

Chapter 2 Cell Biology of prokaryote

Section 1 Overview of cell structure

Features of prokaryotic cell

1. Their genetic material (DNA) is not enclosed within a membrane and they lack other membrane – bounded organelles.
2. Their DNA is not associated with histidine.
3. Their cell walls almost always contain the complex polysaccharide peptidoglycan.
4. They have 70S ribosome.
5. They are very small!

Section 2 Cell wall

The cell wall of the bacterial cell has a complex, semi-rigid structure that surrounds the underlying, fragile plasma (cytoplasmic) membrane .

- Almost all prokaryotes have cell walls.

1 Structure of cell wall

Bacteria can be divided into two major groups called gram-positive and gram-negative, which was based on a special staining procedure, the Gram staining.

A Gram-positive cell wall

The Gram-positive cell wall has a peptidoglycan layer that is relatively thick (ca. 40 nm) and comprises approximately 90% of the cell wall. The cell walls of most Gram-positive eubacteria also have teichoic acids.

Structure of Peptidoglycan of Gram-positive

Peptidoglycan is composed of two sugar derivatives, N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), which are connected by β 1-4 bond, and a small group of amino acids consisting of L-alanine --- D-glutamic acid ---L- lysine ---D-alanine, which are connected to N-acetylmuramic acid (NAM).

B Gram-negative cell wall

The Gram-negative cell wall consists of a thin layer peptidoglycan and an outer membrane. The peptidoglycan layer is attached to the outer membrane via lipoproteins. The outer membrane contains phospholipid on its inner surface and lipopolysaccharide (LPS) on its outer surface. The space between the outer membrane and the cytoplasmic membrane is called the periplasmic space. Teichoic acids do not occur in Gram-negative bacterial cell walls.

2 Gram staining

The difference between gram-positive and gram-negative bacteria is due to the physical nature of their cell walls. If the cell walls are removed from gram-positive bacteria, they become gram negative.

The Mechanism and procedure of Gram Staining

The peptidoglycan seems to act as a permeability barrier preventing loss of crystal violet. Gram-negative peptidoglycan is very thin, not as highly cross-linked, and has larger pores. Alcohol treatment may extract enough lipid from the gram negative wall to further increase its porosity as well. For these reasons, alcohol more readily removes the purple crystal violet-iodine complex from gram-negative bacteria.

3 Functions of cell wall

- A It protects the cell.
- B It keeps the shape of the cell.
- C It performs as molecular sieve.
- D It performs as a pivot of a flagellum, and it is involved in pathogeny of pathogen and infection of phage.

Protoplast Formation

Peptidoglycan can be destroyed by certain agents, for instance lysozyme that breaks the 1,4-glycosidic bonds between N-acetylglucosamine and N-acetylmuramic acid in the molecule. Protoplast appears when cell walls are removed from the cells.

Section 3 Cytoplasmic membrane

Cytoplasmic membrane, or cell membrane, plasma membrane, is a soft, fragile and flexible semi-permeable membrane, which underlines cell wall and surrounds cytoplasm.

1 Structure of cytoplasmic membrane

A. The typical cytoplasmic membrane of prokaryotic and eukaryotic cells is a lipid bilayer, as illustrated here showing the orientations of the hydrophilic (tan spheres) and hydrophobic (black) ends of phospholipids that make up this structure.

B. Colorized electron micrograph of the cytoplasmic membrane (CM) of the bacterium *Bacillus subtilis* reveals the characteristic railroad track appearance of this lipid bilayer.

Membranes contain both lipids and proteins, although the exact proportions of lipid and protein vary widely.

Proteins are embedded on or through the cytoplasmic membrane.

Mesosome.

Mesosome may be involved in wall formation during division or play a role in chromosome replication and distribution to daughter cells. It may also be involved in secretory processes

2 Functions of cytoplasmic membrane

A It is an elective control of transportation of nutrition and metabolite into or out cell.

B. It is a tructure that can keep the ordinary osmotic pressure of the cell.

C. It is the site where components of cell wall, peptidoglycan and teichoic acid, and capsule polysacharride are synthesized.

D. It is the base for energy-generation.

E. It is the site where basal body is located and flagella obtain energy for rotation.

Section 4 Prokaryon

Prokaryon, also called nucleoid, bacterial chromosome, is the primitive nucleus in prokaryote, without the surrounding of membrane and regular shape.

1 Structure and size of prokaryon

The prokaryon is usually a single covalently closed circular DNA macromolecule, except in *Streptomyces* where it is linear and *Rhodobacter sphaeroides*, which has two separate chromosomes.

2 Reproduction of bacteria

A Procedure of reproduction

Bacteria are normally reproduced by binary fission. The inward growth of the septum divides the parent cell to produce two equal-sized progeny cells.

B Colony

Colony is the group of bacterial cells that reproduces on or in solid medium and forms visible shapes. If the colony is resulted from a single cell, it is called a clone.

Features of colonies(shape, color, size...) can be used as the factors to classify microbes.

Bacteria with flagella form larger, flatter colonies with irregular edges.

Bacteria without flagella, especially cocci, form smaller, thicker, hemisphere-shaped colonies with regular round edges.

Bacteria with capsules form larger and clear colonies with smooth surfaces. Bacteria with spores form dry colonies with rough surfaces.

When a number of colonies are joined together, they are called bacterial lawn.

Rapid bacterial reproduction in liquid media often causes turbidity.

Aerobic bacteria often form pellicle or scum on the surface of liquid media.

3 Plasmid

Plasmids is the small extra-chromosomal circular DNA molecule in prokaryotic cells.

Stringent replication control; Relax replication control.

Plasmids don't contain the genetic information for the essential metabolic activities of the cell, but they generally do contain genetic information for special features.

Major types of plasmids

R plasmids

Col plasmids

F plasmids

Ti (Tumor inducing) plasmids

Ri (Root inducing) plasmids

Mega

Other plasmids

Function of prokaryon and plasmid: to carry and pass on genetic information.

Section 5 Ribosome and inclusion body

1 Ribosome

There are about 10000 ribosomes in a procaryotic cell, and the number changes in different growth stage.

All eucaryotic and procaryotic cells contain ribosomes, which function as the sites where protein synthesis occurs.

Ribosomes are composed of two subunits.

Procaryotic ribosomes are called 70S ribosomes, and those of eucaryotic cells are known as 80S ribosomes

2 Inclusions

Within the cytoplasm of procaryotic (and eucaryotic) cells are several kinds of reserve deposits, known as inclusion bodies. Some inclusions are common to a wide variety of bacteria, whereas others are limited to a small number of species and therefore serve as a basis for identification.

Among the more prominent bacterial inclusions are the following:

Carbon polymers

Phosphate polymers

Sulfur Granules

Gas Vacuoles

PHB is a lipidlike compound - one of the most common inclusion bodies in prokaryotic organisms. It is commonly found as storage material and unique to bacteria.

Glycogen is a starchlike polymer of glucose subunits. Glycogen granules are usually smaller than PHB granules.

Many microorganisms accumulate granules of polyphosphate, which are large reserves of inorganic phosphates that can be used in the synthesis of ATP.

Some bacteria, including many photosynthetic bacteria, accumulate elemental sulfur granules as a result of their metabolism.

The formation of gas vacuoles by aquatic bacteria provides a mechanism for adjusting the buoyancy of the cell. Many aquatic cyanobacteria use their gas vacuoles to move up and down in the water column.

Section 6 Components external to the cell wall

1 Flagellum

Flagellum is the long motile filament on the surface of bacteria.

Many prokaryotes are motile, and this ability to move independently is usually due to a special structure, the flagellum (plural, flagella).

Motility allows the cell to reach different regions of its environment. In the struggle for survival, movement to a new location may mean the difference between survival and death of the cell. But, as in any physical process, cell movement is closely tied to an energy expenditure, and the movement of flagella is no exception.

Flagella are arranged differently on different bacteria. In polar flagellation the flagella are attached at one or both ends of the cell.

Occasionally a tuft (group) of flagella may arise at one end of the cell, which is an arrangement called lophotrichous.

In peritrichous flagellation the flagella are inserted at many places around the cell surface (peri means "around").

The type of flagellation, polar or peritrichous, is often used as a characteristic in the classification of bacteria.

The filament of bacterial flagella is composed of subunits of a protein called flagellin.

The base of the flagellum is different in structure from that of the filament. There is a wider region at the base of the flagellum called the hook. The hook consists of a single type of protein and functions to connect the filament to the motor portion of the flagellum.

The basal body is anchored in the cytoplasmic membrane and cell wall. The basal body consists of a small central rod that passes through a system of rings, L-ring, P-ring, S-M ring.

In gram-negative bacteria, an outer ring is anchored in the lipopolysaccharide layer (L-ring) and another in the peptidoglycan layer (P-ring) of the cell wall, and an inner pair of rings (S-M ring) is located within the cytoplasmic membrane.

In gram-positive bacteria, which lack the outer lipopolysaccharide layer, only the inner pair of rings is present.

Surrounding the inner ring and anchored in the cytoplasmic membrane are a pair of proteins called Mot. These proteins actually drive the flagellar motor causing a torque that rotates the filament. A final set of proteins, called the Fli proteins function as the motor switch, reverse rotation of the flagella in response to intracellular signals.

The movement of a procaryotic flagellum results from rotation of its basal body and is similar to the movement of the shaft of an electric motor. Bacterial cells can alter the speed and direction of rotation of flagella and thus are capable of various patterns of motility.

2 Fimbriae and pili

Fimbriae (pl-fimbria), thinner, shorter and more numerous filaments on the surface of a bacterium. They can be evenly distributed over the entire surface of the cell. They can number anywhere from a few to several hundred per cell. Fimbriae allow a cell to adhere to surfaces including the surfaces of other cells.

Pilus(pl-pili): long filament on the surface of a bacterium that can be used as the pass-way for the transfer of DNA from donor cell to recipient cell.

They are usually longer than fimbriae and number only one or two per cell. However, they have the same structure and chemical components as fimbriae.

3 Glycocalyx

Glycocalyx is gel-like material covering the surface of some bacteria, which includes,

Capsule, which is in a steady state, and

Slime Layer, which is in a unsteady state.

Zoogloea is a group of cells that are contained in a capsule.

Many prokaryotic organisms secrete on their surfaces of slimy or gummy materials. A variety of these structures consist of polysaccharide, and a few consist of protein. The terms capsule and slime layer are frequently used to describe polysaccharide layers.

Section 7 Endospore

1 Endospore and its structure

Endospore, also called spore, is the round resistant dormant structure formed in the latter phase of the bacterial growth.

2 Physical, chemical and biological properties of endospore

1) Physical and chemical properties

* In its cortex, abundant DPA is found

(dipicolinic acid) which is combined with calcium ions.

* It has lower water content – only 10-30% of the water content of the vegetative cell.

* It has low pH value, and contains small acid-soluble spore proteins (SASPs)

* It has high refractility.

2) Biological properties

It is highly resistant to heat, drying, radiation and chemical disinfectant.

3 Endospore formation

Sporulation involves a very complex series of events in cellular differentiation.

Bacterial sporulation does not occur when cells are dividing exponentially but only when growth ceases owing to the exhaustion of an essential nutrient. Thus, cells of *Bacillus* cease vegetative growth and begin sporulation when a key nutrient such as the carbon or nitrogen source becomes limiting. The process is described as the following,

a, Axial filament formation

b, Septum formation

c, Engulfment of forespore

d, Cortex formation

e, Coat synthesis

f, Completion of coat synthesis, Increase in refractility and heat resistance

g, Lysis of sporangium, spore liberation

Parasporal crystal, or spore-companioned crystal, is the intracellular crystals of toxic glycoproteins produced by several *Bacillus* species, most notably *B. popilliae* and *B. thuringiensis*, when they sporulate.

4 Spore germination

There are 3 steps in spore germination,

1) Activation – Usually results from treatments like heating.

2) Germination – Breaks spore's dormant state. This process is characterized by spore swelling, loss of resistance to heat and other stresses, loss of refractility and increase in metabolic activity.

3) Outgrowth – The spore protoplast makes new components and develops once more into an active bacterium.