Microbial Cell Structure and Function

The Cell Envelope

The Cytoplasmic Membrane

The cytoplasmic membrane is physically rather weak but is an ideal structure for its major cellular function: selective permeability. In order for a cell to grow, nutrients must be transported inwards and waste products outwards. Both of these events occur across the cytoplasmic membrane. A variety of proteins located in the cytoplasmic membrane facilitate these reactions, and many other membrane proteins play important roles in energy metabolism.

Bacterial Cytoplasmic Membranes

The cytoplasmic membrane of all bacterial and eukaryal cells is a phospholipid bilayer containing embedded proteins. The cytoplasmic membrane is only 8–10 nanometers wide but can be resolved easily by transmission electron microscopy. Phospholipids are composed of both hydrophobic (water-repelling) and hydrophilic (water-attracting) components. In *Bacteria* and *Eukarya*, the hydrophobic component consists of fatty acid "tails" and the hydrophilic component consists of a glycerophosphate (a glycerol molecule bound to a phosphate) and one of several other functional groups (such as sugars, ethanolamine, or choline) also bonded to the phosphate. This type of membrane structure is called a *lipid bilayer*, or a *unit membrane*.

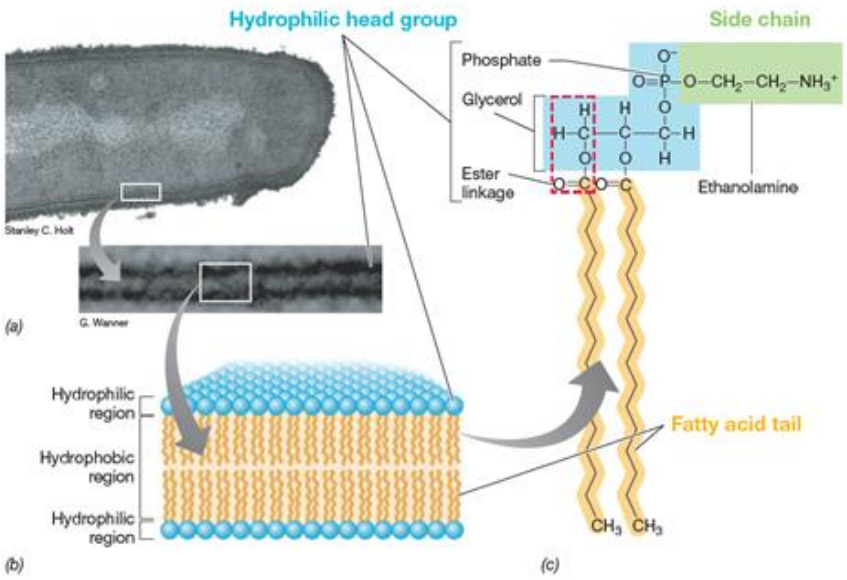


Figure: **Phospholipid bilayer membrane.**

A variety of proteins are attached to or integrated into the cytoplasmic membrane; Proteins significantly
embedded in the membrane are called *integral* membrane proteins. Many, though not all, integral membrane proteins extend completely across the membrane, and

these are called *transmembrane* proteins. By contrast, *peripheral* membrane proteins are more loosely attached. Some peripheral membrane proteins are lipoproteins, proteins that contain a hydrophobic lipid tail that anchors the protein into the membrane. Other peripheral membrane proteins have residues that associate with the hydrophilic head groups of phospholipids, or they associate indirectly with membranes by binding to other proteins anchored in the membrane. Peripheral membrane proteins typically interact with integral membrane proteins in important cellular processes such as energy metabolism and transport.

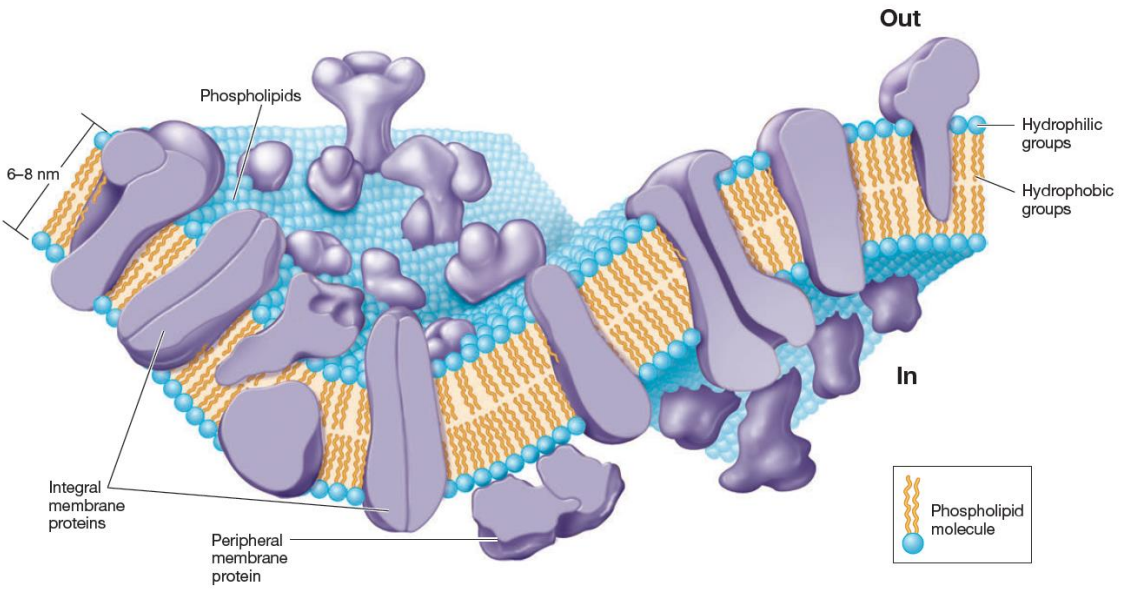
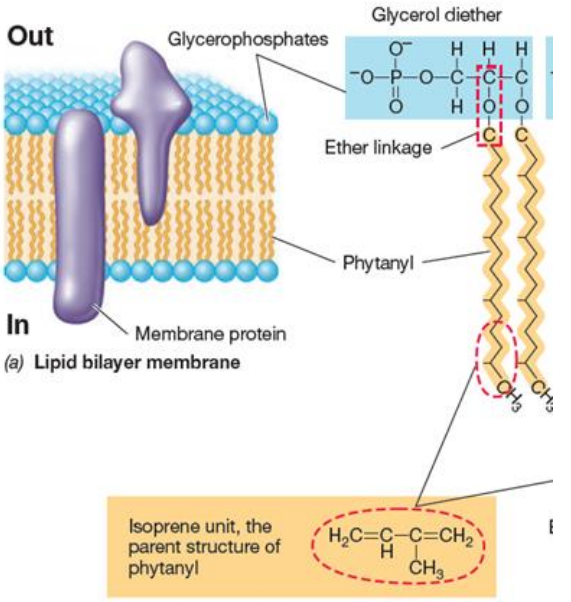


Figure: **Structure of the cytoplasmic membrane.**

Archaeal Cytoplasmic Membranes

The cytoplasmic membrane of *Archaea* is structurally similar to those of *Bacteria* and *Eukarya*, but the chemistry is somewhat different. In the lipids of *Bacteria* and *Eukarya*, the hydrophobic *fatty acid* tails are bound to glycerol by *ester* linkages; in contrast, the lipids of *Archaea* have hydrophobic *isoprenoid* (rather than fatty acid) tails, which are bound to glycerol by *ether* bonds.

The hydrophobic region of archaeal membranes is formed from repeating units of the five-carbon hydrocarbon *isoprene*, rather than from fatty acids.



Cytoplasmic Membrane Function

The cytoplasmic membrane has at least *three* major functions. First, it is the cell's permeability barrier, preventing the passive leakage of solutes into or out of the cell. Second, the cytoplasmic membrane anchors several proteins that catalyze a suite of key cell functions. And third, the cytoplasmic membrane of *Bacteria* and *Archaea* plays a major role in energy conservation and consumption.

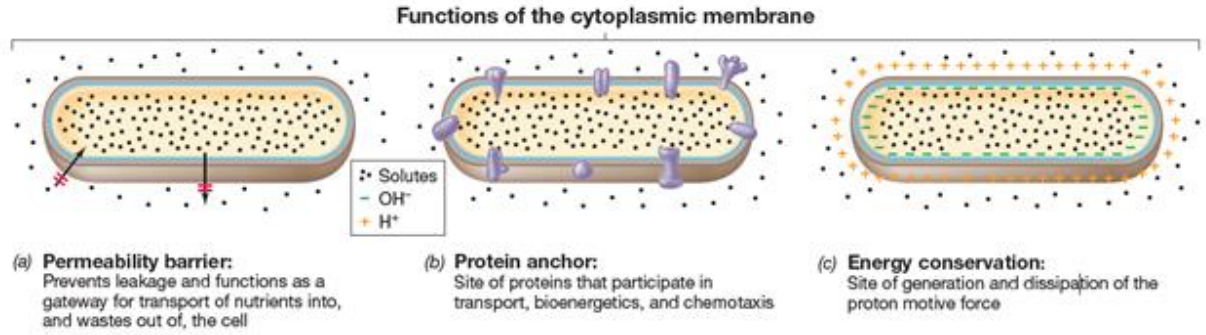


Figure: The major functions of the cytoplasmic membrane.

The cytoplasmic membrane is a barrier to the diffusion of most substances, especially polar or charged molecules. Because the cytoplasmic membrane is so impermeable, most substances that enter or leave the cell must be carried in or out by *transport proteins*. These are not simply ferrying proteins but instead function to *accumulate* solutes against the concentration gradient, a process that diffusion alone cannot do. Transport, which requires energy, ensures that the cytoplasm has sufficient concentrations of the nutrients it needs to perform biochemical reactions efficiently.

Transport proteins typically display high sensitivity and high specificity. If the concentration of a solute is high enough to saturate the transporter, which often occurs at the very low concentrations of nutrients found in nature, the rate of uptake can be near maximal. Some nutrients are transported by a low-affinity transporter when the nutrient is present at *high* external concentration and by a separate, typically higher-affinity, transporter when the nutrient is present at *low* concentration.

In addition to its permeability and transport functions, the cytoplasmic membrane of *Bacteria* and *Archaea* is a major site of both energy conservation and energy consumption. the cytoplasmic membrane can be energized when protons (H+) are separated from hydroxyl ions (OH-) across the membrane surface. This charge separation creates an energized state of the membrane called the *proton motive force*, analogous to the potential energy present in a charged battery. Dissipation of the proton motive force can be coupled to several energy-requiring reactions, such as transport, cell locomotion, and the biosynthesis of ATP. In eukaryotic microbial cells, although transport across the cytoplasmic membrane is just as necessary as it is in prokaryotic cells, energy conservation

takes place in the membrane systems of the cell's key organelles, the mitochondrion (respiration) and chloroplast (photosynthesis).

Transporting Nutrients into the Cell

Active Transport and Transporters

Active transport is the process by which cells accumulate solutes against the concentration gradient. Three basic mechanisms of active transport are found in prokaryotic cells. **A simple transport system** consists only of a transmembrane transport protein, **group translocation** employs a series of proteins in the transport event, and **ABC transport systems** consist of three components: a binding protein, a transmembrane transporter, and an ATP-hydrolyzing protein. Each of these transport systems is energy-driven, be it from the proton motive force, ATP, or some other energy-rich compound.

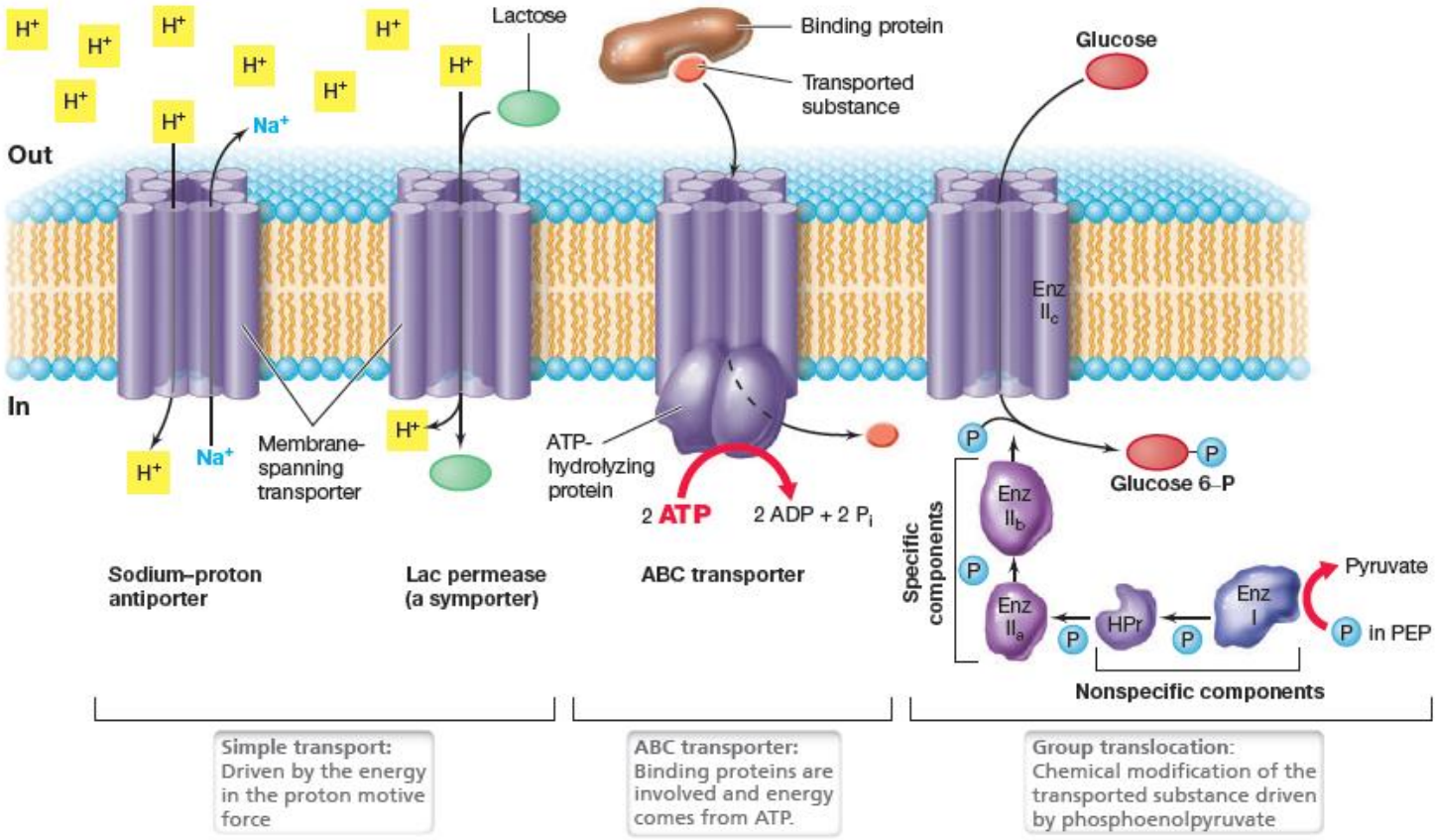


Figure 2.6 The three classes of transport systems. Transmembrane transporters are generally composed of a polypeptide that has 12 α-helices (each shown as a cylinder) that aggregate to form a channel through which solutes can cross the membrane. In simple transport the movement of a solute is coupled with the dissipation of an electrochemical gradient such as the proton motive force. ABC transporters have three components: a binding protein that has high affinity for a substrate, a transmembrane protein channel, and a cytoplasmic ATP-hydrolyzing protein, which supplies the energy required to drive substrate transport. In group translocation, the substance transported is chemically modified upon entering the cell. For example, the glucose group translocation system has five proteins: Enzyme (Enz) I, Enzymes II_a, II_b, and II_c, and HPr. A phosphate cascade occurs from phosphoenolpyruvate (PEP) to Enz II_c, and the latter protein actually transports and simultaneously phosphorylates the sugar. Proteins HPr and Enz I are nonspecific and participate in the transport of any sugar, while the three components of Enz II are specific for a particular sugar. Note how simple transporters and the ABC system transport substances without chemically modifying them, whereas group translocation results in chemical modification (in this case phosphorylation) of the transported substance.

The cell wall

The cell envelopes of most *Bacteria* can be classified as being either *gram-positive* or *gram-negative* based on their organization and cell wall structures. The cell envelope of a gram-positive cell typically contains a cytoplasmic membrane and a thick cell wall, whereas a gram-negative cell has a cytoplasmic membrane, a thin cell wall, an outer membrane, and a periplasm, which is a compartment between the cytoplasmic and outer membranes.

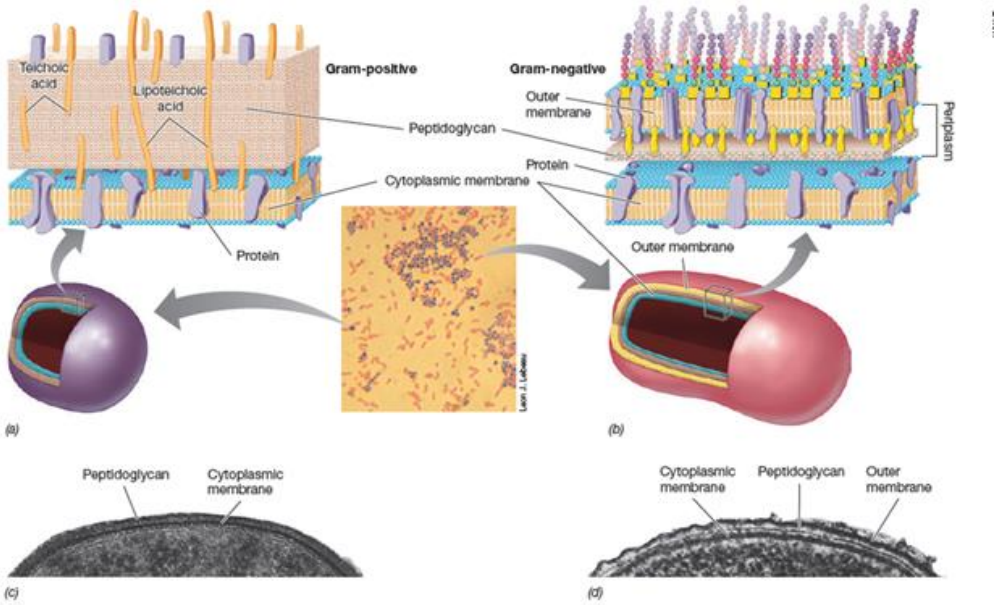


Figure: Cell envelopes of *Bacteria.* *(a, b)* Schematic diagrams of gram-positive and gram-negative cell envelopes; The photo of Gram-stained bacteria in the center shows cells of *Staphylococcus aureus* (purple, gram-positive) and *Escherichia coli* (pink, gram-negative).

Bacterial Cell Walls

The cell walls found in *Bacteria* contain a rigid polysaccharide called peptidoglycan that confers structural strength on the cell. Peptidoglycan is found in all *Bacteria* that contain a cell wall, but it is unique to *Bacteria* and is not found in *Archaea* or *Eukarya*. The sugar backbone of peptidoglycan is composed of alternating repeats of two modified glucose residues called *N-acetylglucosamine* and *N-acetylmuramic acid* joined by a β-1,4 linkage. Attached to the latter residue is a short peptide side chain. The amino acid composition of this peptide side chain can vary considerably between bacterial species. In *Escherichia coli* this peptide contains the amino acids l-alanine, d-alanine, d-glutamic acid, and diaminopimelic acid (DAP), though in other bacteria, l-lysine can be substituted for DAP. The presence of d stereoisomer amino acids, d-alanine and d-glutamic acid, is an unusual feature of peptidoglycan since proteins are always constructed of l-amino acids. These constituents are connected in an ordered way to form the *glycan tetrapeptide*, and long chains of this basic unit form peptidoglycan.

Strands of peptidoglycan run parallel to each other around the circumference of the cell. The peptide side chains of adjacent peptidoglycan strands are cross-linked together by covalent peptide bonds, and in this way, the peptidoglycan forms one single enormous molecule. In gram-negative bacteria, the crosslinks form primarily between the amino group of DAP on one glycan strand and the carboxyl group of the terminal d-alanine on the adjacent glycan strand.
The cell wall in the gram-negative cell envelope is 2–7 nm thick consisting primarily of a single layer of peptidoglycan, though it can be up to three layers thick in some places. The peptidoglycan mesh so formed is flexible and porous, but strong enough to resist turgor pressure and prevent rupture of the cytoplasmic membrane and cell lysis. Additional strength against osmotic lysis in gram-negative bacteria is provided by the outer membrane.

The typical bacterial gram-positive cell envelope contains a thick peptidoglycan cell wall, which can measure 20 to 35 nm in thickness and is usually much thicker than the wall of gram-negative organisms. As much as 90% of the gram-positive cell envelope can consist of peptidoglycan. Whereas the gram-negative cell wall typically contains only a single layer of peptidoglycan, the gram-positive cell wall can be 15 or more layers thick. The peptidoglycan of the gram-positive cell wall is stabilized three-dimensionally by peptide cross-links, which form between adjacent peptidoglycan strands both horizontally and vertically. In gram-positive bacteria, peptide cross-links often contain a short peptide "interbridge," the kinds and numbers of amino acids in the interbridge varying between species. In the gram-positive bacterium *Staphylococcus aureus*, for example, the interbridge often consists of five glycines.

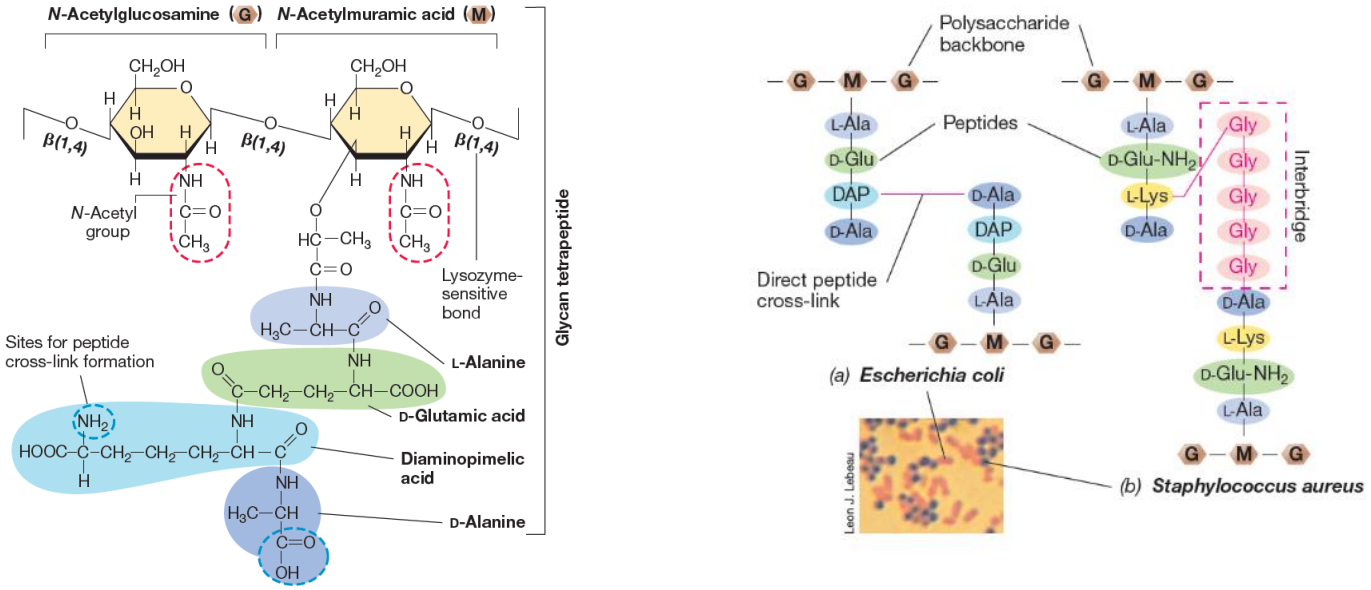


Figure: Structure of the repeating unit in peptidoglycan, the glycan tetrapeptide.

In addition to peptidoglycan, many gram-positive bacteria produce acidic molecules called teichoic acids embedded in their cell wall. Teichoic acids are composed of glycerol phosphate or ribitol phosphate with attached molecules of glucose or d-alanine (or both). Individual alcohol molecules are then connected through their phosphate groups to form long strands, and these are then covalently linked to peptidoglycan.

Some teichoic acids are covalently bonded to membrane lipids rather than to peptidoglycan, and these are called *lipoteichoic* acids. Peptidoglycan can be destroyed by *lysozyme*, an enzyme that cleaves the glycosidic bond between *N*-acetylglucosamine and *N*-acetylmuramic acid. This weakens the peptidoglycan and can cause cell lysis. Lysozyme is present in human secretions including tears, saliva, and other bodily fluids, and functions as a major line of defense against bacterial infection. Many antibiotics, including penicillin, also target peptidoglycan. Whereas lysozyme destroys preexisting peptidoglycan, penicillin blocks the formation of peptide cross-links, which compromises the strength of the peptidoglycan, leading to cell lysis.

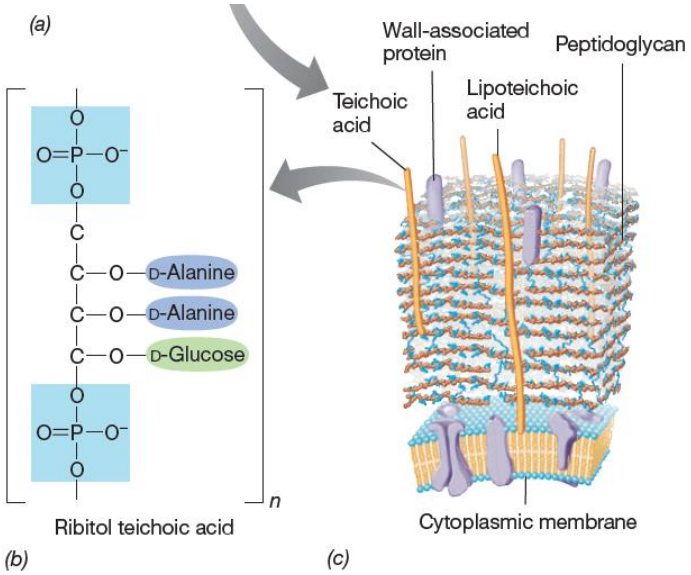


Figure: Structure of the gram-positive bacterial cell wall.
(b) Structure of a ribitol teichoic acid.

Archaeal Cell Walls

The cell envelopes of *Archaea* differ from those of *Bacteria by lacking of* peptidoglycan. In addition, *Archaea* typically lack an outer membrane. One consequence of these differences is that the Gram stain reaction is not very useful for predicting the structures of archaeal cell envelopes and so we typically do not use the terms gram-positive and gram-negative to describe cells of *Archaea*. Most *Archaea* lack a polysaccharide-containing cell wall and instead have an *S-layer.* which is a rigid protein shell that functions to prevent osmotic lysis just as does the bacterial cell wall.

While some *Archaea* do have cell walls, these walls have unique chemical structures not found in *Bacteria*. For example, the cell walls of certain methane-producing *Archaea* (methanogens) contain a polysaccharide called *pseudomurein*, which is structurally similar to peptidoglycan (the term *murein* is from the Latin word for "wall" and was an old term for peptidoglycan). The backbone of pseudomurein is formed from alternating repeats of *N*-acetylglucosamine (also present in peptidoglycan) and *N*-acetyltalosaminuronic acid; the latter replaces the *N*-acetylmuramic acid of peptidoglycan. Pseudomurein also differs from peptidoglycan in that the glycosidic bonds between the sugar derivatives are b-1,3 instead of b-1,4, and the amino acids are all of the L- stereoisomer. pseudomurein is immune from destruction by both lysozyme and penicillin, molecules that destroy peptidoglycan.

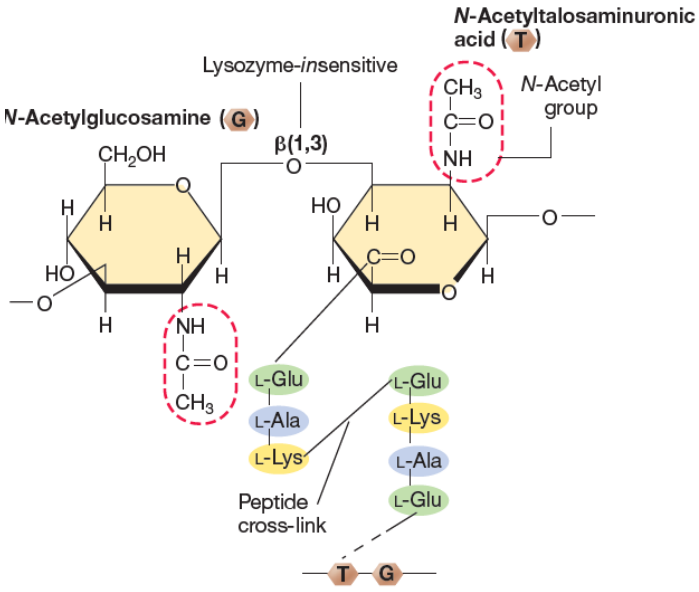


Figure: Pseudomurein. Structure of pseudomurein, the cell wall polymer of *Methanobacterium* species.

LPS: The Outer Membrane

Most of the gram-negative cell envelope is composed of the outer membrane. The outer membrane and cytoplasmic membrane are similar in that they both contain phospholipid and protein, but a major difference is that the outer membrane also contains polysaccharide molecules covalently bound to lipids. another major difference between the cytoplasmic and outer membranes is that the outer membrane contains *porins*, which are transmembrane proteins that allow for the nonspecific transport of solutes. Hence, the outer membrane is far more permeable than is the cytoplasmic membrane.

LPS molecules have several unique functions: They can facilitate surface recognition, they are important virulence factors for some bacterial pathogens, and they contribute to the mechanical strength of the cell.

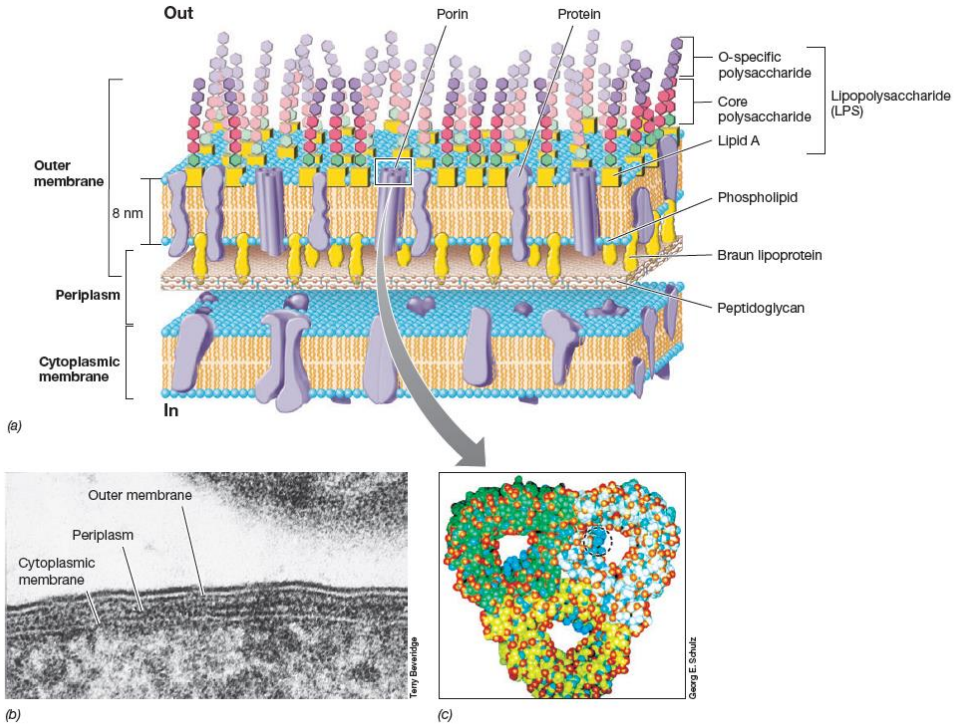


Figure: The gram-negative bacterial cell envelope. *(a)* Arrangement of lipopolysaccharide, lipid A, phospholipid, porins, and Braun lipoprotein in the outer membrane. *(c)* Molecular model of porin proteins showing their hollow pores that allow solute transport across the outer membrane. The view of the porin is perpendicular to the plane of the membrane.

Structure and Activity of LPS

LPS contains a polysaccharide that consists of two components, the *core polysaccharide* and the *O-specific polysaccharide*. In *Salmonella* species, where LPS has been well studied, the core polysaccharide consists of ketodeoxyoctonate (KDO), various seven-carbon sugars (heptoses), the hexose sugars glucose and galactose, and *N*-acetylglucosamine. Connected to the core is the O-specific polysaccharide, which typically contains galactose, glucose, the hexoses rhamnose and mannose, and one or more dideoxyhexoses, abequose, colitose, paratose and tyvelose, which are rarely found elsewhere in nature. Variations in sugar content of the O-polysaccharide contribute to the wide variety of antigenic types between species and even strains of Gram-negative bacteria.

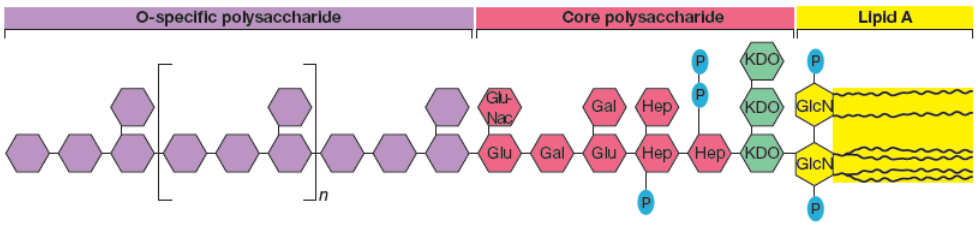


Figure: Structure of bacterial lipopolysaccharide. The chemical structures of lipid A and polysaccharides can vary among gram-negative *Bacteria*, but the major components (lipid A–KDO–core–O-specific) are typically invariant. The O-specific polysaccharide is highly variable among species. KDO, ketodeoxyoctonate; Hep, heptose; Glu, glucose; Gal, galactose;

GluNac, *N*-acetylglucosamine; GlcN, glucosamine; P, phosphate. Glucosamine and the lipid A fatty acids are linked through the amine groups of GlcN.

These sugars are connected in four- or five-membered sequences, which often are branched. When the sequences repeat, the long O-specific polysaccharide is formed. Within the outer membrane, these negatively charged polysaccharides can be linked together tightly when adjacent LPS molecules mutually form ionic bonds to divalent cations (such as Ca2+ and Mg2+). The presence of these ionic bonds confers considerable strength to the outer membrane, which rivals the gram-negative cell wall in its mechanical strength.

The lipid portion of the LPS, called *lipid A*, is not a typical glycerol lipid; instead, the fatty acids are bonded through the amine groups from a disaccharide composed of glucosamine phosphate.

The disaccharide is attached to the core polysaccharide through KDO. Fatty acids typically found in lipid A include caproic (C6), lauric (C12), myristic (C14), palmitic (C16), and stearic (C18) acids. LPS replaces much of the phospholipid in the outer half of the outer membrane, and although the outer membrane is technically a lipid bilayer, its many unique components distinguish it from the cytoplasmic membrane. The outer membrane is anchored to the peptidoglycan layer by the *Braun lipoprotein*, a molecule that spans the gap between the LPS layer and the peptidoglycan layer.

An important biological activity of LPS is its toxicity to animals. Common gram-negative pathogens for humans include species of *Salmonella, Shigella*, and *Escherichia*, among many others, and some of the gastrointestinal symptoms these pathogens elicit are due to their toxic outer membrane components. Toxicity is specifically linked to the LPS layer, in particular, to lipid A, evidenced by the fact that injection of purified lipid A into an experimental animal will elicit the same inflammatory responses as intact LPS. LPS (and consequently lipid A) are called endotoxin because of the association with the cell wall of bacteria. LPS molecules can dissociate from the surfaces of Gram-negative bacteria. The O-polysaccharide is hydrophilic and may allow diffusion or delivery of the toxic lipid in the hydrophilic (in vivo) environment. Lipid A molecules are detected at picomolar levels by the innate immune system of animals. During severe infections by Gram-negative bacteria, macrophages and endothelial cells are greatly stimulated by the released LPS, resulting in excess production of cytokines and inflammatory mediators. These products resulting from macrophage activation are thought to be responsible for the complications of Gram-negative sepsis. However, the inflammatory response is primarily protective in nature in that host defenses are recruited and stimulated to counter infection. If infection is not held in check, the overwhelming number of bacteria can then stimulate an excessive host response with ensuing damage.

LPS first binds the serum LPS binding protein. Subsequently, this complex binds to the membrane receptor, CD14, present on certain host cells such as macrophages. More recently a role for members of the human toll-like receptor (TLR) family in LPS recognition has been proposed, suggesting that the LPS receptor is multimeric, comprised of at least CD14 and one or more TLRs, primarily TLR-4

The Periplasm and Porins

The outer membrane is impermeable to proteins and other very large molecules. In fact, a major function of the outer membrane is to prevent cellular proteins whose activities must occur outside the cytoplasm from diffusing away from the cell. These extracellular proteins reside in the periplasm, a space of about 15 nm located between the *outer surface* of the cytoplasmic membrane and the *inner surface* of the outer membrane.

The periplasm may contain several different classes of proteins. These include hydrolytic enzymes, which function in the initial degradation of polymeric substances; binding proteins, which begin the process of transporting substrates chemoreceptors, which are proteins that govern the chemotaxis response; and proteins that construct extracellular structures (such as peptidoglycan and the outer membrane) from precursor molecules secreted through the cytoplasmic membrane. Most periplasmic proteins reach the periplasm by way of a protein-exporting system present in the cytoplasmic membrane.

The outer membrane is relatively permeable to small molecules because of proteins called *porins* that function as channels for the entrance and exit of solutes. Porins are unique to the outer membrane of *Bacteria* and should not be confused with *aquaporins*, which are a different class of proteins (aquaporins facilitate water transport across the cytoplasmic membrane). Several porins are known, including both specific and nonspecific classes.

Nonspecific porins form water-filled channels through which most very small hydrophilic substances can pass. By contrast, specific porins contain a binding site for one or a group of structurally related substances. Porins are transmembrane proteins composed of three identical polypeptides; the proteins are arranged to form channels through which solutes can diffuse.

A few *Bacteria* and *Archaea* lack cell walls altogether. These include in particular the mycoplasmas and other pathogenic *Bacteria* that grow within a host cell, and *Archaea* such as *Thermoplasma* and its relatives.

Lacking a cell wall, these cells would be expected to contain unusually tough cytoplasmic membranes. For example, most mycoplasmas contain *sterols* in their cytoplasmic membranes; these molecules function to add strength and rigidity to the membrane as they do in the cytoplasmic membranes of eukaryotic cells.

Mycoplasmas may also have little need for a cell wall because they experience little osmotic pressure when living within the cytoplasm of another cell. In addition, the loss of peptidoglycan may help mycoplasmas evade the host immune system because host defenses recognize bacterial cell wall components as one of many signals of bacterial invasion.

Cell Surface Structures

Capsules and Slime Layers

The terms *capsule* and *slime layer* are used to describe a sticky coat of polysaccharide formed outside of the cell envelope. If the polysaccharide layer is organized in a tight matrix that excludes small particles and is tightly attached to the cell, it is called a capsule. if the surface layer is easily deformed and loosely attached, it will not exclude particles and is more difficult to see microscopically. Such a loosely attached polysaccharide coat is called a *slime layer*.

Outer surface layers have several functions. Surface polysaccharides assist in the attachment of microorganisms to solid surfaces. Pathogenic microorganisms that enter the body by specific routes usually do so by first binding to specific surface components of host tissues; this binding is often facilitated by bacterial cell surface polysaccharides. When the opportunity arises, many bacteria will bind to solid surfaces, often forming a thick layerof cells called a *biofilm* . Extracellular polysaccharides play a key role in the development and maintenance of biofilms as well. Besides attachment, outer surface layers have other functions.

These include contributing to the infectivity of a bacterial pathogen and preventing dehydration. For example, the causative agents of the diseases anthrax and bacterial pneumonia—*Bacillus anthracis* and *Streptococcus pneumoniae*, respectively—each contain a thick capsule of either protein (*B. anthracis*) or polysaccharide (*S. pneumoniae*). Encapsulated cells of these bacteria avoid destruction by the host's immune system because the immune cells that would otherwise recognize these pathogens as foreign and destroy them are blocked from doing so by the bacterial capsule. In addition to this role in disease, bacterial outer surface layers bind water, and this helps protect the cell from desiccation in periods of dryness.

Fimbriae, Pili, and Nanowire

Pili are thin (2–10 nm in diameter) filamentous structures made of protein that extend from the surface of a cell and can have many functions. Short pili that mediate attachment are often called *fimbriae.* Pili enable bacterial cells to stick to surfaces, including animal tissues, or to form pellicles (thin sheets of cells on a liquid surface) or biofilms on solid surfaces.

Pili, by allowing bacteria to attach to other cells, often contribute to the virulence of pathogens.

Pili can enable bacteria to adhere to surfaces and this function can allow pathogens to target and invade specific host tissues. However, pili are diverse and they can have several other important functions as well. For example, *conjugative pili* facilitate genetic exchange by causing cell-to-cell attachment during a process called *conjugation*.

In addition, *electrically conductive pili* (also known as ***nanowires***, can conduct electrons toward or away from the cell and in so doing play an important role in the energy metabolism of diverse microbes.

Lastly, a type of pili called *type IV pili* not only facilitate adhesion but also support an unusual form of cell movement called *twitching motility* in certain bacterial species. Twitching motility allows cells to move along a solid surface. In twitching motility, pili are extended away from the cell, attach to a surface, and are subsequently retracted, dragging the cell forward. ATP supplies the energy necessary for extension and retraction of the pilus. On rod-shaped cells that move by twitching, type IV pili are present only at the cell poles. Type IV pili assist in infectivity by certain pathogens, including the gram-negative bacteria *Vibrio cholerae* (cholera) and *Neisseria gonorrhoeae* (gonorrhea) and the gram-positive bacterium *Streptococcus pyogenes* (strep throat and scarlet fever). The twitching motility of these organisms assists them in locating specific sites for attachment to initiate the disease process.