

# فسيولوجيا الأحياء الدقيقة

# Microbial Physiology

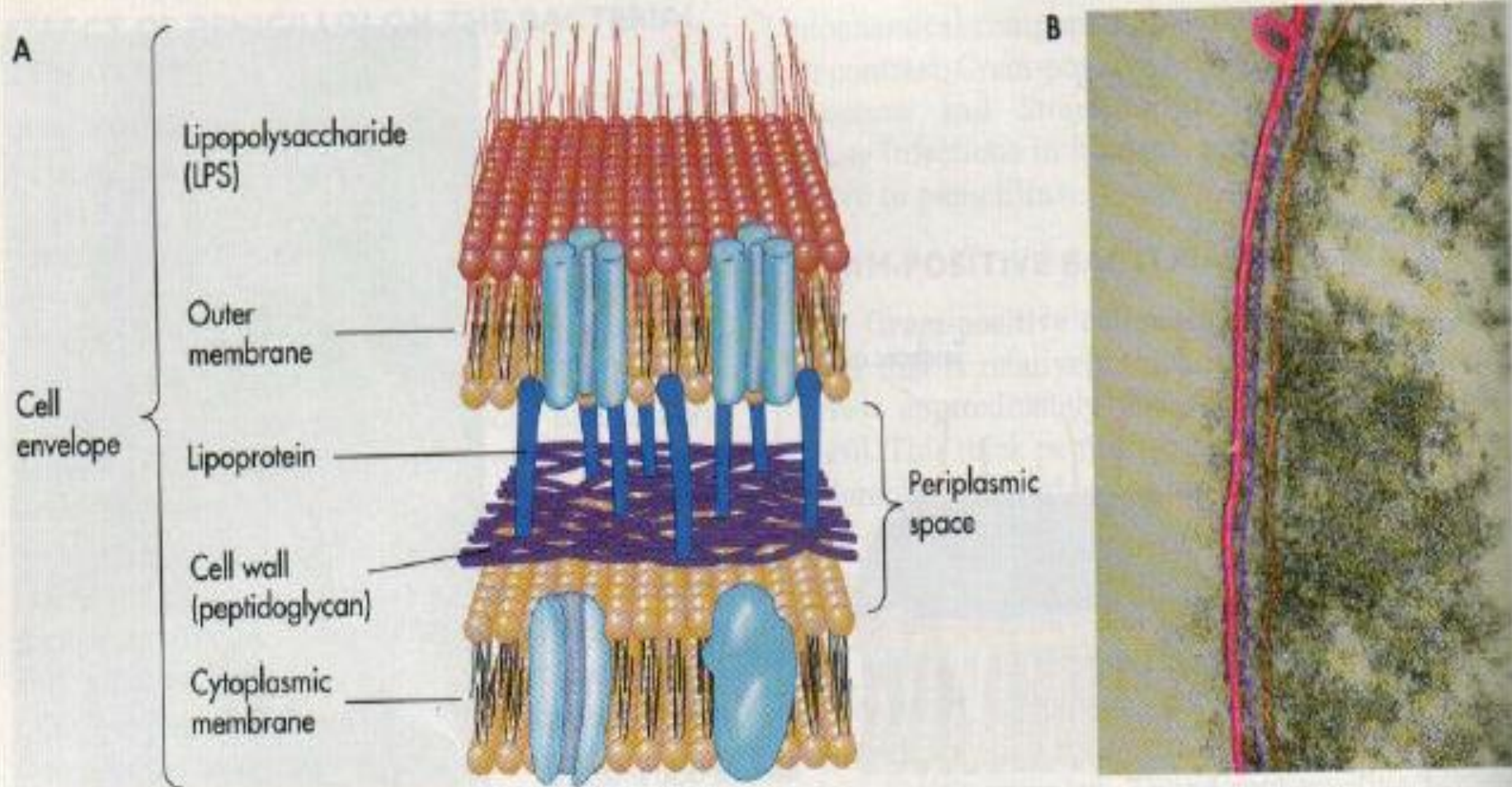
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مكتب ٢ ب ٤٥

مقدمة

Introduction-L5

# Gram Negative Cell Wall Synthesis

- **The peptidoglycan layer:**
  - Produced in an identical fashion as the gram-positive bacteria.
  - The undecaprenyl phosphate lipid carrier is used.
  - NAG and NAM-pentapeptide is assembled in the cytoplasm.
- The outer membrane phospholipids are assembled in the cytoplasm and inserted first into the inner membrane.
- Diffusion into the outer membrane occurs via the continuity of the inner and outer membrane (Bayer Junctions).
- Lipo-polysaccharides (LPS) are an important feature of the gram-negative cell wall.



**Fig. 3-21 Gram-negative Bacterial Cell Wall.** **A**, The Gram-negative cell wall is a thin layer attached to an outer membrane via lipoproteins. The outer membrane contains phospholipid (tan) on its inner surface and lipopolysaccharide (LPS) (red) on its outer surface. There is also peptidoglycan (purple) between the two membranes, which is anchored to the outer membrane by lipoprotein (blue). The area between the outer membrane and the cytoplasmic membrane is called the periplasm or periplasmic gel. **B**, Colorized micrograph of the cell wall of the Gram-negative bacterium *Escherichia coli*. (110,000 $\times$ .) The outer membrane encloses the peptidoglycan. The entire cell wall surrounds the cytoplasmic membrane.

# Gram Negative Cell Wall Synthesis

- **Lipopolysaccharides (LPS):**

- Made up of three units:

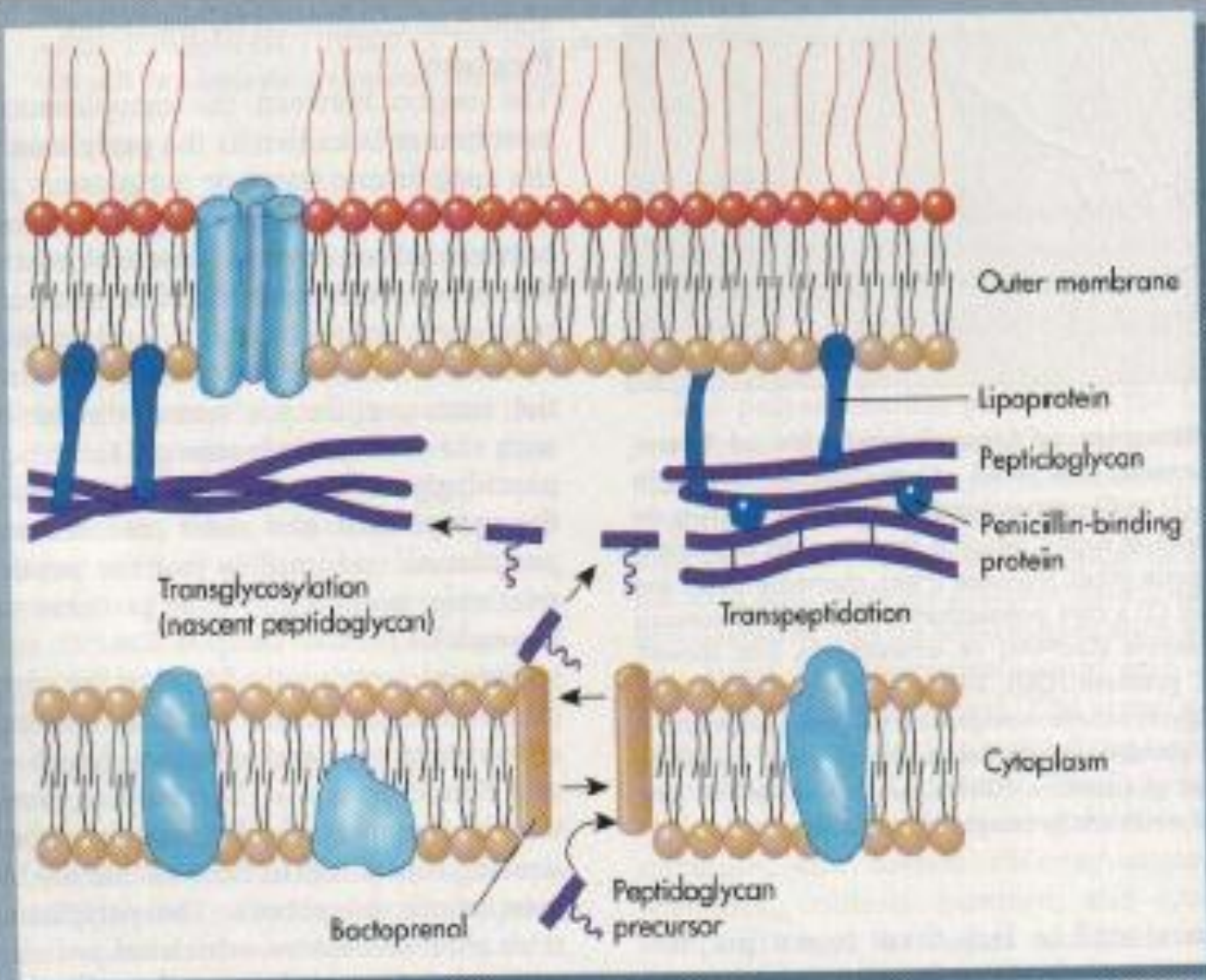
- ✓ Lipid A (the part embedded into the membrane).
    - ✓ The core polysaccharide (essentially the same G-).
    - ✓ The O-antigen (a variable polysaccharide region).

- Two parallel processes occur in the cell membrane synthesize the Lipopolysaccharides (**LPS**).

- The precursors for both processes are assembled at the inner membrane.
  - Lipid A acts as a carrier as well as the primer site for the core polysaccharide addition.
  - O-antigen is synthesized on the lipid carrier undecaprenyl phosphate.

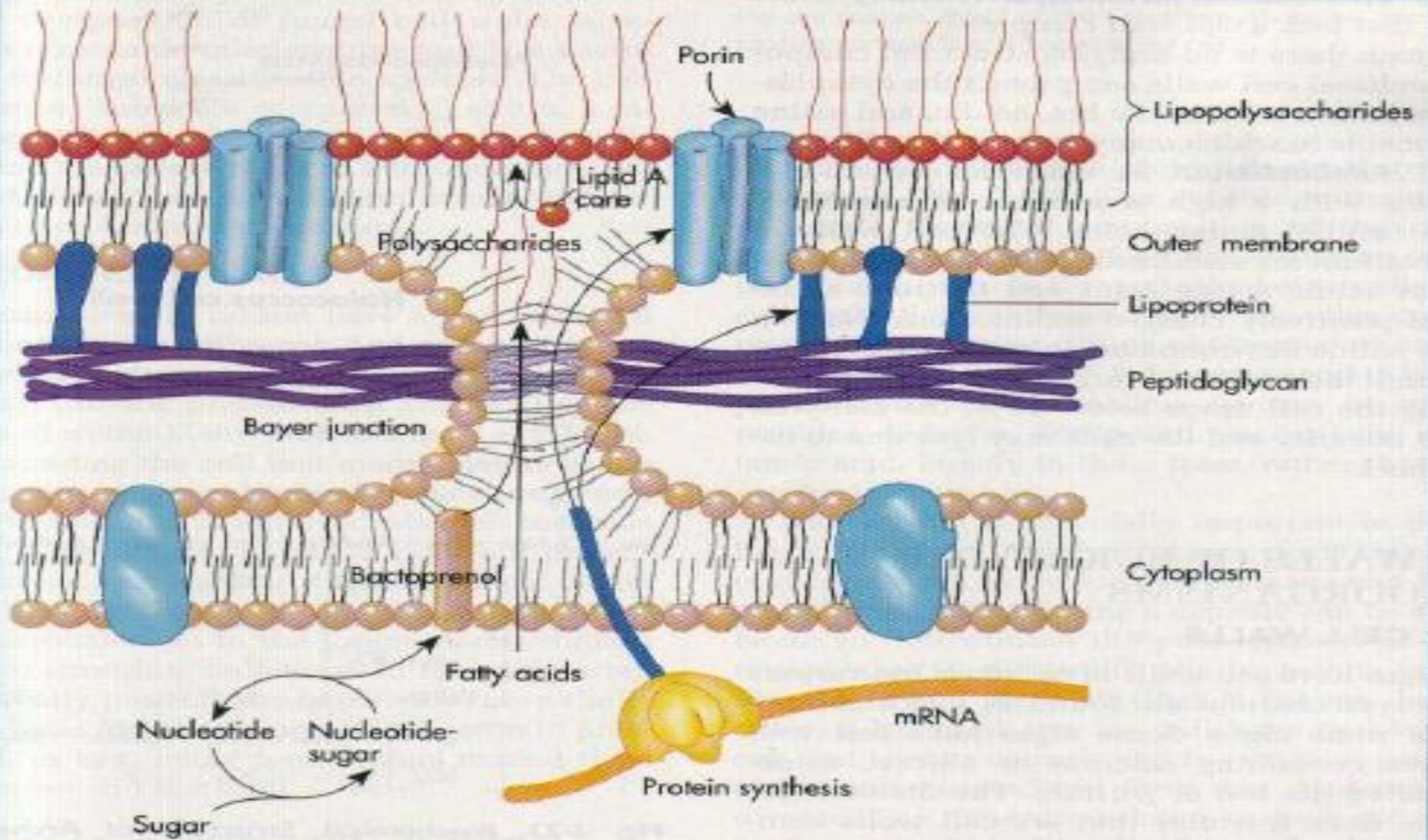
# Gram Negative Cell Wall Synthesis

- Two parallel processes occur in the cell membrane synthesize the Lipopolysaccharides (**LPS**).
  - The precursors for both processes are assembled at the inner membrane.
  - Lipid A acts as a carrier as well as the primer site for the core polysaccharide addition.
  - O-antigen is synthesized on the lipid carrier undecaprenyl phosphate.
  - **Transported to the outer membrane.**
  - **The O-antigen is added to the Lipid A-core polysaccharide by a transfer enzyme.**

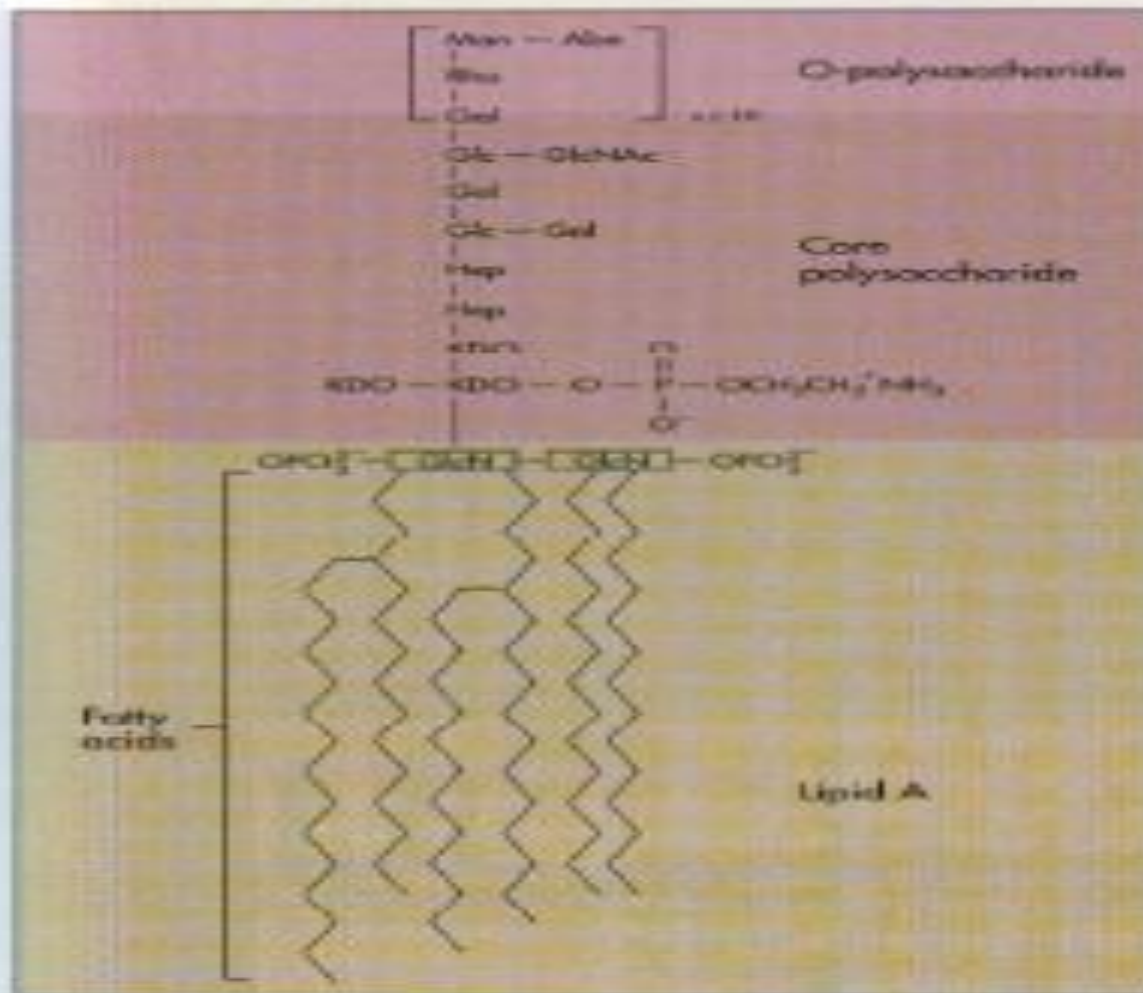


### Assembly of Gram-negative Bacterial Cell Walls

**A, Peptidoglycan Assembly in Gram-negative Bacteria.** Cell wall precursors are assembled from the cytoplasm in the cytoplasmic membrane. They are translocated to the outside of the cytoplasmic membrane by  $C_{55}$ -carrier lipid (bactoprenol) and linked together to form nascent peptidoglycan by penicillin-binding proteins (PBPs). The nascent peptidoglycan becomes incorporated into the cell wall layer by transpeptidation performed by other PBPs.



**B, Outer Membrane Assembly in Gram-negative Bacteria.** The Gram-negative bacterial outer membrane is assembled by three different mechanisms: (1) Phospholipids (tan) are synthesized in the cytoplasmic membrane. (2) Lipopolysaccharide (LPS) (red) is assembled by enzymes in the cytoplasmic membrane and (3) bactoprenin, which translocates some of the growing polysaccharide chains to the outside of the membrane (as in peptidoglycan assembly). The Lipid A-core becomes attached to these translocated polysaccharide chains to form LPS. Proteins are synthesized on membrane-bound ribosomes and are secreted through the cytoplasmic membrane. The mechanism of movement of phospholipids, lipopolysaccharides and proteins from the cytoplasmic membrane to the outer membrane is not understood.



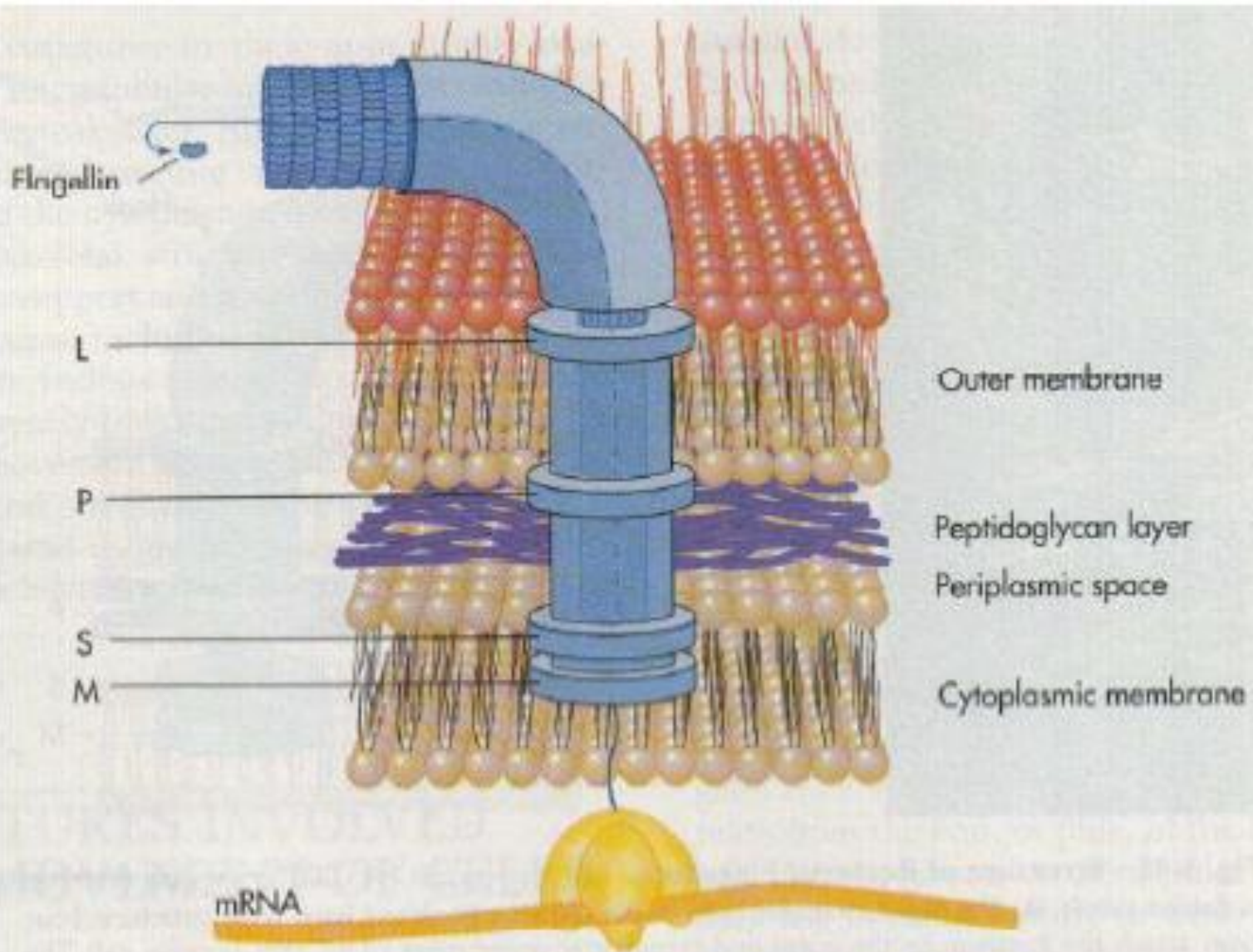
**Fig. 3-22 Structure of Lipopolysaccharide of Gram-negative Bacterial Cell Wall.** Lipopolysaccharide (LPS) is composed of (1) an O-polysaccharide portion that is a long repeating sequence of sugars, which is strain specific and usually contains abequeose (Abe), mannose (Man), rhamnose (Rha), and galactose (Gal); (2) a core polysaccharide that typically contains N-acetylglucosamine (GlcNAc) or ethanolamine (not shown), glucose (Glc), galactose (Gal), 2-keto-3-deoxyoctulosonic acid (KDO) and L-glycero-D-mannoheptose (Hep); and (3) the lipid A portion that contains repeat polysaccharide units containing phosphorylated glucosamine (GlcN) that is esterified to fatty acids, some of which are hydroxylated fatty acids.



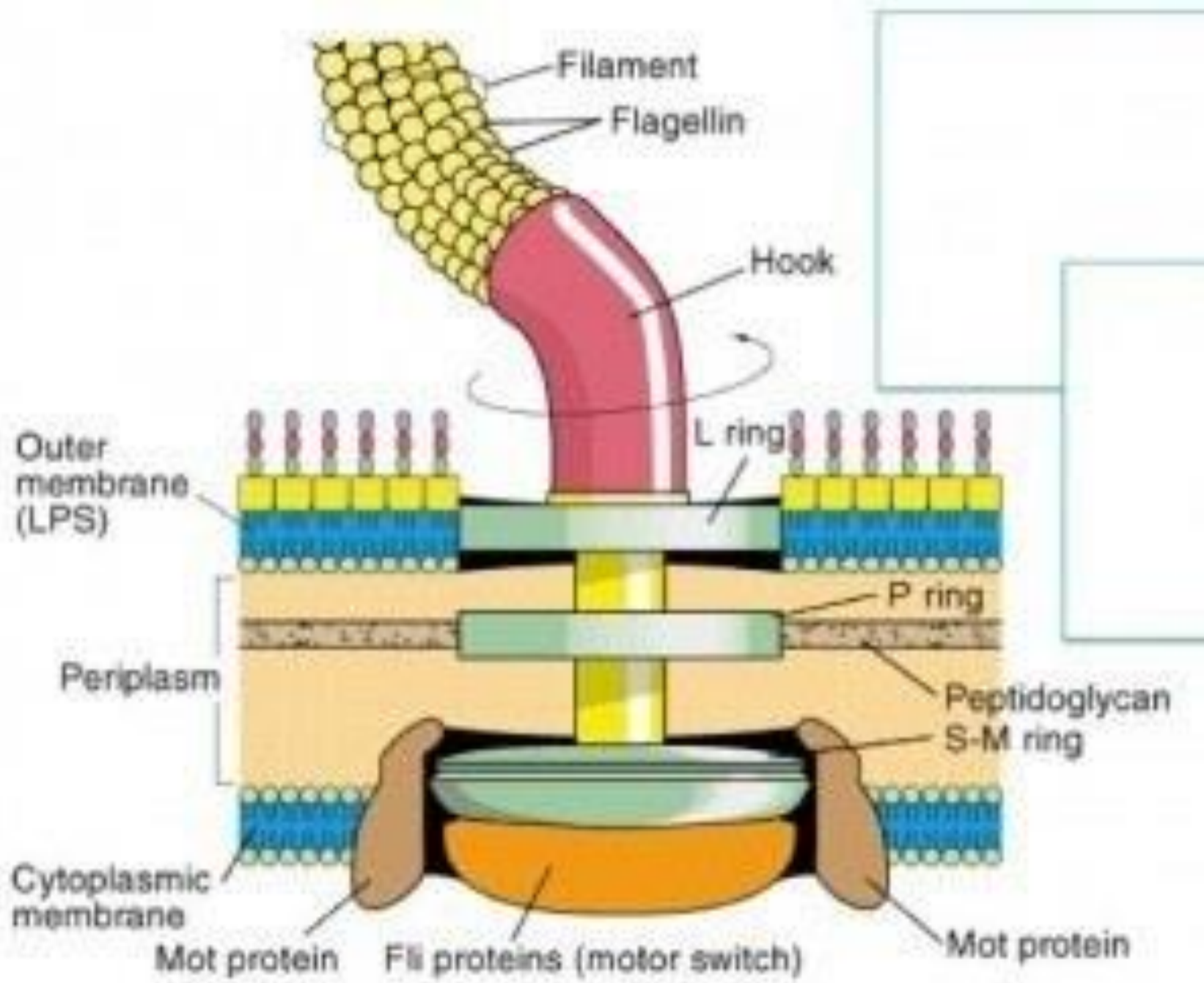
# Flagella Synthesis

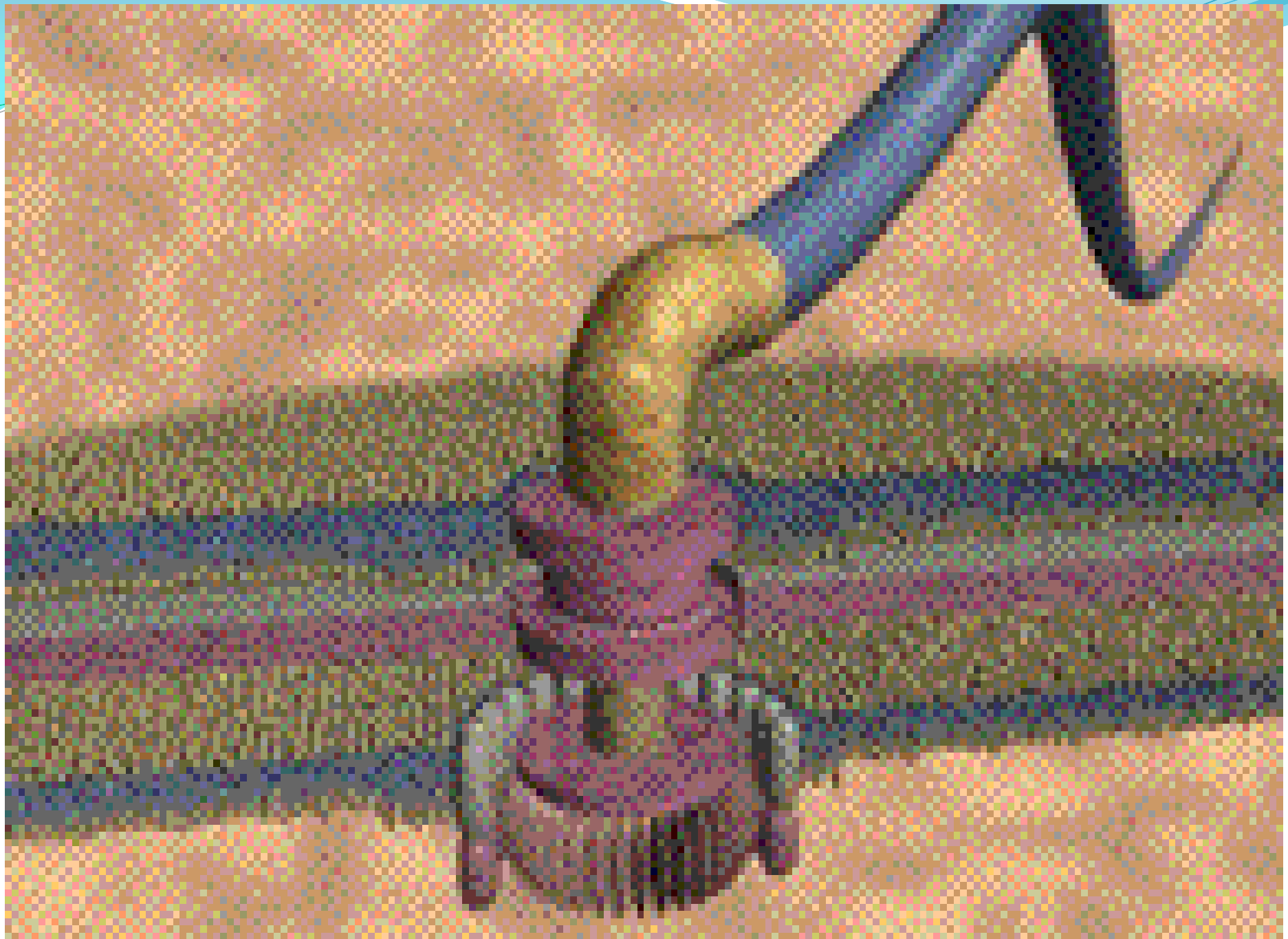
## • Flagella Assembly:

- The synthesis involves over 40 genes.
- Comprised of a basal body, a hook, and a tail consisting of many protein sub-units called “Flagellin”.
- The basal body consists of a number of rings
  - 2 in gram positive.
  - 4 in gram negative.
- A rod that the flagella attach to.
- Bottom-up process:
- Starting with the M (motor).
- S (stator) ring (both are inserted into the cytoplasmic membrane).
- The P, then L rings- acts like bearings (G-).
- The hook is formed and Flagellin proteins are added to the tail of the flagella (can rotate 1200 rpm).



**Fig. 3-46 Assembly of Bacterial Flagellum.** The assembly of the filament of the bacterial flagellum occurs by exporting flagellin protein units through a central hollow core. The flagellin is self-assembled at the tip of the growing filament. Only after the filament reaches its full length and the assembly process is complete does the flagellum become functional.





# Pili and Fimbriae

- Their assembly is less known.
- **Pili:**
  - Straight protein rods.
  - Involved in DNA transfer between cells.
  - Consist of of pilin proteins.
  - Hollow tube is too small for the pilin protein to be added.
  - Pilin proteins are synthesized in the cytoplasm.
  - Co-translationally translocated across the membrane.
- **Fimbriae:**
  - Special proteins attached to the end of the rods called “adhesions”.
  - Aid in the attachment process.

# QUESTIONS??

