

BBYCT-131
BIODIVERSITY
(MICROBES, ALGAE, FUNGI
AND ARCHEGONIATES)

Block

1

MICROBES

UNIT 1

Viruses: General Account and Economic Importance	7
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UNIT 2

Viruses: Replication	35
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UNIT 3

Bacteria: General Account and Economic Importance	56
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UNIT 4

Bacteria: Reproduction	88
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BIODIVERSITY (MICROBES, ALGAE, FUNGI AND ARCHEGONIATES)

You will study about the world of microbes and non-flowering plants in this elective course namely: CBCS-Botany, Paper I–Biodiversity (Microbes, Algae, Fungi and Archegoniates).

This course will reveal to you how diverse these organisms are in their body organization and mode of reproduction. You will also be able to appreciate the organizational and reproductive strategies they adopt to thrive successfully in their diverse habitats, both aquatic and terrestrial. You will also be exposed to the ecological significance and importance to human welfare of these organisms.

However, some of the groups of organisms such as microbes, fungi and even the true algae, though studied traditionally by botanists are no longer considered to be true plants. In spite of this fundamental distinction between plants and non-plant organisms, their inclusion as part of botany text is partly justified because they share certain similarities in their ecology, morphology, physiology and methods of reproduction.

One of the most important event that could have occurred in the evolution of all kinds of organisms had been the transfer of life from an aquatic environment to the land habitat. How and when did it actually happen is still an unsolved mystery? We shall, however, trace and discuss the significant and probable strategies adopted by organisms during and after this path breaking event. You should also remember that the most obvious, most evolved plant group, the flowering plants, represented by about 2,60,000 species is not part of this course. This observation alone should make you comprehend the enormity of diversity of plant life.

This course is a 4 credit course. The overall organisation of the study of UGC-CBCS Core Botany, Paper I – Biodiversity (Microbes, Algae, Fungi and Archegoniates) is as follows:

COURSE BBYCT-131 BOTANY, PAPER I – BIODIVERSITY (Microbes, Algae, Fungi and Archegoniates)

Block 1	Microbes (4 Units)
Block 2	Algae (4 Units)
Block 3	Fungi (3 Units)
Block 4	Bryophytes (4 Units)
Block 5	Pteridophytes (3 Units)
Block 6	Gymnosperms (4 Units)

Objectives

After studying Paper I, Biodiversity course, you will be able to:

- appreciate the diversity in structure, numbers and distribution of life forms;
- discuss the origins, evolution and interrelationships among different groups;
- classify the various groups in major broad categories;
- describe how they reproduce;
- discuss the role of these groups of plants in ecosystem and in human welfare;
- study the life cycles of certain selected genera; and
- correlate the importance and strategies adopted by primitive archegoniates during transfer to land habitat.

BLOCK 1 : MICROBES

The first block of course Biodiversity (Microbes, Algae, Fungi and Archegoniates) deals with bacteria and viruses. In the first two units you will study about viruses while next two units will describe bacteria. Life on the planet Earth can be classified into three domains: Archaea, Bacteria, and Eukaryota. The Archaea and Bacteria are prokaryotic single-celled organisms whose cells do not have a nucleus. The cells of Eukaryota have a nucleus and membrane-bound organelles. All the animals, plants, fungi and protozoa are included in the domain Eukaryota.

Bacteria were the first organisms on earth; they have lived on this planet for a period of at least 3.7 billion years out of the 4.6 billion years the earth has existed. The simplest and the most abundant microorganisms living on earth today are bacteria, and are thought to be the most ancient. Biologists think that they closely resemble the first organisms that evolved on earth. Microorganisms are living organisms (such as a bacteria, archaea, fungi, viruses) that are too small to be seen with naked eye but visible under a microscope.

First unit is the introduction to the world of viruses. Viruses are the only forms which infects all the three domains of life (i.e. Archaea, Bacteria and Eukaryota). Viruses can be defined as small obligate, intracellular particles seen only by electron microscope that infect and take over the host cell to replicate. Viruses blur the boundary between inanimate (non-living) and life (living). All viruses are acellular entities and are made of genetic material (DNA or RNA) inside a protein coat. The viruses have the ability to defy the germ theory, which led to their discovery. The structure of viruses and function of each component along with the properties of viruses has been discussed in detail. Viruses are economically important as they cause diseases in humans (Swine flu), animals (Foot and Mouth Disease) and crops (Tobacco mosaic Disease). They also play an important role in medicine (vaccine production, cancer therapy, phage therapy).

In the second unit you will study about replication of viruses. The viruses cannot reproduce on their own and therefore, require machinery of the host cell for synthesis of the viral genome and proteins. To replicate, a virus has to first enter the host cell and then replicate. In this unit the steps involved in the process of viral replication namely: attachment/adsorption, penetration and uncoating, biosynthesis, assembly and release, will be studied. We have selected replication cycle of one DNA virus (T phage) and one RNA virus (TMV) for detailed studies. These would help you know of the process of replication, especially lysogenic and lytic cycles of viral replication.

Third unit is an introduction about the extremely diverse groups of microorganisms exhibiting different morphologies and physiologies. Bacteria are ubiquitous on earth. In this unit there is a brief discussion on the structure and morphology of bacteria and description of various organelles found in bacterial cell. Bacteria have different shapes and can be classified by their shapes. Life on earth could not exist without bacteria because they make possible many of the essential functions of ecosystems, including the fixing the nitrogen from the atmosphere, and decomposition of organic matter in many aquatic communities. Bacteria play vital role both in productivity and in cycling of the substances essential to all other life-forms. The unit also includes economic importance of bacteria in our life and also their harmful effects.

Fourth unit talks about various aspects of bacterial reproduction: asexual and sexual. You will study about asexual reproduction of bacteria through fission, endospore, or cyst formation. Fission or binary fission is trait of bacteria and is shared only by some yeast.

Sexual reproduction occurring in bacteria is quite different from the one encountered in eukaryotes, but it does involve genetic recombination. The genetic transfer and recombination in bacteria takes place via mechanisms of conjugation, transformation and transduction.

Objectives

After studying this block, you should be able to:

- appreciate the discovery of viruses and describe their structural components;
- know the classification criteria of viruses and differentiate between DNA virus (T phage) and RNA virus (TMV);
- enlist the economic importance of viruses;
- describe different ways of viruses replication and differentiate between lysogenic and lytic cycle of replication;
- apprehend the history of discovery of bacteria and list the characteristic features of bacteria;
- classify bacteria on the basis of shapes and identify the structure and morphology of bacteria;
- know about the economic importance of bacteria;
- describe various types of reproduction in bacteria and differentiate between asexual and sexual reproduction in bacteria; and
- compare and contrast between bacterial conjugation, transduction and transformation.

VIRUSES: GENERAL ACCOUNT AND ECONOMIC IMPORTANCE

Structure

- | | |
|----------------------------------|--|
| 1.1 Introduction | 1.6 Other Virus-Like Things :
Sub Viral Particles |
| Objectives | Viriods |
| 1.2 Discovery | Prions |
| 1.3 General Account | 1.7 Economic Importance |
| Virus Size | Economic Losses due to Viral Diseases |
| Structure of Viruses | Economic Benefits of Viruses |
| The Virus Symmetry | 1.8 Summary |
| Viral Envelope | 1.9 Terminal Questions |
| The Viral Nucleic Acid | 1.10 Answers |
| Viral Enzymes and other Contents | Glossary |
| Viral Taxonomy | |
| 1.4 DNA Virus (T Phage) | |
| 1.5 RNA Virus (TMV) | |

1.1 INTRODUCTION

We all, have suffered from symptoms of common cold that include, runny nose, sneezing, feeling feverish (but not having fever) at some point of our life. The doctor does not prescribe an antibiotic (effective against bacterial infections) for the common cold as common cold occurs due to an infection of virus (Rhinovirus). Viruses are unique class of infectious agents. They infect all types of organisms. According to the Carl Woese system of classification, the tree of life consists of three domains: **Archaea, Bacteria, and Eukaryota** (Fig. 1.1). The Archaea and Bacteria are prokaryotic single-celled organisms whose cells have no definite nucleus. Cells of eukaryota possess a nucleus and membrane-bound organelles. The animals, plants, fungi and protozoa are included in the eukaryota. You will be surprised to know that viruses are the only form of life which infects all the three domains of life (i.e. Archaea, Bacteria and Eukaryota). **Viruses can be defined as small obligate, intracellular particles seen only by electron microscope that infect and take over the host cell to replicate.**

In this village

*It looks as if frosting
continuously*

For, the plant I saw

In the field of summer

*The color of the
leaves were yellowing*

(Written by Empress
Koken in AD 752 and
translated by T. Inouye)

The plant, identified
as *Eupatorium
lindleyanum*, has
been found to be
susceptible to tomato
yellow leafcurl virus,
which causes a
yellowing disease.

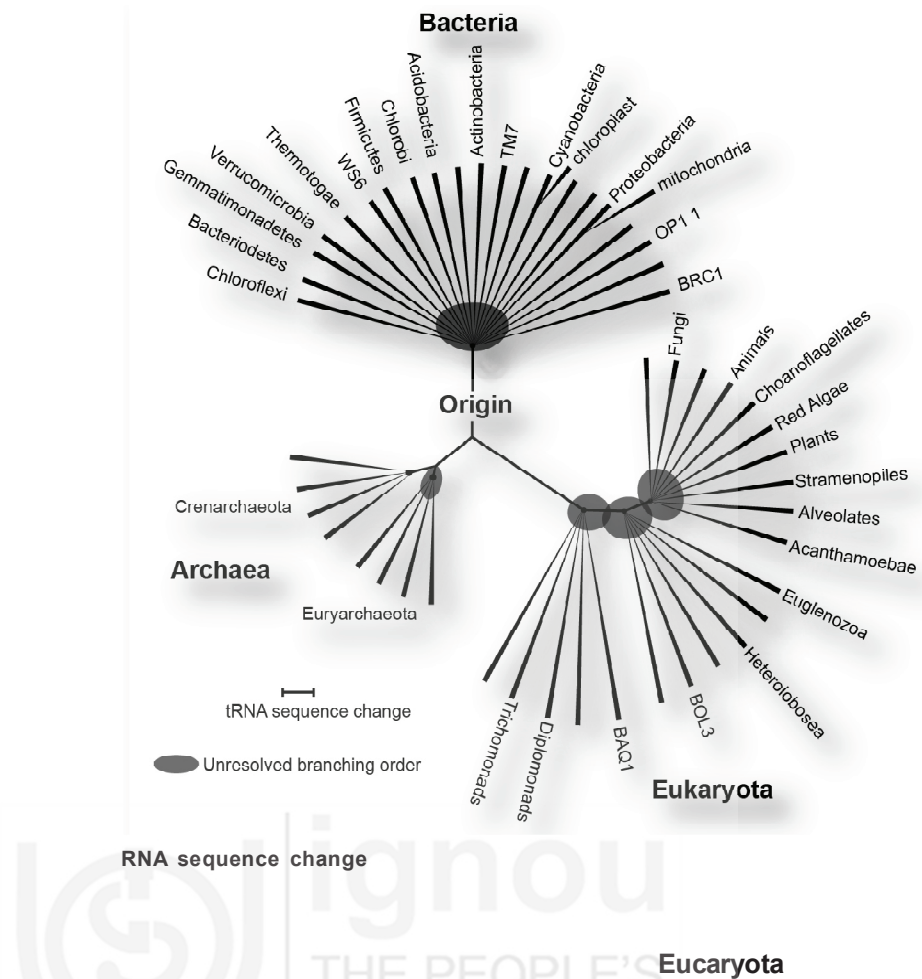


Fig. 1.1: Three domains of Life.

Viruses blur the boundary between inanimate (non-living) and life (living). Anything is considered living if it is made of cells, reproduces, grows, metabolises, responds and adapts to the environment *e.g.*, bacteria, fungi, protist, human beings, plants, animals etc. All viruses are **acellular entities** and are made of genetic material (DNA or RNA) inside a protein coat. Viruses do not have the ability to grow and multiply in any artificial media as they are metabolically inert (inactive) and can be metabolically active only when inside a living host cell. They multiply or are metabolically active only inside an animal, plant or microbial cell. This ability of the viruses to infect all forms of life makes them a major force for evolution as each virus has the ability to introduce new DNA or RNA through shuffling and redistribution of genes into any other life form. Thus, it can be said that viruses have played a key role in shaping the history of life on earth.

In the first section of this unit you will learn how the viruses were discovered and the role tobacco growers of Europe played in this fascinating discovery. You will study how the ability of the viruses to defy the germ theory led to their discovery. You will also learn how inferences drawn from experiments by scientists led to certain concepts of virology. The structure of viruses and function of each component along with the properties of virus, will be studied in detail. Viruses are important due to the economic impact they have by causing diseases in humans (Swine flu), animals (Foot and Mouth Disease) and crops (Tobacco mosaic Disease) and the role they play in medicine (vaccine production, cancer therapy, phage therapy). In this unit, focus will also be on their economic importance.

Objectives

After studying this unit, you will be able to:

- ❖ describe how viruses were discovered;
- ❖ describe and draw the structural components of viruses;
- ❖ differentiate between helical, icosahedral and binal symmetries;
- ❖ discuss the classification criteria of viruses and list examples of bacteriophages, animal and plant viruses;
- ❖ draw and differentiate between DNA virus (T phage) and RNA virus (TMV); and
- ❖ enlist the economic importance of viruses.

1.2 DISCOVERY

The credit for the discovery of virus goes to the invention of **Chamberland-Pasteur filter** (Fig. 1.2). Chamberland-Pasteur filter was developed in 1884 in Paris by Charles Chamberland, who worked with Louis Pasteur (Father of Modern Microbiology). It consisted of unglazed porcelain “candles”, with pore sizes of 0.1-1 micron (100-1000 nm), which had the ability to completely remove/filter all bacteria or any other cells known at the time, from a liquid suspension. Through this simple invention a whole new science – Virology was established.

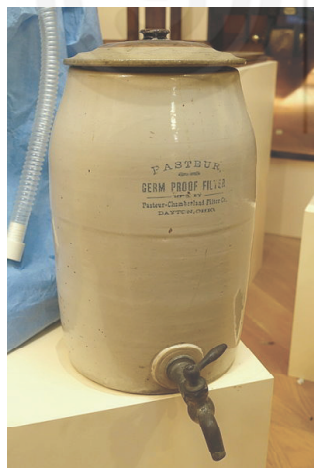


Fig. 1.2: The credit for the discovery of virus goes to the invention of Chamberland-Pasteur filter.

At the end of the nineteenth century the **germ theory** of disease was formulated by Robert Koch (Founder of Modern Bacteriology). The **germ theory** states that infectious diseases are caused by microorganisms, the causative agents of disease can be seen with the aid of a microscope, cultivated on a nutrient medium, and can be retained/filtered by bacterial filters.

At about the same time i.e., the end of the 19th century tobacco growers in Europe were worried because the presence of mosaic like pattern on tobacco leaves was causing poor crop yield and heavy losses. Some of the farmers even stopped growing tobacco and a search for the causative agent responsible for mosaic like pattern on tobacco leaves began. A German

scientist, Adolf Meyer (1883) did an experiment where he rubbed the sap extracted from diseased leaves onto healthy plants, the latter also developed the disease. Meyer was unable to isolate the causative microbe present in the sap. Therefore, he concluded that the causative agent may be very small bacteria which could not be seen by a microscope. Two scientists **Dimitri Iwanowski** and **Martinus Beijerinck** played a crucial role in the discovery of the causative agent of this puzzling disease (Fig. 1.3).

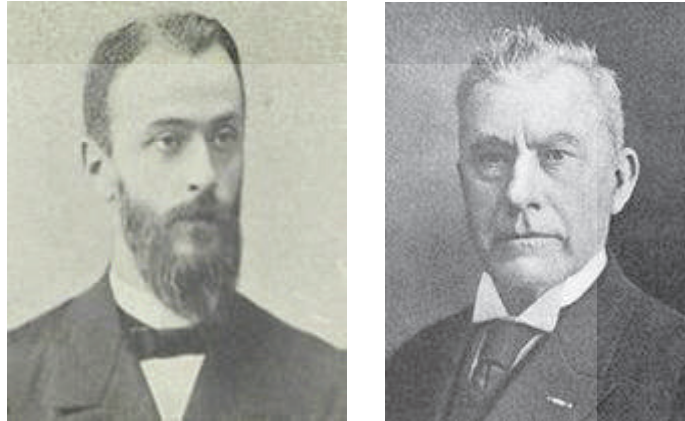


Fig. 1.3: Dimitri Iwanowski (1892) and Martinus Beijerinck (1898) are credited for the discovery of viruses.

In 1892 **Dimitri Iwanowski** became the first person to show that the causative agent of the mosaic disease of tobacco plants, visible as a discoloration of the leaf, passed through a bacteria-proof filter, and could not be seen under light microscope. Dimitri Iwanowski performed an experiment to isolate the causative agent of Tobacco Mosaic Disease (TMD). He crushed the leaves of the TMD infected plants, and filtered the mixture through a bacteria-proof filter candle. To his surprise, the sap, which had passed through the bacteria-proof filter candle was infective. From his observation, Iwanowski concluded that TMD was not caused by bacteria, but by something smaller than the bacteria that could pass through these bacteria proof filters. Dimitri Iwanowski was, however somewhat unconvinced by his discovery and doubted the efficacy of his bacterial filters.

Another microbiologist **Martinus Beijerinck** repeated the same set of experiments in 1898 and observed that the causative agent of TMD passed through the bacterial filters and validated the discovery of Iwanowski. Moreover, he observed that the filtered agent could not be cultivated in non-living media as is expected for bacteria. He believed that TMD was caused by a new form of infectious agent which was smaller and simpler than bacteria. He termed it *contagium vivum fluidum*, or virus meaning poison.

At the same time, **Friedrich Loeffler and Paul Frosch** (Fig. 1.4) were working on foot-and-mouth disease (FMD), a disease of cloven-hoofed animals (eg. cow, buffalo etc) which is characterized by vesicular lesions in and around the mouth, on the feet and on the teats and causes high morbidity and reduces milk production. Loeffler and Frosch isolated the extract from the vesicles of infected animals and filtered it through bacterial filters. They diluted the filtered extract having the causative agent. On dilution of the filtered vesicle extract, the concentration of causative agent in the filtered vesicle extracts

decreases. They then injected the diluted filtered vesicle extract in healthy animals. The healthy animals, developed FMD, even after dilution. Loeffler and Frosch again isolated the extract from the vesicles of infected animals and filtered it through bacterial filters. The filtered extract having the causative agent was again diluted and injected in healthy animal. They observed that foot-and-mouth disease could be transmitted from animal to animal even after dilution. Loeffler and Frosch from their experiments concluded that the causative agent must be reproducing as it was infective even after dilution and so could not be a bacterial toxin but something living. Loeffler and Frosch were able to rule out that the causative agent of FMD was not a toxin and were able to discover the first animal virus.



Fig. 1.4: In 1898, Friedrich Loeffler and Paul Frosch showed for the first time that animal virus was responsible for foot-and-mouth disease in cattle.

Bacteriophages, the virus infecting bacterial cells were discovered independently by **Fredrick Twort** in England in 1915, and by **Félix d'Hérelle** at Pasteur Institute in Paris in 1917 (Fig.1.5). **Fredrick Twort** had been trying to grow viruses in the artificial media, which seems a futile path of research to us now, as we now know that viruses can only grow inside living cells and not on artificial media. Apparently, he never succeeded in growing the filter-passing agent on artificial media. He did, however, observe something else. He had inoculated an agar medium with some fluid (having vaccinia virus) commonly used for smallpox vaccinations. He noticed that although the vaccinia virus did not grow, however Micrococcus, a type of bacteria did grow. The bacteria, however, appeared to be afflicted with some disease – “inoculated agar tubes often showed watery-looking areas, and in cultures that grew micrococci it was found that some of these colonies could not be subcultured, but if kept they became glassy and transparent”. This phenomenon, came to be known as the glassy transformation of Twort. Twort, made some very interesting observations about the glassy transformation: (i) The affected colonies would not grow on any medium. (ii) Examination of the glassy areas revealed only minute granules and no bacteria. (iii) If a pure culture of micrococcus was touched with a small portion of one of the glassy colonies, the growth at the point touched started to become transparent and gradually made the whole colony transparent. (iv) After filtration of the glassy material through a Chamberland candle, it retained its ability to cause the “glassy transformation.” (v) The “transformation” could be conveyed to fresh cultures

for an indefinite number of generations. Fredrick Twort published a report of a glassy transformation of bacterium *Micrococci*. Twort noticed that the *Micrococcus* colonies were being lysed by the ultrafilterable infectious agent. Twort referred to it as “the bacteriolytic agent”. This was the first evidence of a **bacteria virus**. In 1917 **d’Herelle’s** rediscovered this phenomenon with his first communication on bacteriophage, “An Invisible Microbe Antagonistic to Dysentery Bacteria”. The main observations reported were that an agent capable of killing the dysentery bacterium (*Shigella*) appeared in the intestines of persons recovering from dysentery and, that this agent could be filtered through a Chamberland filter and still be active; that it could be serially transmitted and hence must be a “living germ”. Since the virus was incapable of multiplying except at the expense of living bacteria, he called his virus a *bacteriophage* (literally a bacterium eater) or *phage* for short. This is derived from the Greek word “phagein” which means to eat. Thus the first definition of these new agents, the viruses, was presented entirely in negative terms: they could not be seen, could not be cultivated in the absence of cells, and most important of all, were not retained by bacteria-proof filters.



Fig. 1.5: Frederick Twort (1915) independently discovered bacteriophage.

In 1935 **Wendell Stanley** for the first time crystallized a virus, the tobacco mosaic virus (TMV, Fig. 1.6) The crystallized virus when injected inside host cell also showed infective properties proving that viruses could exist as living entities in living cells and as non living entities (crystals) outside living cells. Crystallisation of virus opened up the debate whether viruses are living or non living or are just on the threshold of life (see Box).



Fig. 1.6: In 1935 Wendell Stanley (1904-1971), a young chemist purified a virus in the form of needle-shaped crystals with the chemical properties of a protein.

BOX 1.1: Are viruses living or non living?

The controversy whether viruses are living or non living is continuing since TMV was first crystallized.

Viruses are considered living as :

1. They possess genetic material (DNA or RNA) and protein;
2. They can replicate only inside the host cell. They use the cellular machinery of the cell (ribosomes, tRNA, enzymes etc) to replicate its genome and synthesize protein coat;
3. Even the crystallized virus conserve the living properties; and
4. They are transmitted from infected host to uninfected host.

Viruses are considered non living as :

1. Viruses are unable to show living properties outside a cell;
2. They can be crystallized;
3. Viruses can replicate inside host but cannot grow; and
4. They do not have metabolic machinery.

Box 1.2 : Invention of vaccine by Edward Jenner against smallpox (a viral disease)

Vaccine for a viral disease, smallpox which had devastated mankind for centuries was developed even before the discovery of viruses. In May 1796, Edward Jenner found a young dairymaid, Sarah Nelms, who had fresh cowpox lesions on her hands and arms. On May 14, 1796, using matter from Nelms' lesions, he inoculated an 8-year-old boy, James Phipps. Subsequently, the boy developed mild fever and discomfort. Nine days after the procedure he felt cold and had lost his appetite, but on the next day he was much better. In July, 1796, Jenner inoculated the boy again, this time with matter from a fresh smallpox lesion. No disease developed, and Jenner concluded that protection was complete and the first vaccine for a viral disease was developed.

SAQ 1

Which among the following statement are true:

- i) Viruses can be defined as large obligate, intracellular particles seen only by light microscope that have to infect and take over host cell to replicate.
- ii) Bacteriophages infect bacteria.
- iii) First animal virus was discovered by Twort and d'Herelle.
- iv) Wendell Stanley for the first time crystallised virus.

1.3 GENERAL ACCOUNT

Viruses are **non-cellular** life forms and are obligate intracellular parasites. Viruses exist in two phases i.e. extracellular and intracellular. In extracellular phase, the complete virus particle is called **virion**, which remains in an inert state i.e., it does not carry out any metabolic activity. In intracellular phase, however, the virus replicates by using genome and metabolic machinery of the host cell. Viruses do not have organelles, cell wall, cytoplasm, nucleus or nucleoid. Viruses have few or no enzyme of their own for metabolism. The viruses lack enzymes that synthesize proteins, nucleic acid and those generating ATP. So a virus in order to survive and multiply has to use the enzymes of the host cell. The fact that **viruses cannot multiply outside the host cell** has considerable medical importance for the development of antiviral drugs. **Antiviral drugs** as the name indicates destroy the virus.

You by now know that the virus can survive only if it is inside the cell. Let us now discuss the structural features of viruses.

1.3.1 Viral Size

Viruses are so small in size that most of them cannot be seen under the light microscope as resolution of a light microscope is limited to about 200 nm. Their size ranges from 0.02 to 0.3 μm (20 to 300 nm) in diameter (Fig. 1.7). Although most viruses are smaller than bacteria (polio virus, which is one of the smallest one, is about 28 nm in diameter), some of the larger viruses (such as the smallpox virus which is about 200 nm in diameter) are about the same size as very small bacteria (mycoplasma).

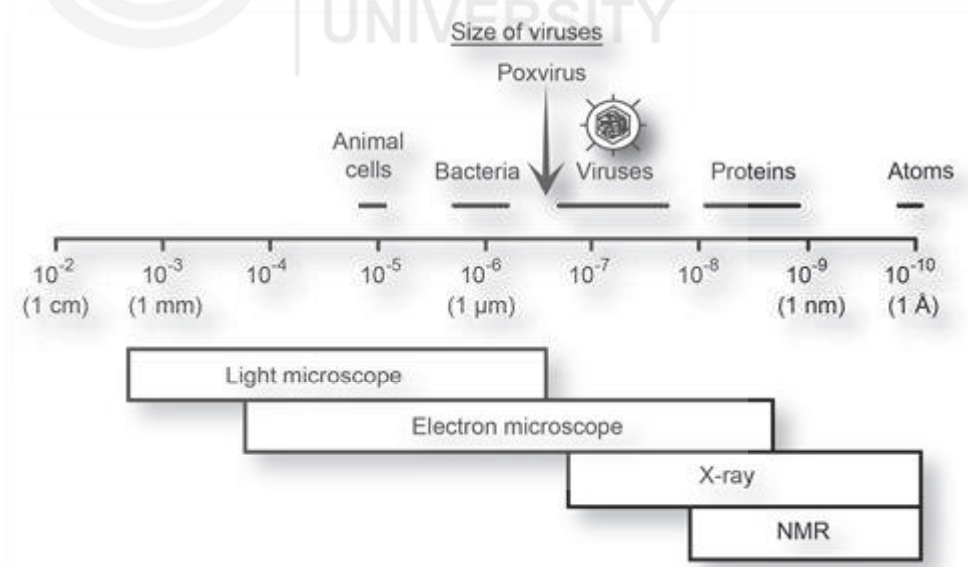


Fig. 1.7: Comparative sizes of animal cells, bacteria, viruses, proteins.

1.3.2 Structure of Viruses

The viruses are made up of only two components: a) protein (capsid) and b) nucleic acid (viral genome) (Fig. 1.8). The capsid and the viral genome are together called the **nucleocapsid**. The nucleic acid (DNA or RNA) is the infectious part of the virus or the part that causes diseases in the host organism.

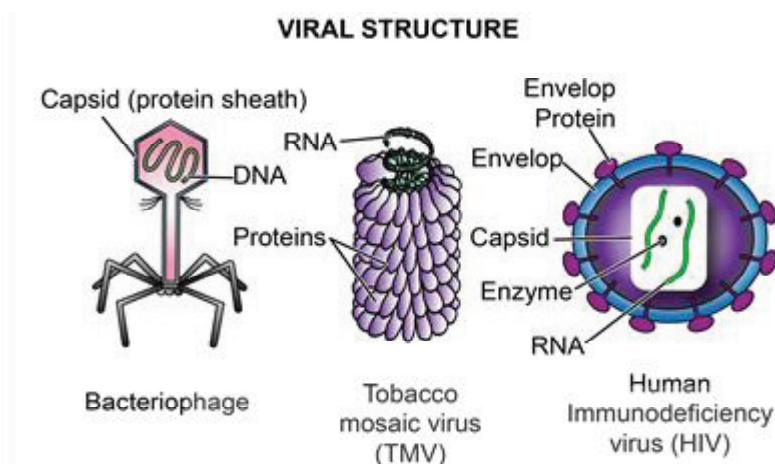


Fig. 1.8: Structure of Bacteriophage; Tobacco mosaic virus and HIV virus.

The Capsid

Capsid is a protein coat that protects the viral genome against chemical and physical damage. It also helps the virus to enter into the host cell. Capsid helps in assembly and packaging of the viral genome during viral replication (will be discussed in detail in the next unit). One of the important functions of capsid is that it induces a protective (immune) response in host. The capsid acts as antigen in the host and invokes an antibody-based immune response which is the first layer of defence of the host from viral infection. Antibodies lock onto the outer surface proteins of a virus and prevent it from entering host cells.

The capsid is constructed from a number of protein molecules, the **structural subunits** that are called **protomers**. These protein molecules are arranged around the genome in a precise and repetitive pattern. Some viruses have single type of protomers in their capsid. Many other viruses contain different types of structural subunits (protomers) that assemble to form larger structural subunits of the protein coat and are called **capsomeres**. Based on their shape and structure, nucleocapsid also gives symmetry to the virus.

1.3.3 The Virus Symmetry

The capsomeres as you know are the building blocks of capsid. These capsomeres are responsible for the three different symmetry groups of viruses.

The viruses fall into three symmetry groups :

1. **Helical symmetry:** The helical structure is cylindrical in shape (Fig. 1.9). The nucleic acid (DNA or RNA) and the capsomeres are wound together in form of a helix. This virus structure has a capsid with a central cavity or hollow tube. The hollow tube is made by proteins arranged in a circular fashion, creating a disc like shape. The disc shapes are attached helically forming a tube with the nucleic acid in the middle. Viruses having helical symmetry are long rods. The length of the helical capsid is determined by the length of the viral nucleic acid and diameter is dependent upon the size, shape and interactions of the protomers. Examples of a virus with a helical symmetry are the tobacco mosaic virus, influenza virus etc.

2. **Cubical / icosahedral symmetry:** An icosahedral (*icosa* means 20 in Greek) is a polygon with 20 facets and 12 corners (Fig. 1.9). Each facet in an icosahedron is an equilateral triangle. The icosahedral forms of nucleocapsid have the shape of a regular polyhedron with triangular faces and vertices. Icosahedral capsids are constructed from knob-shaped capsomeres. Each capsomere is generally made up of five or six protomer subunits. Capsomeres called pentamers (pentons) have five subunits and those with six subunits are called hexamers (hexons). This is the most optimal way of forming a closed shell using identical protein sub-units. The genetic material (DNA or RNA) is fully enclosed inside the capsid. Examples of viruses with an icosahedral structure are some plant and bacterial RNA viruses, many of which have identical protomers in the pentamers and hexamers of their capsids. Adenoviruses are animal viruses whose capsid has 252 capsomeres and its pentamers are made of different protein subunits.
3. **Complex symmetry:** These viruses do not show purely helical or cubical symmetry but have some extra features or are a combination of cubical and helical symmetry and may have a complex outer wall or head-tail morphology (Fig. 1.9). The head-tail morphology structure is unique to viruses that infect only bacteria and are known as bacteriophages. The head of the virus has an icosahedral shape with a helical shaped tail.

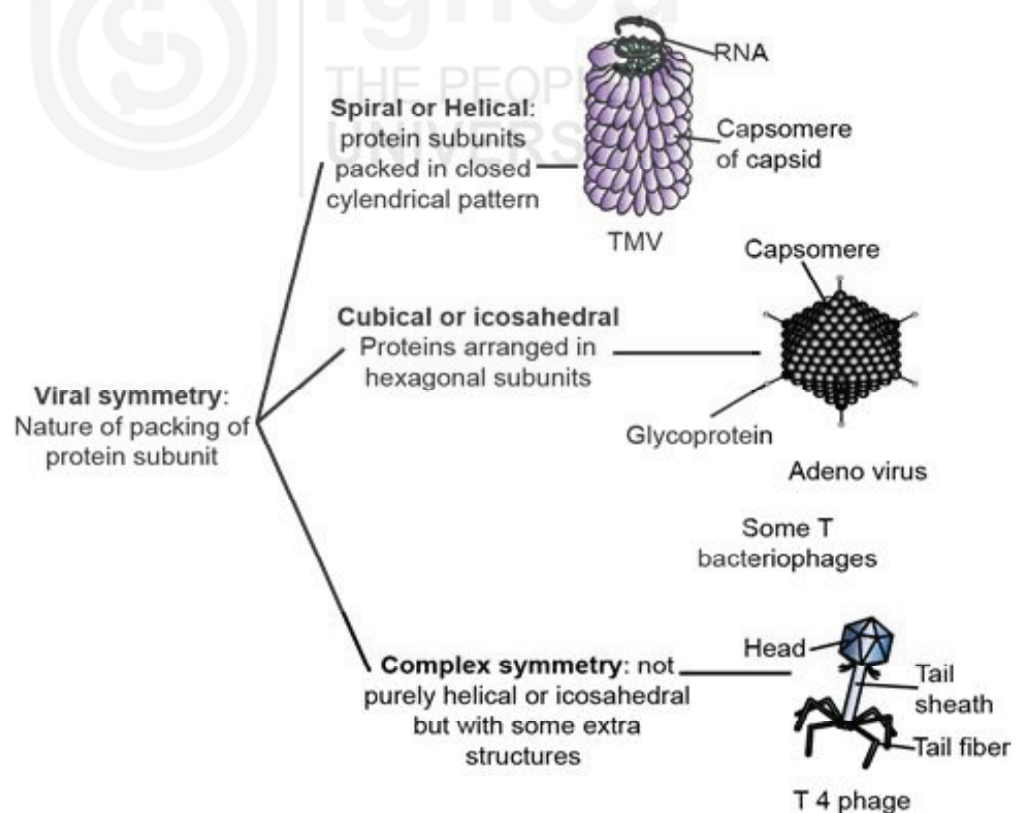


Fig. 1.9: The three different symmetric groups of virus.

The Poxvirus is one of the largest viruses in size and has a complex structure. They do not have a clear morphology, and are neither helical nor icosahedral. They lack clearly identifiable capsids but have several coats surrounding their double stranded DNA.

1.3.4 Viral Envelope

Envelope is the outer membranous layer that surrounds the nucleocapsid of certain viruses and such viruses are called **enveloped viruses**. Many animal viruses and some plant and bacterial viruses are enveloped. Animal viruses like herpesvirus and togavirus are enveloped icosahedral viruses. The envelope is made of carbohydrates, lipids and proteins and are acquired from the host cell during replication. Viruses which do not have an envelope are called **naked viruses or non-enveloped viruses**.

Since envelope is a flexible membranous structure such viruses are usually pleomorphic i.e., of varying shape. Their nucleocapsid can be helical or icosahedral. A layer of protein called the **matrix** is present between nucleocapsid and envelope. This matrix protein is responsible for holding the nucleocapsid to the envelope. The envelope of the animal virus is formed from cell and nuclear membrane of the host cell while the virus is exiting the cell via budding. Envelope proteins are coded by viral genes. On the viral envelope, special glycoproteins called **spikes or peplomers** are present. These spikes provide binding sites which help the virus to attach to specific receptors on the host cells. They can also be used for identification of viruses as they differ among viruses.

The infectivity of enveloped viruses is mostly dependent on the viral envelope. The enveloped viruses are not able to infect host cells if the viral envelope is destroyed. Enveloped viruses are susceptible to the action of lipid solvents such as ether, chloroform and detergents. This susceptibility of enveloped virus to lipid solvents helps us to control their spread or transmission whereas most naked viruses are resistant to them.

Thus, the five structural forms of viruses that occur in nature are: **naked icosahedral** e.g. Adenovirus, picorna virus, hepatitis A virus; **naked helical** e.g. Tobacco mosaic virus, bacteriophage M13; **enveloped icosahedral** e.g. Rubella virus, Yellow fever virus, Herpes virus; **enveloped helical** e.g. Influenza virus, and **complex virus** e.g. Pox virus, Vaccinia virus, T4 bacteriophages.

1.3.5 The Viral Nucleic Acid

The viral nucleic acid or genome is made of only one type of nucleic acid either deoxy ribonucleic acid (DNA) or ribonucleic acid (RNA). The DNA and RNA are made of nucleotides which are the structural units of nucleic acids. The viral nucleic acid is usually linear or circular and in some cases it exists as segment (influenza virus). Segmented genome occurs in fragments, as two or more pieces of nucleic acids and codes for various proteins. Each segment is believed to function as an individual gene. Genome size also varies to a great extent. Most DNA viruses have double stranded DNA (dsDNA) whereas most RNA viruses have single stranded RNA (ssRNA) as their genomic material. Most of the plant viruses are usually ssRNA viruses.

BOX 1.3: Structure of nucleotide

Each nucleotide has three parts:

- i) A nitrogenous base – Purines and pyrimidines. The nitrogen containing bases are cyclic compounds made up of carbon, hydrogen, oxygen and nitrogen atoms. The purines are bases with double ring structures namely adenine (A) and guanine (G). The pyrimidines are single ring structures namely cytosine (C), thymine (T), and uracil (U). In the DNA purine A, G and pyrimidine T, C are present. In the RNA purine A, G and pyrimidine U, C are present.
- ii) A pentose sugar called deoxyribose or ribose sugar.
- iii) A phosphate group (Phosphoric acid).

The two strands of DNA or RNA are held together by weak hydrogen bonds between the nitrogenous bases of the opposite strand.

Classification of Viruses on basis of Nucleic Acid (BALTIMORE CLASSIFICATION)

The viruses are usually classified into two groups on the basis of their nucleic acid/genome (DNA or RNA) and then split into separate families based on characteristics such as strand type (double- stranded and single stranded).

- i) Viruses with DNA as their genetic material are called **DNA viruses**; and
- ii) Viruses with RNA as genetic material are called **RNA viruses**.

The DNA and RNA viruses are further subgrouped as:

- a) Class I double stranded (ds) DNA viruses having double strand of DNA e.g. Adenoviruses; Herpesviruses; Poxviruses, etc. (Fig. 1.10).

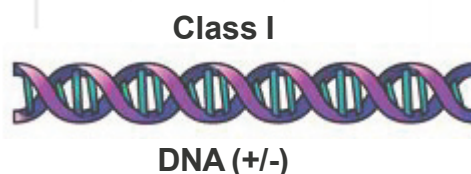


Fig. 1.10: Class I double stranded (ds) DNA viruses.

- b) Class II single stranded (ss) DNA viruses having single strand of DNA e.g. Parvoviruses (Fig. 1.11).

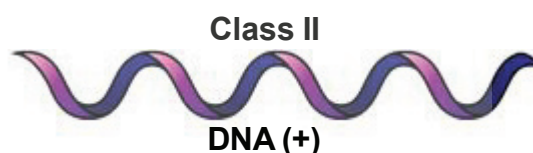


Fig. 1.11: Class II single stranded (ss) DNA viruses.

- c) Class III contains viruses that have double stranded (ds) RNA genomes (e.g. Reoviruses; Birnaviruses) (Fig. 1.12).

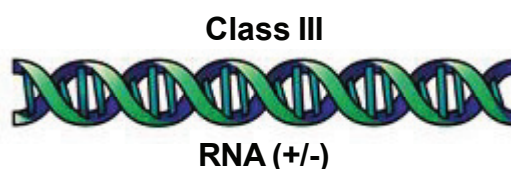


Fig. 1.12: Class III double stranded (ds) RNA virus.

- d) Class IV contains viruses with single stranded (ss) RNA genomes (positive (+) sense). In this type of virus, the base sequence of RNA molecule is identical with that of mRNA. It means that in the host cell, viral RNA acts like mRNA and can be directly translated by the host ribosome e.g. Picornaviruses; Togaviruses, etc. (Fig. 1.13).

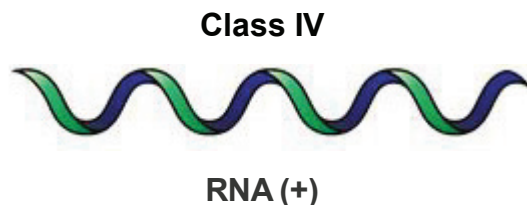


Fig. 1.13: Class IV single stranded (ss) RNA virus.

- e) Class V contains viruses that have single stranded (ss) RNA genomes which are complementary in base sequence to the mRNA (negative (–) sense) RNA viruses, e.g. Orthomyxoviruses, Rhabdoviruses, etc. (Fig. 1.14).

In the host cell, negative sense viral RNA acts as a template for transcription to make complementary mRNA, which is then translated by the host ribosome. Negative sense RNA viruses also carry RNA polymerase needed for transcription of their RNA.

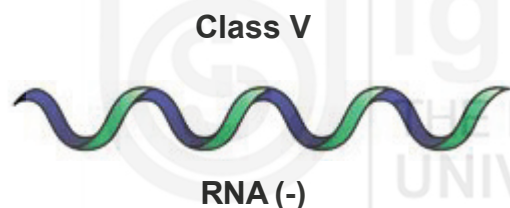


Fig. 1.14: Class V single stranded (ss) RNA virus.

- f) Class VI contains viruses that have single stranded (ss) RNA genomes that with help of reverse transcriptase enzyme convert to double stranded (ds) DNA and then form mRNA (e.g. Retroviruses) (Fig. 1.15).

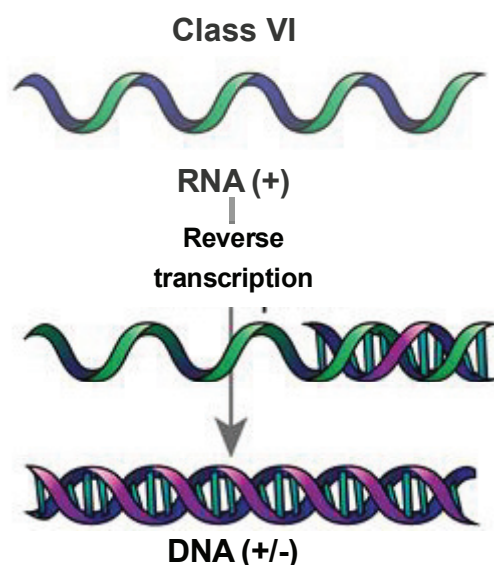


Fig. 1.15: Class VI single stranded (ss) RNA genomes with reverse transcriptase enzyme.

- g) Class VII contains viruses that have double stranded (ds) DNA genome convert to positive (+) sense single stranded (ss) RNA and then with help of reverse transcriptase form double stranded (ds) DNA and then transcribe to mRNA (e.g. Hepadnaviruses) (Fig. 1.16).

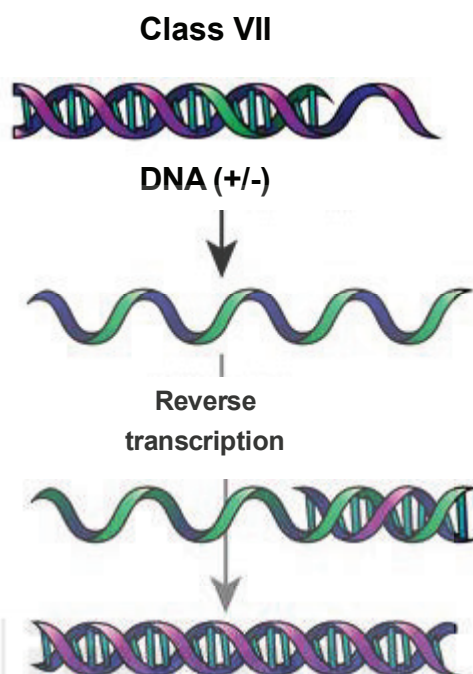


Fig. 1.16: Class VII with double stranded (ds) DNA genome with reverse transcriptase.

1.3.6 Viral Enzymes and Other Contents

Neuraminidase (NA) was the first viral enzyme to be discovered. You have already studied that virus cannot survive and multiply outside the host cell. For the virus to spread, it has to be released from the host cell and enter another cell. Neuraminidase is required by the virus so that it can be released from the host cell. Neuraminidase cleaves the sialic acid groups from the glycoproteins of the host cell surface. This results in the release of progeny viruses and the spread of the virus from the host cell to uninfected surrounding cells (Fig. 1.17).

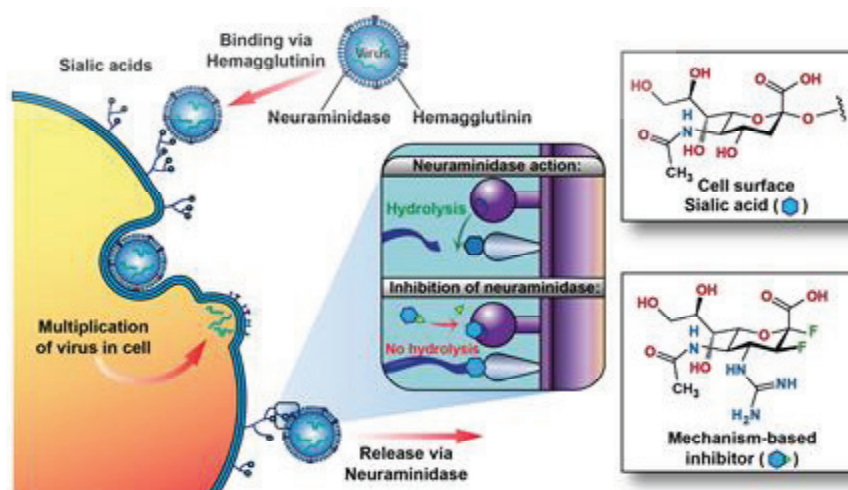


Fig. 1.17: Mechanism of action of Haemagglutinin and neuraminidase.

Haemagglutinin (HA), a glycoprotein has also been isolated from viruses. Haemagglutinin helps the virus to enter the host cell. HA binds with groups from the glycoproteins of the host cell surface which is responsible for binding the virus to cells and causing infection in the new cell (Fig. 1.17).

Another important enzyme present in viruses is the viral transcriptase (DNA dependent RNA polymerase). Viral transcriptase is required for the synthesis of mRNA from double strand of DNA or single strand of DNA or double stranded RNA (Fig. 1.18).

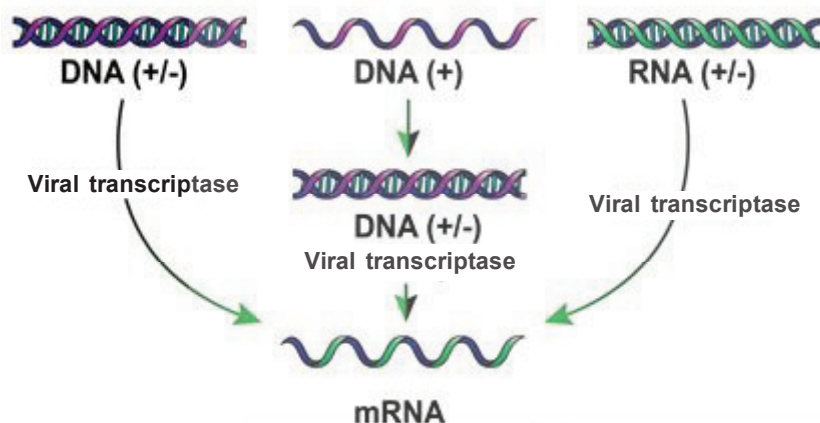


Fig. 1.18: Mechanism of action of viral transcriptase.

Some viruses also have an enzyme reverse transcriptase which synthesizes DNA from single stranded RNA (Fig. 1.19).

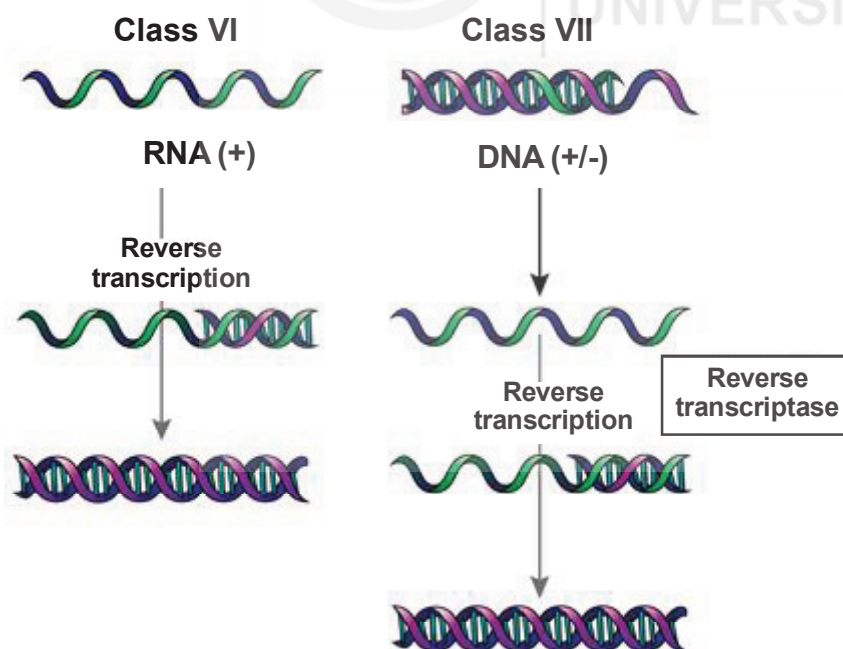


Fig. 1.19: Mechanism of action of viral reverse transcriptase.

Another enzyme discovered is replicase (i.e. RNA-dependent RNA polymerase). Replicase is required for the formation of complementary RNA from single stranded RNA (Fig. 1.20).

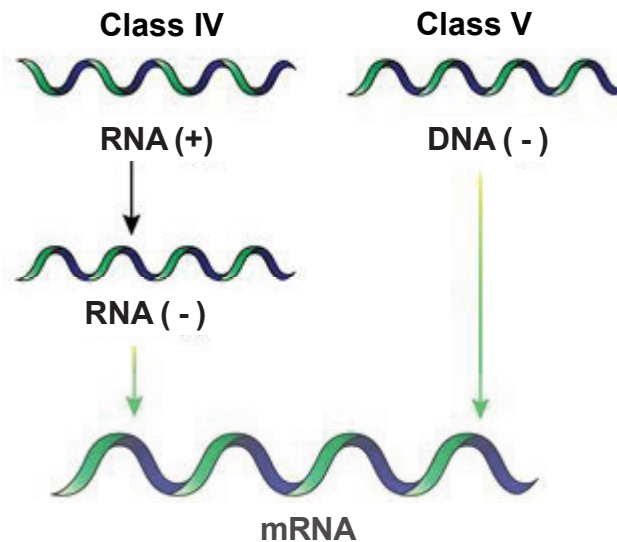


Fig. 1.20: Mechanism of action of viral replicase.

1.3.7 Viral Taxonomy

The uniform classification system for classifying viruses has been developed by International Committee for Taxonomy of Viruses (ICTV). The number of viruses and their taxonomic categories are continuously expanding.

The family name of viruses ends with **viridae**; subfamily name is **virinae**, and genus (and species) name is **virus**. For example, in the family Poxviridae, subfamily Chordopoxvirinae, the genus Orthopoxvirus contains several species of poxvirus including cowpox, vaccinia and variola major (causative agent of small pox).

The taxonomic designation for HIV is family Retroviridae, genus Lentivirus and species Human Immunodeficiency Virus (HIV).

Viral species is a group of viruses that have same genome and same relationships with the specific organisms. Division of viruses into different taxonomic groups is based on various characteristics such as type of host organism, structure and composition of virion (nucleic acid, capsid, envelope, enzymes) strategies of replication, immunological relationship with the host, types of diseases caused and modes of transmission, and viral genome sequences. Viruses are host specific which means each virus is capable of infecting only certain species of host cells. Host specificity is determined by the attachment of viruses to a specific cell, which depends on the following:

- Specific structure of virion capsid or envelope.
- Presence of specific receptor sites on host cell surface, which can be recognized only by specific virions.

1.4 DNA VIRUS (T-PHAGE)

You have already learnt that viruses having DNA as their genetic material are known as DNA viruses. A DNA virus can have single stranded DNA (ssDNA) or double stranded DNA (dsDNA) as genetic material. Viruses with

single-stranded DNA (ssDNA) genomes are very small. None of the known ssDNA viruses has a lipid envelope. Viruses with double-stranded DNA genomes show variation in their genome size. It is interesting to note that virtually no known double-stranded DNA viruses infect higher plants except the reverse transcriptase viruses. Most of the known bacteriophages (95%) are tailed phages with double-stranded DNA genomes. Large DNA viruses of bacteria, archaea, and eukaryotes appear to be evolutionarily linked. Although little sequence conservation can be identified between the T4 phage of bacteria, the halophage of archaea, the viruses of the family Phycodnaviridae which infect algae, and the herpes viruses of vertebrate eukaryotes, all show similarities in their DNA polymerase types, capsid structures, and capsid assembly, consistent with a common ancestor.

T Phage Virus

Among all the criteria to classify bacteriophages, structural morphology and nucleic acid properties are considered important. The major morphological groups of bacteriophages include: viruses with contractile tail, viruses with non contractile tails, tailless icosahedral phages and filamentous phages. T-phages (T1 through T7) where T stands for the 'type' are a specific class of large, complex bacteriophages with icosahedral heads, double-stranded DNA, with a characteristic contractile tail structure (Fig. 1.21). T-phages have double stranded DNA and so belong to Class I of viruses (double stranded DNA virus). The most commonly studied T-phages are T4 and T7, both of whom infect *E. coli*. As all viruses, the bacteriophage uses the host cell to replicate and make more bacteriophages. When the bacterium is lysed, the newly made viruses are released to infect other *E. coli* and to repeat the cycle again. The T even phages (T2, T4, etc.) are also referred to as lytic phages because they always lyse and kill the host bacterium cell.

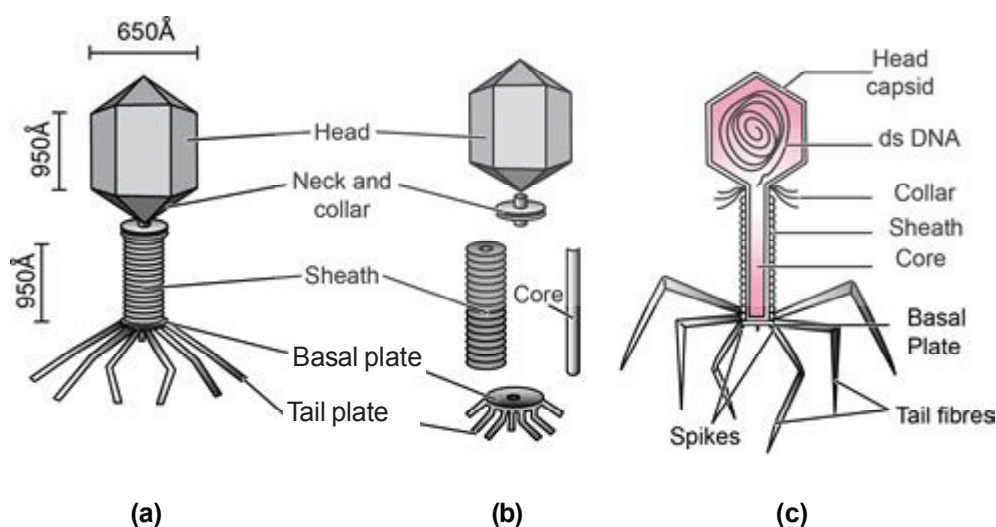


Fig. 1.21: (a) T4 Bacteriophage; (b) Parts; (c) LS of a virion.

Structure: The bacteriophage, T-even phage has a tadpole like structure with a icosahedral head connected to a helical tail through a short collar. The head is composed of about 2000 capsomeres forming icosahedral symmetry. The

icosahedral head encloses a tightly packed double stranded (ds) DNA (50 nm long). The tail of the bacteriophage includes the tail sheath, basal plate and tail fibers (Fig. 1.21a), which are made of different proteins. The tail has an inner hollow tube called core, surrounded by a contractile sheath which consists of 24 annular rings (Fig. 1.21b). The distal end of the tube is connected to a hexagonal basal plate with spike or tail spin at each corner. Six long, flexible tail fibers arise from the basal plate. (Fig. 1.21c). The long tail fibers are used by the bacteriophage to attach itself to the bacterium so that the virus can insert its genetic material inside of the host cell to begin the replication process.

1.5 RNA VIRUS (TMV)

You have already learnt that viruses having RNA as their genetic material are known as RNA viruses. The virus for its survival and replication requires a host cell. The genetic material of all the host cells is DNA. The host cell has the machinery to replicate DNA but does not have the ability to replicate RNA. However, the host cell has the necessary machinery to transcribe DNA to messenger RNA. So for RNA viruses to replicate must have a mechanism necessary to produce more genetic material i.e. RNA for new viruses.

For certain RNA viruses, the RNA is replicated by a viral enzyme (replicase) which is contained in the virus. In some RNA viruses, the viral RNA genome codes for the enzyme (replicase). The enzyme replicase is synthesized in the host cell with help of the host cellular machinery. In other viruses a reverse transcriptase contained in the virus converts the viral RNA into DNA, which is then replicated by the host cell. Among the plant viruses, Tobacco Mosaic virus (TMV) has been studied most extensively. TMV is used in plant molecular biology especially for plant transformations, and therefore will be studied in detail.

Tobacco Mosaic Virus (TMV)

TMV is historically important as it was the first virus to be discovered by Dmitri Ivanowsky and the first virus to be crystallized by Wendal Stanley (See section 1.2). TMV infects tobacco and other plants like tomato, spinach and pepper and causes considerable damage to plantations. Plant cell walls must be damaged for the virion to enter the cell and cause infection. The chloroplasts of TMV infected leaves become yellow, due to loss of chlorophyll as a result of viral infection. The disease is termed as Tobacco mosaic disease because the infected leaves develop a characteristic mosaic pattern i.e. yellowish chlorotic infected lesions alternating with green regions. The mosaic disease is characterized by presence of mixed light green and yellow patches on leaves by making a mosaic pattern.

Structure: TMV is a positive sense (+) single stranded (ss) RNA virus belonging to Class IV with helical symmetry (Fig. 1.22). It is long thin rod shaped particle measuring about 300 nm in length and 15 to 17 nm in diameter. The tube is hollow and composed of protein capsomeres arranged helically around the central core. The central core is 4 nm in diameter and runs through the entire length of the virus. A complete rod of TMV contains

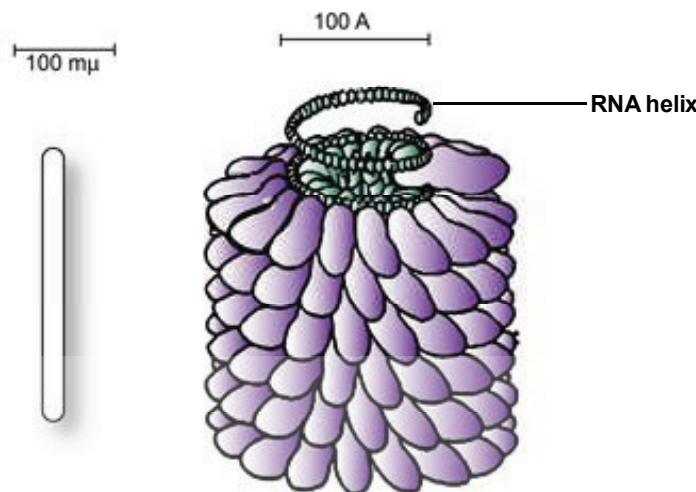


Fig. 1.22: Tobacco mosaic virus (TMV) showing protein subunits tagged on the RNA helix.

about 2130 capsomeres. There are about 16 capsomeres in each helical turn. The subunit proteins of the capsid along with the RNA form a close spiral. There is no envelope external to the capsid.

The positive sense (+) ssRNA is helically coiled. Its genome is a single copy of positive strand RNA that contains 6,395 nucleotide bases and has a cap at 5' terminus. The RNA molecule folds into tRNA like structure at its 3' end. TMV genome encodes for only four kinds of proteins: two are components of RNA polymerase and the other two are components of viral capsid.

1.6 OTHER VIRUS-LIKE THINGS: SUBVIRAL PARTICLES

These are virus related agents (sometimes called atypical viruses) that are smallest known pathogens capable of causing diseases. Two type of such virus like agents studied so far are viroids and prions.

1.6.1 Viroids

Viruses may be often referred to as the smallest infectious things. But there are some smaller contenders also. Some of the agents of plant disease lack even a viral coat and are merely small strings of plain, or "naked," RNA. They are believed to be a more primitive version of ordinary viruses. These particles with a small, circular, single stranded RNA that do not contain any protein or capsid are called **viroids**. The RNA molecule does not contain any protein coding gene. In spite of being just RNA molecule without any protective coverings, viroids are not degraded by enzymes. The reason is that they have a very tight secondary structure as a result of extensive internal base pairing. Thus they look like rods, resembling double stranded molecule with closed ends. Agricultural researchers have found that viroids cause plant diseases like potato spindle tuber, tomatoes, citrus exocortis, and cucumber pale fruit disease. Potato spindle Tuber viroid (PSTV) infects both potato and tomato. There can be serious agricultural impact due to diseases caused by viroids. Recently a viroid has also been linked to hepatitis D.

1.6.2 Prions

Prions (proteinaceous infectious particles) are infectious particles that have only proteins and do not have DNA or RNA. Prions are proteins that have the ability to transmit diseases, a finding that has defied scientific expectations. There is still much debate about how they work, but scientists think these rogue proteins direct the host to create abnormal proteins that can cause serious neurological disease in animals and humans. Scientists suggest that they spread when a prion comes into contact with the normal version of the protein and causes the normal protein to change shape and become a prion, too. Unlike viruses the prions are resistant to a wide range of chemical and physical treatment like heat, ultraviolet radiations, formaldehyde etc.

Box 1.4: Prion diseases

Do you recall hearing about Mad Cow Disease? This is an ailment that affects the animals' brains and is also called bovine spongiform encephalopathy (BSE) because it makes the brain appear like a sponge. Prion disease represents a group of conditions that affect the nervous system in humans and animals. In people, these conditions impair brain function, causing changes in memory, personality, and behavior; a decline in intellectual function (dementia); and abnormal movements, particularly difficulty with coordinating movements (ataxia). Creutzfeldt-Jakob disease : Classic CJD is a human prion disease. It is a neurodegenerative disorder with characteristic clinical and diagnostic features. This disease is rapidly progressive and always fatal. The disease leads to death usually within 1 year of onset of illness.

1.7 ECONOMIC IMPORTANCE

Viruses are extremely small sized infectious agent that lead to massive economic losses. Though viruses are important as pathogens, they play a major beneficial role in medicine research and diagnostics.

1.7.1 Economic Losses Due to Viral Diseases

Plant Diseases

Crop losses as a result of disease caused by plant-infecting viruses come second only to fungal diseases. However plant viruses are much more difficult to control. The economically important food crops are severely affected by viral diseases resulting in economic losses because of decrease in grain production, poor quality seed, and extra expenditure for phytosanitation and disease control. As the plant cell wall is thick, penetration of viruses into plant cell is difficult. For the virus to enter the cell the plant cell wall has to be mechanically damaged and this is caused by either insects or animals. In the majority of the virus infecting crops, transmission of plant viruses is by insects like aphids, leafhoppers etc. The insects act as vectors by picking up viruses on their mouth parts and transmitting it to plants. Pesticides are used to control the vectors which also increase the production cost.

There are basically two kinds of infections caused by plant viruses- the mosaic disease and the curling and dwarfing.

Few of the plant disease caused by plant viruses are Tobacco Mosaic Virus, Potato Virus and Cauliflower Mosaic Virus, Mosaic virus of cucumber and lettuce, yellow disease of Potato, Tomato bunchy top disease, Cotton leaf curl Viruses (LCV), Necrotic Diseases and tumours.

Animal Diseases

The annual economic loss due to foot and mouth disease (FMD), a disease caused by viruses in India is huge. The foot and mouth disease caused by Picorna virus is highly contagious and can spread rapidly in cloven-footed animals viz. cattle, buffaloes, goats, sheep and pigs, and is endemic in India. Economic losses caused by the FMD are because of loss/decrease in milk production, decrease in the body weight leading to reduced yield of meat and extra expenditure of vaccination. In addition to this, the milk and milk products, meat, hide and skin are not accepted by countries free from the disease causing reduction in export.

Other economically important viral diseases are avian influenza or bird flu (Influenza virus), rabies (Rhabdoviruses), bovine viral diarrhoea (Pestivirus), new castle disease in poultry (paramyxovirus) etc.

Human Viral Diseases

The distressing effects of viral diseases such as AIDS, influenza, dengue, polio, diarrhoea, smallpox, and hepatitis are well known. There are some emerging viral infections which affect the health systems of countries and loss of wealth on health. Among these is the sudden emergence of the Ebola virus which causes intensive bleeding inside and outside the body. Another such disease is caused by coronavirus that causes severe acute respiratory syndrome (SARS). Vaccination remains the preferred strategy for controlling viral diseases.

1.7.2 Economic Benefits of Viruses

Vaccine production: Vaccines provide immunity against viral diseases.

The conventional vaccines synthesized using live attenuated viruses (weakened in force or effect) or killed viruses are easy to produce and economic.

Gene therapy: We all know that a gene is a region of DNA that encodes a functional RNA or protein product. A defective gene produces a defective/non functional protein product and causes disease. Gene therapy is the introduction of functional gene into human cells to correct defective genes by replacing them. Gene therapy is largely used in cancer treatment e.g., bone marrow transplant in blood cancer.

Cancer therapy: Some viruses that cause cancer are called **oncoviruses**. Viruses can be directly used to prevent cancer by being the source of anti cancer vaccines, e.g., vaccines against hepatitis B virus (causes hepatic cancer) and human papillomavirus (cervical cancer) are commercially available.

Bacteriophage therapy: You have already studied that bacteriophages are bacteria eating viruses. Therefore bacteriophages can be used for destruction of bacterial pathogens. This therapy has been used to successfully treat staphylococcal and *E. coli* infections and appears to be very promising.

Role of viruses in research: Viruses have been extensively used for research as they infect all three domains of life (bacteria, archaea and eukaryota). Viruses have the unique ability to insert genetic content into host cells, therefore are commonly used as vector in recombinant DNA technology and research procedures. Bacteriophages are the routinely used viral vectors in genetic engineering. The lambda and M13 phage are the most commonly used *E. coli* phages.

Virus-based diagnosis: Viruses have a major role in diagnostic techniques like southern blotting, cloning, DNA and RNA sequencing of diseases etc. which are commonly employed in diagnosis. Viruses are used as vectors in these techniques.

Viral biopesticides: They are much less significant than bacterial pesticides. Research and development on various viruses as potential bio-control agents is still going on. Baculoviruses are the most important group of viruses used as biopesticides.

SAQ 2

In the following statements choose alternate correct word given in parenthesis.

- i) The therapy in which viruses can be used for destruction of bacterial pathogens is (gene therapy/ bacteriophage therapy).
- ii) Rabies is caused by (Rhabdoviruses/Picornavirus).
- iii) Tobacco Mosaic virus (TMV) is a (DNA virus/RNA virus).
- iv) Enzyme required by the virus so that it can be released from the host cell (Hemagglutinin/Neuraminidase).
- v) Positive (+) sense single stranded RNA virus are classified under (Class V Class IV) Baltimore Classification.
- vi) A layer of protein present between nucleocapsid and envelop is called the (Matrix/Spike).

1.8 SUMMARY

In this unit you have learnt that

- Viruses can be defined as small obligate, intracellular particles seen only by electron microscope that have to infect and take over host cell to replicate.

- Two scientists Dimitri Iwanowski and Martinus Biejernick played a crucial role to discover viruses.
- Loeffler and Frosch were able to discover the first animal virus.
- Fredrick Twort and d Herelle discovered bacteriophages (bacteria eating viruses).
- Wendell Stanley for the first time crystallized virus (TMV).
- Viruses cannot survive and multiply outside the host cell.
- The viruses are made up of only two components a) protein (capsid) and sb) nucleic acid (viral genome).
- The viral genome is protected against chemical and physical damage by the protein coat of the virus particle called **capsid**.
- The capsid and the viral genome are together called the **nucleocapsid**.
- The building blocks of capsid are protein subunits called **capsomeres**..
- On the viral capsid, special capsid proteins called spikes are present are binding sites of the virus so that it can attach to specific receptors on the host cells.
- These capsomeres are responsible for the three different symmetry groups of viruses: Icosahedral, helical and complex symmetry.
- In some viruses with conventional icosahedral or helical structure, the nucleocapsid is surrounded by a flexible loose fitting membrane made up of proteins and lipids which are acquired from the host cell during replication, known as the **viral envelop** and such viruses are called **enveloped viruses**. Viruses which do not have an envelop are called **naked viruses or non-enveloped virus**.
- The viral nucleic acid or genome is made of either de-oxy ribonucleic acid (DNA) or ribonucleic acid (RNA).
- Viruses with DNA as their genetic material are called **DNA viruses** and viruses with RNA as genetic material are called **RNA viruses**.
- The DNA and RNA viruses are further subgrouped into 7 classes (Baltimore classification).
- T-phages (T1 through T7; T standing for type) are a specific class of large, complex bacteriophages with icosahedral heads, double-stranded DNA, with a characteristic head and tail structure.
- Tobacco mosaic virus has a helical symmetry with positive sense (+) single stranded (ss) RNA in the middle.
- Viruses are economically important as they cause losses from diseases and play a major beneficial role in medicine research and diagnostics.

1.9 TERMINAL QUESTIONS

1. Write short notes on
 - i) Prions
 - ii) Virioids
 - iii) Virus classification
 - iv) Viral envelope
 - v) Economic importance of viruses
2. With help of diagram describe the structure of viruses.
3. Differentiate between RNA and DNA virus.

1.10 ANSWERS

Self-Assessment Questions

1.
 - i) False
 - ii) True
 - iii) False
 - iv) True
2.
 - i) Bacteriophage therapy
 - ii) Rhabdo virus
 - iii) RNA virus
 - iv) Neuraminidase
 - v) Class IV
 - vi) Matrix

Terminal Questions

1.
 - i) Prions: Subsection 1.6.2.
 - ii) Virioids: Subsection 1.6.1.
 - iii) Virus classification: Subsection 1.3.5.
 - iv) Viral envelope: Subsection 1.3.4.
 - v) Economic importance of viruses: Section 1.8.
2. With help of diagram describe the structure of viruses: Section 1.3.
3. Differentiate between RNA and DNA virus: Sections 1.4 and 1.5.

Acknowledgement of Figures

- Fig.1.2 https://upload.wikimedia.org/wikipedia/commons/thumb/9/93/Pasteur_Germ_Proof_Filter%2C_c._1890%2C_Pasteur-Chamberland_Filter_Co.%2C_Dayton%2C_Ohio_-_Museum_of_Science_and_Industry_%28Chicago%29_-_DSC06633.JPG/401px-Pasteur_Germ_Proof_Filter%2C_c._1890%2C_Pasteur-Chamberland_Filter_Co.%2C_Dayton%2C_Ohio_-_Museum_of_Science_and_Industry_%28Chicago%29_-_DSC06633.JPG
- Fig.1.3 https://upload.wikimedia.org/wikipedia/commons/thumb/8/8f/Dmitry_Iosifovich_Ivanovsky.jpg/225px-Dmitry_Iosifovich_Ivanovsky.jpghttps://upload.wikimedia.org/wikipedia/commons/thumb/c/c7/Martinus_Willem_Beijerinck.jpg/300px-Martinus_Willem_Beijerinck.jpg
- Fig.1.4 http://3.bp.blogspot.com/_GifmgL3NWN0/SWh-II2Yf_I/AAAAAAAAABM8/_3mtNtGZPU4/s320/History+of+Viruses+pic+15.jpg
- Fig 1.5 http://talk2bio.com/wp-content/uploads/2017/01/Twort_Frederick.jpg
- Fig.1.6 http://centennial.rucars.org/centennial/assets_public/images/48_photo1.jpg, http://centennial.rucars.org/centennial/assets_public/images/48_photo2.jpg

GLOSSARY

- Antiviral** : A compound that inhibits the growth and reproduction of viruses.
- Antagonist** : An agent that acts in physiological opposition.
- Capsid** : The protein covering of a virus particle.
- Cancer** : A disease in which abnormal cells divide uncontrollably and destroy body tissue.
- Capsomeres** : Substructures of virus particles. Composed of aggregates of polypeptide chains that interact to form the basic structural units of the capsid.
- Cloven hoof** : A **cloven hoof**, cleft **hoof**, divided **hoof** or split **hoof** is a **hoof** split into two toes. This is found on members of the mammalian order Artiodactyla. Examples of mammals that possess this type of **hoof** are cattle, deer, antelopes, gazelles, goats, and sheep.
- DNA** : Deoxyribonucleic acid, the chemical structure that contains the genetic information of an organism. The double helical structure is made of two strands consisting of deoxyribose and phosphate and is held together by bonds between purine and pyrimidine bases which project inward from two chains and form the genetic code.

Gene	: A sequence of genetic material that provides the information to make a specific protein.
Genome	: The entire genetic information of an organism.
Hexamer	: Any of the clusters of six protein subunits (capsomeres) in icosahedral virus capsids.
Envelope	: A lipid casing that surrounds the capsid that covers a virus. A viral envelope assists the virus in infiltrating the cells of the host organism.
Eukaryotes	: Organisms whose cells possess a membrane-bound structure called a nucleus that contains the genetic material (DNA).
Foot-and-Mouth Disease	: Foot-and-Mouth Disease (FMD) is a highly contagious viral vesicular disease of cloven-hoofed animals.
Matrix protein	: A type of protein that connects the components of the viral envelope to the nucleus of the virus.
Neucleocapsid	: The composition of a virus that includes the DNA, RNA, and the capsid protein cover.
Negative-sense RNA	: Negative-sense RNA Viral RNA with a base sequence complementary to that of messenger RNA (mRNA). The RNA must be converted to positive-sense RNA by a viral enzyme called RNA polymerase before translation into protein can occur. Viruses that have a negative-sense RNA genomes include influenza and Ebola.
Neuraminidase	: A protein found on the surface of influenza viruses that is needed for the virus to exit the host cell and infect more cells. The action of this protein is inhibited by the class of antiviral drugs that includes the drug Tamiflu®. In the system using for naming influenza subtypes (H1N1, for example), the N stands for neuraminidase.

Peptide	: Short chain of linked amino acids. Amino acids are the building blocks of proteins.
Phage Therapy	: Phage therapy or viral phage therapy is the therapeutic use of bacteriophages to treat pathogenic bacterial infections.
Polyhedron	: A solid figure with many plane faces, typically more than six
Positive-sense RNA	: Viral RNA that has the same base sequence as mRNA which allows it to function as a template for protein synthesis during viral replication.
Protein	: Components of cells and viruses that play structural and functional roles in cells.
RNA	: Ribo nucleic acid, a chemical structure that is related to DNA, but has only one strand and a somewhat different chemical composition. RNA performs a variety of functions in the cell and can act as a messenger to carry the genetic code from the DNA to other parts of the cell. RNA can also serve as the genetic material of some viruses.
Spike	: Surface projection of varying lengths spaced at regular intervals on the viral envelope, also called peplomers. Consist of viral glycoproteins.
Tobacco mosaic virus	: Tobacco mosaic virus is a positive-sense single stranded RNA virus that infects a wide range of plants, especially tobacco and other members of the family Solanaceae.
Virions	: A virus particle, which invades the cells of a host organism, causing infection.
Vaccine	: A preparation of killed or weakened microorganisms that is administered to produce or increase immunity to a particular disease.
Vesicles	: Vesicles are small, fluid-filled sacs that can appear on your skin.

Virus	: A microscopic particle that is made up of genetic material (either DNA or RNA) and protein that can replicate only inside living cells.
Virus-like particle	: A particle assembled from multiple copies of the capsid protein that, like a virus, can produce an immune response, but unlike a virus, is not infectious because it does not contain genetic material.



VIRUSES : REPLICATION

Structure

2.1	Introduction	2.5	Lysogenic Cycle
	Objectives	2.6	Replication of RNA Virus (TMV)
2.2	Replication (General Account)	2.7	Summary
2.3	Replication of Bacteriophage : DNA Virus (T-phage)	2.8	Terminal Questions
		2.9	Answers
2.4	Lytic Cycle		Glossary

2.1 INTRODUCTION

In the previous unit you have studied that viruses cannot replicate on their own and for replication they require machinery of the host cell for synthesis of the viral genome and proteins.

As the virus (virion) **cannot replicate outside the host** it has to first enter the host cell and then replicate. Viral replication results in many progenies. When these progenies leave the host cell they repeat the cycle, infect other cells, and spread the infection. The viral nucleic acid (DNA or RNA) possesses genes, needed for synthesis of new viruses. These genes carry information and help in the formation of virus's structural component such as capsid proteins, and few enzymes required in the virus life cycle. Some of the viral enzymes are encoded by the viral DNA/RNA however these are synthesized and are functional only when the virus is inside the host cell. The larger viruses with DNA having more genes may also contain one or few preformed enzymes, which usually help the virus to penetrate the host cell or in some cases may help in the replication of the viral genome.

The key to control viral infections lies in our understanding of details of viral replication cycle.

In this unit we will study the steps involved in the process of viral replication namely: Attachment/Adsorption, penetration and uncoating, biosynthesis, assembly and release. We will also discuss the replication cycle of one DNA virus (Tphage) and one RNA virus (TMV). Also in this unit emphasis will be given on the lysogenic and lytic cycle of viral replication.

Objectives

After reading this unit, you will be able to:

- ❖ know the importance and different ways in which viruses replicate;
- ❖ understand and differentiate between lysogenic and lytic cycle of replication; and
- ❖ distinguish between DNA virus (T phage) and RNA virus (TMV) replication.

2.2 REPLICATION (GENERAL ACCOUNT)

One of the most unique events in nature is the process of virus replication. The two experiments that laid the foundation of our understanding on replication in viruses are:

- Ellis and Delbruck experiment
- Hershey and Chase experiment

Ellis and Delbruck experiment

In 1939 EL Ellis and Max Delbruck demonstrated virus replication. Their experiment is referred as single burst or one step growth curve.

To study viral replication, Ellis and Delbruck added bacteriophages (bacteria viruses) to a culture of rapidly growing bacteria. After a few minutes the mixed culture (bacteria and virus) was diluted so that interaction between phage particle and bacteria became minimum. Samples of mixed culture were taken at different time intervals and plated on lawns of bacteria (bacteria covering the entire surface of the agar in a petri plate) (Fig. 2.1a) for presence of phage particles. After incubation small craters – “plaque” or “hole” in a monolayer of bacterial cells were observed on the lawn of bacteria (Fig. 2.1b). At the start of the experiment, the plaque count was relatively constant for samples plated during first hour but after that it increased hundred-fold within a few minutes. During the first phase of infection, the ‘latent period’ no progeny particles were liberated from the infected cells. Then lysis of bacterial cells occurred resulting in release of newly synthesized bacteriophages. The average number of bacteriophages released per bacterium was called **the burst size**. There was an increase in concentration of phage particle with time (Fig. 2.1c).

This was the first experiment to show the three essential phases of virus replication:

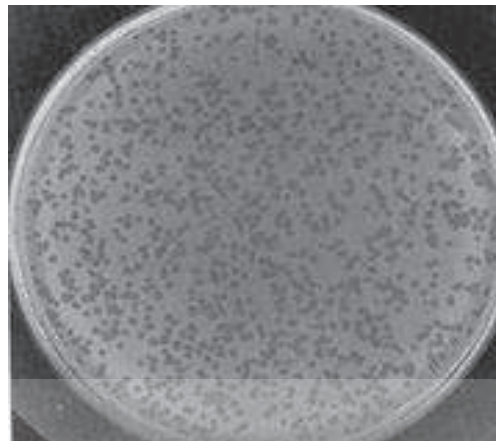
- i) Initiation of infection
- ii) Replication and expression of virus
- iii) Release of mature virus from infected cells

Hershey and Chase Experiment

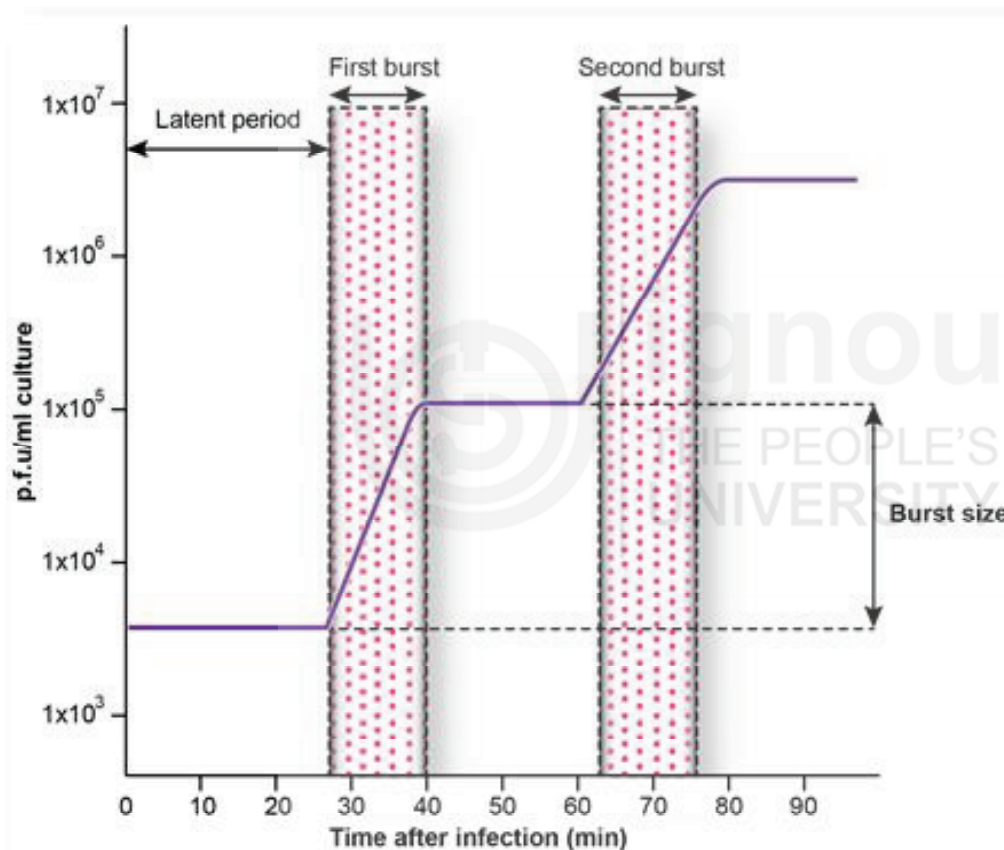
The second key experiment on virus replication using bacteria was performed by Alfred Hershey and Martha Chase in 1952. They worked with bacteriophage T2, which infects *Escherichia coli*. The bacteriophage T2 consists of an outer protein coat with DNA inside. Hershey and Chase grew cultures of *E. coli* in media containing radioactive S-35, sulfur in sulfate form. Sulfur is an important constituent in the amino acids cysteine and methionine, which makes part of



(a)



(b)



(c)

Fig. 2.1: (a) Bacterial Lawn; (b) Plaque forming units in bacterial lawn; (c) Graph showing an increase in concentration of phage particle with time. The Single Burst Experiment (Ellis and Delbruck, 1939).

the protein coat. An another set of *E. coli* culture in media containing radioactive P-32, phosphorous in phosphate form (the element phosphorus is present in a DNA molecule but not present in any of the 20 amino acids from which proteins are made). So Hershey and Chase had one set of *E. coli* with radioactive S-35 sulfur and another set of *E. coli* with radioactive P-32 phosphorous. These bacterial cells with radioactive sulfur and radioactive phosphate were infected with bacteriophage T2 phages separately (Fig. 2.2/2.3a). These progeny phages in *E. coli* cultured on media with radioactive S-35 had radioactive sulfur atoms, incorporated into their protein coats. But DNA of these phages was

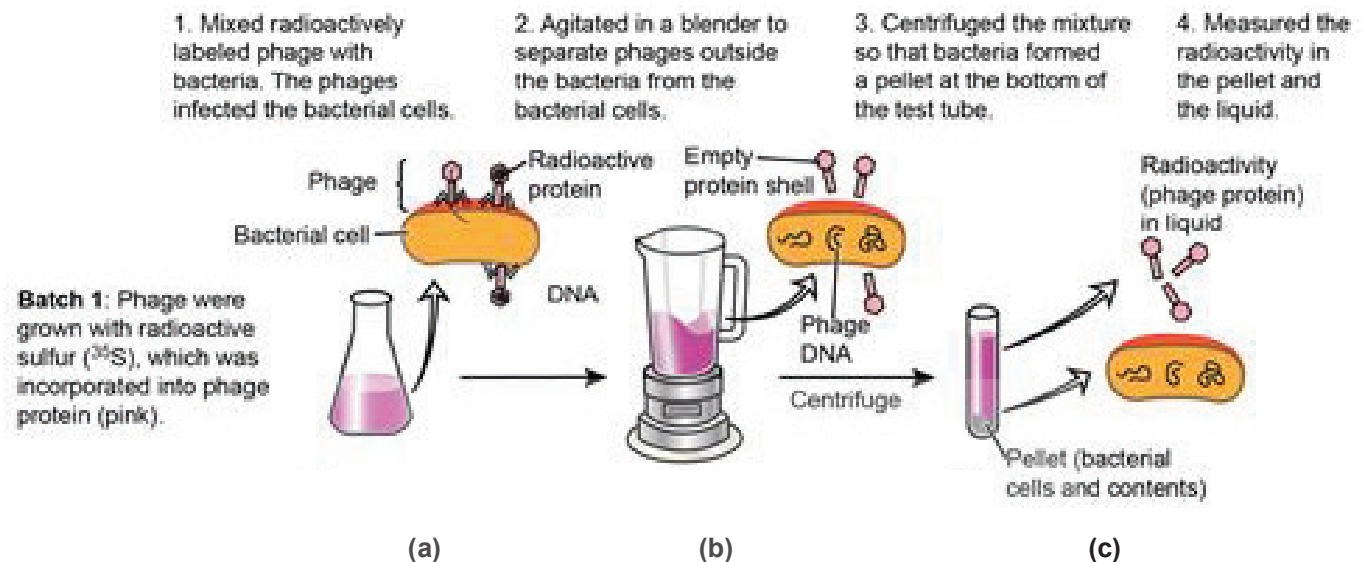


Fig. 2.2: The Hershey and Chase Experiment where phages were grown with radioactive S-35.

non-radioactive. On the other hand, the progeny phages in *E. coli* cultured on media with radioactive phosphorus (P-32), had P-32 incorporated into the DNA.

New cultures of nonradioactive *E. coli* were infected with S-35 and P-32 bacteriophage T2, respectively. After a few minutes post infection, the infected culture was centrifuged to remove the virus coats (Fig. 2.2b, 2.3b). The nonradioactive bacteria infected with bacteriophages T2 containing P-32 (radioactive), became radioactive and, much of the radioactivity was passed on to the next generation of bacteriophages T2 (Fig. 2.3c). However, in the nonradioactive bacteria infected with bacteriophage T2 labeled with S-35, practically no radioactivity could be detected (as the virus coat was removed during centrifugation) in the infected cells (Fig. 2.2c).

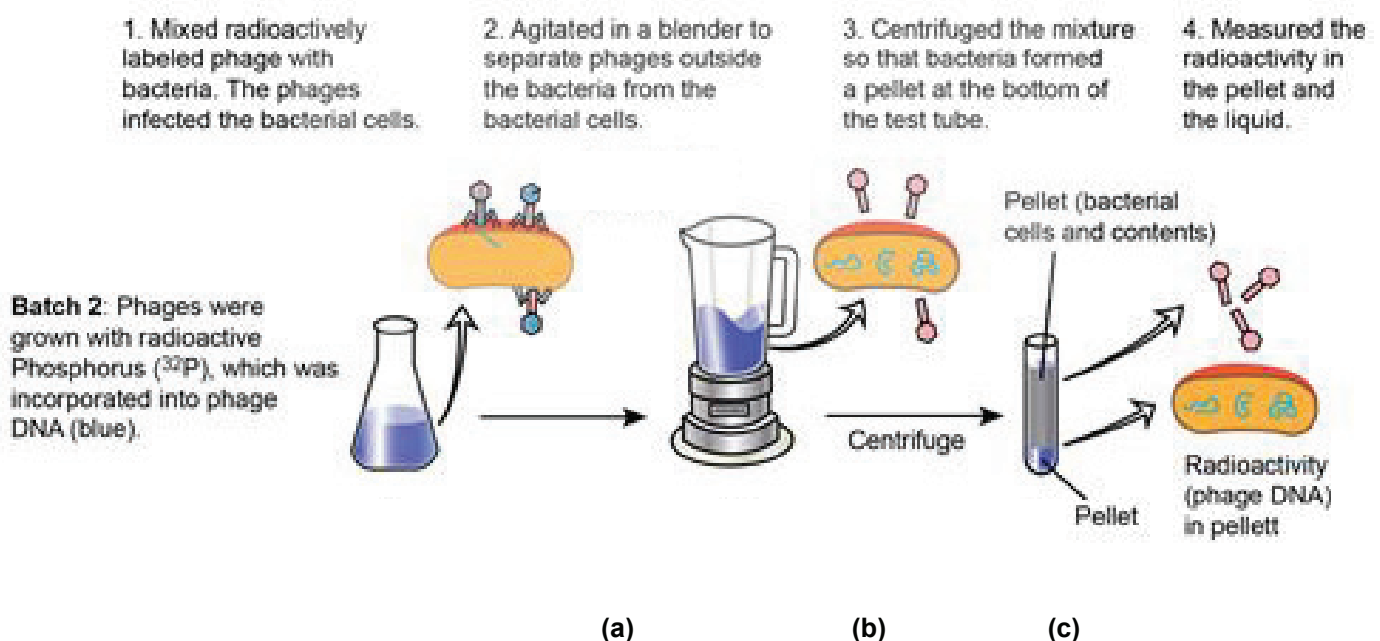


Fig.2.3: The Hershey and Chase Experiment where phages were grown with radioactive P-32.

From these experiments, they concluded that the DNA component of the bacteriophages T2 was injected into the bacterial cell while the protein component remained outside and that DNA was the genetic material of T2 bacteriophage.

The above experiments were instrumental in our understanding of viral replication.

Viral Replication

A virus uses life processes of host cell for synthesis of viral protein and DNA/ RNA to replicate. The viral replication causes biochemical and structural changes in the host cell, which may lead to, either changes in cell function or destruction of the cell. When we suffer from common cold, we are infected by the common cold virus known as rhinovirus, some of our cells die through lysis (bursting) or apoptosis (programmed cell death or “cell suicide”), releasing all progeny virions at once. In some cases of virus infection, such as HIV (Human Immunodeficiency Virus), the virus leaves the infected cells of the immune system by a process known as budding, where the cell does not undergo lysis and thus is not immediately killed. However, the damage to the cells makes it impossible to function normally, even though the cells remain alive for a period of time.

Steps of Viral Replication

The viral replication is broadly divided into five steps, namely:

- 1) Attachment/Adsorption
- 2) Penetration and Uncoating
- 3) Biosynthesis
- 4) Assembly
- 5) Release

1. Attachment/Adsorption

Since the viruses are **obligate parasites** they need to enter the host cell. To enter the host cell the virus has to first come into contact with a host cell and then has to breach/break the barrier i.e. the cell membrane in animal cells, and the cell wall in plant cell. Viruses are highly specific and can only attack particular cells of a particular organism meaning that viruses can affect only certain organisms or tissues. For example, HIV can infect the immune cells of humans, polio virus attacks the nerve cells, Hepatitis B virus (HBV) attacks the liver cells. Plant virus will not affect an animal cell and animal virus will not affect a plant cell. Specificity of the virus is due to affinity/attraction of viral surface proteins to proteins on the surface of the host cell (just like the lock and key mechanism of enzyme and substrate). You have already read in Section 1.3.4 that protein coat of the virus particle has capsid, spikes on naked or enveloped virus which helps in attachment with the host membrane.

Bacteriophages and animal viruses bind to cellular receptors on the surface of bacteria or animal cell. A well studied example of the virus-receptor interaction is that of influenza virus. The hemagglutinin protein form a glycoprotein spikes on the surface of influenza virus. This spike binds with sialic acid groups from the glycoproteins of the host cell surface (Fig. 2.4).

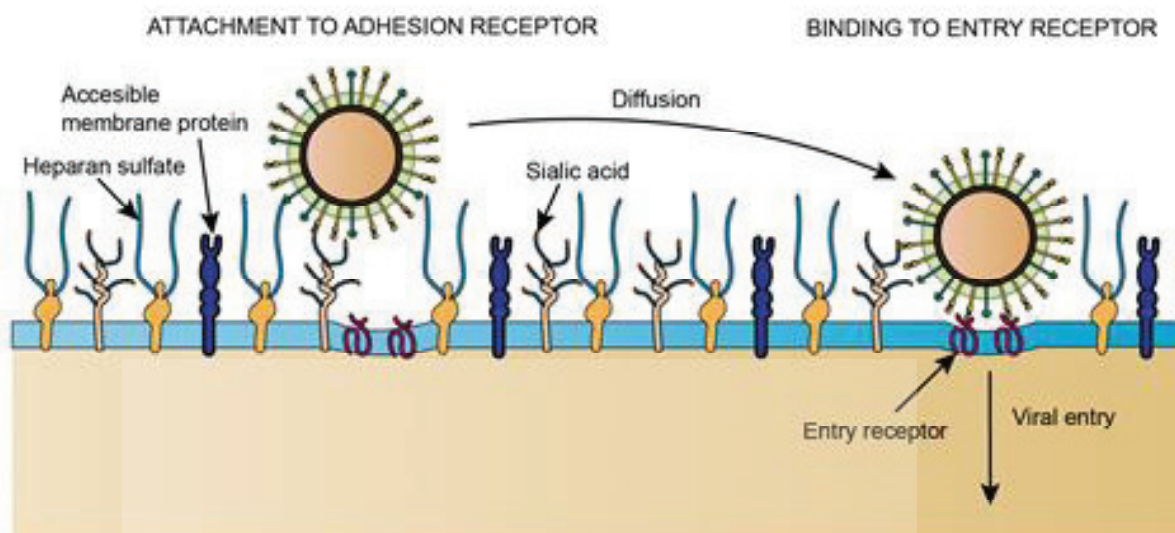


Fig. 2.4: Entry of virus into animal cell.

Entry of plant viruses into plant cell is difficult as the outer layer of plants are composed of protective layer of waxes and pectin and each cell is surrounded by thick wall of cellulose. To date no plant virus has been found which enters the plant cell with the help of receptors. The plant viruses rely on breach of the integrity of cell wall to enter the host cell.

2. Penetration and Uncoating

Penetration

Once the virus attaches to the cell membrane, it penetrates the host cell either by endocytosis or by fusion of virus envelope:

- i) Endocytosis [endo (internal/within) + cytos (referring to cell)], involves the taking in of matter by a living cell by invagination of its membrane. This is probably the most common mechanism of virus entry into the cell. The enveloped or naked viruses adsorb to the receptor on the host cell surface and are taken into the cell by a process, endocytosis. In this process the plasma membrane of the host cell surrounds the whole naked virion particle forming a vesicle, which enters the cytoplasm. In case of enveloped viruses, the envelope fuses with the vesicle membrane. The viruses then dissolve and rupture the vesicle membrane resulting in the release of the nucleocapsids into the cytoplasm (Fig. 2.5).
- ii) Fusion of the virus envelope: Several viruses are able to directly fuse their envelope with cell plasma membrane without being internalized by endocytosis. After getting attached to the host cell receptor, the envelope of the virion gets adsorbed to the host cell surface and fuses with the plasma membrane of the host cell. The nucleocapsid is then released into the cell cytoplasm (Fig. 2.6).

Uncoating

Once the nucleocapsid enters the cell cytoplasm of the host cell, viral genome must be separated from its protein coat for replication. This process of release of viral genome is called **uncoating**. In majority of the viruses, the

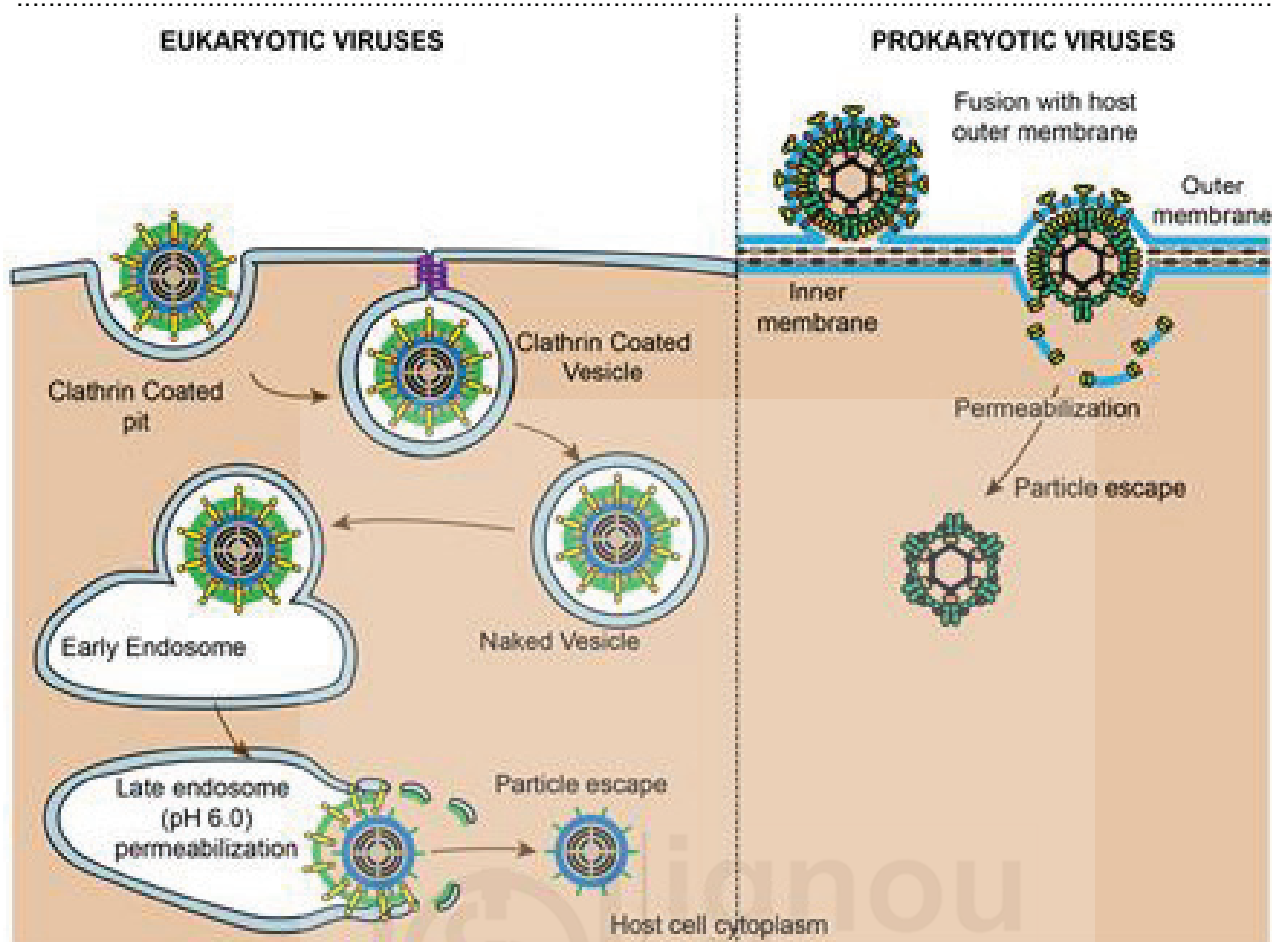


Fig. 2.5: Virus penetration of cell membrane of host by endocytosis.

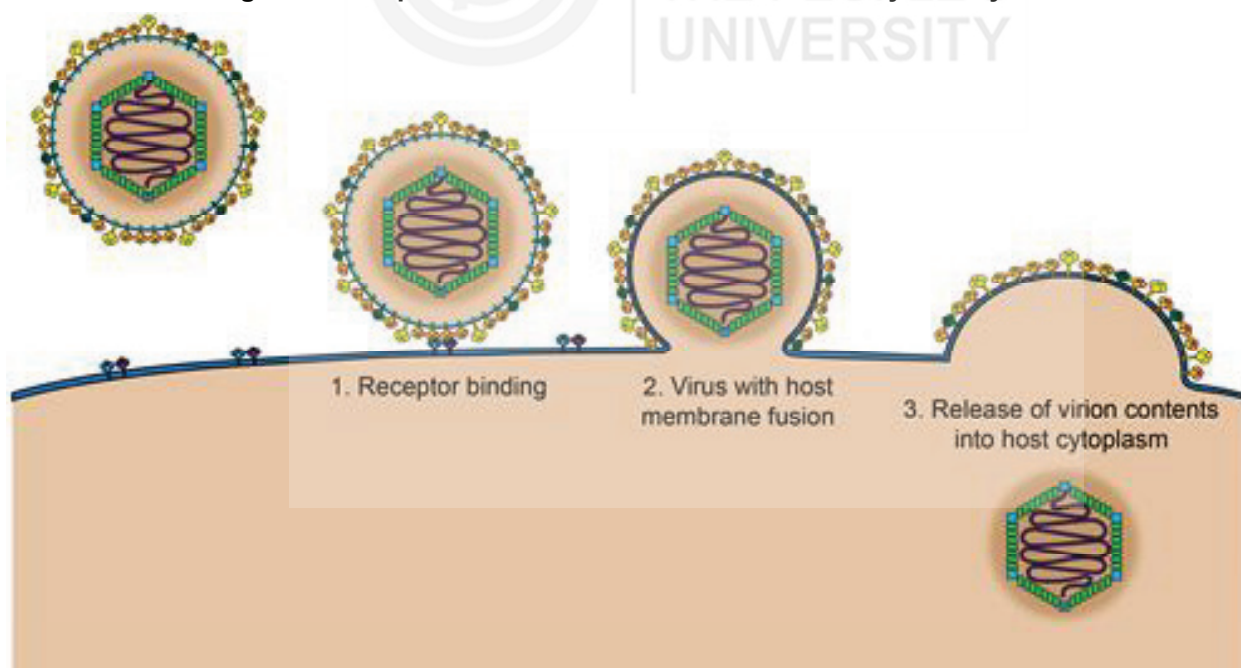


Fig. 2.6: Penetration of virus into the host cell by fusion of host cell membrane and viral cell envelope.

proteolytic enzymes (enzymes which break down the proteins) digest the capsid and carry out uncoating. The proteolytic enzymes used for uncoating either are coded by viral genome or is from the host cells. Some viruses like picorna viruses do not undergo uncoating. Picorna virus penetrates the host

cell by endocytosis. They directly release their nucleic acid in the host cytoplasm through a membrane pore present in the endocytic vesicle. After uncoating the released viral nucleic acid takes control over the host cell metabolic machinery.

3. Biosynthesis

After entering the host cytoplasm the synthesis of viral genome and viral protein starts with help of host cellular machinery. The enzymes of host cells are used for viral genome synthesis and the host cell ribosome and translational machinery is used for protein synthesis. The replication strategy of any virus depends on the nature of its genetic material. We have studied in the previous unit that viruses can be divided into seven groups (Baltimore Classification) on the basis of their genetic material.

DNA Viruses

Generally the genomic DNA of DNA viruses is replicated in the nucleus of the host cell. The DNA viruses first enters the host cell's nucleus so that they can replicate and form two daughter strands. The central mechanism of DNA synthesis in virus is similar to DNA synthesis in any cell.

The synthesis of new DNA strand requires a template, an enzyme which is a host cell enzyme DNA dependent DNA polymerase or DNA polymerase. The DNA polymerase uses DNA as template and nucleotides to form a new strand of DNA. The capsid and other viral protein are synthesized in the cytoplasm of host cell. These proteins migrate to the nucleus where it assembles with the newly formed viral DNA to form the nucleocapsid. The nucleocapsid then is transported from the nucleus along the endoplasmic reticulum for its release. Pox virus is an exception as all their components are synthesized in the cytoplasm of the host cell.

RNA Viruses

Multiplication of RNA virus occurs in the host cytoplasm and involves formation of mRNA and viral RNA molecules. The enzyme, RNA dependent RNA polymerase, is required for the synthesis of new RNA. RNA dependent RNA polymerase is not present in the infected host cell, so are encoded by the virus and are either transported into the cell at the time of infection or are synthesized very soon after the infection has begun. Frequently, the polymerases involved in RNA replication are referred to as "replicases" to differentiate them from the polymerases involved in transcription. However, both processes are carried out by the same enzyme exhibiting different synthetic activities at different times in the infectious cycle.

To study the biosynthesis of genetic material three representative examples of one DNA virus, one RNA virus and one RNA virus, which replicates with help of reverse transcriptase are being illustrated.

Class I double stranded (ds) DNA viruses (having double strand of DNA eg. Adenoviruses; Herpesviruses; Poxviruses, etc.)

In the nucleus the DNA of double stranded (ds) DNA viruses is used for two things: one as a template for replication and secondly as template for

transcription *i.e.* synthesis of mRNA (Fig. 2.7). The mRNA formed on entry of the virus into the cell is called “early” mRNA. These “early” mRNA molecules are then used as a template for synthesis of proteins (translation). These proteins help the virus in taking over or controlling the host cell’s machinery. The double-stranded DNA is replicated using the host cell’s DNA polymerase. After DNA replication has occurred there are a lot of newly made viral daughter DNA which are now available as template for mRNA synthesis. The mRNAs formed after viral DNA replication has occurred are called “late mRNAs”. The “late” mRNAs are then translated to form structural proteins. These structural proteins are used for encapsulating double stranded DNA. The newly replicated ds DNA is then enclosed within the protein capsule. The protein capsule with newly replicated ds DNA then moves to the cytoplasm, to be released from the host cell.

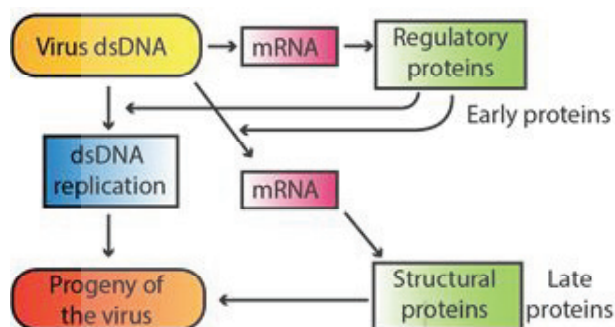


Fig. 2.7: Replication of double stranded (ds) DNA in Class I viruses.

Class IV contains viruses with ssRNA genomes of the same (positive) sense as mRNA and which can be translated (eg. Picornaviruses; Togaviruses etc.).

In the first step of Positive (+) Strand RNA viral replication, viral RNA acts as mRNA. This viral RNA binds to host cell’s ribosomes and is translated into early proteins. One of the early proteins synthesized is the viral replicase or RNA dependent RNA polymerase. The viral replicase uses RNA as a template to make more RNA. Positive (+) strand RNA serves as templates for synthesis of complementary full length (-) strand RNA’s. The Positive (+) strand RNA and complementary (-) strand RNA’s together form a double-stranded RNA replicative complex. The newly made negative-sense RNA serves as template for synthesis of positive (+) strand RNA’s. These single strands of positive-sense RNA are then used as mRNA to make the structural proteins (Fig. 2.8).

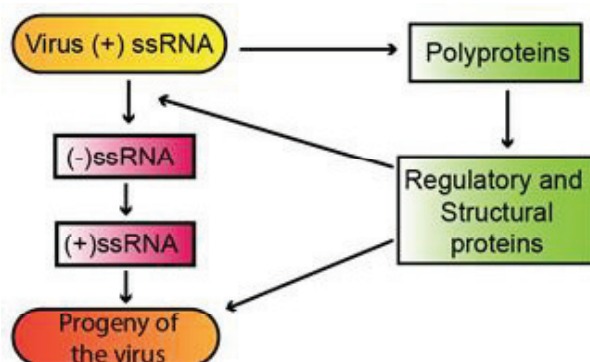


Fig. 2.8: Replication of single stranded (ss) RNA in Class IV viruses.

Class VI contains viruses that have ssRNA genomes which with help of reverse transcriptase enzyme convert to dsDNA and then form mRNA (eg. Retroviruses).

An important element of replication of retroviruses is an enzyme called reverse transcriptase (RT). After infecting a cell, the virus synthesizes viral DNA from viral RNA utilizing the reverse transcriptase. The process by which DNA is copied from an RNA template is known as reverse transcription. Viral genomic RNA serves as template for production of DNA copy. This is produced by an RNA-dependent DNA polymerase (reverse transcriptase) which is part of the virus and enters the host cell with the virus. The double-stranded DNA then migrates to the nucleus and integrates with the host cell genome. This integrated DNA may either remain silent or be transcribed into RNA. The transcribed RNA may be full-length RNA or shorter. The shorter RNAs serve as template for synthesis of accessory and structural proteins. Full-length RNA transcripts are packaged into forming virions (Fig. 2.9).

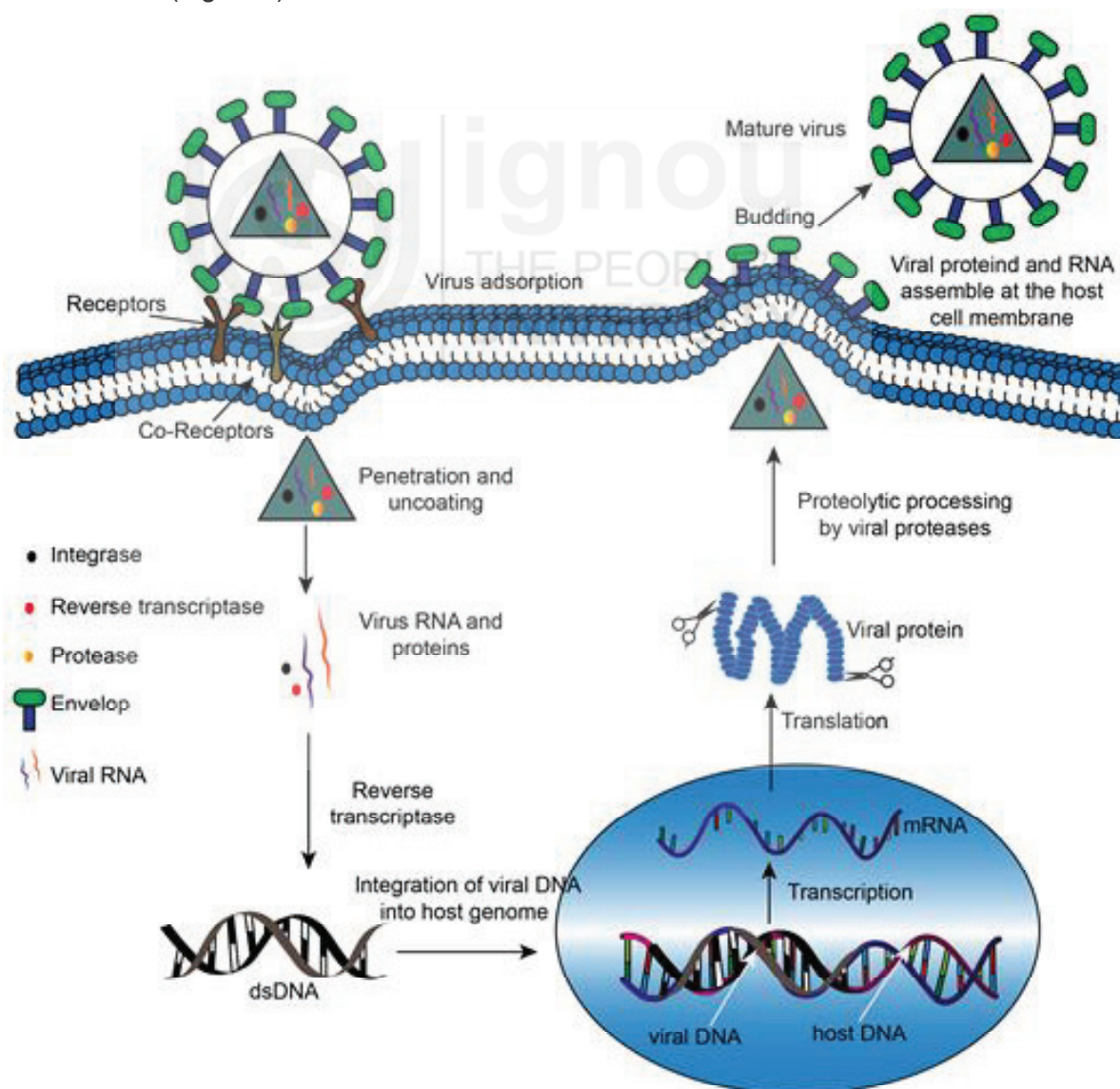


Fig. 2.9: Replication of single stranded (ss) RNA in Class VI viruses.

The replication strategy of different viruses is different. It is based on the genetic material present in the virus. Fig. 2.10 summarises the different strategies adopted by viruses (Baltimore classification).

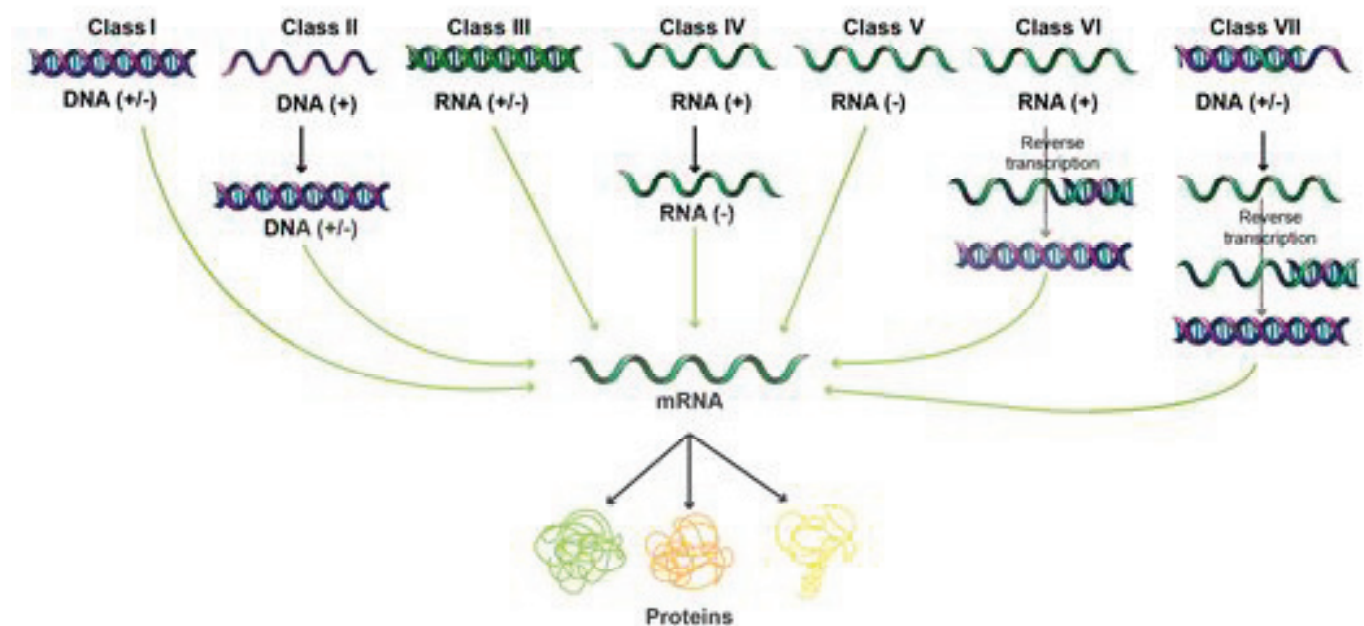
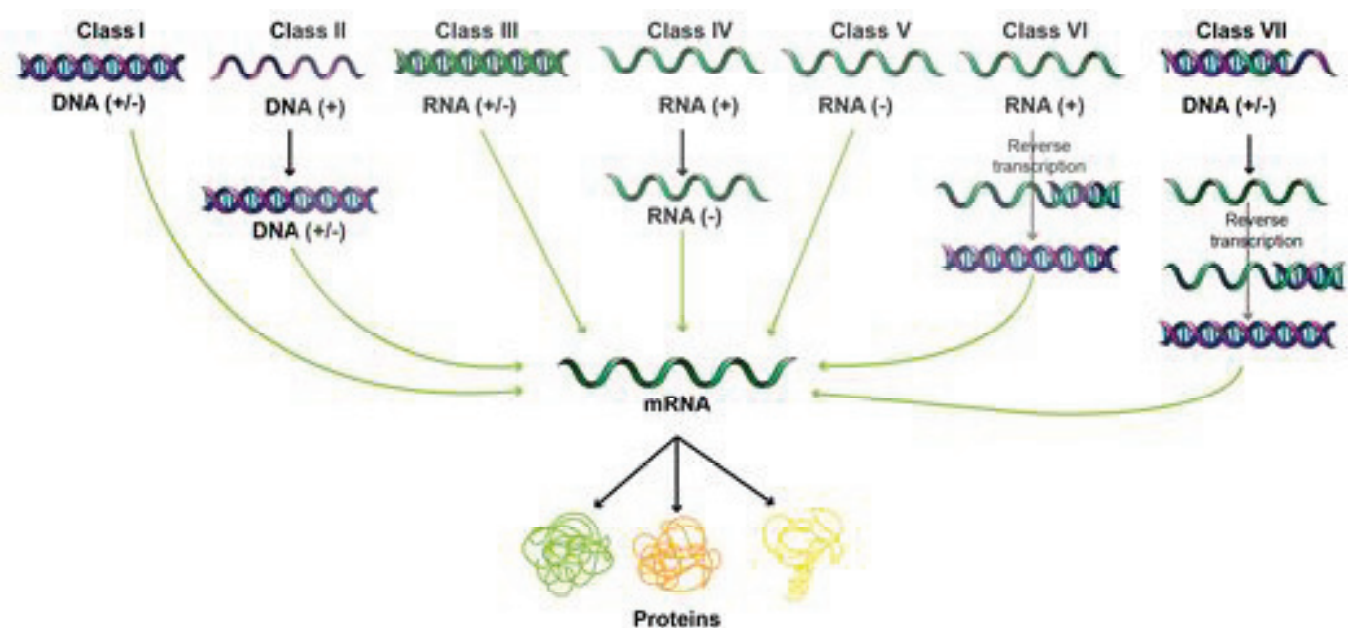


Fig. 2.10: The replication strategy of viruses (Baltimore Classification) on the basis of their genetic material depends on the nature of its genetic material.

SAQ 1

Complete the following flow chart:



4. Maturation and Assembly

During maturation, assembly of the viral components takes place in the host cell cytoplasm. The assembly process involves collection of all components (virus-encoded proteins and nucleic acid synthesized in the host cell) necessary for formation of mature virus at a particular site (Fig. 2.11). For some viruses there is an additional aspect when assembly takes place in the nucleus and proteins synthesized in the cytoplasm have to be moved to the nucleus.

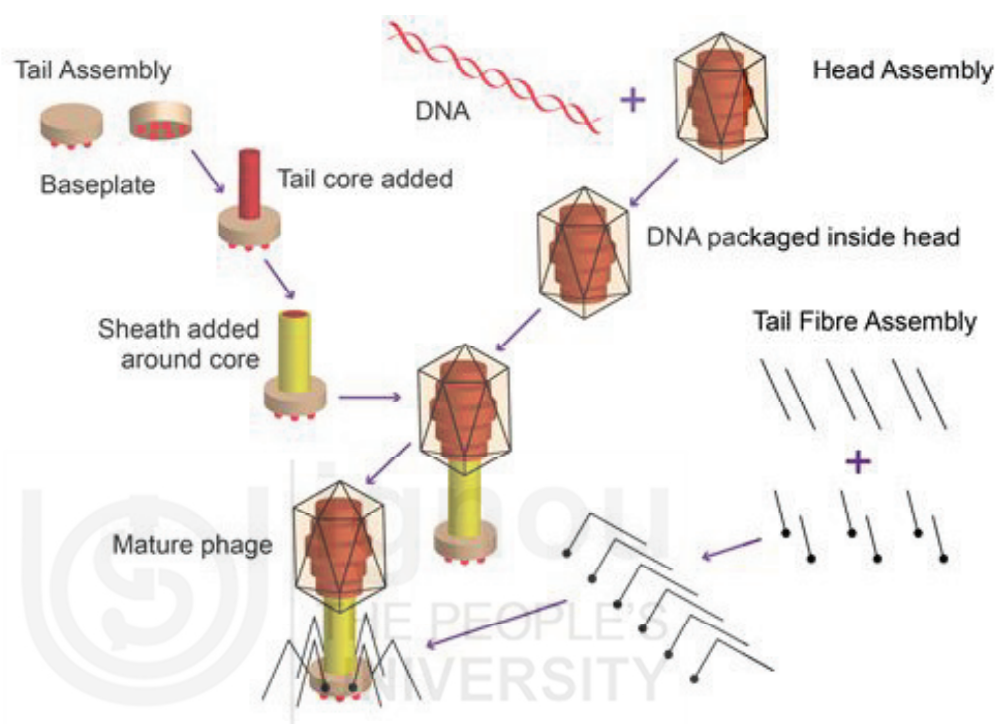


Fig. 2.11: T4 Phage assembly (simplified scheme).

The assembly of virus takes place mainly by three mechanisms:

- i) The various components combine spontaneously to form particles in a process of self assembly.
- ii) In some viruses, specific virus-encoded proteins which are not part of virus structure are first removed and then assembly takes place.
- iii) Virus particle may also be assembled from precursor proteins (pro-proteins or pro-peptides), an inactive protein (or peptide) that can be turned into an active form by post-translational modification, which are then modified, usually by proteolytic cleavage, for assembly to take place.

The final step of assembly for enveloped virus is the acquisition of the envelope by synthesis of envelope proteins and its incorporation in nuclear, cytoplasmic, or plasma membrane.

5. Release

The viruses are released as a result of lysis of dead cells or by budding/exocytosis from the host cell.

- i) **Cell lysis:** For lytic virus (some enveloped and naked virus) the release is a simple process. The infected cells' membrane ruptures which results in cell lysis and release of the virus. Host cell die because entire host macromolecular synthesis is stopped and only viral nucleic acids and viral proteins are synthesized. As a result cell death and subsequently lysis occurs releasing the viruses. Mostly naked virions are released by cell lysis.
- ii) **Budding:** Once the nucleocapsid is assembled, some viruses push through the plasma membrane, forcing a portion of the membrane around the virion resulting in an envelope, or fuse with the plasma membrane, releasing the virion. This process is called budding. In this process the host cell is not destroyed during release because the ruptured plasma membrane can be repaired after budding has taken place (Fig. 2.12).

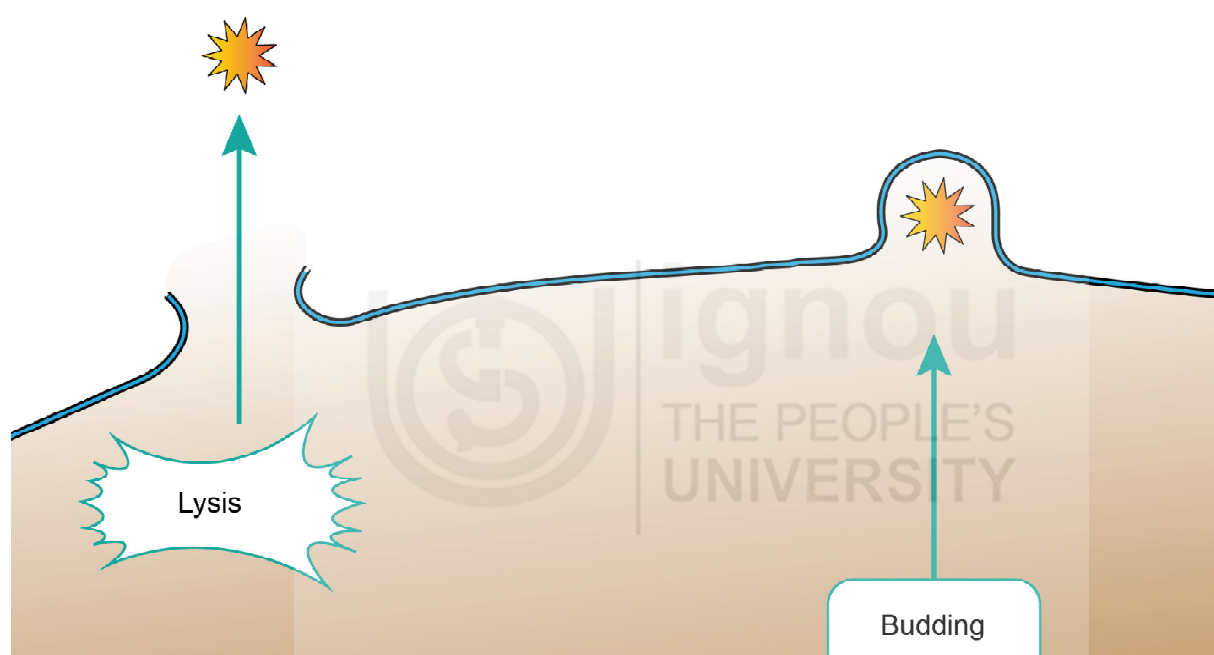


Fig. 2.12: Release of virus from cell.

2.3 REPLICATION OF BACTERIOPHAGE: DNA VIRUS (T-PHAGE)

In *E. coli*, lytic infections are caused by phages known as the T-phages, while lysogenic infections are caused by the phage lambda.

On the basis of replication methods, there are two main types of bacteriophages: lytic or virulent type and lysogenic (temperate) or avirulent type. So we can say bacterial cells can undergo one of two types of infections by viruses termed lytic infections and lysogenic (temperate) infections. Lytic phage infects the bacterial cells and multiplies within the infected cell producing large number of progeny phages. Progeny phage particles are released after the host cell bursts or its lysis occurs at the end of incubation period. This process is termed as **lytic cycle**. Temperate phage undergoes lysogenic cycle wherein progeny particles are not produced. The phage DNA gets integrated into the bacterial chromosome and is replicated along with the

bacterial chromosome. Lysis of host cell does not occur in this case and the phenomenon of this stable relationship between phage and its host is called **lysogeny**. However, temperate phages may sometimes spontaneously become virulent and bring about the lysis of the host cell releasing their progenies.

2.4 THE LYTIC CYCLE

The bacteriophages of the T-even group (T2, T4 etc) are virulent viruses as they lyse the host cell during lytic cycle of infection. To study the lytic cycle phage T4 replication in *E. coli* will be used as a model (Fig. 2.13). Recall the structure of T4 phage (Section 1.4).

Attachment/Adsorption: When the phage and bacterial cells randomly collide, the tail fibres of phage base plate gets attached to its specific complementary molecular component called receptor site on the surface of bacterial cells by the formation of weak bonds. This is an irreversible process, which means that attached phages cannot get separated from their host cells.

Penetration: After attachment the tail of phage releases lysozyme which dissolves portion of the bacterial cell wall forming a pore. The tail sheath contracts so that the tail core is pushed through the cell wall and comes in contact with bacterial plasma membrane. When it reaches the cell membrane of the bacteria, the protein plug blocking the tail core is removed and DNA is ejected through the core into the cytoplasm and the capsid remains outside the bacterial wall. Mechanism of phage attachment and nucleic acid penetration are more or less similar in both lytic and lysogenic cycles.

Biosynthesis: The viral genome after entering the host cytoplasm, uses the cells' ribosomes and translation machinery to synthesize new phage proteins (head, tails and tail fibers). Once viral genome is in the host cell, it takes over the metabolic machinery of the host cell and directs the cell to make only viral nucleic acids and viral proteins. Host DNA gets degraded and the nucleotides as well as the host cell's enzymes are used for synthesizing copies of phage DNA. Phage DNA is transcribed to mRNA, which directs the synthesis of phage enzymes and capsid proteins on the host cell's ribosomes. The newly synthesized enzyme, DNA polymerase replicates the phage DNA (Fig. 2.13).

Maturation and Assembly: The phage proteins and the copies of viral genome spontaneously self assemble to form virus particle. Phage head and tails are assembled separately from viral proteins. Each head is then packed with the viral DNA and gets attached to the tail. Tail fibers are then added to form mature infective phages.

Release: In the final stage of the lytic multiplication cycle, the lysozyme, encoded by phage genome, causes the lysis of the wall of the host bacterium and the phages are released from the ruptured bacterial shell (Fig. 2.13).

The time taken from attachment to release is called the **burst time**. The number of virions synthesized and released from one infected host bacterial cell is called **burst size** or **viral yield**.

The lytic cycle is called a productive infection, as many virions are produced.

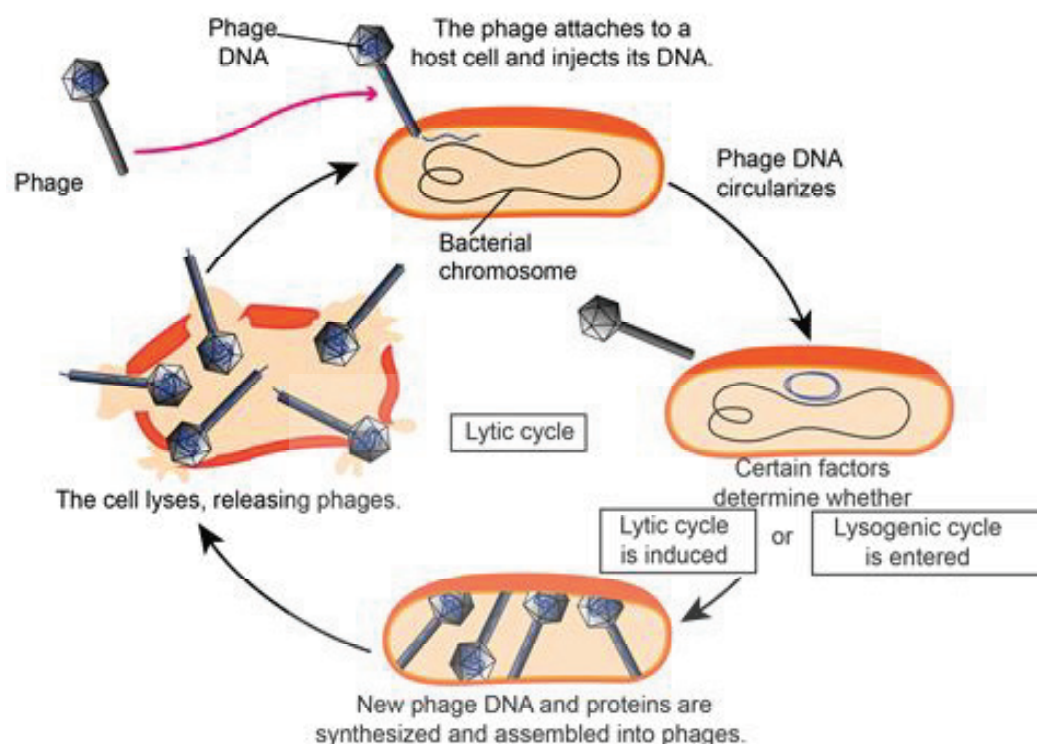


Fig. 2.13: Lytic Cycle.

2.5 THE LYSOGENIC CYCLE

Lambda (λ) phage of the bacteria *E. coli* is the most widely studied example of lysogeny. The phages whose choice of replication cycle is lysogenic are called lysogenic phage (temperate phage) and the bacteria in which it occurs is called a lysogenic cell.

In the lysogenic cycle the λ phage gets adsorbed to the bacterial cell surface and penetrates its linear DNA genome into the cell. In the host cell cytoplasm, the phage DNA forms a circle (circularizes) and gets inserted into the circular DNA (chromosome of bacterium). The viral DNA integrated with bacterial DNA is called prophage and the combination of the bacterium and temperate phage is called a lysogen. Two genes present on prophage produce two repressor proteins and repress the transcription of all other prophage gene. The prophage gets replicated along with the bacterial chromosomes during reproduction and remains latent in the bacterial progenies. As the prophage does not lyse the bacterial cell, the infection is referred to as **latent infection**. When the bacterial cell becomes "stressed" due to UV radiations, presence of chemicals, the prophage excises itself from the bacterial chromosome and is released in the cytoplasm. This disassociation is called **induction**. After induction lytic cycle is initiated, lyses of the bacterial cell occurs and new λ phages are released (Fig. 2.14).

There are three implications of lysogeny. One, the lysogenic cell becomes immune to infection by another phage of same type, a process called lysogenic conversion. However, the lysogen can be infected by other types of temperate or virulent phages. Secondly the lysogenic cells may exhibit different properties than the non-infected host cell. For example the bacteria

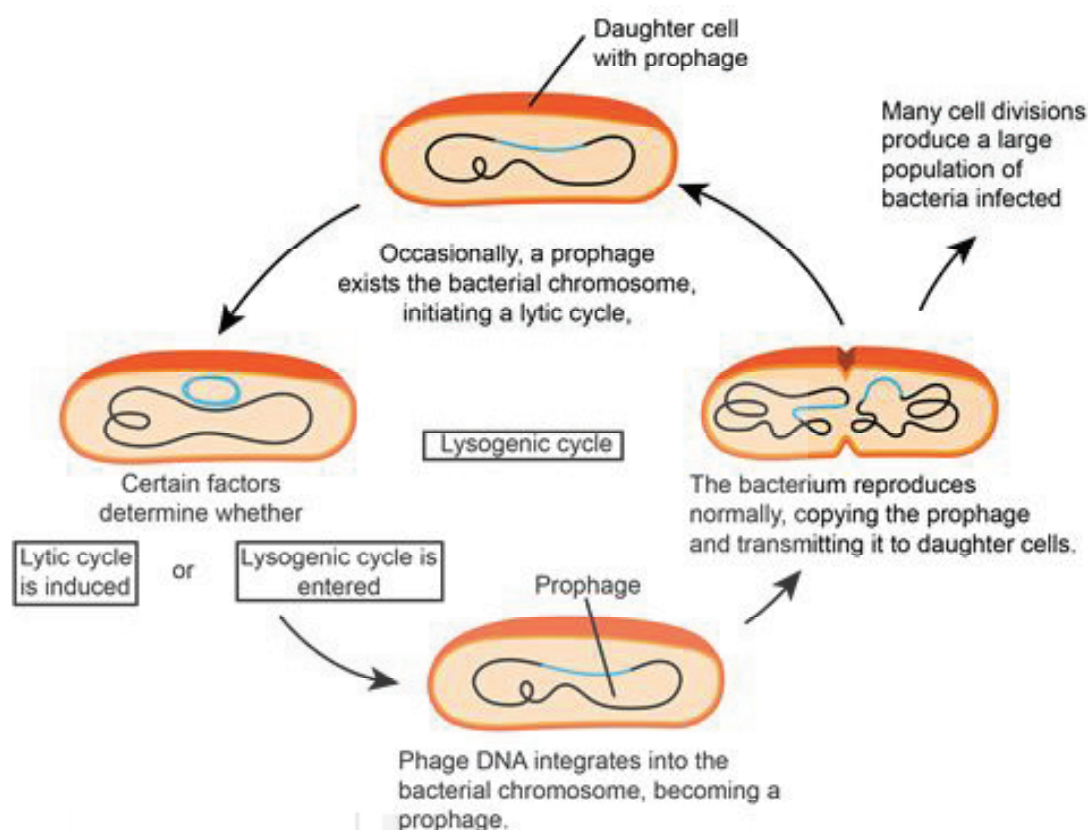


Fig. 2.14: Lysogenic cycle.

Corynebacterium diphtheriae, that causes diphtheria and *Clostridium botulinum*, the causative agent of botulinum contain prophages each of which has a gene which codes for the production of a toxin. The disease producing properties of these bacteria are related to these toxins, as without the prophages these bacteria do not cause diseases. Thirdly, lysogeny enables the specialized transduction. In this process when the prophage is excised from host bacterial chromosomes during its lytic cycle, the adjacent genes of bacterial chromosome from its either side may remain attached to this phage DNA. As a result the phage particle packages the bacterial DNA segment along with its own DNA in its capsid and carries the same to another bacterial cell it infects.

2.6 REPLICATION OF RNA VIRUS (TMV)

Recall the structure of TMV (Section 1.5). The TMV enters the cell through cell sap via abrasions/wounds on the host cell surface as it does not have any specialized organ for attachment as observed in bacteriophage. Once the virus enters the plant cell the viral genome i.e. (+) ssRNA is released into the cell after uncoating takes place. Uncoating occurs by dissolution of protein coat (capsid) by the host cell enzymes.

The (+) ssRNA acts as mRNA and replicase gene is translated with help of host cell ribosomes. The newly formed replicase enzyme is used to replicate the viral genome. (+) ssRNA acts as a template and replicates with help of replicase to form a complementary (–) ssRNA. The (+) ssRNA and complementary (–) ssRNA form a (±) double stranded RNA called the replicative intermediate (RI). The complementary (–) ssRNA of replicative

intermediate is used as template for synthesis of more (+) ssRNA. This (+) ssRNA is used as mRNA and translated with help of host cell machinery to form capsid proteins. Once enough capsid proteins are produced at an optimal level (+) ssRNA formed is used as viral genome (Fig. 2.15).

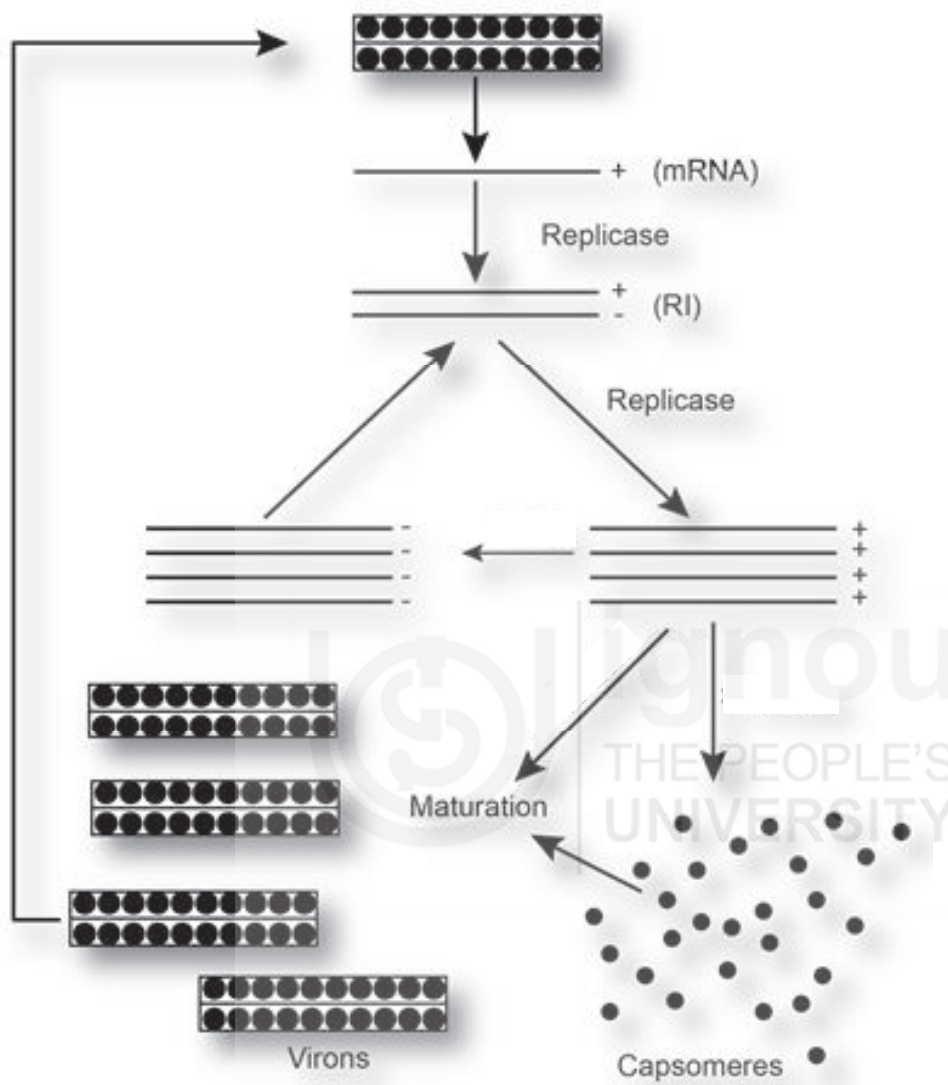


Fig. 2.15: Replication of Tobacco Mosaic virus.

The TMV viral genome (+) ssRNA and capsid protein combine spontaneously to form particles in a process of self assembly.

SAQ 2

Fill in the blanks:

- i) The virus penetrates the host cell by two main mechanism: fusing of envelope and
- ii) The process in which various viral components combine spontaneously to form particles is called

- iii) The bacteriophages of the T-4 lyse the host cell during cycle of infection.
- iv) A process by which viral genome is pushed out into the cytoplasm by break of capsid is called
-

2.7 SUMMARY

In this unit you have learnt that

- A virus particle (virion) cannot replicate outside the host as it lacks the desired cellular machinery that are required to reproduce.
- Viral replication results in many progeny, and these progeny leave the host cell to infect other cells in the organism or spread the infection.
- Virus replicate by a 5 step replication: attachment; penetration and uncoating; biosynthesis; assembly and release.
- The virus-host cell receptor interaction helps in the attachment of virus to the host. Plant viruses enter plant cells by breach in plant cell wall.
- The virus penetrates the host cell by two main mechanism: endocytosis and fusion of envelope. Uncoating is a process by which viral genome is pushed out into the cytoplasm by break of capsid.
- The strategy for biosynthesis of genetic material of viruses depends on the nature of its genetic material (DNA or RNA).
- The assembly of virus takes place mainly by three mechanisms: by a process of self assembly, specific virus-encoded proteins are first removed (not part of virus structure) and then assembled, and assembled from precursor proteins, which are then modified, usually by proteolytic cleavage, to form the infectious virion.
- All viruses escape the cells by one or two mechanisms: budding and cell lysis.
- Bacterial cells can undergo one of two types of infections by viruses termed lytic infections and lysogenic (temperate) infections.

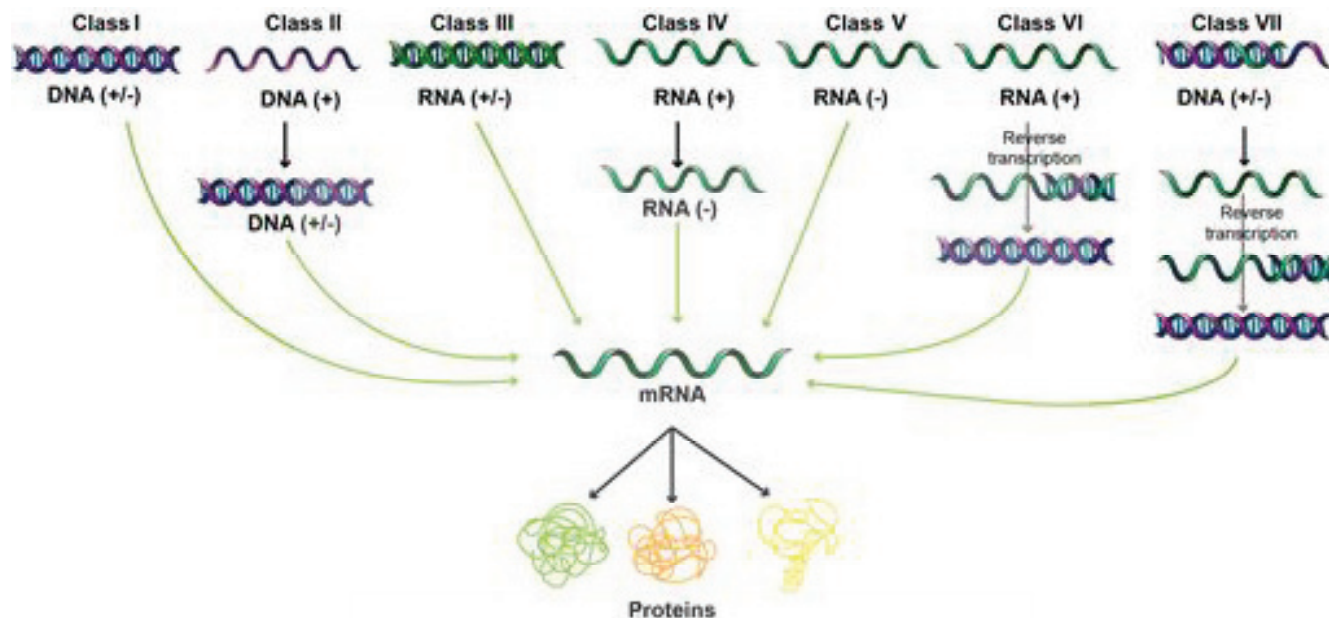
2.8 TERMINAL QUESTIONS

1. With help of diagram discuss viral replication (General Account).
2. Differentiate between lytic and lysogenic viral replication.
3. Give in detail the biosynthesis of DNA viruses.
4. Discuss the replication of TMV.

2.9 ANSWERS

Self-Assessment Questions

1.



2. i) Endocytosis
- ii) Assembly
- iii) Lytic
- iv) Uncoating

Terminal Questions

1. Section 2.2
2. Section 2.4 and 2.5
3. Section 2.2, (3) Biosynthesis
4. Section 2.6

Acknowledgement of Figures

Fig. 2.1a. https://docs.google.com/document/d/1iRxjQQ6jCTcabxPR9SourfbelM2OOyZS8C8_1nIC8Vw/edit

Fig. 2.1b. http://4.bp.blogspot.com/_FN1wR3ASuIU/Sbwlc4qaobI/AAAAAAAAARs/XZ4uwi3e3vo/s400/phage-lambda-plaque.gif

GLOSSARY

- Assembly** : The gathering and replication of viruses within a cell by using the metabolism of the host organism.
- Attachment** : The condition where the capsid proteins of the virus bind to certain receptors of the host organism.

Bacteriophage	: A virus that must infect bacteria in order to reproduce itself.
Burst size	: The burst size of a phage is the average number of new phage produced by a single infected bacterium during the lytic growth cycle. This number varies widely for well-studied phages, from only a few to over one hundred.
DNA polymerase	: One of a class of enzymes of complex structure that catalyze the synthesis of a new strand of DNA complementary to the template strand in a primer-dependent reaction.
dsRNA	: Double-stranded RNA.
Endocytosis	: The process of incorporation of viruses or large molecules into a cell by formation of a specific vesicle at the cell surface that engulfs the material and transports it into the cell's interior.
Endoplasmic reticulum	: The complex membrane system in eukaryotic cells that is continuous with the nucleus. It is the site of lipid and membrane synthesis as well as the synthesis of proteins destined to be secreted or remain membrane associated in the cell.
Gene Expression	: An activity where information from a gene is made into functional gene material.
Incubation period	: The time between initial infection and the onset of notable symptoms of a disease.
Genome Replication	: The reproduction of genetic material, particularly that in the structure of DNA.
Latent Infection	: A viral infection that exists in dormancy and does not exhibit symptoms.
lysogeny	: Ability of certain bacteriophages (notably, bacteriophage λ) to integrate its genome into that of the host bacteria and remain associated as a genetic passenger as the bacteria replicate.
Maturation	: The phase during replication at which a virus becomes infectious.
Penetration	: The process of the virus entering the cell of the host organism, causing infection.
Plaque	: A defined area of cell destruction resulting from virus infection in vitro.
Plaque-forming unit (PFU)	: A unit of infectious virus determined by the ability of the virus to form a plaque or area of lysed cells on a "lawn" of susceptible cells.

- Prophage** : Prophage is a bacterial virus the genome of which is residing in a bacterial host in a passive state, not expressing many viral functions or producing progeny virions. It may be incorporated into the bacterial DNA or it may be a separately replicated molecule within the bacterial cytoplasm. In some cases toxin genes present in prophage genomes have gene expression signals that allow their independent function when the viral genome is otherwise passive.
- Receptor** : A specific type of molecule found on a cell membrane that a virus is able to attach to.
- Release** : The process of the death of a host cell that discharges a virus.
- Reverse transcriptase** : An enzyme, originally discovered in retroviruses, that uses single stranded RNA as a template for the synthesis of a cDNA sequence; can also use DNA as a template, and can degrade RNA from a DNA–RNA hybrid. This latter activity is termed RNase H activity.
- Temperate phage** : Temperate phage is capable of establishing a lysogenic state in a susceptible bacterial host. This is a quiescent state in which virus replication does not occur. If this option is not available to the phage, the phage is referred to as “lytic”.
- Transcription** : The enzymatic synthesis of RNA from either a complementary DNA template.
- Uncoating** : A condition when the protein capsid of the virus is unsheathed due to enzymes of the cells of the host organism.

BACTERIA: GENERAL ACCOUNT AND ECONOMIC IMPORTANCE

Structure

3.1 Introduction	3.6 Protoplasm
Objectives	Cell Membrane
3.2 Discovery	Cytoplasm
Pre Twentieth Century	Genome
Transition into 20 th Century	Plasmids
3.3 Prokaryotic Cell and Eukaryotic Cell	Ribosomes
	Microcompartments
3.4 General Characteristics	Gas Vesicles
Size	Magnetosomes
Shape of Bacteria	3.7 Economic Importance of Bacteria
Arrangement of Bacterial Cell	3.8 Summary
3.5 Cell Wall and its Adherents	3.9 Terminal Questions
Cell Wall	3.10 Answers
Surface Adherents	Glossary

3.1 INTRODUCTION

Bacteria in fossils form have been found in geological strata about 3.5 billion year ago and they are almost three times as old as any organism known to have existed on the planet earth. Approximately 5000 species of bacteria are recognized today and you can find them in every possible habitat that you can think, they are found in plants, animals, every type of soil and water, also in ice caps, hot springs, hyper saline environments and in atmospheres rich in toxic gases like methane or hydrogen sulfide that would kill most other organisms. Bacteria, the simplest organisms living on earth, are too small to be seen with the unaided eye, and are the most abundant of all organisms.

The bacteria are an extremely diverse group of microorganisms exhibiting different types of morphology and physiology. They are also the only

organisms with prokaryotic cellular organisation. Life on earth can not exist without bacteria because they make possible many of the essential functions of ecosystems, including fixing nitrogen from the atmosphere, decomposition of organic matter in many aquatic communities. Bacteria play a vital role both in productivity and in cycling the substances essential to all other life-forms. Bacteria are ubiquitous on earth.

Objectives

After studying this unit, you will be able to:

- ❖ appreciate the history of discovery of bacteria;
- ❖ list the characteristic features of bacteria;
- ❖ classify bacteria on the basis of shapes;
- ❖ identify the structure and morphology of bacteria and describe various organelles found in bacterial cell;
- ❖ appreciate the economic importance of bacteria; and
- ❖ know about the harmful effects of bacteria.

3.2 DISCOVERY

Microorganisms are omnipresent and have been found in different micro environments for billions of years. However, prior to the invention of the microscope, there was no scientific proof of the existence of microorganisms. Though, scattered reports from different parts of the world, over a span of centuries, were available on the possible existence of microbes. Let us start with an overview of milestone events that made the history of microbiology (Table 3.1). Period from 1857 to 1910 in the history of microbiology is named as the Golden Age of Microbiology. Discoveries during this period led to the establishment of microbiology as a science.

Table 3.1: Some important events in the development of microbiology

Year	Investigator(s)	Events
1676	Anton van Leeuwenhoek	First observation of very little animalcules now recognized as protozoa
1684	Anton van Leeuwenhoek	Discovery of bacteria
1798	Edward Jenner	Vaccination with cowpox for preventing small pox
1857	Louis Pasteur	Lactic acid fermentation
1860	Louis Pasteur	Role of yeast in alcoholic fermentation
1861	Louis Pasteur	Proof of biogenesis
1864	Louis Pasteur	Pasteurization
1867	Joseph Lister	Antiseptic principles in surgery

Year	Investigator(s)	Events
1876	Robert Koch	<i>Bacillus anthracis</i> as the causative agent of anthrax
1881	Robert Koch	Methods for study of bacteria in pure culture
1882	*Robert Koch	Discovery of <i>Mycobacterium</i> , cause of tuberculosis
1884	Robert Koch	Koch's postulates
1884	*Elie Metchnikoff	Description of phagocytosis
1884	Hans Christian Gram	Gram staining technique
1885	Louis Pasteur	Vaccination against rabies in humans
1887	Richard J. Petri	Petri dish (plate) developed
1889	Sergei Winogradsky	Nitrification and chemolithotrophy
1889	Martinus W. Beijerinck	Crystallised virus, isolated <i>Rhizobium</i>
1890	*Emil von Behring	Antitoxins for diphtheria
1892	Dmitri Ivanovsky	Virus, the causative agent of tobacco mosaic disease
1901	Martinus W. Beijerinck	Enrichment culture method Term Virus
1910	*Paul Ehrlich	Chemotherapeutic agents, drug for syphilis
1928	Frederick Griffith	Transformation in bacteria
1929	*Alexander Fleming, Ernst Chain, Howard W. Florey	Miracle drug penicillin from <i>Penicillium notatum</i> , effective against bacterial infection
1935	*Wendell M. Stanley, Northrop and Sumner	Crystallized tobacco mosaic virus
1941	*George Beadle and Edward Tatum	One gene-one enzyme hypothesis
1944	Oswald Avery, Colin MacLeod, Maclyn McCarty	Explanation of Griffith's work: DNA is genetic material
1944	Selman Waksman	Discovery of streptomycin
1953	*James Watson, Francis Crick	Molecular structure of DNA
1961	*Francois Jacob, Jacques Monod	Concept of an Operon
1970	*Daniel Nathans, Hamilton O. Smith, Werner Arber	Restriction enzymes used for genetic engineering

Year	Investigator(s)	Events
1975	Georges Kohler, Cesar, Milstein	Monoclonal antibodies
1977	Fred Sanger, Steven Niklen, Alan Coulson	Methods for sequencing DNA
1978	*Peter Mitchell	Chemiosmotic mechanisms
1982	*Aaron Klug	Structure of tobacco mosaic virus
1983	Luc Montagnier	Discovery of HIV, the cause of AIDS
1986	Benjamin Hall, Gustav Ammerer	Hepatitis B vaccine produced by genetic engineering
1994	R. J. Cano	Cultured 40 million year old bacteria
1996	Andre Goffeau <i>et al</i>	Yeast genome sequenced
1998	James B. Kaper <i>et al</i>	Discovered 2 circular chromosomes in <i>Vibrio cholerae</i>
1999	Frederek R. Blattner, <i>et al</i>	<i>Escherichia coli</i> genome sequenced
2000	Edward Delong	Discovery of marine archaea
2002	Jeronimo Cello. Paul A.V., Eckard Wimmer	Synthesis of infectious poliovirus from basic chemical building blocks
2004	J. Craig Venter, <i>et al</i>	First large scale environmental genome: shot gun sequencing of the Sargasso Sea

*Awarded Nobel Prize

The Roman philosopher, Lucretius (98-55 BC) and a physician Girolamo Fracastoro (1478-1553) suggested that invisible living creatures caused diseases. An Italian called Frances Stelluti made observations on bees and weevils between 1625 and 1630, by using a microscope. The English scientist Robert Hooke built a compound microscope and observed the minute compartments in the slices of cork through it and named these compartments as cells. He also observed and reported seeing elongated stalks, in around 1664, which were in fact, the fruiting structures of fungi. Visualization of microorganisms was made easier by the development of more advanced microscopes as well as techniques like staining.

3.2.1 Pre Twentieth Century

Microbiology did not exist as an area of science in the nineteenth century, although human beings had been practicing microbiology from prehistoric times. Beer and wine production was common. Foods were preserved by

drying and addition of salt. However, no one knew the reason behind any of these processes. Diseases used to spread and acquire epidemic proportions, but man did not know the cause of these diseases. It was the dedicated efforts of a few people, some not even trained to be scientists like Anton van Leeuwenhoek, and others who had scientific training like Louis Pasteur, Robert Koch and Martinus Beijerinck that microbiology blossomed into a science.



Fig. 3.1: Anton van Leeuwenhoek.

June 16, 1675
recording of Anton van Leeuwenhoek: "I discovered, in a tiny drop of water, incredibly many very little animalcules, and these of diverse sorts and sizes. They moved with bending, as an eel always swims with its head in front, yet these animalcules swam as well backwards as forwards, though their motion was very slow."

Anton van Leeuwenhoek

The credit for **discovering the microbial world** goes to a Dutch merchant, Anton van Leeuwenhoek (1632-1723) (Fig. 3.1), who had no university education. He is known as Father of microbiology. He was a draper, haberdasher and also the official wine taster of Delft, a place in Holland. To begin with, he made lenses to magnify and inspect the cloth pieces. Later, to satisfy his own quest for knowledge, he began constructing simple microscopes with double convex glass lenses held between two silver or glass plates. His microscopes could magnify around 50 to 300 times. He eventually made more than 250 microscopes. Beginning in 1673, Leeuwenhoek started sending detailed letters written in Dutch, describing his observations to the Royal Society in London. Most of these letters were translated in English and published in the *Proceedings of the Royal Society*. He examined samples of river water, pepper infusions, saliva, feces etc. It was clear from his descriptions that he was the first person who saw both bacteria and protozoa, as we know them today, under his microscope. He called the microscopic forms of life 'animalcules' as he thought they were tiny living animals.

Louis Pasteur



Fig. 3.2: Louis Pasteur.

Until nineteenth century, the most common topic of scientific debate was regarding the theory of spontaneous generation (called abiogenesis). This theory proposed that living organisms could arise spontaneously from nonliving matter. It had been observed that there was a connection between the growth of microorganisms in the organic broth and onset of the chemical changes. The chemical changes in broth were due to **fermentation** (souring of carbohydrates in grapes that lead to the production of wine: the process of conversion of sugars into alcohol in the absence of air) and putrefaction (decomposition of meat proteins). The question was where did microorganisms come from? Italian physician Francesco Redi (1626-1697) was among the first to provide evidence against spontaneous generation. He carried out an experiment where he took meat in three containers; one was uncovered, second was sealed and the third was covered with a fine gauze. Needless to say, flies laid eggs on the uncovered meat and not on the covered meat. Flies also laid eggs on the gauze covering, which prevented or delayed the contact of eggs with meat and hence no initial appearance of maggots. Hence, he proved that maggots (fly larvae) came from the eggs of flies and not from the decaying meat (Fig. 3.3).

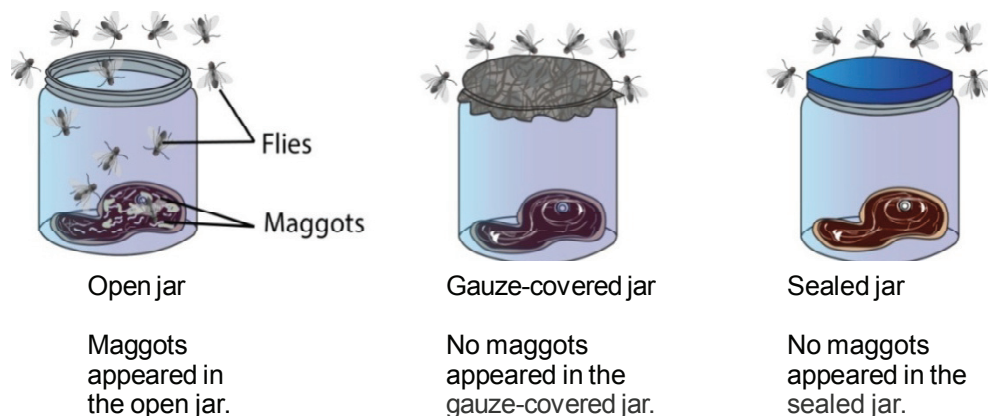


Fig. 3.3: Experiment conducted by Redi that refuted spontaneous generation in maggots. Maggots did not arise from the second jar, as eggs did not come in contact with the meat at all.

A further blow to the concept of spontaneous generation came from the work of an Italian scientist Lazzaro Spallanzani (1729-1799) who was a priest. He demonstrated that by sealing flasks that contained meat broth, and had been heated for long period to destroy microbes, no microbial growth was observed and that these sealed flasks could be stored indefinitely. However, those who supported spontaneous generation argued that sealing the flasks led to the elimination of oxygen and hence Spallanzani's experiments were not considered to be foolproof. The debate finally ended with the experimental work of the French scientist Louis Pasteur (Fig. 3.2) (1822-1895).

Pasteur placed nutrient solutions in long-necked flasks and heated their necks in a flame, drawing them in the shape of a swan's neck i.e., S-shaped curves. He heated the flasks to boil the nutrient solutions and allowed them to cool. Air could reenter the flask through the capillary curved neck but its shape prevented bacteria and other microorganisms from reaching the liquid broth, as they remained trapped in the curved neck. The broths remained sterile and could be stored indefinitely unless the swan-shaped necks were broken or the flasks were tipped and liquid came in contact with the air in the neck of the flask (Fig. 3.4).

The fact that air could enter the flasks and yet no growth was seen in the solutions contained in them disproved the theory of spontaneous generation. The swan-necked flask is now called as Pasteur flask. Some of such flasks used for original work that were sealed later on, still show no sign of contamination (Fig. 3.4). The work led to the fact that appearance of microorganisms in the nonliving nutrient materials like nutrient solutions was due to the microbes already present in the air or in the solutions. Thus, the controversy over spontaneous generation gave way to the **Theory of Biogenesis**, which says that living cells can arise only from pre-existing living cells.

Louis Pasteur was a chemist by training and a staunch patriot at heart, who always tried to solve the problems being faced by French manufacturers in their industrial processes. One such problem he tackled was of souring of alcohol produced from the beets. He compared samples from vats, both with good wine and sour wine under a microscope and observed budding cells of the fungus yeast in good wine and rod-shaped bacteria in sour wine. He thus,

Vats are containers used for fermentation of wine that were earlier made of high quality wood. These are now being replaced by stainless steel ones.

The wine that is suitable for consumption is good wine while sour wine is vinegar.

The carbohydrate in grapes or other fruits are converted to alcohol by the yeast (*Saccharomyces cerevisiae*). This alcohol is further converted to acetic acid if there is contamination of the wine by *Acetobacter* or *Gluconobacter*.

Attenuation is a procedure in which the pathogenicity of a given organism is reduced or abolished.

Virulence is the capacity of pathogen to cause disease. It is broadly defined in terms of the severity to the disease produced by the pathogen. Virulence may be made to vary by attenuation.

proved that these organisms determined the course of the **chemical process of fermentation**. Yeast cells led to the production of alcohol while rod-shaped bacteria produced lactic acid that made the wine sour.

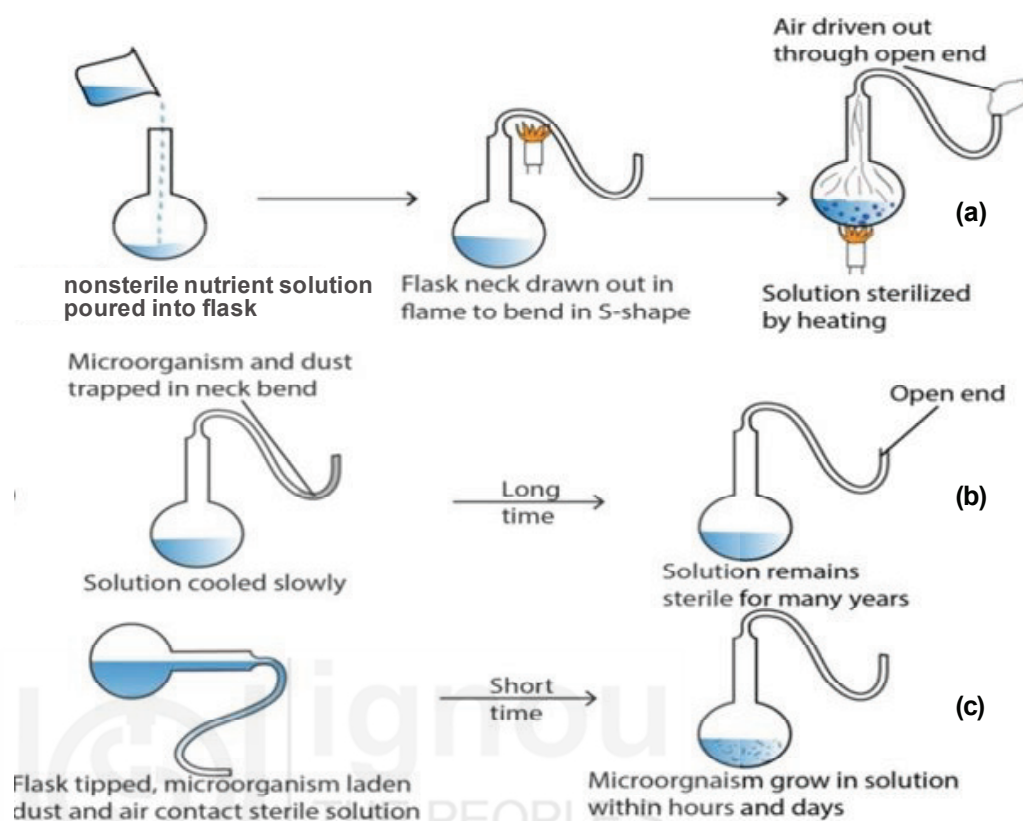


Fig. 3.4: The experiment of Pasteur that disproved the theory of spontaneous generation. (a) The long neck of the flask is bent into S-Shaped curve and the contents are sterilized (b) No microbial growth occurs if the flask remains upright. (c) On bending the flask, microorganisms trapped in the neck come in contact with the nutrient liquid and grow rapidly.

This discovery established that some microorganisms could carry out anaerobic metabolism. It also hinted that the nature of **microbial metabolism (aerobic or anaerobic transformation of organic matter)** decided the formation of an end product. In 1857 Pasteur showed that souring of milk was also the result of microbial action. In the year 1860 he recommended heating of wine and beer at moderate temperatures (a process now known as **pasteurization**) to control microbial contamination. He also sowed the seed of doubt among the minds of scientists, that bacteria could also be the cause of diseases. This was the initiation of the **concept of Germ Theory of Disease** that emphasised the role of microorganisms in the development of infectious diseases.

The other outstanding contribution of Pasteur was the development of vaccine by using attenuated culture of virulent bacteria. Incubating these bacterial cultures for long intervals between transfers produced attenuated cultures. He also developed an attenuated anthrax vaccine by treating cultures with potassium dichromate and by incubating the bacteria at 40°C to 43°C. For

developing the rabies vaccine, he injected the virus in the heterologous host, the rabbit. After the death of the infected rabbits, the brain and spinal cord were removed, dried and powdered to be used as a vaccine. Pasteur administered increasingly virulent doses of the attenuated virus thirteen times, over a period of 10 days, to a nine-year-old boy, Joseph Meister, who had been bitten by a rabid dog. The boy survived and the concept of vaccines got a strong foothold. It was in appreciation of his **development of vaccines** that people from all over the world contributed to the establishment of the Pasteur Institute in Paris.

Robert Koch

As early as the sixteenth century, it was thought that there was something that got transmitted from a diseased person to a healthy person, and that 'something' could make the healthy person sick. Even though it was thought that microorganisms were responsible for infectious diseases, there was no proof in favour of this belief. The work of Robert Koch (1843-1910), a German physician (Fig. 3.5), laid the foundation of the **Germ Theory of Disease**. Koch worked on the disease anthrax, which is caused by the bacterium *Bacillus anthracis* in cattle and in humans. He found that the blood of an animal dying of anthrax always showed bacteria, and if the blood from a diseased mouse was injected into a healthy mouse, it also suffered from anthrax. He then took blood from the second mouse and injected it into another healthy mouse. He repeated this for twenty times and was always successful in transmitting the disease and always detected bacilli in the blood of dying animals. His criteria for proving the role of microorganisms as causative agents of an infectious disease are known as **Koch's postulates** which are summarized below:

- A specific microorganism is present in every diseased animal but absent in a healthy animal.
- The suspected microorganism can be isolated from the host and grown in a pure culture. The same disease must result when the isolated microorganism is inoculated into a healthy but susceptible host.
- The same microorganism must be isolated from the diseased host and cultured again under laboratory conditions. It should be identical to the original microorganism.

Koch further cultivated bacteria outside the bodies of animals, in media such as beef serum and found that 'cultured' bacteria were as effective in causing the disease as bacteria from diseased animals. He emphasized the importance of **pure culture** and devised several ways of obtaining pure cultures. One well-known way is isolation of single colonies on solid media, where colonies can be grown from a single microbial cell or a spore. He initially used sterile surfaces of cut and boiled potatoes. But as all bacteria did not grow well on potato, he looked for other alternatives. He added gelatin to nutrient fluids to solidify but realized that many bacteria digested the gelatin. It also melted at temperatures above 28°C. Fannien Hesse, the wife of his assistant, Walther Hesse, suggested the use of **agar** as a solidifying agent in the medium. Agar melts at 100°C and solidifies at 40°C. Another assistant, Richard Petri developed the **Petri dish** for holding solid culture media under



Fig. 3.5: Robert Koch.

Culture is the growth of microorganisms on or in a medium. A medium (plural - media) is a mixture of nutritional substances. Mixed culture contains different kinds of microbial populations grown together.

Pure culture refers to a culture where all the cells are descendents of the same cell or spore of a microorganism, and thus, genetically identical.

Fannien Eilshemius Hesse, used agar as a solidifying agent to harden the jelly.

Agar as solidified agent was an instant success and till date it is essential in the laboratory work related to microorganisms.

aseptic conditions. In 1882, Koch isolated the bacillus (*Mycobacterium tuberculosis*) that causes tuberculosis. In those times tuberculosis was one of the largest killers of mankind. The etiology of tuberculosis was unknown and Koch's aim was to pinpoint the microbe responsible for this disease. In order to achieve this, he used all the techniques devised by him and his associates, such as microscopy, staining of tissues, pure culture isolation and animal inoculation. He stained the tissue samples of infected person with alkaline methylene blue and Bismarck brown. He observed bright blue, rod-shaped cells of *M. tuberculosis* in the infected tissues. He succeeded in growing the pure cultures of *M. tuberculosis* and clearly established the cause of tuberculosis by infecting guinea pigs with *M. tuberculosis* and again isolating the bacilli from diseased animals. Koch also prepared 'tuberculin', a substance that is being useful in the diagnosis of tuberculosis. Koch was awarded the Nobel Prize in 1905 for his work. He also visited the Veterinary Research station, Mukhteswar, Uttaranchal during British rule, where British used to breed horses.

Martinus Beijerinck

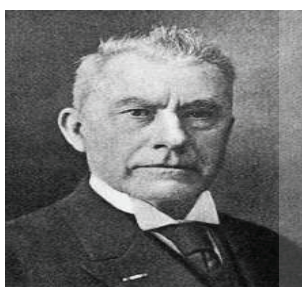


Fig. 3.6: Martinus Beijerinck.

The earliest developments in the field of microbiology that gained worldwide attention were in medical microbiology (as we learnt through the works of Louis Pasteur and Robert Koch). However, other scientists were quick to tap the potential uses of microbes. One such area was ecology that examines the interactions of microorganisms with their biotic and abiotic surroundings. Martinus Beijerinck (1851-1931) (Fig. 3.6) studied symbiotic and non-symbiotic aerobic nitrogen fixation carried out by bacteria. He isolated the nitrogen fixing, free-living, aerobic bacterium *Azotobacter* and also the bacterium *Rhizobium* that forms nodules on the roots of leguminous plants and fixes nitrogen symbiotically. He showed how these bacteria could use atmospheric nitrogen for synthesizing their cell constituents. These microbes thus, help to maintain the supply of chemically combined nitrogen (such as NO_2 , N_2O , NO_3 , NH_3) on which rest of the life forms are dependent.

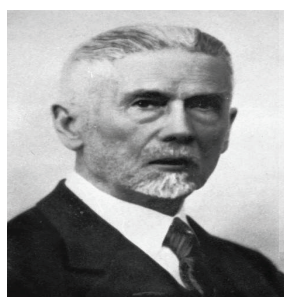


Fig. 3.7: Sergei Winogradsky.

Beijerinck also worked with sulphate-reducing bacteria. He found that the bad odour from the canals in his town in summer time was due to bacteria that could change sulphates to H_2S , which smelled like rotten eggs. Along with Russian microbiologist Sergei Winogradsky (1856-1953) (Fig. 3.7), Beijerinck developed the **enrichment culture technique**, which was a step towards the **development of selective culture media**. With the help of these techniques, we can isolate microorganisms with desirable qualities from a mixed population. For instance, if we need to isolate bacteria from soil that produce enzymes, which are stable at alkaline pH and high temperatures, the medium having pH in alkaline range is inoculated with the soil sample and incubated at high temperature, by doing so repeatedly, alkalophilic and thermophilic bacteria from soil sample will grow selectively. These bacteria produce thermostable enzymes, which work optimally at alkaline pH and high temperature.

The works of Winogradsky and Beijerinck were mainly responsible for establishing the **biogeochemical role of microbes** in cycling of matter such as in carbon, nitrogen and sulphur cycles. Microbes play an important role in

the turnover of matter on earth as many species of microorganisms can carry out chemical transformations which plants and animals are not able to perform.

Beijerinck was first to characterise **viruses**. Prior to this, virus was the general term used for poisons and infectious agents. Beijerinck used the term virus for the specific disease causing molecules that get incorporated into the cells and borrow the metabolic and replicative machinery for their use from the infected cells i.e., the host cell. Subsequent findings in the area established the branch **virology**.

Elie Metchnikoff

The Russian Zoologist Elie Metchnikoff (1845-1916) (Fig. 3.8) contributed very significantly to the field of **immunology**. It was believed that noncellular molecules in the blood were responsible for the immunity against the diseases. Metchnikoff in 1880s proved that there were certain cells in the body that were able to ingest the microbes. He called such cells as **phagocytes**, which means cell eating. Identifying the role of cellular phagocytes in defense of the body against microbial infections was first step in the understanding of immunity. In 1908 Metchnikoff was awarded Nobel Prize for his pioneering work on phagocytosis and immunology.



Fig. 3.8: Elie Metchnikoff.

SAQ 1

- a) Write true (**T**) for correct and false (**F**) for incorrect statements in the space provided against each.
- i) Anton van Leeuwenhoek called the motile, microscopic forms that he observed, as animalcules. []
 - ii) Louis Pasteur observed yeast cells in good wine. []
 - iii) Pasteur developed a vaccine for pneumonia. []
 - iv) Robert Koch founded the germ theory of disease. []
 - v) Winogradsky worked on the bacterium *Bacillus anthracis* to study symbiotic nitrogen fixation process. []
- b) i) What is pasteurization?
- ii) How did Joseph Meister survive after being bitten by a rabid dog?

3.2.2 Transition into 20th Century

The works of Pasteur and Koch spearheaded many advances in the field of microbiology eventually establishing it as a subject of science. There was a shift towards two separate directions: basic and applied Microbiology. On the applied side, the focus shifted towards the discovery of compounds that could fight pathogens and help in controlling infections. Achievements of various contributors helped towards the establishment of different fields in microbiology such as chemotherapy, virology, immunology and microbial genetics, that are prolific research areas today. In this subsection, we will study the works of some of the early contributors in these areas.

Joseph Lister



Fig. 3.9: Joseph Lister.

Surgery was greatly benefited by the introduction of anesthesia in 1840 and the speed of operations was no longer of prime importance. However, post surgical infections remained a serious concern. Joseph Lister (1827-1912) (Fig. 3.9), a British scientist thought that sepsis could also be the result of microbial infections of the exposed tissues. He prevented microbes from infecting the wounds with the help of an antiseptic, carbolic acid. He soaked bandages in carbolic acid that was used as an antiseptic, to dress the wounds on one set of patients while he did not use any antiseptic on another set of patients. The patients who had received antiseptic treatment recovered soon, while those who did not get any antiseptic treatment, showed post-surgical infections. He used carbolic acid air spray in addition to its direct application and also used heat-sterilized instruments for surgery. This approach was extremely successful and the incidence of post surgical infections went down remarkably ever since. This was indirect evidence establishing the role of microorganisms in the diseases. Lister is credited with first **aseptic technique** and was awarded *Order of Merit* for his work on the prevention of spread of infection about 37 years after he introduced the use of carbolic acid.

Paul Ehrlich



Fig. 3.10: Paul Ehrlich.

Paul Ehrlich (1854-1915) (Fig. 3.10) was born in Breslau (now in Poland) and became a medical doctor there. He was interested in chemical dyes that react specifically with organs, tissues and cells. He began testing them for therapeutic properties. His aim was to find a magic bullet that is, a chemical that would specifically kill pathogens and leave the host cells intact. His findings that certain dyes stained only the microorganisms and not the animal cells, suggested that some chemicals might selectively kill the microbes and not the host cells. From 1880 to 1896 he worked in Koch's laboratory and later became the founder director of an institute, which he dedicated to the synthesis of chemical compounds. He synthesised hundreds of such compounds and in 1909 he synthesised his 606th compound, dihydroxy-diamino arsenobenzene dihydrochloride, which he called salvarsan. Both salvarsan and neosalvarsan, which was his 914th compound, were very effective against syphilis. His 418th compound (arsenophenyl glycine) was found to be effective against sleeping sickness. Ehrlich coined the term chemotherapy. He shared the Nobel Prize with Elie Metchnikoff in 1908 for the work on antibody formation against toxins.

Alexander Fleming



Fig. 3.11: Alexander Fleming.

Alexander Fleming (1881-1955) (Fig. 3.11) was a Scottish physician in London's St. Mary's hospital. He was working with *Staphylococcus aureus* and observed that one of the plates was contaminated with a mould. Around the mould was a zone of clearance where the *Staphylococcus* was unable to grow. He identified the mould as *Penicillium notatum*. He cultivated *P. notatum* in broth and added a drop of this broth to a culture of *Staphylococcus*. The culture of *Staphylococcus* disintegrated. He also found that other Gram-positive bacteria were also susceptible to the mould.

Fleming inferred that *Penicillium* was producing some substance that inhibited the growth of bacteria. He named this substance as **penicillin** but was unable to purify it. The report on his work attracted attention of the scientific world ten years later when H.W. Florey and E.Chain of Oxford University came across his work, purified penicillin and conducted chemical trials that led to the discovery of the 'miracle drug'. Florey, Chain and Fleming received the Nobel Prize in 1945.

Selman Waksman

Selman Waksman was a soil microbiologist, who was born in Ukraine and moved to United States in 1910 (Fig. 3.12). He along with his co-workers at Rutgers University in New Jersey showed that various bacteria of the actinomycetes group produced antibacterial substances. He coined the term **antibiotic** in 1941 to describe actinomycin and other similar products which he had isolated. He discovered streptomycin, which was produced by the bacterium *Streptomyces griseus* in 1943. This proved to be another wonder drug because unlike penicillin, antibiotics obtained from actinomycetes are broad-spectrum drugs. Streptomycin led to major breakthrough in the treatment of tuberculosis. In the same decade neomycin, chloramphenicol and chlortetracycline were also isolated by Waksman and others.



Fig. 3.12: Selman Waksman.

SAQ 2

- i) Match the items given in column I with those in column II.

Column I

- a) Robert Koch
- b) Fannie Hesse
- c) Beijerinck
- d) Paul Ehrlich
- e) Joseph Lister
- f) Alexander Fleming

Column II

- i) *Azotobacter*
- ii) Antiseptic
- iii) Salvarsan
- iv) Pure culture
- v) Penicillin
- vi) Agar

- ii) Write true (T) for correct and false (F) for incorrect statements in the space provided against each

- a) Microbial world was discovered by Anton van Leeuwenhoek. []
- b) The vaccine from attenuated culture of virulent bacteria was developed by Louis Pasteur. []
- c) The foundation of germ theory of disease was laid down by Koch. []
- d) According to Koch's postulate a specific microorganism is present in every diseased animal and healthy animal. []

3.3 PROKARYOTIC CELL AND EUKARYOTIC CELL

The cell is the basic structural unit of all living organisms. This is a self contained unit separated from its surrounding by cytoplasmic membrane that serves as its limiting boundary. It contains hereditary material and various organelles. The cell, whether bacterial, archaeal or eukaryotic uses ATP, for energy for various kinds of work. The organizational patterns among living cells of prokaryotes i.e., bacteria and archaea, are different from eukaryotic organisms such as protists, fungi, plants and animals.

Prokaryotic cells are smaller than eukaryotic cells and simpler in structure (Fig. 3.13). The cell is bound by a plasma membrane and interior of the cell is filled with a thick, jelly like fluid called cytosol in which all the cellular components are suspended. The cell contains tiny structures called ribosomes which make proteins. Most prokaryotes have fairly rigid, chemically complex cell wall which protects the cell and helps to maintain the shape. Thus a bacterial cell has a complex cell envelope that encloses cell protoplasm and its DNA which is coiled into region called the nucleoid (nucleus like), but no membrane surrounds the DNA. Prokaryotic bacteria can be photosynthetic and known as cyanobacteria which you will study in detail in Block 2 (Algae).

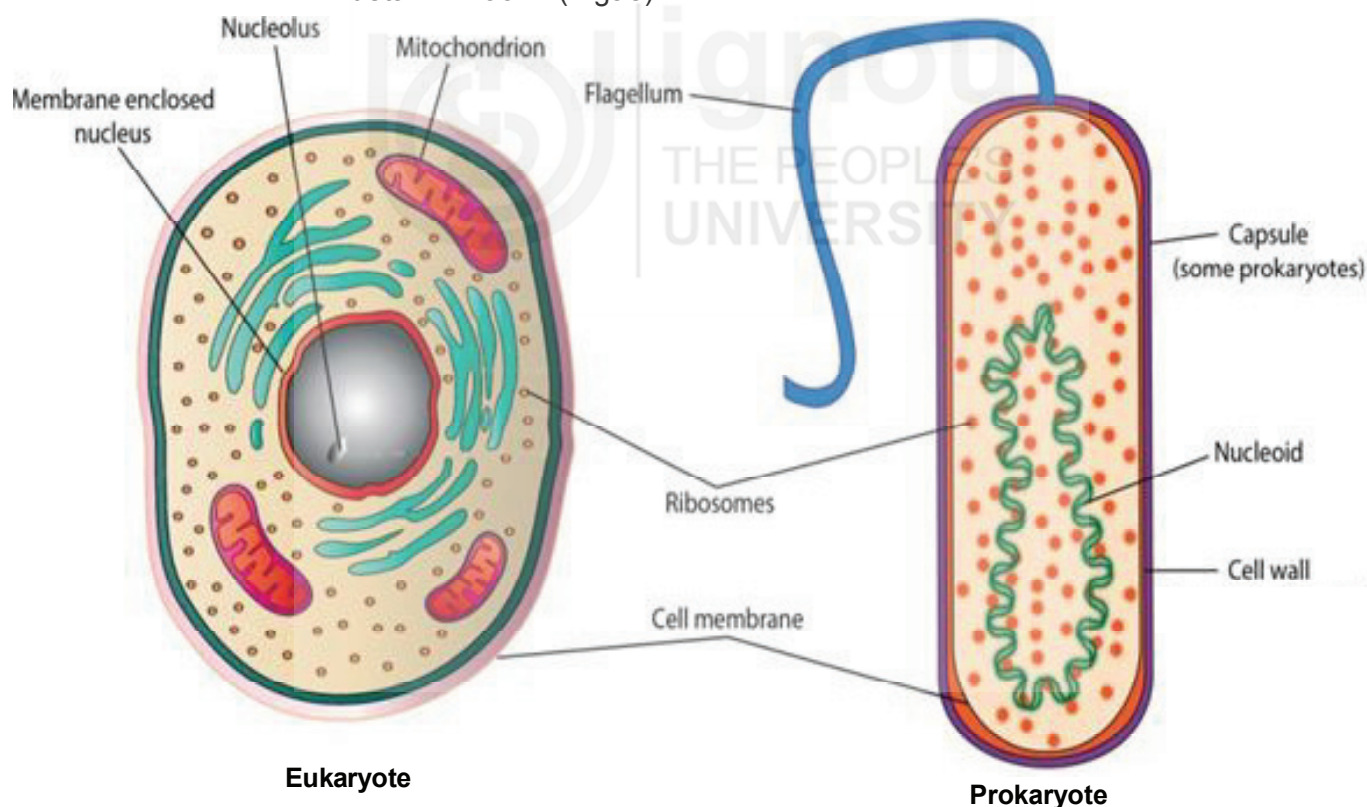


Fig. 3.13: Eukaryotic and Prokaryotic cell.

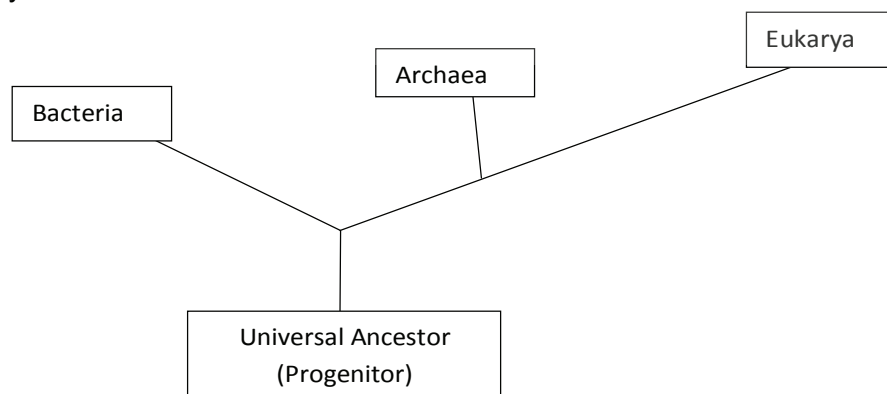
In contrast, the eukaryotic cell does have a well defined nucleus. In fact all eukaryotic cells at some time of their life cycle have a nucleus but bacterial and archaeal cells never possess a true nucleus. Eukaryotic means “true nucleus” while prokaryotic means “before the nucleus”.

Main differences between bacterial, archaeal and eukaryotic cells are given in Table 3.2.

Table 3.2: Comparison of Bacterial, Prokaryotic, Archaeal and Eukaryotic Cells

Structure	Prokaryotic Bacterial Cell	Prokaryotic Archaeal Cell	Eukaryotic Cell
Cytoplasmic membrane	+	+	+
Nucleus containing a nuclear membrane surrounding DNA	-	-	+
DNA arranged as true chromosome with associated histone proteins	-	-	+
Ribosomes	70S	70S	80S
Cell wall	+	+	±
Cell wall constituents	peptidoglycan	pseudopeptidoglycan	If present then cellulosic
Internal organelles	-	-	+
Chloroplasts	-	-	±
Mitochondria	-	-	+
Endoplasmic reticulum	-	-	+
Golgi apparatus	-	-	+
Vacuoles	-	-	±
Flagella	+	+	+
9+2 microtubular arrangement	-	-	+

It is now revealed that there were three principal lines of evolution that formed three separate domains of cellular evolution: bacterial cells, archaeal cells and eukaryotic cells (Fig. 3.14). Archaea and bacteria are the only organisms with prokaryotic cell. They have evolved from a common progenitor cell along distinct lines of evolution. Archaea though are similar to bacteria in terms of structural organization but on a molecular basis are equally related to eukaryotes.

**Fig. 3.14: Phylogenetic Tree Showing Evolutionary Paths of Bacteria, Archaea and Eukaryotes.**

3.4 GENERAL CHARACTERISTICS

The unit of measurement in bacteriology is the micron or micrometer (μm).

1 micron (μ) or micrometer (μm) = a millionth part of a meter or a thousandth of a millimeter.

1 millimicron ($\text{m}\mu$) or nanometer (nm) = one thousandth of a micron or one millionth of a millimeter.

1 Angstrom unit ($\text{\AA}$) = one tenth of a nanometer.

You know the difference between a bacterial and eukaryotic cell. Now let us study some morphological characteristics of the bacterial cell such as: size, shape and their cell arrangement etc.

3.4.1 Size

Bacteria are very small in size which varies from $0.2\ \mu\text{m}$ to $2.0\ \mu\text{m}$. Cocci measure somewhere between 0.5 to $3.0\ \mu\text{m}$; bacilli range from 0.2 to $2.0\ \mu\text{m}$ diameter and 0.5 to $20.0\ \mu\text{m}$ in length. Vibrios and spirilla vary from 0.2 to $2.0\ \mu\text{m}$ in diameter and 0.5 to $100\ \mu\text{m}$ in length. Spirochaetes range from $0.1\ \mu\text{m}$ to $3.0\ \mu\text{m}$ in diameter and from 0.5 to $250\ \mu\text{m}$ in length.

By naked eye we can see a body of upto $200\ \mu\text{m}$ diameter while bacteria of medical importance generally measure 0.2 - $1.5\ \mu\text{m}$ in diameter and about 3 - $5\ \mu\text{m}$ in length.

3.4.2 Shape of Bacteria

Bacteria are classified into several types depending on their shape, (Fig. 3.15):

- **Coccus:** (Cocci- Pl.) (from kokkos meaning berry) are spherical, or nearly spherical.
- **Bacillus:** (Bacilli- Pl.) (from baculus meaning rod) are relatively straight, rod shaped (cylindrical) cells. In some of the bacilli, the length of the cells may be equal to width. Such bacillary forms are known as coccobacilli and have to be carefully differentiated from cocci.
- **Vibrios:** Vibrios are curled or comma-shaped rods and derive the name from their characteristic vibratory motility.
- **Spirillum:** (Spirilla-Pl.) Spirillum are rigid spiral or helical forms.
- **Spirochetes:** Spirochetes (from *speira* meaning coil and *chaite* meaning hair) are flexuous spiral forms.
- **Mycoplasma:** Mycoplasma is cell wall deficient bacteria and hence do not possess a stable morphology. They occur as round or oval bodies and interlacing filaments.

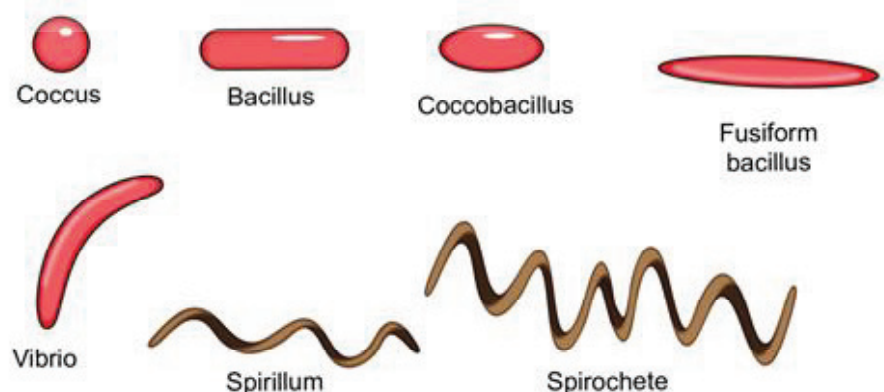


Fig. 3.15: Shapes of bacteria.

3.4.3 Arrangement of Bacterial Cells

Pathogenic bacteria species appear as spheres (cocci), rods (bacilli), and spirals. Bacteria sometimes show characteristic cellular arrangement or grouping (Fig. 3.16). The type of cellular arrangement is determined by the plane through which binary fission takes place and also by the tendency of the daughter cells to remain attached even after division.

Cocci Arrangement

- i) **Diplococci:** Cocci may be arranged in pairs (diplococci) when cocci divide and remain together.
- ii) **Long chains:** Long chains (genus *Streptococcus*, *Enterococcus*, and *Lactococcus*) when cells adhere after repeated divisions in one plane.
- iii) **Grape like clusters:** Grape like clusters (genus *Staphylococci*) when cocci divide in random planes.
- iv) **Tetrads:** Square groups of four cells (tetrads) when cocci divide in two planes as in members of the genus *Micrococcus*.
- v) **Cubical packets:** Cubical packets of eight cells (genus *Sarcina*) when cocci divide in three planes.

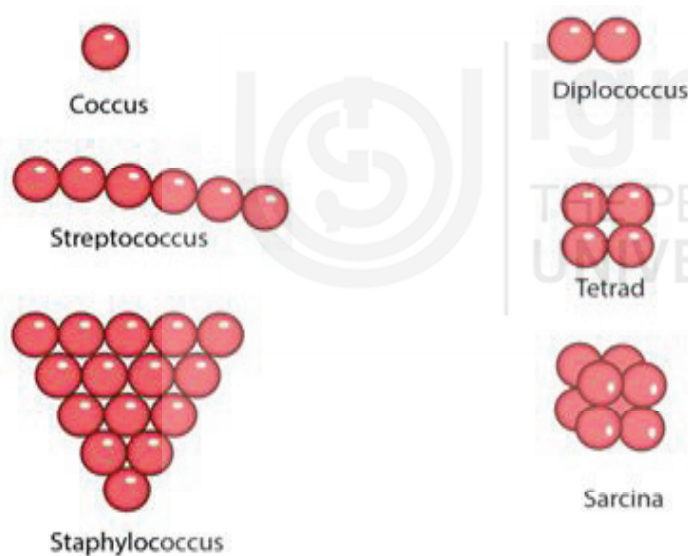


Fig. 3.16: Arrangement of bacterial cell.

Bacilli Arrangement

Bacilli split only across their short axis; therefore, the patterns formed by them are limited. The shape of the rod's end often varies between species and may be flat, rounded, cigar-shaped, or bifurcated. Some bacilli too may be arranged in chains (Streptobacilli). Others are arranged at various angles to each other, resembling the letter V, presenting a cuneiform or Chinese letter arrangement and is characteristic of *Corynebacterium diphtheria*.

SAQ 3

- a) Draw a bacterial cell and label it properly.
- b) Differentiate between a bacterial cell and an eukaryotic cell
- c) Show bacterial shape and bacterial arrangement only by giving diagrams.

3.5 CELL WALL AND ITS ADHERENTS

Bacterial cell wall is a strong, firm and flexible structure which gives shape to the bacteria which you have already studied. It is somewhat porous so it can help the flow of small molecules to and fro from the bacterial cell. In this section you will study in detail about the structure and function of cell wall and various types of adherents on bacterial cell (Fig.3.17).

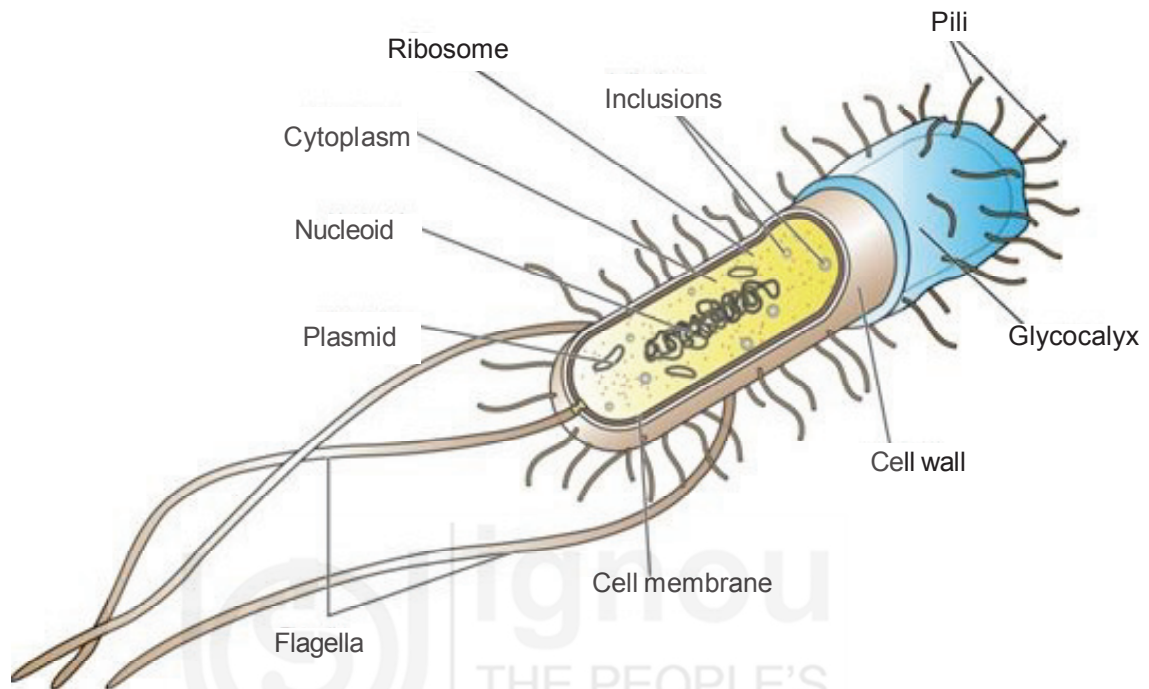


Fig. 3.17: Single bacterial cell.

3.5.1 Cell Wall

Most of the bacterial cells have a semi-rigid cell wall. It determines the shape of the bacterial cell. It is relatively porous so it can facilitate the flow of small molecules to and from the cytoplasmic membrane. The cell wall covers the entire cell surface and thus acts as an exoskeleton to protect the cell from injury and damage. Along with cytoplasmic cytoskeleton, it helps to maintain the shape of the cells and reinforce the cell envelope against the high intracellular water pressure pushing against the cell membrane. In the absence of such a cell wall bacterial cell would rupture or undergo osmolysis.

The cell walls of bacteria and cyanobacteria differ from plants in being made up of mucopeptide (=peptidoglycan) and not cellulose. This difference provides a site where bacterial pathogens can be attacked by antibiotics without damaging the diseased eukaryotic plant or animal cells.

Mucopolysaccharide is a polymer made up of alternating units of NAG (N-acetyl glucosamine) and NAM (N-acetyl muramic acid) joined by β , 1-4 linkages. NAG and NAM are amino sugars. The mucopeptide chains are laterally linked by short chains of amino acids like lysine, which originate at the carboxyl group of the muramic acid molecules and are essential for cross-linking of the amino acid chains.

The cell walls of Gram +ve and Gram –ve bacteria differ in their chemical composition. The wall of the Gram +ve bacteria is homogeneous containing 85 per cent or more of mucopeptide and simple polysaccharides, like teichoic acids (*teichos* = wall) which are polymers of ribitol and glycerol phosphates. Teichoic acids serve as antigens and also regulate entry of ions. The cell wall of Gram –ve bacteria contains only 3 to 12 per cent mucopeptide, the rest being lipoprotein and lipopolysaccharide. The wall of Gram –ve bacteria can be only seen in electron microphotographs, which appears to be tripartite, i.e., three-layered. It is formed by an **inner** cytoplasmic membrane, **middle** periplasmic space containing mucopeptide and an **outer** membrane. The wall of Gram –ve bacteria functions like a sieve. The outer membrane contains proteins, called **porins**, which form aqueous channels through which small molecules can pass. The differences between cell walls of Gram +ve and Gram –ve bacteria are shown in Table 3.3.

Table 3.3: Differences in the cell walls of Gram +ve and Gram –ve bacteria.

Points	Gram positive	Gram negative
Appearance in electron micro-photographs	Homogeneous layer	3-layered
Chemical composition	Mucopptide forms major portion of the wall (85 per cent of the dry weight), rest being simple polysaccharides, like teichoic acids. Do not contain lipids	Mucopptide acid forms only 3-12 per cent of the total dry weight; major portion contributed by lipoprotein, lipopolysaccharide complex. Thus, their wall has high lipid content. No teichoic acids.
Rigidity	Much rigid due to presence of greater amount of mucopeptides	Less rigid due to plastic nature of lipoprotein and lipopolysaccharides
Outer membrane	Absent	Present
Periplasmic space	Absent	Present
Result of enzymic digestion	Protoplast	Sphaeroplast

Functions of the cell wall:

- to impart shape and rigidity to the cell.
- it supports the weak cytoplasmic membrane against the high internal osmotic pressure of the protoplasm (ranges from 5 and 25 atm).
- maintains the characteristic shape of the bacterium.
- it takes part in cell division.
- also functions in interaction (e.g. adhesion) with other bacteria and with mammalian cells.
- provide specific protein and carbohydrate receptors for the attachment of some bacterial viruses.

3.5.2 Surface Adherents

Many bacterial species secrete an adhering layer of polysaccharides, or a complex of polysaccharides and small proteins, called the **glycocalyx** (*glycol* = “sweet”; *calyx* = “coat”). The layer can be thick and covalently bound to the cell, in which case it is known as a **capsule**. A thinner, loosely attached layer is referred to as a **slime layer**. Colonies containing cells with a glycocalyx appear moist and glistening.

The glycocalyx serves as a buffer between the cell and the external environment. Because of its high water content, the glycocalyx can protect cells from desiccation. Another major role of the glycocalyx is to allow the cells to stick to surfaces. The glycocalyx of *Vibrio cholerae*, for example, permits the cells to attach to the intestinal wall of the host. *Streptococcus mutans*, an important agent of tooth decay, has a slime layer containing a mass of tangled fibers of a polysaccharide called **dextran**.

Flagella

A bacterium could be non motile or **atrichous** when flagellum is not present e.g. *Staphylococcus*, *Streptococcus*. Bacteria are termed motile when cells possess one or more flagella (Singular: flagellum). When flagellum (flagella) is attached to one or both ends then it is **polar** (e.g. *Vibrio*, *Pseudomonas*) or when distributed all over the cell surface it is known as **peritrichous** (e.g. *Escherichia coli*). The length of flagellum ranges from 10µm to 20µm.

And many times they are longer than the diameter of the cell. Each flagellum is made-up of a helical filament, hook and basal body anchored in the cell envelope (Fig.3.18). They are different from the flagella or cilia of eukaryotes in lacking the 9+2 structure. The flagella is a cylindrical, hollow strand made up of protein molecules called flagellin which are structurally similar to the proteins of hair and muscles. The basal body is anchored in the plasma membrane; the hook penetrates the wall and the filament is the part that you can see in the stained slides. The cell wall is necessary for the flagellar movement.

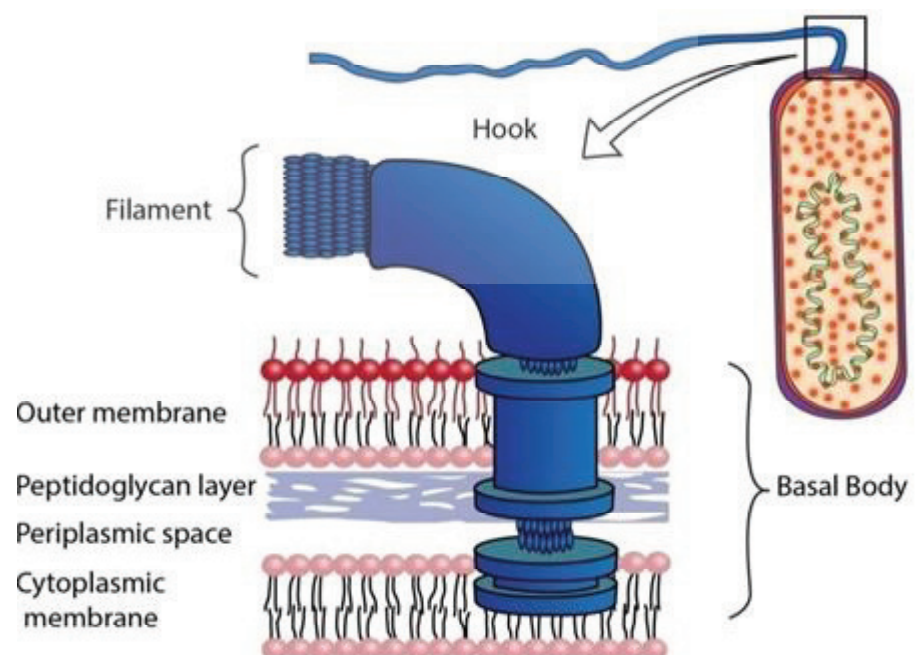


Fig. 3.18: Structure of Flagella.

The basal body is an assembly of more than 20 different proteins forming a central rod and set of enclosing rings. Gram-positive cells have a pair of rings embedded in the cell membrane and one ring in the cell wall, while gram negative cells have a pair of rings embedded in the cell membrane and another pair in the cell wall.

The basal body represents a powerful biological motor or rotary engine that generates a propeller-type rotation of the rigid filament. The energy for rotation comes from the diffusion of protons (hydrogen ions; H^+) into the cell through proteins associated with the basal body. This energy is sufficient to produce up to 1,500 rpm by the filament, driving the cell forward.

The flagella act like propellers to push the cell forward through the fluid, whether in the ocean water, broth in a culture tube, or the fluids in the human gut. When the flagellum or a bundle of flagella rotates anticlockwise, the cell moves straight ahead in what is called a “run”. These runs can last a few seconds and the cells can move up to 10 body lengths per second (the fastest human can run about 5-6 body lengths per second. So motility is tremendous for bacterial cells in a molasses-like environment in human gut). A reversal of flagellar rotation (clockwise rotation) causes the cell to “tumble” randomly for a second as the flagella become unbundled and uncoordinated. Tumbling results in a change of direction, so when the flagella again rotate anticlockwise, another run occurs sending the cell off in a new, random direction. When searching for nutrients (e.g., simple sugars, amino acids) in an environment, a flagellated bacterial cell can manipulate its runs and tumbles so that it moves towards the nutrient (attractant). This process is called **chemotaxis**.

One additional type of flagellar organisation is found in the spirochetes, the group of gram negative, coiled bacterial species. The cells are motile by flagella that extend from one or both poles of the cell but fold back along the cell body. Such **endoflagella** lie in an area called the “periplasm”. Motility results from the torsion generated on the cell by the normal rotation of the flagella, in other words, the cell swims in a corkscrew motion.

Pili

Pili (singular pilus; pilus means hair) or fimbriae (see Fig. 3.17) are short, straight, several in number, thin fibre like structures which protrude from the surface of most gram – negative bacterial species and recently have also been found in many gram – positive species. These rigid fibers which are composed of a protein called pilin act as scaffolding onto specific adhesive molecules, called adhesins are attached at the tip. The primary function of pili is to attach the bacterial cell to other objects. This involves the sticking of bacteria to various surfaces as well to one another. As human pathogen pili act as virulence factor because they allow pathogens to attach to tissue and /or to resist attack by phagocytic white blood cells.

Some bacterial species produce F pilus (**fertility pilus**) which is involved in bacterial mating and during this process they are found only on those cells that donate DNA to the other cell. F pili are longer than attachment pili and only

Similar types of motile behavior are seen in photosynthetic organisms moving toward light (“phototaxis”) or other cells moving toward oxygen gas (“aerotaxis”).

Microbiologists often use term “pili” interchangeably with “fimbriae”.

one or a few are produced on a cell. These conjugation pili contact with suitable recipient bacterial cell facilitating the transfer of DNA from donor to recipient bacterial cells. This process is called “conjugation”.

Pili are also helpful in locomotion by attaching themselves to a surface and then retracting and pulling the bacterial cell forward. This movement is called either “twitching motility” or “gliding motility”.

SAQ 4

- Differentiate between flagella and pili.
 - Describe the functioning of flagella.
-

3.6 PROTOPLASM

In the previous section you have studied about the cell wall of the bacterial cell. The cell wall encloses the protoplasm which consist of cytoplasm, ribosomes, mesosomes, granules, nucleoid (genome), and plasmids. The major constituents of the cytoplasm are water, carbohydrates, salts, amino acids and enzymes. Now we will describe them in detail, see Fig. 3.17.

- cell membrane
- cytoplasm
- genome
- plasmid
- ribosomes
- microcompartments
- gas vesicles
- magnetosomes

Each bacterium has certain unique nucleic acid sequences; this allows detection of the organism by nucleic acid based analyses.

3.6.1 Cell Membrane

The bacterial cell membrane is about 7nm in thickness, and made up of 32-40% of phospholipid (by weight) and ~60% of protein. The membrane is quite fluid in nature and with olive oil like consistency. This membrane keeps control on what gets into and out of the cell. The membrane provides a critical barrier between the cell interior and environment. The cell membrane is also involved in energy production including respiration and photosynthesis. A specialised form of energy production including nitrification and methane oxidation also occurs close to the membrane.

3.6.2 Cytoplasm

The cell membrane delimits cell cytoplasm, which consists of metabolites and nutrients in a solution. The major constituent of the cytoplasm is water, which acts as a solvent for carbohydrates, salts, amino acids, and enzymes. Within the protoplasm you will find the nucleoid (genome), plasmid, microcompartments, gas vesicles, magnetosomes, ribosomes, mesosomes, and granules.

3.6.3 Genome

The bacterial cells do not have a true nucleus or a nuclear membrane. Instead, the DNA is folded into a dense area of material called the **nucleoid**. It is represented as a sub compartment in the cytoplasm where the DNA aggregates and usually there is a single chromosome in a cell. The genome is not organised into chromosomes but represented by a circular, long DNA molecule attached to the cell membrane; but sometimes a closed loop of DNA and protein also exists. DNA is also known as chromosome and consists of a single circular molecule of double-stranded DNA. Though length of a cell may be 2 μm and the total length of the DNA molecule can be as long as 1200 μm . The DNA contains essential hereditary information for cell growth, metabolism and reproduction. DNA contains all the genetic material necessary for the metabolism, growth, and survival of the cell.

The photosynthetic bacteria possess, inner to protoplasmic membrane numerous **lamellae** (thylakoids) and vesicles, which constitute the photosynthetic apparatus.

Mesosomes are extensions of the plasma membrane which simultaneously initiate bacterial genome-replication and septum formation during cell division. In addition to the chromosome, many bacteria contain smaller accessory pieces of DNA known as **plasmids**.

3.6.4 Plasmids

Plasmids are stable extrachromosomal DNA molecules that exist as closed loops containing 5 to 100 genes and are a tenth the size of the chromosome. There can be one or more plasmids in a cell and have similar or different genes. Plasmids replicate independently of the chromosome and can be transferred between cells during recombination. Plasmids normally code for unique characteristics that can aid the cell in some specific circumstances but are not vital for the survival of the cell. They also represent important vectors in industrial technologies used in genetic engineering. Some plasmids possess genes for disease causing toxins or have genes for chemical antibiotic resistance.

3.6.5 Ribosomes

One of the universal structures in all cells is the ribosome. There are thousands of these nearly spherical particles in the cell cytoplasm, which gives it a granular appearance. In addition, there also are ribosomes loosely associated with the cell membrane. Their relative size is measured by how fast they settle when spun in a centrifuge and measured in Svedberg units (S). Bacterial and archaeal cells have 70S ribosomes composed of 30S and 50S subunits.

These ribosomes occur either free in cytoplasm or are associated with membrane. Free ribosomes make soluble proteins that are used in the cell, while the ribosomes associated with membrane produce proteins for the cell envelope and for secretion.

Some antibiotics, such as streptomycin and tetracycline, prevent bacterial and archaeal ribosomes from carrying out protein synthesis. In addition, the division of prokaryotes into the Bacteria and Archaea was based, in part, on sequencing the ribosomal gene from the small subunit (16 S RNA).

3.6.6 Microcompartments

Recently, some bacterial species have been found to contain microcompartments. The microcompartments appear to be unique to the bacteria and consist of a polyprotein shell 100 to 200 nm in diameter. These are localized areas where enzymes more directly interact with their substrates or potentially isolate harmful reaction products.

3.6.7 Gas Vesicles

In some aquatic and marine bacterial species gas vesicles are found which used to achieve buoyancy. These vacuoles are filled with air to decrease the density of the cell, which generates and regulates their buoyancy and position in the water column.

3.6.8 Magnetosomes

The magnetosome is an example of an actual membrane-bound compartment found in some aquatic bacterial species. These are membrane – bound vesicles containing Fe_3O_4 in magnetotactic bacteria. The organelle is used like a compass to navigate aquatic environments and locate preferred areas having little or no oxygen gas.

Magnetosomes have been found in meteorites from Mars which are associated with bacillus shaped structures and it is believed to represent bacterial life on Mars.

SAQ 5

Match the items given in Column I with those in Column II. Write your answers in the boxes provided.

Column I

- i) Cell wall
- ii) Slime layer
- iii) Cell membrane
- iv) Bacterial ribosome
- v) Plasmid

Column II

- a) RNA and protein
- b) circular molecules of DNA
- c) mucopeptide
- d) dextran
- e) 60% of protein

3.7 ECONOMIC IMPORTANCE OF BACTERIA

Microorganisms have a major impact on human life by their beneficial and harmful activities since time immemorial and the pathogenic microbes have been a cause of worry for the society. AIDS, Leprosy, Meningitis, etc. have caused large scale morbidity and death. Bacteria are also responsible for number of plant diseases which lead to enormous economic losses but they also have lots of useful activities. Besides, they also play a huge role in the ecological stability of the environment. Bacteria are the natural scavengers on the earth. They help in decaying of any dead or waste matter on the surface of the earth and in the soil.

Further, they degrade any chemical or biochemical fallen on the soil and thereby detoxify the valuable soil thus make it fit for growth of plants and animals and safe survival on the earth. Even the enormous amount of chemicals we put in our surroundings and other waste are degraded over a period of weeks in the soil. Otherwise the soil and water would be toxic and unable for further use. They provide macro and micro-nutrients required by primary producers in a marine food chain and sustain marine animals.

For decades bacteria have been used for generation of specific products with industrial and medical uses. In this section we will describe the uses of bacteria in various fields of human life. Bacteria play an important role in agriculture, food industry, pharmaceutical industry, leather industry and tobacco industry. Some of these are:

Role in Food Industry

Bacteria play important role in fermentation. So they are used for manufacturing butter, yogurt, cheese, cakes and beer. Alcoholic beverages are produced by fermentation of carbohydrate substrate (present either in grains or fruits) by yeast (*Saccharomyces cerevisiae*). However after fermentation, the aging of wine (done to impart flavour) is carried out by bacteria. This involves a secondary fermentation called malolactic acid fermentation. This is done to convert malic acid (highly acidic and sour in taste) into lactic acid and carbon dioxide. Lactic acid is moderately acidic and imparts good flavour to the wine. The bacteria involved are *Leuconostoc oenos*, *L. plantarum* etc. Bacteria are also used for production of wine/ vinegar which uses bacteria like *Acetobacter* and *Glucanacetobacter* which oxidize ethanol to acetic acid. For production of Scotch whisky, the bacterium *Lactobacillus delbrueckii* is used after initial fermentation.

Fermented Food

In Japan and China, a number of fermented meat and fish products are consumed. Sausages, hams, salami etc are fermented using *Pediococcus cerevisiae* and *Lactobacillus plantarum*.

Sauerkraut, a fermented product of cabbage is consumed worldwide. The fermenting bacteria include *Leuconostoc mesenteroides*, *Lactobacillus plantarum* and *Lactobacillus brevis*. Kimchii, a food made by cabbage and other vegetables is made by lactic acid bacteria and is common in Korea.

Pediococcus cerevisiae, *Leuconostoc mesenteroids*, *Enterococcus faecalis* and *Lactobacillus plantarum* are important in fermentation reactions leading to generation of specific flavours in pickles.

Probiotics are similar to curd consisting usually of live microorganisms which are taken orally to promote good health and used as health supplements.

Cheese

Commercial variety of cheeses is available and formation of this entire cheese making involves lactic acid fermentation of milk. *Lactococcus lactis* or

Lactococcus cremoris are used for fermenting the milk. The milk proteins on coagulation form curd. The common bacterial strains which are used for ripening include *Leuconostoc cremoris*, *Lactobacillus casei*, *Lactobacillus bulgaricus* etc.

Role in Pharmaceutical Industry

Many antibiotics are obtained from bacteria such as streptomycin, terramycin etc. Recombinant bacteria are used to synthesize insulin, growth factors and other pharmaceutical products.

Antibiotics

Antibiotics are compounds produced by microorganisms which either kill the target pathogen or inhibit its growth. One of the most common antibiotics produced by bacteria is streptomycin. Streptomycin is a broad spectrum antibiotic produced by *Streptomyces griseus*. This bacterium also produces antibiotics like kanamycin, neomycin and tobramycin.

Vaccines

Bacteria are used to produce vaccines by either separating their antigens or sometimes even as dead or alive one form that lacks pathogenic character. TB-vaccine is against tuberculosis (*Mycobacterium tuberculosis*) where dead bacteria of TB are administered to build up resistance to tuberculosis in humans. Once administered, these pathogens cannot cause disease. But the body will be able to produce antibodies to kill any infection of *Mycobacterium*. Inactivated whole agent bacterial vaccines have been formulated for *Vibrio cholerae*, *Pneumococcus*, *Bordetella* and *Salmonella*.

Hormones

Naturally occurring strains of bacteria produce a variety of compounds of medical significance-hormones. Bacterial genes can be manipulated to produce a much larger number of useful molecules. Genes coding for the synthesis of useful products can be transferred through a vector into a bacterial cell using the procedures of recombinant DNA technology.

Energy Production

The entire world is facing the problem of shortage of energy. Bacteria are used to produce biogas. Biogas is used as alternative resource of energy. Organic waste can be converted into usable forms of energy, fertilizers and other products by microorganisms through the process of biodegradation.

Mining Industry

Bacteria are also used for the extraction of metals from mines. Now recombinant bacteria have been prepared which selectively extract metals from raw ore. The microbes such as *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans* are involved in biomining to produce acid, which solubilises the metal from their ore bearing rocks.

Bioremediation

Bioremediation is the process of adjustment of environment to enhance the degradation or transformation of environmental pollutants by micro organisms.

Bacteria can degrade a variety of organic compounds thus they are used in waste processing and bioremediation. Bacteria are capable of digesting the hydrocarbons in petroleum therefore they are often used to clean up oil spills and are also used for the bioremediation of industrial toxic waste.

Biological Control

The commonly used bacteria for biological control are *Bacillus thuringiensis* (also called BT), It is a Gram-positive, soil dwelling bacterium. Subspecies of this bacterium are used as Lepidopteran-specific insecticides. Because of their specificity, these pesticides are regarded as environmentally friendly. It has little or no effect on humans, wildlife, pollinators and most other beneficial insects. Bacteria are used as pesticides for biological control. It is most effective and cheap method of controlling pests.

Biofertilizers

Biofertilizers contain live beneficial microorganisms and certain plant nutrients (amino acids, growth hormones, trace elements etc) which promote plant growth. They are ecofriendly, cost effective and renewable and commonly mixed with soil to help plant growth.

As you know that nitrogen is a vital nutrient required for growth of living organisms. The atmospheric nitrogen is fixed by bacteria and converted into usable form. Nitrogen fixing bacteria such as *Rhizobium*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Enterobacter*, *Klebsiella* live in root nodules of leguminous plant symbiotically and helps in fixing atmospheric nitrogen. Similarly, *Nitrosomonas* and *Nitrococcus* convert ammonium salt to nitrites. These bacteria are quite effective in increasing yields of crop plants when applied in appropriate concentration on seed or in soil.

Textile, Leather and Tobacco industry

Some bacteria like *Clostridium* are used for retting of hemp, jute and flax. These bacteria separate the fibers. These fibers are used for making clothes and ropes. Bacteria are used for preparing commercial leather. Bacteria are used for drying tobacco leaves. These leaves are used for preparing cigarettes.

You have studied all the beneficial activities of bacteria but they are also harmful to human life and plants, now we will describe the harmful effect of bacteria.

Bacterial Diseases

Bacteria as Plant Pathogens

Almost every kind of plant is susceptible to one or more kinds of bacterial disease. These bacterial diseases cause heavy economical losses in terms of agricultural and horticultural deterioration (Table 3.4). The symptoms of these plant diseases vary, but they are commonly manifested as spots of various sizes on the stems, leaves, flowers, or fruits. Other common and destructive diseases of plants, including blights, soft rots, and wilts, also are associated with bacteria. Fire blight, which destroys pears, apple trees, and related plants, is one of the most destructive among bacterial diseases. Most

of the bacterial diseases are caused by the group of rod-shaped bacteria known as pseudomonads.

Table 3.4: Some important diseases of plants

S.No.	Pathogen	Disease
1.	<i>Ralstonia solanacearum</i> (Aerobic, Gram negative rods; former <i>Pseudomonas gladiicola</i>)	Ring rot (Wilt) of potato
2.	<i>Burkholderia gladiicola</i> (Aerobic, Gram negative rods; former <i>Pseudomonas gladiicola</i>)	Onion rot
3.	<i>Xanthomonas axonopodis</i> pv. <i>citri</i> (Aerobic, Gram negative rods; former <i>Xanthomonas citri</i>)	Citrus canker
4.	<i>X. campestris</i> pv. <i>malvacearum</i>	Angular leaf spot of cotton
5.	<i>Rhizobium tumefaciens</i> (Aerobic, Gram negative rods; former <i>Agrobacterium tumefaciens</i>)	Crown gall of dicot plants
6.	<i>Pectobacterium carotovora</i> pv. <i>carotovora</i> (Aerobic, Gram negative rods; former <i>Erwinia carotovora</i>)	Soft rot of carrot
7.	<i>E. amylovora</i> pv. <i>amylovora</i> (facultative anaerobic, Gram negative rods)	Fire blight of pear and apple
8.	<i>Xylella fastidiosa</i> (Gram negative rods with rippled wall; formerly <i>Rickettsia</i> -like organism)	Pierce's disease of grape vine
9.	* <i>Candidatus liberobacter</i> (Gram negative very small rods)	Citrus greening
10.	<i>Clavibacter sepedonicum</i> (Non-spore forming, Gram positive rods of irregular shapes; former <i>Corynebacterium sepedonicum</i>)	Ring rot (Wilt) of potato
11.	<i>Rathayibacter tritici</i> (Non-spore forming, Gram positive rods of irregular shapes; former <i>Corynebacterium tritici</i>)	Yellow ear rot (tendu) of wheat
12.	<i>Streptomyces scabies</i> (Gram positive filamentous organism)	Scab of potato
13.	<i>Spiroplasma citri</i> (wall less pleomorphic bacteria; former mycoplasma like organism)	Citrus stubborn disease, corn stunt, and mulberry dwarf

* Since these organisms are not yet cultured (or cultured on complex media), though well-characterized by molecular methods, they are given the status of a *Candidatus* genus.

Bacteria cause many diseases in humans, including cholera, leprosy, tetanus, bacterial pneumonia, whooping cough, diphtheria and lyme disease (Table 3.5). Members of the genus *Streptococcus* are associated with scarlet fever, rheumatic fever, pneumonia, and other infections. Tuberculosis (TB), another bacterial disease, is still a leading cause of death in humans.

Table 3.5: Some bacterial diseases of humans

S. No.	Disease	Pathogen
1.	Actinomycoses	<i>Actinomyces Israeli</i>
2.	Anthrax	<i>Bacillus anthracis</i>
3.	Bacterial meningitis	<i>Haemophilus influenzae</i> , <i>Neisseria meningitis</i> , <i>Streptococcus pneumoniae</i> , <i>Listeria monocytogenes</i>
4.	Botulism	<i>Clostridium botulinum</i>
5.	Cholera	<i>Vibrio cholera vibrio</i>
6.	Conjunctivitis	<i>Haemophilus aegypticus</i>
7.	Dental caries	<i>Streptococcus mutans</i>
8.	Diphtheria	<i>Corynebacterium diphtheriae</i>
9.	Food poisoning	<i>Staphylococcus aureus</i> , <i>S. pyogenes</i> , <i>Clostridium perfringenes</i> , <i>C. botulinum</i>
10.	Gas gangrene	<i>C. perfringenes</i> and others
11.	Gonorrhoea	<i>Neisseria gonorrhoeae</i>
12.	Leprosy	<i>Mycobacterium leprae</i>
13.	Peptic ulcers	<i>Helicobacter pylori</i>
14.	Plague (black death)	<i>Yersinia pestis</i>
15.	Shigillosis (dysentery)	<i>Shigella</i> sp.
16.	Syphilis	<i>Treponema pallidum</i>
17.	Tetanus	<i>Clostridium tetani</i>
18.	Trachoma	<i>Chlamydia trachomatis</i>
19.	Tuberculosis	<i>Mycobacterium tuberculosis</i>
20.	Typhoid	<i>Salmonella typhi</i>
21.	Typhus (epidemic)	<i>Rickettsia prowazeki</i> , <i>R. typhi</i>
22.	Whooping cough	<i>Yersinia enterocolitica</i>

3.8 SUMMARY

- The milestone events in the history of microbiology which started in year 1676 to 2004. In 1676 Anton Leeuwenhoek observed of very small animalcules and discovered bacteria in 1684.
- Bacteria are prokaryotic microorganisms that do not contain true nucleus and are unicellular and do not exhibit true branching.
- Most bacteria have cell walls that consist of a network of polysaccharide molecules connected by polypeptide cross-links.
- A bacterial cell does not possess specialised compartments or a membrane-bounded nucleus, but it exhibits a nucleoid region where the bacterial DNA is located.
- Bacteria are classified based on the shape as cocci, bacilli, vibrio, and spirilla and they are classified as diplococci, streptococci, tetrads and cubical packets based on arrangements.
- Bacterial cell has cell wall, inner protoplasm and other components which include plasmids, ribosomes, microcompartments, gas vesicles, and magnetosomes.
- Bacteria have a noteworthy impact on human life because of their beneficial and harmful activities. Now it is possible to manipulate microbes for benefit of mankind by using the latest advances in biological sciences and technology. Bacteria plays major role in food industry, medicine, environmental management and agriculture.

3.9 TERMINAL QUESTIONS

1. Differentiate between the bacterial cell and archaeal cell.
2. List the functions of cell wall.
3. Differentiate between cell wall of Gram +ve and Gram –ve bacteria.
4. What are plasmids and what is their importance?
5. What are magnetosomes?
6. Discuss the characteristics of bacterial ribosomes.
7. Discuss economic importance of bacteria.

3.10 ANSWERS

Self-Assessment Questions

1. a) i) True; ii) True; iii) False; iv) True; v) False
b) i) Pasteurisation is the process of disinfecting liquids, especially dairy products by heating the liquid at mild temperatures.
ii) Joseph Meister had been inoculated 13 times with the attenuated virus of rabies. This led to the development of an immune response by his body system, i.e., his body produced antibodies against the virus, allowing him to survive the infection.

2. i) Robert Koch – Pure culture; Fannine Hesse – Agar; Beijerinck – *Azotobacter*; Paul Ehrlich – Salvarsan; Joseph Lister – Antiseptic; Alexander Fleming – Penicillin
 ii) a) T, b) T, c) T, d) F
3. a) See Section 3.3, Fig. 3.8
 b) See Section 3.3 Prokaryotic and Eukaryotic cell
 c) See Figs. 3.15 and 3.16
4. a) See section 3.5, Subsection 3.5.2
 b) See Subsection 3.5.2
5. (i) c, (ii) d, (iii) e, (iv) a, (v) b

Terminal Questions

1. See Section 3.3, Table 3.2
2. See Section 3.5, Function of cell wall
3. See Section 3.5, Table 3.3
4. See Section 3.5, Subsection 3.6.4
5. See Subsection 3.6.8 Magnetosomes
6. See Section 3.5, Subsection 3.6.5 Ribosomes
7. See Section 3.7, Economic importance of bacteria

Acknowledgement of Figures

- Fig. 3.1 : Anton van Leeuwenhoek
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- Fig. 3.3 : Louis Pasteur
<http://www.historylearningsite.co.uk/wp-content/uploads/2015/03/pasteur3.gif>
- Fig. 3.5 : Robert Koch http://a1.files.biography.com/image/upload/c_fit,cs_srgb,dpr_1.0,h_1200,q_80,w_1200/MTE5NTU2MzE2MjgzMzA3NTMx.jpg
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- Fig. 3.7 : Sergi Winogradsky
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- Fig. 3.8 : Elie Metchnikoff
<http://www.nature.com/nl/journal/v4/n1/images/nl0103-3-l1.jpg>
- Fig. 3.9 : Joseph Lister
[https://upload.wikimedia.org/wikipedia/commons/5/50/Joseph_Lister,_1st_Baron_Lister_\(1827_%E2%80%93_1912\)_surgeon_Wellcome_L0002075.jpg](https://upload.wikimedia.org/wikipedia/commons/5/50/Joseph_Lister,_1st_Baron_Lister_(1827_%E2%80%93_1912)_surgeon_Wellcome_L0002075.jpg)
- Fig. 3.10 : Paul Ehrlich
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- Fig. 3.11 : Alexander Fleming
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- Fig. 3.12 : Selman Waksman
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- Fig 3.15 : Shape of bacteria.
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<http://theamazingmedicine.blogspot.in/2013/07/microbiology-iv-domain-bacteria.html>

GLOSSARY

- Abiogenesis** : The concept which promotes the view that life arises spontaneously from non-living matter.
- Alkalophilic** : Bacteria that thrive at very high pH.
- Biofertilizer** : Fertilizers which contain live beneficial microbes which help increase nutrient content of soil. e.g., Nitrogen-fixing bacteria-rich soil; vermicpost
- Biofuel** : Alternate source of energy, generated by using plants or microbes, from readily available, cheap substrates like plant residues/municipal wastes etc; ethanol from fermentation of sugars/cellulose etc.
- Biogas** : Inflammable mixture of gases used for generating energy from wastes (such as municipal or industrial wastes, generally contain CH₄, CO₂, hydrogen etc.
- Biogenesis** : The concept that states that a life originates from a pre-existing life; opposite of spontaneous origin of life hypothesis.
- Chemolithotrophs** : Microbes that obtain energy through chemical oxidation and use inorganic compounds as source of electrons.
- Canker** : Plant diseases, or conditions of diseases, that interfere with translocation of water and minerals to crown of the plant.
- Fermentation** : A mode of energy-yielding metabolism which involves a sequence of oxidation-reduction reactions in which an organic substrate usually carbohydrates and the organic compounds derived from the substrate serve as the primary electron donor and the terminal electron acceptor, respectively.
- Fimbriae** : Structure involved in cell-to-cell contact between mating bacteria.
- Flagellum** : Flexible, relatively long appendage on cells used for locomotion.

Glycocalyx	:	Specialized bacterial structure with an attachment function composed of a mass of tangled fibers of polysaccharides or branching sugar molecules surrounding a cell or colony of cells.
Pasteurisation	:	Reduction in number of microbes by exposure to raised temperatures but not necessarily killing the microbes in sample; a kind of heat treatment that is lethal for causal agent of a large number of milk-transferable diseases; to prevent deterioration of milk.
Phagocytosis	:	The process in which particulate matter is ingested by a cell, involving the engulfment of that matter by the cell membrane.
Probiotics	:	Curd-like dairy products which contain microbes and are taken orally to promote good health and also used as health supplement.
Susceptible	:	The likelihood of an organism getting infected if exposed to the causative agent.
Thermophilic	:	Microbes showing optimal to higher growth at temperatures higher than 45°C.
Vaccination	:	The administration of a vaccine to stimulate the immune response to protect an individual from a pathogen or toxin.
Vaccine	:	A preparation of antigens used for vaccination.

BACTERIA : REPRODUCTION

Structure

4.1 Introduction	4.3 Genetic Recombination
Objectives	Conjugation
4.2 Asexual	Transduction
Binary Fission	Transformation
Endospore	4.4 Summary
Exospore	4.5 Terminal Questions
Cyst	4.6 Answers
	Glossary

4.1 INTRODUCTION

Bacteria can reproduce by asexual reproduction through fission, endospore, or cyst formation. Fission or binary fission is trait of bacteria and is only shared by some yeast. Sexual reproduction that occurs in bacteria is quite different from the one encountered in eukaryotes, but it does involve genetic recombination. The genetic transfer and recombination in bacteria could take place via mechanisms of conjugation, transduction and transformation.

Objectives

After studying this unit, you will be able to:

- ❖ list various types of reproduction in bacteria;
- ❖ differentiate between asexual and sexual reproduction in bacteria;
- ❖ appreciate the variety of genetic mechanisms that operate in bacteria; and
- ❖ compare and contrast between bacterial conjugation and transduction.

4.2 ASEXUAL REPRODUCTION

The genetic material in bacteria is organized as a continuous circular strand of DNA. This circle of DNA is localized in an area called the **nucleoid**. They lack a definite nucleus unlike eukaryotes. Bacterial cells possess one or more plasmids.

Plasmids are extrachromosomal circular DNA strands that can replicate independently, but they themselves are not responsible for reproduction.

4.2.1 Binary Fission

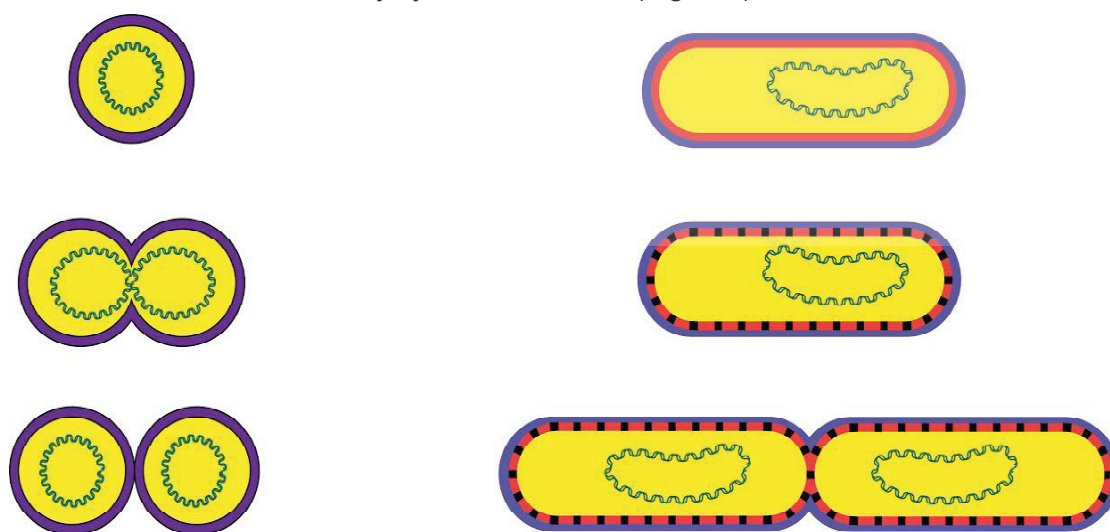
In bacterial cells asexual reproduction occurs through transverse binary fission. The mother cell develops a transverse cell wall and then divides into two daughter cells. This transverse wall is formed centripetally due to an ingrowth of the cell wall. One cell doubles in size and splits into half to produce two identical daughter cells. The time it takes, for a bacterial cell to grow and divide into two is called the **doubling time**. During favourable conditions, the doubling time of some bacteria can be as short as 20 minutes. Most bacterial species show a doubling time between one and four hours. When the daughter cells formed by binary fission do not separate, a chain of bacteria may be formed.

DNA Replication

The DNA or bacterial chromosome is circular and it replicates itself resulting in production of two circular chromosomes. The chromosome is duplicated at a site that traverses the whole chromosome and is called **replicating fork**. At this point two strands become four and in both daughter chromosomes one strand comes from the parent and other is newly formed (semi conservative). As the new double strands are formed, each origin point moves away from the cell wall attachment toward the opposite ends of the cell.

Cell Division

A peripheral ring of plasma membrane invaginates and grows centripetally to form a double membraned septum. The wall material is deposited between the two membranes of the septum. The two events i.e., septum formation and the cross wall synthesis occur concurrently. The cell division is followed by separation of the daughter cells as two unicellular bacteria by dissolution of the matrix between the newly synthesized walls (Fig. 4.1).



Streptococci

Escherichia coli

Fig. 4.1: Binary fission in bacteria.

During this process, all the cellular constituents increase in number in such a way that each daughter cell gets not only a complete chromosome but also sufficient copies of all other macromolecules and monomers including ions. In the spherical forms (cocci), divisions may occur in any plane, but in rod-shaped forms (bacilli), divisions occur perpendicular to the long axis of the cell. In cocci, divisions occur as follows (Fig. 4.2).

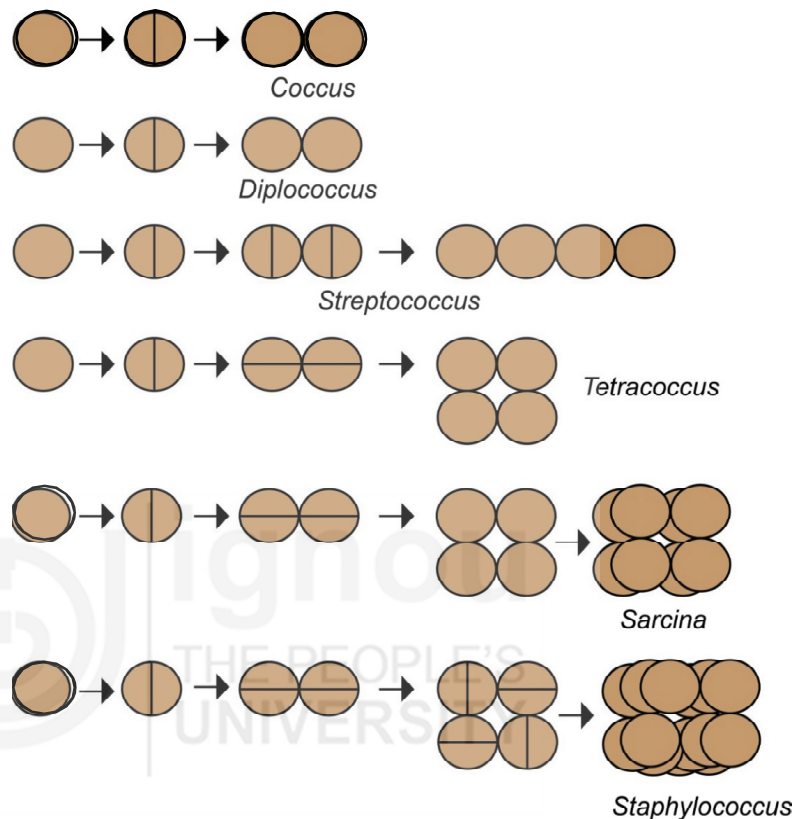


Fig. 4.2: Binary fission in cocci. Various forms of cocci are formed by the successive divisions in more than one plane.

Division in One Plane

1. If two divisions occur in the same plane, without separation of daughter cells, a pair of cells is formed; e.g., *Diplococcus*.
2. When more than two divisions occur in the same plane, a chain of cells results; e.g., *Streptococcus*.

Division in More Than One Plane

1. Two divisions at right angles result in groups of 4 cells; e.g., *Tetrads*.
2. Three divisions each at right angles results in cubical structure; e.g., *Sarcina*.
3. Many irregular divisions in all planes result in a cluster of cells e.g., *Staphylococcus* (Fig. 4.2).

4.2.2 Endospore

Bacteria can reproduce asexually through endospore formation (Fig. 4.3b). During this process an actively growing bacterium becomes dormant,

heat-resistant and non-growing cell. Endospore formation is an example of cellular differentiation. It never occurs when the bacteria are in exponential phase of growth. Such differentiation gets induced when essential nutrients get exhausted. For example, if glucose is added to the medium during endospore differentiation, the sporulation events stop by inhibiting the enzymes that help sporulation. Infact, growth and sporulation events are opposite processes. Sporulation is regulated at both transcription and translation level. The presence of different kinds of m-RNA's during growth and sporulation periods suggests that transcription has major regulatory role in endospore differentiation. The genera *Bacillus* and *Clostridium* and a few cocci and spirilla form endospores. A comparison of an actively growing bacterium cell and an endospore is provided in Table 4.1.

Table 4.1: Comparison of an active bacterium cell and an endospore cell

Item under comparison	An active bacterium	An endospore
Structure	Typical Gram (+)ve cell	Thick spore coat
Microscopic observation	Non-refractile	refractile
Calcium	low	high
Polysaccharides	high	absent
Crystalline protein	absent	high
Sulfur amino acid	low	high
Enzymatic activity	high	low
Metabolites	high	low
Macromolecule synthesis	high	low/absent
m-RNA	present	low/absent
Irradiation resistance	low	high
Resistance to chemicals	low	high
Stainability to dye	stainable	only with special methods
Lysogeny	sensitive	resistant

4.2.3 Exospore

Exospores are desiccation-resistant resting cells formed by certain bacteria, especially methanotrophs: type II, e.g., *Methylosinus* and *Methylobacterium*. They are produced by budding from one pole of the cell and, when mature, have a complex wall and a fibrous capsule. Such exospores are refractile and exhibit no metabolic activity. After budding, the mother cell is incapable of growth and division, even when provided with essential nutrients for growth.

4.2.4 Cyst

The cysts are also produced by certain Methanotrophs type II, e.g., *Methylocystis* and almost all the *Methylocystis* type I genera and *Azotobacteria* (Fig. 4.3 a).

Methanotrophic organisms are all Gram-(ve), strict aerobes, grow more rapidly under reduced oxygen tension. They are unicellular rods, vibrios, cocci or pyriform cells. They are often motile by means of polar flagella. They all form some types of resting structures. Most methanotrophs are obligate methophiles, (that grow on methane as substrate but, incapable of growth on compounds with C-C bonds).

During cyst formation, the entire vegetative cell enlarges, becomes spherical, and additional cell walls are deposited. Extensive accumulation of poly- β -hydroxyl butyrate may occur. Cysts are also desiccation resistant.

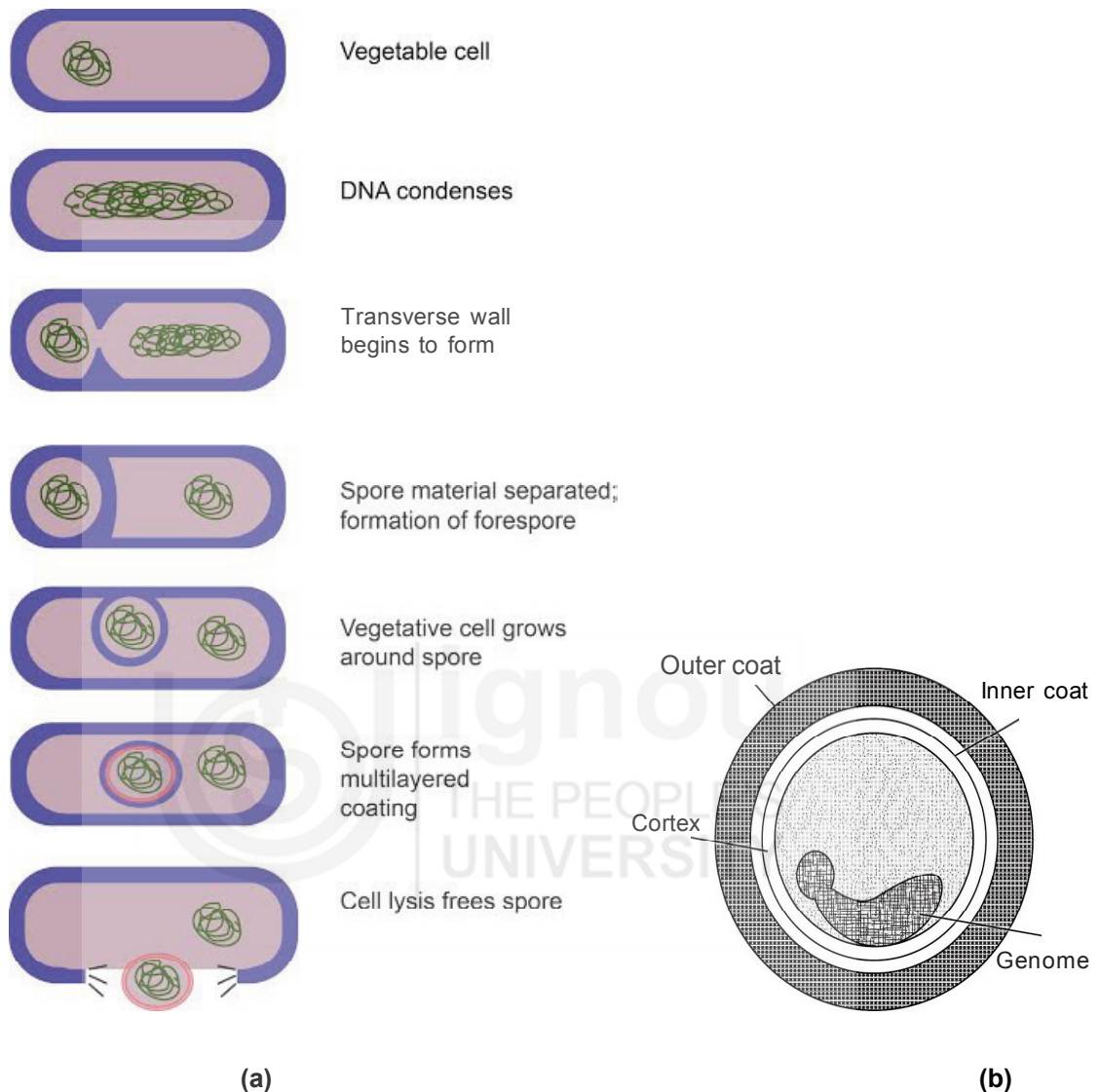


Fig. 4.3: Endospore and cyst in bacteria; (a) Cyst formation and germination and (b) An endospore.

SAQ 1

Choose the correct alternative from the ones provided in the paranthesis:

- During binary fission in a bacterial cell, the new cell wall formation is (centripetal /centrifugal).
- In *Staphylococcus*, the plane of all division is (any plane/at right angles).
- Addition of glucose to the culture medium (enhances/inhibits) differentiation of bacterial endospores.
- The bacterium *Azotobacter* produces (cyst/exospores).

4.3 GENETIC RECOMBINATION

The ability to exchange genes within a population is a nearly universal attribute of eukaryotes. It involves fusion of two haploid gametes to form a diploid zygote i.e., whole segments of genes of both the parents are exchanged. In prokaryotes, however, such event(s) almost never occurs. The molecular details of genetic exchange during sexual reproduction in prokaryotes reveal that only a small portion (segment) of the genome from one cell (the donor) is transferred to another cell (the recipient). It results in formation of an incomplete zygote (termed as merozygote). A merozygote contains the complete genetic compliment of the recipient (the endogenote), but, with very few exceptions, only a portion of the genetic compliment of the donor (the exogenote).

Moreover, in no known case is genetic exchange among prokaryotes an obligatory step in the organism's life cycle. Rather, it is an occasional process. The three mechanisms of genetic exchanges in bacteria are:

Conjugation

Transduction

Transformation

Conjugation

During conjugation, genetic exchange occurs between cells in direct contact with one another by a process, predominantly encoded by plasmid-borne genes. Usually, only the plasmid itself by this process is transferred from donor to recipient. Occasionally, chromosomal genes are also transferred (Fig. 4.4a).

Transduction

In the case of transduction, DNA is transferred from one prokaryote cell to another as a consequence of rare formation of an aberrant phage virion in which some or all its normal compliment of DNA is replaced by bacterial DNA (donor DNA). When such a phage virion attaches to and introduces this DNA into another bacterial cell (the recipient), genetic exchange is affected (Fig. 4.4b).

Transformation

In transformation, DNA is transferred from cells into the surrounding medium, and the recipient cells incorporate it into themselves from the solution (Fig. 4.4c).

4.3.1 Conjugation

The process by which the genetic material from one bacterium is transformed to and recombined with that of another when they are in contact with each other is called **conjugation**. In the process one cell acts as a donor and the other as a recipient, and recombinants could be scored in the recipient cells.

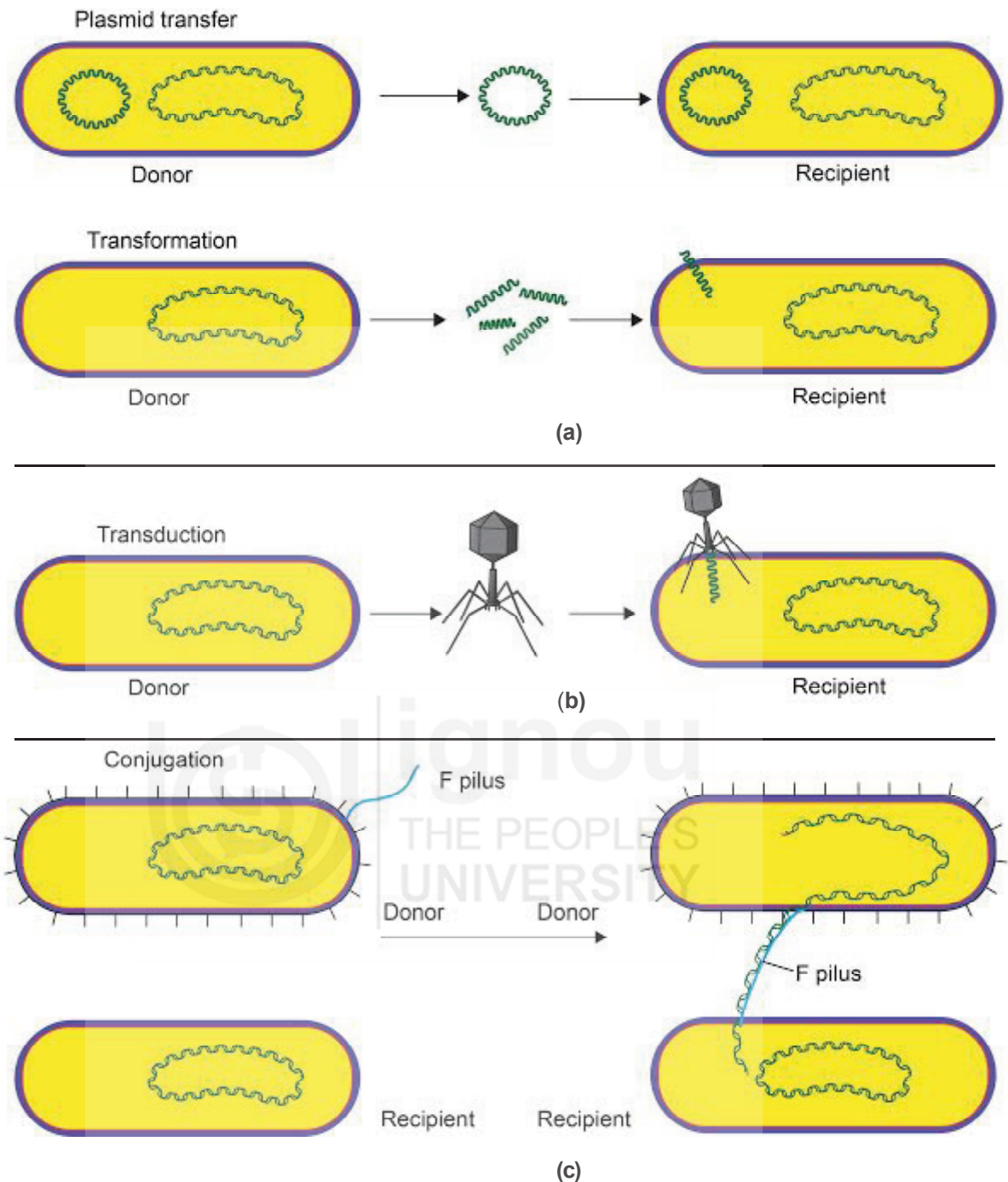


Fig. 4.4: Types of genetic combination in bacteria; (a) Conjugation; (b) Transduction; (c) Transformation.

Discovery

Joshua Lederberg and Edward Tatum (1946) obtained some mutant strains of the bacterium, *Escherichia coli* by exposing them to x-ray and ultraviolet irradiation. These mutants failed to grow on the minimal medium (a medium which supplies all the minimal nutrients except growth factors), and were labelled **auxotrophs**. In contrast, the organisms that grow on the minimal medium are known as **prototrophs**. The experiment of Lederberg and Tatum that led to the discovery of conjugation in bacteria is depicted in the Fig. 4.5.

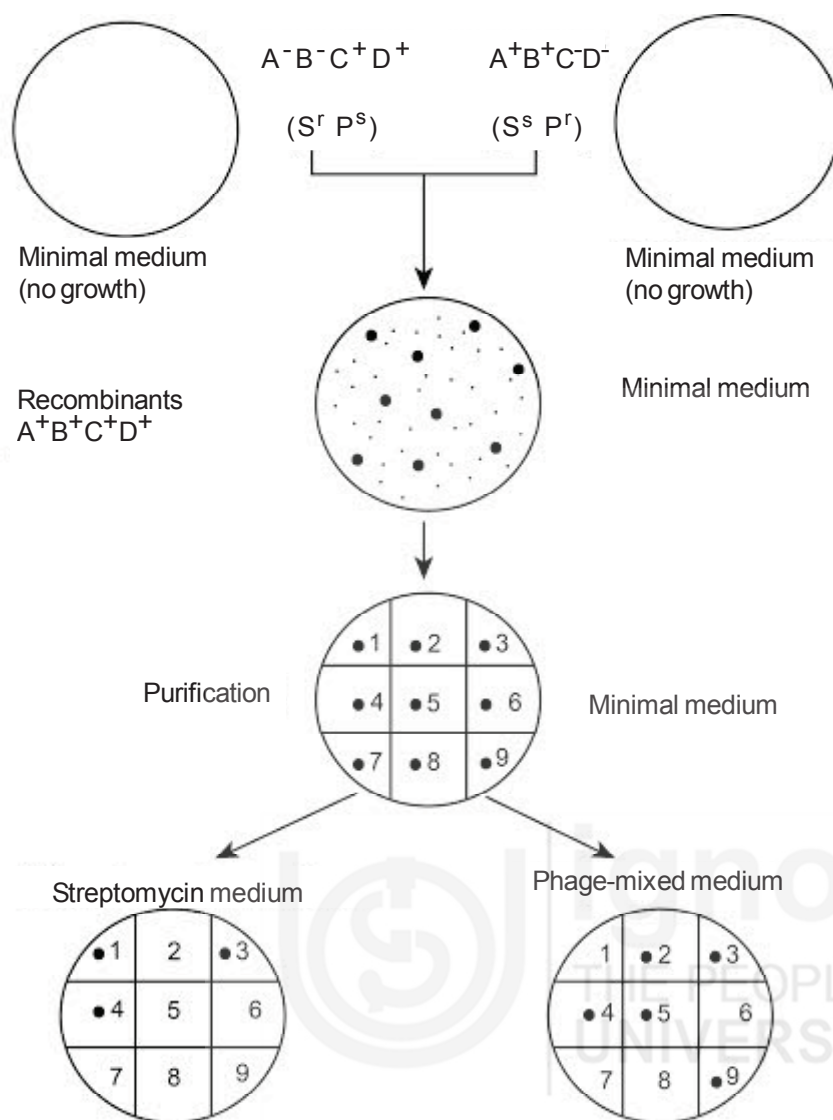


Fig. 4.5: The experiment of Lederberg and Tatum suggesting recombination in *E. coli*.

They chose one parental strain ($A^-B^-C^+D^+S^rP^s$) that requires factors A and B for growth and this strain was resistant to streptomycin and sensitive to phage (a virus), and another parental strain ($A^+B^+C^-D^-S^sP^r$) required growth factor C and D for growth while being sensitive to streptomycin and resistant to phage. Both the strains were unable to grow on minimal media (without any growth factor).

Interestingly, when both the strains were mixed and then spread on the same minimal medium, colonies appeared, i.e., they grew even in the absence of any growth factors A,B,C,D. How was it possible? It could be only because of the development of recombinant bacteria which must be $A^+B^+C^+D^+$. When these recombinants were tested for streptomycin and phage resistance, they also exhibited appearance of new markers (combinations). None of the parents were double sensitive/double resistant to streptomycin or phage. But, when plate was observed it showed that the colonies 3 and 4 were double resistant, S^rP^r , while colonies 6, 7 and 8 were double sensitive S^sP^s .

Mechanism of Conjugation

The independent experiments of Lederberg, Hayes and Wollman led to our understanding of the mechanism of such recombinations in *E. coli* strains. They conclusively proved that transformation was not involved but the physical contact between the two different kinds of strains was involved.

During conjugation two bacterial cells come together and the donor cell directly transfers plasmid DNA to the recipient cell. There was horizontal gene transfer between cells of various bacterial genera. The antibiotic resistance could be transferred between antibiotic-resistant and sensitive species.

It was demonstrated that in gram negative species like *E. coli* strain the donor cell requires an **F Factor**, (a transmissible plasmid having 100 genes). The F factor is now known to be a small circular DNA molecule (equal in size to the DNA of a bacteriophage) which is independent and lies free in the cytoplasm. During cell to cell contact the F factor containing donor cell is known as F^+ and produces a conjugation pilus that makes contact with the recipient F^- cell which does not have F factor. The pilus shortens itself to bring the two cells closer and then a conjugation bridge connects the cytoplasm of both the cells together.

After the formation of bridge the F^+ factor DNA replicates and passes through the bridge to the recipient cell. As soon as the HGT (Horizontal Gene Transfer) occurs, a new complementary strand to replace the transferred DNA strand (to the recipient cell) is formed in the donor cell. Such new strand formation is through DNA synthesis. After the complete transfer of plasmid DNA to the recipient cell, the two cell separate.

Now, in the recipient cell the new single- stranded DNA is a template for synthesis of complementary strand which forms the F factor, and this recipient cell itself becomes donor cell (F^+) and ready to conjugate with another F^- recipient cell. The transfer of F factor does not involve bacterial chromosomes (Fig. 4.6).

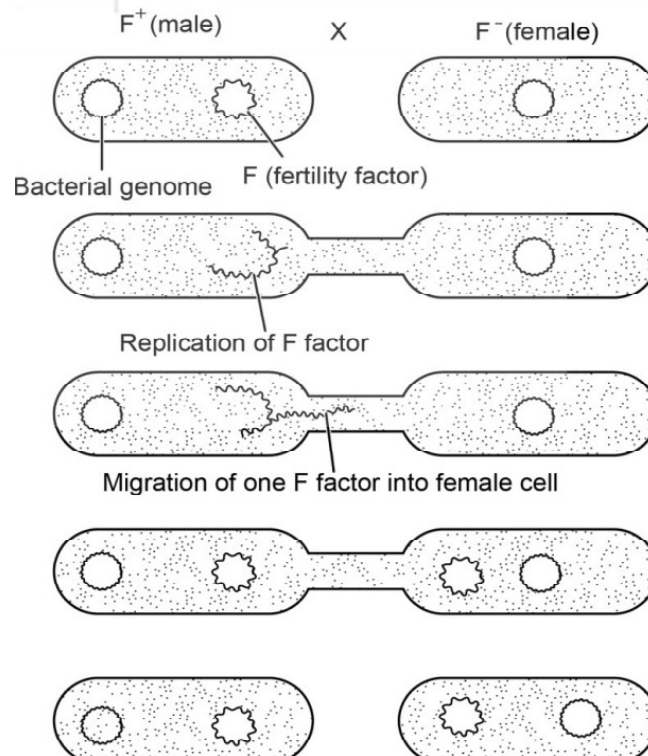


Fig. 4.6: Bacterial Conjugation – conjugation between F^+ and F^- bacteria resulting in conversion of F^- into F^+ .

Whenever a single cell of F^+ type is present in a population of F^- cells, within a matter of few hours all the F^- are converted to F^+ cells. During the process of bacterial conjugation, after the formation of a conjugation tube between F^+ and F^- cells, a copy of the plasmid DNA moves to F^- cell converting it to F^+ (Fig. 4.6). At the end of conjugation, every recipient cell becomes an F^+ cell thereby showing the transfer of F factor through the conjugation tube during each mating.

High Frequency of Recombination (Hfr) Strains

The F plasmid can exist in two states – it either lies free in the bacterial cytoplasm or can be integrated with the bacterial chromosomes. You will appreciate that when it is inserted with chromosomes; the F^+ male becomes a **Hfr** male and is called **High frequency of recombination (Hfr)** strain. Hfr cell can conjugate just like an F^+ cell. Once the cells are connected, the replicated single-stranded DNA starts to pass through the conjugation bridge into the recipient cell. Rarely the whole chromosome enters the recipient cell because this process is interrupted by the movements that break the bridge between cells before the transfer of complete chromosome. The transferred DNA replaces a similar piece of recipient's chromosomes and the recipient cell is known as a recombinant F^- cell because whole F factor did not cross into the recipient cell. The partial diploid as a result of DNA transfer is called Merozygote.

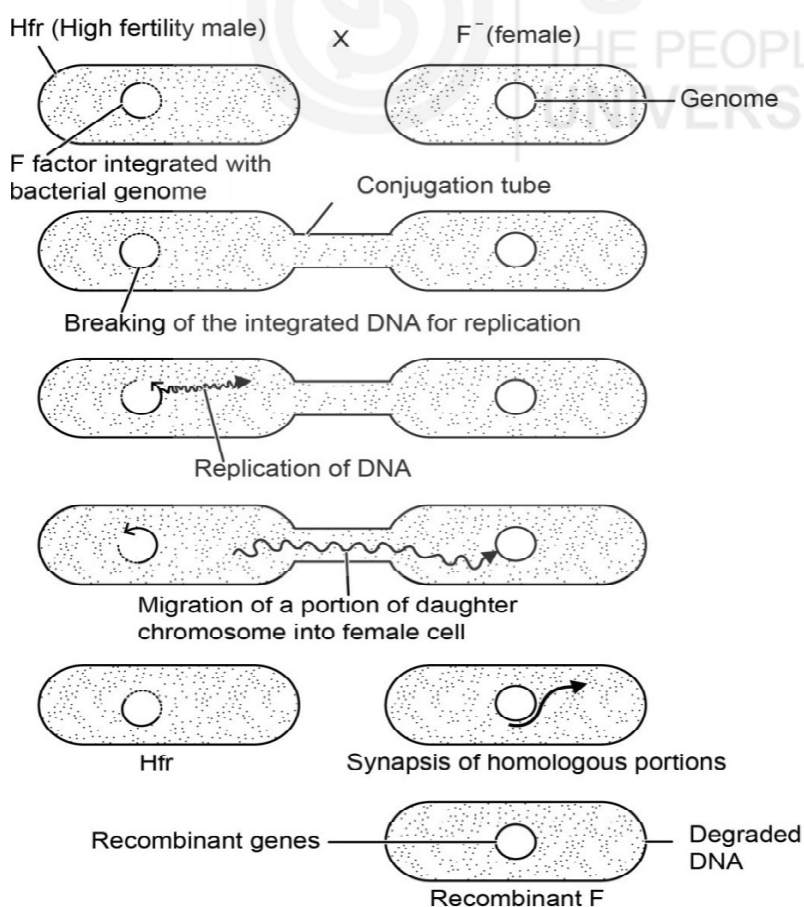


Fig. 4.7: Bacterial Conjugation – conversion of F^+ male into Hfr $^-$ high fertility male.

One question that would interest you is that why Hfr cells do not transfer the fertility factor? It was found that in various Hfr strains, the F plasmid DNA integrates into bacterial chromosome at different points and at the time of conjugation, the circular bacterial chromosome breaks open at the point where the F factor is integrated with it. In an $Hfr \times F^-$ mating (Fig. 4.7), it is the bacterial chromosome and the genes present in it which are the ones to be transferred first to the F^- cell. The F factor would be the last segment to be transferred. In practice, conjugation process does not last long enough to allow the entire chromosome to enter into an F^- cell. And this explains why the recipient cells namely F^- cells when mated with Hfr cells do not acquire the fertility factor.

4.3.2 Transduction

Joshua Lederberg and his student Norton Zinder in 1951 started looking for recombination in *Salmonella typhimurium* – the mouse typhoid bacterium. They used the same technique which Lederberg and Tatum had used with *E. coli*. They obtained nutritionally deficient mutants (auxotrophs) which failed to grow on the minimal medium. When a mixture of two mutants were plated together, recombinants appeared in some but not with other strains. When they analysed the cause they came across a new type of gene exchange, which involved mediation of bacteriophages. Lederberg and Zinder described this method of gene transfer as **Transduction** (Fig. 4.8).

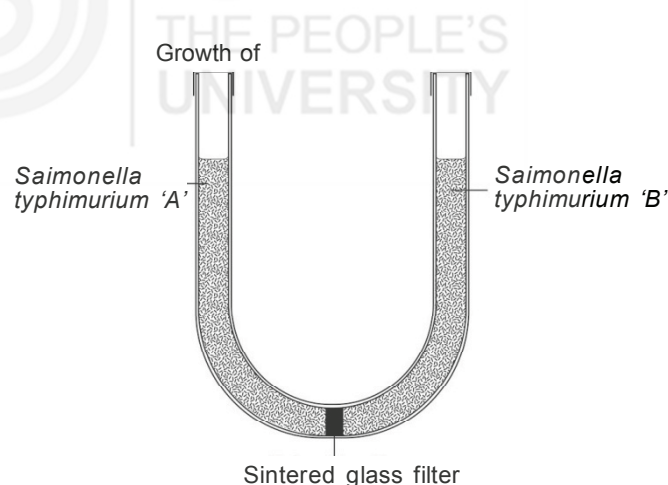


Fig. 4.8: The Transduction (U tube) experiment of Zinder and Lederberg.

A new type of gene exchange Transduction is third type of Horizontal gene Transfer ((HGT) but it requires a virus to carry a bacterial DNA fragment from donor to recipient cell. The virus participating in transduction is called a bacteriophage (bacteria eater) or simply phage. There are two types of Transduction.

Generalised Transduction

In this type of transduction, a random fragment of bacterial DNA gets unintentionally packed into a phage rather than with phage DNA. Such phage is called a defective particle. This defective particle attaches to a recipient cell and ejects the donor DNA into the cell where this DNA can recombine with a section of the recipient's DNA.

They conducted an experiment which ruled out this genetic transfer through conjugation. They took an U-tube which had a sintered glass filter with two-arms. No bacteria could pass through this filter. They filled two arms 'A' and 'B' with two different strains or auxotrophs of *Salmonella typhimurium*. In this set-up, while media in two arms could move freely across the filter, the two bacterial strains could not. Interestingly, recombinants appeared in the arm 'A' only. Addition of an enzyme DNAase, that degrades DNA could not prevent such recombination. Hence, mechanism of transformation was ruled out. They explained this observation by transduction (Fig. 4.8).

It was found that the strain 'A' was a lysogenic strain, which contained a **temperate** phage (Fig. 4.9).

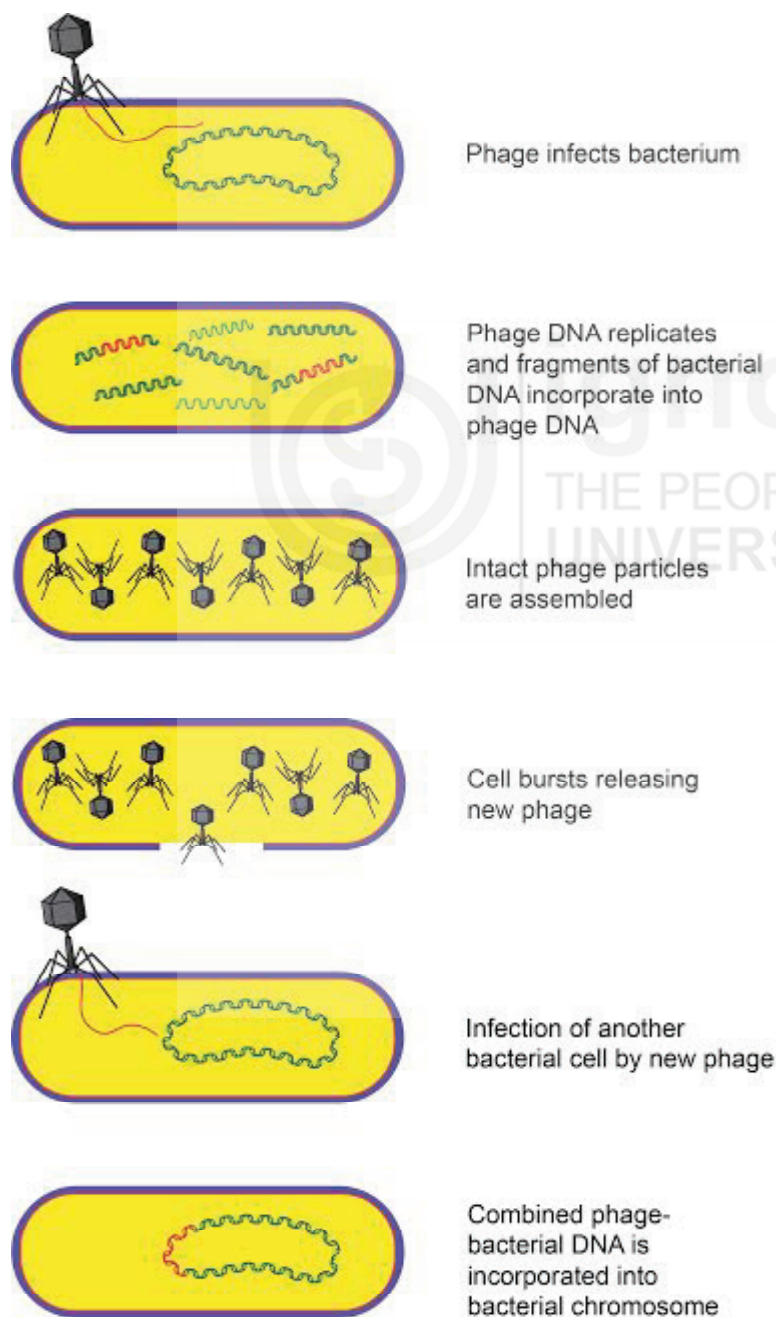


Fig. 4.9: Generalised transduction by temperate phage.

Some of the bacteria present in medium always get lysed, releasing these phages in the medium. These phages are small enough to permit them to pass through the filter while intact bacteria could not pass the arm 'A' to through the filter to the strain 'B' present in the other arm. The bacteria in arm 'B' were sensitive to these temperate phages. During the lytic cycle of strain 'B' of bacteria, some portion of bacterial DNA was incorporated into the DNA fragments of phage particles. Such changed phages were called **transducing phages**. Such phages could attack the bacterial strain 'A' in arm 'A'. Thus, the segments of DNA of strain 'B' were passed on to the DNA of bacterium present in arm 'A' even in absence of any physical contact of two bacterial strains. It resulted in genome of the recipient being modified (Fig. 4.9).

Specialised Transduction

In this type of transduction, only specific bacterial genes are transferred. Here the transferring agents are again defective phages because they are missing a few phage genes needed for virus replication (Fig. 4.10). Such a transducing phage can infect another cell and transfer its genes along with the any other bacterial genes which can integrate into recipient cell chromosomes. The recipient cell which has acquired genes from the original donor cell is now considered transduced.

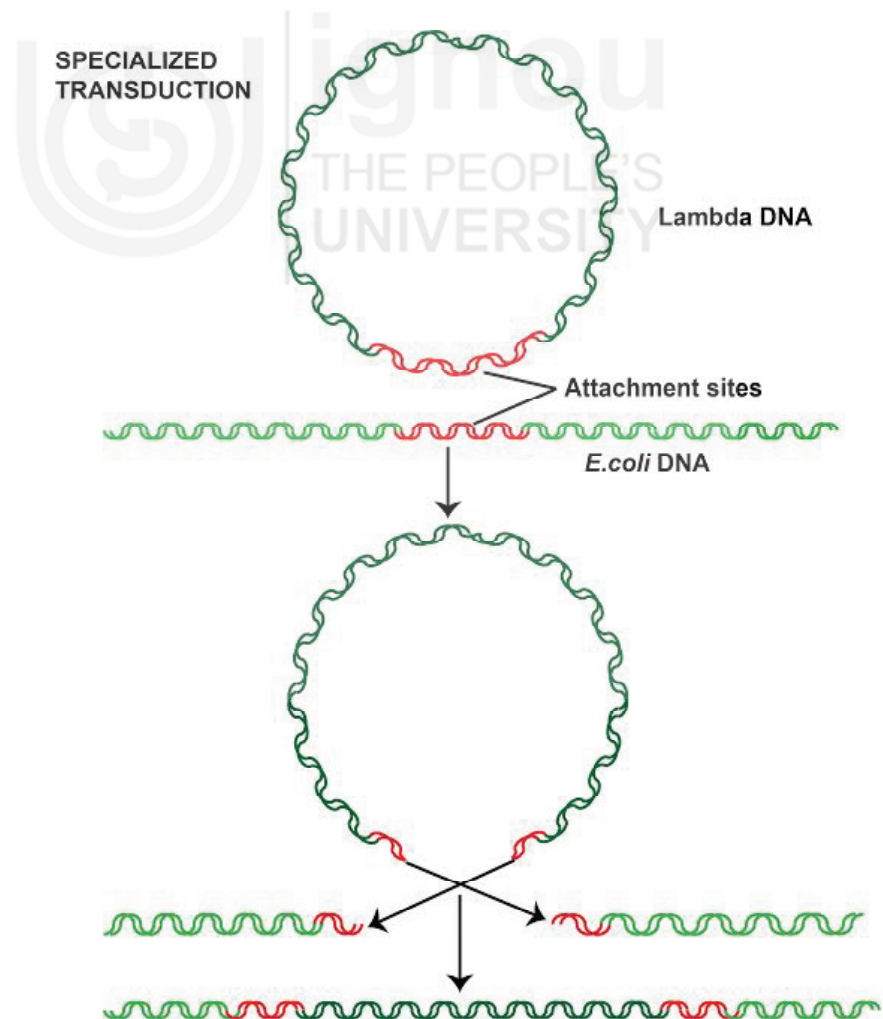


Fig. 4.10: Specialised Transduction.

Abortive Transduction

Sometimes the bacterial DNA brought by the phage, does not integrate with the genome of the recipient bacterial cell and instead, express itself independently. It does not replicate and passes on during binary fission to any one of the two daughter cells as it is neither integrated with the chromosomes nor can replicate itself.

4.3.3 Transformation

Transformation may be defined as a process of uptake of DNA fragments from the surrounding environment and its expression in the recipient cells. In other words, in transformation a free DNA molecule is transferred from a donor to a recipient bacterium. Many bacteria also take up DNA molecules from the surroundings. Generally transformation occurs naturally among gram negative and gram positive bacteria.

In 1928, the first demonstration of bacterial transformation was done with *Streptococcus pneumoniae* by Fred Griffith (Fig. 4.11). The cells of *S. pneumoniae* possess a gummy capsule of polysaccharides, which provides a glistening and smooth appearance to the colonies on a solid culture medium.

These cells were called 'S' cells. After prolonged cultivation on artificial medium, some cells lost the ability to form the capsule. These cells consisted

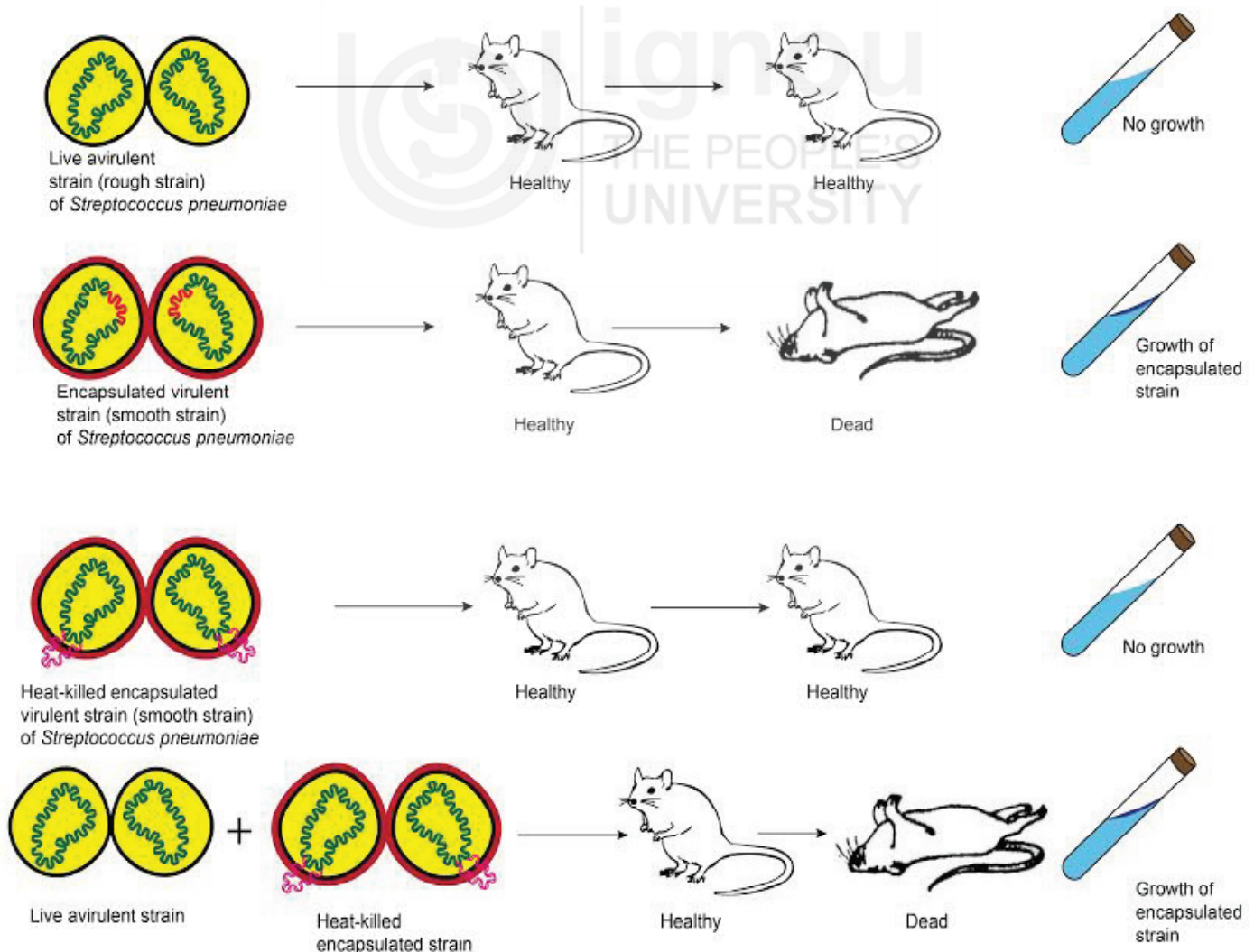


Fig. 4.11: Griffith's experiment with mice and *Streptococcus pneumoniae*.

of wrinkled and rough surface and were called 'R' cells. These bacteria lost their virulence with the loss of their capsule. The capsule provides them virulence as it prevents the *Pneumococci* from being engulfed and destroyed by the neutrophils and macrophages of the body. Mice injected with a few S cells, Type II *Pneumococci* (*Pneumococci* occur in different types I, II, III, and so on), soon had their bodies teeming with descendant cells of the same type.

Griffith did an experiment and observed that when living R cells (harmless) and dead S cells (which also should have been harmless) were injected together, the mouse became ill and living S cells could be recovered from its body. Injection of living R-I cells and dead S-II cells resulted in death of the mouse with its body filled with living S-II *Pneumococci*. So, something in the dead S-II cells had made a permanent change in the phenotype of the R-I cells (Fig 4.11). This process is known as *transformation*. The ability of a cell to be transformed depends on its competence. The cells that are in a state in which they can be transformed by DNA present in their environment are said to be **competent**, which can be defined as the ability of a recipient bacterium to take up DNA from the environment i.e., must have binding sites for DNA at the surface, and must not have DNase, that degrades DNA. Competence is an interesting property that varies among bacteria, very few bacteria genera are competent or capable of taking up DNA naturally.

In a significant number of bacteria the competent state is attained through its chromosomal genes and signalled by certain environmental conditions. Certain bacteria do not become competent under ordinary conditions, but they can be made competent by a variety of highly artificial treatments, such as exposure to high concentration of divalent cations. Such system of transformation has been termed artificial transformation.

Streptococcus pneumoniae (a Gram (+)ve bacterium) and *Haemophilus influenzae* (a Gram (–) ve bacterium) are examples of those bacteria that encode a capacity for natural transformation. *Escherichia coli* does not possess capacity for natural transformation. However, it can be artificially transformed through plasmid technology.

SAQ 2

Fill in the blanks with appropriate word(s):

- In a merozygote the complete genetic complement belongs to the
- DNA is transferred from surrounding into the cell of a bacterium during the mechanism of
- J. Lederberg and E. Tatum described the mode of genetic recombination in bacteria.
- When a plasmid integrates with bacterial chromosome, it converts the cell into strain.

4.4 SUMMARY

- In bacteria, the genetic material is organized as a continuous circular strand of DNA, present in the area called nucleoid.
- Such cells may contain extrachromosomal circular DNA strands that can replicate independently. These are called plasmids.

- Bacteria exhibit both asexual and sexual reproduction.
- Asexual reproduction is through binary fission, endospore, exospores or by cyst formation.
- During binary fission, each daughter cell gets a complete chromosome and sufficient copies of all essential macromolecules, in addition to monomers and ions. The wall formation during fission is centripetal.
- The plane of fission in cell wall as well as non-separation of daughter cells is responsible for the formation of coccus, *diplococcus*, *streptococcus*, *tetracoccus*, *sarcina* and *staphylococcus*.
- A dormant, heat-resistant and non-growing cell develops during endospore formation.
- Exospores and cysts are desiccation-resistant resting cells especially formed by some methanotrophs.
- Sexual reproduction in bacteria is not an obligatory step in the life of a bacterium. The mechanism of genetic exchange in bacteria could be: conjugation, transformation, and transduction.
- Conjugation involves genetic-exchange between two bacterial cells through direct physical contact, principally through plasmids, a F-Factor.
- During transformation, DNA is transferred from cells to the surrounding medium and then the recipient cell incorporates it.
- During transduction, the DNA is transferred from one bacterial cell to another through a phage virion.

4.5 TERMINAL QUESTIONS

1. Describe the mechanism of binary fission in bacteria.
2. Differentiate between transformation, transduction and conjugation in bacteria.
3. Describe the experiments of Lederberg and Zinder that led to the discovery of transduction in bacteria.
4. Define F⁺, F⁻ and Hfr strains of bacteria.
5. Write a short note on endospores of bacteria.

4.6 ANSWERS

Self-Assessment Questions

1. a) centripetal
b) any plane
c) inhibits
d) cyst
2. a) recipient
b) transformation
c) conjugation
d) Hfr⁺

Terminal Questions

1. See Subsection 4.2.1
2. See Section 4.3 (Genetic recombination)
3. Refer to Subsection 4.3.2 (Fig. 4.8)
4. Refer Subsection 4.3.1
5. See Subsection 4.2.2 and Fig. 4.3

GLOSSARY

Auxotroph	: A mutant of prototroph, which fails to grow on the minimal medium (a nutritional mutant).
Binary Fission	: Centripetal invagination of cell wall in a bacterial cell resulting in the formation of two identical daughter cells. Both the formation of a septum and chromosome division are triggered by a chromosome.
Competance	: A state of a bacterium in which it can be transformed by DNA in its environment.
Complete medium	: The medium which supplies the deficient growth the substance(s) for the auxotroph.
Cyst	: Transformed vegetative desiccation - resistant bacterial cell. It possesses additional cell wall, rich in poly- β -hydroxybutyrate.
Endospore	: A dormant, heat-resistant, non-growing asexual reproductive unit formed by a bacterium.
Exospore	: The desiccation-resistant resting cells formed by certain bacteria produced as a bud, from one pole of the cell, it possesses complex wall and fibrous capsule.
F-factor	: A transmissible plasmid present in a donor cell of bacterium, during the process of conjugation.
F⁻-cell	: The recipient cell during the conjugation, which is devoid of the F-factor.
F⁺- cell	: F-Factor containing donor cell that produces pilus to make a contact with the recipient F ⁻ - cell.
Hfr-Cell	: F-factor when gets inserted with chromosome of F ⁺ cell it transforms F ⁻ - male into Hfr male (high frequency recombination) strain.
Plasmid	: A small circular DNA molecule which is autonomous and lies free in cytoplasm of a bacterium, functions as F- factor during conjugation events.
Minimal Medium	: A medium which supplies all the minimum nutritional requirements except the growth factors.
Prototroph	: An organism that grows on the minimal medium.

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