Baltimore system of classification.

Virus classification is the process of naming viruses and placing them into a taxonomic system. Much like the classification systems used for cellular organisms, virus classification is the subject of ongoing debate and proposals. This is mainly due to the pseudo-living nature of viruses, which is to say they are non-living particles with some chemical characteristics similar to those of life. As such, they do not fit neatly into the established biological classification system in place for cellular organisms.

Classification of Viruses:

The International Committee on Taxonomy of viruses (ICTV) established in 1966, authorizes and organizes the taxonomic classification of viruses. It recognizes the taxa below kingdom; those of order family, subfamily, genus, and species. Therefore the virus classification starts at the level of order and follows as with the taxon suffixes given in italics.

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

On the basis of shared properties viruses are grouped at different hierarchical levels of order, family, subfamily, genus and species. More than 30,000 different virus isolates are known today and grouped in more than 3,600 species, in 164 genera and 71 families. Viral morphology provides the basis for grouping viruses into families. A virus family may consist of members that replicate only in vertebrates, only in invertebrates, only in plants, or only in bacteria. Certain families contain viruses that replicate in more than one of these hosts. This section concerns only the 21 families and genera of medical importance.

Criteria Used in Virus Classification (Baltimore classification)

Baltimore classification (first defined in 1971) is a classification system that places viruses into one of seven groups depending on a combination of their nucleic acid (DNA or RNA), strandedness (single-stranded or double-stranded), Sense, and method of replication. Named after David Baltimore, a Nobel Prize-winning biologist, these groups are designated by Roman numerals and discriminate viruses depending on their mode of replication and genome type. Other classifications are determined by the disease caused by the virus or its morphology, neither of which are satisfactory due to different viruses either causing the same disease or looking very similar. In addition, viral structures are often difficult to determine under the microscope.
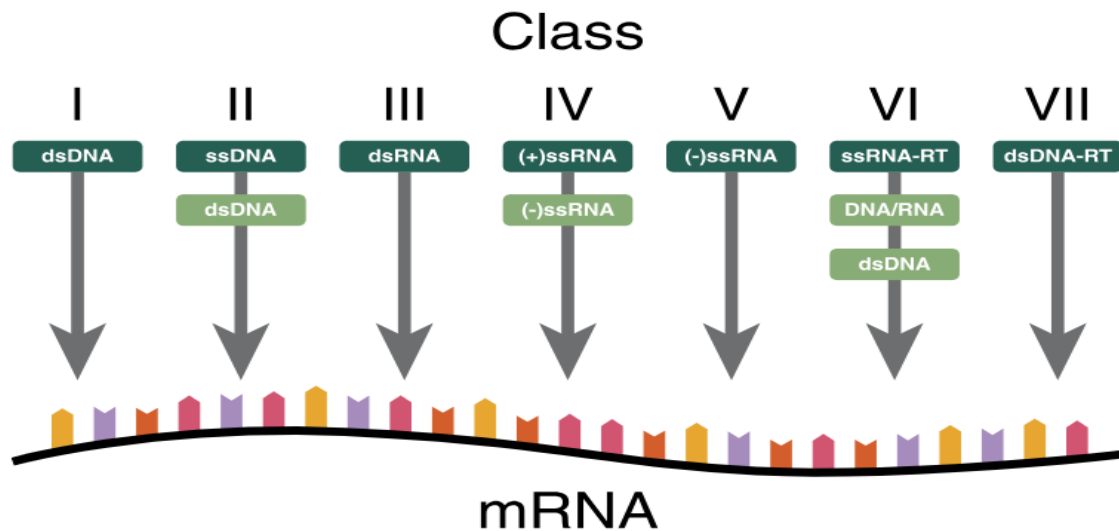
Class

Figure: **Viral classes**: Baltimore classification of viruses

Classifying viruses according to their genome means that those in a given category will all behave in a similar fashion, offering some indication of how to proceed with further research. Viruses can be placed in one of the seven following groups:

1. I: dsDNA viruses (e.g. Adenoviruses, Herpesviruses, Poxviruses)
2. II: ssDNA viruses (+)sense DNA (e.g. Parvoviruses)
3. III: dsRNA viruses (e.g. Reoviruses)
4. IV: (+)ssRNA viruses (+)sense RNA (e.g. Picornaviruses, Togaviruses)
5. V: (−)ssRNA viruses (−)sense RNA (e.g. Orthomyxoviruses, Rhabdoviruses)
6. VI: ssRNA-RT viruses (+)sense RNA with DNA intermediate in life-cycle (e.g. Retroviruses)
7. VII: dsDNA-RT viruses (e.g. Hepadnaviruses)

Q.9. write about Cultivation of Viruses

Since the viruses are obligate intracellular parasites, they cannot be grown on any inanimate culture medium Viruses can be cultivated within suitable hosts, such as a living cell The primary purposes of viral cultivation are:

 1. To isolate and identify viruses in clinical specimens

2. To prepare viruses for vaccines

3. To do detailed research on viral structure, multiplication cycles, genetics, and effects on host cells

Methods to cultivate viruses

Generally three methods are employed for the virus cultivation

1. Inoculation of virus into animals

2. Inoculation of virus into embryonated eggs

3. Tissue culture

1. Animal inoculation:

The earliest method for the cultivation of viruses causing human diseases was inoculation into human volunteers. Reed and colleagues (1900) used human volunteers for their pioneering work on yellow fever. Due to serious risk involved, human volunteers are used only when no other method is available and when the virus is relatively harmless.

Landsteiner and Popper (1909) used monkeys for the isolation of poliovirus. However due to their cost and risk to handlers monkeys find only limited application in virology. Theiler (1903) used white mice and extended the scope of animal inoculation greatly. Mice are still the most widely employed animals in virology.

Laboratory animals play an essential role in studies of viral pathogenesis Live animals such as monkeys, mice, rabbits, guinea pigs, ferrets are widely used for cultivating virus Mice are the most widely employed animals in virology
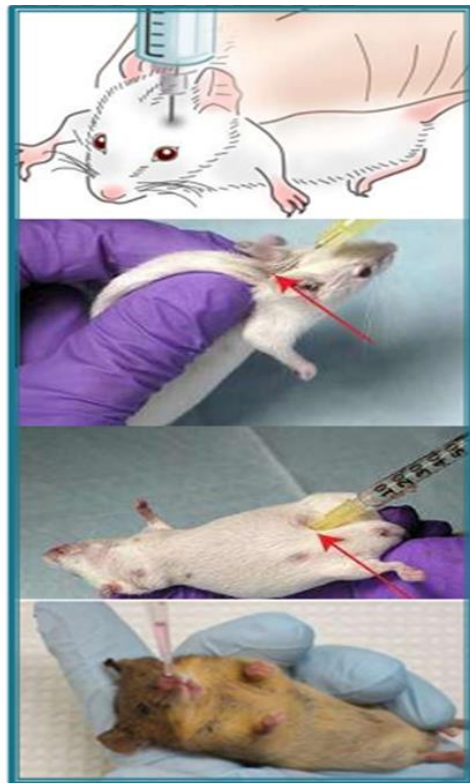
The different routes of inoculation in mice are:

Intracerebral,

 subcutaneous

intraperitoneal or intranasal

 After the animal is inoculated with the virus suspension, the animal is: observed for signs of disease, visible lesions or is killed so that infected tissues can be examined for virus.

Disadvantages

Cost Maintenance , Interference of immune system, Individual variations , Difficulty in choosing of animals for particular virus .\

Inoculation of Virus into Embryonated Eggs

Goodpasture and Burnet 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 being used Eggs provide a suitable means for:

◦ The primary isolation and identification of viruses

◦ The maintenance of stock cultures

◦ and the production of vaccines

Chicken, duck, and turkey eggs are the most common choices for inoculation The egg used for cultivation must be sterile and the shell should be intact and healthy. Rigorous sterile techniques must be used to prevent contamination by bacteria and fungi from the air and the outer surface of the shell.

Routes of viral inoculation

An embryonated egg offers various sites for the cultivation of viruses The different sites of viral inoculation in embryonated eggs are:

1. Chorioallantoic membrane(CAM)
2. Amniotic Cavity
3. Allantoic Cavity
 4. Yolk sac
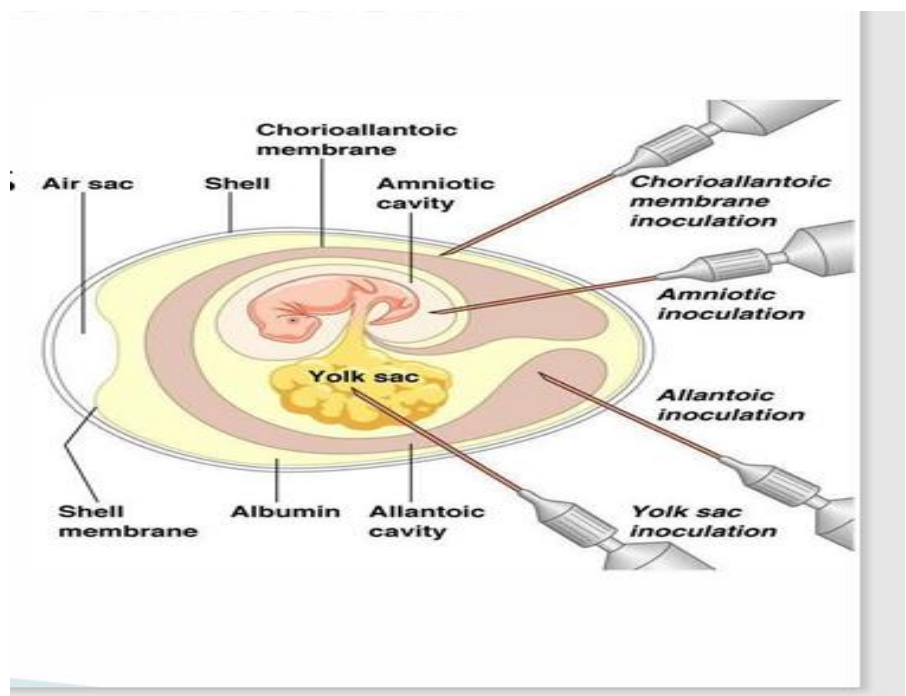
Chorioallantoic membrane:

This method has been widely used in veterinary virology Many viruses grow readily or can be adapted to grow on the CAM Viruses produce visible foci or 'pocks', inclusion bodies, oedema or other abnormalities Each infectious virus particle forms one pock Viruses which can be grown include: ◦ Herpes viruses ◦ and poxviruses
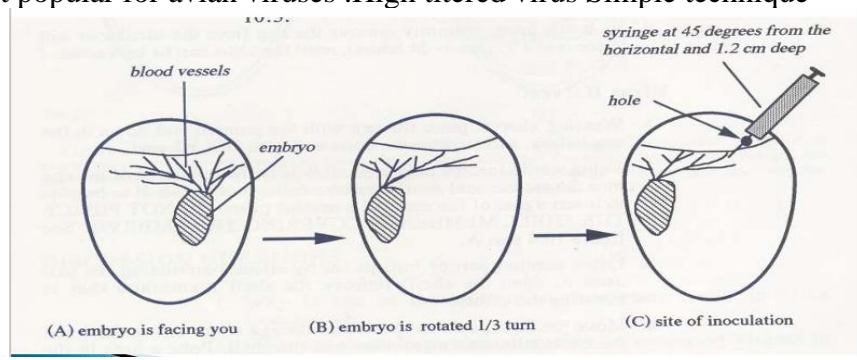
Chorioallantoic sac inoculation
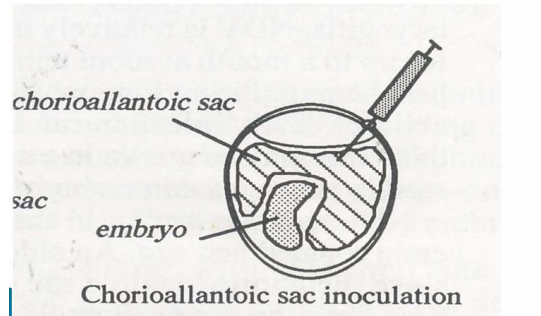
Amniotic sac:

Primary isolation of influenza and mumps viruses.Growth of virus detected by haemagglutination.

Allantoic route:

It is the most popular for avian viruses .High titered virus Simple technique

(A) embryo is facing you   (B) embryo is rotated 1/3 turn   (C) site of inoculation

Yolk sac inoculation

It is also a simplest method for growth and multiplication of virus. Mostly mammalian viruses are isolated using this method. Immune interference mechanism can be detected in most avian viruses. This method is also used for the cultivation of some bacteria like Chlamydiae and Rickettsiae .

Tissue culture Methods:

There are three types of tissue culture:-

Organ culture.

Explant culture

Cell culture.

Organ culture:- Small bits of organs can be maintained in vitro for days and weeks, preserving their original architecture and function.

Organs culture are useful for the isolation of some viruses which appear to be highly specialised parasite of certain organs.

For example, the tracheal ring organ culture is employed for the isolation of coronavirus, a respiratory pathogen. Explant culture:- Fragments of minced tissue can be grown as 'explants' embedded in plasma clots. They may also be cultivated in suspension.- This method is now seldom employed in virology.- Adenoid tissue explant culture were used for the isolation of adenoviruses.

Cell culture:-

This is the type of culture routinely employed for growing viruses.

Tissue are dissociated into the component cell by the action of proteolytic enzyme.-

The cells are washed , counted and suspended in a growth medium.-

Such media will enable most cell types to multiply with a division time of 24-48 hrs.-

The cell suspension is dispensed in bottles, tubes or petridishes.

The cell adhere to the glass surface and on incubation, divide to form a confluent monolayer sheet of cells covering the surface within about a week.

Based on their origin, chromosomal characters and the number of generation through which they can be maintained, cells cultures are classified into three types.

Primary cell cultures

These are normal cells obtained from fresh organs of animals or human being and cultured.- They are capable of only limited growth in culture and cannot be maintained in serial culture.- e.g. monkey kidney cell culture. human embryonic kidney.-- chick embryo cell culture.- They are commonly employed for primary isolation of viruses and in preparation of vaccine

Diploid cell culture

These are cells of single type, contain the same number of chromosome as the parent cells and are diploid.The diploid cell strains can be subculture for limited number of times. After about 50 serial passage they undergo senescence.They are also employed for the production of viral vaccine. Eg. human embryonic lung cell strain WI-38.

Continuous cell lines

These are cells of single type capable of infinite growth in vitro. They are derived from cancer cells. They are termed continuous cell lines as they can be serially cultivated indefinitely. The continuous cell line have been derived from human cancer such as HeLa, Hep 2 and KB cells(human carcinoma of nasophyranx). Continuous cell line are maintained either by serial subculture or by storing in deep freeze at -70 so that these can be used when necessary

Q. 10. General features of Viral Replication

Multiplication Within the Host Cell

Viral replication is the term used to indicate the formation of biological viruses during the infection process in the target host cells. Viruses must first penetrate and enter the cell before viral replication can occur. From the perspective of the virus, the purpose of viral replication is to allow reproduction and survival of its kind. By generating abundant copies of its genome and packaging these copies into viruses, the virus is able to continue infecting new hosts.

Replication between viruses is varied and depends on the type of genes involved. Most DNA viruses assemble in the nucleus; most RNA viruses develop solely in cytoplasm. Viral populations do not grow through cell division, because they are acellular. Instead, they hijack the machinery and metabolism of a host cell to produce multiple copies of themselves, and they assemble inside the cell.

The life cycle of viruses differs greatly between species but there are six common basic stages:

Attachment is a specific binding between viral capsid proteins and specific receptors on the host cellular surface. This specificity determines the host range of a virus. For example, HIV can infect only a limited range of human leukocytes. Its surface protein, gp120, specifically interacts only with the CD4 molecule – a chemokine receptor – which is most commonly found on the surface of CD4+ T-Cells. This mechanism has evolved to favor those viruses that infect only cells within which they are capable of replication. Attachment to the receptor can fore the viral envelope protein to undergo either changes that result in the fusion of viral and cellular membranes, or changes of non-enveloped virus surface proteins that allow the virus to enter.

Penetration follows attachment. Virions enter the host cell through receptor-mediated endocytosis or membrane fusion. This is often called *viral entry*. The infection of plant and fungal cells is different from that of animal cells. Plants have a rigid cell wall made of cellulose,

and fungi one of chitin, so most viruses can get inside these cells only after trauma to the cell wall. However, nearly all plant viruses (such as tobacco mosaic virus) can also move directly from cell to cell, in the form of single-stranded nucleoprotein complexes, through pores called plasmodesmata. Bacteria, like plants, have strong cell walls that a virus must breach to infect the cell. However, since bacterial cell walls are much less thick than plant cell walls due to their much smaller size, some viruses have evolved mechanisms that inject their genome into the bacterial cell across the cell wall, while the viral capsid remains outside.

Uncoating is a process in which the viral capsid is removed: This may be by degradation by viral or host enzymes or by simple dissociation. In either case the end-result is the release of the viral genomic nucleic acid.

Replication of viruses depends on the multiplication of the genome. This is accomplished through synthesis of viral messenger RNA (mRNA) from "early" genes (with exceptions for positive sense RNA viruses), viral protein synthesis, possible assembly of viral proteins, then viral genome replication mediated by early or regulatory protein expression. This may be followed, for complex viruses with larger genomes, by one or more further rounds of mRNA synthesis: "late" gene expression is, in general, of structural or virion proteins.
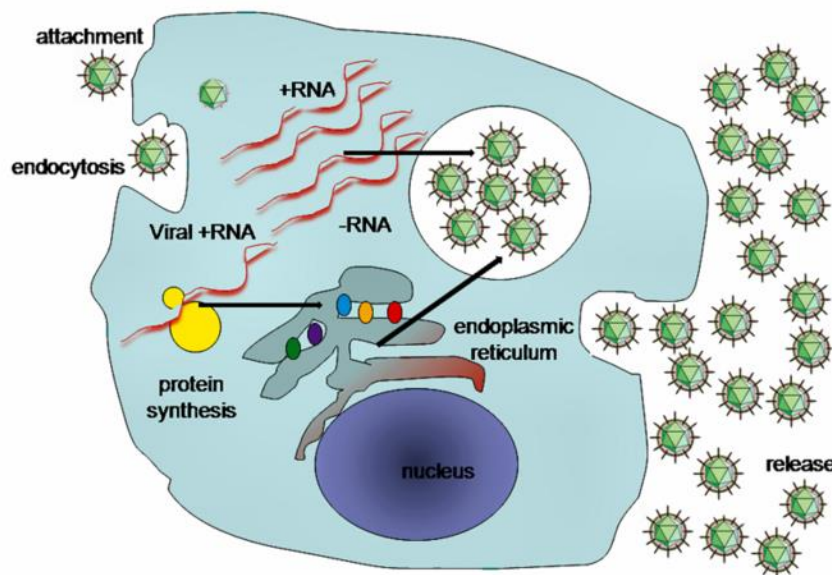
Hepatitis C virus: A simplified diagram of the Hepatitis C virus replication cycle.

Following the structure-mediated self-assembly of the virus particles, some modification of the proteins often occurs. In viruses such as HIV, this modification (sometimes called maturation) occurs after the virus has been released from the host cell.

Viruses can be released from the host cell by lysis, a process that kills the cell by bursting its membrane and cell wall if present. This is a feature of many bacterial and some animal viruses. Some viruses undergo a lysogenic cycle where the viral genome is incorporated by genetic recombination into a specific place in the host's chromosome. The viral genome is then known as a *provirus* or, in the case of bacteriophages a *prophage*. Whenever the host divides, the viral genome is also replicated. The viral genome is mostly silent within the host; however, at some point the provirus or prophage may give rise to active virus, which may lyse the host cells. Enveloped viruses (e.g., HIV) typically are released from the host cell by budding. During this

process the virus acquires its envelope, which is a modified piece of the host's plasma or other internal membrane. The genetic material within virus particles, and the method by which the material is replicated, varies considerably between different types of viruses.

Q.11. Replication of T4, lambda, HIV

Model organisms of virulent viruses that have been extensively studied include virus T4 and other T-even bacteriophages which infect Escherichia coli and a number of related Bacteria.

The lytic cycle is one of the two cycles of viral reproduction, the other being the lysogenic cycle. The lytic cycle is typically considered the main method of viral replication, since it results in the destruction of the infected cell. A key difference between the lytic and lysogenic phage cycles is that in the lytic phage, the viral DNA exists as a separate molecule within the bacterial cell, and replicates separately from the host bacterial DNA. The location of viral DNA in the lysogenic phage cycle is within the host DNA, therefore in both cases the virus/phage replicates using the host DNA machinery, but in the lytic phage cycle, the phage is a free floating separate molecule to the host DNA.

The lytic cycle is a six-stage cycle. In the first stage, called "penetration," the virus injects its own nucleic acids into a host cell. Then the viral acids form a circle in the center of the cell. The cell then mistakenly copies the viral acids instead of its own nucleic acids. Then the viral DNA organize themselves as viruses inside the cell. When the number of viruses inside becomes too much for the cell to hold, the membrane splits and the viruses are free to infect other cells. Some viruses escape the host cell without bursting the cell membrane; instead, they bud off from it by taking a portion of the membrane with them. Because it otherwise is characteristic of the lytic cycle in other steps, it still belongs to this category, although it is sometimes named the Productive Cycle. HIV, influenza and other viruses that infect eukaryotic organisms generally use this method.
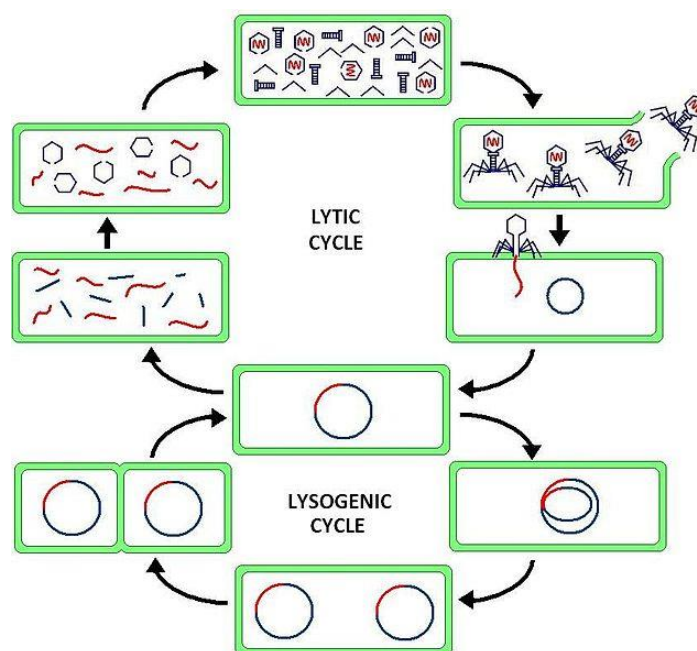
Cycles of viral reproduction: Comparison of the bacteriophage lysogenic and lytic cycles.

T-4 bacteriophage is a bacteriophage that infects E. coli bacteria. Its double-stranded DNA genome is about 169 kbp long and is held in an icosahedral head, also known as a capsid. T4 is a relatively large phage, at approximately 90 nm wide and 200 nm long (most phages range from 25 to 200 nm in length). Its tail fibres allow attachment to a host cell, and the T4's tail is hollow so that it can pass its nucleic acid to the cell it is infecting during attachment. T4 is capable of undergoing only a lytic lifecycle and not the lysogenic lifecycle.

The T4 Phage initiates an E. coli infection by recognizing cell surface receptors of the host with its long tail fibers (LTF). A recognition signal is sent through the LTFs to the baseplate. This unravels the short tail fibers (STF) that bind irreversibly to the E. coli cell surface. The baseplate changes conformation and the tail sheath contracts causing GP5 at the end of the tail tube to puncture the outer membrane of the cell. The lysozyme domain of GP5 is activated and degrades the periplasmic peptidoglycan layer. The remaining part of the membrane is degraded and then DNA from the head of the phage can travel through the tail tube and enter the E. coli.

The lytic lifecycle (from entering a bacterium to its destruction) takes approximately 30 minutes (at 37 °C) and consists of:

- Adsorption and penetration (starting immediately)
- Arrest of host gene expression (starting immediately)
- Enzyme synthesis (starting after 5 minutes)
- DNA replication (starting after 10 minutes)
- Formation of new virus particles (starting after 12 minutes)

After the life cycle is complete, the host cell bursts open and ejects the newly built viruses into the environment, destroying the host cell. T4 has a burst size of approximately 100-150 viral particles per infected host. Complementation, deletion, and recombination tests can be used to map out the rII gene locus by using T4. These bacteriophage infect a host cell with their information and then blow up the host cell, thereby propagating themselves.

Replication of HIV

Human immunodeficiency virus (HIV) is a lentivirus (a member of the retrovirus family) that causes acquired immunodeficiency syndrome (AIDS). AIDS is a condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive. HIV can infect dendritic cells (DCs). DCs are one of the first cells encountered by the virus during sexual transmission. They are currently thought to play an important role by transmitting HIV to T-cells when the virus is captured in the mucosa by DCs. HIV enters macrophages and T cells by the adsorption of glycoproteins on its surface to receptors on the target cell. This is followed by fusion of the viral envelope with the cell membrane and the release of the HIV capsid into the cell.
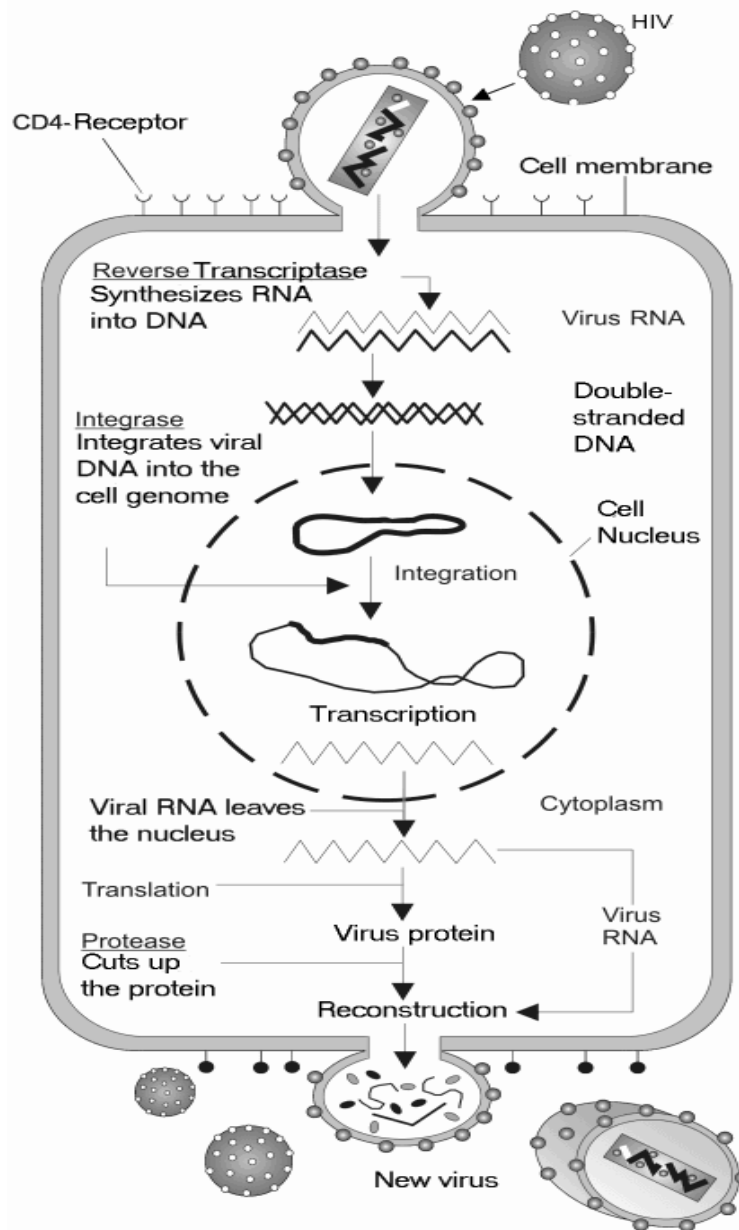
Figure: *HIV Replication: Steps in the HIV Replication Cycle: Fusion of the HIV cell to the host cell surface.Cell Entry, HIV RNA, reverse transcriptase, integrase, and other viral proteins enter the host cell.Viral DNA is formed by reverse transcription.Viral DNA is transported across the nucleus and integrates into the host DNA.New viral RNA is used as genomic RNA to make viral proteins.New viral RNA and proteins move to cell surface and a new, immature, HIV virus forms.Virus maturation and protease release of individual HIV proteins.*

Shortly after the viral capsid enters the cell, an enzyme called reverse transcriptase liberates the single-stranded (+)RNA genome from the attached viral proteins and copies it into a complementary DNA (cDNA) molecule. The process of reverse transcription is extremely error-prone, and the resulting mutations may cause drug resistance or allow the virus to evade the body's immune system. The reverse transcriptase also has ribonuclease activity that degrades the viral RNA during the synthesis of cDNA, as well as DNA-dependent DNA polymerase activity that creates a sense DNA from the antisense cDNA. Together, the cDNA

and its complement form a double-stranded viral DNA that is then transported into the cell nucleus.

This integrated viral DNA may then lie dormant, in the latent stage of HIV infection. To actively produce the virus, certain cellular transcription factors need to be present. The most important of these is NF-κB (NF kappa B), which is upregulated when T-cells become activated. This means that those cells most likely to be killed by HIV are those currently fighting infection. During viral replication, the integrated DNA provirus is transcribed into mRNA, which is then spliced into smaller pieces. These small pieces are exported from the nucleus into the cytoplasm, where they are translated into the regulatory proteins Tat (which encourages new virus production) and Rev.

As the newly produced Rev protein accumulates in the nucleus, it binds to viral mRNAs and allows unspliced RNAs to leave the nucleus, where they are otherwise retained until spliced. At this stage, the structural proteins Gag and Env are produced from the full-length mRNA. The full-length RNA is actually the virus genome; it binds to the Gag protein and is packaged into new virus particles. The final step of the viral cycle, assembly of new HIV-1 virions, begins at the plasma membrane of the host cell. The Env polyprotein goes through the endoplasmic reticulum and is transported to the Golgi complex. There, it is cleaved by HIV protease and processed into the two HIV envelope glycoproteins, gp41 and gp120. These are transported to the plasma membrane of the host cell where gp41 anchors gp120 to the membrane of the infected cell. The Gag (p55) and Gag-Pol (p160) polyproteins also associate with the inner surface of the plasma membrane along with the HIV genomic RNA as the forming virion begins to bud from the host cell.

Maturation occurs either in the forming bud or in the immature virion after it buds from the host cell. During maturation, HIV proteases cleave the polyproteins into individual functional HIV proteins. This cleavage step can be inhibited by protease inhibitors. The various structural components then assemble to produce a mature HIV virion. The mature virion is then able to infect another cell.

Q. 12. Replication of Polio and Influenza
POLIO VIRUS

- 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 monopartite, linear ssRNA(+) genome of 7.2-8.5 kb, polyadenylated, composed of a single ORF encoding a polyprotein.
- The virus particles lack a lipid envelope, and their infectivity is insensitive to organic solvents.

Replication of Polio Virus

- 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 nascently 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.
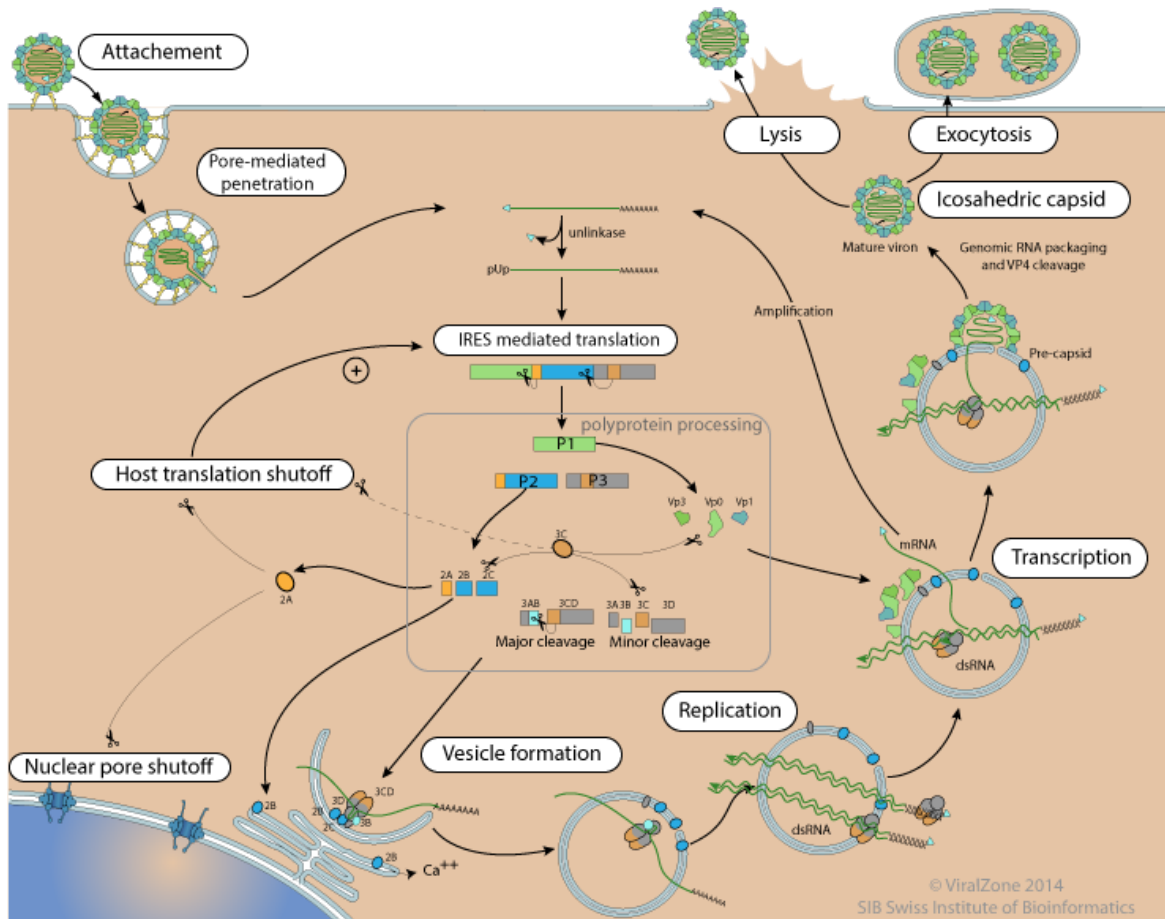
Replicative Cycle of Influenza

Influenza A follows the typical life cycle of most influenza viruses. The infection and replication is a multi-step process:

- Binding to and entering the cell
- Delivering the genome to a site where it can produce new copies of viral proteins and RNA
- Assembling these components into new viral particles
- Exiting the host cell

Influenza viruses bind through hemagglutinin onto sialic acid sugars on the surfaces of epithelial cells, typically in the nose, throat, and lungs of mammals, and the intestines of birds (Step 1 in infection figure). After the hemagglutinin is cleaved by a protease, the cell imports the virus by endocytosis.
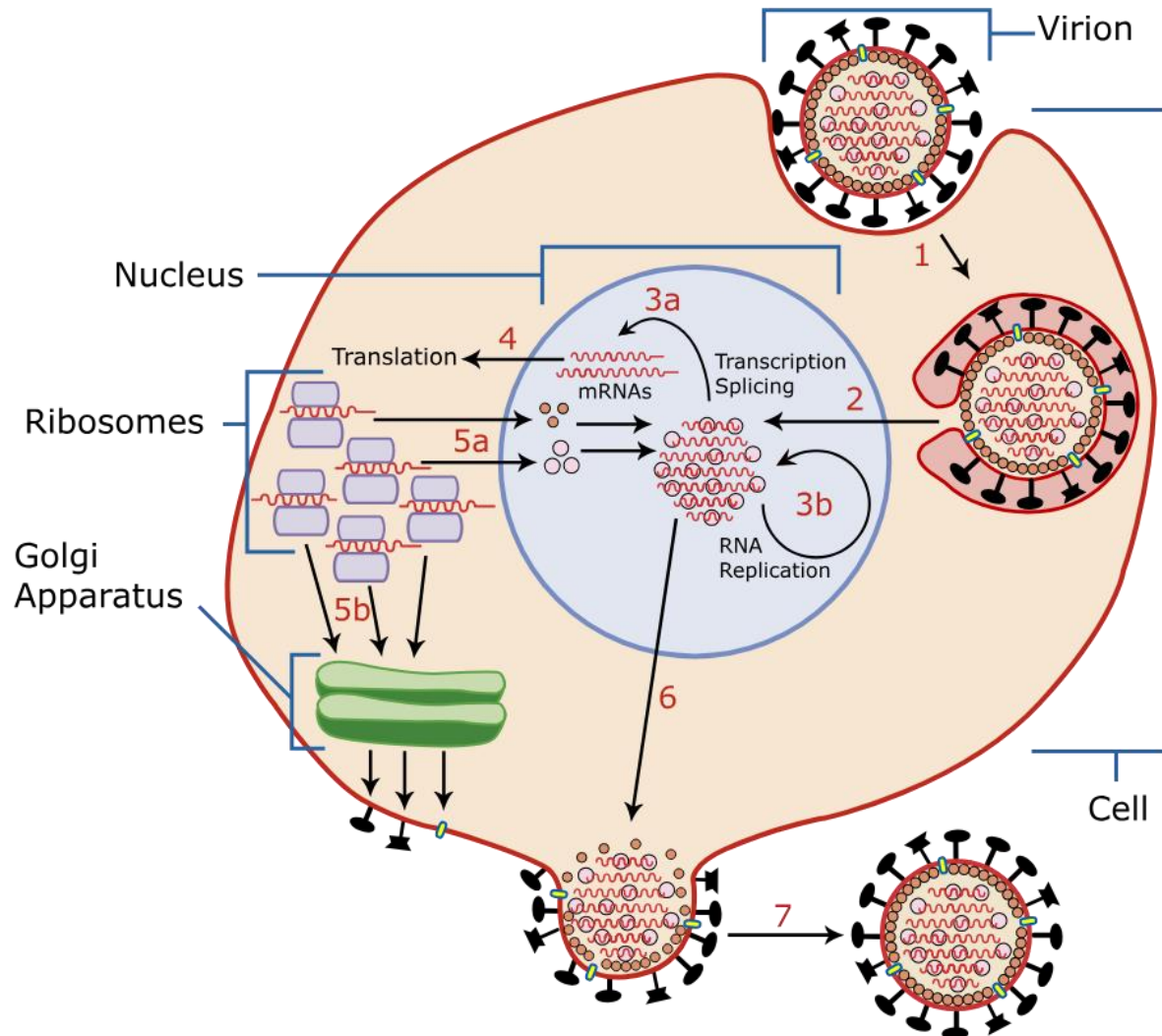

Figure: *Influenza replication cycle: Host invasion and replication cycle of an influenza virus. Step 1: Binding Step 2: Entry Step 3: Complex formation and transcription Step 4: Translation Step 5: Secretion Step 6: Assembly Step 7: Release*

The intracellular details are still being worked out. It is known that virions converge to the microtubule organizing center, interact with acidic endosomes, and finally enter the target endosomes for genome release. Once inside the cell, the acidic conditions in the endosome cause two events to happen:

1. The hemagglutinin protein fuses the viral envelope with the vacuole's membrane.
2. The M2 ion channel allows protons to move through the viral envelope and acidify the core of the virus, which causes the core to dissemble and release the viral RNA and core proteins.

The viral RNA (vRNA) molecules, accessory proteins, and RNA-dependent RNA polymerase are then released into the cytoplasm (Step 2 in figure). These core proteins and vRNA form a complex that is transported into the cell nucleus, where the RNA-dependent RNA polymerase begins transcribing complementary positive-sense vRNA (Steps 3a and b in figure).

The vRNA either enters into the cytoplasm and translated (Step 4) or remains in the nucleus. Newly synthesized viral proteins are either secreted through the Golgi apparatus onto the cell surface (in the case of neuraminidase and hemagglutinin, Step 5b) or transported back into the nucleus to bind vRNA and form new viral genome particles (Step 5a).

Other viral proteins have multiple actions in the host cell—including degrading cellular mRNA and using the released nucleotides for vRNA synthesis, and also inhibiting translation of host-cell mRNAs.

Negative-sense vRNAs that form the genomes of future viruses, RNA-dependent RNA polymerase, and other viral proteins are assembled into a virion. Hemagglutinin and neuraminidase molecules cluster into a bulge in the cell membrane. The vRNA and viral core proteins leave the nucleus and enter this membrane protrusion (Step 6). The mature virus buds off from the cell in a sphere of the host phospholipid membrane, acquiring hemagglutinin and neuraminidase with this membrane coat (Step 7). As before, the viruses adhere to the cell through hemagglutinin; the mature viruses detach once their neuraminidase has cleaved sialic acid residues from the host cell. Drugs that inhibit neuraminidase, such as oseltamivir, therefore prevent the release of new infectious viruses and halt viral replication. After the release of new influenza viruses, the host cell dies.

13. Write an essay on Viroids and Prions

Structure Of Viroids

Viroids differ from virus in structure and form. These consists of solely short strands of circular, and single-stranded RNA without the protein coats.

The plants that are infected by viroids are responsible for the crop failures and also cause the loss of millions of dollars in agricultural revenue every year. Some of the plants that are affected by these pathogens are potatoes, tomatoes, cucumbers, chrysanthemums, coconut palms, avocados, etc.

Viroids were first discovered by T.O. Diener in the year 1971. It was first examined in the potato spindle tuber viroid caused a huge loss to the potato industry.

Viroids are the plant parasites like transcriptional machinery of the **cell organelles** such as the nucleus or the chloroplast since they are known to be non-coding. These replicate by the process of RNA–RNA transcription. They mainly infect the epidermis of the hosts after causing mechanical damage to the cell wall of the plant.

Characteristic Features Of Viroids

Some of the characteristic features of viroids are given below-

- Viroids contain only RNA.
- These are known to be smaller in size and infect only the plants.
- These are among the smallest known agents causing infectious disease.
- Viroids are species of nucleic acid with relatively low molecular weight and a unique structure.

- They reproduce within the host cell which they affect and cause variations in them causing death.
- Viroids are mainly classified into two families namely Pospiviroidae- nuclear viroids and Avsunviroidae- chloroplastic viroids.
- Viroids are said to move in an intracellular manner, cell to cell through the plasmodesmata, and long distance through the phloem.

Viroid Diseases

Some of the diseases that are caused by the infection of viroids in plants are citrus exocortis, cucumber pale fruit, and chrysanthemum stunt. These **infectious diseases** are spread by the propagation of seeds in plants by cutting, tubers, etc and also by mishandling the contaminated implements. Hepatitis- D is caused in humans by viroid-like particles.

The symptoms that are caused by the infection of viroid in plants include stunting of growth, stem necrosis, deformation of the leaves and fruits, and at last causing the death of the plant.

Most of the viroids are said to infect the plants, including coconut and apple trees. The (PSTV) potato spindle tuber viroid causes significant crop damage to the potato yields causing the tubers to elongate and then crack. The other common type of viroid infection symptoms includes stunting and leaf epinasty.

Prions are infectious protein particles responsible for a group of transmissible and/or inherited neurodegenerative diseases including Creutzfeldt-Jakob disease, kuru, and Gerstmann-Straussler-syndrome in humans, as well as scrapie in sheep and goats, and bovine spongiform encephalopathy (mad cow disease) in cattle and in humans (where it is called new variant Creutzfeldt–Jakob disease humans). The infections are often referred to as transmissible spongiform encephalopathies.
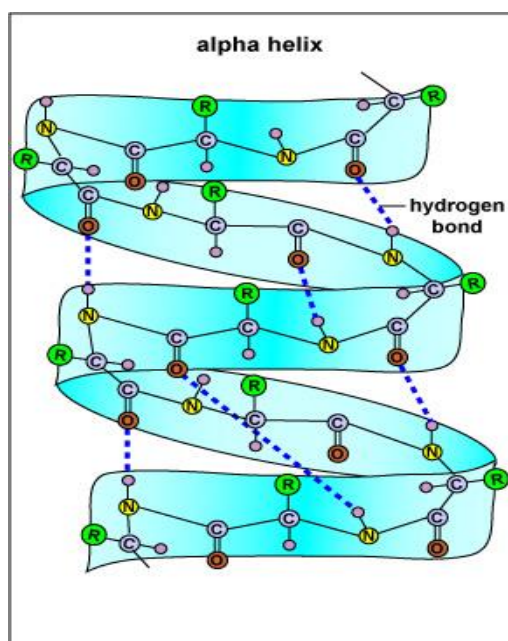

Figure : Secondary Structure of a Protein or Polypeptide Alpha Helix. The secondary structure of a protein or polypeptide is due to hydrogen bonds forming

between an oxygen atom of one amino acid and a nitrogen atom of another. There are two possible types of secondary structure: an alpha helix and a beta sheet. In the case of an alpha helix, the hydrogen bonding causes the polypeptide to twist into a helix. With a beta sheet the hydrogen bonding enables the polypeptide to fold back and forth upon itself like a pleated sheet.

Most evidence indicates that the infectious prion proteins are modified (misfolded) forms of normal proteins coded for by a host gene in the brain. It is thought that the normal prion protein, expressed on stem cells in the bone marrow and on cells that will become neurons, plays a role in the maturation of neurons. In the case of the disease scrapie, the normal prion protein in an animal without the disease has alpha-helices in the proteins secondary structure (Figure hile the scrapie prion protein in diseased animals has beta-sheets for the secondary structure (Figure 10.5.2). When the scrapie prion protein contacts the normal protein it causes it to change its configuration to the scrapie beta-sheet form. This suggests that the conversion of a normal prion protein into an infectious prion protein may be catalyzed by the prion protein itself upon entering the brain. Inherited forms may be a result of point mutations that make the prion protein more susceptible to a change in its protein structure.
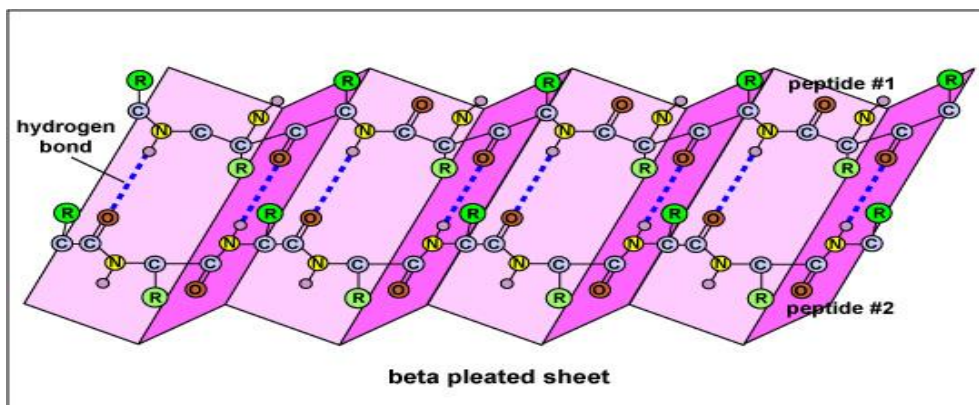

Figure 10.5.2: Secondary Structure of a Protein or Polypeptide Beta Pleated Sheet. The secondary structure of a protein or polypeptide is due to hydrogen bonds forming between an oxygen atom of one amino acid and a nitrogen atom of another. There are two possible types of secondary structure: an alpha helix and a beta sheet. In the case of an alpha helix, the hydrogen bonding causes the polypeptide to twist into a helix. With a beta sheet the hydrogen bonding enables the polypeptide to fold back and forth upon itself like a pleated sheet.

There is growing evidence that other probable protein misfolding diseases initiated by prions include Alzheimer's disease, Hunington's disease, Parkinson's disease, frontotemporal dementias, amyotrophic lateral sclerosis, and certain cancers.

Q.14. Give a outline Concept of Oncogenes and Proto Oncogenes

Humans have about 20,000 genes, which determine everything from hair and eye color, to blood type. Cancer is considered a genetic disease because it is caused by changes, or mutations, in genes that control the way cells grow and multiply.

A combination of mutations involving the following genes are frequently involved in cancer development:

Oncogenes are mutated forms of normal genes (called proto-oncogenes) that promote cell growth. Once mutated, oncogenes trigger "gain-of-function" activities, which may promote cancer development or make cancer more difficult to treat.

Tumor suppressor genes normally manage cell growth and prevent tumor development. Mutations in these genes trigger "loss of function," which may promote tumor development and growth.

DNA repair genes are genes that fix mistakes in DNA or trigger cells that cannot be fixed to die. Mutations in DNA repair genes lead to more mistakes within the cell, which can promote tumor growth.

This article takes a closer look at the role of oncogenes in cancer, along with how they differ from tumor suppressor genes and DNA repair genes. It also provides examples of oncogenes and the cancers they can cause.

What Are Oncogenes?

Proto-oncogenes are normal cellular genes that help cells grow, divide, and stay alive. Every person has them.In most people, proto-oncogenes never mutate into oncogenes. If a mutation does occur, the gene can start to "turn on" in an uncontrolled manner, at which point it is called an oncogene.Unlike proto-oncogenes, an oncogene will not turn off when it should. As a result, cells can grow out of control, potentially leading to the growth of a Examples of Oncogenes. Oncogenes most frequently linked to cancer include:

MUC16: An oncogene that is mutated in about 19% of all tumors. It has been found in cancers of the pancreas, breast, lung, ovaries, and more.[5]

PIK3CA: An oncogene that is mutated in about 12% of all tumors. It has been found in cancers of the breast, lung, stomach, ovary, brain, colon, rectum, and more.[6]

KRAS: An oncogene that is mutated in about 11% of all tumors. It has been found in cancers of the lung, colon, rectum, pancreas, and more.[7]

What Causes Oncogenes to Activate?

Oncogenes may be activated due to inherited causes, or they can activate upon short or prolonged exposure to carcinogens (cancer-causing agents) in the environment.

Carcinogens

Environmental carcinogens both occur naturally and are generated by humans. Known carcinogens include:[9]

Ultraviolet (UV) rays from the sun
Certain viruses, including human papillomavirus (HPV), hepatitis B virus (HBV), and Epstein-Barr virus (EBV)
Arsenic, often in contaminated plants or water
Aflatoxins, a fungi found on corn, peanuts, tree nuts, and other plants
Asbestos, a group of minerals found in construction and building materials, such as vinyl flooring and insulation, as well as contaminated rocks and soil[10]
Formaldehyde, found in building insulation, household glues, paints, and lacquers, and preservatives used in some medicines, cosmetics, dishwashing liquids, and fabric softeners[11]

Alcoholic beverages
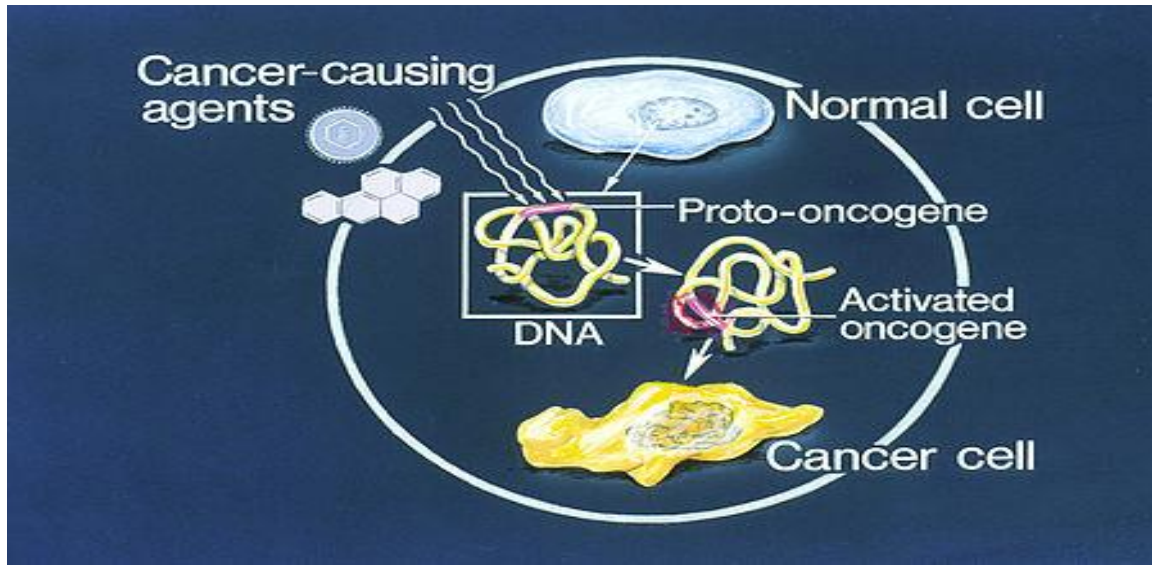Coal emissions
Engine exhaust and diesel
Ionizing radiation, found in natural sources like soil and vegetation, and manmade sources like x-ray machines
Outdoor air pollution
The consumption of processed meat
Welding fumes
Estrogen-only menopausal therapy.

Q.15. write about Applications of Viruses in Biotechnology

Viruses, traditionally seen as agents of disease, have found numerous applications in biotechnology. These applications range from gene therapy to molecular biology tools. Here are some key areas where viruses are utilized:

● Gene Therapy: Viruses, particularly retroviruses and adenoviruses, are employed as vectors to deliver therapeutic genes into target cells. These viral vectors are engineered to carry the desired gene into the host cell's genome, correcting genetic disorders or enabling the expression of therapeutic proteins.
● Vaccine Production: Viruses play a crucial role in the production of vaccines. Attenuated or inactivated viruses are used to induce an immune response without causing disease, thereby providing immunity against specific pathogens. Examples include the flu vaccine and the measles vaccine.
● Molecular Cloning: Viral vectors are utilized in molecular cloning techniques to introduce foreign DNA into host cells for replication and expression. Bacteriophages, such as lambda phage, are commonly used in bacterial cloning systems.
● Gene Editing: Viruses are employed in gene editing techniques such as CRISPR-Cas9. Researchers use viral vectors to deliver CRISPR components into target cells, allowing precise modification of the host genome for various purposes, including disease modeling and gene therapy.
● Phage Therapy: Bacteriophages, viruses that infect bacteria, are explored as an alternative to antibiotics for combating bacterial infections. Phage therapy involves

using specific bacteriophages to target and kill pathogenic bacteria, offering a potential solution to antibiotic resistance.

- Viral Display Systems: Viruses are engineered to display foreign peptides or proteins on their surfaces. These viral display systems, such as phage display, enable the selection of peptides or antibodies with desired properties, facilitating drug discovery and development.
- Viral Vectors in Gene Delivery: Viral vectors, including lentiviruses and adeno-associated viruses (AAVs), are widely used for delivering genetic material into mammalian cells for research and therapeutic purposes. They offer efficient transduction and stable gene expression in a variety of cell types.
- Viral Nanoparticles: Viruses can be engineered into nanoparticles for various applications, including drug delivery and imaging. Their small size, high surface area-to-volume ratio, and ability to target specific cells make them promising tools in nanomedicine.
- Viral Assays for Research: Viruses serve as essential tools in various assays and experiments for studying cell biology, virology, and immunology. Techniques such as viral plaque assays and viral neutralization assays are fundamental for quantifying viral infectivity and assessing immune responses.
- Viral Vectors in Agriculture: Viral vectors are explored for applications in plant biotechnology, including crop improvement and gene editing. Plant viruses can be engineered to deliver genes of interest into plant cells, enabling traits such as disease resistance and enhanced yield.

In conclusion, viruses have diverse applications in biotechnology, ranging from therapeutic interventions to fundamental research tools. Continued advancements in viral engineering and understanding of viral biology are expected to expand the scope of their applications in various fields.