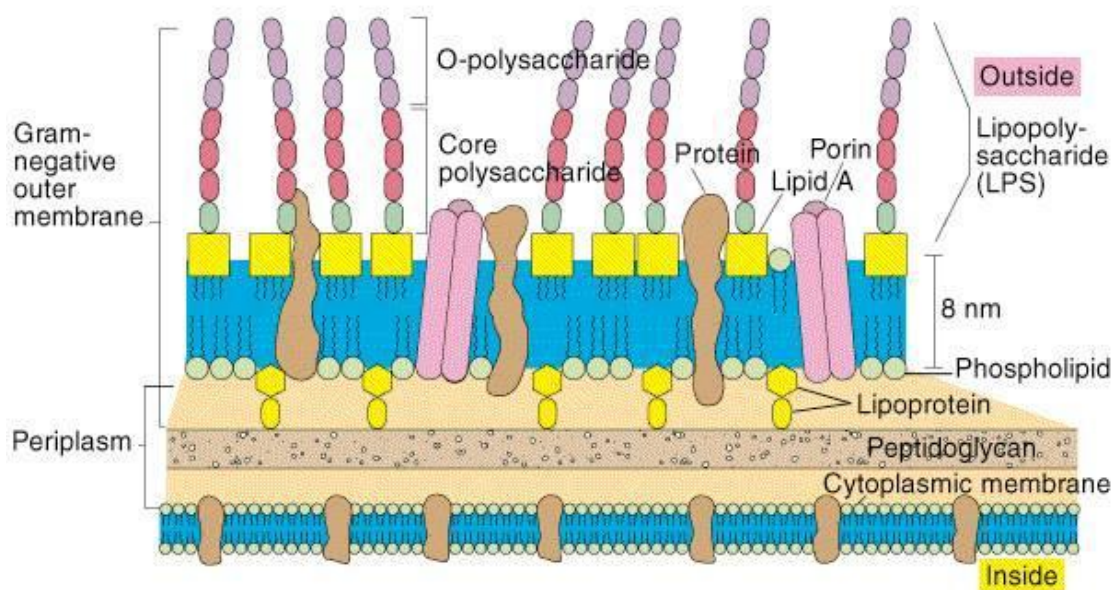


## Prokaryotic Cell Structure II

### C- Special Components of Gram-Negative Cell Walls

Gram-negative cell walls contain three components that lie outside of the peptidoglycan layer: **lipoprotein**, **outer membrane**, and **lipopolysaccharide**.



#### 1. Outer membrane

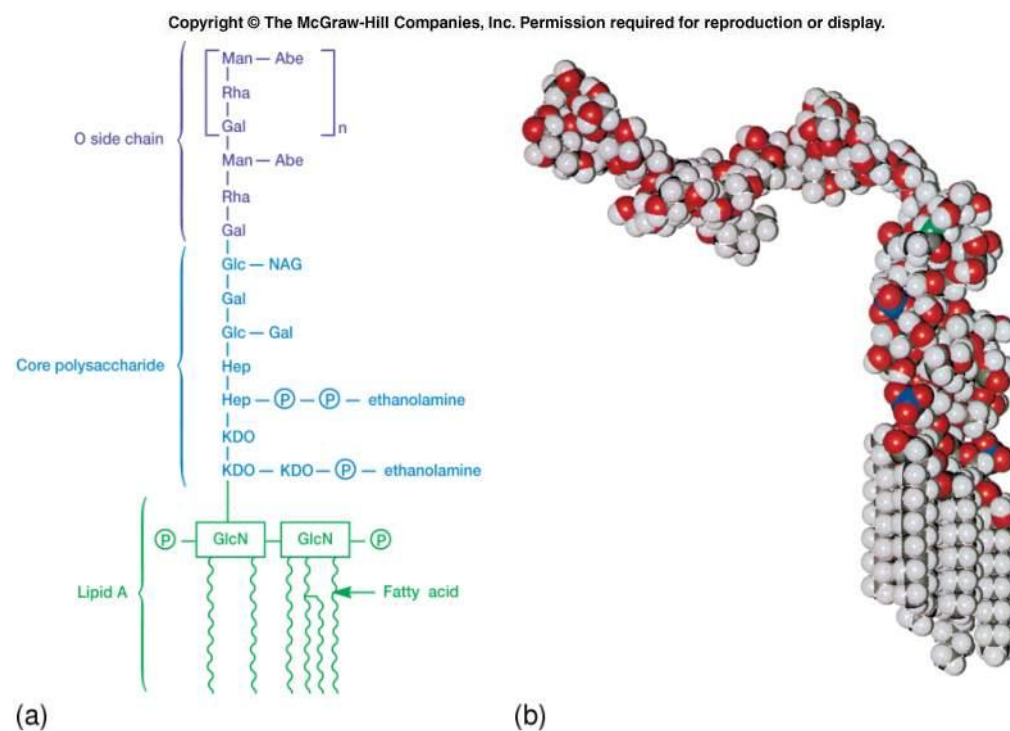
The outer membrane is chemically distinct from all other biological membranes. It is bilayer structure; its inner leaflet resembles in composition that of the cell membrane while its outer leaflet contains a distinctive component, a **lipopolysaccharide (LPS)**. The outer membrane has special channels, consisting of protein molecules called **porins**, that permit the passive diffusion of low molecular-weight hydrophilic compounds like sugars, amino acids, and certain ions.

## 2. Lipopolysaccharide (LPS)

The LPS of gram-negative cell walls consists of a complex glycolipid, called **lipid A**, that consists of phosphorylated glucosamine disaccharide unite to which are attached a number of long-chain fatty acids.

The **polysaccharide core** is similar in all gram-negative species that have LPS and includes two characteristic sugars, **ketodeoxyoctanoic acid (KDO)** and a **heptose**. The repeat units are usually linear trisaccharides or branched tetra-or pentasaccharides. The repeat unit is referred to as the **O antigen**.

LPS, which is extremely toxic to animals, has been called the **endotoxin** of gram-negative bacteria because it is firmly bound to the cell surface and is released only when the cells are lysed. When LPS is split into lipid A and polysaccharide, all of the toxicity is associated with the former.



### 3. Lipoprotein

The lipoprotein contains 57 amino acids, representing repeats of a 15-amino-acid sequence; it is peptide-linked to DAP residues of the peptidoglycan tetrapeptide side chains. Its function is to stabilize the outer membrane and anchor it to the peptidoglycan layer.

### 4. The periplasmic space

The space between the inner and outer membranes, called the **periplasmic space**, contains the peptidoglycan layer and a gel-like solution of proteins.

### D- The Acid-Fast Cell Wall

Some bacteria, notably tubercle bacillus (*M. tuberculosis*) and its relatives have cell walls that contain large amounts of **waxes**, complex branched hydrocarbons (70-90 carbons long) known as **mycolic acids**. The cell wall is composed of peptidoglycan and an external asymmetric lipid bilayer; the inner leaflet contains other extractable lipids. Some compounds penetrate the lipid domains of the cell wall albeit slowly. This hydrophobic structure renders these bacteria resistant to many harsh chemicals including detergents and strong acids. If a dye is introduced into these cells by brief heating or treatment with detergents, it cannot be removed by dilute hydrochloric acid, as in other bacteria. These organisms are therefore called **acid-fast**.

### E- Cell Walls of the Archaea

The Archaea do not have cell walls like the Bacteria. Some have a simple S-layer often comprised of glycoproteins. Some Archaea

have a rigid cell wall composed of polysaccharides or a peptidoglycan called **pseudomurein**. The pseudomurein differs from the peptidoglycan of bacteria by having L-amino acids rather than D-amino acids and disaccharide units with an  $\alpha$ -1 $\rightarrow$ 3 rather  $\beta$ -1 $\rightarrow$ 4 linkage. Archaea that have a pseudomurein cell wall are gram-positive.

## F- Crystalline Surface Layers

Many bacteria, both gram-positive and gram-negative bacteria as well as archaeobacteria, possess a two-dimensional crystalline, subunit-type layer lattice of protein or glycoprotein molecules (**S-layer**) as the outermost component of the cell envelope. The function of the S-layer is uncertain but is probably protective.

## G- Enzymes that Attack Cell Walls

The  $\beta$ -1 $\rightarrow$ 4 linkage of the peptidoglycan backbone is hydrolyzed by the enzyme **lysozyme**, which is found in animal secretions (tears, saliva, nasal secretions) as well as in egg white. Gram-positive bacteria treated with lysozyme in low-osmotic-strength media lyse; if the osmotic strength of the medium is raised to balance the internal osmotic pressure of the cell, free spherical bodies called **protoplasts** are liberated. The outer membrane of the gram-negative cell wall prevents access of lysozyme unless disrupted by an agent such as ethylene-diaminetetraacetic acid (EDTA), a compound that chelates divalent cations; in osmotically protected media, cells treated with EDTA-lysozyme form **spheroplasts** that still possess remnants of the complex gram-negative wall, including the outer membrane.

Bacteria themselves possess a number of **autolysins**, hydrolytic enzymes that attack peptidoglycan, including **muramidases**, **glucosaminidases**, **endopeptidases**, and **carboxypeptidases**. These enzymes catalyze the turnover or degradation of peptidoglycan in bacteria. Their activity is most apparent during the dissolution of dead cells (autolysis).

Enzymes that degrade bacterial cell walls are also found in cells that digest whole bacteria, e.g. protozoa and the phagocytic cells of higher animals.

### H- Protoplasts, Spheroplasts, & Forms

Removal of the bacterial wall may be accomplished by hydrolysis with lysozyme or by blocking peptidoglycan synthesis with an antibiotic such as penicillin. In osmotically protective media, such treatments liberate **protoplasts** from gram-positive cells and **spheroplasts** (which retain outer membrane and entrapped peptidoglycan) from gram-negative cells.

If such cells are able to grow and divide, they are called **L forms**. Some L forms can revert to the normal bacillary form upon removal of the inducing stimulus. Thus, they are able to resume normal cell wall synthesis. Others are stable and never revert. The factor that determines their capacity to revert may again be the presence of residual peptidoglycan, which normally acts as a primer in its own biosynthesis.

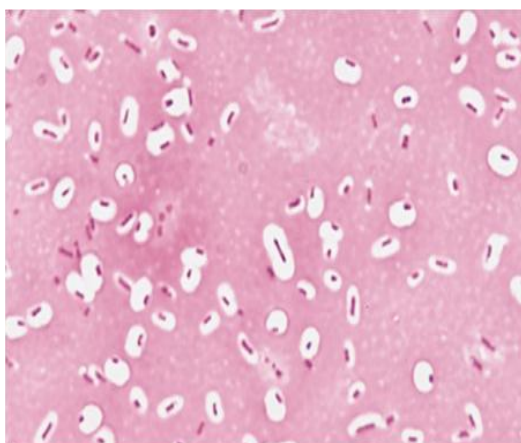
### I- The Mycoplasmas

The **mycoplasmas** are cell wall-lacking bacteria containing no peptidoglycan. There are also wall-less Archaea, but they have been

less well studied. Mycoplasmas lack a target for cell wall-inhibiting antimicrobial agents (e.g. penicillins and cephalosporins) and therefore resistant to these drugs. Some, like *Mycoplasma pneumonia*, an agent of pneumonia, contain sterols in their membranes. The difference between L forms and mycoplasmas is that when the murein is allowed to reform, L forms revert to their original bacteria shape, but mycoplasmas never do.

## Capsule & Glycocalyx

Many bacteria synthesize large amounts of extracellular polymer when growing in their natural environments. The extracellular material is polysaccharide. The terms **capsule** and **slime layer** are frequently used to describe polysaccharide layers; the more inclusive term **glycocalyx** is also used. Glycocalyx is defined as the polysaccharide-containing material lying outside the cell. A condensed, well-defined layer **closely surrounding the cell** that excludes particles, such as India ink, is referred to as a capsule. If the glycocalyx is **loosely associated with the cell** and does not exclude particles, it is referred to as a slime layer. The glycocalyx plays a role in the adherence of bacteria to surfaces in their environment, including the cells of plant and animal hosts.



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