

Chapter 3 - Prokaryotic Cell Structure and Function

General Terms

- ◆ Two distinct groups of microbes comprise the prokaryotes
 - * Bacteria (“true bacteria”)
 - * Archaea (“ancient bacteria”) [Chapter 20]
- ◆ The term “**prokaryote**” is refers to both of the above groups
- ◆ The term “**bacteria(ium)**” is used in specific reference to the Eubacteria

Overview of Cell Structure

- ◆ Bacteria come in a variety of shapes (morphologies) and arrangements
 - * Cocci (s., coccus) - roughly spherical cells
 - Individual cells
 - Diplococci - two cells
 - Streptococcus - chain of cocci
 - Staphylococcus - cluster (“grape-like”) of cocci
 - Tetrads - square groups of four cells
 - Cubical - packet of eight cells
 - * Bacilli (s., bacillus) - rod-shaped cells
 - Individual cells
 - Streptobacillus - chain of rods
 - Coccobacilli - so short and wide that they resemble both cell types
 - Vibrio - curved, comma like rod
 - * Spirilla (s., spirillum) - long rods that are spiral in shape, yet rigid
 - * Spirochete(s) - long rods that are helical in shape, yet flexible
 - * Filamentous - thread-like cells (**hyphae** [s., **hypha**] that form a network termed a **mycelium**)
 - * Various other shapes - square, flat, etc.
 - * **Pleomorphic** bacteria - multiple shapes
- ◆ Bacteria also vary in size
 - * Typical bacteria, like *Escherichia coli*, range in size from 1-2 μm in width and 2-6 μm in length
 - * Smallest bacteria, e.g., *Mycoplasma* sp., are about 0.3 μm in diameter
 - * Nanobacteria (less than 0.14-0.20 μm in diameter) - do they exist?

- * Very large bacteria - a few species are much larger than the typical eucaryotic cell (10-50 μm in diameter)
 - Some spirochetes reach 500 μm in length
 - Cyanobacterium *Oscillatoria* - 7 μm in diameter (about the size of a red blood cell)
 - *Epulopiscium fishelsoni* (*Acanthurus nigrofuscus*) - typically 200-500 μm in length, but can reach a size of 80 μm x 600 μm (a volume about a million times that of *E. coli*)
 - *Thiomargarita namibiensis* - a spherical bacterium that ranges between 100 μm and 750 μm in diameter (about 100 times larger in volume than *E. fishelsoni*)

Cell (Plasma) Membrane

- ◆ Chief point of contact with the cell's environment
- ◆ Usually have a higher protein content than those of eucaryotes
- ◆ Fulfills many of the functions normally conducted by specialized organelles in eucaryotic cells
- ◆ Functions of plasma membrane
 - * Retains cytoplasm
 - * Serves as a selectively permeable barrier
 - * Assists in the transport of nutrients, wastes, and other cellular protein products
 - * Site of many crucial metabolic processes
 - Respiration
 - Photosynthesis
 - Lipid and cell wall synthesis
 - * Contains receptors that detect and respond to chemicals in the environment
- ◆ Comprised of a lipid bilayer
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 - Enables the formation of a lipid bilayer
 - Many are also phospholipids - phosphate is the charged, hydrophobic portion
 - * Lipid composition varies with temperature in order to keep the membrane fluid
- ◆ Sterols are not present (unlike in eucaryotic cells), but contain a related type of molecule - a **hopanoid** - which presumably help in stabilizing the plasma membrane

- ◆ **Fluid Mosaic Model** - currently accepted model of membrane structure which distinguishes between two types of proteins
 - * Peripheral - loosely connected
 - * Integral - embedded amphipathic proteins
 - Hydrophobic portion - buried within the membrane
 - Hydrophilic portion - extend beyond the membrane surface and often have carbohydrates attached
- ◆ **Mesosomes** - invaginations of the plasma membrane, probably artifacts
- ◆ Some invaginations of the cell membrane are extensive and complex in photosynthetic and nitrifying bacteria, serving as a membrane surface for metabolic activities

Cytoplasmic Matrix

- ◆ Part of the **protoplast**, i.e., all parts of the cell within the plasma membrane
- ◆ Substance lying between the plasma membrane and the nucleoid
 - * Mostly water (approximately 70%)
 - * Featureless, but contains ribosomes and other proteins
 - * Highly organized
- ◆ Inclusion bodies - granules of organic or inorganic material typically visible using a light microscope
 - * Some are used for storage
 - * Some reduce osmotic pressure by collecting molecules as particulates
 - * Some are enclosed in a *single-layered* membrane
 - * Vary in composition and quantity
- ◆ Types of organic inclusion bodies
 - * Carbon storage reservoir
 - Glycogen
 - Poly- β -hydroxybutarate
 - * Lipid storage bodies
 - * Cyanophan granules - produced by cyanobacteria for storage of proteins that serve as nitrogen sources
 - * Carboxysomes - polyhedral bodies containing the enzyme ribulose-1,5-bisphosphate carboxylase used in the fixation of CO₂ (making carbohydrates)
 - * Gas vacuole - used by photosynthetic bacteria for buoyancy and comprised of proteinaceous gas vesicles

- ◆ Inorganic inclusion bodies
 - * Polyphosphate granules (**volutin**) - sometimes called **metachromatic granules** due to their appearance when stained
 - Energy reserve
 - Synthesis of cellular materials
 - * Sulfur granules
 - * Magnetosomes - iron-containing granules that orient the movement of certain bacteria in accord with earth's magnetism
- ◆ Ribosomes - site of protein synthesis
 - * Comprised of protein and ribonucleic acid (RNA; ribosomal RNA [rRNA])
 - * Smaller than eucaryotic ribosomes (70S, 14-15 nm by 20 nm)
 - * Comprised of two subunits - 30S and 50S

Nucleoid

- ◆ Chromosome located in an irregularly shaped region termed a "**nucleoid**"
 - * Single chromosome - some bacteria have more than one chromosome
 - * Circular (some bacteria have a linear chromosome)
 - * Comprised of double-stranded deoxyribonucleic acid (DNA)
- ◆ Nucleoid is not membrane bound with the exceptions of two genera of the phylum Planctomycetes
- ◆ Chromosome in *E. coli* measures about 1400 μm in length
- ◆ RNA and non-histone proteins are used to efficiently pack the chromosome in an extensively looped and coiled fashion
- ◆ Plasmids - many bacteria contain these
 - * Small, double-stranded DNA molecules
 - * Self (independently) replicating
 - * Not required for growth or reproduction
 - * May carry genes that give the bacterium a selective advantage, e.g, drug resistance, new metabolic properties, virulence factors, etc.

Cell Wall

- ◆ Rigid layer that lies outside the plasma membrane
- ◆ Functions
 - * Gives cells their shape (exceptions - mycoplasmas (eubacteria) and certain Archaea do not have cell walls)

- * Protects cells from osmotic lysis
- * May contribute to pathogenicity
- * Protects the cell from toxic substances
- * Site of action for several antibiotics
- ◆ Cell envelope - all structures, including the cell wall, outside the cell membrane
- ◆ The composition of the cell wall determines if a bacterium is Gram positive or Gram negative
 - * Gram-positive bacteria
 - 20-80 nm thick wall
 - comprised of a homogeneous layer of **peptidoglycan (murein)** lying outside the plasma membrane
 - * Gram-negative bacteria
 - 2-7 nm thick peptidoglycan layer
 - 7-8 nm thick outer membrane
 - Periplasmic space - area between the plasma membrane and the cell wall containing a loose network of peptidoglycan in a gel-like substance (**periplasm**)
 - ❖ Contains enzymes for nutrient acquisition, peptidoglycan synthesis, and the neutralization of toxic substances
 - ❖ Certain bacteria have enzymes involved in energy production located here
 - Some Gram-positive bacteria may secrete periplasmic substances and enzymes (**exoenzymes**), though they lack a visible periplasmic space
- ◆ Archaea may be either Gram positive or negative because they have distinctive cell walls that differ in structure and composition (e.g., they lack peptidoglycans)
- ◆ Peptidoglycan
 - * Polymer of alternating residues of two sugar derivatives - *N*-acetylglucosamine and *N*-acetylmuramic acid
 - * Also contain three amino acids not found in proteins (resist peptidase digestion)
 - D-glutamic acid
 - D-alanine
 - *meso*-diaminopimelic acid (sometimes substituted with L-lysine)
 - * Polymers linked by a peptide interbridge

◆ Gram-positive cell walls

- * Thick homogeneous peptidoglycan layer also contains large amounts of **teichoic acid** that are covalently attached to
 - *N*-acetylmuramic acid
 - Plasma membrane (lipoteichoic acids)
- * Teichoic acids
 - Extend to cell wall surface giving Gram-positive cells a negative charge
 - No known function (maintenance of the wall?)

◆ Gram-negative cell walls

- * More complex than in Gram-positive cells
- * Outer membrane contains **Braun lipoprotein** that is also covalently attached to peptidoglycan layer
- * Adhesion sites are present - fused areas of outer and plasma membrane that strengthen the cell wall
- * Contain lipopolysaccharides (LPSs) - extend outward from the outer membrane
- * LPS comprised of
 - Lipid A - toxic, acts as an endotoxin
 - Core polysaccharide - contributes to the negative charge of the bacterial surface
 - O side chain - antigenic
- * Outer membrane
 - Protects cell
 - Permeable to small molecules due to the presence of porin proteins that form channels
 - Prevents loss of the periplasmic components

◆ Mechanism of the Gram stain

- * Gram-positive reaction - decolorization shrinks the pores of the thick peptidoglycan layer, thereby trapping the crystal violet-iodine complex giving the cell a purple color
- * Gram-negative reaction - peptidoglycan layer is thinner, has large pores, and decolorization removes lipids from the cell wall, thereby increasing porosity and releasing the crystal violet-iodine complex

◆ Osmotic protection

- * Solutes are usually more concentrated inside the bacterial cytoplasm (**hypertonic**) than in its environment (**hypotonic**)
- * During **osmosis** (the movement of water across a membrane), water will move from an area of dilute concentrations to an area of higher concentrations

- * In an enclosed system, such as a bacterium, water then would move into the cell creating pressure on the membrane
- * The osmotic pressure then could cause the cell to undergo **lysis** if there were not a cell wall
- * Alternatively, if a bacterium is placed in hypertonic environment, as opposed to its cytoplasm, water would flow out of the cell causing **plasmolysis**, the shrinking of the plasma membrane inward
- * The importance of cell wall in protecting the bacterium from lysis can be demonstrated experimentally
 - Remove the peptidoglycan with treatment by lysozyme (digestive enzyme) or penicillin (inhibitor of peptidoglycan synthesis)
 - Produces a **protoplast** (Gram-positive bacterium) or **spheroplast** (Gram-negative bacterium - has both plasma and outer membranes)
 - Subjecting cells to dilute (hypotonic) solution will induce lysis due to influx of water

Protein Secretion

- ◆ Prokaryotes have developed at least 5-6 methods to transport proteins from the cytoplasm through the plasma membrane and cell wall
- ◆ Sec-dependent pathway (general secretion pathway [GSP])
 - * Most common
 - * Translocates proteins across the membrane or integrates them into it
 - * A signal peptide is made at the beginning of a protein (preprotein) as it is synthesized on the ribosome
 - * Chaperones bind to preprotein to keep it from folding and help it reach the **translocon** - site of secretion in the plasma membrane
 - * Transferred through the membrane at the translocon via the SecYEG complex of proteins (involves use of cellular energy)
 - * When preprotein emerges on the other side of membrane, the signal peptide is removed
 - * The protein then folds into its proper shape and carries out its function
- ◆ Type I Secretion (ABC Secretion Pathway)
 - * Sec-independent
 - * Member of the ATP-binding cassette (ABC) family of transport systems
 - * Uses ATP (cellular energy) to drive the protein through the membrane-embedded domains
 - * In Gram-negative cells, these domains pass through both the plasma membrane and inner membrane

- ◆ Type II Secretion
 - * Sec-dependent pathway
 - * Used by Gram-negative bacteria to transport proteins across outer membrane
- ◆ Type III Secretion
 - * Used by Gram-negative bacteria to secrete virulence factors and inject them into host cells
 - * Contact between host and bacterium stimulates the process and often involves activation by a low calcium ion level
 - * Four types of proteins are secreted by this system
 - Those required for secretion
 - Secretion regulators
 - Proteins that aid in the insertion of secreted proteins into the host cell
 - Effector proteins that alter host cell functions

External Wall Components

- ◆ Glycocalyx
 - * Network of polysaccharides extending from the surface of bacteria and other cells
 - * Technically, this definition includes capsules and slime layers
- ◆ Capsule
 - * Well-organized layer typically composed of polysaccharides
 - * Not easily removed from the cell's surface
 - * Not required for growth or reproduction in laboratory cultures (but may be needed for these functions *in vivo*)
 - * Functions of a capsule
 - Help resist phagocytosis
 - Protect against desiccation
 - Exclude bacterial viruses and some toxins
 - Aids in attachment to surfaces
- ◆ Slime layer
 - * Loosely organized layer of polysaccharides external to the cell surface
 - * Easily removed by washing the cell
 - * Functions in the same fashion as a capsule

◆ S-Layer

- * Layer of regularly organized protein or glycoprotein attached to the bacterial cell surface

- * Functions

- Protection from pH/ion fluctuations, osmotic stress, enzymes, and predation
- Maintain cell shape and envelop rigidity
- Promote adhesion to surfaces
- Protects against complement and phagocytosis

◆ Fimbriae (s., fimbria)

- * Short, fine hairlike appendages

- * Composed of helical tubes of proteins

- * Used for attachment to surfaces

- * Not involved in motility

◆ Pili (s., pilus)

- * Similar to fimbriae

- * Hollow tubes of the protein subunit, pilin

- * Not involved in motility

- * Required for bacterial mating (conjugation)

◆ Flagella (s., flagellum)

- * Long, slender rigid structures used for motility

- * Composed of the protein flagellin

- * Arranged in different patterns on a bacterial cell depending upon the species

- Monotrichous - located at one polar end
- Amphitrichous - located at both polar ends
- Lophotrichous - multiple flagella at one end
- Peritrichous - surround the whole surface

- * Movement of bacteria involves the rotation of flagella (*not* a whiplash motion)

- * Some bacteria move by use of other mechanisms

- Axial filaments - type of periplasmic flagellum
- Gliding motility - no visible external structures used in coasting along a surface

Bacterial Endospore

- ◆ An endospore is a resistant dormant structure formed by some Gram-positive bacteria
- ◆ Highly resistant to
 - * Heat
 - * Ultraviolet light
 - * Gamma radiation
 - * Disinfectants
 - * Desiccation
- ◆ Structure of a bacterial endospore is complex and consists of the following
 - * Exosporium - thin, delicate coat
 - * Spore coat - thick layers of proteins
 - Helps make endospore impermeable/resistant
 - Contains enzymes for germination
 - * Cortex - peptidoglycan layer
 - * Cell wall - surrounds the core
 - * Core - contains ribosomes and nucleoid, but is metabolically inactive
- ◆ Why are endospores so resistant?
 - * Answer remains unsolved
 - * Possible mechanisms include
 - Calcium-dipicolinate and acid-soluble protein stabilization of DNA inside endospore
 - Protoplast dehydration
 - Presence of DNA repair enzymes
 - Spore coat resistant to attack by enzymes and chemicals
- ◆ Spore formation (sporogenesis, sporulation)
 - * Begins when growth ceases due to the lack of nutrients
 - * Seven stage process
 - * Takes as few as 10 hours to complete
 - * Location of endospore inside vegetative cell is species dependent
- ◆ The transformation of endospores into vegetative cells requires three steps
 - * Activation - process that prepares the endospore for germination
 - * Germination - breaking of the dormancy, characterized by, among other things, endospore swelling, rupture of the spore coat, and an increase in metabolic activity
 - * Outgrowth - emergence of the protoplast and development of a vegetative cell