UNIT 2

Cytology

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2.5 Eukaryotic cell : General structure of plant cell and animal cell
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molecules present in our cells must be very similar to those molecules that were present in our primitive cellular ancestors who lived more than 3 billion years ago.

3. **Cells possess genetic program:** Organisms are built according to the information encoded in a cluster of genes. The vast amount of information is packaged into a set of chromosomes that occupy space in the cell nucleus. The discovery of mechanism by which cells use their genetic information to accomplish such functions is the greatest achievement of biotechnology.
4. **Cells are capable to reproduce themselves:** Cells reproduce by division, a process in which the "mother" cell is divided into two "daughter" cells.
5. **Cells acquire and utilize energy:** The source of energy for all living organisms is the electromagnetic radiation coming from the sun. In plants, the light energy trapped by photosynthetic cells is converted into chemical energy which is stored in the form of energy rich compounds called carbohydrates like sucrose and starch. Animal cells utilize energy from these carbohydrates. The energy currency of cell is a energy rich compound called ATP.
6. **Cells are able to respond stimuli:** The cells respond to stimuli in several ways. A unicellular organism moves towards the source of nutrients. In multicellular plant or animal, an individual cell also responds to stimuli. Cells of multicellular organisms possess receptor for hormone which co-ordinate the physiology of all organs.
7. **Cells carry out chemical reaction:** All chemical changes that take place into cells require enzymes. The sum of total chemical reactions is known as metabolism.

2.3 COMPARATIVE ACCOUNT OF PROKARYOTIC AND EUKARYOTIC CELL

Prokaryotic and eukaryotic cells are distinguished on the basis of several characteristics (Table 2.1).

2.4 PROKARYOTIC CELL: GENERAL STRUCTURE OF BACTERIAL CELL

All bacteria including eubacteria, mycoplasma, actinomycetes, rickettsia and archaea have prokaryotic cell. The ultrastructure of a typical bacterial cell is shown in Fig. 2.1.

2.4.1 Flagella and Other Appendages

Several bacteria possess long filamentous appendage called flagella that confers motility. Flagella are generally found only in bacilli, but exceptionally detected from cocci *Nitrosomonas mobilis*.

Table 2.1: Comparative account of prokaryotic and eukaryotic cells

	Prokaryotic	**Eukaryotic**
Size	Small (0.2–2 μm diameter)	Large (10–100 μm)
Nucleus	Nuclear membrane absent Nucleoli absent	True nucleus consisting of nuclear membrane and nucleoli present
Cell organelles	Absent	Present [Examples: lysosomes, Golgi complex, endoplasmic reticulum, etc.]
Flagella	Simple	Complex microtubules show 9 + 2 arrangement
Cell wall	Usually present, chemically complex (typical bacterial cell wall is made up of Peptidoglycan)	Present in plant cells. But absent in human and protozoan cells
Plasma Membrane	No carbohydrate and generally lacks sterols High proportion of protein is present	Carbohydrate and sterols are present Comparatively less proportion of protein is present
Cytoplasm	Cytoskeleton absent	Cytoskeleton present
Ribosome	70 S ribosome present	80 S ribosome present in the cytoplasm, 70 S ribosome present in mitochondria and chloroplasts
Chromosome	Single circular DNA, histone proteins absent	Multiple linear chromosomes with histones proteins
Cell Division	Binary fission (Amitosis)	Mitosis
Reproduction	Meiosis absent	Meiosis present
Example	Bacteria	Algae, fungi, protozoa, plants and animals

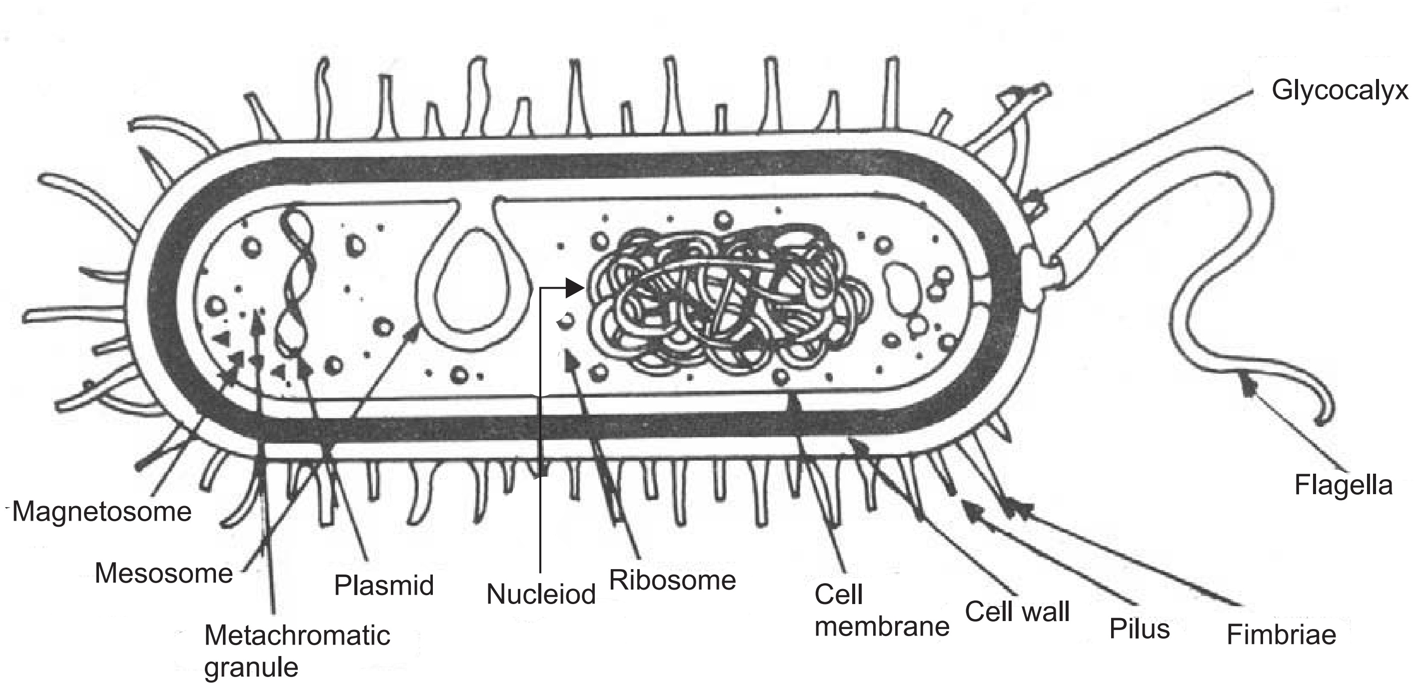

Fig. 2.1: Ultrastructure of bacteria

Size: The length of flagellum is approximately 70 µm (several times larger than bacterial cell itself) and the diameter is 0.01 µm–0.02 µm (10 to 20 nm). It cannot reveal under light microscope but can reveal only under electron microscope.

Arrangement of flagella: The number and arrangement of flagella vary from bacteria to bacteria; they are generally constant for each species. The arrangement pattern of flagella in different types of bacteria is given below (Fig. 2.2):

1. **Monotrichous:** Bacterium has a single flagellum at one end of the cell, e.g., *Vibrio cholerae*
2. **Lophotrichous:** Bacterium has two or more flagella at one end of the cell, e.g., *Alcaligenes faecalis*.
3. **Amphitrichous:** Bacterium has at least one flagellum at each end of cell, e.g., *Spirillum volutans.*
4. **Peritrichous:** Bacterium has numerous flagella distributed all over the surface of cell, e.g., *Escherichia coli.*

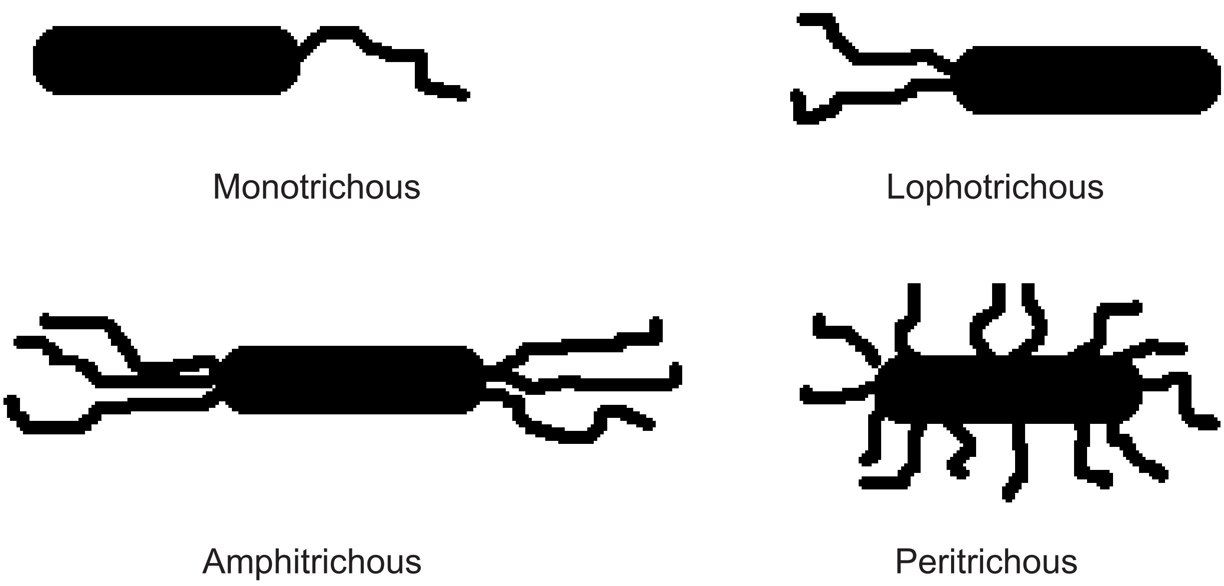

Fig. 2.2: Types of bacteria depending on flagellar arrangements.

Ultra-structure of flagella

The flagellum has following three basic parts:

1. Filament
2. Hook
3. Basal body

Filament is a long outermost region of flagellum protruding out of cell surface. It is thin and cylindrical with constant diameter. The filament is made up of protein monomers called "flagellin" that has molecular weight ranging from 20,000 to 40,000.

The filament is attached to a slightly wider and shorter **hook**; the second part of the flagellum. It is a curved part that joins filament with basal body. It is made up of different types of proteins.

The **basal body** is composed of a small central rod inserted into a series of rings. Gram-negative bacteria contain four rings as L-ring, P-ring, S-ring and M-ring. The L-ring is embedded in **L**ipo-polysaccharide layer of outer membrane, P-ring in the **P**eptidoglycan layer, S-ring is present just above the cytoplasmic membrane (**S**emi position of Membrane) and **M**-ring is present within the Cytoplasmic **M**embrane. These rings are placed one above the other around the **central rod**. Gram-positive organisms have only S- and M-rings in the basal body. Gram-positive cells have thick cell wall hence two rings are sufficient to anchor the flagella, besides that extra support is also provided by a thick peptidoglycan layer (Fig. 2.3).

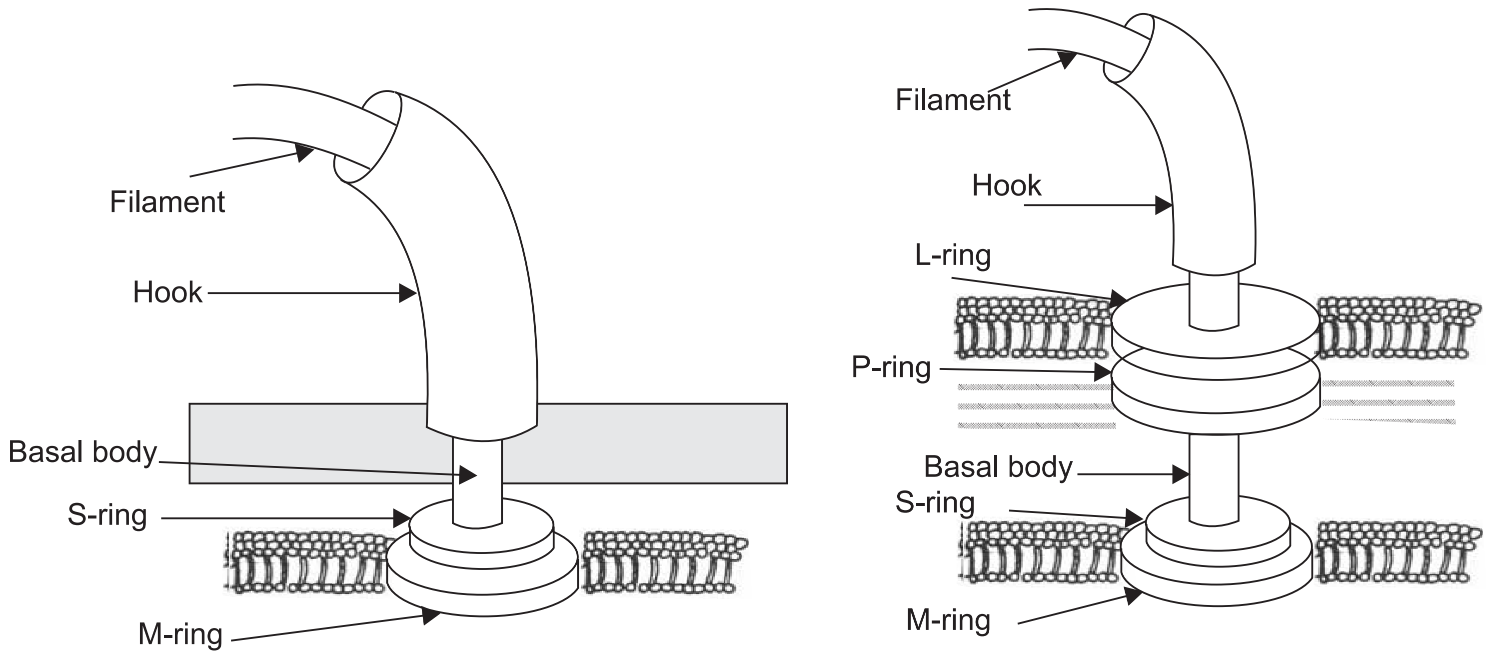

Fig. 2.3: Ultra structure of bacterial flagella.
(A) Gram-positive bacteria (B) Gram-negative bacteria

Functions of Flagella

Flagella confer motility to bacterial cell. The advantage of motility is that the bacterium moves towards a favourable environment or away from the adverse one. The movement of a bacterium towards or away from a particular stimulus is called taxis. The bacterial movement towards a chemical stimulus is called chemotaxis; movement towards the light is called phototaxis. If a suspension of bacteria is placed on slide under a cover slip, aerobic organisms accumulate at the edges of cover slip, while anaerobic organisms accumulate at the centre. This phenomenon is called aerotaxis. Flagella impart antigenicity to bacterial cell, whereas filament is associated with H-antigen.

Detection of motility

1. Microscope—It is possible to observe motile, living bacteria using phase contrast microscope and also with compound microscope by adjusting proper contrast.
2. Culture medium—On semisolid medium, motile microbes can swim throughout the plate. The presence of turbidity—‘swarming’ throughout the plate is a positive test for the presence of motility.

3. Electron microscope—The high magnification and resolution of the electron microscope along with common staining practices make the motility easily visible.
4. Staining—Flagella can be coated with stains like basic fuchsin. The binding of the dye adds extra width to the structure, making it visible even under the light microscope.
5. Antibody staining—The antibodies, which recognize flagellin, are available. By attaching a fluorescent dye to the antibody and using a fluorescence microscope, it is possible to detect the flagella.

Mechanism of flagellar movement

Flagella are semi-rigid, helical rotors, which rotate either clockwise or anticlockwise around their long axis. Their movement is enhanced by relative rotation of S and M rings. In Gram-positive motile bacteria, only S and M rings are present. It indicates that only these rings are involved in motility. L and P rings give mechanical support, as Gram-negative cell wall is thin.

Endoflagellum (Axial Filament)

It is the flagella present within the outer sheath. It arises at one end and is present spirally around the cell. The rotation of filament leads to the movement of bacteria. This motion is just like corkscrew motion. Endoflagellum is generally detected in spirochaetes e.g., *Treponema pallidum.*

Other appendage structures

Pilli are longer than fimbriae and there are only a few per cell (1 to 2 per cell). They help in gene transfer and attachment. They are known to be receptors for certain bacterial viruses. The pilli are also referred as sex pili. The sex pilus (or F-pilus) is involved in conjugation process occuring in certain bacteria (e.g., *E. coli*). Donor bacteria attaches to a recipient via the sex pilus. Then a copy of small fragment of the donor bacteria's genome passes through the sex pilus into the recipient. The drug resistance in many different species of bacteria spreads by this mechanism.

Fimbriae are shorter and straighter than flagella and are more numerous. The number ranges from few to hundred. Not all bacteria synthesize fimbriae. Fimbriae do not function in motility, but are thought to be important in attachment to surfaces. *Neisseria gonorrheae* (the causative agent of gonorrhea) have fimbriae that helps it to adhere to the urogenital tract. The microbe is more virulent when it is able to synthesize fimbriae.

2.4.2 Capsule

An extracellular slimy or mucilaginous layer external to cell wall formed by several bacteria is called capsule.

It literally means 'sugar coat'. This structure is not essential for viability of cell. The glycocalyx, well-organised and firmly attached to cell wall is called Capsule.

While the glycocalyx, unorganised and loosely attached to cell wall is called slime layer.

Size

Capsule layer may be thin or thick, which varies from species to species. The thin layer having a size less than 0.2 μm is called microcapsule (such structure is not detected by light microscope); the thick layer having a size more than 0.2 μm up to 10 μm is called macrocapsule.

Composition of capsule

Water is the principal component of bacterial capsule (98%). The commonest organic constituent of bacterial capsule is polysaccharide. It may homopolysaccharide or heteropolysaccharide. Several bacteria containing peptides/protein are also reported.

Acetobacter xylinum	Cellulose
Streptococcus pyogenes	Hyaluronic acid
Bacillus anthracis	D- glutamic acid
E. coli	Colanic acid
*Salmonell*a	Colanic acid

Functions of capsule

1. Protection from desiccation
2. Protection from bacteriophages
3. Antiphagocytic factor
4. Promotes stability by preventing cell aggregate formation.

Detection of Capsule—Capsule can be detected by staining technique like Manuals method and Hiss method. It can also be detected by quellung reaction.

2.4.3 Cell Wall

Complex semi-rigid structure, just outside the plasma membrane, which determines the shape of bacteria is called cell wall.

Cell wall is an essential structure for the survival of bacterial cells. Exceptionally *Mycoplasma* species and several archaea do not have cell wall.

Although the presence of cell wall is a unique characteristic of bacteria, two types of cell walls are detected in bacteria—Gram positive and Gram negative. These categories are based on differential staining response due to difference in chemical composition of cell wall. Gram positive and Gram negative cells share one thing in common that is unique to bacteria—peptidoglycan.

Structure of Gram Positive Cell Wall

The size of Gram positive cell wall ranges from 20–80 nm. It constitutes one thick layer. The cell wall of Gram positive bacteria is stronger than the cell wall of Gram negative bacteria. The components found in Gram positive cell wall are peptidoglycan and teichoic acid.

Peptidoglycan—It is a thick rigid layer. It is composed of two sugars—N-acetyl glucosamine (NAG) and N-acetyl muramic acid (NAM). Both sugars are covalently by β (1–4) linkage. Generally, a side chain of four amino acids is attached to NAM. The amino acid composition of side chain varies from species to species, but the most commonly found side chain contains four amino acids—L-alanine, D-alanine, D-glutamic acid, and diamino-pimelic acid (DPA). Notice that the presence of D-isomers of amino acid in bacteria is rare and an exceptional example in biological world. Generally, living system is made up of L-isomer (Fig. 2.4).

Teichoic acid—Another component of the Gram-positive cell wall is teichoic acid. It is is a polymer of glycerol or ribitol joined by phosphate groups.

It present in two forms—lipoteichoic acid and wall-teichoic acid. The lipoteichoic acid bridges peptidoglycan and plasma membrane, while wall-teichoic is acid present within the peptidoglycan layer.

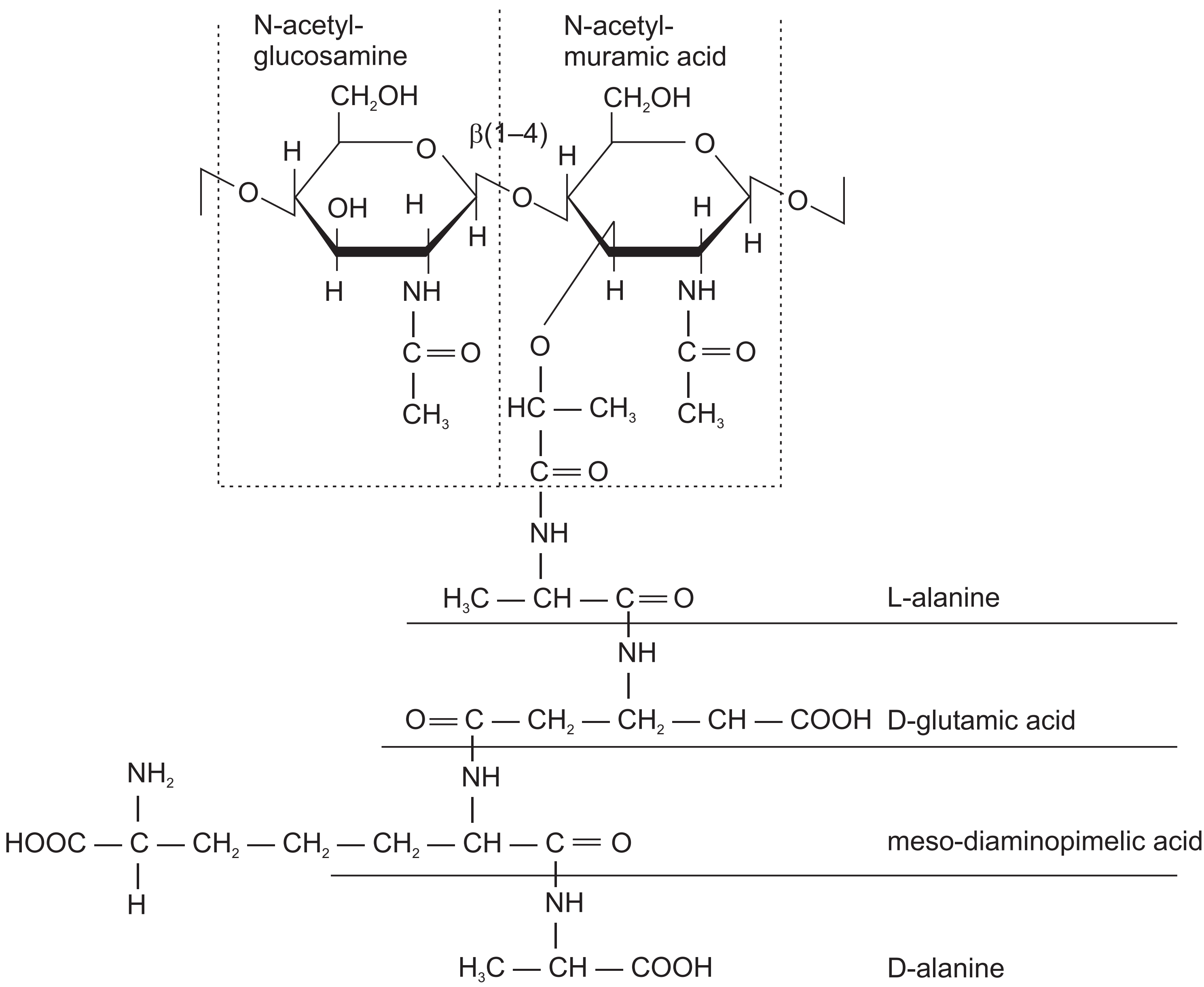

Fig 2.4: Structure of peptidoglycan

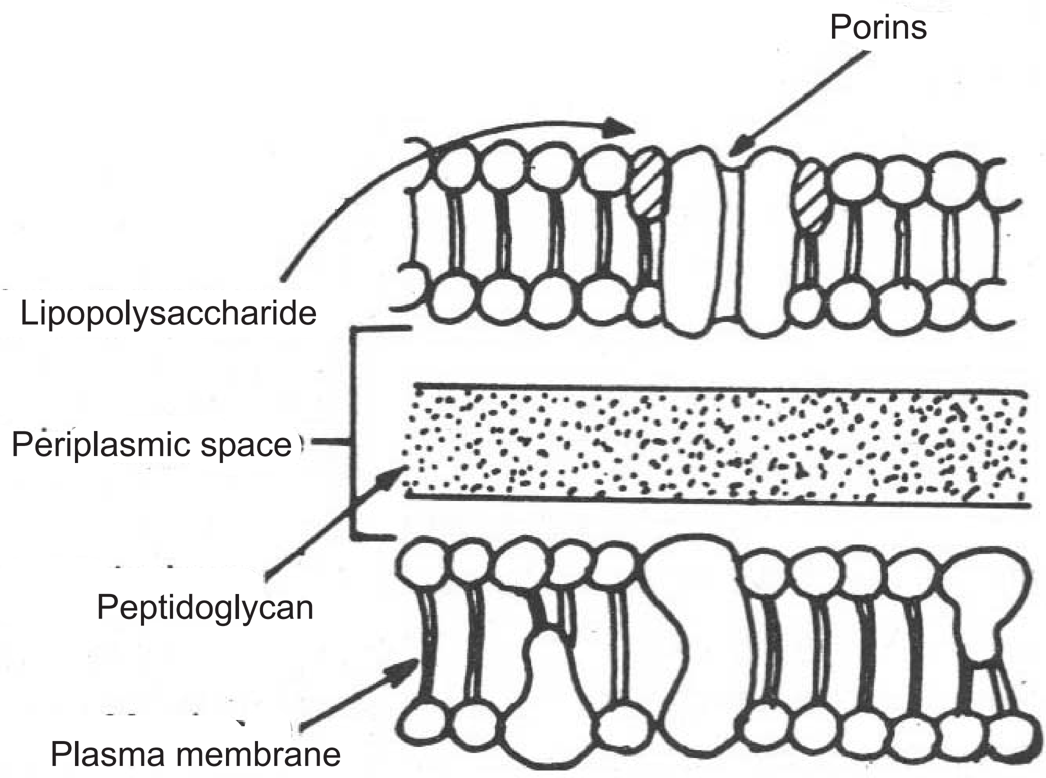

Fig. 2.6: Ultrastructure of Gram-negative cell wall

the periplasmic space for transport across the cytoplasmic membrane. Larger or hydrophobic molecules cannot penetrate the outer membrane.

C. Periplasmic space: It is the space between outer membrane and plasma membrane. The size varies between 1 mm and 7 mm. The fluid filled in this space is called periplasm. It includes enzymes and several proteins. These proteins function to transport the nutrients into the cell. Some examples of periplasmic enzymes are hydrolytic enzymes (phosphatases—degrade phosphate containing compounds, proteases—degrade proteins and peptides, endonucleases—degrade nucleic acids). Binding proteins—recognize specific solutes and transport them across the membrane (e.g., sugars, amino acids, inorganic ions and vitamins). When the cell is immersed in a solution having a high solute concentration, it causes water to flow out of the cell. To protect themselves, bacteria synthesize small molecules to balance the osmotic stress. These are called compatible solutes.

Peptidoglycan

The thin layer of peptidoglycan has a thickness of 2–7 nm. It has same structure like the peptidoglycan present in Gram-positive cell wall, but the peptidoglycan in Gram-negative cell contains less cross-linking (Fig. 2.6)

Structure of acid-fast cell wall

Several bacteria have different composition as compared to Gram-positive and Gram-negative cell wall. These bacteria do not get decolourised after staining even upon the application of a strong decolourizer like acid-alcohol mixture. This property is known as acid-fastness. Such bacteria are called acid-fast bacteria. These bacteria contain mycolic acid and other waxy material along with peptidoglycan in their cell wall, e.g., Mycobacterium *sp.* and several *Actinomycetes.*

Functions of cell wall

1. It determines the shape of bacteria.
2. It provides strength to bacterial cell.
3. Cell wall confers pathogenicity to several pathogens. (Example—Mycobacterium *sp.*)
4. It provides protection from toxic substances.
5. In case of Gram-negative bacteria, the outer membrane is barrier to several harmful substances like antibiotics (e.g., penicillin), enzymes (e.g., lysozyme) and heavy metals.

Concept of Spheroplast and Protoplast

Spheroplast—If the cell wall is partially removed by artificial means, the cell is called spheroplast. When the Gram-negative cell is treated with EDTA, the outer membrane is removed, such a cell is called spheroplast.

Protoplast—If the cell wall is completely removed artificial means or by mutation, the cell is called protoplast. If the Gram-positive cell is treated with lysozyme, the cell wall is completely removed. Such a cell is called protoplast.

2.4.4 Plasma Membrane

It is a thin structure made up of proteins and phospholipids. It encloses the cytoplasm and controls transport across the cell.

Plasma membrane is a sheet-like structure. The thickness of bacterial plasma membrane is between 5–10 nm. Membranes are made up of lipids and proteins and also contain trace amounts of carbohydrate. Bacterial membrane contains high proportion of proteins compared to eukaryotic cell. Although sterols are absent in the membrane but it contains pentacyclic sterol-like molecules called hopanoids. These hopanoids stabilise the membrane. Membrane lipids have both hydrophilic and hydrophobic moieties. The proteins present in the membrane serve as channels, receptors and enzymes. The lipid-bilayer creates suitable environment for the orientation and activity of proteins. The lipid and proteins are held together by non-covalent interactions. Both sides of membrane differ from each other. Plasma membrane is electrically polarised. The membrane potential plays an important role in transport, energy conversion, etc (Fig. 2.7).

2.4.5 Endospore

It is a heat resistant structure first discovered by Ferdinand Cohn. Under the conditions of limited supply of nutrients or water, certain bacteria produce specialized "resting" cell body called endospore. Although endospores are detected in Gram-

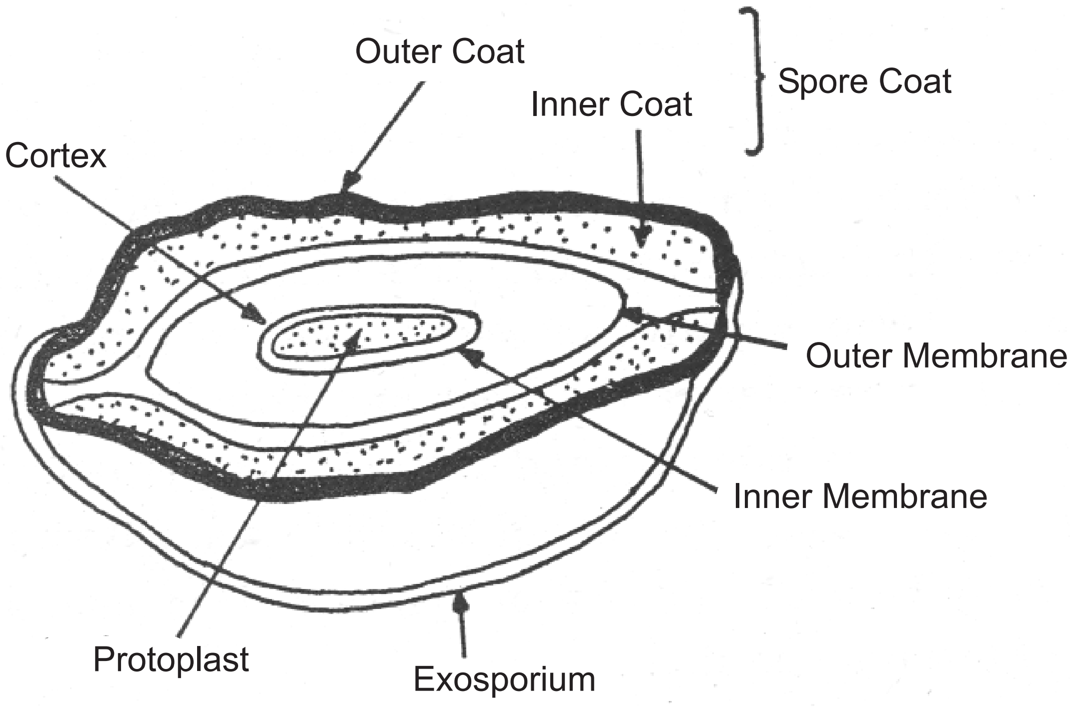

Fig. 2.8: Ultrastructure of bacterial endospore

amino acids. They are unaffected by chemical treatment and confer resistance to spore from chemicals (Fig. 2.8)

Significance of Endospore

Endospores are resistant to heat and chemicals, drying, freezing and radiation. The main ecological role of endospores appears to be survival in the dry state in a non-nutrient environment.

The comparison between vegetative cell and endospore is mentioned in Table 2.2.

Table 2.2: Comparison between vegetative cell and endospore

Vegetative cell	**Endospore**
Usually stains Gram positive. Readily stained by ordinary stains.	Not readily stained by ordinary stains.
Sensitive to various physical and chemical agents (heat radiation, disinfectant and antibiotic).	Resistant to various physical and chemical agents (heat radiation, disinfectant and antibiotic).
Relatively low sulfur containing amino acid.	High sulfur containing amino acid and calcium.
Calcium ions and dipicolinic acid (DPA) absent.	Calcium ions and DPA is present.
Metabolically active.	Metabolically inactive (activities are negligible).

2.4.6 70 S Ribosome

Ribosomes are granular small bodies made up of protein and rRNA. They are involved in protein synthesis. Ribosomes give a typical granular appearance to the cytoplasm. Bacterial cells contain 70 S ribosomes. Their number varies from 5000–

Table 2.3: Bacterial cell inclusion

Cell inclusion	Composition	Significance	Example of microbes
Metachromatic granule	These are polyphosphate granule	Reserve source of phosphate	*Spirillum volutins, Corynebacteriam diphtheriae*
Lipid granules	Polyhydroxy Butyrate granules	Reserve source of lipid	*Bacillus, Lactobacillus*
Carboxysomes	Ribulose 1,5 diphosphate carboxylase	Important enzyme of photosynthesis	*Nitrifying bacteria, Cyanobacteria, and Thiobacillus*
Gas vacuoles	Gas ($CO_{2,}$ H_2S)	Maintain buoyancy to receive oxygen, light and nutrients	*Cyanobacteria, anoxygenic Halobacteria*
Magnetosome	Iron oxide (Fe_3 O_4), magnetite	Orients the cell to proper environment	*Aquaspirillum magnetotacticum*

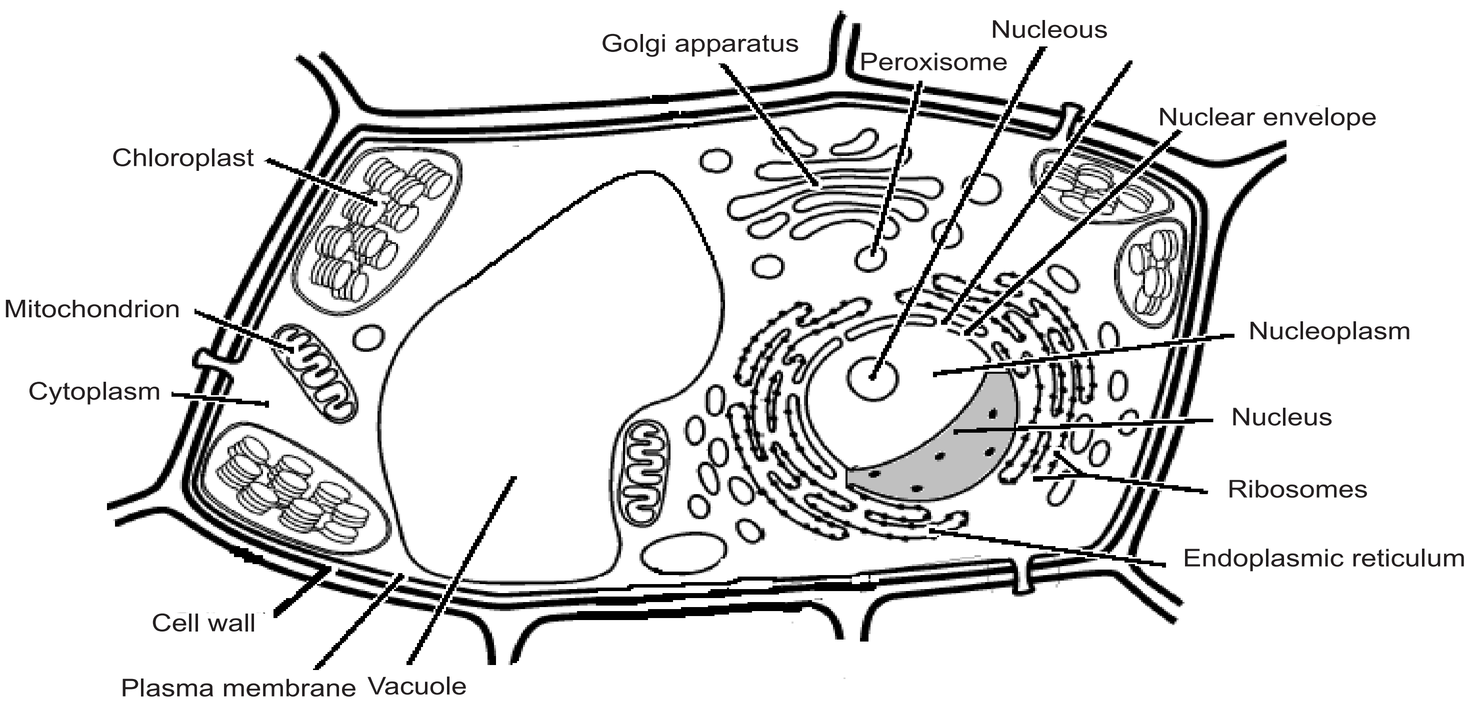

Fig. 2.9: Ultrastructure of plant cell

2.5.1 Flagella and Cilia

The eukaryotic cell can move with the help of flagella or cilia or both or by internal cytoplasmic changes i.e., pseudopodia.The flagellated eukaryotic cell includes zoospores of lower fungi, flagellated protozoa, numerous algae and sperm cell. Generally, cilia are more in number than flagella. The length of cilia is less than that of flagella.

Structure—The flagellar or cilliary basal body gives rises to the ‘quxoneme’, the microtubule arrangement common to cilia and flagella. The cross-section of flagellum shows typical “9+2” arrangement of microtubules.

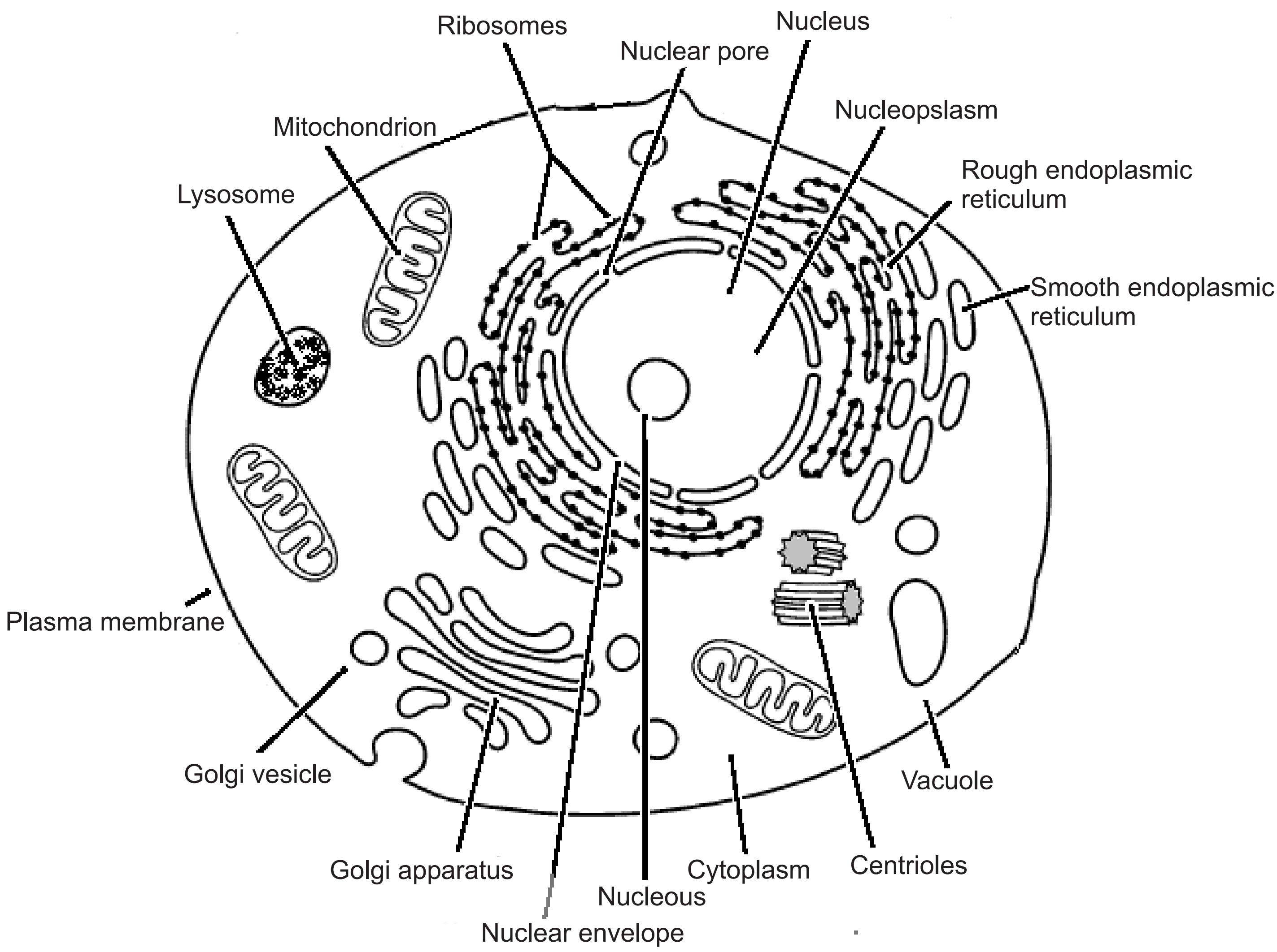

Fig. 2.10: Ultrastructure of animal cell

2.5.2 Cell Wall

The cell wall is absent in animals and protozoa. Cell wall constitutes a framework, which provides mechanical support to the cell. The plant cell wall is mainly composed of cellulose. There are three general regions of cell wall—middle lamellae, primary wall and secondary wall. In several cells, a fourth layer, the tertiary cell wall, is also present. Middle lamella is an intracellular matrix made up of pectin and lignin. The primary wall is composed of cellulose and pectin. The secondary cell wall, generally present in non-growing cells, is made of cellulose, hemi-cellulose and lignin. Few plant cells have tertiary cell wall, which is unique in composition having xylan instead of cellulose. The function of cell wall is to provide mechanical strength and confer shape to the cell.

2.5.3 Cytoplasm

Cytoplasm is a colourless, viscous, homogenous fluid present inside the cell in which cell organelles are enclosed. It is also called cytoplasmic matrix. It is the "environment" of the organelles and the location of many important biochemical processes. The cytoplasmic fluid is also called cytosol. 'The movement of organelles in cytoplasm is called cytoplasmic streaming'. The summary of prokaryotic cell components are given in Table 2.4 below.

25 nm in diameter. They consist of two different protein subunits named actin and tubulins.

Functions

1. Cytoskeleton is directly involved in movements such as muscle contration and during change in the shape of developing vertebrate embryo.
2. Cytosleleton provides machinery for cyclosis in cytoplasm.
3. Microfilaments play an important role in muscle contraction, cytokinesis, cell movement, and other cellular functions and structure.
4. Intermediate filaments consist of major structural proteins of skin and hair. The scaffold that holds Z discs and myofibrils instead of muscle generally function as important structural components of many animal cells and tissues.
5. Microtubules are important component of cilia, flagella the mitotic spindle and other cellular structure.

2.5.5 Endoplasmic Reticulum (ER)

Endoplasmic reticulum is an irregular network of branching and fusing membranous tubules, around 40–70 nm in diameter. It has many flattened sacs called cisternae. The ER may connect to nucleus and extend up to plasma membrane.

Their are two types of ER, depending on the presence/absence of membrane-attached ribosomes.

A. Rough ER / Granular ER—The RER has a number of ribosomes attached to the membrane (Fig. 2.11).

B. Smooth ER / Agranular ER—The smooth ER does not possess ribosomal granules on its surface. The ER creates an extensive network of membrane channels throughout the cytoplasm, with connections to the nuclear and plasma membrane.

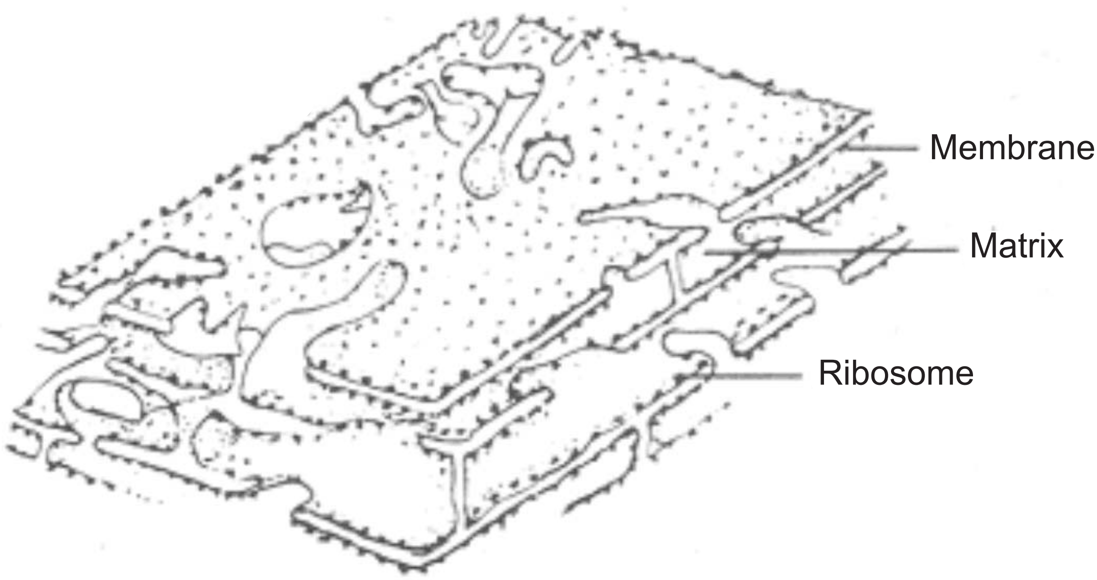

Fig. 2.11: Rough endoplasmic reticulum

40 S. The ribosome is attached to ER by 60S subunit. Several ribosomes are attached to single mRNA and simultaneously translate its message into protein. This structure is called polyribosome or polysomes. Although the eukaryotic cell possesses 80 S ribosomes, mitochondria and chloroplast possess 70S ribosomes. The function of ribosomes is protein synthesis.

2.5.8 Golgi Apparatus/Golgi complex/dictyosomes (Fig. 2.13)

The Golgi apparatus is a membrane-bound organelle composed of flattened, sac-like cisternae present one upon the another. The thickness of each sac/cisternae is 20–30 nm. The cisternae has a definite polarity i.e., the two ends of a cisternae, called 'cis' and 'trans' are quite different from one another. A number of fungi and ciliate protozoa lack a well-formed Golgi apparatus and it is made up of a single stack of cisternae, which are called 'dictyosomes'.

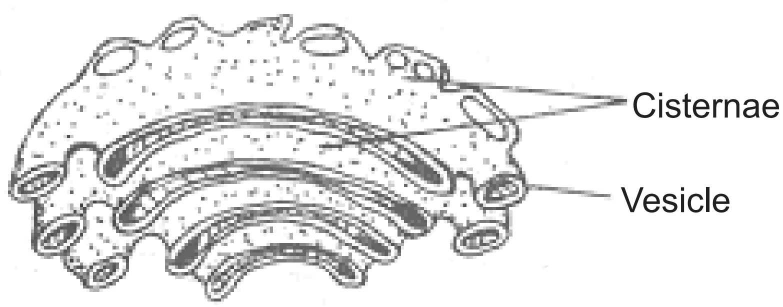

Fig. 2.13: Ultrastructure of Golgi apparatus

Functions:

1. Golgi apparatus is the site of synthesis of lipoproteins, glycoproteins and various polysaccharide derivates that are essential for the synthesis of various cell components.
2. Secretory vesicles are important for the construction of structures external to cell membrane, such as cell wall.
3. Golgi complex packages the hydrolytic enzymes into lysosomes, which cause cytoplasmic digestion, i.e., phagocytosis and pinocytosis.
4. Golgi apparatus plays an important role in acrosome formation in sperm.

2.5.9 Lysosome (lysis-breaking; some-body)

Lysosome is a membrane bound organelle found only in eukaryotic cell that contains hydrolytic enzymes. If the lysosome accidently breaks inside the cell, the enzyme contents will destruct the cell. Hence, lysosome is also called "the suicide bag". Hydrolytic enzymes are synthesised by the ribosomes of RER, which are then transferred to Golgi apparatus where they are packed into lysosome.

The hydrolytic enzymes are proteases, nucleases, glycosidase, sulfatases, lipase and phosphatases.

Functions:

1. Phagocytosis and endocytosis—The lysosome destroys the foreign organic particles (e.g., bacteria) by a process called "Phagocytosis", and

2.5.11 Plastid

Plastid is a cell organelle primarily involved in the formation and storage of carbohydrates. These are smaller bodies found in the cytoplasm of plant and algal cells. Schimper classified the plastids depending on the functions and pigment content as given in Table 2.5 below.

Table 2.5: Schimper's classification of plastid

	Type	Occurrence	Function	Pigment
I.	**Chromoplast**			
	A. Chloroplast	Green algae, higher plants	Photosynthesis	Chlorophyll
	B. Phenoplast	Brown algae, diatoms	Absorb light	Fucoxanthin
	C. Rhodoplast	Blue-green algae	Absorb light	Phycoerythrin
	D. Blue green chromoplast	Blue-green algae	Photosynthesis	Phycocyanin
II.	**Leucoplast**			
	A. Amyloplast	Endosperm	Storage [Starch]	None
	B. Elaioplast	Monocotyledon plants	Storage [Oil]	None
	C. Aleuroplast	Seeds	Storage [Protein]	None

Biologically, the most important plastid is chloroplast. In the eukaryotic cell of plant and algae a cell organelle is present, which is the site of photosynthesis. It called chloroplast. The size of chloroplast is 2–4 μ × 5–10 μ, but some contain a single, huge chlorophyll occupying nearly half volume of the cell. Chloroplast is

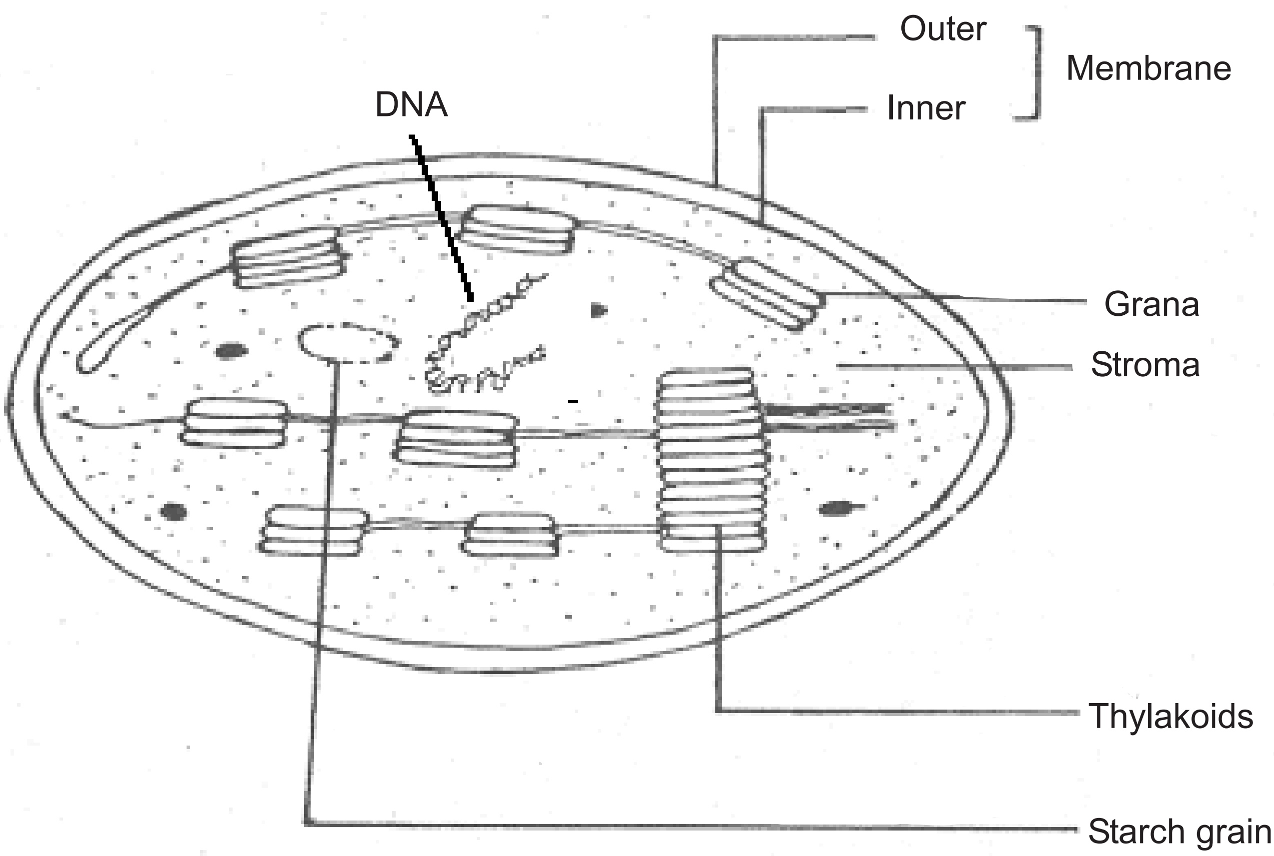

Fig. 2.15: Ultrastructure of chloroplast

vacuoles is not fixed. The single membrane of vacuole is called tonoblast. Some vacuoles act as a storage house of food material e.g., yeast vacuoles store polyphosphate, amino acid, uric acid, etc. Sometimes vacuoles are involved in cell exocytosis. In some cases, vacuole surrounds the food source and allows digestion by following its fusion with lysosomes. The summary of eukaryotic structures and their functions are given in Table 2.6.