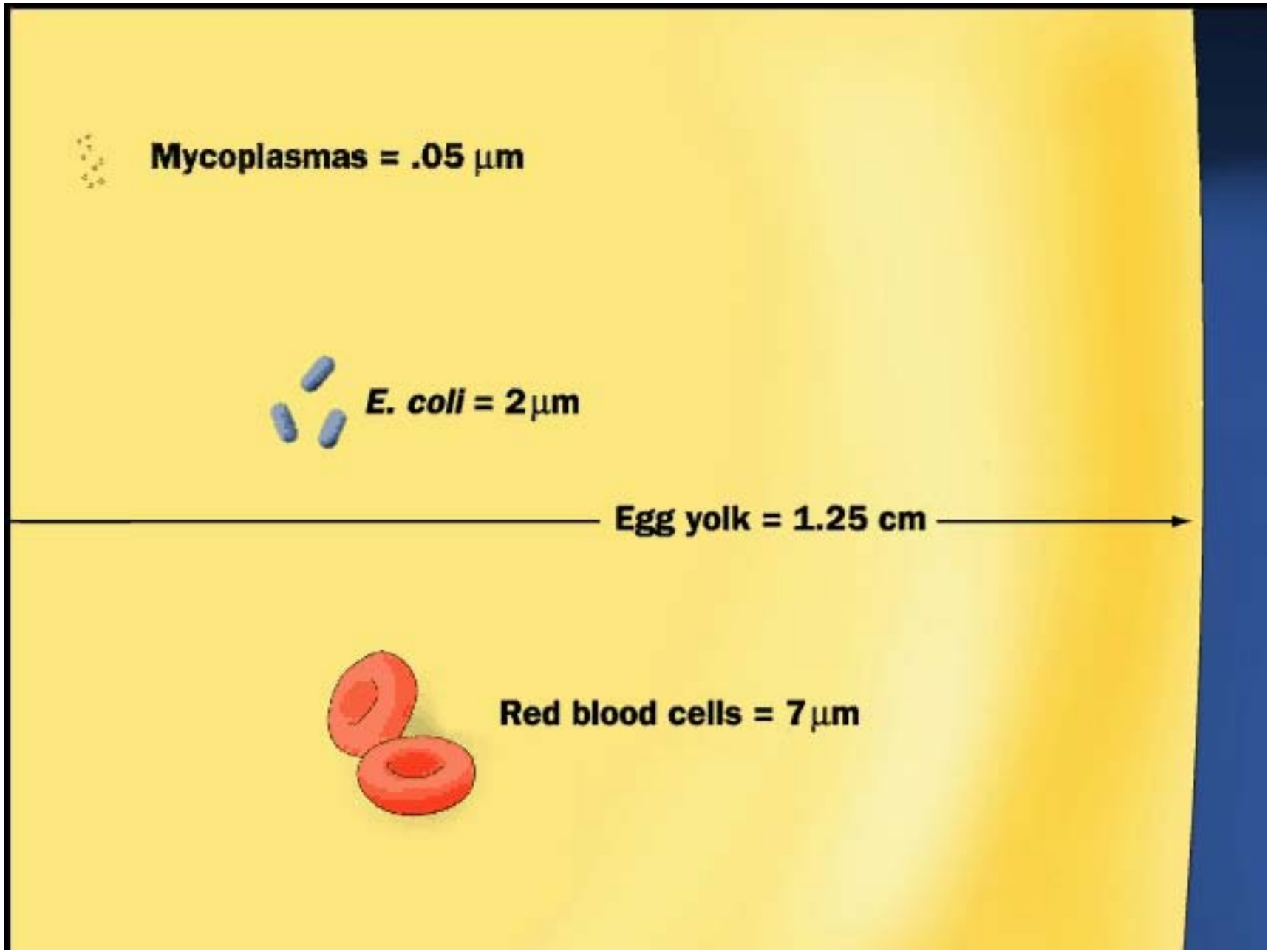
Prokaryotes (bacteria) and Gram Staining

Gram positive and

Gram negative

Why are we learning this?

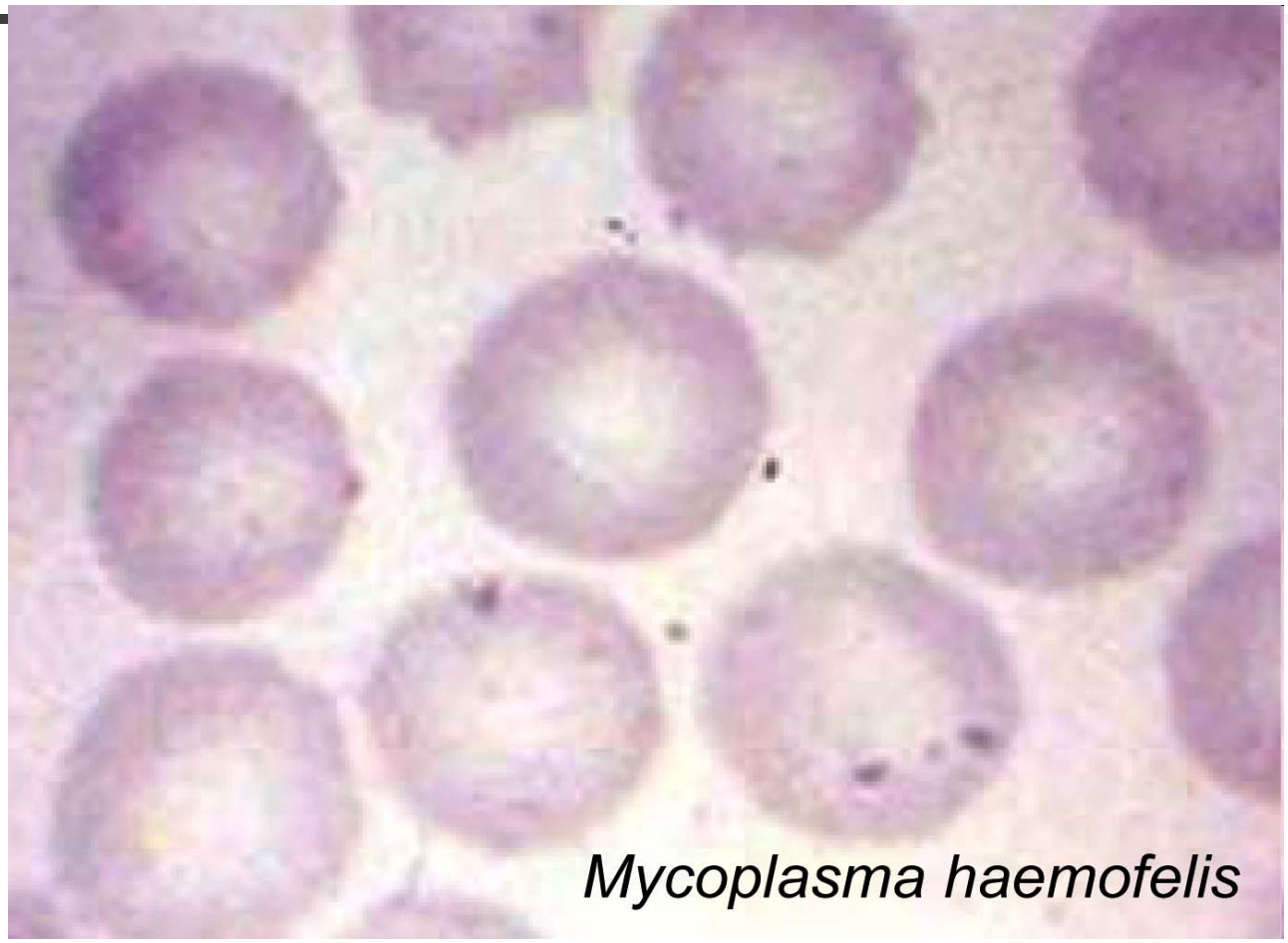
- We will be monitoring our bioreactors for bacterial contamination.
- We sometimes are culturing *E.coli* as the organism of choice and in this case we want to make sure it is not contaminated.


Mycoplasmas = .05 μm
E. coli = 2 μm
Egg yolk = 1.25 cm
Red blood cells = 7 μm

Mycoplasmas

- Mycoplasmas small prokaryotes without a cell wall.
- Huge contamination problem in industry and research laboratories doing **Cell** culture
- Treatment of contaminated cultures is quite problematic, usual solution is abandoning the culture.

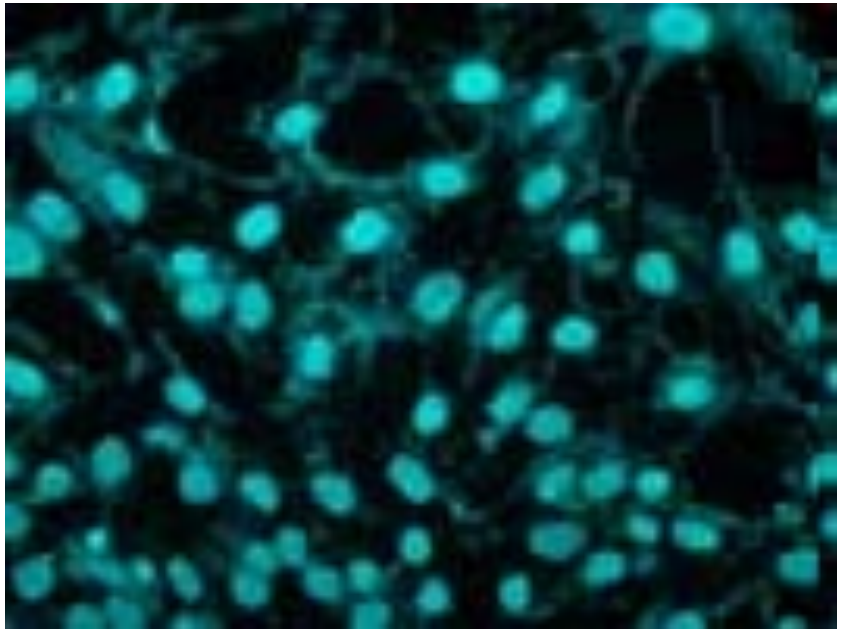
Mycoplasma haemofelis in a blood film from an infected cat.

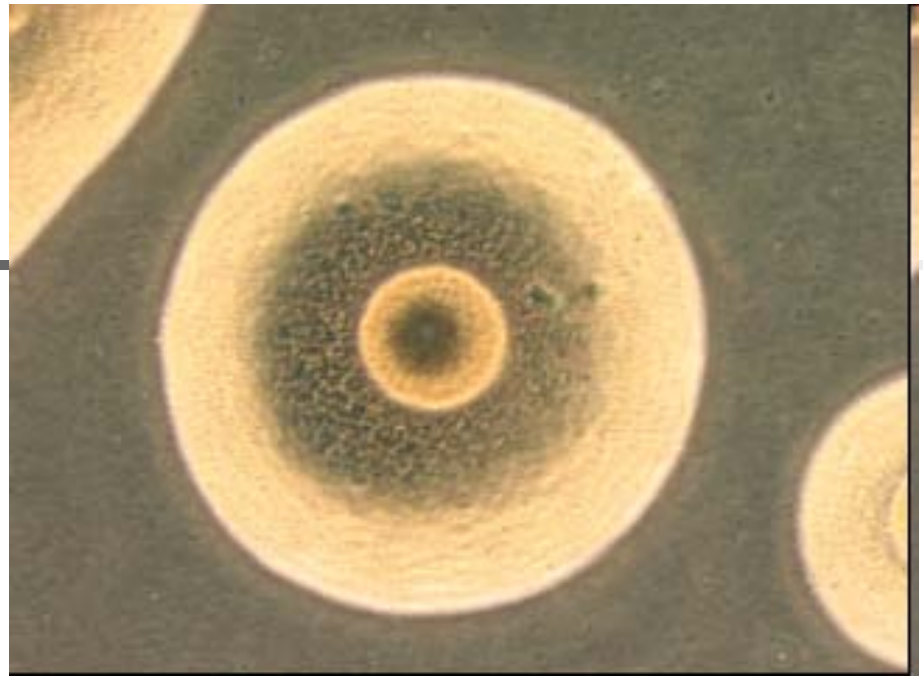


Methods of Detection for Mycoplasma

- **PCR (Polymerase Chain Reaction) Test**
- **Culture on Selective media**
- **Hoechst DNA stain**

Hoechst DNA stain test

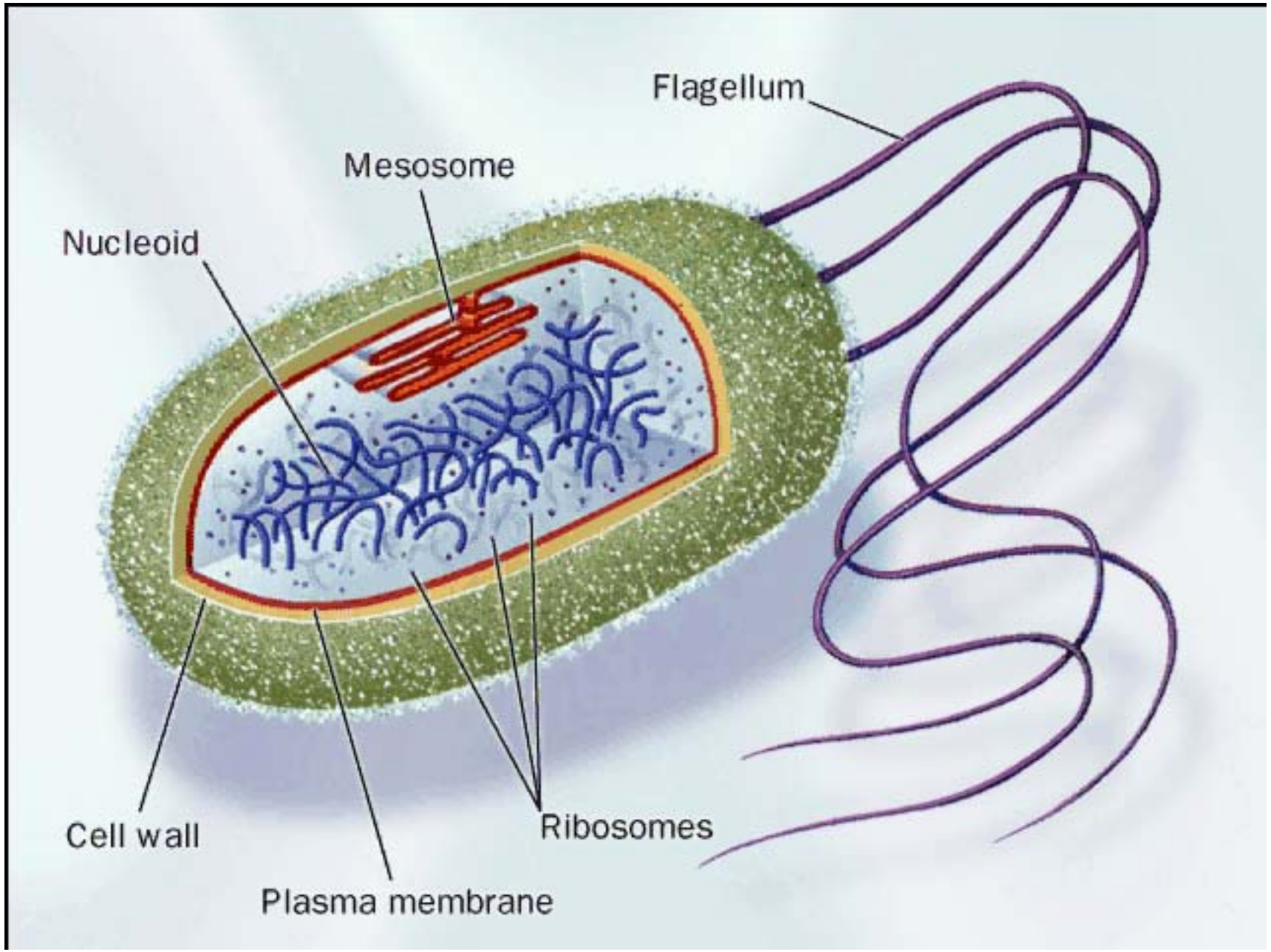


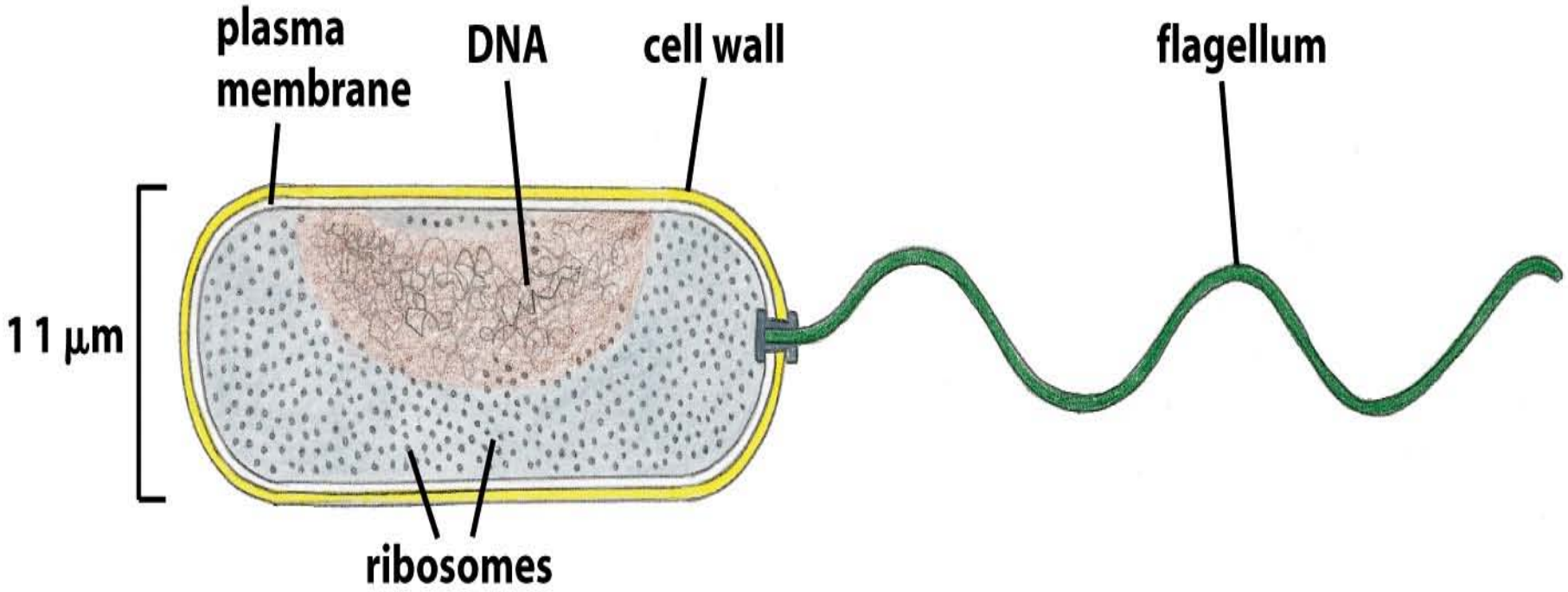


Colonies of Mycoplasma exhibit a distinctive "fried egg" morphology when viewed under a plate microscope. The assay requires a 28 day test interval before a definitive result can be obtained.

Generalized bacterial information

- Single celled organisms
- No nucleus or membrane bound organelles
- Used extensively in biotechnology
- Also a major contaminate of cultures

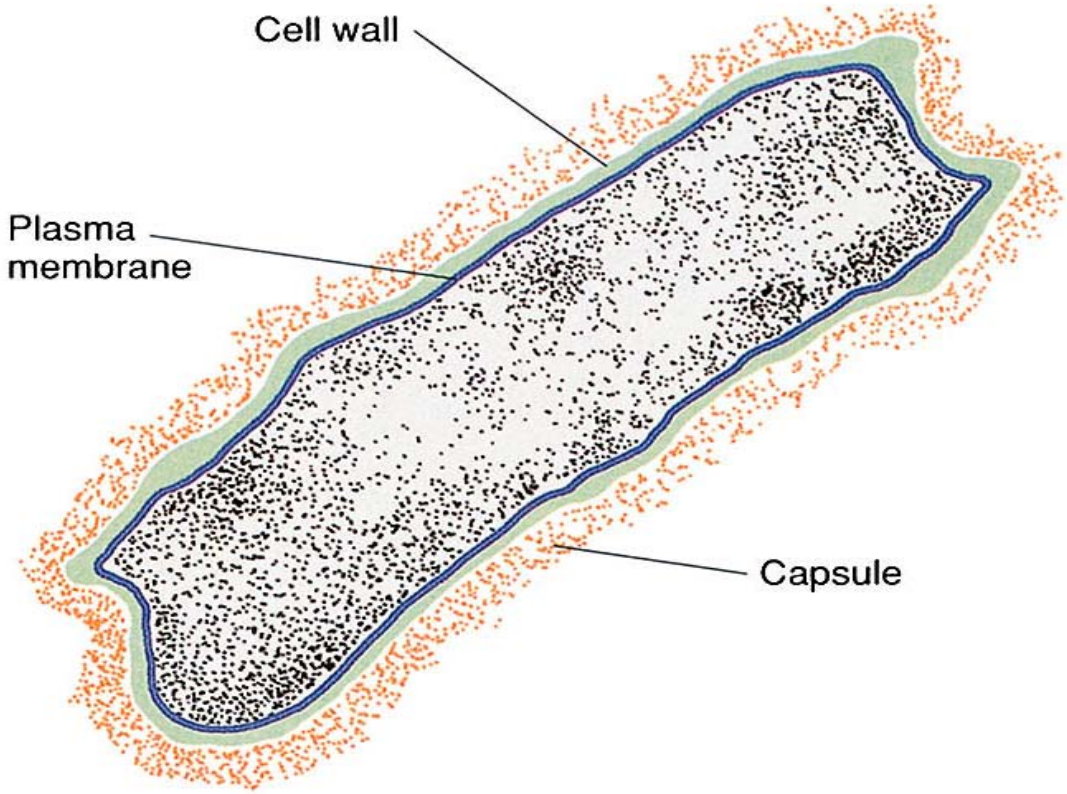

Flagellum
Mesosome
Nucleoid
Cell wall
Plasma membrane
Ribosomes



Fi Figure 1-18a Molecular Biology of the Cell 5/e (© Garland Science 2008)

Terms

- **Cytoplasm:** Cellular contents inside the plasma membrane- water, nutrients, building blocks for the cell.
- **Outer membrane:** Seen in gram negative bacterial cells. Endotoxins are lipopolysacchrides found in this membrane.
- **Capsule (Slime Layer):** this is a polysacchride layer around the cell. Some evidence that they help in avoiding the immune system.



http://www.lhup.edu/smarvel/seminar/fall_2004/dolan-ward/index.htm

Terms

- **Cell Wall**: The function of the cell wall is to give shape and rigidity to the cell. Here we find peptidoglycan and structurally similar molecules.
- **Plasma membrane:** This is the membrane that surrounds the cell keeping the cytoplasm in and the external environment out.

Terms

- **Ribosomes**: Protein complex and RNA which makes other proteins in the cell.
- **Nucleoid:** The region inside the cell where the bacterial chromosome is housed.
- **Mesosome:** folding of the plasma membrane.
- **Flagellum:** Method of bacterial locomotion.

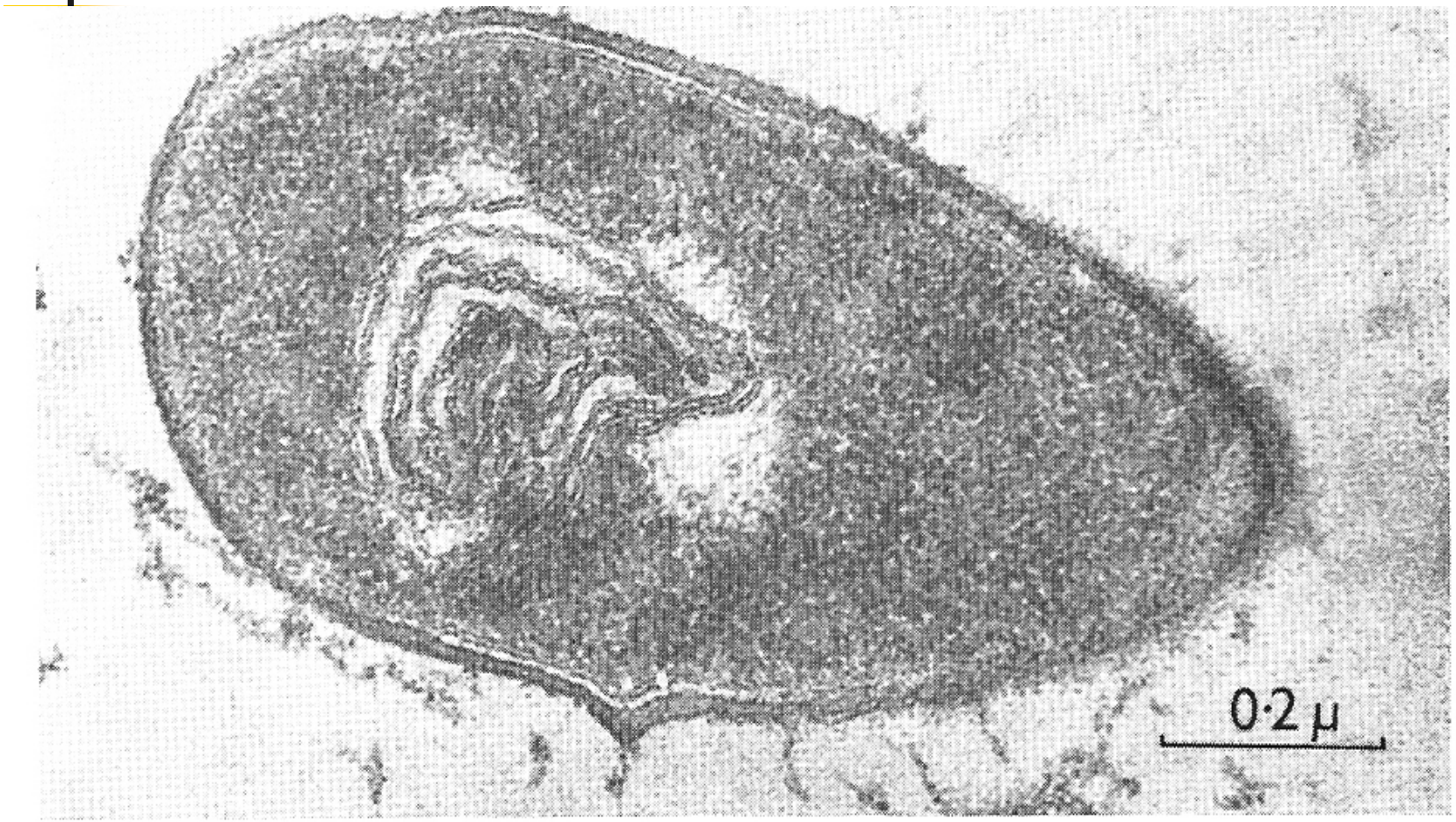
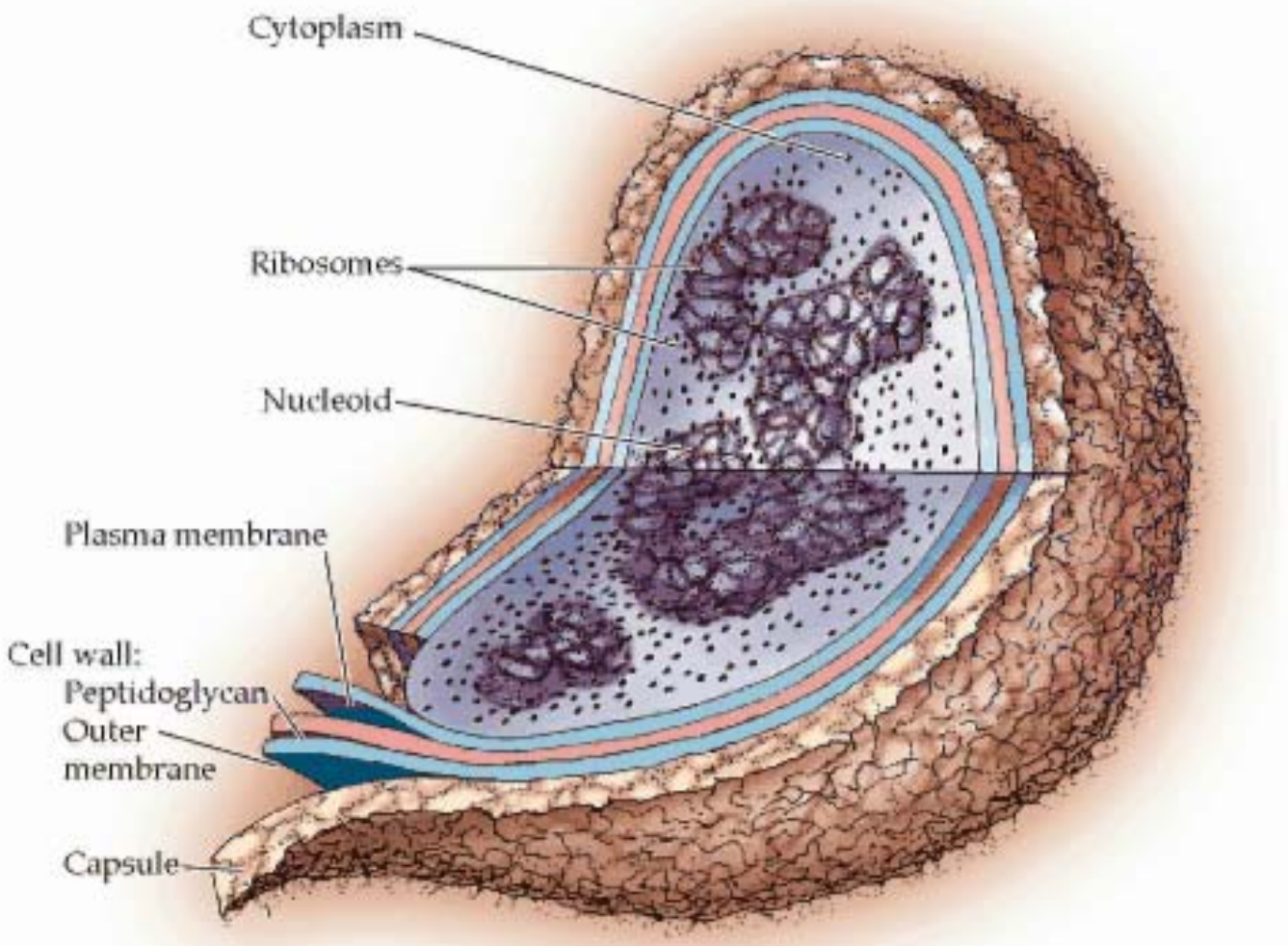
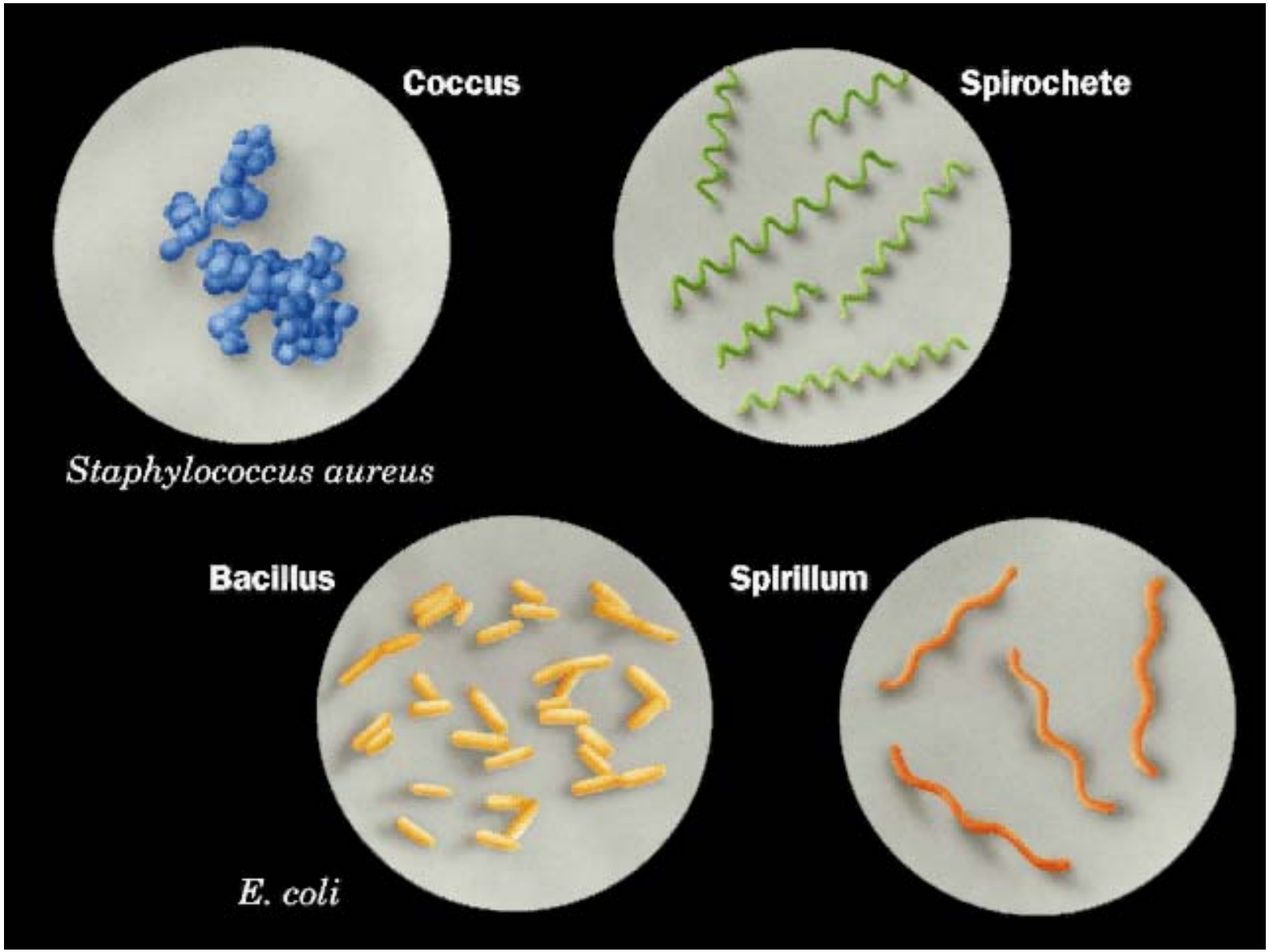


FIGURE 42. Mesosomes of *Bacillus subtilis* sectioned in the region of the nucleus. The internal structure takes the form of (a) vesicles and (b) whorls of membranes.

Bacterial anatomy and physiology



Bacteria can have different shapes

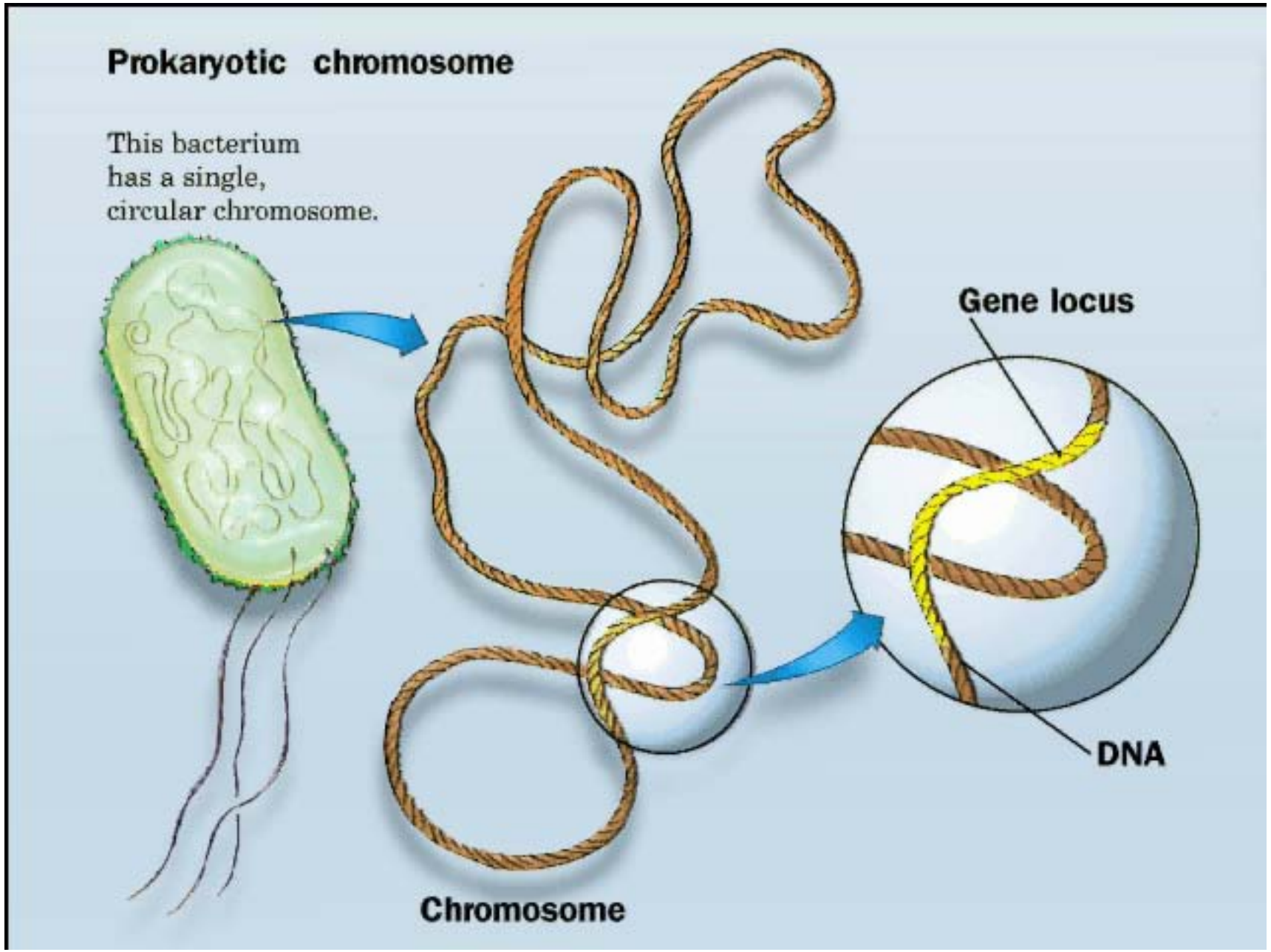


Four shapes of bacteria

- Rod (bacillus)
- Circular (coccus)
- Spiral (spirillium)
- Corkscrew (spirochete)

Genetic Material

- Bacterial chromosome is circular and attaches to the plasma membrane of the bacterial cell.
- Plasmids small DNA loops transfer characteristics such as antibiotics resistance
- In eukaryotes the chromosomes are linear and are contained in the nucleus.

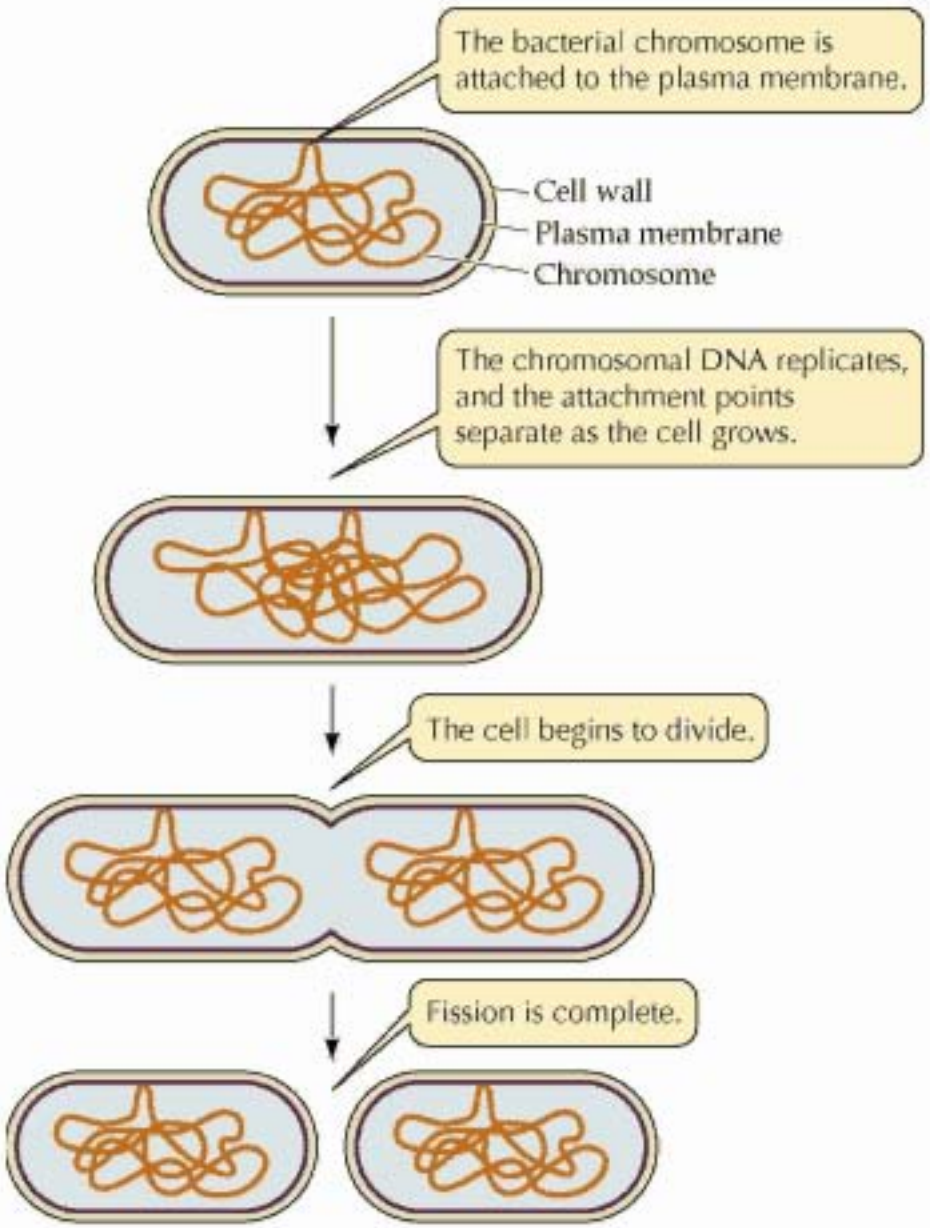

Prokaryotic chromosome
This bacterium has a single, circular chromosome.
Gene locus
DNA
Chromosome

Binary Fission

- Bacterial chromosomes are attached to the plasma membrane.
- The DNA is replicated and the second chromosome is attached to the membrane.
- Membrane is added between the two replicated chromosomes.

Binary Fission

- Membrane begins to pinch in and begins to divide.
- End up with two bacterial cells that are genetically identical.


The bacterial chromosome is attached to the plasma membrane.
Cell wall
Plasma membrane
Chromosome
The chromosomal DNA replicates, and the attachment points separate as the cell grows.
The cell begins to divide.
Fission is complete.

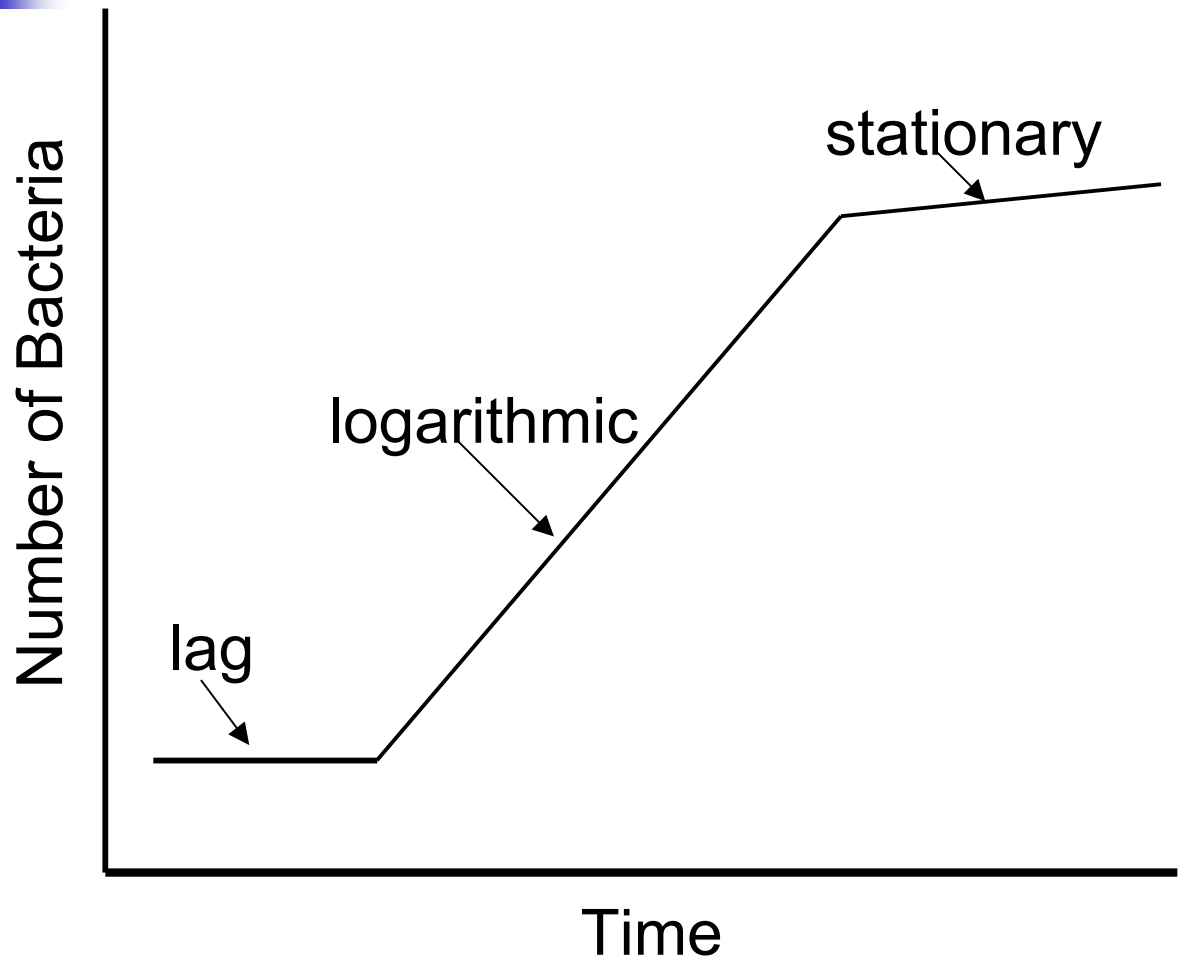
Bacterial Growth

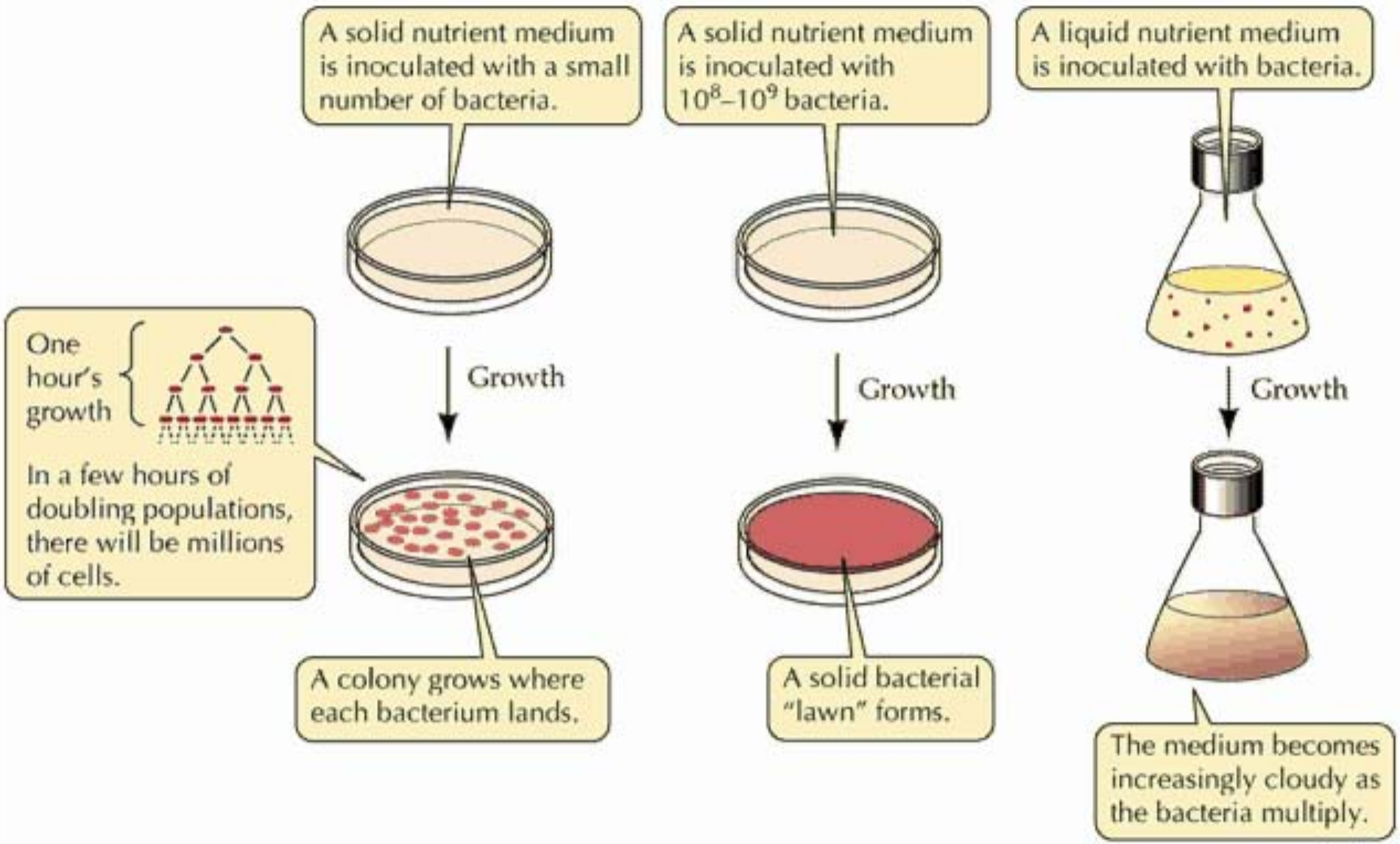
- Bacterial growth is very rapid.
- *E. coli* divides can divide every twenty minutes.
- The bacterial culture starts out slow this is called the **lag phase**.

Bacterial Growth

- Next the bacteria start to grow rapidly, which is the **logarithmic phase.**
- Finally the bacterial culture runs out of room and nutrition, this phase of growth is called the **stationary phase**. This is when the culture does not continue to increase in number.

Bacterial Growth



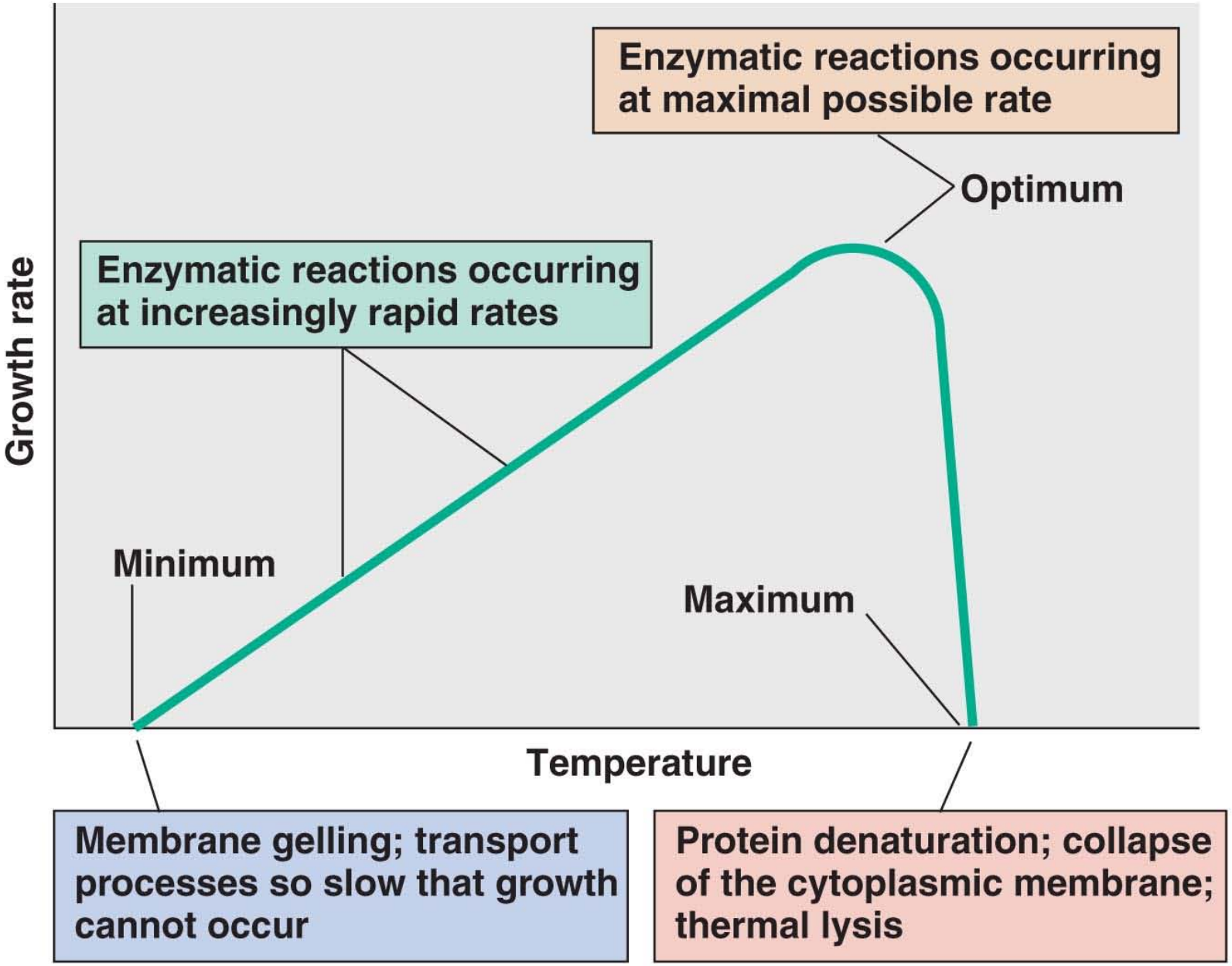

A solid nutrient medium is inoculated with a small number of bacteria.
A solid nutrient medium is inoculated with 10^8–10^9 bacteria.
A liquid nutrient medium is inoculated with bacteria.
Growth
Growth
Growth
One hour's growth
In a few hours of doubling populations, there will be millions of cells.
A colony grows where each bacterium lands.
A solid bacterial "lawn" forms.
The medium becomes increasingly cloudy as the bacteria multiply.

Bacteria can live in different environments

- Acidophiles
- Thermophiles
- Anaerobes
- Aerobes
- Halophiles

Cardinal temperatures

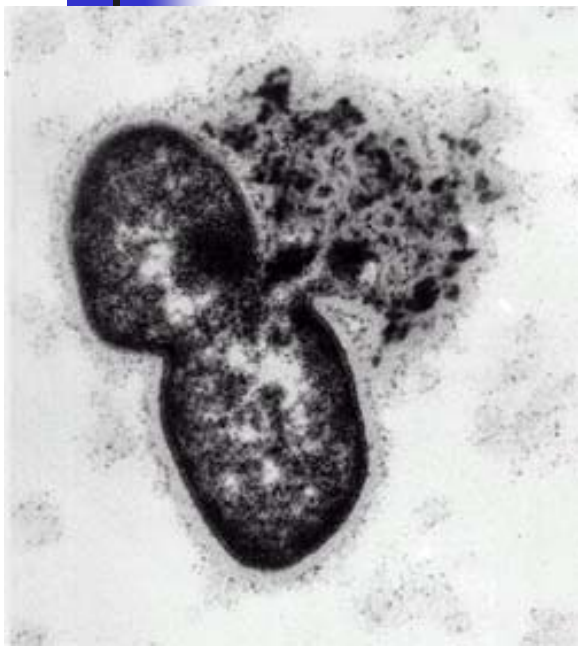
- Minimum growth temperature
  - Lowest temperature at which an organism will grow
  - Below this temp.→nutrient transport difficulty due to the fact that membrane gels and transport too slow
- Optimum growth temperature
  - Temperature at which an organism grows best
  - Metabolic enzyme reactions occurring at maximum rate
- Maximum growth temperature
  - Highest temperature at which an organism will grow
  - Above this temp.→protein denaturation; membrane collapse, and lysis


Enzymatic reactions occurring at maximal possible rate
Optimum
Enzymatic reactions occurring at increasingly rapid rates
Growth rate
Minimum
Maximum
Temperature
Membrane gelling; transport processes so slow that growth cannot occur
Protein denaturation; collapse of the cytoplasmic membrane; thermal lysis

Different Bacteria have different antibiotic sensitivity

- Cell membrane
- Cell wall synthesis
- Folic acid biosynthesis
- DNA gyrase
- DNA polymerase
- Protein synthesis, 30S inhibitors
- Protein synthesis, 50S inhibitors

antibiotics

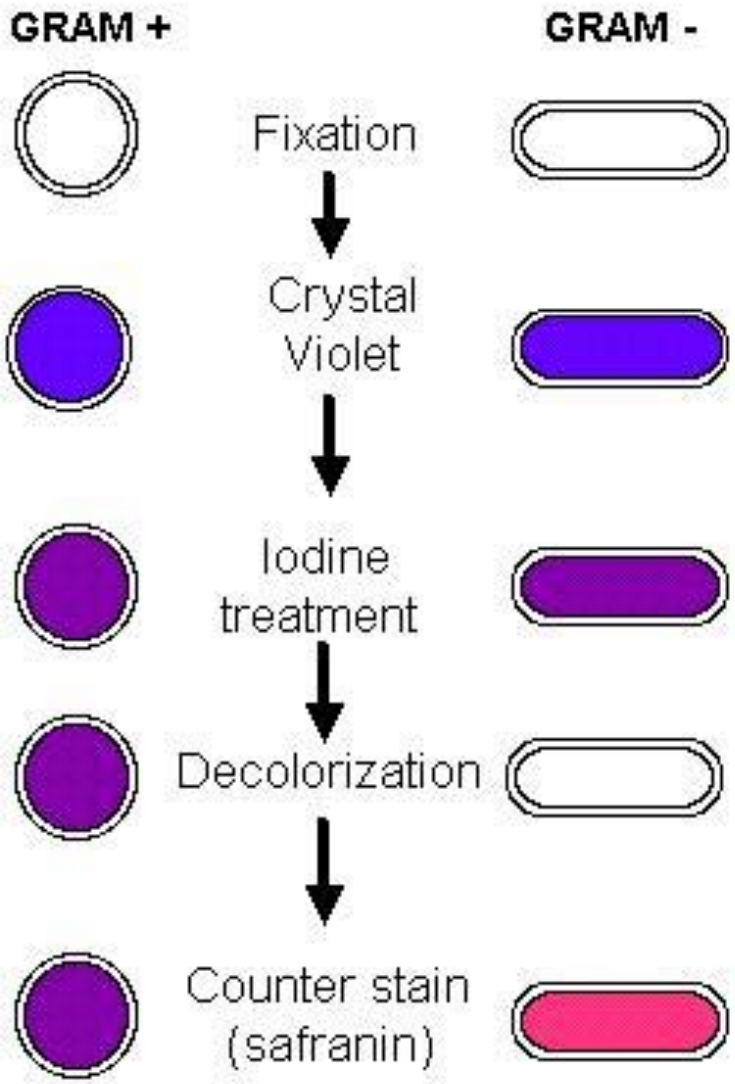


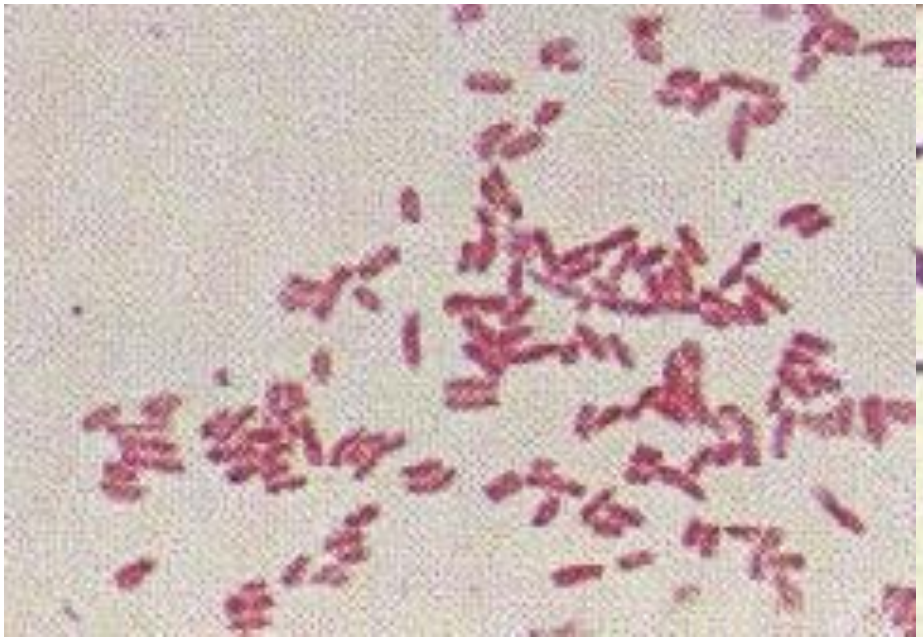
- Many antibiotics such as penicillin work by disrupting cell wall synthesis
- The cell walls become weak and eventually allow the cell to burst

Gram Stain

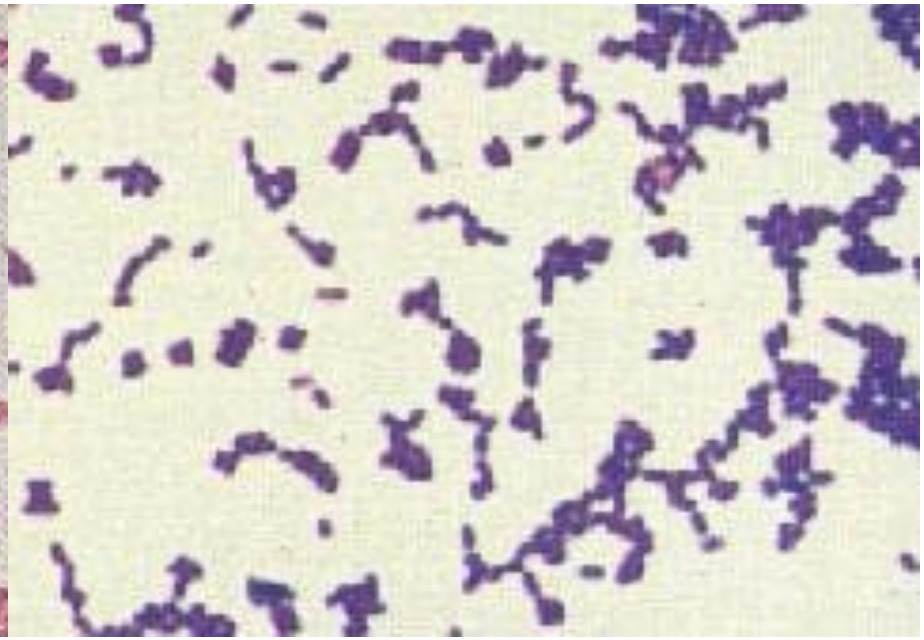
- This test allows scientists to differentiate between gram negative and gram positive bacteria
- Gram negatives will stain a red
- Gram positives will stain a purple

Gram Stain


GRAM +
GRAM -
Fixation
Crystal Violet
Iodine treatment
Decolorization
Counter stain (safranin)



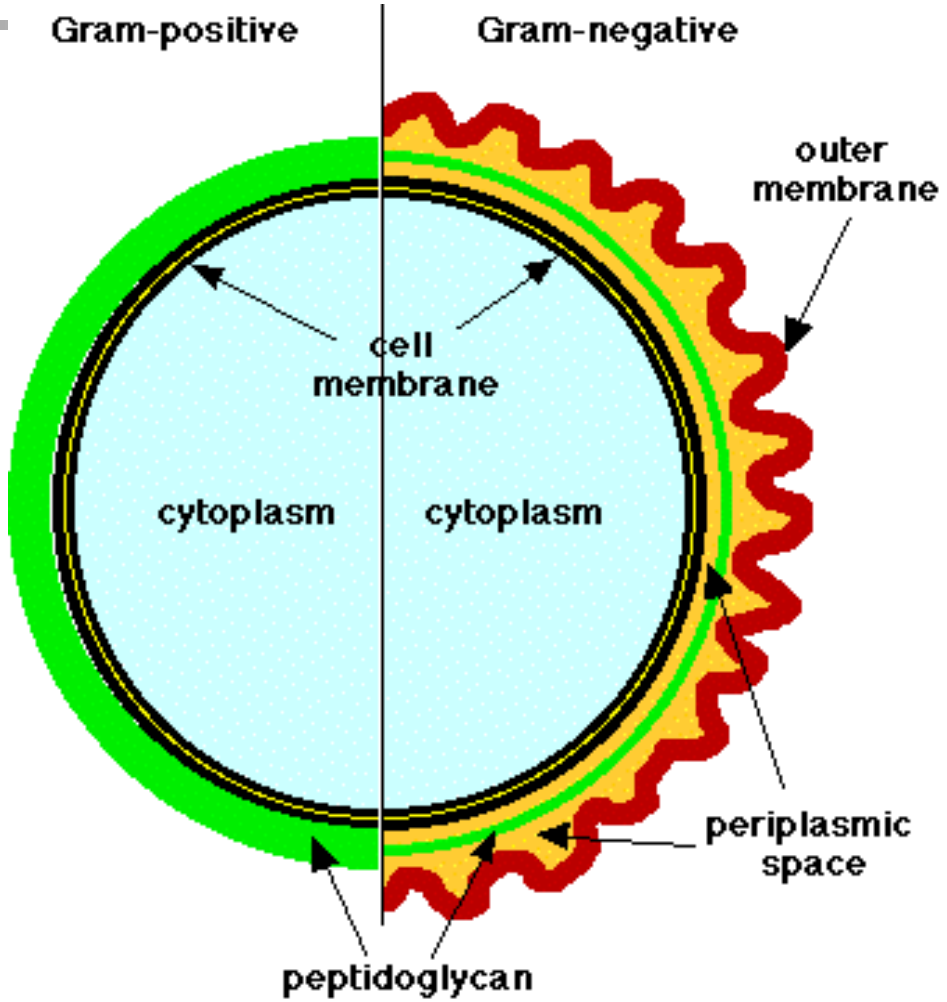
Gram Positive Bacteria



Gram Negative Bacteria

Bacterial Cell wall types

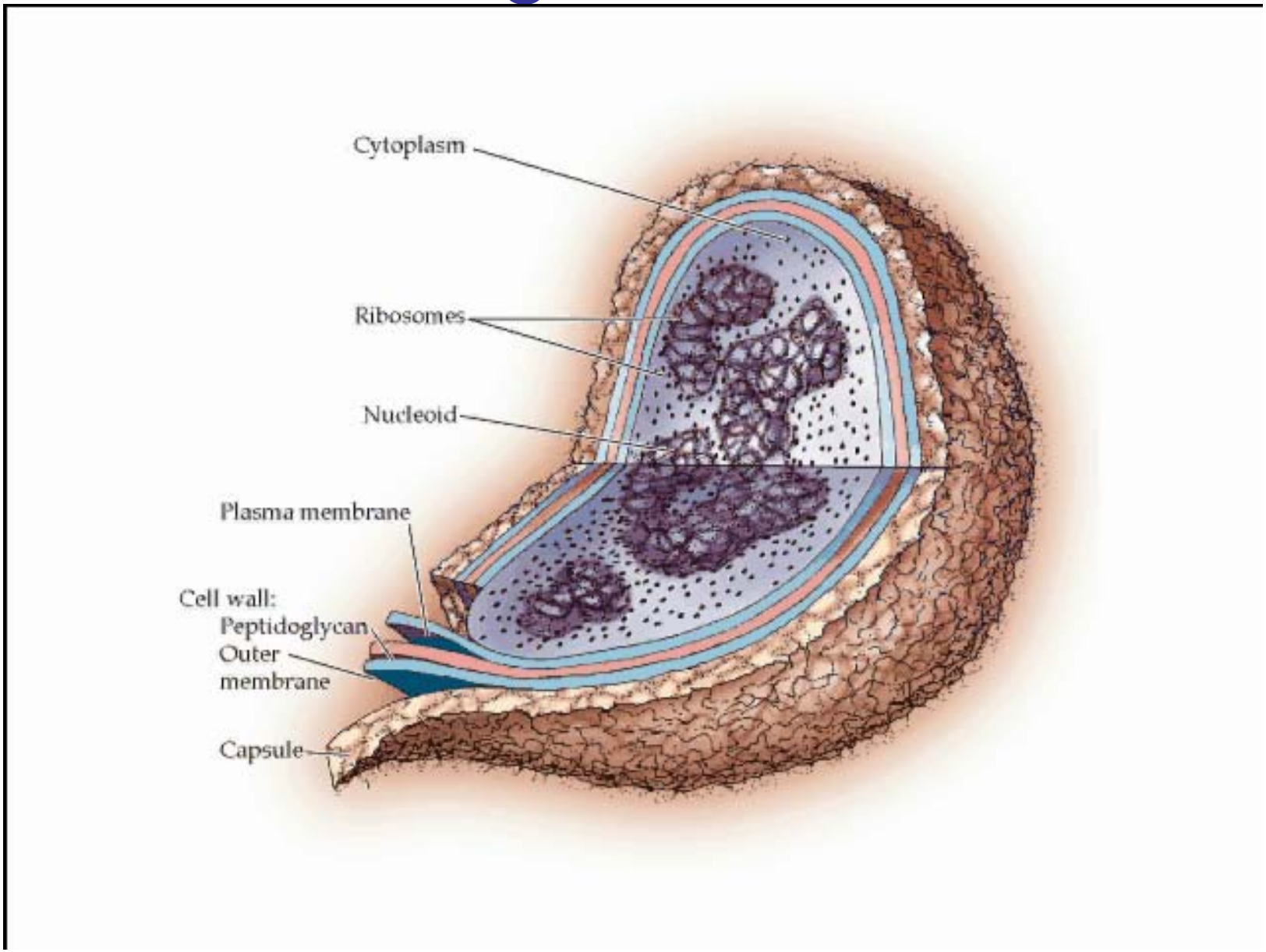
- Gram Negative vs. Gram Positive



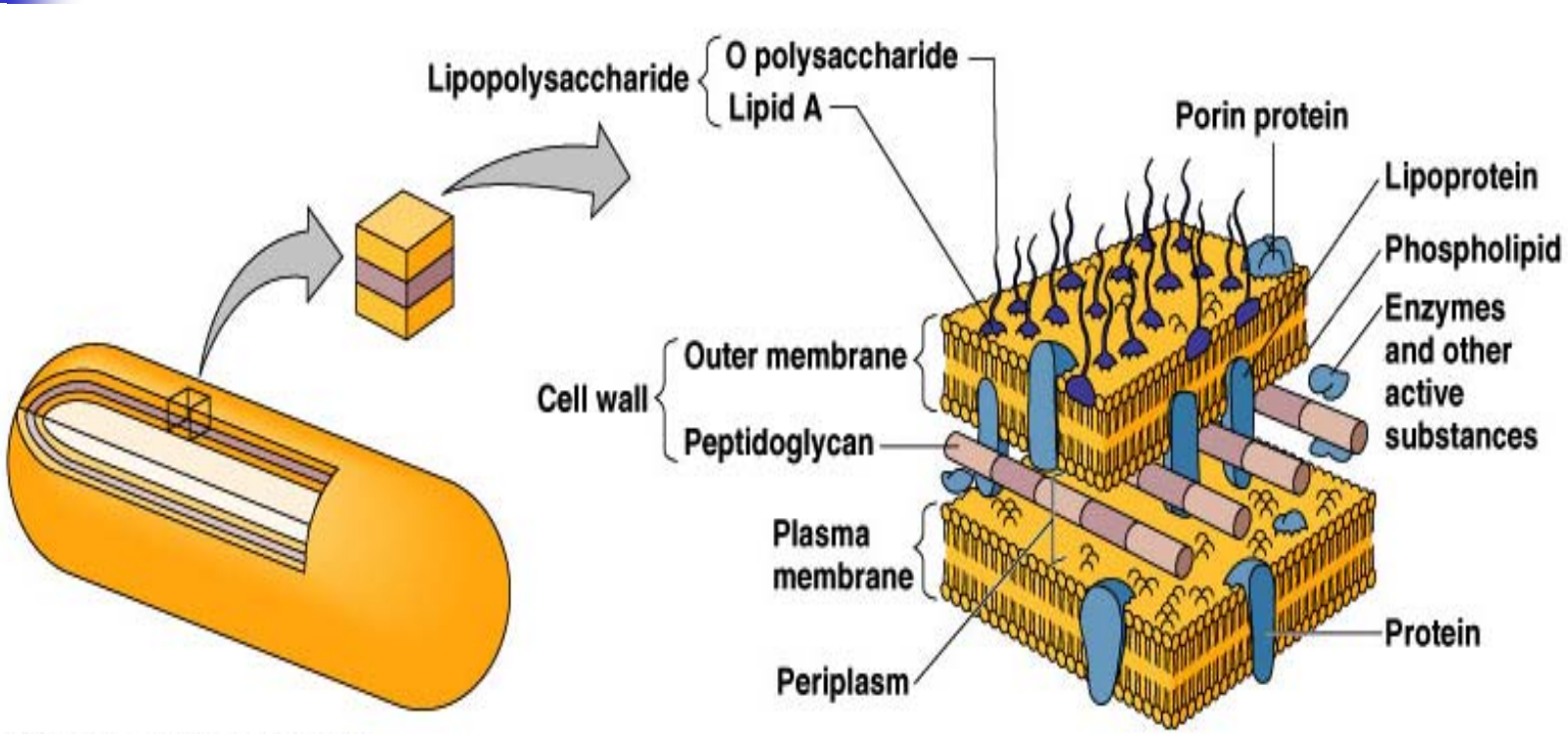
Gram negative

- Thinner cell wall
  - Less peptidoglycan
- Two membranes
- Many are pathogenic
- Examples:
  - E.Coli
  - Salmonella

Gram negative bacteria

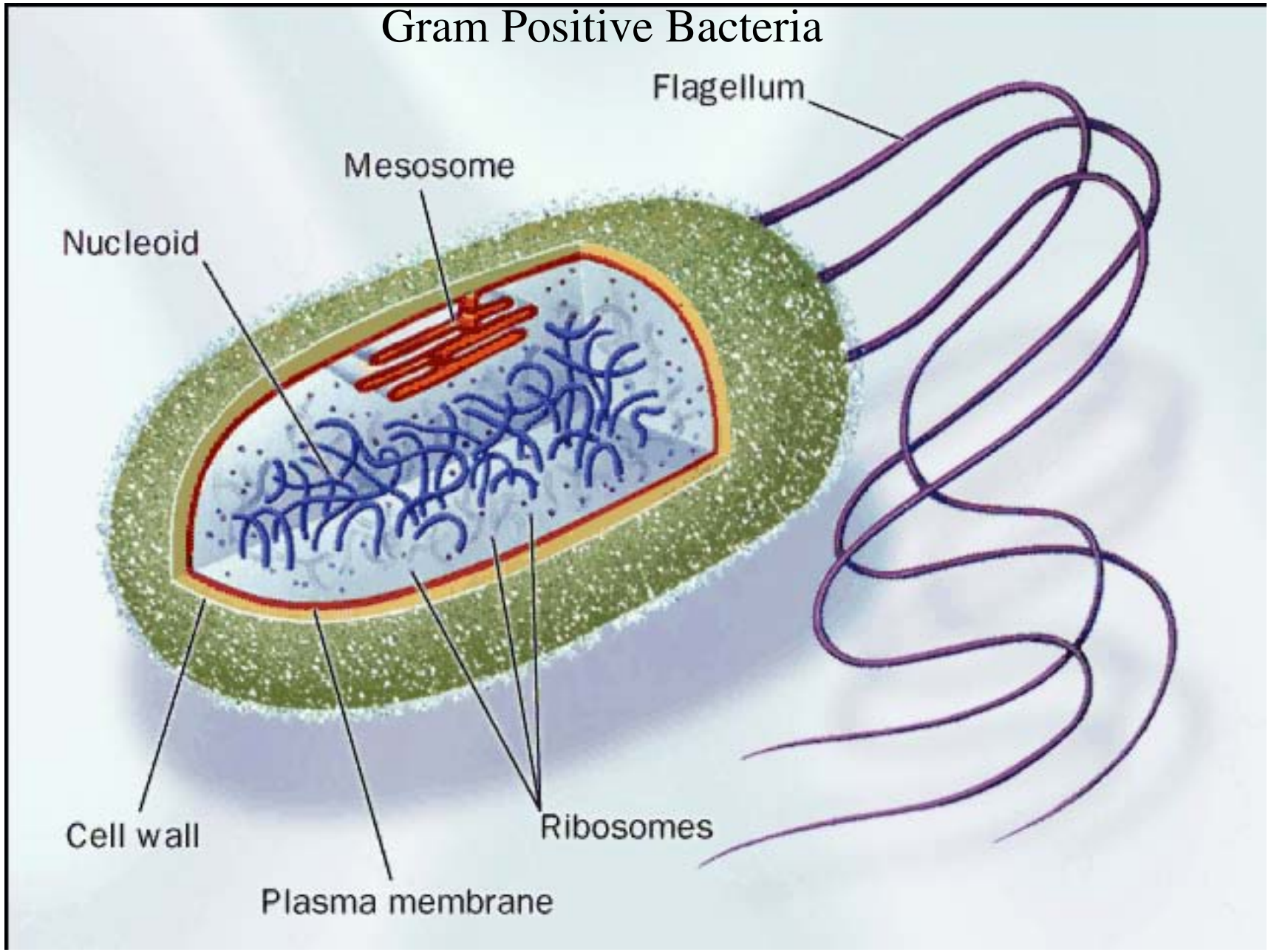


Gram Negative Cell Wall



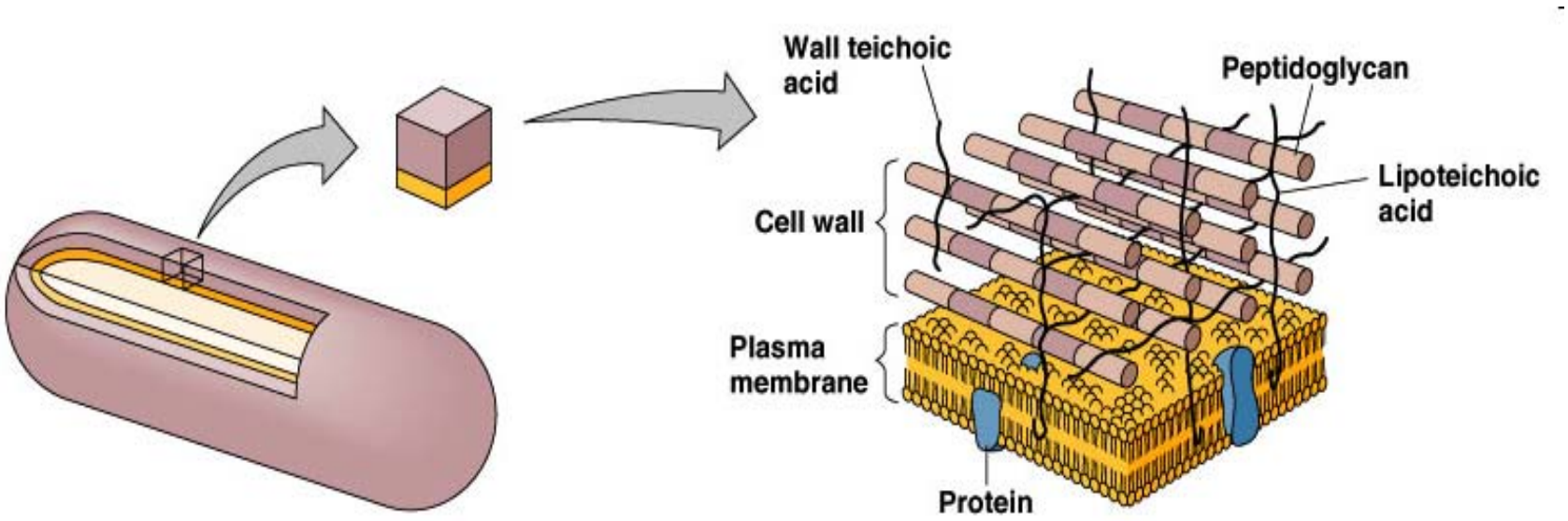
(c) Gram-negative cell wall

Gram Positive Bacteria



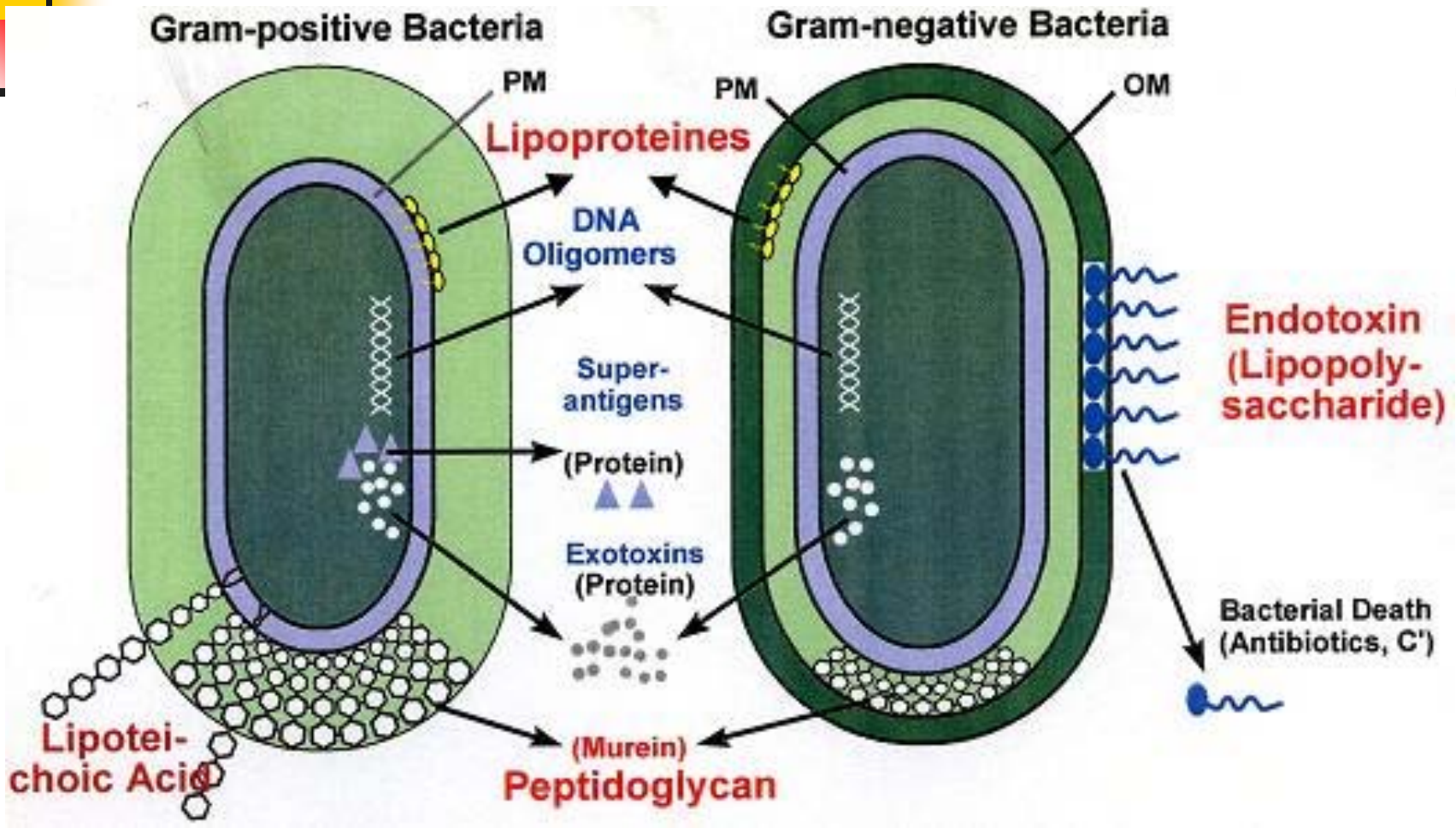
Gram positive

- Thicker cell wall
- Only one membrane



(b) Gram-positive cell wall

Comparison of Cell Walls



Difference between Gram positive and Gram negative

- Cell wall is thicker in a gram positive bacteria.
- Gram positive bacteria has only the inner plasma membrane and no outer membrane.
- Gram positive bacteria stain blue while gram negative stain red.

General Summary of Bacteria

- Lack organelles
- Nucleus is lacking
- Smaller ribosomes
- Endotoxins are problematic
- Linked transcription and translation
- Can make simple proteins

Control of Bacterial Contamination

- Cleaning- removing debris and residues that can be nutrients for microbes
- Chemical disinfectants such as 70% IPA or EtOH, bleach
- Treatment with Gamma Irradiation or UltraViolet light (UV) irradiation
- Treatment with Gases Ethylene oxide or propylene oxide (common in spice trade, hospitals for equipment)

The Three Main Cell Types used in Fermenation

- Tools of the Trade:
- Bacteria (*E. coli*-**Prokaryote):**
  - Used for insulin and growth hormone, DNA products
  - We will use it for GFP.
- Yeast (*P. pastoris, S. cerevisiae*-**Eukaryote**):
  - Use in food processing (brewing , baking)
  - We will use it for HSA.
- Mammalian Cells (CHO, BHK, Hybridomas **-Eukaryote**):
  - Antibody products , large complex glycosylated proteins
  - Too expensive for us to use, however extensively used in the industry.

Fermentor

- In the fermentor we will try and duplicate optimum conditions.
- Increase density as far as possible.
- Will also be inducing the formation of the GFP protein by using arabinose.

The advantages of E.coli or prokaryotes as a host cell

- Can be grown to high numbers in short time
- Can be grown in economical simple defined media
- Robust organisms withstand high agitation without shearing

Disadvantages of Prokaryotes

- Inability to glycosylate proteins
- Proteins are sometimes expressed in refractile bodies that need resolubilisation and refolding
- Inherent endotoxin contamination
- Folding of complex proteins is often problematic