Bacterial Structure and Function

Lecture 8

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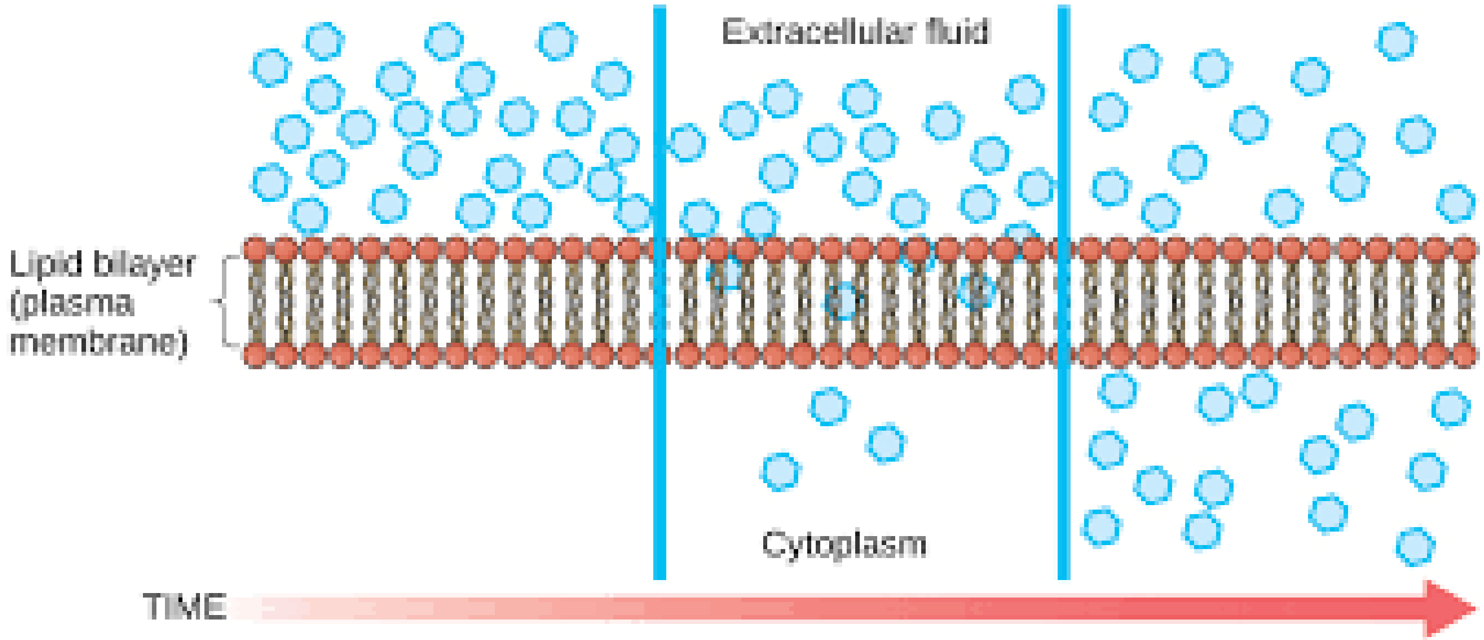
Uptake of Nutrients

- Microbes can only take in dissolved particles across a selectively permeable membrane
- Some nutrients enter by passive diffusion
- Microorganisms use transport mechanisms
  - Facilitated diffusion – all microorganisms
  - Active transport – all microorganisms
  - Group translocation – *Bacteria* and *Archaea*
  - Endocytosis – *Eukarya* only

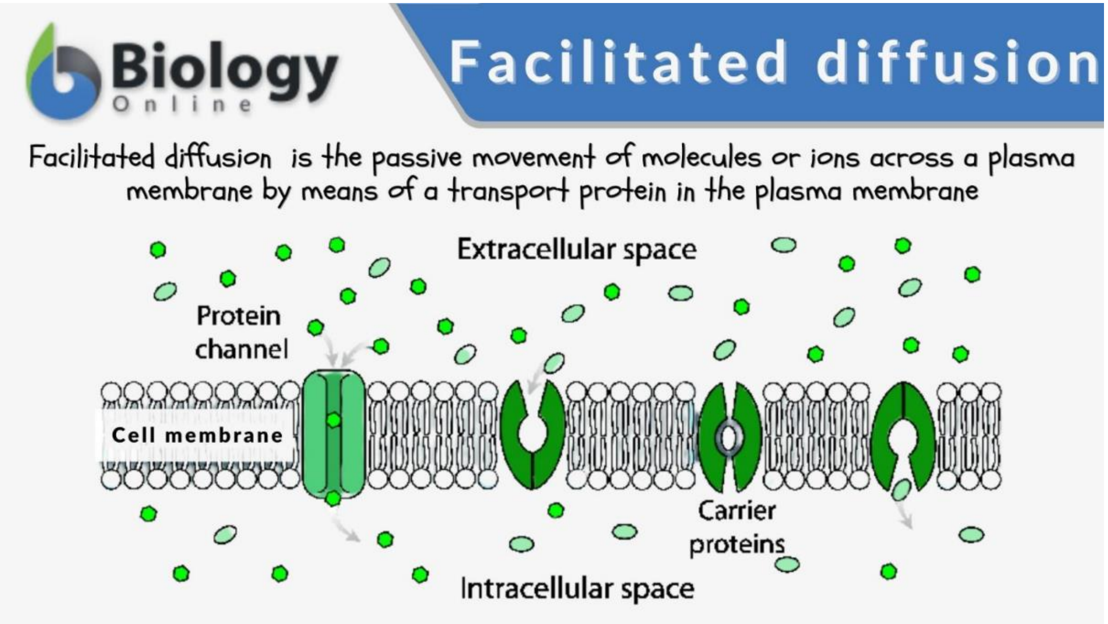
Passive Diffusion

- Molecules move from region of higher concentration to one of lower concentration between the cell's interior and the exterior
- H_2O, O_2, and CO_2 often move across membranes this way



Facilitated Diffusion

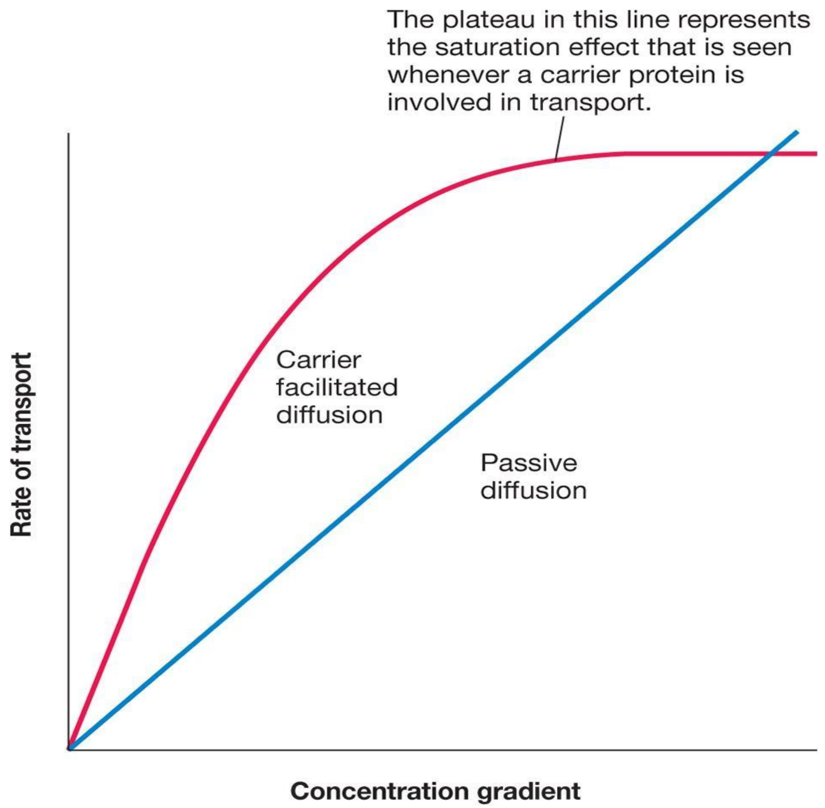
- Similar to passive diffusion
  - Movement of molecules <u>is not</u> energy dependent
  - Direction of movement is from high concentration to low concentration
  - Size of concentration gradient impacts rate of uptake



- Differs from passive diffusion
  - uses membrane bound carrier molecules (permeases)
  - smaller concentration gradient is required for significant uptake of molecules
  - effectively transports

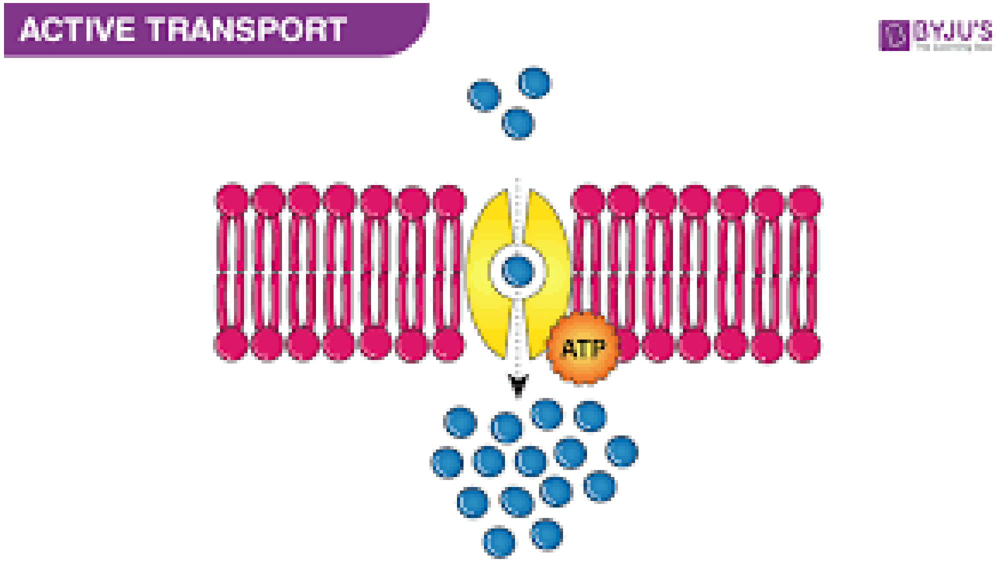
  glycerol, sugars, and amino acids
- more prominent in eukaryotic cells than in bacteria or archaea

Facilitated Diffusion…



Active Transport

- Energy-dependent process
  - ATP or proton motive force used
- move molecules against the gradient concentrates molecules inside cell
- involves carrier proteins (permeases)
  - carrier saturation effect is observed at high solute concentrations



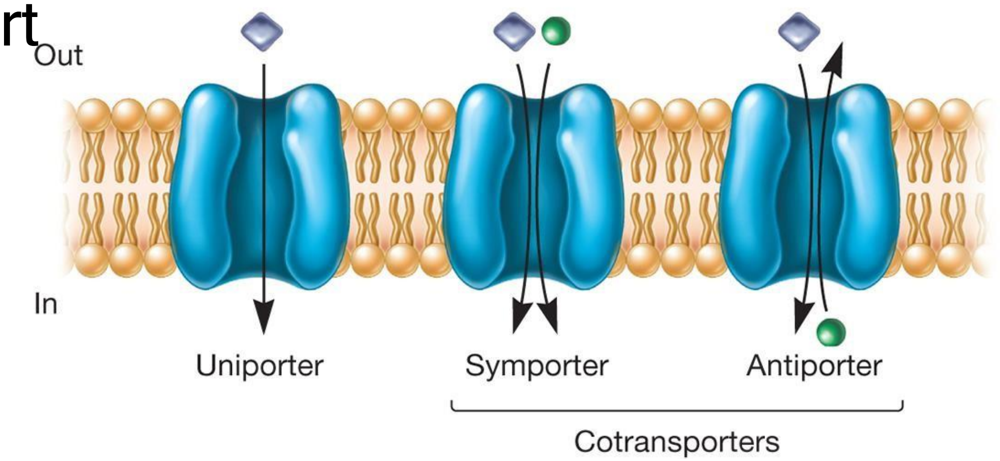
Active Transport

❖ Two types:

- Primary and secondary active transport both move molecules against their concentration gradients.
- **Primary active transport** directly uses chemical energy, such as ATP, to move substances across a membrane.
- **Secondary active transport** uses the energy stored in an electrochemical gradient, which was established by primary active transport, to move a different molecule against its gradient.

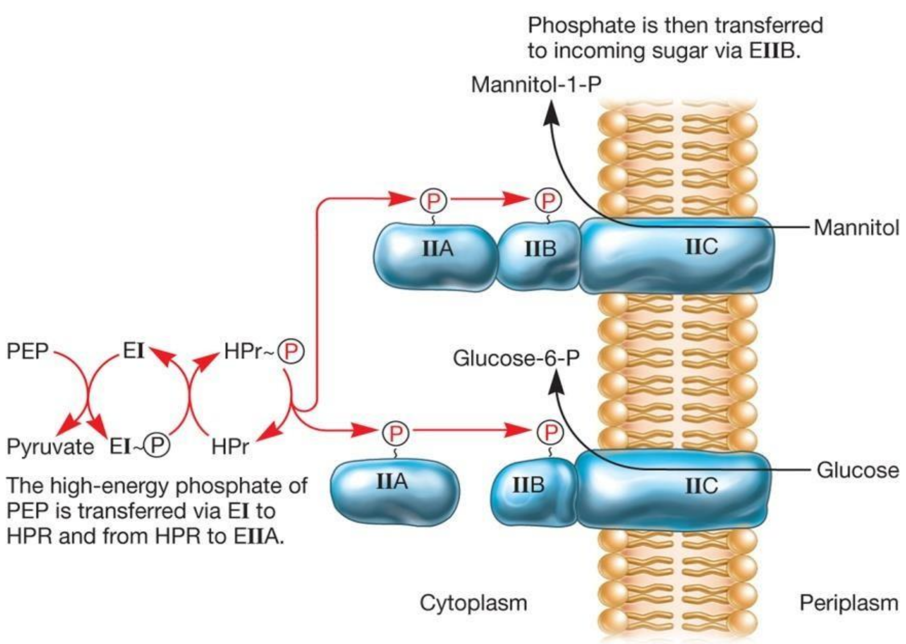
Secondary Active Transport

- Major facilitator superfamily (MFS)
- Use ion gradients to cotransport substances
  - Protons
  - Symport - two substances both move in the same direction
  - Antiport - two substances move in opposite directions
  - All of these transporters can also transport small, uncharged organic molecules like glucose.



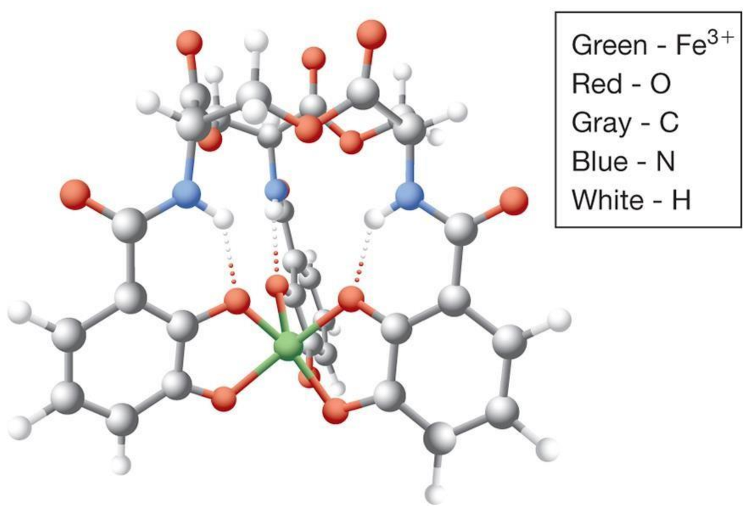
Group Translocation

- <u>Energy dependent</u> transport, that chemically modifies molecule as it is brought into cell
- Best known translocation system is <u>phosphoenolpyruvate</u>: sugar phosphotransferase system (PTS)
- Not all bacteria have this system



Iron Uptake

- Microorganism s require iron
- Ferric iron is very insoluble so uptake is difficult
- Microorganisms secrete **siderophores** to aid uptake
- Siderophore small, high-affinity iron-chelating compounds.
- Complex is then transported into cell



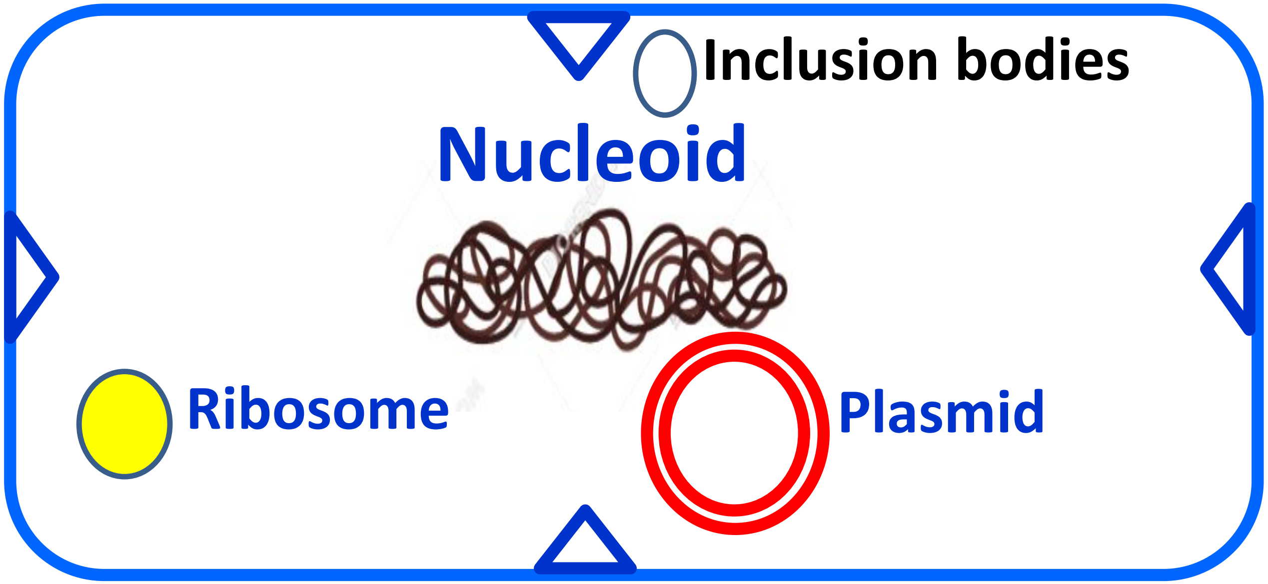
Bacterial Cytoplasmic Structures

- Intracytoplasmic membranes

  - Cytoskeleton
  - Inclusions
  - Ribosomes
  - Nucleoid
  - Mesosome
  - Plasmids

Intracytoplasmic

Cell Membrane



Mesosome

Protoplast and Cytoplasm

- Protoplast - is a whole bacterial cell that has its cell wall removed, and therefore includes the cytoplasm and the plasma membrane enclosing it.
- Cytoplasm – is the internal fluid of a bacterial cell.

The Cytoskeleton

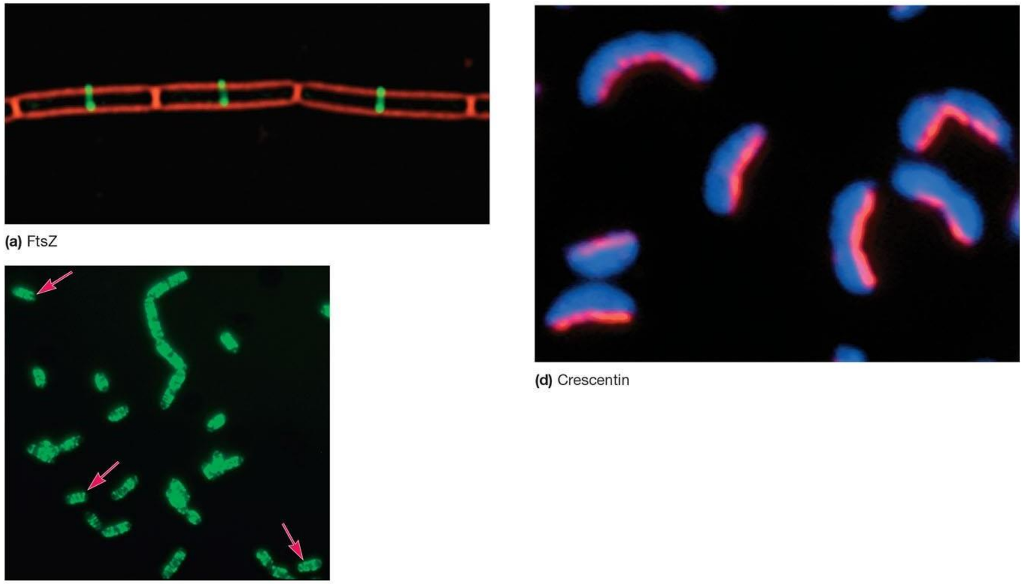
- Homologs of all 3 eukaryotic cytoskeletal elements (Microfilaments, Intermediate filaments, Microtubules)have been identified in bacteria
- Functions are similar as in eukaryotes

Table 3.2 Bacterial Cytoskeletal Proteins

Type	Function	Comments
Tubulin Homologues		
FtsZ	Cell division	Widely observed in bacteria and archaea
BtubA/BtubB	Unknown	Observed only in *Prosthecobacter* spp.; thought to be encoded by eukaryotic tubulin genes obtained by horizontal gene transfer
TubZ	Possibly plasmid segregation	Encoded by large plasmids observed in members of the genus *Bacillus*
Actin Homologues		
MamK	Positioning magnetosomes	Observed in magnetotactic species
MreB/Mbl	Helps determine cell shape, may be involved in chromosome segregation, localizes proteins	Most rod-shaped bacteria
ParM	Plasmid segregation	Plasmid encoded
Intermediate Filament Homologues		
CreS (crescentin)	Induces curvature in curved rods	*Caulobacter crescentus*
Unique Bacterial Cytoskeletal Proteins		
MinD	Prevents polymerization of FtsZ at cell poles	Many rod-shaped bacteria
ParA	Segregates chromosomes and plasmids	Observed in many species, including *Vibrio cholerae, C. crescentus,* and *Thermus thermophilus*

Best Studied Examples

- FtsZ - many bacteria
  - forms ring during septum formation in cell division
- MreB - many rods
  - maintains shape by positioning peptidoglycan synthesis machinery
- CreS - rare, maintains curve shape

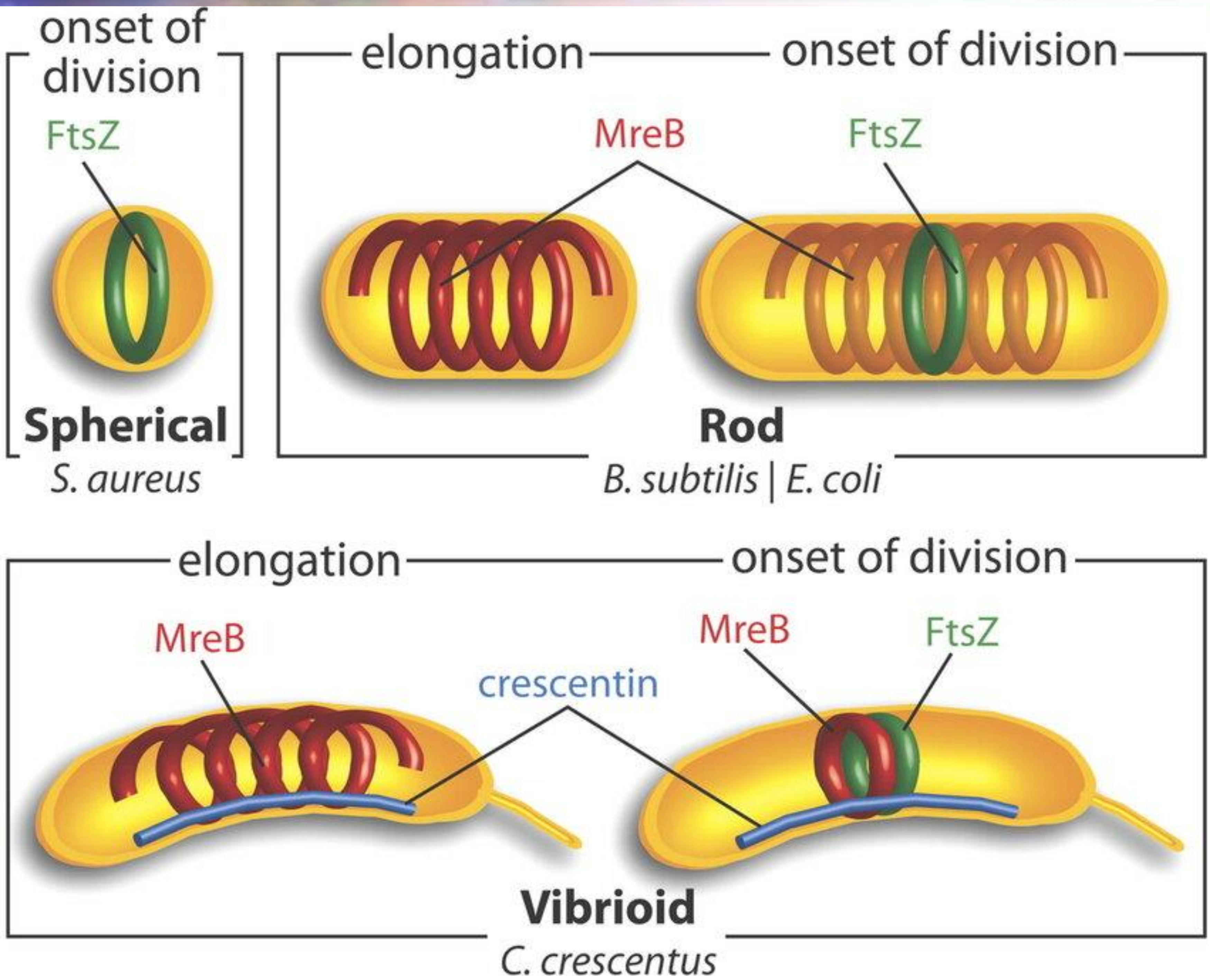


(a) FtsZ

(b) Mbl

(d) Crescentin

a: Dr. Joseph Pogliano; b: Image courtesy of Rut Carballido-Lo'pez and Jeff Errington; d: Dr. Christine Jacobs-Wagner



onset of division
FtsZ
Spherical
S. aureus
elongation
onset of division
MreB
FtsZ
Rod
B. subtilis | E. coli
elongation
onset of division
MreB
crescentin
MreB
FtsZ
Vibrioid
C. crescentus

Inclusions

- Granules of organic or inorganic material that are <u>stockpiled</u> by the cell for future use
- <u>**Some** are enclosed by a single-layered membrane</u>
  - membranes vary in composition
  - some made of proteins; others contain lipids
  - may be referred to as *microcompartments*

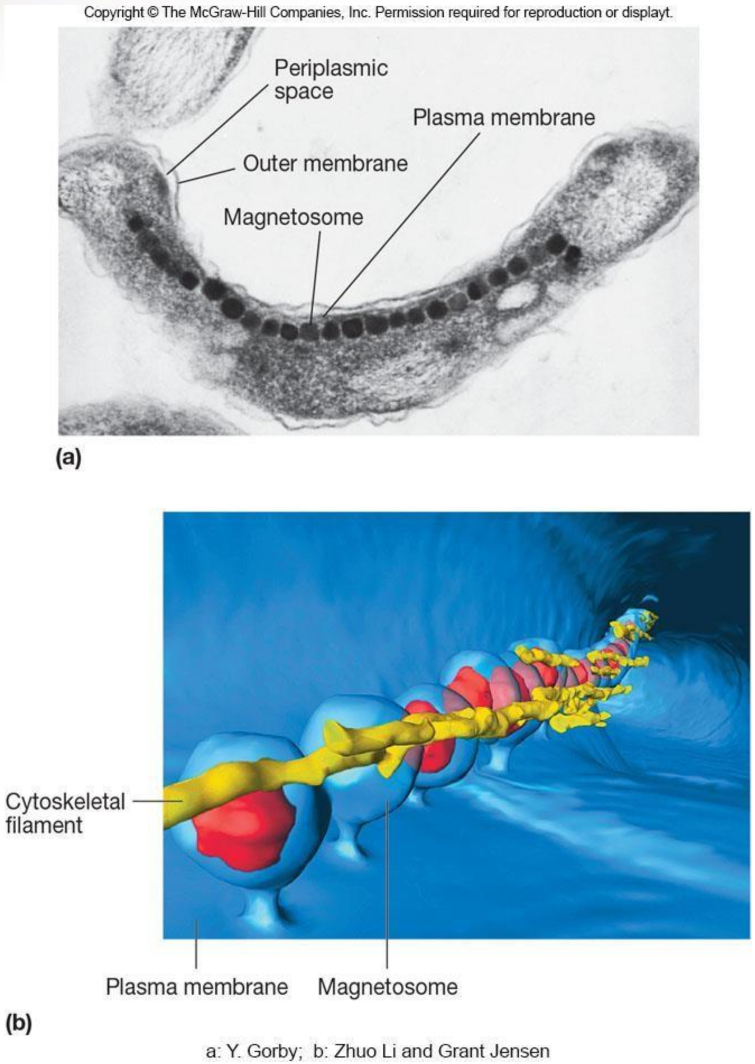
Storage Inclusions

- Storage of nutrients, metabolic end products, energy, building blocks

- Glycogen storage
- Carbon storage
  - poly-β-hydroxybutyrate (PHB)
- Phosphate - (**Volutin** granules) also known as metachromatic granules

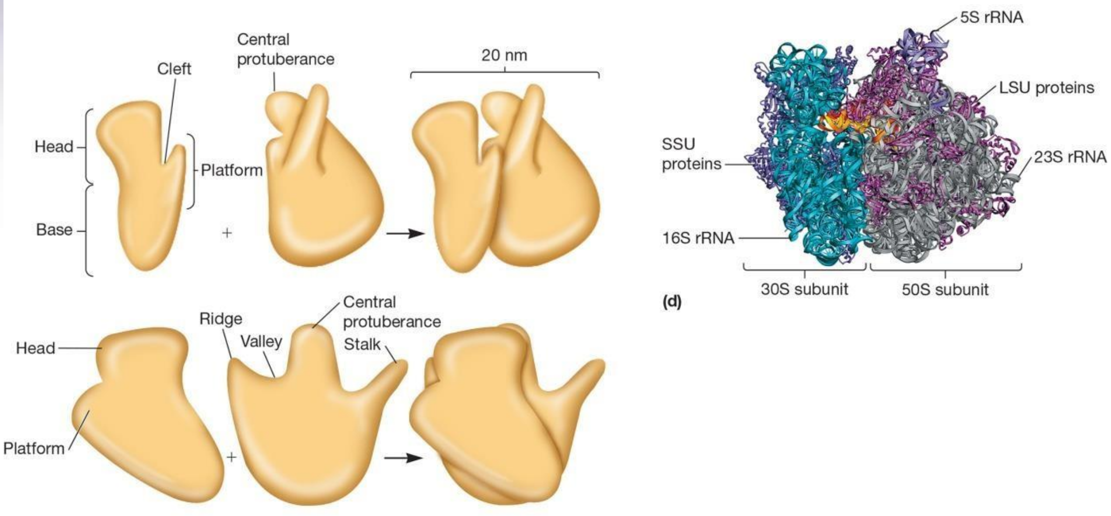
  * ***Corynebacterium diphtheriae***
- Amino acids - cyanophycin granules

Other Inclusions



- Magnetosomes
  - found in aquatic bacteria
  - magnetite particles for orientation in Earth's magnetic field
  - cytoskeletal protein **MamK**
    - helps form magnetosome chain

Ribosomes



(a) 30S subunit (b) 50S subunit (c) 70S ribosome

Some antibiotic effect on Ribosomes

- **Streptomycin**
- **Erythromycin**

- Complex protein/RNA structures
  - sites of protein synthesis
  - bacterial and archaea ribosome = 70S
  - eukaryotic (80S) S = Svedburg unit
- Bacterial ribosomal RNA (rRNA)
  - 16S small subunit
  - 23S and 5S in large subunit

The Nucleoid

- Usually not membrane bound (few exceptions)

  * *Planctomycetes* bacteria

- Location of chromosome and associated proteins

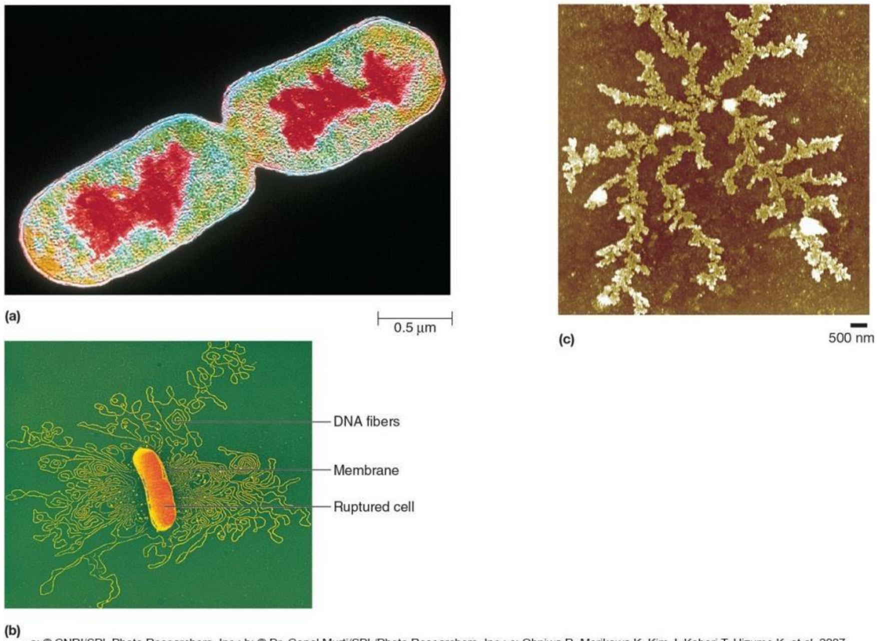
❖**Single chromosome**

❖ **Circular**

❖**dsDNA**

❖**1mm in length**

❖Supercoiling and nucleoid proteins (different from histones) aid in folding

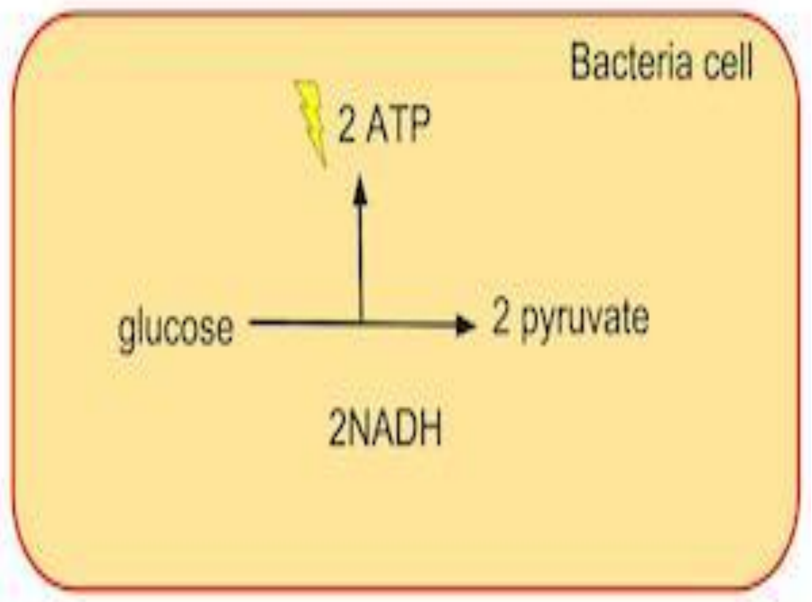


a: © CNRI/SPL Photo Researchers, Inc.; b: © Dr. Gopal Murti/SPL/Photo Researchers, Inc.; c: Ohniwa R. Morikawa K, Kim J, Kobori T, Hizume K, et al. 2007. *Microsc. Microana.* 13:3–12. Reprinted with the permission of Cambridge University Press

Mesosomes

Respiration enzyme

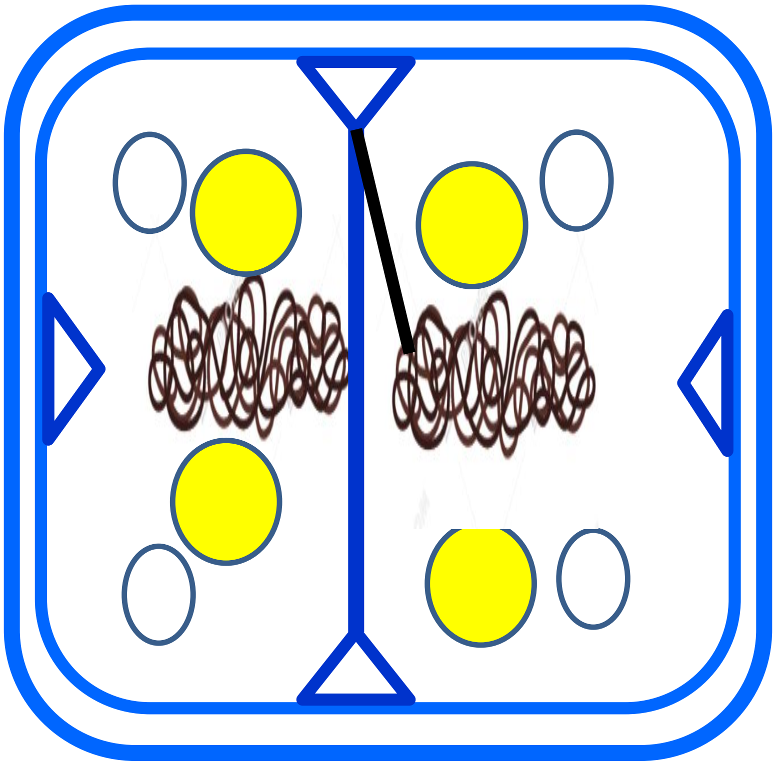
(Making energy) (Like Mitochondria)



(Mesosomes)

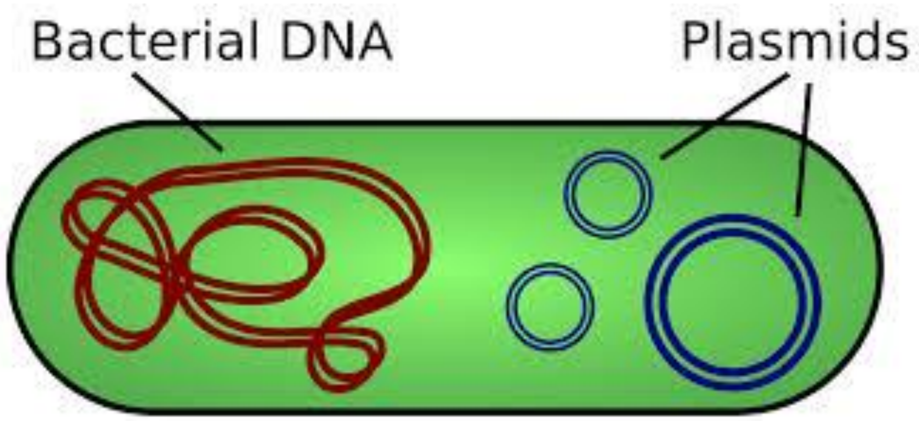
Cell division

- ✓ Separate DNA
- ✓ Septal mesosome



Plasmids

- Extrachromosomal dsDNA
  - found in bacteria, archaea, some fungi
  - usually small, closed circular DNA molecules
- Carrying genes for toxic products
- Carrying genes for drug resistant.
- Not essential for bacteria growth and survival.



The Bacterial Endospore

- A bacterial endospore is a dormant, tough, and non-reproductive structure.
- usually occurs in Gram-positive bacteria
- produced by certain bacteria, such as *Bacillus* and *Clostridium* species, to survive harsh conditions.
  - heat
  - radiation
  - chemicals
  - Desiccation
  - Nutrient deplition.

Endospore Structure

An endospore consists of the following 5 layers:

1. **Core:** It consists of nucleoid. It is a gel-like dehydrated state (10-25% water) and provides heat resistance to the endospore.
   - ✓ **Calcium dipicolinate**: enhances the heat resistance of endospores.
   - ✓ **Small acid soluble proteins (SASP)** protect DNA from UV radiation, desiccation and drying. Also provide nutrition and energy for spore germination.

2. Spore wall:

- ✓ Surrounding the core. It contains normal **peptidoglycan**

3. Cortex:

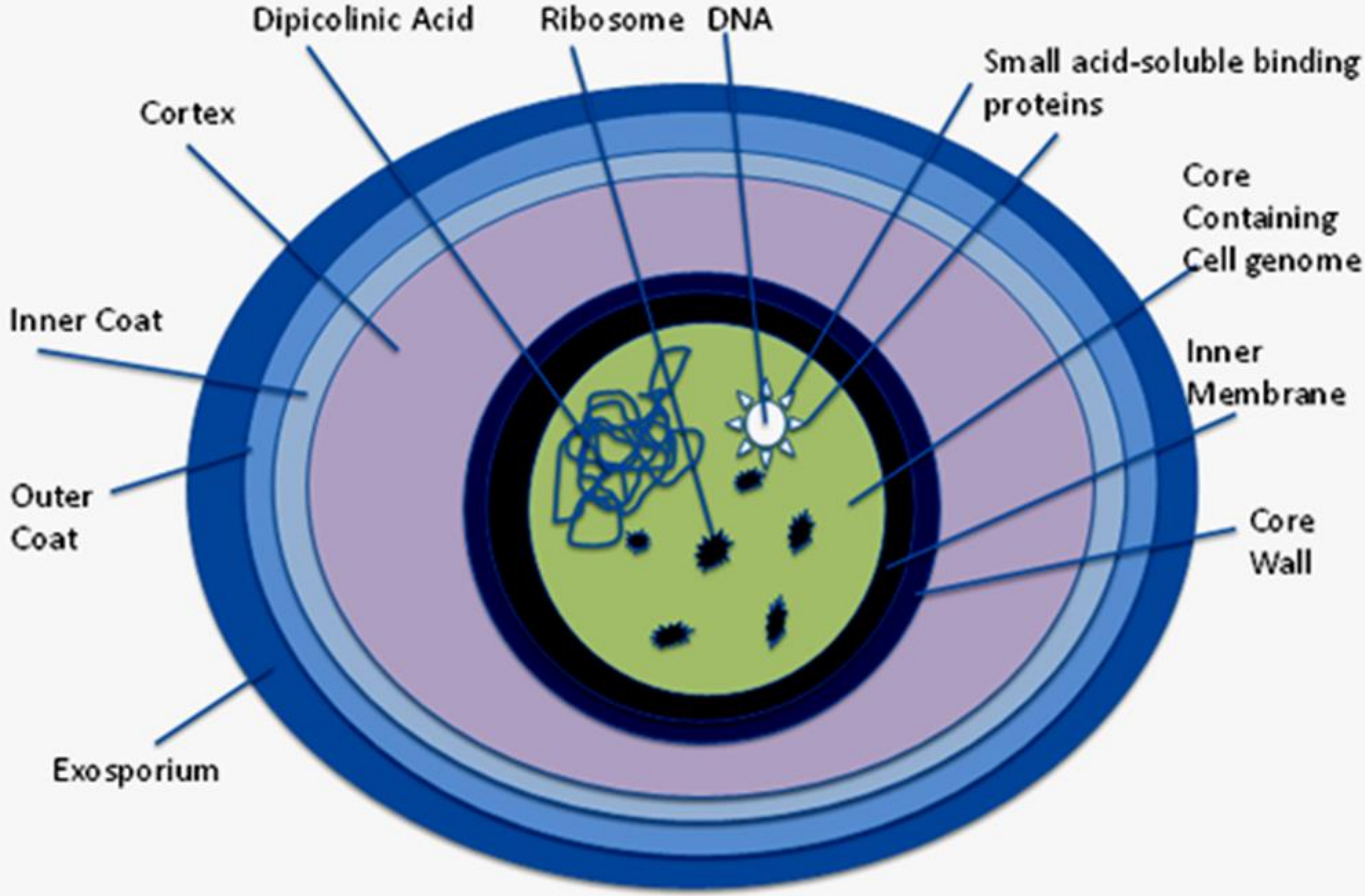
- ✓ Cortex lies outside the spore wall. It is the thickest layer of the spore envelope. unusual layer of **peptidoglycan**.
- ✓ Sensitive to lysozyme.

4. **Spore coat:**

- ✓ layered membrane that encloses the cortex. It consists of spore specific keratin like **proteins**. The proteins are rich in cysteine and hydrophobic amino acids. resistant to antibacterial chemical agents.

5. Exosporium:

- ✓ It is the outermost layer covering the spore coat. It is made up of lipoprotein and some carbohydrate.


Dipicolinic Acid
Ribosome
DNA
Small acid-soluble binding proteins
Cortex
Core Containing Cell genome
Inner Coat
Inner Membrane
Outer Coat
Core Wall
Exosporium

Sporulation

Sporulation Stages in Bacteria (e.g., *Bacillus subtilis*)

Stage 0: Normal Vegetative Cell

1. Regular metabolic activity before sporulation begins.

Stage I: Axial Filament Formation

1. Bacterial chromosome elongates into a thread-like axial filament.
2. Attached to the membrane via mesosomes.
3. Cell elongates and utilizes stored para-hydroxy benzoic acid (PHBA) for sporulation.

Stage II: Asymmetric Septation

1. The cell membrane folds inward asymmetrically, forming a **forespore** with a portion of DNA.

Stage III: Engulfment

1. The mother cell membrane engulfs the forespore, resulting in a double-membrane-bound structure inside the cytoplasm.

Stage IV: Cortex Synthesis

1. A thick **cortex** of specialized peptidoglycan forms between the forespore membranes.
2. Mother cell DNA breaks down and dehydration begins.
3. Exosporium (outermost layer) synthesis starts.

Stage V: Coat Deposition

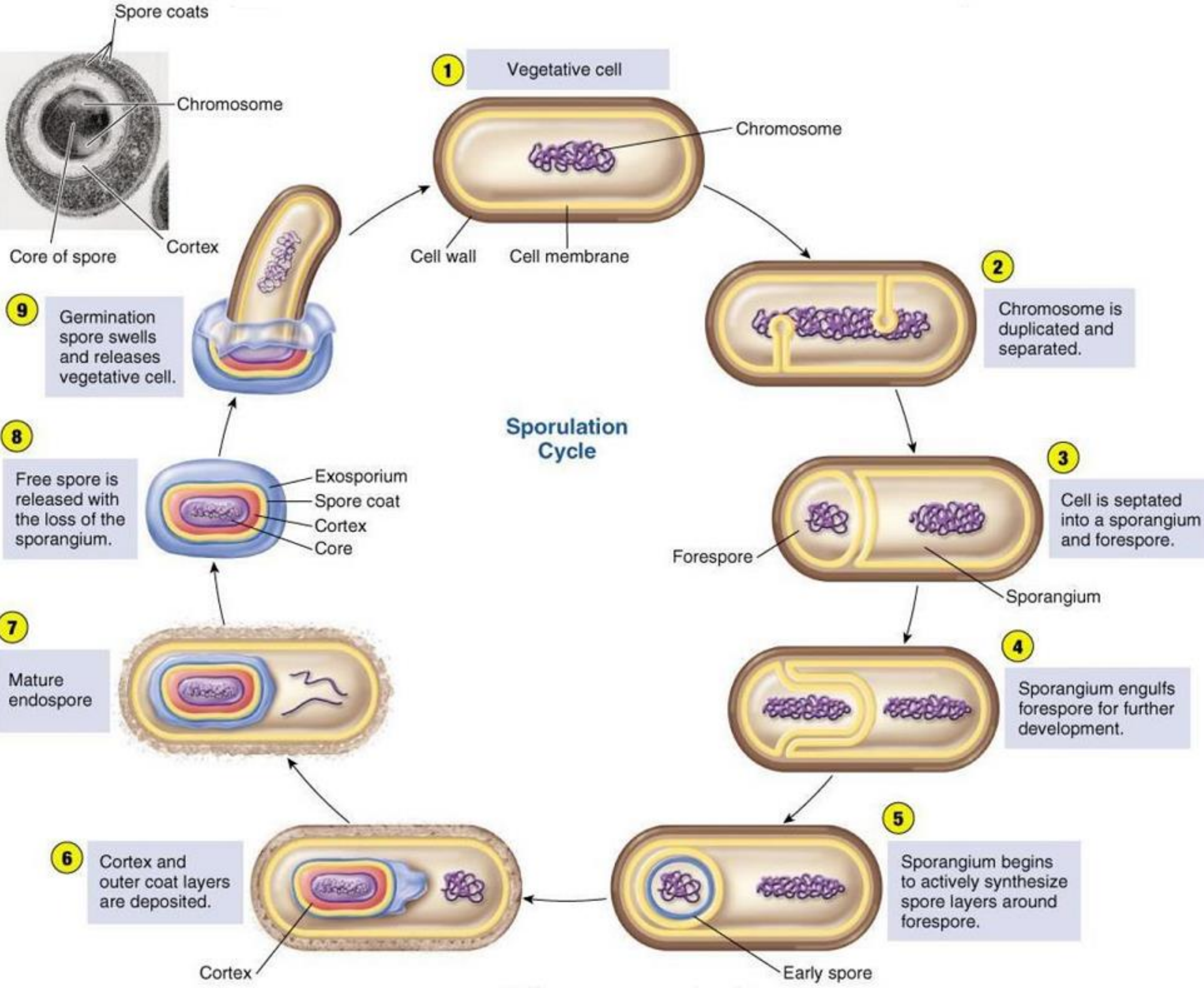
1. Dipicolinic acid and SASPs form **calcium dipicolinate** for heat resistance.
2. Cytoplasm further dehydrates.
3. A protective **spore coat** is deposited.

Stage VI: Maturation

1. Unique spore proteins replace vegetative enzymes.
2. The **endospore** matures and becomes highly resistant.

Stage VII: Release

1. The **mother cell lyses**, releasing the mature endospore into the environment.


Spore coats
Chromosome
Core of spore
Cortex
1
Vegetative cell
Chromosome
Cell wall
Cell membrane
2
Chromosome is duplicated and separated.
3
Cell is septated into a sporangium and forespore.
Forespore
Sporangium
4
Sporangium engulfs forespore for further development.
5
Sporangium begins to actively synthesize spore layers around forespore.
Early spore
6
Cortex and outer coat layers are deposited.
Cortex
7
Mature endospore
8
Free spore is released with the loss of the sporangium.
Exosporium
Spore coat
Cortex
Core
9
Germination spore swells and releases vegetative cell.
Sporulation Cycle

Formation of Vegetative Cell

Endospore Germination Process

1.Activation

1. Triggered by heat, abrasion, acid, or sulfhydryl compounds in a nutrient-rich medium.
2. Damages the spore coat, preparing it for germination.

2.Initiation

1. Environmental signals (effector molecules) bind to spore receptors.
2. **Autolysin** enzymes degrade the cortex.
3. Water is absorbed, calcium dipicolinate is released, and spore components break down.

3.Outgrowth

1. The endospore swells as biosynthesis resumes (DNA, RNA, proteins).
2. A new **vegetative cell** emerges and grows under favorable conditions.