For human cells, the plasma membrane is enough, but microorganism cells need more…… What?

MICROBIOLOGY

厦门大学
生命科学学院
SCHOOL OF LIFE SCIENCES XIAMEN UNIVERSITY

Lecture 2

(Chapter 3)

Bacterial Structure

张连茹

Outline

1. Cell wall(peptidoglycan)-close to membrane(3.4)
2. Capsule and slime layer-out of the wall(3.5)
3. Bacterial cytoplasmic structures(3.6)
4. Flagella, fimbriae and pili-stretch out(3.7)
5. Bacterial Motility and Chemotaxis(3.8)
6. Endospore(3.9)

生|命|科|学|学|院
SCHOOL OF LIFE SCIENCES XIAMEN UNIVERSITY

MICROBIOLOGY

Lecture 2
Episode 1

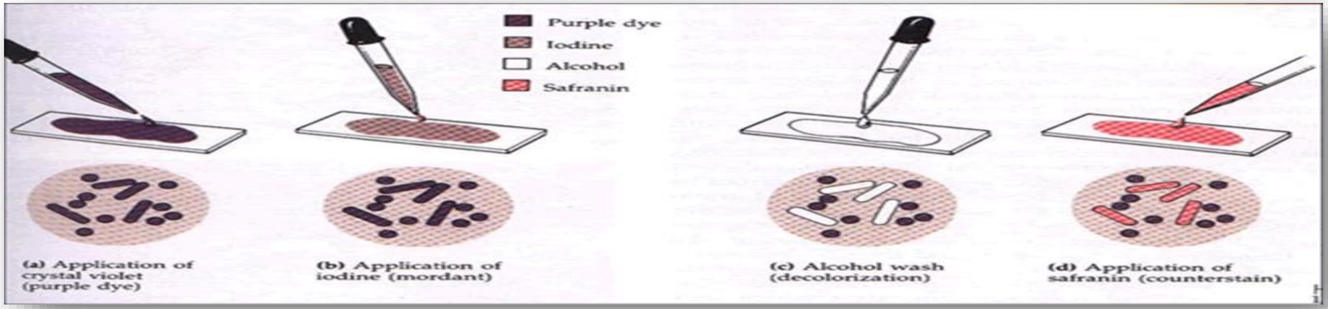
3.4 Cell Wall(peptidoglycan)

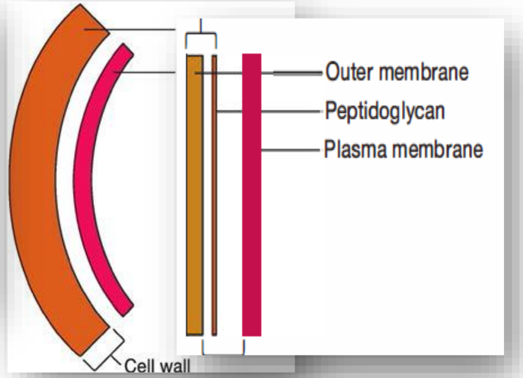
1. Peptidoglycan structure
2. Gram stain and the difference between Gram positive and Gram negative
3. Bacteria that lack peptidoglycan or cell walls

3.4 Bacterial Cell Walls

3.4.1 Overview of bacterial cell wall structure

- **After Christian Gram developed the Gram stain in 1884, most bacteria could be divided into two major groups （+/-） based on their response to the Gram-staining procedure .**





- **Typical negative bacteria: E. coli ;**
  Typical positive bacteria: Bacillus subtilis
- **Why?**
- **Peptidoglycan**+outer membrane/**Peptidoglycan**
- **What is a peptidoglycan(PTG) ?**

3.4.2 Peptidoglycan Structure

PTG (murein):Meshlike(网状) polymer of identical subunits forming long strands

Building block:

- **1) Two sugars:**

***N*-acetylglucosamine (NAG)**

***N*- acetylmuramic acid(NAM)**

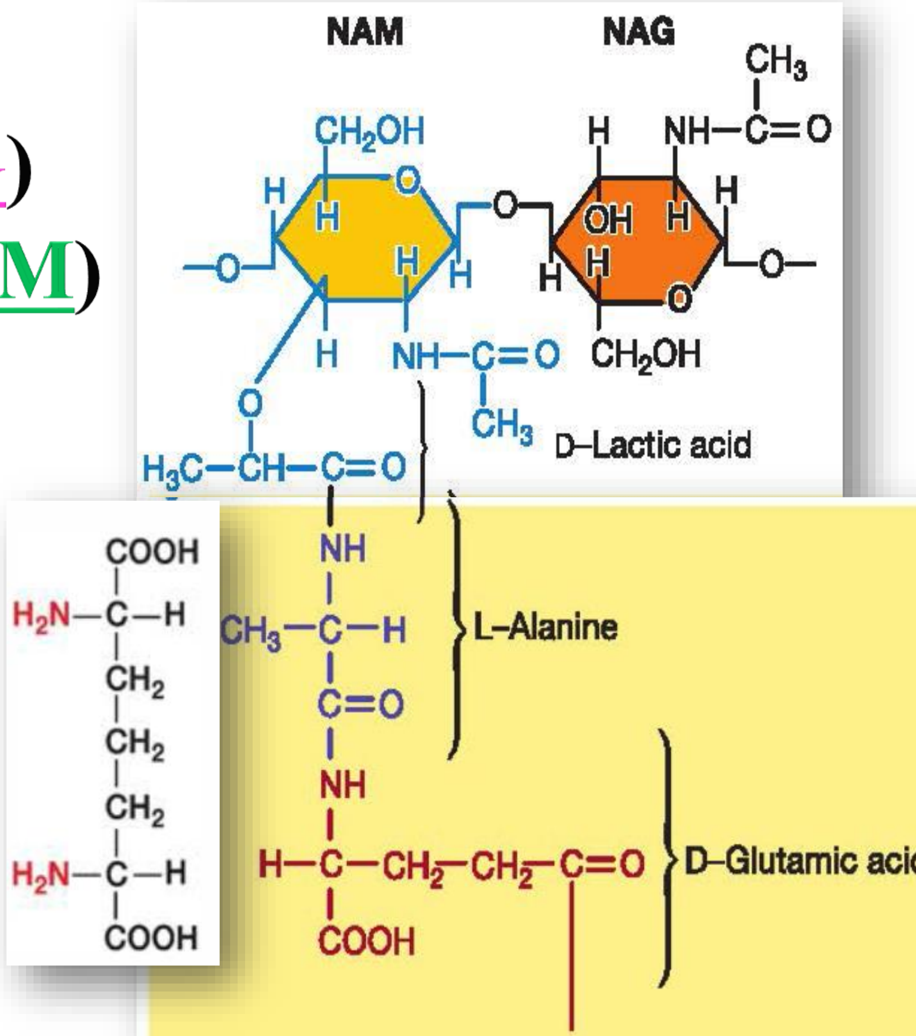
- **2) Tetrapeptide:**

L-(ala,lys, gln);

D-(glu,ala) ?;

DAP:meso-diaminopimelic acid

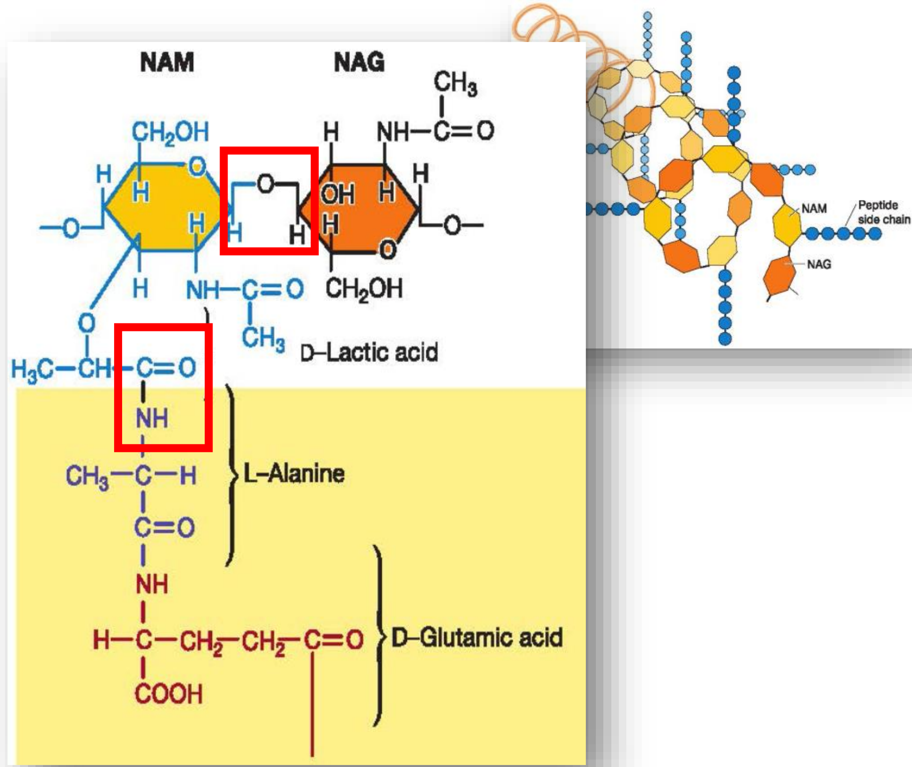
How to connect?

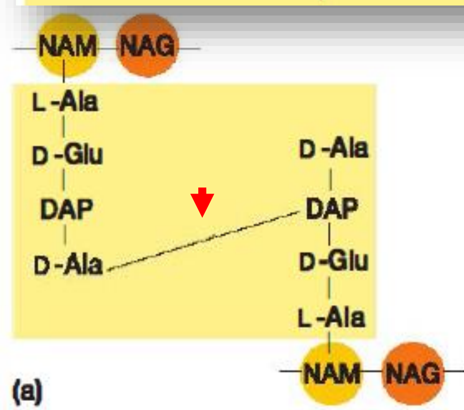


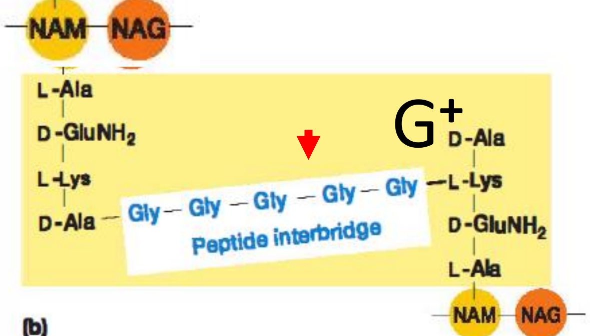
3.4.2 Peptidoglycan Structure

Linkage:

- **PTG strands have a helical shape(NAM(1,4)NAG)**
- **PTG chains are crosslinked by peptides for strength(NAM-L-ala)**
  - **Interbridges may form PTG sacs – interconnected networks**
  - **Various structures occur- (D-ala)-DAP/(D-ala)-(Gly)$_5$-(L-lys)interbridge**



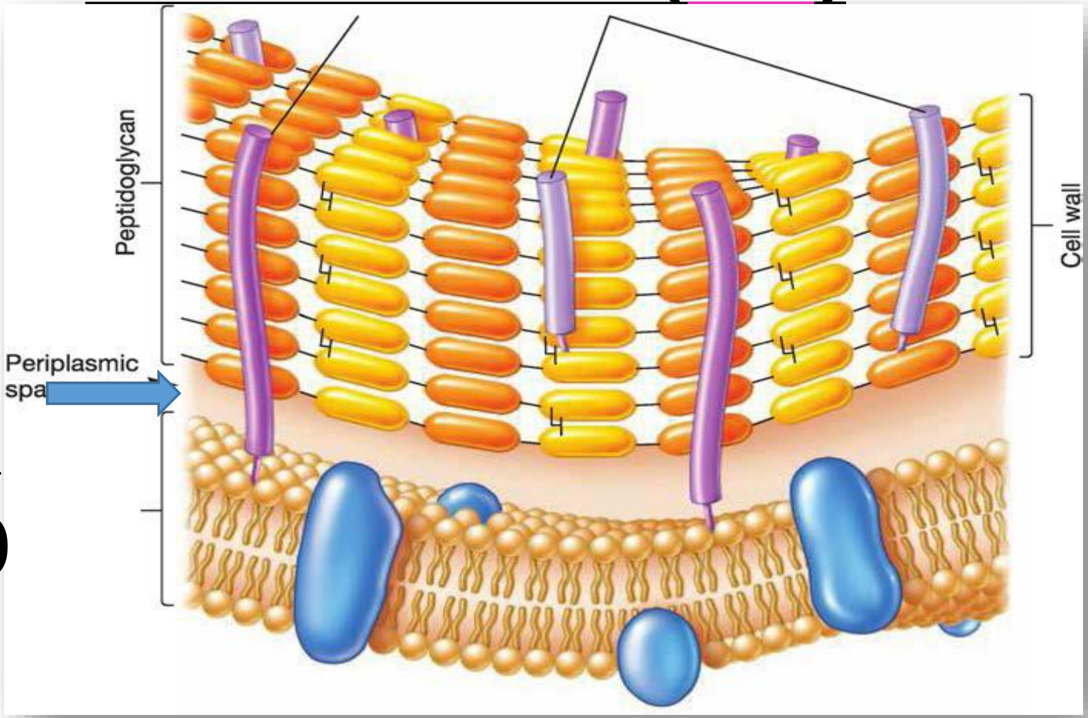




- **How to identify G$^+$ or G$^-$?**

3.4.3 Typical Gram-Positive Cell Walls

- **Most have thick PTG and large amounts of other polymers such as teichoic acids (TA)**

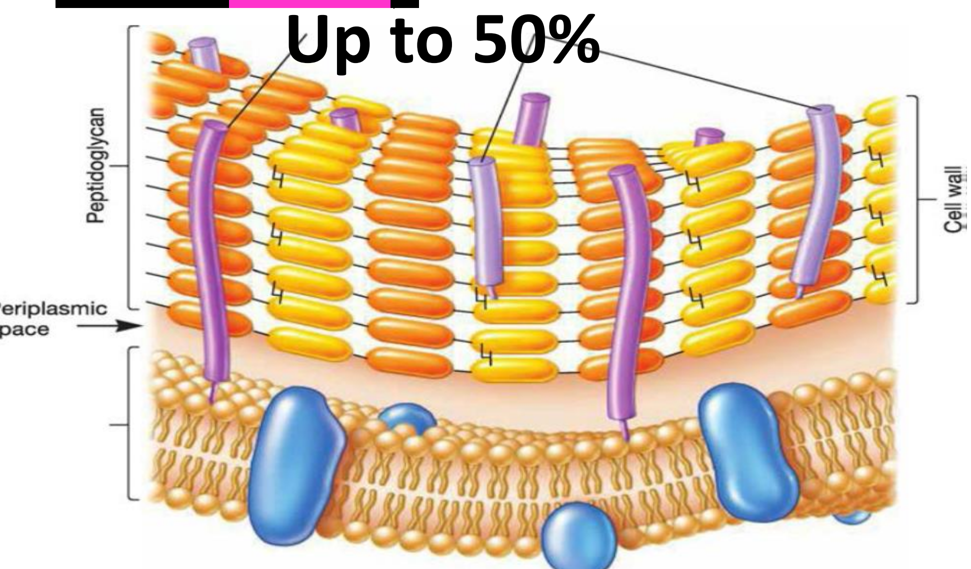


The periplasmic space is so narrow that it is often not visible. (exoenzymes)

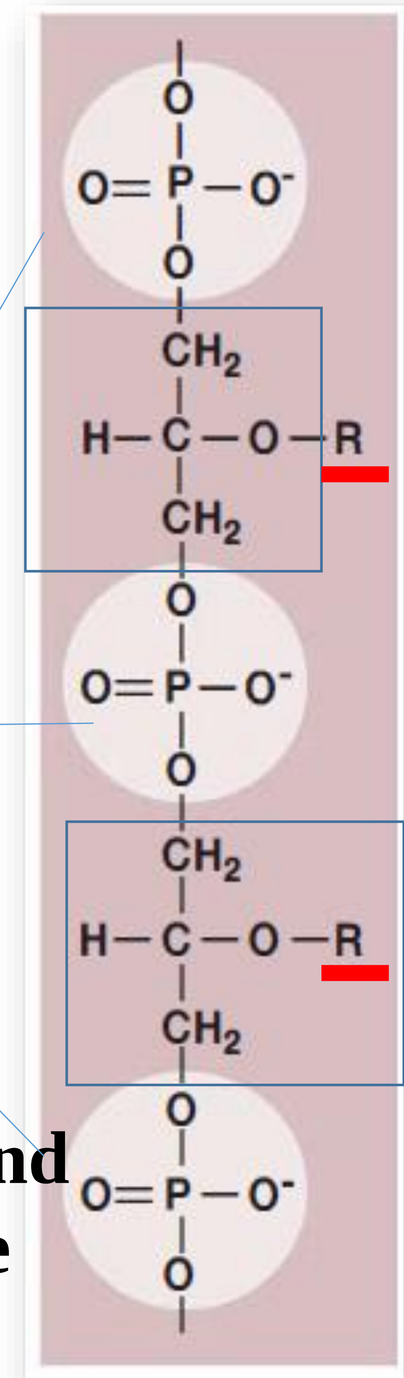
- **What is a TA?**

3.4.3 Typical Gram-Positive Cell Walls

- **TAs are polymers of glycerol or ribitol joined by phosphate groups**
- **TA are covalently connected to PTG or plasma membrane lipids(Lipoteichoic acid,LTA).**



Negative charges

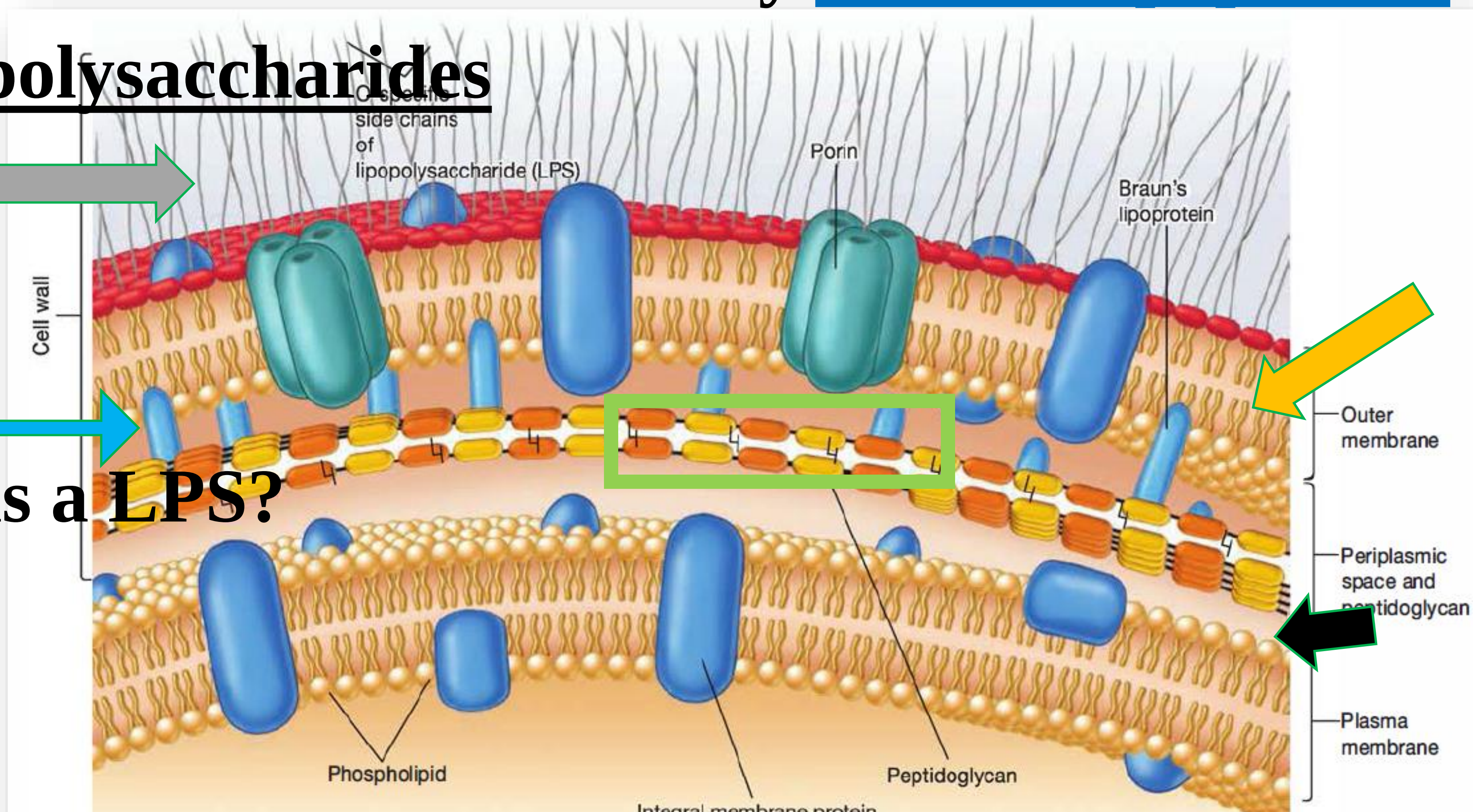


They help create and maintain the structure and protect the cell from harmful substances in the environment.

- **How about of G⁻?**

3.4.3 Typical Gram-Negative Cell Walls

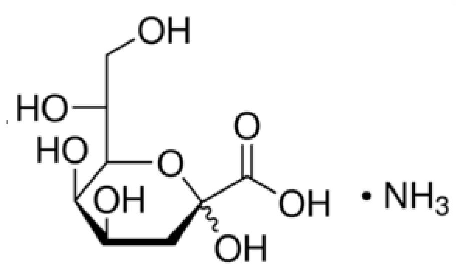
- The **PTG** layer is very **thin** (2 to 7 nm) and sits within the **wide periplasmic space**(metabolism).
- The **outer membrane** lies outside the thin PTG layer. It is linked to the cell by **Braun's lipoprotein**
- **Lipopolysaccharides** (LPSs)



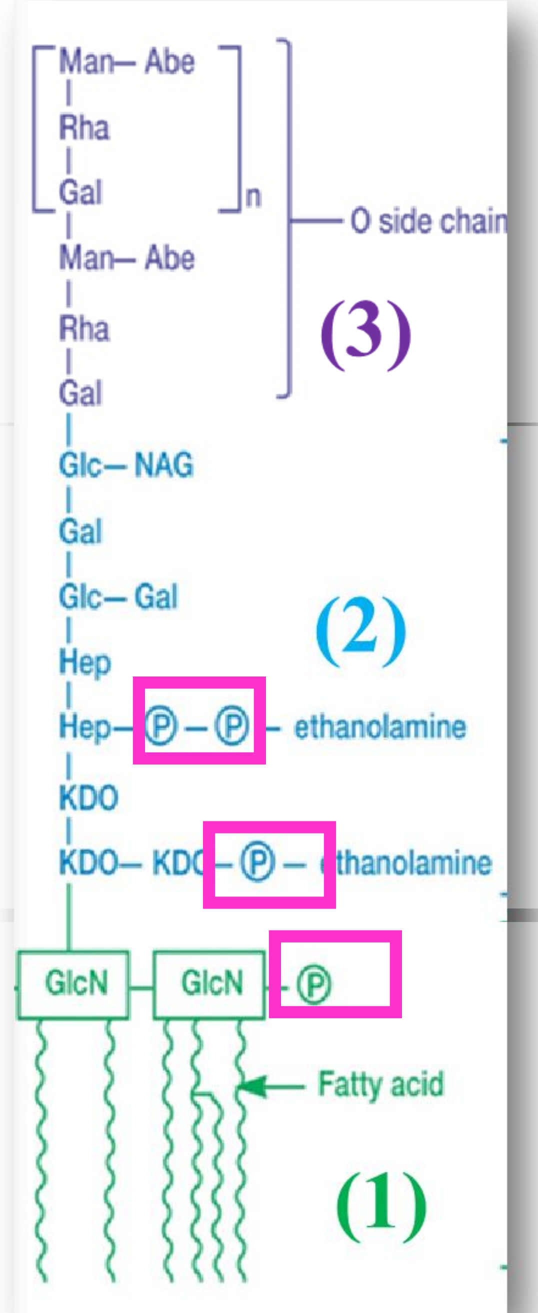
What is a LPS?

3.4.3 Typical Gram-Negative Cell Walls

- **LPS**:
- **(3) the O side chain or O antigen extending outward from the core.**
- **(2) the core polysaccharide: Joined to lipid A ,10sugars(KDO) (-)**
- **2-keto-3-deoxyoctonate**



- **(1) lipid A, contains two GlcN, three fatty acids and phosphate or pyrophosphate attached.**
  . (*Salmonella* spp)



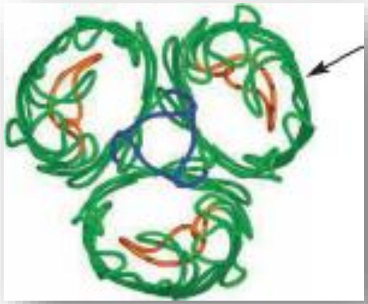
3.4.3 Typical Gram-Negative Cell Walls

LPS function:

- **(1) It contributes to the negative charge on the bacterial surface, create a permeability barrier.**
- **(2) LPS also plays a role in protecting pathogenic bacteria from host defenses. For example, G^- bacteria using the O antigen, such as *E. coli* O157; here the O side chain is the antigenic type number 157.**
- **(3) The lipid A portion of LPS is called endotoxin.**

Outer membrane

More permeable-pore protein



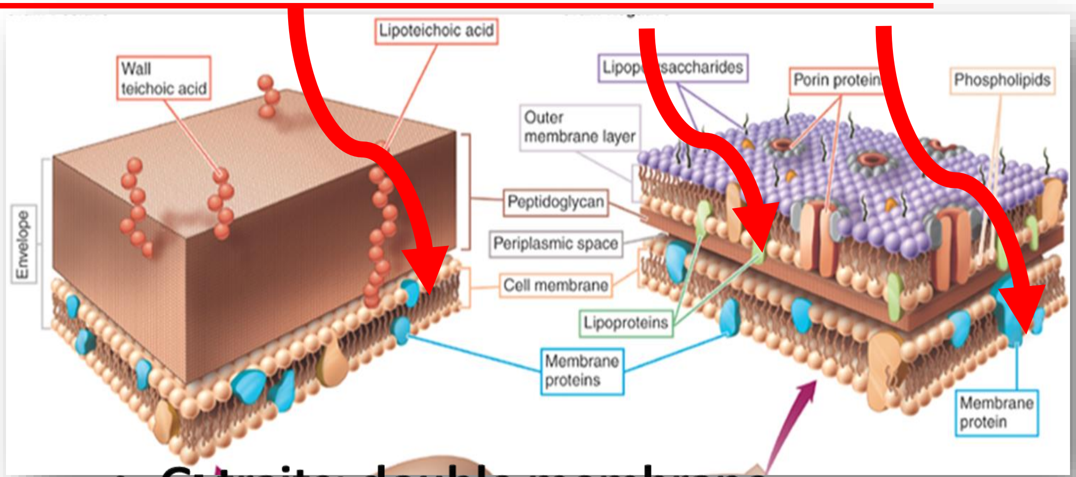
3.4.4 Mechanism of Gram Staining

- What is the procedure of Gram stain?
- How to prove cell wall is the main factor for Gram stain?
- What is the main difference of G+ and G- in cell wall?
- Try to explain the mechanism of Gram stain.

Summary

Mononderms and Diderms

- <u>G⁺</u> traits
- <u>PTG thick</u>, with <u>TA</u>
- Periplasmic space <u>is narrow</u>, with exoenzyme……
- Inter-bridge-$(Gly)_5$ between pentapeptides
- Amino acid(L-Lys)



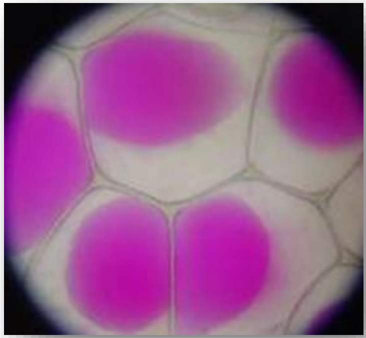
- <u>G⁻</u> traits: double membrane
- <u>PTG thin</u>, with <u>LPS</u>
- Periplasmic space <u>is wide</u>,
- Direct Crosslink-between pentapeptides
- DAP

- The phyla *Firmicutes* and *Actinobacteria* are <u>G^+</u> (Monoderms); Others are negative strains(Diderms).
- So, is it much better than the Gram (±)?

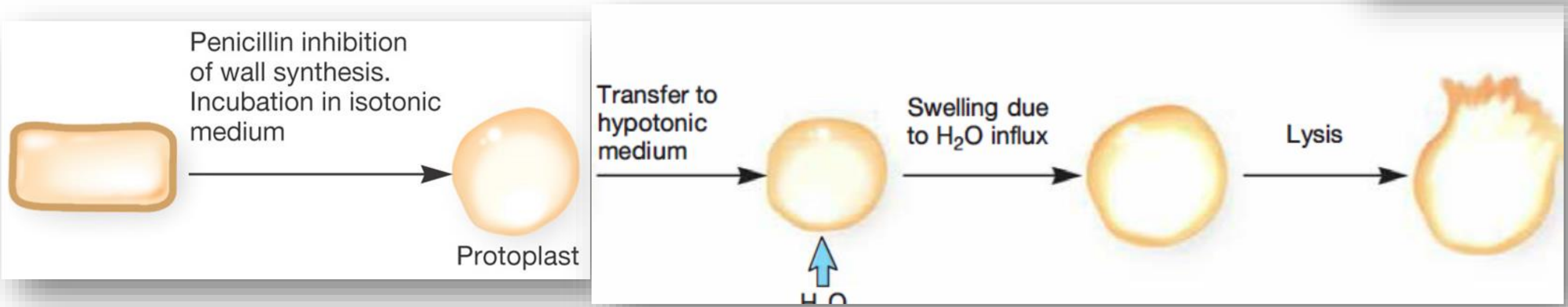
- **What is a consequence of lysis cell wall?**

- **How to lyse or destroy cell wall?**

3.4.5 Cell Walls and Osmotic Protection



Hypotonic(低渗):swells-
Hypertonic（高渗): Plasmolysis(质壁分离)
In isotonic solution(等渗）？

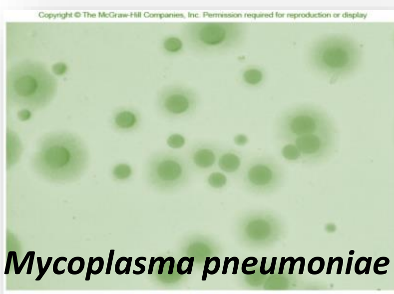


- **Breaks the bond between NAM-NAG (β-1,4 linkage)-lysozyme; Inhibits peptide bridge-penicillin**
- **Protoplasts(原生质体) G^+ ;**
- **Spheroplasts（球质体) (with little wall or out membrane) (G^-)?**

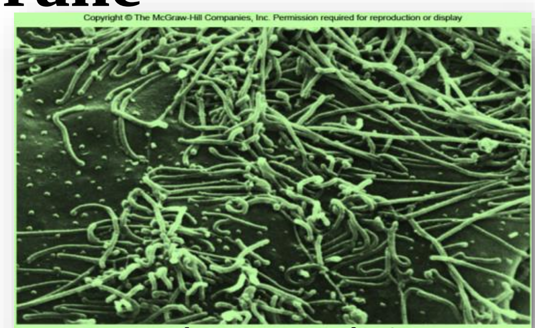
- **Do they take place spontaneously in bacteria?**

3.4.6 Bacteria that Lack Peptidoglycan or Cell Walls

- **Some stain G^- do not have PTG in their cell walls: phyla *Chlamydiae*(initial body) and *Planctomycetes*, but with outer membrane. Shaped.**
- **Mycoplasma（in nature）（G+？）**
  - **Does not produce a cell wall (pleomorphic)**
  - **Plasma membrane more resistant to osmotic pressure**
  - **Sterols may stabilize plasma membrane**



Mycoplasma pneumoniae

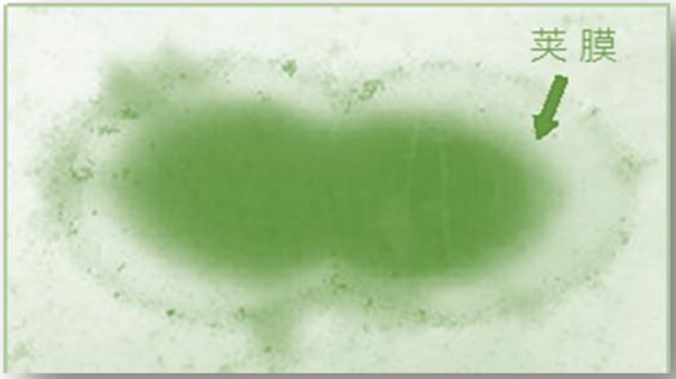


Ureaplasma urealyticum

L-form bacteria: cell wall-deficient (CWD) bacteria, are strains of bacteria that lack cell walls (in lab). They are likely to develop resistance for antibiotics.(?)

3.4 Bacterial Cell Walls- Summary

- **The cell wall is the layer that lies just outside the plasma membrane.(location)**
- **It helps maintain cell shape and protect the cell from osmotic lysis; it can protect the cell from toxic substances; and in pathogens, it can contribute to pathogenicity. (function)**
- **Cell walls are so important that most bacteria have them. (essential)**
- **Those that do not have other features that fulfill cell wall function-lack cell wall (but)**
- **Mycoplasma; *Chlamydiae* and *Planctomycetes***
- **L-form bacteria;**
- **Protoplasts; Spheroplasts**



MICROBIOLOGY

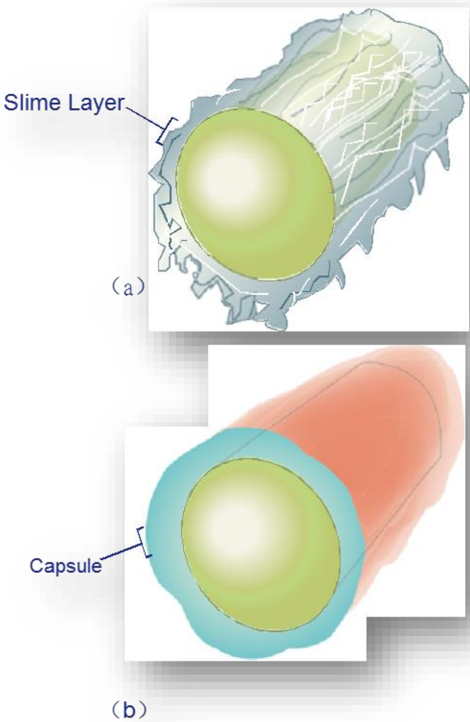
Lecture 2
Episode 2

3.5 Cell envelope layers outside the cell wall

张连茹

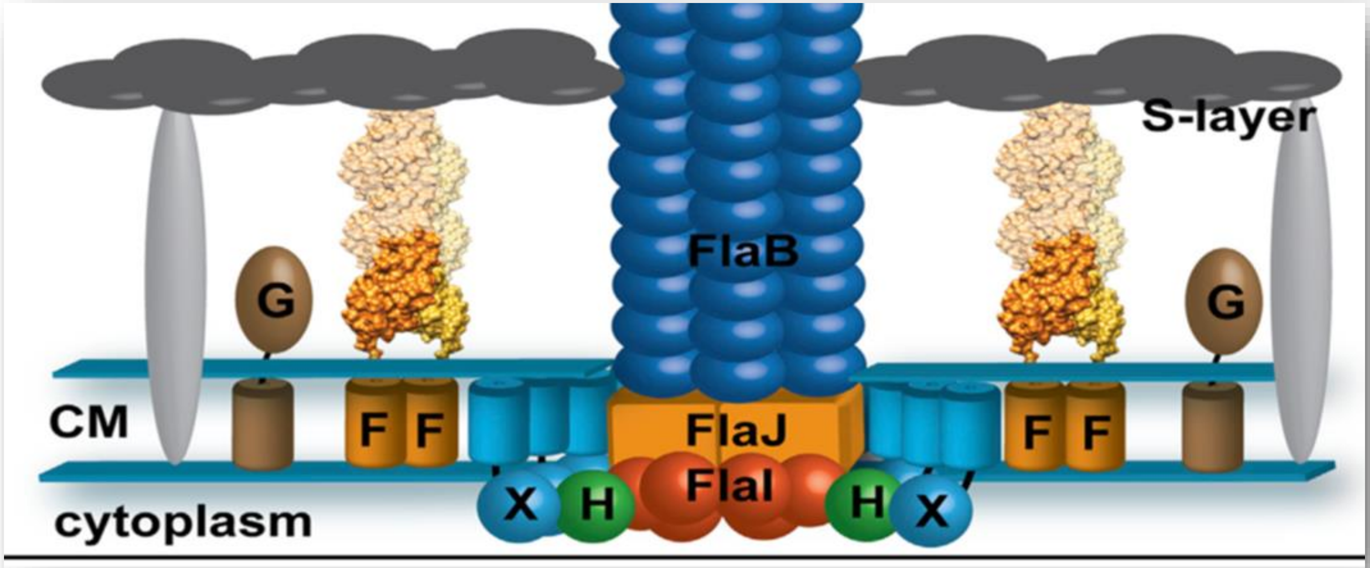
3.5.1 Capsules and slime layer

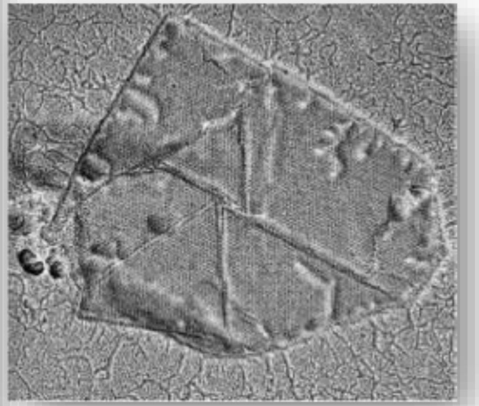
- **<u>Glycocalyx</u> – surface coating, made of sugars and/or proteins**
  - **Slime layer:loosely organized and attached(Gliding bacteria)**
  - **Capsule: highly organized, tightly attached**



3.5.2 S-Layers

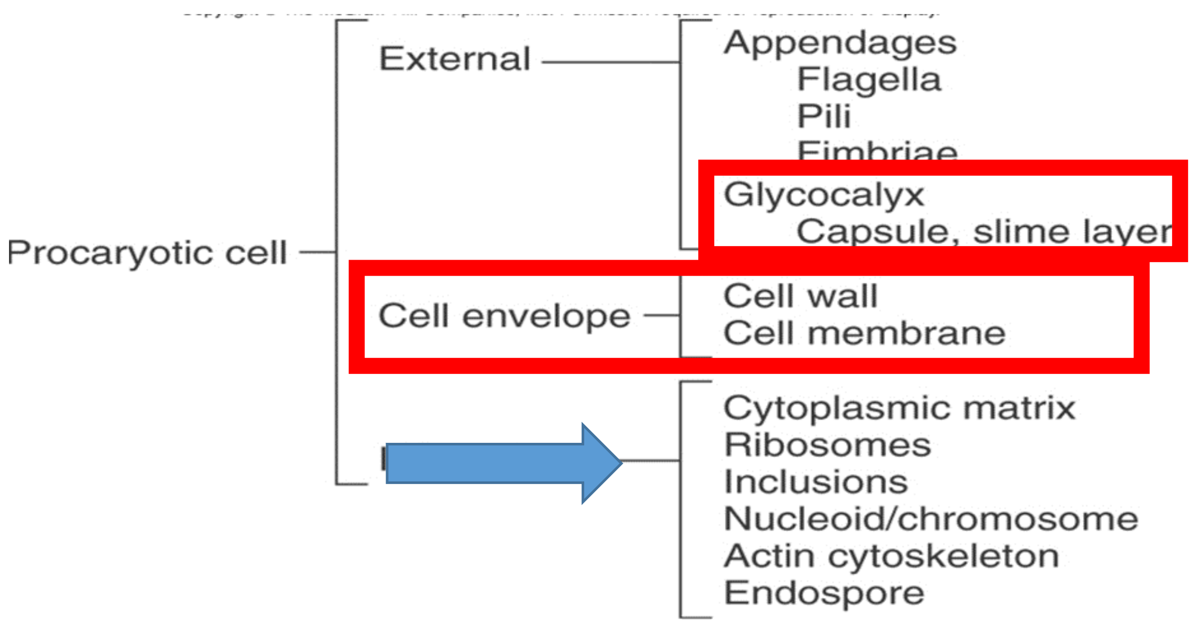
- **Many bacteria have a regularly structured layer called a S-layer on their surface.**
- **It is composed of protein or glycoprotein.**
- **The S-layer adheres directly to the outer membrane of G-; it is associated with the peptidoglycan surface of G+.**
- **S-layer proteins can self-assemble**
- **Structure 23, 863–872, May 5, 2015 (in Archaea)**





Summary

- **Outside coat: capsule and slime layer(glycocalyxes); S-layer**
- **Protect cells from dehydration and nutrient loss**
- **Inhibit killing by phagocytosis**
- **Attachment - formation of biofilms**
- **Exclude viruses and detergents**
- **Pathogenicity**



MICROBIOLOGY

Lecture 2
Episode 3

3.6 Bacterial cytoplasm

What is you opinion about of Bacterial cells were <u>bags of water </u>in which structures <u>floated</u>?

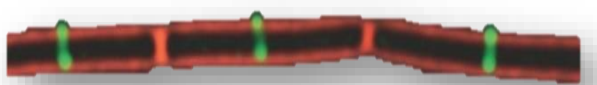
张连茹

3.6 Bacterial Cytoplasm

- Dense gelatinous solution of sugars, amino acids, and salts
- 70-80% water
  - serves as solvent for materials used in all cell functions
- **Do other`s cellular structures floated in this cytoplasm?**

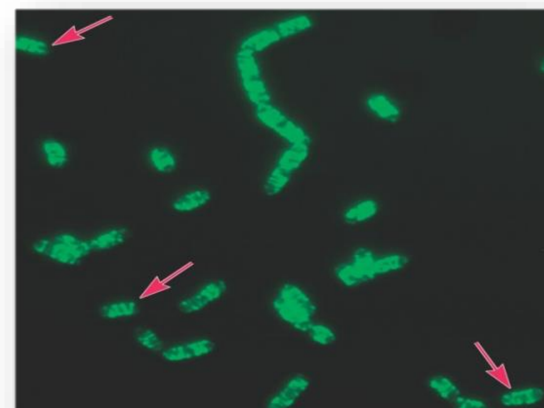
3.6.1 Bacterial Cytoskeleton

- **Homologs of all 3 eukaryotic cytoskeletal elements have been identified in bacteria**
- Evidence 1

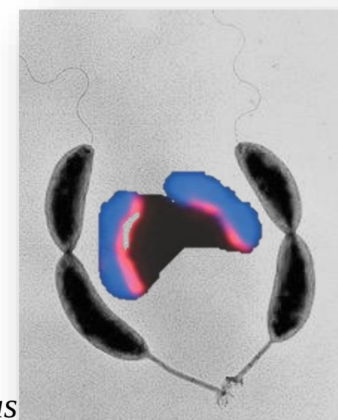


Tubulin(FtsZ)

Actin(MreB)



Filament (CreS)

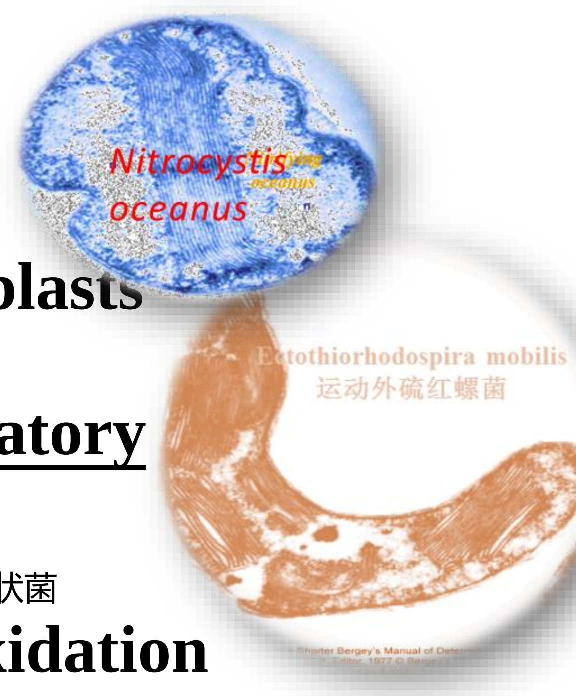


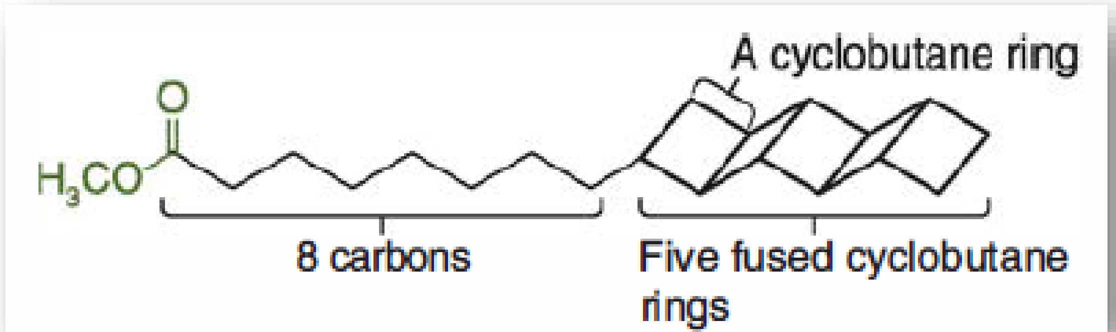
Caulobacter crescentus

They participate in cell division, localize proteins to certain sites in the cell, and determine cell shape.

3.6.2 Intracytoplasmic Membranes

- **Although members of Bacteria do not contain complex membranous organelles, but**
- **<u>Intracytoplasmic Membranes</u>**
- **Observed in many <u>photosynthetic bacteria</u>**
    - **Analogous to <u>thylakoids</u>类囊体of chloroplasts**
    - **Reactions centers <u>for ATP formation</u>**
- **Observed in many bacteria with high <u>respiratory</u> activity**_(Nitrocystis oceanus)_
- **Anammoxosome**厌氧氨氧化体**in *Planctomycetes***浮霉状菌
    - **organelle – site of anaerobic ammonia oxidation**
- **The anammoxosome membrane contains an unusual group of lipids called <u>ladderane lipids</u>**



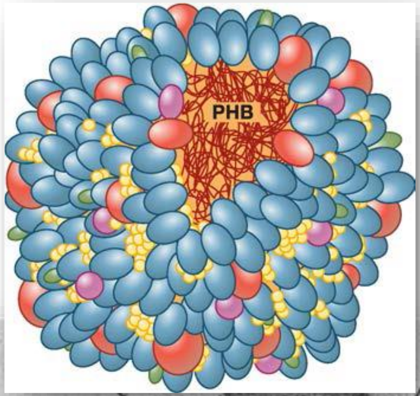


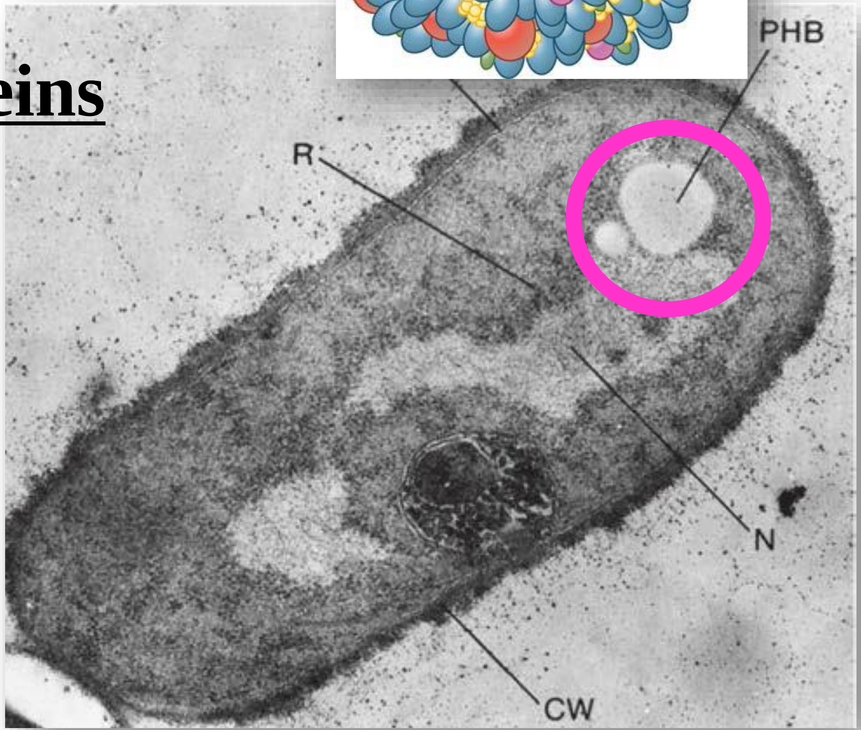
3.6.3 Inclusions

The first bacterial inclusions were discovered in the late 1800s.

- Intracellular **storage** bodies or to reduce osmotic pressure by tying up molecules in particulate form.

3.6.3.1 Storage Inclusions

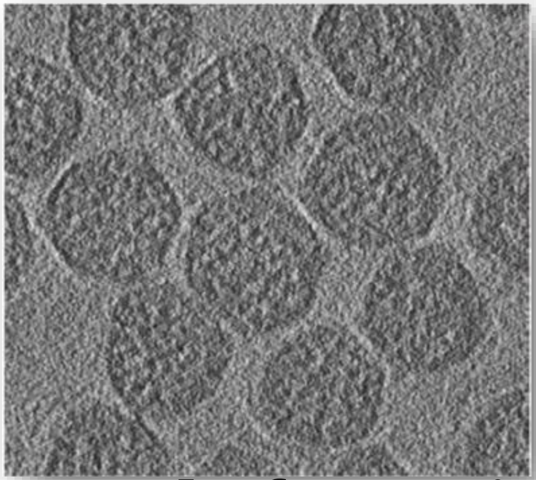




- Poly-beta-hydroxybutyrate(PHB),
- It is surrounded by a single-layered shell composed of proteins and a small amount of phospholipids
- In 1925, was isolated
- PHB/PHA- biodegradeable plastics

3.6.3.2 Microcompartments-functional inclusion

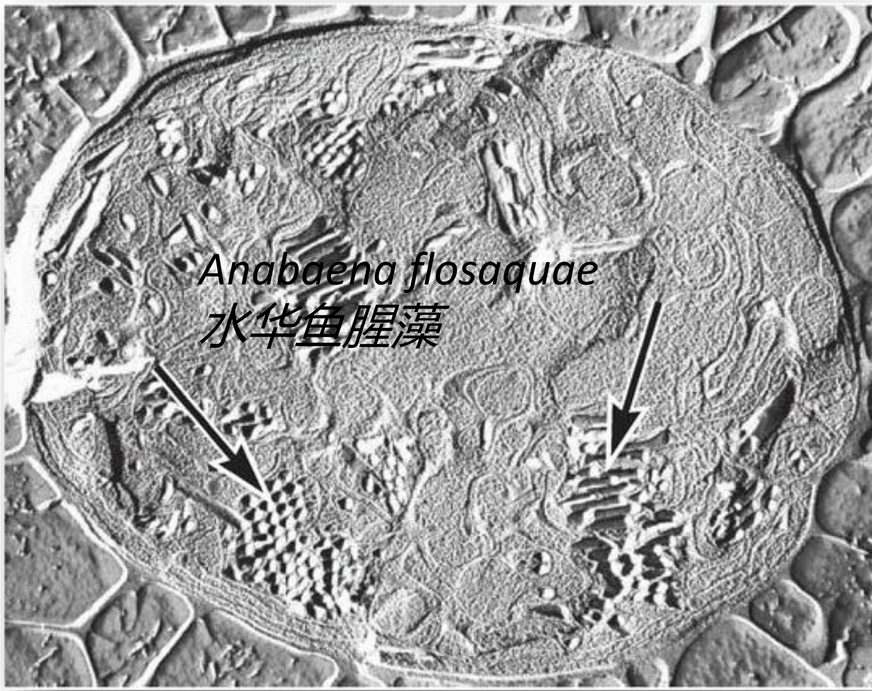
- **They are relatively large polyhedrons formed by one or more different proteins.**
- **Enclosed within the protein shell are one or more enzymes.**
- **Carboxysomes-fixed CO_2**

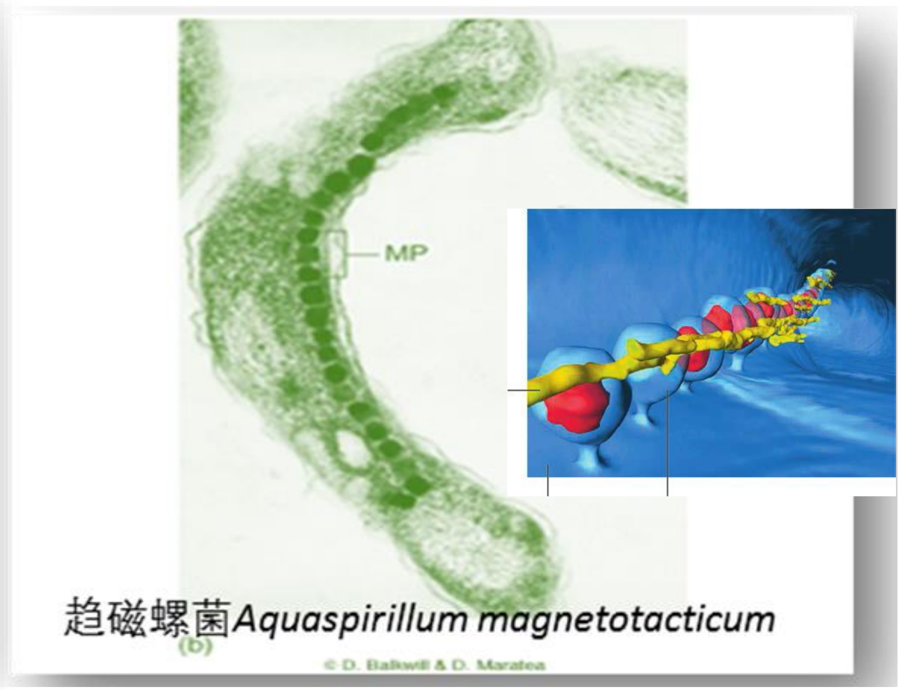


- **Their polyhedral coat is composed of proteins and is about 100 nm in diameter. Enclosed by the shell is the enzyme carbonic anhydrase碳酸酐酶, which converts carbonic acid into CO_2, ,then turn to -COOH**

3.6.3.3 Other Inclusions

- Gas vacuoles
- **Magnetosomes**: Fe_3O_4
- Both are involved in bacterial movement.



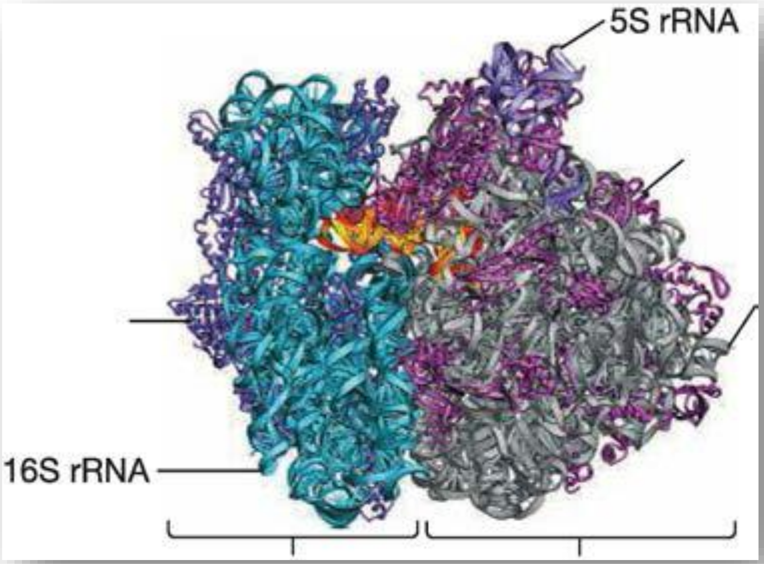


The gas vacuole provides buoyancy to some aquatic bacteria, many of which are photosynthetic.

For the cell to move properly within a magnetic field, magnetosomes must be arranged in a chain

[3.6.3.4 Bacterial Ribosomes]

- **Ribosomes are the site of protein synthesis, and large numbers of them are found in nearly all cells.**

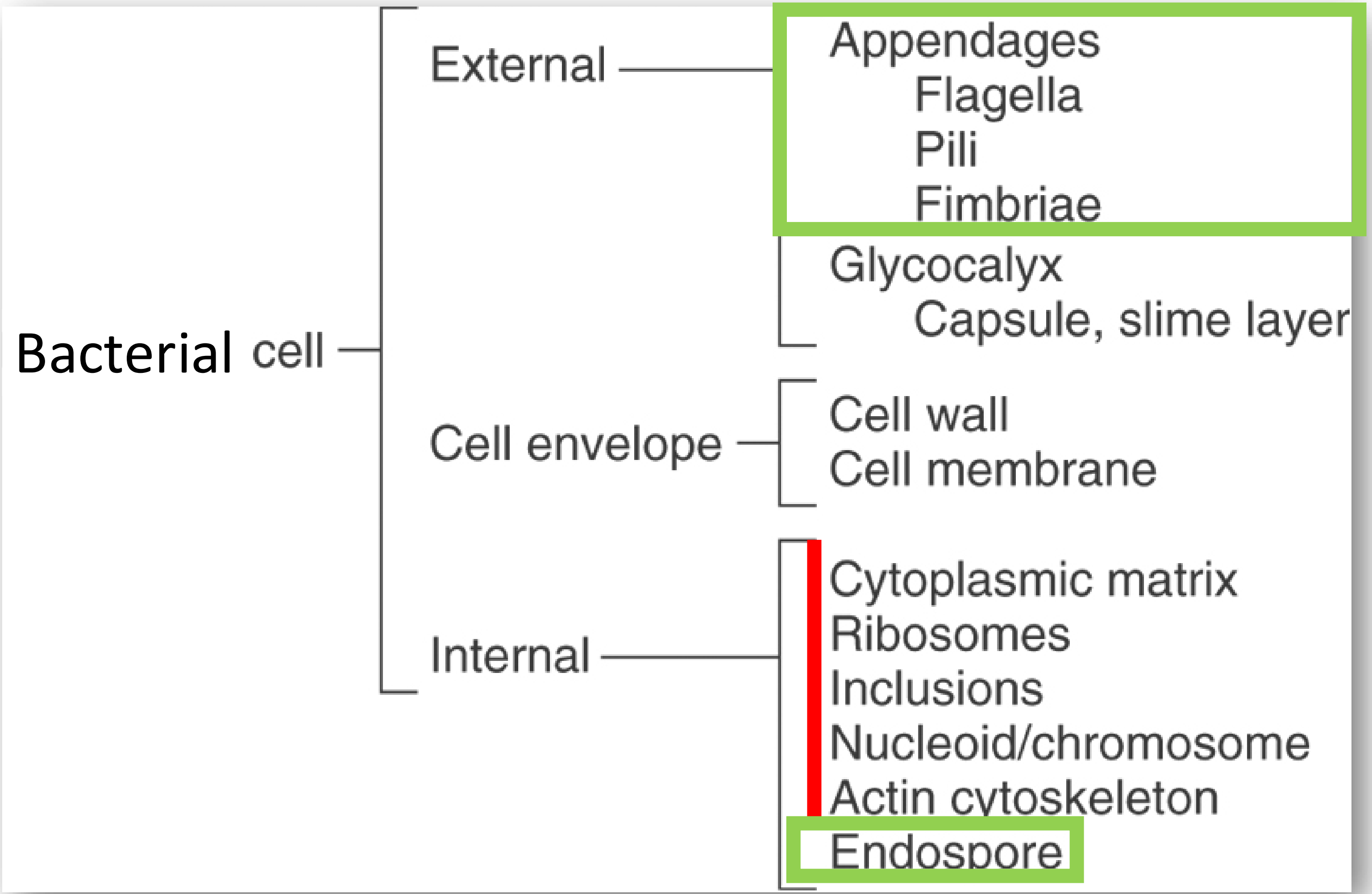


- **Bacterial ribosomes are called <u>70S</u> ribosomes.**
- **The small subunit contains [16S rRNA]+21Proteins;**
- **The large subunit contains 23S, 5S rRNA and 34 Proteins.**

3.6.3.5 Bacterial –DNA

- **Chromosome(DNA+NAPs)**
- **Plasmids are small, double-stranded DNA molecules(circular) that can exist independently of the chromosome.**
- **Duplicated and passed on to offspring**
- **Not essential to bacterial growth and metabolism**
- **May encode antibiotic resistance, tolerance to toxic metals, enzymes and toxins**
- **Episome: plasmid integrated into host DNA**
- **Curing:** The loss of a plasmid is called curing.
- **Curing treatments are acridine mutagens, ultraviolet and thymine starvation, antibiotics, and growth above optimal temperatures.**
- **Used in genetic engineering- readily manipulated and transferred from cell to cell.**

Summary: Bacterial cytoplasmic



Do bacteria move on the surface of Petri dish ?

厦门大学 生命科学学院
SCHOOL OF LIFE SCIENCES XIAMEN UNIVERSITY

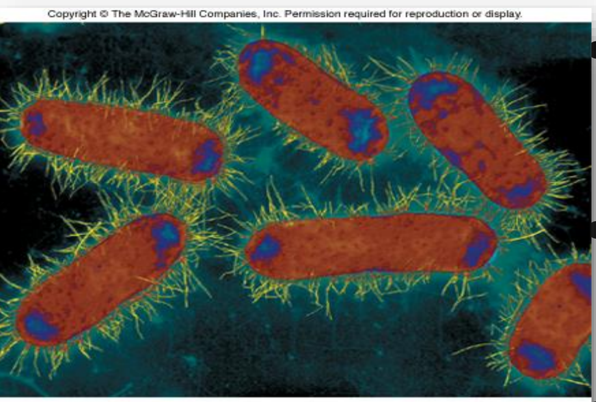
MICROBIOLOGY

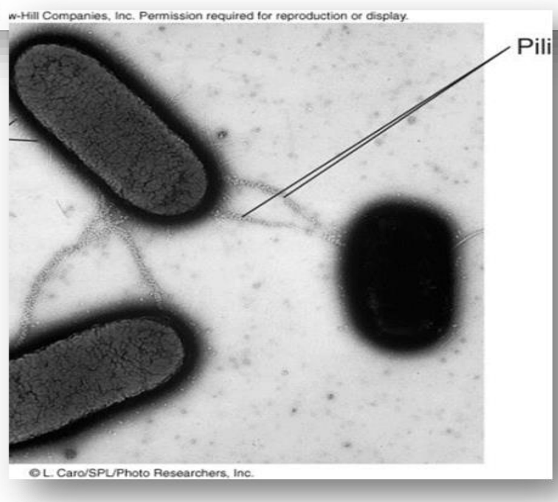
Lecture 2
Episode 4
3.7 External Structures

- **Many bacteria have structures that extend beyond the cell envelope, these external structures can function in protection and horizontal gene transfer.**

张连茹

3.7.1 Bacterial Pili and Fimbriae （伞毛/纤毛/菌毛）





- **Fine, hairlike appendages from the <u>cell surface (1,000 fimbriae)</u>**
- **Function in <u>adhesion</u> to other cells and surfaces(G+)**
- **Type IV pili, are involved in motility and the uptake of DNA during the process of bacterial transformation.**

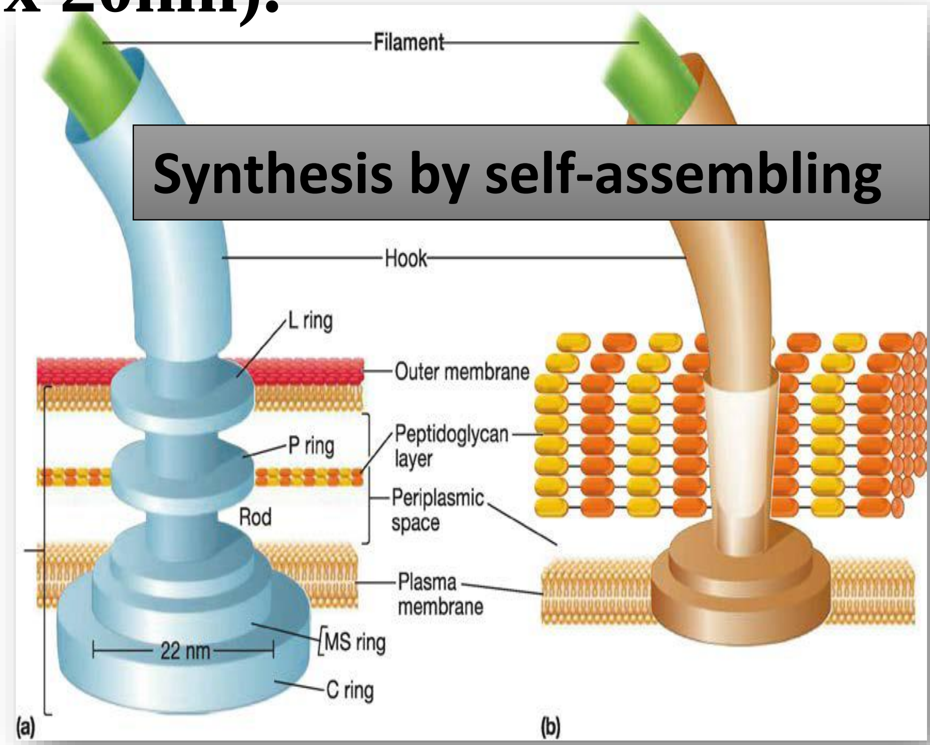
The difference of Sex pili(10)and fimbriae

- **<u>Larger</u> than other pili or fimbriae in diameter.**
- **Function to join bacterial cells for partial DNA transfer called <u>conjugation</u>**

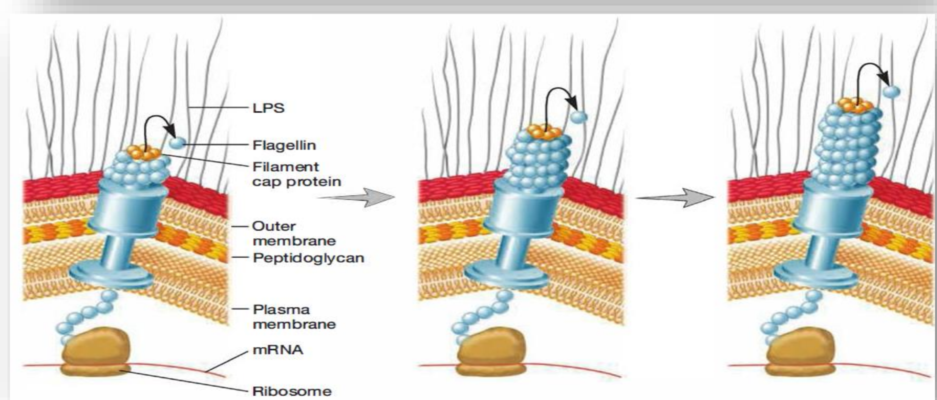
3.7.2 Bacterial Flagella

Many motile bacteria move by use of flagella, threadlike locomotor appendages(20um x 20nm).

- **<u>Filament</u> – long, thin, helical structure composed of <u>protein</u> flagellin**
- **<u>Hook</u>- curved sheath**
- **<u>Basal body</u> – stack of rings firmly anchored <u>in cell wall</u>**

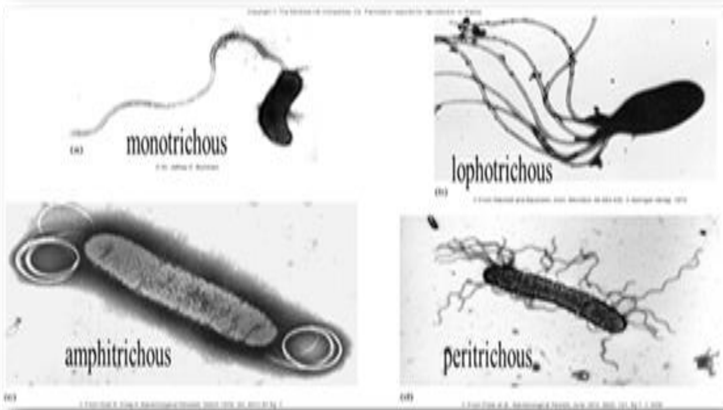


Synthesis by self-assembling



3.7.2 Bacterial Flagella

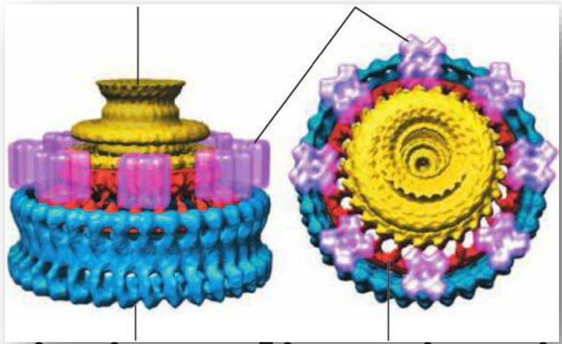
Flagellar Distribution-are useful in identifying bacteria.

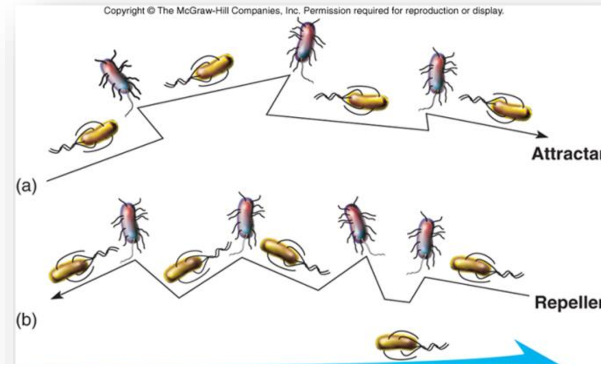


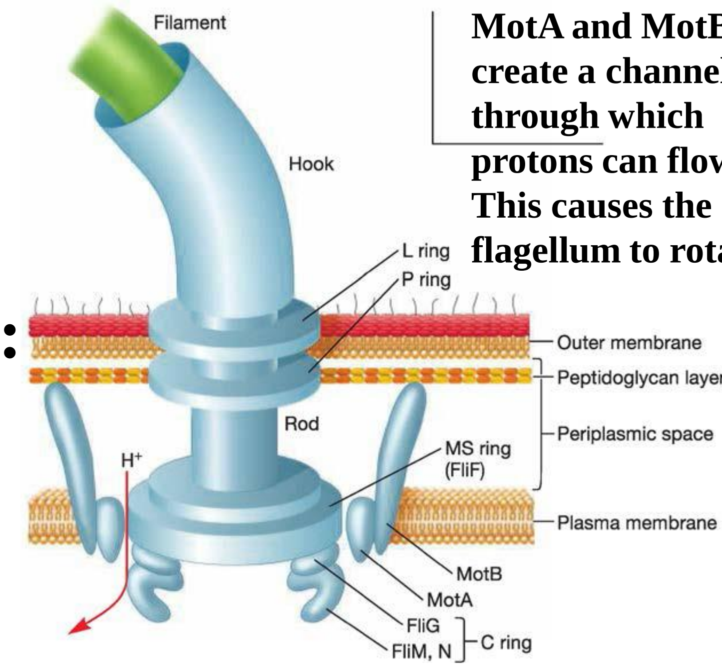
- **Monotrichous bacteria** (trichous means hair) have one flagellum
- **Amphitrichous bacteria** (amphi means on both sides) have a single flagellum at each pole.
- **Lophotrichous bacteria** (lopho means tuft) have a cluster of flagella at one or both ends
- **Peritrichous (peri means around), flagella are spread evenly over the whole surface**

3.8 Bacterial Motility and Chemotaxis

- **Signal sets flagella into rotary motion:rotates 360°**
- **counterclockwise – results in smooth linear direction – run**
- **clockwise - tumbles**







MotA and MotB create a channel through which protons can flow. This causes the flagellum to rotate.

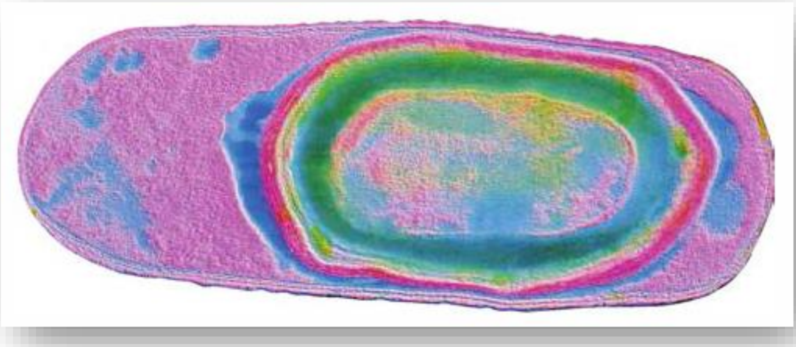
Guide bacteria in a direction in response to <u>experimental stimulus</u>:

<u>Chemical stimuli</u> – chemotaxis(趋化性);

<u>Light stimuli</u> – phototaxis(趋光性)

tracking microscope

MICROBIOLOGY



Lecture 2
Episode 5

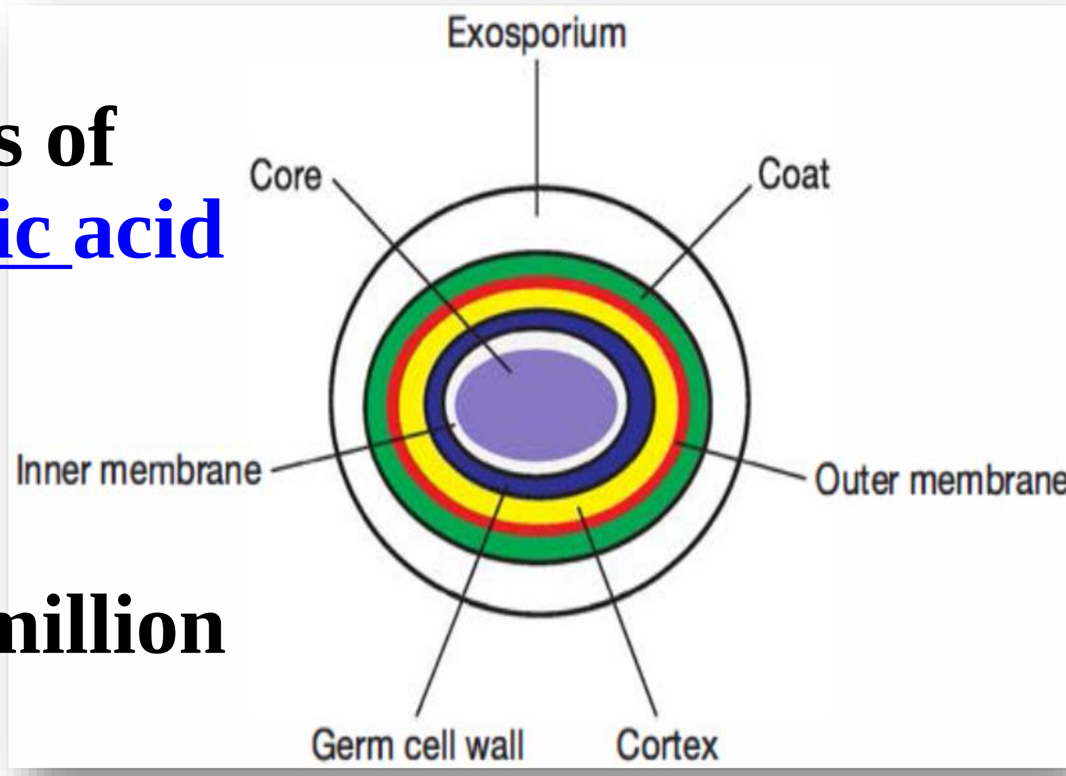
3.9 Bacterial Endospores

- Dormant cells formed within a so-called mother cell, are fascinating bacterial structures.

张连茹

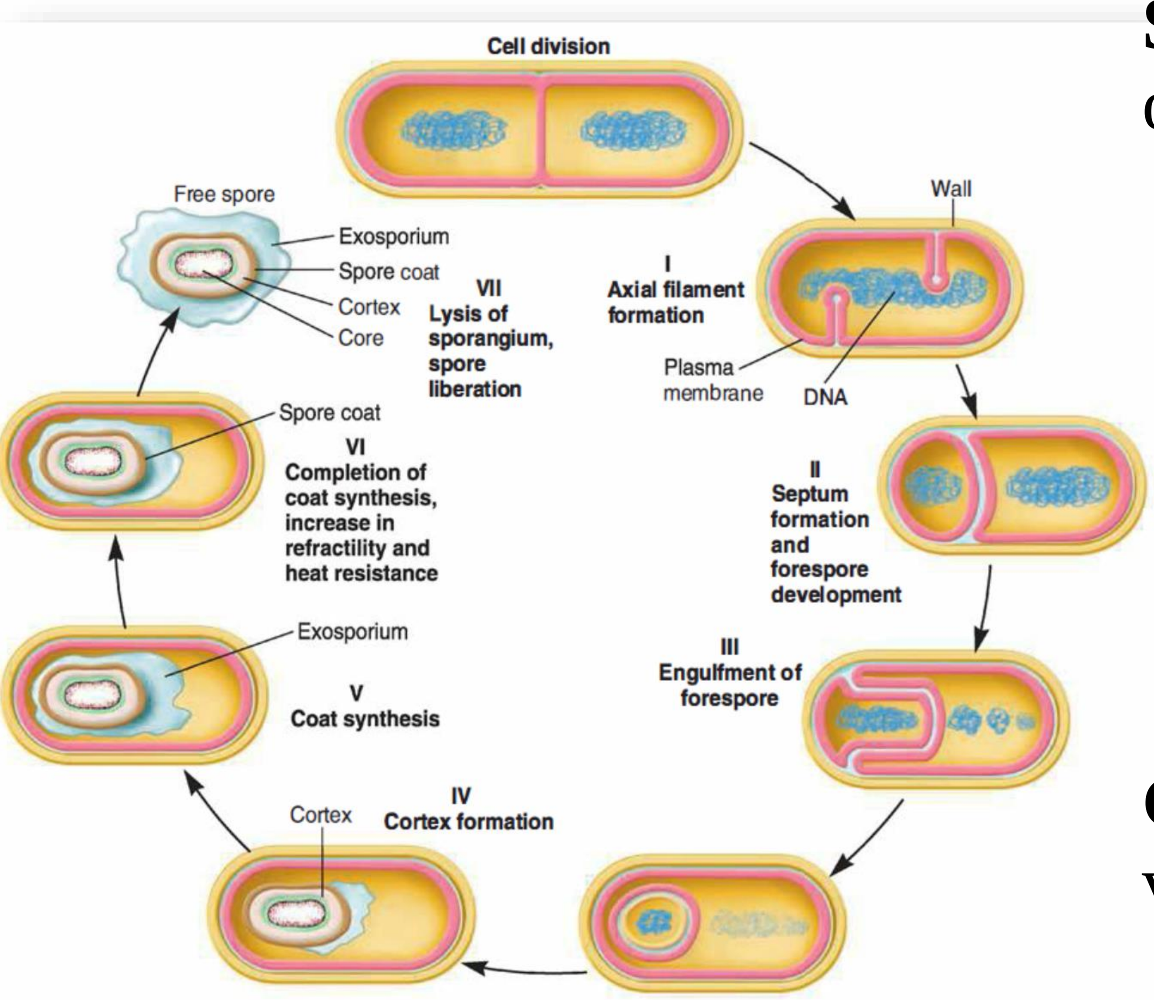
3.9. Bacterial Endospores

Inert, resting, cells produced by some G^{+} genera: *Clostridium*梭菌, *Bacillus and* *Sporosarcina*芽孢八叠球菌属**within the phylum *Firmicutes***



- **Resistance: high levels of calcium and dipicolinic acid**
- **Dehydrated（dry), metabolically inactive Longevity verges on immortality - 25,250 million years.**
- **Pressurized steam at 120ºC for 20-30 minutes will destroy**

3.9. Bacterial Endospores

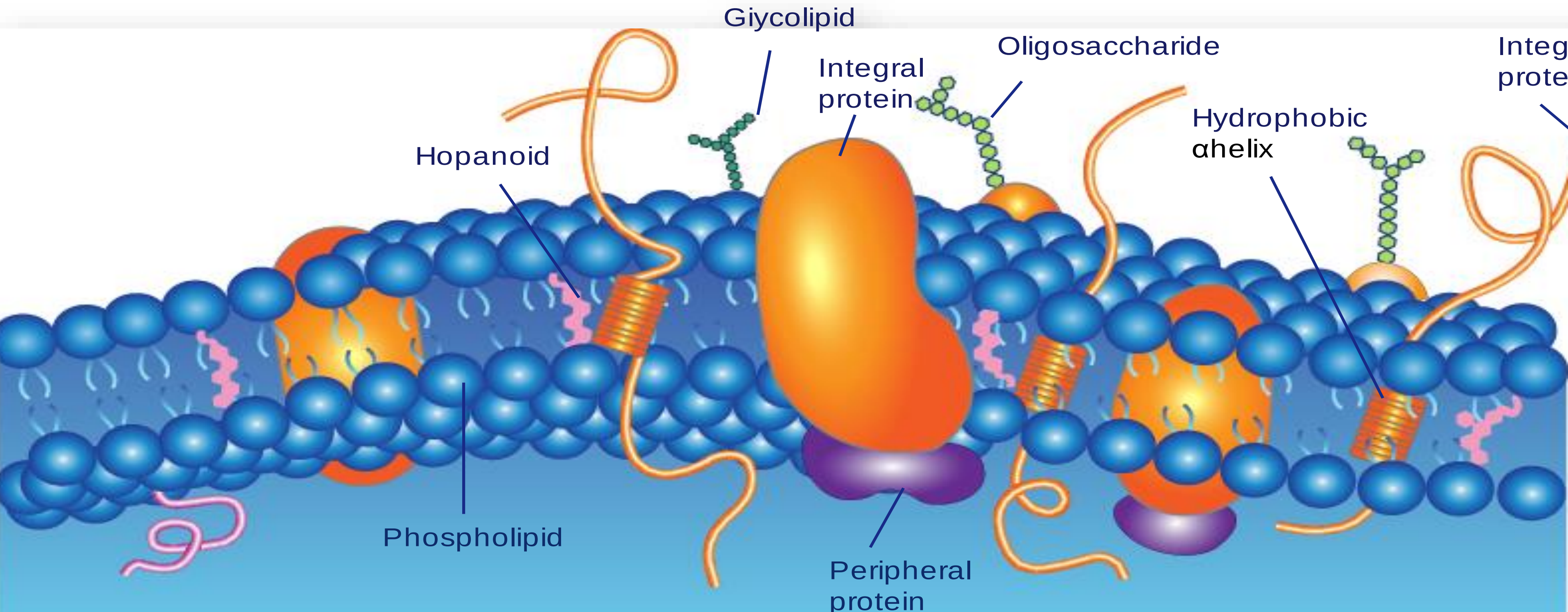


Sporulation -formation of endospores

- **Hardiest** of all life forms
- **Withstands** extremes in heat, drying, freezing, radiation,chemicals
- **Not** a means of **reproduction**

Germination- return to vegetative growth

Bacterial cytoplasmic structures



Cytoplasm; Inclusions

Cytoskeleton

Nucleoid and **plasmids** Ribosomes

endospores

Summary

- **Major prokaryotic cell structures**
- **The difference of bacterial plasma membrane and cell wall**
- **The structure of G- and G+ bacterial cell walls and the Gram reaction**
- **The external structures such as capsules, fimbriae, and flagella; the various arrangements of bacterial flagella**
- **The concept of the bacterial endospore and survive**
- **The s-layer**

Thanks!

Next-Archaeal Cell Structure

Discussion:

1. Prokaryotes（What is your opinion about of “The Prokaryote Controversy”? Please use three or more evidences to support your opinion）

2. Both bacteria and archaea can have S-layers. How does their use as components of the cell envelope differ?

3. Identify three features that distinguish archaeal plasma membranes from those of bacteria.

4. Provide two examples that illustrate the similarity of archaea to bacteria; list two examples of their similarity to eukaryotes.

5. Identify two other molecules that could be used to determine if a microbe having a typical prokaryotic architecture is a bacterium or an archaeon.

6. Compare and contrast nutrient uptake mechanisms observed in bacteria and archaea