CHAPTER

3

Cell

A close-up view of *E.coli* (yellow) in the human intestine (pink).

The cell is the basic unit of organization or structure of all living matter. Within a selective and retentive semipermeable membrane, it contains a complete set of different kinds of units necessary to permit its own growth and reproduction from simple nutrients. It has always been quite difficult to define a cell. Different cell biologists have defined the cell differently as follows : **A.G. Loewy** and **P. Siekevitz** (1963) have defined a cell as "*a unit of biological activity delimited by a semipermeable membrane and capable of self-reproduction in a medium free of other living systems*". **Wilson** and **Morrison** (1966) have defined the cell as "*an integrated and continuously changing system.*" **John Paul** (1970) has defined the cell as "*the simplest integrated orgainization in living systems, capable of independent survival*."

All these definitions have excluded the viruses (see 'Exception of Cell Theory' in Chapter 1). A virus is neither an organism nor a cell, yet it consists of a core of nucleic acid (DNA or RNA) enclosed in an external mantle of protein. In the free state viruses are quite inert. They become activated only when they infect a living host cell and in the process only the nucleic acid core enter the host's cell. The nucleic acid which is the genetic substance, takes over the metabolic activity of the host cell and utilises the cell machinery for the formation of more viruses, ultimately killing the host cell. In a way, thus, viruses are cellular parasites that cannot reproduce by itself. But, because viruses are primitive and simpler units of life, therefore, they should be discussed prior to other cells.

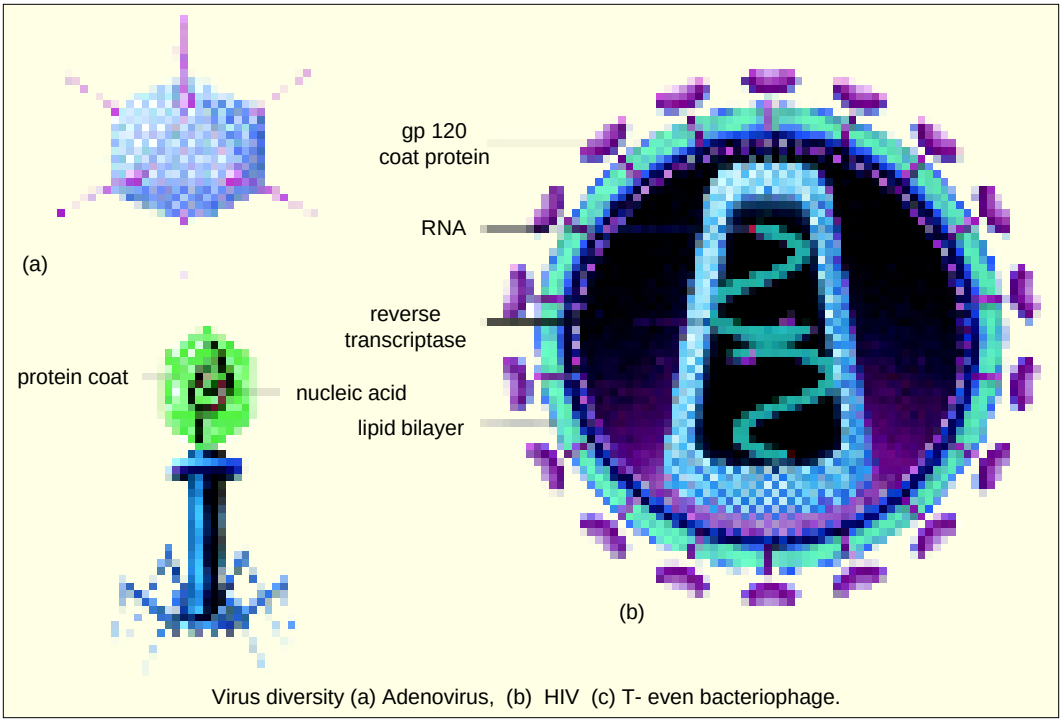
VIRUSES

Viruses (L., venoum or poisonous fluid) are very small submicroscopic biological entities which though lack cellular organization (*viz.*, plasma membrane and metabolic machinery) possess their own genetic material, genetically determined macromolecular organization and characteristic mode of inheritance. For their multiplication, they essentially require the presence of some host cell, *i.e.*, they are obligate cellular parasites of either bacteria, plants or animals.

Structure

Viruses are quite a varied group (Fig. 3.1). They range in between 30 to 300 nm or 300 to 3000 A° in size, so they can be observed only by electron microscopy and X-ray crystallography. They have a regular geometrical and macromolecular organization. Basically an infectious virus particle (called **virion**) is composed of a **core** of only one type of nucleic acid (DNA or RNA) which is wrapped in a protective coat of protein, called **capsid**. The capsid consists of numerous **capsomeres**, each having a few **monomers** or **structural units**. Each structural unit is made up of one or more polypeptide chains. The capsomeres are of different shapes such as hollow prism, hexagonal, pentagonal, lobular or any other shape. The specific arrangement of capsomeres in the capsid determines the shape of a virion. Viruses have the following three different types of symmetry :

1. Icosahedral symmetry. Many viruses have spherical, cubical or polygonal shape which is basically **icosahedral** or 20-sided. Icosahedral symmetry depends on the fact that the assembly of the capsomeres causes the capsid of the virus to be at a state of minimum energy (**Caspar** and **Klug**, 1962). An icosahedral capsid comprises both **penta-meres** (*i.e.*, capsomeres containing 5 structural units) and **hexameres** (*i.e.*, capsomeres having 6 structural units). In an icosahedral virus the minimum number of capsomeres is 12 or its multiple such as 32, 42, 72, 92, 162, 252, 362, 492, 642 and 812. For example, the total number of capsomeres of different icosahedral viruses are : (1) Bacteriophage ϕ (phi) × 174 = 12 pentameres; (2) Turnip yellow mosaic virus or TYMV = 32 capsomeres; (3) Poliovirus = 32 capsomeres; (4) polyoma virus and papilloma virus = 72 capsomeres; (5) Reovirus



Virus diversity (a) Adenovirus, (b) HIV (c) T- even bacteriophage.

= 92 capsomeres; (6) Herpes virus = 162 capsomeres; (7) Adenovirus = 252 capsomeres; and (8) Tipula iridescent virus = 812 capsomeres. In all of these icosahedral viruses, only 12 capsomeres are pentameres, occupying 12 corners of five-fold symmetry, while the rest are hexameres (Fig. 3.2). Since a polyhedron of 20-sided icosahedron basically has triangular faces, it is also known as **deltahedron** (**Alberts** *et al.*, 1989).

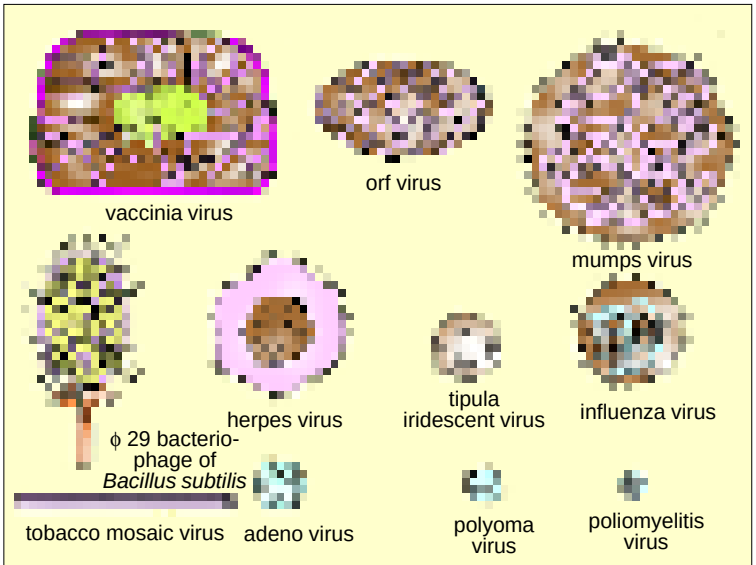


Fig. 3.1. Different kinds of viruses.

2. Helical or cylindrical symmetry. The rod-shaped helical capsid of viruses such as tobacco mosaic virus (TMV), bacteriophage M13 and influenza virus, consists of numerous identical capsomeres arranged into a helix because they are thicker at one end than the other.

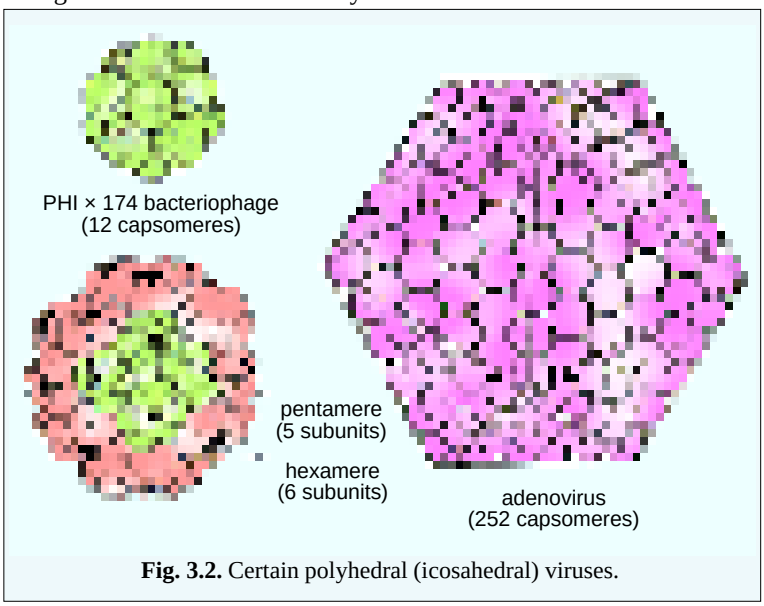


Fig. 3.2. Certain polyhedral (icosahedral) viruses.

3. Complex symmetry. Viruses with complex shaped capsids are of two shapes : those without identifiable capsids (*e.g.*,pox viruses such as vaccinia, cowpox, extromelia and Orf viruses) and those with tadpole-shaped structures in which each part has different sort of symmetry (*e.g.*, T-even phages of *E.coli*; T_2 phage has an icosahedral head, helical tail sheath, hexagonal end plate and rod-shaped tail fibres). Some viruses such as rabies virus are bullet-shaped.

Some viruses such as herpes virus, influenza virus, mumps virus and Semliki forest virus are surrounded by a 100 – 150 A^0 thick spiked membrane. This membrane contains lipid bilayer of plasma membrane from which projects the virus-specific protein molecules or spikes. It is not made by or specified by the virus itself but is derived from the plasma membrane of the host cell (*i.e.*, animal cell).

Are Viruses Living Entities ?

There is no clear answer to this question, because there is no single definition of life which will satisfy everyone. If life is defined as being cellular, then viruses are not alive. If life is defined as being

capable of making new life directly through its own metabolic efforts, then viruses are not living. However, if life is defined as being able to specify each new generation according to its own genetic instructions, then viruses are living systems.

In fact, virus multiplication is very different from cell replication mechanisms. Cells produce their own chromosomes, proteins, membranes and other constituents and these materials are partitioned into progeny cells after a division process in the parent cell. As stated in the cell theory, cells arise only from other cells. Viruses do not give rise directly to new viruses. Instead, they must sabotage the biosynthetic machinery of their host cell so that virus-specific proteins and nucleic acids are made, according to viral genetic information. Eventually virus particles are assembled from newly-made molecules in the host cell and are released when the host cell bursts. They may then initiate new cycles of infection in other host cells. Thus, viruses borrow metabolism and a sheltering membrane from their host, but they provide the genetic instructions that ensure continuity of their species from generation to generation. Since viruses are entirely dependent on living cells for their replication, they cannot be a precellular form in evolutionary terms, but should be viewed as pieces of cellular genetic material which have gained some degree of individual autonomy (see **Bradbury** *et al.,* 1981).

Naming and Classification

Viruses are not named according to the method of binomial nomenclature like other organisms (Binomial nomenclature is the Linnean system of classification requiring the designation of a **binomen** (L., *bi* = twice + *nomen* = a name), the genus and species name, for every species of bacteria, blue green algae, plants and animals). Viruses tend to be named in a random fashion according to the disease caused (*e.g.*, poliomyelitis virus), the host organism (*e.g.*, bacterial viruses or bacteriophages, plant viruses and animal viruses), or some coded system (*e.g.*, T_1, T_2, P_1 phages). Recently, with increase in knowledge of viral biochemistry and molecular biology, various specific characteristics such as nature of nucleic acid (DNA or RNA), the symmetry of capsid, the number of capsomeres, etc., are now being used in viral classification. However, we will stick to the following conventional classification of viruses which is based on the type of the host cell :

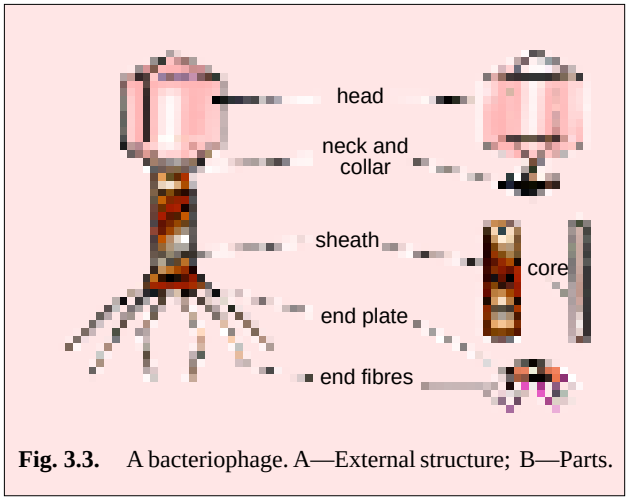
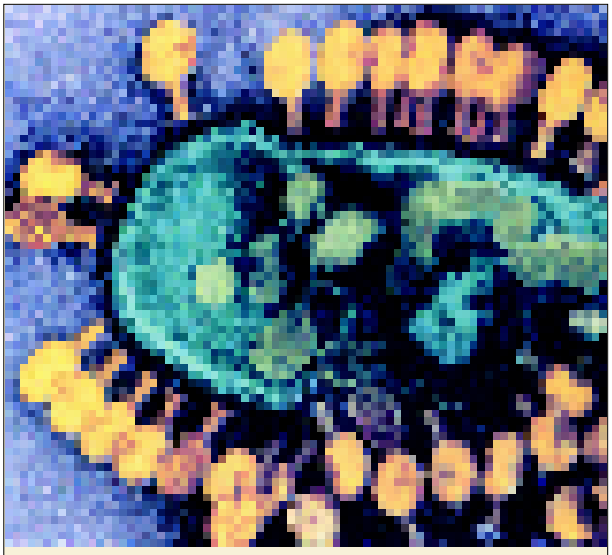


Fig. 3.3. A bacteriophage. A—External structure; B—Parts.

A. Bacterial viruses or bacteriophages. Viruses that parasitize the bacterial cells, are called **bacteriophages** or **phages** (phage means 'to eat'). The phages have specific hosts and they are of variable shapes, sizes and structures. The most widely studied phages are T-even bacteriophages such as T_2, T_4, T_6, etc., which infect the colon bacillus, *Escherichia coli* and are also known as **coliphages** (T for "type". The plural word phages refers to different species; the word phage is both singular and plural and in the plural sense refers to particles of same type. Thus, T_4 and T_7 are both phages, but a test tube might contain either $1T_4$ phage or 100 T_4 phage; see **Freifelder,** 1985).

T_4 bacteriophage is a large-sized tadpole-shaped complex virus (Fig. 3.3). Its capsid comprises of an icosahedral **head** (1250 A^0 length and 850 A^0 width; 2000 capsomeres), a short neck with **collar** bearing 'whiskers' and a long helical **tail**. The tail is made up of a thick and hollow **mid-piece**, a hexagonal **base plate** or **end plate** to which are attached six **spikes** and six long **tail fibres**. The mid-

piece consists of a central hollow **core** and a spring-like **contractile sheath** which comprises 24 rings of hexameres and remains helically arranged around the core. The T_4 genome or chromosome is a single DNA molecule which is 60 µm long, linear, double-stranded and tightly-packed within the head of the phage. Phage DNA contains more than 1,66,000 nucleotide pairs and encodes more than 200 different proteins (*i.e.*,proteins involved in DNA replication and in the assembly of head and tail). For example, T_4 phage DNA codes for at least 30 different enzymes (*e.g.*, helicases, topoisomerases, DNA polymerases, DNA ligases, etc.) all of which ensure rapid replication of phage chromosome in preference to DNA of *E.coli* (host cell). Further, during DNA replication, an unusual nitrogen base, called **5- hydroxymethylcytosine** is incorporated in place of cytosine in the phage DNA. This unusual base makes phage DNA recognisable from that of host DNA and selectively protects it from the nuclease enzymes. The nucleases are encoded in T_4 phage genome to degrade only the DNA of host cell. Some other phage proteins alter host cell's RNA polymerase enzymes, so that they transcribe different sets of T_4 genes at different stages of viral infection according to the phage's needs (see **Alberts** *et al*., 1989).



T_4 phage attached to the wall of its host.

Life cycle of the bacteriophage. Bacteriophages may have the following two types of life cycles: (1) **Lytic cycles**, in which viral infection is followed by **lysis** (bursting and death) of the host cell and release of new infective phages, *e.g.*, virulent phages such as T_4 and all other T-even coliphages. (2) **Lysogenic cycles**, in which infection rarely causes lysis, *e.g.*, temperate phages such as P_1 and lambda (λ) phages.

1. Lytic cycle of a virulent phage. Life cycle of a T_4 bacteriophage (Fig. 3.4) involves the following steps: 1. **Attachment** or **adsorption** of phage to bacterial (host) cell. 2. **Injection** or **penetration** of viral genetic material (DNA) into the host cell. 3. **Eclipse period**, during which synthesis of new phage DNA and protein coats takes place. 4. **Assembly** of phage DNA into protein coats.5. **Lysis** of host cell and **release** of the infective progeny phages. Such a phage is called **virulent** or **lytic phage** since it has infectiousness and it causes death of host cell by lysis.

The adsorption of the phage to its host is made possible by a reaction of chemical groups on the two during a random collision. Reactive groups (called **adsorption protein** or **pilot protein**; **Kornberg**, 1974) at the end of the tail of the phage can join with a complementary set of chemical groups (a **receptor site**) in the cell wall of the bacterium. During adsorption, long tail fibres of the phage are first to contact and attach to the cell. They help to position the phage's tail perpendicularly to the cell wall. Once the phage is attached to its prospective host, injection can take place involving a movement of phage DNA from its position inside the head of the phage through the hollow core of the tail into the bacterium. Entry is made possible by a hole punched in the bacterial cell wall, either by contraction of outer sheath of tail or by the action of enzymes carried by phage tail, or both. The protein coat or capsid of the phage remains outside the cell. Once inside the host cell, the phage DNA becomes a **vegetative phage**, *i.e.*, phage genes take over the metabolic machinery of the cell and direct it to produce replicas of the infecting virus. Although the cell continues to procure raw materials and

energy from the environment, the phage genes allow only viral components to be built. Further, either the normal ability of the host DNA to control the cell is lost, or the host DNA is completely destroyed by early products of the viral genes. Thus, phage DNA is both replicated and transcribed; first the enzymes needed for synthesis of phage DNA are translated, then the capsid proteins are translated. Phage particles are assembled around condensed cores of the complete phage nucleic acid (by self-assembly method). At last lytic enzymes which have been coded by phage DNA, break open the bacterium and release the new phage particles which diffuse in the surrounding in search of new host (see **Mays**, 1981).

2. Lysogenic cycle. Certain bacteriophages such as P_1 and lambda (λ) phages, have entirely different pattern of life cycle than the virulent phages. This pattern is called **lysogeny** and is characterized by delayed lysis after phage infection. A virus with this capacity is called **temperate virus**. The infected host cell is said to be lysogenic because dormant virus may at any time become active and begin directing the synthesis of new virus particles.

In lysogeny, the process of adsorption and nucleic acid injection are quite similar to a lytic cycle of virulent phages, although different phages recognise different bacterial cell surface receptors. The next step, however, is unique to lysogeny. The nucleic acid is neither extensively replicated nor extensively transcribed. The virus generally expresses one or a few genes which code for a **repressor protein** that turns off (*i.e.*, represses) the expression of the other genes of the virus. In consequence, virus is not replicated, but phage DNA remains in the bacterium, being replicated in such a way that when the lysogenic bacterium divides, each daughter cell receives at least one phage genome in addition to the bacterial genome. There are two styles to this persistence of phage DNA : the phage chromosome may exist as a fragment of DNA outside the host's chromosome (*i.e.*, in

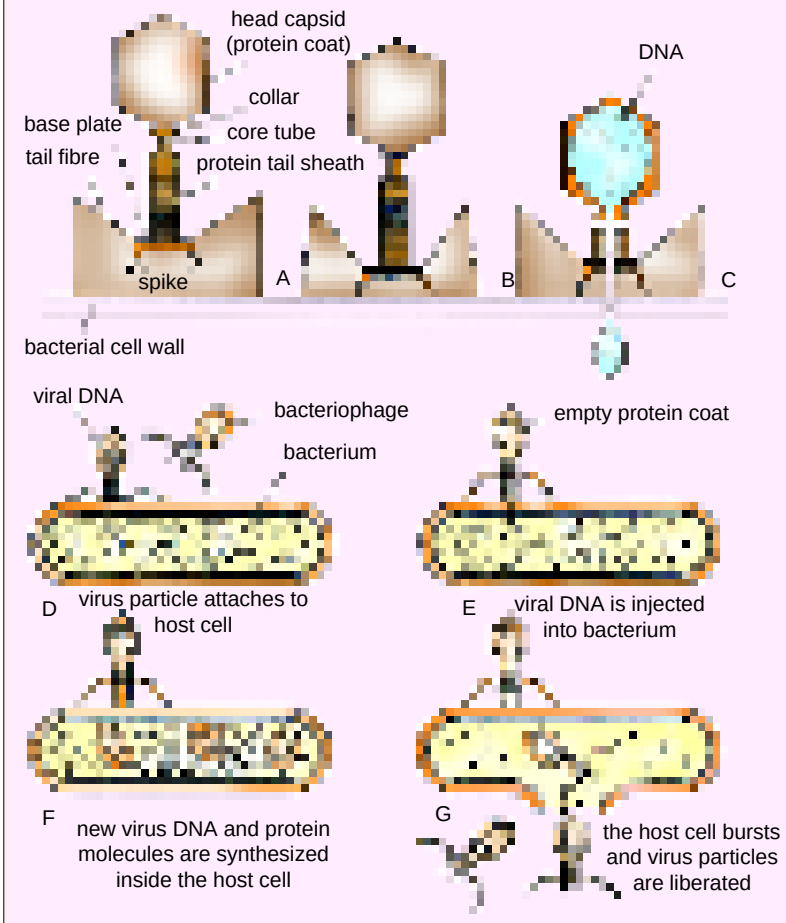


Fig. 3.4. A,B,C — Mode of attachment of a T-even phage (bacteriophage) on a bacterial cell wall and injection of DNA into the bacterium (host cell); D,E,F and G — Steps of viral reproduction inside the host cell.

the host's cytoplasm) essentially as a plasmid* (*e.g.*, P_1 bacteriophage) or it may attach itself to the host's chromosome as an episome* (*e.g.*, lambda phage). Thus, in case of lambda phage the DNA first becomes circular due to joining of its both cohesive ends and then is integrated into the circular DNA molecule of the bacterium. Such an integrated and dormant viral genome is often termed as **provirus** or **prophage**. The infection of *E. coli* cell with lambda phage and its consequent integration and adoption of lysogeny, renders that cell immune to further attack by phage of the same type.

The calm lysogenic period is ended by some type of shock (*e.g.*, temperature changes, UV irradiation or conjugation of a lysogenic bacterium with a non-lysogenic bacterium) to the lysogenic culture. A shock evidently inactivates the repressor of the phage so that all the phage genes can be expressed. Then the lysogenic bacterium is ruined, for the phage DNA that the host bacterium is harbouring enters the lytic phase. It replicates, transcribes, translates, assembles viron particles and lyses the bacterium (Fig. 3.5).

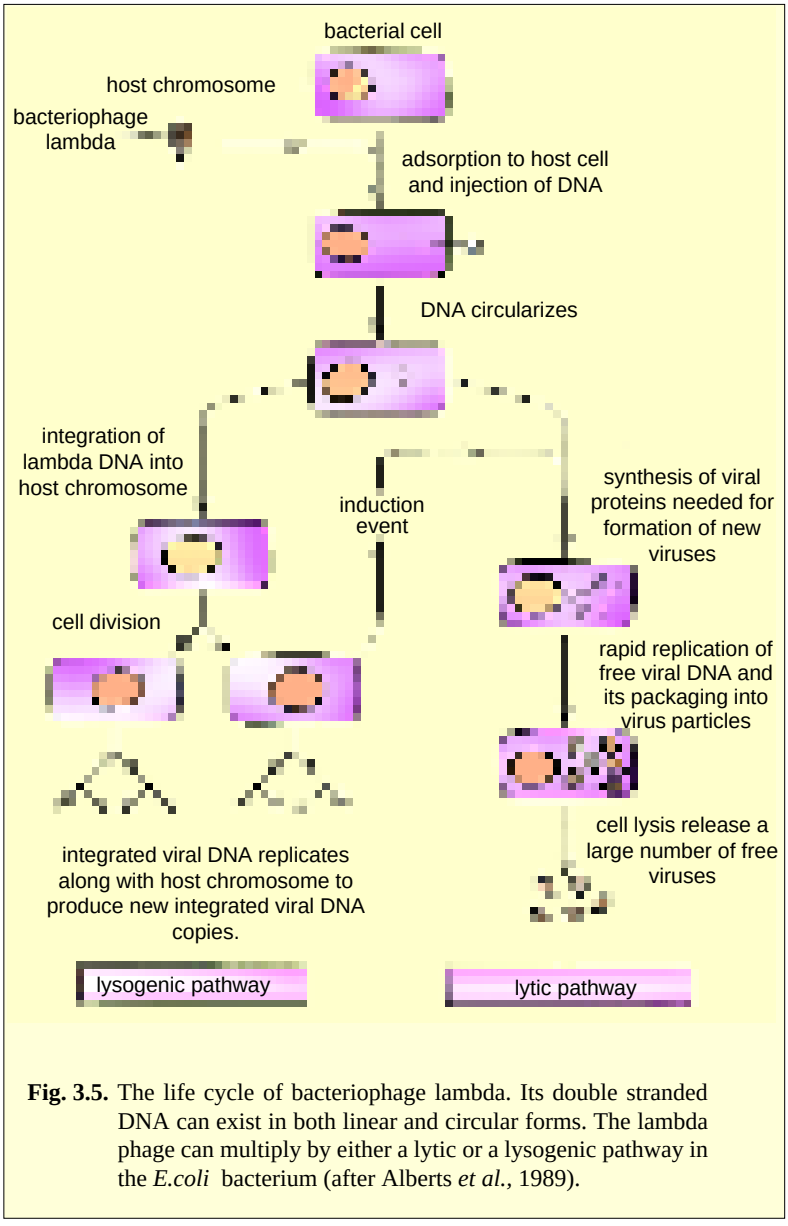


Fig. 3.5. The life cycle of bacteriophage lambda. Its double stranded DNA can exist in both linear and circular forms. The lambda phage can multiply by either a lytic or a lysogenic pathway in the *E.coli* bacterium (after Alberts *et al.*, 1989).

B. Plant viruses. The plant viruses parasitize the plant cells and disturb their metabolism and cause severe diseases in them. All plant viruses consist of ribonucleoproteins in their organization. The important plant

* **Episome** is an extrachromosomal, circular, transposable, closed DNA molecule which can exist either integrated ito the bacterial chromosome or separately and autonomously in the cytoplasm. **Plasmid** is that bit of autonomous genetic material of bacteria (*i.e.*, circular DNA) that exists only extrachromosomally and cannot be integrated into the bacterial chromosome (DNA) (see **Sheeler** and **Bianchi,** 1987). Now only the term plasmid is used for all kinds of extrachromosomal autonomous transposable circular DNA fragments (see **Alberts** *et al.*, 1989; **Burns** and **Bottino**, 1989).

viruses are tobacco rattle virus (TRV), tobacco mosaic virus (TMV), potato virus, beet yellow virus (BYV), southern bean mosaic virus (SBMV) and turnip yellow viruses (TYV). Among plants, few hundred viral diseases are caused, *e.g.*, mosaic diseases of tobacco, cabbage, cauliflower, groundnut and mustard; black-ring spot of cabbage; leaf roll of tomato; leaf curl of papaya, cotton, bean and soyabean; yellow disease of carrot, peach; little-leaf of brinjal. These diseases are spread mainly by insects such as aphids, leaf hoppers and beetles.

Tobacco mosaic virus (TMV). TMV is the most extensively studied plant virus. It was discovered by **Iwanowski** (1892) and obtained in a pure state (*i.e.*, in paracrystalline form) by **Stanley** (1935). **Bawden** and **Pirie** (1937) extensively purified TMV and showed it to be a nucleoprotein containing RNA. **H.Fraenkel-Conrat** experimentally demonstrated that RNA is the genetic substance of TMV.

TMV is a rod-shaped, helically symmetrical RNA virus (Fig. 3.6). Each virus particle is elongated, cigarette-like in shape having the length of 3000A^0 (300 nm) and diameter of 160 A^0 (16 nm; see **De Robertis** and **De Robertis, Jr.**, 1987). In each rod of TMV, there are about 2130 identical elliptical protein subunits or capsomeres. The capsomeres are closely packed and arranged in a helical manner around the RNA helix, forming a hollow cylinder. Thus, there is a hollow core (axial hole) of about 40A^0 (4nm) diameter which runs the entire length of the rod and contains the RNA molecule. The RNA molecule does not occupy the hole but is deeply embedded in the capsomeres. RNA of TMV is a single-stranded molecule consisting of 6500 nucleotides and is in the form of a long helix extending the whole length of viral particle. Lastly, there are about 16 capsomeres in each helical turn. Each capsomere contains about 158 amino acids and has a molecular weight of 18000 daltons. The whole TMV capsid has all amino acids found in other plant proteins.

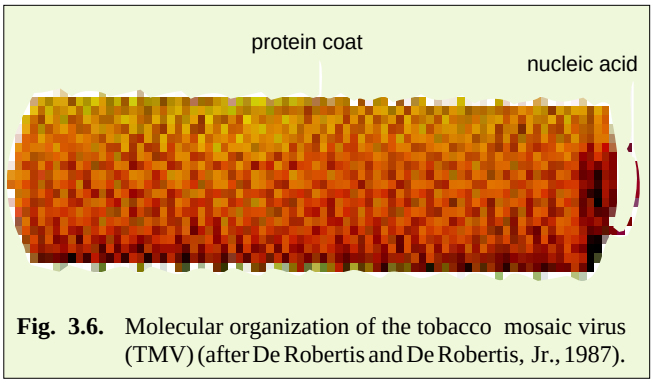


Fig. 3.6. Molecular organization of the tobacco mosaic virus (TMV) (after De Robertis and De Robertis, Jr., 1987).

TMV infects the leaves of tobacco plant. It is transmitted and introduced into the host cell by some vector or by mechanical means such as rubbing, transplanting and handling. Once inside the host cell, the viral RNA directs the metabolic systems of host to synthesize its own proteins and to replicate (multiply) its RNA molecule. All the raw materials for RNA replication and capsomere biosynthesis are derived from the host cell. Ultimately when numerous viral particles are formed by self-assembly method inside the host cell, they are released after lysis of the cell. Recently, it was found that plant viruses exploit the route of plasmodesmata to pass from cell to cell. For example, TMV is found to produce a 30,000 dalton protein called $\mathbf{P_{3O}}$ which tends to enlarge the plasmodesmata in order to use this route to pass or spread its infection from cell to cell (see **Alberts** *et al.*, 1989).

C. Animal viruses. The animal viruses infect the animal cells and cause different fatal diseases in animals including man. Generally, they have a polyhedron or spherical shape and genetic material in the form of DNA or RNA. The protein coat or capsid of animal viruses is surrounded by an envelope.

Certain common viral infections of human beings are : common cold, influenza, mumps, measles, rubella (German measles), chickenpox, small pox, polio, viral hepatitis, herpes simplex, viral encephalitis, fever blisters, warts and some types of cancer. Among livestock and fowl, viruses cause encephalitis, foot and mouth disease, fowl plague, Newcastle disease, pseudorabis, hog cholera and a variety of warts and other tumors. A virus usually displays some specificity for a particular animal group.

Poliomyelitis is a most extensively studied RNA-containing animal virus. The polio virus has comparatively very simple organization. It consists of a protein shell built up of 60 structurally equivalent asymmetric protein subunits of approximately 60 A^0 diameter, packed together in such a way that they form a spherical shell of about 300 A^0 in diameter. The shell or capsid encloses a single stranded RNA molecule of 5,200 nucleotides.

Herpes virus is another most extensively studied animal virus. It possesses a DNA containing core embedded in a regular icosahedral **capsid** (162 capsomeres) and an outer **envelope** of lipids, proteins and carbohydrates. The DNA molecule of herpes virus is a single, linear, double-stranded having a molecular weight of 10^8 daltons and codes for about 100 average sized protein molecules.

Life cycle of animal viruses. Like the bacteriophages, animal viruses have two types of life cycles or growth : **1. Permissive growth** which permits an animal virus to multiply lytically and kill the host cell. **2. Non-permissive growth** which permits an animal virus to enter the host cell but does not allow it to multiply lytically. In some of these non-permissive cells, the viral chromosome either becomes integrated into genome of the host cell, where it is replicated along with the host chromosome or it forms a plasmid — an extrachromosomal circular DNA molecule — that replicates in a controlled fashion without killing the cell.

The modes of infection or entry of animal viruses inside the host cells have been well investigated for the RNA-containing viruses such as influenza virus and Semliki forest virus. Influenza virus has segmented genome (Fig. 3. 7). Each segment is a template for the synthesis of a different single mRNA. The RNA core is protected by an icosahedral capsid. The nucleocapsid (RNA + capsid) of influenza virus is ultimately surrounded by a phospholipid bilayer membrane in which are embedded the following two types of viral glycoproteins or **spikes** : **1. Large spikes** of trimers (*i.e.*, each unit of three monomers) of **hemagglutinin** or **Ha protein.** At the time of adsorption or attachment of the virus to the host cell, these HA spikes bind to virus-specific receptors on the surface or plasma membrane of the host cell. **2. Small spikes** of multimers of **neuraminidase** or **NA protein.** These NA spikes remove those virus-loaded receptors from the host's surface which have not entered the host cell through the process of receptor-mediated endocytosis. Thus, NA spikes cause the viruses to desorb from the host cell and make them free to infect other host cells.

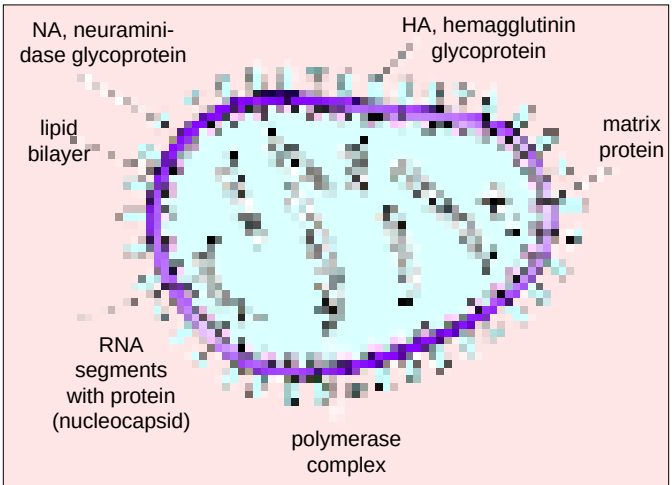


Fig. 3.7. The influenza viron (after Watson *et al.*, 1987).

Infection of influenza virus is initiated when the virus binds to specific receptor proteins on the plasma membrane of target host cell. These RNA viruses are taken in by the host cell by **receptor-mediated endocytosis** and are delivered to the **endosomes** (which are special vesicles having acidic medium or low pH between 5 to 5.5 and recycle the receptors; see Chapter 5). The low pH in endosome activates a **fusogenic protein** or **fusogen** in the viral envelope that catalyzes the fusion of membranes of the influenza virus and the endosome. This allows the escaping of intact viral particle into the cytosol of the host. Thus, both the viral protein and viral nucleic acid penetrate into the host cell. Ultimately viral RNA comes out from the viral capsid and gets attached to ribosomes to start the process of viral multiplication (Fig. 3.8).

Usually, the animal viruses are released from the host cell by rupturing (lysis) and subsequent death of host cells. However, sometimes viral particles are pinched off as buds from the cell surface and they retain the host's plasma membrane around them, *e.g.*, influenza virus, Semliki forest virus, retroviruses such as AIDS virus. AIDS or acquired immune deficiency syndrome is caused by **HIV-1** or Human immunodeficiency virus type -I.

Viroids. Viroids are small RNA circles, only 300 to 400 nucleotides long, lacking AUG codon (the signal for the start of protein synthesis). They are replicated autonomously despite the fact that they do not code for any protein. Having no protein coat or capsid, they exist as naked RNA molecules and pass from plant to plant only when the surfaces of both donor and recipient cells are damaged so that there is no membrane barrier for the viroid to pass (see **Alberts** *et al.*, 1989).

The term viroid was coined by **Diener** (1971) who discovered the first viroid, called **potato spindle tuber viroid** or **PSTV**. Viroids form a class of subviral pathogens which cause infections and diseases in many plants and also in animals (see **Sheeler** and **Bianchi**, 1987). Some of the plant diseases which are caused by the viroids are the following :

1. Potao spindle tuber (Its viroid contains 359 nucleotides in single and circular RNA molecule);
2. Citrus exocortis (Its viroid contains 371 nucleotides in RNA molecule);
3. Chrysanthemum stunt;
4. Chrysanthemum chlorotic mottle;
5. Cucumber pale fruit;
6. Hope stunt; and
7. Tomato plant macho (see **Sharma**, 1990).

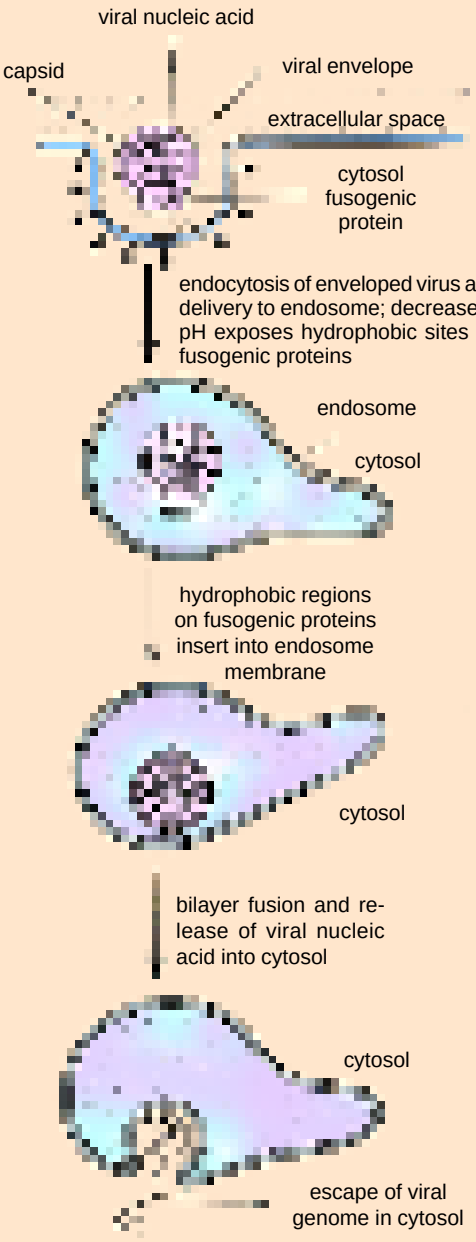


Fig. 3.8. Schematic diagram to show how fusogenic proteins on the surface of many enveloped viruses are thought to catalyze the fusion of viral and endosomal membranes (Alberts *et al.*, 1989).

Prions. Prions are described as 'rod-shaped' proteinaceous particles thought to cause a number of diseases in animals such as **Scrapie disease** of central nervous system of goats and sheep (in which animals scrape or scratch themselves against some gate post or similar object). Prions are also found to cause a Scrapie-like disease, called **Creutzfeldt-Jakob disease** of nervous system of humans and **Kuru** disease of brain of cannibalistic tribes of New Guinea. Prions were named by **S.B.Prusiner** (1984). They can survive

heat, ionizing and UV radiations, and chemical treatment that normally inactivates viruses (*i.e.*, they are resistant to inactivation by phenol or nuclear enzymes).

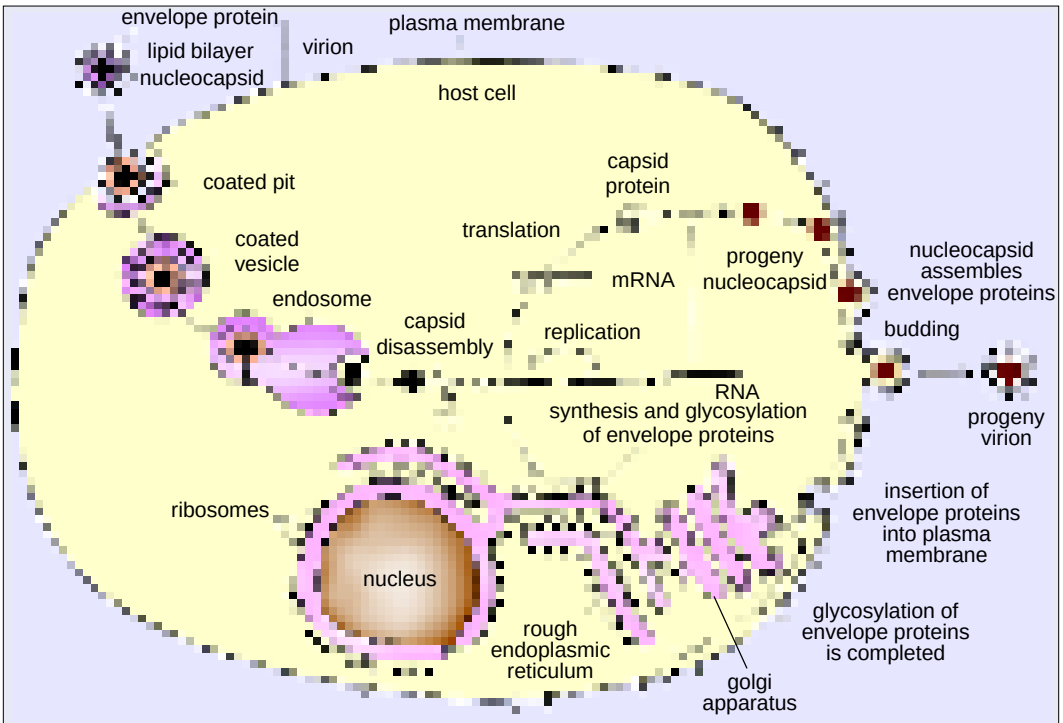


Fig. 3.9. Life cycle of Semliki forest virus : An animal virus containing a single-stranded RNA genome, capsid and spiked envelope (after Alberts *et al.*, 1989).

The protein comprising a prion has a molecular weight between 50,000 to 100,000, corresponding to a particle size that is 100 times smaller than the smallest virus (see **Sheeler** and **Bianchi**, 1987). Though nothing is still clear regarding the mode of replication, multiplication of prions, yet, it is thought that prion protein somehow serves as template to perform ‘ reverse translation’, *i.e.*, from protein → RNA → DNA, thus, turning the central dogma of molecular biology on its head (see **Banerjee**, 1987; **Sharma**, 1990).

CELLS OF CELLULAR ORGANISMS

The body of all living organisms (bacteria, blue green algae, plants and animals) except viruses has **cellular organization** and may contain one or many cells. The organisms with only one cell in their body are called **unicellular organisms** (*e.g.*, bacteria, blue green algae, some algae, Protozoa, etc.). The organisms having many cells in their body are called **multicellular organisms** (*e.g.*, most plants and animals). Any cellular orgainsm may contain only one type of cell from the following types of cells :

A. Prokaryotic cells ; B. Eukaryotic cells.

The terms prokaryotic and eukaryotic were suggested by **Hans Ris** in the 1960’s.

PROKARYOTIC CELLS

The prokaryotic (Gr., *pro* = primitive or before; *karyon* = nucleus) are small, simple and most primitive. They are probably the first to come into existence perhaps 3.5 billion years ago. For example, the **stromatolites** (*i.e.*, giant colonies of extinct cyanobacteria or blue green algae) of

Western Australia are known to be at least 3. 5 billion years old. The **eukaryotic** (Gr., *eu* =well; *karyon* = nucleus) cells have evolved from the prokaryotic cells and the first eukaryotic (nucleated) cells may have arisen 1.4 billion years ago (**Vidal,** 1983).

The prokaryotic cells are the most primitive cells from the morphological point of view. They occur in the bacteria (*i.e.*, mycoplasma, bacteria and cyanobacteria or blue-green algae). A prokaryotic cell is essentially a **one-envelope system** organized in depth. It consists of central nuclear components (*viz.*, DNA molecule, RNA molecules and nuclear proteins) surrounded by cytoplasmic ground substance, with the whole enveloped by a plasma membrane. Neither the nuclear apparatus nor the respiratory enzyme system are separately enclosed by membranes, although the inner surface of the plasma membrane itself may serve for enzyme attachment. The cytoplasm of a prokaryotic cell lacks in well defined cytoplasmic organelles such as endoplasmic reticulum, Golgi apparatus, mitochondria, centrioles, etc. In the nutshell, the prokaryotic cells are distinguished from the eukaryotic cells primarily on the basis of what they lack, *i.e.*, prokaryotes lack in the nuclear envelope, and any other cytoplasmic membrane. They also do not contain nucleoli, cytoskeleton (microfilaments and microtubules), centrioles and basal bodies.

Bacteria

The bacteria (singular bacterium) are amongst the smallest organisms. They are most primitive, simple, unicellular, prokaryotic and microscopic organisms. All bacteria are structurally relatively homogeneous, but their biochemical activities and the ecological niches for which their metabolic specialisms equip them, are extremely diverse.

Bacteria occur almost everywhere : in air, water, soil and inside other organisms. They are found in stagnant ponds and ditches, running streams and rivers, lakes, sea water, foods, petroleum oils from deeper regions, rubbish and manure heaps, sewage, decaying organic matter of all types, on the body surface, in body cavities and in the internal tracts of man and animals. Bacteria thrive well in warmth, but some can survive at very cold tops of high mountains such as Alps or even in almost boiling hot springs. They occur in vast numbers. A teaspoonful of soil may contain several hundred million bacteria. They lead either an autotrophic (photoautotrophic or chaemoautotrophic), or heterotrophic (saprotrophic or parasitic) mode of existence. The saprophytic or saprotrophic species of bacteria are of great economic significance for man. Some parasitic species of bacteria are pathogenic (disease producing) to plants, animals and man.

(a) Cells of *Pseudomonas* (cylindrical); (b) *Streptococcus* (spherical) (c) *Spirilla* (twisted shape)

Bacteria have a high ratio of surface area of volume because of their small size. They show high metabolic rate because they absorb their nutrients directly through cell membranes. They multiply at a rapid rate. In consequence, due to their high metabolic rate and fast rate of multiplication, bacteria produce marked changes in the environment in a short period.

1. Size of bacteria. Typically bacteria range between 1µm (one micrometre) to 3 µm, so they are barely visible under the light microscope. The smallest bacterium is *Dialister pneumosintes* (0.15 to 0.3µm in length). The largest bacterium is *Spirillum volutans* (13 to 15µm in length).

2. Forms of bacteria. Bacteria vary in their shapes. Based on their shape, bacteria are classified into the following groups :

(1) Cocci (singular coccus). These bacteria are spherical or round in shape. These bacterial cells may occur singly (**micrococci**); in pairs (**diplococci**, *e.g.*, pneumonia causing bacterium, *Diplococcus pneumoniae*); in groups of four (**tetracocci**); in a cubical arrangement of eight or more (**sarcinae**); in irregular clumps resembling bunches of grapes (**staphylococci**, *e.g.*, boil causing bacterium, *Staphylococcus aureus* or in a bead-like chain (**streptococci**, *e.g.*, sore throat causing bacterium, *Streptococcus pyogenes*) (Fig. 3.10).

(2) Bacilli (singular, bacillus). These are rod-like bacteria. They generally occur singly, but may occasionally be found in pairs (**diplobacilli**) or chains (**streptobacilli**). Bacilli cause certain most notorious diseases of man such as tuberculosis (*Mycobacterium* or *Bacillus tuberculosis*), tetanus (*Clostridium tetani*), typhoid (*Salmonella* or *Bacillus typhosus*), diphtheria (*Corynebacterium diphtheriae*), leprosy (*Mycobacterium leprae*), dysentery and food poisoning (*Clostridium botylinum*). Certain well known diseases of the animals are also caused by bacilli, *e.g.*, anthrax (*Bacillus anthracis*) and black leg (*Clostridium chauvei*).

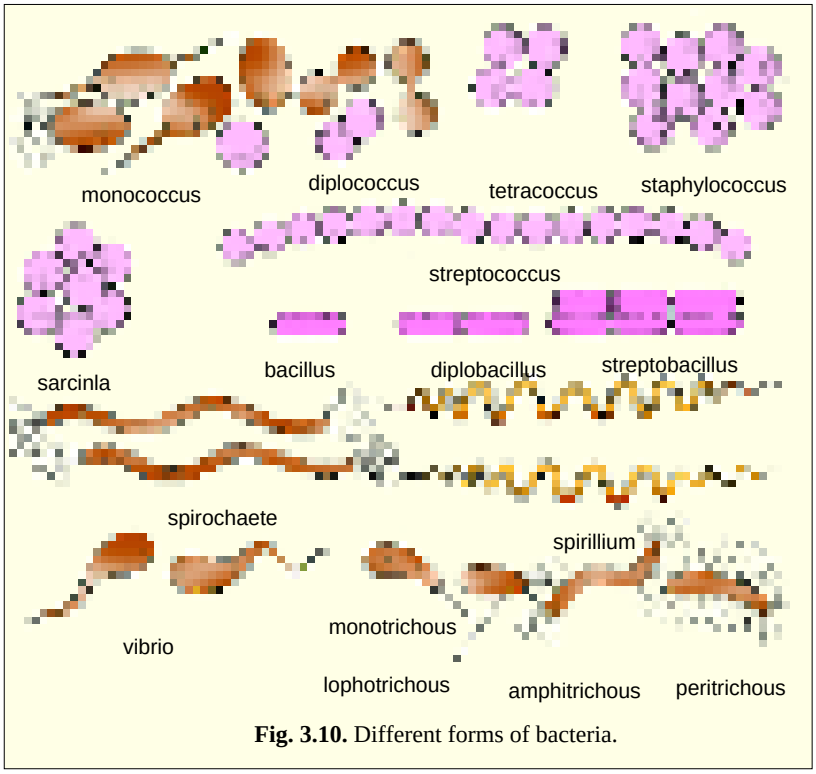


Fig. 3.10. Different forms of bacteria.

(3) Spirilla (singular, spirillum). These are also called **spirochetes**. These are spiral-shaped and motile bacteria (Fig. 3.10). Spirilla cause human disease such as syphilis (*Treponema pallidum*).

(4) Vibrios (singular vibrio). These are comma-shaped or bent-rod like bacteria (Fig. 3.10). Vibrios cause human disease such as cholera (*Vibrio cholerae*).

3. Gram negative and Gram positive bacteria. On the basis of structure of cell wall and its stainability with the Gram stain, the following two types of bacteria have been recognized : Gram positive and Gram negative bacteria. The Gram staining method is named after **Christian Gram** who developed it in Denmark in 1884. In this technique, when heat-fixed bacteria are treated with the basic dye, crystal violet, they become blue or purple. Such blue stained cells are treated with a mordant (*i.e.*, agent that fixes stains to tissues) such as iodine (*i.e.*, potassium iodide or KI solution) and ultimately washed with some organic solvent such as alcohol. Some bacteria retain the blue colour, while others lose it and stay colourless. The former are **Gram positive bacteria** (*e.g.*, *Bacillus subtilis*, *Staphylococcus*, etc.) and the latter are **Gram negative bacteria** (*e.g.*, *Escherichia coli*, *Simonsiella*,

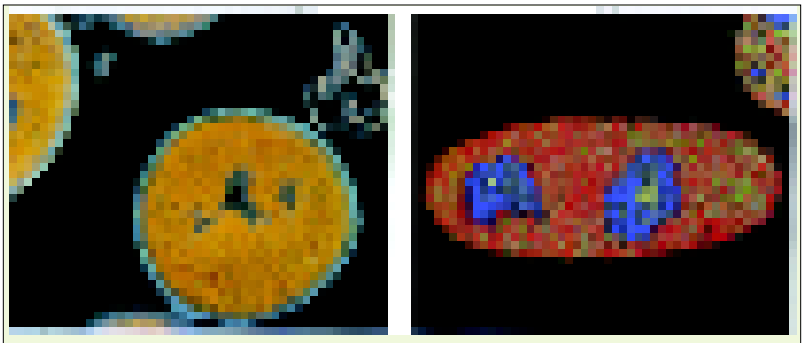
cyanobacteria, etc.). Colourless Gram negative bacteria may thereafter be stained pink with safranin stain for their better microscopic visibility.

The long search for the chemical basis of this differentiating staining reaction ended in 1950's when it was detected that cell wall of Gram negative bacteria has high lipid content which tends to be dissolved away by alcohol. The alcohol then can enter the cell and leach out the stain, whereas the cell walls of Gram positive bacteria form a barrier (*i.e.*, peptidoglycan layers) that prevent the penetration of the solvent inside the cell.

4. Structure of bacteria. Structural details of a bacterial cell can only be seen with an electron microscope in very thin sections. A typical bacterial cell has the following components:

A. Outer covering. The outer covering of bacterial cell comprises the following three layers:

I. Plasma membrane. The bacterial protoplast is bound by a living, ultrathin (6 to 8 nm thick) and dynamic plasma membrane. The plasma membrane chemically comprises molecules of lipids and proteins which are arranged in a **fluid mosaic pattern**. That is, it is composed of a bilayer sheet of **phospholipid** molecules with their polar heads on the surfaces and their fatty-acyl chains (tails) forming the interior. The **protein** molecules are embedded within this lipid bilayer, some spanning it, some exist on its inner side and some are located on its external or outer side. These membrane proteins serve many important functions of the cell. For example, the transmembrane proteins act as **carriers** or **permeases** to carry on selective transportation of nutrients (molecules and ions) from the environment to the cell or vice versa. Certain proteins of the membrane are involved in oxidative metabolism, *i.e.*, they act as enzymes and carriers for electron flow in respiration and photosynthesis leading to phosphorylation (*i.e.*, conversion of ADP to ATP). The bacterial plasma membrane also provides a specific site at which the single circular chromosome (DNA) remains attached. It is the point from where DNA replication starts. The first stage in nuclear division involves duplication of this attachment, followed by a progressive bidirectional replication of DNA by two replication forks.



Pictures showing bacterial membrane -- blue in coci (left) and fine red outline in bacillus (right).

Plasma membrane intrusions. Infoldings of the plasma membrane of all Gram-positive bacteria and some Gram-negative bacteria give rise to the following two main types of structures:

(I) Mesosomes (or **chondrioids**). They are extensions of the plasma membrane within the bacterial cell (*i.e.*, cytoplasm) involving complex whorls of convoluted membranes (Fig. 3.11). Mesosomes tend to increase the plasma membrane's surface and in turn also increase their enzymatic contents. They are seen in chemoautotrophic bacteria with high rates of aerobic respiration such as *Nitrosomonas*, and in photosynthetic bacteria such as *Rhodopseudomonas* where they are the site of photosynthetic pigments. Mesosomes are involved in cross-wall (septum) formation during the division of cell.

(2) Chromatophores. These are photosynthetic pigment-bearing membranous structures of photosynthetic bacteria. Chromatophores vary in form as vesicles, tubes, bundled tubes, stacks, or thylakoids (as in cyanobacteria).

II. Cell wall. The plasma membrane is covered with a strong and rigid cell wall that renders mechanical protection and provides the bacteria their characteristic shapes (the cell wall is absent in *Mycoplasma*). The cell wall of bacteria differs chemically from the cell wall of plants in that it contains proteins, lipids and polysaccharides. It may also contain chitin but rarely any cellulose.

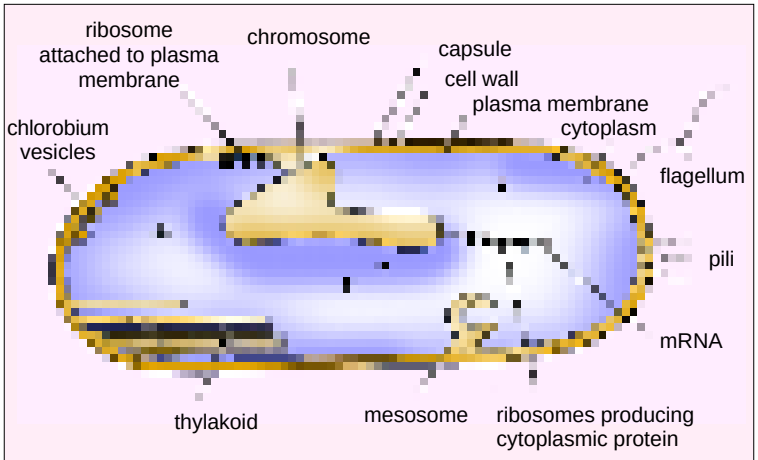


Fig. 3.11. A diagram of structures seen in the prokaryotic (bacterial) cells (after Sheeler and Bianchi, 1987; King, 1986).

Electron microscopy has revealed the fact that the cell wall of Gram-negative bacteria comprises the following two layers : 1. Gel, proteoglycan or peptidoglycan (*e.g.*, murein or muramic acid) containing **periplasmatic space** around the plasma membrane and 2. The **outer membrane** which consists of a lipid bilayer traversed by channels of **porin** polypeptide. These channels allow diffusion of solutes. The lipids of lipid bilayer are phospholipids and lipopolysaccharides (LPS). LPS have antigenic property and anchor the proteins and polysaccharides of the surrounding capsule (see **King**, 1986). The cell wall of Gram positive bacteria is thicker, amorphous, homogeneous and single layered. Chemically it contains many layers of peptidoglycans and proteins, neutral polysaccharides and polyphosphate polymers such as teichoic acids and teichuronic acids.

III. Capsule. In some bacteria, the cell wall is surrounded by an additional slime or gel layer called **capsule**. It is thick, gummy, mucilaginous and is secreted by the plasma membrane. The capsule serves mainly as a protective layer against attack by phagocytes and by viruses. It also helps in regulating the concentration, and uptake of essential ions and water.

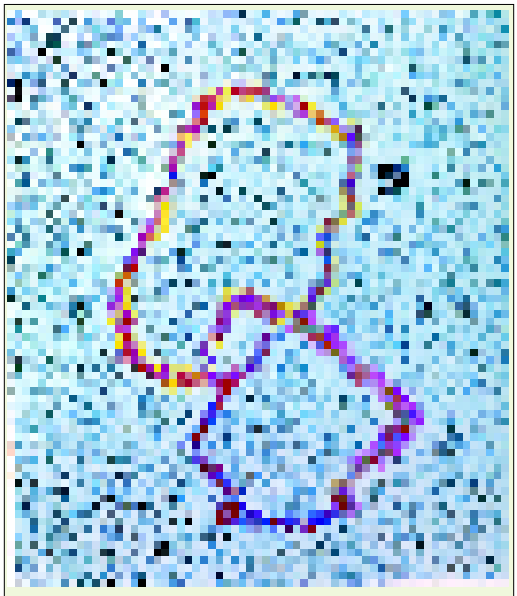
B. Cytoplasm. The plasma membrane encloses a space consisting of **hyaloplasm, matrix** or **cytosol** which is the ground substance and the seat of all metabolic activities. The cytosol consists of water, proteins (including multifunctional enzymes), lipids, carbohydrates, different types of RNA molecules, and various smaller molecules. The cytosol of bacteria is often differentiated into two distinct areas : a less electron dense nuclear area and a very dense area (or dark region). In the dense cytoplasm occur thousands of particles, about 25 nm in diameter, called **ribosomes**. Ribosomes are composed of ribonucleic acid (RNA) and proteins and they are the sites of protein synthesis. Ribosomes of bacteria are 70S type and consist of two subunits (*i.e.*, a larger 50S ribosomal subunit and a small 30S ribosomal subunit). Non-functional ribosomes exist in the form of separated subunits which are suspended freely in the cytoplasm. During protein synthesis many ribosomes read the codes of single mRNA (messenger RNA) molecules and form **polyribosomes** or **polysomes**.

Reserve materials of bacteria are stored in the cytoplasm either as finely dispersed or distinct granules called **inclusion bodies** or **storage granules**. There are three types of reserve materials. First, there are organic polymers which either serve as reserves of carbon, as does **poly-β-hydroxybutyric acid**, or as stores of energy, as does a polymer of glucose, called **granulose** (*i.e.*, glycogen). Second, many bacteria contain large reserves of inorganic phosphate as highly refractile granules of metaphosphate polymers known as **volutin**. The third type of reserve material is elemental **sulphur**, formed by oxidation from hydrogen sulphide. It occurs as an energy reserve in the form of spherical droplets in certain sulphur bacteria.

C. Nucleoids. In bacteria the nuclear material includes a single, circular and double stranded DNA molecule which is often called **bacterial chromosome**. It is not separated from the cytosol by the nuclear membranes as it occurs in the eukaryotic cells. However, the nuclear material is usually concentrated in a specific clear region of the cytoplasm, called **nucleoid**. A nucleoid has no ribosome and nucleolus. The bacterial chromosome is permanently attached to the plasma membrane at one point, and when isolated often carries a number of membrane component with it. Bacterial chromosome does not contain histone proteins, however, chromosomes of some species are found to contain small quantities of a small heat-stable (HU) proteins that may be analogous to eukaryotic histones.

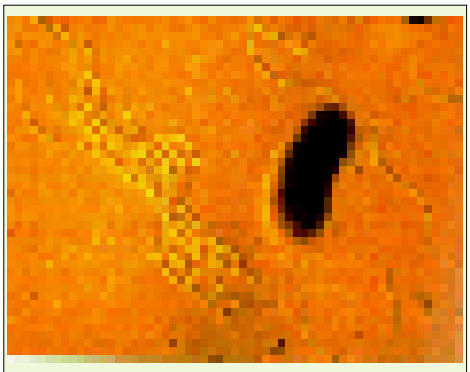
All three classes of RNA (*i.e.*, mRNA, tRNA, and rRNA) are formed (transcribed) by the activity of the single RNA polymerase (RNAP) species in prokaryotes. The messenger RNA formed at the chromosome is directly available for translation without processing, and so ribosomes may attach to the beginning of the mRNA strand and commence translation, while the other end of the mRNA is still being formed by transcription from DNA. Proteins for use within the cell are synthesized at cytoplasmic ribosomes; but ribosomes responsible for the synthesis of membrane proteins or proteins destined for export from the cell to form either the cell wall or secretory products, are attached to the plasma membrane. The resulting exportable polypeptides are ejected directly into or through the membrane as they are formed.

Plasmids. Many species of bacteria may also carry extrachromosomal genetic elements in the form of small, circular and closed DNA molecules, called **plasmids**. Some plasmids are merely **bacteriophage (viral) DNA** which may alternatively be incorporated within the chromosome. Other plasmids may be separated parts of the normal genome from the same or a foreign cell, and may recombine with the main chromosome. One function of some of these plasmids (called **colcinogenic factors**) is the production of antibiotically active proteins or **colicins** which inhibit the growth of other strains of bacteria in their vicinity. Some plasmids may act as **sex** or **fertility factors** (**F factor**) which stimulate bacterial conjugation. **R factors** are also plasmids which carry genes for the resistance to one or more drugs such as chloramphenicol, neomycin, penicillin, streptomyocin, sulphonamides and tetracyclines.



TEM of plasmids in bacterial cytoplasm.

D. Flagella and other structures. Many bacteria (*e.g.*, *E. coli*). are motile and contain one or more **flagella** for the cellular locomotion (swimming). Bacterial flagella are smaller than the eukaryotic flagella (*i.e.*, they are 15 to 20 nm in diameter and up to 20 µm long) and are also simpler in organization. A bacterial flagellum consists of a helical tube containing a single type of protein subunit, called **flagellin**. The flagellum is attached at its base, by a short flexible **hook** that is rotated, like a propeller of ship, by the flagellar rotatory "**motor**" (*i.e.*, basal body; Fig. 3.12). The flagellar motor comprises four distinct parts : rotor (M ring), stator, bearing (S ring) and rod. The '**rotor**' is a protein disc integrated into the plasma membrane.



The bacterial flagellum.

It is driven by energy stored in the transmembrane proton H^+ gradient (not by ATP breakdown; see **Jones**, 1986) and rotates rapidly (~ 100 revolutions/second) in the lipid bilayer against another protein disc, called the '**stator**'. A **rod** links the 'rotor' to a hook and flagellum, thereby causing them to rotate. The protein "**bearing**" serves to seal the outer membrane of the cell wall as the rotating rod passes through it. The `stator' and `bearing' remains stationary (**Berg**, 1975; **Adler**, 1976).

According to the number and arrangement of the flagella in a bacterial cell, following four types of flagellation patterns have been recognized : **(1) Monotrichous**. There is a single flagellum at one pole of the cell. **(2) Lophotrichous**. There are several flagella at one pole. **(3) Amphitrichous**. The cell bears at least one flagellum at each pole. **(4) Peritrichous**. There are flagella all over the surface of cell (Fig. 3.10). Flagella-like **axial filaments** are the characteristics of some spirochetes which move like snakes through the environment. The axial filaments do not project away from the cell but are wrapped around the cell surface.

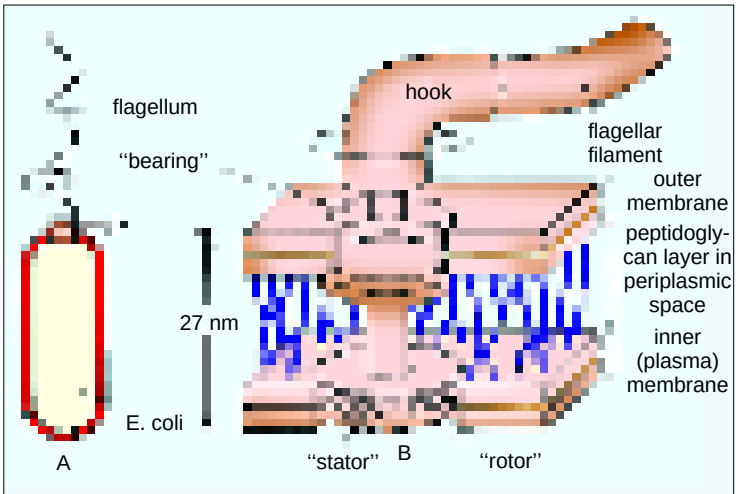
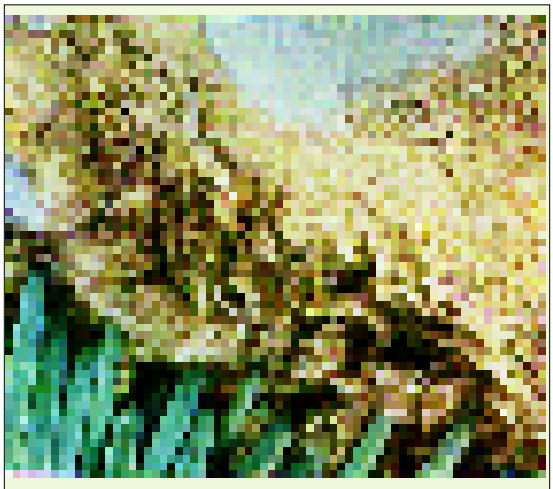


Fig. 3.12. Schematic drawing of the flagellar rotatory 'motor' of *E. coli* (after Alberts *et al.*, 1989).

Fimbriae or pili. Some bacteria (mostly Gram negative bacilli) contain non-flagellar, extremely fine, appendages called **fimbriae** (**Dugid** *et al.*, 1955) or **pili** (singular **pilus**; **Brinton**, 1959). Pili are non- motile but adhesive structures. They enable the bacteria to stick firmly to other bacteria, to a surface or to some eukaryote such as mold, plant and animal cells including red blood cells and epithelial cells of alimentary, respiratory and urinary tracts. Pili help in conjugation (*e.g.*, long F-pili or **sex pili** of male bacteria); in the attachment of pathogenic bacteria to their host cells (*e.g.*, attachment of gonorrhea- causing coccus, *Neisseria gonorrhoeae*, to the epithelial cells of the human urinary tract) and in acting as specific sites of attachment for the bacteriophages. Pili are known to be coded by the genes of the plasmid.

Spinae. Some Gram positive bacteria have tubular, pericellular and rigid appendages of single protein moiety, called **spinin**. They are called **spinae** and are known to help the bacterial cells to tolerate some environmental conditions such as salinity, pH, temperature, etc.

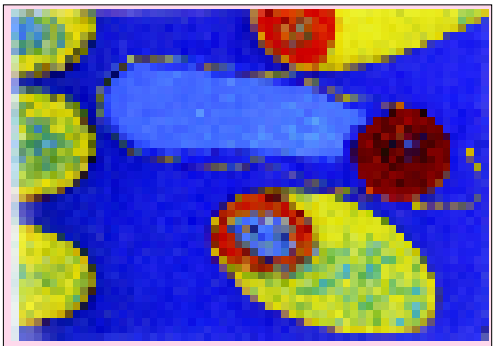
5. Nutrition in bacteria. Bacteria show wide diversity in their nutrition. Some are chemosynthetic, some are photosynthetic, but most of them are heterotrophic. Heterotrophic bacteria are mostly either saprophytic or parasitic. Parasitic bacteria live on the body of plants and animals and with few exceptions, most bacteria are pathogenic.



Bacteria inhabiting mouth – These bacteria possess a slime layer that allows them to cling to tooth enamel, where they can cause tooth decay unless they are removed by their chief antagonist, a toothbrush (seen here as green bristles).

Modes of respiration of bacteria are both aerobic and anaerobic. Some of the end products of bacterial anaerobic respiration are useful to man, so, they are used in the manufacture of various foods such as butter, cheese and vinegar. *Pseudomonas* is a gram negative heterotrophic aerobic form which can decompose (biodegrade) a wide variety of organic compounds such as hydrocarbons. So it is used in reducing water pollution due to petroleum spillage.

6. Reproduction in bacteria. Bacteria reproduce asexually by **binary fission** and **endospore** formation and sexually by **conjugation**. In the binary fission, the cell divides into two genetically identical daughter cells. During this process, the single circular chromosome first makes a copy of itself (*i.e.*, it duplicates) and daughter chromosomes become attached to the plasma membrane. They separate as the bacterial cell enlarges and ultimately the formation of a cross wall between the separating daughter chromosomes, divides the parent cell into two daughter cells.



Resistant endospores, here coloured red, have formed inside bacteria of the germs (*clostridium*).

Under unfavourable ecological conditions, many bacteria (*e.g.*, *Clostridium*, *Bacillus*, etc.) form spores which are not reproductive units but represent an inactive state. In endospore formation, a part of the protoplasmic material is used to form an impermeable coat or cyst wall around the chromosome along with some cytoplasm. The rest of the cell degenerates. The spore being metabolically inert can survive an unsuitable temperature, pH and drought. Under favourable conditions, spores imbibe water, become metabolically active again and germinate.

TEM of *E.coli* bacteria conjugating.

Bacterial conjugation is simplest form of sexual reproduction known. It was first of all observed in *E.coli* by **Laderberg** and **Tatum** in 1946. During the process of conjugation, a F^+ or **donor** bacterium (equivalent to male) passes a piece of DNA or plasmid containing **fertility** or **F gene** to the F^- or **recipient** bacterium (equivalent to female). The donor's plasmid passes through the sex pilus of donor cell to the recipient. Following the conjugation, the progenies of the recipient express some of the characteristics of the donor. Thus, bacterial conjugation is a means of making new genetic combinations or **recombinations** which are expressed in the progeny.

Examples of Prokaryotic Cells

The following three types of prokaryotic cells are well studied ones :

1. Mycoplasma or PPLO. Among living organisms that have the smallest mass, are small bacteria called **mycoplasmas** which produce infectious diseases in animals including humans. Mycoplasmas are unicellular, prokaryotic, containing a plasma membrane, DNA, RNA and a metabolic machinery to grow and multiply in the absence of other cells (*i.e.*, they are capable of autonomous growth). They can be cultured *in vitro* like any bacteria, forming **pleomorphic** (Gr., *pleo* = many; *morphe* = forms) **colonies**, *i.e.*, depending on the type of culture medium, mycoplasmas tend to form different shaped colonies such as spheroid (fried - egg-shaped), thin, branching filaments, stellate, asteroid or irregular. They differ from the bacteria in the following respects :

1. Mycoplasmas are filterable through the bacterial filters (this fact was first demonstrated by **Iwanowsky** in 1892).

2. They do not contain cell wall and mesosomes.

3. Like the viruses and animal cells, they are resistant to antibiotics such as penicillin which kills bacteria by interfering with cell wall synthesis (see **Ambrose** and **Easty**, 1979).

4. Their growth is inhibited by tetracyclines and similar antibiotics that act on metabolic pathways.

Mycoplasmas were discovered by French scientists, **E. Nocard** and **E.R. Roux** in 1898 while studying pleural fluids of cattle suffering from the disease **pleuropneumonia** (*i.e.*, an infectious disease of warm blooded animals producing pleural and lung inflammation). Similar organisms were later isolated from other animals such as sheep, goats, dogs, rats, mice and human beings and were named as **pleuropneumonia - like organisms** (**PPLO**). PPLO were later on included under the genus *Mycoplasma* by **Nowak** (1929) and these organisms are now commonly called **mycoplasmas**. **W.V.Iterson** (1969) has placed PPLO in the group **Mycoplasmatceae** of bacteria. Currently mycoplasmas are considered as the simplest bacteria (see **Alberts** *et al.*, 1989). However, some cell biologists still prefer to place PPLO in between the viruses and bacteria (see **Sheeler** and **Bianchi**, 1987).

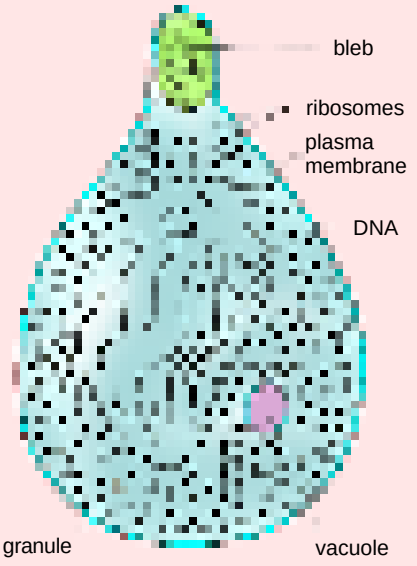
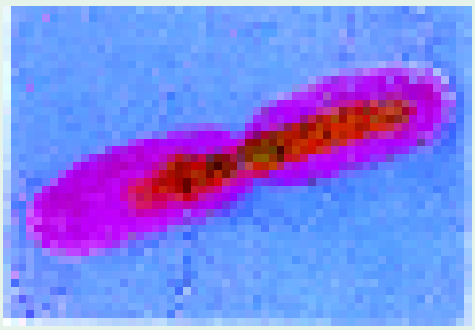


Fig 3.13. A schematic diagram of typical PPLO cell.

Mycoplasmas are mostly free-living, saprophytes or parasites. For example, *Mycoplasma laidlawii* (0.1 µm in diameter) is saprophytic and is found in sewage, compost, soil, etc. *Mycoplasma gallisepticum* (0.25 µm in diameter) is parasitic and pathogenic; it is the parasite of cells and cell exudates of respiratory organs of warm-blooded animals causing in them various chronic respiratory diseases.

Mycoplasmas range in size (diameter) from 0. 25 to 0.1 µm. Thus, they correspond in size to some of the large viruses. The spherical cell of a mycoplasma is bounded at its surface by a 75 A^0 thick plasma membrane which is composed of molecules of proteins and lipids, but there is no cell wall. Internally the cell's composition is more or less diffuse. The only microscopically discernible features within the cell are its genetic component, the DNA and the ribosomes. The DNA molecule is contained in a membraneless and clear nucleus-like region and it is a double helix which may exist either as the linear strands or a single circular molecule. The nuclear region is surrounded by numerous (50 to 100) 70S type ribosomes existing either freely or in the polysomes (Fig. 3.13). A variety of other cytoplasmic inclusions, such as vacuoles and granules, have also been detected, but their functions are not known. At one side of the cell occurs **bleb** (localised collection of fluid) of ill understood function.As in other prokaryotes, there is no intracellular membranous structure.

The PPLO cells contain many enzymes which may be required for DNA replication, the transcription of different kinds of RNA molecules and translation involved in protein synthesis, and also in the biosynthesis of adenosine triphosphate (ATP) by anaerobic breakdown of sugars. Unlike viruses, they are free living and do not require host cells for their duplication. PPLO reproduce by binary fission, budding, formation of small spore-like bodies and by growth of large branched filaments that ultimately fragment.



E.coli as seen in a electronmicrograph.

2. *Escherichia coli.* *E. coli* is a Gram negative, monotrichous, symbiotic bacillus of colon of human beings and other vertebrates. It is heterotrophic and non-pathogenic bacteria producing some vitamins (*e.g.*, vitamin K) for human use. Some strains of *E. coli* are known to recognise and bind specifically to

sugar-containing target cells on the surface of gut lining of mammals (*e.g.*, D-mannose residues of epithelial cells of human gut or colon; **King**, 1986). *E. coli* is one of the best studied bacteria. It has served well in the field of molecular biology, since this bacterium is particularly easy to grow in an artificial medium where it divides every 20 minutes at 37^0C under optimal conditions. Thus, a single cell become 10^9 bacteria in about 20 hours.

The prokaryotic cell of *E. coli* (Fig. 3.14) is about 2µm long and 1µm wide. The cytoplasm of the bacterium is bounded by a typical fluid mosaic **plasma membrane.** External to the plasma membrane occurs the rigid and protective **cell wall** which has a complex organization; it comparises following two structures :

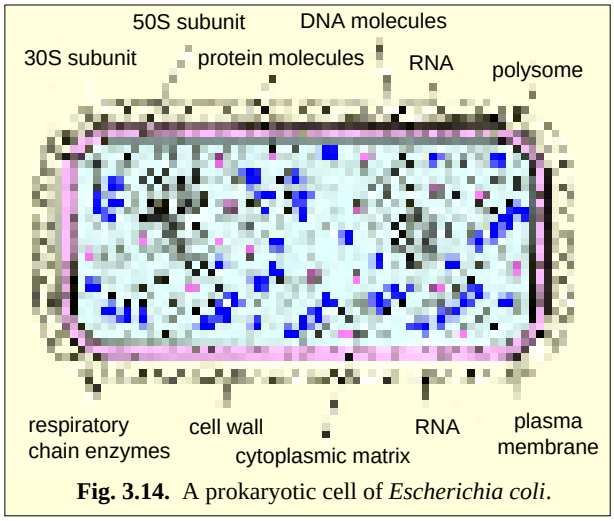


Fig. 3.14. A prokaryotic cell of *Escherichia coli*.

1. External membrane which is a lipid bilayer traversed by numerous **porin channels** that allow the diffusion of solutes. Each porin channel is formed by 6 to 8 subunits, each having three suspended hydrocarbon chains (Fig. 3.15). The porin is a polypeptide and it spans the full thickness of outer membrane. 2. Both membranes–the plasma membrane and external membrane of the cell wall – are separated by the **periplasmatic space.** This space contains a grid or reticulum of peptidoglycans. Some porin subunits remain attached to the peptidoglycan grid (Fig. 3.16).

The plasma membrane serves as a molecular barrier with the surrounding medium. It comprises a variety of transport proteins, called **permeases** which control the entrance and exit of small molecules and ions. It contributes to the establishment of bacterial protoplasm. *E. coli* has both oxygen-requiring (aerobic) and non-oxygen-requiring (anaerobic) respiratory machinery for the breakdown of sugar and contains a special group of proteins called the **electron transport chain** for the generation of stored energy in the form of ATP molecules. *E.coli* lacks mitochondria, and respiratory chain enzymes such as cytochromes, enzymes of Krebs cycle, NADH, acid phosphatase, etc.,are attached to inner face of the plasma membrane.

All genes of *E.coli* are contained on a single supercoiled, double-stranded, circular **DNA molecule**, which occurs in a clear zone of cytoplasm, called **nucleoid**, and is attached to the plasma membrane at one point. The total length of the DNA circle is about 1300 µm, comprising about 4.7 x 10^6 nucleotide pairs; this is enough DNA to code for about 4000 different proteins (see **Alberts** *et al.*, 1989). The DNA of *E.coli* is naked, lacking histones, but certain polyamines may be bound to some of its phosphates. Electron microscopy of isolated chromosome of *E. coli* has shown that DNA is folded into a series of **looped domains**, *i.e.*, about 45 loops radiate out from a dense proteinaceous **scaffold**. The DNA of loops is in the so-called supercoiled conformation in

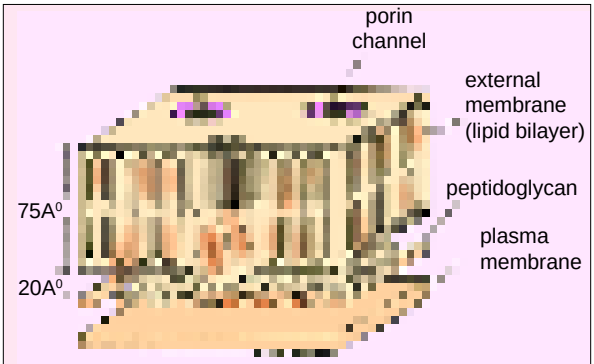
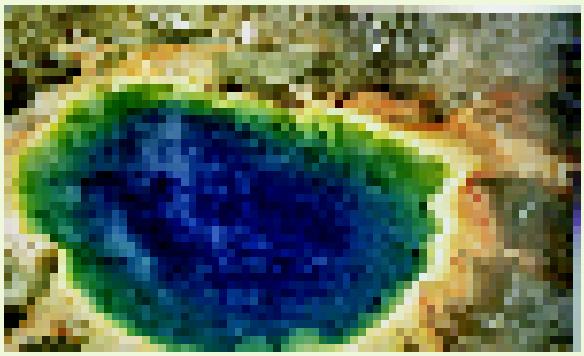


Fig. 3.15. Ultrastructure of the cell wall of a Gram-negative bacterium (after De Robertis and De Robertis, Jr., 1987).

which the double helix is itself twisted (**Schmid**, 1988). The enzyme **DNA gyrase** is responsible for the DNA supercoiling (it is inhibited by the drug called **coumermycin**; see **Freifelder**, 1985).

The colloidal cytoplasmic matrix of *E. coli* contains about 5000 distinguishable components, ranging from water to DNA (*i.e.*, three types of RNA, enzymes, glycogen, amino acids, monosaccharides and various other small molecules). Surrounding the DNA is dark dense region of matrix containing 20,000 to 30,000 70S type **ribosomes**, each existing in the form of their two subunits. During protein synthesis numerous complete ribosomes read the codes of mRNA molecules to form the polysomes (Fig. 3.14)

Cyanobacteria living inside the hairs of these polar bears are responsible for the unusual greenish colour of their coat.



Some bacteria thrive in extreme conditions like this hot spring.

3. Cyanobacteria or blue-green algae. The Gram-negative cyanobacteria or oxyphotobacteria (*i.e.*, oxygen yielding photosynthetic blue green algae) are one of the most successful and primitive (3.5 billion year old) groups of organisms on earth. They even inhabit the steaming hot springs and the undersides of icebergs.

Cyanobacteria form another group of prokaryotes which include about 1500 species (85 genera and 750 species are found in India; see **Sharma**, 1992).

Cyanobacteria occur as individual cells, as small clusters or colonies of cells, or as long, filamentous chains. They lack flagella but are able to perform movement by rotatory motion or gliding over a gelatinous layer secreted through the cell surface.

A typical cell of a blue green alga is composed of outer cellular coverings and cytoplasm. The **outer cellular coverings** include an outermost **gelatinous** or **slimy layer**, **the capsule**, a middle **cell wall** and an innermost lipoproteinous **plasma membrane.** The cell wall of blue green algae resembles the cell wall of bacteria and contains an **outer bimolecular membrane** of phospholipids, lipoproteins and lipopolysaccharides, and a grid of peptidoglycans (muramic acid) in the **periplasmatic space** existing in between cell

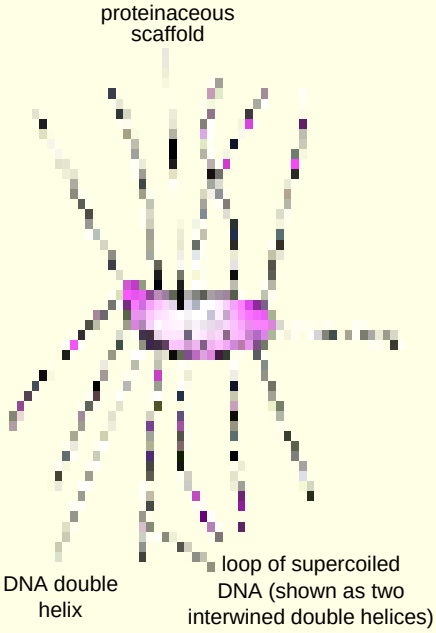


Fig. 3.16. Schematic representation of the chromosome of *E.coli*, showing only 12 of the 45 supercoiled loops (after King, 1986).

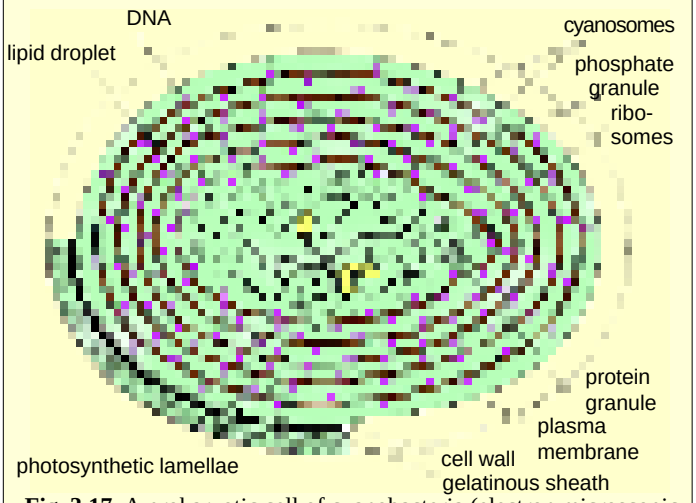
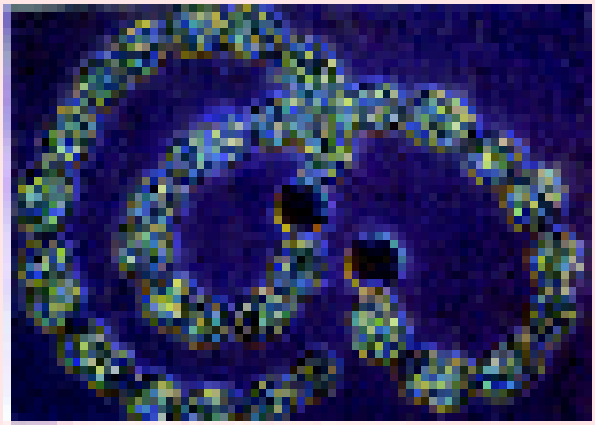


Fig. 3.17. A prokaryotic cell of cyanobacteria (electron microscopic view).

wall and plasma membrane. The **cytoplasm** of cyanobacteria appears more organized than that of other bacteria. The matrix extends throughout the cell. The cytoplasm (or protoplast) is differentiated in two regions : 1 Outer or peripheral pigmented region, the **chromoplasm** having photosynthetic lamellae or **thylakoids**. 2. Inner or central colourless region called **centroplasm** or **DNA plasm** having DNA and crystalline granules (Fig. 3.17).

Because the metabolism of the blue green algae is based on photosynthesis, therefore, the cells of them contain the photosynthetic pigment, *viz*., the **chlorophyll** and **carotenoid**. In addition to these pigments, these algae contain certain unique pigments collectively called **phycobilin**; one of the phycobilin is blue and called **phycocyanin**, while other type of phycobilin is red and called **phycoerythrin**. The photosynthetic pigments (chlorophylls and carotenoids) occur in flattened sacs called **lamellae** which remain arranged in parallel array. In between the lamellae occur certain granules of 400A^0 diameter. These granules contain phycobilin pigments and are called **cyanosomes** or **phycobilisomes**. They are attached to the outer lamellar membrane surface (**Berns**, 1983). Being earliest oxygenic photosynthesizers of earth, they made early earth's atmosphere aerobic providing the conditions favourable for the evolution of aerobic bacteria and eukaryotes.

The two subunits of 70S ribosomes of cyanobacteria are freely distributed in the cytoplasm and form polyribosomes during protein synthesis. As in all prokaryotes, the DNA molecule of blue green algae is circular, double- stranded helix and occurs in the centroplasm. This area (nucleoid) is not bound by the nuclear membrane and it does not contain a nucleolus.

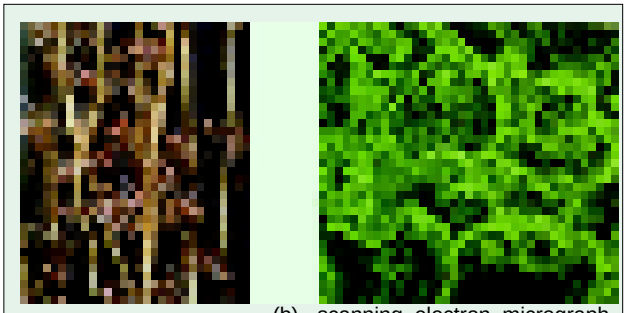


Nostoc - a cyanobacteria.

Cyanobacteria also contain a variety of **inclusions** in its cytoplasm. Membrane-bound inclusions are the gas vacuoles and the carboxysomes. **Gas vacuoles** are gas-filled cavities which are located in the inner part of chromatoplasm. They occur commonly in planktonic species such as *Nostoc, Anabaena, Phormidium, Calothrix, Galaeotrichia,* etc. Gas vacuoles serve the function of flotation or buoyancy. **Carboxysomes** contain enzymes involved in carbon dioxide fixation.

The cytoplasm of blue green algae also contains a variety of membrane-free inclusions such as **(1) cyanophycin granules** which are located in chromatoplasm and are protein storage products,

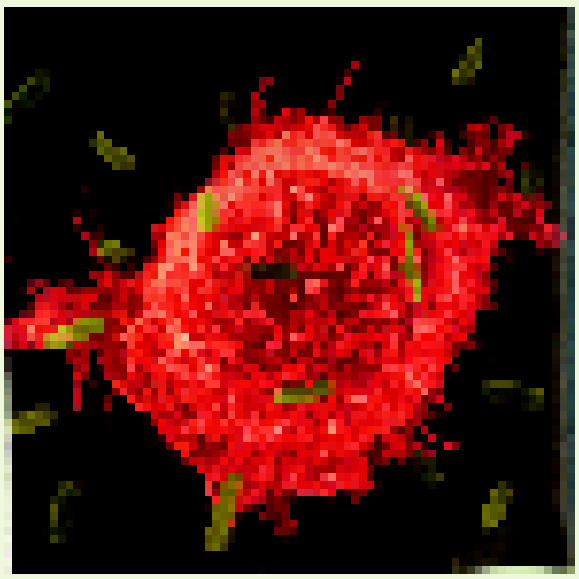
containing large amount of arginine amino acid (**Fogg**, 1951) or copolymers of alanine and aspartic acid (**Simon**, 1971); **(2) myxophycean starch** which is the main food storage compound; (3) **polyglucon granules,** polyhedral bodies, lipid droplets, polyphosphate bodies etc., are some other cytoplasmic inclusions of cyanobacteria.



(a) Root-nodules of a leguminous plant. (b) scanning electron micrograph shows the nitrogen-fixing bacteria inside cells within the nodules.

Lastly, many cyanobacteria (about 20 species) tend to fix atmospheric nitrogen as ammonia, *e.g.*, *Anabaena, Nostoc, Mastigocladus,* etc. Under aerobic condition nitrogen fixation is done principally in special type of cells called **heterocysts,** as in *Nostoc* (**Donze**, 1971; **Carr**, 1976).

EUKARYOTIC CELLS



The white blood cell (in red) is eukaryotic and the bacterial cells (in green *E.coli)* are prokaryotic.

The eukaryotic cells (Gr., *eu*=good, *karyotic*=nucleated) are essentially **two envelope systems** and they are very much larger than prokaryotic cells. Secondary membranes envelop the nucleus and other internal organelles and to a great extent they pervade the cytoplasm as the endoplasmic reticulum. The eukaryotic cells are the true cells which occur in the plants (from algae to angiosperms) and the animals (from Protozoa to mammals). Though the eukaryotic cells have different shape, size, and physiology; all the cells are typically composed of plasma membrane, cytoplasm and its organelles, *viz.*, mitochondria, endoplasmic reticulum, ribosomes, Golgi apparatus, etc., and a true nucleus. Here the nuclear contents, such as DNA, RNA, nucleoproteins and nucleolus remain separated from the cytoplasm by the thin, perforated nuclear membranes. Before going into the details of cell and its various components, it will be advisable to consider the general features of different types of eukaryotic cells which are as follows:

Cell Shape

The basic shape of the eukaryotic cell is **spherical**, however, the shape is ultimately determined by the specific function of the cell. Thus, the shape of the cell may be **variable** (*i.e.*, frequently changing the shape) or **fixed**. Variable or irregular shape occurs in *Amoeba* and white blood cells or leucocytes (In fact, leucocytes are spherical in the circulating blood, but in other conditions they may produce pseudopodia and become irregular in shape). Fixed shape of the cell occurs in almost all protists (*e.g., Euglena, Paramecium*), plants and animals. In unicellular organisms the cell shape is maintained by tough plasma membrane and exoskeleton. In a multicellular organism, the shape of the cell depends mainly on its functional adaptations and partly on the surface tension, viscosity of the protoplasm, cytoskeleton of microtubules, microfilaments and intermediate filaments, the mechanical

action exerted by adjoining cells and rigidity of the plasma membrane (*i.e.*, presence of rigid cell wall in plant cells). The shape of the cell may vary from animal to animal and from organ to organ. Even the cells of the same organ may display variations in the shape. Thus, cells may have diverse shapes such as **polyhedral** (with 8, 12 or 14 sides; *e.g.*, squamous epithelium); **flattened** (*e.g.*, squamous epithelium, endothelium and the upper layers of the epidermis); **cuboidal** (*e.g.*, in thyroid gland follicles); **columnar** (*e.g.*, the cells lining the intestine); **discoidal** (*e.g.*, red blood cells or erythrocytes);**spherical** (*e.g.*, eggs of many animals); **spindle shaped** (*e.g.*, smooth-muscle fibres); **elongated** (*e.g.*, nerve cells or neurons); or **branched** (*e.g.*, chromatophores or pigment cells of skin). Among plants, the cell shape also depends upon the function of the cell. For example, cells such as glandular hairs on a leaf, the guard cells of stomata and root hair cells have their special shape.

Cell Size

The eukaryotic cells are typically larger (mostly ranging between 10 to 100 µm) than the prokaryotic cells (mostly ranging between 1 to 10 µm). Size of the cells of the unicellular organisms is larger than a typical multicellular organism's cells. For example, *Amoeba proteus* is biggest among the unicellular organisms; its length being 1 mm (1000 µm). One species of *Euglena* is found up to 500 µm (0.5 mm) in length. *Euplotes* (a freshwater ciliate) is 120 µm in length. Another ciliate, *Paramecium caudatum* is from 150 to 300 µm (0.15 to 0.3 mm) in length. Diatoms have a length of 200 µm or more. The single-celled alga, *Acetabularia* which consists of a stalk and a cap is exceptionally large-sized and measures up to 10 cm in height.

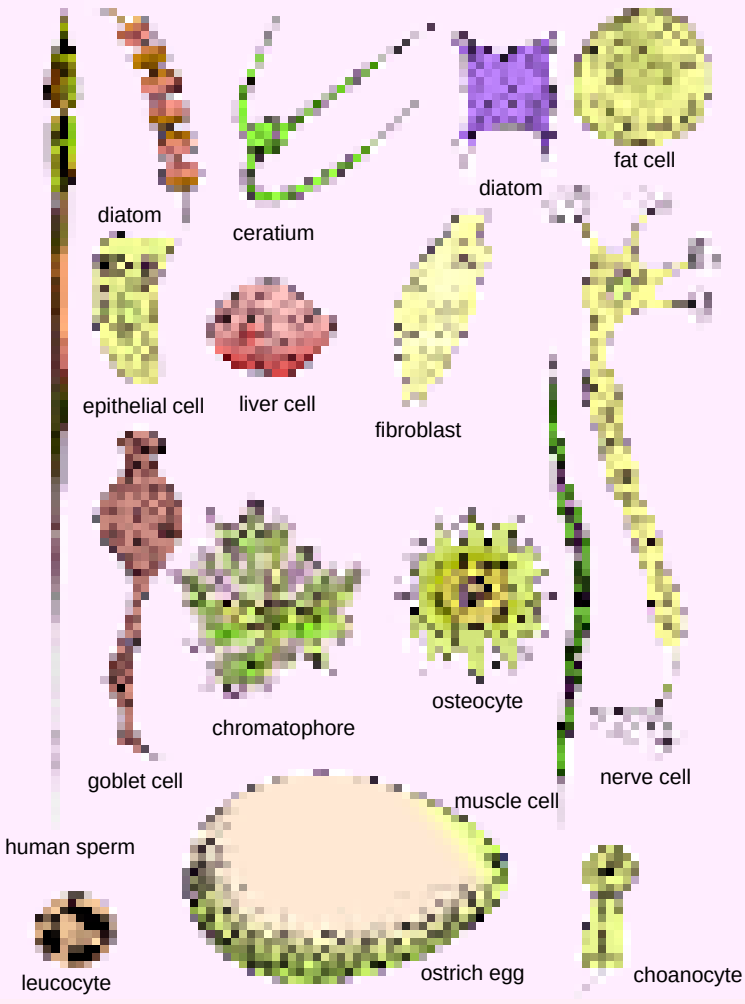


Fig. 3.18. Various types of eukaryotic cells showing different shapes.

The size of the cells of multicellular organisms ranges between 20 to 30 µm. Among animals, the smallest cells have a diameter of 4 µm (*e.g.*, polocytes); human erythrocytes being 7 to 8 µm in diameter. Largest animal cell is the egg of ostrich, having a diameter of 18 cm (its yolk or deutoplasm is about 5 cm in diameter); though, some nerve cells of human beings have a meter

long "tails" or axons. Among the multicellular plants, the largest cell is the ovule of *Cycas* (see **Dnyansagar**, 1988). The fibre cells (*i.e.*, sclerenchyma cells) of Manila hemp are over 100 cm in length.

Cell Volume

The volume of a cell is fairly constant for a particular cell type and is independent of the size of the organism. (This is called the **law of constant volume**.) For example, kidney or liver cells are about the same size in the bull, horse and mouse. The difference in the total mass of the organ or organism depends on the number, not on the volume of the cells. Thus, the cells of an elephant are not necessarily larger than those of other tiny animals or plants. The large size of the elephant is due to the larger number of cells present in its body.

If a cell is to be efficient, the ratio of volume to surface should be within a limited range. An increase in cell volume is accompanied by a much smaller expansion in the surface area of the cell (In fact, volume increases as cube of radius, while surface area increases as square of radius). In other words, a large cell has a proportionately smaller surface and a higher volume : surface ratio than a smaller cell. Further, a large cell volume has to accommodate many organelles simultaneously limiting the exchange of information and materials through the surface. This problem is partially overcome by developing a cylindrical shape or by forming numerous extensions (*e.g.*, microvilli) of the plasma membrane. It is also for this reason that metabolically active cells, tend to be smaller in size.

Cell Number

The number of cells present in an organism varies from a single cell in a **unicellular organism** (Protists such as protozoa and protophyta) to many cells in multicellular organisms (Most plants, fungi and animals). The number of cells in the multicellular organisms usually remains correlated with size of the organisms and, therefore, small-sized organism has less number of cells in comparison to large-sized organisms. For example, a human being weighing about 80 kg may contain about 60 thousand billion cells in his body. This number would be more in certain other multicellular organisms.

Further, the number of cells in most multicellular organisms is indefinite, but the number of cells may be fixed in some multicellular organisms. For example, in rotifers, number of nuclei in the various organs are found to be constant in any given species. This phenomenon of cells or nuclear constancy is called **eutely**. In one species of rotifer, **Martini** (1912) always found 183 nuclei in the brain, 39 in the stomach, 172 in the cornea epithelium, and so on (see **Hickman**, **Sr**., *et al.* 1979). Among plants, colonial green algae exhibit cell constancy. For example, the green alga, *Pandorina* has a colony consisting of 8, 16 or 32 cells. Likewise, another green alga, *Eudornia*, has 16, 32 or 64 cells in its colony.

Structure

An eukaryotic cell consists of the following components : A. Cell wall and plasma membrane; B. Cytoplasm; and C. Nucleus.

Fig. 3.19 and Fig. 3.21 respectively show the ultrastructure or finer details of a typical animal cell and a typical plant cell which have been revealed by the electron microscope.

Cell Wall and Plasma Membrane

1. Cell wall. The outermost structure of most plant cells is a dead and rigid layer called **cell wall**. It is mainly composed of carbohydrates such as cellulose, pectin, hemicellulose and lignin and certain fatty substances like waxes. Ultrastructurally cell wall is found to consist of a microfibrillar network lying in a gel-like matrix. The microfibrils are mostly made up of cellulose. There is a pectin-rich cementing substance between the walls of adjacent cells which is called **middle lamella**. The cell wall which is formed immediately after the division of cell, constitutes the **primary cell wall**. Many kinds of plant cells have only primary cell wall around them. Primary cell wall is composed of pectin,

hemicellulose and loose network of cellulose microfibrils. In certain types of cells such as phloem and xylem, an additional layer is added to the inner surface of the primary cell wall at a later stage. This layer is called **secondary cell wall** and it consists mainly of cellulose, hemicellulose and lignin. In many plant cells, there are tunnels running through the cell wall called **plasmodesmata** which allow communication with the other cells in a tissue.

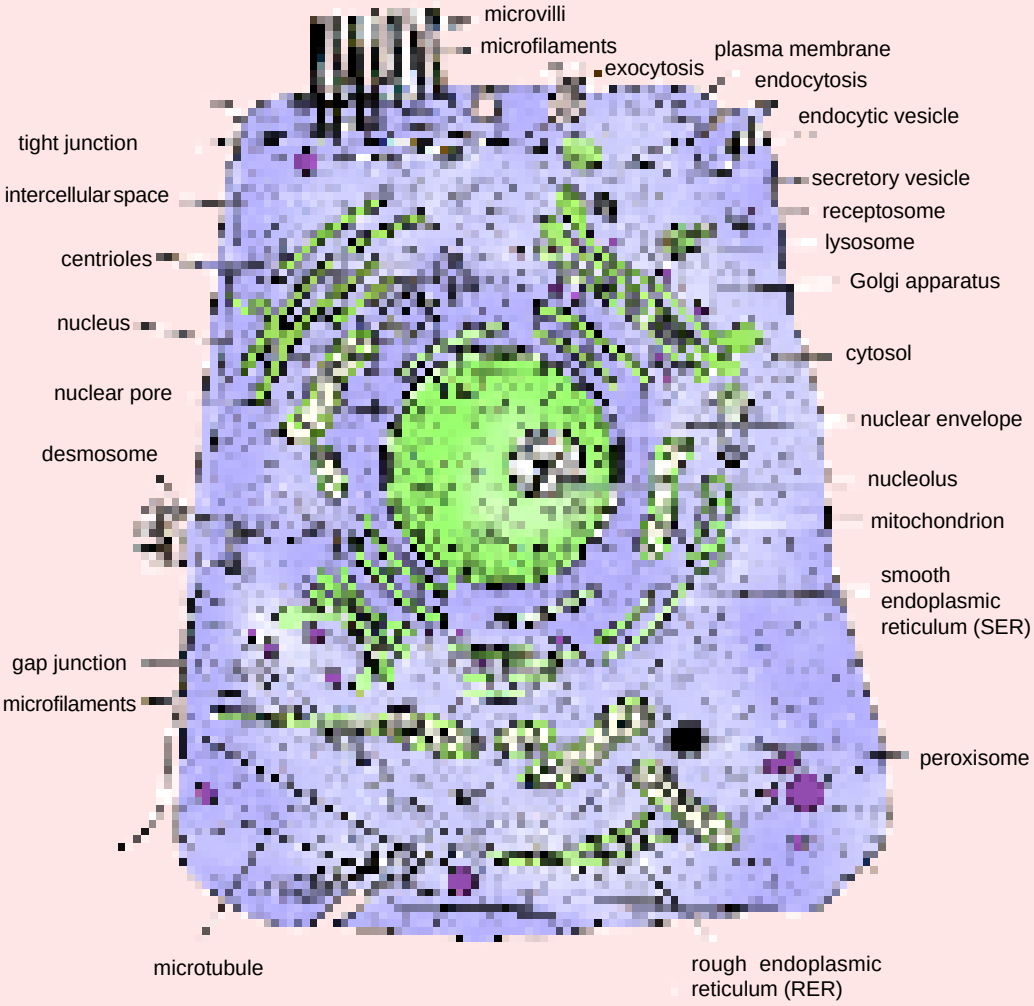


Fig. 3.19. Ultrastructure of a typical animal cell as seen in the electron microscope.

The cell wall constitutes a kind of exoskeleton that provides protection and mechanical support to the plant cell. It determines the shape of plant cell and prevents it from desiccation.

2. Plasma membrane. Every kind of animal cell is bounded by a living, extremely thin and delicate membrane called **plasmalemma, cell membrane** or **plasma membrane**. In plant cells, plasma membrane occurs just inner to cell wall, bounding the cytoplasm. The plasma membrane exhibits a tri-laminar (*i.e.*, three-layered) structure with a translucent layer sandwiched between two dark layers. At molecular level, it consists of a continuous bilayer of lipid molecule (*i.e.*, phospholipids and cholesterol) with protein molecules embedded in it or adherent to its both surfaces. Some carbohydrate molecules may also be attached to the external surface of the plasma membrane, they

remain attached either to protein molecules to form **glycoproteins** or to lipids to form **glycolipids**. The plasma membrane is a **selectively permeable membrane**; its main function is to control selectively the entrance and exit of materials. This allows the cell to maintain a constant internal environment (**homeostasis**). Transport of small molecules such as water, oxygen, carbon dioxide, ethanol, ions, glucose, etc., across the plasma membrane takes place by various means such as osmosis, diffusion and active transport. The process of active transport is performed by special type of protein molecules of plasma membrane called **transport proteins** or **pumps**, consuming energy in the form of ATP molecules. For bulk transport of large-sized molecules, plasma membrane performs **endocytosis** (*i.e.*, endocytosis, pinocytosis, receptor-mediated endocytosis and phagocytosis) and **exocytosis** both of these processes also utilise energy in the form of ATP molecules.

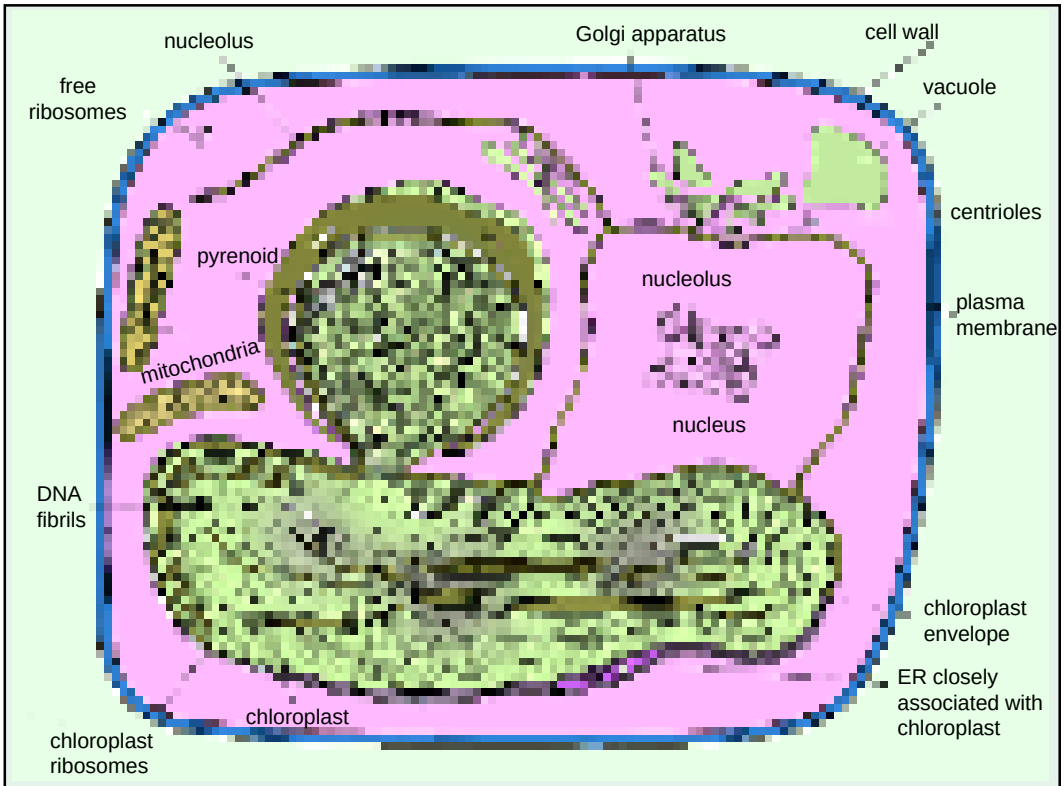


Fig. 3.20. Relationships among organelles in a hypothetical brown alga. Note that the flat sacs within the plastid are arranged in extended parallel arrays other than separated into grana and stroma systems as in higher plants.

Various cell organelles such as chloroplasts, mitochondria, endoplasmic reticulum and lysosomes are also bounded by membranes similar to the plasma membrane. All the cellular membranes have a basic trilaminar **unit membrane** construction. However, their structure and extent of activity are mainly depended on the relative proportion of their constituent protein and lipid molecules. Thus, membranes which are metabolically highly active, *e.g.*, those of mitochondria and chloroplasts have a greater proportion of proteins and more granular appearance than those membranes which are relatively less active, *e.g.*, myelin sheath of certain nerve fibres.

Cytoplasm

The plasma membrane is followed by the cytoplasm which is distinguished into following structures :

A. Cytosol. The plasma membrane is followed by the colloidal organic fluid called **matrix** or **cytosol**. The cytosol is the aqueous portion of the **cytoplasm** (the extranuclear protoplasm) and of the **nucleoplasm** (the nuclear protoplasm). It fills all the spaces of the cell and constitutes its true **internal milieu**. Cytosol is particularly rich in differentiating cells and many fundamental properties of cell are because of this part of the cytoplasm. The cytosol serves to dissolve or suspend the great variety of small molecules concerned with cellular metabolism, *e.g.*, glucose, amino acids, nucleotides, vitamins, minerals, oxygen and ions. In all type of cells, cytosol contains the soluble proteins and enzymes which form 20 to 25 per cent of the total protein content of the cell. Among the important soluble enzymes present in the matrix are those involved in glycolysis and in the activation of amino acids for the protein synthesis. In many types of cells, the cytosol is differentiated into following two parts : (i) **Ectoplasm** or **cell cortex** is the peripheral layer of cytosol which is relatively non-granular, viscous, clear and rigid. (ii) **Endoplasm** is the inner portion of cytosol which is granular and less viscous.

Cytoskeleton and microtrabecular lattice. The cytosol of cells also contains **fibres** that help to maintain cell shape and mobility and that probably provide anchoring points for the other cellular structures. Collectively, these fibres are termed as the **cytoskeleton**. At least three general classes of such fibres have been identified. 1. The thickest are the **microtubules** (20 nm in diameter) which consists primarily of the **tubulin** protein. The function of microtubules is the transportation of water, ions or small molecules, cytoplasmic streaming (cyclosis), and the formation of fibres or asters of the mitotic or meitotic spindle during cell division. Moreover, they form the structural units of the centrioles, basal granules, cilia and flagella. 2. The thinnest are the microfilaments (7 nm in diameter) which are solid and are principally formed of **actin** protein. They maintain the shape of cell and form contractile component of cells, mainly of the muscle cells. 3. The fibres of middle order are called the **intermediate filaments** (**IFs**) having a diameter of 10 nm. They having been classified according to their constituent protein such as **desmin filaments**, **keratin filaments**, **neurofilaments**, **vimentin** and **glial filaments**.

Recently, cytoplasm has been found to be filled with a three-dimensional network of interlinked filaments of cytoskeletal fibres, called **microtra-becular lattice** (**Porter** and **Tucker**, 1981). Various cellular organelles such as ribosomes, lysosomes, etc., are found anchored to this lattice. The microtrabecular lattice being flexible, changes its shape and results in the change of cell shape during cell movement.

B. Cytoplasmic structures. In the cytoplasmic matrix certain non-living and living structures remain suspended. The non-living structures are called **paraplasm** or **inclusions**, while the living structures are membrane bounded and are called **organoids** or **organelles**. Both kinds of cytoplasmic structures can be studied under the following headings :

(a) Cytoplasmic inclusions. The stored food and secretory substances of the cell remain suspended in the cytoplasmic matirx in the form of refractile granules forming the cytoplasmic inclusions. The cytoplasmic inclusions include oil drops, triacylglycerols (*e.g.*, fat cells of adipose tissue), yolk granules (or **deutoplasm**, *e.g.*, egg cells), secretory granules, glycogen granules (*e.g.*, muscle cells and hepatocytes of liver) and starch grains (in plant cells).

(b) Cytoplasmic organelles. Besides the separate fibrous systems, cytoplasm is coursed by a multitude of internal membranous structures, the organelles (literally the word organelle means a tiny organ). Membranes close off at specific regions of the eukaryotic cells performing specialized tasks : oxidative phosphorylation and generation of energy in the form of ATP molecules in mitochondria; formation and storage of carbohydrates in plastids; protein synthesis in rough endoplasmic reticulum; lipid (and hormone) synthesis in smooth endoplasmic reticulum; secretion by Golgi apparatus; degradation of macromolecules in the lysosomes; regulation of all cellular activities by nucleus; organization of spindle apparatus by centrosomes and so forth. Membrane-bound enzymes catalyze

reactions that would have occurred with difficulty in an aqueous environment. The structure and function of some important organelles are as follows:

1. Endoplasmic reticulum (ER). Within the cytoplasm of most animal cells is an extensive network (reticulum) of membrane-limited channels, collectively called **endoplasmic reticulum** (or ER). Some portion of ER membranes remains continuous with the plasma membrane and the nuclear envelope. The outer surface of **rough ER** has attached ribosomes, whereas **smooth ER** do not have attached ribosomes. Functions of smooth ER include **lipid metabolism** (both catabolism and anabolism; they synthesize a variety of phospholipids, cholesterol and steroids); **glycogenolysis** (degradation of glycogen; glycogen being polymerized in the cytosol) and **drug detoxification** (by the help of the **cytochrome P-450**; **Darnell** *et al.*, 1986).

On their membranes, rough ER (RER) contain certain ribosome-specific, transmembrane glycoproteins, called **ribophorins I** and **II**, to which are attached the ribosomes while engaged in polypeptide synthesis. As a growing secretory polypeptide emerges from ribosome, it passes through the RER membrane and gets accumulated in the lumen of RER. Here, these polypeptide chains undergo tailoring, maturation, and molecular folding to form functional secondary or tertiary protein molecules. RER pinches off certain tiny protein-filled vesicles which ultimately get fused to cis Golgi. RER also synthesize membrane proteins and glycoproteins which are cotranslationally inserted into the rough ER membranes. Thus, endoplasmic reticulum is the site of biogenesis of cellular membranes.

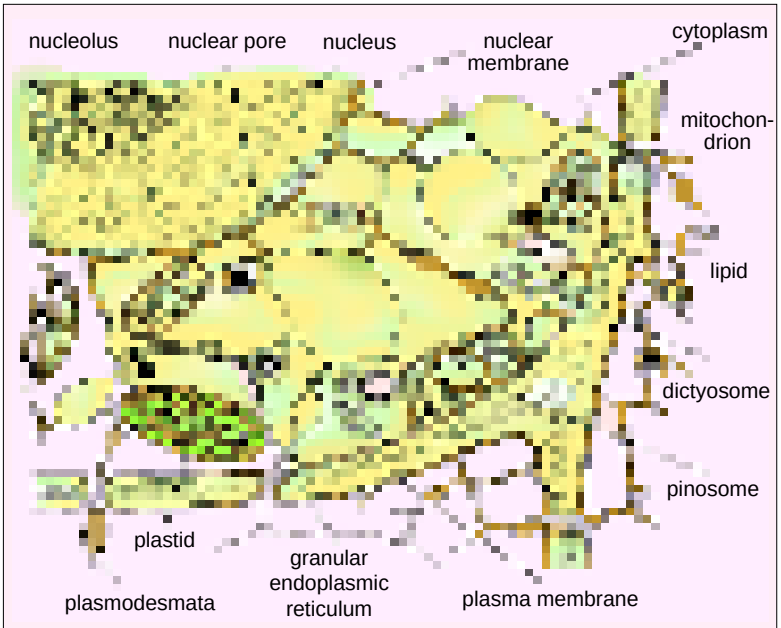


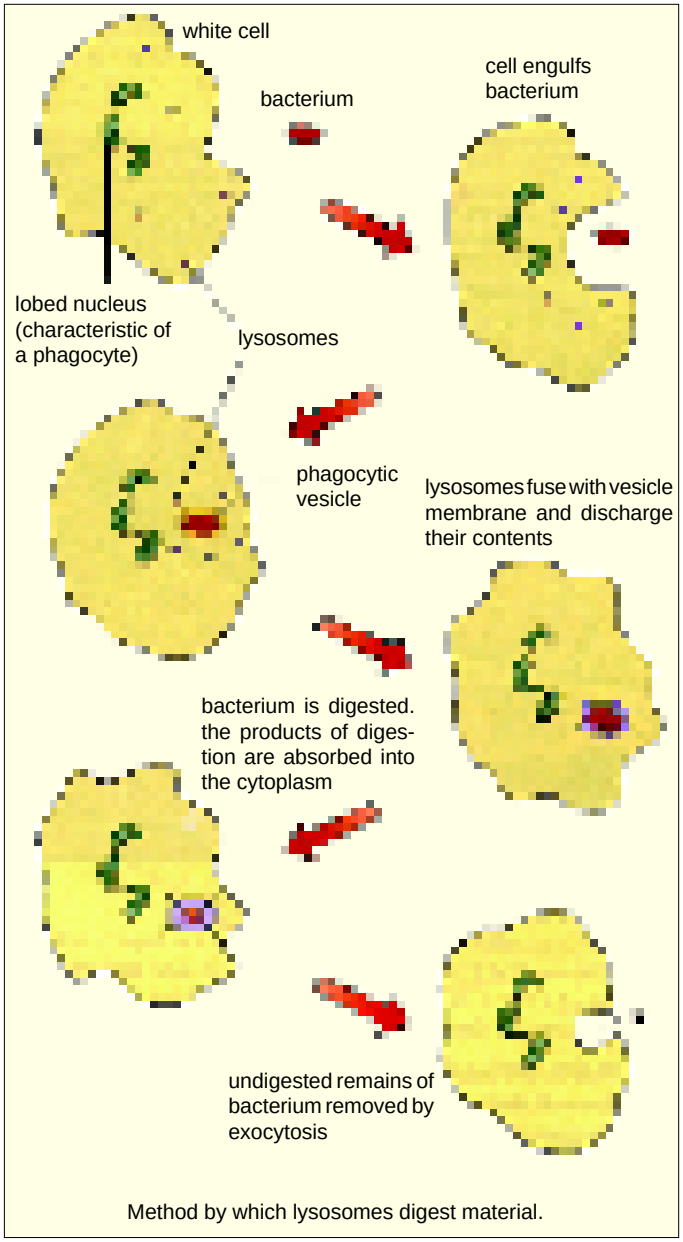
Fig. 3.21. Ultrastructure of plant cell (showing small portion of cell).

2. Golgi apparatus. It is a cup-shaped organelle which is located near the nucleus in many types of cells. Golgi apparatus consists of a set of smooth **cisternae** (*i.e.*, closed fluid-filled flattened membranous sacs or vesicles) which often are stacked together in parallel rows. It is surrounded by spherical membrane bound **vesicles** which appear to transport proteins to and from it.

Golgi apparatus consists of at least three distinct classes of cisternae : **cis Golgi, median Golgi** and **trans Golgi**, each of which has distinct enzymatic activities. Synthesized proteins appear to move in the following direction : rough ER→ cis Golgi→ median Golgi →trans Golgi→secretory vesicles/ cortical granules of egg/ lysosomes or peroxisomes. Thus, the size and number of Golgi apparatus in a cell indicate the active metabolic, mainly synthetic, state of that cell. Plant cells contain many freely distributed sub-units of Golgi apparatus, called **dictyosomes**, secreting cellulose and pectin for cell wall formation during the cell division.

Generally, Golgi apparatus performs the following important functions : 1. The packaging of secretory materials (*e.g.*, enzymes, mucin, lactoprotein of milk, melanin pigment, etc.) that are to be discharged from the cell. 2. The **processing** of proteins, *i.e.*, glycosylation, phosphorylation,

sulphation and selective proteolysis. 3. The synthesis of certain polysaccharides and glycolipids. 4. The sorting of proteins destined for various locations (*e.g.*, lysosomes, peroxisomes, etc.) in the cell. 5. The proliferation of membranous element for the plasma membrane. 6. Formation of the acrosome of the spermatozoa.



Method by which lysosomes digest material.

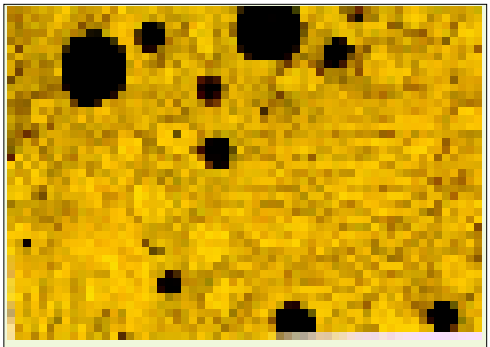
3. Lysosomes. The cytoplasm of animal cells contains many tiny, spheroid or irregular-shaped, membrane-bounded vesicles known as **lysosomes**. The lysosomes are originated from Golgi apparatus and contain numerous (about 50) hydrolytic enzymes (*e.g.*, **acid phosphatase** that is cytochemically identified) for intracellular and extracellular digestion. They digest the material taken in by endocytosis (such as phagocytosis, endocytosis and pinocytosis), parts of the cell (by autophagy) and extracellular substances. Lysosomes have a high acidic medium (pH 5) and this acidification depends on ATP- dependent **proton pumps** which are present in the membrane of lysosomes and which accumulate protons (H^+) inside the lysosomes. Lysosomes exhibit great **polymorphism**, *i.e.*, there are following four types of lysosomes : primary lysosomes (storage granules), secondary lysosomes (digestive vacuoles), residual bodies and autophagic vacuoles. The lysosomes of plant cells are membrane-bounded storage granules containing hydrolytic digestive enzymes, *e.g.*, large **vacuoles** of parenchymatous cells of corn seedlings, **protein** or **aleurone bodies** and **starch granules** of cereal and other seeds.

4. Cytoplasmic vacuoles. The cytoplasm of many plant and some animal cells (*i.e.*, ciliate protozoans) contains numerous small or large-sized, hollow, liquid-filled structures, the **vacuoles**. These vacuoles are supposed to be greatly expanded endoplasmic reticulum or Golgi apparatus. The **vacuoles** of animal cells are bounded by a lipoproteinous membrane and their function is the storage, transmission of the materials and the maintenance of internal pressure of the cell.

The vacuoles of the plant cells are bounded by a single, semipermeable membrane known as

tonoplast. These vacuoles contain water, phenol, flavonols, anthocyanins (blue and red pigment), alkaloids and storage products, such as sugars and proteins.

5. Peroxisomes. These are tiny circular membrane-bound organelles containing a crystal-core of enzymes (such as urate oxidase, peroxidase, D-amino oxidase and catalase, *e.g.*, liver cells and kidney cells). These enzymes are required by peroxisomes in **detoxification** activity, *i.e.*, in the metabolism or production and decomposition, of hydrogen peroxide or H_2O_2 molecules which are produced during neutralization of certain superoxides—the end products of mitochondrial or cytosolic reactions. Peroxisomes are also related with β-oxidation of fatty acids and thermogenesis like the mitochondria and also in degradation of the amino acids. In green leaves of plants, peroxisomes carry out the process of **photorespiration**.



TEM of dark staining peroxisomes from the liver cell of a rat.

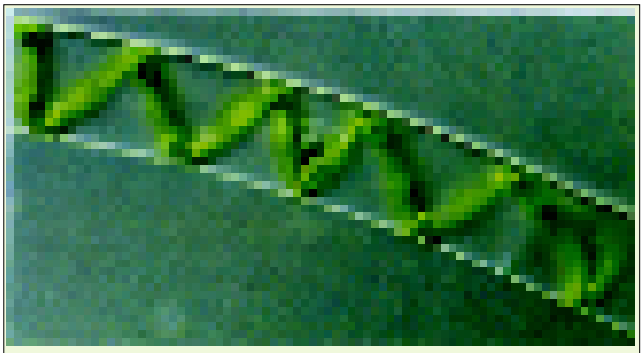
6. Glyoxysomes. These organelles develop in a germinating plant seed (*e.g.*, castor bean or *Ricinus*) to utilize stored fat of the seed (*i.e.*, to metabolise the triglycerides). Glyoxysomes consist of an amorphous protein matrix surrounded by a limiting membrane. The membrane of glyoxysomes originates from the ER and their enzymes are synthesized in the free ribosomes in the cytosol. Enzymes of glyoxysomes are used to transform the fat stores of the seed into carbohydrates by way of **glyoxylate cycle**.

7. Mitochondria. Mitochondria are oxygen-consuming ribbon-shaped cellular organelles of immense importance. Each mitochondrion is bounded by two unit membranes. The outer mitochondrial membrane resembles more with the plasma membrane in structure and chemical composition. It contains **porins**, proteins that render the membrane permeable to molecules having molecular weight as high as 10,000. Inner mitochondrial membrane is rich in many enzymes, coenzymes and other components of electron transport chain. It also contains **proton pumps** and many **permease** proteins for the transport of various molecules such as citrates, ADP, phosphate and ATP. Inner mitochondrial membrane gives out finger-like outgrowths (**cristae**) towards the lumen of mitochondrion and contains tennis-racket shaped **F_1 particles** which contain ATP-ase enzyme for ATP synthesis.

Mitochondrial matrix which is the liquid (colloidal) area encircled by the inner membrane, contains the soluble enzymes of Krebs cycle which completely oxidize the **acetyl-CoA** (an end product of cytosolic glycolysis and mitochondrial oxidative decarboxylation) to produce CO_2, H_2O and hydrogen ions. Hydrogen ions reduce the molecules of NAD and FAD, both of which pass on hydrogen ions to respiratory or electron transport chain where oxidative phosphorylation takes place to generate energy- rich ATP molecules. Since mitochondria act as the 'power-houses' of cells, they are abundantly found on those sites where energy is earnestly required such as sperm tail, muscle cell, liver cell (up to 1600 mitochondria), microvilli, oocyte (more than 300,000 mitochondria), etc. Mitochondria also contain in their matrix single or double circular and double stranded DNA molecules, called **mt DNA** and also the 55S ribosomes, called **mitoribosomes**. Since mitochondria can synthesize 10 per cent of their proteins in their own protein-synthetic machinery, they are considered as **semi-autonomous organelles**. Mitochondria may also produce heat (brown fat), and accumulate iron-containing pigments (Heme ferritin), ions of Ca^{2+} and HPO_4^{2-} (or phosphate; *e.g.*, osteoblasts of bones or yolk proteins).

8. Plastids. Plastids occur only in the plant cells. They contain pigments and may synthesize and accumulate various substances. Plastids are of the following types: **1. Leucoplasts** are colourless

plastids of embryonic and germ cells lacking thylakoids and ribosomes. **2. Amyloplasts** produce starch. 3. **Proteinoplasts** accumulate protein. 4. **Oleosomes** or **elaioplasts** store fats and essential oils. 5. **Chromoplasts** contain pigment molecules and are coloured organelles. Chromoplasts impart a variety of colours to plant cells, such as red colour in tomatoes, red chillies and carrots, various colours to petals of flowers and green colour to many plant cells. The green coloured chromoplasts are called **chloroplasts**. They have chlorophyll pigment and are involved in the photosynthesis of food and so act like the kitchens of the cell.

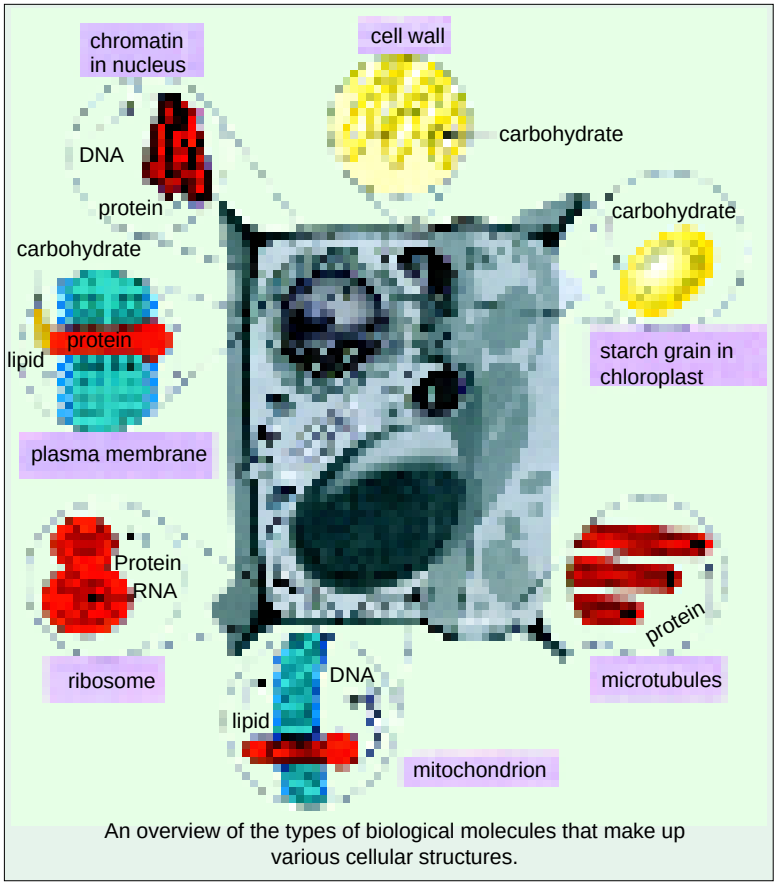


The ribbon - like chloroplast as seen in the *Spirogyra.*

Chloroplasts have diverse shapes in green algae but are round, oval or discoid in shape in higher plants. Like mitochondria, each chloroplast is bounded by two membranous envelopes, both of which have no chlorophyll pigment. However, unlike mitochondria there occurs third system of membranes within the boundary of inner membrane, called **grana**. The grana form the main functional units of chloroplast and are bathed in the homogeneous matrix, called the **stroma**. Stroma contains a variety of photosynthetic enzymes and starch grains. Grana are stacks of membrane-bounded, flattened discoid sacs, arranged like neat piles of coins. A chloroplast contains many such interconnected grana on which are located various photosynthetic enzymes and the molecules of green pigment chlorophyll and other photosynthetic pigments to trap the light energy. They contain DNA, ribosomes and complete protein synthetic machinery.

9. Ribosomes. Ribosomes are tiny spheroidal dense particles (of 150 to 200 A^0 diameter) that contain approximately equal amounts of RNA and proteins. They are primarily found in all cells and serve as a scaffold for the ordered interaction of the numerous molecules involved in protein synthesis. Ribosome granules may exist either in the **free state** in the cytosol (*e.g.*, basal epidermal cells) or **attached** to RER (*e.g.*, pancreatic acinar cells, plasma cells or antibodies-secreting lymphocytes, osteoblasts, etc.). Ribosomes have a sedimentation coefficient of about **80S** and are composed of two subunits namely **40S** and **60S**. The smaller 40S ribosomal subunit is prolate ellipsoid in shape and consists of one molecule of 18S ribosomal RNA (or rRNA) and 30 proteins (named as S_1, S_2, S_3, and so on). The larger 60S ribosomal subunit is round in shape and contains a **channel** through which growing polypeptide chain makes its exit. It consists of three types of rRNA molecules, *i.e.*, 28S rRNA, 5.8 rRNA and 5S rRNA, and 40 proteins (named as L_1, L_2, L_3 and so on).

10. Microtubules and microtubular organelles. With rare exceptions, such as human erythrocyte, microtubules are found in the cytoplasm of all types of eukaryotic cells. They are long fibres (of indefinite length) about 24 nm in diameter. In cross section each microtubule appears to have a dense wall of 6 nm thickness and a light or hollow centre. In cross section, the wall of a microtubule is made up of 13 globular subunits, called **protofilaments**, about 4 to 5 nm in diameter. Chemically, microtubules are composed of two kinds of protein subunits : **α-tubulin (tubulin A)** and **β-tubulin** (**tubulin B**), each of M.W. 55,000 daltons. The wall of a microtubule is made up of a helical array of repeating α and β tubulin subunits. Assembly studies have indicated that the structural unit is an **αβ dimer** of 8 nm length. Thus, in each microtubule, there are 13 protofilaments, each composed of αβ dimers that run parallel to the long axis of the tubule. The repeating unit is an αβ heterodimer which is arranged 'head to tail' within the microtubule, that is $\alpha\beta \rightarrow \alpha\beta \rightarrow \alpha\beta$. Thus, all microtubules have a defined **polarity** : their two ends are not structurally equivalent.



An overview of the types of biological molecules that make up various cellular structures.

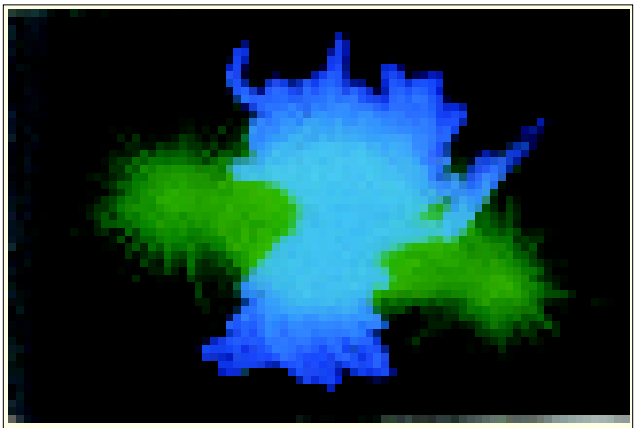
Microtubules undergo reversible assembly-disassembly (*i.e.*, polymerization–depolymerization), depending on the need of the cell or organelles. Their polymerization is regulated by certain **MAPs** or **microtubule-associated proteins** (*e.g.*, Tau protein). The assembly of microtubules involves preferential addition of subunits (αβ dimers) to one end of tubule, called **A end** (or **net assembly end**); the other end of the tubule is called **D end** (or **net disassembly end**). Such an assembly involves the hydrolysis of GTP to GDP. Thus, assembly of tubulin in the formation of microtubules is a specifically oriented and programmed process. Centrioles, basal bodies and centromeres of chromosomes are the sites of orientation for this assembly. Calcium and **calmodulin** (an acidic protein having four Ca^{2+} binding sites) are some other regulating factors in the *in vivo* polymerization of tubulin. Certain drugs such as **colchicine** and **vinblastin**, are found to block the polymerization of tubulin.

The following cell organelles are derived from special assemblies of microtubules :

(1) Cilia and flagella. Ciliary and flagellar cell motility is adapted to liquid media and is executed by minute, specially differentiated appendices, called **cilia** and **flagella**. Both of these organelles have very similar structure; they differ mainly in size and number (*i.e.*, flagella are longer and fewer in number, while cilia are short and numerous). Cilia are used for locomotion in isolated cells, such as certain protozoans (*e.g.*, *Paramecium*). or to move particles in the medium, as in air passages and oviduct. Flagella are generally used for locomotion of cells, such as the spermatozoon and *Euglena* (protozoan). All cilia and flagella are built on a common fundamental plan : a bundle of microtubules called the **axoneme** (1 to 2 nm in length and 0.2 μm in diameter) is surrounded by a membrane that is part of the plasma membrane. The axoneme is connected with the basal body which is an intracellular granule lying in the cell cortex and which originates from the centrioles. Each axoneme is filled with **ciliary matrix**, in which are embedded two central **singlet** microtubules, each with the 13 protofilaments and nine outer pairs of microtubules, called **doublets**. This recurring motif is known as the 9 + 2 array. Each doublet contains one complete microtubule, called the **A subfibre,** containing all the 13 protofilaments. Attached to each A subfibre is a **B subfibre** with 10 protofilaments. Subfibre A has two **dynein arms** which are oriented in a clockwise direction. Doublets are linked together by **nexin links**. Each subfibre A is also connected to the central microtubules by **radial spokes** terminating in fork-like structures, called **spoke knobs** or **heads**.

Propulsion by both cilia and flagella is caused by bending at their base. Cilia move by a whip-like **power stroke** fueled by hydrolysis of ATP, followed by a **recovery stroke.** Flagellar movement is also powered by ATP hydrolysis. In contrast to cilia, they generally move by waves that emanate from the base and spread outward toward the tip.

2. Basal bodies and centrioles. Basal bodies and centrioles are similar in structure and function; both act as nucleating centres from which microtubules grow. **Centrioles** are cylinders that measure 0.2 µm × 0.5 µm. This cylinder is open on both ends, unless it carries a cilium or flagellum (then it is called **basal body** or **kinetosome**). The wall of a centriole has nine groups of microtubules arranged in a circle. Each group, called **blade** is a **triplet** formed of three tubules — A, B, and C that are skewed toward the centre. Tubule A has 13 protofilaments, while tubules B and C have only 10 protofilaments each. There are no central microtubules in the centrioles and no dynein arms like the cilia; however, triplets are linked by connectives. The **procentriole** (or daughter centriole) is formed at right angles to the centriole and is located near the proximal end of the centriole. Both centrioles are found in a specially differentiated region the **centrosome, cell centre** or **centrosphere**. The centrosome is juxtanuclear (L., juxta = near) and firmly attached to the nuclear envelope. At the time of cell division two pairs of centrioles are formed and form the spindle of microtubules which help in the separation and movement of chromosomes during concluding stages of cell divisions.

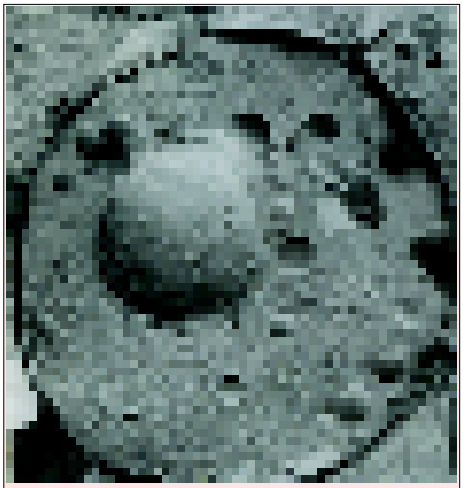


Mitotic spindle is constructed primarily of microtubules.

C. Nucleus

The nucleus is centrally located and spherical cellular component which controls all the vital activities of the cytoplasm and carries the hereditary material the DNA in it. The nucleus consists of the following three structures :

1. Chromatin. Nucleus being the heart of every type of eukaryotic cell, contains the **genes**, the hereditary units. Genes are located on the **chromosomes** which exist as **chromatin network** in the non- dividing cell, *i.e.*, during interphase. The chromatin has two forms : **1. Euchromatin** is the well-dispersed form of chromatin which takes lighter DNA-stain and is genetically active, *i.e.*, it is involved in gene duplication, gene transcription (DNA- dependent RNA synthesis) and **phenogenesis** or phenotypic expression of a gene through some type of protein synthesis. **2. Heterochromatin** is the highly condensed form of chromatin which takes dark DNA-stain and is genetically inert. Such type of chromatin exists both in the region of centromere (called **constitutive heterochromatin**) and in the sex chromatin (called **facultative heterochromatin**) and is late-replicating one.



Nucleus.

Chemically, the chromatin contains a single DNA molecule, equal amount of five basic types of histone proteins, some RNA molecules and variable amount of different types of acidic proteins. In fact, the chromatin has its unit structures in the form of **nucleosomes**. The chromatin binds strongly to the inner part of nuclear lamina, a 50 to 80 nm thick fibrous lamina lining the inner side of the nuclear envelope. Nuclear lamina is made up of three types of proteins, namely **lamin A**, **B** and **C**. Lamin proteins are homologous in structure to IF proteins and serve the following functions : 1. They anchor parts of interphase chromatin to the nuclear membrane. They tend to interfere with chromatin condensation during interphase of cell cycle. 2. Lamins may play a crucial role in the assembly of interphase nuclei after each mitosis.

2. Nuclear envelope and nucleoplasm. Nuclear envelope comprises two nuclear membranes— an **inner nuclear membrane** which is lined by nuclear lamina and an **outer nuclear membrane** which is continuous with rough ER. At certain points the nuclear envelope is interrupted by structures called **pores** or **nucleopores.** Nuclear pores contain octagonal **pore complexes** which regulate exchange between the nucleus and cytoplasm. The number of nucleopores is found to be correlated with the transcriptional activity of the cell. For example, in the frog *Xenopus laevis* oocytes (which are very active in transcription) have 60 pores/ μm^2 (and up to 30 million pore complexes per nucleus) , whereas frog's mature erythrocytes (inactive in transcription) have only about 3 pores/μm^2 (and a total of only 150 to 300 pores per nucleus) (**Scheer**, 1973).

The nuclear envelope binds the **nucleoplasm** which is rich in those molecules which are needed for DNA replication, transcription, regulation of gene actions and processing of various types of newly transcribed RNA molecules (*i.e.*, tRNA, mRNA and other types of RNA).

3. Nucleolus. Nucleus contains in its nucleoplasm a conspicuous, darkly stained, circular sub-organelle, called **nucleolus**. Nucleolus lacks any limiting membrane and is formed during interphase by the ribosomal DNA (rDNA) of **nucleolar organizer** (**NO**). Nucleolus is the site where ribosomes are manufactured. It is here where ribosomal DNA transcribes most of rRNA molecules and these molecules undergo processing before their step-wise addition to 70 types of ribosomal proteins to form the ribosomal sub-units.

Table 3-1. Differences between prokaryotic and eukaryotic cells (Source : Maclean and Hall, 1987).

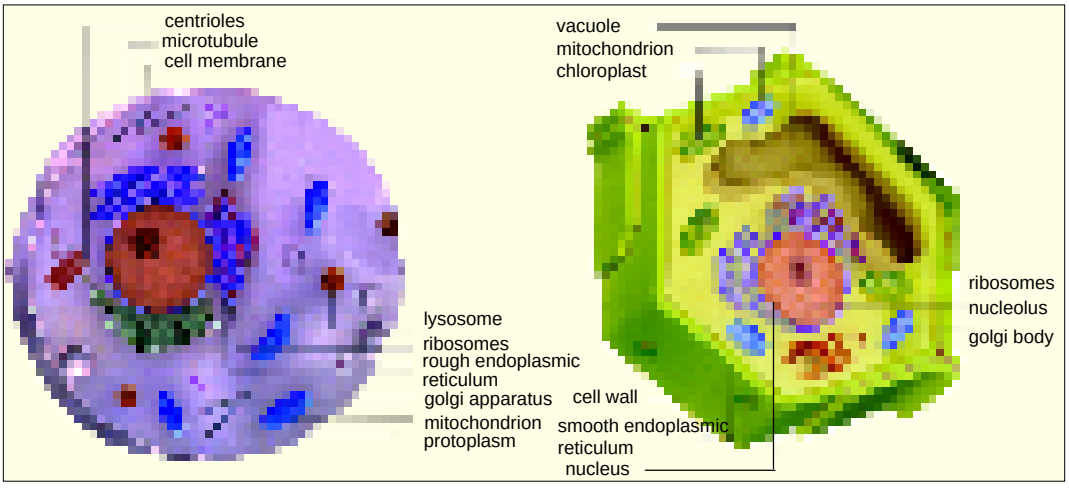
Feature	Prokaryotic cell	Eukaryotic cell
1. Size	Mostly 1-10 µm	Mostly 10-100 µm
2. Multicellular forms	Rare	Common, with extensive tissue formation
3. Cell wall	Present in most but not in all cells	Present in plant and fungal cells only
4. Plasma membrane	Present	Present
5. Nucleus	Absent	Present
6. Nuclear membranes	Absent	Present
7. Chromatin with histone	Absent	Present
8. Genetic material	Circular or linear, double -stranded DNA : genes are not interrupted by intron *	Linear double-stranded DNA : genes frequently interrupted by intron sequences, especially in higher eukaryotes
9. Nucleoli and mitotic apparatus	Absent	Present
10. Plasmids	Commonly present	Rare
11. Cellular organelles :		
(i) Mitochondria	Absent	Present

* Intron is an intervening sequence of nucleotides in DNA, located within a gene that is not included in the mature mRNA.

Feature	Prokaryotic cell	Eukaryotic cell
(ii) Endoplasmic reticulum	Absent	Present
(iii) Vacuoles	Absent	Present
(iv) Lysosomes	Absent	Present
(v) Chloroplasts	Absent	Present (only in plants)
(vi) Centrioles	Absent	Present (absent in higher plants)
(vii) Ribosomes	Present (70S)	Present (80S)
(viii) Microtubules	Absent	Present
(ix) Flagellae	Simple structure composed of the protein flagellin	Complex 9 + 2 structure of tubulin and other protein
12. Respiration	Many strict anaerobes (oxygen fatal)	All aerobic, but some facultative anaerobes by secondary modifications
13. Metabolic patterns	Great variations	All share cytochrome electron transport chains, Krebs cycle oxidation, Embden-Meyerhof glucose metabolism or glysolysis
14. Photosynthetic enzymes	Bound to plasma membrane as composite chromatophores	Enzymes packaged in plastids bound by membrane
15. Sexual system	Rare : if present one way (and usually partial); transfer of DNA from donor to recipient cell occurs.	Both sexes involved in sexual participation and entire genomes transferred; alternation of haploid and diploid generations is also evident

Table 3-2. **The cells of animals and plants have the following differences :**

Animal cell	Plant cell
1. Animal cells are generally small in size.	1. Plant cells are larger than animal cells.
2. Cell wall is absent.	2. The plasma membrane of plant cells is surrounded by a rigid cell wall of cellulose.
3. Except the protozoan *Euglena* no animal cell possesses plastids.	3. Plastids are present.
4. Vacuoles in animal cells are many and small.	4. Most mature plant cells have a large central sap vacuole.
5. Animal cells have a single highly complex and prominent Golgi apparatus.	5. Plant cells have many simpler units of Golgi apparatus, called dictyosomes.
6. Animal cells have centrosome and centrioles.	6. Plant cells lack centrosome and centrioles.



REVISION QUESTIONS

1. What are the viruses ? Write an essay on the viruses.
2. Give the life cycle of a virus.
3. What is a lysogenic phage ?
4. Describe the structural peculiarities of prokaryotic organization.
5. Write an essay on the bacteria.
6. Draw a well-labelled diagram of an animal cell as seen by the electron microscope. Comment upon the functions of nucleus, mitochondria, ribosome and microtubules.
7. Give an account of the structure of a typical animal cell.
8. Draw a labelled diagram of a typical plant cell as seen through an electron microscope. Describe the functions of specific structures of plant cells only.
9. Compare the characteristics of prokaryotic and eukaryotic cells.
10. Write short notes on the following :
    (i) Bacteriophage; (ii) Viroids; (iii) Prions; (iv) TMV; (v) PPLO; (vi) Bacteria; (vii) Blue green algae (Cyanobacteria).
11. Write differences between the following :
    (i) Viruses and bacteria;
    (ii) Prokaryotic cells and eukaryotic cells;
    (iii) Animal cells and plants cells.
12. “Structural complexity of eukaryotes is reflected in their subcellular structures”. Discuss.