

## Prokaryotic Cell Structure and Function:

Prokaryotic organisms are much less complex than eukaryotic cells, but have some features in common. This presentation will begin with prokaryotic structures found outside the cell membrane, and will work inward from there. Structures visible on the cell model are similar in appearance to those associated with some eukaryotic organisms (though not usually found together on a single cell); however, although the long, slender appendages are flagella, the shorter, hair-like structures are not cilia. **Prokaryotic cells do not have cilia.**

### A. Structures found outside the cell membrane:

1. **Flagella** – The flagella (singular = flagellum) of prokaryotic cells (bacteria specifically) are made up of proteins called **flagellin proteins** or **flagellins**. These globular proteins polymerize to form linear strands that wrap around one another forming the walls of the flagellum (the core is hollow). Prokaryotic flagella are **not surrounded by the cell membrane** and are **not supported by microtubules**. Instead, each flagellum is attached to the cell membrane and the cell wall by a series of complex ring-like structures. That portion of a flagellum associated with the rings extends through the membrane and the wall like an axel attached to several "wheels". Outside the wall, it makes a 90° bend and then extends lengthwise away from the cell. The **motion of bacteria flagella is rotary, i.e., they spin**. Movement is powered by ion flow (usually H<sup>+</sup>, but sometimes Na<sup>+</sup>) across the cell membrane.

Prokaryotic flagella are not visible when live bacteria are viewed using light microscopes because they are too thin; however, they can be observed if treated with stain reagents increasing their thickness. Flagellar number and arrangement varies between bacterial species, and is sometimes useful in identification. Some commonly encountered arrangements are indicated below, and include the root word trich, meaning hair-like.

**Atrichous** = Cells having no flagella (a = without).

**Monotrichous** = Cells having only one flagellum (mono = one).

**Peritrichous** = Cells having flagella arranged more or less uniformly all over their surfaces (peri = around).

**Amphitrichous** = Cells having one or more flagella at both ends (amphi = both ways or two ways).

Flagella allow bacteria to swim through their environments in search of food materials, light, etc., so are involved in **taxis** (directed movement). If the bacteria are pathogens, flagella can aid their dispersal, so may increase their ability to cause disease symptoms. Certain viruses may access bacteria through their flagella, so flagella can also serve as virus entry ports. The ability to form flagella is dependent on environmental conditions; therefore, even though cells are genetically capable of forming flagella, they may not do so.

2. **Fimbriae and Pili** – Fimbriae (singular = fimbria) are short, hair-like appendages found outside the cell walls of certain types of bacteria (especially Gram-negative cells). They are made of

**fimbrin proteins** and are often quite numerous, covering the entire cell surface. Although fimbriae look similar to cilia in scanning electron photomicrographs, they are not used for swimming. **Fimbriae are used primarily for attachment.** They allow bacteria to bind to the surfaces of rocks, sticks, leaves, etc. in their environments or in the case of pathogenic forms, they allow for attachment to host cells. Pathogenic bacteria such as *Neisseria gonorrhoeae* cannot cause disease if they are unable to bind to the surfaces of their host cells.

**Pili** (singular = pilus) are longer and less numerous than fimbriae and are made of **pilin proteins**. Pili allow bacteria to bind other cells of the same species and facilitate genetic exchange, i.e., the passage of DNA from one cell to another. Pili involved in this gene transfer activity are called "F" (fertility) or **sex-pili**.

3. **Glycocalyx** – The glycocalyx is a layer found outside the cell wall of certain types of bacteria. It is usually made up of polysaccharide, but may contain protein and can be thick or thin depending on the availability of nutrient materials in the environment. If the glycocalyx is dense and well organized, it is called a **capsule** while if it is loose and poorly organized it is called a **slime layer**. The colonies of bacteria forming glycocalyxes are often wet looking (slimy) and will sometimes drip from agar surfaces into the lids of inverted culture plates.

The primary function of the glycocalyx is food storage. Because bacteria live in environments prone to "feast or famine" conditions, they frequently collect **reservoirs of stored food** materials outside their walls when conditions are favorable, and then use these to sustain themselves during long periods with poor nutrient availability. Capsules cause the surfaces of bacteria to be sticky, facilitating their **attachment to smooth surfaces** such as tooth enamel. The capsules formed by *Streptococcus mutans*, a common inhabitant of the human mouth, are largely responsible for the formation of dental plaque, a soft, whitish, gooey material readily removed with dental floss. Capsules provide a certain amount of **protection** to cells forming them, both from predators in their environments and desiccation. Because capsule-forming bacteria are generally ignored by phagocytic WBCs involved in defending us against pathogens, the presence of a glycocalyx also **increases the pathogenicity** (disease-causing ability) of bacteria.

4. **Cell wall** – The cell wall is a rigid layer (usually) found outside the cell membrane of most bacteria cells. In the case of bacteria commonly encountered in the clinical setting, it is composed of a unique material called **peptidoglycan** (described in detail during the Gram stain lab). The peptidoglycan layer is thick in Gram-positive cell walls and much thinner in those of Gram-negative cells. The walls of Gram-negative cells are also equipped with an outer membranous layer absent from Gram-positive cells. Differences in wall composition made evident by stain reactions (Gram-positive = purple Vs Gram-negative = pink) influence the responses of bacteria to environmental conditions. **Gram-positive bacteria** with their thick peptidoglycan walls **are more resistant to damage from physical factors** such as heat, radiation, desiccation and pressure than are Gram-negative cells; therefore, pasteurization will kill Gram-negative cells more readily than Gram-positive forms. **Gram-negative cells**, protected by an extra membrane layer outside their thin peptidoglycan wall, **are more resistant to chemicals** such as basic dyes,  $\beta$ -lactam antibiotics and lysozyme. Media containing basic dyes are frequently used to select for Gram-negative bacteria because Gram-positive cultures will not grow on them.

5. **Periplasmic space** – The periplasmic space (peri = around) is a potential space existing outside the cell membrane but inside the outer membrane of the Gram-negative cell wall (though a similar potential space exists outside the cell membrane and inside the peptidoglycan layer of the Gram-positive cell wall). It is a region where enzymes and wall components released by the cell membrane tend to accumulate, so is **involved in storage** of these materials.
6. **Periplasmic flagella** – Periplasmic flagella (also known as endoflagella or axial filaments) are flagella found within the periplasmic space of **Spirochetes**, long, slender, spiral-shaped bacteria with flexible peptidoglycan walls. Periplasmic flagella arise from both ends of a typical spirochete and wrap around the cell surface inside the outer membrane. The rotary motion of these flagella causes the entire cell to spin creating a swimming motion similar to a corkscrew.

The Cell Membrane, described in detail in an earlier section, serves here as a boundary between external and internal cell structures. Recall that the prokaryotic cell membrane is involved in ATP synthesis and contains enzymes involved in other physiological processes. The cell membranes of some prokaryotic cells fold inward forming regions with extensive surface area and therefore high concentrations of enzymes. Two of these structures are described below.

1. **Mesosomes** – Mesosomes are membranous folds extending into the cytoplasm of some prokaryotic cells. They contain enzymes involved in ATP synthesis via oxidative phosphorylation so are analogous to the cristae of mitochondria. Mesosomes also apparently attach to the covalently closed, circular DNA molecules of prokaryotic cells and aid in chromosome separation during cell division. This is significant because prokaryotic cells do not contain microtubules and so cannot form a spindle apparatus.
2. **Thylakoids** – Thylakoids are long, slender membranous structures formed by the inward folded cell membranes of certain bacteria capable of using light as an energy source (i.e., cyanobacteria, green and purple sulfur bacteria and others). The pigments involved in capturing light energy as well as the enzymes involved in photophosphorylation are bound to the membranes of thylakoids.

## **B. Structures found inside (internal to) the cell membrane:**

1. **Cytoplasm** – The cytoplasm of a prokaryotic cell includes all the protoplasm surrounded by the cell membrane. Because prokaryotes are not nucleated, there is no nucleoplasm surrounded by a nuclear envelope. The cytoplasm forms the bulk of the cell, but is not compartmentalized by membranous organelles. Structures common in eukaryotes such as the endoplasmic reticulum, Golgi apparatus, lysosomes, peroxisomes, vacuoles, vesicles and mitochondria are lacking. A variety of structures are suspended in the cytoplasm, but these are not surrounded by membranes made of lipid bilayers with integral and peripheral proteins (some may be surrounded by thin membranes made up of protein or other materials).
2. **Nucleoid** – The nucleoid (nucleoid = nucleus-like) or nuclear region is the control center of the prokaryotic cell and contains an extremely dense accumulation of ccc-DNA (sometimes more than one segment) accompanied by small amounts of RNA and protein. It is not surrounded by a membrane or nuclear envelope, so is **not a true nucleus**. Like the nucleus of eukaryotic cells,

the nucleoid contains most of the cell's DNA and controls physiological processes by determining which enzymes the cell can make. It contains homogeneous proteins known as **nucleoid proteins** that are functionally similar to the histones of eukaryotic cells, but nucleosomes are not formed within prokaryotes and neither are nucleoli. Most of the RNA present within the nucleoid is messenger-RNA (m-RNA).

3. **Plasmids** – Plasmids are small, extrachromosomal loops of DNA (cccDNA) carrying genes not essential to cell function under most circumstances. Plasmids replicate themselves independently of the cell's chromosome and frequently occur in relatively large numbers; sometimes more than 100 per cell. Their size is variable and the genes they carry code for a variety of different proteins. Some examples of frequently described plasmids include:  
**F factor plasmids** – Plasmids known as F factor (F = fertility), carry genes coding for the production of sex pili and the transfer of DNA through conjugation.  
**R factor plasmids** – Plasmids known as R factor (R = resistance) carry genes coding for resistance to various antimicrobial drugs. For example, many bacteria carry genes coding for  $\beta$ -lactamase, an enzyme that degrades  $\beta$ -lactam antibiotics (such as penicillins). Bacteria carrying R factor plasmids are resistant to antimicrobial drugs, i.e., are not harmed/controlled by them.  
**Col-plasmids** – Plasmids called Col-plasmids (found in Gram-negative bacteria identified as *Escherichia coli*) carry genes coding for the production of proteins called **colicins**. These proteins can be released by *E. coli* cells and will kill other closely related cells. The *E. coli* living in your gut produce colicins that can kill other strains of *E. coli*, thus protecting you from infection.

The ability of *Pseudomonas* species to catabolize a wide range of unusual organic compounds is determined by genes carried on plasmids.

4. **Ribosomes** – The ribosomes of prokaryotic cells, like those of eukaryotic cells are small, granular bodies **made up of ribosomal-RNA (r-RNA) and protein**. Unlike eukaryotic ribosomes, those in prokaryotic cells are 70S instead of 80S and are made up of 50S and 30S subunits. **Ribosomes are the site of protein synthesis** and are numerous within actively growing cells. Most of the RNA present in prokaryotic cytoplasm is r-RNA.
5. **Inclusions** – Prokaryotic cells may contain a variety of structures known as inclusions. Although eukaryotic inclusions are usually considered to be non-living, this description seems less appropriate for those found in prokaryotes. Some example inclusions are listed below:
  - a. **Carboxysomes** – Carboxysomes are inclusions containing enzymes involved in "fixing" carbon dioxide (CO<sub>2</sub>) into organic compounds. Although not all prokaryotes capable of using CO<sub>2</sub> from the air contain carboxysomes, many of them do.
  - b. **Poly  $\beta$ -hydroxybutyrate granules (PHB granules)** – PHB granules contain nutrient materials stored as polymers during periods when cells are not actively growing. Although important to cells as **nutrient reserves**, PHB granules are used by researchers to capture recombinant proteins produced within genetically engineered cells, and are also being considered for use in the production of biodegradable plastics.

- c. **Metachromatic granules** – Metachromatic granules are accumulations of stored **phosphate reserves** (polyphosphates). Since phosphates are required for the production of ATP, metachromatic granules are sometimes considered to be energy reserves. They tend to stain red in the presence of methylene blue.
- d. **Gas vacuoles** – The gas vacuoles (gas vesicles) found within certain prokaryotic cells are not true vacuoles, but are hollow cylinders surrounded by a thin layer of protein. They are found in aquatic bacteria and are used to **regulate buoyancy** within water environments.
- e. **Magnetosomes** – Magnetosomes are inclusions made up of crystalline metals (usually iron or magnetite) contained within folds of the cell membrane. They are involved in the detection of magnetic fields in bacteria capable of magnetotaxis.
- f. **Sulfur granules** – Sulfur granules are particles of elemental sulfur formed within cells capable of using hydrogen sulfide (H<sub>2</sub>S) as an electron donor. Electrons pulled away from H<sub>2</sub>S can be passed to pigment molecules replacing those removed by exposure to light (a phenomenon explained later). The remaining sulfur atoms can bind to form elemental sulfur (S<sub>8</sub>).

### C. Specialized cell types:

A number of structures associated with prokaryotic cells are most accurately considered as specialized cell types even though they may be formed within or from other cells. Some examples of specialized cell types are included below.

1. **Endospores** – Endospores are dormant structures produced within certain types of bacteria including the genera *Bacillus* and *Clostridium*. They are not reproductive structures, but are highly resistant to damage and allow cells to survive periods of unfavorable conditions. Endospores are unlike vegetative cells (the cells forming them) in a number of ways including:
  - a. They contain **little or no water** and are **metabolically inactive**.
  - b. They contain **higher levels of DNA** than active cells and **almost no RNA**.
  - c. They contain **high levels of calcium and dipicolinic acid** (pyridine-2,6-dicarboxylic acid).
  - d. Each endospore is surrounded by **two layers of membrane** and **two layers of wall-like material** (a **cortex** of modified peptidoglycan and a **spore coat** formed of multi-layered hydrophobic protein).
  - e. They are **highly resistant to damage caused by physical factors** such as heat, pressure, radiation, desiccation and are also **resistant to toxic chemicals** including antimicrobial drugs.
  - f. Endospores can **remain inactive but potentially viable for long periods of time** (e.g., 500-1000 years in lake sediments; millions of years in amber).

Endospores are formed inside vegetative cells through a process called **sporulation**. A cell containing an endospore or forming an endospore is called a **sporangium**. The sporulation process can be divided into a number of stages or steps as indicated below:

- a. The cells **DNA is replicated** (copied) and condensed.
- b. The **cell membrane invaginates forming a septum** between the chromosomes toward one end of the cell. This divides the cytoplasm into two unequal parts or protoplasts, the smaller one being the forespore or prespore.

- c. The **larger protoplast engulfs the smaller one** (endocytosis occurs), and the resulting forespore is surrounded by two layers of membrane.
- d. A modified form of **peptidoglycan is deposited** outside the inner spore membrane, **forming the cortex**.
- e. Layers of **hydrophobic protein are deposited** outside the outer spore membrane **forming the spore coat**. At this time also, calcium-dipicolinate (**dipicolinic acid**) **accumulates** within the developing endospore.
- f. The endospore matures, developing its characteristics as described above. Eventually the sporangium deteriorates and an **exospore** is released.

A sample of *Bacillus* cells stained with malachite green or carbol fuchsin (as practiced in the laboratory), will typically contain mature spores within cells as well as numerous exospores. Endospore shape and location within the sporangium is variable and can be useful for identification purposes. The formation of an endospore sometimes causes swelling of the sporangium and sometimes not.

The process involved when an exospore develops into a new vegetative cell is called **germination**. It requires a **triggering event, or activation** of the spore that may involve aging, temperature change, pH change, or exposure to certain chemicals. During activation, the exspore undergoes internal changes in molecular configuration, but does not lose its resistance to environmental stress. During germination, the exspore takes on water and becomes metabolically active. The levels of calcium and dipicolinic acid inside decrease, outer coverings are degraded, and a new cell grows out. The outgrowth phase occurring after the cortex and spore coat are degraded is somewhat similar (at least in appearance) to the growth of a new seedling from a germinating plant seed (hence the name).

2. **Heterocysts and Akinetes** – Heterocysts and akinetes are specialized cells formed by certain types of bacteria called **cyanobacteria** (formerly known as blue-green algae). **Heterocysts** are modified vegetative cells that function as anaerobic compartments rich in **enzymes involved in nitrogen fixation**. They are typically somewhat larger, have thicker coverings than vegetative cells and are internally modified to fix nitrogen most efficiently. They communicate with vegetative cells through their cell membranes, and the two are interdependent. Heterocysts cannot reproduce.
 

**Akinetes** are specialized cells that often develop adjacent to or near heterocysts. They are larger than vegetative cells, have thick walls, granular cytoplasm and are less active metabolically. Akinetes are more resistant than vegetative cells to extreme temperatures and desiccation; allowing cyanobacteria populations to survive cold winters and possibly dry summer months.
3. **Conidia (conidiospores)** – Conidia are reproductive structures produced by filamentous bacteria (the term is also applied to reproductive structures produced by certain fungi). They appear as cocci arranged in long chains at the ends of thread-like filaments. The colonies of bacteria forming conidia are typically dry and powder-like in surface texture, like a powdered sugar doughnut.
4. **Sphaeroplasts and Protoplasts** – Sphaeroplasts and protoplasts are spherical, fragile cells formed by removing the peptidoglycan walls from bacteria maintained in isotonic environments.

Sphaeroplasts are formed from Gram-negative cells and maintain a partial wall, i.e., the outer membrane of their cell wall. Protoplasts are formed from Gram-positive cells and lack cell walls. These specialized cell types are sometimes developed for research purposes, but require careful handling because they are sensitive to changes in osmotic pressure.