Course: Medical Microbiology, PAMB 650/720 2007/2008

Lecture 35: The external layers of vegetative cells and spores

Faculty: Dr. Alvin Fox, Phone: 733 3288, E-mail: afox@med.sc.edu, Office: C-19, Blg. 2

Suggested reading: Murray, Fifth Edition, Chapter 3

KEY WORDS

Cell envelope	Lipopolysaccharide (endotoxin)
Cell wall	Teichoic acid
Cell membrane	Teichuronic acid
Outer membrane	Lipoteichoic acid
Peptidoglycan	Mycolic acid
Braun lipoprotein	Undecaprenol (bactoprenol)
Porins	Endospore (spore)

The structure of Gram negative, Gram positive and acid-fast cell envelopes will be discussed. The composition and function of unique cell envelope macromolecules and their biosynthesis will be described. In addition endospores, whose cell envelope is unusual, will be discussed.

The cell envelope: defined as the cell membrane and cell wall plus an outer membrane if one is present. The cell wall consists of the peptidoglycan layer and attached structures. Most bacterial cell envelopes fall into two major categories: Gram positive and Gram negative. This is based on Gram staining characteristics that reflect major structural differences between the two groups. Other types of cell wall are found in a few bacterial species (neither Gram positive nor Gram negative).

The peptidoglycan is a single bag shaped highly cross-linked macromolecule that surrounds the bacterial cell membrane, and provides rigidity. It is huge (billions in molecular weight; compare proteins which are thousands in molecular weight). Peptidoglycan consists of a glycan (polysaccharide) backbone consisting of N-acetyl muramic acid and N-acetyl glucosamine with peptide side chains containing D- and L- amino acids and in some instances diaminopimelic acid. The side-chains are cross-linked by peptide bridges. These peptide bridges vary in structure among bacterial species. Muramic acid, D-amino acids and diaminopimelic acid are not synthesized by mammals. PG is found in all eubacteria except *Chlamydia* and *Mycoplasma*.

The Gram positive cell envelope: Covalently bound to the thick peptidoglycan is teichoic acid (backbones is usually polymers of ribitol phosphate or glycerol phosphate) or teichuronic acid (glucuronic acid- containing polysaccharides). The latter are synthesized under phosphate-limiting conditions. These negatively charged molecules concentrate metal ions from the surroundings. In some instances neutral polysaccharides are present instead. Teichoic acids can also direct autolytic enzymes to sites of peptidoglycan digestion (autolysis). This is needed to insert sections of cell wall for growth and division. Lipoteichoic acid is primarily associated with the cell membrane. The lipoteichoic acid bind autolysins keeping them away from the cell wall until needed in biosynthesis.

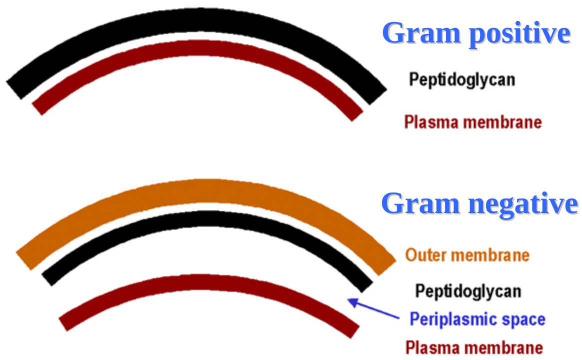
The Gram negative cell envelope: Covalently linked to the thin peptidoglycan is the Braun lipoprotein which binds the outer membrane to the cell wall. Like other membranes the outer membrane contains proteins and phospholipids. Unlike other membranes it contains additional molecules (lipopolysaccharide, LPS). LPS is important to the bacterial cell since it helps to provide a permeability barrier, including hydrophobic substances. LPS consists of three regions: an outer O antigen, a middle core and an inner lipid A region. The core contains several sugars (heptoses and ketodeoxyoctonic acid) not found commonly in nature and lipid A contains β hydroxy fatty acids (uncommon in nature). The molecule displays endotoxin activity. Porins in the outer membrane help form channels to allow passage of small hydrophilic nutrients (such as sugars) through the outer membrane.

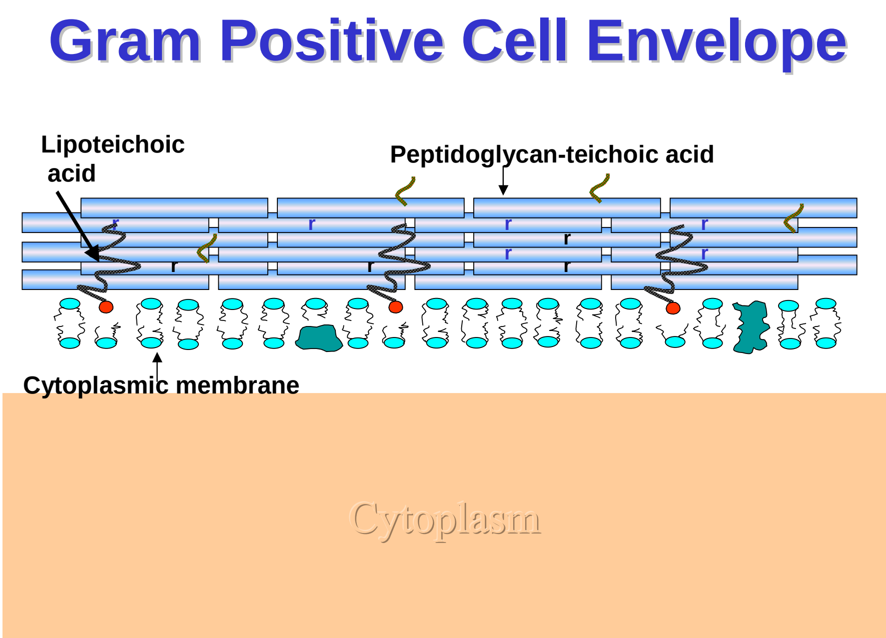
Acid-fast and related bacteria (mycobacteria, nocardia and corynebacteria): These cell envelopes resemble Gram positive bacteria in having polysaccharide covalently bound to the peptidoglycan. Additional mycolic acids (long, branch chained fatty acids) are covalently linked to this polysaccharide. Other mycolic acid containing compounds and complex lipids form a thick waxy membranous layer outside the peptidoglycan layer, i.e. there is an outer membrane (as found in Gram negative bacteria).

Synthesis of cell envelope macromolecules

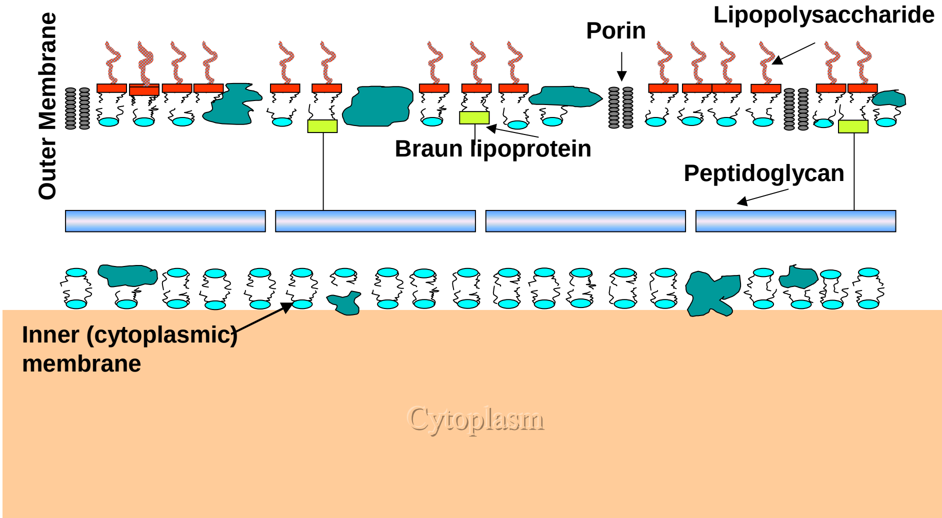
Peptidoglycan: The precursor subunit (muramyl pentapeptide attached to uridine diphosphate, UDP) is synthesized in the cytoplasm and passed to the cell membrane. The subunit is moved enzymatically from the nucleotide to a lipid carrier (undecaprenol/bactoprenol) and built into a completed subunit (disaccharide pentapeptide with attached bridge peptide). The synthesis of the peptide side-chain and bridge does not employ ribosomes. The completed subunits are then exported to the cell wall. After release of the monomer, the undecaprenol is re-circulated in the cell membrane and used again. The glycan backbone of the existing cell wall is enzymatically broken (by autolysins) to allow insertion of the newly synthesized subunit. If these enzymes are overactive the cell wall becomes degraded and the high osmotic pressure of the cell bursts the cytoplasmic membrane killing the cell ("autolysis"). Cross-linking of the peptide side-chain of the inserted subunit to the existing chain then occurs enzymatically (penicillin binding proteins). Completed subunits of teichoic and teichuronic acids are also synthesized in the cell membrane (on lipid carriers) before transport and insertion into the existing cell wall.

Simplified diagram of the cell envelope of Gram positive and Gram negative bacteria



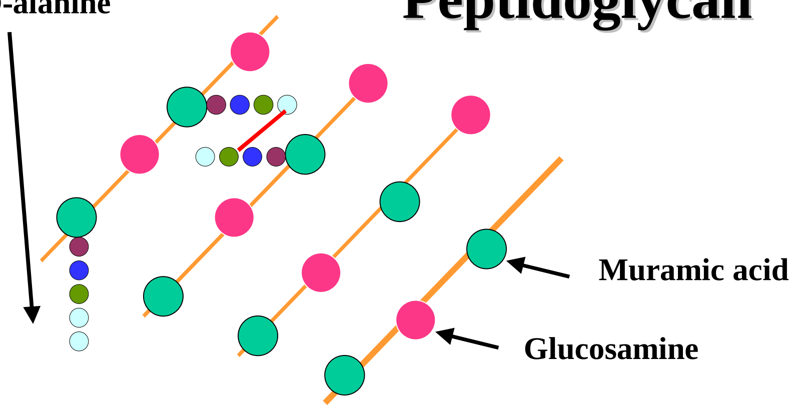


Gram Negative Cell Envelope

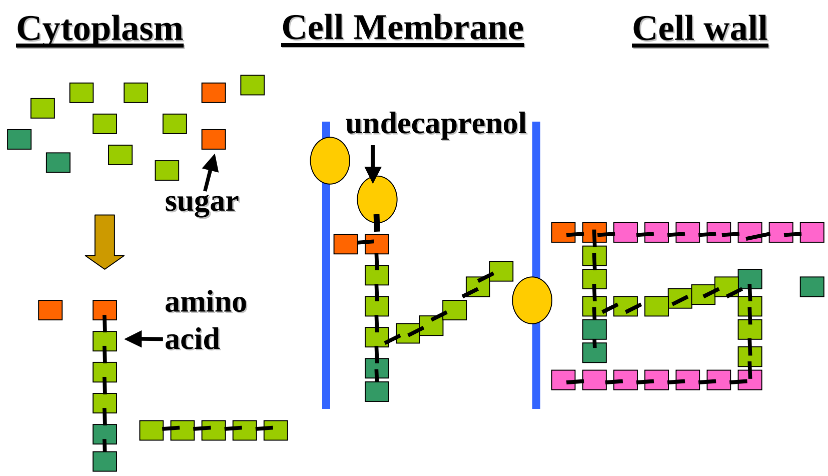


L-alanine
D-glutamic acid
L-lysine/Diaminopimelic acid
D-alanine
D-alanine

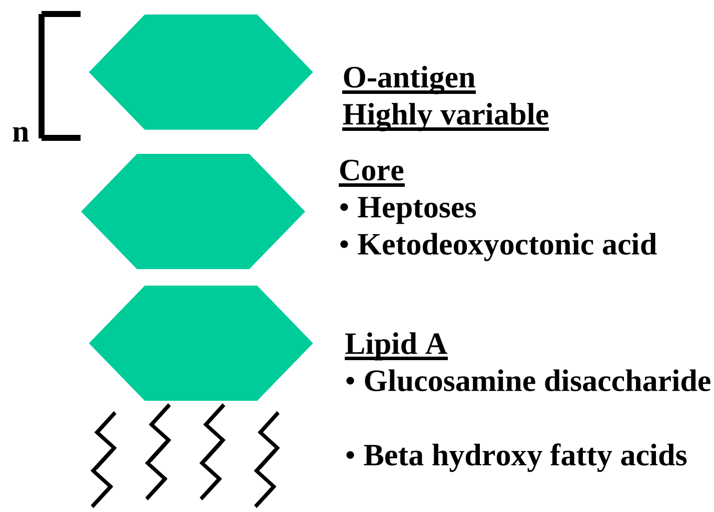
Peptidoglycan



Peptidoglycan synthesis



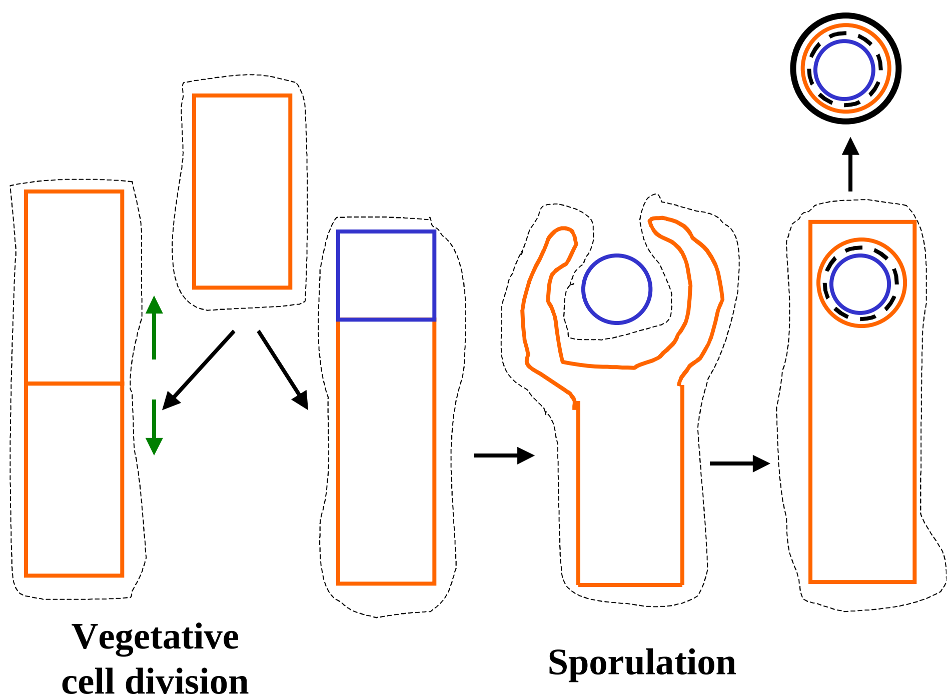
Lipopolysaccharide



Lipopolysaccharide: Lipid A is assembled in the cell membrane and the core sugars attached sequentially. O-antigen subunits are independently synthesized (on a lipid carrier as in peptidoglycan synthesis). The fully synthesized O-antigen is then attached to the lipid A-core (generating lipopolysaccharide) in the cell membrane before passage/insertion into the outer membrane.

Endospores: These modified Gram positive bacterial cells have an unusual cell envelope that contains a cell membrane and an outer membrane. Normally in bacterial replication, as cells divide, a septum forms dividing the mother cell into two roughly equally sized daughters. When sporulation occurs cell division is unequal and the larger so-called "mother cell" envelops the daughter cell. The cell membrane of the daughter constitutes the inner membrane of the spore and the cell membrane of the mother forms the outer membrane.

The peptidoglycan layer is less cross-linked than in most bacterial cells and contains a dehydrated form of muramic acid (lactam). The spore peptidoglycan is referred to as a cortex and is found between the two membranes. A thick protein coat makes the bacterial spore highly resistant to chemical agents. An exosporium (made up of glycoprotein) surrounds the coat.



The endospore