SACRAMENTO STATE
Leadership begins here.

Chapter 4:
Prokaryote cell biology

Chapter 6: *Gram Stain*

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Fall 2006
Lectures: MW Noon
Office Hours: Wednesdays 9 AM
Most Mondays

All bacteria are Prokaryotes

- *No **nucleus** or other **membrane-bound organelles***
- All are single-cell organisms
- Have a plasma membrane
- Genome is DNA
- Differences between prokaryotic cells & eukaryotic cells are exploited by antimicrobial drugs

TABLE 4.1

Similarities and Differences Between Prokaryotic and Eukaryotic Cells

Characteristic	Prokaryotic Cells	Eukaryotic Cells
Genetic Structures		
★ Genetic material (DNA)	★ Usually found in single circular chromosome	★ Typically found in paired chromosomes
★ Location of genetic information	★ Nuclear region (nucleoid)	★ Membrane-enclosed nucleus
Nucleolus	Absent	Present
Histones	Absent	Present
★ Extrachromosomal DNA	★ In plasmids	★ In organelles, such as mitochondria and chloroplasts, and in plasmids
Intracellular Structures		
Mitotic spindle	Absent	Present during cell division
★ Plasma membrane	★ Fluid-mosaic structure lacking sterols	★ Fluid-mosaic structure containing sterols
★ Internal membranes	★ Only in photosynthetic organisms	★ Numerous membrane-enclosed organelles
Endoplasmic reticulum	Absent	Present
★ Respiratory enzymes	★ Cell membrane	★ Mitochondria
Chromatophores	Present in photosynthetic bacteria	Absent
Chloroplasts	Absent	Present in some
Golgi apparatus	Absent	Present
Lysosomes	Absent	Present
Peroxisomes	Absent	Present
★ Ribosomes	★ 70S	★ 80S in cytoplasm and on endoplasmic reticulum, 70S in organelles
Cytoskeleton	Absent	Present

TABLE 4.1

Similarities and Differences Between Prokaryotic and Eukaryotic Cells

Characteristic	Prokaryotic Cells	Eukaryotic Cells
Extracellular Structures		
Cell wall ★	Peptidoglycan found on most cells	Cellulose, chitin, or both found on plant and fungal cells
External layer ★	Capsule or slime layer	Pellicle, test, or shell in certain protists
Flagella ★	When present, consist of fibrils of flagellin	When present, consist of complex membrane-enclosed structure with "9 + 2" microtubule arrangement
Cilia	Absent	Present as structures shorter than, but similar to, flagella in some eukaryotic cells
Pili	Present as attachment or conjugation pili in some prokaryotic cells	Absent
Reproductive Process		
Cell division ★	Binary fission	Mitosis and/or meiosis
Sexual exchange of genetic material	Not part of reproduction	Meiosis
Sexual or asexual reproduction ★	Only asexual reproduction	Sexual or asexual reproduction

Table 4-1 part 2 Microbiology, 6/e

Prokaryotes: Size & Shape

- **~1 μm (micron) in diameter**
  - Naturally there is *significant* variety in size, particularly length (since not all are spherical)
  - For comparison, human red blood cell ~7.5 μm
- Small size = **Large surface : volume ratio**
  - Example:
    - Spherical bacterium 2 μm diameter
      - » Surface area ~12 μm^2, volume ~4 μm^3
      - » **Surface:volume = 3:1**
    - Euk. cell 20 μm diameter
      - » Surface area ~1,200 μm^2, volume ~4,000 μm^3
      - » **Surface:volume = 0.3:1** (1/10th that of bacterium!)

No internal part of the cell is very far from the surface.
Nutrients can easily and quickly reach all parts of the cell.

The most common bacterial shapes

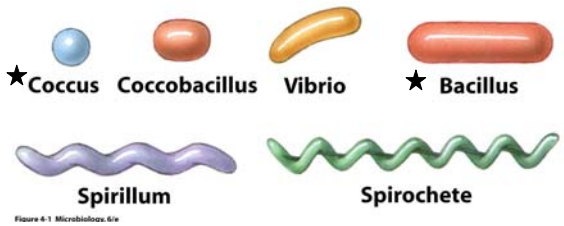


Figure 4-1 Microbiology, 6/e

A particular *shape & arrangement*

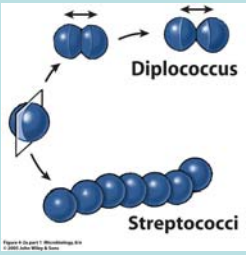
are usually typical for a species; however,

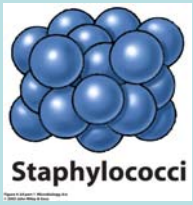
Pleomorphism (shape variation) can be significant in **old** cultures where nutrients are used up, and wastes accumulated

•use fresh cultures for light microscopy & staining!

Arrangements

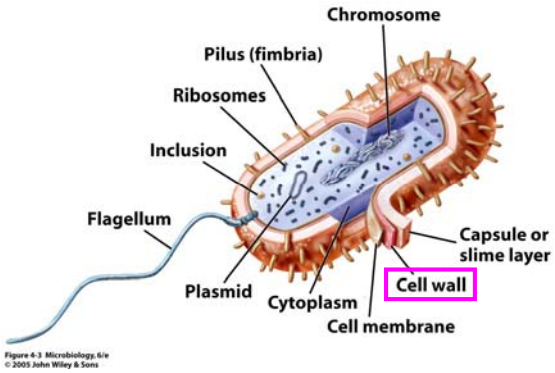
Binary fission without complete separation





diplo-	pairs	**staphylo-**	grapelike clusters
strepto-	chains		

TYPICAL PROKARYOTIC CELL



The Cell Wall

- Lies outside the cell membrane in nearly all bacteria

★ Two important functions:
  - Maintains characteristic cell shape
  - Prevents the cell from bursting when fluids flow into the cell by osmosis

- **Porous**; minor role in regulating transport

Cell Wall & Gram classification

- *Most* bacteria fall into one of two groups:
  Gram-positive or ***Gram-negative***
- Based on results of a differential staining test (the Gram's stain)
- Primary basis for this distinction is *differences in their cell walls*

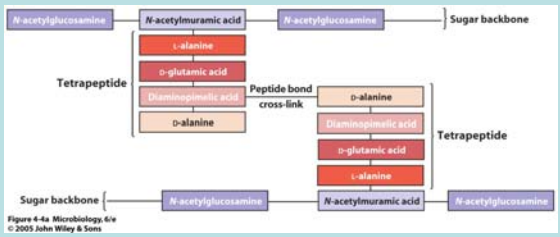
Cell Wall: structure & composition

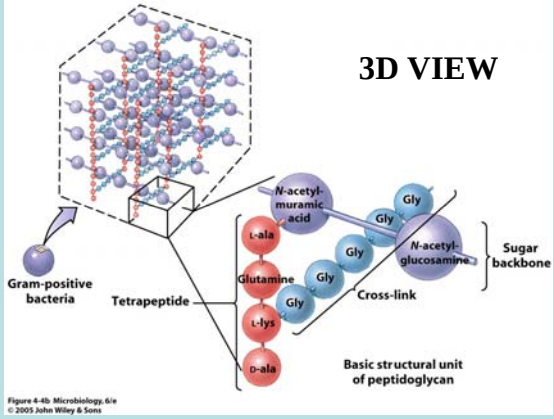
★ *Peptidoglycan* (murein)
- ***Most important*** component
- Polymer (functions as a single, immense molecule)
  - Like multiple layers of chain link fence
  - Gram positive bacteria: may have 40 layers!
  - Made of two alternating sugar subunits:

★ *N-acetylglucosamine* (NAG) alternating with *N-acetylmuramic acid* (NAM)

★ These sugar backbones are covalently *crosslinked* by *tetrapeptides* (4 amino acids)
- Tetrapeptide sequences vary
- Tetrapeptides are themselves crosslinked together by other peptides

PEPTIDOGLYCAN LAYER





Teichoic Acid

★ An additional component found in cell walls of ***Gram-positive*** *bacteria*

- Polymer of glycerol, phosphates, and ribitol (sugar alcohol)
- This polymer extends *beyond* the cell wall
- *Purpose unclear*, but teichoic acid:

1. Provides attachment site for some **bacteriophages** (viruses that infect bacteria)
2. Passageway for movement of ions in/out of cell

Outer Membrane (OM)

★ In *Gram-negative* bacteria

- Is part of cell wall *external* to peptidoglycan

★ *Lipoproteins* embedded in OM are covalently bonded to the peptidoglycan

- Acts as a coarse sieve, has only minor control over transport into & out of the cell

Note this is a *second* lipid bilayer in these cells; it is *in addition to* the normal plasma/cell membrane

Lipopolysaccharide (LPS) or endotoxin

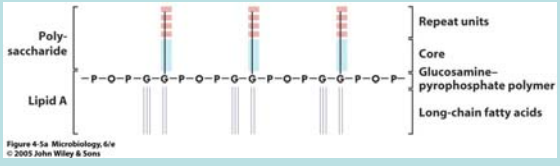
★ Part of the outer membrane of ***Gram-negative*** bacteria

★ Released only when the *cell walls* of Gram-negative bacteria are ***broken down***

★ Consists of polysaccharides and ***Lipid A***

- Polysaccharides extend outward & are vary according to the species, can be used for identification
- Lipid A: toxic

LIPOPOLYSACCHARIDE/ ENDOTOXIN



★ Lipid A causes **shock** (blood pressure collapse due to dilation of blood vessels)

Potentially lethal complication of ***Gram-negative*** infection; antibiotics given *late* in infection can actually make patient *worse*

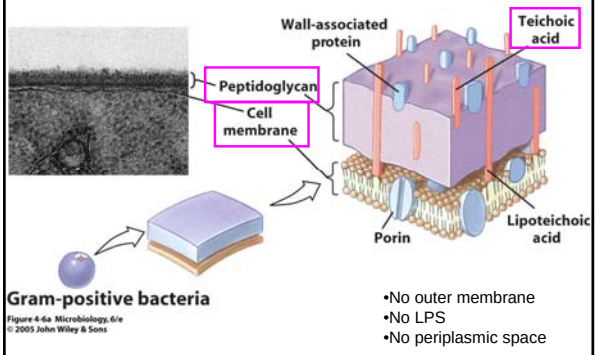
Periplasmic Space

- The gap between the cell wall and the plasma membrane in Gram-negative bacteria
- Active area of cell metabolism
- Contains digestive enzymes and transport proteins (for defense, "eating", & transport)
- Gram-positive bacteria lack both an OM and a periplasmic space

★ Distinguishing Bacteria by Cell Walls

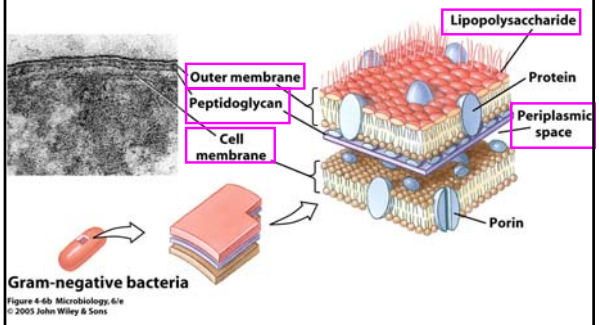
- *Gram-positive* bacteria have a *thick* layer of peptidoglycan
  - + teichoic acid
- *Gram-negative* bacteria have a *more complex* cell wall with a *thin* layer of peptidoglycan
  - Outer membrane
  - Endotoxin/LPS
  - Periplasmic space

Gram positive cell wall



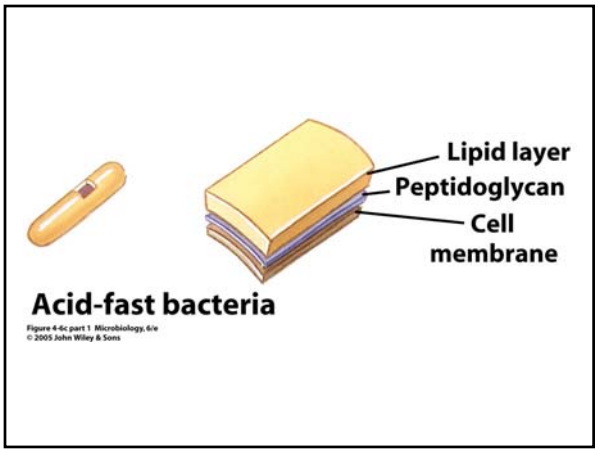
- No outer membrane
- No LPS
- No periplasmic space

Gram negative cell wall



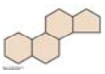
★ Acid-Fast Bacteria

- (Name comes from another staining procedure, not Gram's)
- Genus ***Mycobacterium***, various species including:
  - *M. tuberculosis* TB
  - *M. leprae* leprosy
- Cell wall is *thick* but mainly composed of **lipid**
  - Not much peptidoglycan
- Good defensive barrier but also impedes entry of nutrients & "costly" to manufacture
  - Acid-fast bacteria grow very slowly



Wall-Deficient Organisms

- genus ***Mycoplasma*** have no cell walls
- They are protected from osmotic swelling and bursting by a strengthened cell membrane containing ***sterols*** ★
  - Sterols are typical in eukaryotic cell membranes but rare in prokaryotes



- Difficult to grow in culture; extremely pleomorphic

Cell walls & antibiotics

- Some eukaryotic cells have "cell walls" but these are structurally *unlike* bacterial cell walls
  - No peptidoglycan
  - Cellulose, chitin, or both
- **A distinct chemical difference between prokaryotic & eukaryotic cells can be exploited as a target for antimicrobial therapy (antibiotics):**

★ ***Selective toxicity***

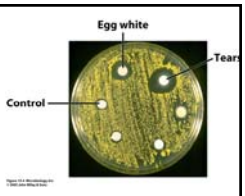
Drug selectively poisons the prokaryote (bacterial invader) not the eukaryote (human or animal host)

- Infections by ***eukaryotic*** microbes & parasites:
  - are difficult to treat because of similarity between host cells & invader
  - Fewer "good" targets for antimicrobial drugs
    (it's harder to find targets for **selective toxicity**)

★ Drugs to treat eukaryotic infections are much *less* selectively toxic
  - have more side effects
  - are less effective

Cell walls & antibacterial agents

The bacterial cell wall is an excellent target for selective toxicity.



- **Lysozyme**:
  - An antibacterial **enzyme** found in tears, and a principle component of egg white
  - ★ ***Hydrolyzes the NAM-NAG glycosidic bonds of peptidoglycan***
  - Kills some Gram-positive bacteria

Peptidoglycan: Breakage of peptide cross links?

- Peptide bond crosslinks in peptidoglycan are generally *unaffected* by natural proteases
- D-stereoisomers of amino acids present
- Enzymes are chiral, are specific for "normal" L-enantiomers

Antibiotics: Inhibitors of cell wall synthesis

Penicillins

★ Interfere with crosslinking of tetrapeptides of peptidoglycan (during synthesis)

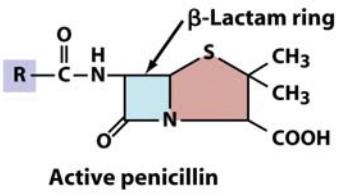
Gram positives most susceptible

★ **Bacteriostatic**: stops growth but doesn't kill bacteria
(vs. **bactericidal**: drug that actually kills bacteria)

β-lactam ring is chemical group crucial for activity

β-Lactam ring

•Found in all natural & semisynthetic penicillins (and cephalosporins)
•**Crucial to activity** of these antibiotics



Active penicillin

Figure 13-7 Microbiology, 6/e
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Antibiotic Resistance

- Microbes which were formerly *sensitive* to an antibiotic can become *resistant*

Antibiotic resistance develops from:

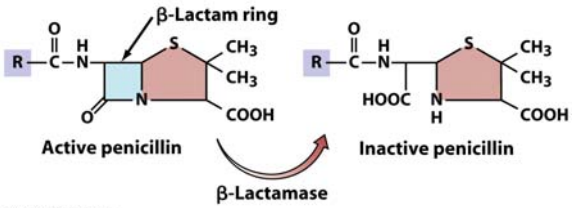
- *Genetic change*
  - Chromosomal or plasmid-mediated

…followed by… ★

- *Natural selection*
  - Overprescription, failure to complete course of therapy
  - Livestock feed

★ β-Lactamases

- Many mechanisms for resistance exist
- Resistance to penicillins often due to production of β-Lactamases
  - Enzymes which hydrolyze the crucial ring structure



How do you treat an infection caused by antibiotic-resistant bacteria?

•Use newer antibiotics (2nd / 3rd / 4th generation)

or be very clever…

★ Augmentin

Antibiotic preparation containing ampicillin (an oral penicillin) + clavulanic acid

Clavulanic acid inhibits β-Lactamase

Often given to children with recurrent ear infections

What happens to bacteria if the cell wall is damaged?

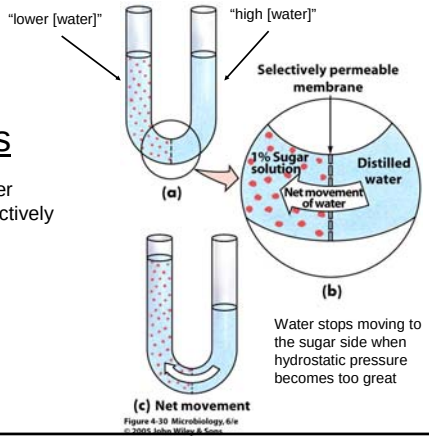
- Gram + bacteria: protoplasts
- Gram – bacteria: spheroplasts

By any name, these cells are highly sensitive to ***osmotic pressure*** and will shrivel or burst unless kept in

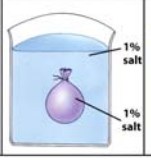
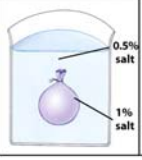
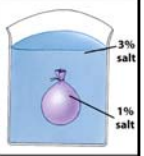
isotonic solution

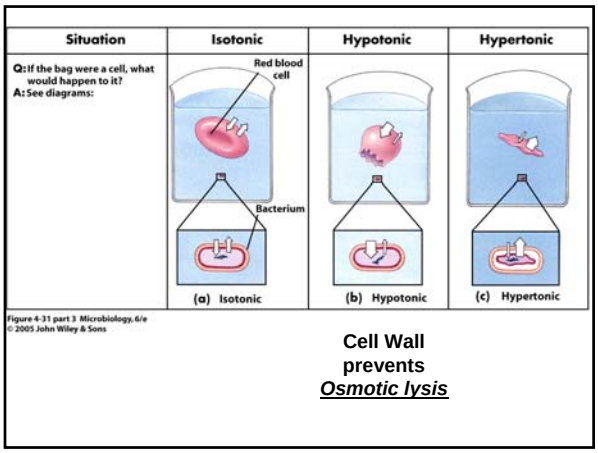
Osmosis

Diffusion of water across a selectively permeable membrane



Water stops moving to the sugar side when hydrostatic pressure becomes too great

Situation	Isotonic	Hypotonic	Hypertonic
A bag, permeable to water but not salt, is placed in a beaker containing one of three different salt solutions			
1% salt, 99% water 0.5% salt, 99.5% water	Relative to the "cell" (bag), the environment is **isotonic** (*same* concentration of dissolved solutes)	Relative to the "cell" (bag), the environment is **hypotonic** (*lower* concentration of dissolved solutes)	Relative to the "cell" (bag), the environment is **hypertonic** (*higher* concentration of dissolved solutes)
Net water movement:	**NONE**	**INTO the "cell"**	**OUT of the "cell"**



Cell Wall prevents
Osmotic lysis

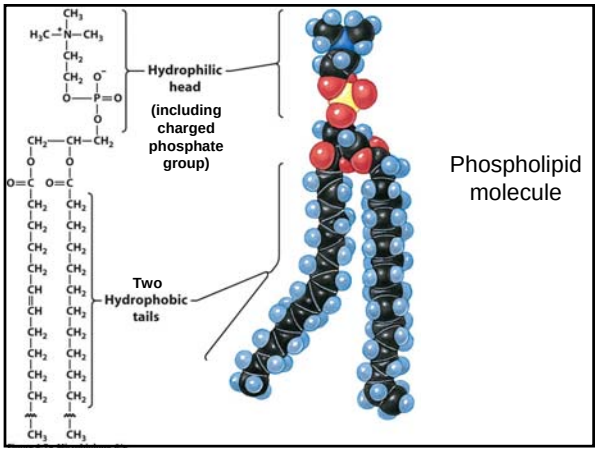
Back to prokaryotic cells…

The Cell Membrane (Plasma Membrane)

- Dynamic, constantly changing (unlike cell wall):

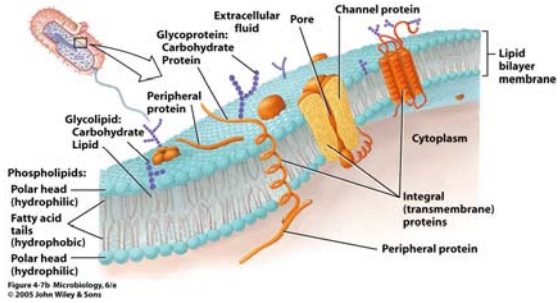
fluid mosaic

- Phospholipid bilayer
  - Hydrophobic interior
  - Hydrophilic exterior
- Associated proteins
  - Transmembrane / integral membrane proteins
  - Peripheral proteins



Phospholipid molecule

The Fluid-Mosaic Model of Membrane Structure



Fluid mosaic: proteins "float like icebergs"

Sterols would be present in *Mycoplasma* (bacteria lacking cell walls) & eukaryotes

Cell membrane: Function

- Regulate movement of materials into & out of the cell
  - Selective permeability
- Respond to chemical signals from the environment

True for both prokaryotes & eukaryotes

Transport & Selective permeability

- ***Diffusion***:
  - All substances spontaneously move from higher concentration to lower until equilibrium is reached
  - This does NOT require energy

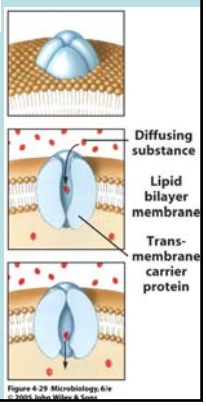
Hydrophobic substances can diffuse freely across a lipid bilayer (cell membrane).

Very small polar substances (water, small ions) diffuse through pores in the membrane

Facilitated Diffusion

- Molecules which cannot pass through a lipid bilayer *can* diffuse into a cell through carrier molecules/protein channels (channels *facilitate* movement)
- No energy required, movement only *down* a concentration gradient (it's still *diffusion*)

Movement *against / up* a concentration gradient requires energy & is called
active transport



Cell membrane: Prokaryotes only

Some functions performed by intracellular organelles in eukaryotes are performed by the cell membrane in bacteria:

- *Synthesize cell wall* components
- Assist with *DNA replication*
- *Secrete* proteins
- ★ Cellular *respiration*
- *ATP* production

Cell membrane

★ **Specific membrane sterols differ *between* eukaryotic species:**
Selective toxicity!

Target for treatment of fungal infections

Mechanisms:

1. Disrupt synthesis of fungal sterols
   - Athlete's foot drugs (clotrimazole, ketoconazole, miconazole)
2. Bind to fungal sterols and make membrane "leaky"

★
- **Amphotericin B**
  - Taken systemically, BAD side effects

Light microscopy: Staining

- Bacteria generally have no color/contrast
- Use ***stains*** to:
  - –Visualize cell morphology, structures
  - –Differentiate species
- Start by preparing a ***smear***
  - –Spread live bacteria (in liquid) onto a slide
  - –Dry, heat fix (kill & attach)
  - –Technique is important:
    - •Too thick, can't see individual cells; too thin, can't find any
    - •Don't destroy cell arrangements & morphology (shape)!

★ **Simple stain** (e.g., crystal violet)
- – One dye only; shows cell size, shape, arrangement
- – Many simple stains use cationic, or basic, dyes which are attracted to the negatively charged cell membrane

★ **Differential stain** (e.g., Gram stain)
- – 2 or more dyes two *distinguish* between different kinds of organisms, or different parts of an organism

- Some stains include a ***mordant*** (a chemical which helps stain retention)

Internal cell structures of prokaryotes

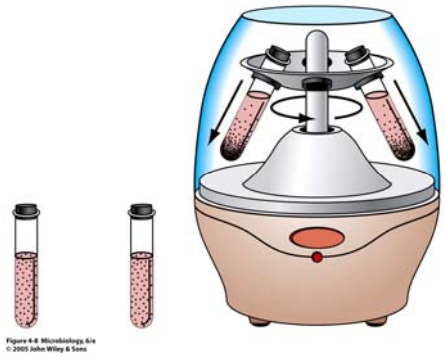
Bacterial cells typically contain (in their cytoplasm):

1. **Ribosomes:** 70S
2. **Nucleoid region:** DNA is not in a nucleus
3. **Inclusions**
4. **Endospores:** only certain bacteria, under certain conditions

Ribosomes

- Consist of ribonucleic acid (RNA) and protein; serve as sites of protein synthesis
- Abundant in the **cytoplasm** of bacteria
- Often grouped in long chains called *polyribosomes*
- ★ **70S in bacteria; 80S in eukaryotes**
  - Large subunit + small subunit
  - Sedimentation rate (Svedberg units): measures rate of migration under centrifugation; proxy for ***size***
  - Target for selective toxicity (antibiotics)

A Centrifuge



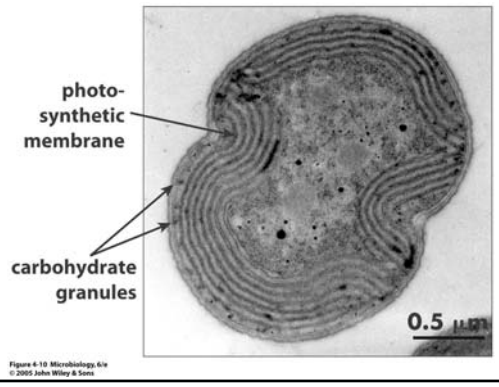
Ribosomes: Selective toxicity

- ***Aminoglycoside antibiotics***
  - e.g. Streptomycin, neomycin, erythromycin, kanamycin
  - {Other classes of antibiotics also act on 70S ribosomes}
- Specifically bind to 70S ribosomes and disrupt **bacterial** protein synthesis ★
  - Eukaryotic 80S ribosomes are not affected
- Mitochondria *can* be affected
  - They have 70S ribosomes

Internal Membrane Systems

- Photosynthetic bacteria and cyanobacteria contain internal membrane systems
- Referred to as **chromatophores**
- Derived from the cell membrane and contain the photosynthetic pigments
- Nitrifying bacteria also have internal membranes

Internal Membrane Systems (Chromatophores)

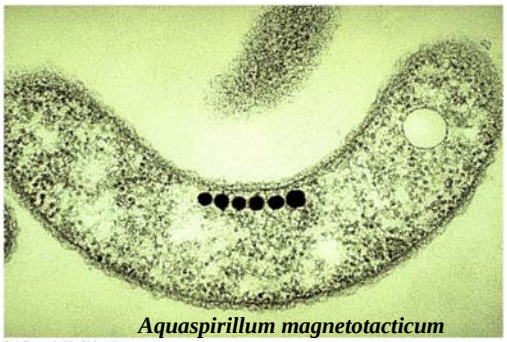


Inclusions

Small bodies within the bacterial cytoplasm:

1. **Granules**: deposits of densely compacted substances (e.g., glycogen or polyphosphate); *not* membrane bound
2. **Vesicles**: ***Specialized*** *membrane-enclosed* structures
   - **Gas**: to regulate depth bacteria float in water
   - **poly-β-hydroxybutyrate** (lipid): energy storage
   - **Magnetosomes:** iron oxide for magnetotaxis

Magnetotactic Bacterium



Aquaspirillum magnetotacticum

Try It Figure 4-1a Microbiology, 6/e
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Endospores

- A specialized resting structure found in bacteria
  - ★genus ***Bacillus*** (aerobic)
    - •common soil bacteria; also **anthrax**
  - ★genus ***Clostridium*** (anaerobic)
    - •Botox; also food poisoning with improper canning methods
- Helps the bacterial cell survive when conditions become unfavorable
- *Highly resistant* to heat, drying, acids, bases, certain disinfectants, radiation, and aging

★**Vegetative cell**: normal, metabolically active bacterium (opposite of spore)

★ Sporulation is *not* reproduction

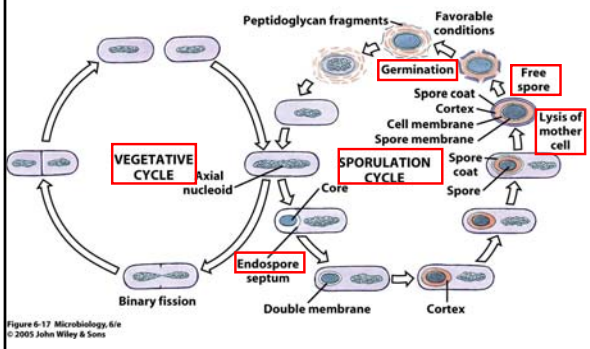

Figure 6-17 Microbiology, 6/e
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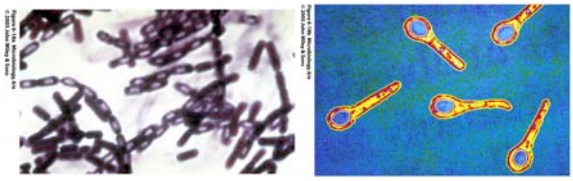
Spore heat resistance:

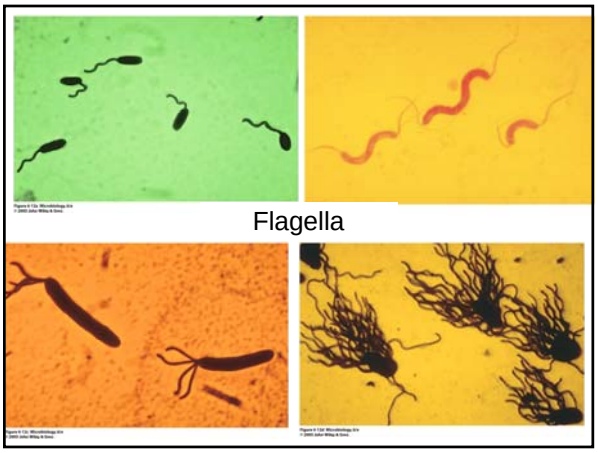
- Large quantity of **Ca^{++} ions**;
- **Dipicolinic acid** present;
- Very **low water** content



Images of endospores

Bacteria: External structures
Flagella

- ~1/2 of known bacteria are ***motile*** (move)
- ***Flagella*** (sing. flagellum)
  - Cell may have one, two, or many flagella
  - Cocci rarely have flagella
- Flagella extend from the cell membrane, through the cell wall, and beyond



Flagella

Flagella

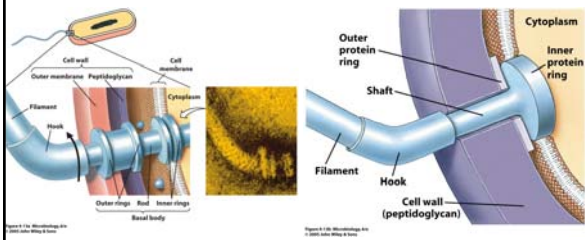
- **Prokaryotic** flagella are **simpler**, **smaller** than eukaryotic flagella (1/10th diameter)
- ***Flagellin***: protein subunit of flagella

★ Structure of flagella in Gram + and Gram – bacteria is different because of differences in the cell walls

FYI:
- Eukaryotic flagella are made of microtubules "9+2" arrangement
- Bacteria do not have cilia

Structures of Two Different Bacterial Flagella

Both have 3 basic components:
1. **Filament**
2. **Hook**
3. **Basal body** (anchor)



Gram-negative bacterium **Gram-positive bacterium**

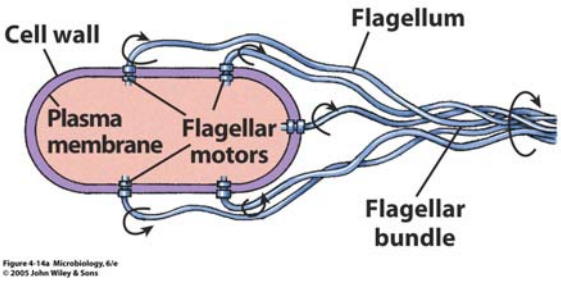


Figure 4-14a Microbiology, 6/e
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Flagellar motion: Runs & Tumbles ★

- Flagella are L shaped because of the "hook"
- Movement occurs when flagella rotate (counterclockwise)
- Straight line movement: **RUN**
  - Runs last avg. 1 second
- When flagella rotate other direction (clockwise): **TUMBLE**
  - No forward progress, *randomizes* direction
  - Tumbles last avg. 0.1 second

To see great little movies of swimming bacteria, visit:
http://www.rowland.harvard.edu/labs/bacteria/index_movies.html

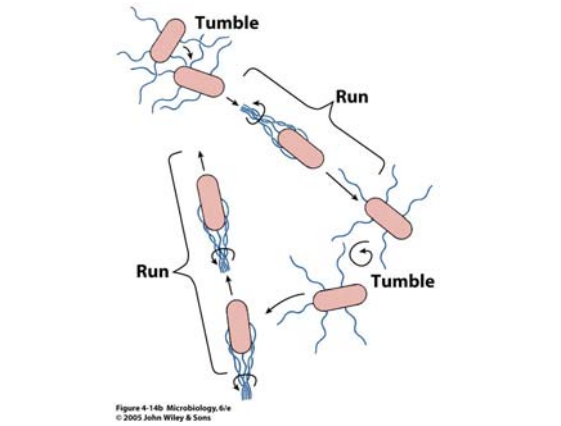

Figure 4-14b Microbiology, 6/e
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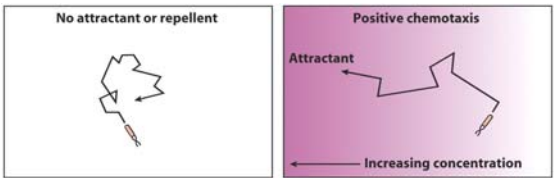
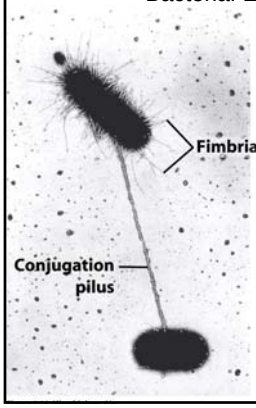
Chemotaxis

- **Nonrandom movement** *toward* (positive chemotaxis) or *away from* (negative chemotaxis) a substance in the environment
- If bacteria "smells" something it likes as it moves,

★ – ***Lengthen runs, shorten tumbles***



Bacteria: External structures: **Pili**



- Tiny, hollow projections
- NOT for movement

Two kinds:

1. **Conjugation pili** (long) ★
2. **Attachment pili (fimbriae)** (short)

Singular: pilus

Conjugation Pili

- Other names: F pili, sex pili
- Function: Connect two cells to **transfer DNA** (*conjugation*)
- NOT a reproductive process

Attachment Pili

- Allow bacteria to adhere to surfaces
- ★Can be an important ***virulence factor***
  - a feature of the organism that enhances its ability to cause disease
    - Some bacteria use pili to adhere to red blood cells, causing *hemagglutination* (clumping)
    - *Neisseria gonorrhoeae*: strains with pili are highly infectious, are able to attach to epithelial cells of the urogenital system

Bacteria: External structures:
Glycocalyx

- General name for polysaccharide-containing layers *external to the cell wall*
- **Capsules** & **Slime Layers**

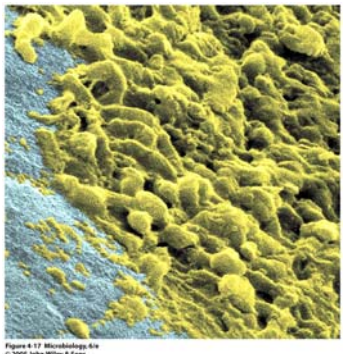
Glycocalyx: Capsules

- **Protective** structure secreted by some bacteria
  - Can protect cell from immune attack/phagocytosis
- Can be a virulence factor
  - *Streptococcus pneumoniae*: needs capsule to adhere to respiratory tract
- Chemical composition varies for each strain of bacteria producing it

Glycocalyx: Slime Layer

- Thinner, less tightly bound than capsule
- Protection, adhesion
- *Streptococcus mutans*: uses slime layer to adhere to teeth, contributing to formation of ***plaque*** (a coating of microbes & organic matter)
  - Once adhered, bacteria metabolize sugars in your diet & produce acids which decay teeth (*dental caries*)
  - "Sugar free" gum contains sugar alcohols (e.g.,sorbitol) that cannot be metabolized by many bacteria

Bacteria growing on teeth



Endosymbiosis

- Prokaryotes were the first life forms on earth (4 billion years ago)
- Eukaryotes didn't appear for another 3 billion years!

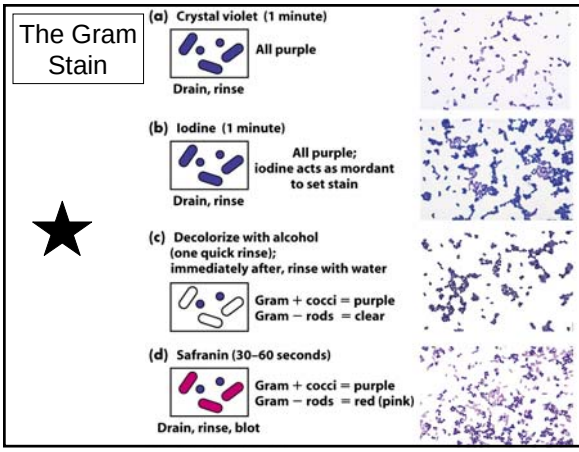
How did eukaryotic cells evolve?

- One cell survived phagocytosis by another, became an ***endosymbiont***
- Engulfed cell protected by the host
- The host acquired new capabilities

★Endosymbiotic theory: Evidence

- Mitochondria & chloroplasts are about same *size* as proks.
- Mitochondria & chloroplasts have their *own DNA*
  - This DNA is *circular*, like prokaryotic chromosome
- Organelles have *70S ribosomes* & synthesize proteins in the way bacteria do, not as it is done under direction of nuclear DNA
- Mitochondria & chloroplasts *divide independently* of the euk. cell cycle, by binary fission

The Gram Stain



★

KOH (potassium hydroxide) Test

- Results correlate with Gram stain
- Strong base damages the cell membranes
  - Gram NEG bacteria: DNA leaks out
  - Gram POS bacteria: DNA stays inside
- Free DNA is viscous, slimy, and *visible* if enough cells are lysed

\+ KOH = viscosity = Gram −

− KOH = no viscosity = Gram +

Relevant reading in Black's Microbiology:

(pages from 6th edition)

- Ch. 4 p. 76-95; 101-111
- Antimicrobials, selective toxicity: ch. 13, p. 352-357; 360; 365-367; 374
- Lysozyme: ch. 19, p. 550
- Staining: Ch. 3 p. 67-73
- Sporulation: ch. 6, p. 158-159
- Tooth decay, ch. 22 p. 646-8