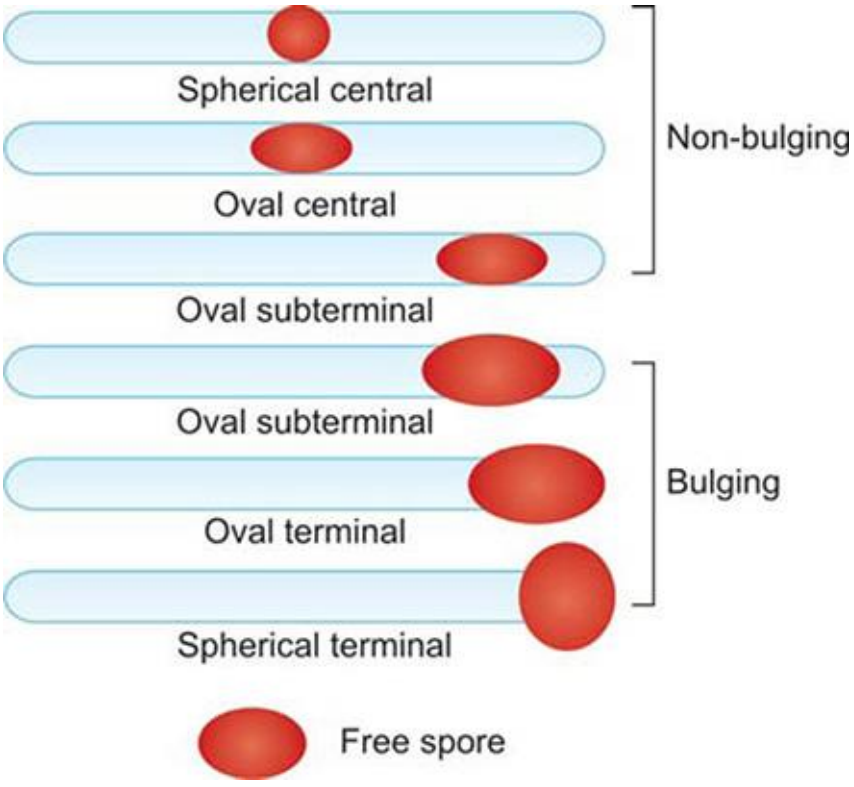
Unit -2 Bacterial Morphology and inner ultrastructure of cell

1. Endospore

Under the conditions of limited supply of Carbon, Nitrogen, Phosphorous etc and under unfavourable environmental condition of Temperature, pH etc, certain bacteria produce endospores. These are resistant to heat, chemicals, drying, freezing and radiations. Endospore shows no detectable metabolism. They can remain in a state of extreme dormancy for long periods and are tolerant to heat, radiations and bactericidal agents.

Species of genera ***Bacillus, Clostridium, Desulfatomaculum, Lactobacillus and Sporosarcina***etc form endospores. Spore may be oval or round, (ellipsoidal or spherical) in shape. Spore may be present at the centre, subterminal or terminal position of the cell.



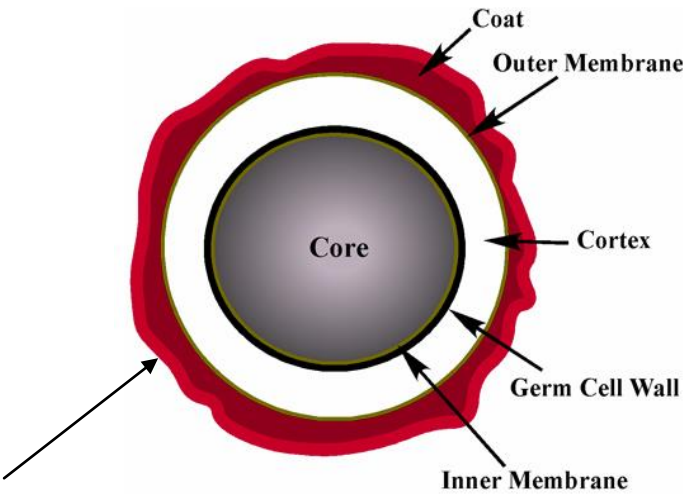
Structure of endospore: -

The main role of endospore is to survive in dry state, in non-nutrient medium. Endospore consists of—

1. Protoplasm or core: -It contains DNA and components of protein synthesis e.g. ribosomes, m-RNA, enzymes and other factors.

2. Envelop: - From inner to outer side, spore envelope consists of—

- ➔ Inner membrane,
- ➔ Cortex
- ➔ Outer membrane
- ➔ Spore coat
- ➔ Exosporium (in some species only)



Inner membrane: - It is bounding spore cytoplasm and nuclear material.

Cortex: - It is multilayer structure, which is present between inner and outer membrane; it consists of many layers of peptidoglycan.

The cortex also contains large amount of Dipicolonic acid and calcium ions in the form of Calcium-dipicolinate.

Outer membrane: - It is the membrane of mother bacterial cell enclosing endospore, which has grown around spore during endospore formation.

Sporecoat: - It is dense layer outside the outer membrane. Sporecoat is made up of protein. This protein provides resistance to spore from physical and chemical factors.

Exosporium: - In some species, an additional covering called exosporium is present. E.g. *Bacillus cereus, Bacillus anthracis.*Exosporium contains proteins, polysaccharide and lipids.

Endospore formation: -

A vegetative growth and division decreases during sporulation and sporulation starts when essential growth factors go down below a certain level.

Sporulation has been divided into number of stages as follows ---

Stage O: - It represents the vegetative cell. Depletion of essential metabolites limits the growth of the vegetative cells and initiates sporulation. An asymmetric cell division occurs, which brings about spore formation, producing a forespore within a mother cell. The vegetative cell contains two nuclear bodies.

Stage – I: - Axial filament formation: - The fusion of two compact nuclear bodies and redistribution of nuclear material from end to end takes place to form an axial chromatin thread called axial filament. Each chromosome may be attached to the cell membrane by a mesosome. The cell undergoes elongation.

Stage II (Spore septum formation): - Inward growth of a double layer of cell membrane takes place near one end of cell. The cell is asymmetrically partitioned by the double membrane, which forms transverse spore septum. The nuclear material divides so that each compartment contains at least one complete chromosome. The spore septum divides the bacterial cell into smaller and larger compartment, each containing DNA. The smaller compartment is called forespore, while the larger compartment is the mother cell.

Stage III (Engulfment of forespore): - The membrane of the large cell invaginates towards the pole of the cell and engulfs the forespore. The forespore is thus enclosed by two concentric sets of membrane, its own (inner forespore membrane) and the membrane derived from the mother cell (outer forespore membrane).

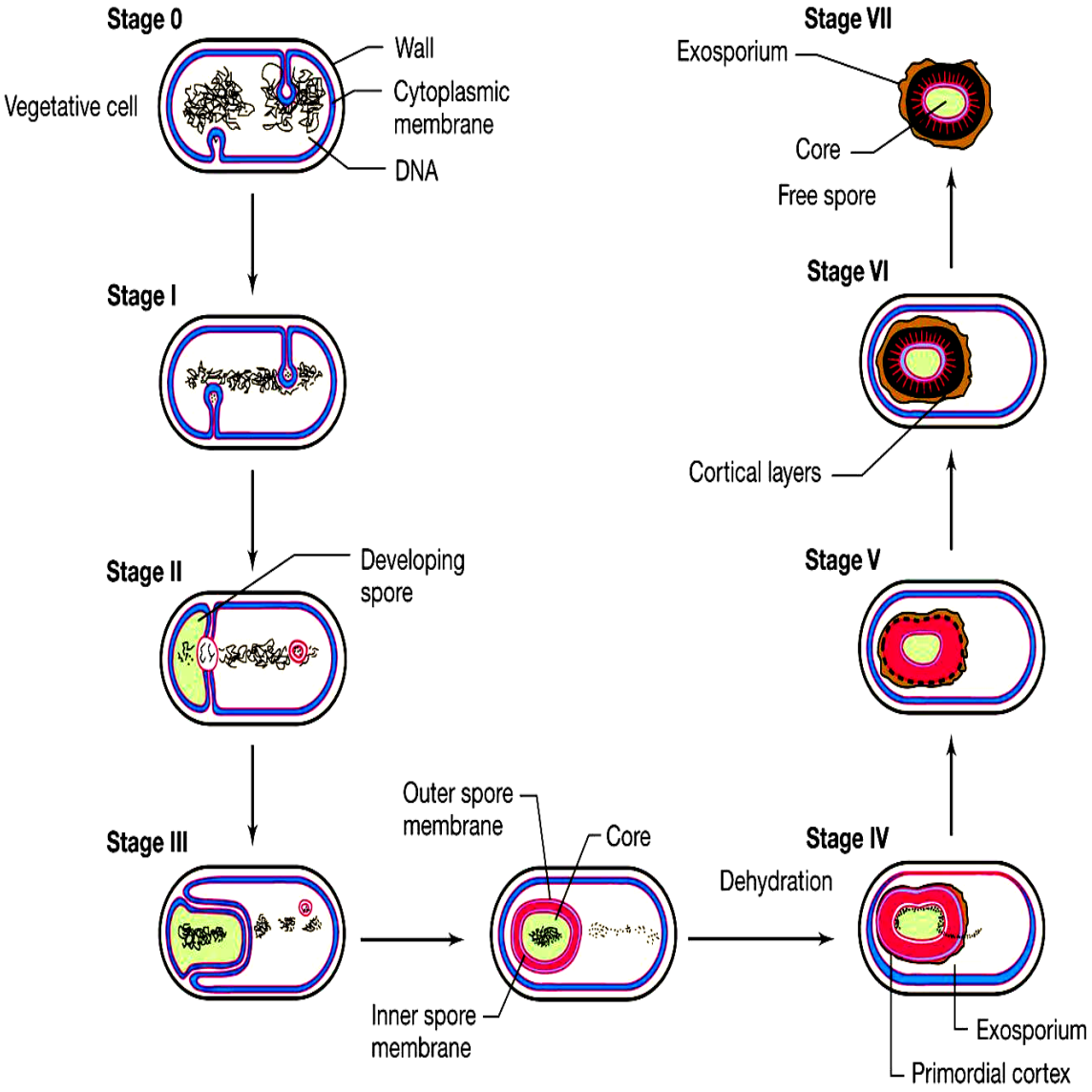
Stage IV (cortex synthesis): - The cortex develops between the inner and outer forespore membranes. In this stage deposition of the primary cell wall and deposition of spore specific peptidoglycan (cortex) outside the primary cell wall takes place.

The forespore also begins to accumulate calcium and Dipicolonic acid (2, 6 pyridine carboxilic acid) and becomes grey in appearance.

Stage V (Early coat synthesis): - Most of the proteinaceoussporecoats are synthesized during this stage. The electron dense sporecoats are formed outside the outer membrane surrounding the cortex. Accumulation of calcium ions and DPA continues and forespore becomes phase white. In *Bacillus cereus* group, a thin exosporium is formed around the sporecoat.

Stage VI (Maturation): - The maturing spore begins to become refractile. Water is withdrawn during spore maturation and is dehydrated.

Stage VII (Lysis and spore liberation): - During this stage spore is liberated by autolysis of mother cell.



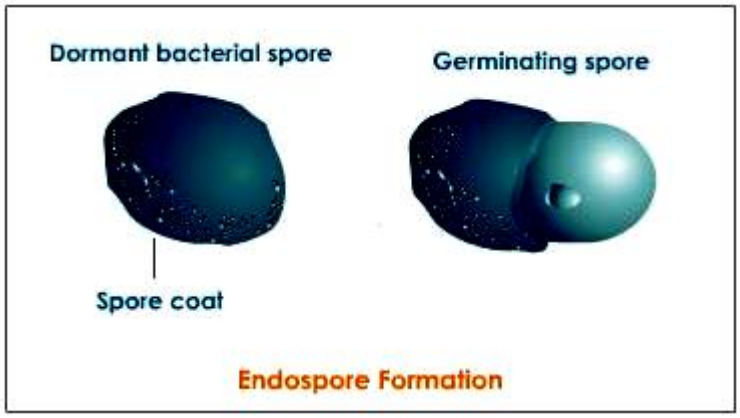
Germination of endospore: -

Spores can remain dormant for several years, although viability of spores decreases with passage of time.

The process by which spore is converted into vegetative cell is called germination. There are three stages of germination.

1. Activation
2. Initiation
3. Outgrowth

1. Activation: - Some bacterial spores germinate spontaneously in a favourable medium. Others remain dormant even if placed in optimal conditions for germination. Activation may be brought about by traumatic agents—such as heat, low pH or --SH compounds. Certain chemicals such as L-alanine, Adenosine, Glucose and some reducing agents can also bring about activation. Heat shock or heat activation appears to be most general mechanism for activation. Heating of spores in an aqueous fluid for 15 to 60 minutes at 65^{o} C results in activation of most spores. Higher temperatures of 105 to 120^{0} C are required for thermophilic spores.



2. Initiation (Germination Proper): - Germination can only takes place after activation. This is a rapid process which is marked by loss of heat resistance and resistance to other harmful agents and initiation of metabolic activity.

30 % soluble materials are liberated outside. The peptidoglycan of cortex undergoes hydrolysis. Calcium dipicolinate is released from the cells. Water is rapidly taken up along with Calcium and Magnesium ions. This causes loss of refractility. The chemical substances, which cause germination of activated spores, include L-alanine, Riboside (Adenosine, Inosine), Potassium nitrate, Glucose, and Various inorganic ions.

Germination can also be brought by physical treatments, which crack the spore coat.

3. Outgrowth or postgerminative development: -

Post germinative outgrowth occurs only if there are favourable conditions of nutrition and growth. Amino acids especially Glutamic acids are essential. Other requirements are minerals, energy sources such as glucose. If germination takes place in a nutrient medium, it is immediately followed by outgrowth. However there is no outgrowth in a starvation medium or in the presence of protein synthesis inhibitors.

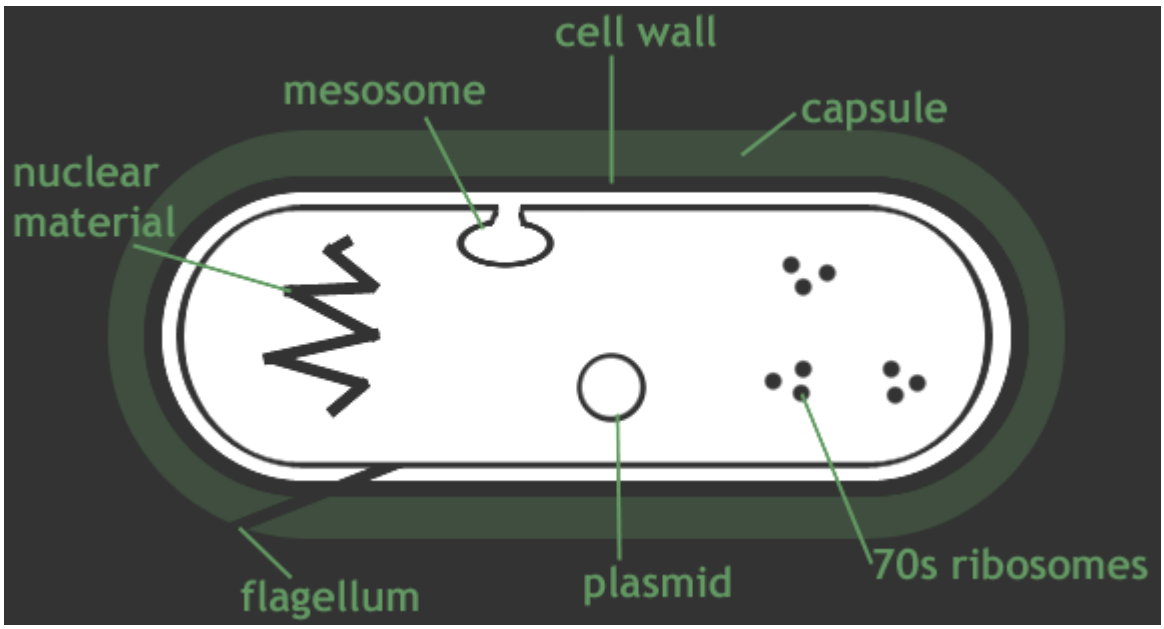
The following changes take place during post germinative development.

i) The spore swells within the sporecoat.
ii) There is rapid synthesis of vegetative cell wall.
iii) The spore wall splits and the vegetative cell emerges from spore coat.
iv) There is elongation of developing cell.
v) There is progressive increase in protein synthesis.
vi) There is increased enzymatic activity.
vii) DNA synthesis begins after about an hour.
viii) The sigma factor activity of vegetative RNA polymerase is restored.

---

3. Mesosomes: -

Mesosomes are complex, localised infoldings of cell membrane. These are more in Gram-positive bacteria. They are particularly well developed in Bacilli.



The Mesosomes consist of an invaginated cell membrane with many vesicles, tubules or lamellar whorls filling the invaginations. The vesicular type and a whorl type appear to be the most commonly seen forms. The lipid component of the Mesosomes appears to be similar to that of the cell membrane but its protein differs. Salton and Owen have suggested that Mesosomes may arise by vesicularisation of the outer half of the lipid bilayer. Although the Mesosomes represent a special cell membrane component, its true location and function is not clear.

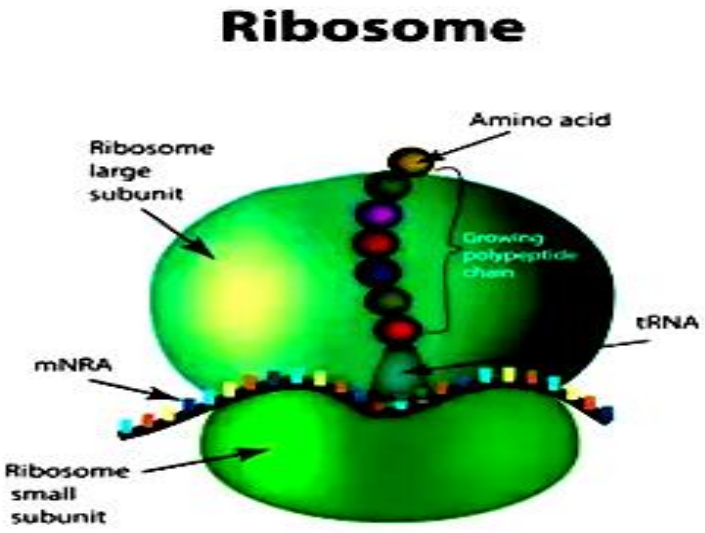
Mesosomes are near to the cell wall septum region hence they may be the sites of some of the wall membrane biosynthetic processes. It also helps during replication of DNA.

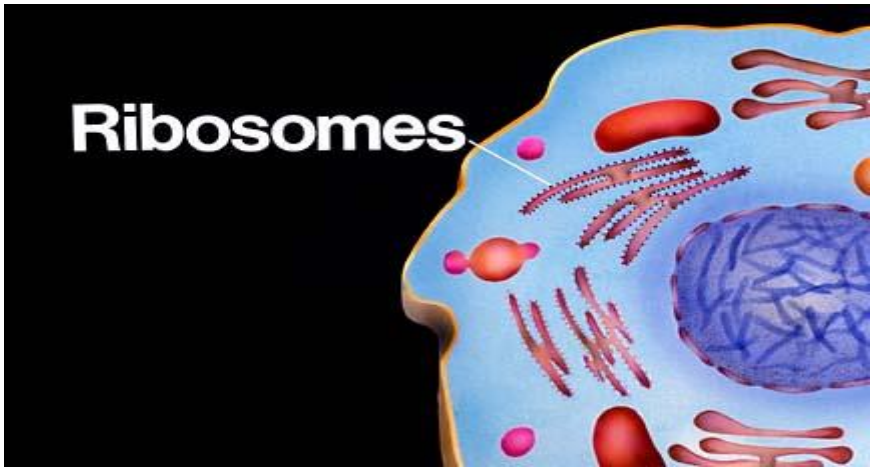
---

3. Ribosomes: -

The bacterial cell contains about 10,000 ribosomes, which constitute about 30 % of weight of the bacterium. Ribosomes are found free in cytoplasm. Prokaryotic ribosomes are around 20 nm (200 Å) in diameter and are composed of 65% rRNA and 35% ribosomal proteins.Prokaryote ribosomes are 70S ribosomes. These consist of larger 50S subunit and smaller 30S subunit. The *E. coli* ribosomes consist of 3 types of RNA (5S, 16S and 23S) and 55 proteins. The 30S subunit consists of 16S r-RNA and 21 proteins. The 50S subunit contains 5S and 23S r-RNA and 34 proteins.

Ribosomes are the workhorses of protein biosynthesis, the process of translating mRNA into protein.





Ribosome structure: -

There are two models explaining ribosome structure.

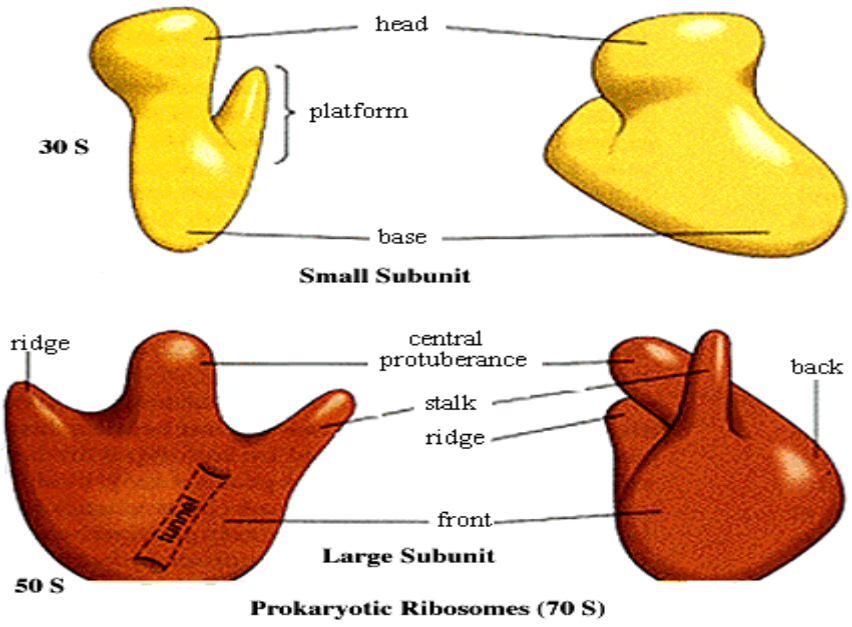
1. Stoffler and Wittmann's model
2. Lake's asymetrical model

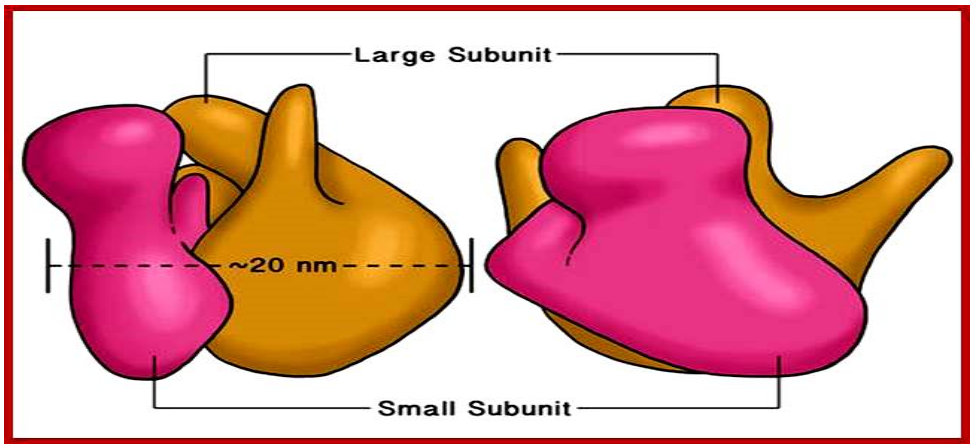
1. Stoffler and Wittmann's model: -

According to this model, 30S subunit has an elongated, slighly bent, prolate shape. It is a bipartite structure. A transverse hollow or cleft devides the 30S subunit into 2 parts.

➔ A smaller head and
➔ A larger body.

The appearance of 30S unit is like telephone receiver. The two lobes protrude unequally and two different extents. The body has elongated extension on both sides. In rear views the dorsal surface either appears plain or shows a shallow dent.





50S subunit: -

Electron microscopy observation of *E. coli* 50S subunit, show a number of shapes—round, kidney shaped circular profiles with a nose and maple leaf structures. These different shapes have been interpreted when 50S structure is seen in different views.

Frontal---> Maple leaf

Lateral---> Kidney shaped

Rear---> Rounded

In frontal view, the 50S subunit appears bilaterally symmetrical and show three protuberances arising from a rounded base. Of these the central protuberance is most prominent. 50S subunit has been compared to an armchair with the rounded base forming a vaulted seat, the central protuberance the back and the lateral protuberance the arms.

70S ribosomes: -

The 30S and 50S subunits are associated to form 70S ribosomes. The frontal face of 30S subunit with its hollow faces the vaulted seat of 50S subunit. The long axis of 30S subunits is oriented transversely to the central protuberance of 50S subunit and the vaulted seat of large subunit.

There are 4 areas of contact between two subunits.

Location of M-RNA: -

The m-RNA binding region is located on the right temporal side of the head of 30S subunit. The m-RNA molecule passes between the right temporal region of 30S subunit and the central protuberance of large subunit.

Location of t-RNA: -

The anticodon loop of t-RNA is located in the m-RNA binding domain and the –CCA end in the regions of proteins L2, L16 and L27.

2. Lake's asymmetrical model: -

In this model 30S unit is considered to be completely asymmetrical and indentation divides the subunit into two unequal parts.

- ➔ Upper 1/3 (head)
- ➔ Lower 2/3 (body)

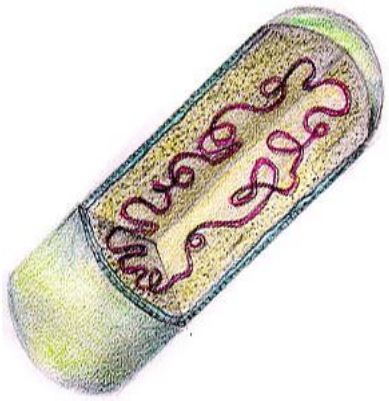
Extending from the low 2/3 is a region called platform. There is a cleft between the platform and the upper 1/3. The cleft is an important functional region. It is the site of the codon-anticodon interaction and the binding site for initiation factors, IF-1, IF-2, and IF-3.

The 50S subunit is also asymmetrical. Lateral projections incline at the angle about 50^0 from a central protuberance. The small subunit is asymmetrically positioned on the large subunit. The platform of the small subunit faces the large subunit. The portion between the 1/3 parts of the 30S subunit is aligned with the notch of large subunit.

---

4. Nuclear material (Nucleoid / Genophore): -

It is of primitive type and also refers to as '**nucleoid'.** It is possible to use coloured or fluorescent stains to describe central DNA rich regions in the bacterial cells. From a lysed protoplast, it can be isolated as a structure consisting of loops of supercoiled DNA together with about 10 % RNA and 10 % protein. It is amorphous, lobular mass of fibrilar, intensely chromatinic material occupies about 10 to 20 % of the cell volume. In electron microscope this body shows diffused area containing fibrous material with no limiting membrane.



The **nucleoid** (meaning *nucleus-like*) is an irregularly-shaped region within the cell of prokaryotes which has nuclear material without a nuclear membrane and where the genetic material is localized. The genome of prokaryotic organisms generally is a circular, double-stranded piece of DNA, of which multiple copies may exist at any time. The length of a genome widely varies, but generally is at least a few million base pairs. In eukaryotes the genome is packed into chromatin and bounded within a membrane-enclosed organelle called the nucleus.

A ***genophore***is the DNA of a prokaryote. This is commonly referred to as a prokaryotic chromosome. The term chromosome is misleading for a

genophore because the genophore lacks chromatin. The genophore is compacted through a mechanism known as supercoiling, whereas a chromosome is compacted via chromatin. The genophore is circular in most prokaryotes, and linear in very few. The circular nature of the genophore allows replication to occur without telomeres. Genophores are generally of a much smaller size than Eukaryotic chromosomes

Experimental evidence suggests that the nucleoid is largely composed of DNA, about 60%, with a small amount of RNA and protein. The latter two constituents are likely to be mainly messenger RNA and the transcription factor proteins found regulating the bacterial genome. Proteins helping to maintain the supercoiled structure of the nucleic acid are known as *nucleoid proteins* or nucleoid-associated proteins and are different from histones of eukaryotic nuclei. In contrast to histones, the DNA-binding proteins of the nucleoid do not form nucleosomes, in which DNA is wrapped around a protein core. Instead, these proteins often use other mechanisms to promote compaction such as DNA looping.

---

5. Reserve food material / Cytoplasmic inclusions

Bacteria have reserved food material stored in cytoplasm. They are referred as granules as they have granule like appearance The food is concentrated organic deposits and also known as cytoplasmic inclusions. Cytoplasmic inclusions are found dispersed in the cytoplasm or sometimes enclosed by membrane.

The reserved food is polymeric, high molecular weight and osmotically inert materials. Being osmotically inert do not decrease or increase osmolarity of cytoplasm and prevent the loss of cytoplasmic contents by cytolysis. The basic requirement of reserved food material as the name itself suggests that they are energy reserves and readily available substrates for metabolic reactions to carry out during stress conditions. There are 5 types of reserve food materials --

i. Non-nitrogenous reserve materials

a) Starch and glycogen granules
b) Poly hydroxybutyric acid granules (PHB)

ii. Nitrogenous reserve material

iii. Phosphate granules / Metachromatic granules / Volutin granules

iv. Sulphur globules

v. Gas vacuoles

The first 3 types are referred as granules as they have granule like appearance.

i. Non-nitrogenous reserve materials

a) Starch and glycogen granules

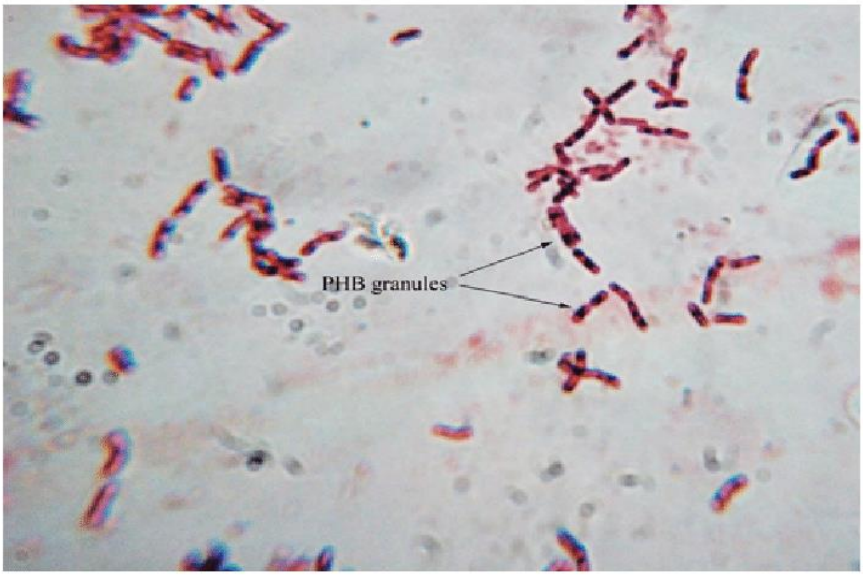
Polyglucan granules: They are also known as iodophilic or polysaccharide granules. They are stained by iodine solution and appear brown or bluish under light microscope. They can be seen as dark round spots by electron microscopy. Polyglucan consists of repeated glucan units with α, 1-4 linkage and α, 1-6 branch points. They are deposited by bacteria themselves inside their cells when simple sugars like glucose, fructose or sucrose are present for polysaccharide (glucan) synthesis. They have been found in *Clostridia and Coliform* group of bacteria; they are very important source of substrate in carbohydrate metabolism during starvation conditions in these bacteria.

b) Poly βhydroxybutyric acid granules (PHB)

Poly-β-hydroxybutyrate (PHB) granules: They are also known as sudanophilic (stained by lipid stain Sudan) or lipid granules. Chemically, they are polyacetides or polyesters. For light microscopic observation, they are stained by Sudan Black B or Nile blue to appear as dark blue dots in cell

cytoplasm against reddish blue background. Electron microscopic observations show them as light round spots.

Generally, PHB granules are formed during lipid synthesis, Acetate or butyrate metabolism, nitrogen deficiency conditions or denitrification. In lipid synthesis, acetyl CoA is condensed to aceto-acetyl CoA and it is further reduced to β-hydroxybutyryl CoA. Polymerization of this compound results in formation of PHB. Poly-β-hydroxybutyrate granules are important source of food during starvation conditions, particularly in soil and rhizosphere environment where nutrient stress is always prevalent. PHB granules are found in almost all species of *Alcaligenes, Bacillus, Rhizobium* and other soil bacteria.



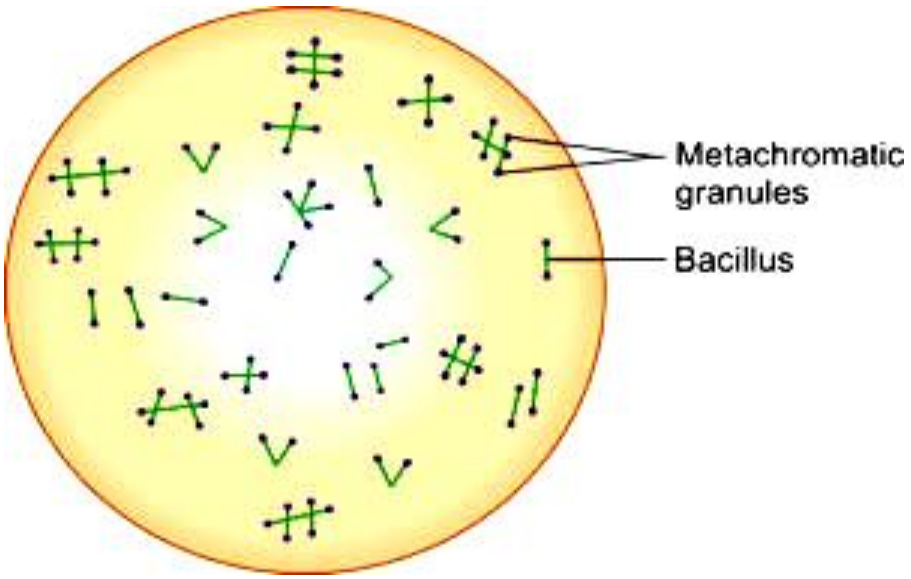
ii. Nitrogenous reserve material

These granules contain proteins and act as protein or amino acid source.

iii. Phosphate granules / Metachromatic granules / Volutin granules

They are also known as Metachromatic or Volutin or Polymetaphosphate granules. They are composed of polyphosphate, RNA and proteins. Their main function is to supply phosphate for nucleic acid synthesis, cell division, energy metabolism and as a source of phosphorous for nutrition. By staining with methylene blue, they represent different

colors like reddish purple, purple, bluish red or maroon under light microscope and hence the name **'metachromatic'.** By electron microscopy, they appear as dark spheres. They are usually detected in old laboratory cultures stored at room temperature or refrigerator. Naturally, they are found in species of *Bacillus, Corynebacterium, Spirillum, and Rhizobium.*



iv. Sulphur globules

Sulphur globules are cytoplasmic globules of elemental sulphur. They are usually found in bacteria growing in environments rich in hydrogen sulfide (H_2S) gas such as hydrothermal vents, thermal geysers, boiling water or sulphur springs. These habitats are always dominated by sulfate reducing (photosynthetic purple and green sulphur) bacteria like *Chromatium* and *Chlorobium*.

They use H_2S as electron donor to reduce carbon dioxide during photosynthesis process. The sulphur globules are also found in sulphur oxidizing bacteria, like extremophile *Thiobacillusthiooxidans* which inhabit sulphur rich environments. They principally oxidize elemental sulphur to sulfates which is then assimilated by plants for synthesis of sulphur containing amino acids. Both sulphur reducing and oxidizing bacteria are integral part of natural elemental sulphur cycle on the Earth.

v . Gas vacuoles:

Aquatic bacteria like *Cyanobacteria* possess gas vacuoles. They are present in the cytoplasm and hence considered as cytoplasmic inclusions. By light microscopy, they appear as bright refractile bodies and by electron microscopy as hollow cylindrical shapes with conical ends and striated protein boundary. Protein boundary is impermeable to water but allows exchange of various gases dissolved in water or at the air-water interface. Vacuoles may get collapsed under gas pressure or can be refilled by gases. Their refractility depends on pressure of internal gas. The main function of gas vacuole is to provide buoyancy to organism in aquatic habitat.

==========

Bacterial cell division

i) Binary fission

In biology, **fission** is the subdivision of a cell (or body, population, or species) into two or more parts and the regeneration of those parts into separate cells (bodies, populations, or species). **Binary fission** produces *two* separate cells, populations, species, etc., whereas **multiple fission** produces *more than two* cells, populations, species, etc.

Binary fission of prokaryotes

Most bacteria reproduce by a relatively simple asexual process called binary fission: each cell increases in size and divides into two cells. During this process there is

i) an orderly increase in cellular structures and components,

ii) replication and segregation of the bacterial DNA,

iii) formation of a septum or cross wall which divides the cell into two progeny cells.

The process is coordinated by the bacterial membrane perhaps by means of mesosomes. The DNA molecule is believed to be attached to a point on the membrane where it is replicated. The two DNA molecules remain attached at points side-by-side on the membrane while new membrane material is synthesized between the two points. This draws the

DNA molecules in opposite directions while new cell wall and membrane are laid down as a septum between the two chromosomal compartments.

When septum formation is complete the cell splits into two progeny cells. The time interval required for a bacterial cell to divide or for a population of bacterial cells to double is called the generation time. Generation times for bacterial species growing in nature may be as short as 15 minutes or as long as several days.