CHARACTERIZATION
OF
PROKARYOTIC CELLS

STRUCTURES
IN
BACTERIA

- At one time the living world was subdivided into plants and animals.

- However, with the development of microscopes, the existence of organisms invisible to the unaided eye was discovered. It was rapidly appreciated that these microorganisms were neither plants nor animals, and in 1866 Haekel proposed a new kingdom, the **Protista**, that contains:
  - **Bacteria**
  - **Fungi**
  - **Protozoa**
  - **Algae**

With study of biological properties of **Protista** it was soon recognized that these microorganisms could be subdivided into two groups based on cell structure:

- **Eukaryotic cells** (Greek for Ătrue nucleusñ)
  - ï (within this group the major subdivisions are the algae, the protozoa and the fungi)
- **Prokaryotic cells** (Greek for Ăprimitive nucleusñ)
  - ï bacteria
- *Eukaryotic cells are structurally more complex than prokaryotic cells, containing a variety of membrane-enclosed organelles.*

- Bacteria, the smallest cells, are visible only with the aid of a microscope.
- The smallest bacteria (*Chlamydia*, *Chlamydophila*, and *Rickettsia*) are only 0.1 to 0.2 μm in diameter, whereas larger bacteria may be many microns in length. Most species are approximately 1 μm in diameter and are therefore visible using the light microscope which has a resolution of 0.2 μm.
- In comparison, animal and plant cells are much larger, ranging from 7 μm (red blood cells) to several feet (the length of certain nerve cells).

EUKARYOTIC CELL STRUCTURE

The nucleus

- The genetic information of the eukaryotic cells, DNA is organized into multiple chromosomes covered with protein (histones).

- The chromosomes in turn are surrounded by a two-layer membrane, of which the outer membrane is continuous with the endoplasmic reticulum.

Endoplasmic reticulum

- The endoplasmic reticulum is a network of membrane-bounded channels.

- The endoplasmic reticulum extends throughout the cell cytoplasm and is subdivided into two types:
  - ï rough,
  - ï smooth.

Endoplasmic reticulum

- The rough endoplasmic reticulum is covered with ribosomes, which are used for protein synthesis.
- One specialized structure of the smooth endoplasmic reticulum is the Golgi complex.
- Proteins from the rough endoplasmic reticulum migrate to the Golgi complex, where they are packaged in vesicles. The vesicles are then transported to the cell surface, where they fuse with cell membrane and release the proteins.

Mitochondria

- The membrane-enclosed mitochondria contain their own DNA and synthetic system and are capable of self-replication.
- Membranes in the mitochondria are the site of the respiratory electron transport system, the primary source of energy in the cell.

Plasma membrane

- The plasma membrane, a lipoprotein structure encloses the cell cytoplasm and regulates transport of macromolecules into and out of the cell.

Cell wall

- The cell wall forms rigid outer barrier that, when present in eukaryotic cells, is most commonly composed of polysaccharides such as cellulose or chitin.

Motility organelles

- Many eukaryotic microorganisms propel themselves through water by means of protein appendages called cilia or flagella (cilia are short, flagella are long).

PROKARYOTIC CELL STRUCTURE

- Prokaryotic cells are smaller and generally less complex than eukaryotic cells, with one exception: the cell envelope is more complex.
- The primary distinguishing characteristics of the prokaryotes are their relatively small size, usually on the order of 1 micrometer in diameter, and the absence of a nuclear membrane.
- The DNA of almost all bacteria is a circle with a length of about 1 mm: this is the prokaryotic chromosome. The specialised region of the cell containing DNA is termed the nucleoid and can be observed by electron microscopy.

Nucleus

- In contrast with eukaryotic cells, the genetic material of bacteria and other prokaryotic cells is diffuse, organized into a single, naked, circular chromosome.
- A nuclear membrane is not present.
- The chromozome is attached to a mesosome, which is important for the replication of the chromosome.

Plasmids

- Plasmids, which are smaller, circular, extrachromosomal DNA, may also be present.

- Plasmids are most commonly found in Gram-negative bacteria, and although not usually essential for cellular survival, they often provide a selective advantage: many confer resistance to one or more antibiotics.

Cytoplasmic structure

- Prokaryotic cells lack mitochondria and chloroplasts.
- The electron transport enzymes are localised in the cytoplasmic membrane.
- Bacteria often store reserve materials in the form of insoluble cytoplasmic granules, which are deposited as osmotically inert, neutral polymers (e.g. metachromatic granules ï they are characteristic features of corynebacteria).

Cytoplasmic structure

- The cytoplasmic membrane is composed of phospholipids and proteins and, in contrast with eukaryotic cells, does not contain sterols (except for *Mycoplasma* species).
- Because prokaryotic cells lack mitochondria and a complex membranous network like the endoplasmic reticulum within the cell cytoplasm, the electron transport enzymes are located in the cytoplasmic membrane.
- The membrane also serves as an osmotic barrier for the cell, contains transport systems for solutes, and regulates transport of cell products to the extracellular environment.

Cytoplasmic structure

- Invaginations of the cytoplasmic membrane form specialized structures called mesosomes.
- There are two types:
  - ï septal mesosomes ï which function in the formation of cross-walls during cell division,
  - ï lateral mesosomes.
- The bacterial chromosome is attached to a septal mesosome.

The major functions of the cytoplasmic membrane are:

- selective permeability and transport of solutes,
- electron transport and oxidative phosphorylation in aerobic bacteria,
- excretion of hydrolytic exoenzymes,
- bearing the enzymes and carrier molecules that function in the biosynthesis of DNA, cell wall polymers, and membrane lipids,
- bearing the receptors and other proteins of the chemotactic and other sensory transduction systems.

Cytoplasmatic structures - conclusion

1. Bacterial chromosome ï unlike eukaryotes, the bacterial chromosome is a single, double-stranded circle that is contained not in a nucleus, but in a discrete area known as the nucleoid.
2. Plasmids.
3. Ribosomes ï the bacterial ribosome consists of 30S+50S subunits, forming a 70S ribosome. This unlike the eukaryotic 80S (40S+60S) ribosome. The proteins and RNA of the bacterial ribosome are significantly different from those of eukaryotic ribosomes and are major targets for antibacterial drugs.
4. Cytoplasmatic membrane.

Cell wall

- The layers of the cell envelope lying between the cytoplasmic membrane and the capsule are referred to collectively as the Ăcell wallñ.

- The cell wall in prokaryotic cells is extremely complex. This rigid structure protects the cell from rupture caused by the high osmotic pressure inside the bacterial cell. The internal osmotic pressure of most bacteria ranges from 5 to 20 atmospheres as a result of solute concentration via active transport.

- Additionally, the cell wall is the site of many of the antigenic determinants that characterize and differentiate microorganisms. Endotoxin activity associated with certain groups of bacteria is also associated with the cell wall.

Cell wall

- Bacteria have historically been subdivided by their reaction with the Gram stain.

- Although both Gram-positive and Gram-negative bacteria have cell walls, their differential staining properties are in large part attributed to the structure of the cell wall.

Cell wall

- The basic structure of the cell wall of Gram-positive bacteria is a thick (15-80 nm) peptidoglycan layer composed of chains of alterating subunits of A-acetylglucoseamine and A-acetylmuramic acid.

- All Gram-positive cell walls also contain teichoic acid bound to the cytoplamic membrane.

Cell wall

- The structure of the cell wall of Gram-negative bacteria is more complex.
- The peptidoglycan layer is thinner, only 1 to 2 nm. Outside the peptidoglycan layer is phospholipid outer membrane (absent in Gram-positive bacteria).
- The area between the outer mebrane and the cytoplasmic membrane is called the periplasmic space. The outer membrane prevents loss of periplasmatic proteins and forms a protective barrier preventing exposure of bacteria to hydrolytic enzymes and toxic substances such as bile in the gastrointestinal tract.

Cell wall

- Embedded in the outsite layer of the outer membrane is lipopolysaccharide. This molecule is the most significant structure in Gram-negative bacteria.
- The lipid component (lipid A ï endotoxin) is responsible for the toxic properties of this group of bacteria.
- The polysaccharide component consists of a core common to all Gram-negative bacilli and a variable terminal segment that is exposed on the outer surface of the bacteria and is the major surface antigen (O antigen).

Capsules and glycocalyx

- Many bacteria synthesize large amounts of extracellular polymer when growing in their natural environments. With one known exception (the poly-D-glutamic acid capsule of *Bacillus anthracis*), the extracellular material is polysaccharide.

- When the polymer forms a condensed, well-defined layer closely surrounding the cell, it is called the capsule. When it forms a loose meshwork of fibrils extending outward from the cell, it is called the glycocalyx.

Capsules and glycocalyx

- The capsule contributes to the invasiveness of pathogenic bacteria.
  - ï Encapsulated cells are protected from the phagocytosis unless they are coated with anticapsular antibody.
  - ï The glycocalyx plays a major role in the adherence of bacteria to surfaces in their environment.

Flagella

- Bacterial flagella are thread-like appendages composed entirely of protein, 12-30 nm in diameter. They are the organs of locomotion.
- Three types of arrangements are known:
  - monotrichous (single polar flagellum),
  - lophotrichous (multiple polar flagella),
  - peritrichous (flagella distributed over the entire cell).
- A bacterial flagellum is made up of a single kind of protein subunit called flagellin. The flagellum is formed by the aggregation of subunits to form a hollow cylindrical structure. The flagellum is attached to the bacterial body by a complex structure consisting of a hook and a basal body.

Pili (fimbriae)

- The pili are short, hairlike structures that can aid in adherence of bacteria to the target cells or facilitate exchange of DNA during bacterial conjugation. They are shorter and finer than flagella, like flagella, they are composed of protein subunits.
- Two classes can be distinquished:
  - ordinary pili (fimbriae), which play a role in the adherence of bacteria to host cells,
  - sex pili, which are responsible for the attachment of donor and recipient cells in bacterial conjugation.

Endospores

- Members of several bacterial genera are capable of forming endospores.

- The two most common are Gram-positive rods (genus *Bacillus* and genus *Clostridium*).

Major characteristics of Eukaryotes and Prokaryotes

Characterictic	Eukaryotes	Prokaryotes
Major group	algae, fungi, protozoa, plants, animals	bacteria
Size (approximate)	> 5 ηm	0,5 ï 3 ηm
Nucleus	classic membrane	no nuclear membrane
Chromosomes	strands of DNA diploid genome	single, circular DNA haploid genome
Mitochondria	present	absent
Golgi bodies	present	absent
Endoplasmatic reticulum	present	absent
Ribosomes (sedimentation coefficient)	80S (60S + 40S)	70S (50S + 30S)
Cytoplasmatic membrane	contains sterols	does not contain sterols
Cell wall	is absent or is composed of chitin	is a complex structure containing proteins, lipids, and peptidoglycan
Reproduction	sexual or asexual	asexual (binary fission)
Movement	complex flagellum, if present	simple flagellum, if present
Respiration	via mitochondria	via cytoplasmatic membrane

Bacterial membrane structures

Structure	Chemical constituents
Cell membrane	phospholipids, proteins, and enzymes involved in generation of energy, membrane potential, and transport
Cell wall Gram-positive bacteria	peptidoglycan
	teichoic acid
	lipoteichoic acid
Cell wall Gram-negative bacteria	peptidoglycan (thinner version of that found in Gram-positive bacteria)
	periplasmatic space (enzymes involved in transport, degradation, and synthesis)
	outer membrane (phospolipids with saturated fatty acids)
	proteins (porins, lipoprotein, transport proteins)
	LPS (lipid A, core polysaccharide, O antigen)
Other structure	
Capsule	polysaccharides and polypeptides
Pili (fimbriae)	pilin, adhesin
Flagellum	proteins, flagellin
Proteins	M protein of streptococci (as an example)

Membrane characteristics of Gram-positive and Gram-negative bacteria

Characteristic	Gram-positive bacteria	Gram-negative bacteria
Outer membrane	-	+
Cell wall	thicker	thinner
LPS	-	+
Endotoxin	-	+
Teichoic acid	often present	absent
Sporulation	some strains	none
Capsule	sometimes present	sometimes present
Lysozyme	sensitive	resistant
Antibacterial activity of penicillin	more susceptible	more resistant
Exotoxin production	some strains	some strains

Staining of cell structure in bacteria

- The cell structures can be observed by optical methods:
  - ï the ligth microscope,
  - ï the electron microscope.

- Stains combine chemically with the bacterial protoplasm. If the cell is not already dead, the staining process itself will kill it. The process is thus a drastic one and may produce artifacts.

The most important staining in microbiology

- the Gram stain
- the acid fast stain
- the flagella stain
- the capsule stain
- the spore stain
- staining of nuclei

Gram Staining

- Basic classification of bacteria is based on the cell wall structure.
- There are 2 main groups: **Gram-positive** and **Gram-negative**.
- Gram staining is a differential staining technique that provides an easy differentiation of bacteria into one of two groups.
- The staining technique was developed by Christian Gram.

Morphology of bacteria

- Bacteria can be distinguished from one another by their morphology:
  - ï size,
  - ï shape,
  - ï arrangement,
  - ï staining characteristics.

Size

- Most bacterial species are approximately 1 micrometer in diameter.

Shape

- Bacteria are difficult to differentiate by size, but they have different shapes:
  - ï spherical bacteria ï cocci
  - ï rod-shaped bacteria ï rods
  - ï a snakelike bacteria ï spirillum

Shapes of Bacteria

- Coccus
  - Chain = Streptoccus
  - Cluster = Staphylococcus
- Bacillus
  - Chain = Streptobacillus
- Coccobacillus
- Vibrio = curved
- Spirillum
- Spirochete

Arrangement

- Some bacteria form aggregates such as the grapelike clusters of *Staphylococcus* species or the diplococcus (two cells together) observed in *Streptococcus* or *Neisseria* species.