Bacterial Biochemistry 1&2

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Why study the bacterial biochemistry

- To detect and identify-Outbreaks
- To identify new drug target sites
- To identify new molecules (drugs)
- To combat antimicrobial resistance (AMR)

(Outline)

- Introduction of Medical Bacterial
- Bacterial Classification
- Bacterial Structure

(Bacterial Classification)

(Phenotypic classification):

- Microscopic morphology
- Macroscopic morphology
- Biotyping
- Serotyping
- Antibiogram patterns
- Phage typing

II
(Bacterial Classification)

(Analytic):

- Cell wall fatty-acid analysis
- Whole cell lipid analysis
- Whole cell protein analysis
- Multifocus locus enzyme electrophoresis

III
(Bacterial Classification)

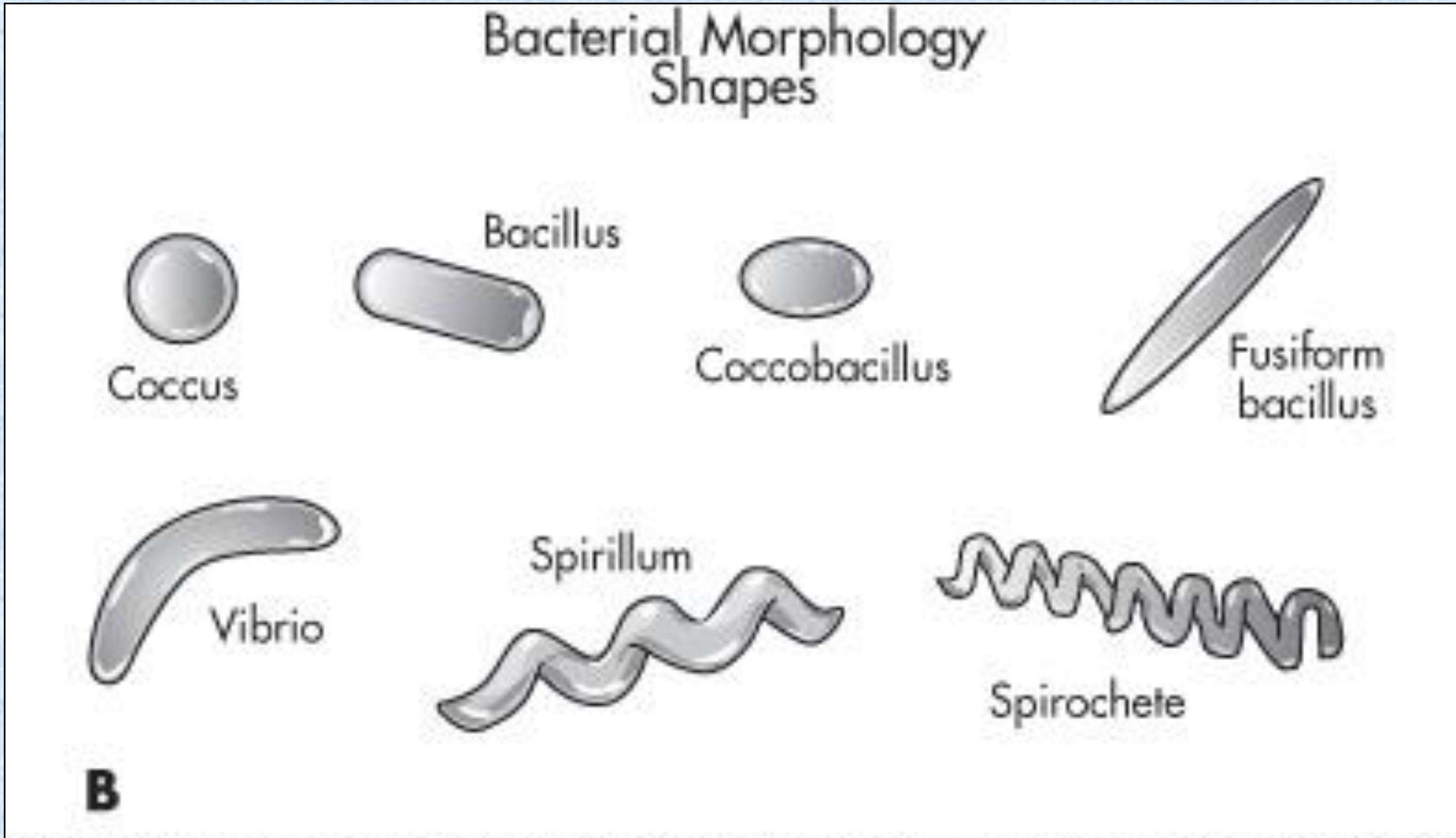
(Genotypic):

- Guanine plus cytosine ratio
- DNA hybridization
- Nucleic acid analysis
- Plasmid analysis
- Chromosomal DNA fragment analysis

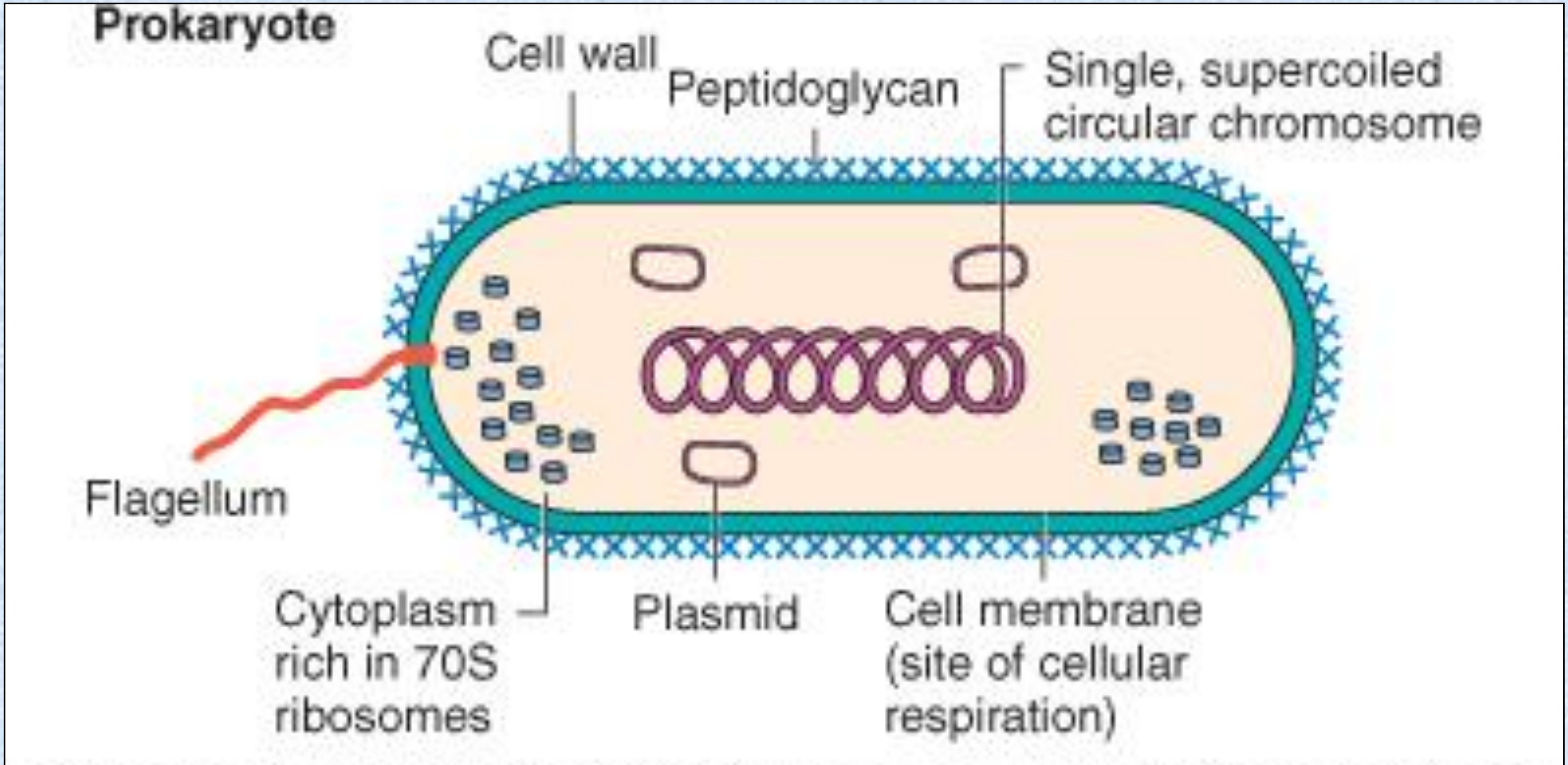
Differences Among Prokaryotes:

Bacteria have different shapes.

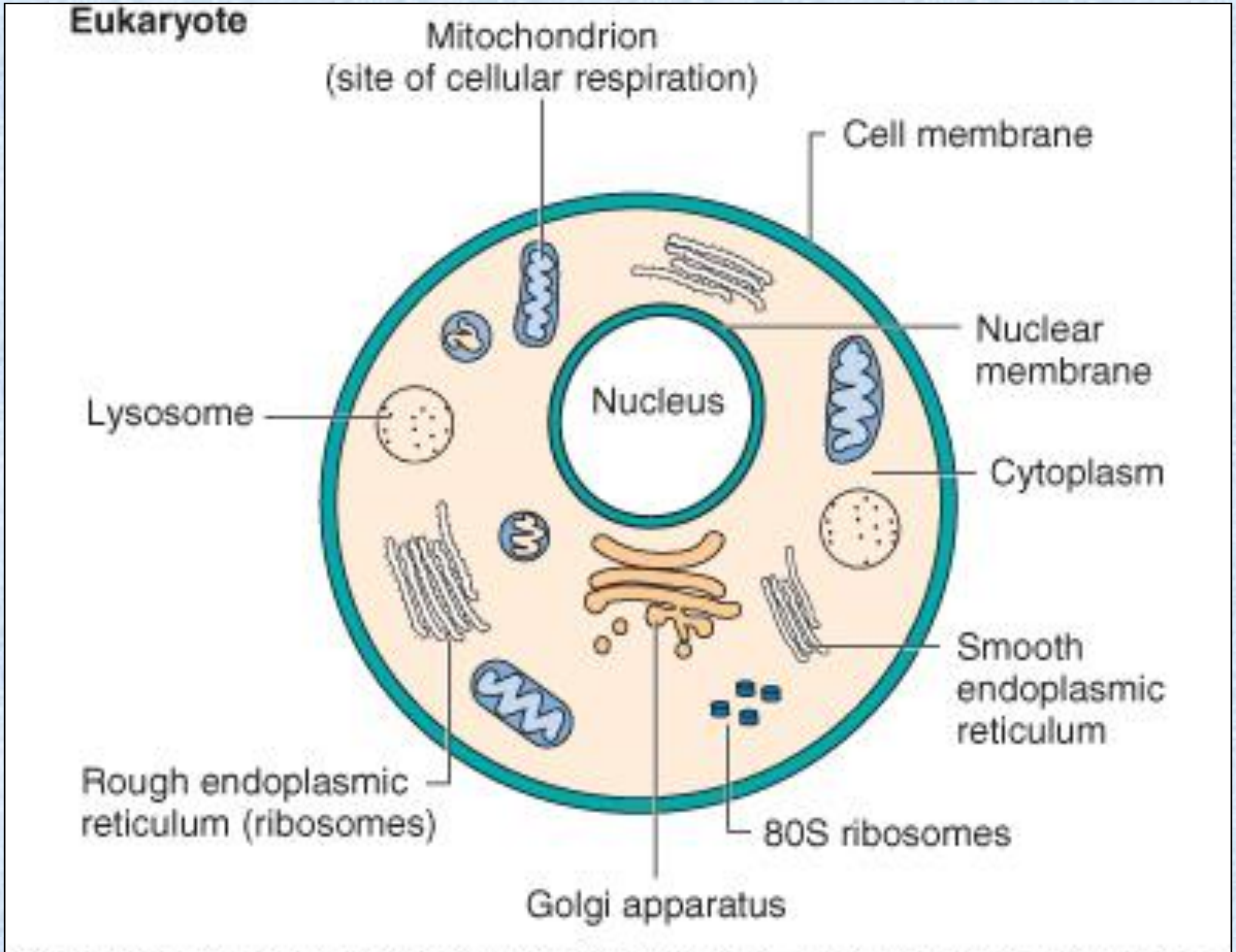
- ♥ **Coccus:**
  - **spherical bacterium**
  - **staphylococcus; grapelike clusters,**
  - **diplococcus; two cells together**
- ♥ **Rod-shaped bacterium: Bacillus**
  - **Escherichia coli : bacillus.**
- ♥ **Spirillum: Snakelike treponeme some bacteria**


Bacterial Morphology
Shapes
Coccus
Bacillus
Coccobacillus
Fusiform
bacillus
Vibrio
Spirillum
Spirochete
B

Prokaryote


Prokaryote
Cell wall
Peptidoglycan
Single, supercoiled circular chromosome
Flagellum
Cytoplasm rich in 70S ribosomes
Plasmid
Cell membrane (site of cellular respiration)

Eukaryote



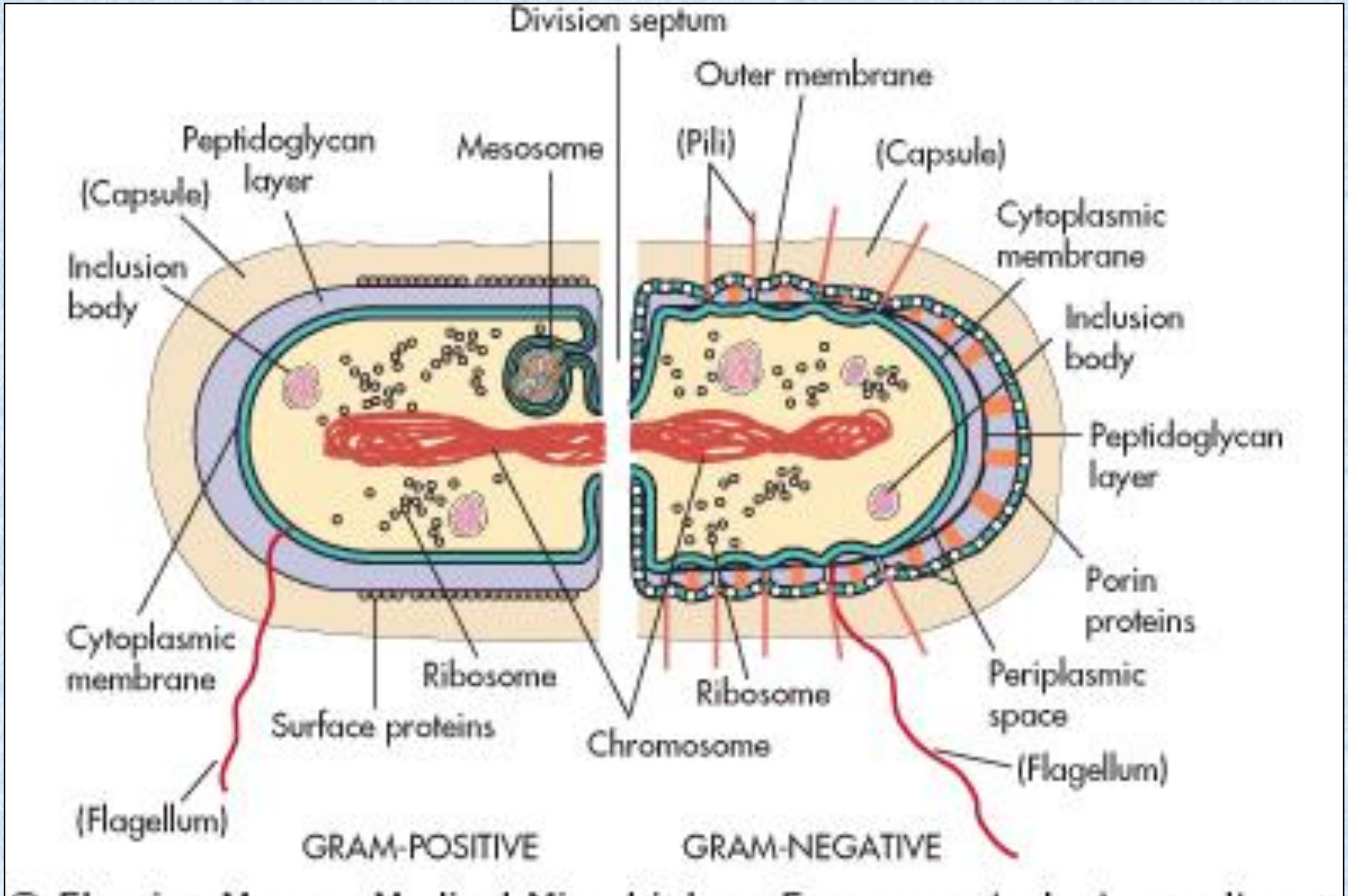
Eukaryote
Mitochondrion
(site of cellular respiration)
Cell membrane
Nuclear
membrane
Nucleus
Lysosome
Cytoplasm
Smooth
endoplasmic
reticulum
Rough endoplasmic
reticulum (ribosomes)
80S ribosomes
Golgi apparatus

Eukaryote vs. Prokaryote

	Eukaryote	Prokaryote
Major groups	Fungi, plants, animals	bacteria
Size	> 5 µm	0.5-3.0 µm
Nuclear structures Nucleus Chromosomes	 Classic membrane Strands of DNA (Diploid)	 No nuclear membrane Circular DNA (Haploid)
Cytoplasmic structures Mito, Golgi, ER Respiration	 + Via mitochondria	 – Via cytoplasmic membrane

Bacterial Ultra-structure

Gram-positive vs. Gram-negative bacteria



Cytoplasmic Structures-I

1. Gram-positive vs Gram-negative bacteria:
   - Similar Internal structures
   - Different External structures.

2. The cytoplasm of the bacteria contains
   - DNA chromosome, mRNA, ribosomes, proteins, and metabolites.

3. The bacterial chromosome
   - A single, double-stranded circle in a discrete area known as the nucleoid.
   - No histones

Cytoplasmic Structures-II

4. Plasmids:
   - Smaller, circular, extrachromosomal DNAs
   - Most commonly found in gram-negative bacteria
   - Not essential for cellular survival
   - Provide a selective advantage:
     many confer resistance to one or more antibiotics.

Cytoplasmic Membrane-I

1. The cytoplasmic membrane
   - A "**lipid bilayer structure**" similar to that of the eukaryotic membranes
   - Contains no steroids (e.g., cholesterol); mycoplasmas are the exception.

2. Involves in **electron transport** and **energy production**, which are normally achieved in the mitochondria.

Cytoplasmic Membrane-II

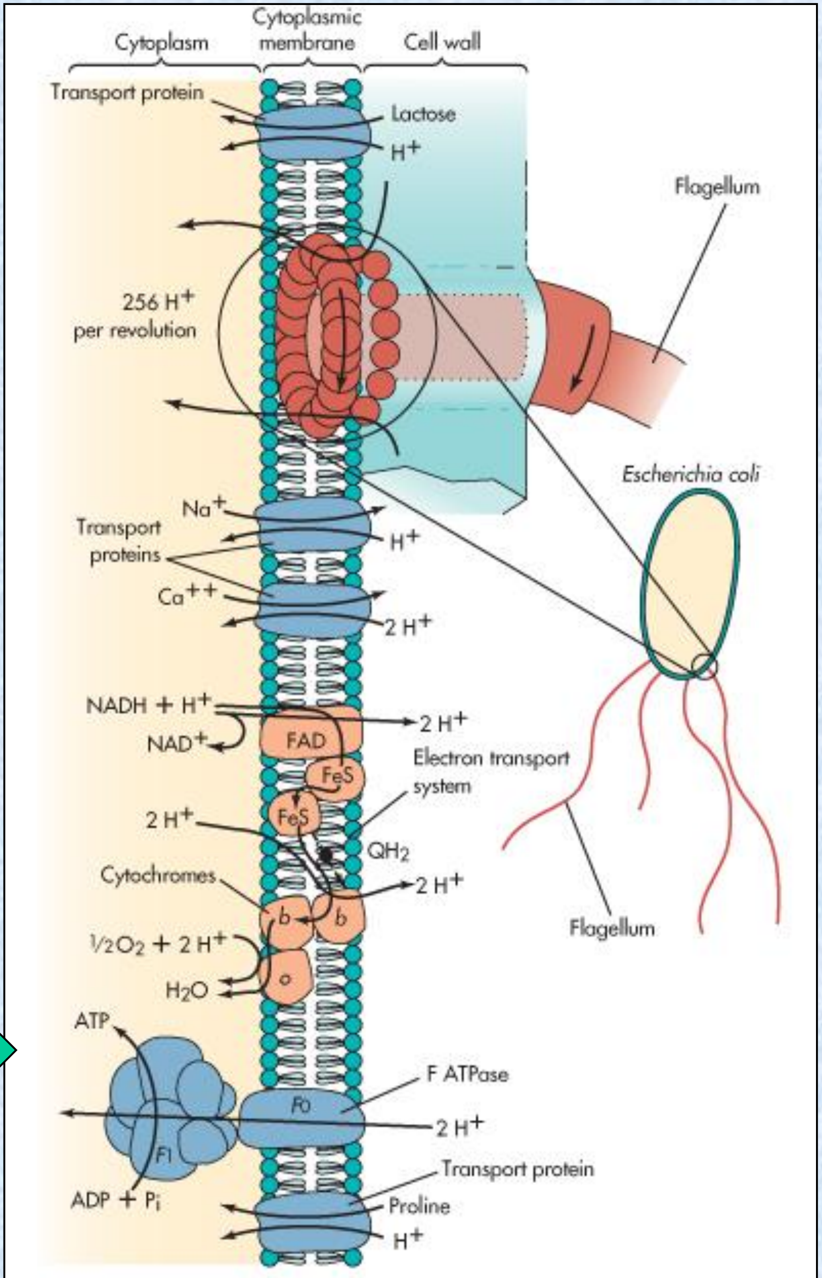
3. Contains transport proteins => exchange metabolites ion pumps => a membrane potential

4. Mesosome
   - A coiled cytoplasmic membrane
   - Acts as an anchor to bind and pull apart daughter chromosomes during cell division.

Bacterial Cytoplasmic Membrane

ATP production machinery

Potential drug targets



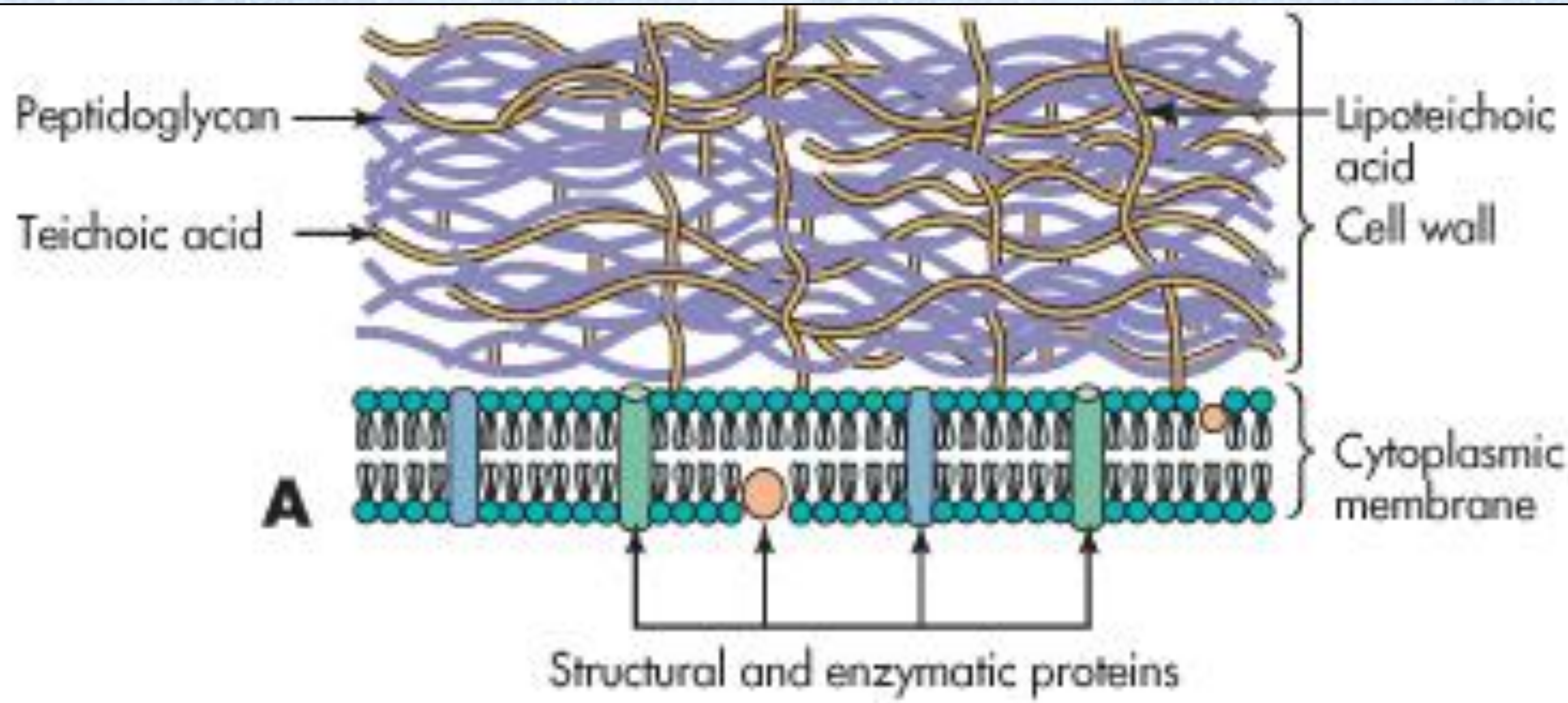
Cell Wall

1. The structure components and functions of the cell wall distinguish gram-positive from gram-negative bacteria.

(A). Gram positive bacteria:

(1). Peptidoglycan (murein, mucopeptide)
(2). Teichoic acid & Lipoteichoic acid
(3). Polysaccharides

(Gram-positive bacterial cell wall)



Functions of Peptidoglycan

1. Essential for the structure, for replication, and for survival in the hostile conditions.
2. Interfere with phagocytosis and has pyrogenic activity (induces fever).
3. Degraded by lysozyme, an enzyme in human tears and mucus

Mouth and eye infections??

Teichoic & Lipoteichoic acid

1. Water-soluble polymers, containing ribitol or glycerol residues joined through phosphodiester linkages.
2. Constitute major surface Ag of those gram-positive species => Bacterial Serotyping
3. Promote attachment to other bacteria as well as to specific receptors on mammalian cell surfaces (adherence).
4. Important factors in virulence, initiate endotoxic-like activities.

Peptidoglycan Synthesis

1. Backbone:
   - N-acetylglucosamine & N-acetylmuramic acid (NAG & NAM)
   - The backbone is the same in all bacterial species.

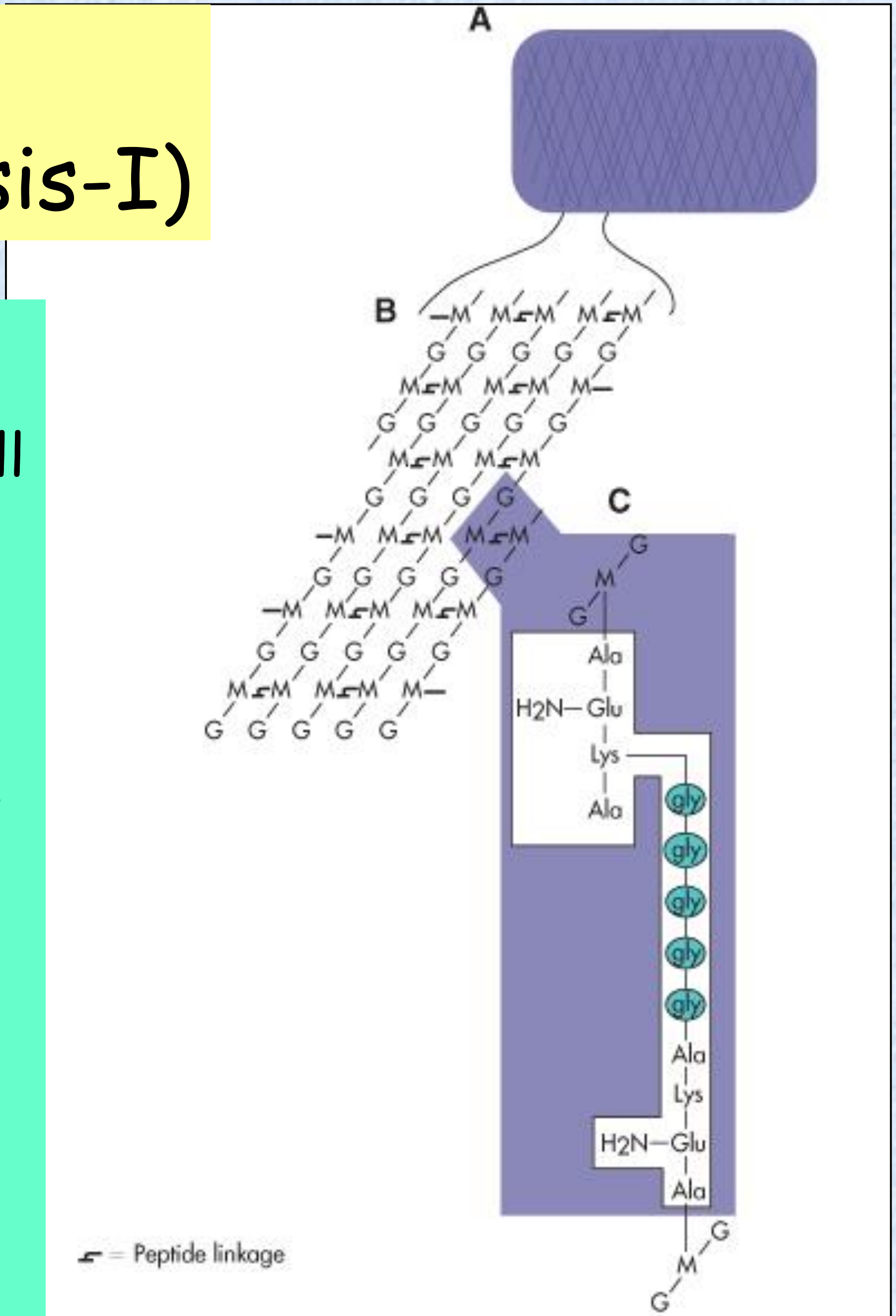
2. Tetrapeptide side chain attach to N-Acetylmuramic acid.

I
(Peptidoglycan Synthesis-I)

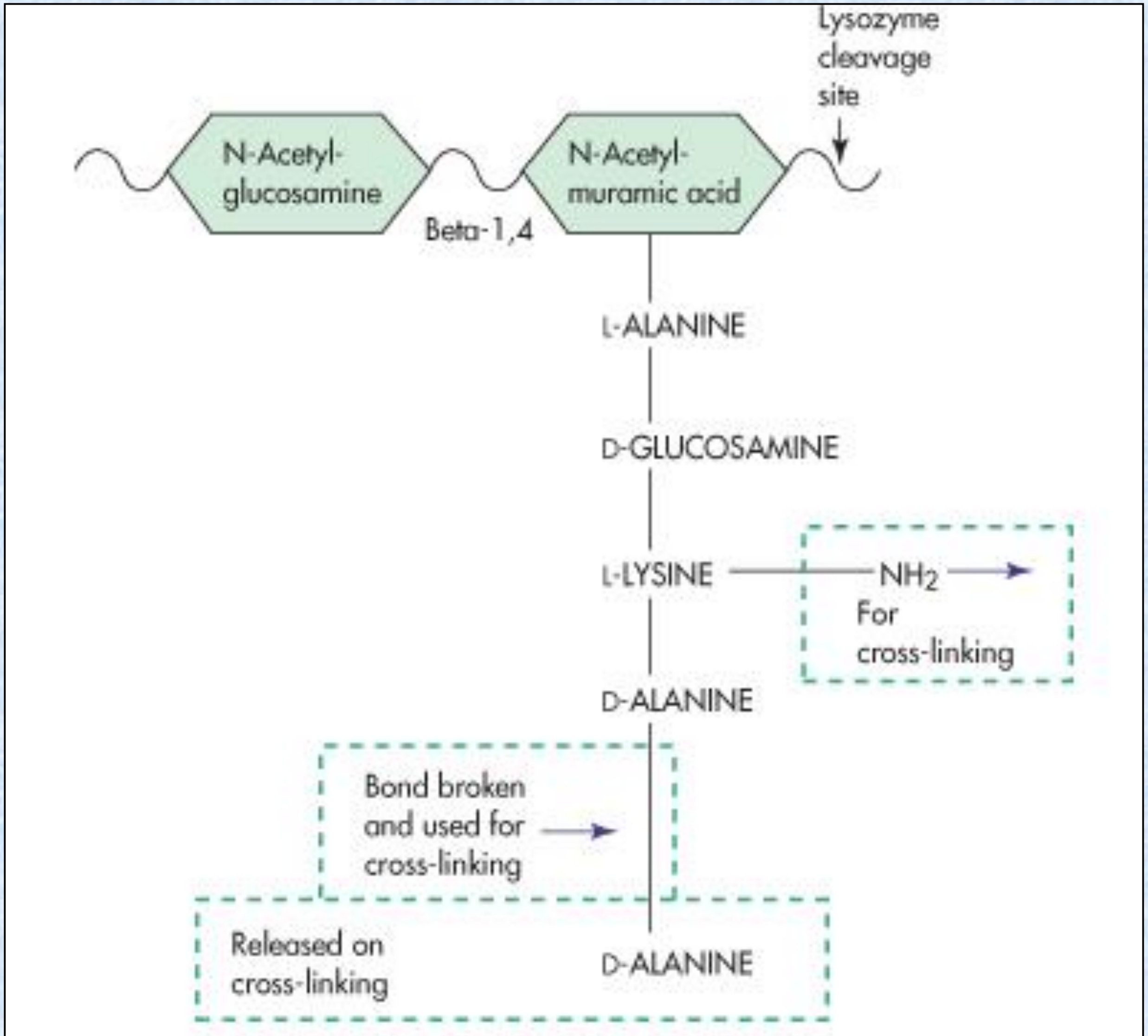
Peptidoglycan

1. A major component of cell wall
2. Forms a "Meshlike layer" consisting:

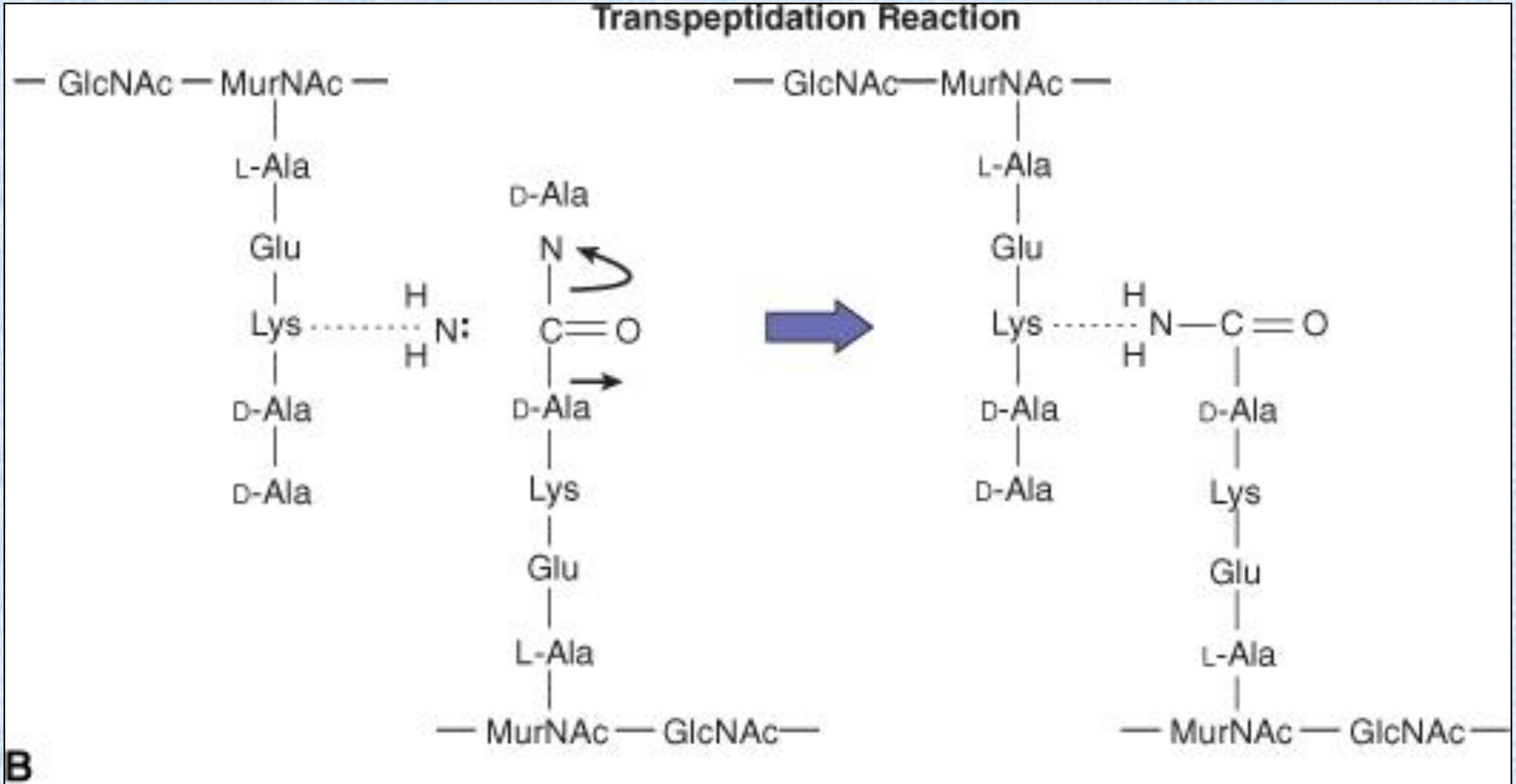
   a polysaccharide polymer cross-linked by Peptide bonds
3. Cross-linking reaction is mediated by:
   - **Transpeptidases**
   - **DD-carboxypeptidases**
   - **Targets of Penicillin**



II
(Peptidoglycan Synthesis-II)



III
(Peptidoglycan synthesis-III)



III
(Peptidoglycan synthesis-III)

Assembly of the peptidoglycan:

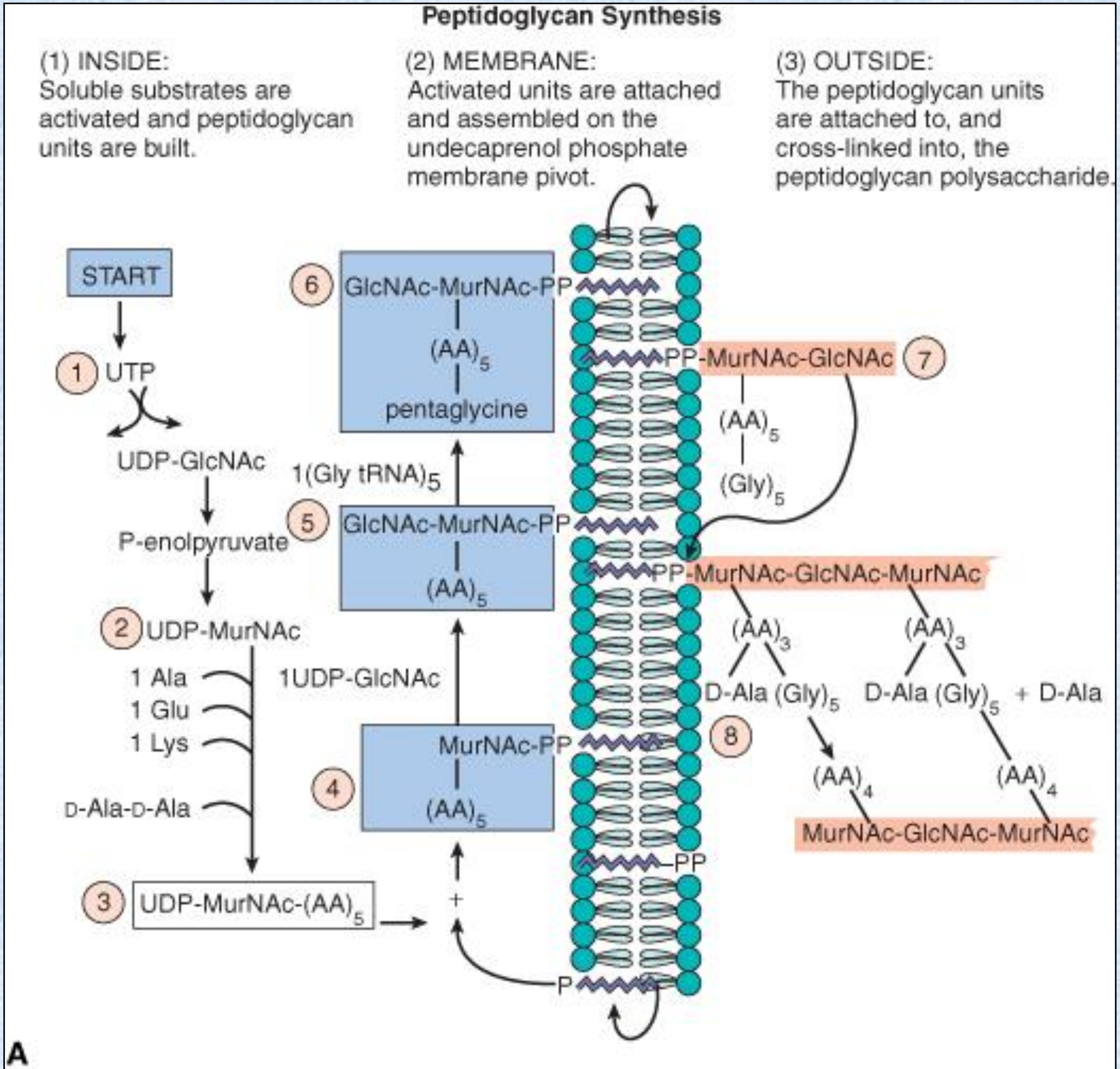
This is a critical step for bacterial survival. The sequence of events is outlined below.

i. Synthesis begins with formation of a water soluble, nucleotide-linked precursor (N-acetylmuramic acid - NAM) also carrying a pentapeptide in the cytoplasm.

ii. The precursor is then linked to a lipid-like carrier in the cell membrane (bactoprenol) and N-acetyl glucosamine (NAG) is added to the NAM. This complex is mobilized across the cytoplasm

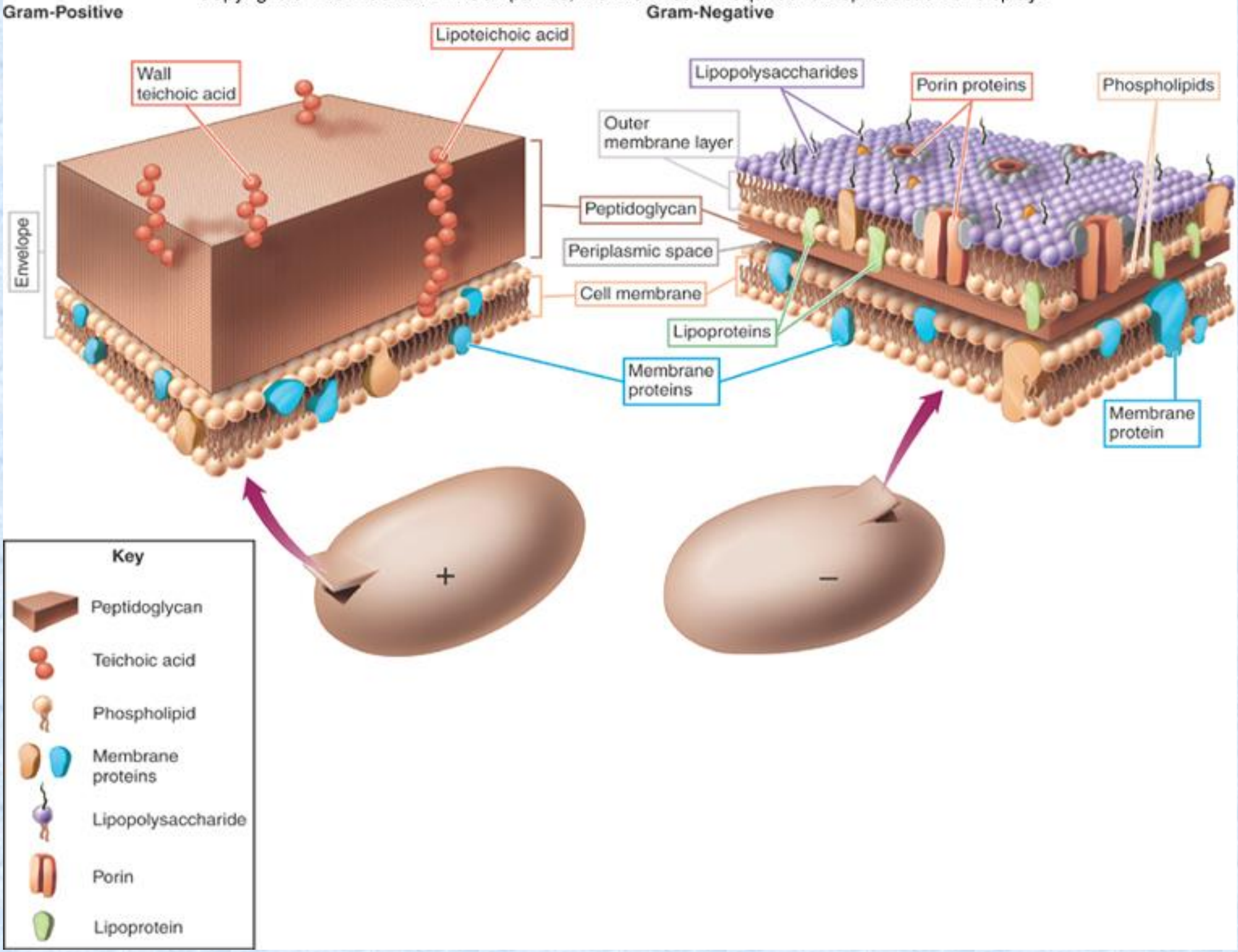
iii. The disaccharide subunit (NAM-NAG) is then added to the end of a glycan strand.

iv. The final step is the transpeptidation reaction catalyzed by a transpeptidase enzyme (also called penicillin binding proteins) that crosslinks the growing strand with others.

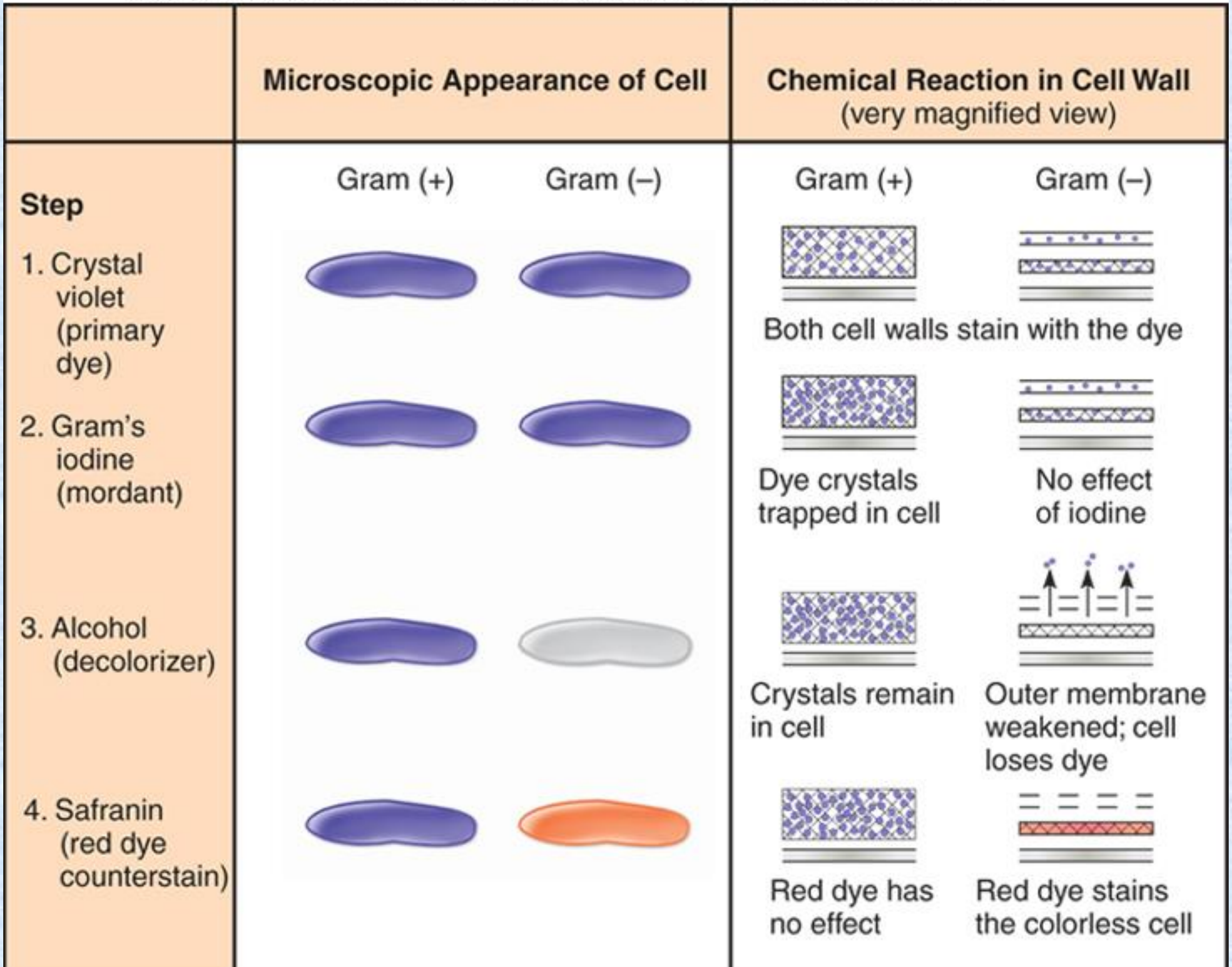

Peptidoglycan Synthesis
(1) INSIDE:
Soluble substrates are activated and peptidoglycan units are built.
(2) MEMBRANE:
Activated units are attached and assembled on the undecaprenol phosphate membrane pivot.
(3) OUTSIDE:
The peptidoglycan units are attached to, and cross-linked into, the peptidoglycan polysaccharide.
START
1 UTP
UDP-GlcNAc
P-enolpyruvate
2 UDP-MurNAc
1 Ala
1 Glu
1 Lys
D-Ala-D-Ala
3 UDP-MurNAc-(AA)5
4 MurNAc-PP
(AA)5
1UDP-GlcNAc
5 GlcNAc-MurNAc-PP
(AA)5
1(Gly tRNA)5
6 GlcNAc-MurNAc-PP
(AA)5
pentaglycine
PP-MurNAc-GlcNAc 7
(AA)5
(Gly)5
PP-MurNAc-GlcNAc-MurNAc
(AA)3
D-Ala (Gly)5
(AA)3
D-Ala (Gly)5 + D-Ala
8
(AA)4
(AA)4
MurNAc-GlcNAc-MurNAc
PP
P
A

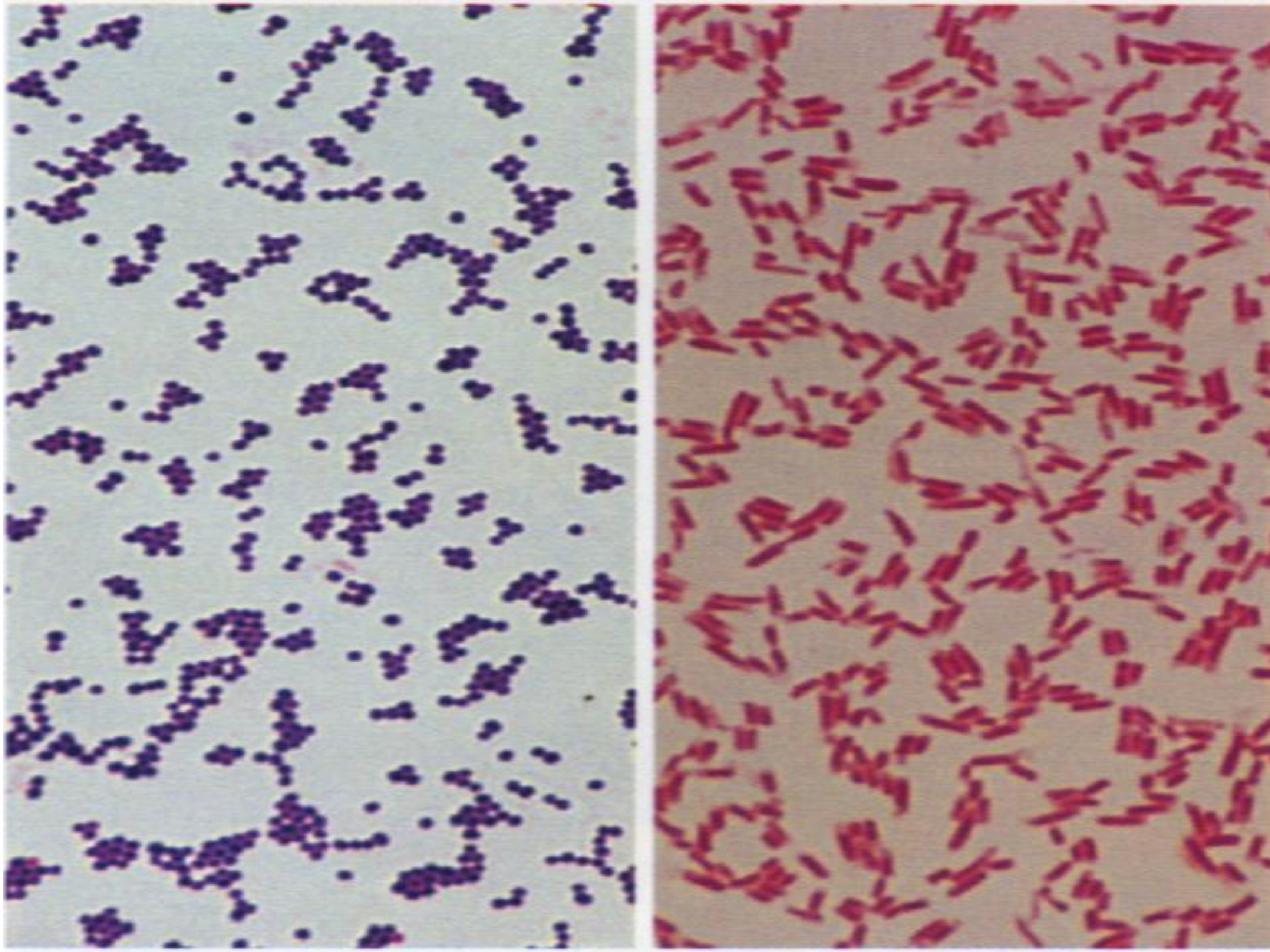
Gram stain

- Gram stain is a powerful, easy test that allows clinicians to distinguish between the two major classes of bacteria and to initiate therapy.
- Bacteria→ heat-fixed →stained with Crystal violet → this stain is precipitated with Gram's iodine → washing with the acetone- or alcohol-based decolorizer →A counterstain, safranin, red
- Gram-positive bacteria, Purple, the stain gets trapped in a thick, cross-linked, meshlike structure.

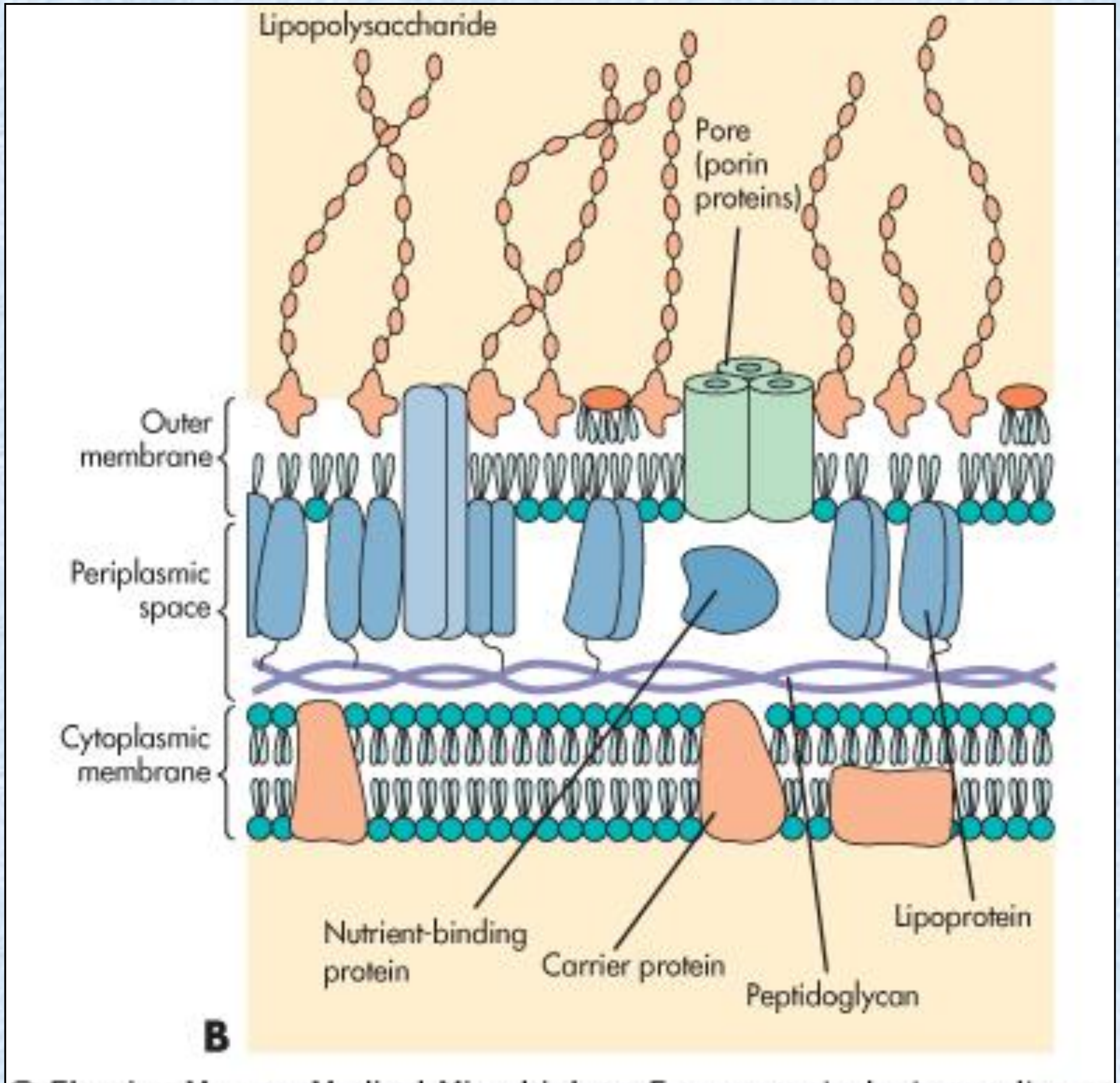

Gram-Positive
Gram-Negative
Lipoteichoic acid
Wall teichoic acid
Lipopolysaccharides
Porin proteins
Phospholipids
Outer membrane layer
Envelope
Peptidoglycan
Periplasmic space
Cell membrane
Lipoproteins
Membrane proteins
Membrane protein
+
–
Key
Peptidoglycan
Teichoic acid
Phospholipid
Membrane proteins
Lipopolysaccharide
Porin
Lipoprotein

Gram stain


Microscopic Appearance of Cell
Chemical Reaction in Cell Wall (very magnified view)
Step
Gram (+)
Gram (–)
Gram (+)
Gram (–)
1. Crystal violet (primary dye)
Both cell walls stain with the dye
2. Gram's iodine (mordant)
Dye crystals trapped in cell
No effect of iodine
3. Alcohol (decolorizer)
Crystals remain in cell
Outer membrane weakened; cell loses dye
4. Safranin (red dye counterstain)
Red dye has no effect
Red dye stains the colorless cell



(Gram-negative bacterial cell wall)



(Gram-negative bacterial cell wall)

1. More complex than gram-positive cell walls.

2. Consists three major parts.
   (1) Outer membrane -Unique
   (2) Periplasmic space
   (3) Cytoplasmic membrane

3. Major Components
   - Lipopolysaccharide (LPS) (Endotoxin)
   - Lipoprotein

Gram (-) bacteria: Outer membrane

1. Unique to Gram-negative bacteria.
   - An "asymmetric bilayer" structure
   - different from any other biologic membrane in the structure of the outer leaflet of the membrane.
2. Maintains the bacterial structure
   a permeability barrier to large molecules (e.g., lysozyme) and hydrophobic molecules.
3. Provides protection from adverse environmental conditions such as the digestive system of the host (important for Enterobacteriaceae organisms).

Gram (-) bacteria: Outer membrane

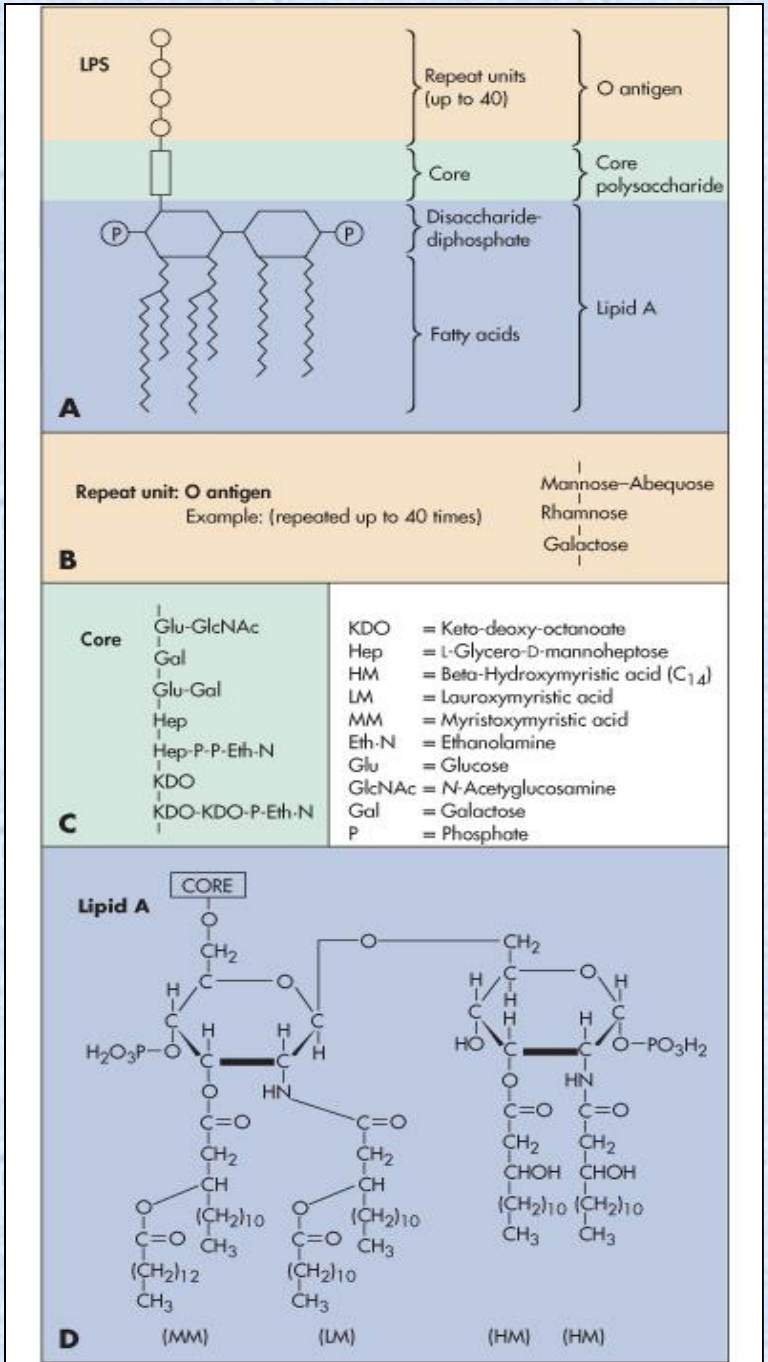
5. The outer membrane is held together by divalent cation (Mg^{+2} and Ca^{+2}) linkages between phosphates on LPS molecules and hydrophobic interactions between the LPS and proteins.

6. These interactions produce a stiff, strong membrane that can be disrupted by antibiotics (e.g., polymyxin) or by the removal of Mg+2 and Ca+2 ions (using ion chelator, eg. EDTA).

Lipopolysaccharide (LPS) (Endotoxin)

1. O antigen
2. Core polysaccharide
3. Lipid A-active component of LPS

1. Induce innate immune response
2. Activate macrophage to secrete cytokines like IL-1, IL-6 & TNF-α



Lipoprotein

1. The outer membrane is connected to the cytoplasmic membrane at adhesion sites and is tied to the peptidoglycan by lipoprotein

2. The lipoprotein is covalently attached to the peptidoglycan and is anchored in the outer membrane.

	Gram +	Gram -
Outer membrane	–	+
Cell wall	Thicker	Thinner
LPS	–	+
Endotoxin	–	+
Teichoic acid	Often present	-
Sporulation	+	–
Lysozyme	Sensitive	Resistant
Penicillin	Sensitive	Resistant
Capsule	Sometimes	Sometimes
Exotoxin	Some	Some

External Structures
Glycocalyx

1. Capsules

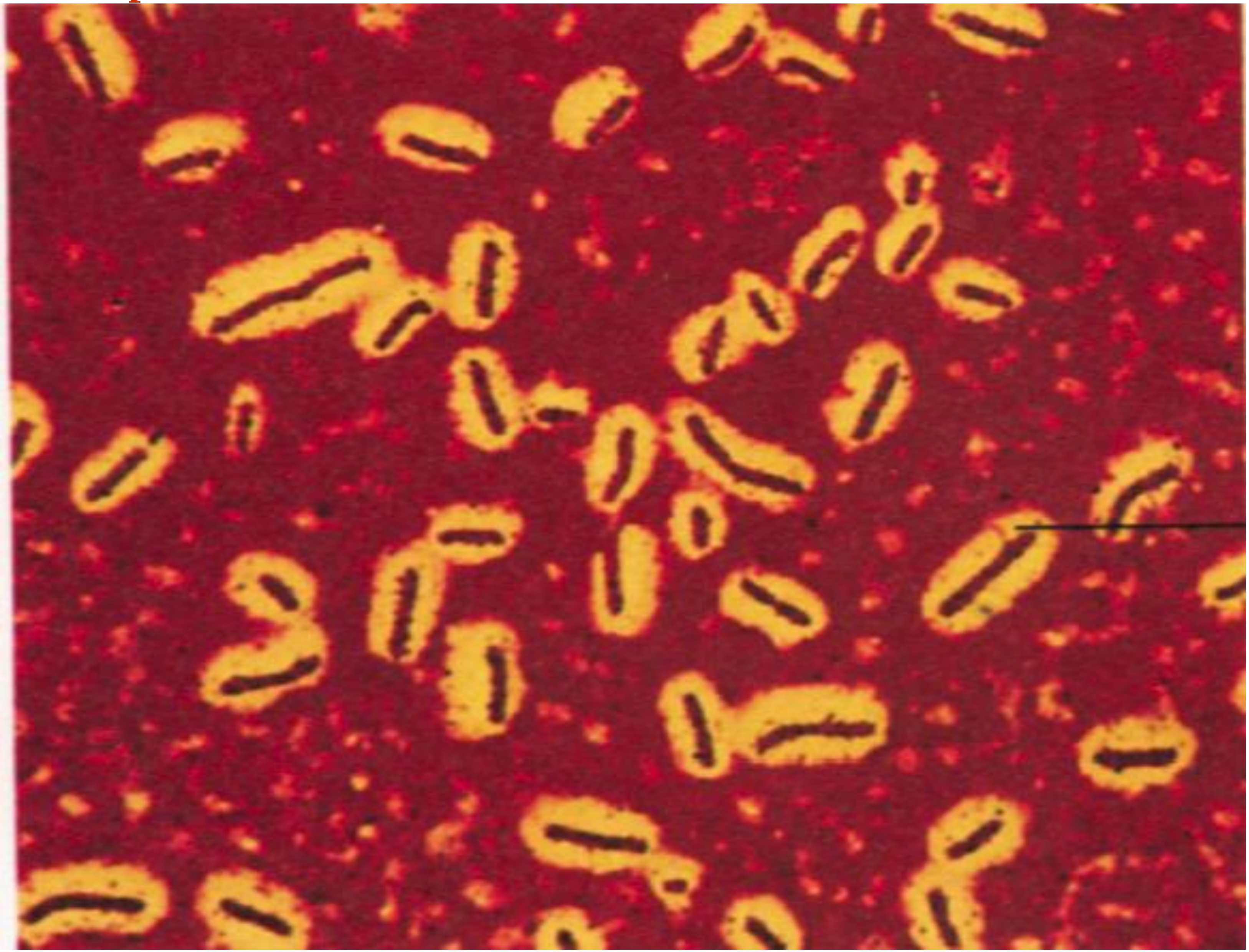
a. Some bacteria are closely surrounded by loose polysaccharide or protein layers called capsules

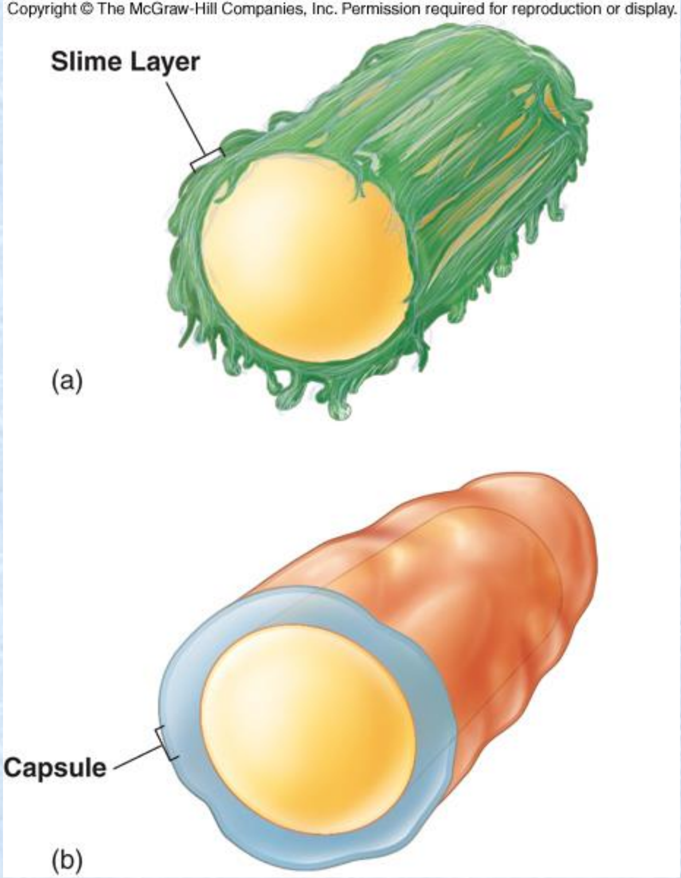
b. Capsules and slimes are unnecessary for the growth of bacteria but are important for survival in the host.

c. The capsule is poorly antigenic and antiphagocytic and is a major virulence factor (e.g., *Streptococcus pneumoniae*).

d. *Bacillus anthracis*: polypeptide

Capsule

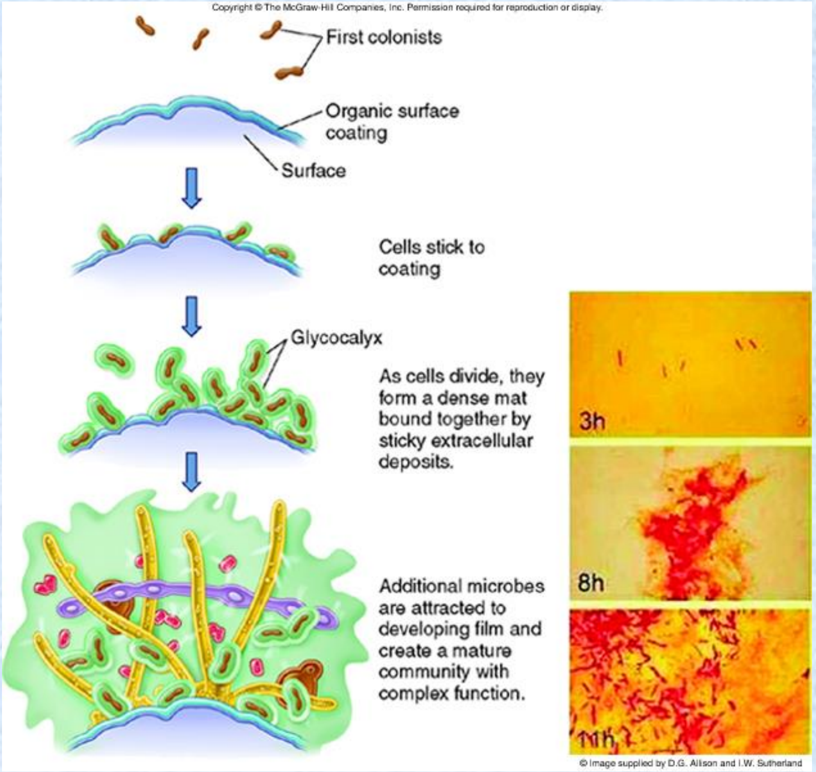




Slime: Use glucose to make plaque
e.g. *Streptococcus mutans*
-Cavities
-Cardiovascular diseases
Mouth and Heart Health are closely related

Capsule:

- Help bacteria adhere
- Prevent phagocytosis
- May aid in triggering endocytosis



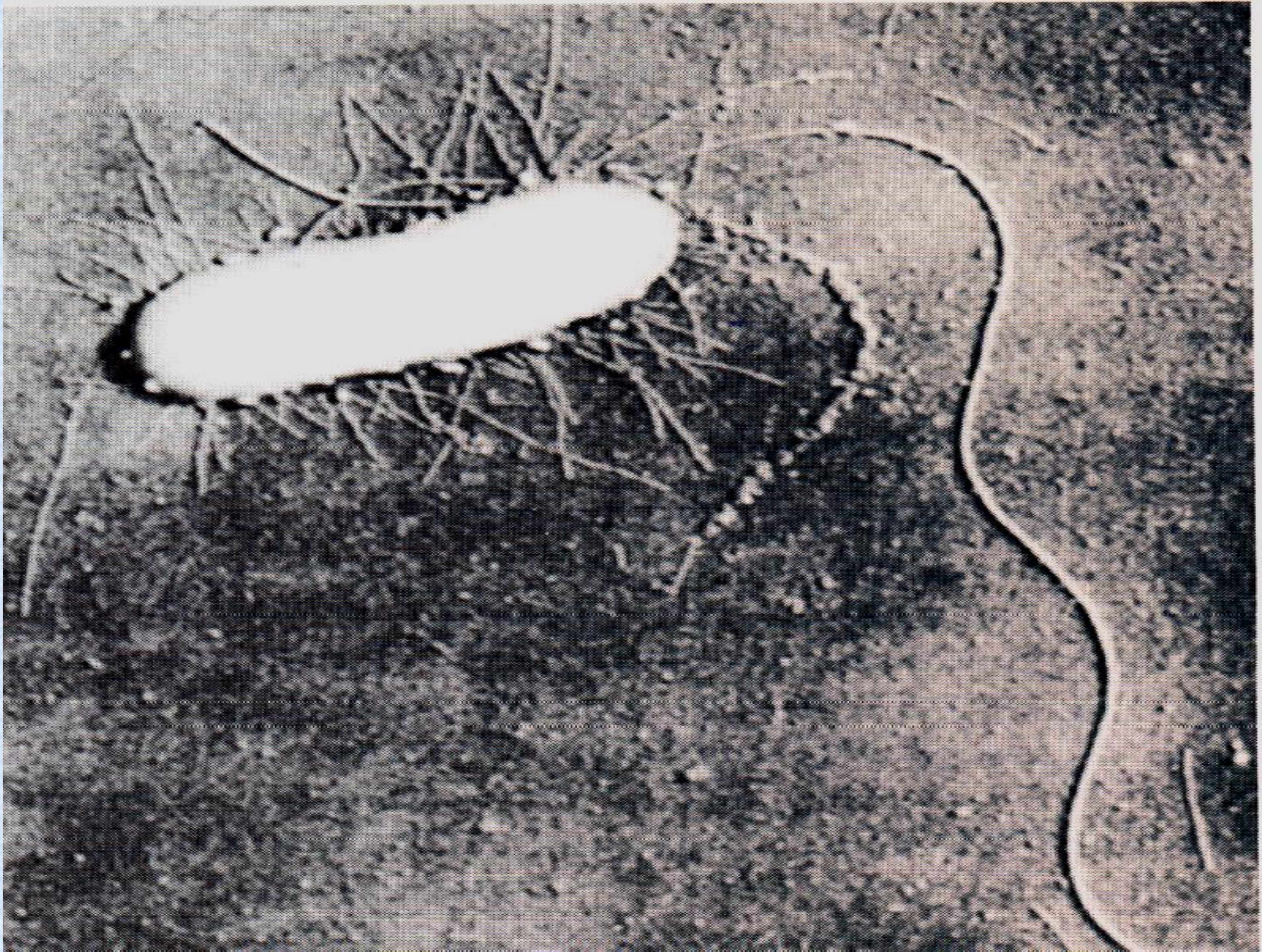
Flagella

1. Ropelike propellers composed of helically coiled protein subunits (flagellin)
   - Anchored in the bacterial membranes through hook and basal body structures.
   - Driven by membrane potential.

2. Flagella provide motility for bacteria, allowing the cell to swim (chemotaxis) toward food and away from poisons.

3. Express Antigenic & strain determinants.

4. Four types of arrangement
   a. Monotrichous: single polar flagellum
   b. Amphitrichous: flagella at both poles.
   c. Lophotrichous: tuft of polar flagella
   d. Peritrichous: Flagella distributed over the entire cell.



Fimbriae (pili): Latin for "fringe"

1. Pili are hairlike structures on the outside of bacteria; they are composed of protein subunits (pilin).

2. Fimbriae can be morphologically distinguished from flagella because they are smaller in diameter (3 to 8 nm versus 15 to 20 nm) and usually are not coiled in structure.

3. They may be as long as 15 to 20 μm, or many times the length of the cell.

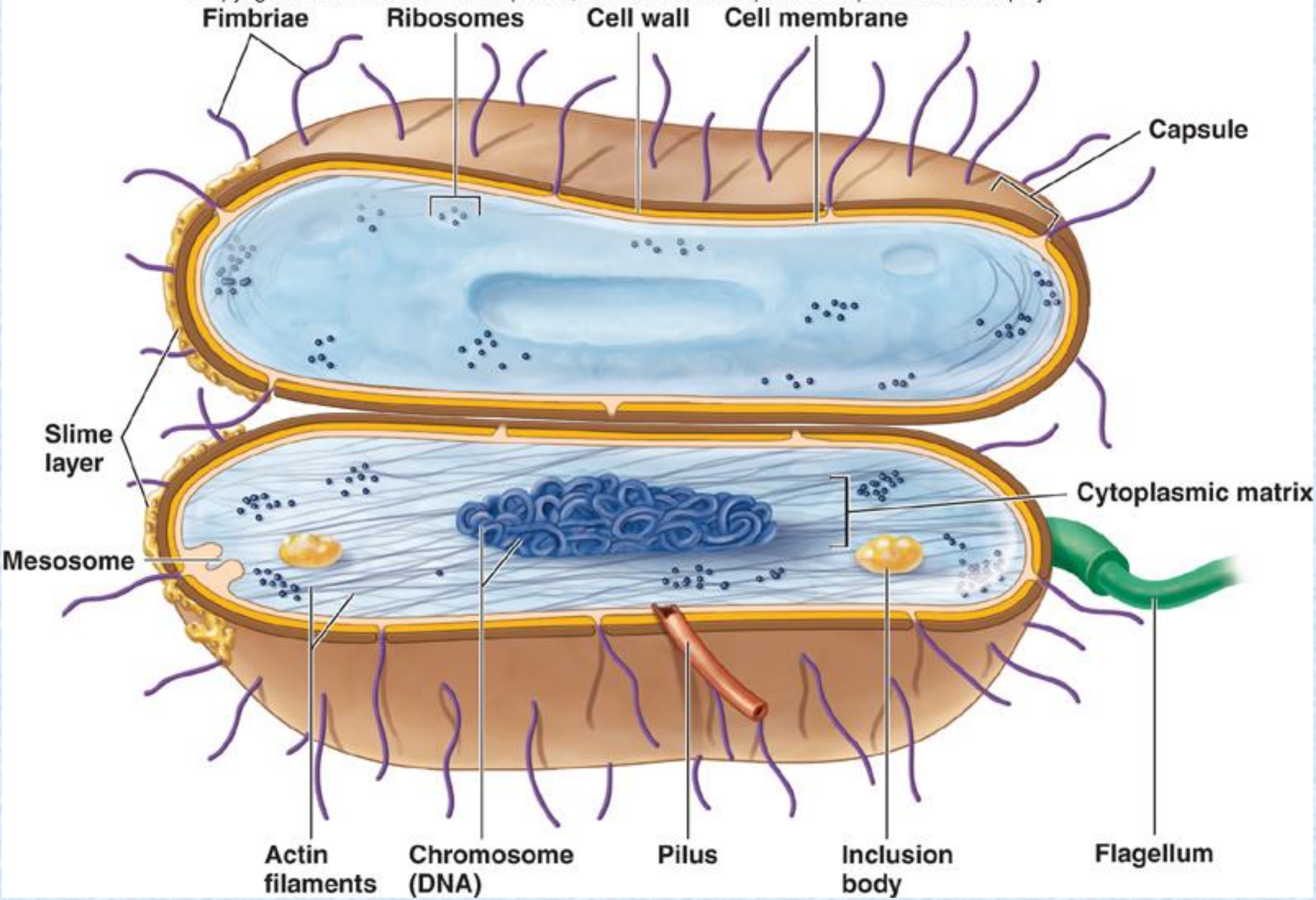
4. Fimbriae promote adherence to other bacteria or to the host (alternative names are adhesins, lectins, evasins, and aggressins).

Fimbriae (pili): Latin for "fringe"

5. As an adherence factor (adhesin), fimbriae are an important virulence factor for *E. coli* colonization and infection of the urinary tract, for *Neisseria gonorrhoeae* and other bacteria.

6. The tips of the fimbriae may contain proteins (lectins) that bind to specific sugars (e.g., mannose).

7. F pili (sex pili) promote the transfer of large segments of bacterial chromosomes between bacteria. These pili are encoded by plasmid (F).


Fimbriae
Ribosomes
Cell wall
Cell membrane
Capsule
Slime layer
Cytoplasmic matrix
Mesosome
Actin filaments
Chromosome (DNA)
Pilus
Inclusion body
Flagellum

Pilus

- Channel for plasmid exchange
- Plasmid provide exchange of DNA, a common route for antibiotic resistance
- Plasmids given or exchanged with others

Spores-I

1. Some gram-positive bacteria, but never gram-negative such as : *Bacillus & Clostridium*

2. Under harsh environmental conditions, such as the loss of a nutritional requirement, these bacteria can convert from a vegetative state to a dormant state, or spore.

3. The location of the spore within a cell is a characteristic of the bacteria and can assist in identification of the bacterium.

Spores-II

4. Dehydrated, multishelled structure that protects and allows the bacteria to exist in "suspended animation ".

5. It contains (a) a complete copy of the chromosome; (b) the bare minimum concentrations of essential proteins and ribosomes; (c) High concentration of Ca^{2+} chelate of DPA (Ca-DPA, dipicolinic acid).
=> DPA appears to be important in spore core dehydration and concomitant spore heat resistance.

6. The structure of the spore protects the genomic DNA from desiccation, intense heat, radiation, and attack by most enzymes and chemical agents.

Spores-III

7. Depletion of specific nutrients (e.g., alanine) from the growth medium triggers a cascade of genetic events (comparable to differentiation) leading to the production of spore.

8. Spore mRNA are transcribed and other mRNA are turned off. Dipicolinic acid(DPA) is produced.

9. Spore structure:

   Core: one copy of DNA and cytoplasmic contents

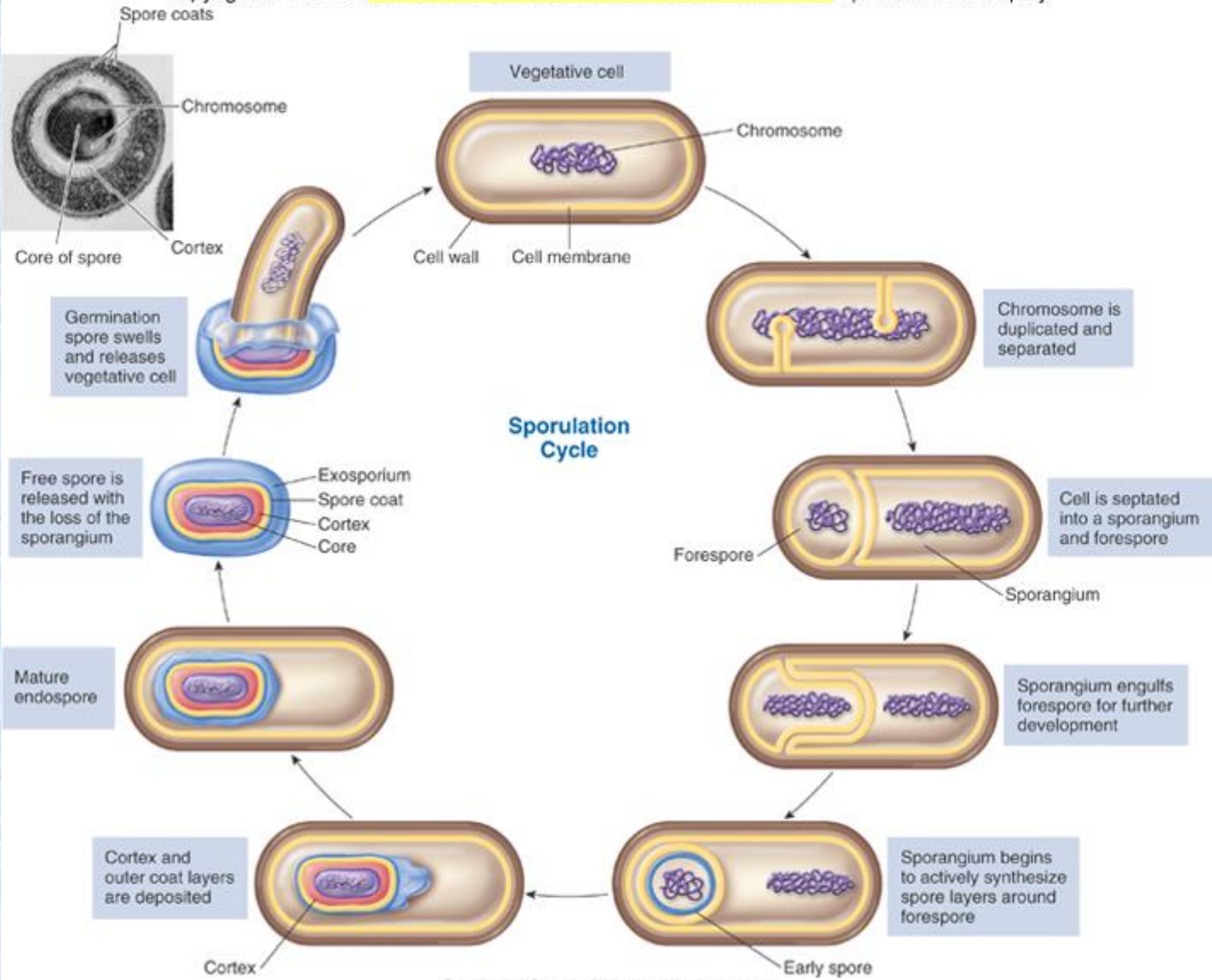
   Inner membrane and Spore wall

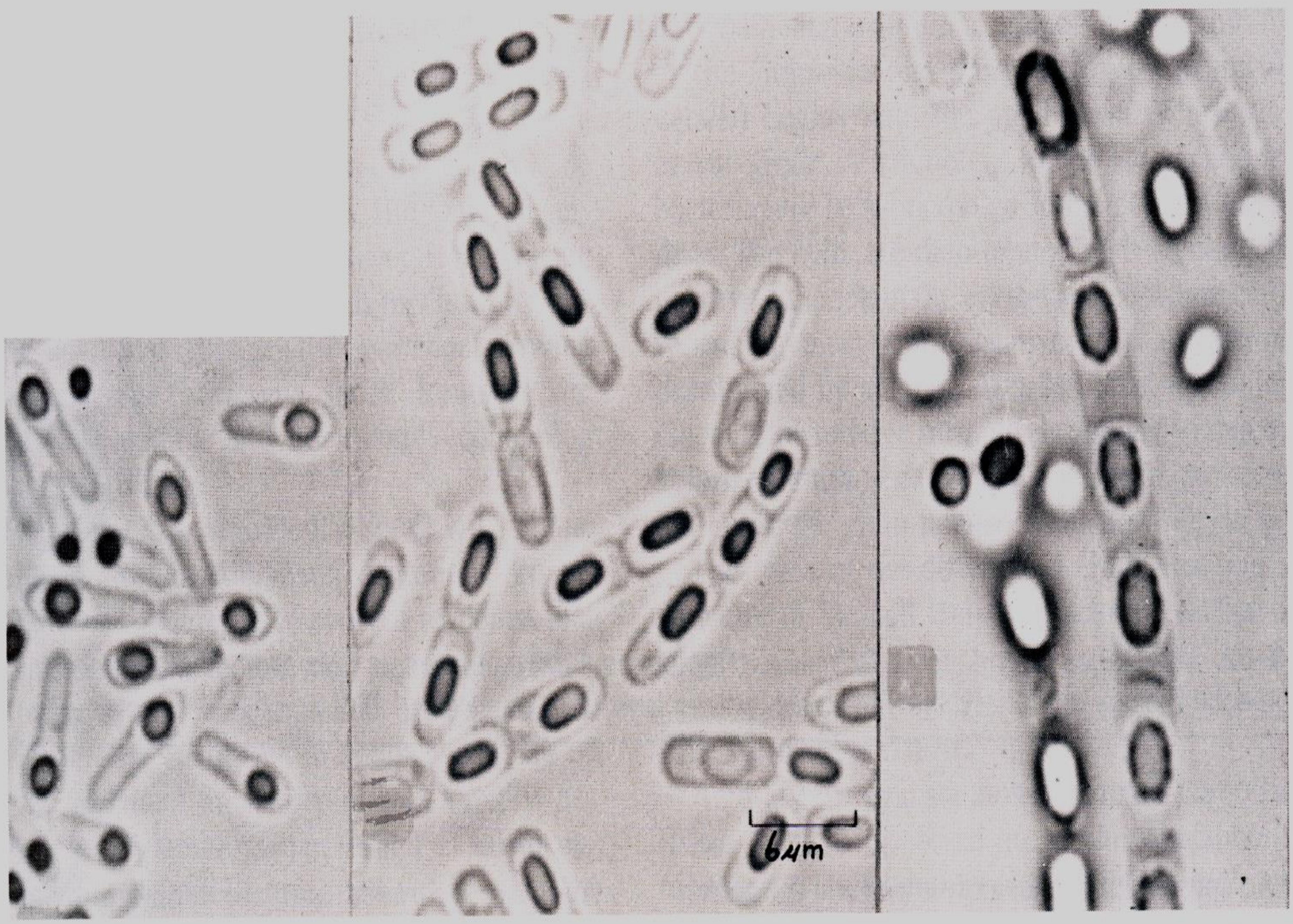
   Cortex: peptidoglycan layer

   Coat: Keratine-like protein which protect the spore.

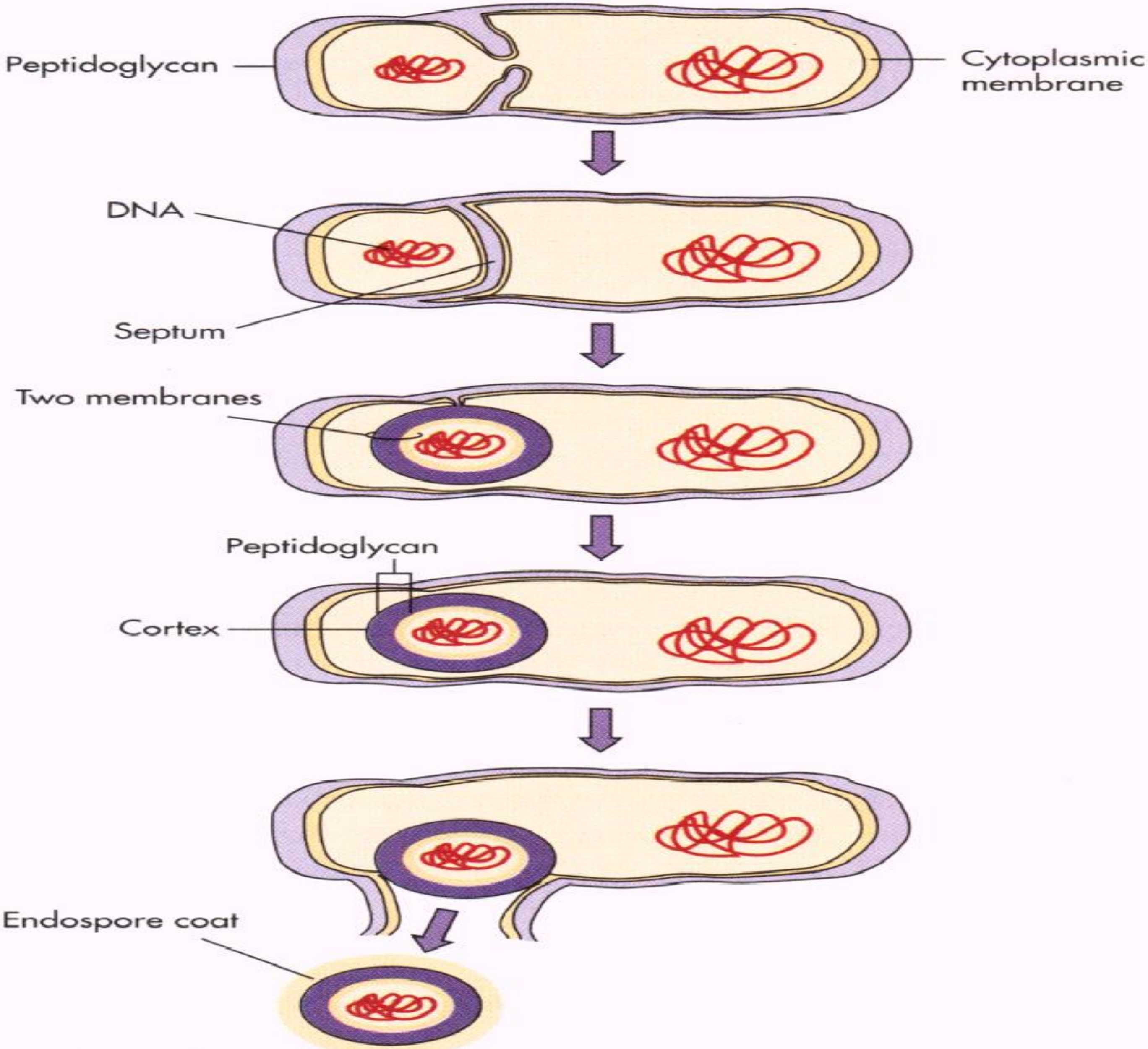
   Exosporium:

Germination





A B C


Peptidoglycan
Cytoplasmic membrane
DNA
Septum
Two membranes
Peptidoglycan
Cortex
Endospore coat

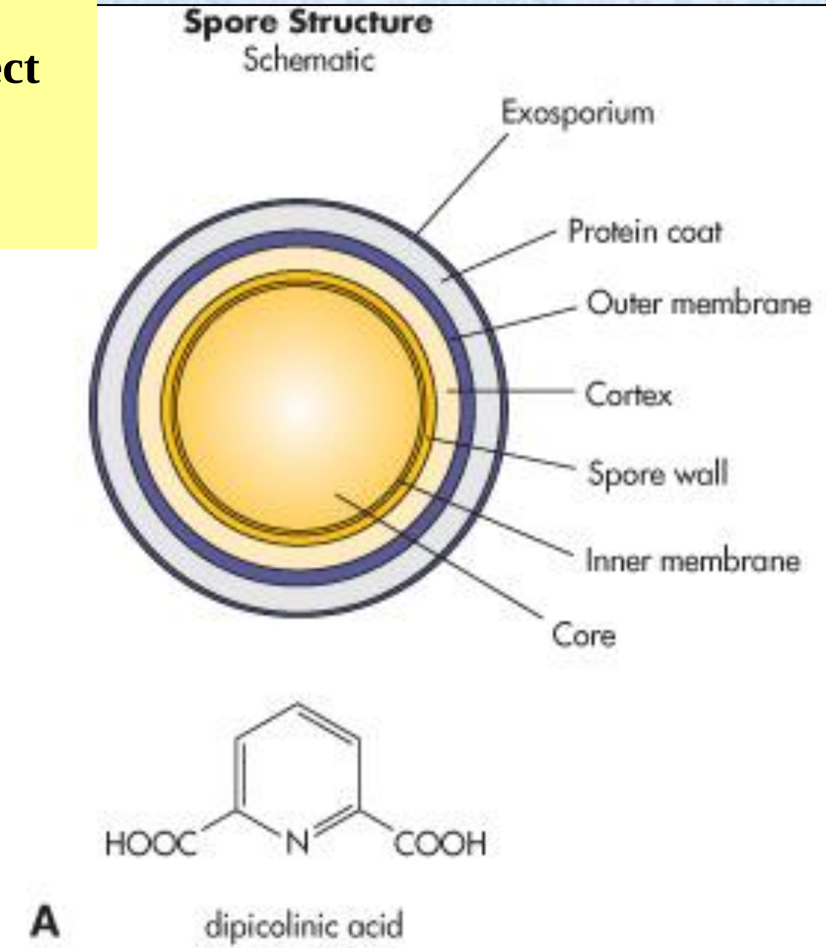
Spore structure:

Core: one copy of DNA and cytoplasmic contents Inner membrane and Spore wall

Cortex: peptidoglycan layer

Coat: Keratine-like protein which protect the spore.

Exosporium:



Bacteria