BIOL 240: General Microbiology

Spring 2020 Rm. 23-203 MW, Feb. 10-12

http://accounts.smccd.edu/staplesn/biol240/

1. **Pre-Lab Writeup #4 due!: ALL of Expt. 5.** Be sure to prepare before each Monday's labs (for BOTH Mon. & Wed.)!! ***(What? Why? How? are we doing in the lab? Question? HYPOTHESIS?)***
2. **QUIZ #2 this week!! First attempt due by Wed. night!**
3. **Study Guides & Lesson Objectives (see slides) due THIS WED. in Lab (Ch. 2b, 4a?)!**
4. ***Extra Credit Opportunity: Starting This Wed: Wed. evenings, 5-6 pm, (2/4-3/25/2020) in Bldg. 6, Room 102 – STEM SPEAKER Series. 1 page summary & reflection due (on CANVAS) the following week.***
   - **https://www.canadacollege.edu/stemcenter/speaker-series.php**
5. **REVIEW SESSIONS for Midterm Exam #1: TODAY & Wed. 2-3pm, & Wed. 8:15am-9:35am… *(Review Sheet is UPDATED!!)***
   - ***Come PREPARED with questions!! ☺ ***PLEASE PLEASE COME!!****
6. ***MIDTERM #1: NEXT WED., 11:10AM. Be ON TIME, with Red (half-sheet) Scantron, and #2 pencil!! Be rested and ready! ☺ Do NOT miss Lab!!!***

1

REVIEW:

1. List the **4 types of macromolecules** in living systems, and describe how the components of each particular ***molecular structure determines its biological function……***
   a) ***How is each Macromolecular polymer synthesized? How is each degraded?***
   b) ***What elements, functional groups and monomers does each have?***
   c) ***What general properties do these components confer?***

OBJECTIVES: Students should be able to: …..

1. Describe, diagram and give examples of each level of **Protein Structure.**
2. **Ch. 4:** Describe **4 extracellular structures unique to prokaryotes**, & explain the function of each.
3. Compare and contrast **5 structural characteristics of the gram positive and gram negative cell walls**. Include illustrations.
4. Compare and contrast **10 differences** between ***"prokaryotic" and eukaryotic cells***. What structures do they share in common? …..
5. Describe the prevailing ***model of cell membrane structure*** and how the macromolecules that form them exemplify **structure determining function**.
6. Compare and contrast the transported molecules, energy balances and membrane molecules required for 2 types each of **passive & active transport**.
7. Recognize and describe the properties and functions of the various **Eukaryotic Organelles**. *(REVIEW!!)*

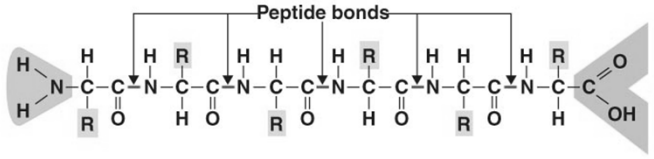
❖ ***These questions are your HOMEWORK between classes!!!***
   - ***DUE (and/or Study Guide questions) WED. at the start of Lab!!***

2

c.) Levels of Protein Structure

1. The ***primary structure*** is a polypeptide chain
   - LINEAR SEQUENCE and number of AA's.
   - ***Covalent peptide bonds*** (-C—N-)
     - ***…. -NCC-NCC-NCC-NCC- ….***

1°



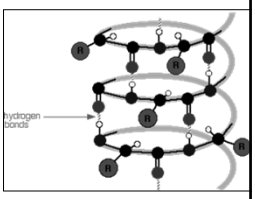
(a) Primary structure:
polypeptide strand

Figure 2.15a

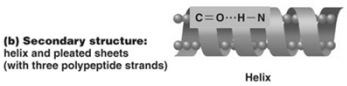
3

Levels of Protein Structure

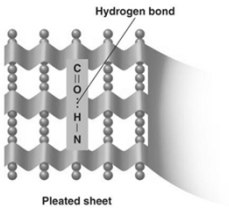
2. The ***secondary structure*** – amino acid chain folds and coils in a regular (α) **helix** or (β) **pleats**.
   - Simple folded/coiled structures
   - Hydrogen-bonding.



2°



(b) Secondary structure:
helix and pleated sheets
(with three polypeptide strands)



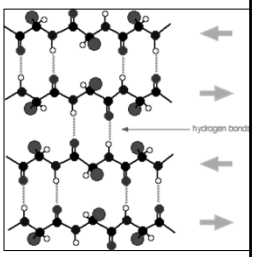


Figure 2.15b

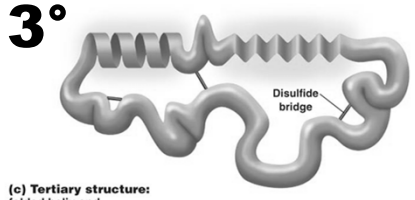
http://www.chemguide.co.uk/organicprops/aminoacids/proteinstruct.html

4

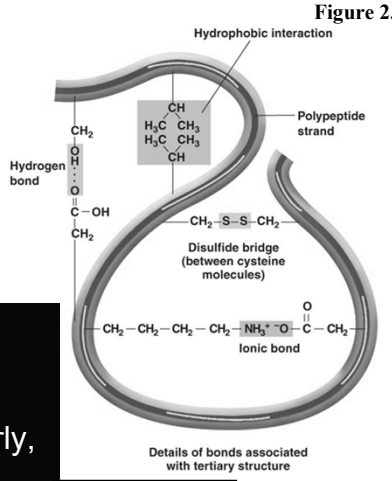
Levels of Protein Structure

Figure 2.15c

3°

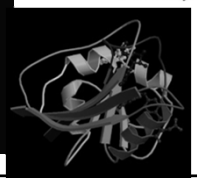


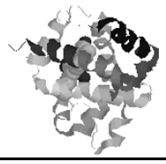
(c) Tertiary structure: folded helix and pleated sheet



Details of bonds associated with tertiary structure

3. The ***tertiary structure*** (3D structure!!):
   - the polypeptide folds irregularly,
   - forming disulfide bonds, H-bonds, hydrophobic interactions, & ionic bonds between amino acids in the chain.





5

Levels of Protein Structure

4. The ***quaternary structure*** consists of two or more polypeptides.
   - ❖ **Two or more (≥ 2), 3D-folded polypeptides** associated into a multi-peptide complex.
     - Same types of bonds as found in tertiary structure connect the separate polypeptides.

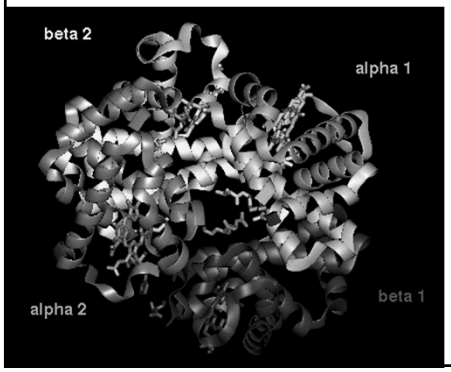
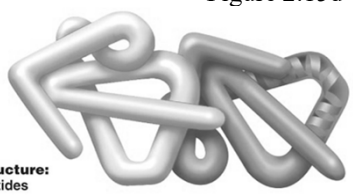


Figure 2.15d

4°



(d) Quaternary structure: two or more polypeptides in their folded states

6

d.) Complex Macromolecules

- **<u>Conjugated proteins</u>** consist of amino acids and other organic molecules:
  1) **Glycoproteins**
  2) **Nucleoproteins**
  3) **Lipoproteins**
  4) **Phosphoproteins …..**

7

4. Nucleic Acids

- Consist of ***<u>nucleotides</u>***.
- Contain information. Some are catalytic!...
- <u>Nucleotides consist of a:</u>
  - ***(Phosphate-Sugar-Base)***
  1. Pentose – 5C sugar (ribose or deoxyribose)
  2. Phosphate group = $\mathbf{PO_4^{2-}}$
  3. Nitrogen-containing base (purine or pyrimidine).

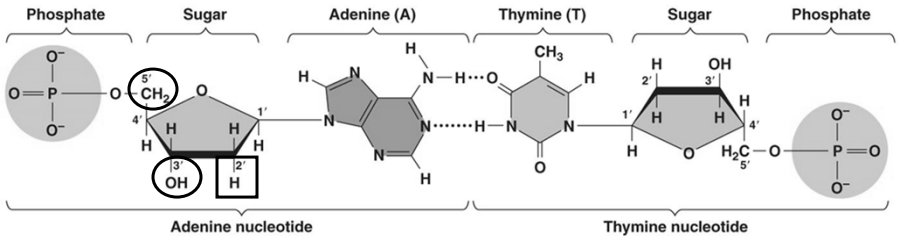


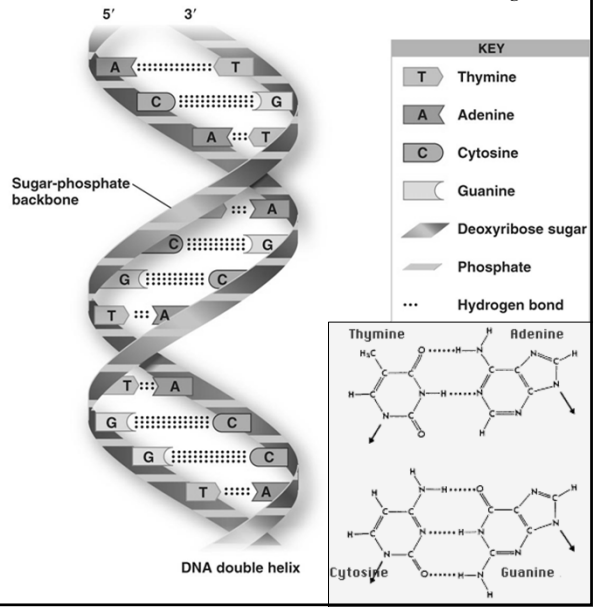
Figure 2.16

8

a.) DNA

1. Has <u>deoxyribose</u>
2. Exists as a double helix
3. Strands assemble **5'Phos. → 3'OH**
4. **A** hydrogen bonds only with **T**
5. **C** hydrogen bonds only with **G**
6. Strands are **ANTIPARALLEL.**

Figure 2.16



9

b.) RNA

1. Has **<u>ribose</u>**.
2. Is single-stranded
3. Strands assemble **5'Phos. → 3'OH**
4. **A** hydrogen bonds only with **U**
5. **C** hydrogen bonds only with **G**
6. Complex 3D folding.

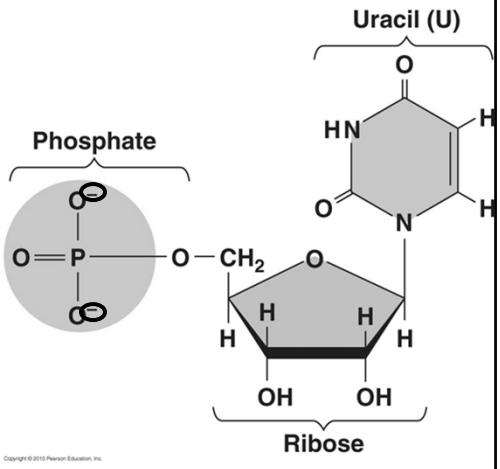
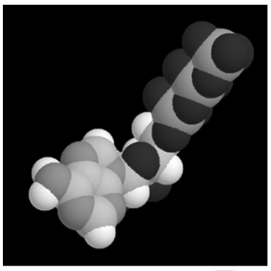


Figure 2.17

10

c.) ATP



- Has ribose, adenine, and 3 phosphate groups

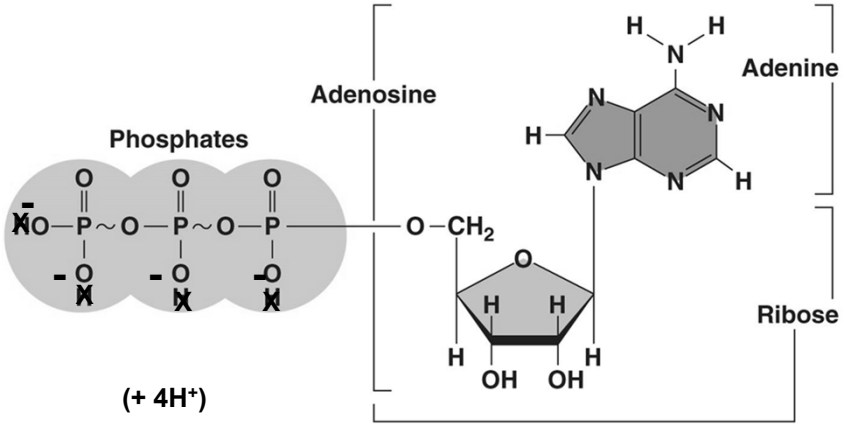
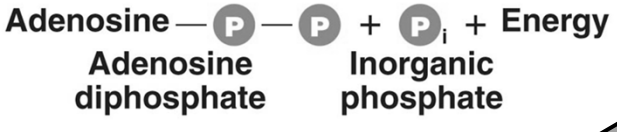


Figure 2.18

11

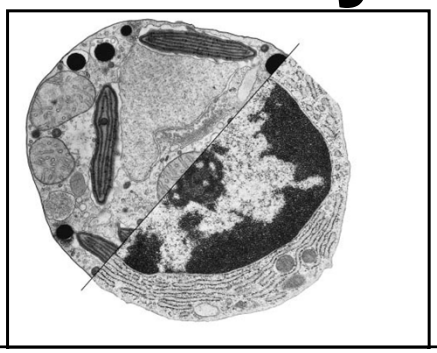
ATP

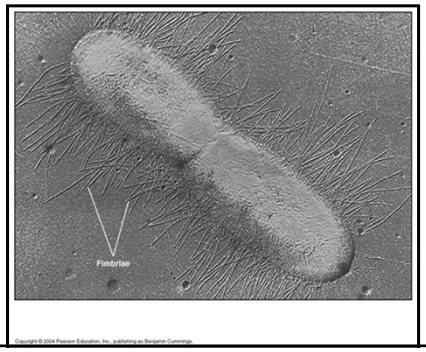
- Is made by dehydration synthesis.
  - Absorbs/requires energy = ***Endergonic***
- Is broken by hydrolysis to liberate useful energy for the cell.
  - Releases energy = ***Exergonic***



12

Chapter 4 Functional Anatomy of Prokaryotic & Eukaryotic Cells

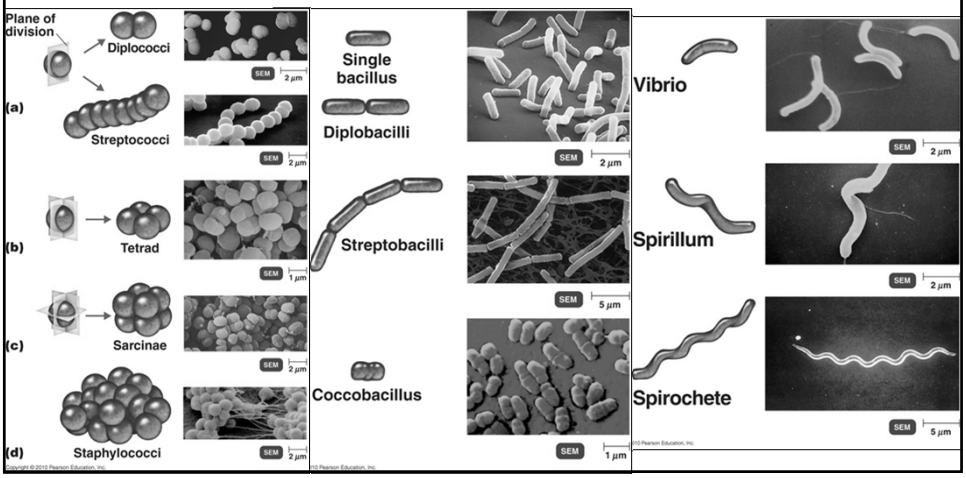




13

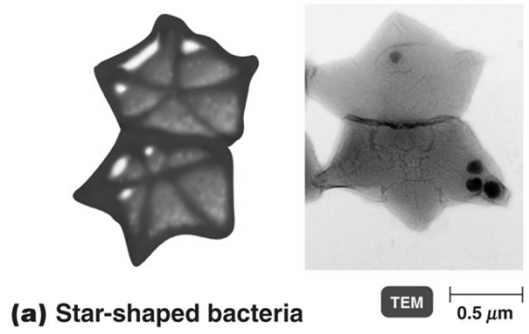
4.1) Prokaryotic Cells

- **Average size: 0.2 -1.0 µm × 2 - 8 µm**
- **Basic shapes:**



14

- Unusual shapes
  - Star-shaped *Stella*
  - Square *Haloarcula*
- Most bacteria are monomorphic (one shape)
- A few are ***pleiomorphic*** (many forms/shapes)



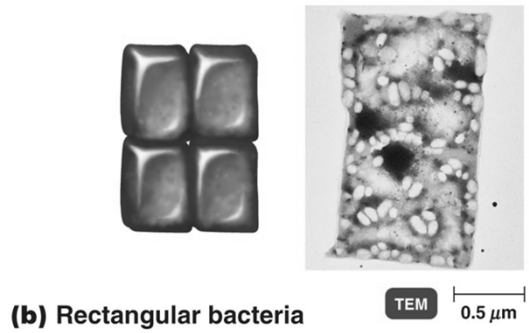
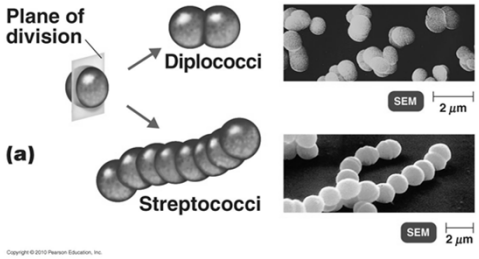


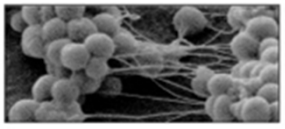
Figure 4.5

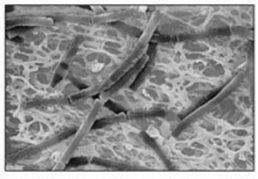
15

Arrangements

- **Pairs**: diplococci, diplobacilli
- **Clusters**: staphylococci
- **Chains**: streptococci, streptobacilli



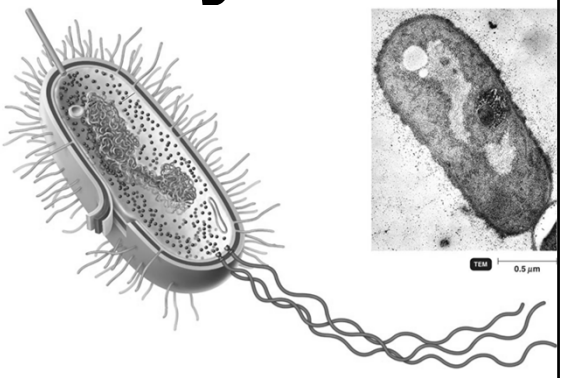




16

A. Glycocalyx

Figure 4.6



- Outside cell wall
- Usually sticky
- A ***capsule*** is neatly organized
- A ***slime layer*** is unorganized & loose
  - Extracellular polysaccharide (EPS) allows cell to attach
  - Capsules prevent phagocytosis

17

B. Flagella

- Outside cell wall
- Made of chains of ***flagellin***
- Attached to a protein ***hook***
- Anchored to the wall and membrane by the ***basal body***

100K rpm!
~human @
120 ft./sec!

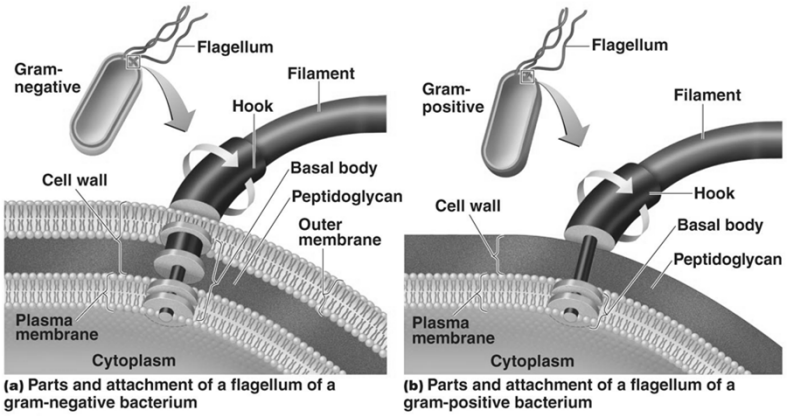


Figure 4.8

18

Flagella Arrangement

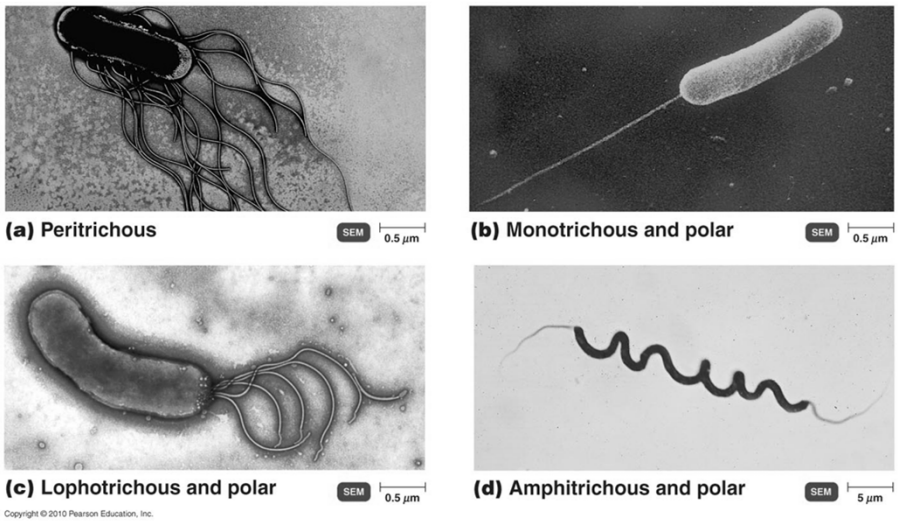
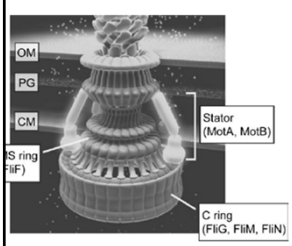


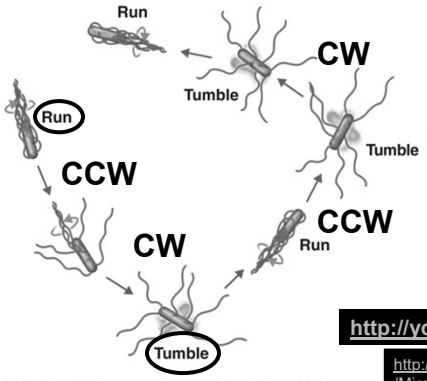
Figure 4.7

19

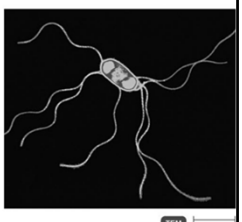
Motile Cells

- Rotate flagella to ***run*** or ***tumble.***
- Move toward or away from stimuli (***taxis***)
- Flagella proteins are H antigens
  - e.g., *E. coli* O157:H7





(a) A bacterium running and tumbling. Notice that the direction of flagellar rotation (blue arrows) determines which of these movements occurs. Gray arrows indicate direction of movement of the microbe.



(b) A *Proteus* cell in the swarming stage may have more than 1000 peritrichous flagella.

http://youtu.be/xEVq7jCT4kw

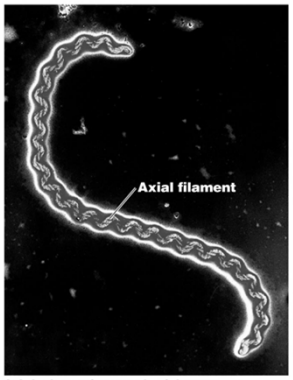
http://web.biosci.utexas.edu/psaxena/MicrobiologyAnimations/Animations/BacterialMotility/PLAY_motility.html

Figure 4.9

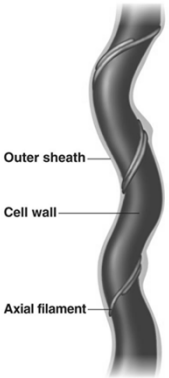
20

B_2. Flagella: ***Axial Filaments***

- ***Endoflagella***
- In **spirochetes**
- Anchored at one end of a cell
- Rotation causes cell to move



(a) A photomicrograph of the spirochete *Leptospira*, showing an axial filament



(b) A diagram of axial filaments wrapping around part of a spirochete. (See Figure 11.24a for a cross section of axial filaments)

http://youtu.be/pUomEHMLHwU

Figure 4.10

21

C. Fimbriae & Pili

1. ***Fimbriae*** allow attachment.
2. ***Pili*** are used to transfer DNA from one cell to another.

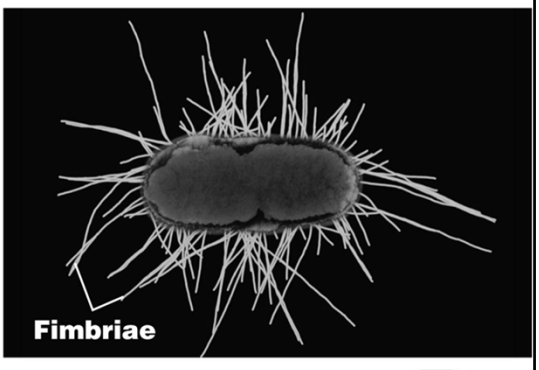


Figure 4.11

22

D. Cell Wall

- Prevents osmotic lysis
- Made of peptidoglycan (in bacteria)

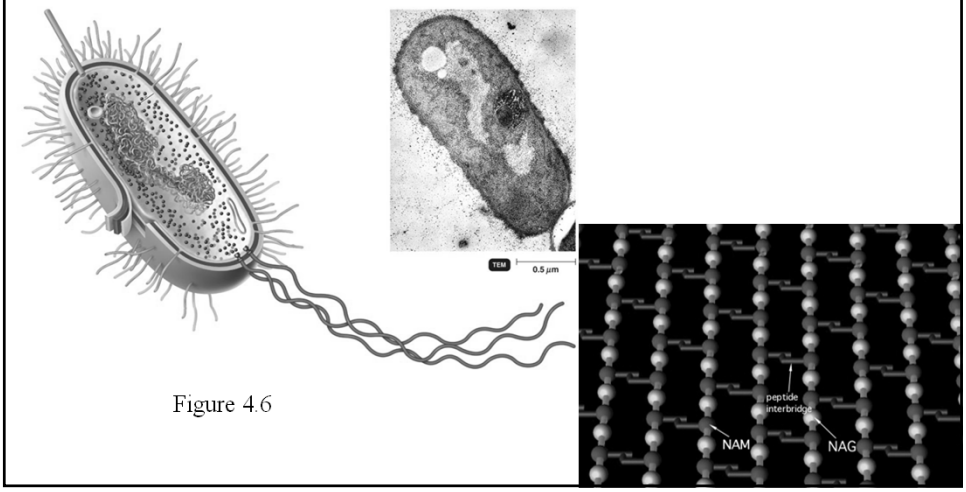
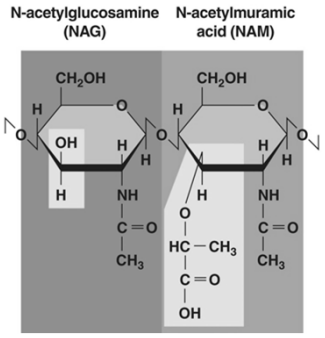


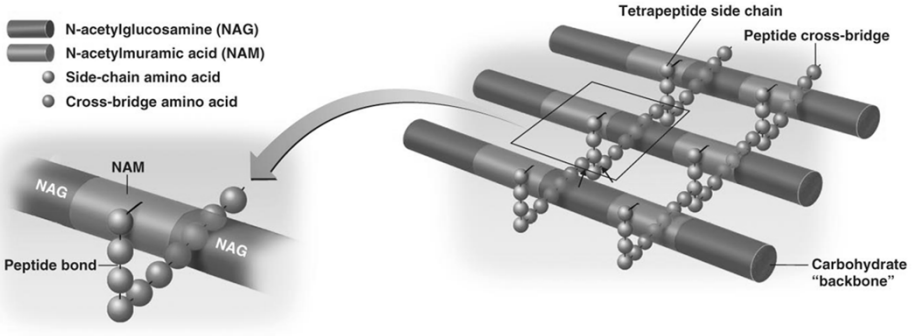
Figure 4.6

23

Peptidoglycan

- Polymer of disaccharide.
  - N-acetylglucosamine (**NAG**) &
  - N-acetylmuramic acid (**NAM**)
- Linked by polypeptides.





(a) Structure of peptidoglycan in gram-positive bacteria

Figure 4.13a

24

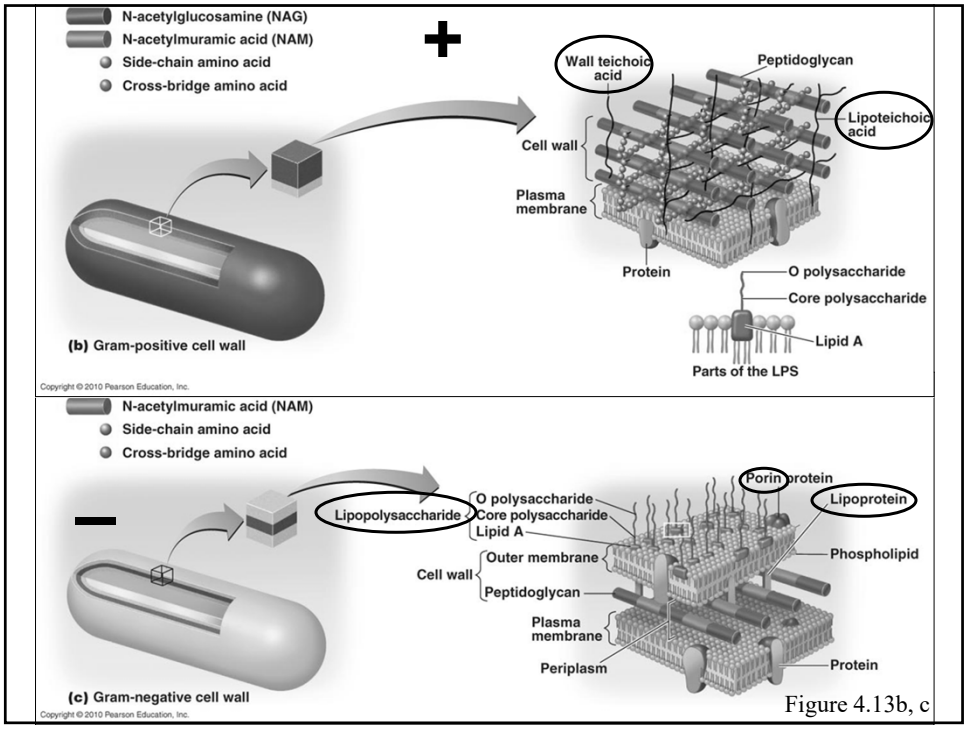


Figure 4.13b, c

25

1. Gram-Positive cell walls

- **Teichoic acids:**
  - ***Lipoteichoic acid*** links to plasma membrane
  - ***Wall teichoic acid*** links to peptidoglycan
- May regulate movement of cations
- Polysaccharides provide antigenic variation

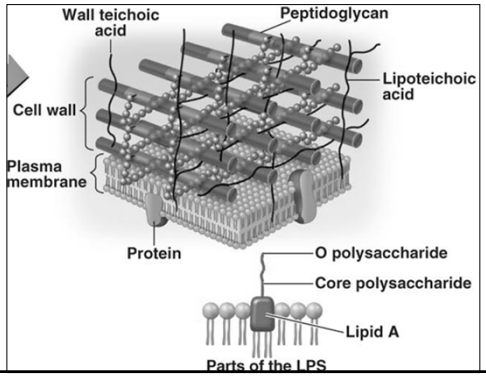


Figure 4.13b

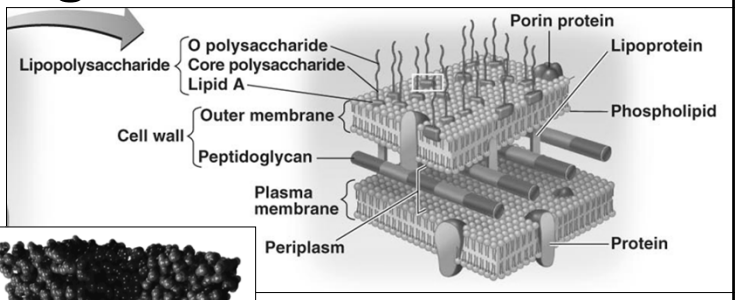
26

2. Gram-Negative Outer Membrane

1. Lipopolysaccharides, lipoproteins, phospholipids.
2. Forms the ***periplasm*** between the outer membrane and the plasma membrane.
3. Protection from phagocytes, complement, antibiotics.
   - **a. O polysaccharide antigen**,
     - e.g., *E. coli* **O157:H7**. (H = flagellum protein antigen)
   - **b. Lipid A** is an endotoxin.
   - **c. Porins** (proteins) form **channels** through membrane

27

Gram-Negative Outer Membrane



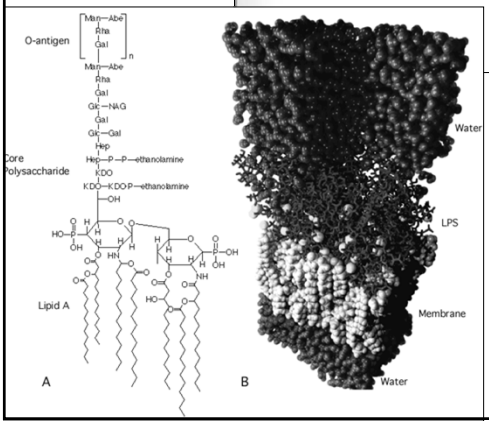


Figure 4.13c

28

Gram-positive Cell Walls	Gram-negative Cell Walls
1. Thick peptidoglycan ***2. Teichoic acids, Lipoteichoic acids*** 3. In acid-fast cells, contains mycolic acid	1. Thin peptidoglycan 2. No teichoic acids 3. Outer membrane – ***Lipopolysaccharide*** • ***Lipid A = endotoxin*** • ***O-antigen = polysacc.***

29

Gram Stain Mechanism

- Crystal violet-iodine crystals form in cell.
- **Gram-positive**
  - Alcohol dehydrates peptidoglycan.
  - CV-I crystals do not leave.
- **Gram-negative**
  - Alcohol dissolves outer membrane and leaves holes (large spaces) in peptidoglycan.
  - CV-I washes out.

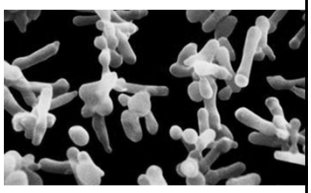
http://faculty.ccbcmd.edu/courses/bio141/labmanua/lab6/images/gram_stain_11.swf

http://highered.mheducation.com/olcweb/cgi/pluginpop.cgi?it=swf::530::530::/sites/dl/free/0073525502/930300/Gram_Stain.swf::Gram%20Stain

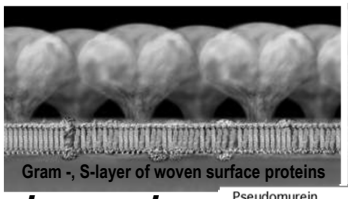
30

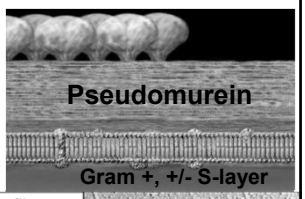
3. Atypical Cell Walls

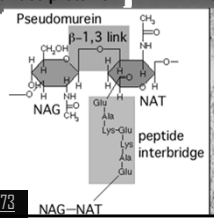
- **Mycoplasmas:**
  - Lack cell walls; smallest bacteria!
  - ***Sterols*** in plasma membrane
    - waxy; fatty; hydrophobic; stabilizes from lysis?



- **Archaea:**
  - Wall-less, or
  - Walls of ***pseudomurein***
    - (lack NAM and D amino acids)
    - ***NAT*** =
      - N-acetylalosaminuronic acid







http://www.microbiologytext.com/index.php?module=Book&func=displayarticle&art_id=73

31

E. Plasma Membrane

1. Phospholipid bilayer
2. Peripheral proteins
3. Integral proteins
   - Transmembrane proteins

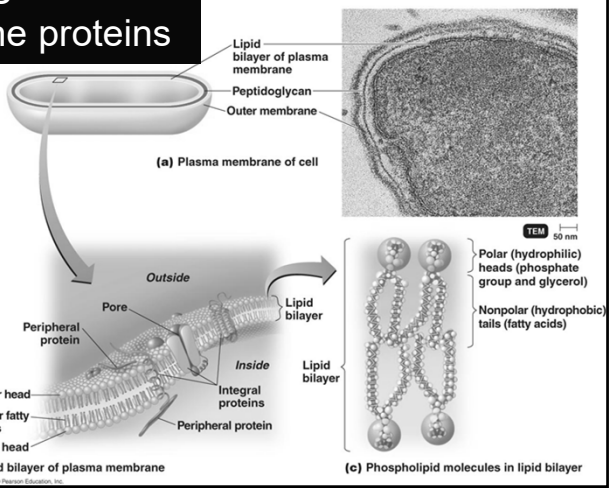


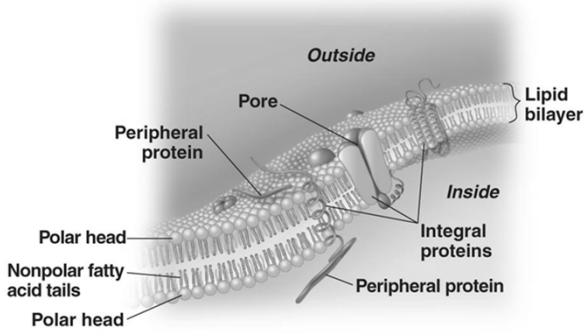
Figure 4.14

32

* ***Fluid Mosaic Model*** *

- Membrane is as viscous as olive oil.
- Proteins move to function.
- Phospholipids rotate and move laterally.

❖ ***"Proteins afloat in a sea of phospholipids"***



(b) Lipid bilayer of plasma membrane

Figure 4.14b

33

Prok. Plasma Membrane

- Selective permeability allows passage of some molecules
- Enzymes for ATP production
- Photosynthetic pigments on foldings called ***chromatophores*** or ***thylakoids***

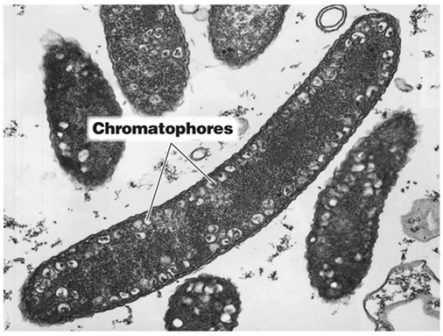
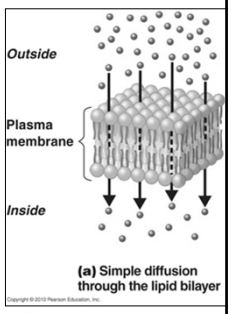


Figure 4.15

34

F. Movement Across Membranes

1.PASSIVE TRANSPORT:



a) Simple diffusion:
Movement of a solute from an area of high concentration to an area of low conc'n. ** ***WITH GRADIENT!!***

b) Facilitated diffusion:
Solute combines with a transporter protein in the membrane.
- ***Carrier-mediated transport***
- ***Channel proteins***

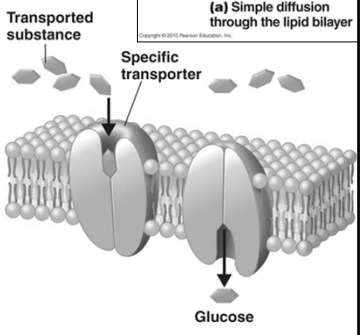


Figure 4.17

35

Movement Across Membranes

- ***Osmosis:*** Movement of water across a selectively permeable membrane
  - **from an area of high [H_2O] to an area of lower [H_2O].**
- **Osmotic pressure:** The pressure needed to stop the movement of water across the membrane.

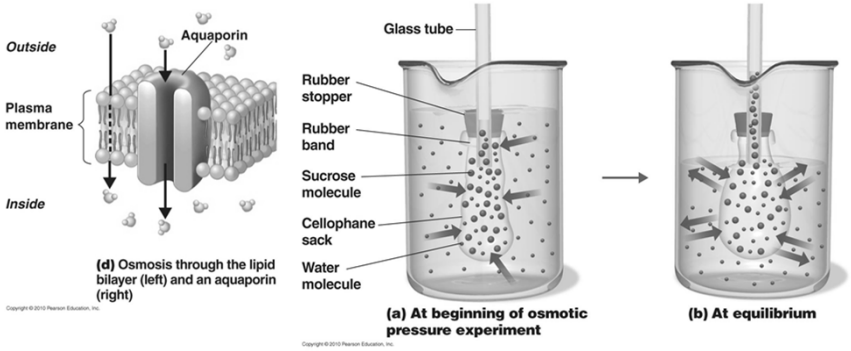
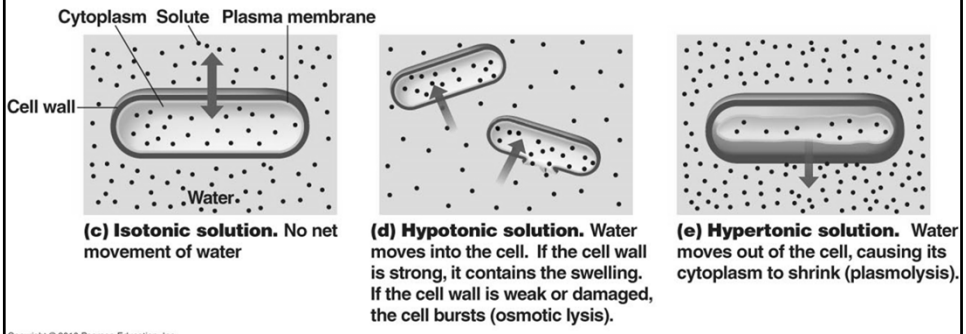


Figure 4.18ab

36

Tonicity *(of **external** solution)*

ISO- = same ***HYPO***- = low ***HYPER***- = high



(c) Isotonic solution. No net movement of water

(d) Hypotonic solution. Water moves into the cell. If the cell wall is strong, it contains the swelling. If the cell wall is weak or damaged, the cell bursts (osmotic lysis).

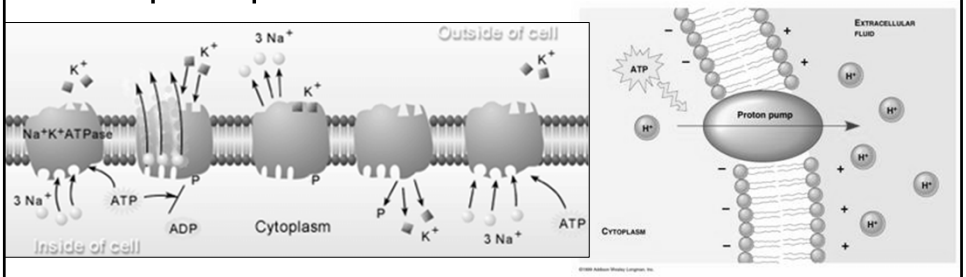
(e) Hypertonic solution. Water moves out of the cell, causing its cytoplasm to shrink (plasmolysis).

Figure 4.18c-e

37

Movement Across Membranes

2. ***Active Transport*** of substances requires a transporter protein and ATP. ***** AGAINST GRADIENT!!***



3. ***Group Translocation*** of substances requires a transporter protein and PEP.
   - Unique to prokaryotes
   - PEP phosphorylates sugar after enters cytoplasm; sugars trapped and concentrated!

4. **Bulk Transport in Euk.: Exocytosis, Endocytosis:**
   - ***Phagocytosis & Pinocytosis.***

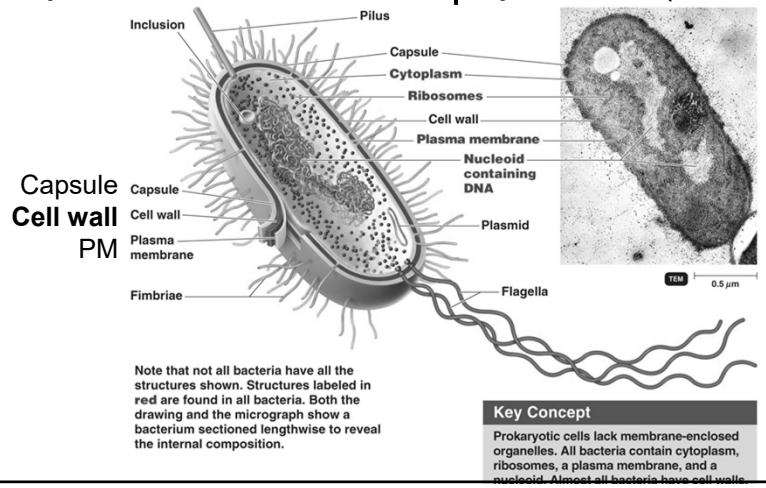
38

G. Cytoplasm

- Cytoplasm is the substance inside the plasma membrane

H. Nuclear Area

- ***"Nucleoid"*** = DNA and associated proteins (***chromosome***)

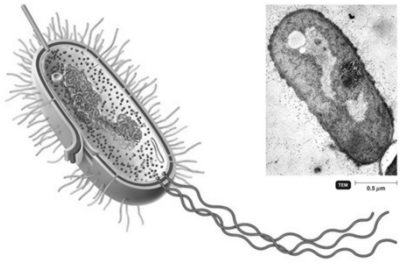


Capsule
Cell wall
PM

Figure 4.6

39

i. Ribosomes



- **"Protein Factories"**

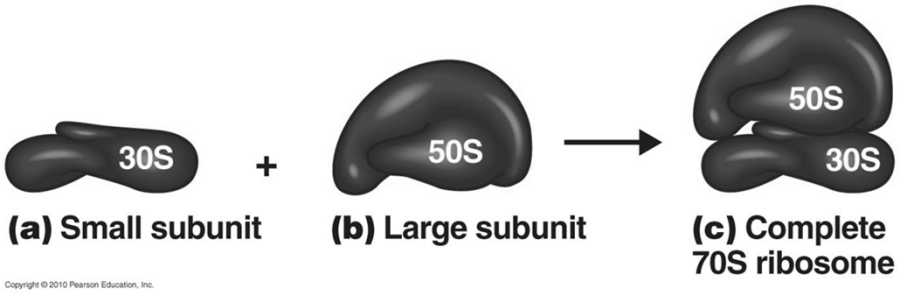
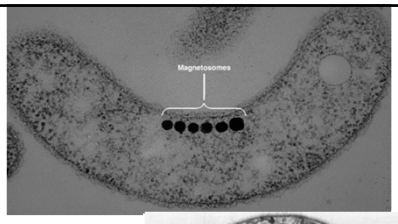


Figure 4.19

40

J. Inclusions



Metachromatic granules (***volutin***)	Phosphate reserves
Polysaccharide granules	Energy reserves
Lipid inclusions	Energy reserves
Sulfur granules	Energy reserves
Carboxysomes	Ribulose 1,5-diphosphate carboxylase for CO_2 fixation (photosynthesis!)
Gas vacuoles	Protein covered cylinders -- buoyancy
Magnetosomes	Iron oxide (destroys H_2O_2)

41

K. Endospores

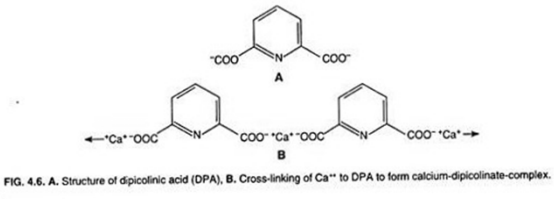


FIG. 4.6. A. Structure of dipicolinic acid (DPA), B. Cross-linking of Ca^{++} to DPA to form calcium-dipicolinate-complex.

- Resting / dormant cells
- Resistant to desiccation, heat, chemicals
  - **Dipicolinic acid + Calcium** in spore coat
- *Bacillus, Clostridium*

- ***Sporulation***: Endospore formation
- ***Germination***: Return to vegetative state

42

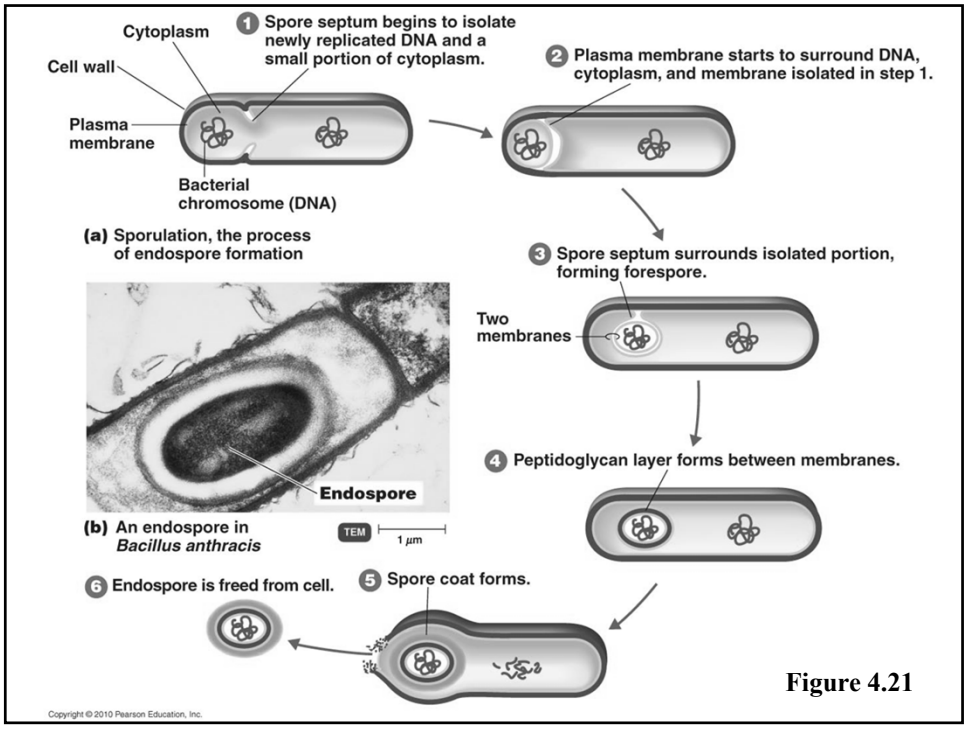


Figure 4.21

43

4.2) Eukaryotic Cells

- **Comparing Prokaryotic & Eukaryotic Cells**
  - Prokaryote comes from the Greek words for ***pre-nucleus***.
  - Eukaryote comes from the Greek words for ***true nucleus***.

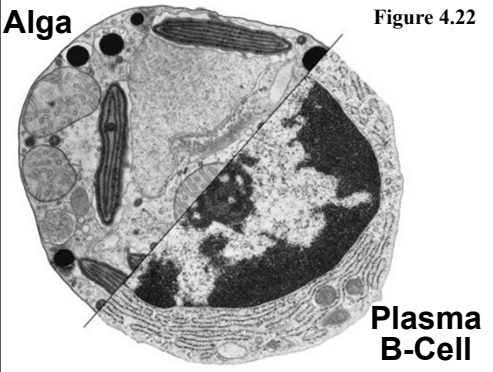
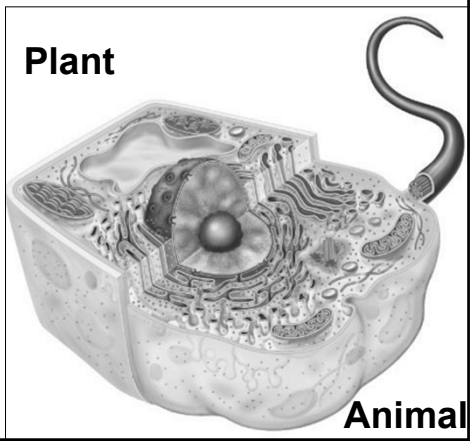


Figure 4.22



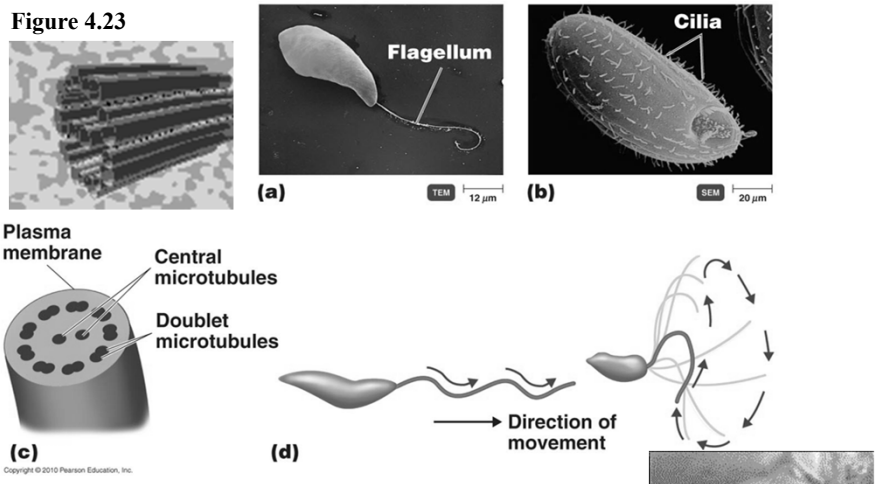
44

Prokaryotic vs. Eukaryotic *Cells*

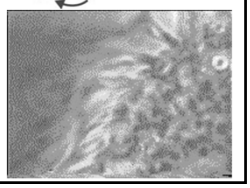
1. No true nucleus	**1. True Nucleus**
2. No memb.-bound organelles	2. Memb.-bound nucleus and other organelles
3. Single, circular chromosome	3. Many, linear chromosomes
4. 70S ribosomes	4. 80S ribosomes
5. Unique cell wall (PG)	5. Plants and Fungi CW's
6. Unique flagella - flagellin	6. Microtubule flagella
7. Outer Membrane (gram -)	7. No Outer Membrane
8. Only unicellular	8. Many spp. Multicellular
9. Small (1-5 μm diameter)	9. Larger (10-100 μm diameter)
10. Divide by binary fission	10. Divide by mitosis/meiosis and cytokinesis
11. Bacteria: no histones	11. Histone-bound chromosomes

45

A. Flagella and Cilia

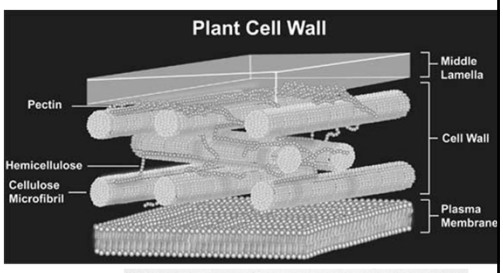


- Microtubules + motor proteins
  - ***Tubulin***
- 9 pairs + 2 -- arrangements

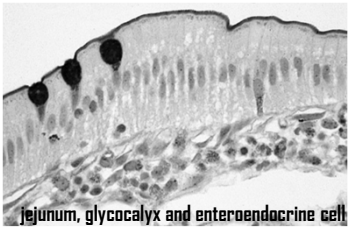


46

B. Euk. Cell Wall



- **Cell wall**
  - Plants, algae, fungi
  - Carbohydrates
- ***Cellulose, chitin, glucan, mannan, pectins***

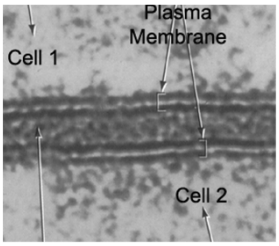


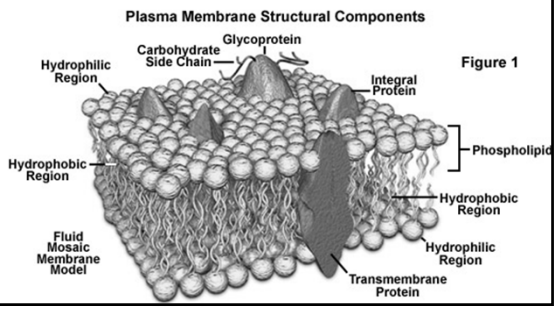
- **Glycocalyx**
  - Carbohydrates extending from animal plasma membrane
  - Bonded to proteins and lipids in membrane

47

C. Euk. Plasma Membrane

1. Phospholipid bilayer
2. **Peripheral proteins**
3. **Integral proteins**
   - Eg: Transmembrane proteins
4. Sterols (animal cells, [mycoplasmas], fungi, plants)
5. Glycocalyx carbohydrates



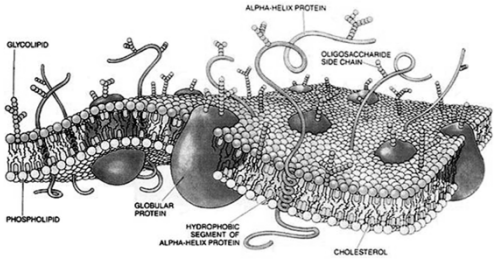


48

Euk. Plasma Membrane

❖ ***Selective permeability allows passage of some molecules***

1. Simple diffusion
2. ***Facilitated diffusion***
3. Osmosis
4. ***Active transport***
5. ***Endocytosis***

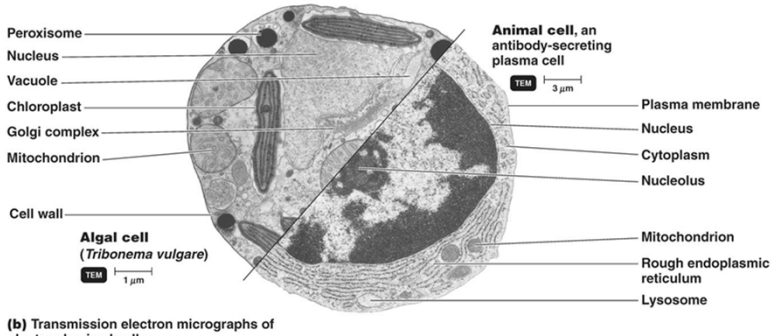


- ***Phagocytosis***: Pseudopods extend and engulf particles.
- ***Pinocytosis***: Membrane folds inward bringing in fluid and dissolved substances.

49

Eukaryotic Cell

Cytoplasm	Substance inside plasma membrane & outside nucleus
Cytosol	Fluid portion of cytoplasm
Cytoskeleton	Microfilaments, intermediate filaments, microtubules
Cytoplasmic streaming	Movement of cytoplasm throughout cells



(b) Transmission electron micrographs of plant and animal cells.

Figure 4.22b

50