# Chapter 3 Lecture Notes: Prokaryotic Cell Structure and Function

I. Overview of Prokaryotic Cell Structure

A. What is a <u>prokaryote</u>? Organism whose cells lack a membrane enclosed nucleus

B. General morphology

1. Size:

Range from nanobacteria (0.05-0.2 µm in diameter) to very large (600 x 80 µm); "Average" = 1 x 4 µm for *E. coli*

Why are bacteria small? Nutrients and wastes are transported in and out the cell via the cytoplasmic membrane. The rate of transport determines the metabolic rates and therefore the growth rates of microbial cells. The smaller the size, the larger the surface area of the cytoplasmic membrane to volume; therefore, the faster the potential growth rate.

| | radius (r) of cell A = 1µm | radius (r) of cell B = 2µm |
|---|---|---|
| Surface area (SA) of cell = $4pr^2$ | $12.6 \mu m^2$ | $50.3 \mu m^2$ |
| Volume (V) of cell = $4/3pr^3$ | $4.2 \mu m^3$ | $33.5 \mu m^3$ |
| Ratio of SA to V | 3.0 | 1.5 |

2. Shape
   a) <u>Cocci</u> – roughly spherical; can be single cells or groups of cells (i.e. diplococci are paired)
   b) <u>Bacilli</u> – rod shaped
   c) <u>Filamentous</u> – form long multinucleate filaments that may branch to produce a network called a mycelium
   d) <u>Spirilla</u> – rigid spirals
   e) <u>Spirochetes</u> – flexible spirals
   f) misc shapes
   g) pleomorphic – variable in shape

C. Cell organization (from inner to outer)
1. Cytoplasm which contains a variety of components
2. Plasma membrane
3. Periplasmic space with periplasm
4. Cell wall
5. Slime layer or capsule
6. Flagella and pili

II. Cytoplasm

Cytoplasmic matrix - substance lying between the plasma membrane and the nucleoid which contains mostly water and a variety of components:

A. Ribosomes – macromolecular protein: RNA structures on which protein synthesis occurs

B. Proteins – various tasks; can be localized to various areas of the cell

C. Inclusion bodies – granules of organic or inorganic materials which may or may not be membrane enclosed

1. Organic materials
    a) glycogen (polymer of glucose) – carbon source
    b) poly-ß-hydroxybutyrate – carbon source
    c) cyanophycin (polypeptides composed of equal amounts of arginine and aspartic acid) – nitrogen source
2. inorganic materials including
    a) polyphosphate (volutin) – phosphate source
    b) iron
    c) sulfur
3. carboxysomes - contain the enzyme ribulose 1,5 bisphosphate carboxylase which is important for incorporation of $CO_2$
4. gas vacuoles

D. Plasmids – small, circular DNA molecules that are independent of the chromosome and contains genes that encode proteins involved in:

1. transfer of DNA from one bacterium to another
2. drug resistance
3. bacteriocins – proteins that kill other bacteria
4. virulence factors
5. metabolism

III. Nucleoid

A. Term used to describe aggregated DNA in prokaryote cell
B. Attached to cell membranes
C. Visible with electron microscope or by staining with Feulgen stain that reacts with DNA
D. Genetic material of prokaryotes

1. always double stranded DNA
2. usually circular
3. haploid (normally 1 copy per cell)
4. looped and coiled to fit in cell with the aid of proteins

IV. <u>Plasma membrane</u> (AKA <u>inner membrane</u> or <u>cytoplasmic membrane</u>)

A. General info: phospholipid bilayer with proteins inserted into it

B. Functions

1. retains cytoplasm (cytoplasm + plasma membrane = protoplast)
2. selectively permeable barrier: Small, neutrally charged molecules ($H_2O$, $O_2$ & $CO_2$) can transverse membrane, but large molecules & ions (glucose) or small charged atoms (protons, H+) require specific transport systems.
3. transport of nutrients and wastes
4. generation of energy; biosynthesis
5. contains receptor proteins for environmental signals

C. Basic components/structure of eubacterial membrane (<u>fluid mosaic model</u>)

1. <u>phospholipid bilayer</u>
   a) the bilayer is composed of two sheets or leaflets each composed of phospholipids
   b) nonpolar hydrophobic fatty acid ends of each of the two sheets of the bilayer interact with each other
   c) polar hydrophilic phosphate ends of the phospholipids face either the aqueous cytoplasm or the periplasm
   d) lipids are frequently found bound to glycerol molecules in ether linkages
2. proteins
   a) <u>integral</u> are inserted in the membrane; amphipathic
   b) <u>peripheral</u> are loosely associated with the membrane (can be dissociated with salt)
   c) Distribution and properties of proteins on each side of the bilayer are different; therefore, the functions of the two bilayers are different
3. <u>hopanoids</u> – rigid, planar pentacyclic molecules similar to cholesterol that stabilize the membranes in some but not all eubacteria

D. Basic structure of archaebacterial membranes

1. lipids are isoprenoids instead of fatty acids
2. ether linkage to glycerol instead of ester
3. some have a monolayer instead of bilayer due to tetraethers
4. The diversity of membranes is related to the diverse habitats in which archaeobacteria live. For example, thermophiles (live at extremely high temperatures) have almost all tetraether, which stabilizes the membrane at high temperatures.

E. Internal membranes

1. no complex internal membranes
2. <u>mesosomes</u>: invaginations in the plasma membrane; function unclear (maybe an artifact of sample preparation)
3. other more extensive and complex plasma membrane foldings to provide larger surface area

V. Cell wall

A. Strong structural layer that lies outside the plasma membrane but does not include capsule or slime layer (cell wall + capsule = cell envelope)

B. Function

1. shape
2. protection from osmotic lysis: pressure inside the cell = 300 lbs/in$^2$
3. can be virulence factor (LPS in gram-negatives)
4. defense against host immune response (LPS in gram-negatives)
5. protection from some toxic substances

C. Overview of chemical composition of eubacterial cell walls

** Note that there are two types of cell walls that are differentiated based on the Gram stain (gram-negative and positive – see below)

1. Peptidoglycan (AKA murein layer)
   a) polymer of two sugar derivatives (glycans): N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM)
   b) several amino acids (peptide) attached to NAM residues
      (1) L-alanine
      (2) D-glutamate
      (3) diaminopimelic acid (DAP) in gram-negatives or lysine in most gram-positives
      (4) D- alanine
   c) connection of polymer strands:
      (1) In gram-negative bacteria: link between amino acid attached to one NAM on one polymer and amino acid on NAM on another polymer
      (2) In gram-positive bacteria: peptide interbridge (typically 5 glycines) that connects the peptides on different polymers
   d) entire cross-linked structure forms a net
   e) rigid enough to provide strength to cell wall yet porous enough to allow components to be transported through
2. Outer membrane (only in gram-negative bacteria – see below)

D. Gram-positive cell walls

1. many layers of peptidoglycan (up to 25)
2. teichoic acid attached to cell wall (specifically to NAM) or to plasma membrane lipids
   a) polymers of glycerol or ribitol which are joined by phosphate groups, have amino acids or sugars attached
   b) may function to negatively charge the cell wall, help ions pass through, provide structure
3. Periplasmic space - very small area between inner membrane and peptidoglycan; contains periplasm (loose network of peptidoglycan, possibly gel-like)

E. Gram-negative cell walls
    1. 1-2 layers of peptidoglycan
    2. Large periplasmic space - area between the inner and outer membrane; contains periplasm (loose network of peptidoglycan, possibly gel-like)
        a) peptidoglycan is located in the periplasm
        b) proteins involved in nutrient acquisition, electron transport, peptidoglycan synthesis, are located in the periplasm
        c) provides protection from toxic substances
    3. outer membrane
        a) lipid bilayer
            (1) inner leaflet composed of phospholipid
            (2) outer leaflet composed of lipopolysaccharide (LPS)
                (a) lipid A – glycolipid (fatty acid residues are connected to a disaccharide composed of N-acetylglucosamine phosphate)
                (b) core polysaccharide – ketodeoxyoctanate, heptoses, glucose, galactose, and N-acetylglucosamine
                (c) O-specific polysaccharide (AKA O-side chain; O antigen) – polymer of 4 –5 sugars that are repeated several times and are specific for each species/strain
        b) proteins
            (1) Braun's lipoprotein – connects outer membrane with the peptidoglycan
            (2) porins – proteins that form a channel in the membrane
            (3) specific transport proteins
        c) functions: immune protection; negative charge; endotoxin; prevents the loss of periplasmic enzymes; prevents toxic compounds from entering

F. Archaebacterial cell walls
    1. Most contain pseudopeptidoglycan which differs from eubacterial in:
        a) NAM is replaced by N-acetylalosaminuronic acid
        b) glycosidic bonds are 1,3 vs. 1,4
        c) L-amino acids in cross-links
    2. Other polysaccharide containing walls
    3. Others have alternative protein covering called S-layer (see below)

G. Basis for Gram stain (gram-positive = purple; gram-negative = pink)
    1. Crystal violet stain
    2. Iodine treatment to retain stain
    3. Ethanol treatment: shrinks pores of the peptidoglycan
        Since gram-positive peptidoglycan is more thick and highly crosslinked, the dye-iodine complex is retained. In contrast, gram-negative peptidoglycan is looser and so dye-iodine stain is NOT retained
    4. Safranin treatment to counterstain cells

H. Wall-less bacteria

Mycoplasmas – plasma membranes are stronger; contain sterols

**Differences Between gram-positive and gram-negative cell walls:**

| | **Gram-positive wall** | **Gram-negative wall** |
|---|---|---|
| Peptidoglycan | Thick layer | Thin layer |
| Peptidoglycan tetrapeptide | Most contain lysine | All contain diaminopimelate |
| Peptidoglycan cross linkage | Generally via peptide interbridge | Direct bonding via tetrapeptides |
| Teichoic acid | Present | Absent |
| Lipoprotein | Absent | Present |
| LPS | Absent | Present |
| Outer Membrane | Absent | Present |
| Periplasmic Space | Very small one | Present |

VI. Optional layers outside wall

A. Slime layers (diffuse and easily removed) and capsules (organized and hard to remove)

1. usually network of polysaccharides extending from the surface (called glycocalyx) but can be made of other materials too
2. functions:
    - a) protection against phagocytosis, dehydration, viruses, detergents
    - b) attachment
    - c) nutrient reserve

B. S-layer – crystalline surface layer composed of outer layer of protein subunits arranged in a regular pattern which

1. functions
    - a) protection
    - b) adherence
    - c) selectively permeable "membrane"
    - d) structure
2. frequently functions as the cell wall in Archaebacteria

VII. Fimbriae and Pili

A. Fimbriae

1. short, fine, numerous hair-like appendages composed of helically arranged proteins
2. function: attachment to surfaces

B. Pili

1. longer and wider and fewer than fimbriae
2. function: attachment; conjugation (bacterial transfer of genetic information)

VIII. Flagella

A. Thread-like, helical appendages

B. Function: movement; chemotaxis

C. Structure:

1. Filament – composed of many subunits of a protein called flagellin which is arranged in a left handed helical manner to form a hollow cylinder
2. Hook – composed of multiple subunits of one protein
3. Extended basal body - motor
    a) 1 rod – hooked to the hook; shaft that transmits torque from the motor to the filament
    b) several rings
        (1) L ring – in LPS of gram-negatives; protects rod
        (2) P ring – in peptidoglycan of gram-negatives; protects rod
        (3) MS ring – in periplasm and plasma membrane; mounting ring for motor
        (4) C ring – in cytoplasm; switching of direction
4. Mot proteins – surround the basal body in the plamsa membrane and drives the motor
5. Fli switch – several proteins that control flagellar rotation in response to signals from the cell

D. Distribution

1. Monotrichous – one flagellum on the bacterium
2. Polar – one flagellum at one end of a bacterium
3. Amphitrichous – one flagellum at each pole of the bacterium
4. Lophotrichous – cluster of flagella at one or both ends
5. Peritrichous – flagella around the whole surface of the bacterium

E. Synthesis and assembly are very complex

F. Mechanism of movement

1. ccw rotation of the flagellum moves the bacterium forward = "run"
2. cw rotation of the flagellum causes the bacterium to either "tumble" in space or to move backward for some species that are monotrichous
3. energy source (proton motive force; 1000 H+ per turn)

IX. The bacterial endospore

A. Special resistant, dormant structure formed by vegetative cells of several gram-positive organisms

B. Resistant to heat, desiccation, radiation, chemicals

C. Functions: Survival

D. Structure

1. Core (contains plasma membrane and cytoplasm of cell)
    a) contains dipicolinic acid complexed with calcium ions
    b) contains SASPs – small acid-soluble spore proteins that protect DNA
    c) dehydrated – only 10-30% water
2. Spore cell wall (core wall)
3. Cortex – loosely cross-linked peptidoglycan
4. Spore coat – several layers of protein which are impermeable to many chemicals
5. Exosporium – thin, protein layer

E. Sporogenesis or sporulation = Spore formation

1. induced by lack of nutrients
2. complex process that requires 200 genes and takes 8-10 hours
    a) Stage 1: DNA becomes denser and forms an "axial filament" of nuclear material
    b) Stage 2: invagination of plasma membrane engulfs the DNA and produce the forespore septum
    c) Stage 3: forespore is engulfed by second membrane
    d) Stage 4: cortex formation between the two membranes; calcium and DPA accumulate
    e) Stage 5: spore coat synthesis around cortex
    f) Stage 6: maturation of spore: development of resistance to heat and chemicals
    g) Stage 7: lysis of cell and release of spore

F. Breaking of the endospore dormant state

1. Activation – reversible process usually from heat treatment that conditions cells so that they will germinate when placed in nutrient rich medium
2. Germination – breaking of spore's resistant state
    a) spore swelling
    b) rupture of the coat
    c) loss of DPA and calcium
    d) degradation of SASPs
3. Outgrowth – synthesis of new components (RNA, proteins, etc)