BIOL 240: General Microbiology

Spring 2020 Rm. 23-203 MW, Feb. 24-26

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

1. **Pre-Lab Writeup #4 due!:** **ALL of Expts. 8 & 9.** Prepare before each Monday's labs (for BOTH Mon. & Wed.)!! ***(What? Why? How? are we doing in the lab? Question? HYPOTHESIS?)***
2. ***0.5-1 hour of OPEN LAB *expected* each week. Goal: 8-16h by May! ☺***
3. **LAB: Growth quantitation, & Fungi!**
4. **QUIZ #3 due by Wed. night!!! (1st attempt!).**
5. **Study Guides & Lesson Objectives (see slides) due NEXT WED. in Lab (Ch. 4b, 5a)!**
6. **MIDTERM EXAM #1 will be returned this week! Review and reexamine the test, & meet with me THIS week if you scored <70%!!**
7. ***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**
   - **NOTE: YOU may upload MULTIPLE Speaker Summaries, but only I can see all of them. You can only see the last upload! It's OK!! ☺**

1

REVIEW:

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.

OBJECTIVES: Students should be able to: …..

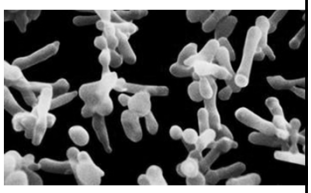
1. Compare and contrast **10 differences** between ***"prokaryotic" and eukaryotic cells***. What structures do they share in common? …..
2. Describe the prevailing ***model of cell membrane structure*** and how the macromolecules that form them exemplify **structure determining function**.
3. Compare and contrast the transported molecules, energy balances and membrane molecules required for 2 types each of **passive & active transport**.
4. Recognize and describe the properties and functions of the various **Eukaryotic Organelles**. *(REVIEW!!)*
5. Illustrate and explain the evidence supporting the predominant **Theory** on the **origin of energetic organelles** in Eukaryotes.
6. Describe **how enzymes speed up chemical reactions**, and how they affect the energy and equilibrium of a reaction.
7. Describe 6 different physical and chemical factors that can **regulate enzyme activity**.
8. Explain how Enzymes and ATP participate in **Energetic Coupling**.
9. Diagram the **investment and release of Energy** and **Carbon** atoms from Glycolysis (through the Citric Acid Cycle).

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

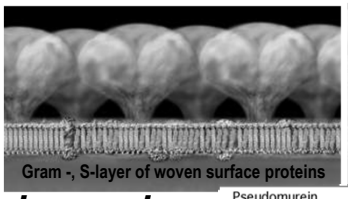
2

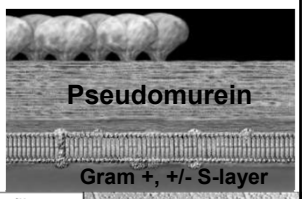
3. Atypical Cell Walls

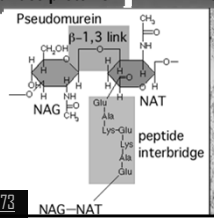
- **Mycoplasmas:**
  - Lack cell walls; smallest bacteria!
  - ***Sterols*** in plasma membrane
    - waxy; fatty; hydrophobic; stabilizes from lysis?



- **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

3

E. Plasma Membrane

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

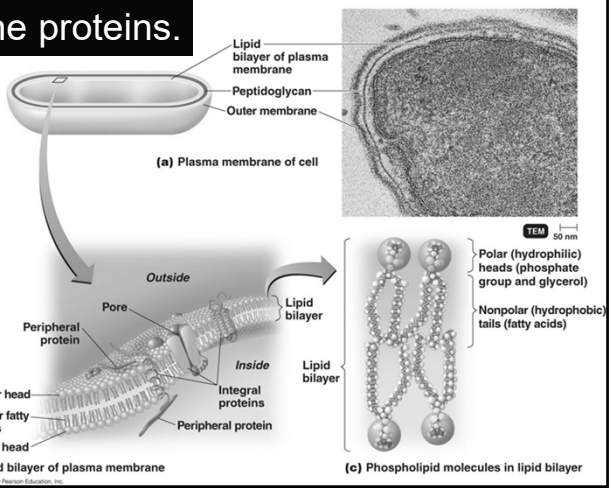


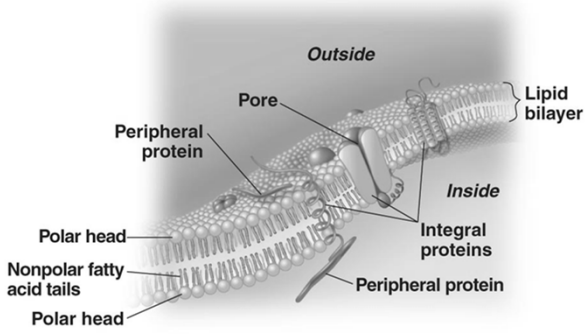
Figure 4.14

4

* ***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

5

Prok. Plasma Membrane

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

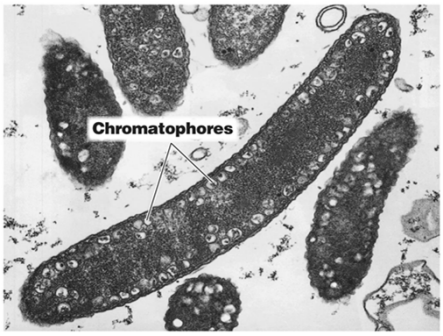


Figure 4.15

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F. Movement Across Membranes

1. PASSIVE TRANSPORT:

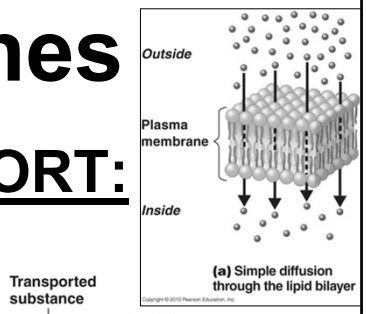
a) Simple diffusion:

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***



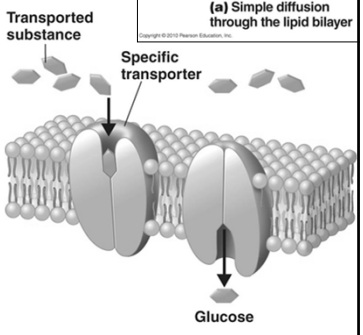
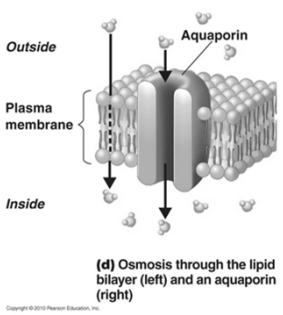


Figure 4.17

7

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.



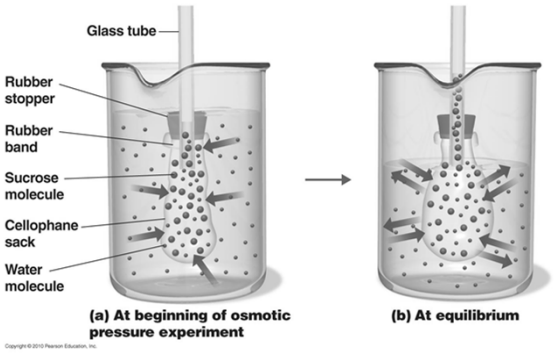
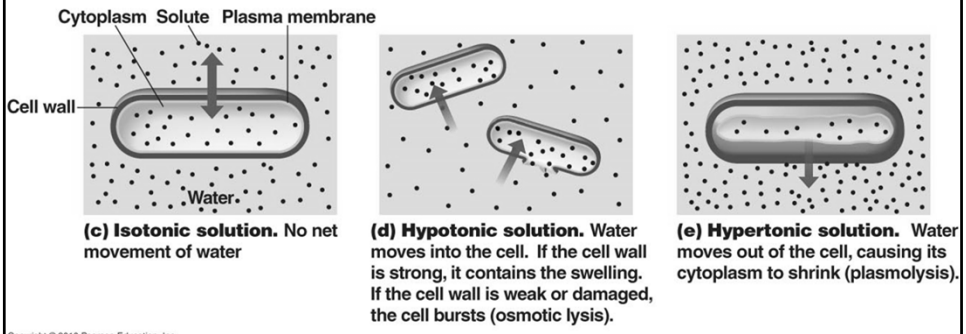


Figure 4.18ab

8

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

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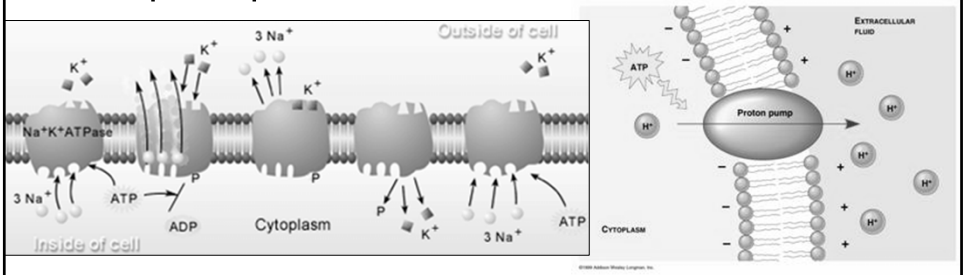
Movement Across Membranes

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.***

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G. Cytoplasm

- Cytoplasm is the substance inside the plasma membrane

H. Nuclear Area

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

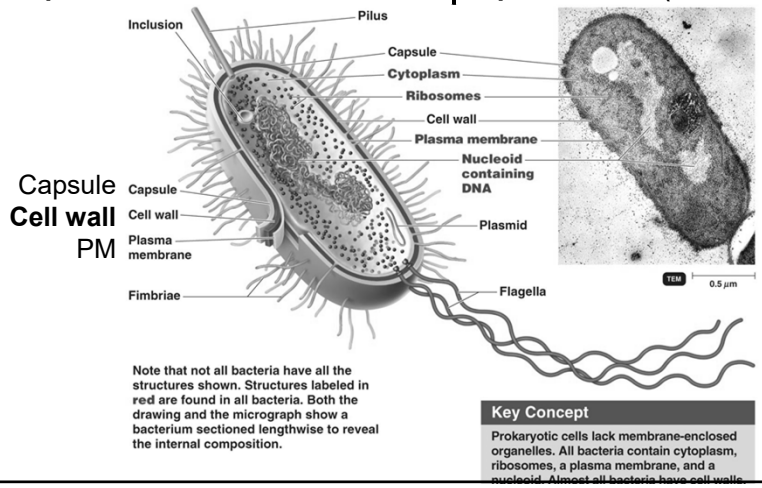

Figure 4.6

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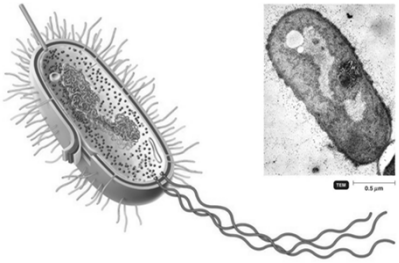
i. Ribosomes



- **"Protein Factories"**

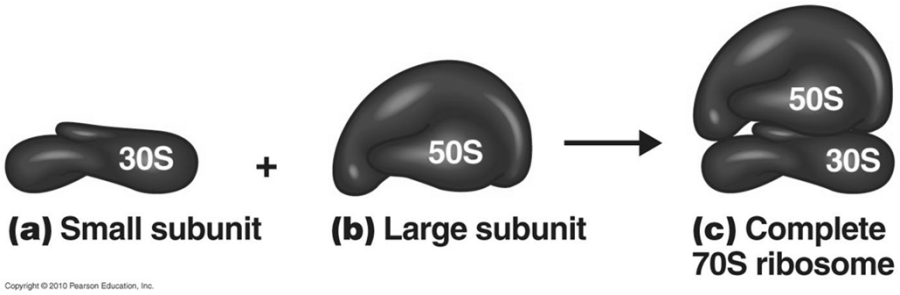


Figure 4.19

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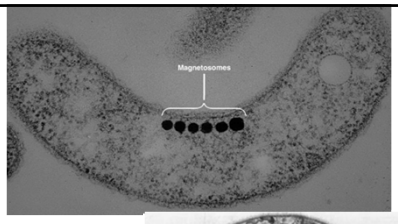
J. Inclusions



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)

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K. Endospores

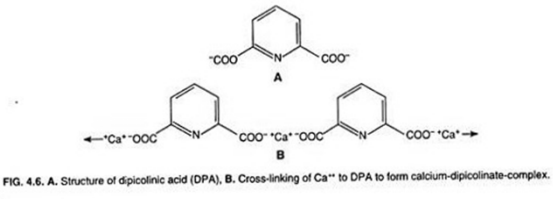

FIG. 4.6. A. Structure of dipicolinic acid (DPA), B. Cross-linking of Ca^{++} to DPA to form calcium-dipicolinate-complex.

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

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

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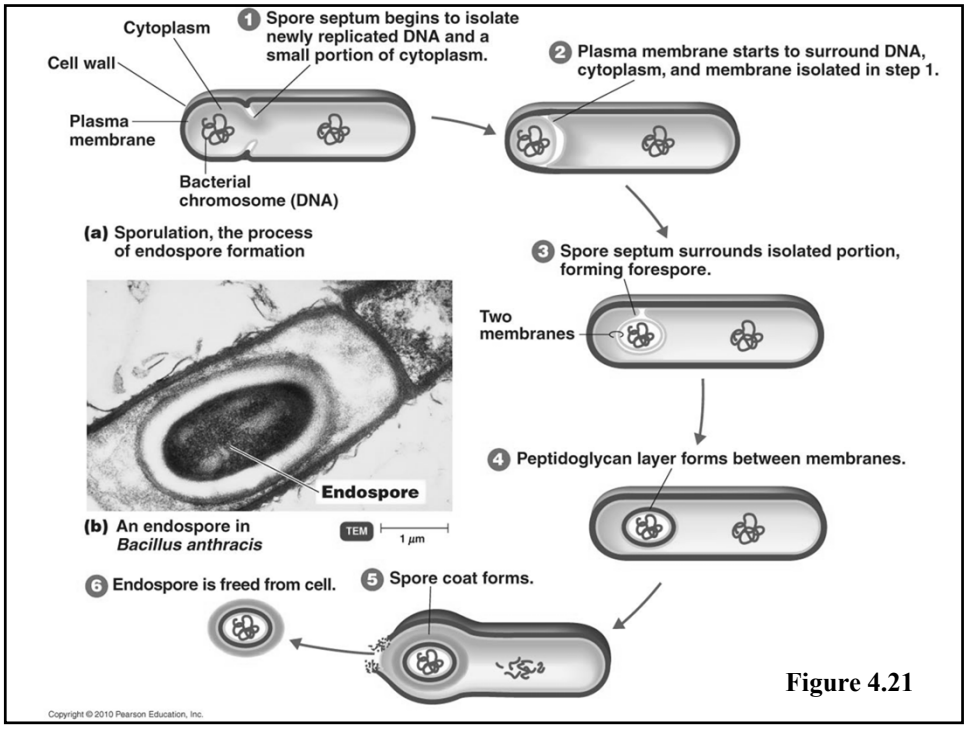

Figure 4.21

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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***.

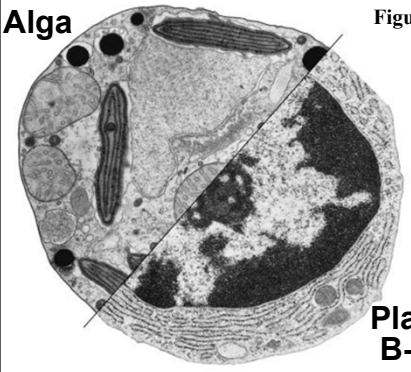
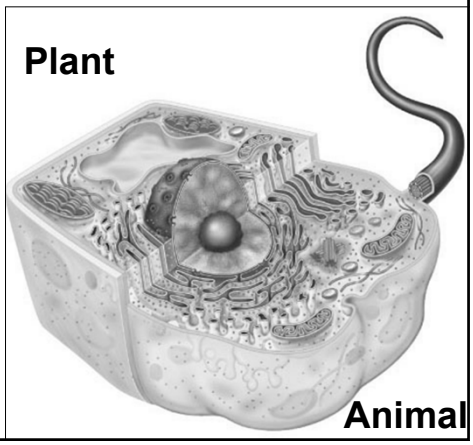


Figure 4.22



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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

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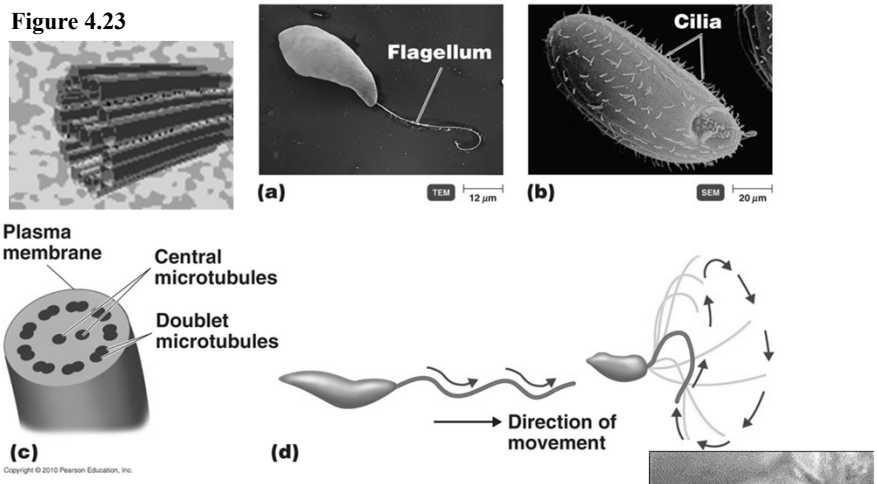
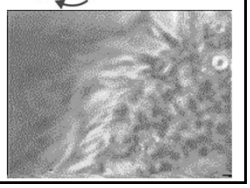
A. Flagella and Cilia



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



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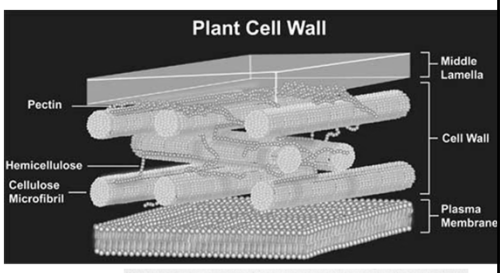
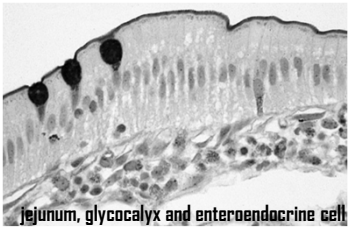
B. Euk. Cell Wall



- **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

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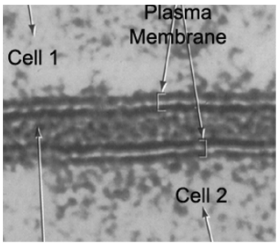
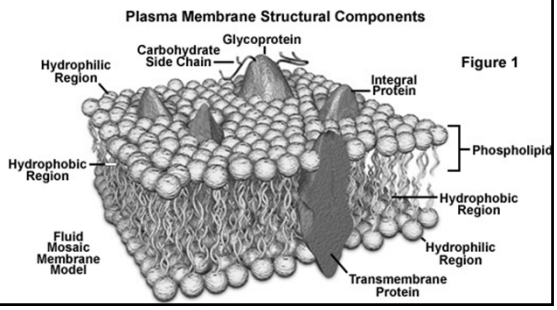
C. Euk. Plasma Membrane

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





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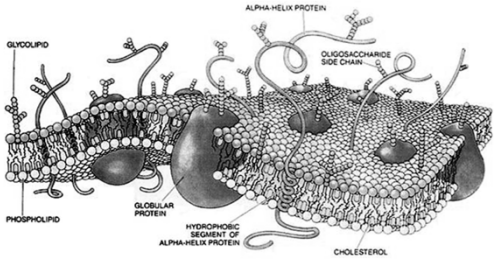
Euk. Plasma Membrane

- ***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.



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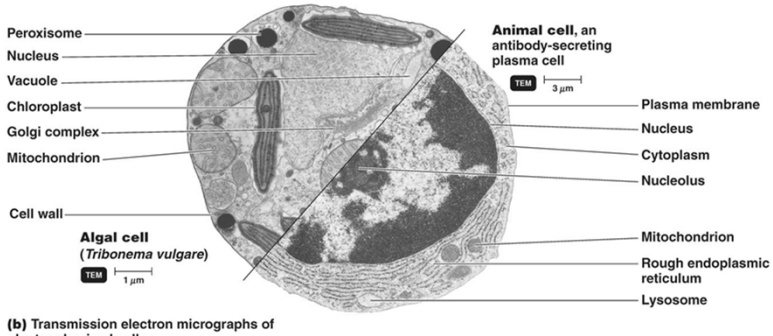
Eukaryotic Cell

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

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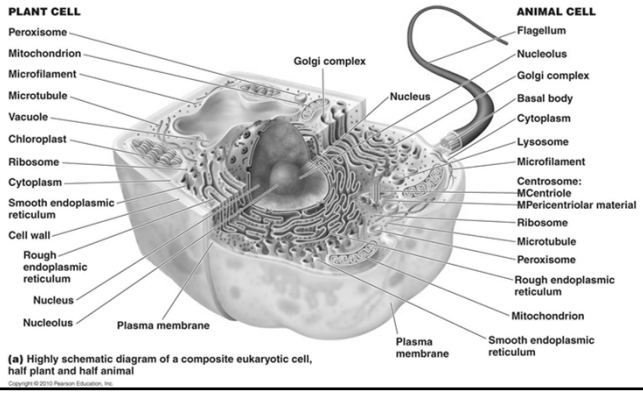
D. Eukaryotic Organelles: NOT membrane-Bound

1. ***Ribosome*** = Protein synthesis
2. ***Centrosome*** = Consists of protein fibers and centrioles
3. ***Centriole*** = Mitotic spindle formation



(a) Highly schematic diagram of a composite eukaryotic cell, half plant and half animal

Figure 4.22a

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E. Eukaryotic Organelles: Membrane-Bound

1. ***Nucleus*** = Contains chromosomes.
2. ***ER*** = Synthesis & transport network.
3. ***Golgi complex*** = Membrane formation and secretion.
4. ***Lysosome*** = Digestive enzymes
5. ***Vacuole*** = Brings food into cells and provides support.
6. ***Mitochondrion*** = Cellular respiration
7. ***Chloroplast*** = Photosynthesis
8. ***Peroxisome*** = Oxidation of fatty acids; destroys H_2O_2.

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(E.) Memb. Bound Organelles:
1. Nucleus ****

1. **Nuclear Pores**
2. **Chromatin**
   - Complex of DNA and protein
   - Histones; nonhistones
3. **Nucleolus**
   - Site of Ribosome synthesis

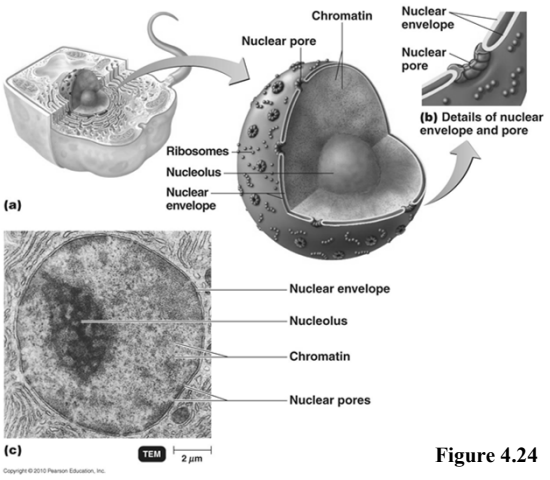

Figure 4.24

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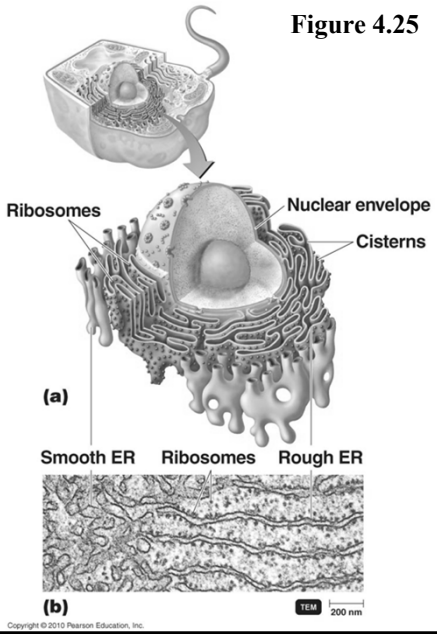
2. Endoplasmic Reticulum

Figure 4.25

a) ***Smooth ER*** – lipid synthesis; detoxification

b) ***Rough ER*** – protein synthesis for export – secretion or PM; protein modification
   - Lipoproteins, glycoproteins



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3. Ribosomes

- **= *Protein factories!!!***
- **80S**
  - Membrane-bound = attached to ER
  - Free – in cytoplasm
- **70S**
  - In chloroplasts and mitochondria

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4. Golgi Complex

- ***Cisterns*** – stacks of membrane "pita pocket" bread
- Vesicles transport proteins and lipids from ER to Golgi for modification and sorting
  - ***"Post Office"*** *of the Cell – package & transport prot & lipid*

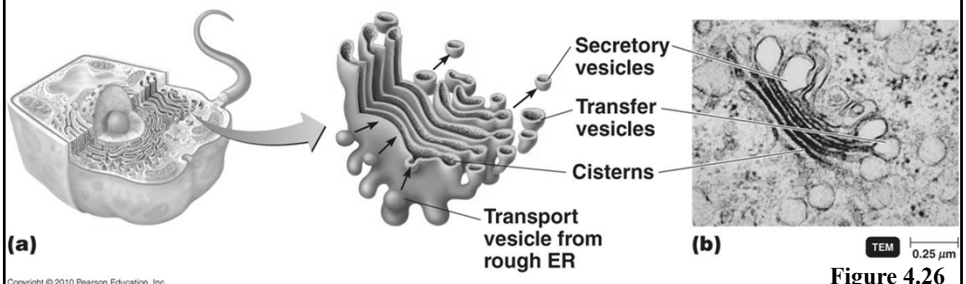

Figure 4.26

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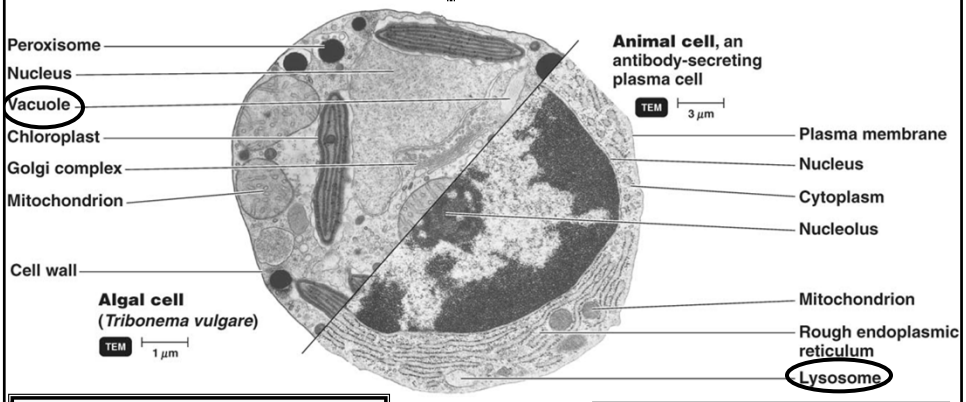
5. Vacuoles;

- **For storage; tonicity (firmness of cell)**

6. Lysosomes

- **For endocytosis**



In plant/algae cells:
- Storage (food, minerals, wastes);
- osmotic pressure -*supports cell!!*

Hydrolytic enzymes:
- **fuse with endocytic vesicles;**
- **intracellular digestion**

Figure 4.22b

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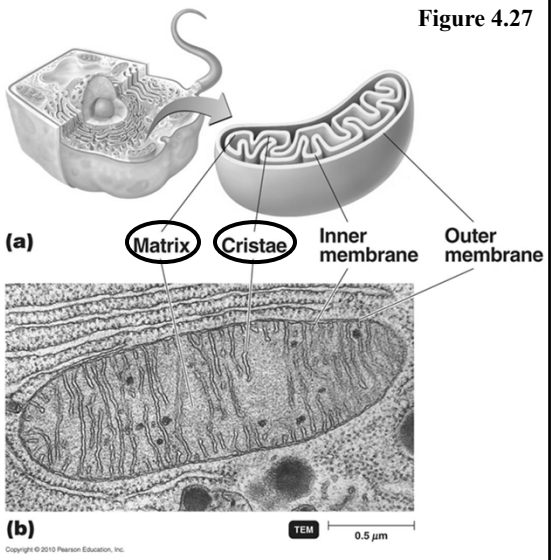
7. Mitochondrion

- ***Powerhouse*** of the cell!
  - O_2 usage
  - ATP & CO_2 production!
- Oxidative respiratory enzymes in **inner membrane** of mitoch.
  - ***Cristae***
  - Double membrane!!

Figure 4.27



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8. Chloroplast

- Site of photosynthesis: Convert light energy to bio-chemical energy
  - ATP, Sugars
- ***Photosynthesis*** = *source of organic carbon and energy for ALL living things!!*
  - Chlorophyll & other pigments (carotenoids…)
- ***Thylakoid membranes*** = site of energy conversion
  - ***Grana*** = stacks of thylakoid sacs
  - ***Stroma*** = fluid matrix
  - Double membrane

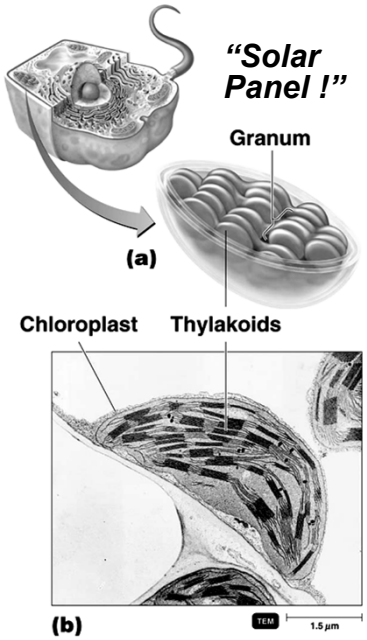

Figure 4.28

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Mitochondria and Chloroplasts: …some early observations…

1. **Have Double-membranes.**
2. **Contain their own DNA and ribosomes.**
3. **Can make some of their own proteins.**
4. **Divide at their own rate ….**

- *Possible scientific explanation??….*

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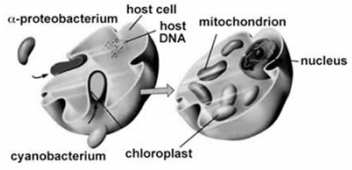
F. Endo-sym-biosis Theory

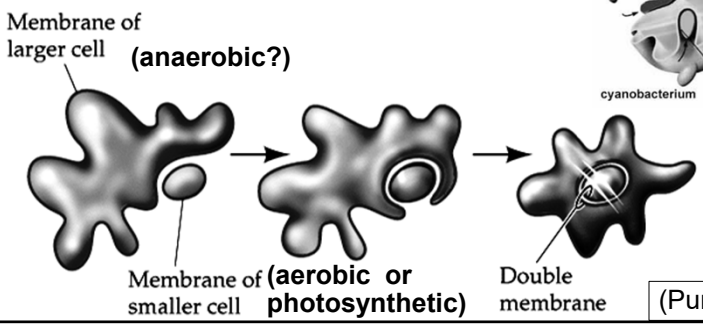
1. The evolution of mitochondria and chloroplasts:

- large prokaryotes engulfed (by "***endocytosis***"), but did not digest, smaller ones → DOUBLE MEMBRANE (from host & endosymbiont)

2. Mutual benefits permitted this symbiotic relationship to evolve into eukaryotic organelles of today

- Home & protection for small cell; food & mineral sources (gatherer).
- New, powerful energy source for larger cell.





(Purves et al, 2001)

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Endosymbiotic Theory

EVIDENCE:

1. Approximately the **same size and shape** of known prokaryotes ("bacteria")
2. Double Membranes
3. Bacteria-like genetic information (chromosomal DNA):
   - **a) Closed, circular DNA** (not linear, like Euk.).
   - b) Encode own: metabolic proteins, prok.-type ribosomes (70S!!)
4. **Prok.-like division** mechanisms.
5. Other very intimate/intracellular symbioses between free-living organisms exist now!!

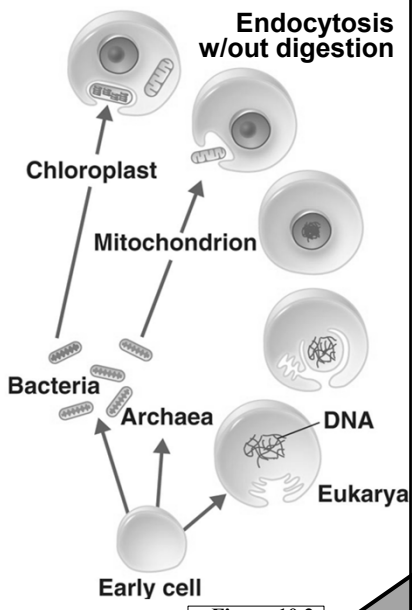

Figure 10.2

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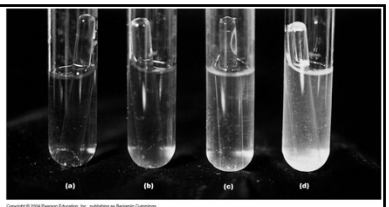
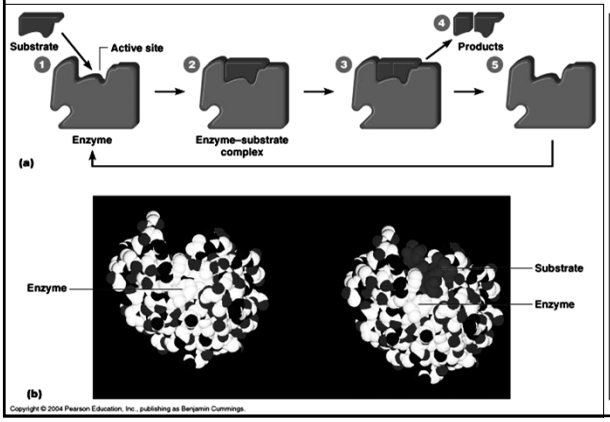
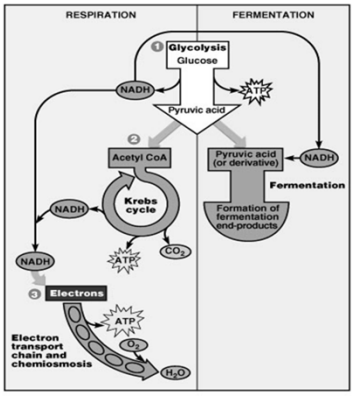
Chapter 5 Microbial Metabolism







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Microbial Metabolism

1. ***Metabolism*** is the sum of the chemical reactions in an organism.
2. ***Catabolism*** is the breaking-down complex molecules; energy-releasing processes.
3. ***Anabolism*** is building up complex molecules from simpler subunits; energy-using processes.

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Microbial Metabolism

- Catabolism provides the building blocks and energy for anabolism.

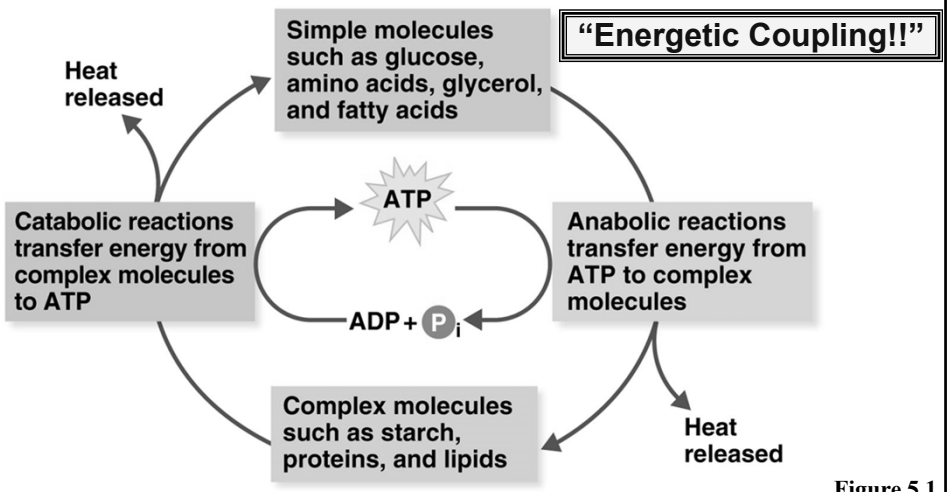

Figure 5.1

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Metabolism

- A ***metabolic pathway*** is a ***sequence of enzymatically catalyzed chemical reactions*** in a cell.

A → B → C → D → E → F
G → H → I

1. Metabolic pathways are determined by enzymes.
2. Enzymes are encoded by genes…….
   Therefore:

➢ ***Genes drive metabolism!!!***

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- The ***Collision Theory:*** chemical reactions can occur when atoms, ions, and molecules collide – allowing exchange of electrons.

- ****Activation energy*** is needed to disrupt electronic configurations.**
- ***Reaction rate*** is the frequency of collisions with enough energy to bring about a reaction.
  - Reaction rate can be increased by ***enzymes*** or by increasing temperature or pressure.

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5.1) Enzymes

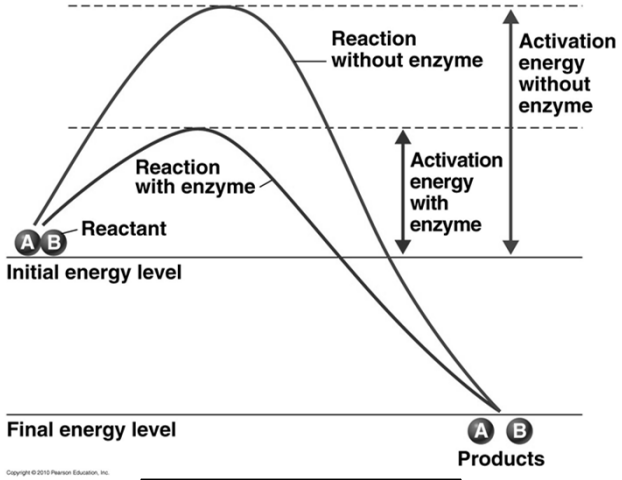

Figure 5.2

FURTHER READING:
http://blog.dearbornschools.org/renkomapbio/2010/10/28/enzymes/

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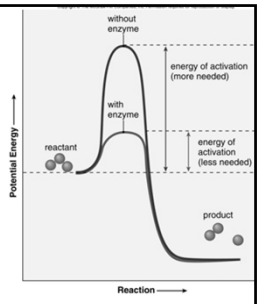
Enzymes



❖ **Biological catalysts**

1) Specific for a chemical reaction;
2) not used up in that reaction; usually protein.
3) Apply:
- **a) tension,**
- **b) temporary charges, or**
- **c) proper orientation/alignment of reactants/bonds to speed up a reaction.**

*4) **Do **NOT** change the **equilibrium** or difference in **energy** between reactants and products, but only speed up **HOW FAST** equilibrium state is reached.***

5) Often catalyze reactions in BOTH directions.

****

- ***Holoenzyme***: Apoenzyme + cofactor
  - ***Apoenzyme****:* protein
  - ***Cofactor****:* Nonprotein component
- ***Coenzyme***: Organic cofactor

****

- ***RIBOZYMES***: = Catalytic RNA's!!
  - RNA that cuts and splices RNA;
  - in peptide synthesis (ribosome).

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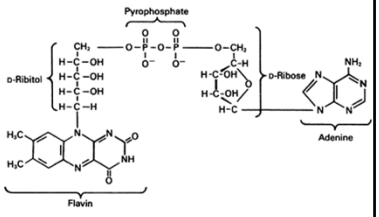
Enzymes & Important Coenzymes

- NAD+
- NADP+ } ***electron carriers***
- FAD



- Coenzyme A – carries 2C units.

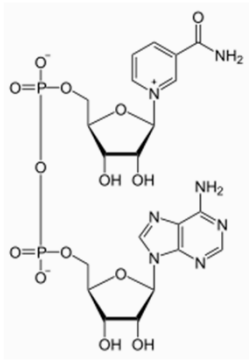
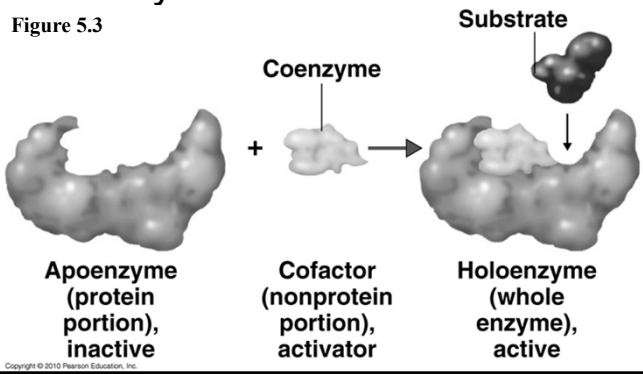


Figure 5.3



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Enzymes

- The turnover number is generally 1-10,000 molecules per second.

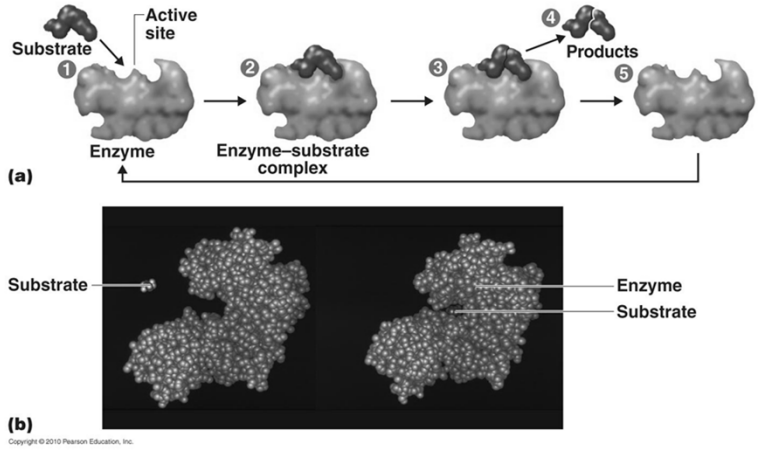

Figure 5.4

http://highered.mcgraw-hill.com/sites/0072495855/student_view0/chapter2/animation__how_enzymes_work.html

http://www.stolaf.edu/people/giannini/flashanimat/enzymes/prox-orien.swf

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5.2) Factors Influencing Enzyme Activity

- Enzymes can be ***denatured*** by temperature and pH. (& high salt, nonpolar solvents….)

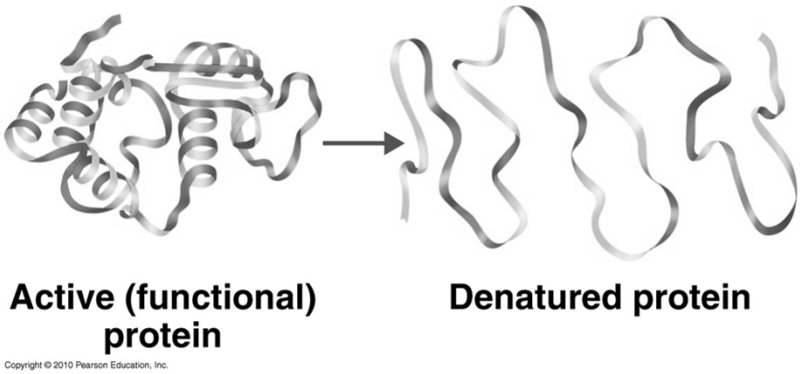

Figure 5.6

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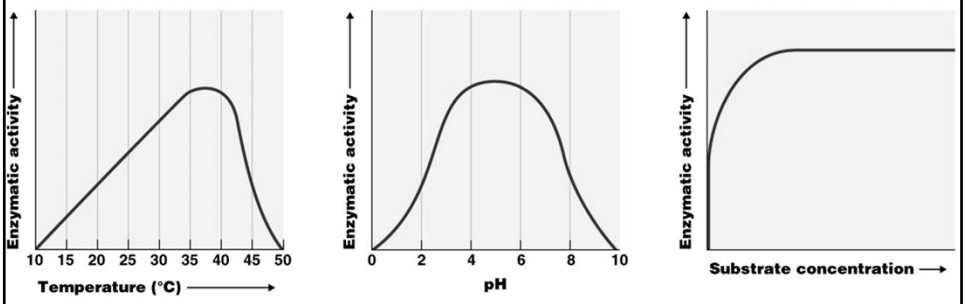
A. Factors Influencing Enzyme Activity: Physical Conditions

- Temperature
- pH
- Substrate concentration



(a) Temperature. The enzymatic activity (rate of reaction catalyzed by the enzyme) increases with increasing temperature until the enzyme, a protein, is denatured by heat and inactivated. At this point, the reaction rate falls steeply.

(b) pH. The enzyme illustrated is most active at about pH 5.0.

(c) Substrate concentration. With increasing concentration of substrate molecules, the rate of reaction increases until the active sites on all the enzyme molecules are filled, at which point the maximum rate of reaction is reached.

Figure 5.5

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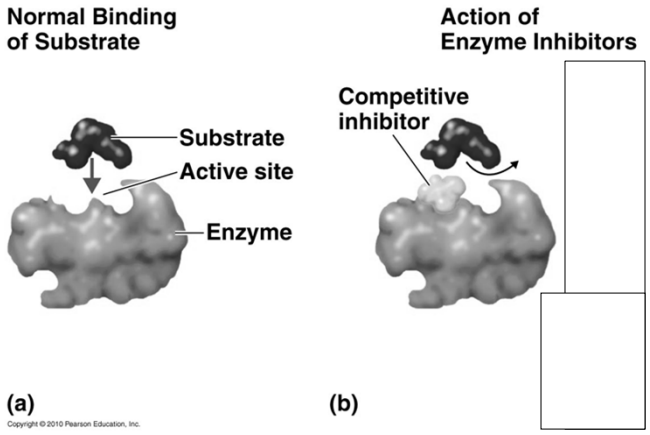
B. Factors Influencing Enzyme Activity: Competitive Inhibition

❖ ***Competitive Inhibition***

Figure 5.7a,b



http://bcs.whfreeman.com/thelifewire/content/chp06/0602001.html

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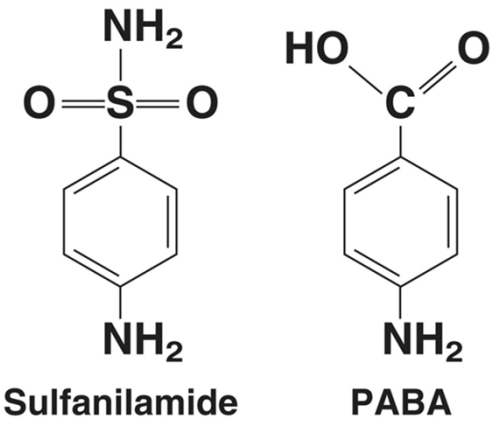
Competitive Inhibition

- Eg: **Sulfanilamide**
- Competes with **PABA**
  - **= *Sulfa Drug***
  - Prevents conversion of PABA to **folic acid**
  - Cells don't grow



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C. Factors Influencing Enzyme Activity: Noncompetitive Inhibition

❖ *Noncompetitive Inhibition*

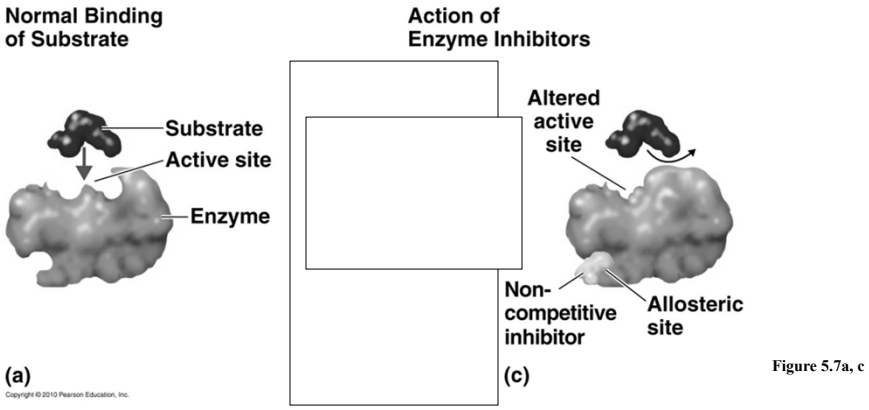

Figure 5.7a, c

➢ ***Allosteric site (regulatory)*** **– "different shape" induced**

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D. Factors Influencing Enzyme Activity

- ***Feedback inhibition***
  - ***Often noncompetitive***
    - ***Allosteric*** site involved
  - Can be competitive.

http://highered.mcgraw-hill.com/sites/0072943696/student_view0/chapter2/animation__feedback_inhibition_of_biochemical_pathways.html

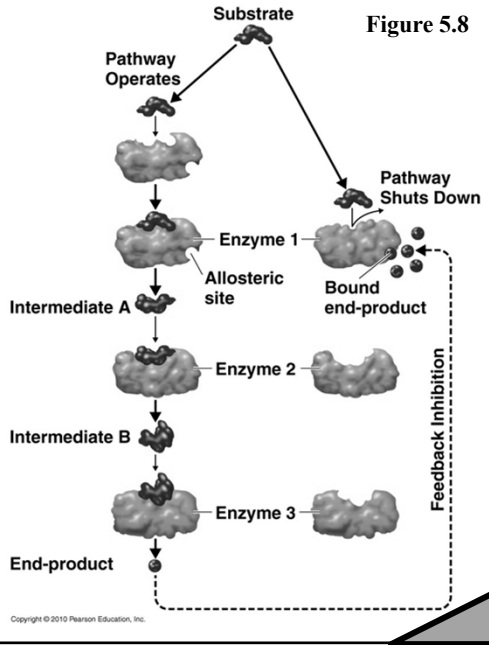

Figure 5.8

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5.3) Oxidation-Reduction

- ***Oxidation*** is the removal of electrons.
- ***Reduction*** is the gain of electrons.
- **Redox reaction** is an oxidation reaction paired with a reduction reaction (always!).

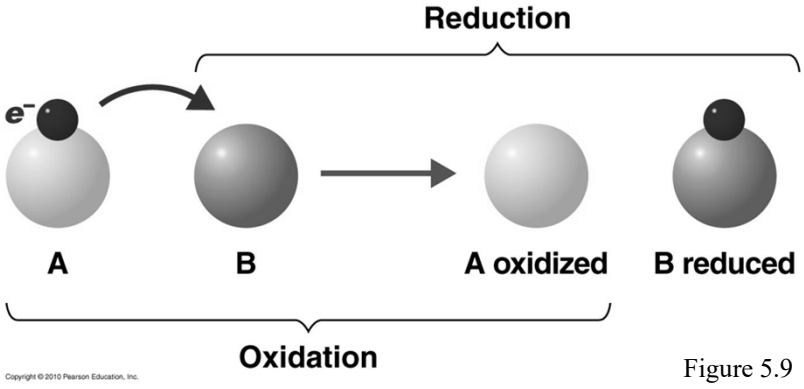

Figure 5.9

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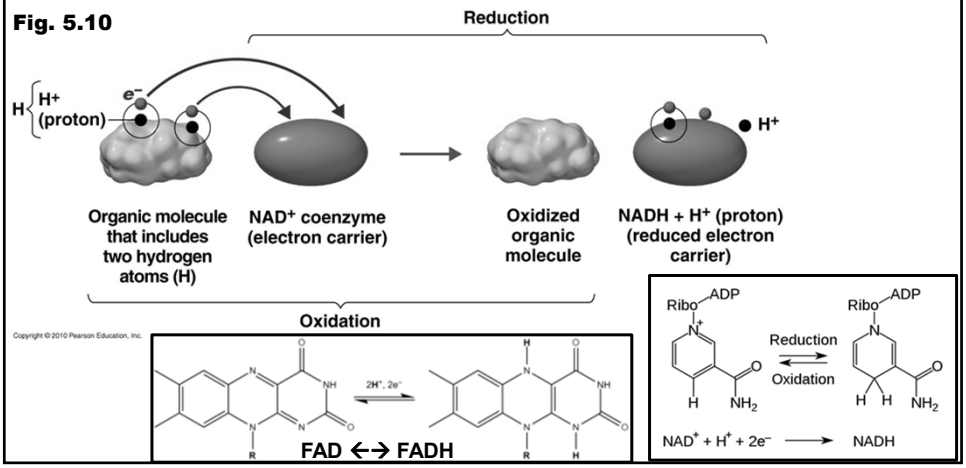
Oxidation-Reduction

- In biological systems, the **electrons** are often associated with **hydrogen atoms**.
- Biological oxidations are often ***dehydrogenations***.

Fig. 5.10



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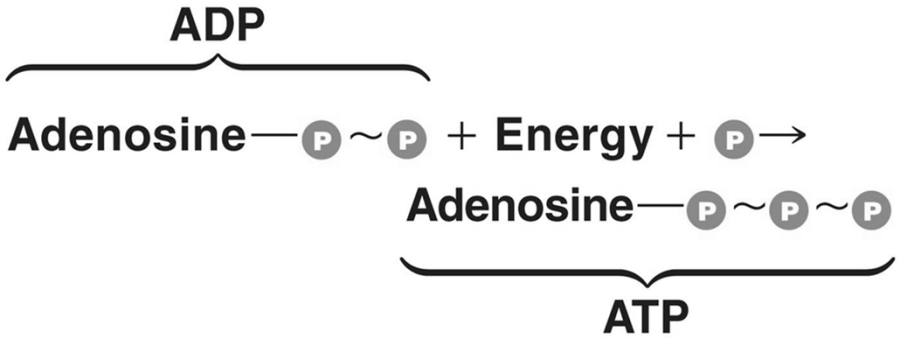
5.4) The Generation of ATP

- ATP is generated by the phosphorylation of ADP.



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