

18BTB101T- Biology

UNIT I

- Introduction
- Cell structure and function
- Genetic information, protein synthesis, and protein structure
- Cell metabolism
- Homoeostasis
- Cell growth, reproduction, and differentiation

| | | | | | | | |
|----------------------------|---------------|-----------------------------|---------------|---------------------|-----|-------------|---------------|
| Code | BBT101 | Name | Biotechnology | Category | | Page Number | 2 0 0 2 |
| Pre-requisite Courses | Nil | Co-requisite Courses | Nil | Progressive Courses | Nil | | |
| Course Offering Department | Biotechnology | Data Book / Codes/Standards | Nil | | | | |

Course Learning Rationale (CLR):

The purpose of learning this course is to:

CLR-1 : Recall the cell structure and function from its organization

CLR-2 : Discuss molecular and biochemical basis of an organism

CLR-3 : Compare enzyme reaction and photosynthesis

CLR-4 : Explain different types of biosensors

CLR-5 : Analyze the different types of bioremediation

CLR-6 : Relate the concept of nervous and immune system pertaining to diseases

Course Learning Outcomes (CLO): At the end of this course, learners will be able to:

CLO-1 : Describe the cell growth, metabolism and reproduction.

Learning

1 2 3

CLO-2 : Explain the concepts and experiments in biochemistry

Level of Thinking (loom)
Expected Proficiency (%)

CLO-3 : Recognize the significance of photosynthesis

Expected Attainment (%)

CLO-4 : Discuss the different methods in enzyme catalytic functions

1 80 80

CLO-5 : Analyze the role of biosensors and its applications

2 85 75

CLO-6 : Explain the concepts of nervous system disorder and the diseases associated with it

2 75 80

3 85 80

2 80 80

Program Learning Outcomes (PLO)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

| Engineering Knowledge | Problem Analysis | Design & Development | Analysis, Design, Research | Modern Tool Usage | Society & Culture | Environment & Sustainability | Ethics | Individual & Team Work | Communication | Project Mgt. & Finance | Life Long Learning | PSO - 1 | PSO - 2 | PSO - 3 |
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| Duration (hour) | 6 | 6 | 6 | 6 | 6 |
|-----------------|---|--|--|---|---|
| S-1 | SLO-1 Basics of cell biology: Relevance to Engineers | Biochemistry: Macromolecules, Biodiversity and its importance | Bioenergetics and metabolism | Molecular machines and motors | Nervous system: History of neuroscience |
| | SLO-2 Cell basic unit of life, Evidence for cell theory | Chemistry of life | Enzymes as biological catalysts, Significance of enzymes | Properties of ATP based protein molecular machines | Glia cells, Neurons |
| S-2 | SLO-1 Cell structure and function | Biochemistry and human biology, DNA replication | Thermodynamics of enzymes | F0F1 ATP synthase motors, Coupling and coordination of motors | Action potential, Organization of nervous system |
| | SLO-2 Genetic Information, Protein structure | Transcription, Protein synthesis | Factors affecting enzyme activity, Effect of inhibitors on enzyme activity | Bacterial flagellar motor, Cytoskeleton | Central Nervous system, Peripheral nervous system |
| S-3 | SLO-1 Cell metabolism | Eukaryotic and prokaryotic protein synthesis difference | Mechanism of enzyme action | Microtubules | Diseases of nervous system |
| | SLO-2 Carbohydrate metabolism, Fatty acid metabolism | Concept of genetic code, Stem cells | Enzyme strategies, Restriction enzymes | Microfilaments, Intermediate filaments | Computer-based neural networks |
| S-4 | SLO-1 Homeostasis | Source of stem cells, Classification of stem cells | NMP kinases, Photosynthesis | Kinesin linear motor, Dynein motor | Immune system |
| | SLO-2 Pathways that alter homeostasis, Cell growth | Human embryonic stem cell, Importance and applications of stem cells | Light reactions, Photosystems | Biosensor | Fluid systems of the body, Innate immune system |
| S-5 | SLO-1 Reproduction | Therapeutic cloning | ATP synthesis in chloroplasts | Resonant biosensors, Glucose biosensors | Cells of innate immune system, Adaptive immunity |
| | SLO-2 Eukaryotic cell division, Mitosis | Regenerative medicine | Calvin cycle | Bio detectors, Biosensor detection in pollutants | Diseases of immune system, Immune engineering |
| S-6 | SLO-1 Meiosis, Cell differentiation | Bone tissue engineering | Significance of photosynthesis | Bioremediation | Cell signaling |
| | SLO-2 Neural crest | Gene therapy | Metabolism, Glycolysis | Bioventing and bio augmentation | Cell-surface receptors |

Introduction

Concept of evolution

- Jean Baptistae Lamarak (1801)
- Charles darwin (1859)

Methods of Science

- The scientific method refers to the model for research developed by Francis Bacon (1561–1626). This model involves the following sequence:

1. Identifying the problem

2. Collecting data within the problem area (by observations, measurements, etc.)

3. Sifting the data for correlations, meaningful connections, and regularities

4. Formulating a hypothesis (a generalization), which is an educated guess that explains the existing data and suggests further avenues of investigation

5. Testing the hypothesis rigorously by gathering new data

6. Confirming, modifying, or rejecting the hypothesis in light of the new findings

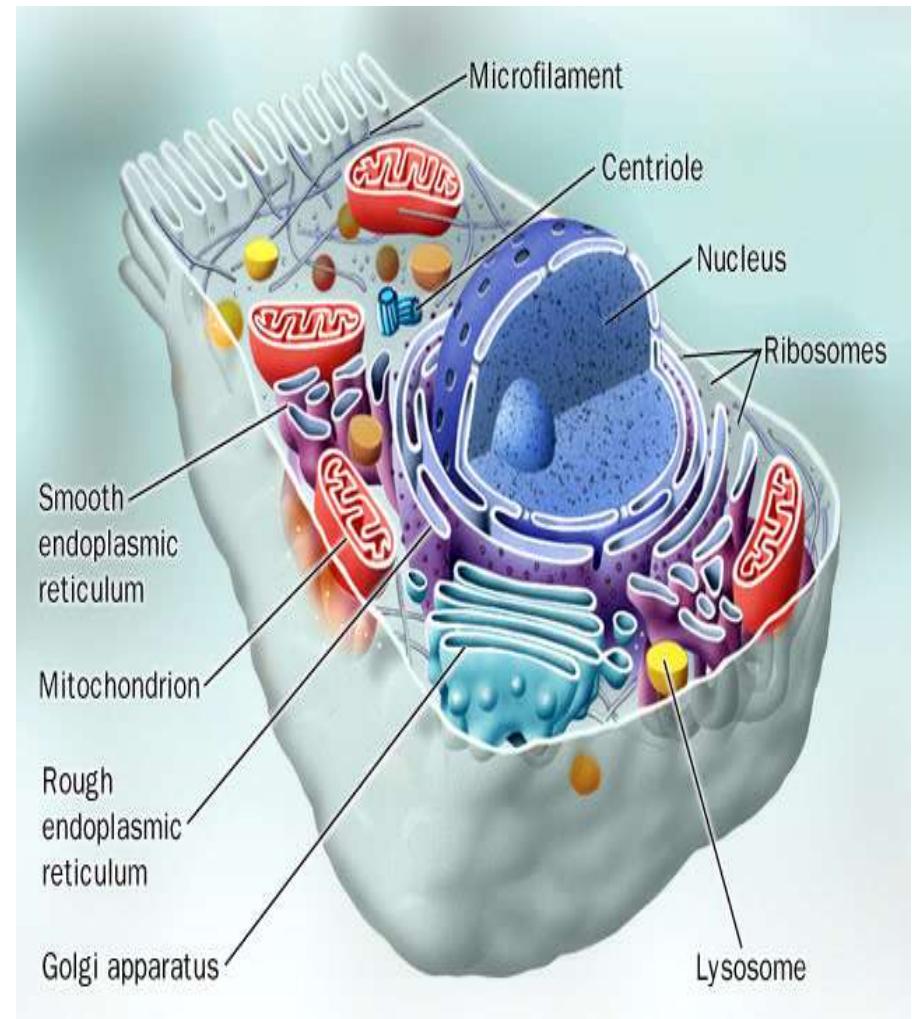


Living Organism

- A living organism may be defined as a complex unit of physicochemical materials that is capable of self-regulation, metabolism, and reproduction.
- Furthermore, a living organism demonstrates the ability to interact with its environment, grow, move, and adapt.

What Are the Main Characteristics of organisms?

1. Made of **CELLS**
2. Require **ENERGY** (food)
3. **REPRODUCE** (species)
4. Maintain **HOMEOSTASIS**
5. **ORGANIZED**
6. **RESPOND** to environment
7. **GROW** and **DEVELOP**
8. **EXCHANGE** materials with surroundings (water, wastes, gases)



Five Kingdoms and their chief characteristics

- Unicellular organisms that lack a nucleus and many of the specialized cell parts, called organelles. Such organisms are said to be prokaryotic (*pro* = “before”; *karyotic* = “kernel,” “nucleus”) and consist of bacteria.
- All of the other kingdoms consist of eukaryotic (*eu* = “true”) organisms, which have cells that contain a nucleus and a fuller repertory of organelles.

| KINGDOM | DISTINGUISHING CHARACTERISTICS | EXAMPLES OF ORGANISMS |
|-------------|---|-----------------------|
| 1. Monera | Single-celled, <i>prokaryotic</i> organisms: cells lack nuclei and certain other specialized parts | Bacteria |
| 2. Protista | Single-celled, <i>eukaryotic</i> organisms: cells contain nuclei and many specialized internal structures | Protozoa |
| 3. Plantae | Multicellular, <i>eukaryotic</i> organisms that manufacture their food | Ferns, trees |
| 4. Fungi | Eukaryotic, plantlike organisms, either single-celled or multicellular, that obtain their food by absorbing it from the environment | Yeasts, molds |
| 5. Animalia | Eukaryotic, multicellular organisms that must capture their food and digest it internally | Fishes, birds, cows |

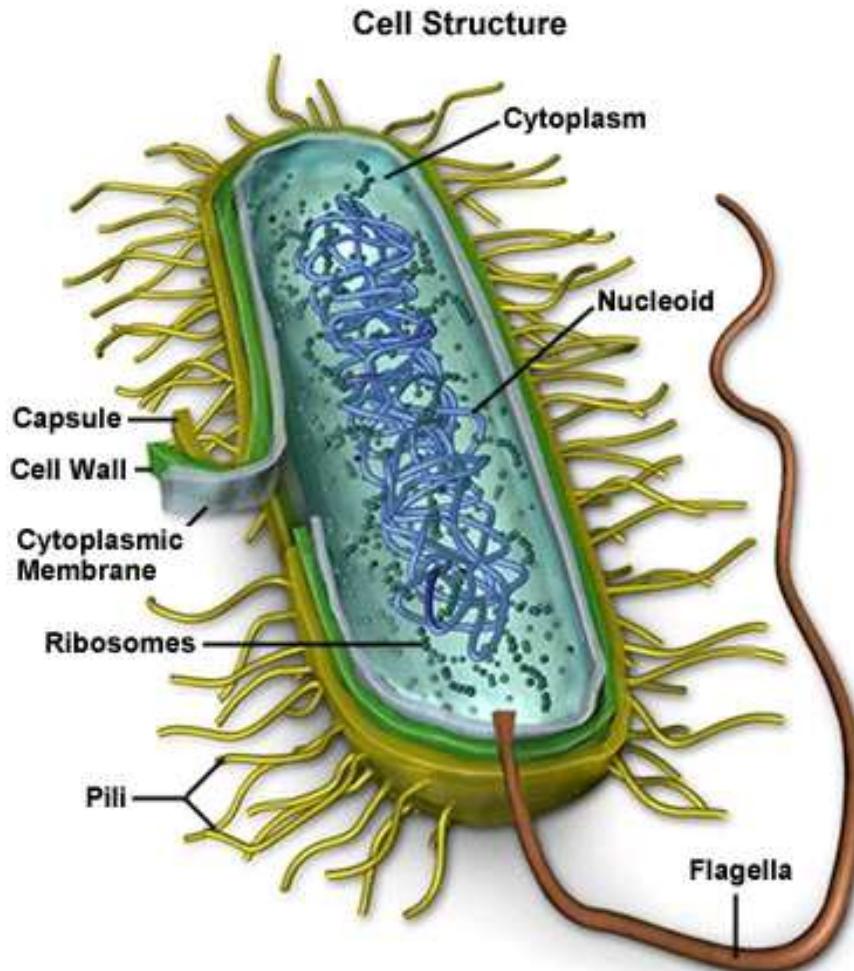
Cell-basic unit of life

- Smallest living form
- Inside the cell some structure transport
- Metabolize
- Respire
- Reproduce (Meiosis)
- Multiply (Mitosis)
- Energy producing
- Keep information

Cell theory

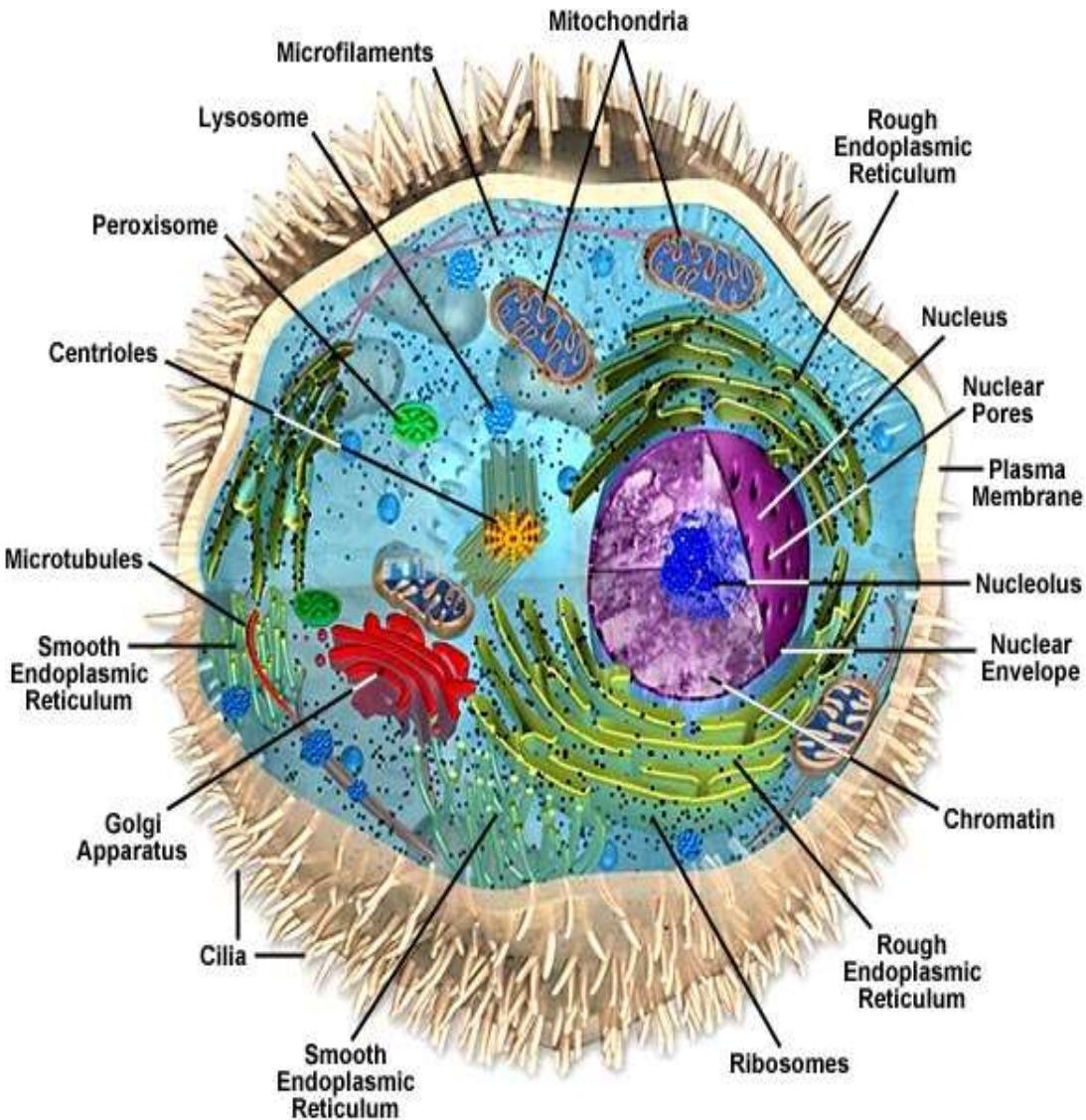
Prokaryotes

- Nucleoid region (center) contains the DNA
- Surrounded by cell membrane & cell wall (peptidoglycan)
- Contain ribosomes (no membrane) in their cytoplasm to make proteins



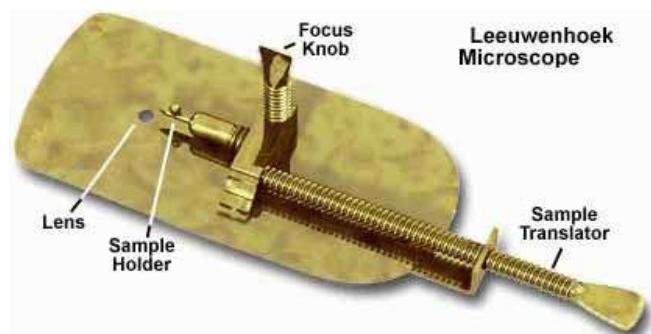
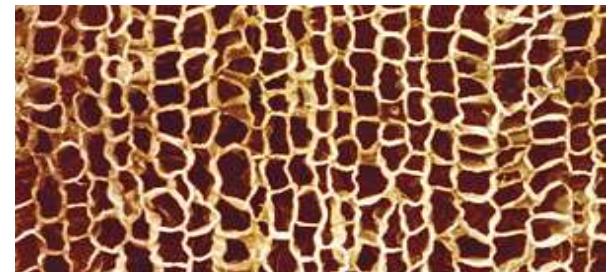
Eukaryotes

- Cells that HAVE a **nucleus and membrane-bound organelles**
- Includes protists, fungi, plants, and animals
- More complex type of cells



Cells and Cell Theory

- In 1665, Robert Hooke used a microscope to examine a thin slice of **cork** (dead plant cell walls). Hooke called them “**CELLS**” because they looked like the **small rooms that monks lived in** called “Cells”
- In 1673, Leeuwenhoek (a Dutch microscope maker), was **first to view organism** (living things)



Cell Theory

- In 1838, a German botanist named **Matthias Schleiden** concluded that all **plants** were made of cells
- In 1839, a German zoologist named **Theodore Schwann** concluded that all **animals** were made of cells

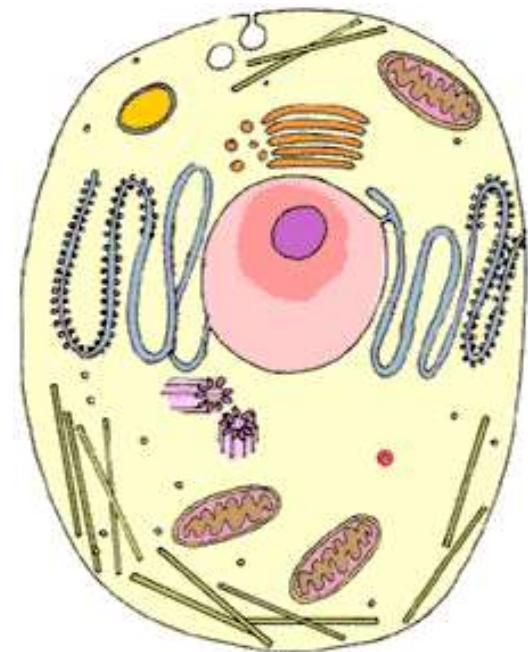
Beginning of the Cell Theory

- In 1855, a German medical doctor named **Rudolph Virchow** observed, under the microscope, **cells dividing**
- He reasoned that **all cells come from other pre-existing cells** by cell division



CELL THEORY

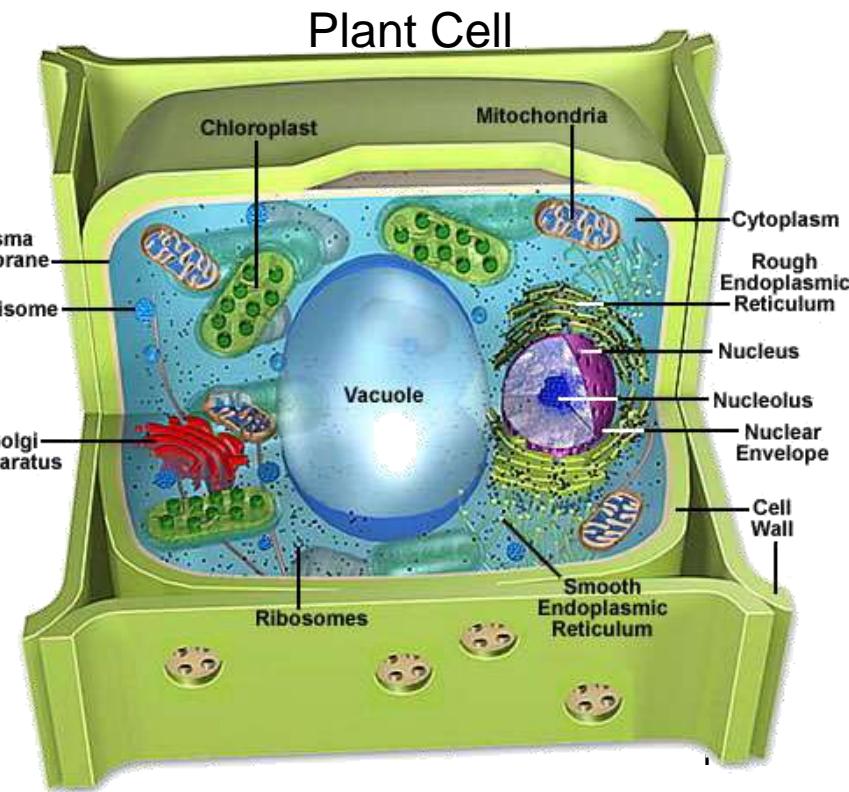
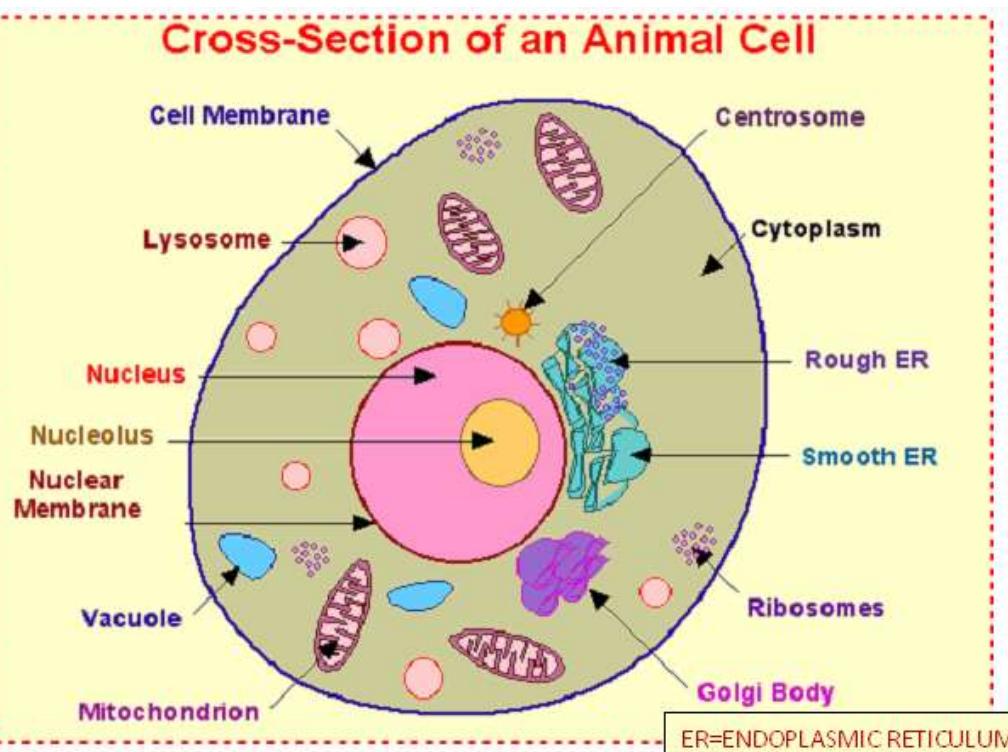
- All living things are made of **cells**
- Cells are the basic unit of **structure and function** in an organism (basic unit of life)
- Cells come from the **reproduction of existing cells** (cell division)



Cell Structure and Function

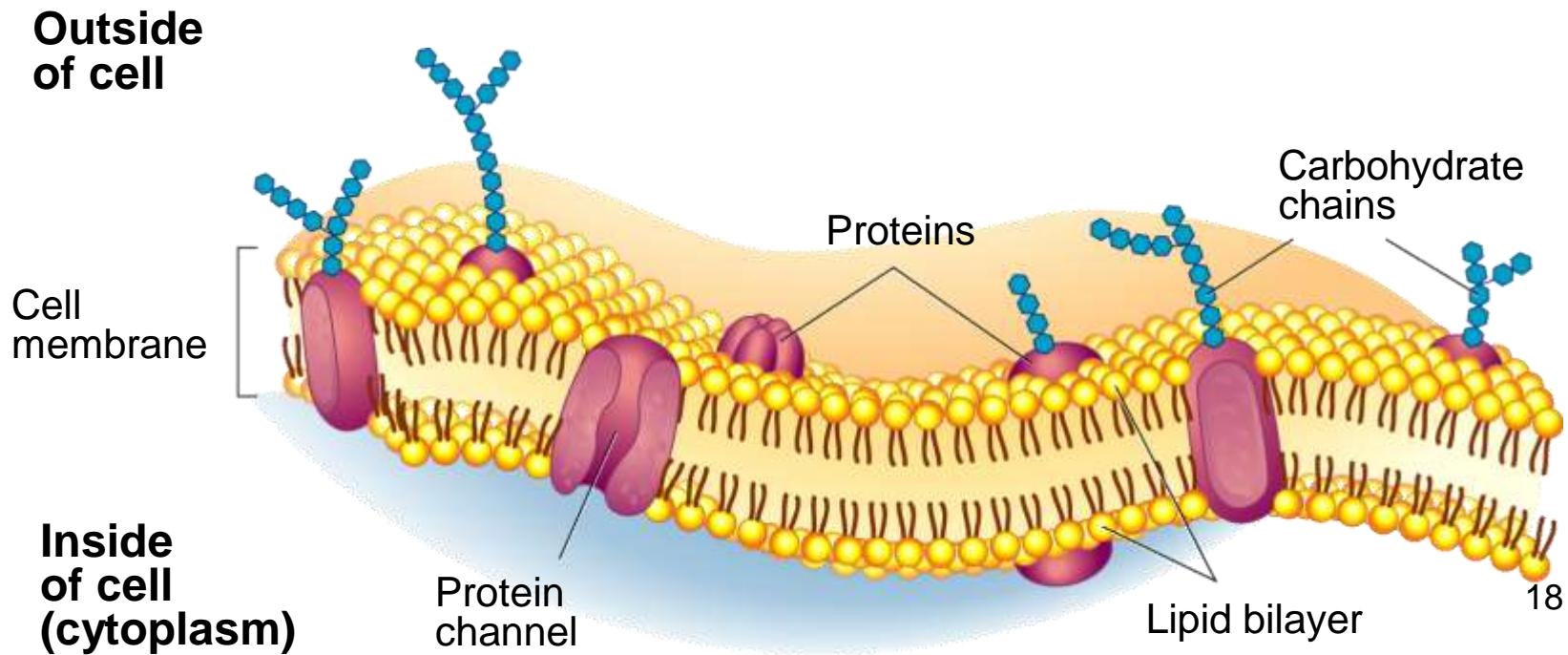
Organelles

- Very small (Microscopic)
- Perform various functions for a cell
- Found in the cytoplasm
- May or may not be membrane-bound



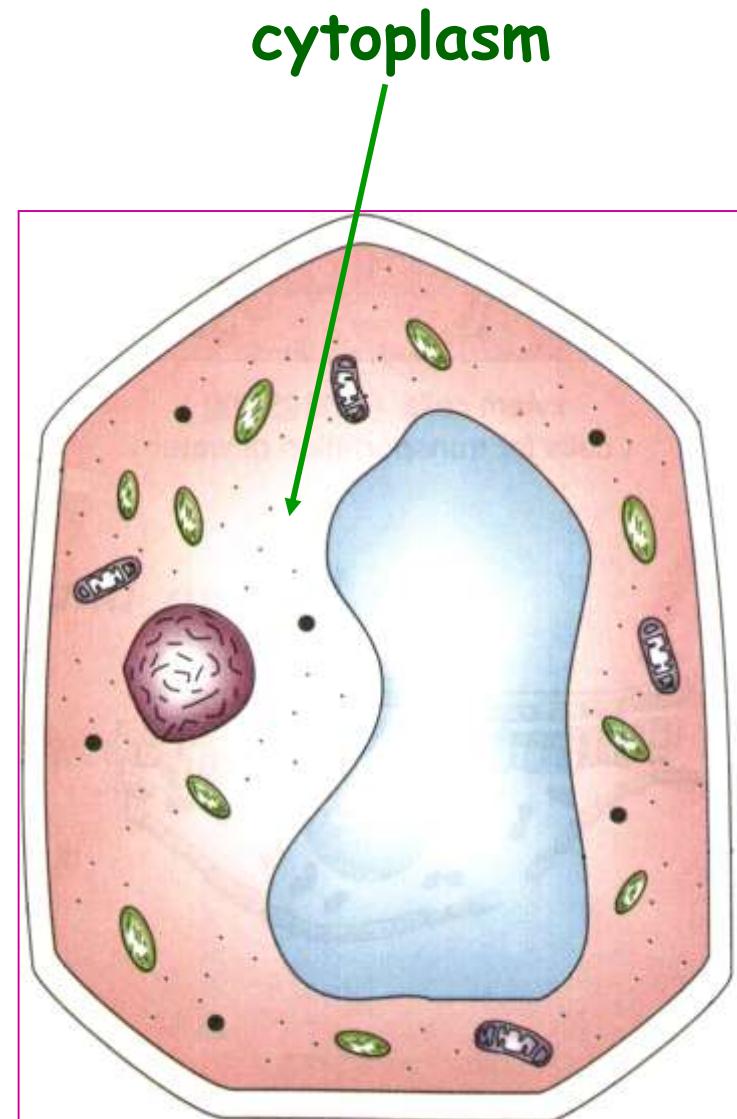
Cell or Plasma Membrane

- Composed of **double layer of phospholipids and proteins**
- Surrounds outside of **ALL cells**
- Controls what enters or leaves the cell
- Living layer



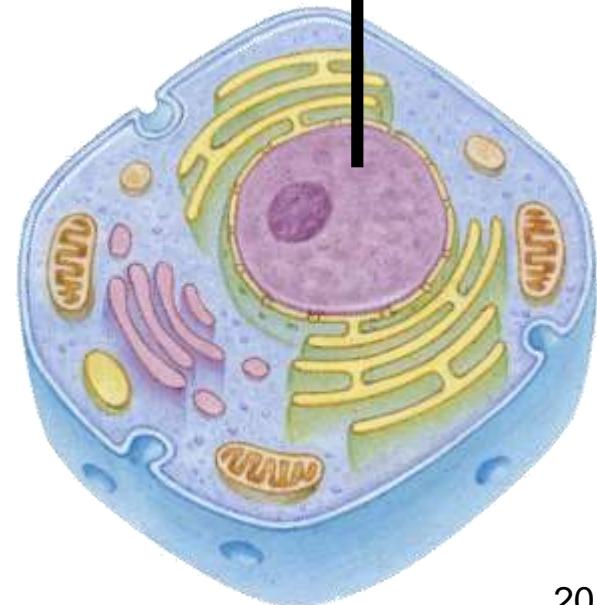
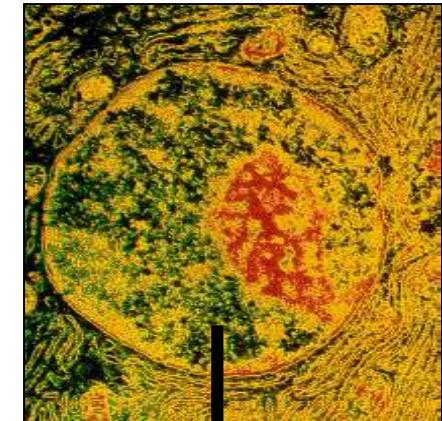
Cytoplasm of a Cell

- Jelly-like substance enclosed by **cell membrane**
- Provides a medium for **chemical reactions** to take place
- Contains **organelles** to carry out specific jobs
- Found in **ALL** cells



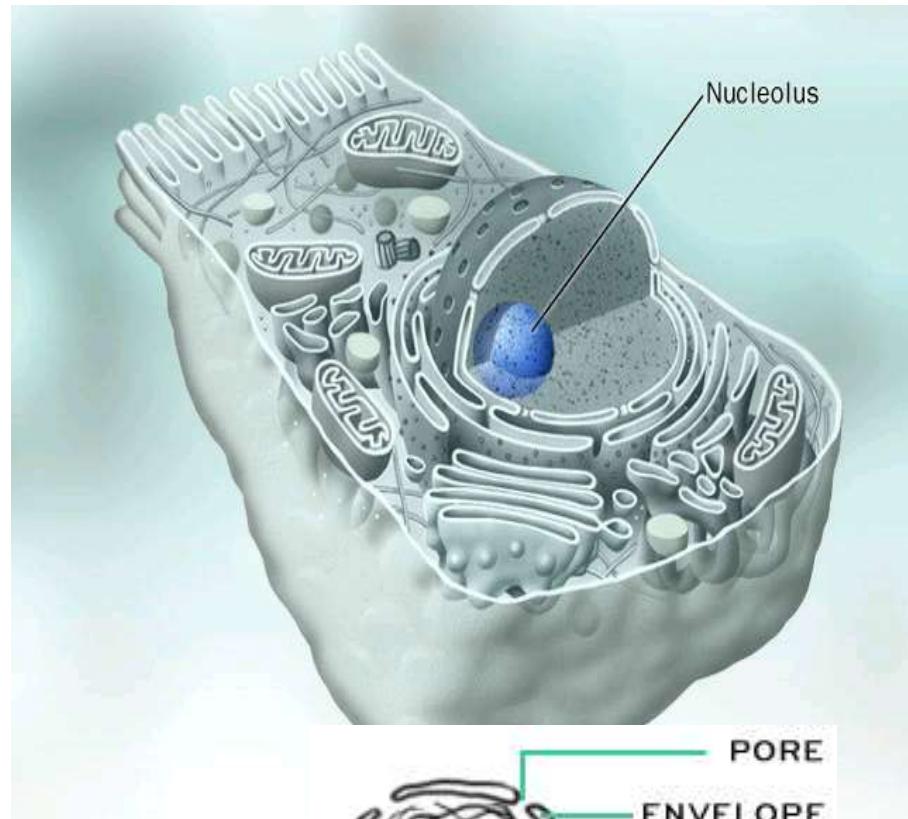
The Control Organelle - Nucleus

- Controls the normal activities of the cell
- Contains the DNA in chromosomes
- Bounded by a nuclear envelope (membrane) with pores
- Usually the largest organelle
- Each cell has fixed number of chromosomes that carry genes
- Genes control cell characteristics



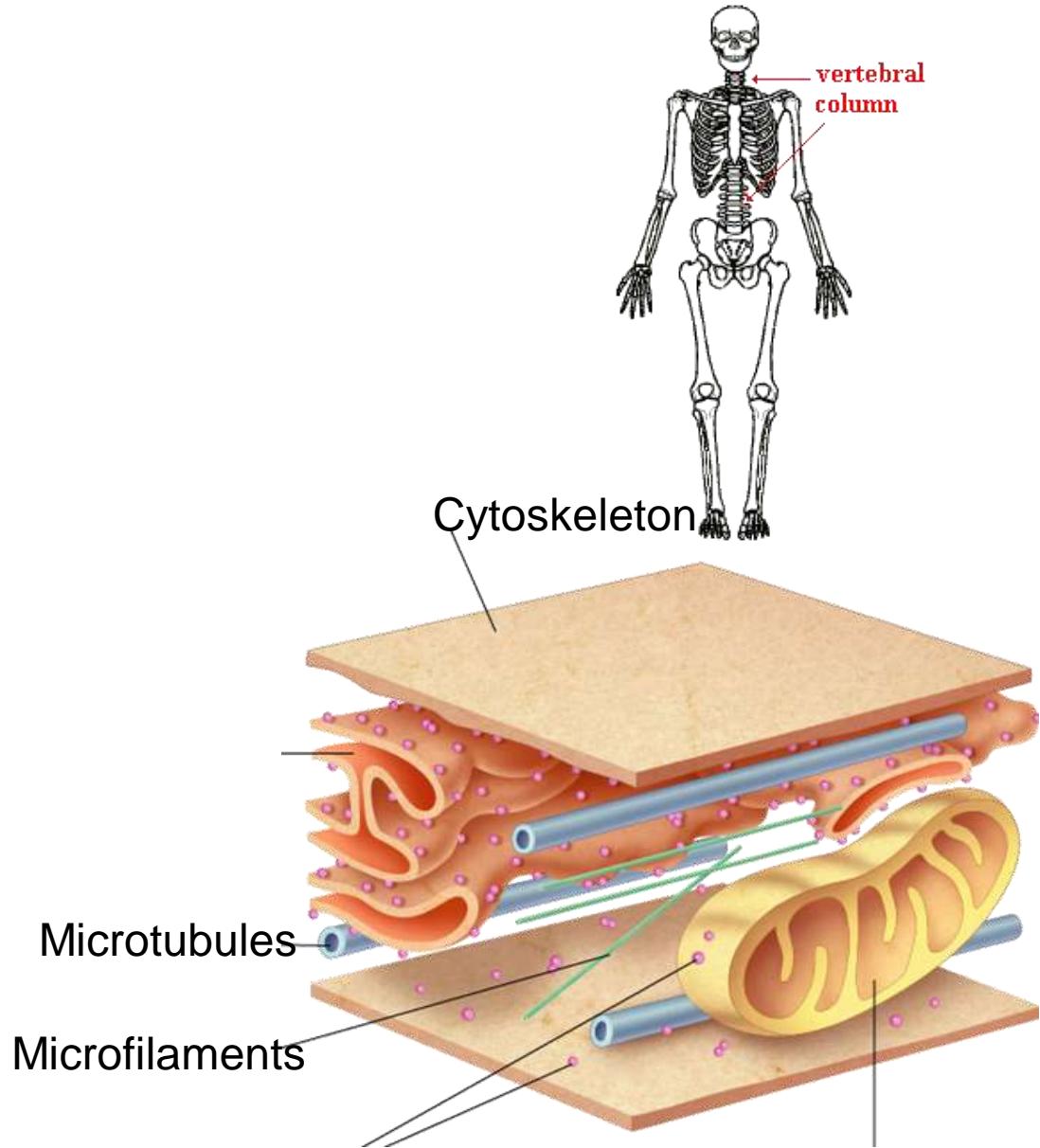
Nucleolus

- Inside nucleus
- Cell may have 1 to 3 nucleoli
- Disappears when cell divides
- Makes ribosomes that make proteins



Cytoskeleton

- Helps cell maintain cell shape
- Also help move organelles around
- Made of proteins
- Microfilaments are threadlike & made of ACTIN
- Microtubules are tube-like and made of TUBULIN



Centrioles



- Found only in **animal** cells
- **Paired** structures near nucleus
- Made of bundle of **microtubules**
- Appear during **cell division** forming **mitotic spindle**
- Help to **pull chromosome pairs apart** to opposite ends of the cell

Mitochondrion (plural = mitochondria)

- “Powerhouse” of the cell
- Generate cellular **energy** (ATP)
- More active cells like **muscle** cells have **MORE** mitochondria
- Both plants & animal cells have mitochondria
- Site of **CELLULAR RESPIRATION** (burning glucose)



MITOCHONDRIA

Mitochondria Inner Structure

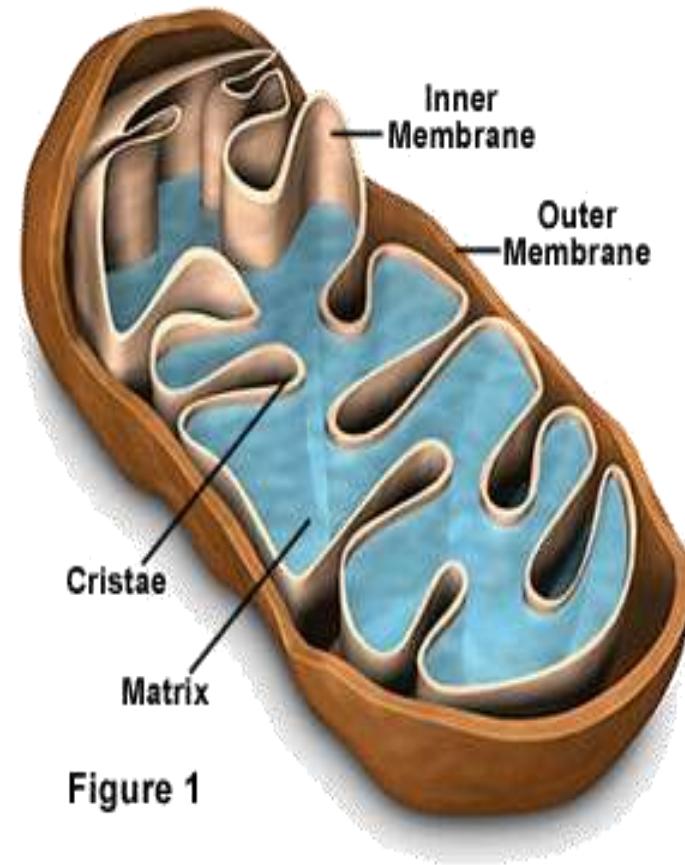
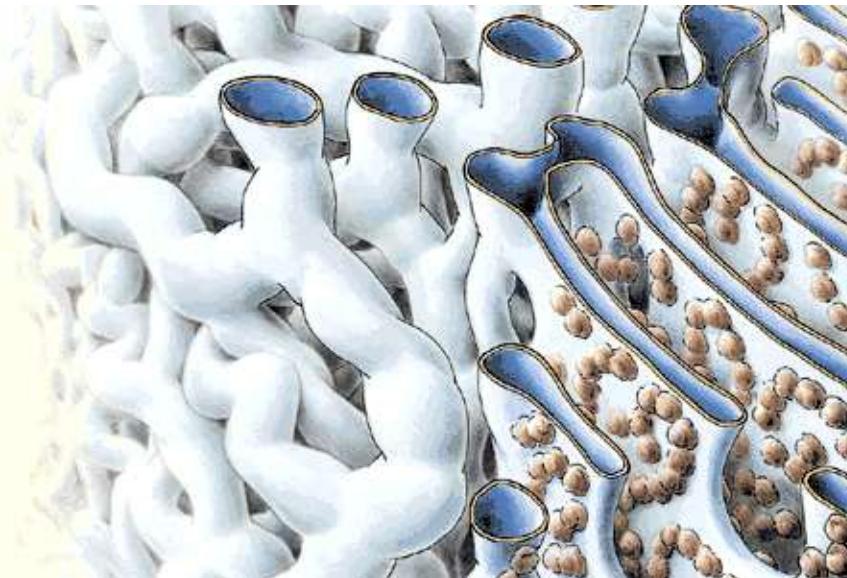


Figure 1

- Surrounded by a **DOUBLE membrane**
- Has its own **DNA**
 - Mitochondria come from cytoplasm in the egg cell during fertilization
 - **Therefore you inherit your mitochondria from your mother!**
- Folded inner membrane called **CRISTAE** (increases surface area for more chemical reactions)
- Interior called **MATRIX**

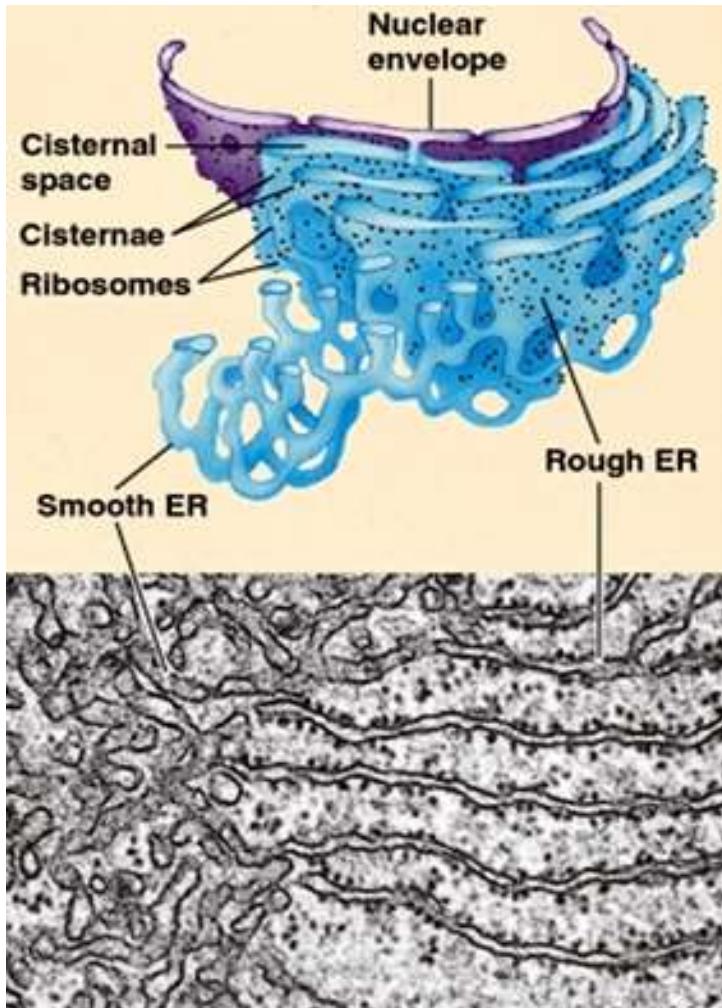
Endoplasmic Reticulum - ER

- Network of **hollow membrane tubules**
- Connects to **nuclear envelope & cell membrane**
- Functions in **Synthesis of cell products & Transport**



Two kinds of ER ---**ROUGH & SMOOTH**

Rough Endoplasmic Reticulum (Rough ER)



- Has **ribosomes** on its surface
- Makes membrane proteins and **proteins for EXPORT** out of cell
- Proteins are made by **ribosomes on ER surface**
- They are then **threaded into the interior of the Rough ER** to be modified and transported

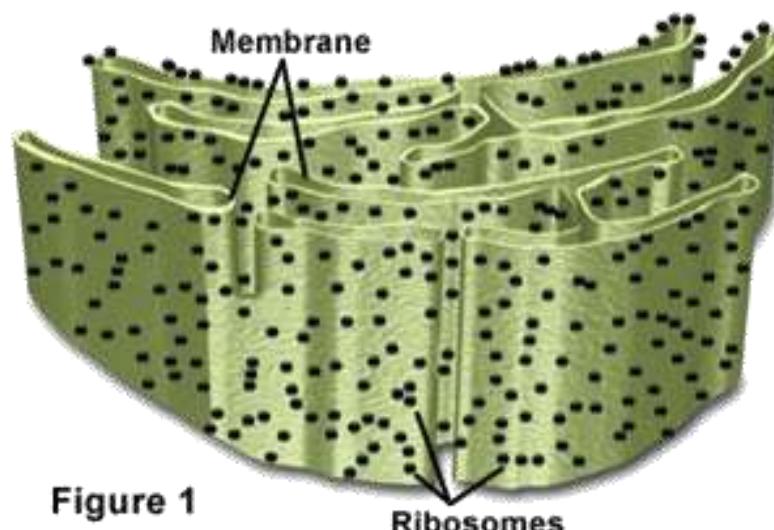
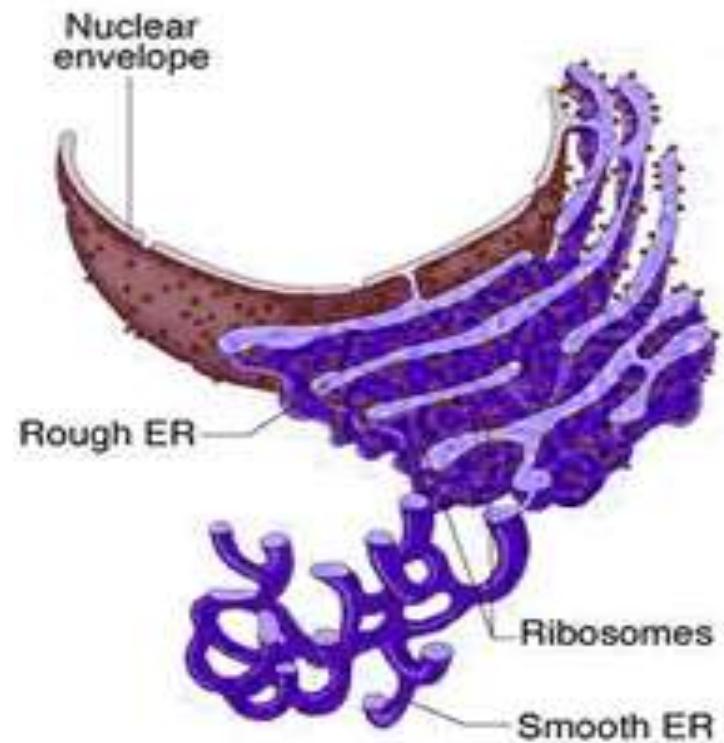


Figure 1

Smooth Endoplasmic Reticulum

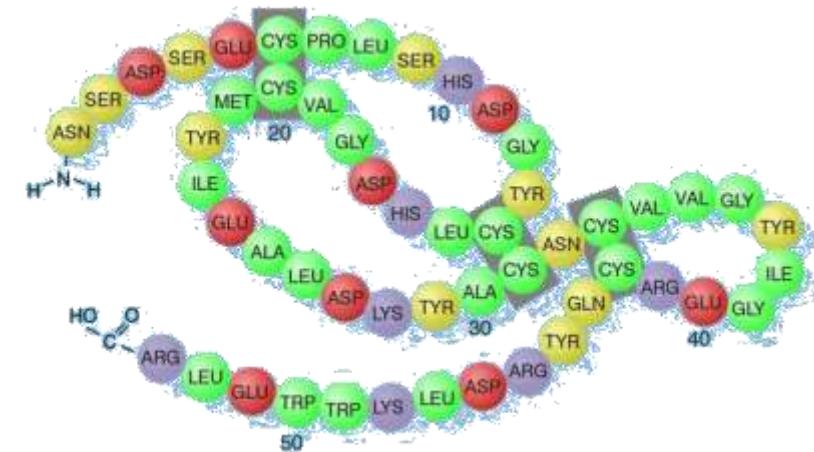
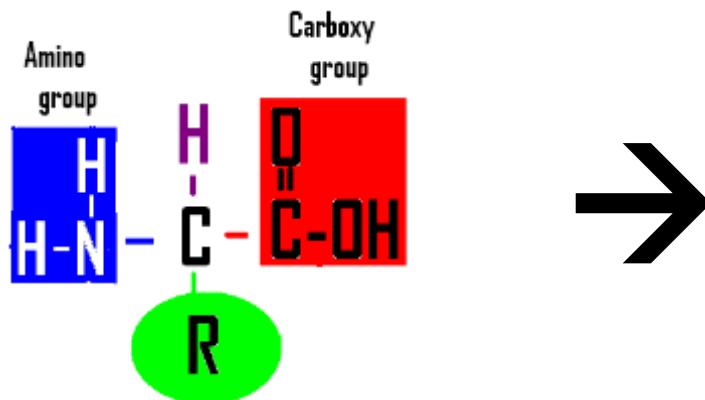
- ***Smooth ER*** lacks ribosomes on its surface
- Is **attached to the ends of rough ER**
- Makes cell products that are **USED INSIDE** the cell
- Makes membrane lipids (**steroids**)
- Regulates calcium (muscle cells)
- Destroys toxic substances (Liver)



Includes nuclear membrane connected to ER connected to cell membrane (transport)

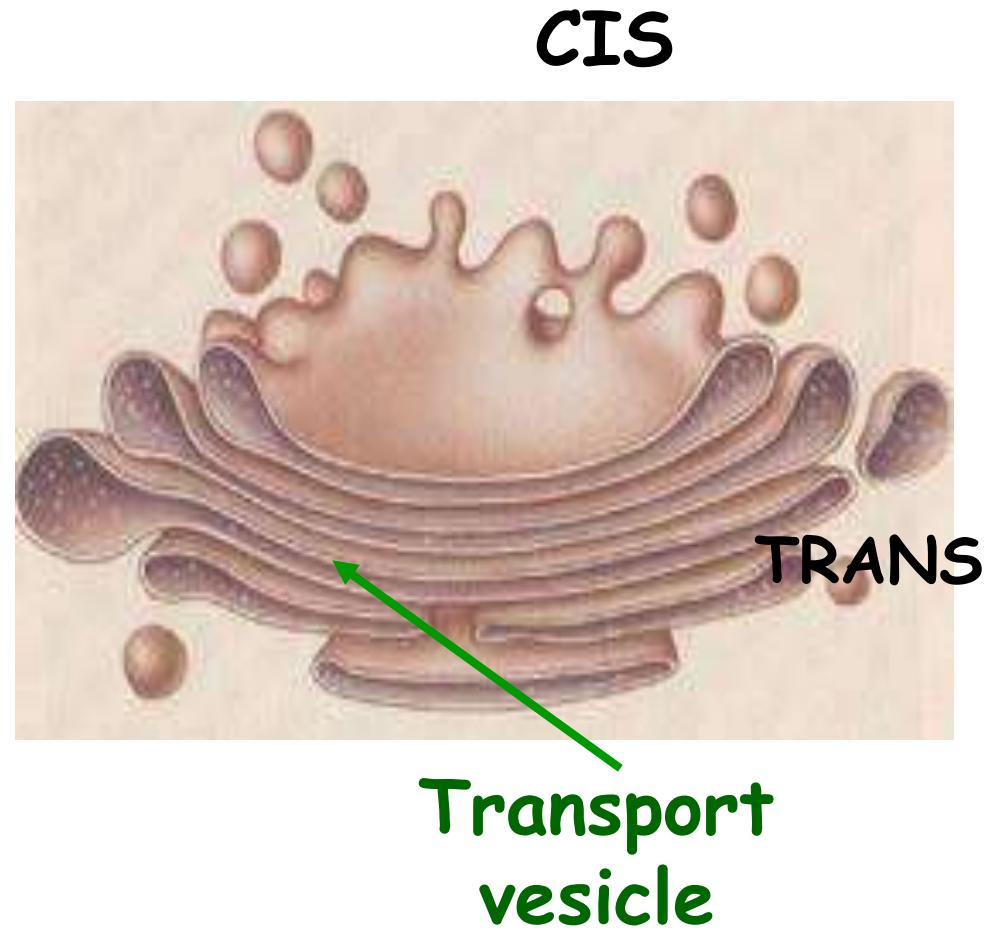
Ribosomes

- Made of **PROTEINS** and rRNA
 - “Protein factories” for cell
 - Join **amino acids** to make proteins
 - Process called **protein synthesis**
 - Can be attached to Rough ER **OR** Be free (unattached) in the **cytoplasm**



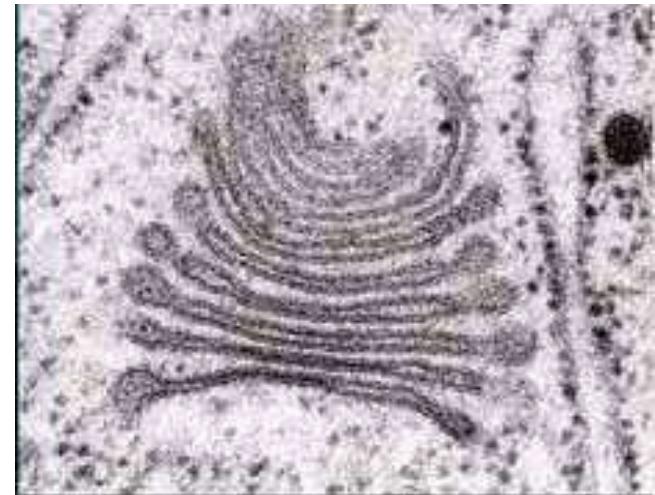
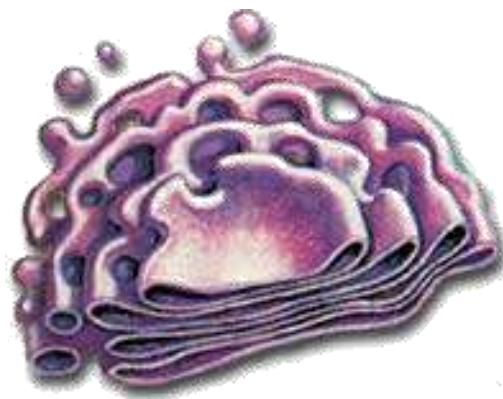
Golgi Bodies

- Stacks of flattened sacs
- Have a shipping side (trans face) and receiving side (cis face)
- Receive proteins made by ER
- Transport vesicles with modified proteins pinch off the ends



Golgi Bodies

Look like a stack of pancakes

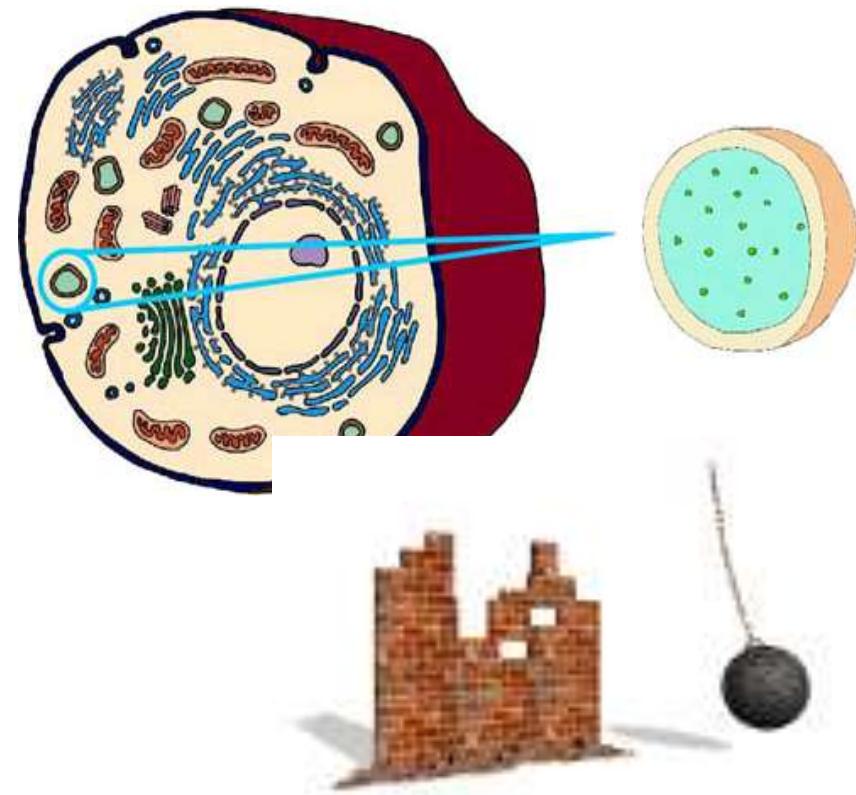


**Modify, sort, & package
molecules from ER
for storage OR
transport out of cell**



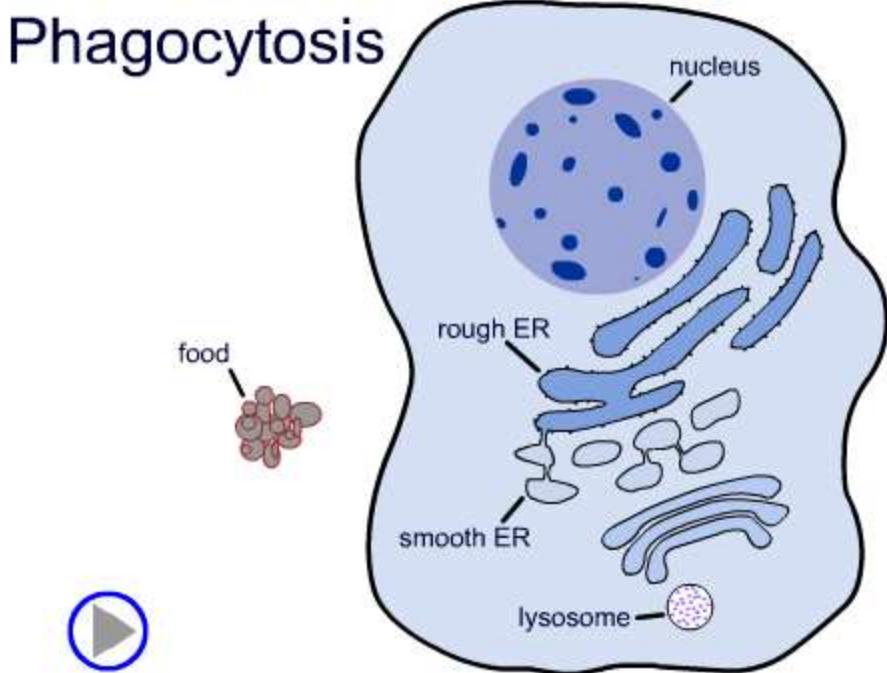
Lysosomes

- Contain **digestive enzymes**
- Break down **food, bacteria, and worn out cell parts** for cells
- Programmed for **cell death (AUTOLYSIS)**
- Lyse (break open) & release enzymes to break down & recycle cell parts)



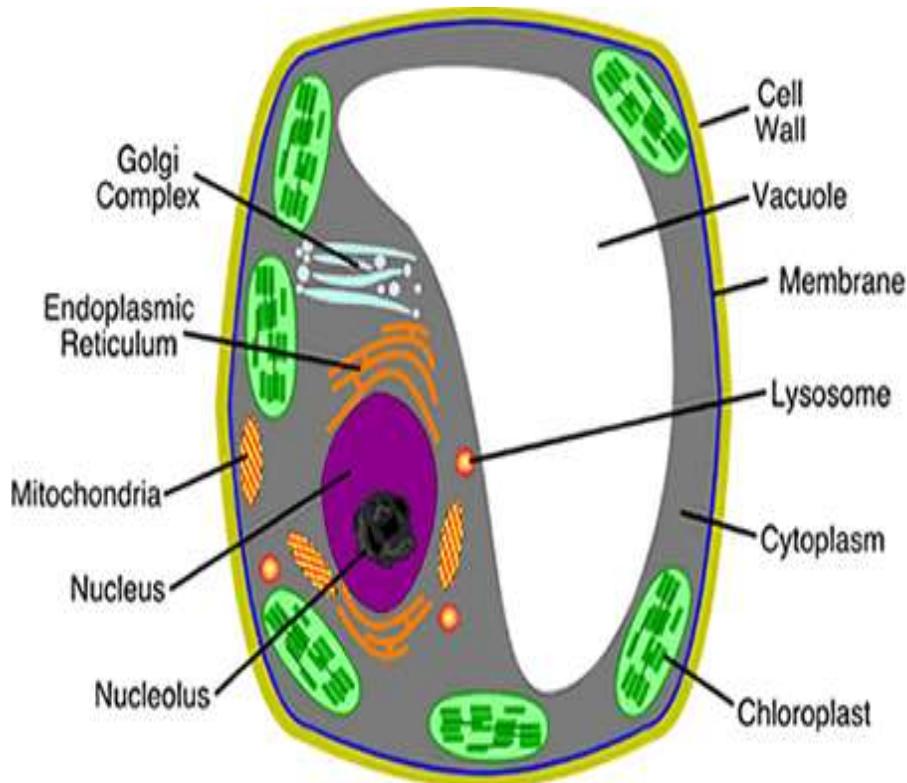
Lysosome Digestion

- Cells take in food by **phagocytosis**
- Lysosomes **digest** the food & get rid of wastes



Vacuoles

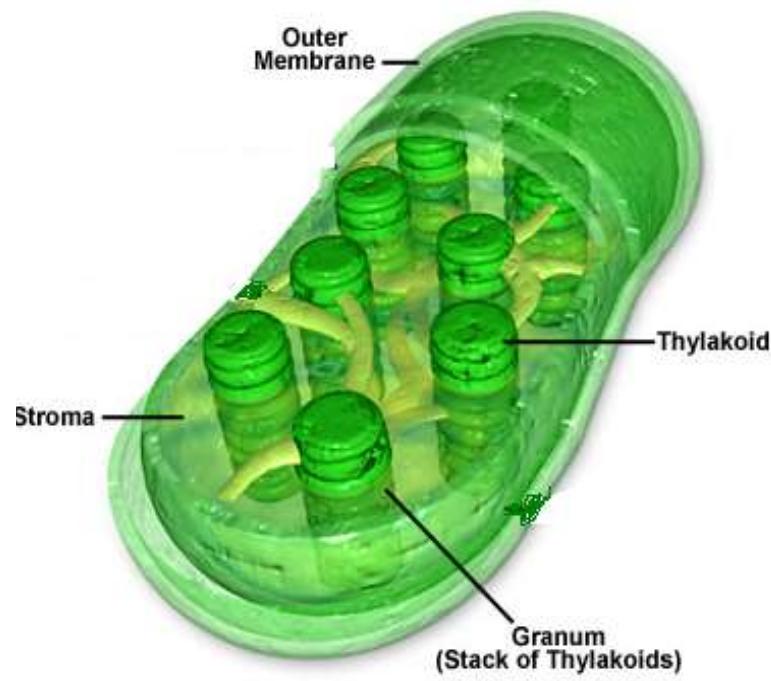
- Fluid filled sacks for storage
- Small or absent in *animal* cells
- *Plant cells have a large Central Vacuole*
- No vacuoles in *bacterial* cells
- In plants, they store **Cell Sap**
- Includes storage of sugars, proteins, minerals, lipids, wastes, salts, water, and enzymes



Chloroplasts

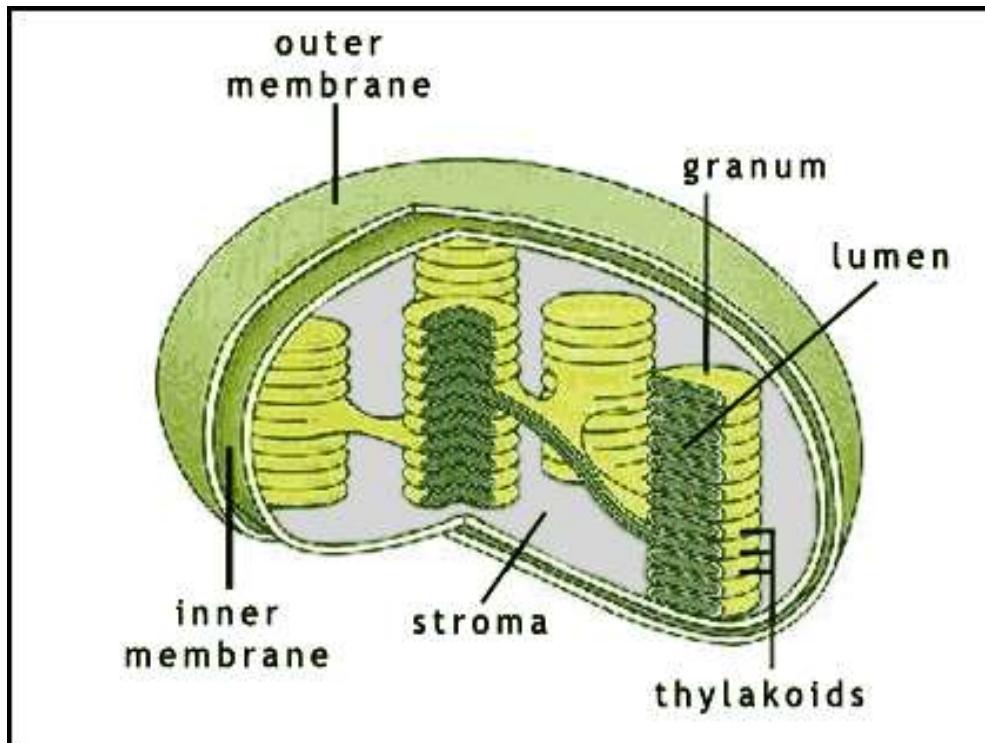
- Found only in **producers** (organisms containing **chlorophyll**)
- Use **energy from sunlight** to make own **food (glucose)**
- Energy from sun stored in the **Chemical Bonds of Sugars**

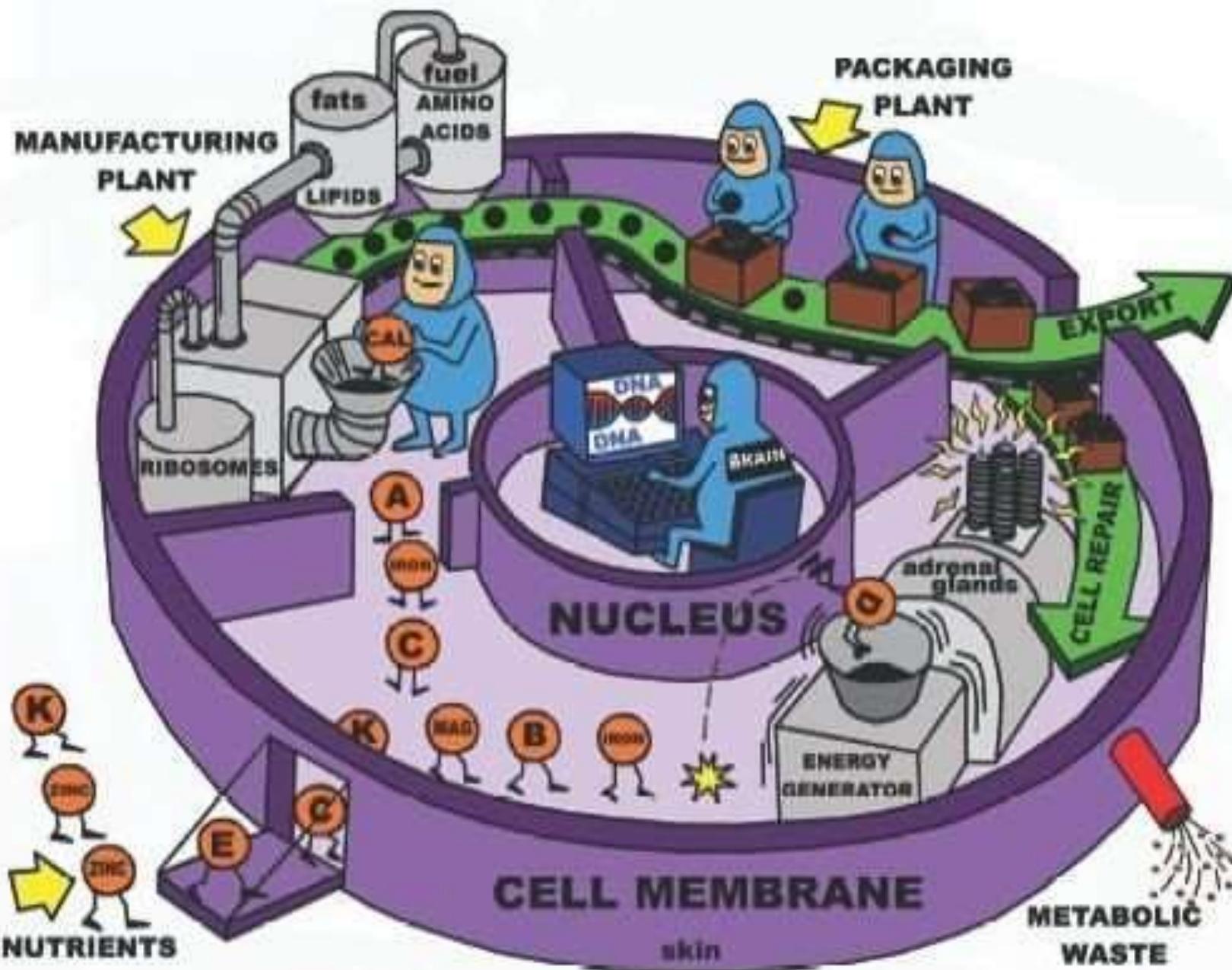
- ❖ Surrounded by **DOUBLE** membrane
- ❖ Outer membrane smooth
- ❖ Inner membrane modified into sacs called **Thylakoids**
- ❖ Thylakoids in **stacks called Grana &** interconnected
- ❖ **Stroma** – gel like material surrounding thylakoids



Chloroplasts

- Contains its own DNA
- Contains enzymes & pigments for Photosynthesis
- Never in animal or bacterial cells
- Photosynthesis – food making process





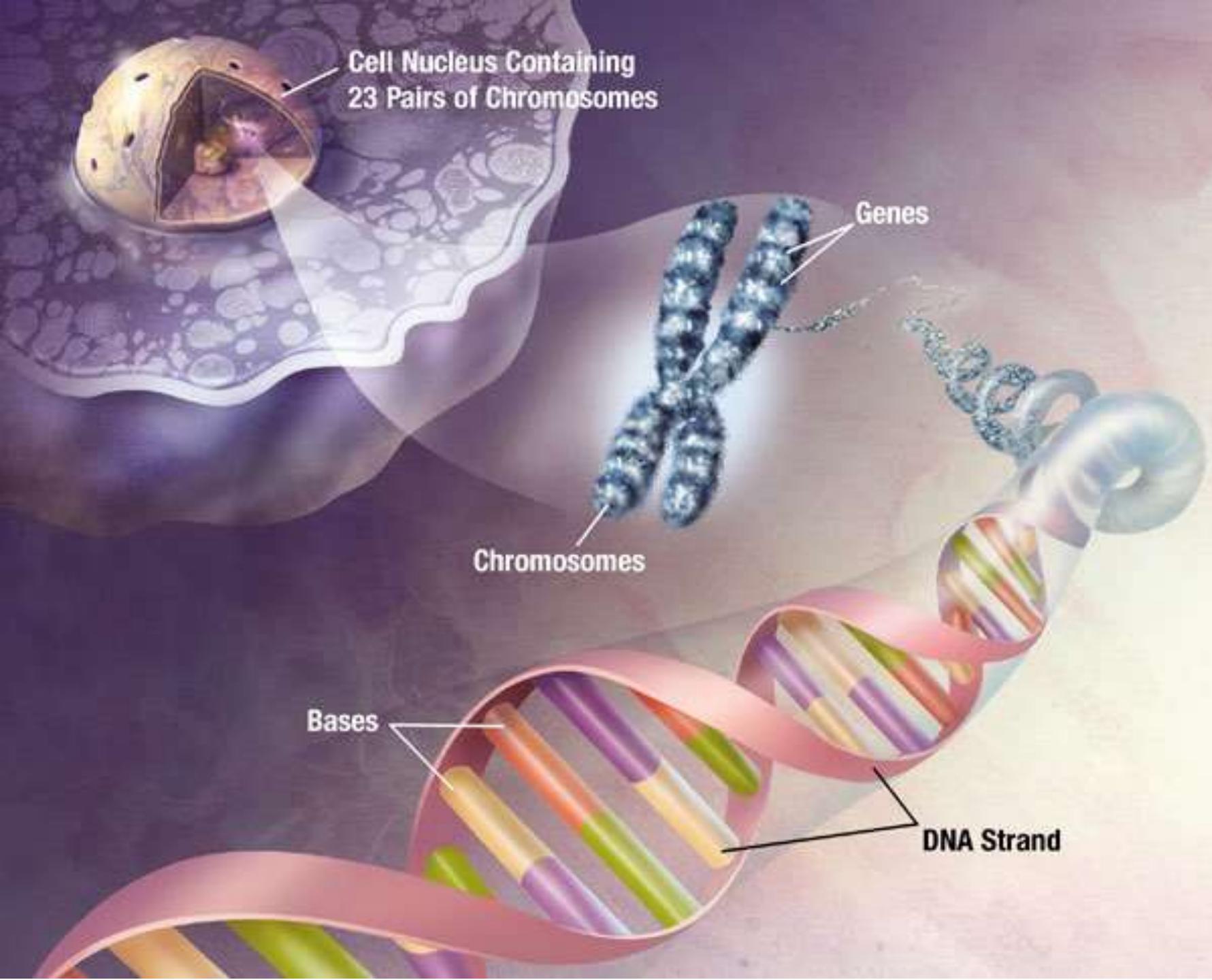


Genetic information, protein synthesis, and protein structure

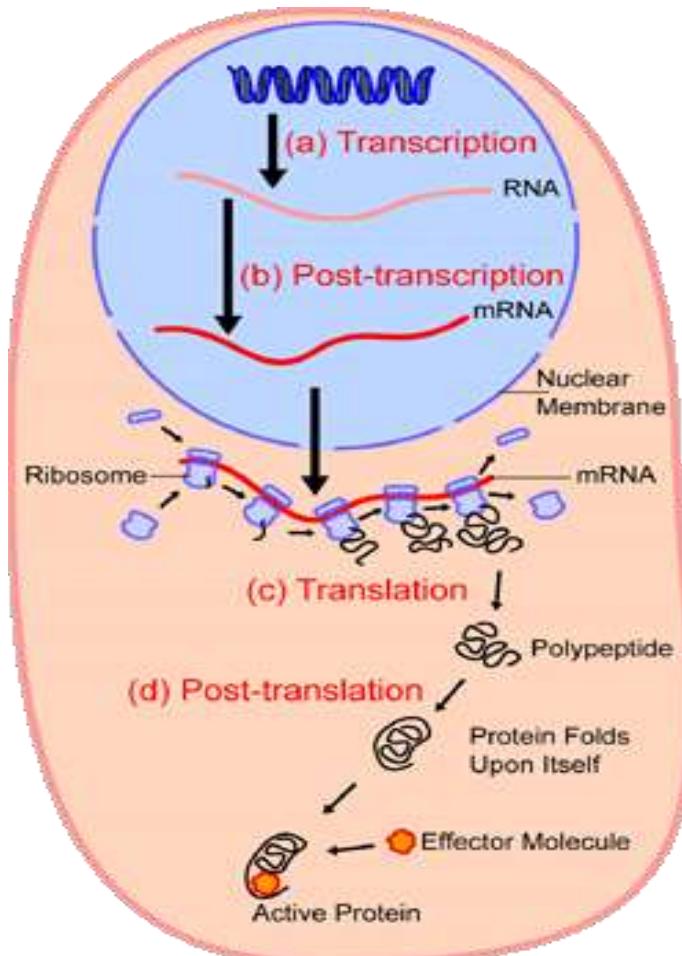
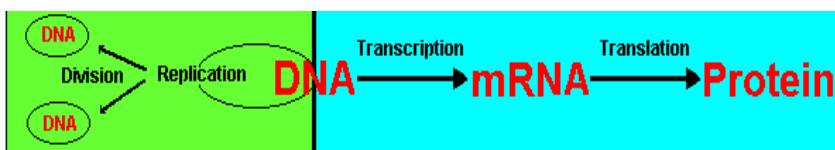
Genetic information

- Genetic information is in the chromosomes found in the nucleus.
 - necessary for reproduction of species and therefore, its propagation on earth.
 - It is coded along the length of a polymeric molecule composed of four types of monomeric units. This polymeric molecule is deoxyribonucleic acid (DNA).
 - It is the chemical basis of heredity which is organised into **genes**, the fundamental units of genetic information. Genes control the synthesis of various types of ribonucleic acid (RNA).

- Nucleic acid is a polynucleotide consisting of nucleotides as the repeating subunits. Each nucleotide is made up of three components are, (i) pentosugar, (ii) nitrogenous base and (iii) phosphate.
- This linkages repeated many times to build up large structures containing hundreds to millions of nucleotides within a single giant molecule.
 - **Pentosugar:** It is a type of cyclic 5 carbon sugar, which connects two phosphate groups. The type of sugar molecule in DNA is deoxyribose, whereas in RNA is ribose.
 - **Nitrogenous base:** Nucleic acids contains 5 major heterocyclic bases, adenine (A), guanine (G), cytosine (C), thymine (T) and uracil (U). First four bases are common in DNA, in case of RNA thymine is replaced with uracil
 - **Phosphate:** A phosphate group is attached to the 5' carbon of the sugar by a phosphodiester linkage. This phosphate group is solely responsible for the strong negative charge of the nucleic acids.



Protein Synthesis



1. DNA unwinds
2. mRNA copy is made of one of the DNA strands.
3. mRNA copy moves out of nucleus into cytoplasm.
4. tRNA molecules are activated as their complementary amino acids are attached to them.
5. mRNA copy attaches to the small subunit of the ribosomes in cytoplasm. 6 of the bases in the mRNA are exposed in the ribosome.
6. A tRNA bonds complementarily with the mRNA via its anticodon.
7. A second tRNA bonds with the next three bases of the mRNA, the amino acid joins onto the amino acid of the first tRNA via a peptide bond.
8. The ribosome moves along. The first tRNA leaves the ribosome.
9. A third tRNA brings a third amino acid
10. Eventually a stop codon is reached on the mRNA. The newly synthesised polypeptide leaves the ribosome.

Proteins

- Proteins are polymers (macromolecules) made of monomers called amino acids
- All proteins are made of 20 different amino acids linked in different orders
- Proteins are used to build cells, act as hormones & enzymes, and do much of the work in a cell

Essential versus Non-essential Amino Acid

Humans need ALL 20 amino acids to be able to make proteins.

| | | |
|---------------|------------|---------------|
| Glycine | Alanine | Serine |
| Cysteine | Proline | Tyrosine |
| Aspartic Acid | Asparagine | Glutamic Acid |
| Glutamine | | |

| | | |
|------------|------------|---------------|
| Valine | Leucine | Isoleucine |
| Threonine | Methionine | Phenylalanine |
| Tryptophan | Lysine | Histidine |

Arginine Essential Amino Acids

Protein Functions in the Body

Storage



- There are many different proteins in your body, and they perform different functions. Proteins functions include:

- Contributing to enzyme activity that promotes chemical reactions in the body
- Signaling cells what to do and when to do it
- Transporting substances around the body
- Keeping fluids and pH balanced in the body
- Serving as building blocks for hormone production
- Helping blood clot
- Promoting antibody activity that controls immune and allergy functions
- Serving as structural components that give our body parts their shapes

Structural



Transport

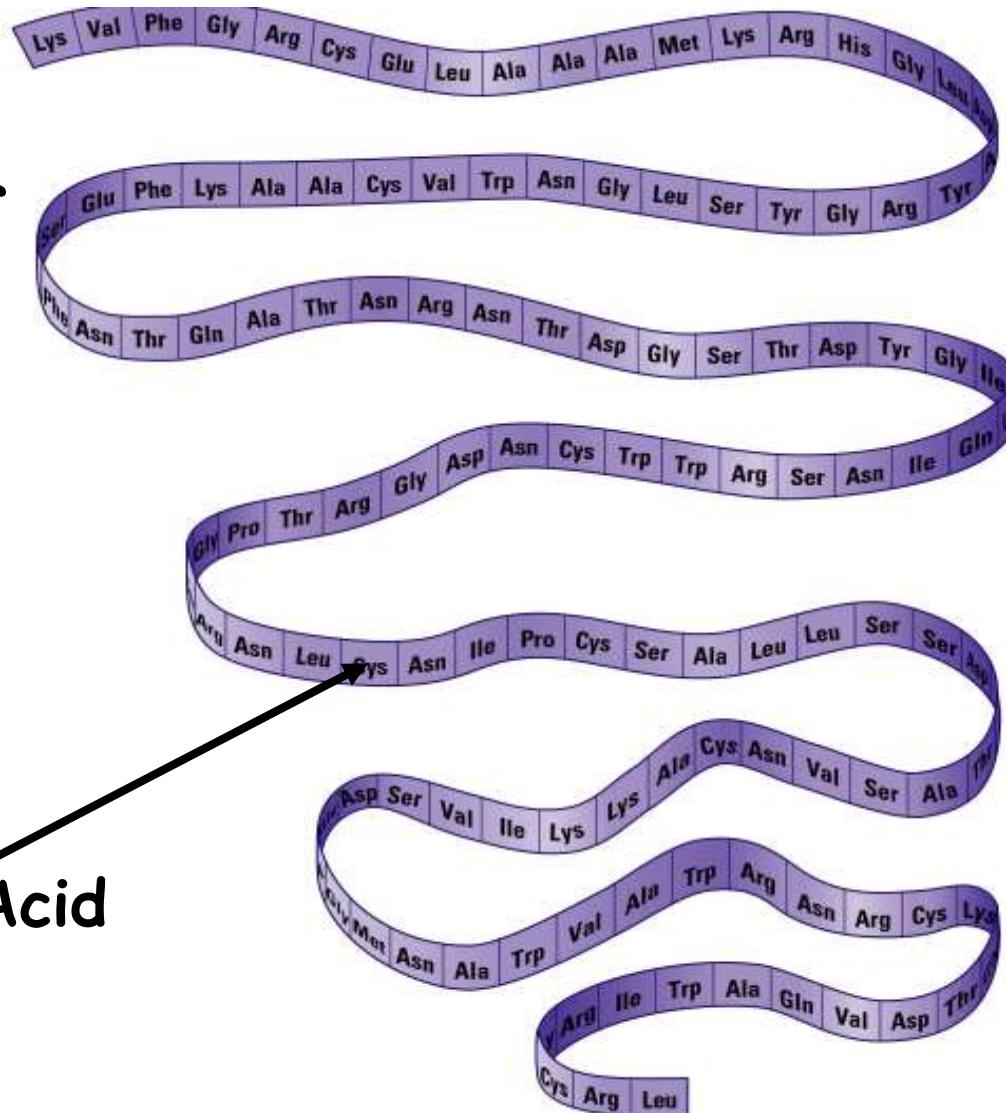


Primary Protein Structure

The **primary** structure is the specific sequence of amino acids in a protein

Called **polypeptide**

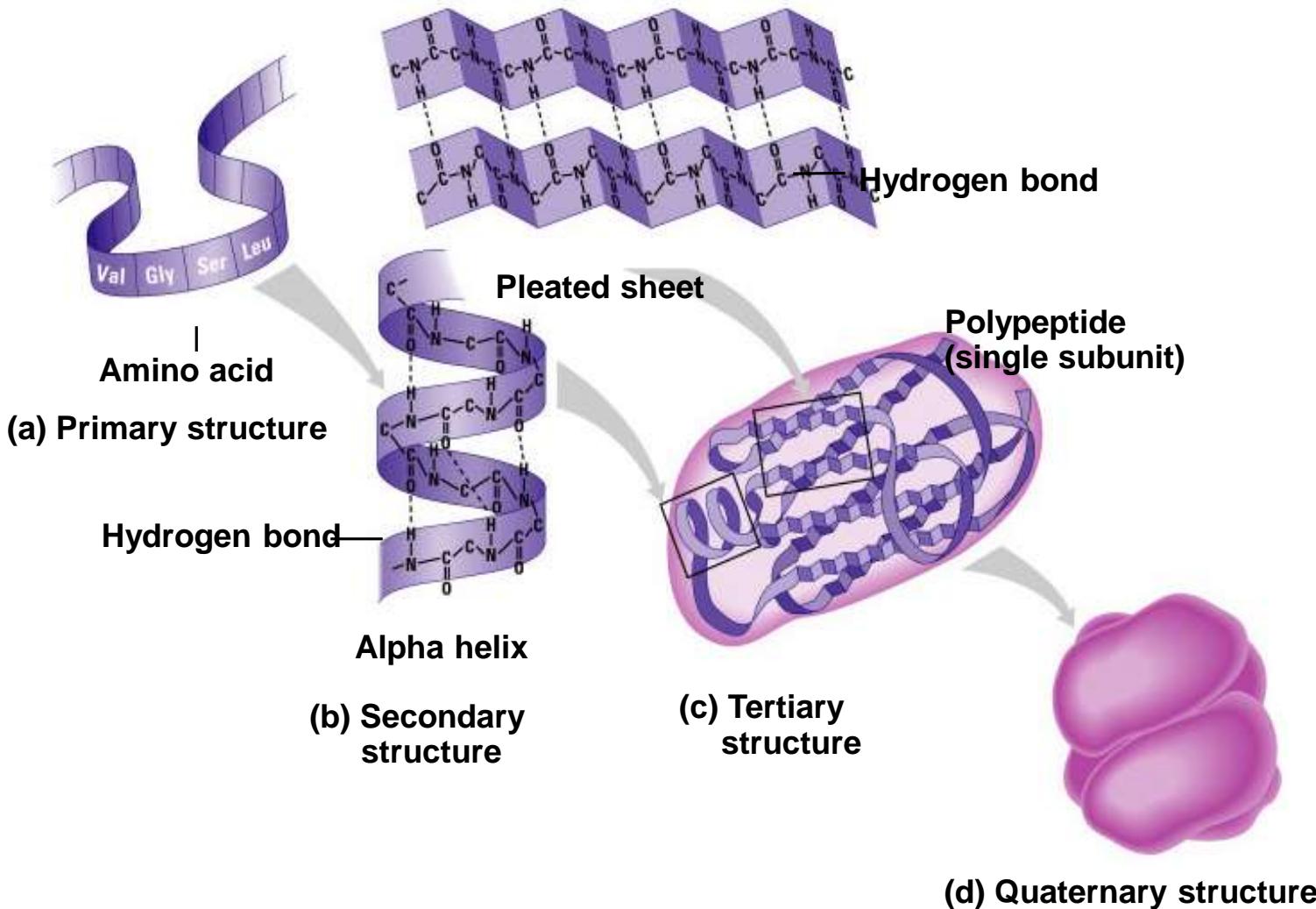
Amino Acid



Protein Structures

- Secondary protein structures occur when protein chains coil or fold
- When protein chains called polypeptides join together, the tertiary structure forms because R groups interact with each other
- In the watery environment of a cell, proteins become globular in their quaternary structure

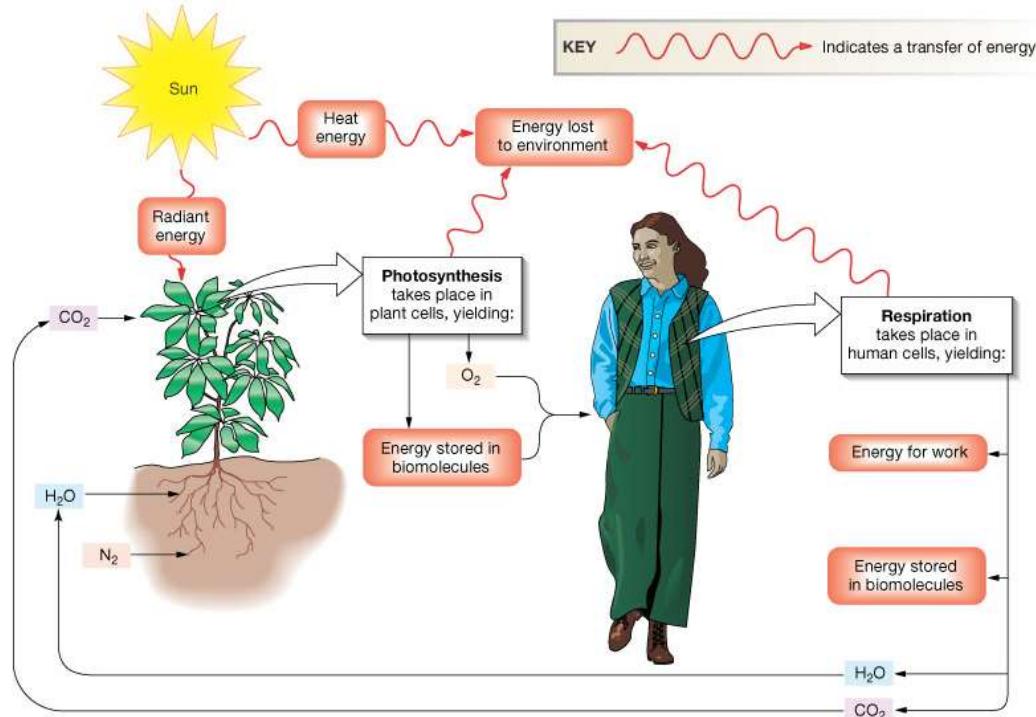
Protein Structures or CONFORMATIONS



Cell metabolism

Cell metabolism

- *Energy* is the ability to do work.
- Living things need to acquire energy; this is a characteristic of life.
- Cells use acquired energy to:
 - Maintain their organization
- Carry out reactions that allow cells to develop, grow, and reproduce



ATP: Energy for Cells

- ATP (*adenosine triphosphate*) is the energy currency of cells.
- ATP is constantly regenerated from ADP (*adenosine diphosphate*) after energy is expended by the cell.
- Use of ATP by the cell has advantages:
 - 1) It can be used in many types of reactions.
 - 2) When $\text{ATP} \rightarrow \text{ADP} + \text{P}$, energy released is sufficient for cellular needs and little energy is wasted.

Function of ATP

- Cells make use of ATP for:
- *Chemical work* – ATP supplies energy to synthesize macromolecules, and therefore the organism
- *Transport work* – ATP supplies energy needed to pump substances across the plasma membrane
- *Mechanical work* – ATP supplies energy for cellular movements

Two types of metabolic reactions

Anabolism

- larger molecules are made
- requires energy

Dehydration synthesis

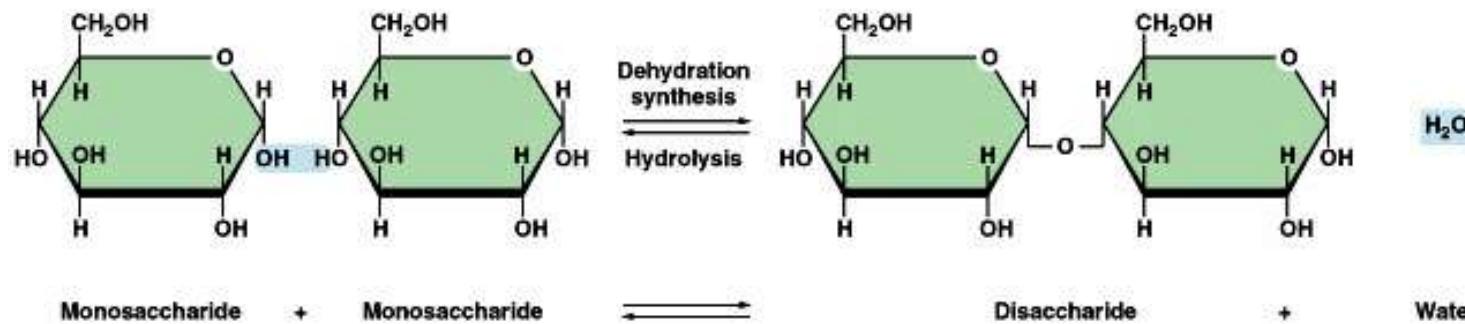
- type of anabolic process
- used to make polysaccharides, triglycerides, and proteins
- produces water

Catabolism

- larger molecules are broken down
- releases energy

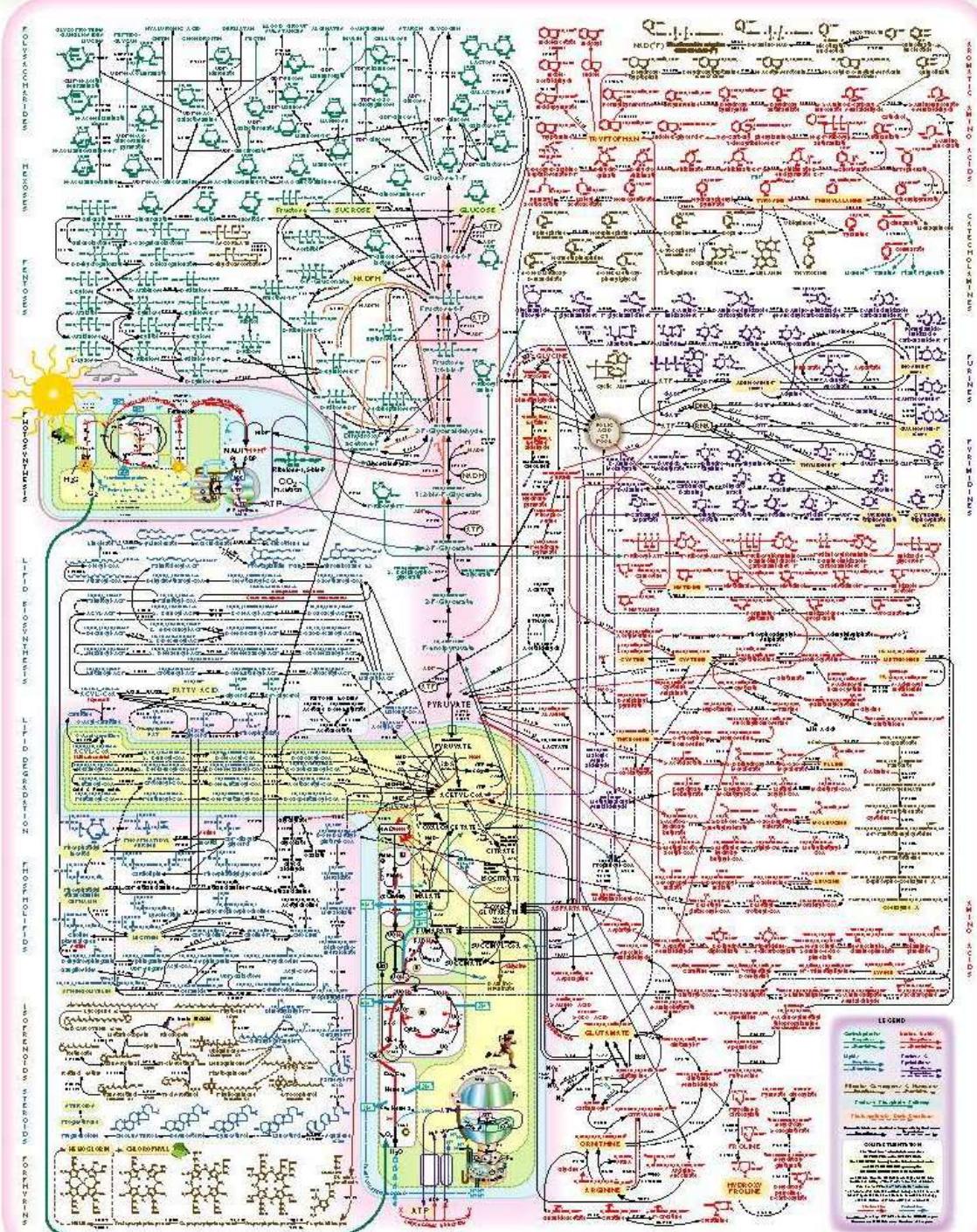
Hydrolysis

- a catabolic process
- used to decompose carbohydrates, lipids, and proteins
- water is used
- reverse of dehydration synthesis



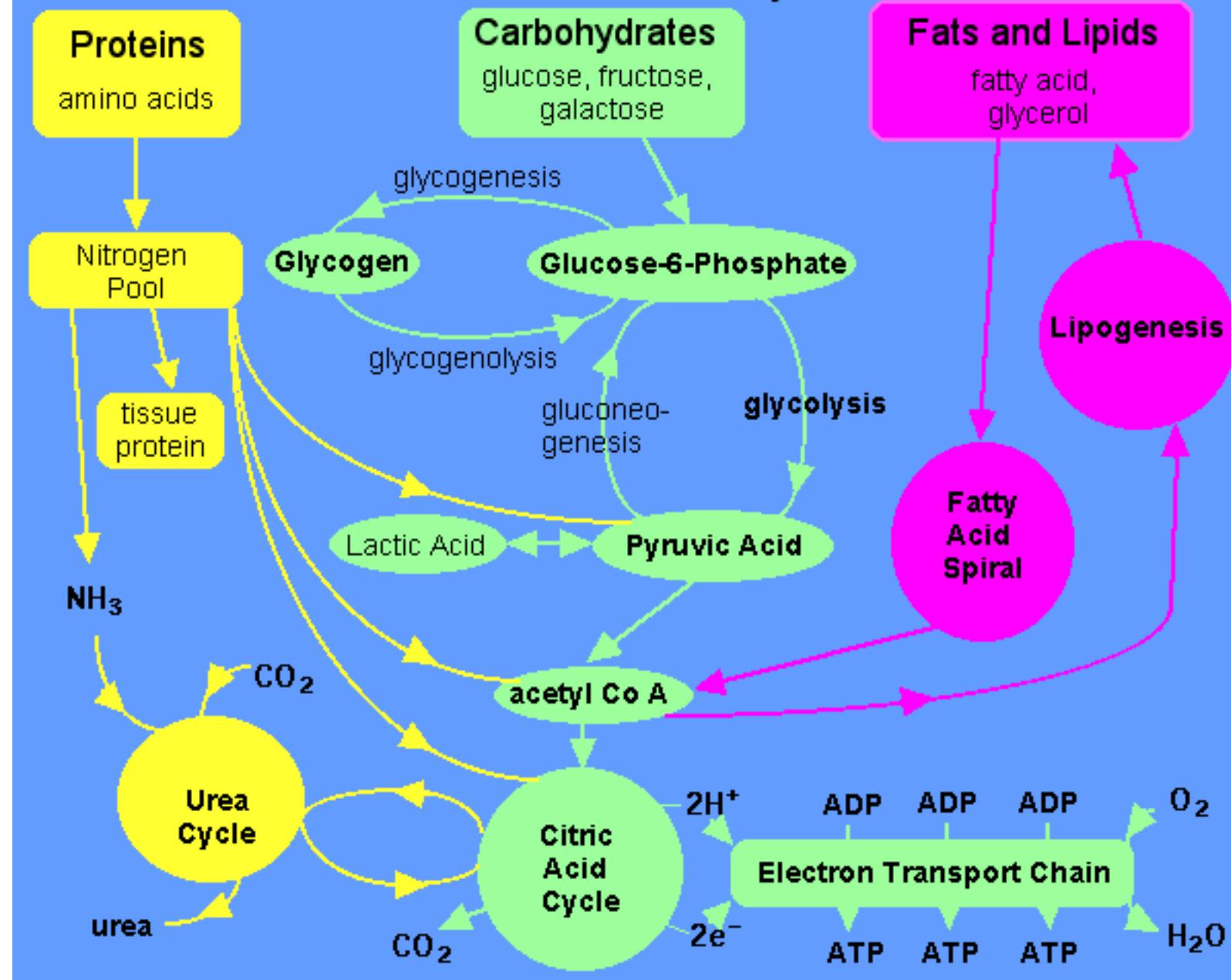
Enzyme Cofactors

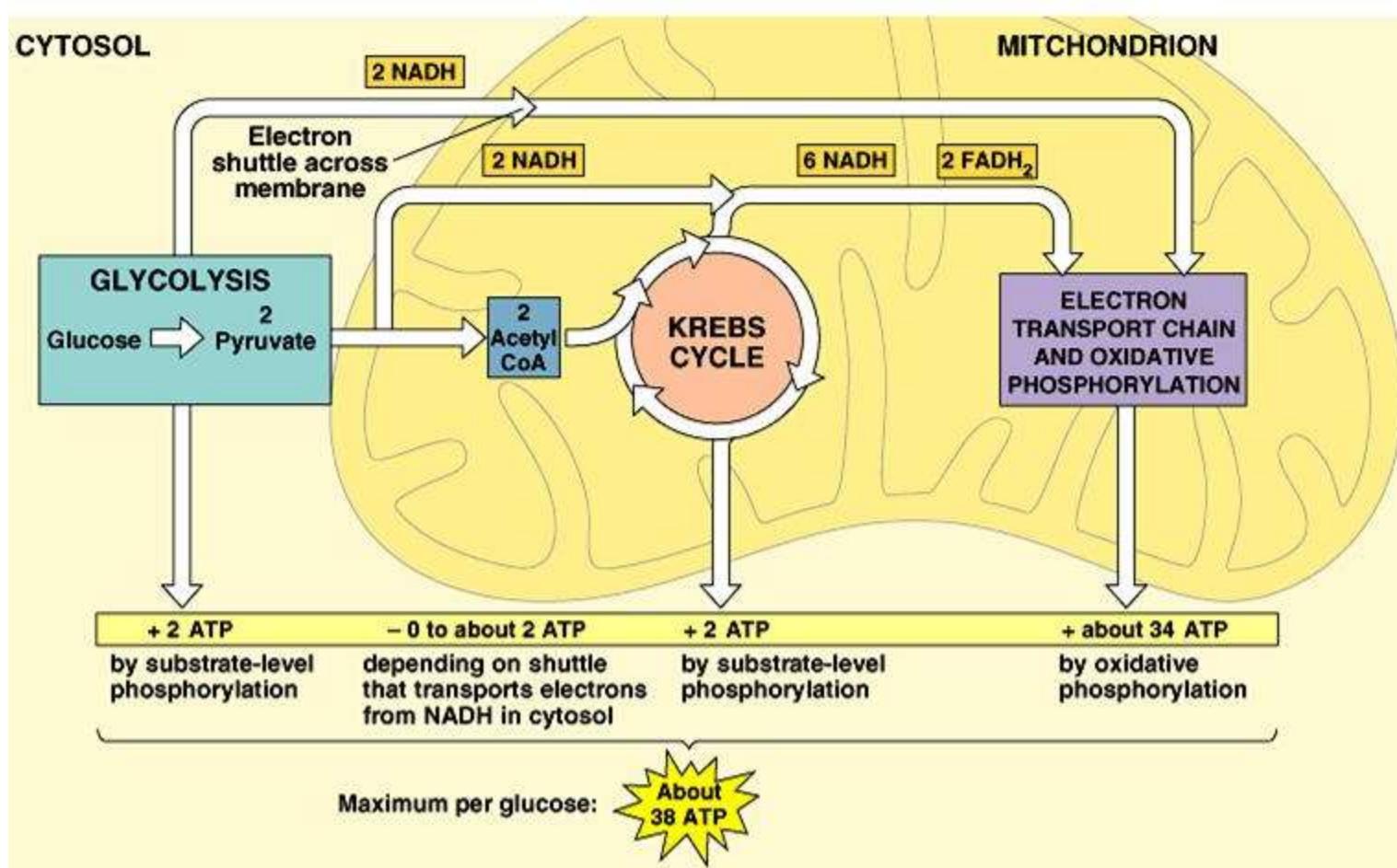
- Presence of enzyme *cofactors* may be necessary for some enzymes to carry out their functions.
- Ions, such as sodium, potassium, calcium, phosphorus, copper, zinc, or iron function are critical to the metabolic reactions
- Organic molecules, termed *coenzymes*, must be present for other enzymes to function.
- Some coenzymes are *vitamins (NADP, FAD, TPP)*.



A metabolic map, indicating the reactions of intermediary metabolism and the enzymes that catalyze them. Over 500 different chemical intermediates, or metabolites, and a greater number of enzymes are represented here.

Metabolism Summary

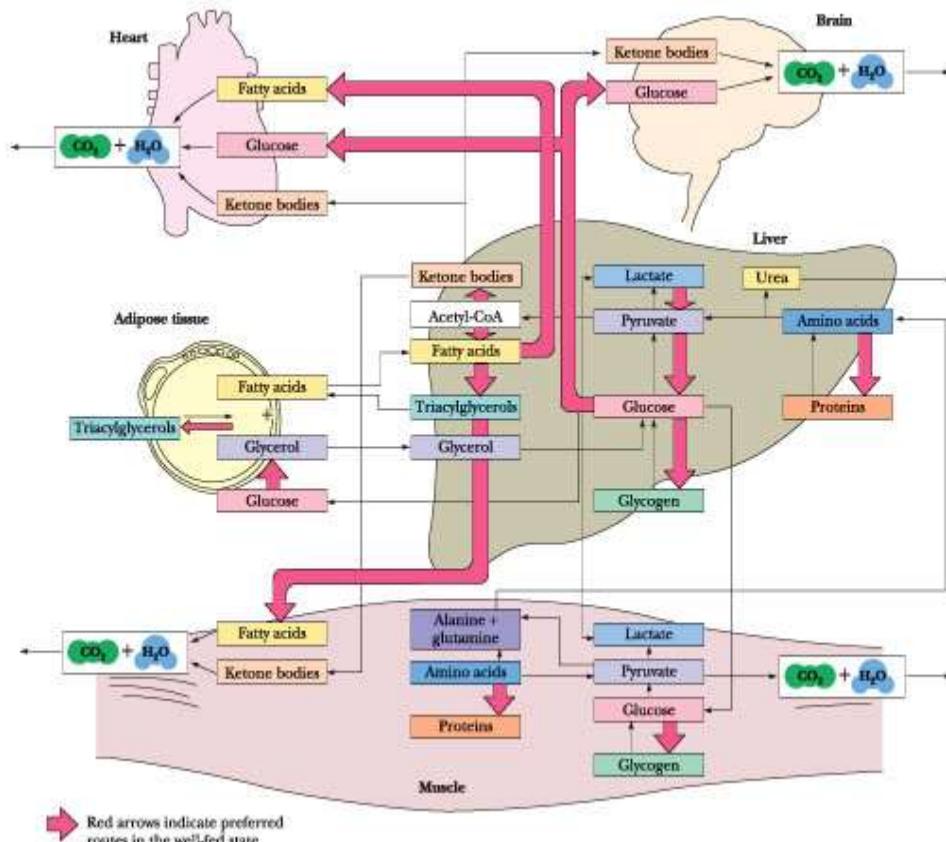




- **Prokaryotic cells** need not carry out ATP/ADP exchange.
- Thus, bacteria have the potential to produce approximately 38 ATP per glucose.

Metabolic relationships among the major human organs: brain, muscle, heart, adipose tissue, and liver

| Organ | Energy Reservoir | Preferred Substrate | Energy Sources Exported |
|--------------------------------------|---------------------------|---|-------------------------------------|
| Brain | None | Glucose (ketone bodies during starvation) | None |
| Skeletal muscle (resting) | Glycogen | Fatty acids | None |
| Skeletal muscle (prolonged exercise) | None | Glucose | Lactate |
| Heart muscle | Glycogen | Fatty acids | None |
| Adipose tissue | Triacylglycerol | Fatty acids | Fatty acids, glycerol |
| Liver | Glycogen, triacylglycerol | Amino acids, glucose, fatty acids | Fatty acids, glucose, ketone bodies |



Red arrows indicate preferred routes in the well-fed state

Homoeostasis

Homoeostasis

Definition : Maintenance of the relative stability of the physical and chemical aspects of the internal environment within a range compatible with cellular function.

Maintaining a constant internal environment with all that the cells need to survive (O_2 , glucose, minerals, ions, and waste removal) is necessary for individual cells. The processes by which the body regulates its internal environment are referred to as *homeostasis*.

Components :

- 1) sensor
- 2) afferent pathway
- 3) integration center or comparator
- 4) efferent pathway
- 5) effector organ(s)

- Physiological control systems are the nervous system, endocrine system, and immune system through feedback mechanisms.

Intrinsic homeostatic systems

- **Nervous System**

- The nervous system maintains homeostasis by controlling and regulating the other parts of the body.

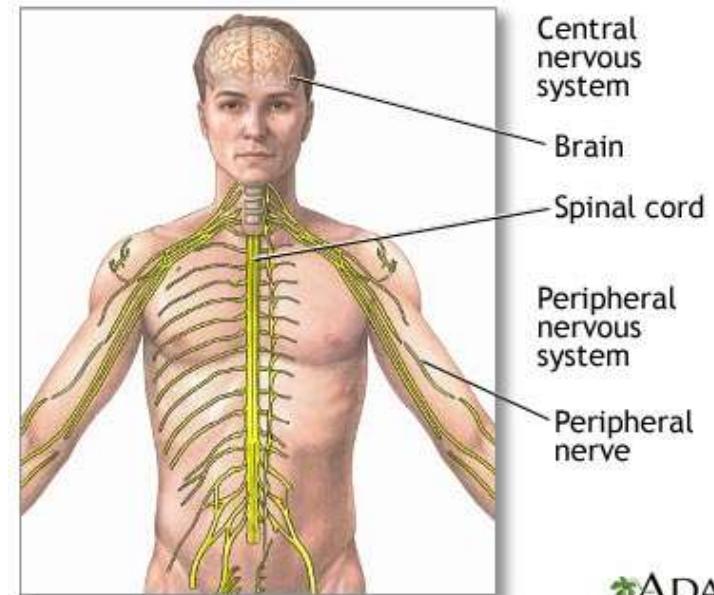
- A deviation from a normal set point acts as a stimulus to a receptor, which sends nerve impulses to a regulating center in the brain. The brain directs an effector to act in such a way that an adaptive response takes place.

- The nervous system has two major portions: the **central nervous system** and the **peripheral nervous system**.

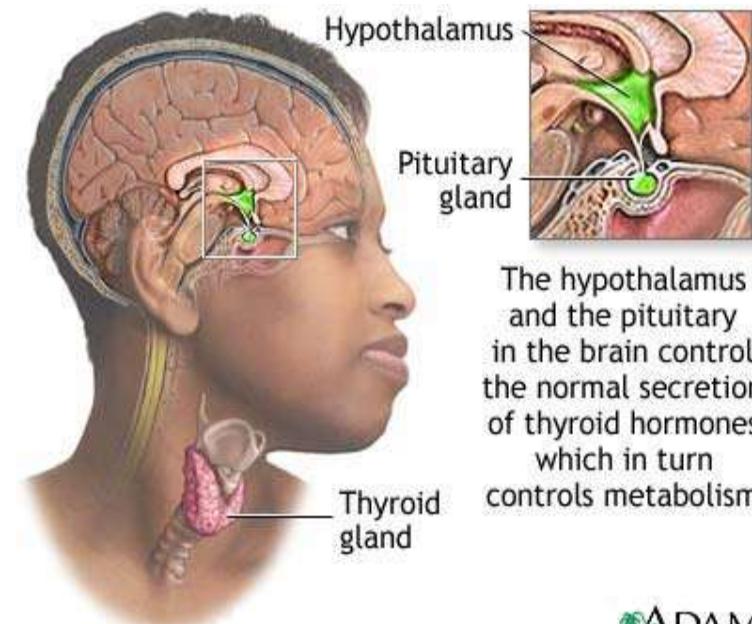
- Regulating centers are located in the central nervous system, consisting of the brain and spinal cord.

- The hypothalamus is a portion of the brain particularly concerned with homeostasis; it influences the action of the medulla oblongata, a lower part of the brain, the autonomic nervous system, and the pituitary gland.

- The peripheral nervous system consists of the spinal nerves. The **autonomic nervous system** is a part of peripheral nervous system and contains motor neurons that control internal organs. It has two divisions, the **sympathetic** and **parasympathetic** systems.

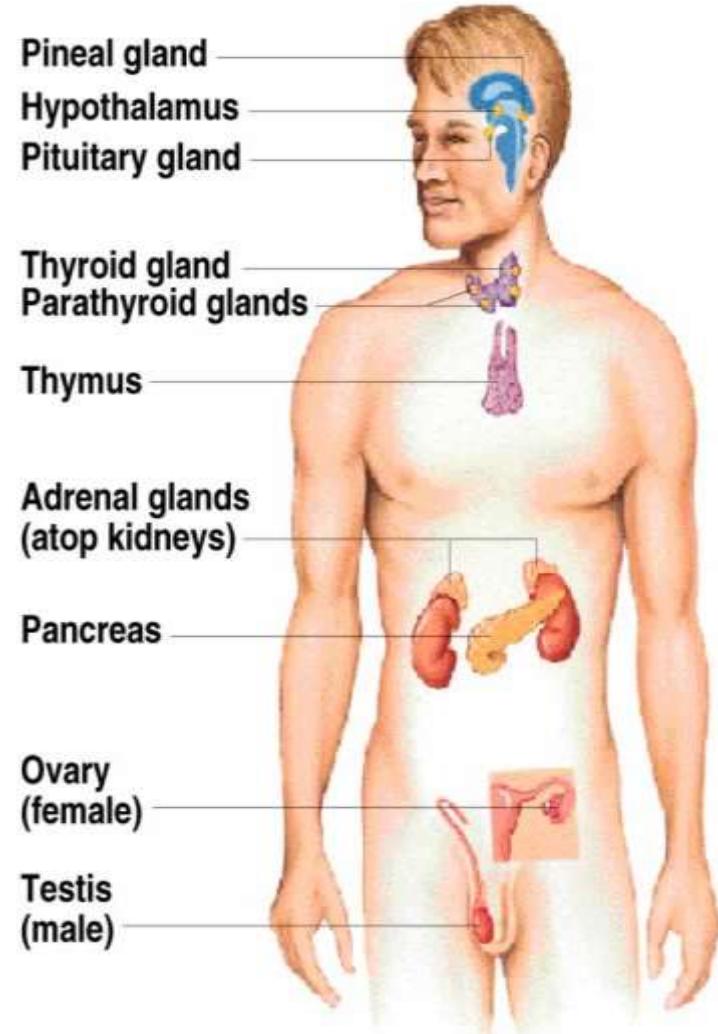


ADAM.

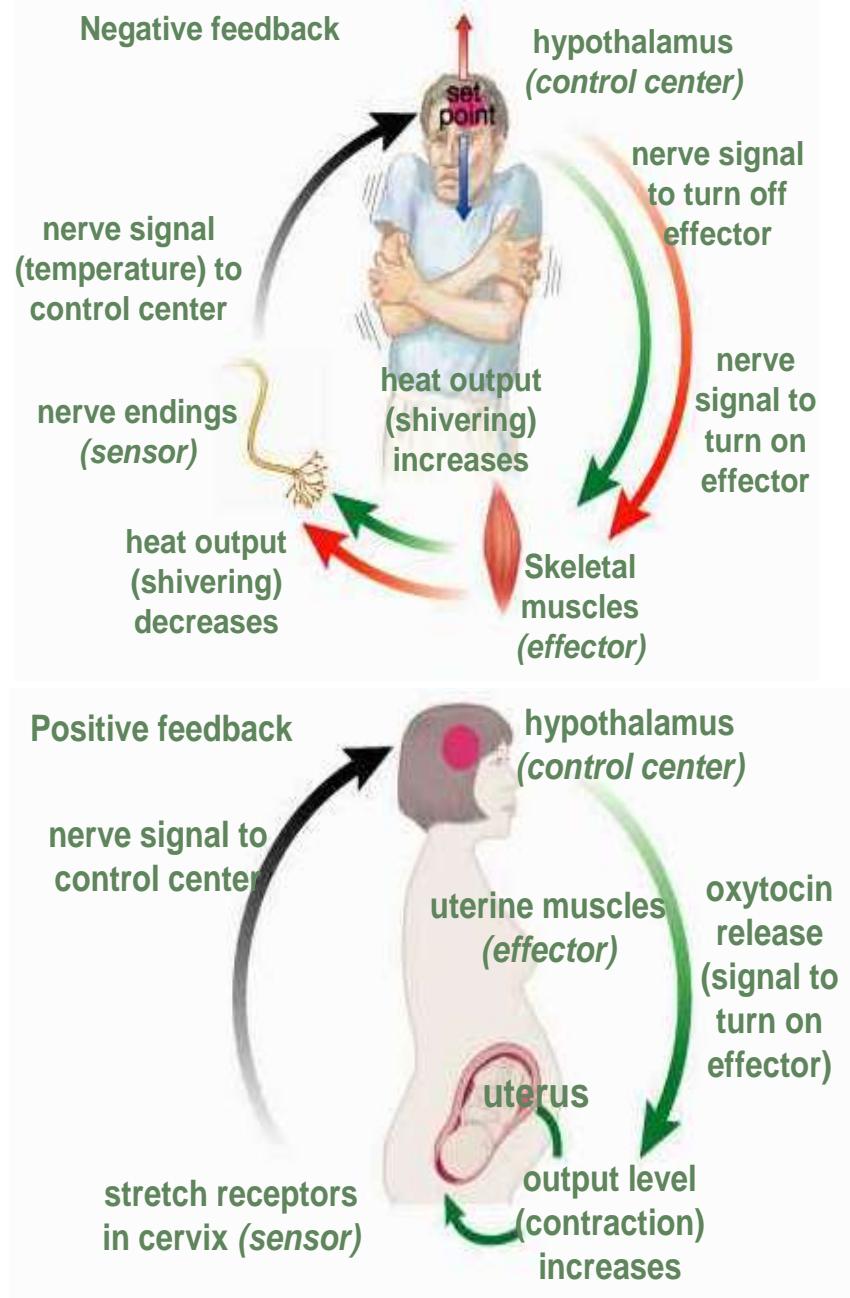


ADAM.

- **Endocrine System**
- The endocrine system consists of glands which secrete special compounds called **hormones** into the bloodstream.
- Each hormone has an effect on one or more target tissues. In this way the endocrine system regulates the metabolism and development of most body cells and body systems.
- For e.g. the endocrine system has sex hormones that can activate sebaceous glands, development of mammary glands, alter dermal blood flow, and release lipids from adipocytes etc besides governing reproduction.
- In the muscular system, hormones adjust muscle metabolism, energy production, and growth.
- In the nervous system, hormones affect neural metabolism, regulate fluid/electrolyte balance and help with reproductive hormones that influence CNS (central nervous system), development and behaviours.
- In the cardiovascular system, hormones regulate heart rate and blood pressure.
- Hormones also have anti-inflammatory effects and control the lymphatic system.



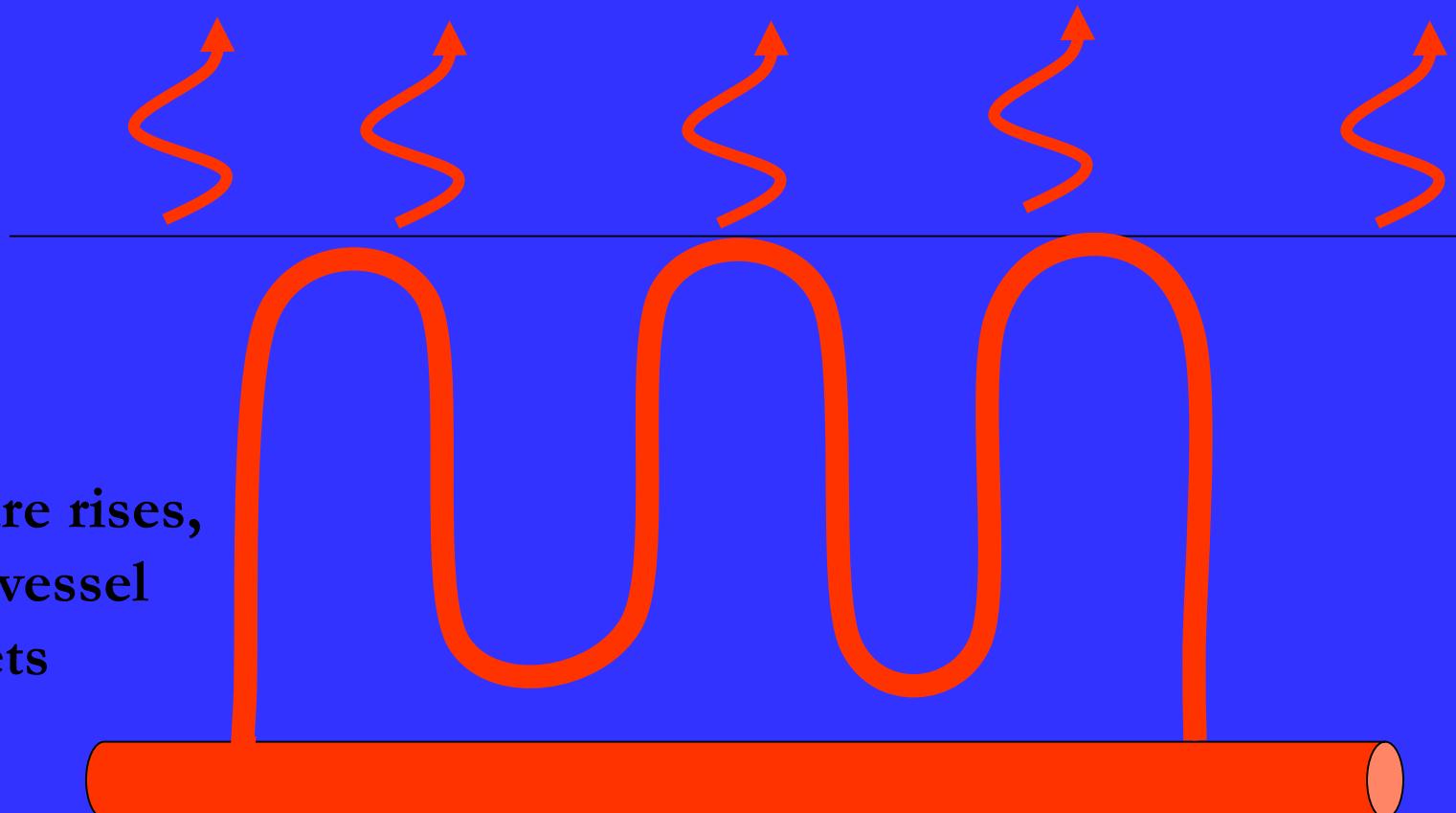
- Negative feedback : a control system that causes the value of a physiological measurement to change in the direction **opposite** to the initial deviation from set point.
- Positive feedback : a control system that causes the value of a physiological measurement to change in the **same** direction as the initial deviation from set point.



This means more heat is lost from the surface of the skin



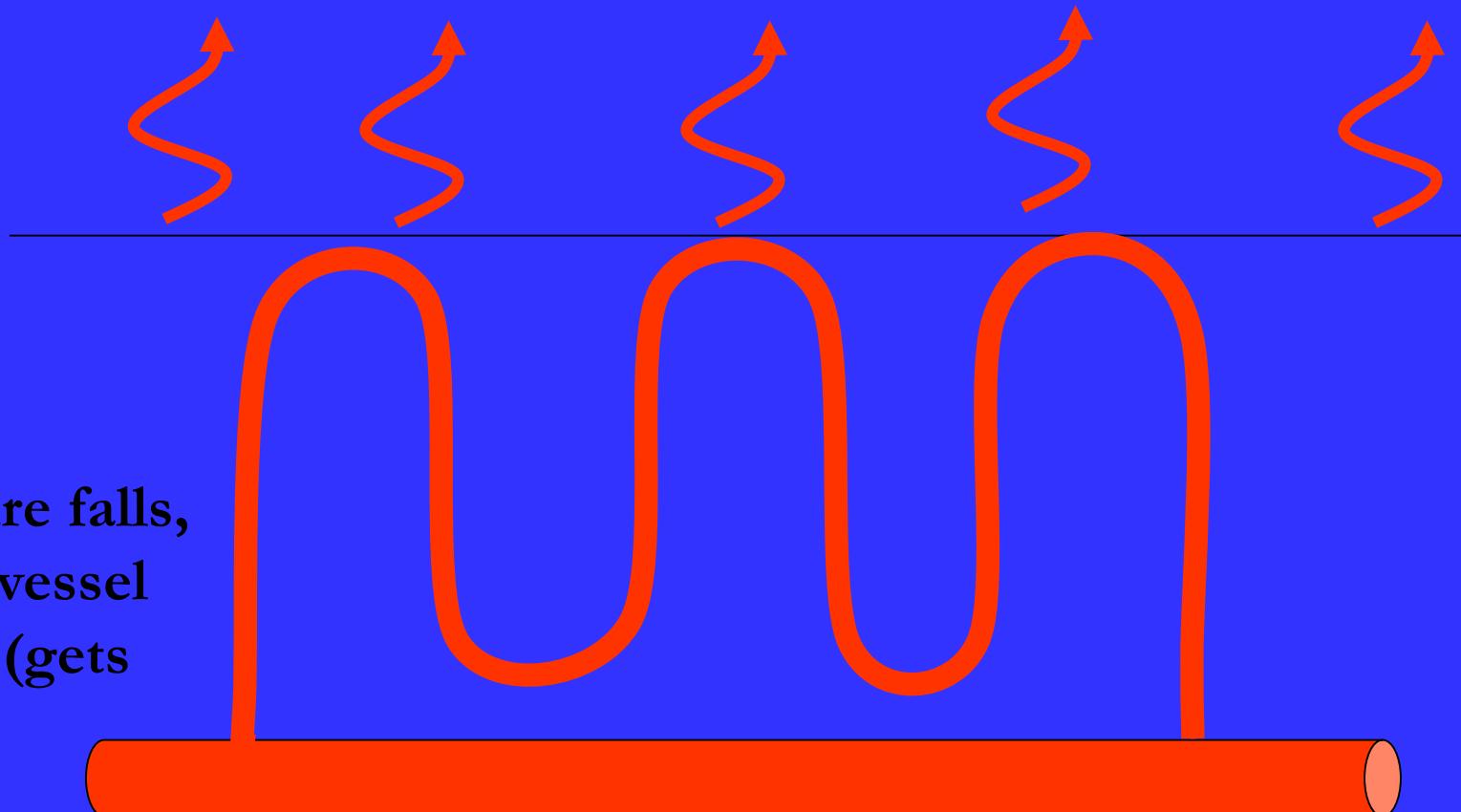
If the temperature rises, the blood vessel dilates (gets bigger).



This means less heat is lost from the surface of the skin



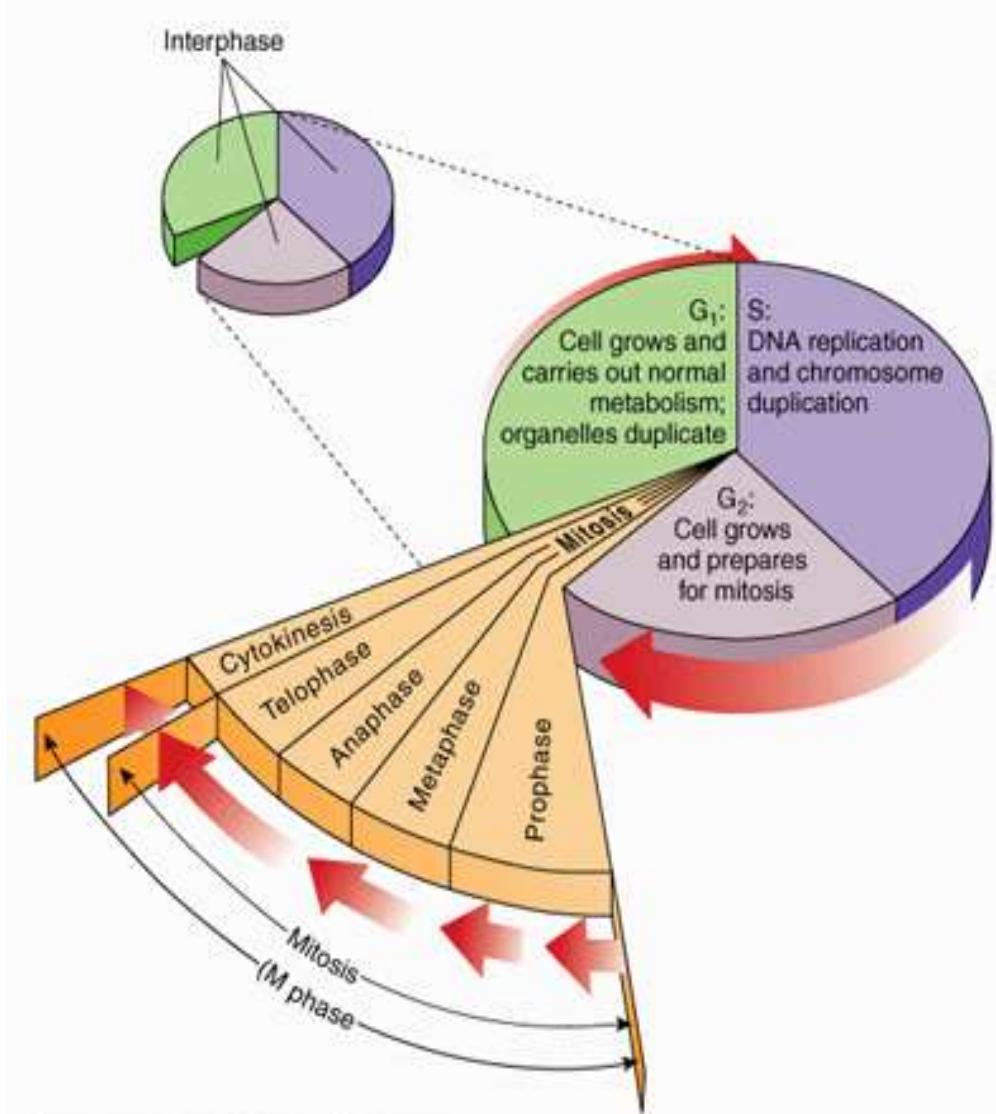
If the temperature falls, the blood vessel constricts (gets shut off).



Cell growth, reproduction, and differentiation

The Cell Cycle

- Mitosis and meiosis are single steps in cell cycle
- G₁, S, G₂, and M phases
 - Cells not in process of dividing are in G₀ phase
 - Chromosomes are duplicated in preparation for the next round of division during interphase

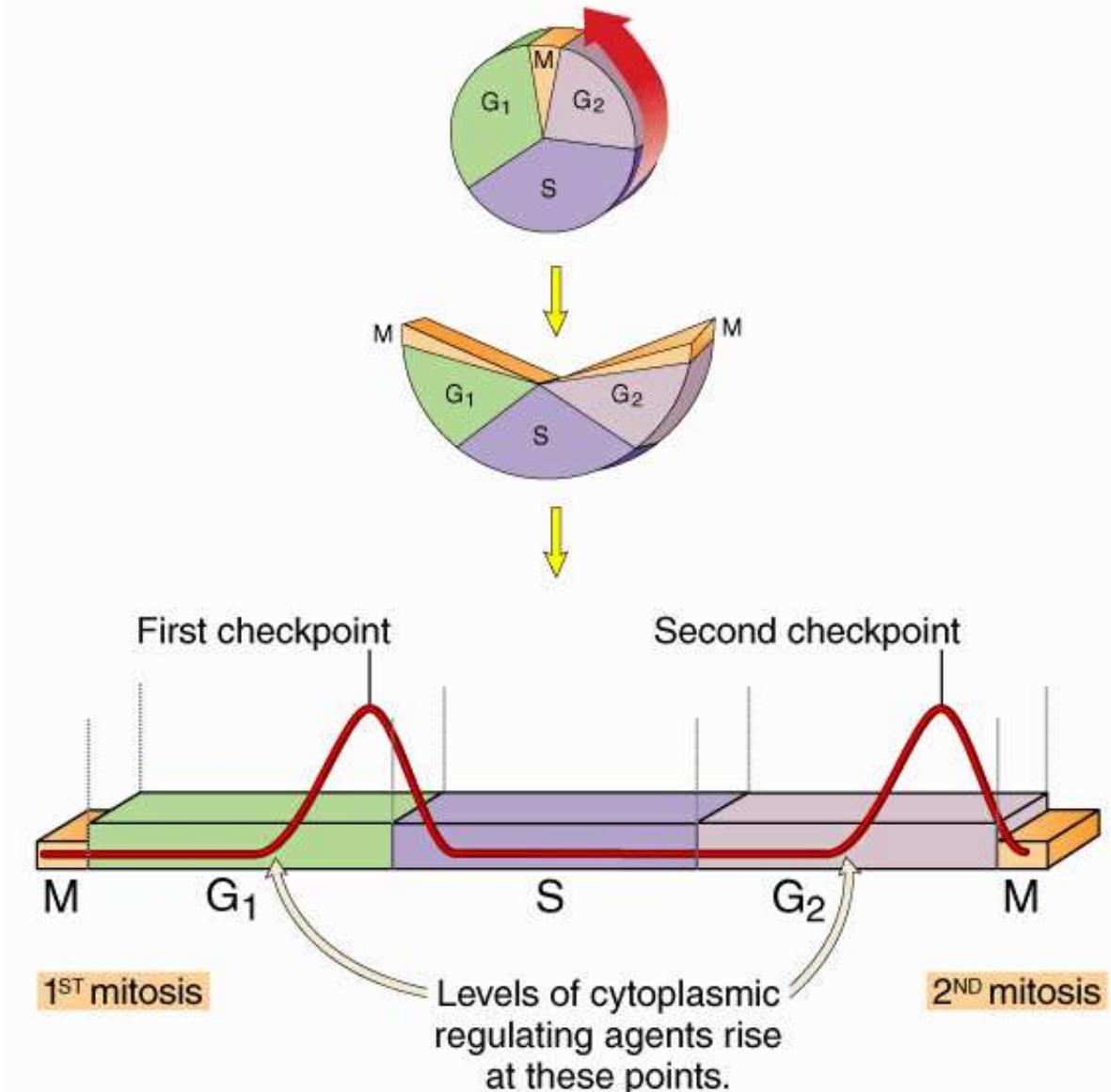


Control of the Cell Cycle

- The stimuli for entering the cell cycle is in the form of growth factors and cytokines that are capable of inducing mitotic divisions
- The cell cycle is highly regulated
 - Proteins whose concentrations rise & fall in a controlled manner
 - Cyclin and cyclin-dependent kinases (cdk)
 - p53 and pRb
 - Inhibitors of cdk
- Internal checkpoints & guardians monitor cell health
- Errors in this process can lead to uncontrollable growth and cancer

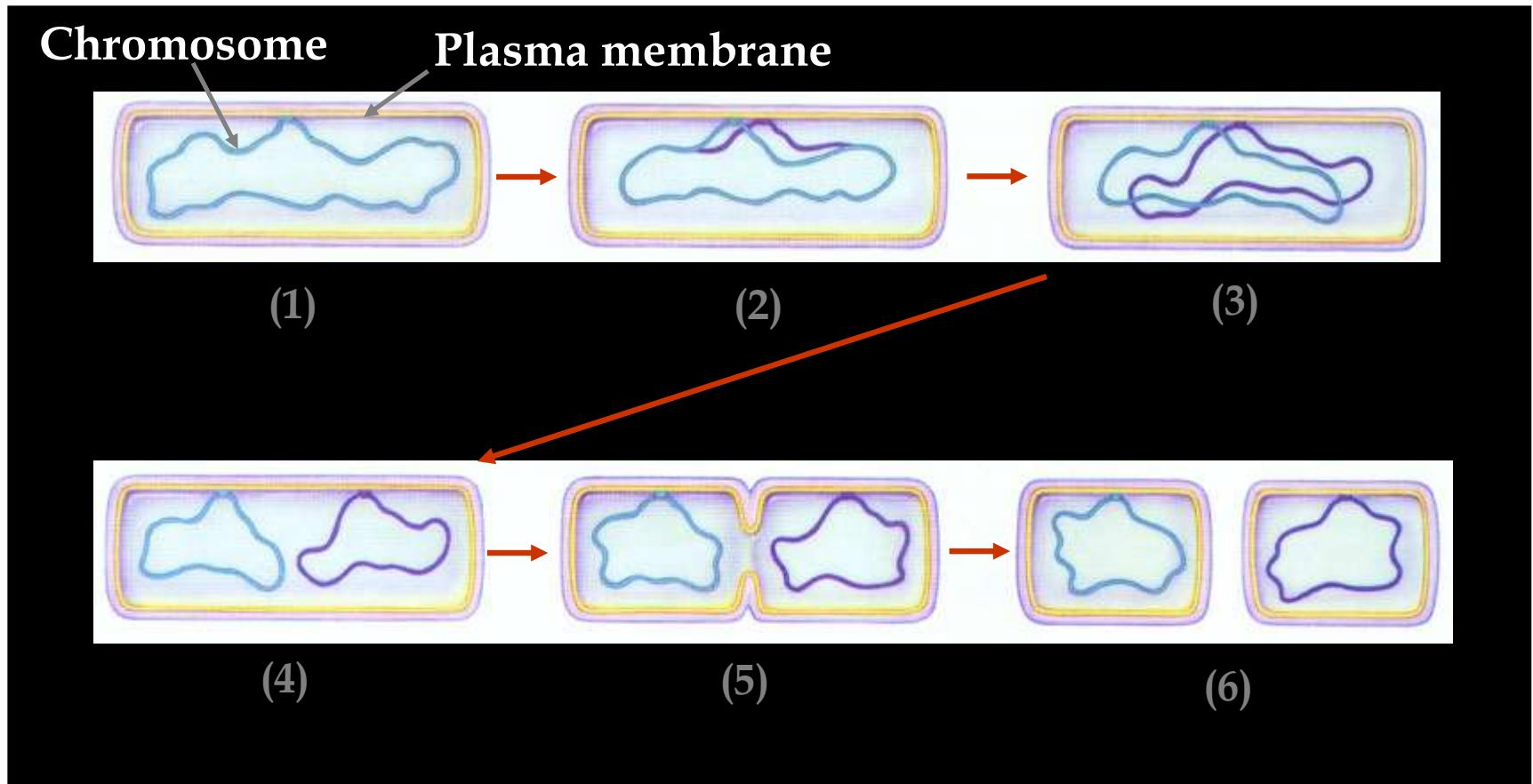
Control of the Cell Cycle

- **Cell cycle control is focused at 3 places:**
- **G1 checkpoint**
- **G2 checkpoint**
- **M checkpoint**
 - Before S phase (DNA synthesis)
 - At transition between G₂ and M phase



Binary fission

Daughter cells are identical copies



Neither mitosis nor meiosis occurs in prokaryotes

Bacteria

Reproduction

Asexual, through binary fission

No true sexual reproduction,
since neither mitosis nor meiosis
exist in prokaryotes

Horizontal transfer of genetic
material

Transformation

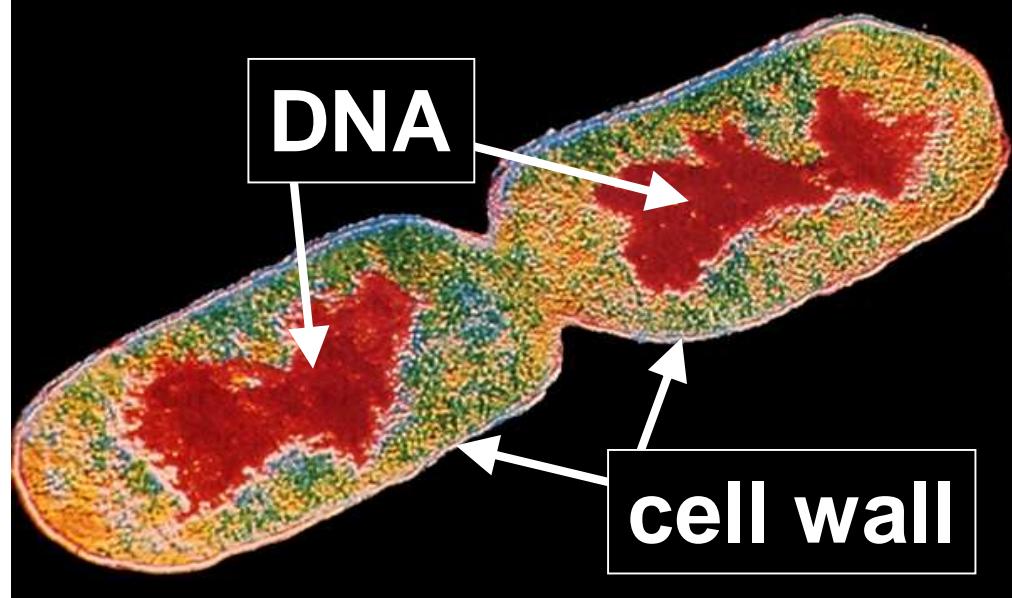
Uptake of genetic material from
the environment

Transduction

Transfer of genetic material
between prokaryotes by viruses

Conjugation

Direct transfer of genetic
material from one prokaryote to
another



Mitosis

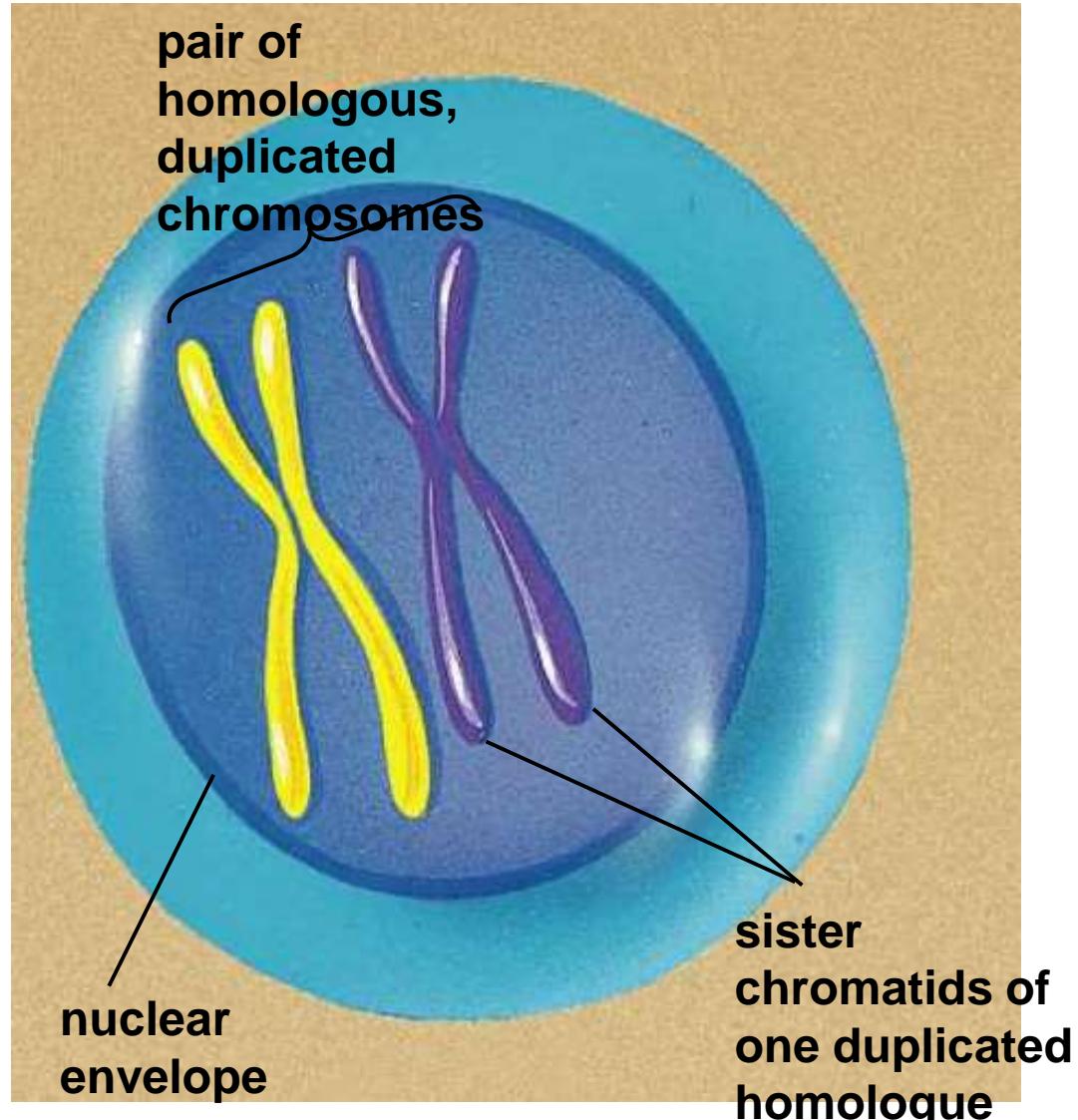
- **Four phases**

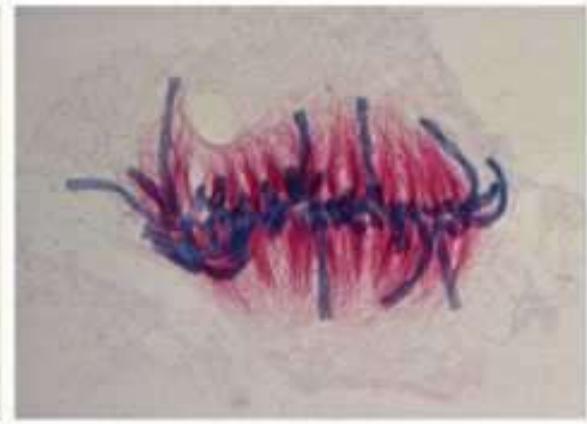
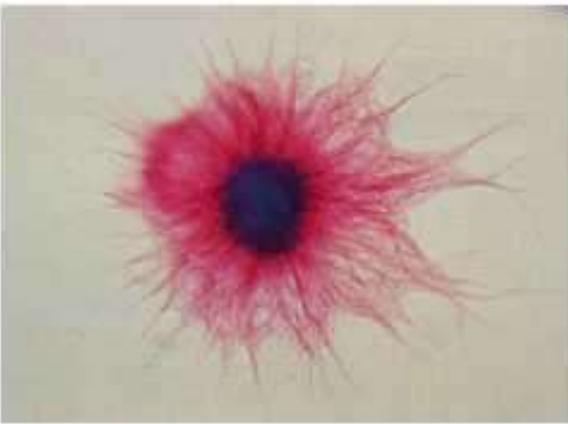
- **1. Prophase:** chromosomes condense, spindle apparatus forms, nuclear envelope breaks down

- **2. Metaphase:** chromosomes line up at equator of cell

- **3. Anaphase:** sister chromatids separate

- **4. Telophase:** new nuclear envelopes form, chromosomes unwind

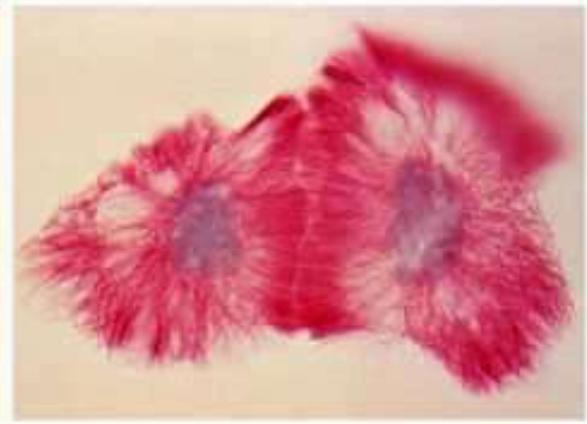
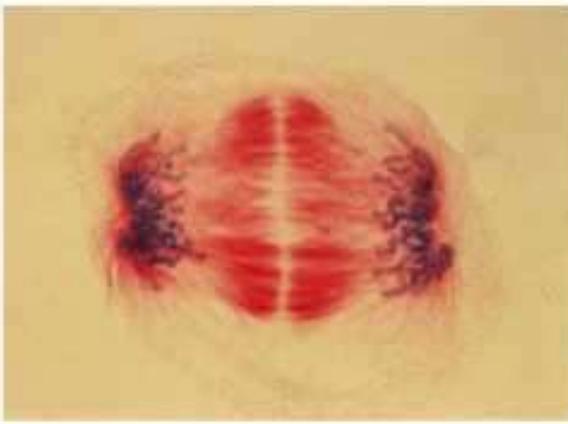




(a) Interphase in a seed cell: The chromosomes (blue) are in the thin, extended state and appear as a mass in the center of the cell. The spindle microtubules (red) extend outward from the nucleus to all parts of the cell.

(b) Late prophase: The chromosomes (blue) have condensed and attached to the spindle microtubules (red).

(c) Metaphase: The chromosomes have moved to the equator of the cell.



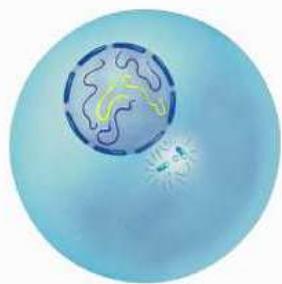
(d) Anaphase: Sister chromatids have separated, and one set has moved toward each pole.

(e) Telophase: The chromosomes have gathered into two clusters, one at the site of each future nucleus.

(f) Resumption of interphase: The chromosomes are relaxing again into their extended state. The spindle microtubules are disappearing, and the microtubules of the two daughter cells are rearranging into the interphase pattern.

Each new nucleus is genetically identical to the parent nucleus

Parent Cell
Chromosomes
have been
replicated



Mitosis



Daughter Cells
Each cell has the same
genetic makeup as the
parent cell



Meiosis

Characteristics of meiosis

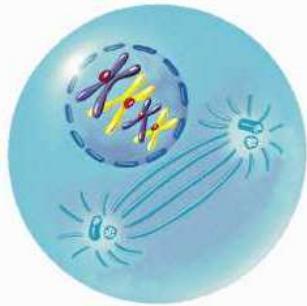
- 1. Occurs in sex cells (germ cells) and produces gametes
- 2. A reductional division resulting in haploid cells
- 3. Involves two sequential divisions resulting in four cells
- 4. Produces cells that are genetically different because of genetic recombination (crossing-over).

Meiosis produces gametes for sexual reproduction

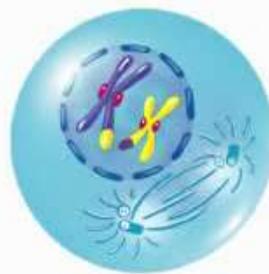
- Multiplies number of cells but also reduces chromosome number in each daughter cell to exactly half the number present before meiosis
- Daughter cells get 1 member of each homologous pair, i.e. 1 allele for each gene
- Mitosis produces 2 daughter cells
- Meiosis produces 4 daughter cells
- All body cells in humans are diploid, *except* gametes
- Cells with 1 member of each homologous pair are haploid

Meiosis

**Parent Cell
(2n)**

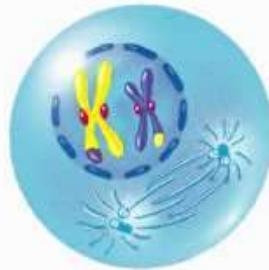


1st division



Daughter Cells (1n)
each chromosome has
2 chromatids

2nd division

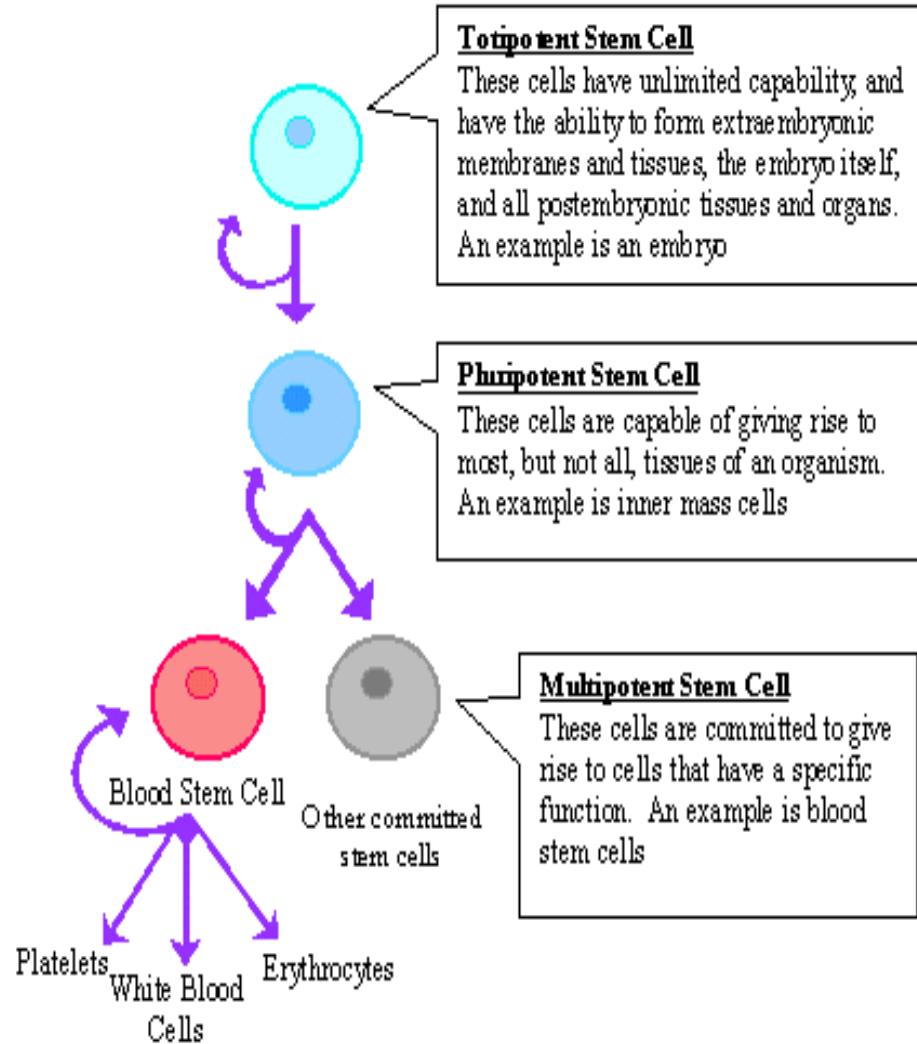


Gamete Cells (1n)

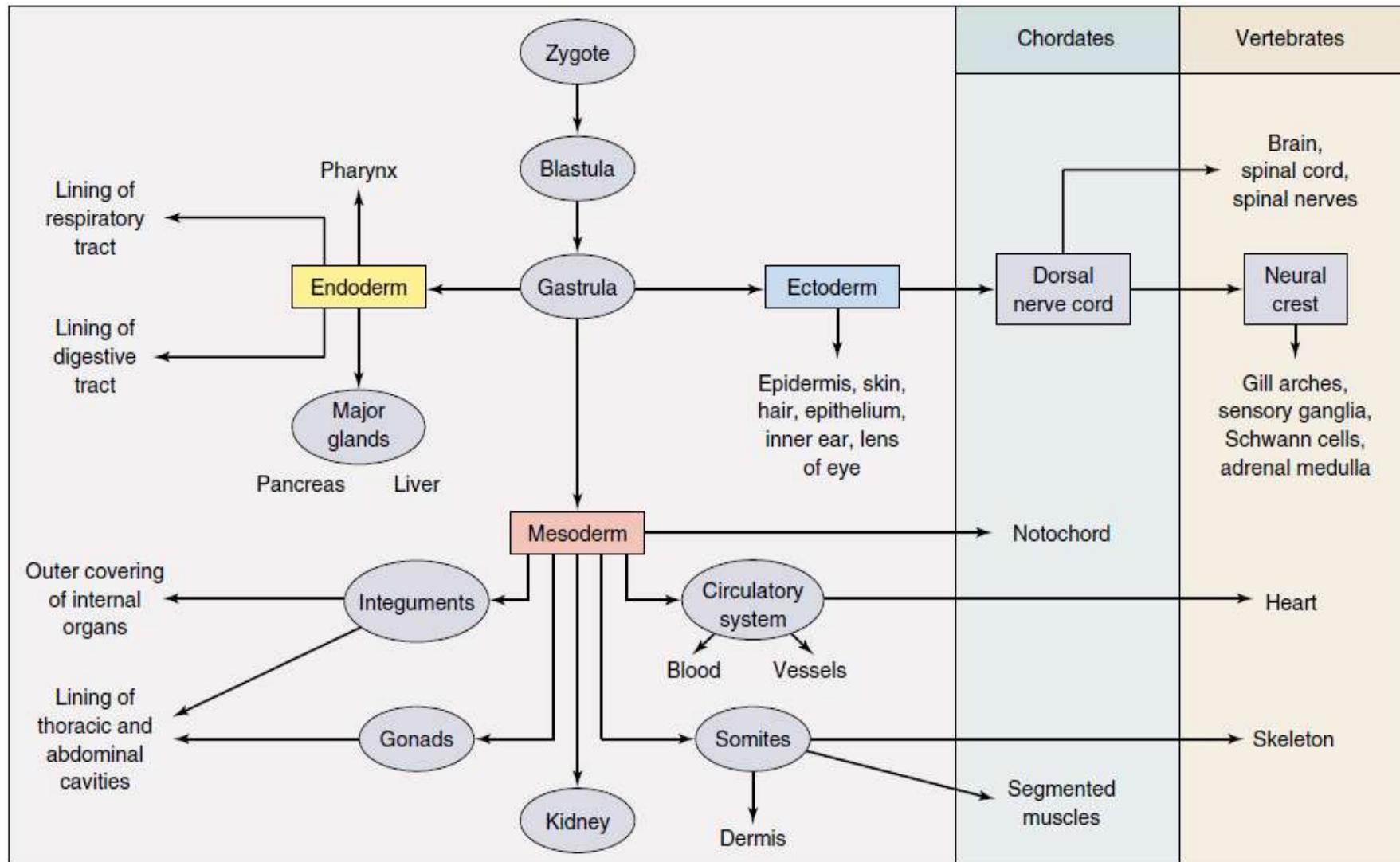


Cell Differentiation

- The process of altering the pattern of gene expression and thus becoming a cell of a particular type is called cell differentiation.
- Presence of chemicals (or other influences) starts altering the decisions as to which genes will be turned on or off.
- The zygote is a **totipotent** cell - its daughter cells can become any cell type. As the development proceeds, some of the cells become **pluripotent** - they can become many, but not all cell types.
- Later on, the specificity narrows down further and a particular stem cell can turn into only a very limited number of cell types, e.g., a few types of blood cells, but not bone or brain cells or anything else. That is why embryonic stem cell research is much more promising than the adult stem cell research.



Differentiation of different tissues and organs



UNIT II

- Biological Diversity
- Chemistry of life: chemical bonds
- Biochemistry and Human biology
- Protein synthesis
- Stem cells and tissue engineering

BIODIVERSITY

Biological diversity - or biodiversity - is the term given to the varieties of life on Earth. It is the result of billions of years of evolution, shaped by natural processes and, increasingly, by the influence of humans.

It forms the web of life of which we are an integral part and upon which we so fully depend.



BIODIVERSITY

- 1) Variety of species
- 2) Genetic differences
- 3) Variety of ecosystems

Genetic diversity



Taxonomic diversity



Community or ecosystem diversity



WHY IS BIODIVERSITY IMPORTANT FOR YOU AND THE WORLD?

**Protecting biodiversity is in our self interest,
providing the goods and services that sustain our
lives including:**

- Provision of shelter and building materials**
- Stabilization and moderation of the Earth's climate**
- Purification of air and water**
- Provision of food, fuel, and fibre**
- Cultural and aesthetic benefits, etc.**



WHAT ARE THE CHALLENGES FACING BIODIVERSITY?

Species have been disappearing at up to 1000 times the natural rate

- An estimated 34,000 plant and 5,200 animal species face extinction, including one in eight birds and one third of all amphibians
- 20% of known bird species have already disappeared
- 41% of mammals are in decline and 28% are under direct threat
- 45% of the Earth's original forests are gone. Forest areas of about four times the size of Belgium are being lost every year.

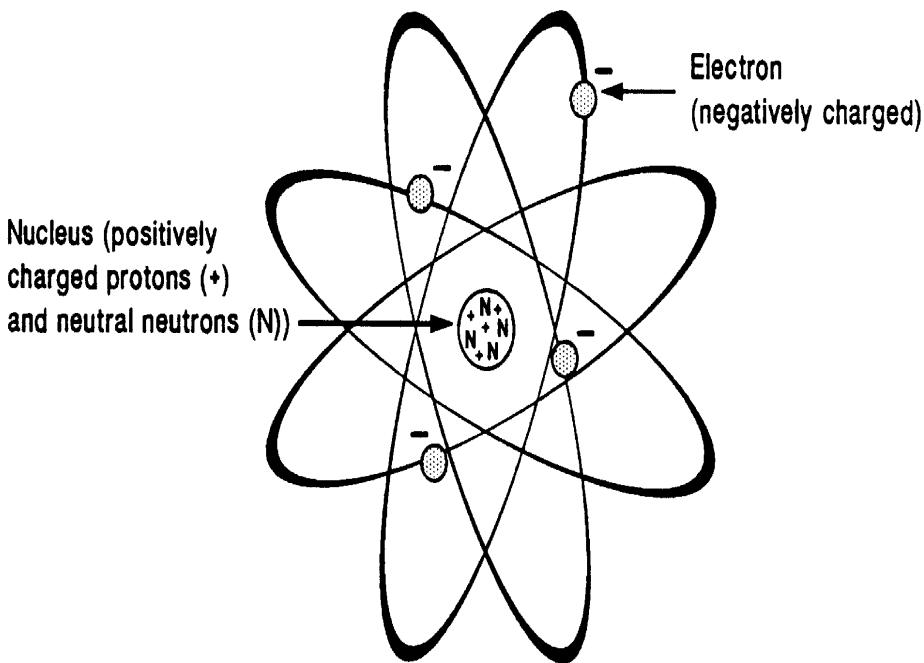


Human activities are creating the greatest wave of extinction since the natural disaster that wiped out the dinosaurs 65 million years ago

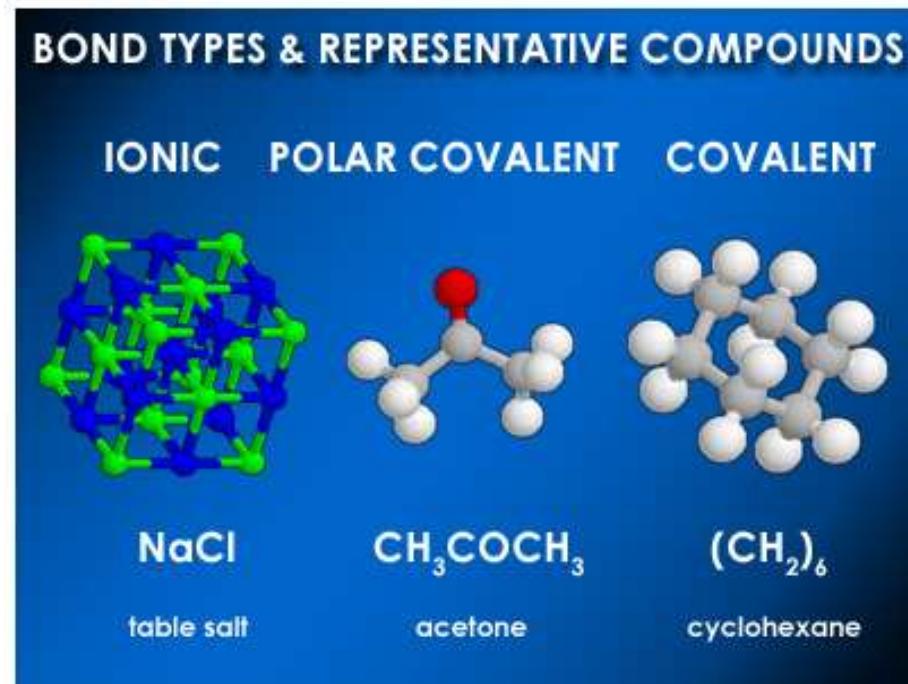
Chemistry of life: chemical bonds

Chemistry of Life

- All matter is built up of simple units called **atoms**.
- Although the word atom means something that cannot be cut (a = “without,” tom = “cut”), these elementary particles are actually made up of many smaller parts, which are themselves further divisible.
- **Elements** are substances that consist of the same kinds of atoms.
- **Compounds** consist of units called molecules, which are intimate associations of atoms (in the case of compounds, different atoms) joined in precise arrangements.

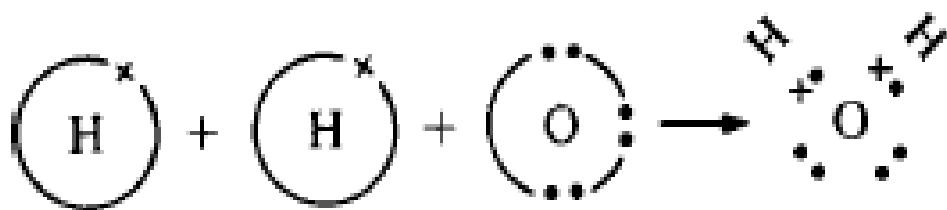


- Atoms interact with one another to form chemical communities. The tightly knit atoms making up the communal molecules are held together by **chemical bonding**.
- One way of achieving this more stable state is for an atom with very few electrons in its outer shell to donate them to an atom with an outer shell that is almost complete.
 - The atom that donates the electrons will then have more protons than electrons and assume a positive charge; it is called a cation. The atom receiving the electrons assumes a negative charge and is called an anion.
 - These two oppositely charged ions are electrostatically attracted to each other and are said to have an **ionic, or polar, bond**.



- A second way in which atoms may join with one another to bring about a filling of their outermost shells is by sharing a pair of electrons.

- The two bonding atoms provide one electron each in creating the shared pair. This pair of electrons forms a **covalent bond** that holds the two atoms together. It is represented by a solid line in the formula of a compound.

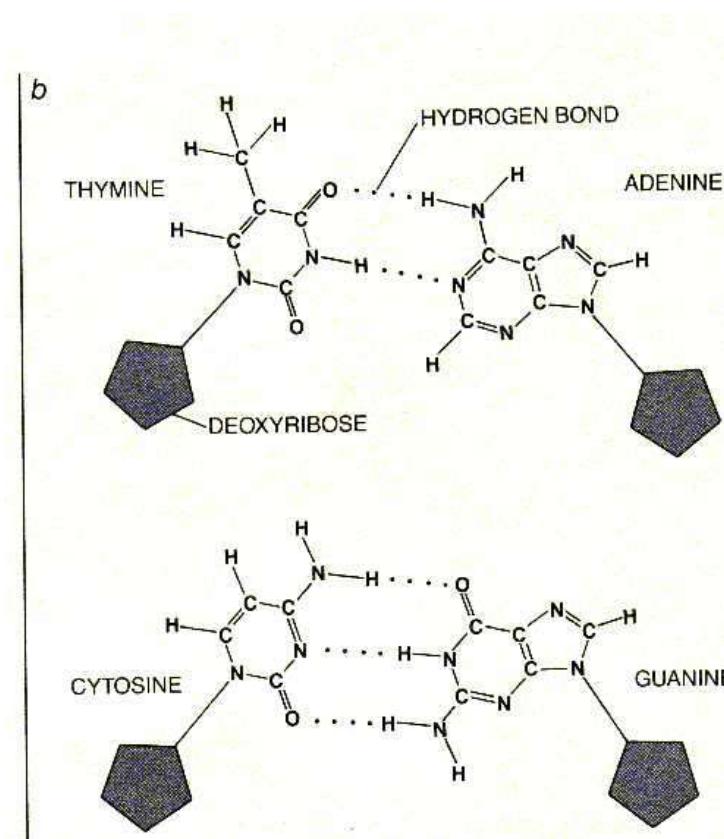
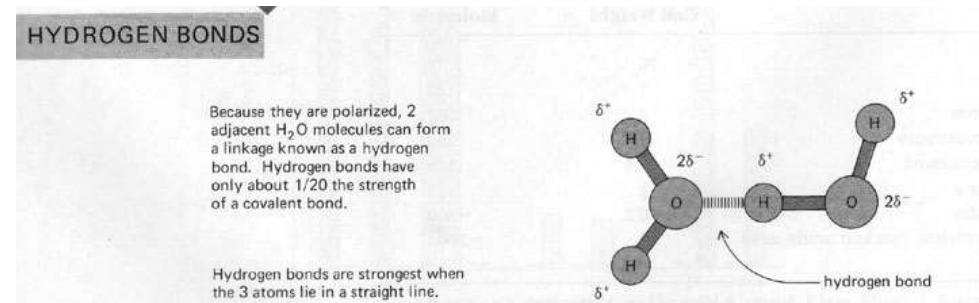


- In many molecules, covalent bonding may occur not just singly (sharing a single pair of electrons), but may involve the formation of double or triple bonds in which two and even three pairs of electrons are shared.

- These double and triple bonds tend to fix the position of the participating atoms in a rigid manner.



- **Non-covalent bonds (ionic, hydrogen)** are much weaker than **covalent bonds** (electron sharing) and so protein shape can be disrupted especially by temperature, pH , ions (salt).
 - It involves more dispersed variations of electromagnetic interactions.
 - Critical in maintaining the three-dimensional structure of large molecules, such as proteins and nucleic acids
 - There are four commonly mentioned types of non-covalent interactions: hydrogen bonds, ionic bonds, van der Waals forces, and hydrophobic interactions.
 - The noncovalent interactions hold together the two strands DNA in the double helix, stabilize secondary and tertiary structures of proteins, and enable enzyme-substrate binding and antibody-antigen association.



Biochemistry and Human biology

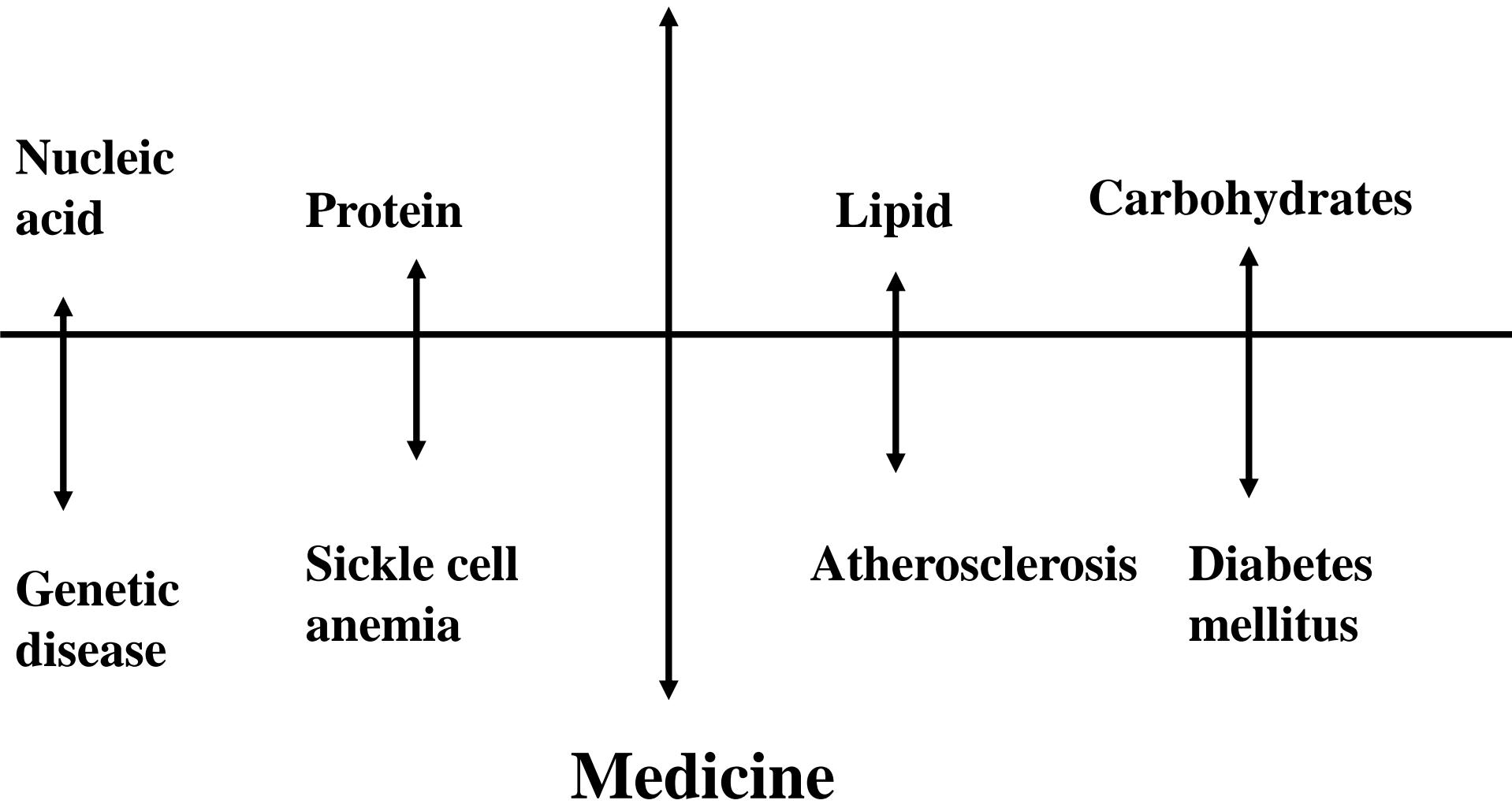
Biochemistry: Where Chemistry & Biology Meet

- **Living things require millions of chemical reactions just to survive.**
- **Metabolism = all the chemical reactions occurring in the body.**
- **Organic molecules:**
 - usually associated with living things.
 - always contain CARBON.
 - are “large” molecules, with many atoms
 - always have covalent bonds (share electrons)

Biochemistry and Human Biology

- **Biochemistry:** Science concerned with the chemical constituents of living cells and with the reaction and process that they undergo.
 - Complete understanding at the molecular level of all the chemical processes associated with living cells
 - An appreciation of the biochemistry of less complex form of life is often direct relevance to human biochemistry
- **Reciprocal relationship between biochemistry and medicine has stimulated mutual advance**
 - Biochemistry studies have illuminated many aspects of health & disease

Biochemistry

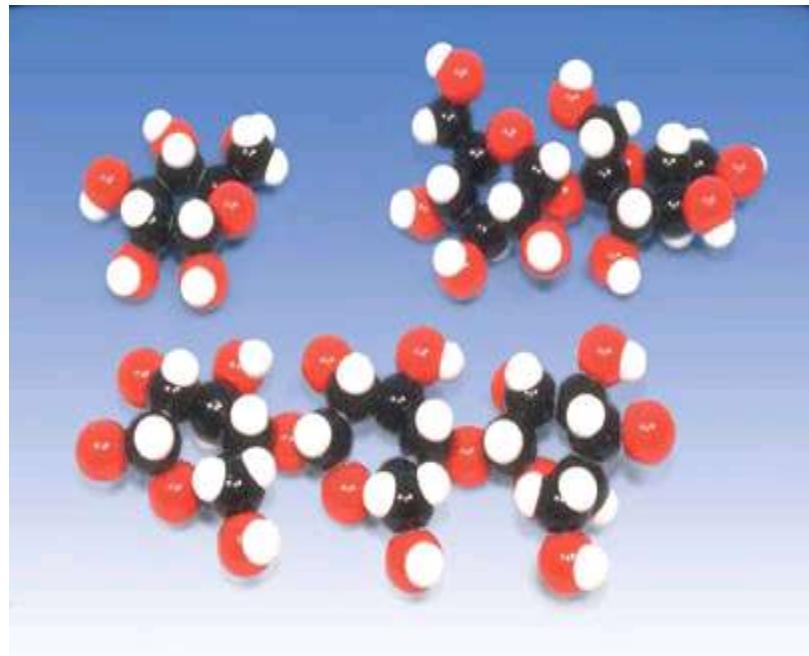


| S. No. | Disease | Causes |
|---------------|---------------------------------|---|
| 1 | Scurvy rickets | deficiencies of vitamins C and D respectively |
| 2 | Atherosclerosis | genetic, dietary, environmental factors |
| 3 | Cystic fibrosis | mutation in the gene coding the CFTR protein (Cystic fibrosis transmembrane conductance regulator, a protein involved in the transport of chloride ions across cell membranes) |
| 4 | Cholera | exotoxin of vibrio cholera |
| 5 | Diabetes mellitus type I | genetic and environmental factors resulting in deficiency of insulin |
| 6 | Phenylketonuria | mainly mutation in the gene coding phenylalanine hydroxylase |

Carbon-based Molecules

- Although a cell is mostly water, the rest of the cell consists mostly of carbon-based molecules

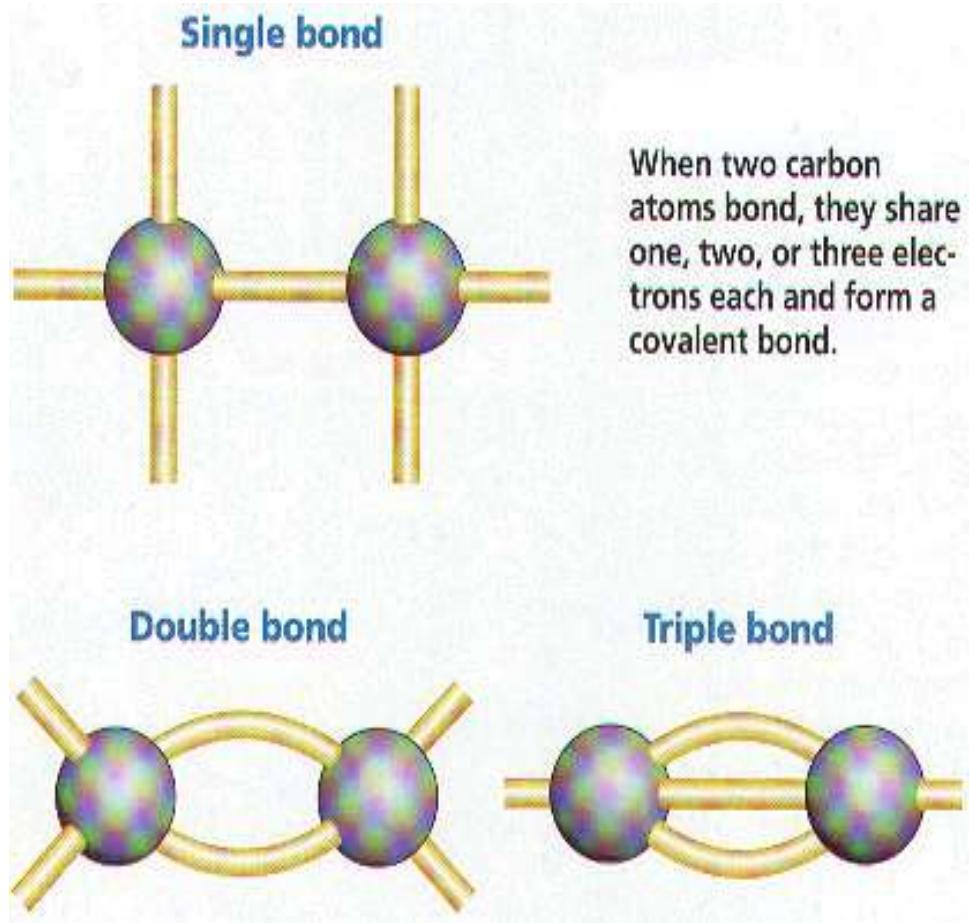
**Organic chemistry is
the study of carbon
compounds**



Carbon is a Versatile Atom

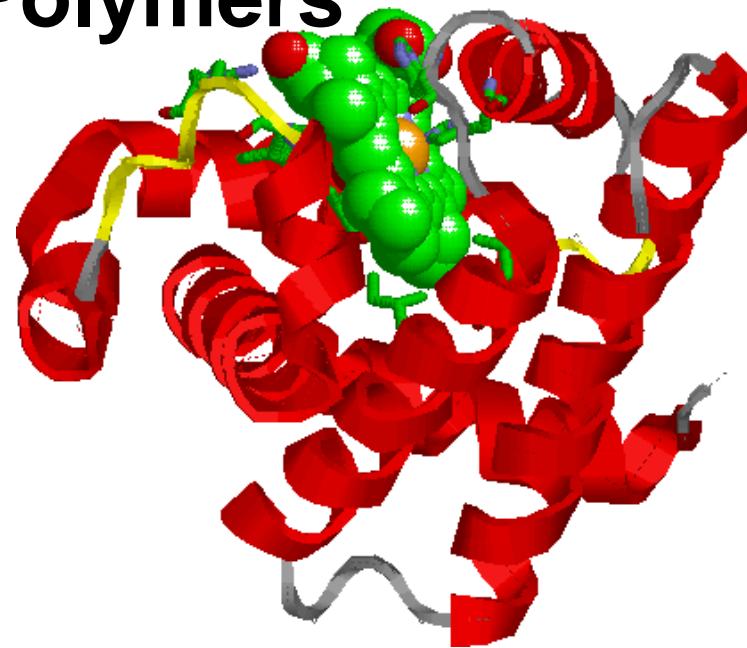
- It has four electrons in an outer shell that holds eight

Carbon can share its electrons with other atoms to form up to four covalent bonds



Giant Molecules - Polymers

- Large molecules are called polymers
- Polymers are built from smaller molecules called monomers
- Biologists call them macromolecules



Macromolecules in Organisms

- There are four categories of large molecules in cells:

Carbohydrates

Lipids

Proteins

Nucleic Acids

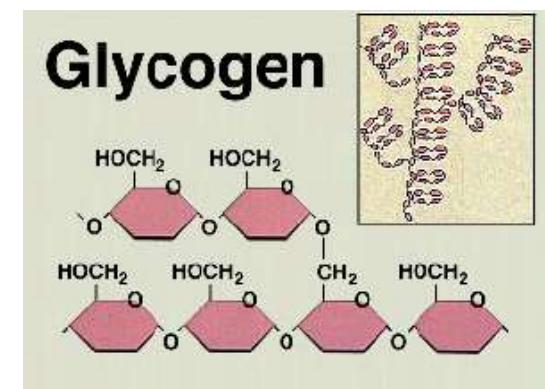
Examples of Polymers

• Proteins



Lipids

Carbohydrates



Nucleic Acids



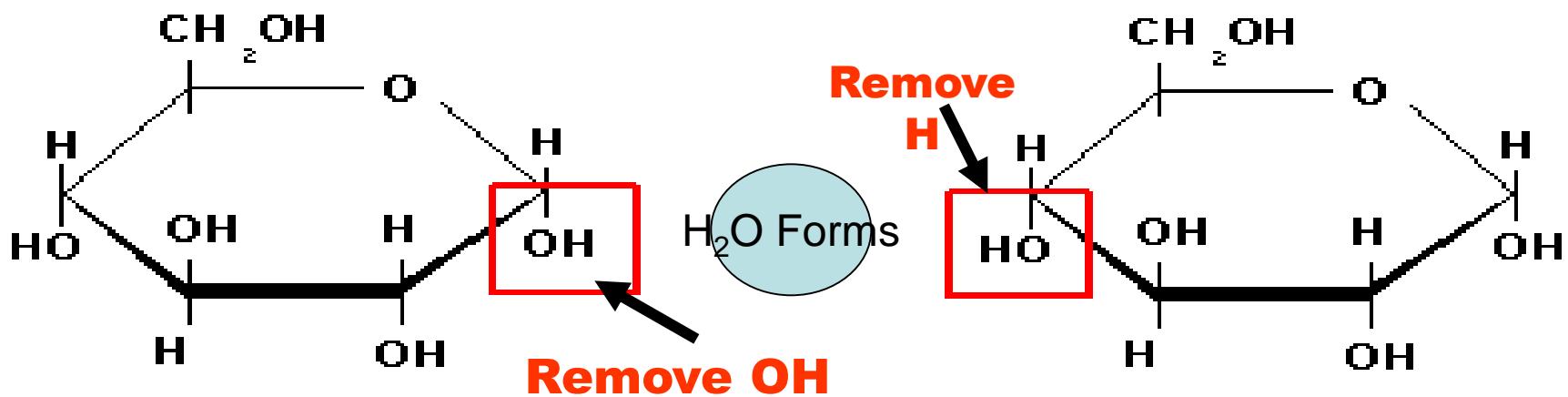
Carbohydrates

- Carbohydrates include:
 - Small sugar molecules in soft drinks
 - Long starch molecules in rice, wheat, pasta and potatoes



Linking Monomers

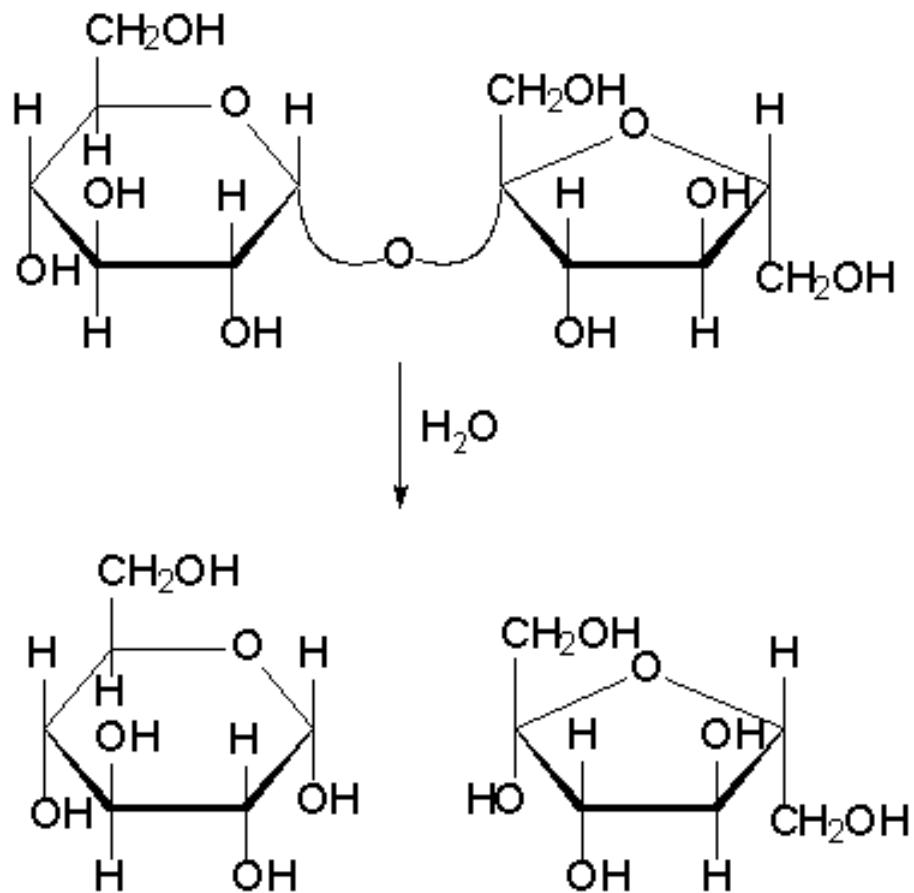
Cells link monomers by a process called condensation or dehydration synthesis (removing a molecule of water)



This process joins two sugar monomers to make a double sugar

Breaking Down Polymers

- Cells break down macromolecules by a process called hydrolysis (adding a molecule of water)



Water added to split a double sugar

Monosaccharides

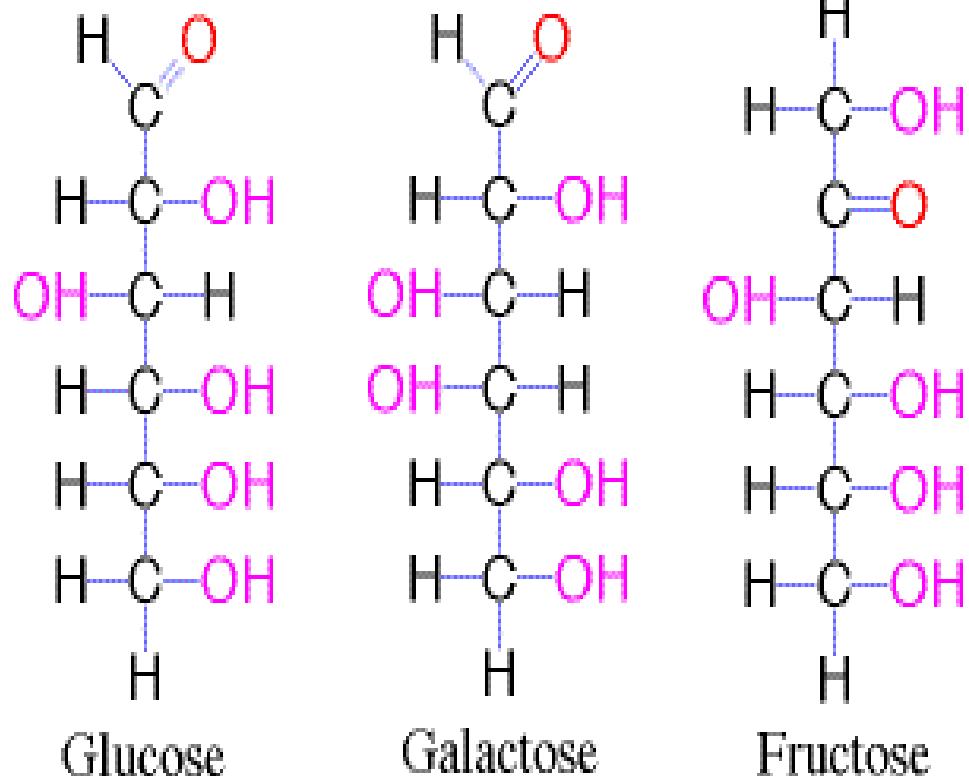
- Called simple sugars

Include glucose, fructose, & galactose

Have the same chemical, but different structural formulas

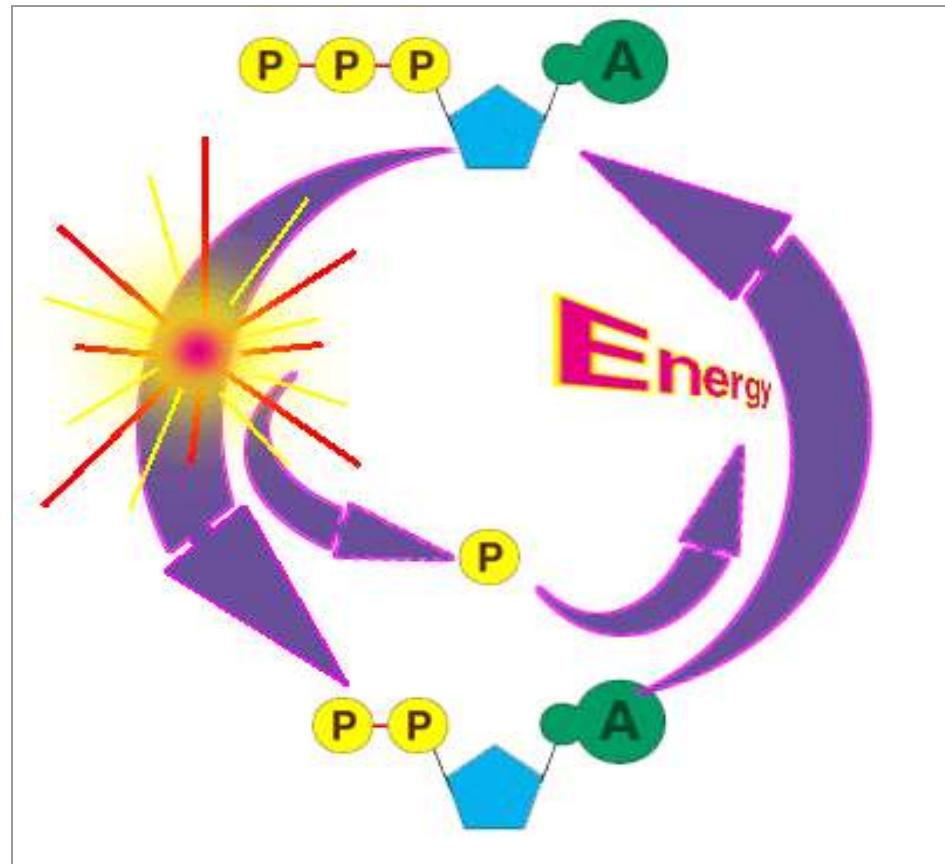


Hexose Sugars



Cellular Fuel

- Monosaccharides are the main fuel that cells use for cellular work



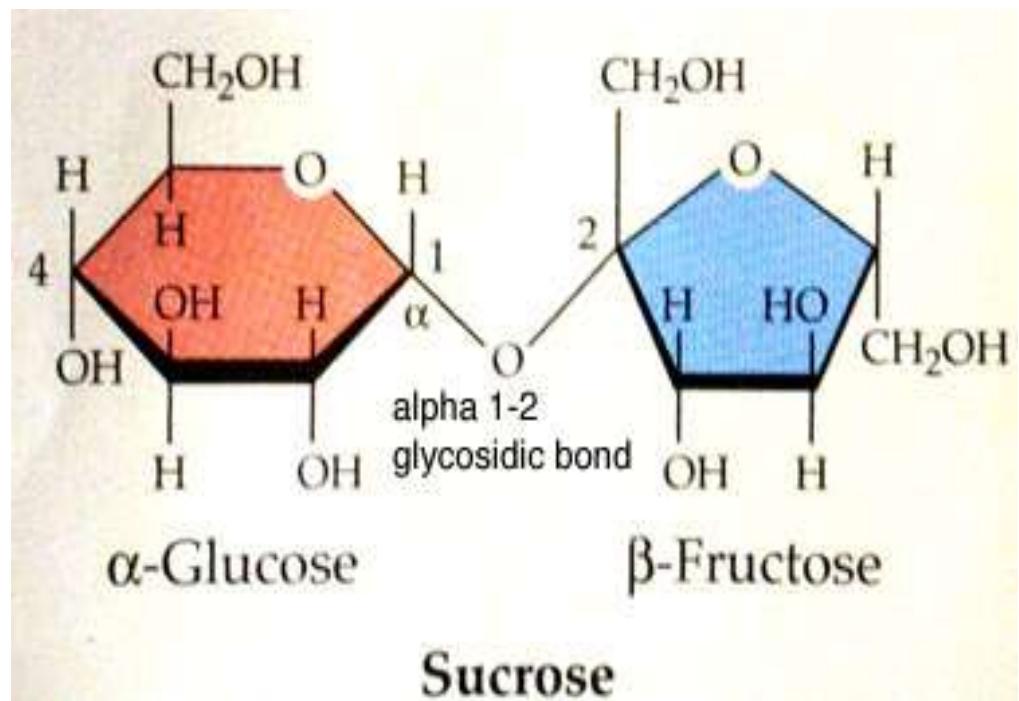
ATP

Disaccharides

- A disaccharide is a double sugar.

They're made by joining two monosaccharides

Involves removing a water molecule (condensation)



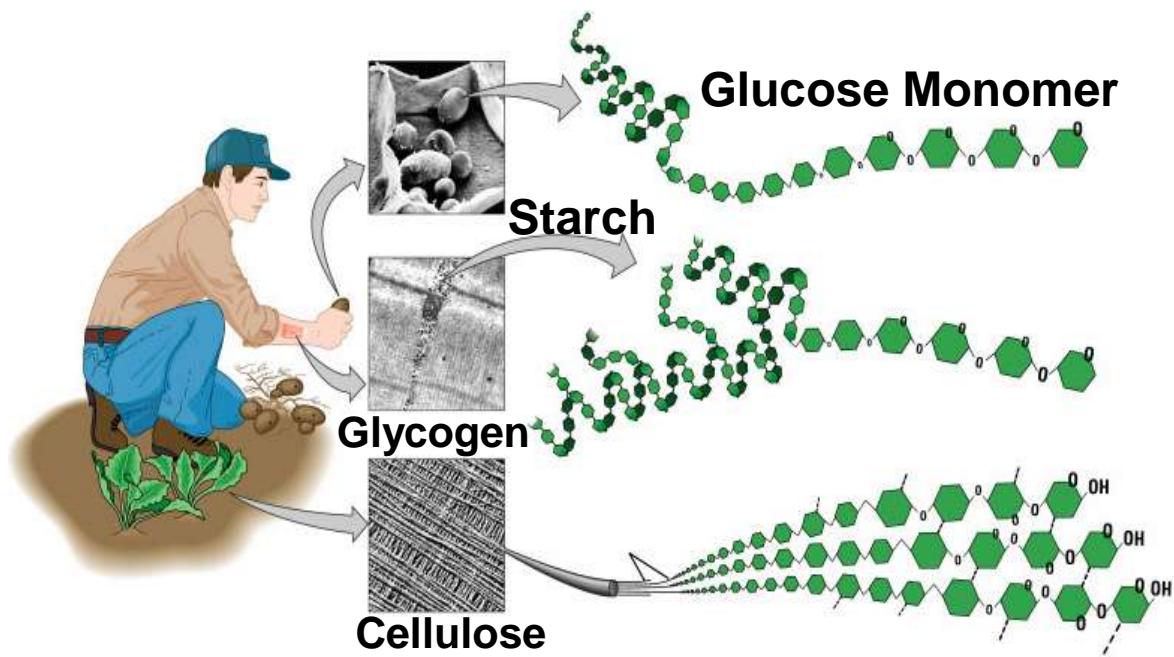
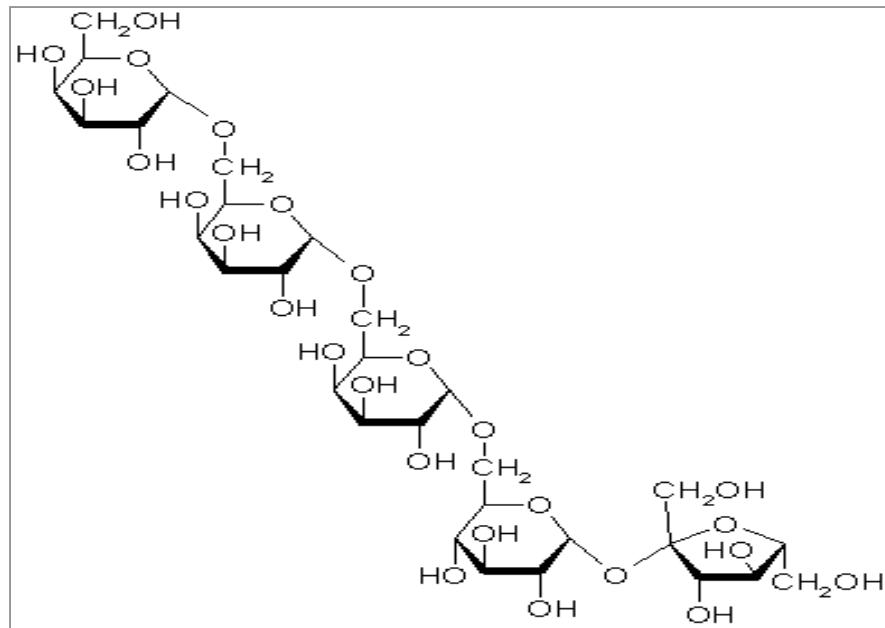
Bond called a GLYCOSIDIC bond

Polysaccharides

- Complex carbohydrates

Composed of many sugar monomers linked together

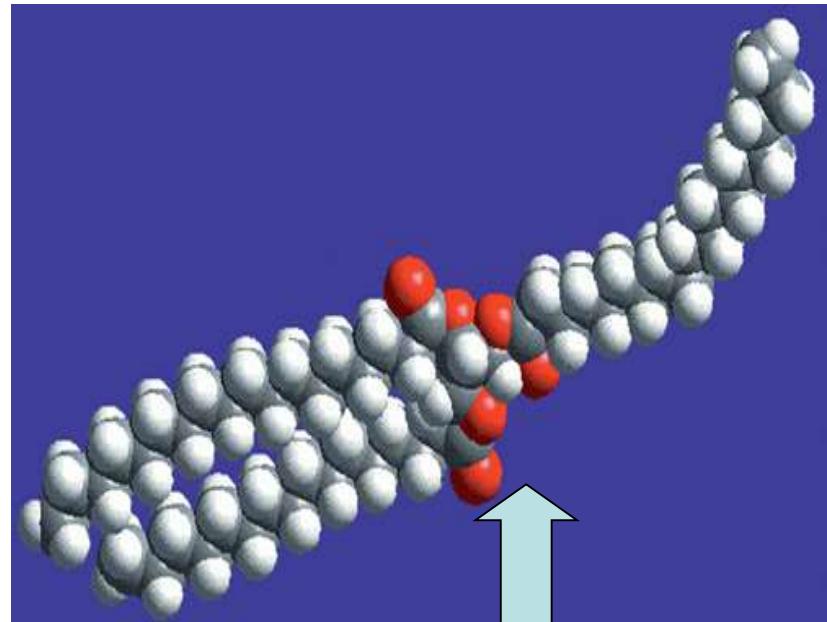
Polymers of monosaccharide chains



Lipids

- Lipids are hydrophobic – "water fearing"
- Do NOT mix with water
- Includes fats, waxes, steroids, & oils

- Fats store energy, help to insulate the body, and cushion and protect organs



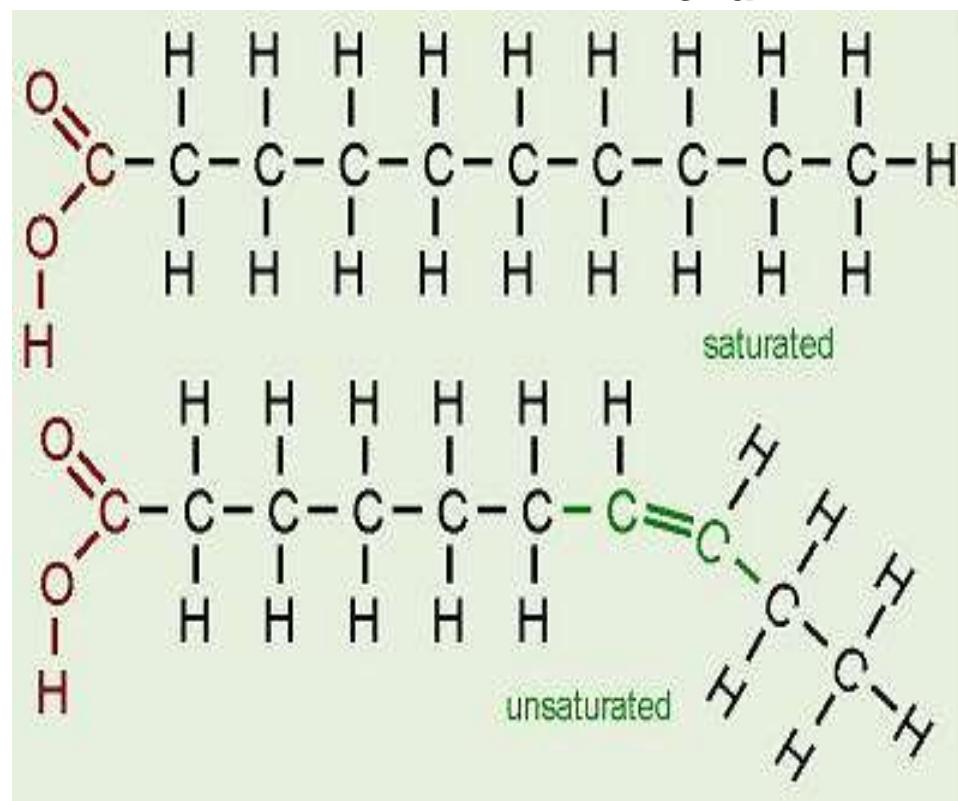
FAT MOLECULE

Types of Fatty Acids

Saturated fatty acids have the maximum number of hydrogens bonded to the carbons (all single bonds between carbons)

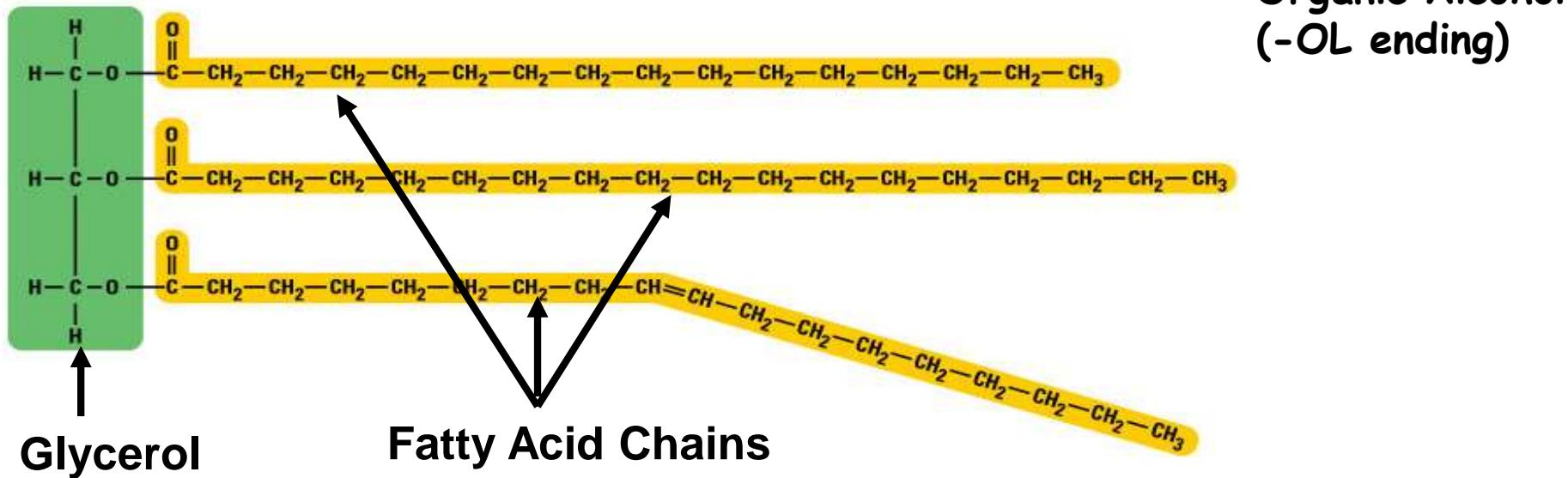
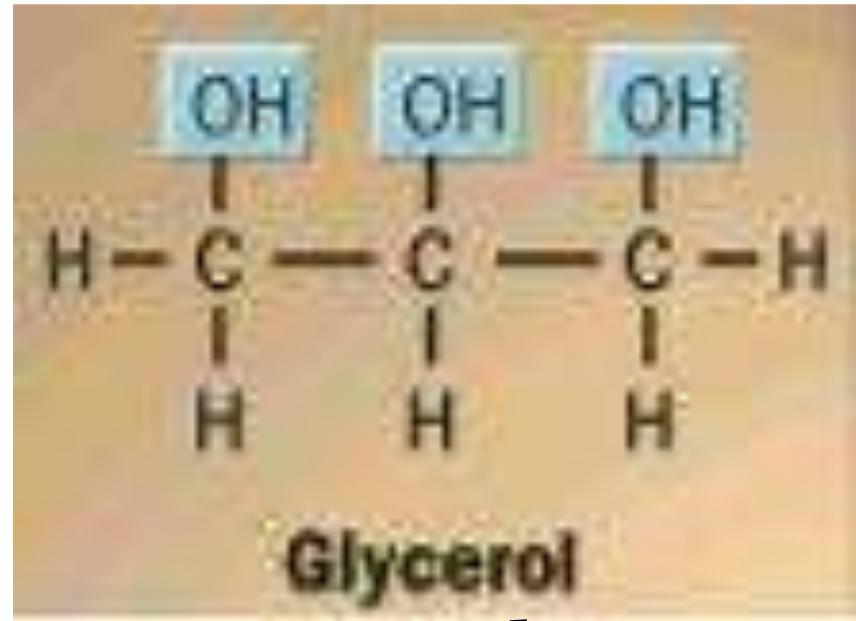
Unsaturated fatty acids have less than the maximum number of hydrogens bonded to the carbons (a double bond between carbons)

Single Bonds in Carbon chain



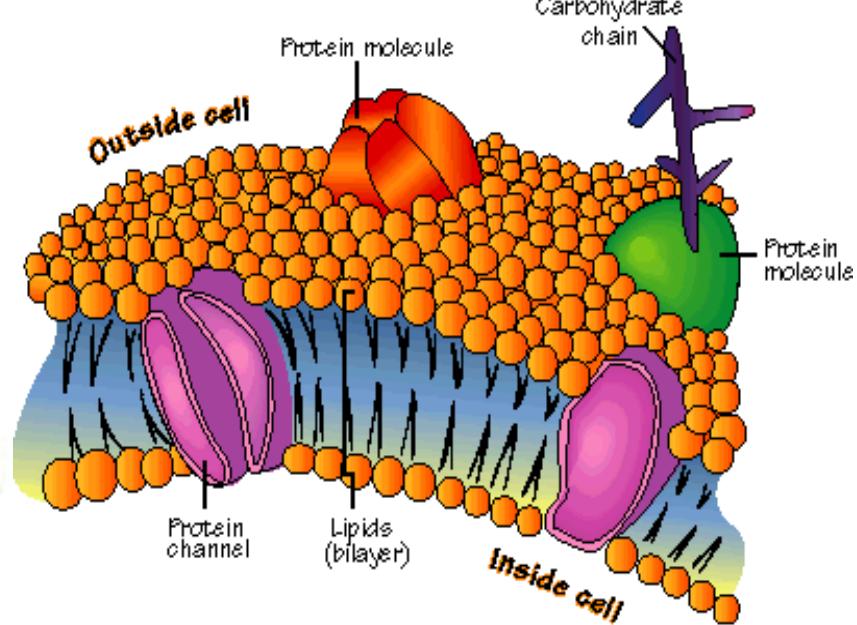
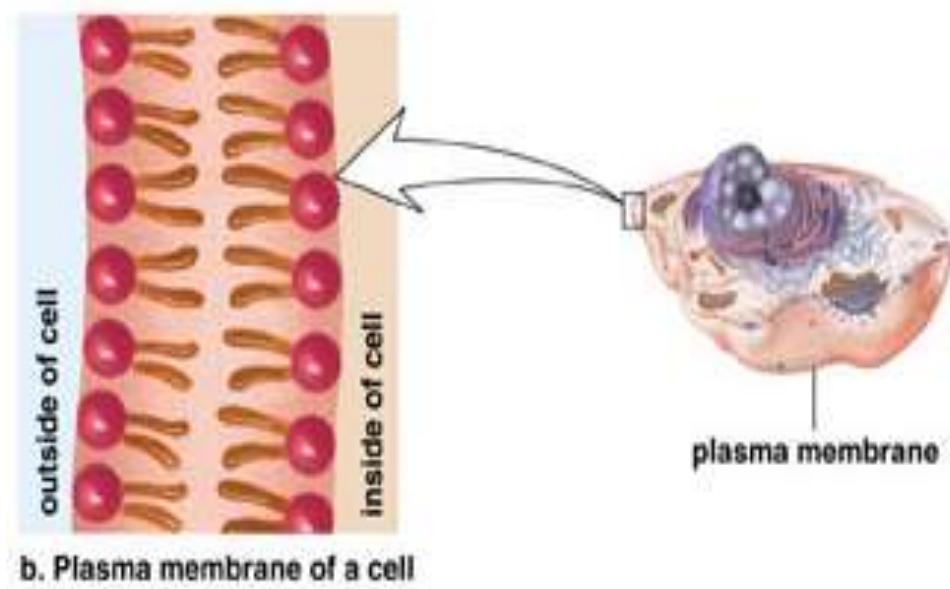
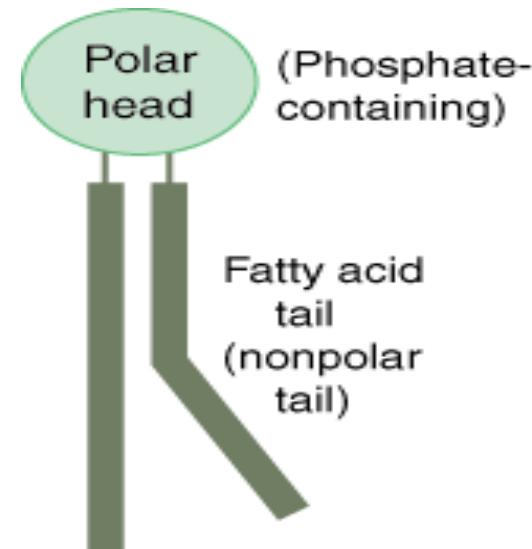
Triglyceride

- Monomer of lipids
- Composed of Glycerol & 3 fatty acid chains
- Glycerol forms the “backbone” of the fat



Lipids & Cell Membranes

- Cell membranes are made of lipids called **phospholipids**
- Phospholipids have a **head** that is polar & attract water (**hydrophilic**)
- Phospholipids also have **2 tails** that are nonpolar and do not attract water (**hydrophobic**)



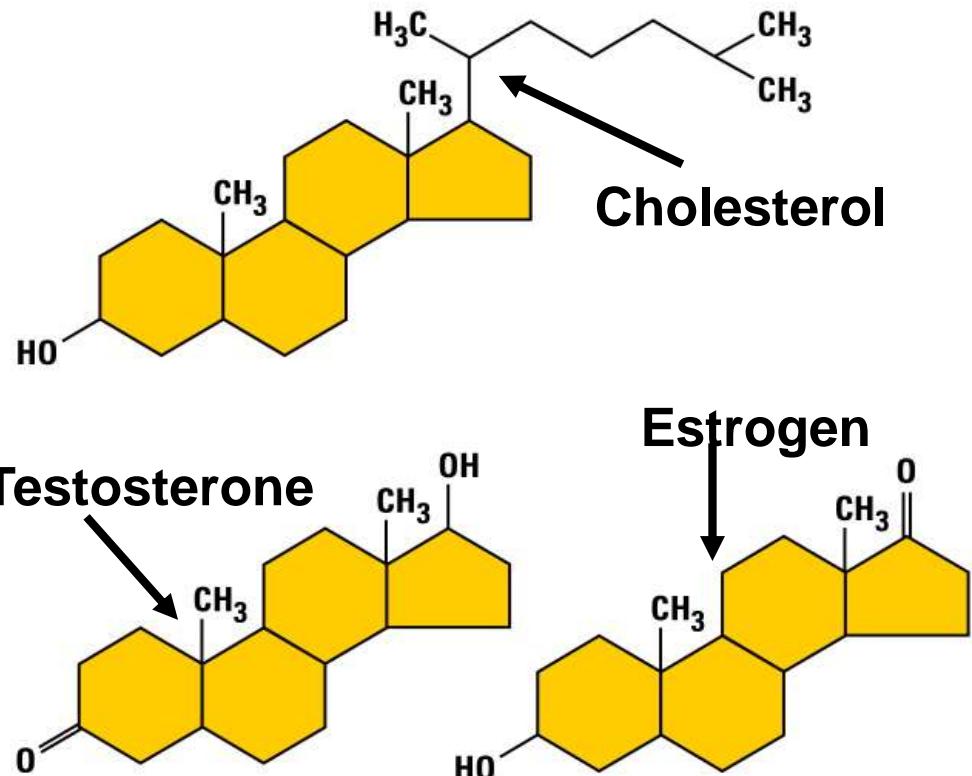
Cell membrane with proteins & phospholipids

Steroids

- The carbon skeleton of steroids is bent to form 4 fused rings

- Cholesterol is the “base steroid” from which your body produces other steroids

- Estrogen & testosterone are also steroids



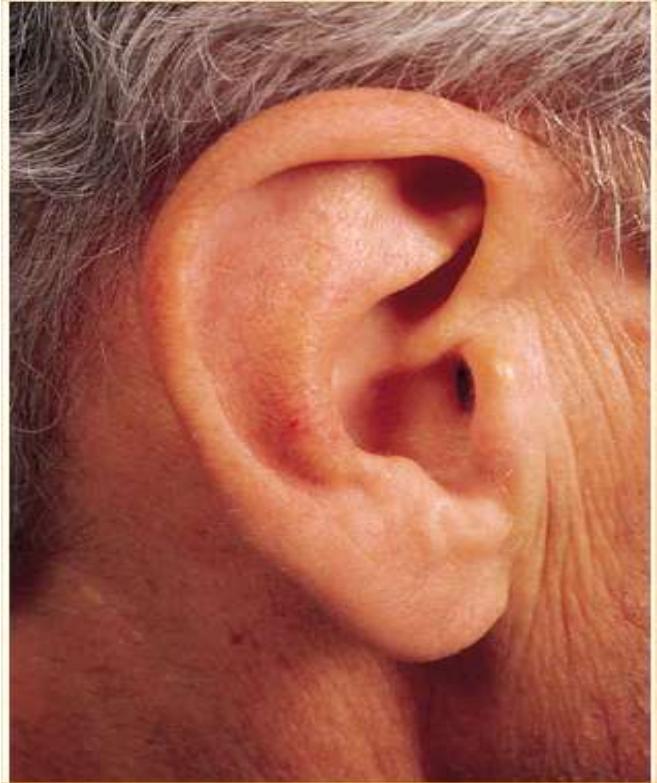
Synthetic Anabolic Steroids

- They are variants of testosterone
- Some athletes use them to build up their muscles quickly
- They can pose serious health risks



Waxes

- A wax is a lipid because of its nonpolar solubility characteristics as well as its extremely hydrophobic (water-hating) properties.
- Waxes are composed of a single, highly complex alcohol joined to a longchain fatty acid in a typical ester linkage.
- Waxes are important structural lipids often found as protective coatings on the surfaces of leaves, stems, hair, skin, etc.
- They provide effective barriers against water loss and in some situations make up the rigid architecture of complex structures such as the honeycomb of the beehive.
- They serve a commercial use as well, in furniture polish, automobile coating compounds, and floor finishes.



Proteins

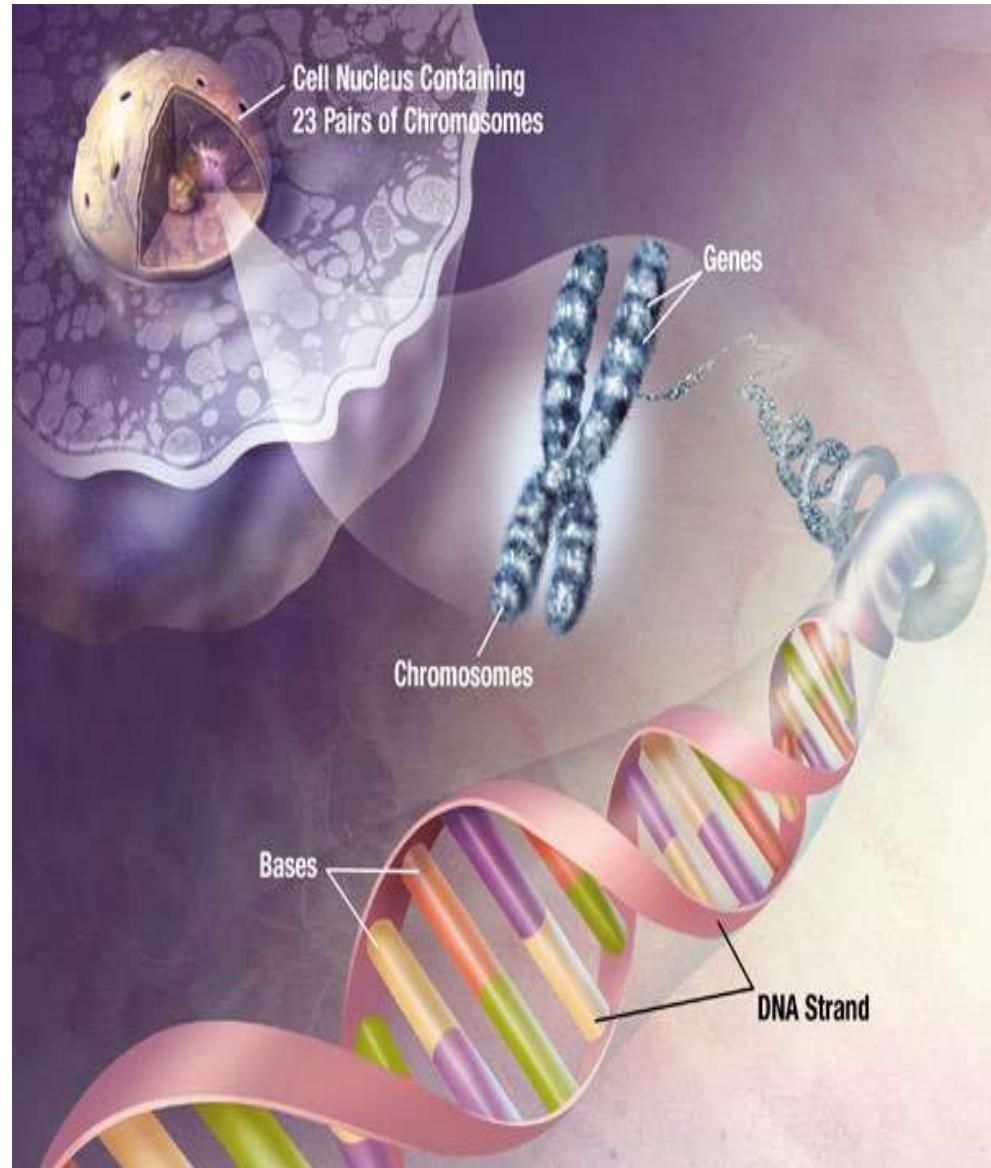
- Proteins are polymers made of monomers called amino acids

All proteins are made of 20 different amino acids linked in different orders

Proteins are used to build cells, act as hormones & enzymes, and do much of the work in a cell

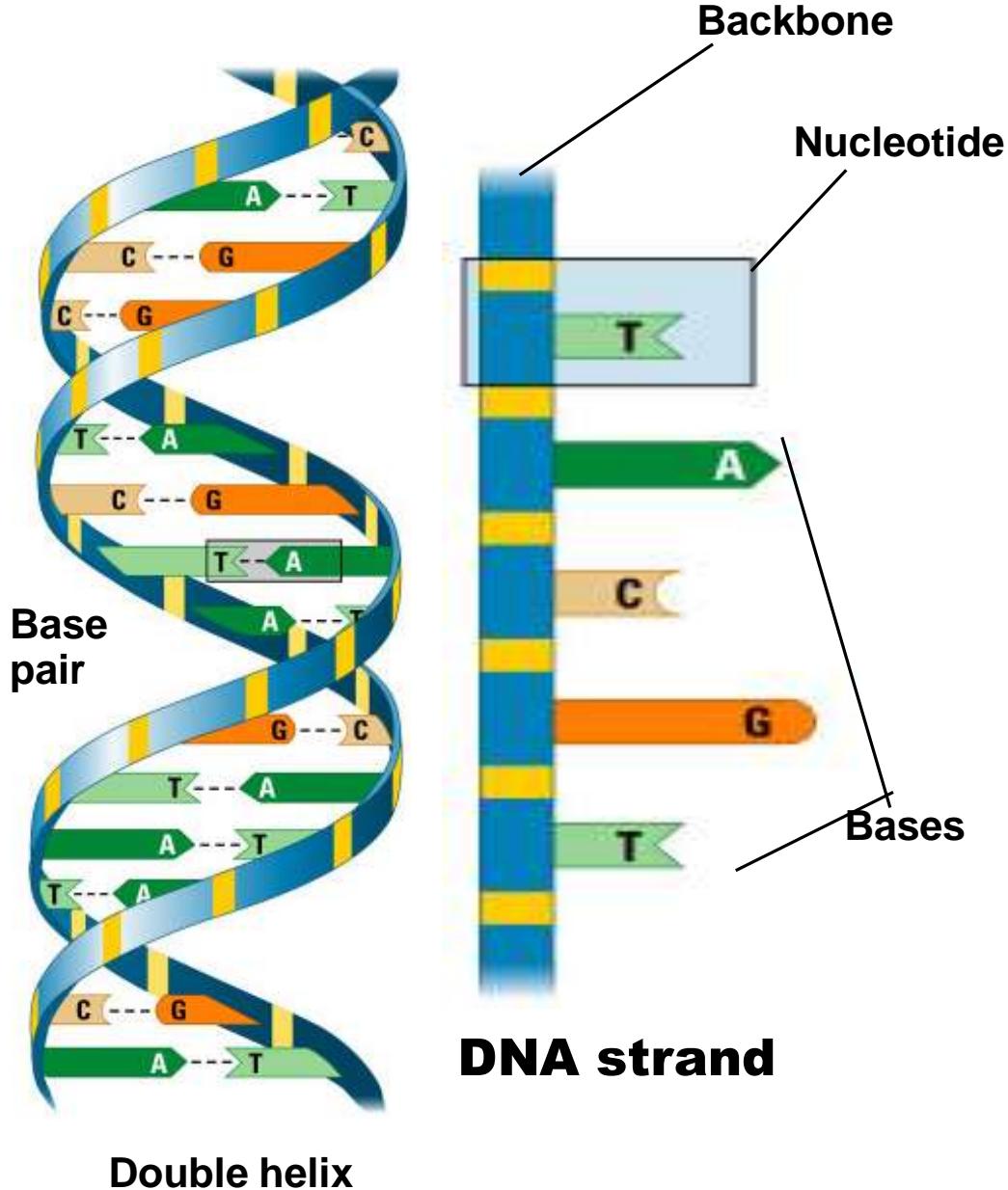
Nucleic Acids

- Store hereditary information
- Contain information for making all the body's proteins
- Two types exist --- DNA & RNA



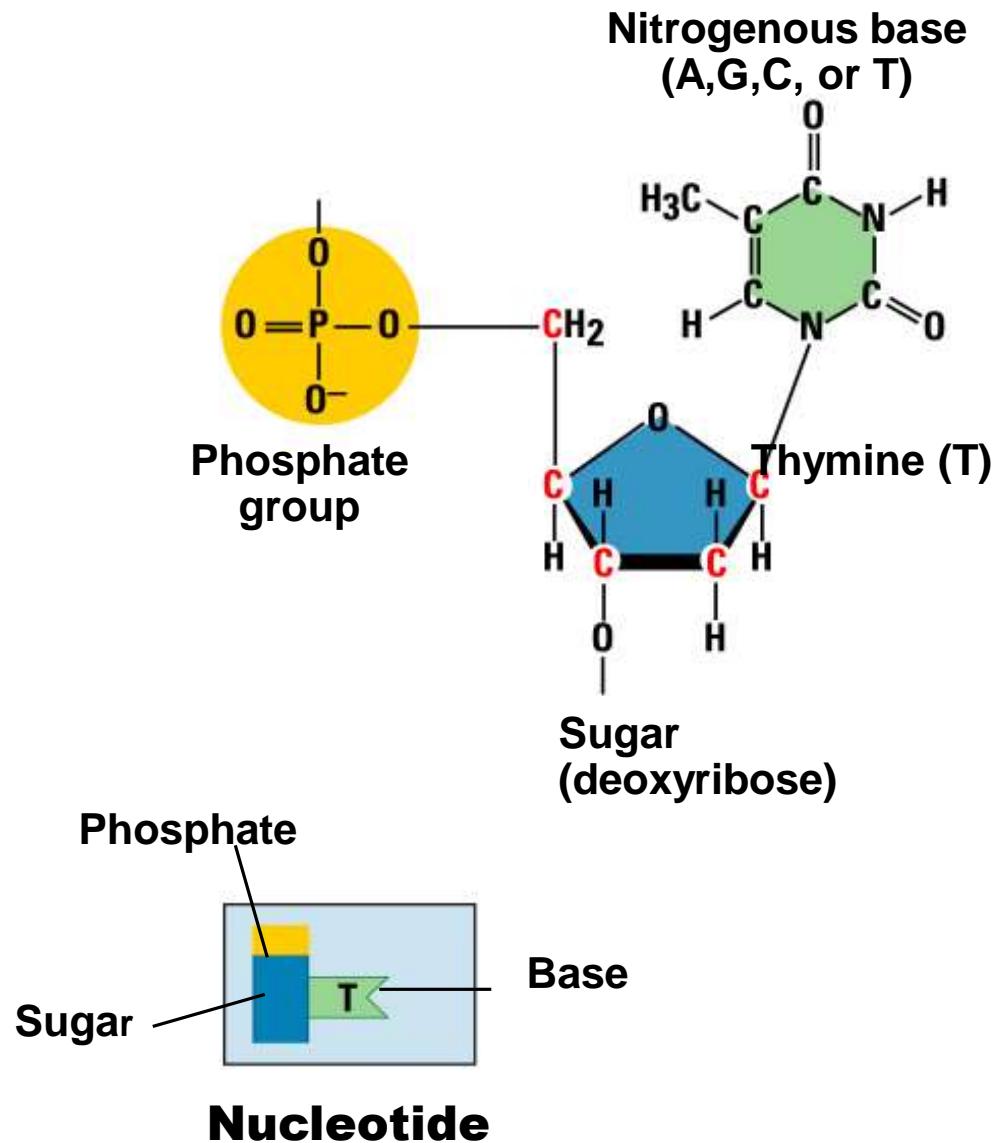
DNA-Deoxyribonucleic acid

- Two strands of DNA join together to form a double helix
- Nucleotides form long chains called DNA
- Nucleotides are joined by sugars & phosphates on the side



Nucleic Acids

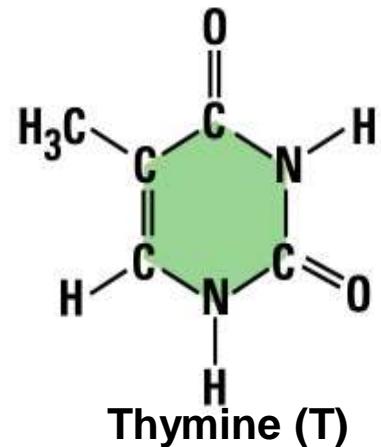
Nucleic acids
are polymers
of nucleotides



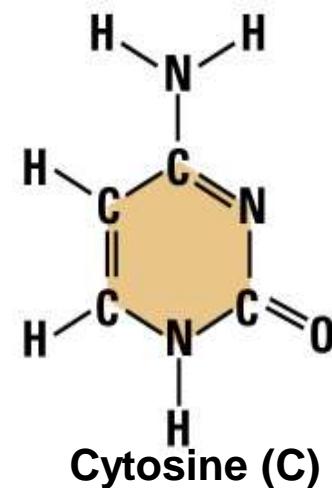
Bases

- Each DNA nucleotide has one of the following bases:

- Adenine (A)

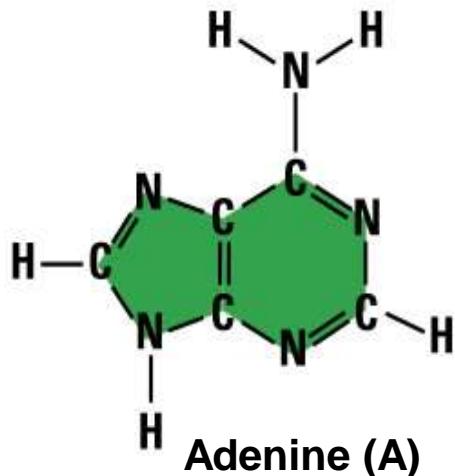


Thymine (T)



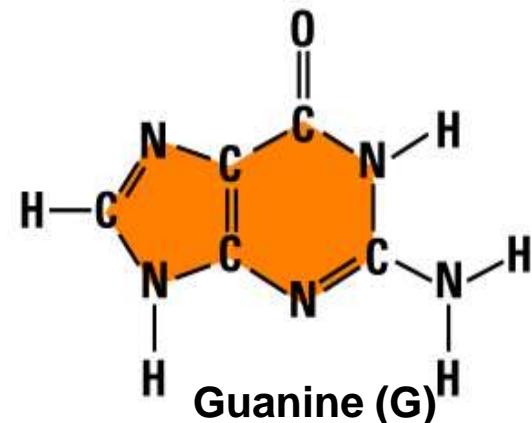
Cytosine (C)

- Guanine (G)



Adenine (A)

- Thymine (T)



Guanine (G)

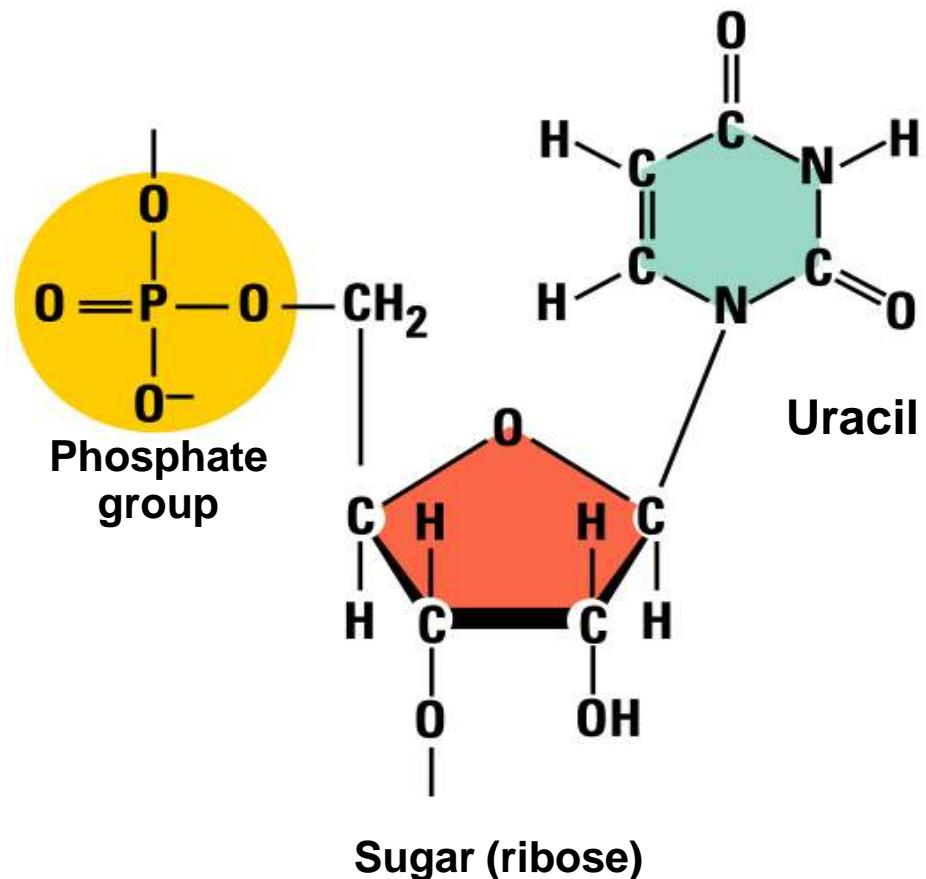
- Cytosine (C)

RNA – Ribonucleic Acid

- Ribose sugar has an extra –OH or hydroxyl group

- It has the base uracil (U) instead of thymine (T)

Nitrogenous base
(A,G,C, or U)



Macromolecules

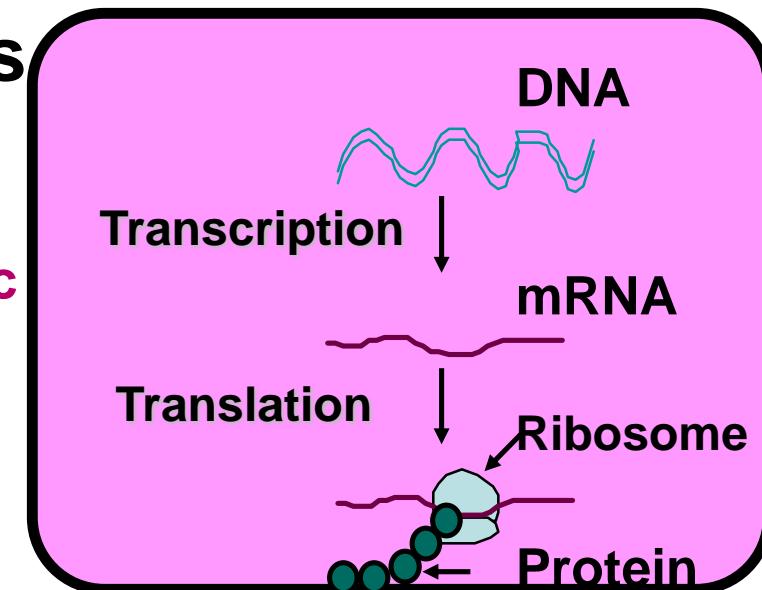
| Biological macromolecule | Function | Monomer | Examples |
|--------------------------|--|--|--|
| Carbohydrates | Dietary energy; storage; plant structure | <p>Monosaccharide</p> | Monosaccharides: glucose, fructose. dissaccharides: lactose, sucrose. Polysaccharides: starch, cellulose. |
| Lipids | Long-term energy storage (for fats); hormones (for steroids) | <p>Components of a fat molecule</p> | Fats, oils, steroids |
| Proteins | Enzymes, structure, storage, contraction, transport, etc. | <p>Amino acid</p> | Lactase (an enzyme), hemoglobin |
| Nucleic acids | Information storage | <p>Nucleotide</p> | DNA, RNA |

Protein Synthesis

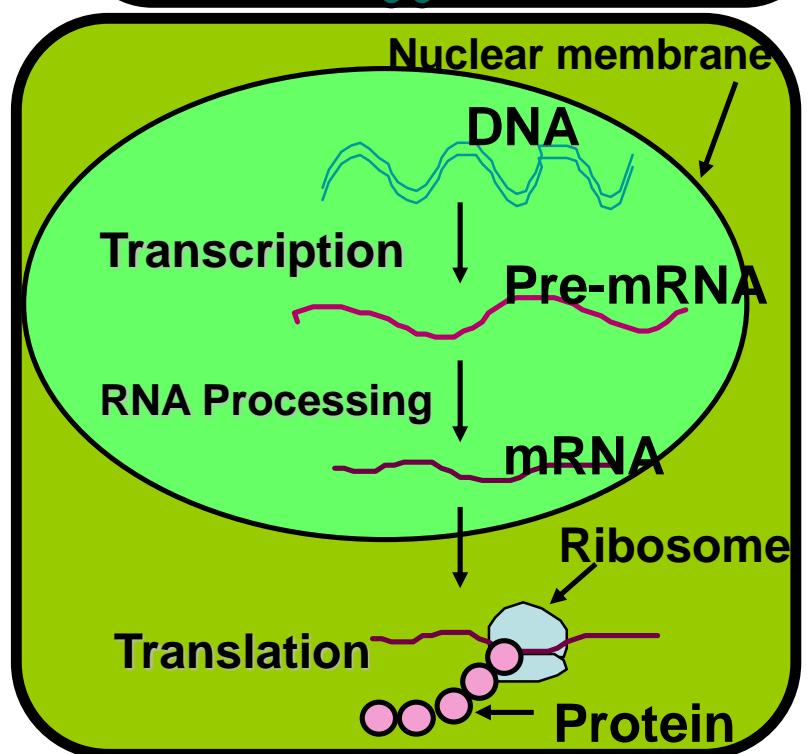
Protein Synthesis

- The production (synthesis) of polypeptide chains (proteins)
- Two phases: **Transcription & Translation**
- mRNA must be processed before it leaves the nucleus of eukaryotic cells

Prokaryotic Cell

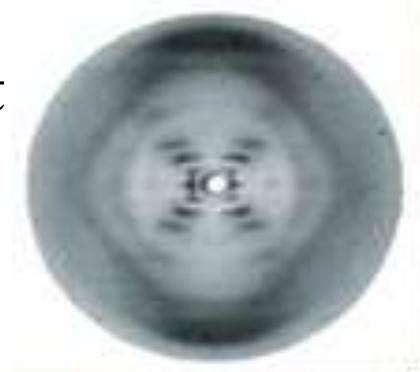
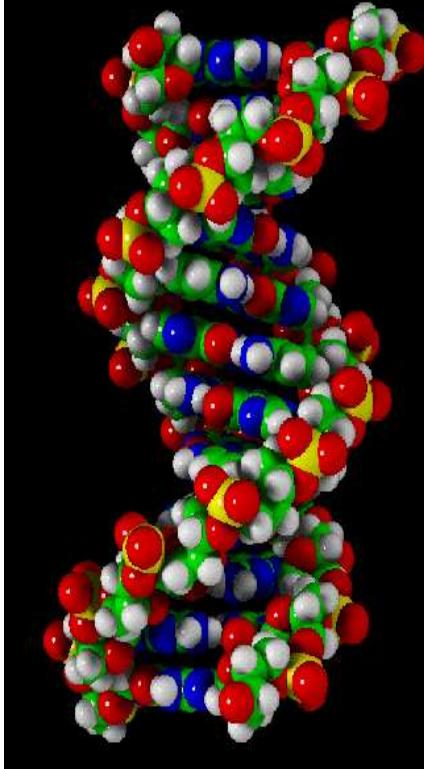


Eukaryotic Cell



Discovery of DNA structure

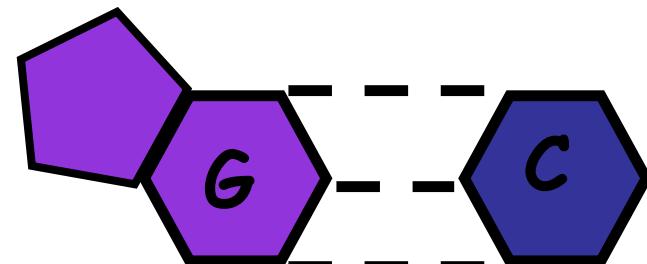
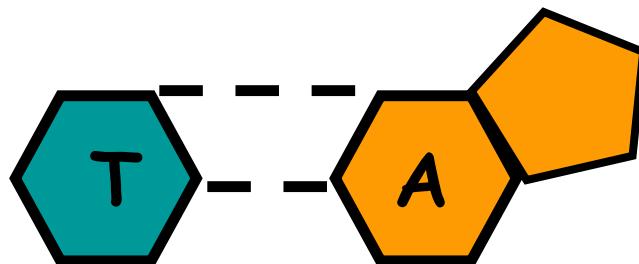
- Walter Sutton discovered **chromosomes** were made of **DNA** and Protein
- However, scientists were **NOT** sure which one (protein or DNA) was **the actual genetic material of the cell**
- Frederick Griffith in 1928 showed the **DNA** was the cell's genetic material
- Rosalind Franklin took diffraction x-ray photographs of DNA crystals
- Watson & Crick in the 1950's built model of DNA



Rosalind Franklin

Discovery of DNA Structure

- Erwin Chargaff showed the amounts of the four bases on DNA (A,T,C,G)
- In a body or somatic cell:
 - A = 30.3%
 - T = 30.3%
 - G = 19.5%
 - C = 19.9%
- Chargaff's rule:
 - Adenine must pair with Thymine
 - Guanine must pair with Cytosine
 - The bases form weak hydrogen bonds



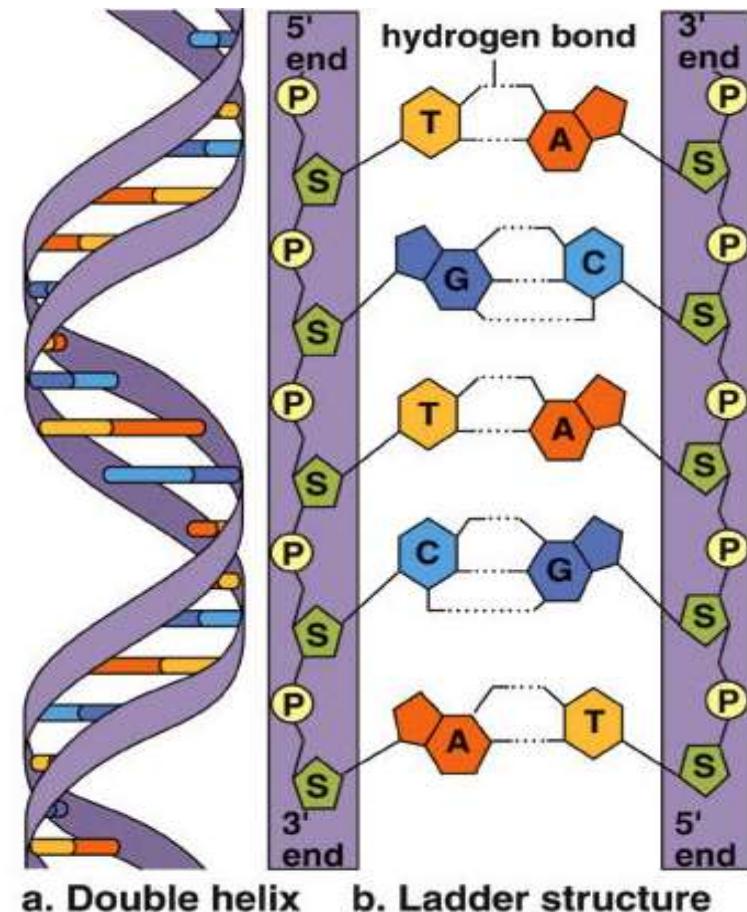
Structure of DNA

- DNA is made of subunits called **nucleotides**
- DNA nucleotides are composed of a **phosphate**, **deoxyribose sugar**, and **a nitrogen-containing base**
- The 4 bases in DNA are: **adenine (A)**, **thymine (T)**, **guanine (G)**, and **cytosine (C)**
- Purines have **single** rings of carbon-nitrogen (G, A)
- Pyrimidines have **double** carbon-nitrogen rings (C, T)
- This is called ***complementary base pairing*** because a **purine** is always paired with a **pyrimidine**

5' to 3' Sugars

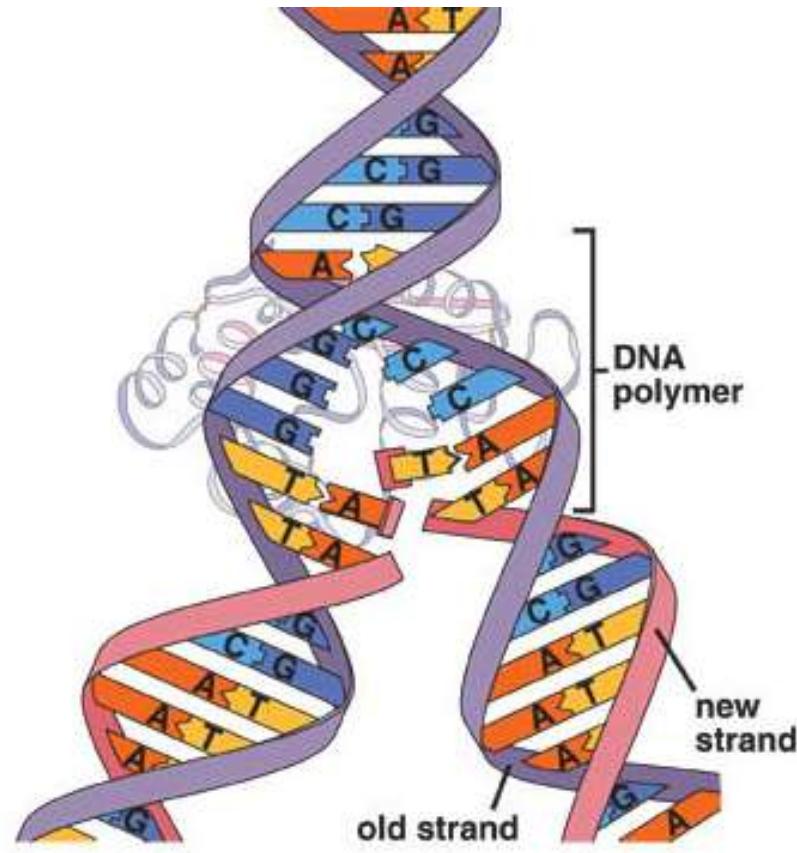
- When the DNA double helix unwinds, it resembles a ladder
- The sides of the ladder are the sugar-phosphate backbones
- The rungs of the ladder are the complementary paired bases
- The two DNA strands are anti-parallel (they run in opposite directions)

Anti-Parallel Strands of DNA



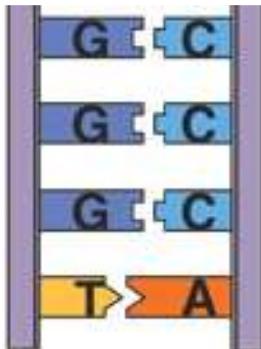
Steps in DNA Replication

- Occurs when **chromosomes** **duplicate** (make copies)
- An **exact copy** of the DNA is produced with the aid of the enzyme **DNA polymerase**
- **Hydrogen bonds** between bases **break** and enzymes “unzip” the molecule
- Each **old strand** of nucleotides serves as a **template** for each new strand
- New nucleotides move into complementary positions are joined by DNA polymerase

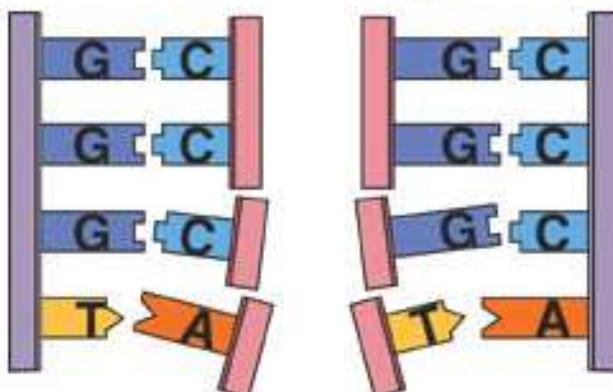


Two New, Identical
DNA Strands Result
from Replication

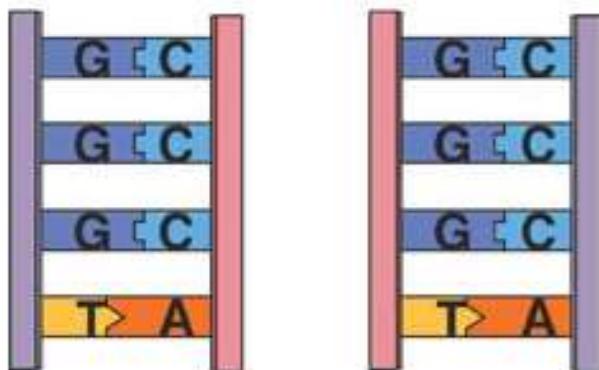
Another View of Replication



Parental DNA molecule contains so-called old strands hydrogen-bonded by complementary base pairing.



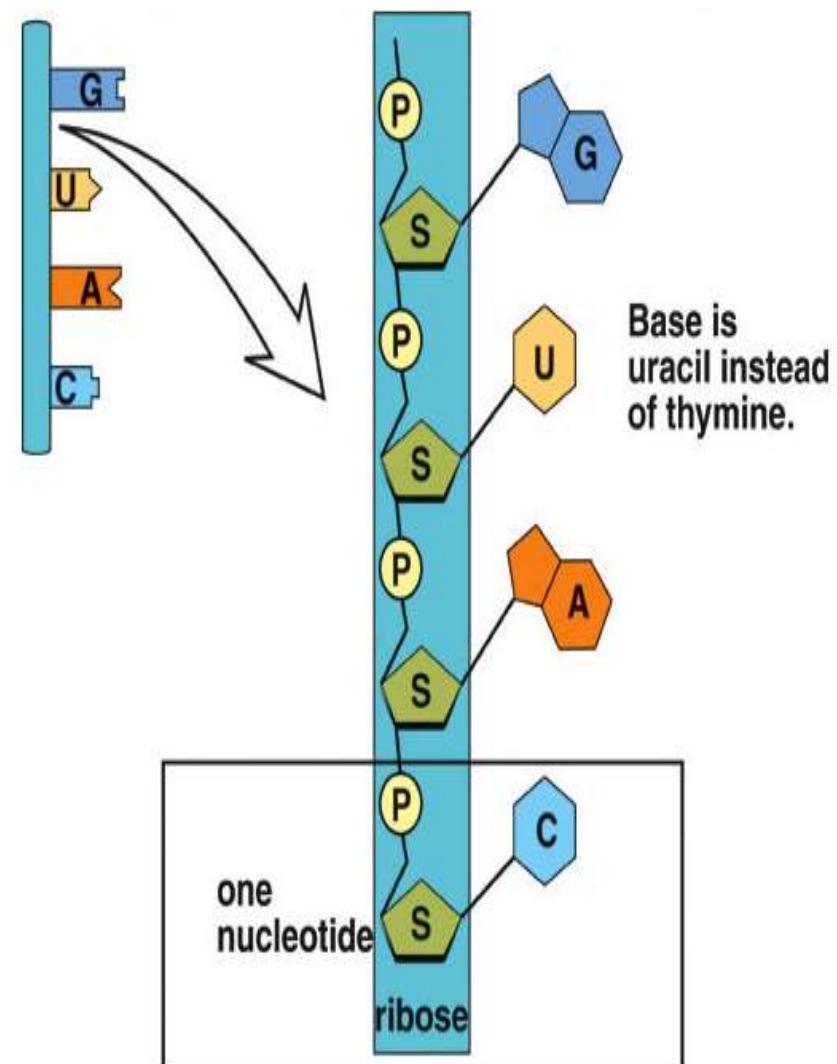
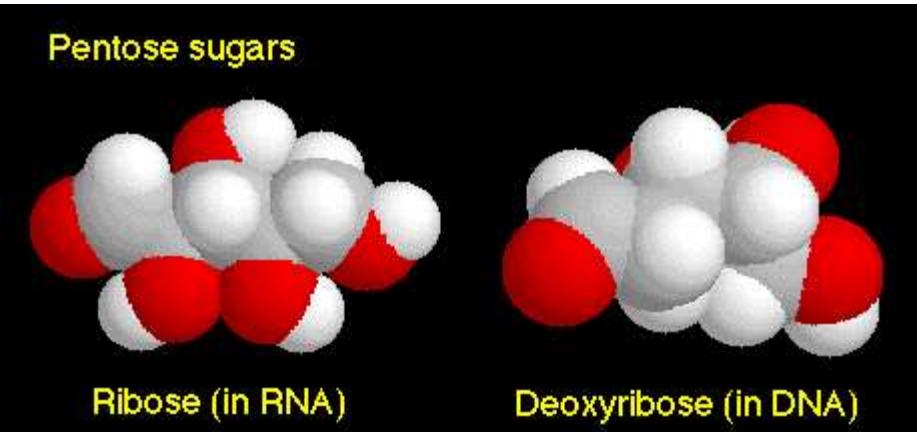
Region of replication.
Parental DNA is unwound
and unzipped. New
nucleotides are pairing
with those in old strands.



Replication is complete.
Each double helix is
composed of an old
(parental) strand and a
new (daughter) strand.

RNA Differs from DNA

1. RNA has a sugar **ribose**
DNA has a sugar **deoxyribose**
2. RNA contains the base **uracil (U)**
DNA has **thymine (T)**
3. RNA molecule is **single-stranded**
DNA is **double-stranded**



Three Types of RNA

- **Messenger RNA (mRNA)** carries genetic information to the ribosomes
(blueprint for the construction of a protein)
- **Ribosomal RNA (rRNA)**, along with protein, makes up the **ribosomes**
(construction site where the protein is made)
- **Transfer RNA (tRNA)** transfers amino acids to the ribosomes where proteins are synthesized
(truck delivering the proper amino acid to the site at the right time)

Genes & Proteins

- Proteins are made of amino acids linked together by peptide bonds
- 20 different amino acids exist
- Amino acids chains are called polypeptides
- Segment of DNA that codes for the amino acid sequence in a protein are called genes

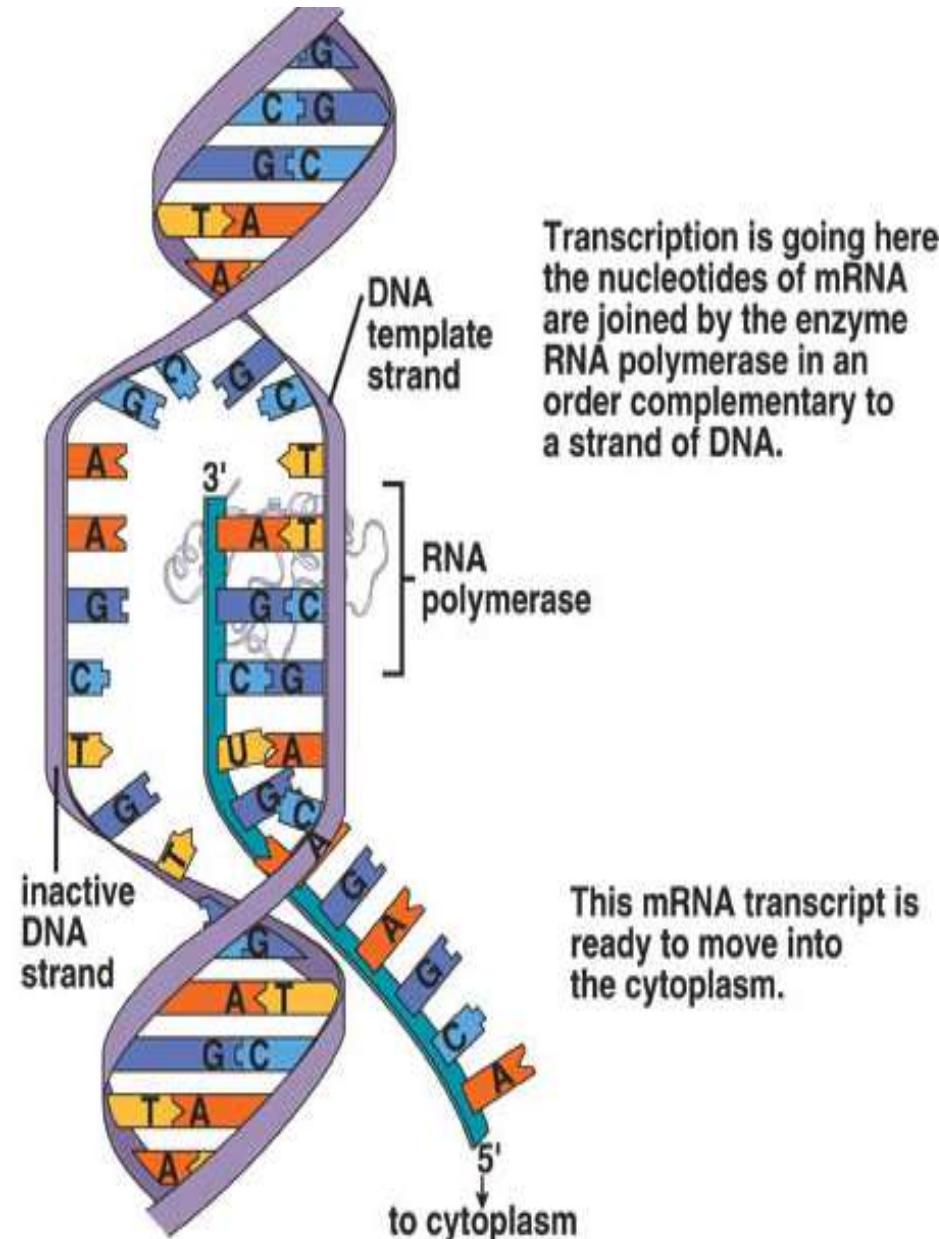
Genetic Code:

- DNA contains a triplet code
- Every three bases on DNA stands for ONE amino acid
- Each three-letter unit on mRNA is called a codon
- Most amino acids have more than one codon!
- There are 20 amino acids with a possible 64 different triplets
- The code is nearly universal among living organisms

| First Base | Second Base | | | | Third Base |
|------------|---------------------------|------------------|-------------------|-------------------|------------|
| | U | C | A | G | |
| U | UUU phenylalanine | UCU serine | UAU tyrosine | UGU cysteine | U |
| | UUC phenylalanine | UCC serine | UAC tyrosine | UGC cysteine | C |
| | UUA leucine | UCA serine | UAA stop | UGA stop | A |
| | UUG leucine | UCG serine | UAG stop | UGG tryptophan | G |
| C | CUU leucine | CCU proline | CAU histidine | CGU arginine | U |
| | CUC leucine | CCC proline | CAC histidine | CGC arginine | C |
| | CUA leucine | CCA proline | CAA glutamine | CGA arginine | A |
| | CUG leucine | CCG proline | CAG glutamine | CGG arginine | G |
| A | AUU isoleucine | ACU threonine | AAU asparagine | AGU serine | U |
| | AUC isoleucine | ACC threonine | AAC asparagine | AGC serine | C |
| | AUA isoleucine | ACA threonine | AAA lysine | AGA arginine | A |
| | AUG (start) methionine | ACG threonine | AAG lysine | AGG arginine | G |
| G | GUU valine | GCU alanine | GAU aspartate | GGU glycine | U |
| | GUC valine | GCC alanine | GAC aspartate | GGC glycine | C |
| | GUA valine | GCA alanine | GAA glutamate | GGA glycine | A |
| | GUG valine | GCG alanine | GAG glutamate | GGG glycine | G |

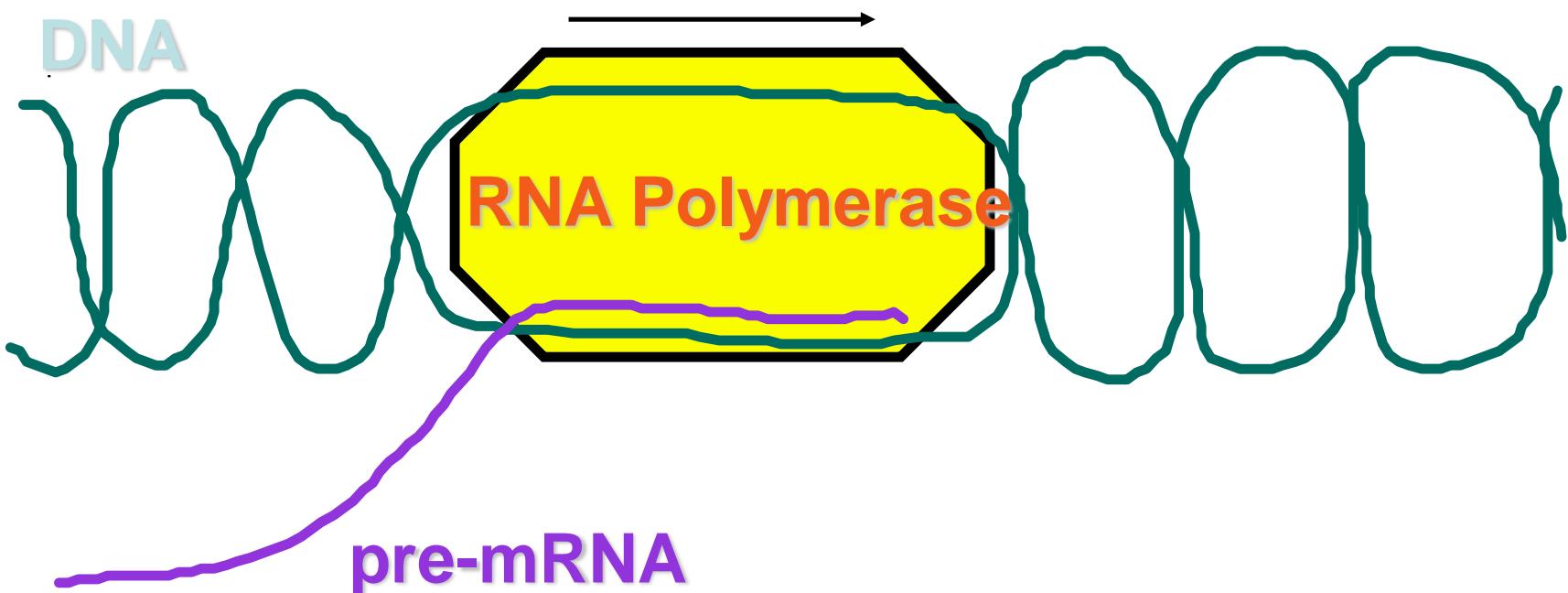
Overview of Transcription

- During **transcription** in the nucleus, a segment of DNA unwinds and unzips, and the **DNA** serves as a **template for mRNA formation**
- **RNA polymerase** joins the RNA nucleotides so that the **codons in mRNA are complementary** to the triplet code in DNA
- The transfer of information in the **nucleus** from a **DNA** molecule to an **RNA** molecule
- Only 1 **DNA** strand serves as the **template**
- Starts at **promoter DNA** (TATA box)
- Ends at **terminator DNA** (stop)
- When complete, **pre-RNA** molecule is released



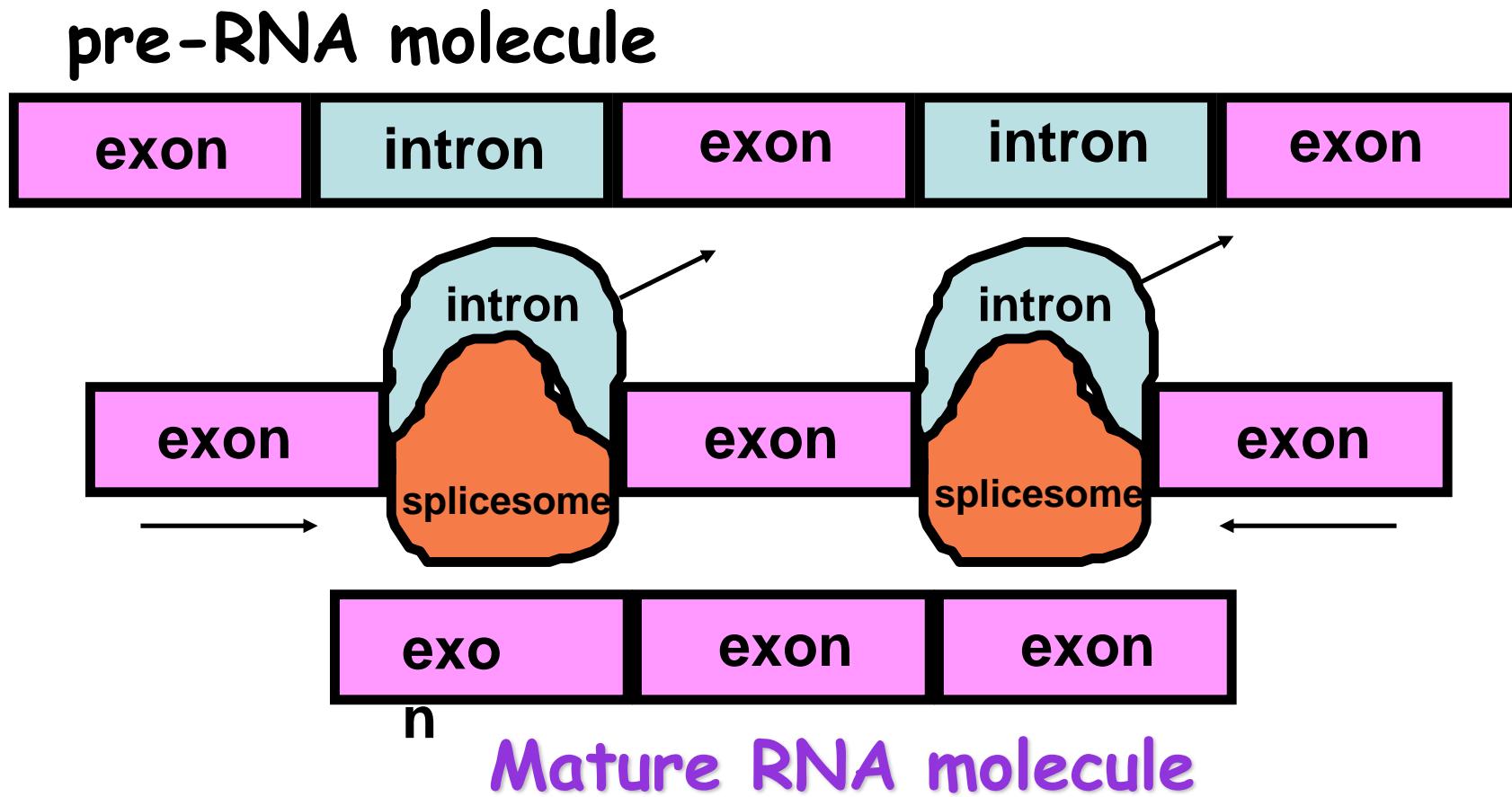
RNA Polymerase

- Enzyme found in the nucleus
- Separates the two DNA strands by breaking the hydrogen bonds between the bases
- Then moves along one of the DNA strands and links RNA nucleotides together



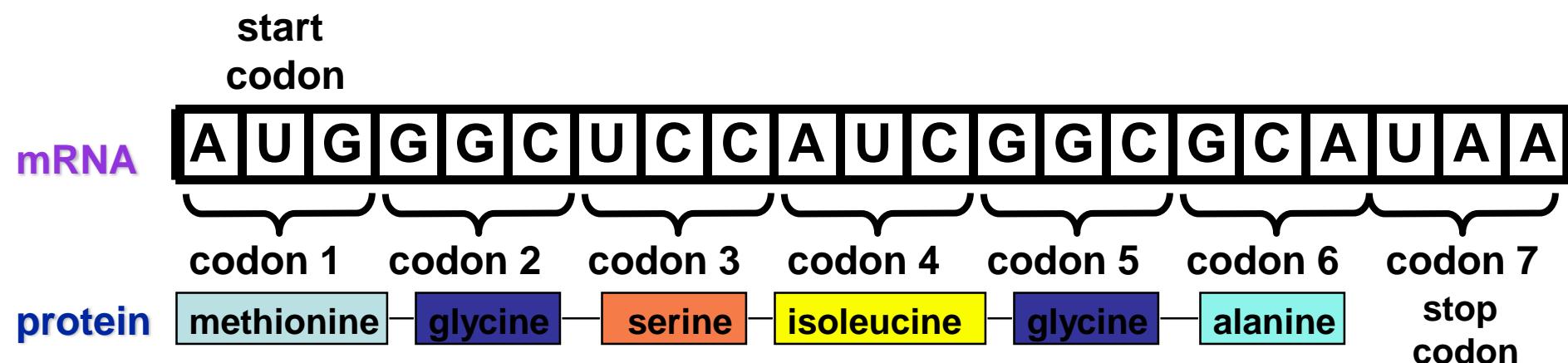
Processing Pre-mRNA

- Also occurs in the **nucleus**
- **Pre-mRNA** made up of segments called **introns & exons**
- Exons code for proteins, while introns do NOT!
- Introns spliced out by **splicesome-enzyme** and exons re-join
- End product is a **mature RNA** molecule that leaves the nucleus to the cytoplasm

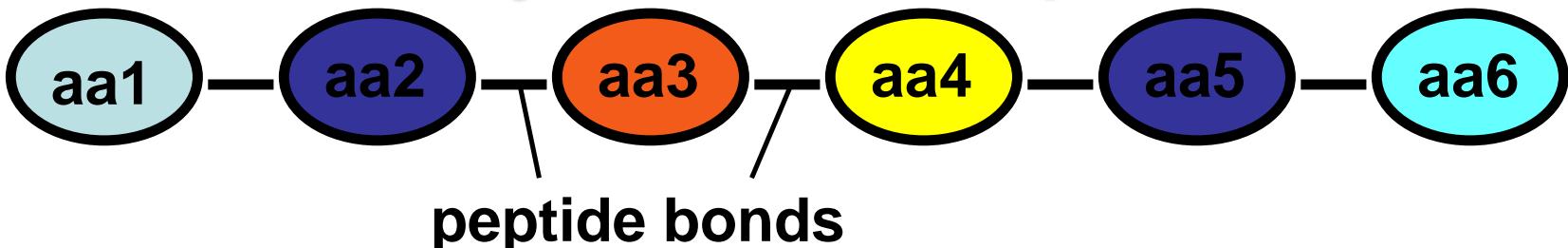


Messenger RNA (mRNA)

- Carries the information for a specific protein
- Made up of **500 to 1000** nucleotides long
- Sequence of 3 bases called **codon**
- **AUG** – methionine or **start codon**
- **UAA, UAG, or UGA** – **stop codons**

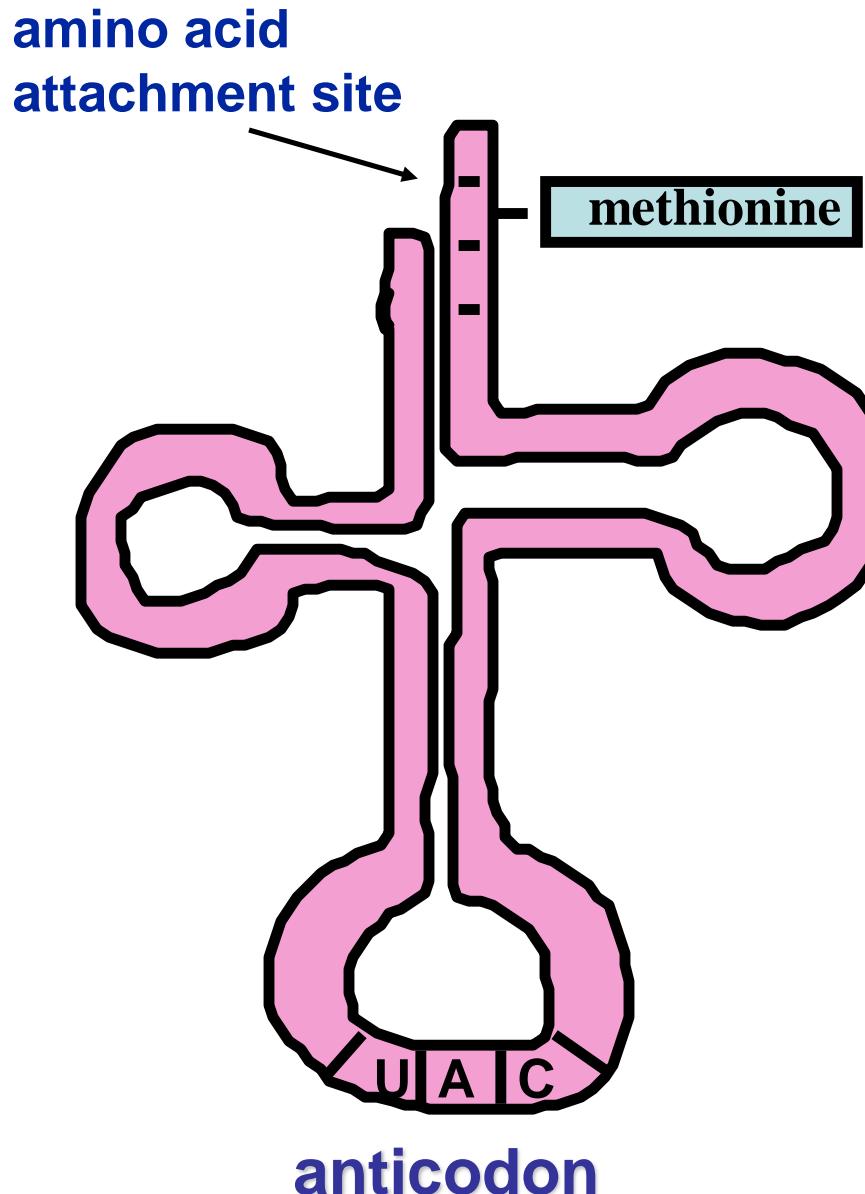


Primary structure of a protein



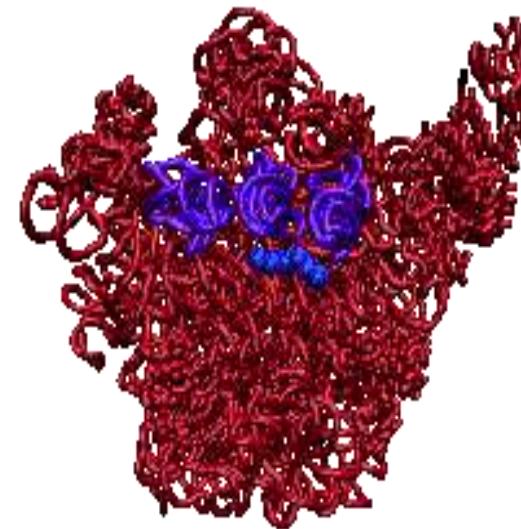
Transfer RNA (tRNA)

- Made up of **75 to 80 nucleotides** long
- Picks up the appropriate **amino acid** floating in the cytoplasm
- Transports **amino acids** to the **mRNA**
- Have **anticodons** that are complementary to **mRNA codons**
- Recognizes the appropriate **codons** on the **mRNA** and bonds to them with H-bonds
- **Four ATP's** are required for each amino acid added to the polypeptide chain: Two to "charge" the tRNA , one to carry the charged tRNA to the ribosome and one to move the ribosome to the next codon.



Ribosomal RNA (rRNA)

- Made up of rRNA is **100 to 3000 nucleotides** long
- Made inside the **nucleus** of a cell
- Associates with **proteins** to form **ribosomes**

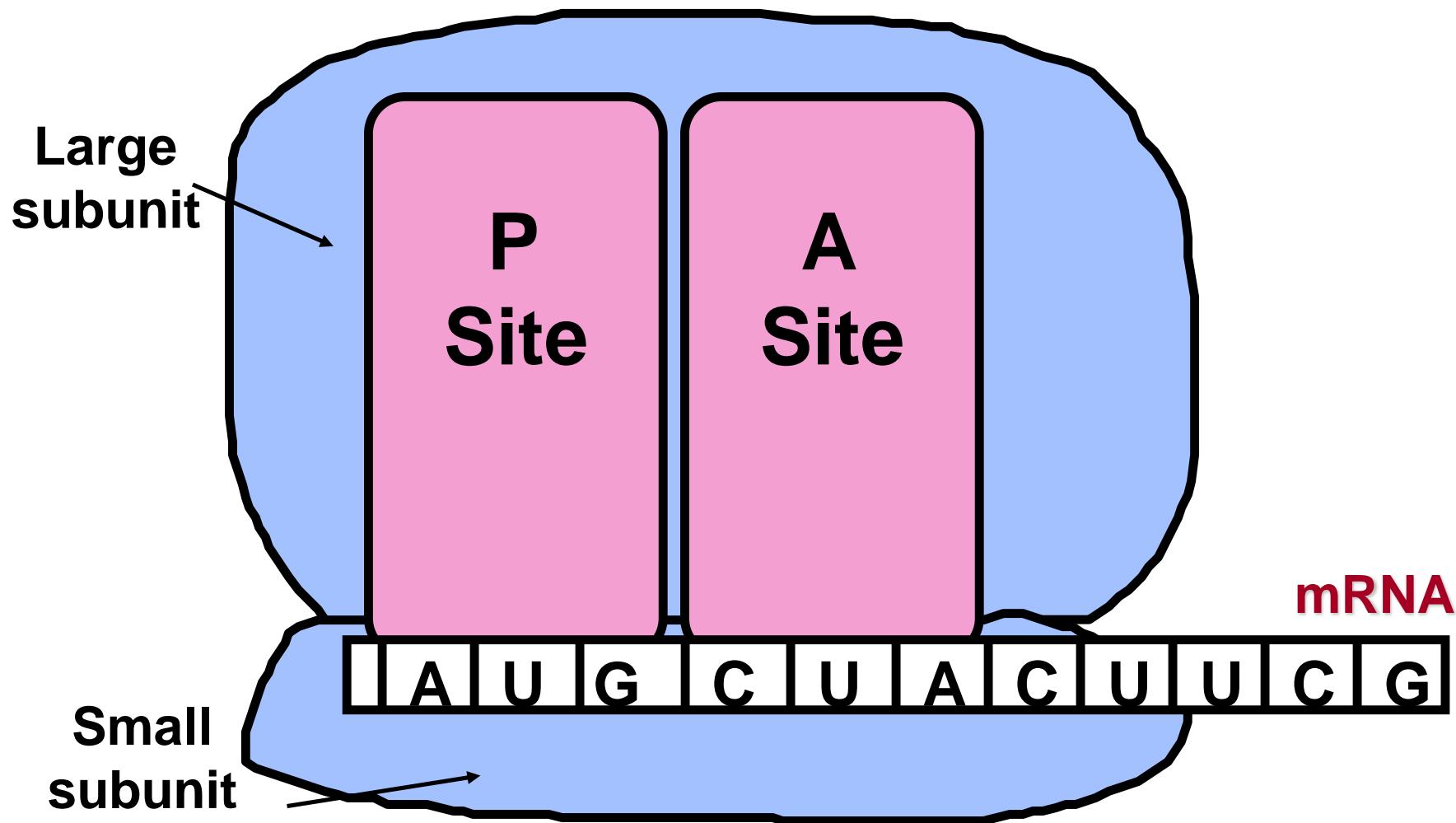


Ribosomes

- Made of a **large and small subunit**
- Composed of **rRNA (40%)** and **proteins (60%)**
- Have **two sites** for tRNA attachment --- **P and A**

Ribosomes

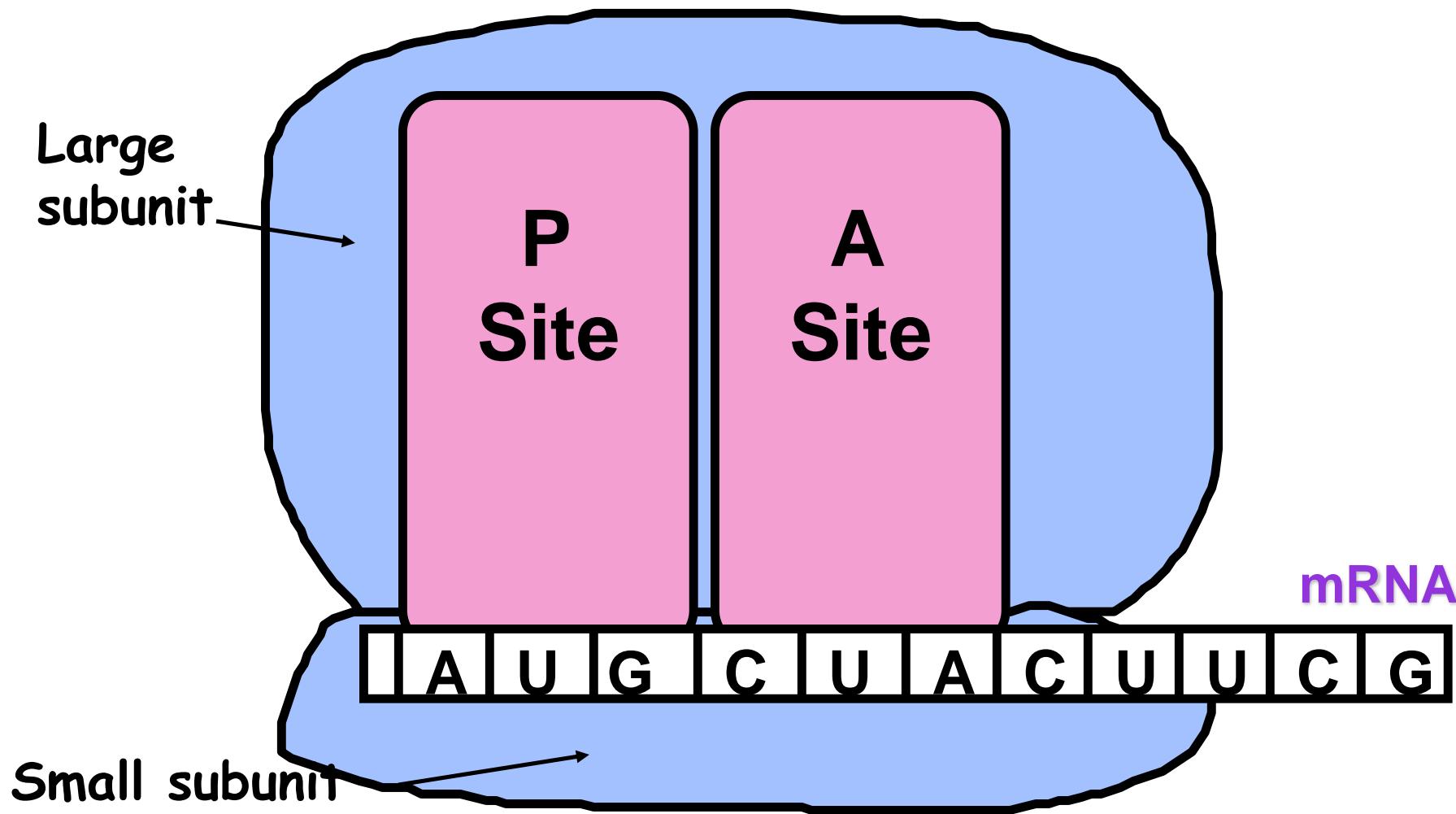
P= Peptide site
A= Amino acid site



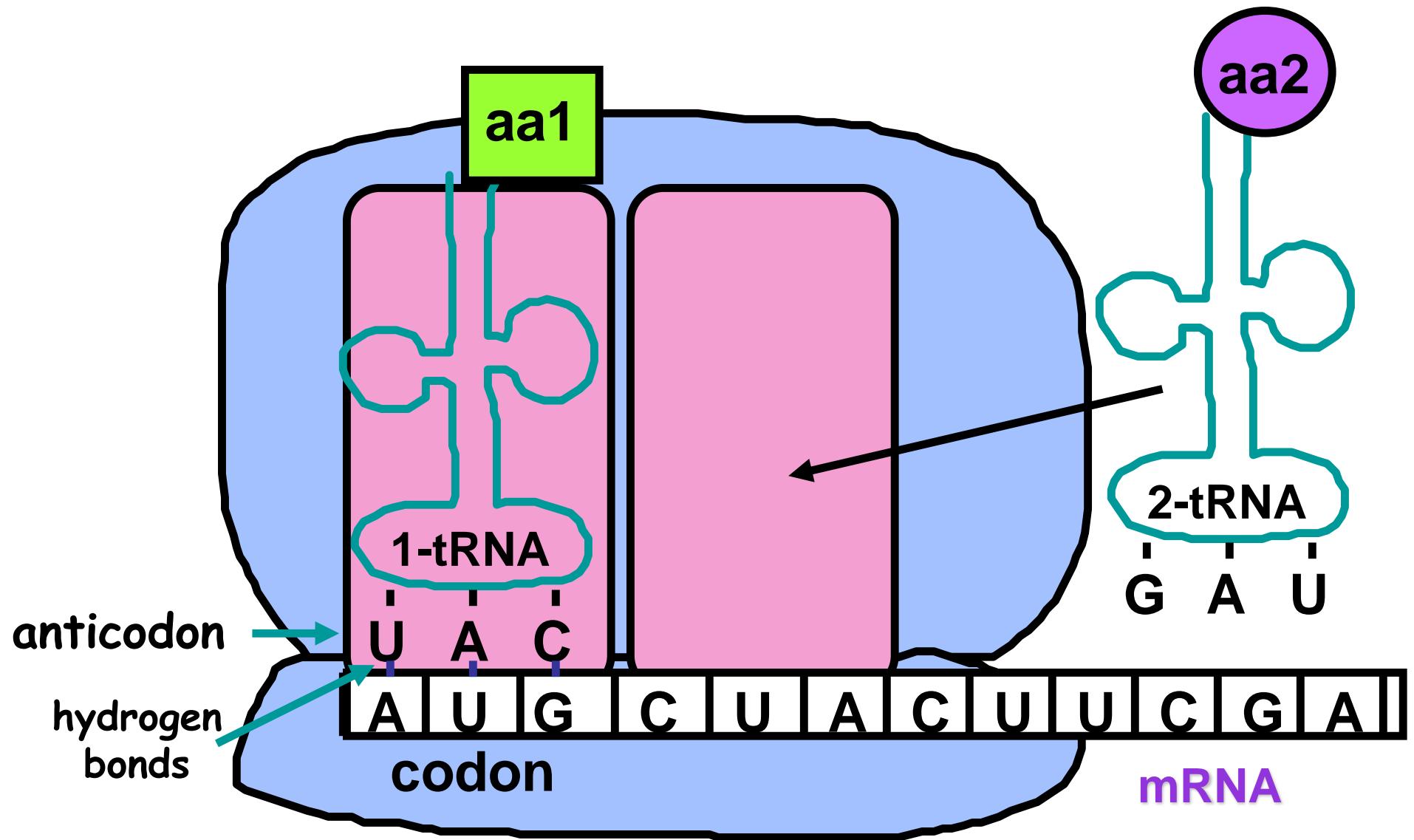
Translation

- **Synthesis of proteins** in the cytoplasm
- **Involves the following:**
 1. mRNA (codons)
 2. tRNA (anticodons)
 3. ribosomes
 4. amino acids
- Three steps:
 1. **initiation:** start codon (AUG)
 2. **elongation:** amino acids linked
 3. **termination:** stop codon (UAG, UAA, or UGA).

mRNA Codons Join the Ribosome

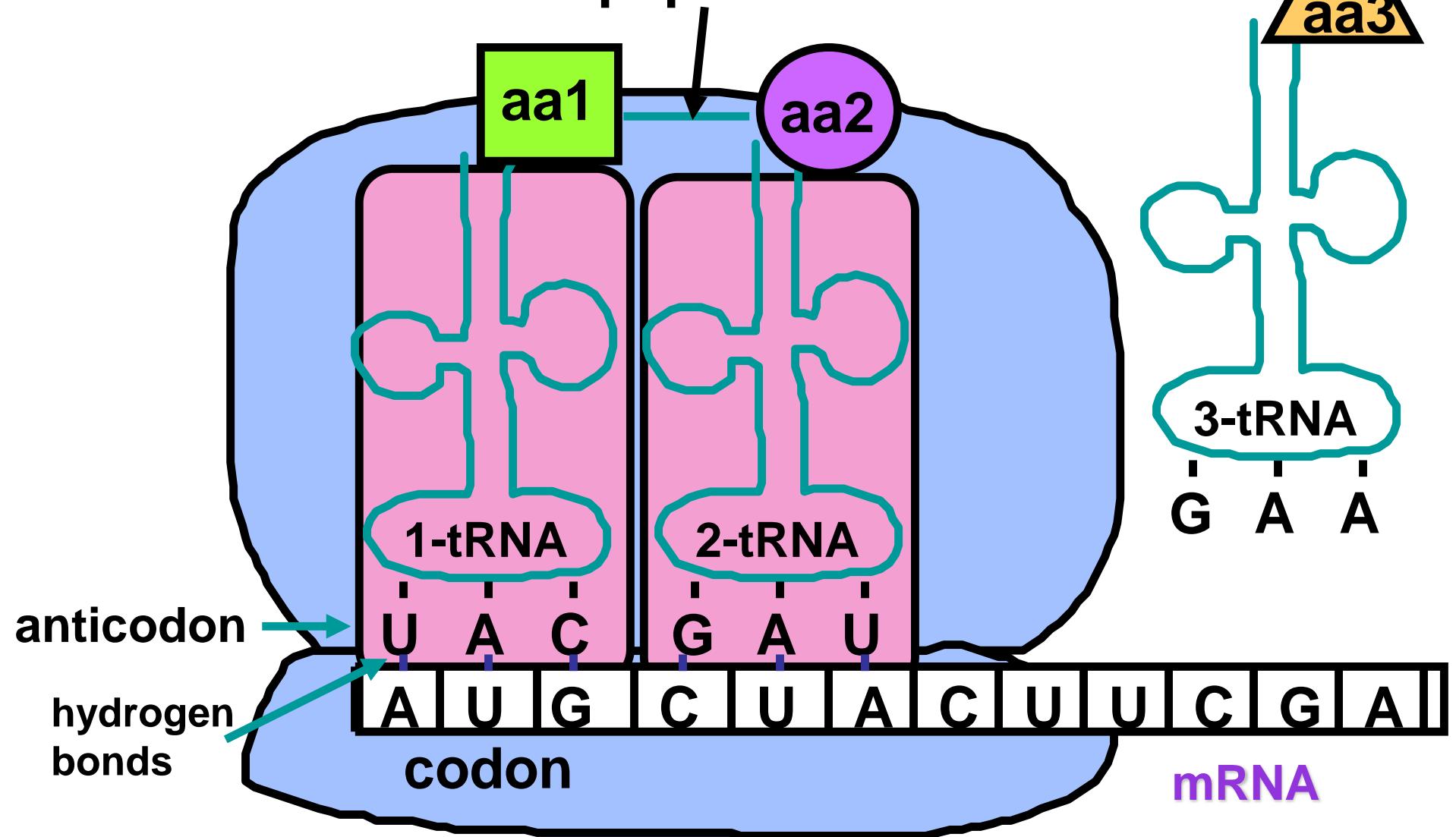


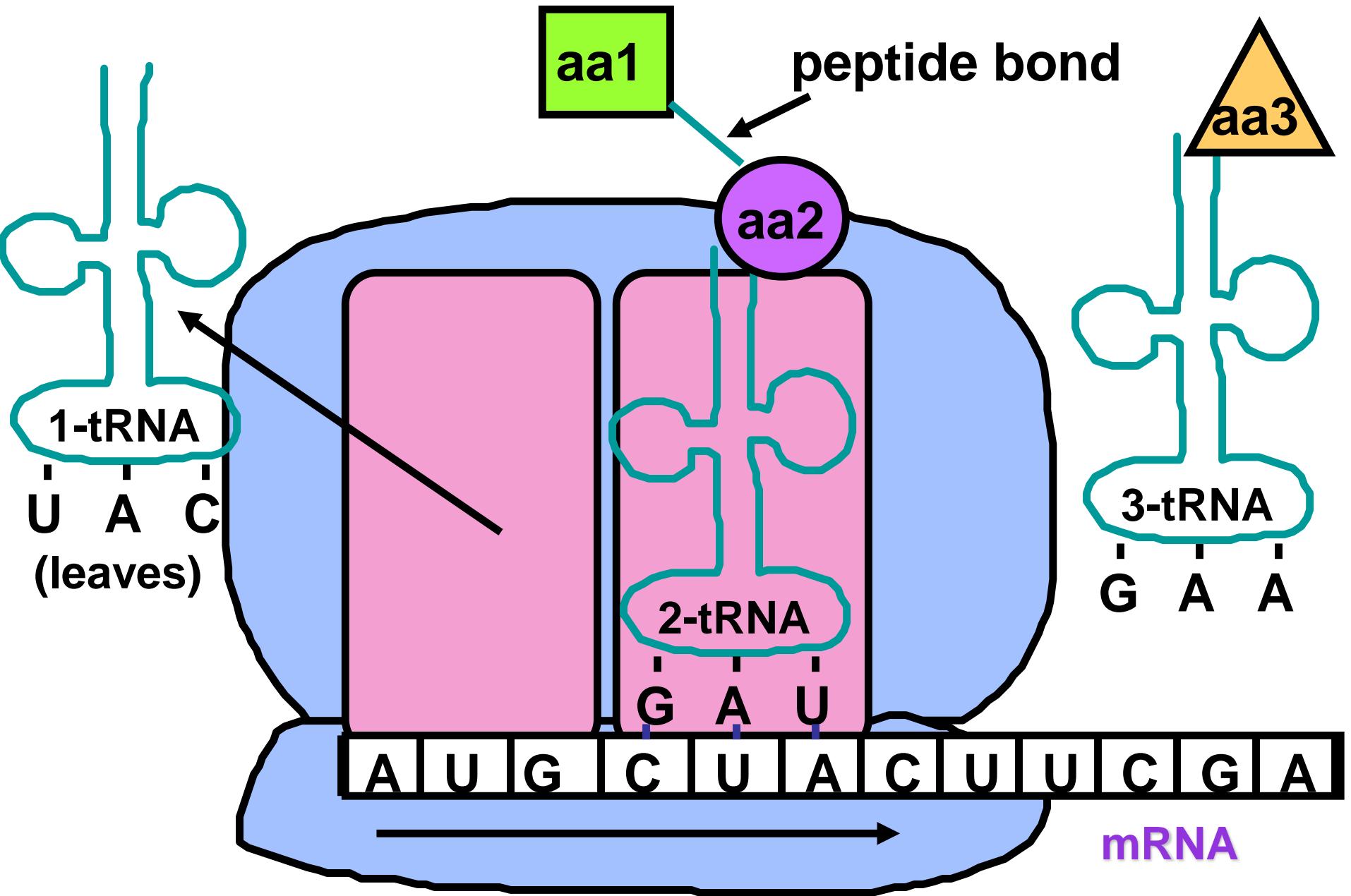
Initiation



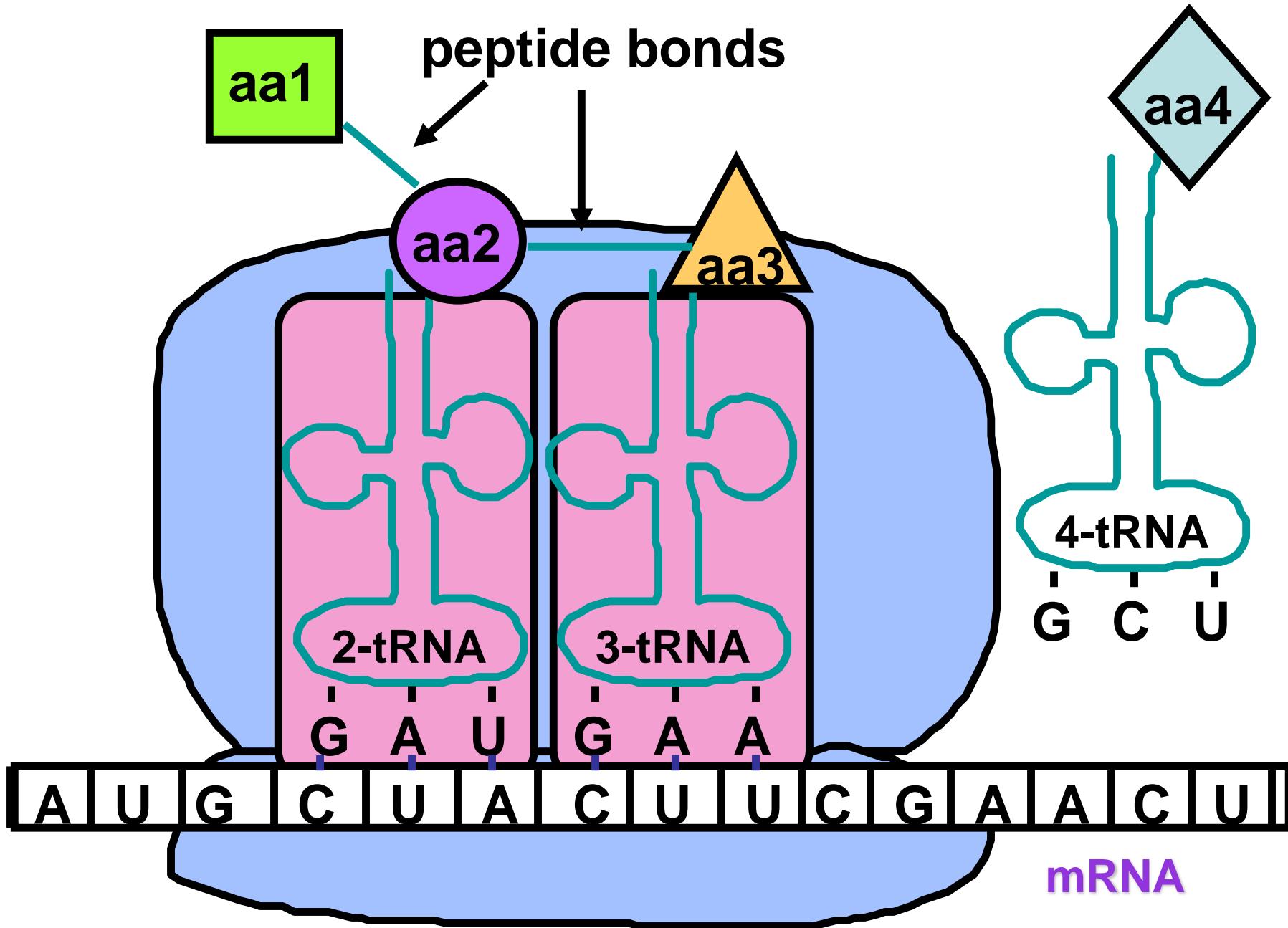
Elongation

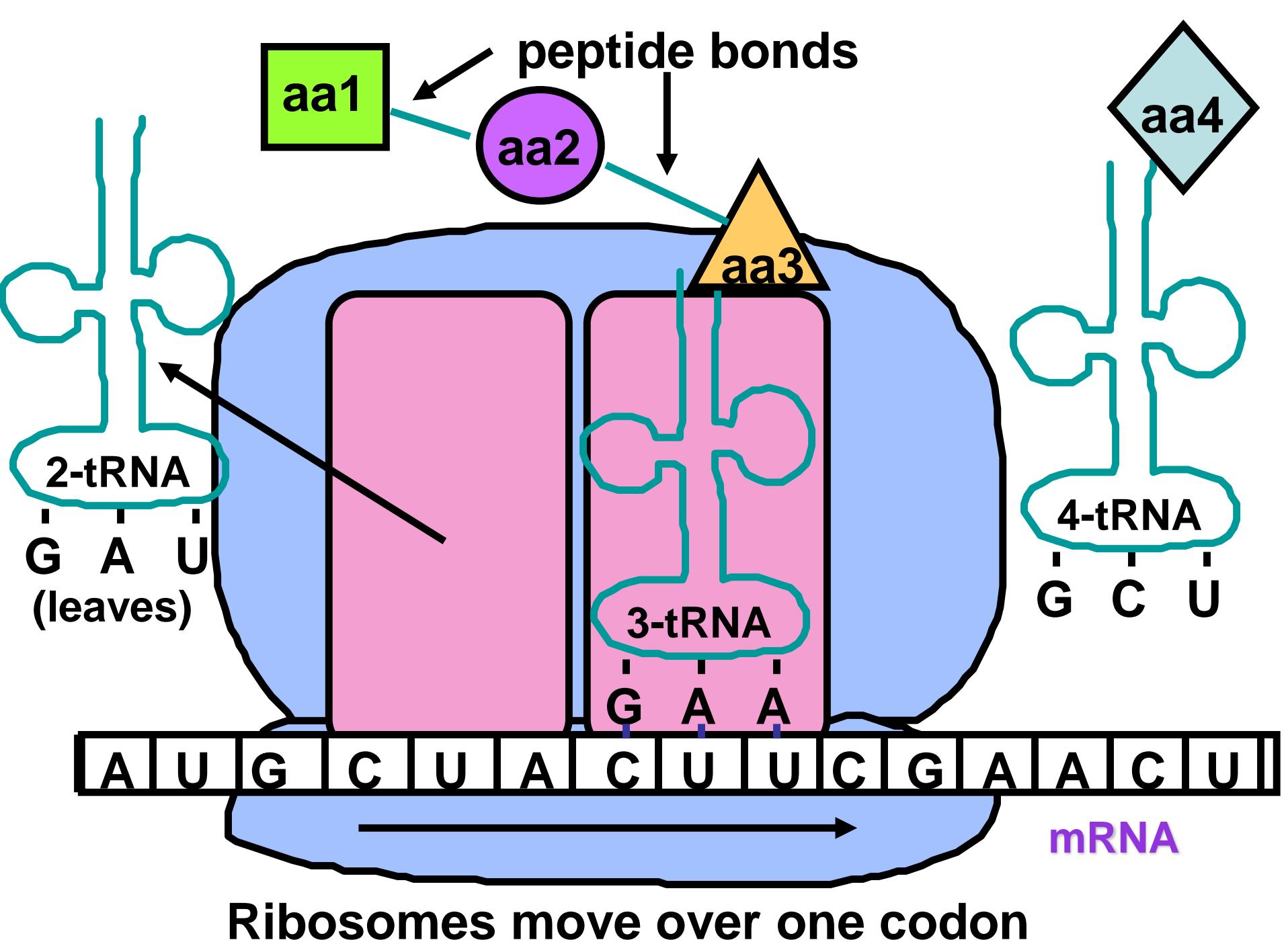
peptide bond

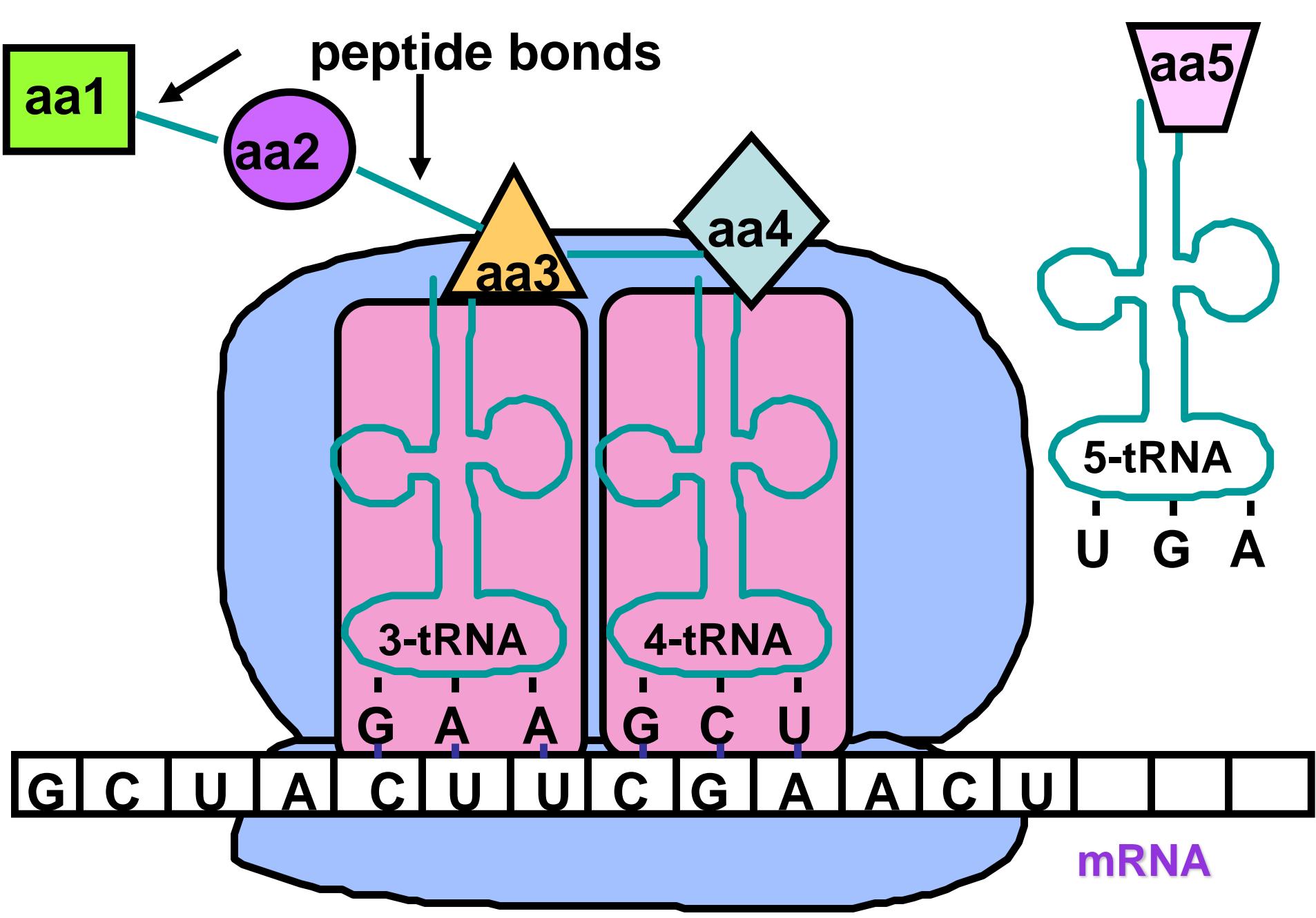


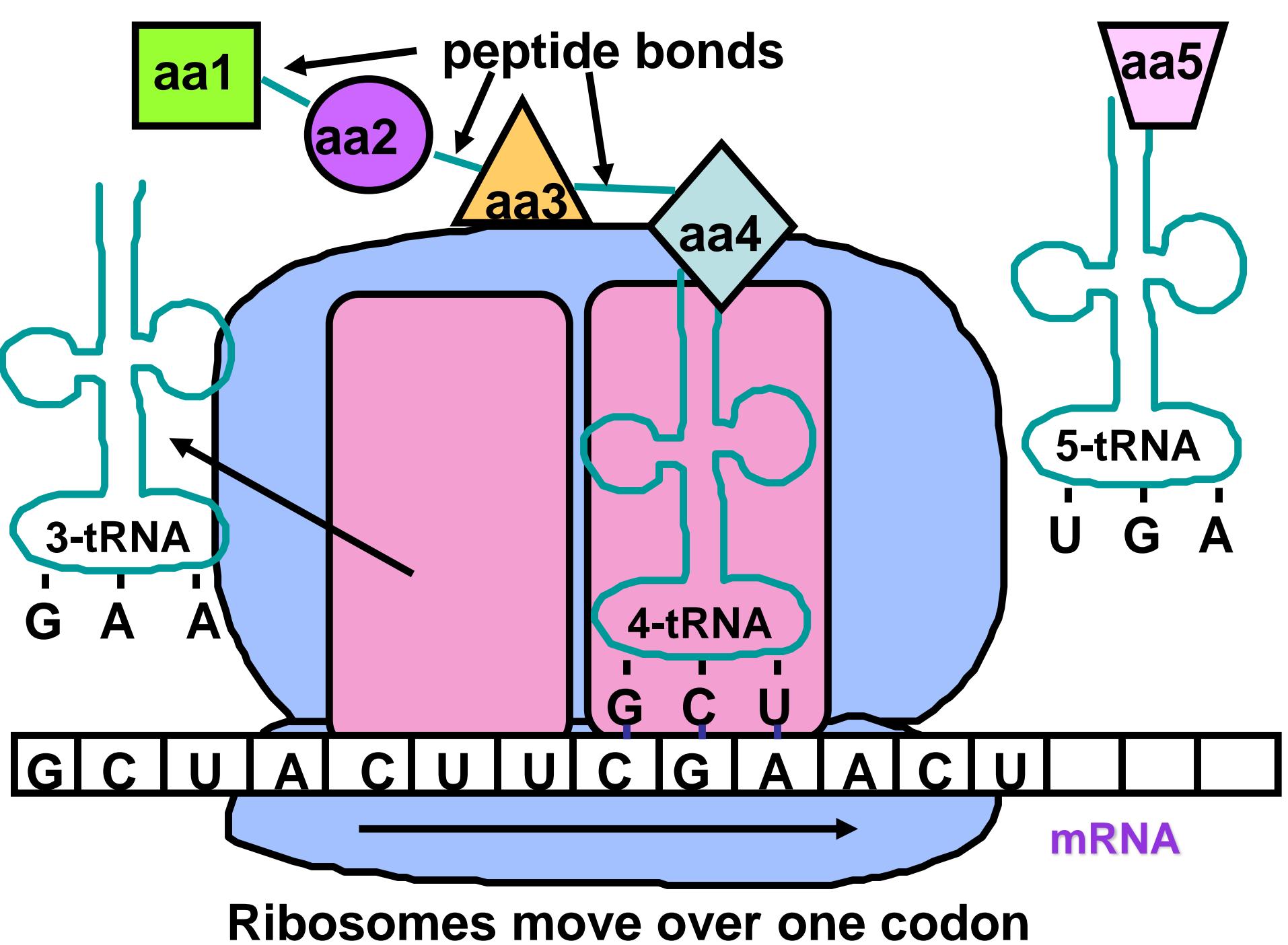


Ribosomes move over one codon

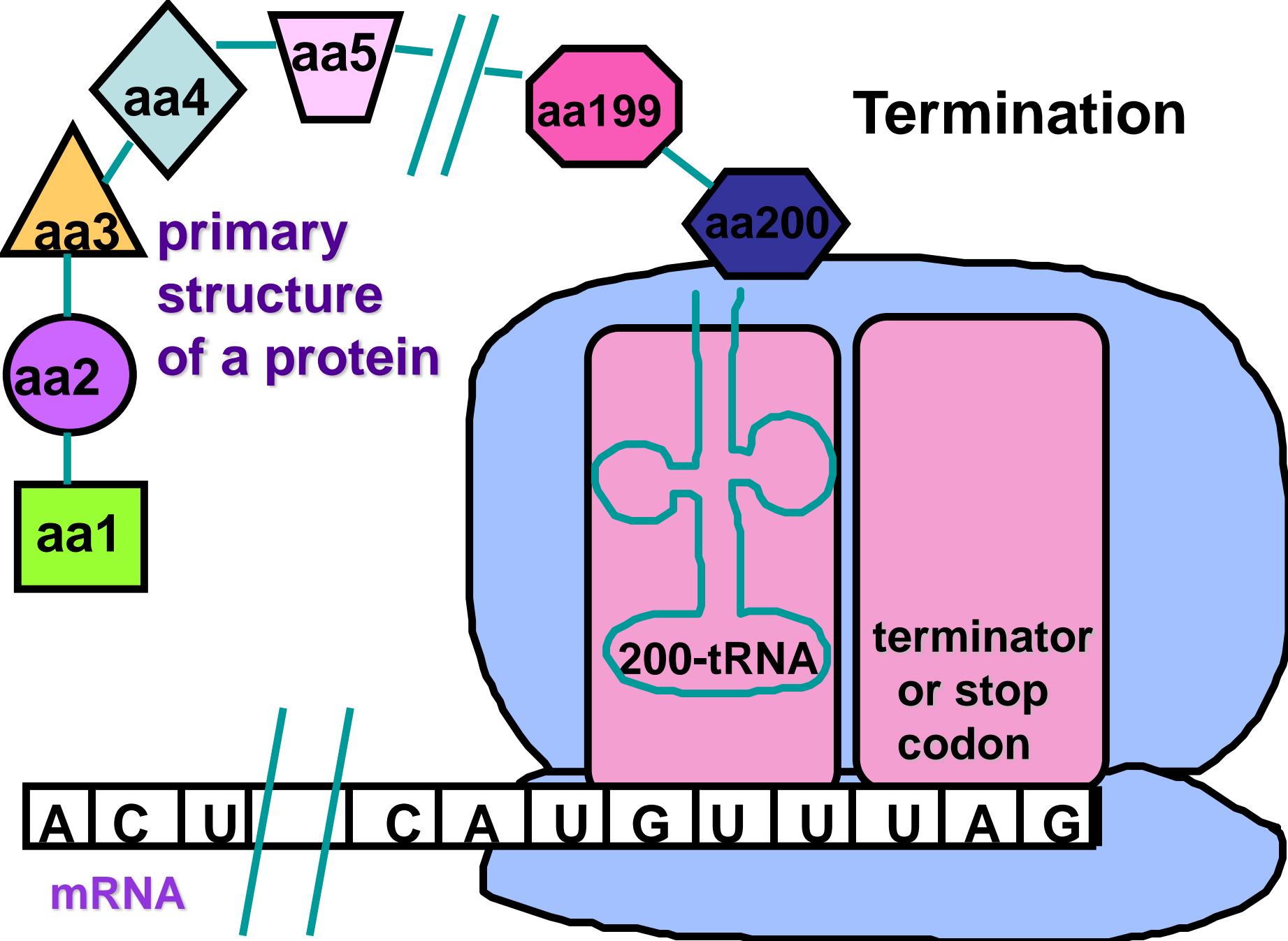






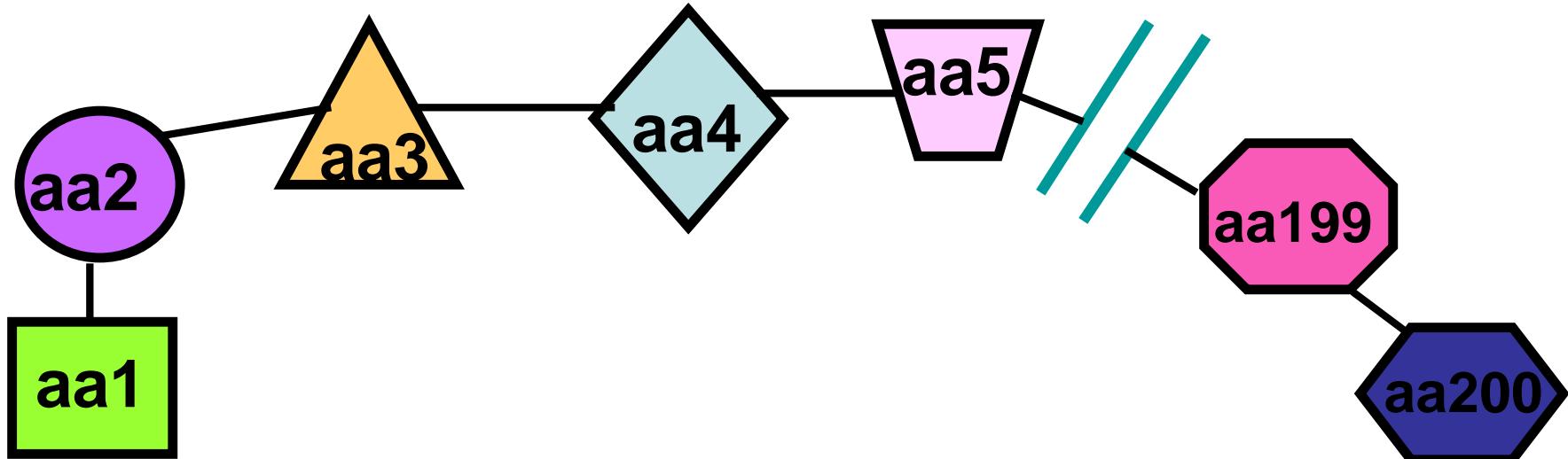


Termination



End Product –The Protein!

- The end products of protein synthesis is a **primary structure** of a protein
- A **sequence of amino acid** bonded together by peptide bonds



Eukaryotic Protein Synthesis Differs from Prokaryotic Protein Synthesis Primarily in Translation Initiation

- The basic plan of protein synthesis in eukaryotes and archaea is similar to that in bacteria.
- Eukaryotic protein synthesis entails more protein components than does prokaryotic protein synthesis, and some steps are more intricate.
- 1. Ribosomes.
 - Eukaryotic ribosomes are larger: consist of a 60S large subunit and a 40S small subunit, which come together to form an 80S particle having a mass of 4200 kd,
 - 40S subunit contains an 18S RNA
 - 60S subunit contains 5S, 5.8S, and 28S
 - Prokaryotic ribosomes have small (in *E. coli*, 30S) and larger (50S) subunits.
 - The 30S unit has 16S rRNA and 21 different proteins.
 - The 50S subunit consists of 5S and 23S rRNA and 34 different proteins.

- 2. *Initiator tRNA*. In eukaryotes, the initiating amino acid is methionine rather than *N*-formylmethionine. However, as in prokaryotes, a special tRNA participates in initiation. This aminoacyl-tRNA is called Met-tRN*A*i or Met-tRN*A*f (the subscript “*i*” stands for initiation, and “*f*” indicates that it can be formylated *in vitro*).
- 3. *Initiation*. The initiating codon in eukaryotes is always AUG. In contrast, a prokaryotic mRNA can have multiple start sites, and it can serve as a template for the synthesis of several proteins. Eukaryotes utilize many more initiation factors than do prokaryotes, and their interplay is much more intricate.
- 4. *Elongation and termination*. Eukaryotic elongation factors EF1 α and EF1 $\beta\gamma$ are the counterparts of prokaryotic EF-Tu and EF-Ts.
 - Termination in eukaryotes is carried out by a single release factor, eRF1, compared with two in prokaryotes. Finally, eIF3, like its prokaryotic counterpart IF3, prevents the reassociation of ribosomal subunits in the absence of an initiation complex.

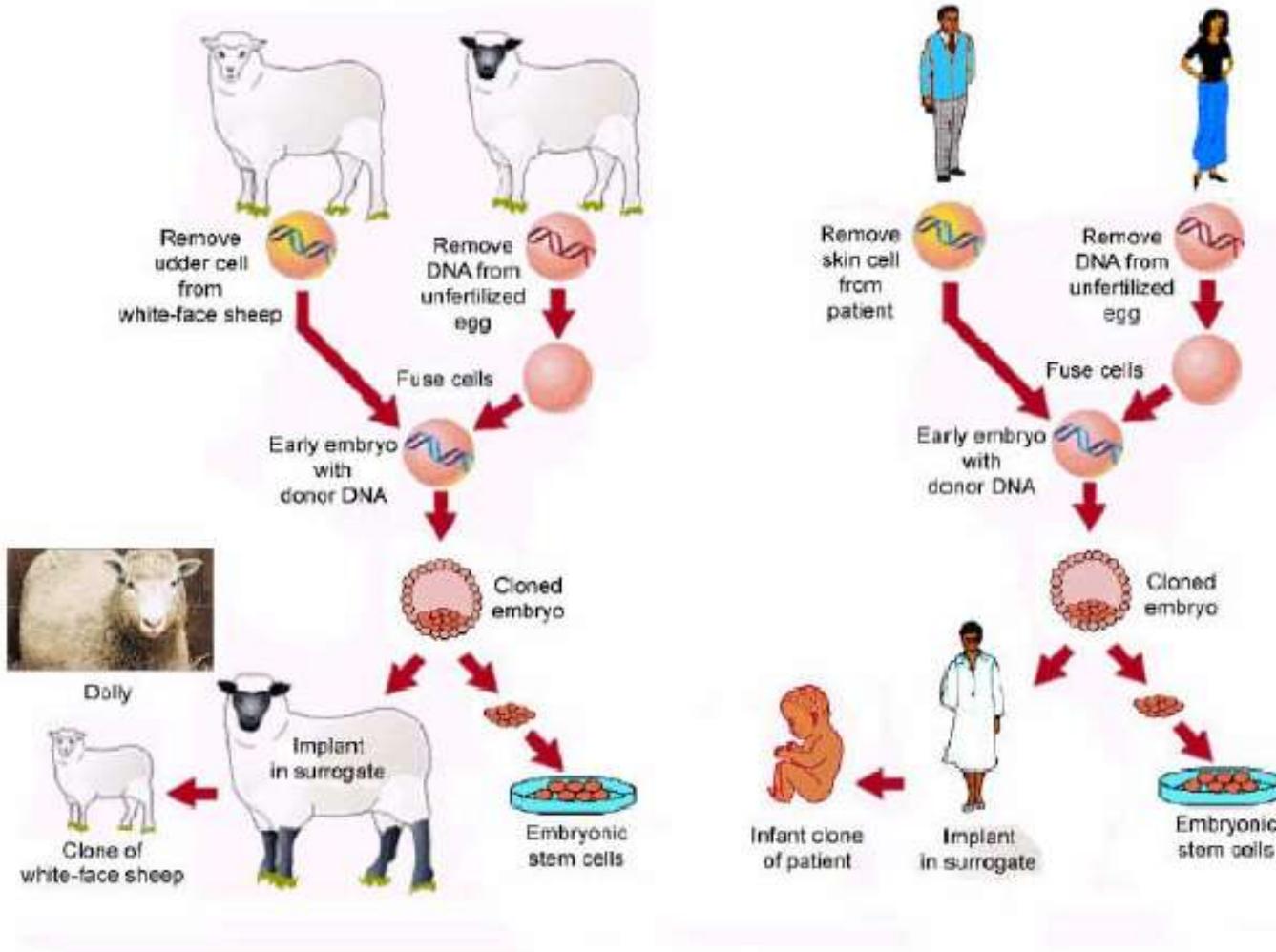
Stem cells and tissue engineering

Stem cell (SC)- Stem Cells are the cells which has the ability to divide for indefinite periods and which can give rise to specialized cells of various tissues of body.

Stem cells history

- **1978 - Stem cells were discovered in human cord blood**
- **1981 - First in vitro stem cell line developed from mice**
- **1998 - Researchers isolated stem cells from human embryos**
- **1999 - First Successful human transplant of insulin-making cells from cadavers**

Importance



Properties

- Two defining property-Ability to **differentiate** into other cells, ability to **self regenerate**
- It can be maintained in the *in vitro* conditions for extended period using artificial medium
- Its karyotype remains stable even after many division
- It can produce any type of adult cells of the organisms.

Sources of stem cells

1. EMBRYONIC STEM CELLS

- Isolated from blastocyst stage of embryos
- Pluripotent (capable of developing into almost all the cell types of the body) in nature

2. ADULT STEM CELLS

- It is present in all the organs, but very little amount
- They are multipotent (ability of a single stem cell to develop into more than one cell type but with ability to differentiate into a closely related family of cells).

Classification of stem cells

Unipotent - cell which can make exact copies of itself indefinitely, can differentiate, and produce same type of cells eg. Adult muscle stem cell

Totipotent- can become any cell type
eg: Morula stage of Embryos

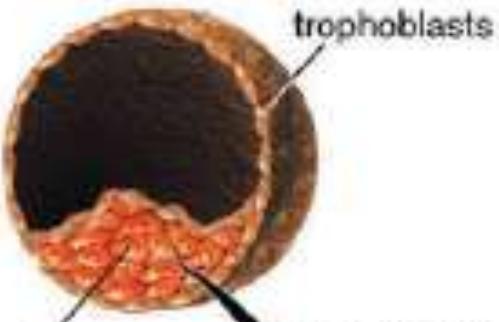
Pluripotent- almost any kind of cell except placenta
eg: Embryonic SC

Multipotent- Produces limited range of cell types
Adult SC: nerve cells, blood cells, muscle cells, bone and skin cells.

Culture of embryonic stem cells

- Collection of embryos from IVF centres
- Isolation of ICM (inner cell mass) from the blastocyst stage
- Transfer ICM to the center of culture plate containing feeder cells and growth medium
- It can be differentiated to any cells by adding specific medium

Blastocyst
(64 to 200 cell stage,
cross-section)

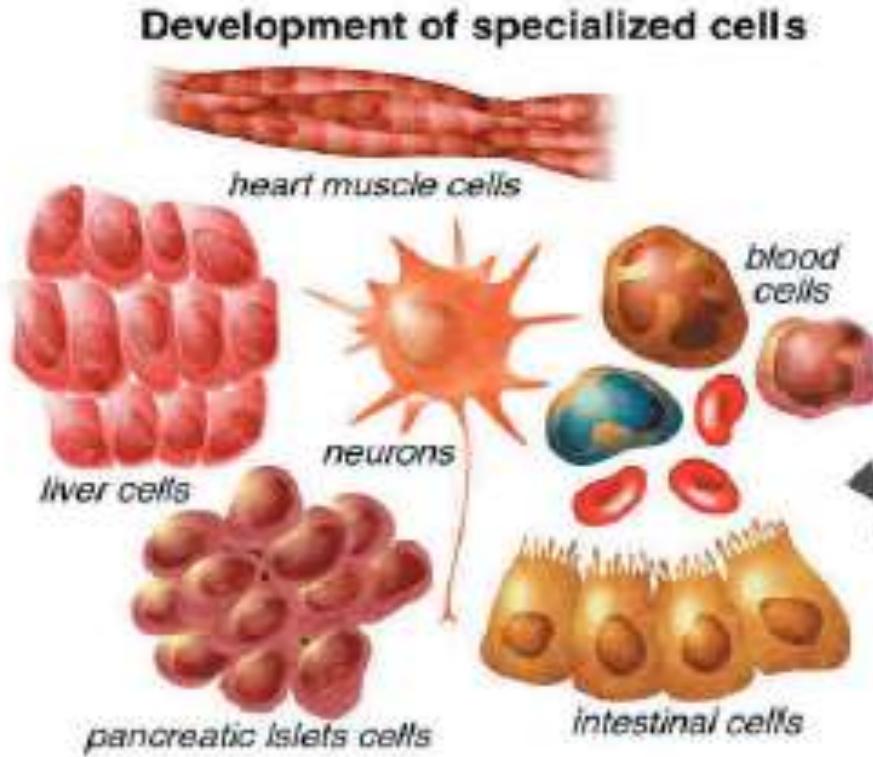


*Propagation
in Culture*



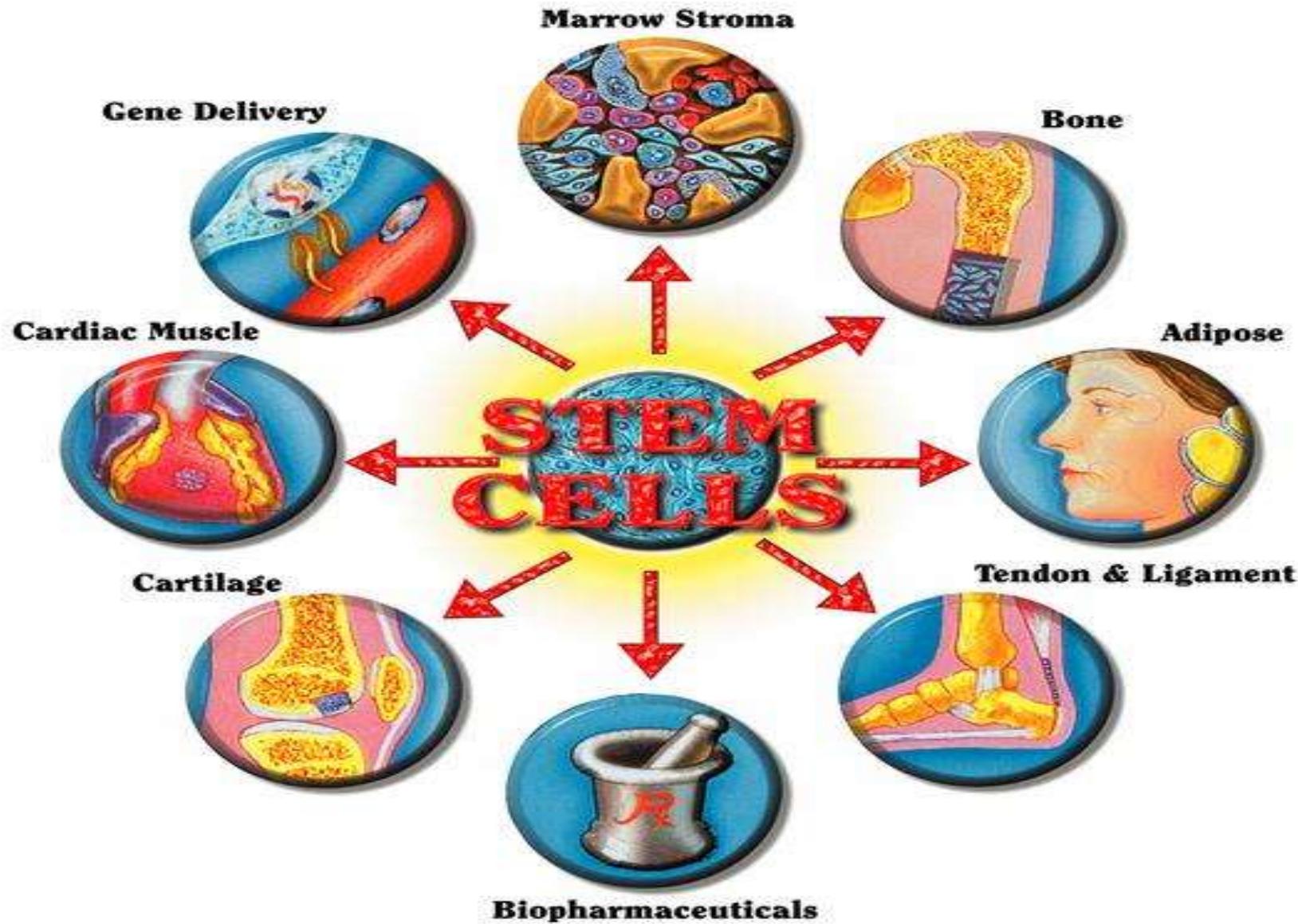
**Pluripotent
embryonic
stem cells**

Differentiation



Applications

- It can be used for neural degenerative diseases (Alzheimer's and Parkinson's disease)
- It can also used to treat diabetes by injecting *in vitro* grown pancreatic islet cells
- It is also used for treating **bone related diseases by injecting *in vitro*** developed osteocytes, chondrocytes and myocytes
- Today most of the diseases get cured through stem cell therapy eg. Muscular dystrophy, Polio.



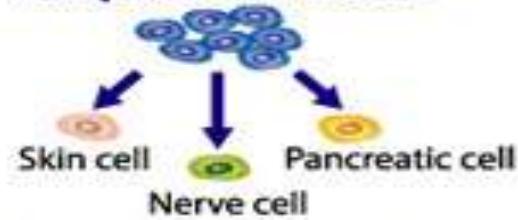
In vitro fertilization



Remove inner cell mass



Pluripotent stem cells



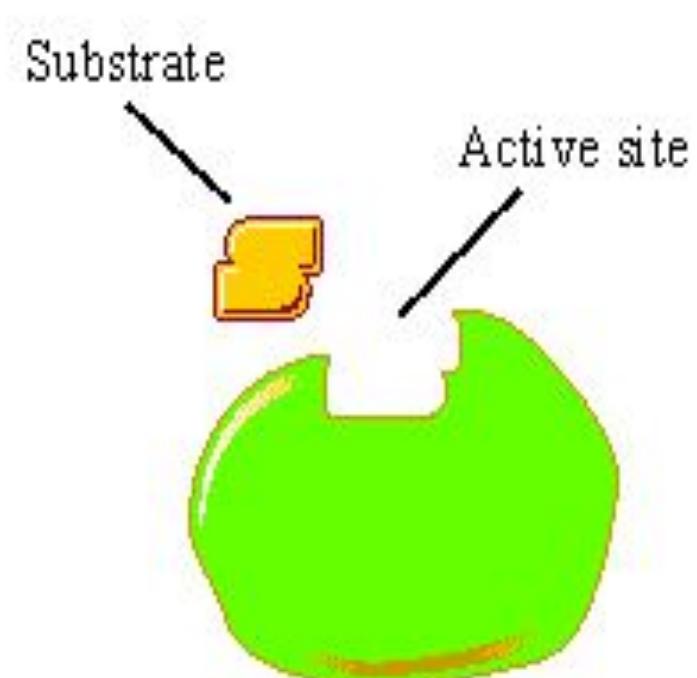
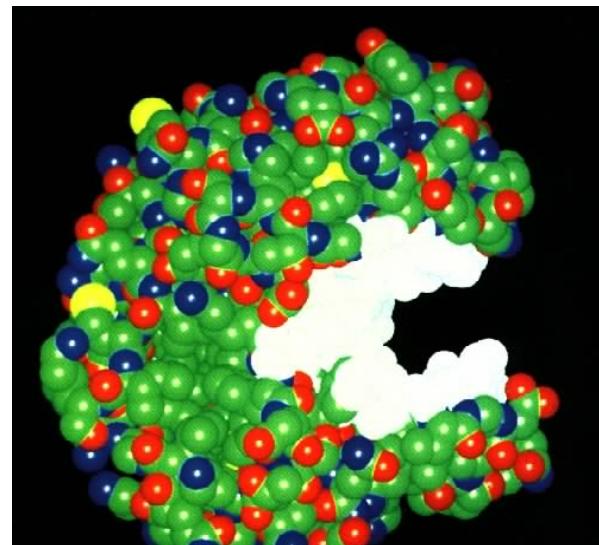
Transplant to patient

Unit III

- Enzymes
- Proteases
- Carbonic anhydrase, Restriction enzymes, and Nucleoside monophosphate kinases
- Photosynthesis
- Biological Energy production

What Are Enzymes?

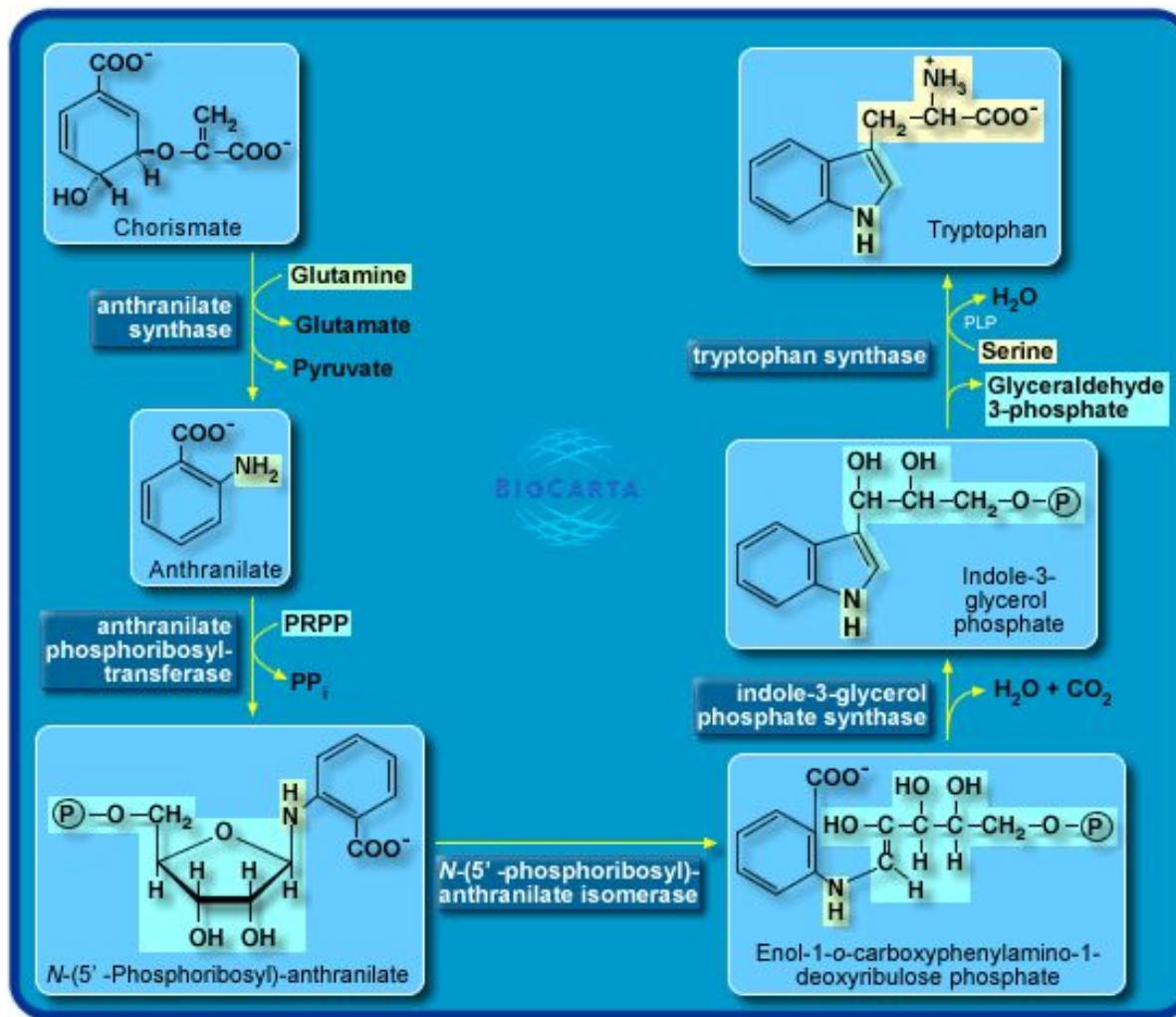
- Most enzymes are **Proteins** (tertiary and quaternary structures)
- Act as **Catalyst** to accelerates a reaction
- Not permanently changed in the process
- Are specific for what they will **catalyze**
- Are **Reusable**
- End in **-ase**
 - Sucrase*
 - Lactase*
 - Maltase*



Why Enzymes?

- Natural catalysts**
- Speed: 10^{16} over un-catalyzed rates!**
- Specificity: only the desired reaction occurs**
- Permit reactions under mild conditions**

Metabolic Pathways



The metabolic pathway that produces tryptophan – an amino acid. This is one of hundreds of metabolic pathways essential for life.

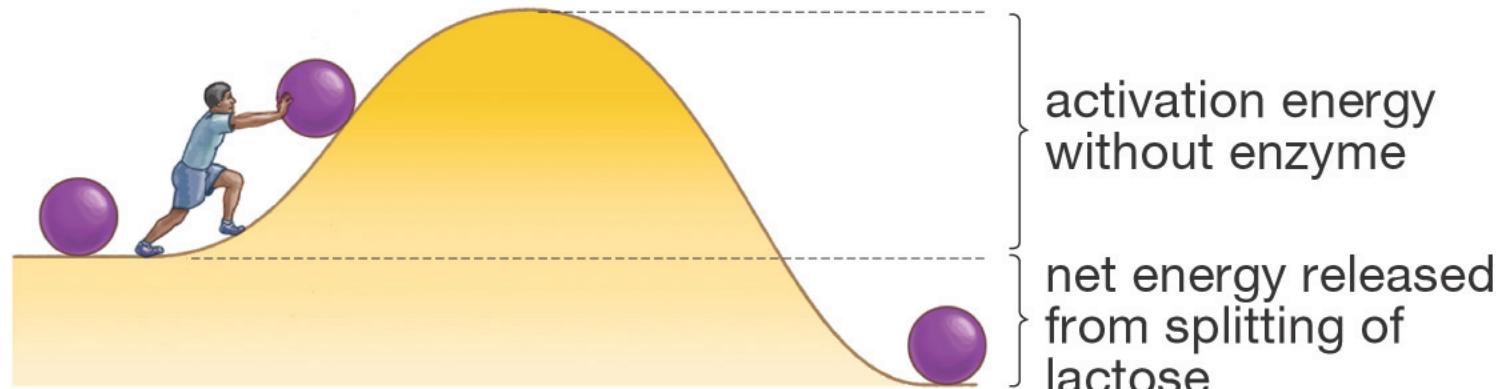
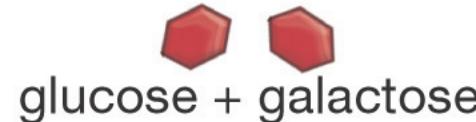
Enzyme Deficiency and Health



Most genetic disorders are due to a deficiency in enzyme function.

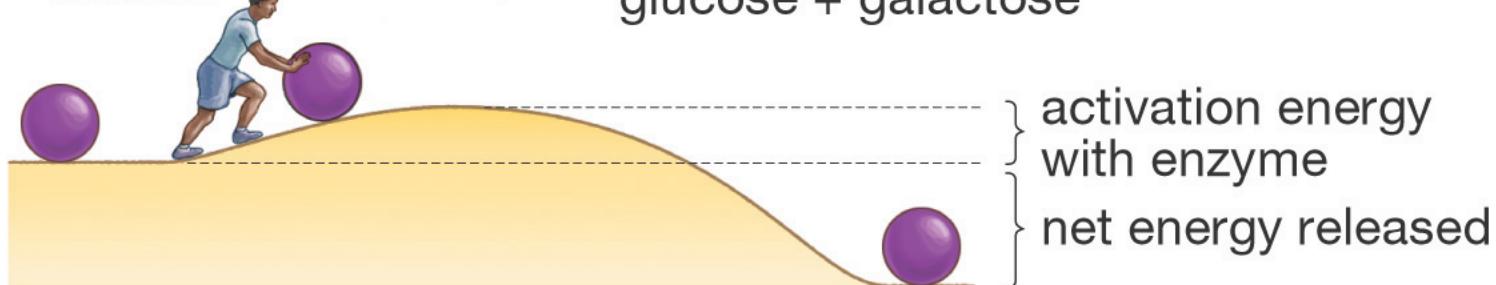
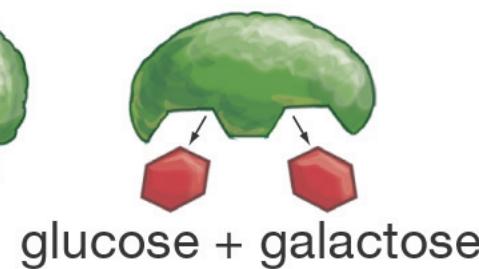
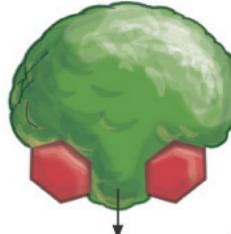
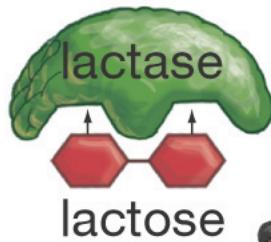
This archival photo shows three children with the enzyme (phenylalanine hydroxylase) deficiency that causes phenylketonuria.

(a) Without enzyme

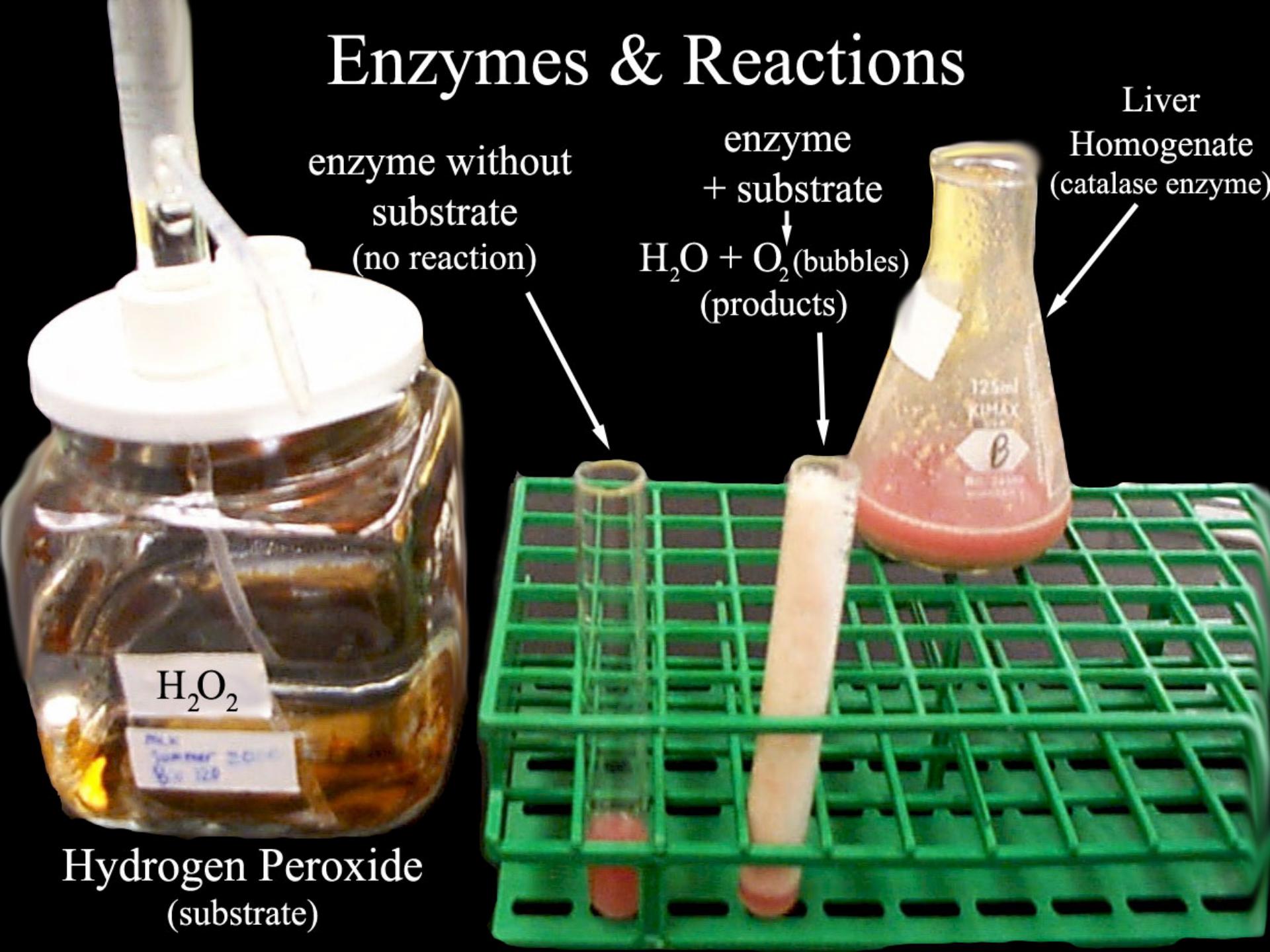


**Enzymes
work by
weakening
bonds
which
lowers
activation
energy**

(b) With enzyme



Enzymes & Reactions

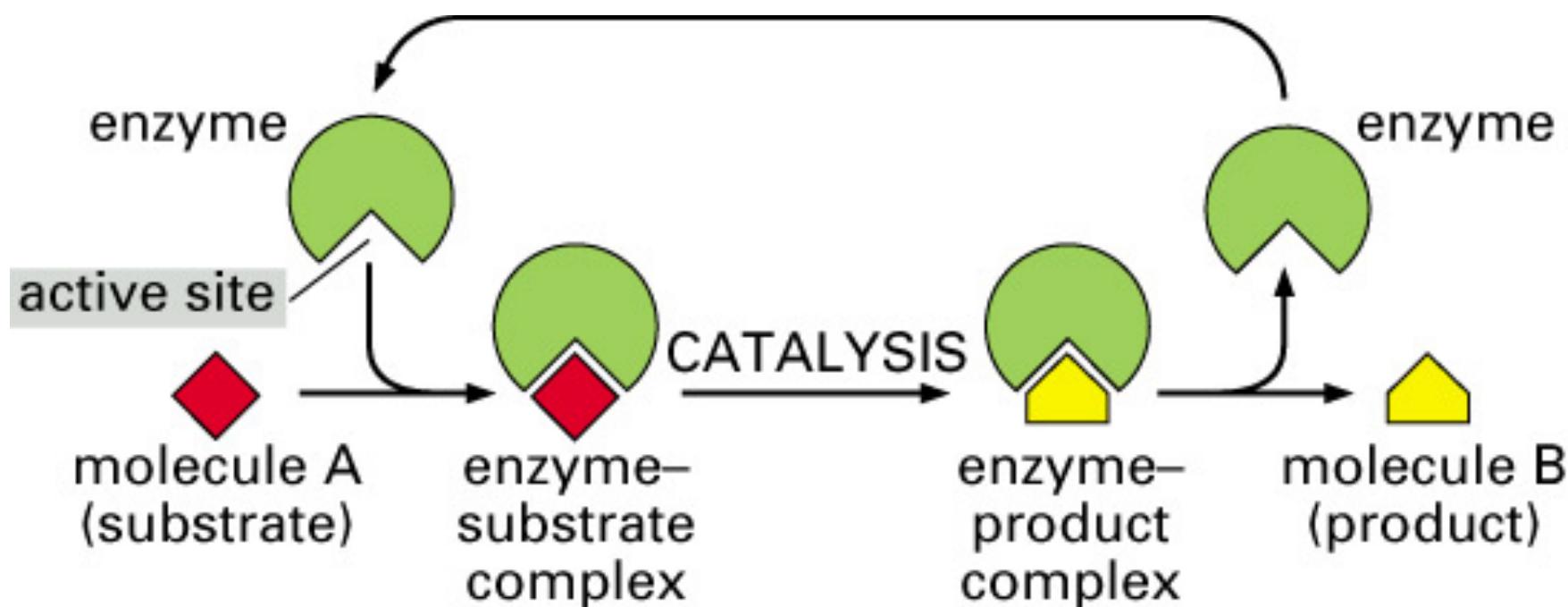


Enzyme Action: Lock and Key Model

Conventionally we say the enzyme (E) acts on the substrate (S) to yield products (P)



Since E is a catalyst it remains unchanged at the end of the reaction



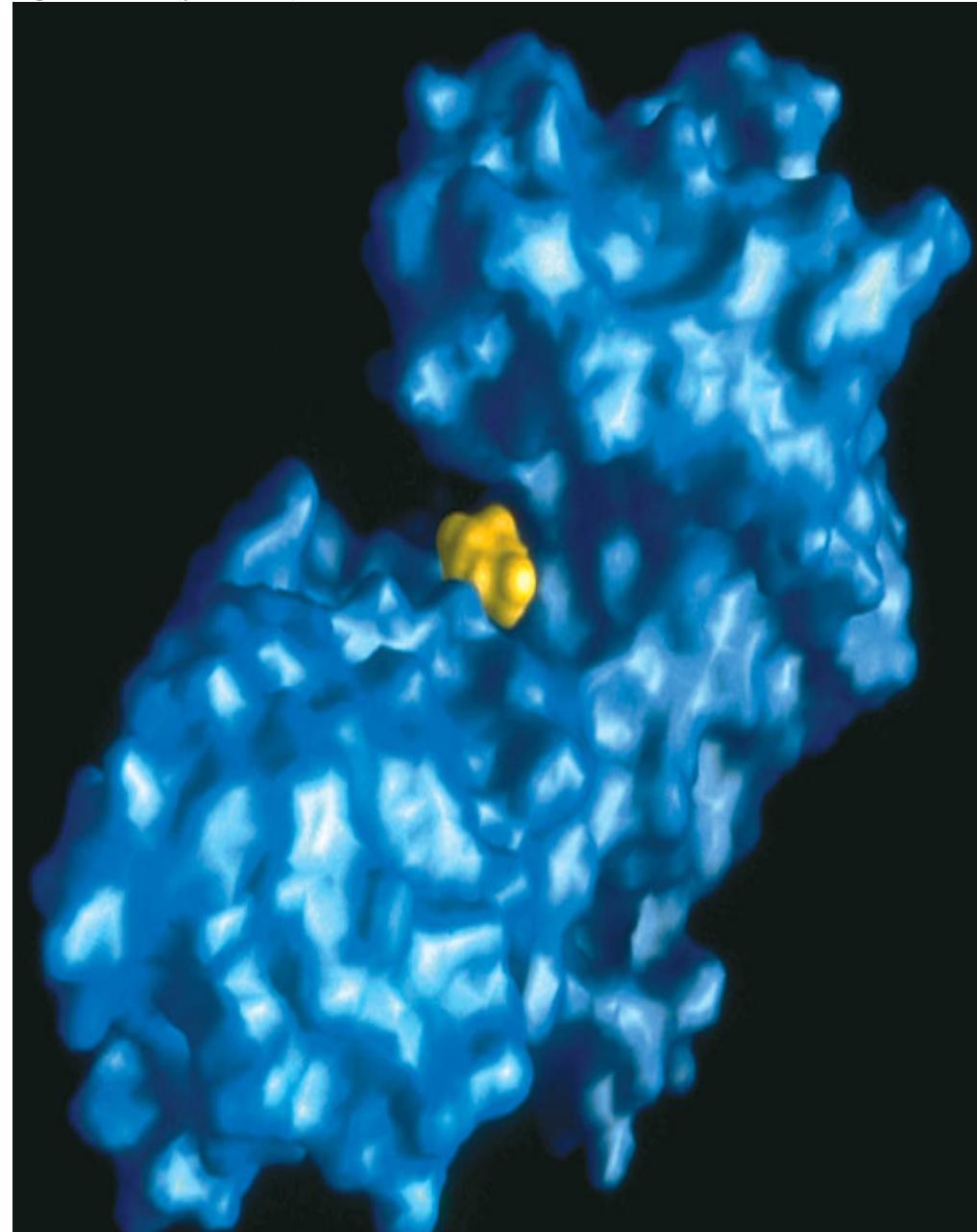
The active site

Typically a pocket or groove on the surface of the protein into which the substrate fits.

The specificity of an enzyme –fit between the active site and that of the substrate.

Enzyme changes shape –tighter induced fit, bringing chemical groups in position to catalyze the reaction.

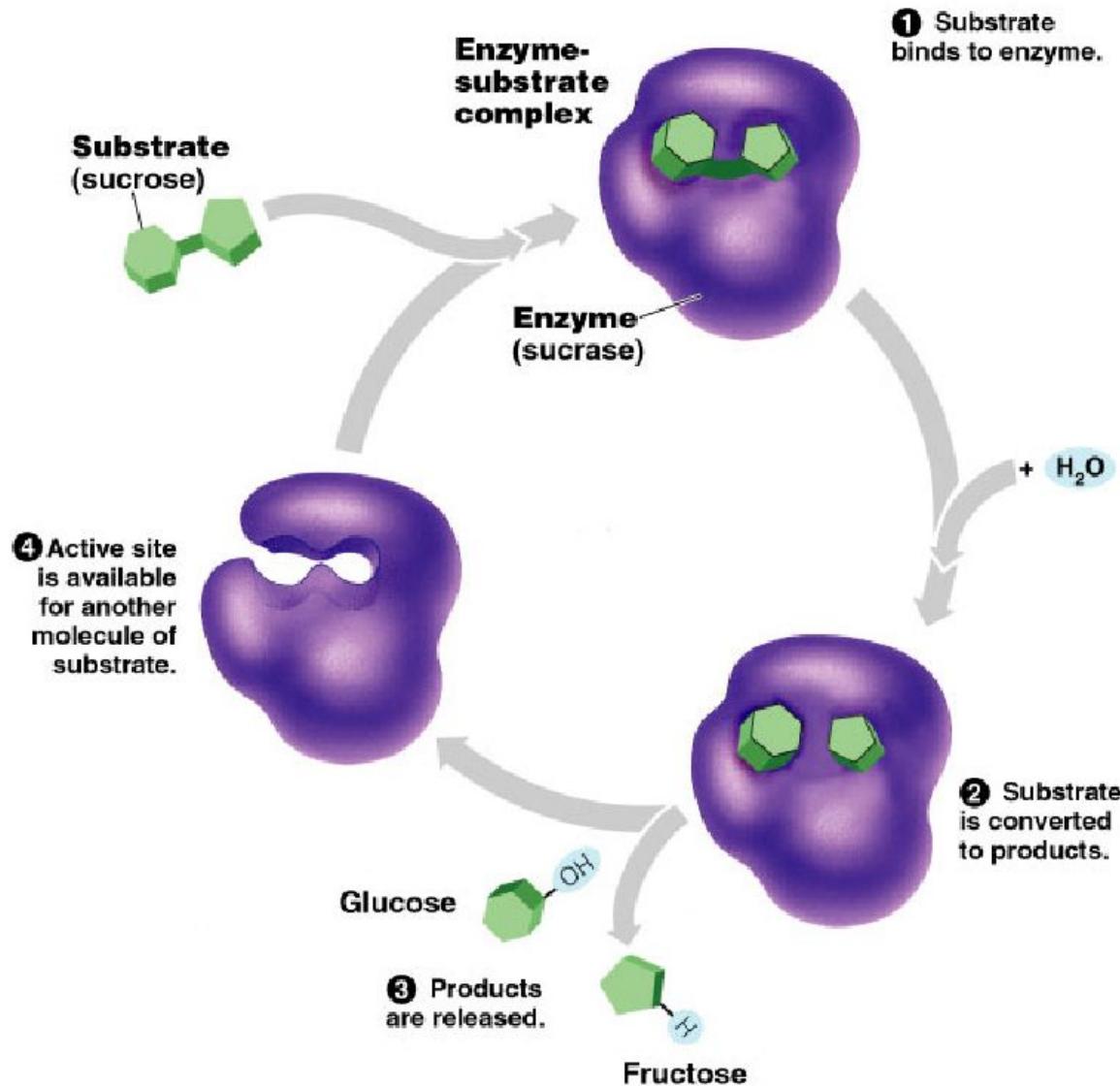
Hexokinase, an enzyme (blue), binding its substrate, glucose (yellow).



Specificity

Enzymes selectively recognize proper substrates over other molecules

Specificity is controlled by structure – the unique fit of substrate with enzyme controls the selectivity for substrate and the product yield



What Affects Enzyme Activity?

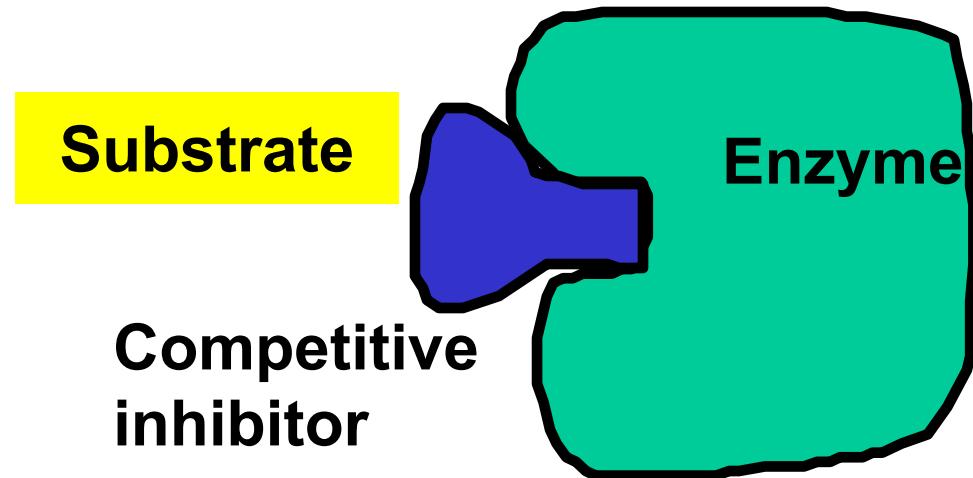
- Three factors:
 1. Environmental Conditions
 2. Cofactors and Coenzymes
 3. Enzyme Inhibitors
- 1. Environmental Conditions:
 1. Extreme Temperature are the most dangerous
 - high temps may denature (unfold) the enzyme.
 2. pH (most like 6 - 8 pH near neutral)
 3. Ionic concentration (salt ions)
- 2. Cofactors and Coenzymes
 - Inorganic substances (zinc, iron) and vitamins (respectively) are sometimes need for proper enzymatic activity.

Example: Iron must be present in the quaternary structure - hemoglobin in order for it to pick up oxygen.

3. Enzyme Inhibitors

Two Types:

a. Competitive inhibitors: are chemicals that resemble an enzyme's normal substrate and compete with it for the active site.



b. Noncompetitive inhibitors: Inhibitors that do not enter the active site, but bind to another part of the enzyme causing the enzyme to change its shape, which in turn alters the active site.



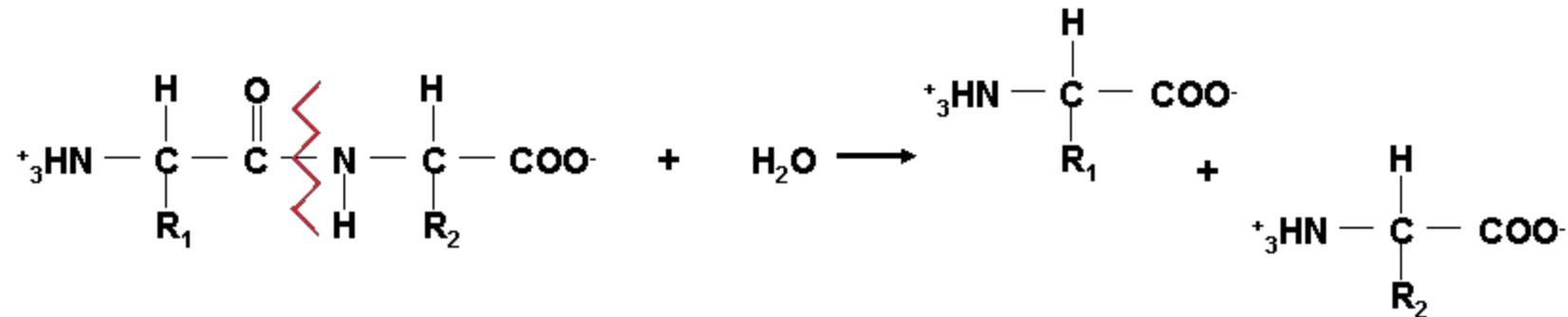
Catalytic Mechanisms

- Acid-Base catalysis
 - Enzyme side chains act as proton donors and acceptors.
- Covalent Catalysis
 - Powerful nucleophilic side chain forms an unstable covalent bond to the substrate.
- Metal ion catalysis
 - By serving as an electrophilic catalyst, generating a nucleophile, and increasing the binding energy between E and S
- Catalysis by approximation
 - When two substrates involved, the reaction rate is enhanced by bringing them together on the active binding site of E.

Proteases

Proteases

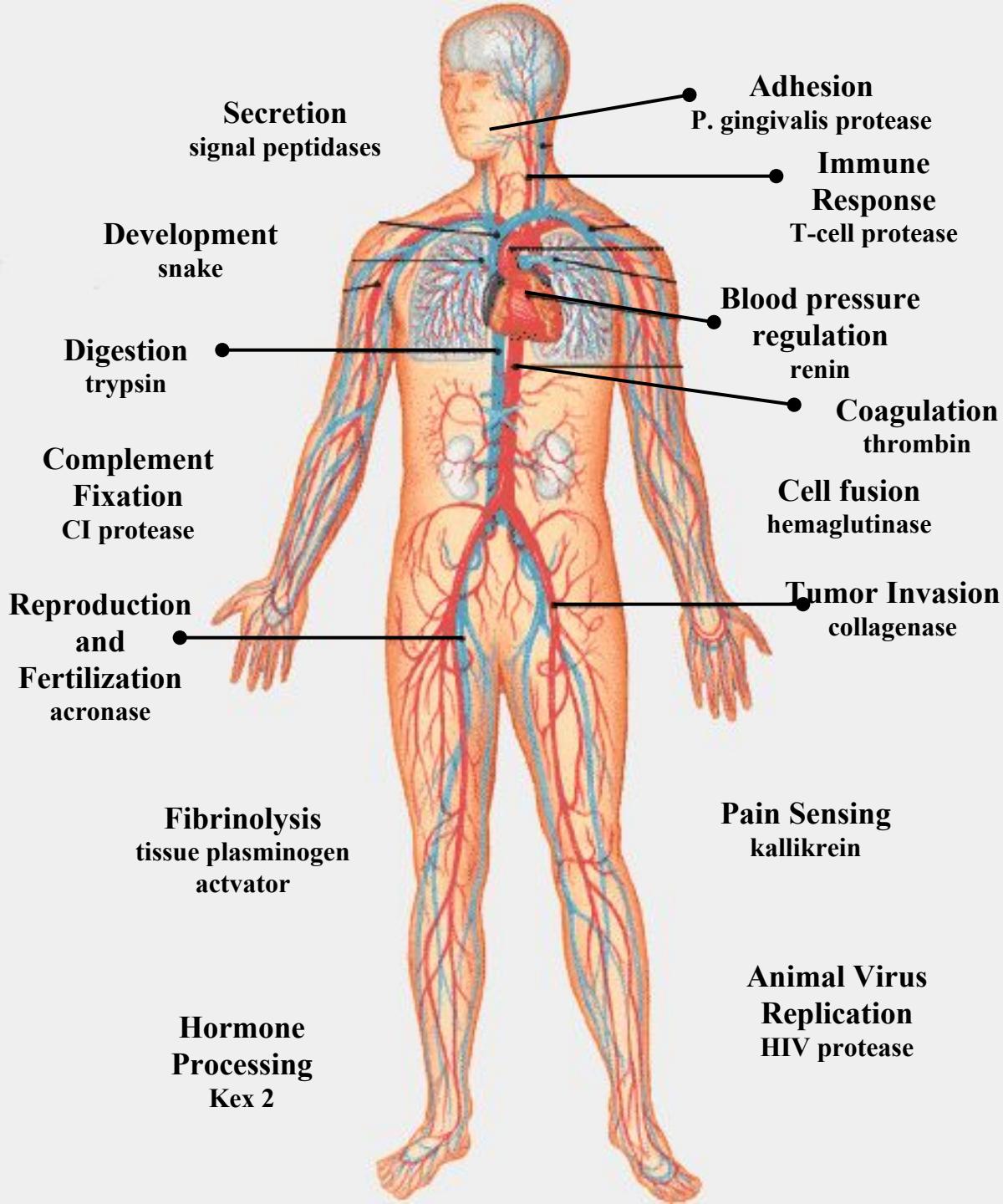
What do proteases do?



Proteases cleave proteins by a hydrolysis reaction – the addition of a molecule of water to a peptide bond

Mechanistic Sets of Proteases

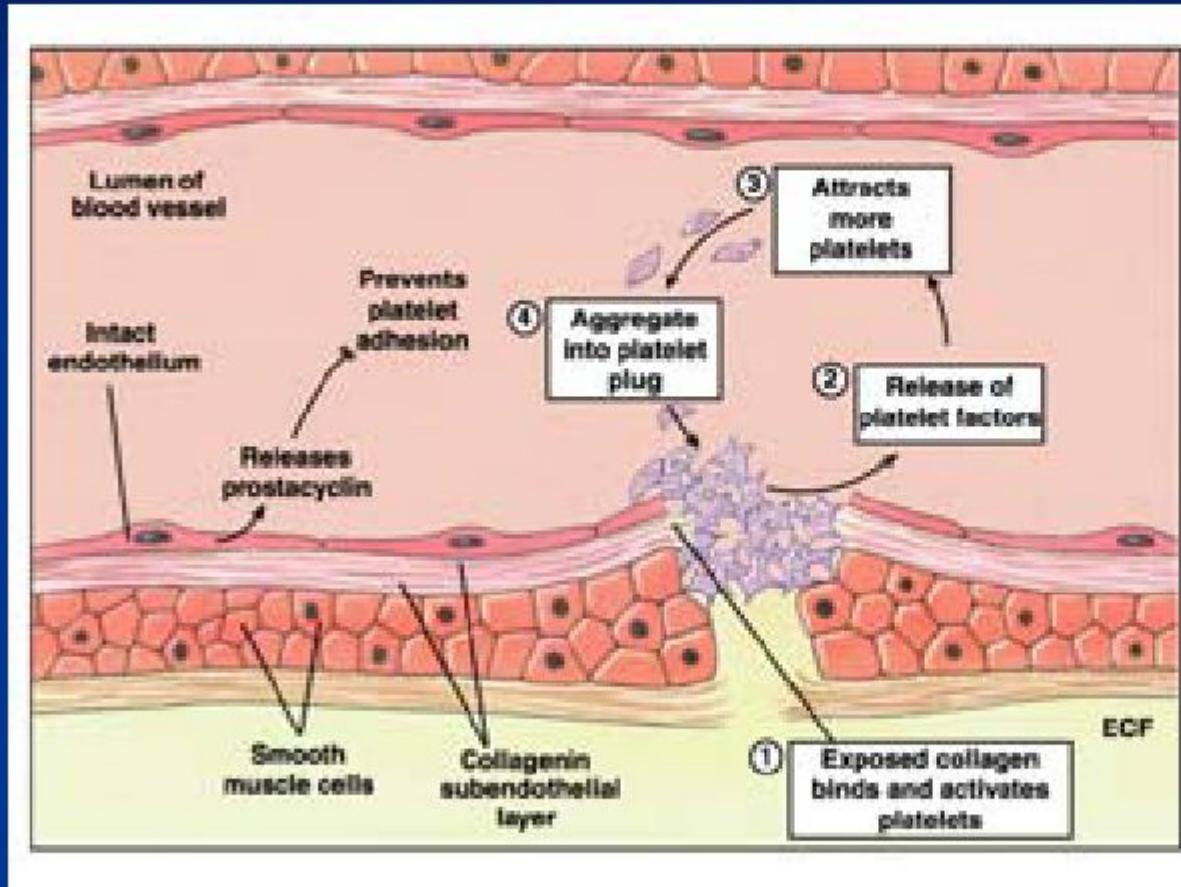
| set | feature | inhibitor | examples | function |
|-------------------|---|------------------|---|--|
| Serine protease | active site serine | fluorophosphates | trypsin thrombin plasmin coccoonase subtilisin acrosin | digestion blood coagulation lysis of blood clots mechanical digestion sperm penetration |
| Cysteine protease | active site cysteine | iodoacetate | papain strept. proteinase cathepsin B | digestion digestion intracell. digestion |
| Acid protease | acidic pH optimum | diazoketones | pepsin chymosin | digestion milk coagulation |
| Metalloproteases | Zn ²⁺ , Zn ²⁺ , Ca ²⁺ | o-phenanthroline | carboxypeptidase thermolysin | digestion digestion |



6 Broad Categories

| <u>Function</u> | <u>Protease</u> |
|-----------------|---|
| Nutrition | trypsin, subtilisin, α -lytic protease |
| Invasion | matrix metallo proteases |
| Evasion | IgA protease |
| Adhesion | P. gingivalis protease |
| Processing | signal peptidase, viral proteases, proteosome |
| Signaling | caspases, granzymes |

Arrest of bleeding is achieved through platelet adhesion and thrombin-related fibrin clotting

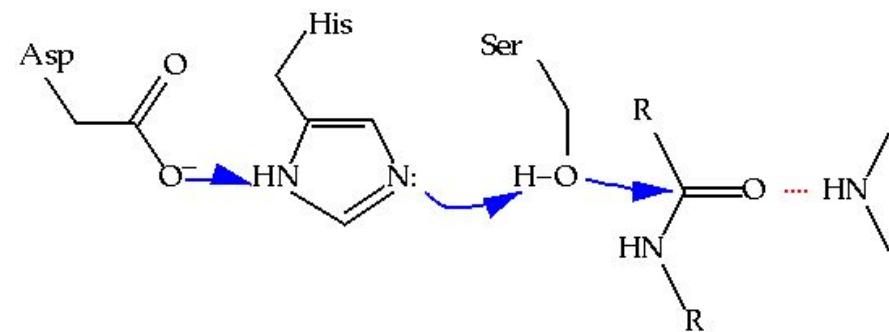
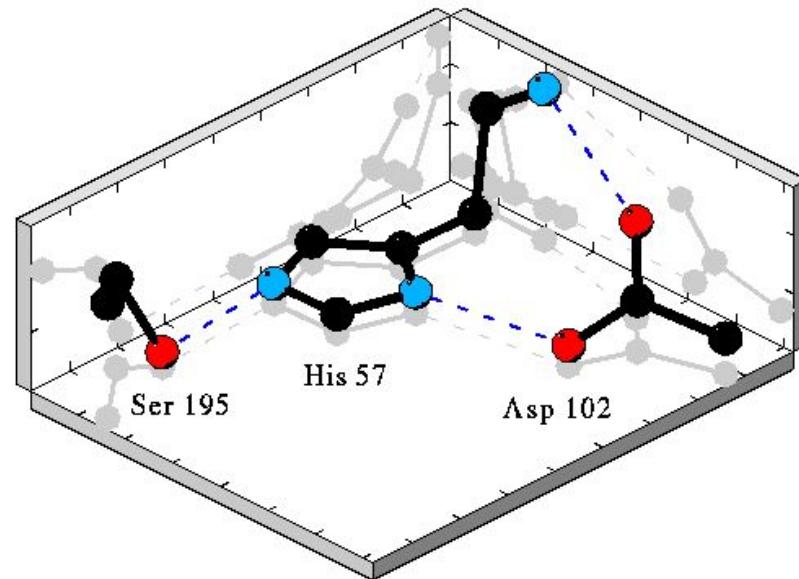


※ Thrombin-induced platelet activation and subsequent aggregation are still a poorly understood mechanism

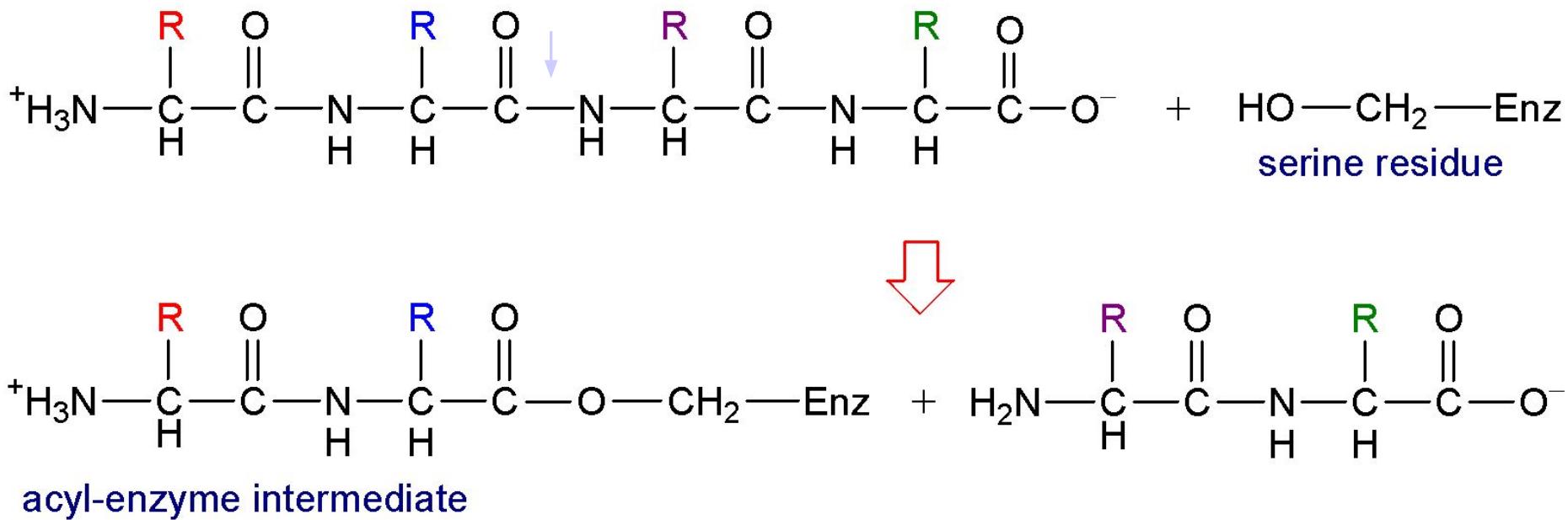
Catalytic triad

The active site in each serine protease includes a **serine** residue, a **histidine** residue, & an **aspartate** residue.

During attack of the serine hydroxyl oxygen, a proton is transferred from the serine hydroxyl to the imidazole ring of the histidine, as the adjacent aspartate carboxyl is H-bonded to the histidine.



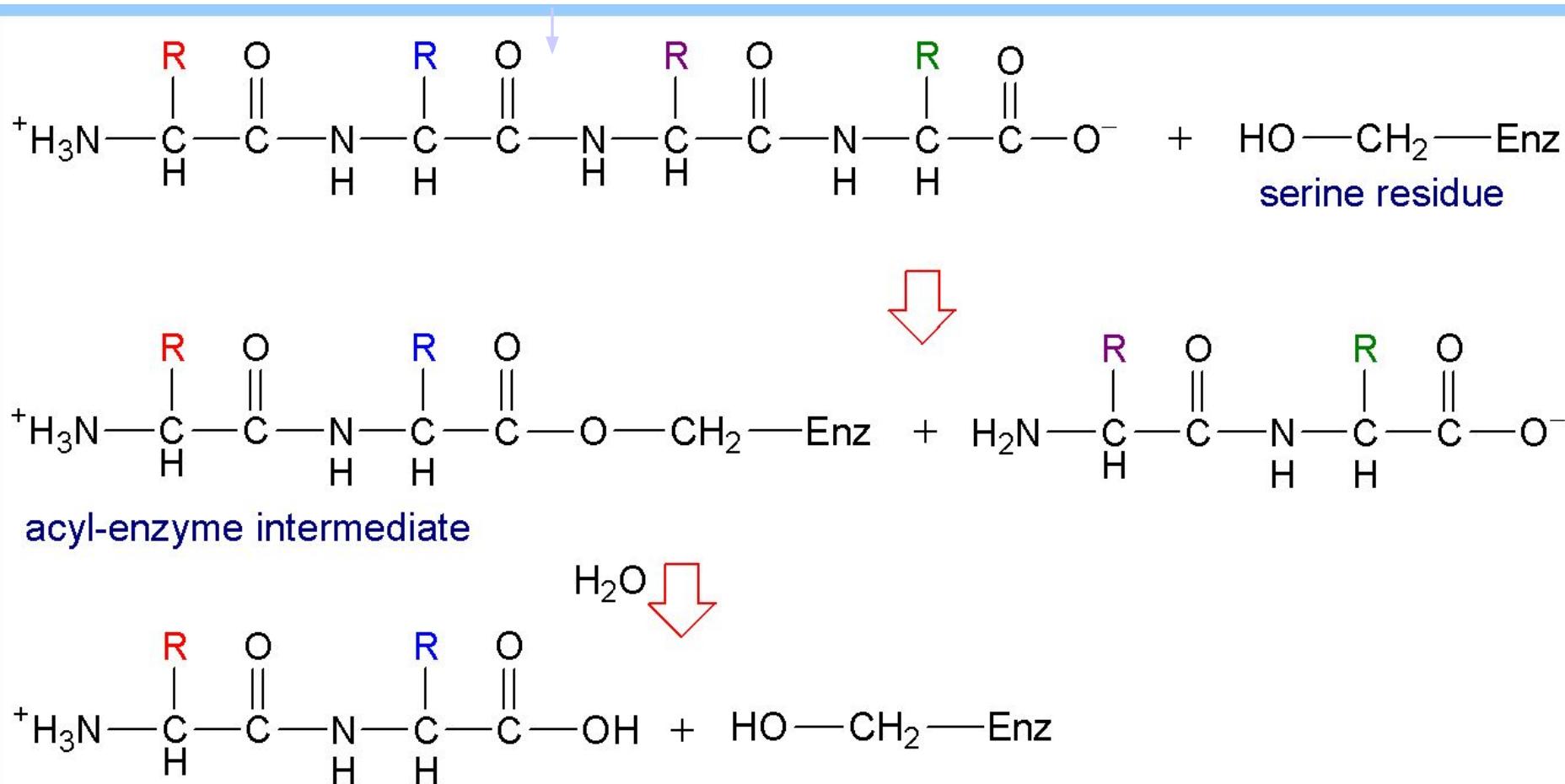
Catalytic Mechanism



During catalysis, there is nucleophilic attack of the **hydroxyl O** of a **serine** residue of the protease on the carbonyl **C** of the peptide bond that is to be cleaved.

An **acyl-enzyme intermediate** is transiently formed.

In this diagram a small peptide is shown being cleaved, while the usual substrate would be a larger polypeptide.

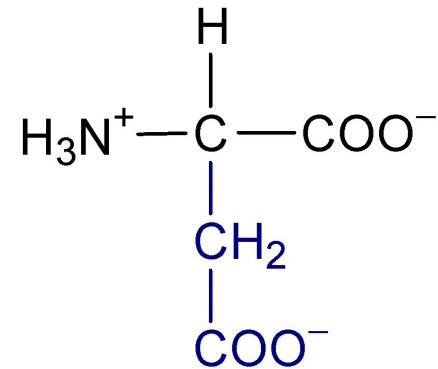


Hydrolysis of the ester linkage yields the second peptide product.

Aspartate proteases include

- the digestive enzyme **pepsin**
- Some proteases found in **lysosomes**
- the kidney enzyme **renin**
- HIV-protease.**

aspartate (Asp)



Two aspartate residues participate in **acid/base catalysis** at the active site.

In the initial reaction, one aspartate accepts a proton from an active site **H₂O**, which attacks the carbonyl carbon of the peptide linkage.

Simultaneously, the other aspartate donates a proton to the oxygen of the peptide carbonyl group.

Zinc proteases (metalloproteases) include:

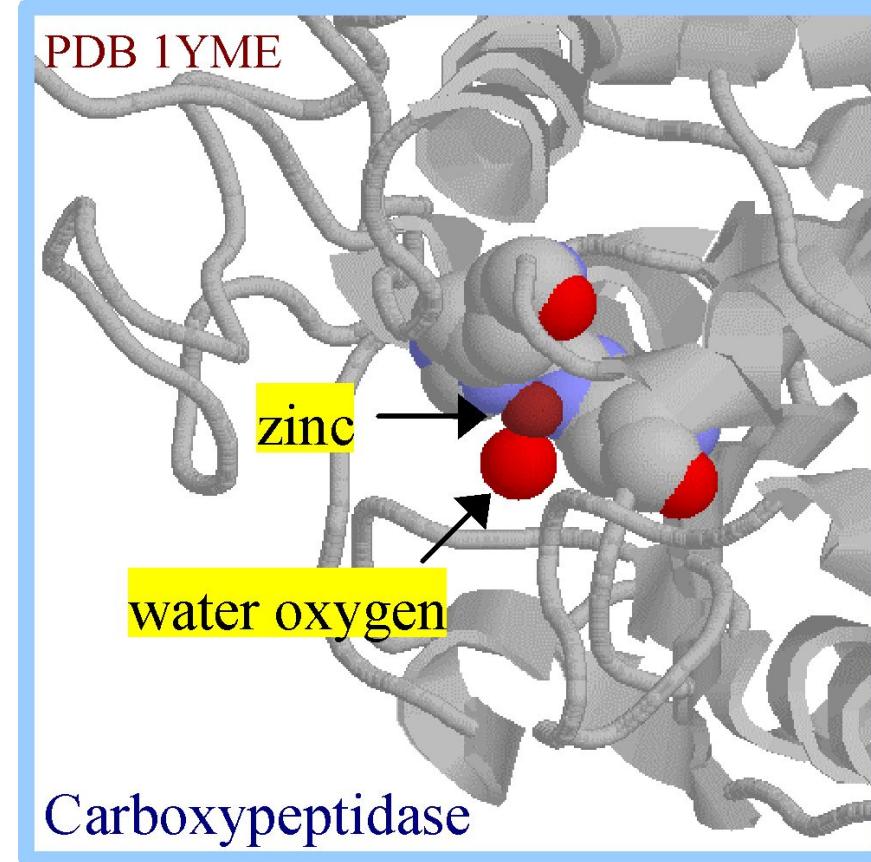
- ◆ digestive enzymes **carboxypeptidases**
- ◆ **matrix metalloproteases (MMPs)**, secreted by cells
- ◆ one **lysosomal** protease.

Some MMPs (e.g., collagenase) are involved in **degradation of extracellular matrix** during tissue remodeling.

Some MMPs have roles in cell **signaling** relating to their ability to release cytokines or growth factors from the cell surface by **cleavage** of membrane-bound **pre-proteins**.

A zinc-binding motif at the active site of a metalloprotease includes two **His** residues whose imidazole side-chains are ligands to the **Zn⁺⁺**.

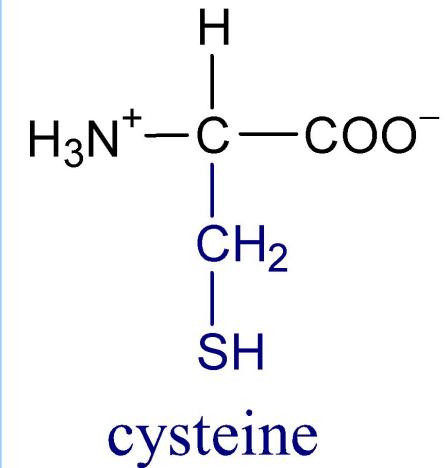
Colors in Carboxypeptidase image at right: **Zn**, **N**, **O**.



During catalysis, the **Zn⁺⁺** promotes nucleophilic attack on the carbonyl carbon by the oxygen atom of a **water** molecule at the active site.

An active site **base** (Glu in Carboxypeptidase) facilitates this reaction by extracting H⁺ from the attacking H₂O.

Cysteine proteases have a catalytic mechanism that involves a cysteine **sulphydryl** group.



Deprotonation of the cysteine SH by an adjacent His residue is followed by nucleophilic **attack of the cysteine S** on the peptide carbonyl carbon.

A **thioester** linking the new carboxy-terminus to the cysteine thiol is an intermediate of the reaction (comparable to acyl-enzyme intermediate of a serine protease).

Cysteine proteases:

- ◆ **Papain** is a well-studied **plant** cysteine protease.
- ◆ **Cathepsins** are a large family of **lysosomal** cysteine proteases, with varied substrate specificities.
- ◆ **Caspases** are cysteine proteases involved in activation & implementation of **apoptosis** (programmed cell death).
Caspases get their name from the fact that they cleave on the carboxyl side of an aspartate residue.
- ◆ **Calpains** are **Ca⁺⁺-activated** cysteine proteases that cleave intracellular proteins involved in cell motility & adhesion.
They regulate processes such as cell migration and wound healing.

Protease Applications in Food Processing

- **Basic rationale:** Proteases are a powerful tool for modifying the properties of food proteins.
 - Improved digestibility
 - Improved solubility
 - Modified functional properties: emulsification, fat-binding, water-binding, foaming properties, gel strength, whipping properties, etc.
 - Improved flavor & palatability
 - Improved processing: viscosity reduction, improved drying, etc.

Protease Applications in Food Processing

- Proteases are also used in a wide range of foods & food processing applications.
- Dairy: milk coagulation, flavor development
- Baking: gluten development
- Fish & seafood processing: fishmeals, enhanced oil recovery, aquaculture
- Animal protein processing: improved digestibility, reduced allergenicity, improved flavor, meat tenderization
- Plant protein processing: improved functionality & processing, generation of bio-active peptides.
- Yeast hydrolysis: flavor compounds.



Nonfood Protease Applications

- Medicine
- Pharmacology & drug manufacture
- Laundry & dishwashing detergents (#1)
- Hard surface cleaning formulations
- Contact lens cleaning formulations
- Waste treatment
- Industrial applications
- Fermentation (fuel EtOH, etc.)
- Chondroitin & heparin production
- Animal feed additives
- Digestive supplements

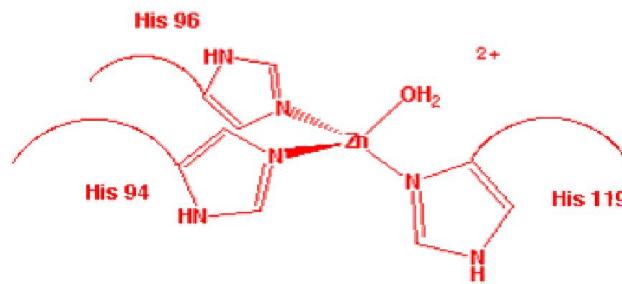


Carbonic anhydrase

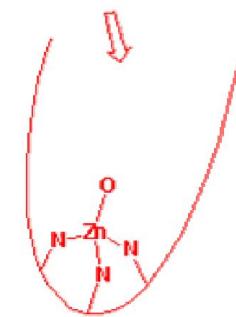
- Carbonic anhydrase, a zinc metallo-enzyme, is widespread throughout the bacterial, plant and animal kingdoms. It is generally considered one of the most efficient enzymes known as it catalyzes the reversible interconversion between CO_2 and the bicarbonate ion.

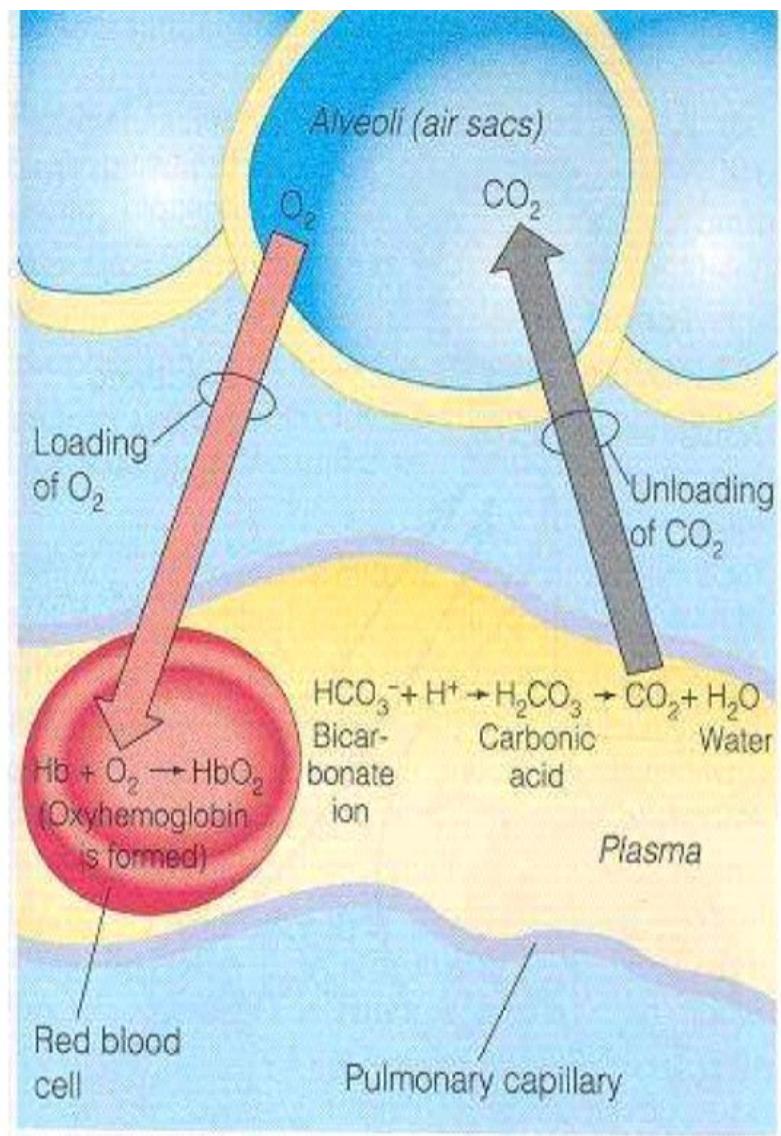
Catalytic Mechanism

The zinc coordination sphere of the "resting" enzyme

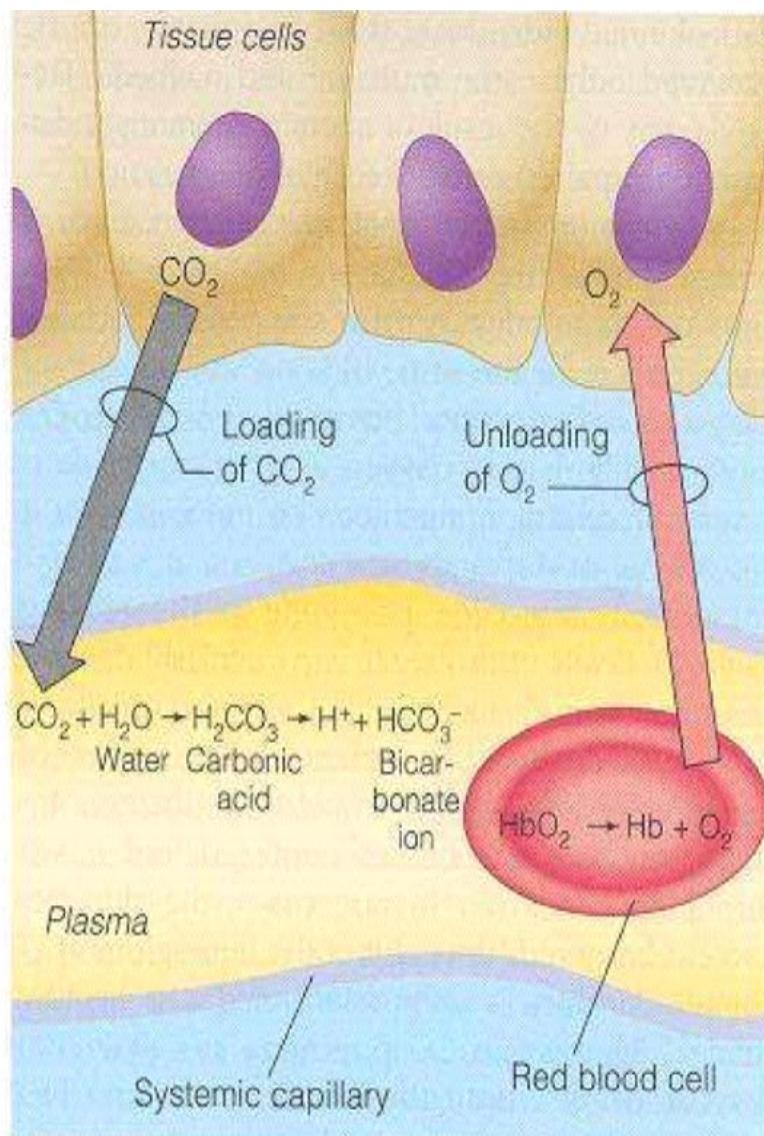


The enzyme "cleft"





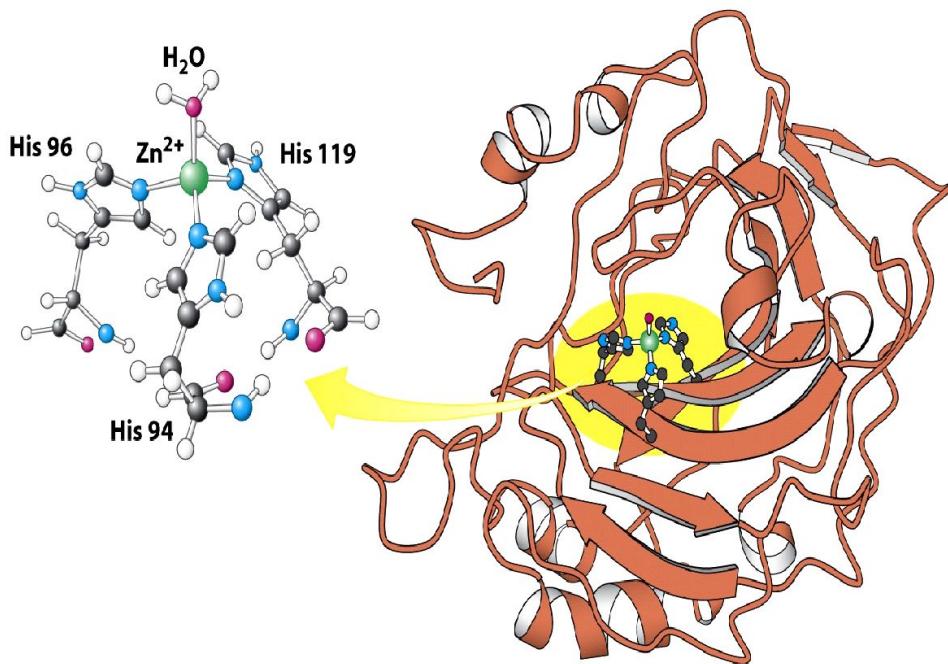
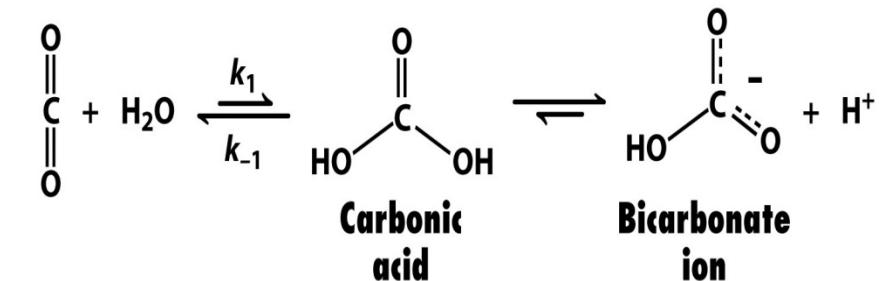
(a)



(b)

Diagrammatic representation of the major means of oxygen (O_2) and carbon dioxide (CO_2) loading and unloading in the body.

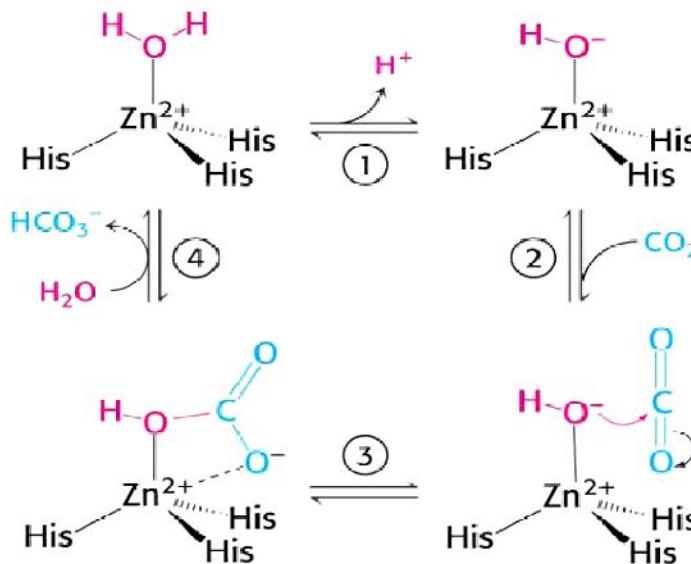
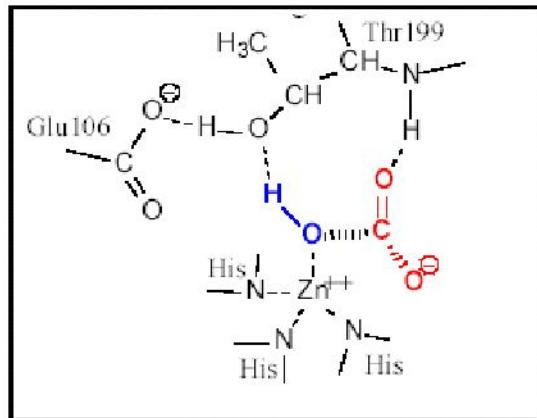
- 1) carbon dioxide
 - a major end product of aerobic metabolism
- 2) carbonic anhydrase as Zn^{2+} metalloenzyme
 - positive charge: strong but kinetically labile bonds
 - more than one oxidation state
 - Zn^{+2} : four or more ligands
 - three histidines + water
- 3) Zinc activation of water



convergent evolution for generation zinc-based active sites

- α -carbonic anhydrase: one conserved histidine
- β -carbonic anhydrase: three conserved histidines
- γ -carbonic anhydrase: trimeric, zinc sites at the interfaces

Reaction Mechanism



- (1) Zn(II) assists in the formation of a nucleophile (hydroxide) with a pK_a of near 7.0
- (2) CO_2 binds to enzyme's active site and is positioned to react with hydroxide
- (3) Hydroxide attacks carbon dioxide converting it to the bicarbonate ion
- (4) Water molecule binds to active site, thus releasing bicarbonate product

Climate Change

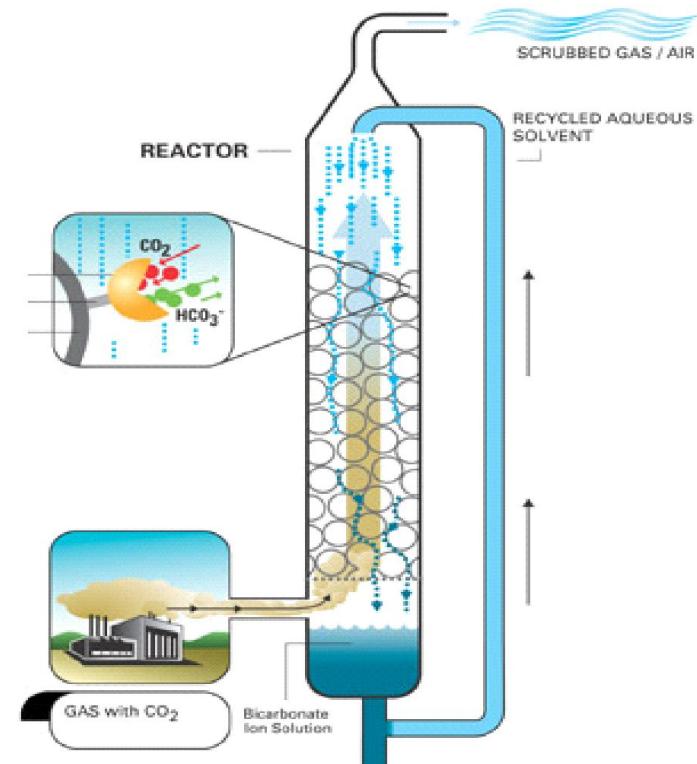
Nature does capture CO₂

- **One more type of air pollution**
 1. Toxic gas pollution (acid rain)
 - Industries, local impacts
 2. Ozone layer depletion (CFC)
 - Industries and households, regional impacts
 3. Air temperature is rising (Greenhouse gases)
 - Industries, transports, agriculture, homes, individuals
 - Global impacts on nature and humans
- **Greenhouse Gases (GHG)**
 - Carbon dioxyde (CO₂)
 - Methane (CH₄)

- **Forests inhale atmospheric CO₂**
 - Not enough trees for man-made CO₂
- **CO₂ capture in human muscles**
 - Cellular CO₂ transformed into bicarbonate
 - Carbonic anhydrase enzyme
- **A new industrial bio-catalyst**
 - Genetically engineered enzyme
 - Allows industrial CO₂ capture

CO₂ Solution Inc. offers an innovative alternative

- CO₂ capture at a large municipal waste incinerator (Quebec City)
- *CO₂ Solution's* Industrial prototype
 - 5 weeks non-stop, 7/24
 - 50% CO₂ capture
 - Permanent sequestration
- Sound basis for larger scale-up



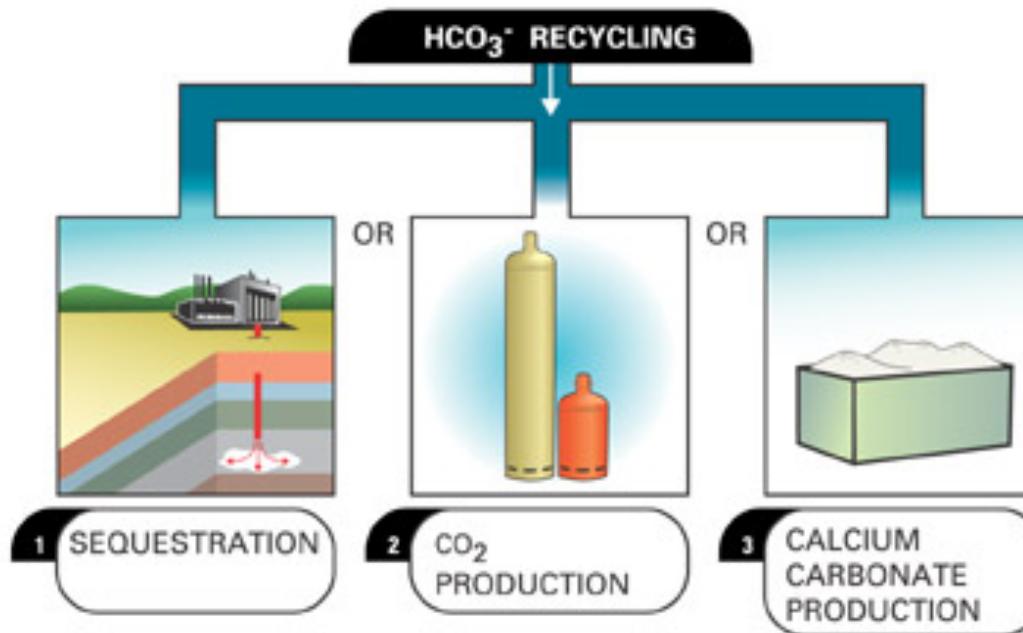
- CO₂ gas is converted into an unharful aqueous by-product, inside the reactor
- Low energy process
- Captured Carbon can be recycled

Sustainable Development

- *CO₂ Solution's* bio-catalysis treatment unit:
 - instantaneously converts harmful CO₂ gas
 - into unharful bicarbonate ion (HCO₃[–]) solution

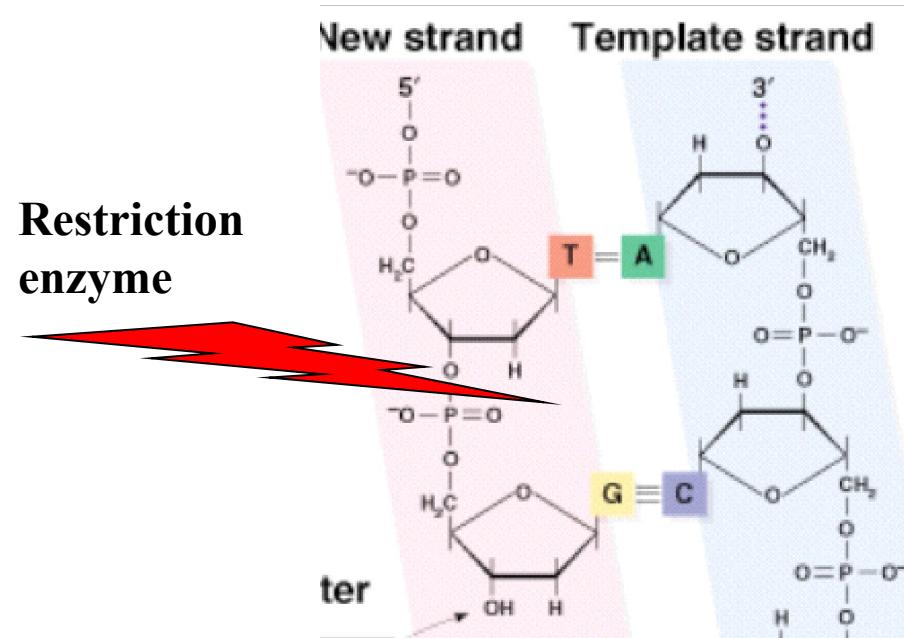
Offers flexible alternatives for CO₂ sequestration

Fossil carbon for energy production can be reused for chemicals



Restriction enzymes

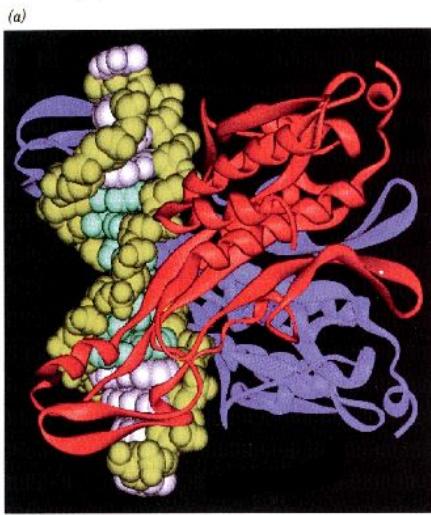
- Recognizes specific base sequences in double-helical DNA and cleave, at specific places, both strands of a duplex containing the recognized sequences.
- Restriction enzymes recognize specific bases pair sequences in DNA called restriction sites and cleave the DNA by hydrolyzing the phosphodiester bond.
- Cut occurs between the 3' carbon of the first nucleotide and the phosphate of the next nucleotide.
- Restriction fragment ends have 5' phosphates & 3' hydroxyls.



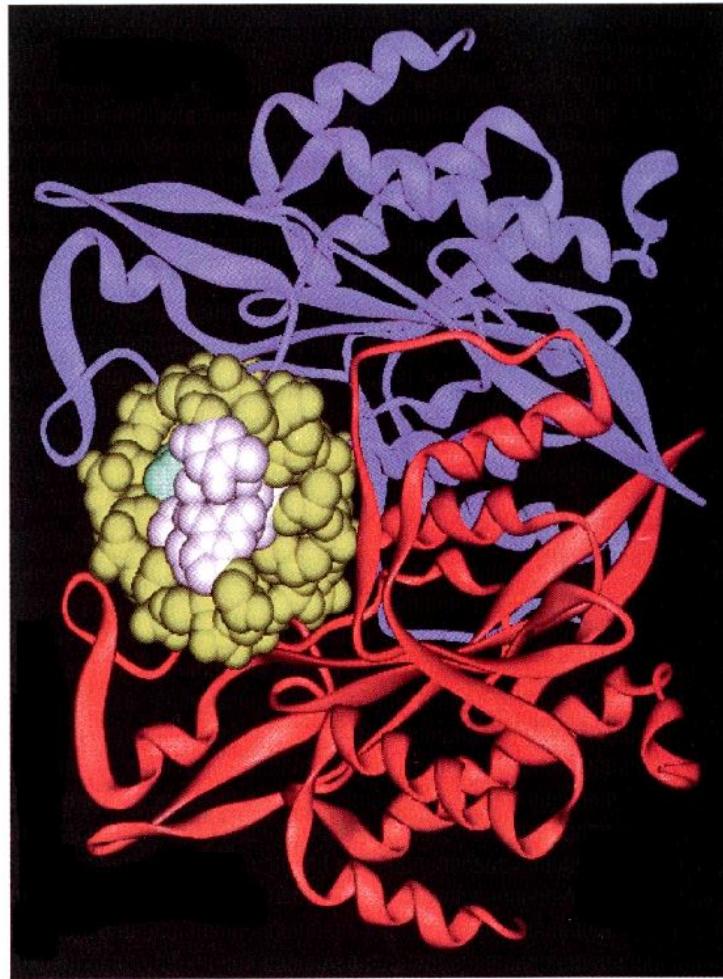
- Most restriction enzymes occur naturally in bacteria.
- Protect bacteria against viruses by cutting up viral DNA.
- Bacteria protects their DNA by modifying possible restriction sites (methylation).
- More than 400 restriction enzymes have been isolated.
- Names typically begin with 3 italicized letters.
 - Enzyme Source
 - *Eco*RI *E. coli* RY13
 - *Hind*III *Haemophilus influenzae* Rd
 - *Bam*HI *Bacillus amyloliquefaciens* H
- Many restriction sites are palindromes of 4-, 6-, or 8-base pairs.
- Short restriction site sequences occur more frequently in the genome than longer restriction site sequences, e.g., $(1/4)n$.

Structure of a restriction endonuclease

Restriction enzymes are usually dimers of identical subunits, analogous to the symmetry of their binding sites in DNA.



b)

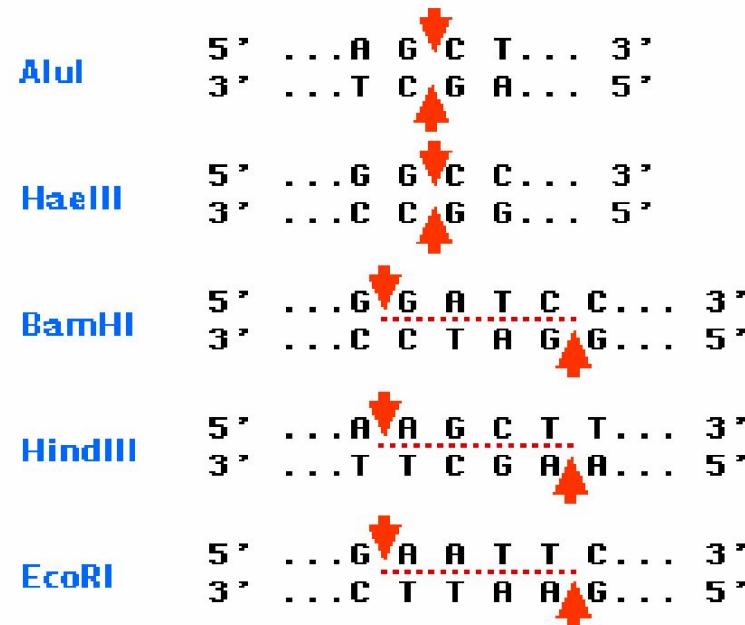


Patterns of DNA cutting by restriction enzymes

Base pairing between overhangs with complementary sequences enables two fragments to be joined by another enzyme DNA Ligase.

A sticky end fragment can be ligated not only to fragment from which it was originally cleaved, but also to any other fragment with a compatible sticky end.

The result is a molecule of recombinant DNA (rDNA)



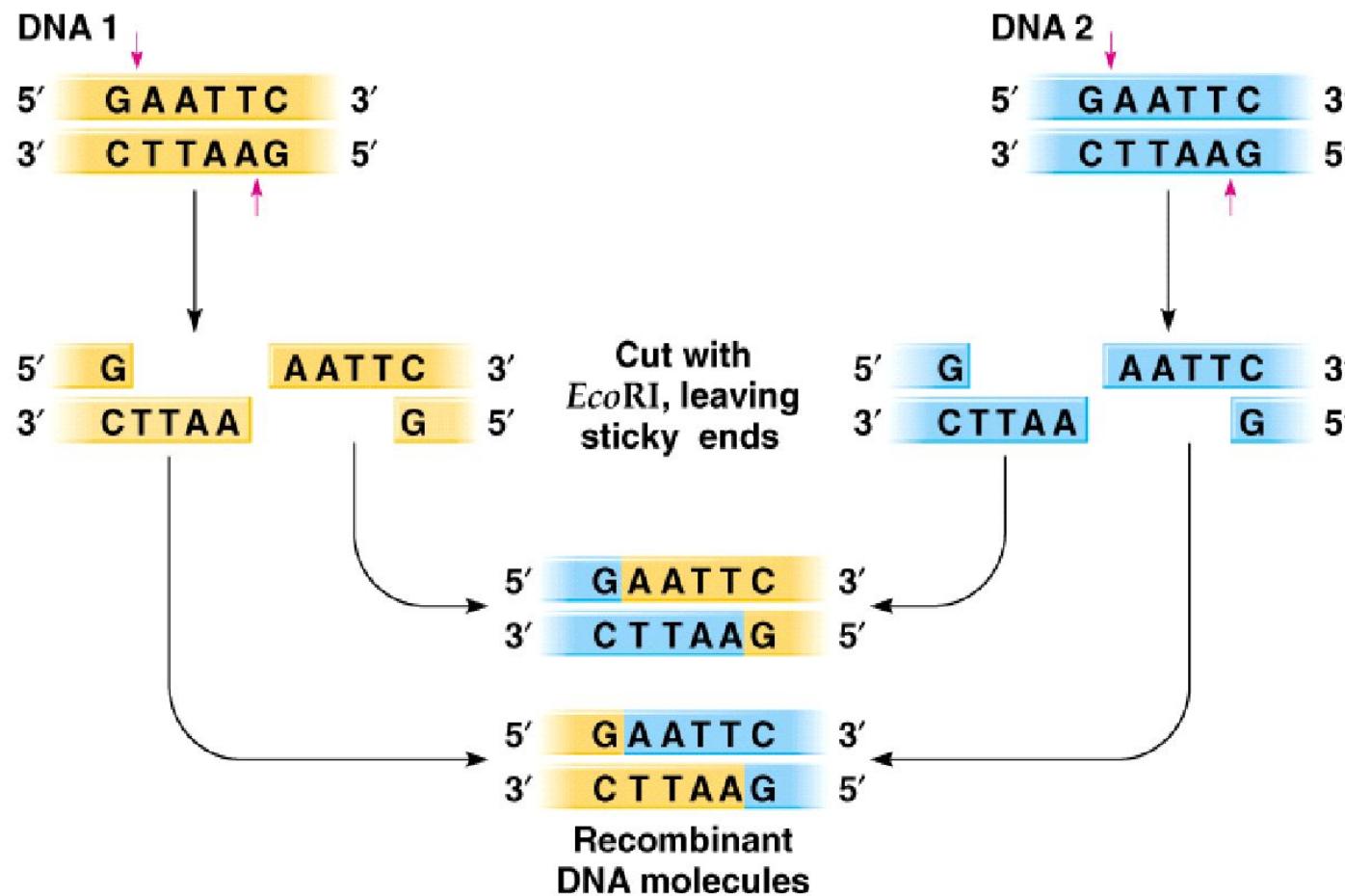
AluI and **HaeIII** produce blunt ends

BamHI **HindIII** and **EcoRI** produce "sticky" ends

Recombinant DNA:

DNA molecules constructed *in vitro*, consisting of DNA from 2 or more sources (i.e. cloning vector with foreign DNA inserted)

Cut and ligate 2 DNAs with *Eco*RI ----> recombinant DNA



Applications of Recombinant DNA technologies

- pharmaceutical products
 - insulin – cheaper and safer compared to animal insulin
 - vaccine sub-unit (against hepatitis B) – safer since will not be infected by pathogens
 - DNA of vaccines against malaria, influenza etc.
- gene therapy
 - replacing defective or missing gene with normal gene using adeno~ and retrovirus as vector
- gene silencing
 - known as RNA interference (RNAi) using dsRNA called short interfering RNA (siRNA) that target specific gene (mRNA) and degrade it
- human genome project
 - 3 billion human genome nucleotides have been sequenced
 - 20,000 – 25,000 genes
 - < 2% encodes function products, 98% intron, transposon (repeating sequence) etc
 - may provide diagnostics and treatments

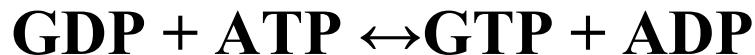
Purine synthesis

Purine monophosphates must be converted to the triphosphate forms before they can be utilized in DNA synthesis. This is done in two steps.

1. The nucleoside monophosphate kinase class of enzymes phosphorylates the monophosphate nucleotides.



2. The nucleoside diphosphate kinases add another phosphate group to the nucleotide diphosphates



Nucleoside monophosphate kinase (NMP kinase)

1) NMP + ATP (or NTP) \rightleftharpoons NDP + ADP (or NDP)

- transfer of a phosphoryl group from ATP to NMP, not to water

- induced fit

2) P-loop structure

- conserved NTP-binding domain: central β sheet surrounded by α helices

- a loop between the first β strand and the first α helix: Gly-X-X-X-X-Gly-Lys

- present in a wide variety of nucleotide-binding proteins

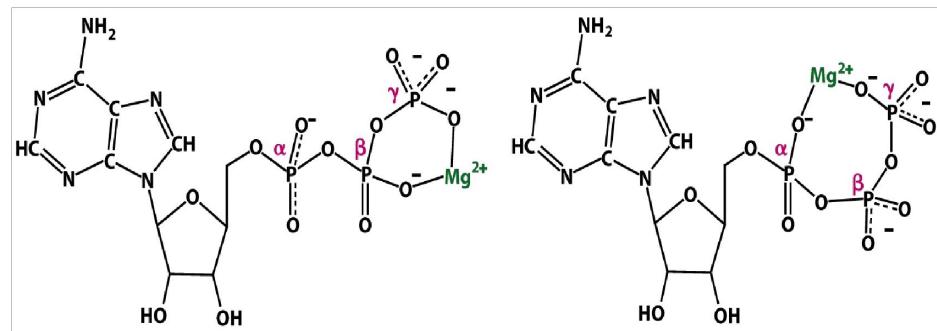
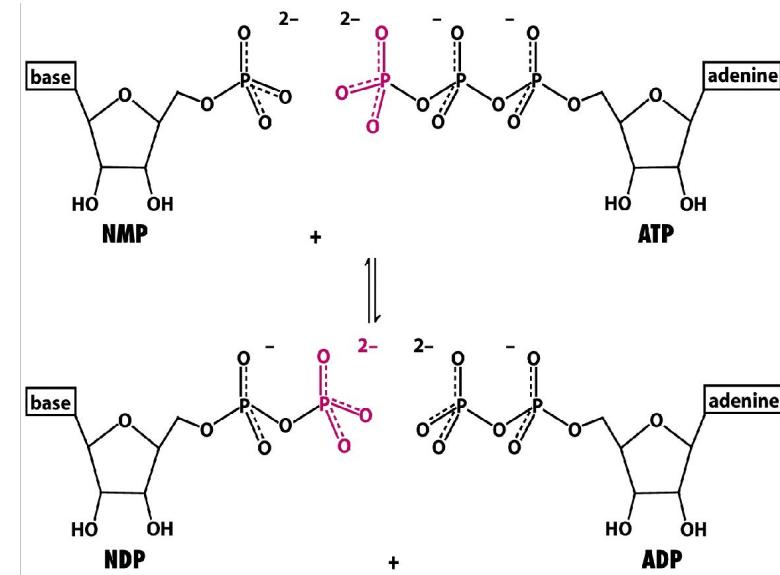
3) magnesium (or manganese) complex of NTP

- requirement of divalent metal ions, Mg²⁺ or Mn²⁺

- neutralization of some negative charges

- well-defined conformations of NTP by coordination: α and β , β and γ , or α and γ .

- more binding energy by interaction with NTP-Mg²⁺ complex

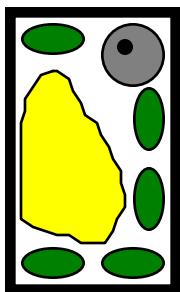


Photosynthesis

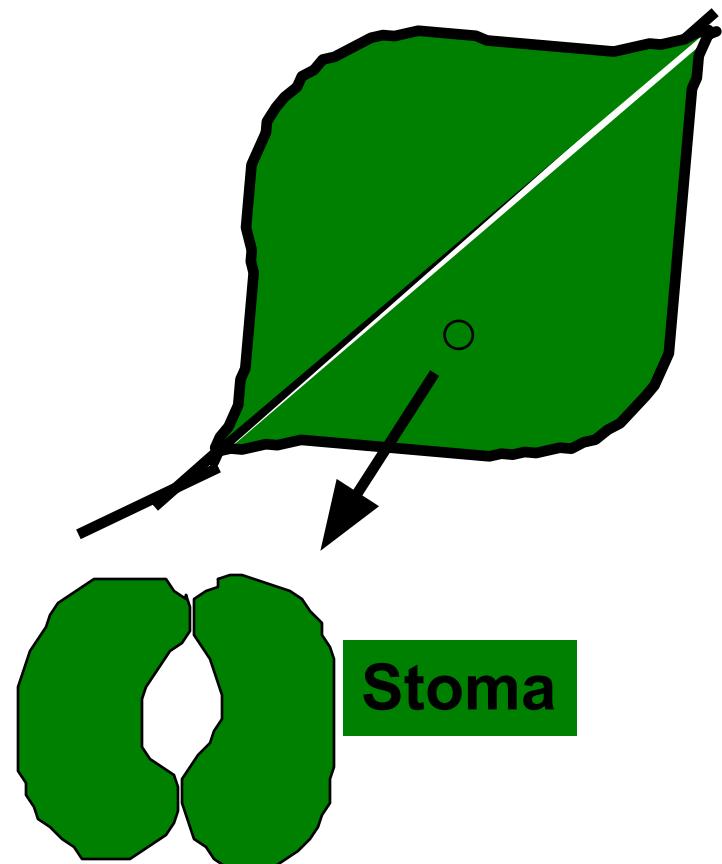
- Autotrophs – produce their own food (glucose) **Plants**
- Process called photosynthesis
- Mainly occurs in the leaves:
 - a. stoma - pores
 - b. mesophyll cells



Mesophyll
Cell →

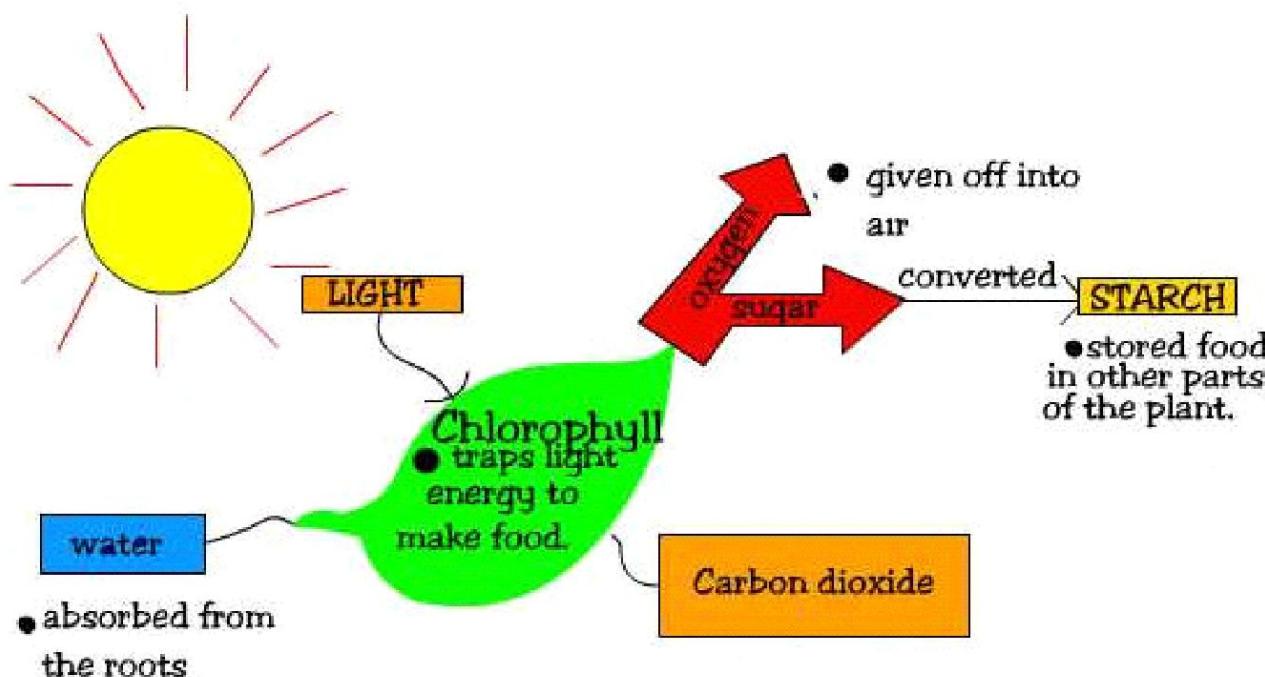
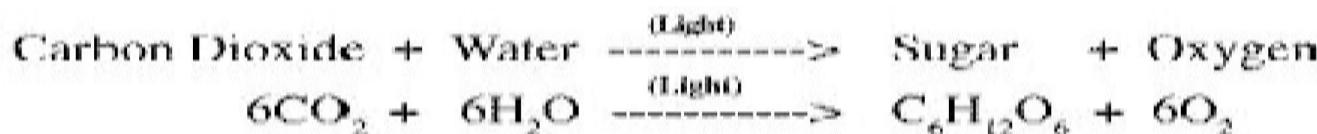
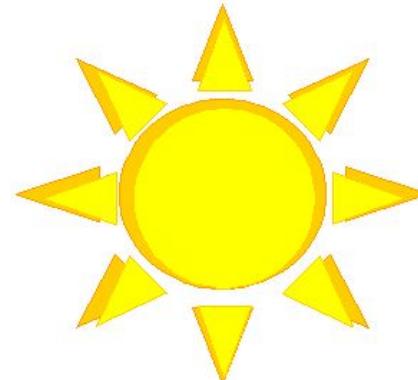


Chloroplast



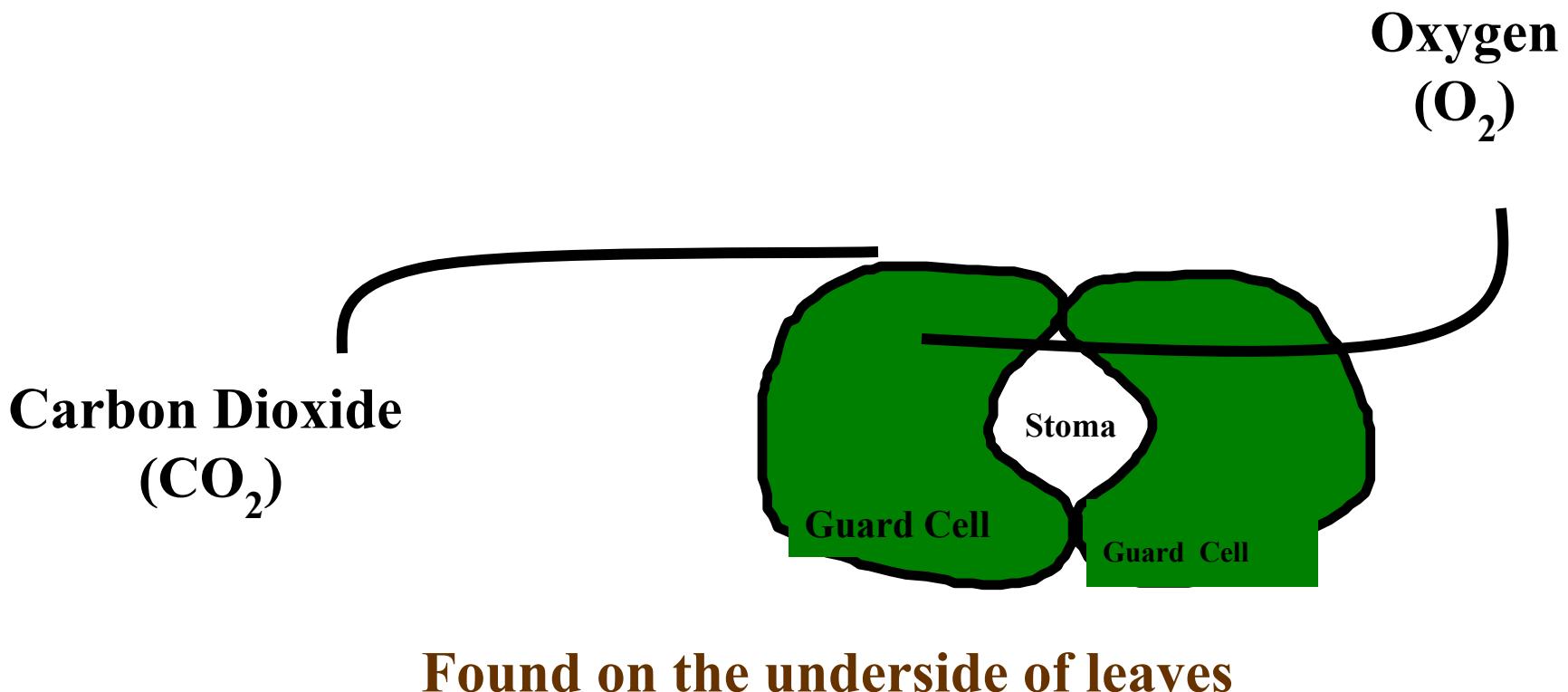
Photosynthesis

- Involves the Use Of light Energy to convert Water (H_2O) and Carbon Dioxide (CO_2) into Oxygen (O_2) and High Energy Carbohydrates (sugars, e.g. Glucose) & Starches



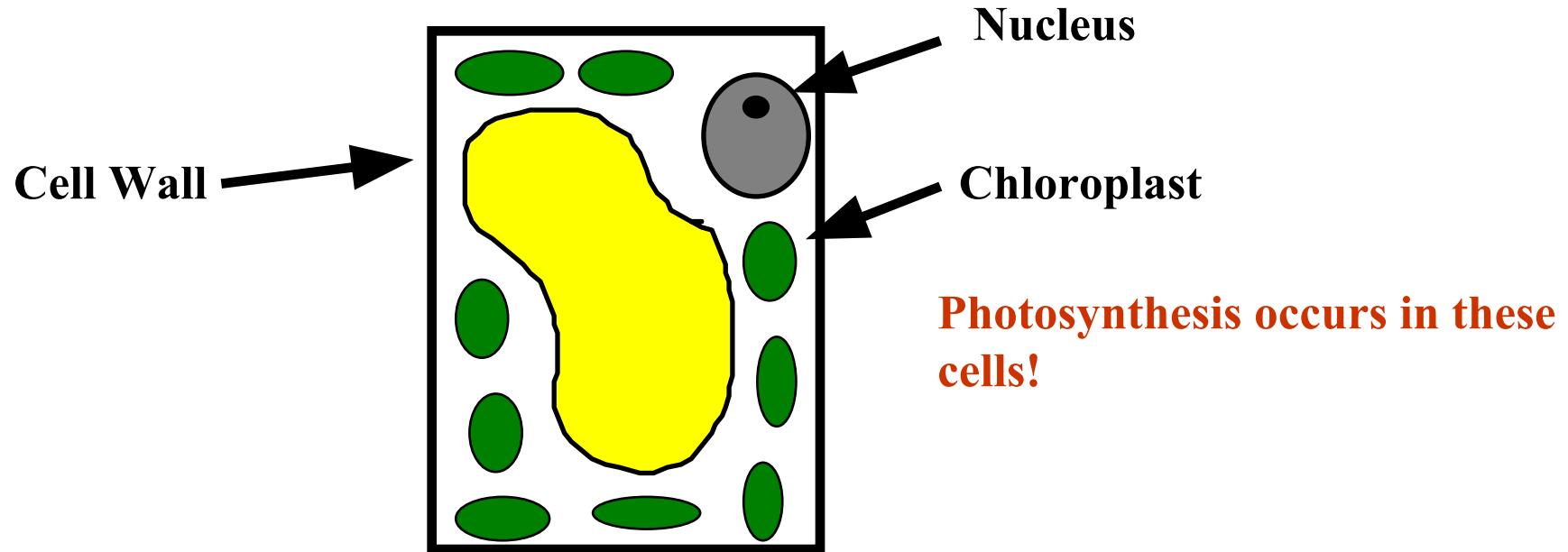
Stomata (stoma)

Pores in a plant's cuticle through which water vapor and gases (CO_2 & O_2) are exchanged between the plant and the atmosphere.



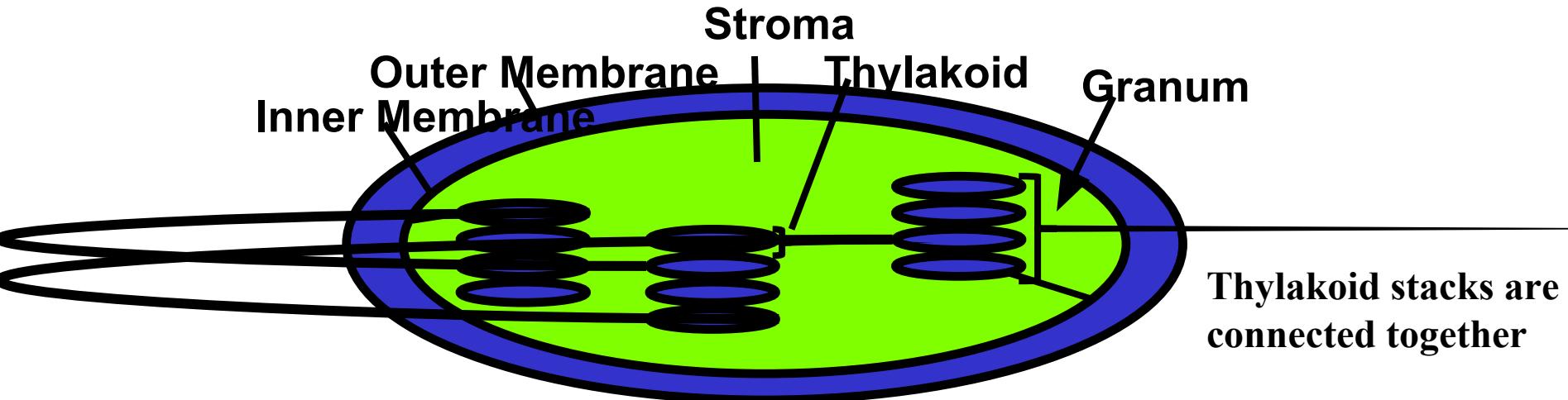
Found on the underside of leaves

Mesophyll Cell of Leaf



Chloroplast

Organelle where photosynthesis takes place.



Chlorophyll



- In addition to water, carbon dioxide, and light energy, photosynthesis requires **Pigments**
- Chlorophyll is the primary light-absorbing pigment in autotrophs
- Chlorophyll is found inside **chloroplasts**
- Located in the **thylakoid membranes**
- Chlorophyll have **Mg⁺** in the center
- Chlorophyll pigments **harvest energy (photons)** by absorbing certain wavelengths (**blue-420 nm and red-660 nm** are most important)
- Plants are **green** because the **green wavelength is reflected**, not absorbed.

Two Parts of Photosynthesis

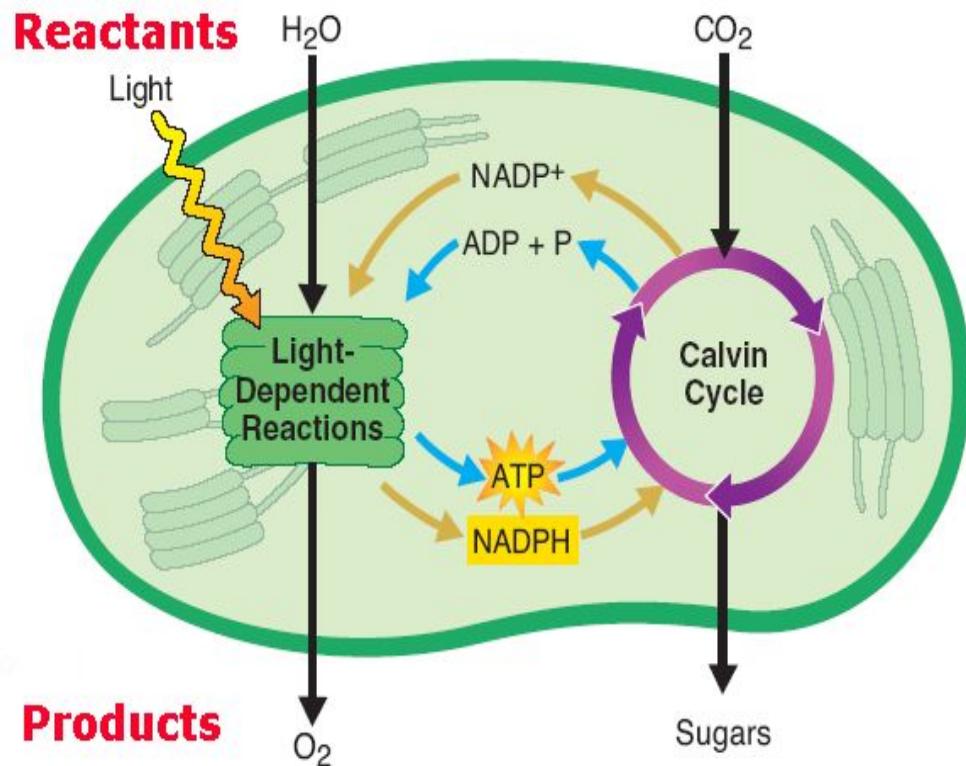
Two reactions make up photosynthesis:

1. Light Reaction or Light-dependent Reaction

- Produces energy from solar power (photons) in the form of ATP and NADPH.
- Occurs in Thylakoid membranes

2. Calvin Cycle or Light-independent Reaction

- Also called Carbon Fixation or C₃ Fixation
 - 3-carbon molecule called Ribulose Biphosphate (RuBP) is used to regenerate the Calvin cycle
- Uses energy (ATP and NADPH) from light reaction to make sugar (glucose).
- Occurs in Stroma



Factors Affecting Photosynthesis

- Amount of available water
- Temperature
- Amount of available light energy



Biological Energy production

Bioenergetics

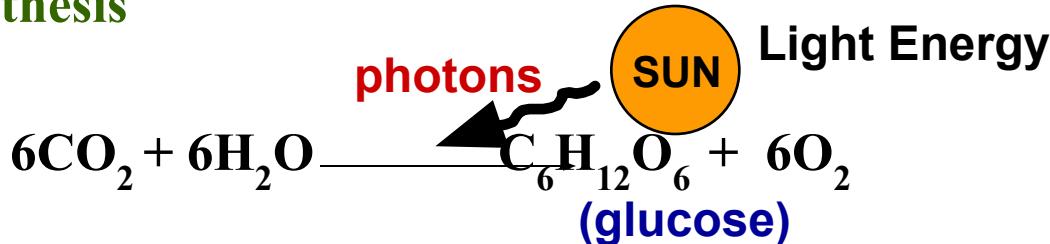
- The study of energy in living systems (environments) and the organisms (plants and animals) that utilize them
- Energy: Required by all organisms
 - May be Kinetic or Potential energy
- Kinetic Energy: Energy of Motion
 - Heat and light energy are examples
- Potential Energy: Energy of position
 - Includes energy stored in chemical bonds



Two Types of Energy Reactions

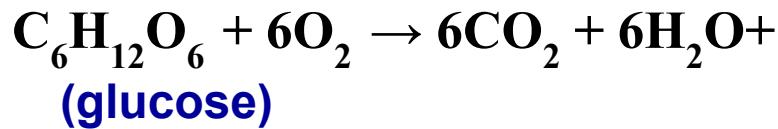
- Endergonic Reactions:

- Chemical reaction that requires a net input of energy.
 - Photosynthesis



- Exergonic Reactions :

- Chemical reactions that releases energy
 - Cellular Respiration



Two Types of Metabolism

- Anabolic Pathways

- Metabolic reactions, which consume energy (endergonic), to build complicated molecules from simpler compounds

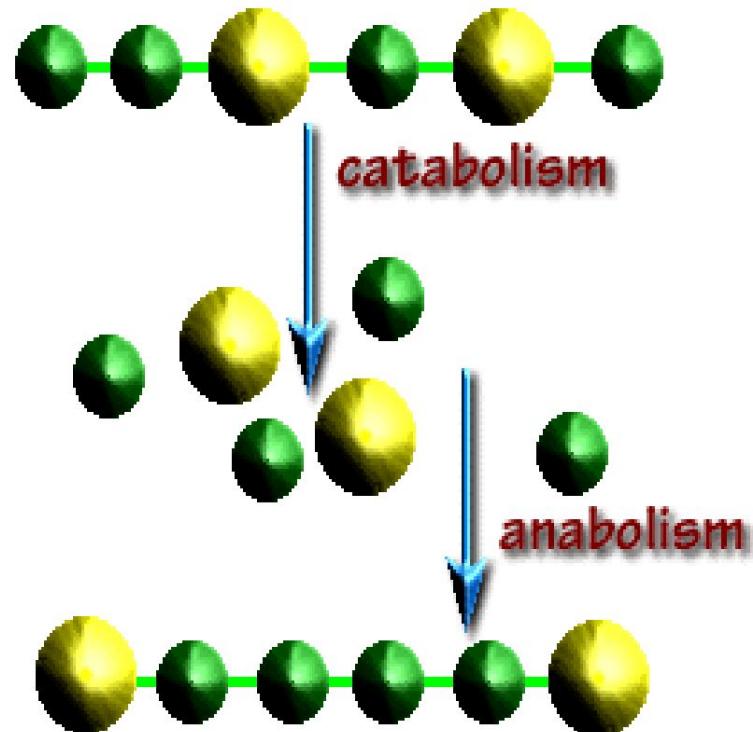
- Photosynthesis

- Catabolic Pathways

- Metabolic reactions which release energy (exergonic) by breaking down complex molecules in simpler compounds

- Cellular Respiration

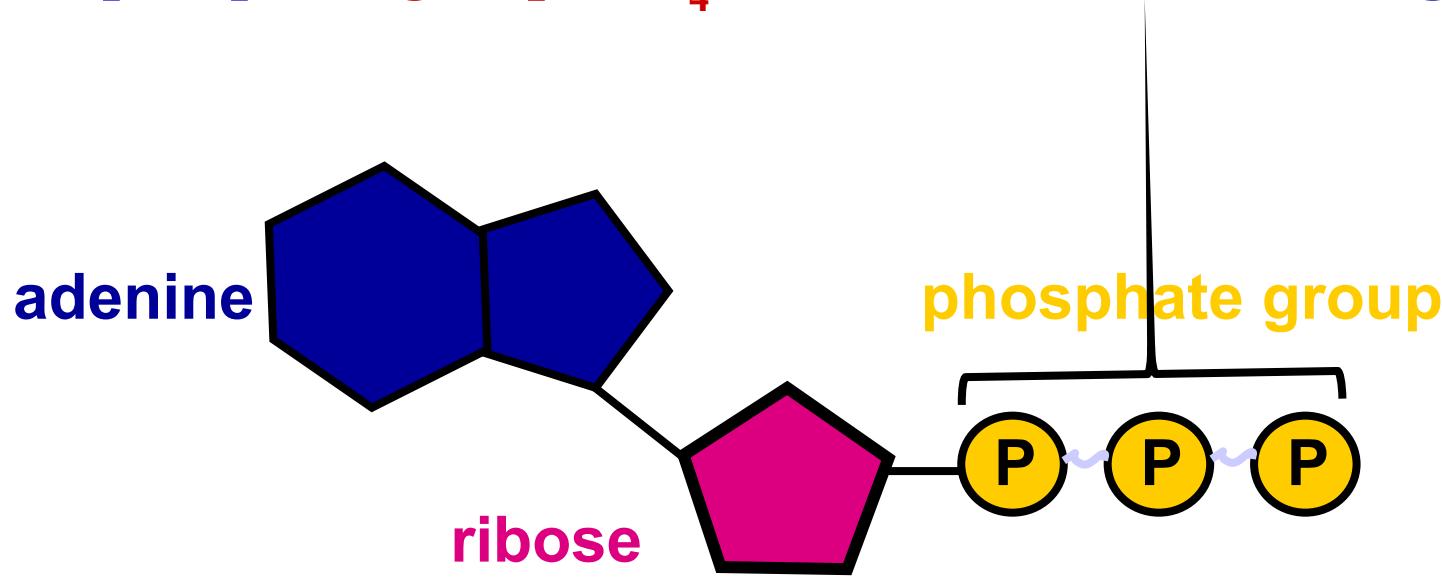
Metabolism
breaking down and building up



Cellular Energy-ATP

- Components:
 1. adenine: nitrogenous base
 2. ribose: five carbon sugar
 3. phosphate group: chain of 3

Three phosphate groups-(two with high energy bonds
Last phosphate group (PO_4) contains the MOST energy



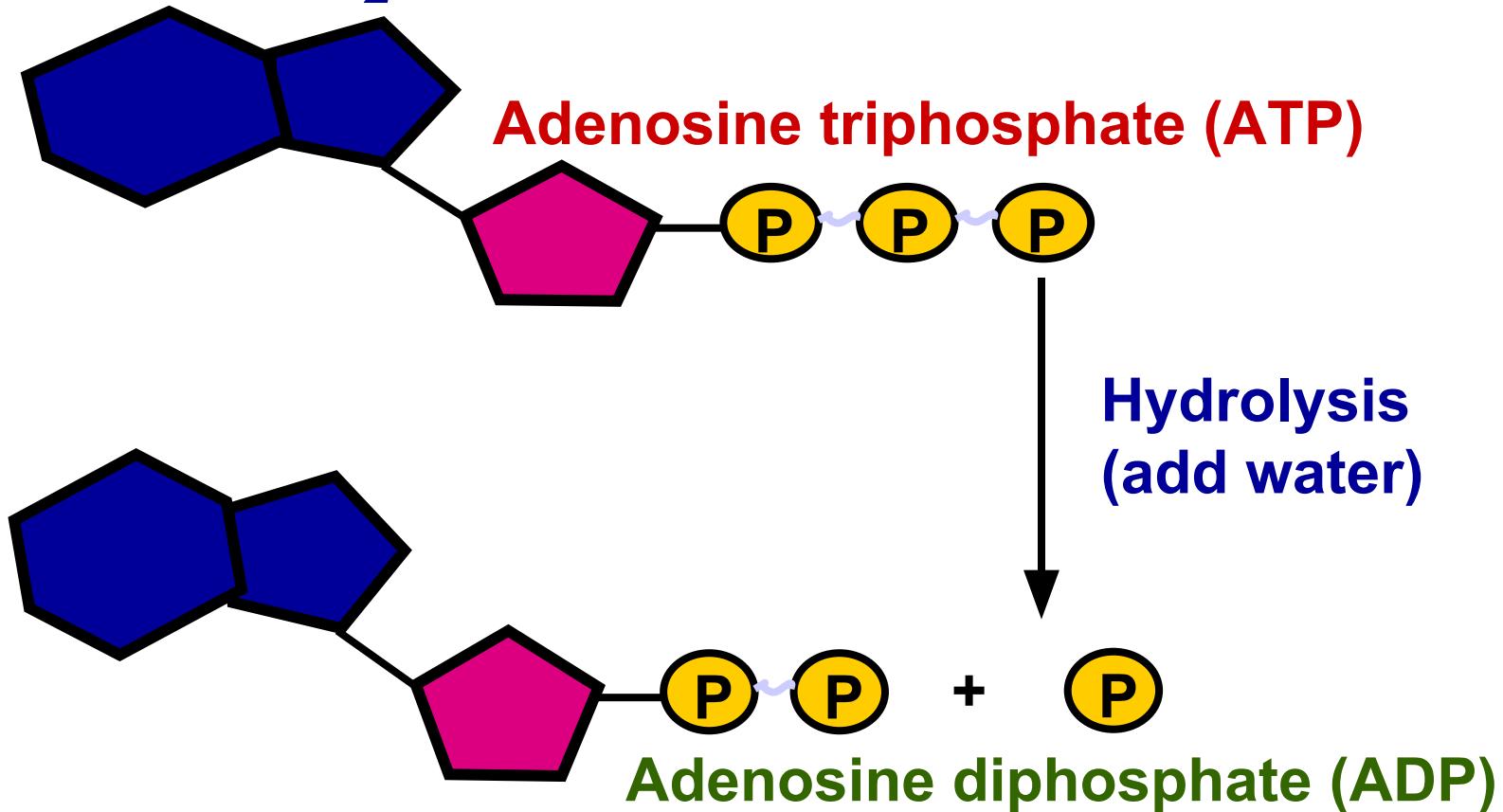
How does ATP work ?

- Organisms use enzymes to break down energy-rich glucose to release its potential energy
- This energy is trapped and stored in the form of adenosine triphosphate(ATP)
- It is estimated that each cell will generate and consume approximately 10,000,000 molecules of ATP per second

ATP - LIKE A RECHARGEABLE BATTERY

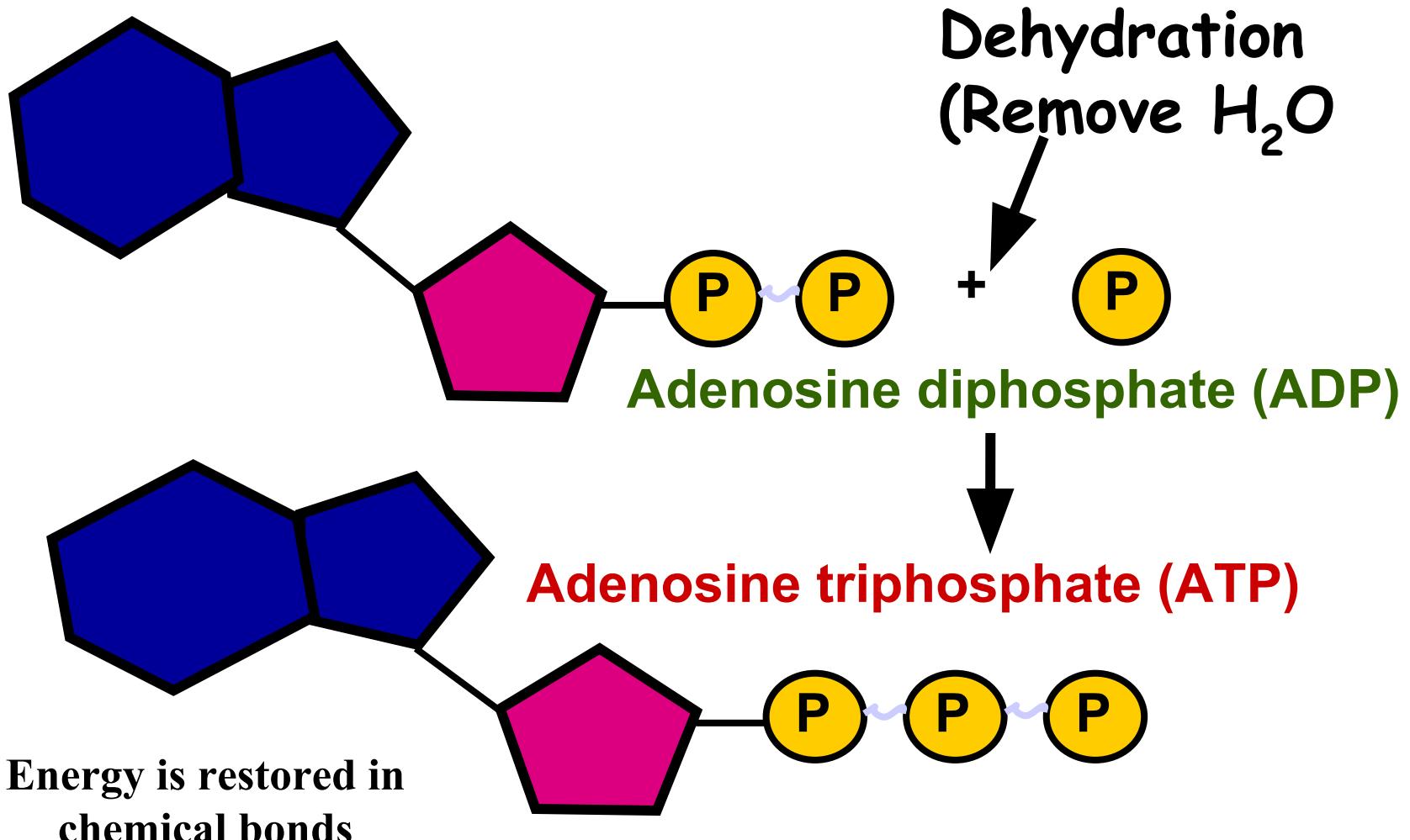
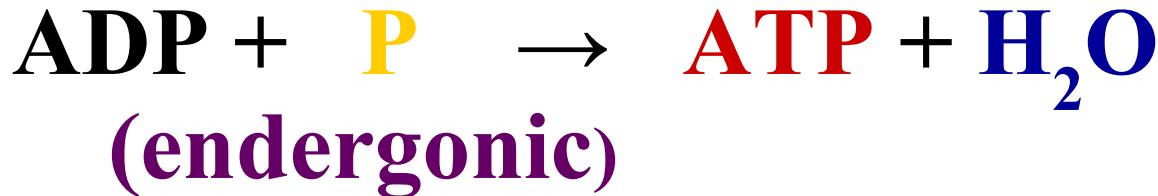


Hydrolysis of ATP



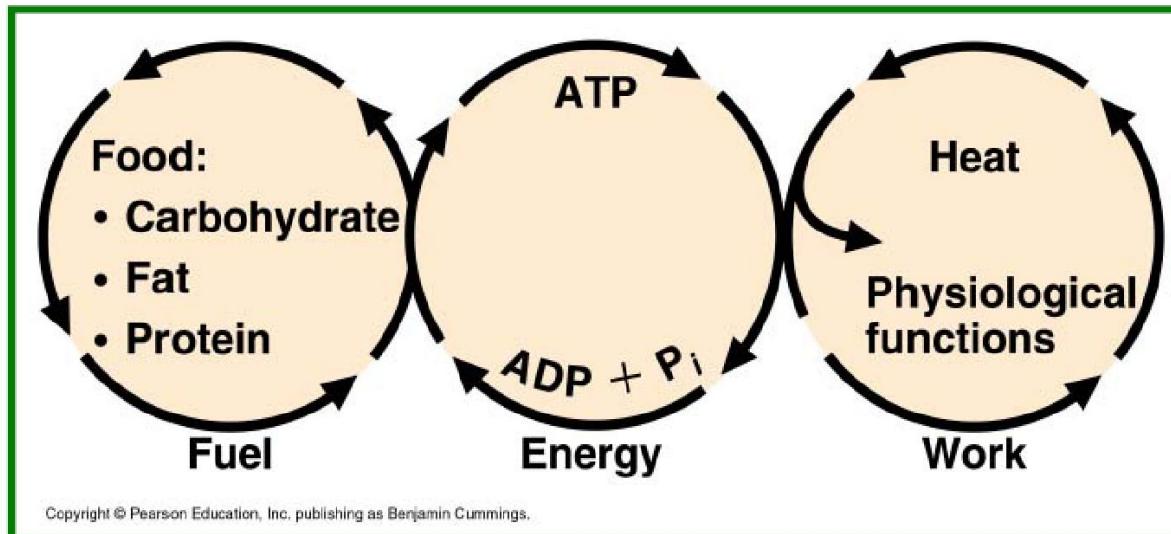
Energy is used by cells

Dehydration of ATP



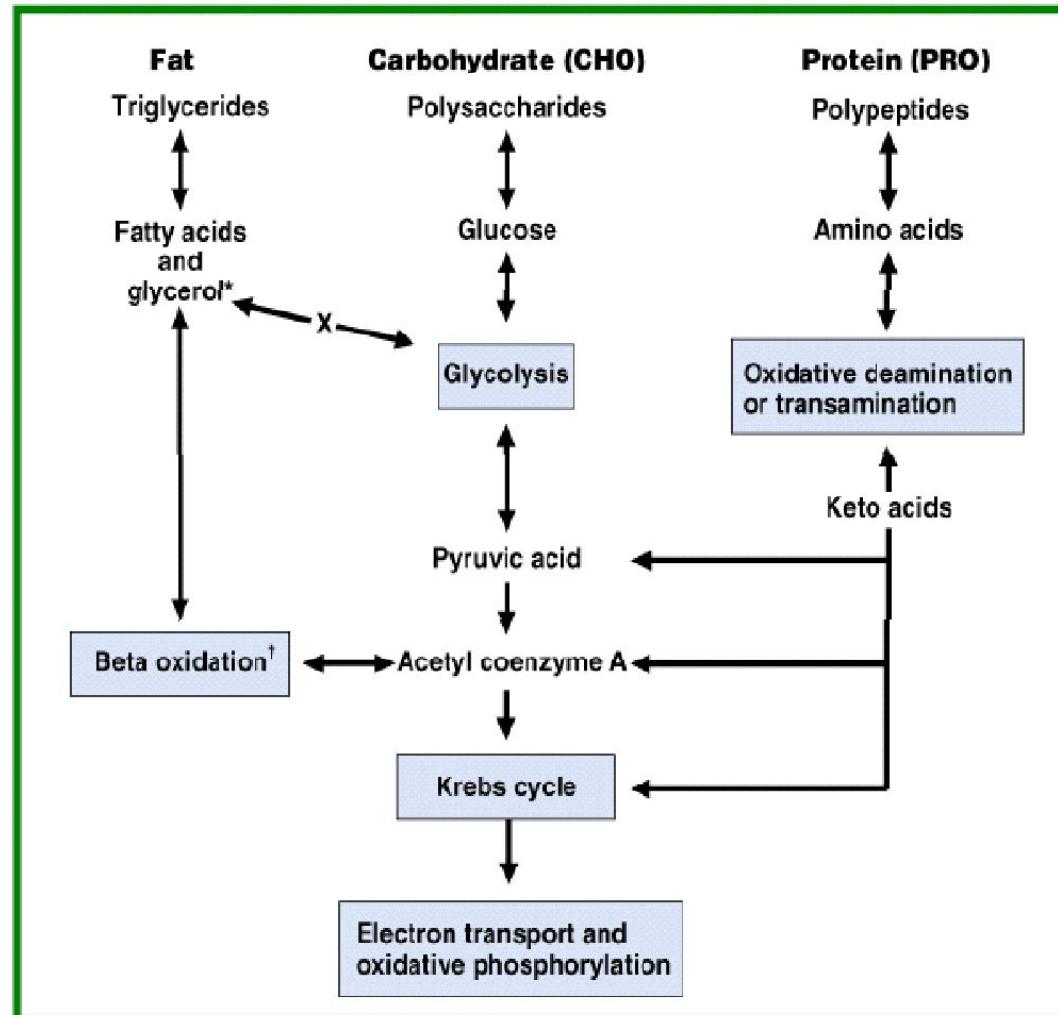
Biological Energy Cycle

- Food + O₂ → CO₂ + H₂O + energy
 - Chemical
 - Mechanical
 - 60-70% of this energy is heat
 - The rest is used for
 - Muscle contraction
 - Cellular operations (respiration)
 - Digestion and absorption
 - Synthesis of new compounds
 - Glandular function



Biological Energy Cycle

- ***Cellular Respiration*** - The process by which cells transfer energy from food to ATP in a stepwise series of reactions; relies heavily upon the use of oxygen
- ***Anaerobic*** - In the absence of, not requiring, nor utilizing oxygen
- ***Aerobic*** - In the presence of, requiring, or utilizing oxygen



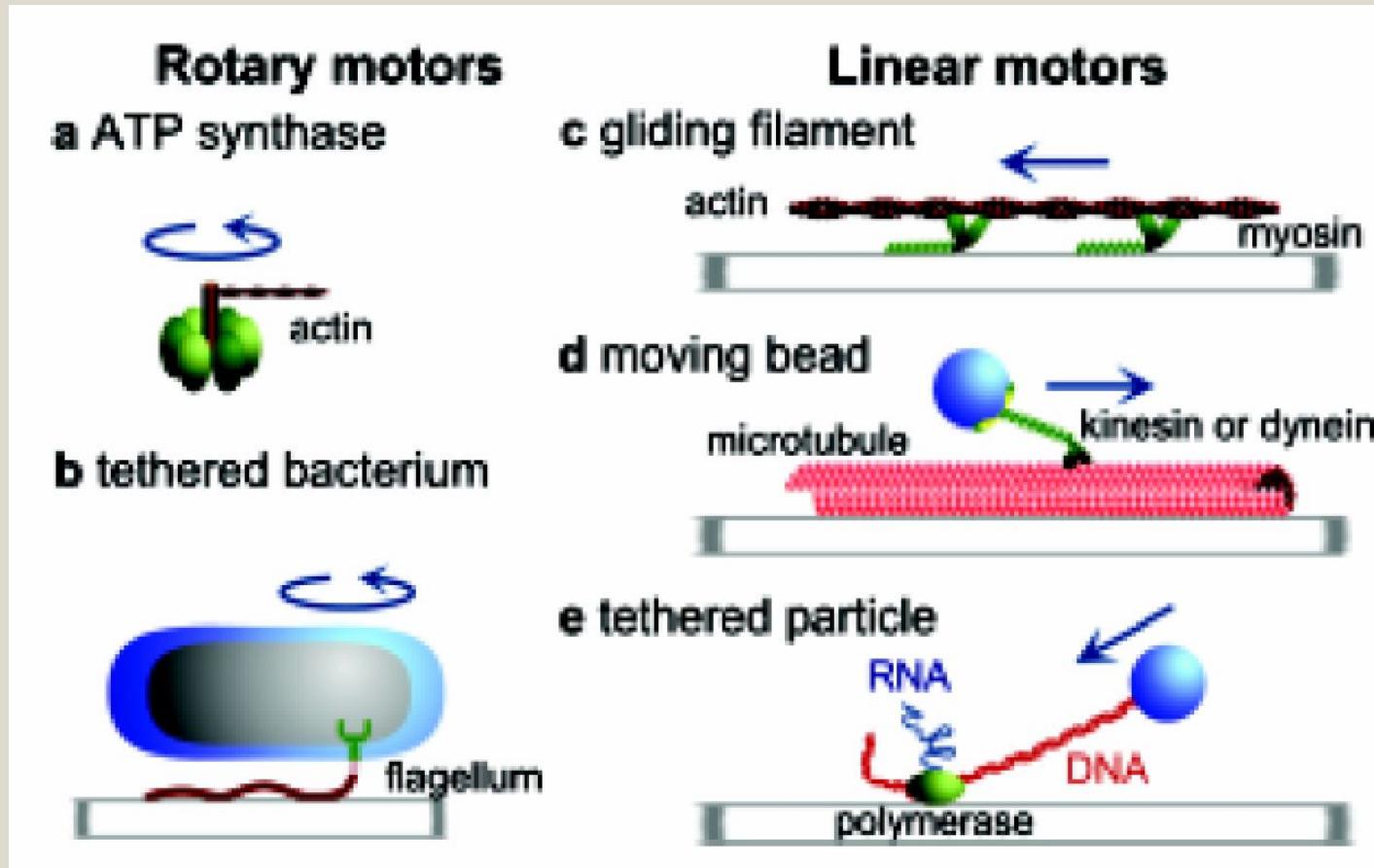
Unit 4 Mechanochemistry

- Molecular Machines/Motors
- Properties of ATP-based Protein Molecular Machines
 - The F0F1-ATP Synthase Motors
 - Coupling and Coordination of Motors
- The Bacterial Flagellar Motor
 - Flagellar motor structure
- Cytoskeleton
- Bioremediation
- Biosensors

Molecular Machines/Motors

- *Molecular machines can be defined as devices that can produce useful work through the interaction of individual molecules at the molecular scale of length.*
- *A convenient unit of measurement at the molecular scale would be a nanometer. Hence, molecular machines also fall into the category of nanomachines.*
- *As our knowledge and understanding of these numerous machines continues to increase, we now see a possibility of using the natural machines, or creating synthetic ones from scratch, by mimicking nature.*

Different types of molecular machines. These machines used by nature for force generation and motion. *They convert chemical energy into mechanical force via conformational changes.*



- Most of the natural machinery is built from proteins.
- These findings help unravel the mysteries associated with the molecular machinery and pave the way for the production and application of these miniature machines in various fields, including medicine, space exploration, electronics and military.
- *We divide the molecular machines into three broad categories—protein based, DNA-based, and chemical molecular motors.*

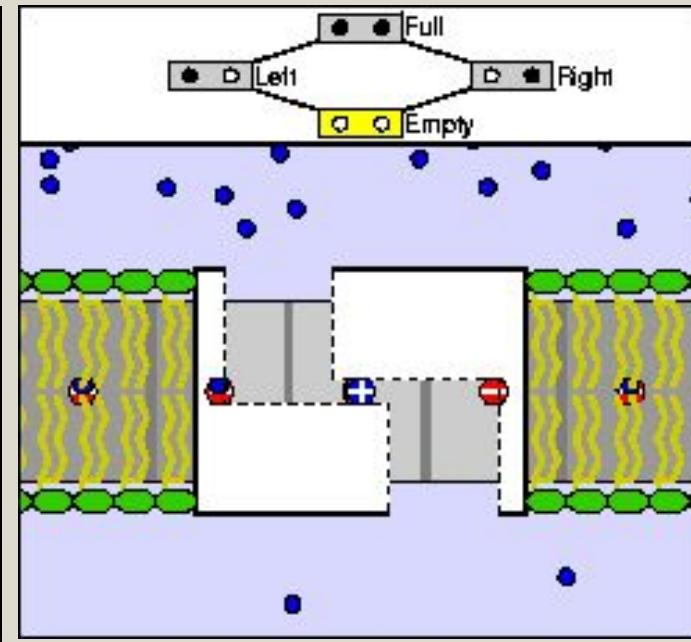
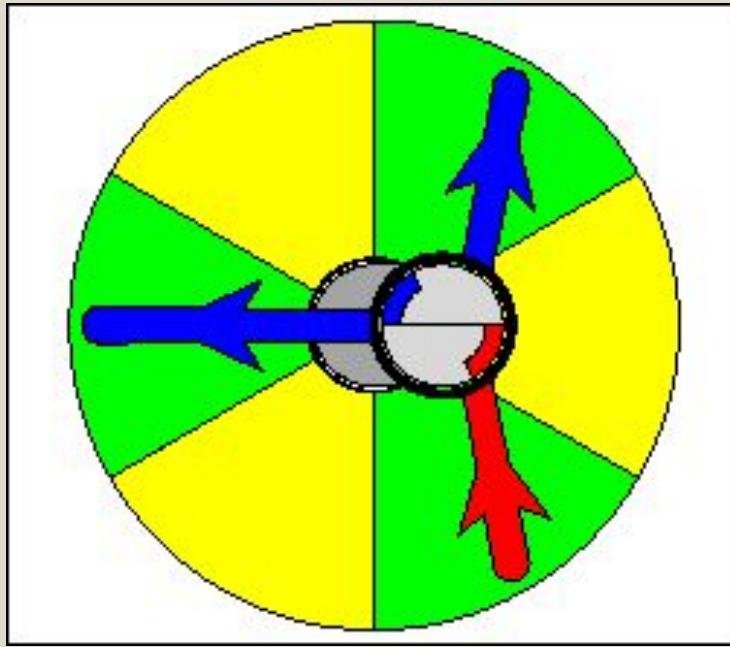
Properties of ATP-based Protein Molecular Machines

- *Two form the F0F1-ATP synthase, and the third one is the bacterial flagellar motor.*
- The protein-based molecular motors rely on an energy-rich molecule known as adenosine triphosphate (ATP),
- The machines described in this section, the F0F1-ATPase, the kinesin, myosin, and dynein superfamily of protein molecular machines, and bacteria flagellar motors all depend, directly or indirectly, on ATP for their input energy.
- These machines have now been segregated out of their natural environment and are seen as energy conversion devices to obtain forces, torques, and motion.
- One disadvantage associated with ATP dependence is that the ATP creation machinery itself could be many times heavier and bulkier than the motors, thereby making it more complex.
- These machines perform best in their natural environment, and in the near future it may be possible to have them as a part of biomimetic molecular machinery.

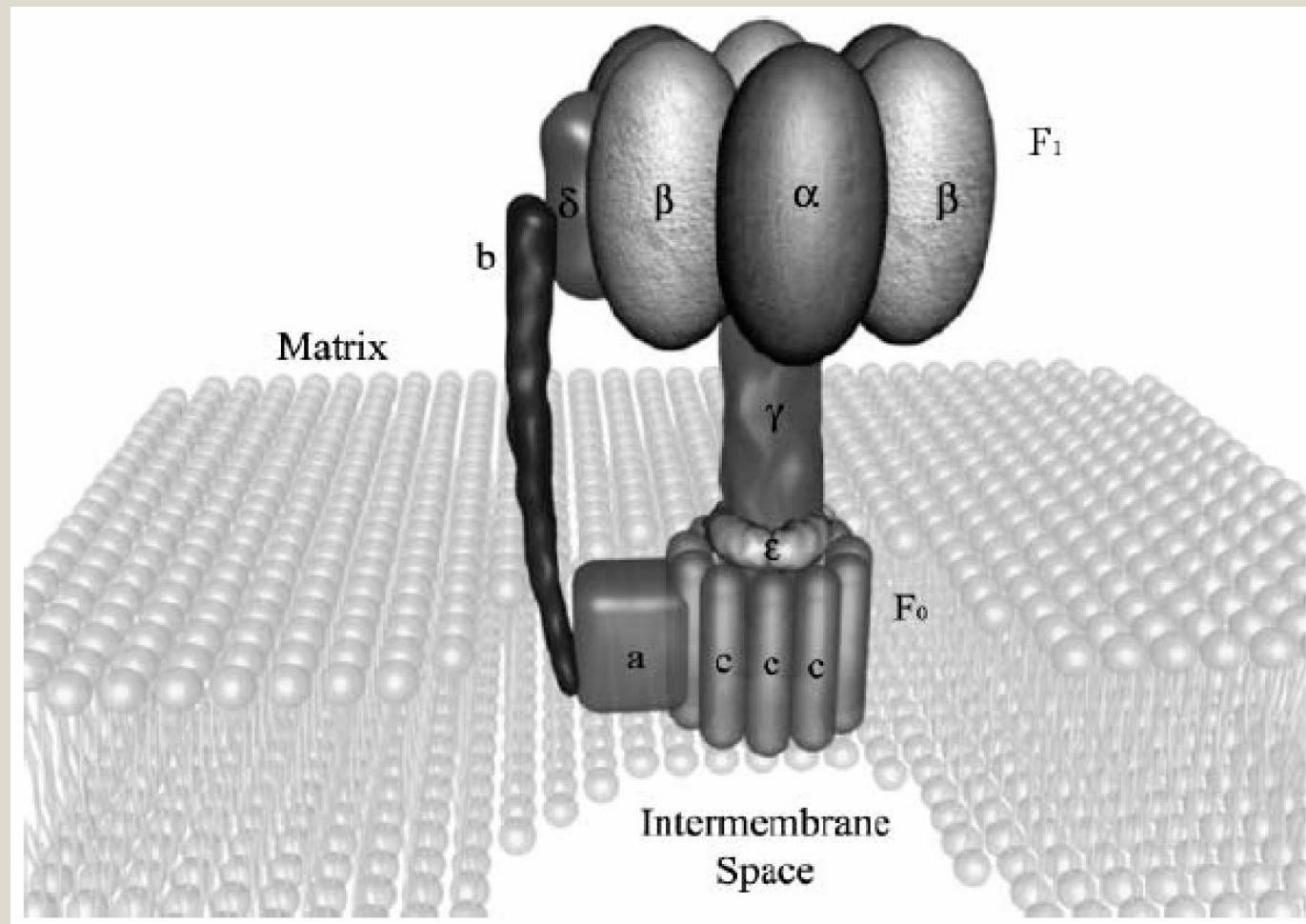
The F0F1-ATP Synthase Motors

- ATP is regarded as the energy currency of biological systems.
- When this currency is utilized (i.e., the energy of the molecule that is used to drive a biological process), the terminal anhydride bond in the ATP molecule has to be split. This leaves adenosine diphosphate (ADP) and a phosphate ion (Pi) as the products, which are recombined to form ATP by a super efficient enzyme motor assembly called the F0F1-ATP synthase (F0F1-ATPase).
- ATP synthase is present inside the mitochondria of animal cells, in plant chloroplasts, in bacteria, and some other organisms.

- ATP synthase consists of two portions: **a membrane-spanning portion, Fo**, comprising the ion channel, and **a soluble portion, F1**, containing three catalytic sites.
- Both Fo and F1 are reversible rotary motors --- perhaps the smallest motors known to science. Fo uses the transmembrane electrochemical gradient to generate a rotary torque to drive ATP synthesis in F1 or, when driven backwards by the torque generated in F1, to pump ions uphill against their transmembrane electrochemical gradient.
- F1 generates a rotary torque by hydrolyzing ATP at its three catalytic sites or, when turned backwards by the torque generated in Fo, as a synthesizer of ATP.

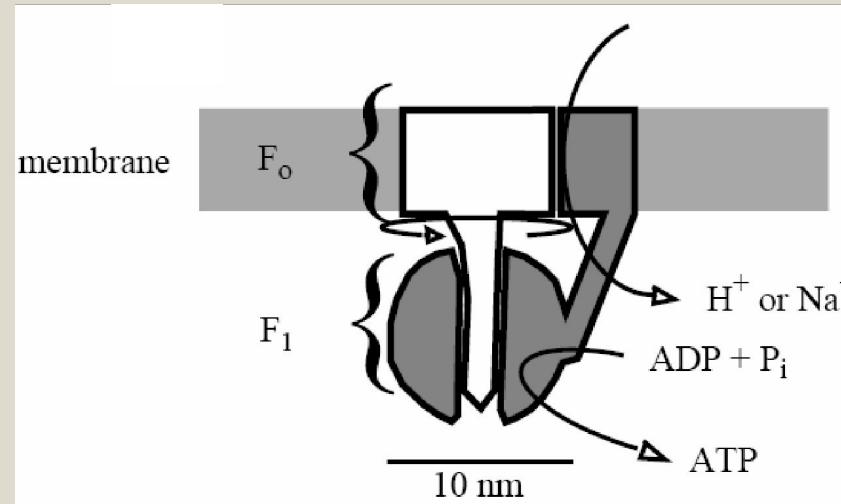


The F0F1-ATPase motors. The F0 motor is embedded in the inner mitochondrial membrane of the mitochondria. F0 is typically composed of *a*, *b*, and *c* subunits as shown. The F1 motor is the soluble region composed of three α -, three β -, one each of γ -, δ - and ϵ -subunits.



Coupling and Coordination of Motors

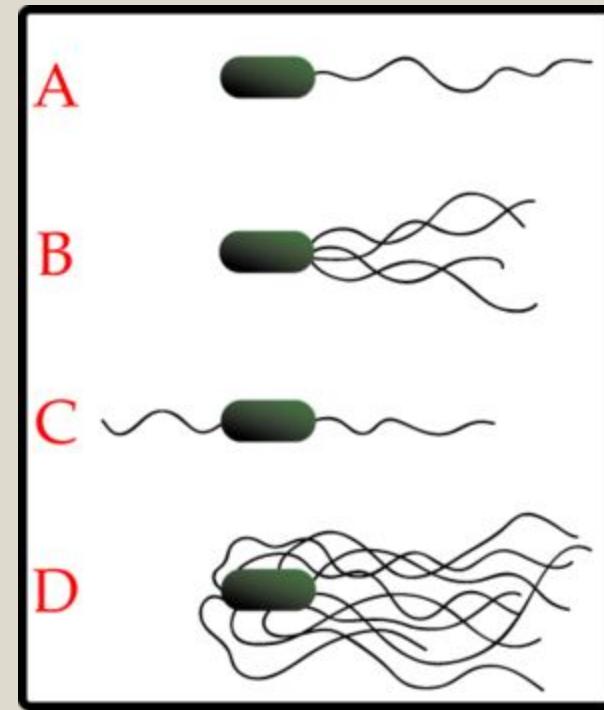
- The ATP synthase is actually a combination of two motors functioning together, the hydrophobic transmembrane F₀-ATPase motor and the globular F₁-ATPase motor



The F₀F₁-ATPase contains two rotary motors: the membrane –bound F₀, driven by the flux of ions across a membrane, and the soluble F₁, driven by ATP hydrolysis. These two motors are coupled by sharing a common motor, drawn in white, and a common stator, drawn dark. The figure shows the F₀F₁-ATPase operating as an ATP-synthase. Rotation of F₀ driven by ion flux drives F₁ in reverse, causing it to synthesize ATP from ADP and inorganic phosphate.

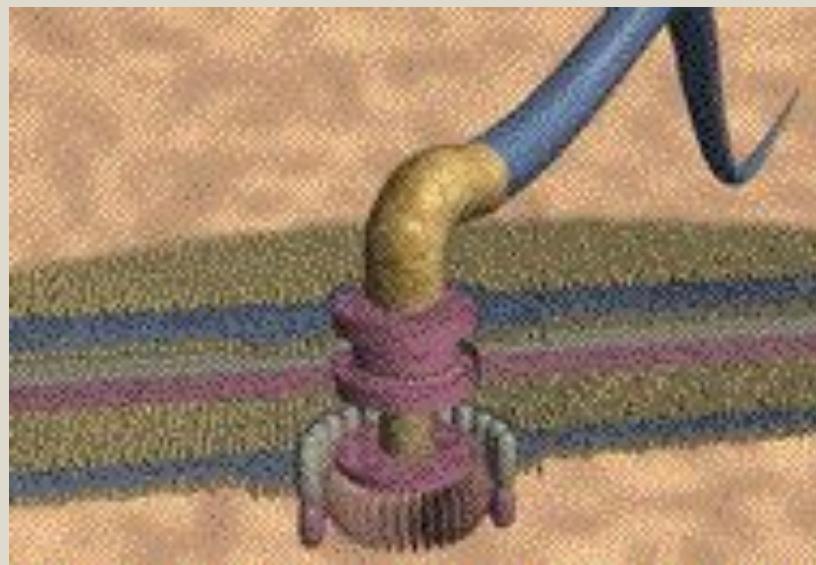
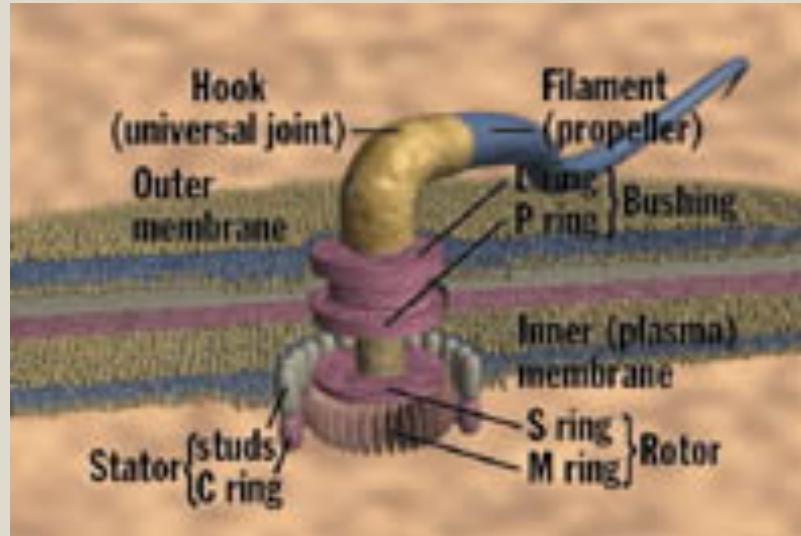
The Bacterial Flagellar Motor

- A flagellum (plural: flagella) is a long, slender projection from the cell body, composed of microtubules and surrounded by the plasma membrane.
- In small, single-cell organisms they may function to propel the cell by beating in a whip-like motion; in larger animals, they often serve to move fluids along mucous membranes such as the lining of the trachea.
- The bacterial flagellar motor is a nanotechnological marvel, no more than 50 nm in diameter, built from about 20 different kinds of parts.



Examples of bacterial flagella arrangement schemes.
A-Monotrichous;
B-Lophotrichous;
C-Amphitrichous; **D-Peritrichous**

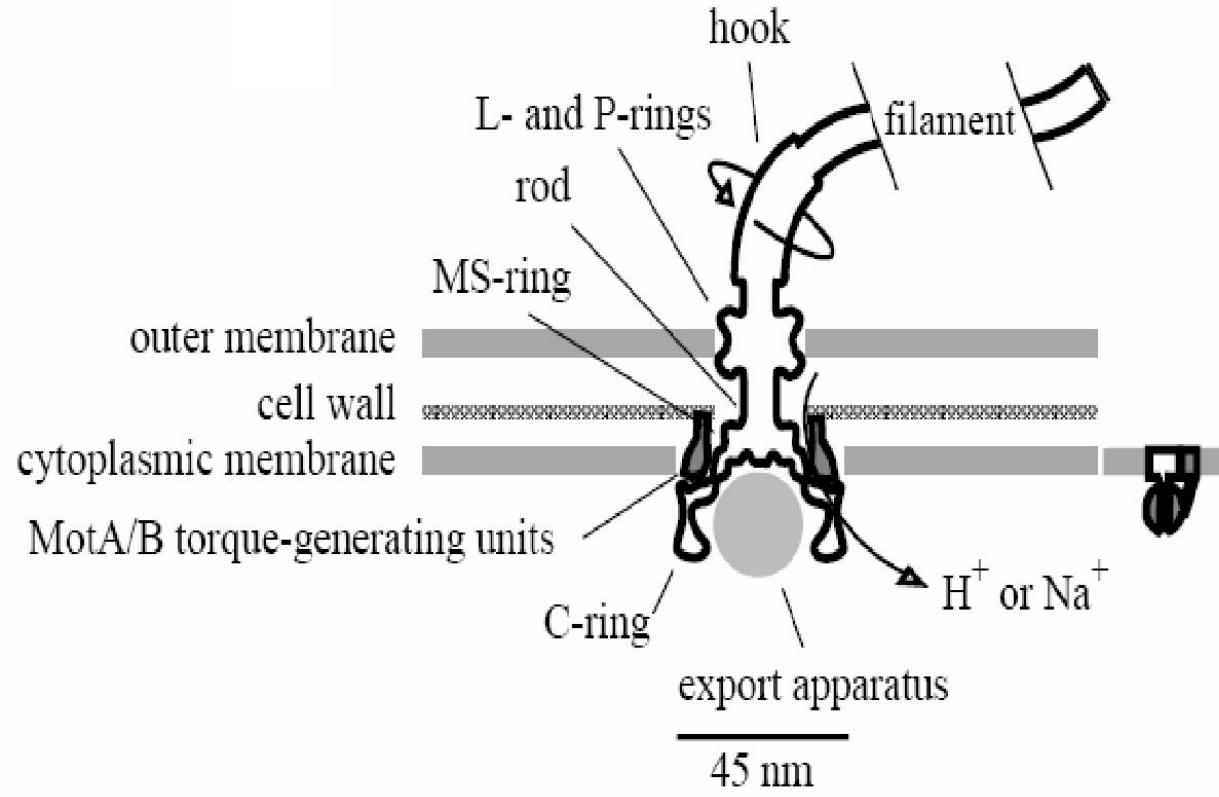
- It spins clockwise (CW) or counterclockwise (CCW) at speeds on the order of 100 Hz, driving long thin helical filaments that enable cells to swim.
- Peritrichously flagellated cells (*peri*, around; *trichos*, hair), such as *Escherichia coli*, execute a random search, moving steadily at about 30 diameters per second, now in one direction, now in another.
- Steady motion requires CCW rotation. Receptors near the surface of the cell count molecules of interest (sugars, amino acids, dipeptides) and control the direction of flagellar rotation.
- If a leg of the search is deemed favorable, it is extended, i.e., the motors spin CCW longer than they otherwise would. This bias enables cells to actively find regions in their environment where life is better.



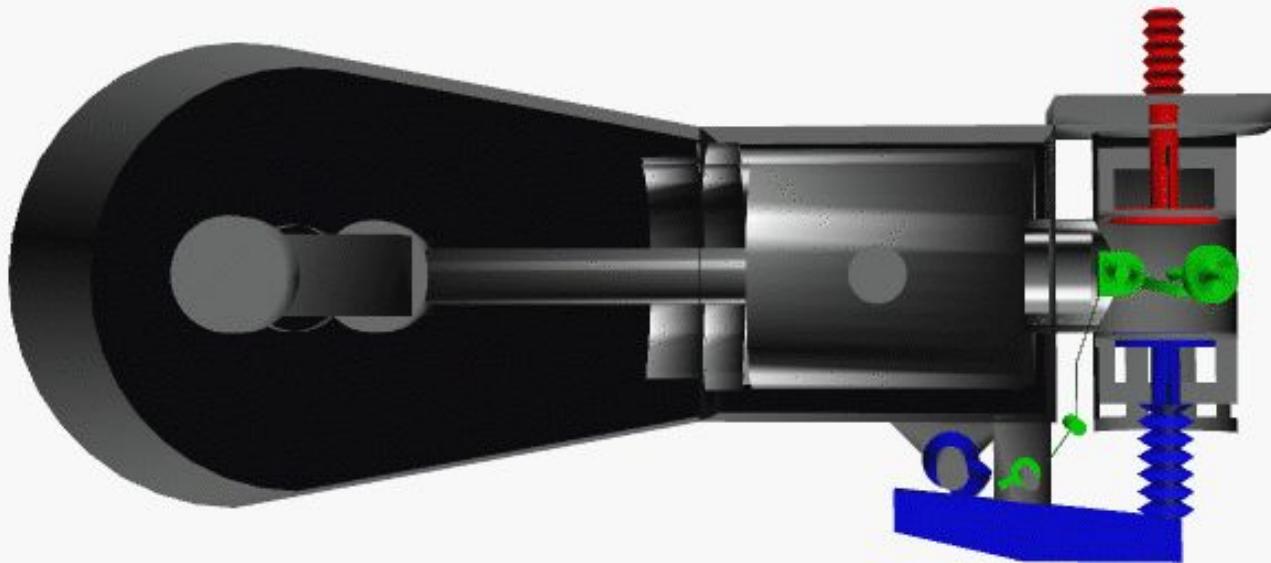
- Thus, the flagellar motor is the output organelle of a remarkable sensory system, the components of which have been honed to perfection by billions of years of evolution.
- A number of bacterial species in addition to *E. coli* depend on flagella motors for motility: e.g., *Salmonella enterica* serovar, Typhimurium (*Salmonella*), *Streptococcus*, *Vibrio* spp., *Caulobacter*, *Leptospira*, *Aquaspirillum serpens*, and *Bacillus*.
- The rotation of flagella motors is stimulated by a flow of ions through them, which is a result of a build-up of a transmembrane ion gradient. There is no direct ATP-involvement; however, the proton gradient needed for the functioning of flagella motors can be produced by ATPase.

Flagellar motor structure

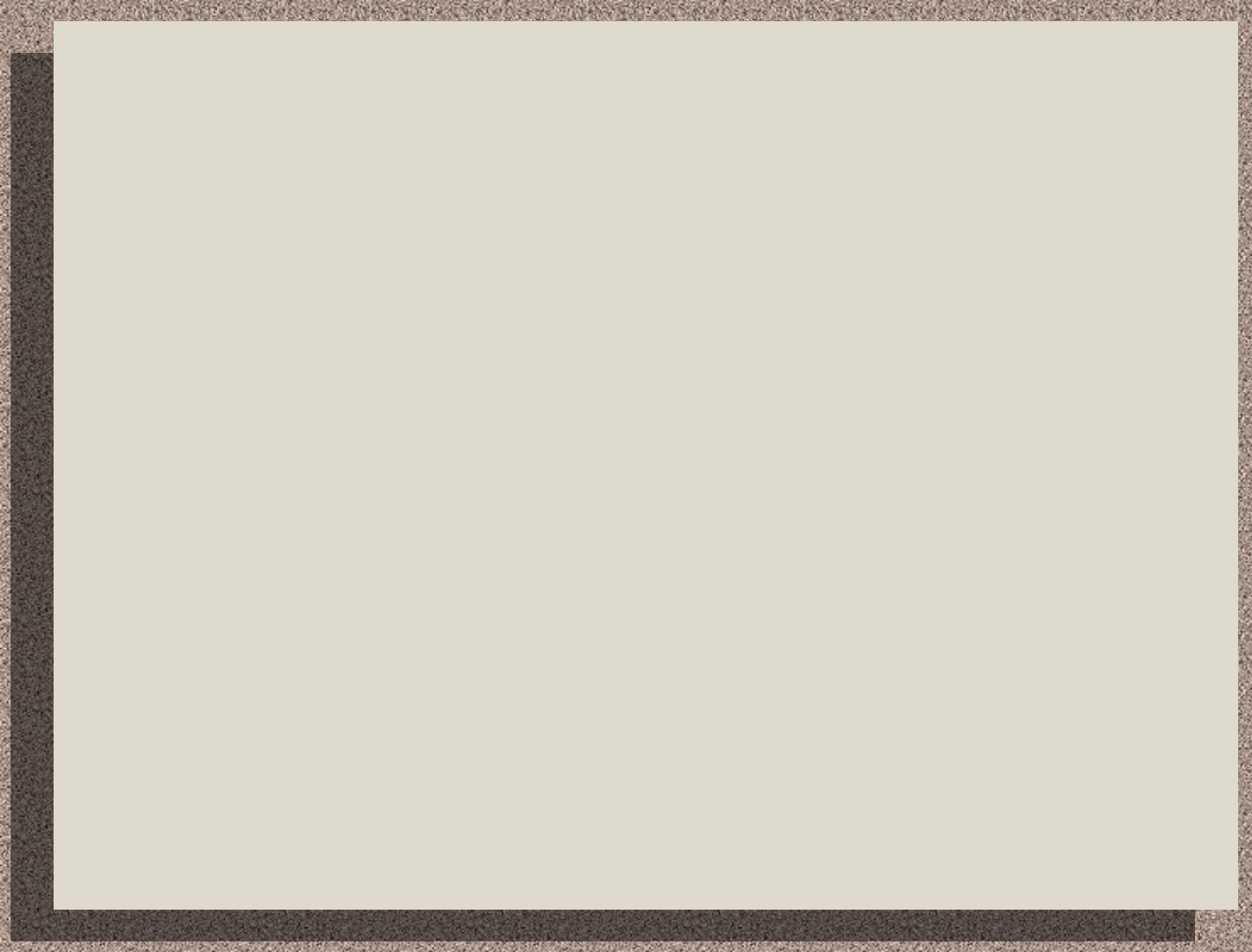
- Bacterial flagella are the only biological structures known that use rotation for the purpose of locomotion.
- Flagella consist of a rotary motor embedded in the cell envelope connected to an extracellular helical propeller. The motor is powered by the flow of ions down an electrochemical gradient across the cytoplasmic membrane into the cell.
- The ions are typically H⁺ (protons), although certain marine and alkalophilic species have motors driven by Na⁺.
- The electrochemical gradient (protonmotive force or sodium motive force) consists of a transmembrane voltage and a concentration difference across the membrane, both of which are maintained by various metabolic processes.
- The rotor shown in white (Figure 5), consists of a series of rings spanning the cell envelope and is attached via the flexible hook to the helical propeller or filament. The stator is a ring of particles in the cytoplasmic membrane, containing the proteins MotA and MotB, and anchored to the peptidoglycan cell



The bacterial flagellar motor. The rotor, drawn in white, consists of a series of rings that span the cell envelope and are attached to the extracellular hook and filament. The stator consists of a ring of torque-generating units containing the proteins MotA and MotB and anchored to the cell wall. Ions flowing through the motor generate torque by means of unknown interactions between the rotor and stator in the vicinity of the C-ring. The F0F1-ATPase is also shown, to the same scale.

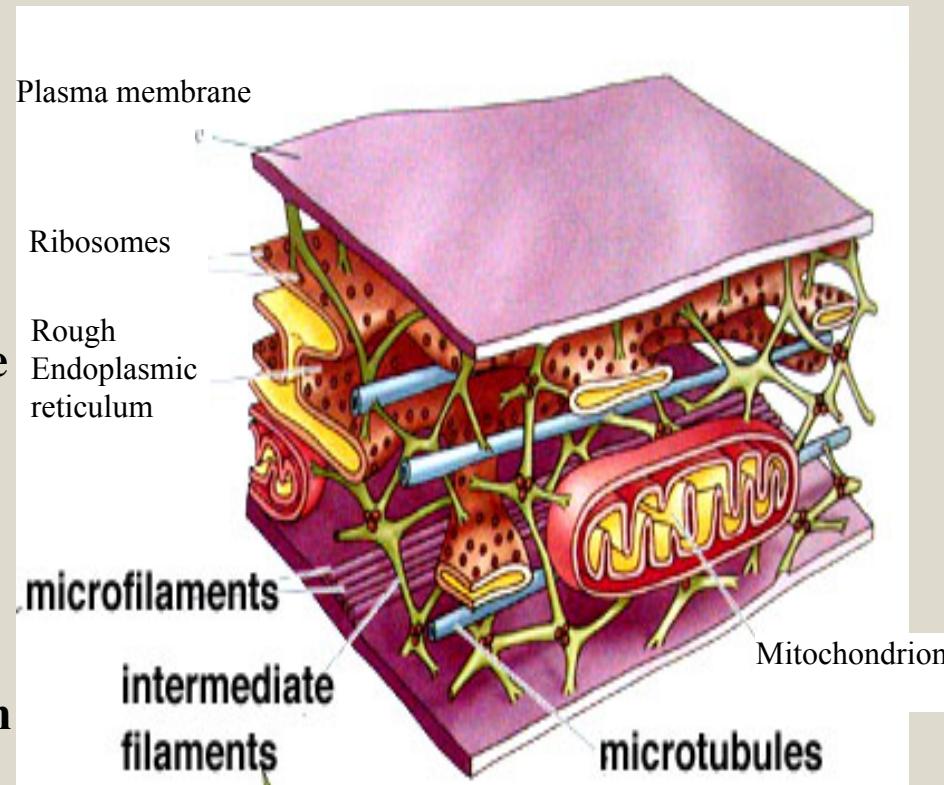


The fuel/air intake system **red**, the electrical system **green**, and the exhaust system **blue**



Cytoskeleton: Microtubules, Microfilaments, and Intermediate filaments

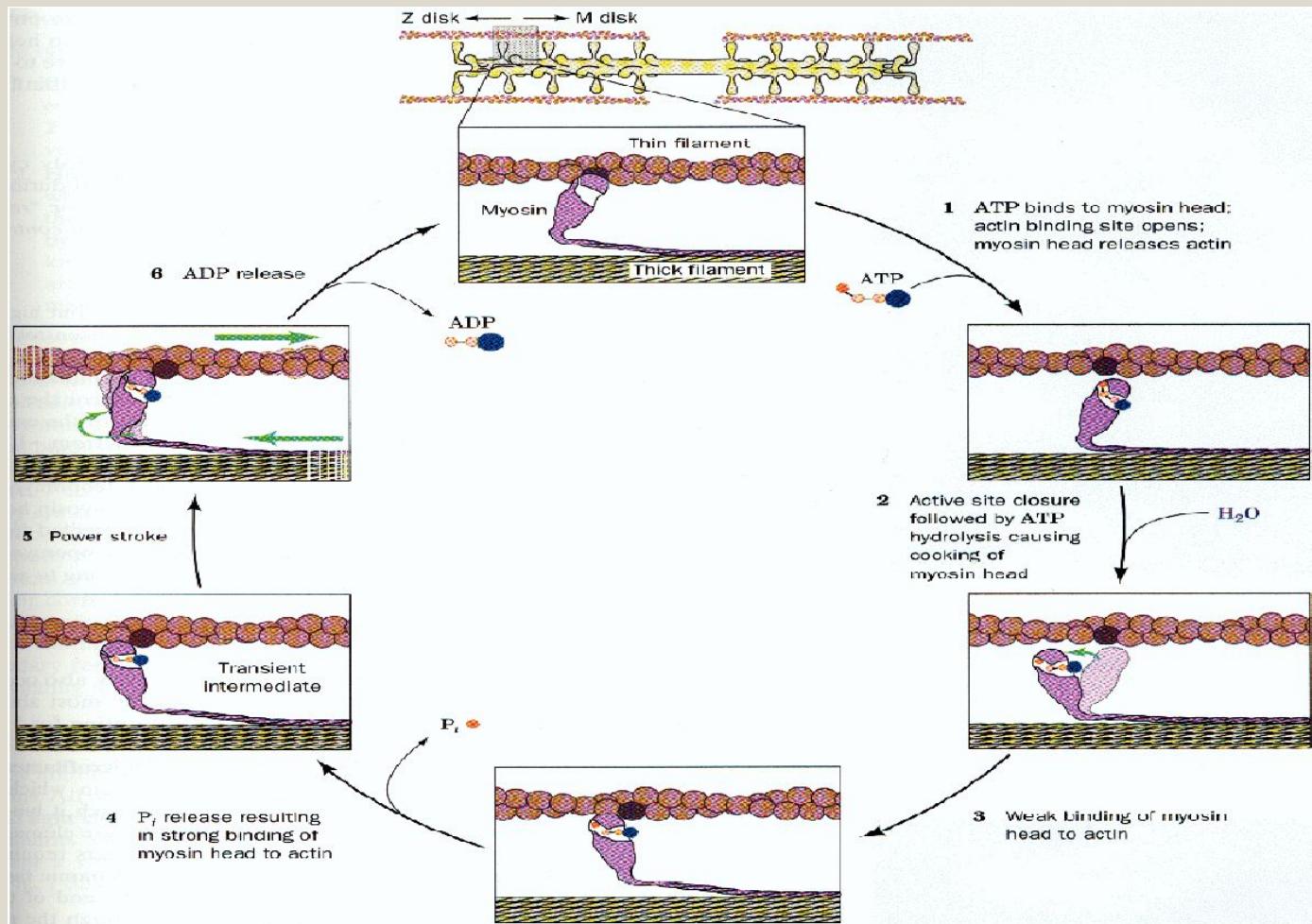
- Cytoskeleton gives the cell shape, structure, and physical organization
- Motor proteins can rearrange the structural elements, or move cell components around the cytoskeleton.
- The cytoskeleton is in constant state of change depending on the requirements of the cell.
- 3 major structural elements of the cytoskeleton
- Microtubules - hollow, rigid cylindrical tubes made from tubulin subunits
- Microfilaments - solid, thinner structures made of actin
- Intermediate filaments - tough, ropelike fibers made of a variety of related proteins



The Myosin Linear Motor

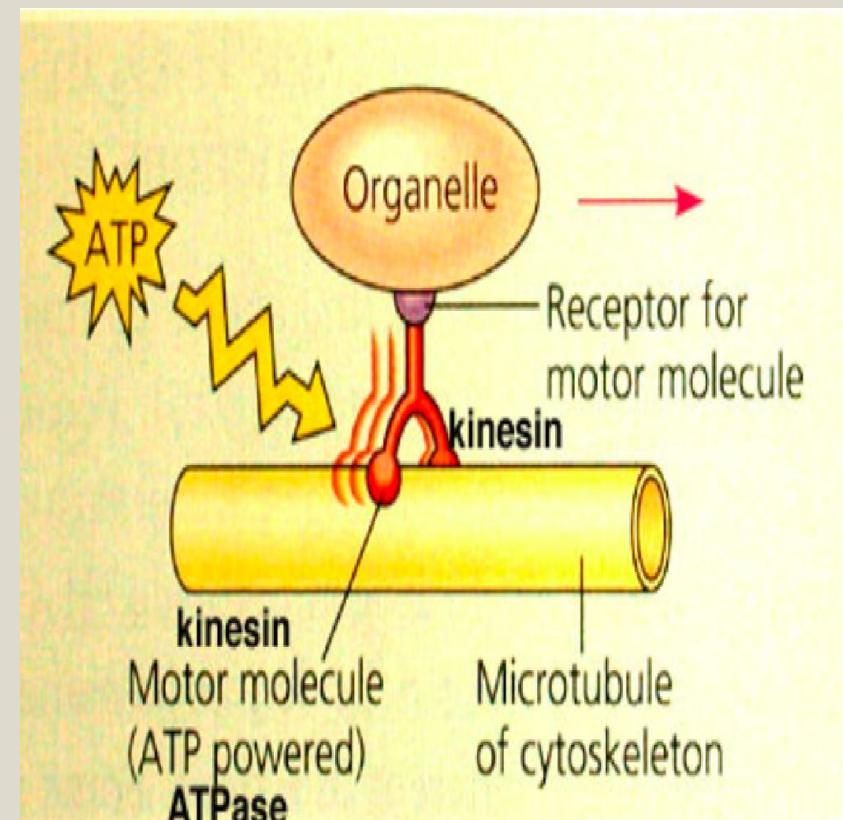
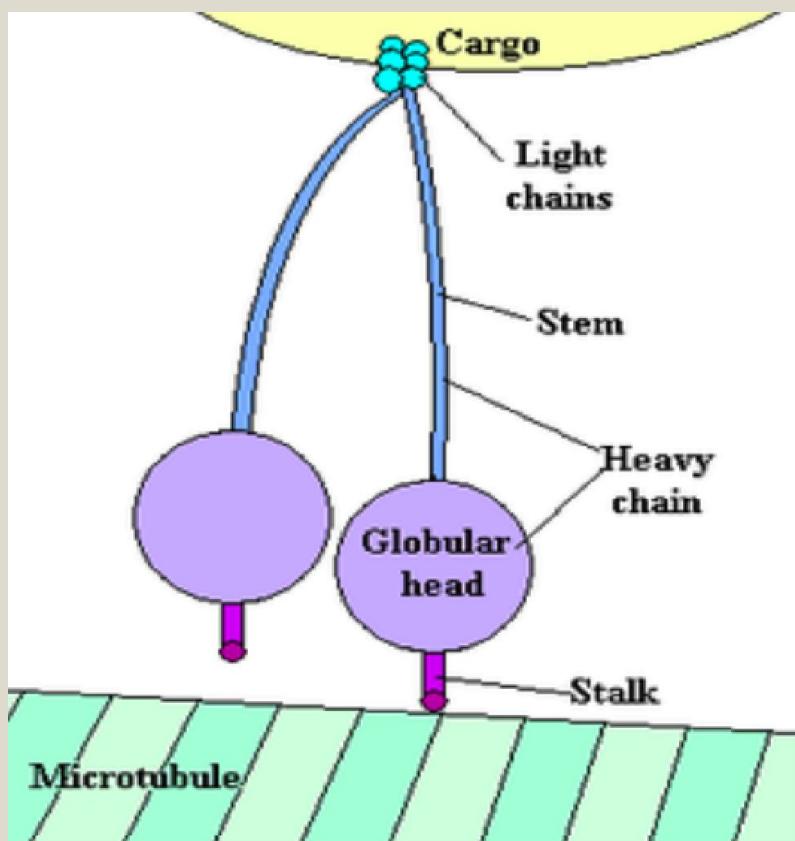
- Myosin is a diverse superfamily of motor proteins.
- Myosin-based molecular machines transport cargoes along actin filaments, the two-stranded helical polymers of the protein actin that are about 5–9 nm in diameter. They do this by hydrolyzing ATP and utilizing the released energy.
- In addition to transport, they are also involved in the process of force generation during muscle contraction, wherein thin actin filaments and thick myosin filaments slide past each other.
- Myosin molecules were first seen (in the late 1950s) through electron microscope protruding out from thick filaments and interacting with the thin actin filaments. Since that time, ATP has been known to play a role in myosin-related muscle movement along actin; however, the exact mechanism was unknown, until it was explained in 1971.

Myosin cross-bridge model of contraction. Myosin uses the energy released from ATP hydrolysis to move along actin.



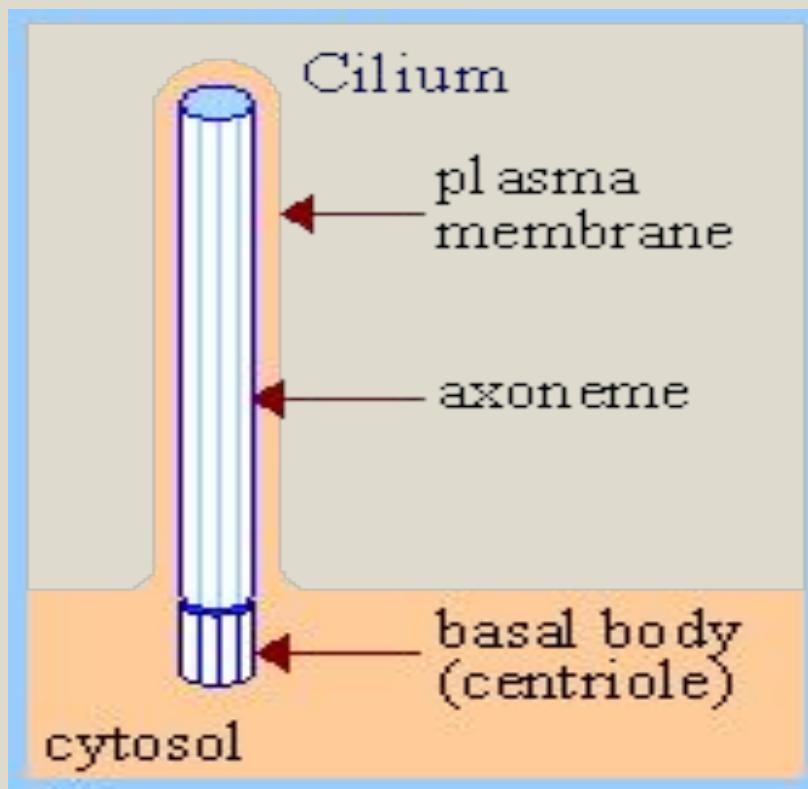
The Kinesin Linear Motor

- The kinesin and dynein families of proteins are involved in cellular cargo transport along microtubules, in contrast to myosin, which transports along actin.
- Microtubules are 25-nm diameter tubes made of protein tubulin and are present in the cells in an organized manner.
- Microtubules have polarity; one end being the plus (fast-growing) end while the other end is the minus (slow-growing) end.
- Kinesins move from the minus end to the plus end of the microtubule, whereas dyneins move from the plus end to the minus end.
- Microtubule arrangement varies in different cell systems. In nerve axons, they are arranged longitudinally such that their plus ends point away from the cell body and into the axon.
- In epithelial cells, their plus ends point toward the basement membrane.
- They extend radially out of the cell center in fibroblasts and macrophages with the plus end protruding outward.
- Similar to myosin, kinesin is also an ATP-driven motor.
- One unique characteristic of the kinesin family proteins is their processivity; they bind to microtubules and literally walk on it for many enzymatic cycles before detaching.



The Dynein Motor

- The dynein superfamily of proteins was discovered in 1965.
- Dyneins exist in two isoforms: cytoplasmic and axonemal.
- Cytoplasmic dyneins are involved in cargo movement, whereas axonemal dyneins are involved in producing bending motions of cilia and flagella.
- Because dynein is a larger and more complex structure than other motor proteins, its mode of operation is not as well known. However, electron microscopy and image processing was used to show the structure of a flagellar dynein at the start and end of its power stroke, which gives some insight into its possible mode of force generation.



Cilia and flagella have a core **axoneme**, a complex of microtubules and associated proteins.

Bioremediation

Definition: Use of living organisms to transform, destroy or immobilize contaminants

Goal: Detoxification of the parent compound(s) and conversion to products that are no longer hazardous to human health and the environment.

Sources of Contamination

- Industrial spills and leaks
- Surface impoundments
- Storage tanks and pipes
- Landfills
- Burial areas and dumps
- Injection wells

Why use Bioremediation?

- No additional disposal costs
- Low maintenance
- Does not create an eyesore
- Capable of impacting source zones and thus, decreasing site clean-up time

Microorganisms

- **Aerobic bacteria:**

- Examples include: *Pseudomonas*, *Alcaligenes*, *Sphingomonas*, *Rhodococcus*, and *Mycobacterium*
- Shown to degrade pesticides and hydrocarbons; alkanes and polyaromatics
- May be able to use the contaminant as sole source of carbon and energy.

- **Methanotrophs:**

- Aerobic bacteria that utilize methane for carbon and energy
- Methane monooxygenase has a broad substrate range
 - active against a wide range of compounds (e.g. chlorinated aliphatics such as trichloroethylene and 1,2-dichloroethane)

- **Anaerobic bacteria:**

- Not used as frequently as aerobic bacteria
- Can often be applied to bioremediation of polychlorinated biphenyls (PCBs) in river sediments, trichloroethylene (TCE), and chloroform

- **Fungi:**

- Able to degrade a diverse range of persistent or toxic environmental pollutants

Forms of Bioremediation

- **In situ Bioremediation**

- Bioventing
- In situ biodegradation
- Biostimulation
- Biosparging
- Bioaugmentation
- Natural Attenuation

- **Ex situ Bioremediation**

- Land farming
- Composting
- Biopiles
- Bioreactors

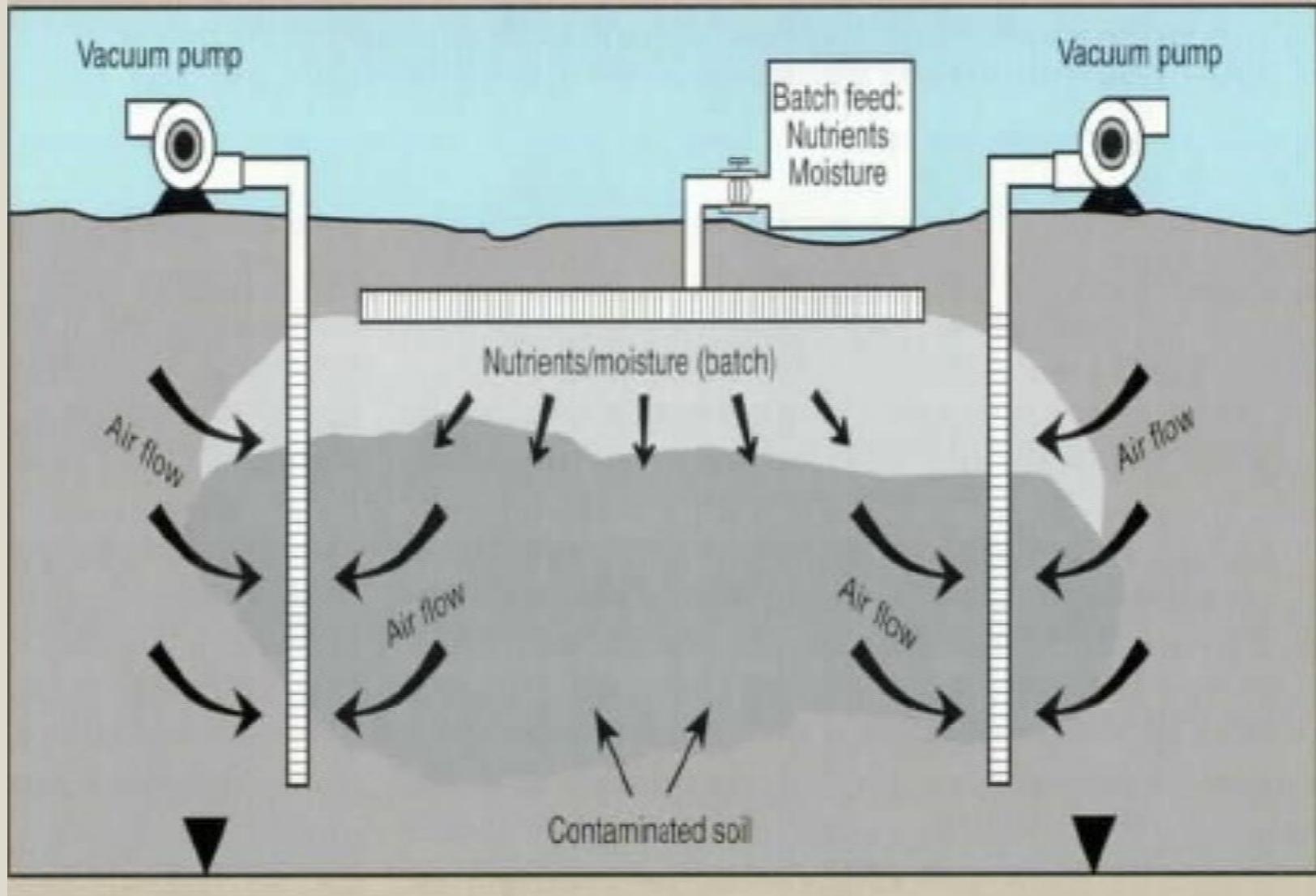
- **Phytoremediation**

- Phytoextraction or phytoaccumulation
- Phytodegradation or phytotransformation
- Phytostabilization
- Rhizodegradation
- Rhizofiltration

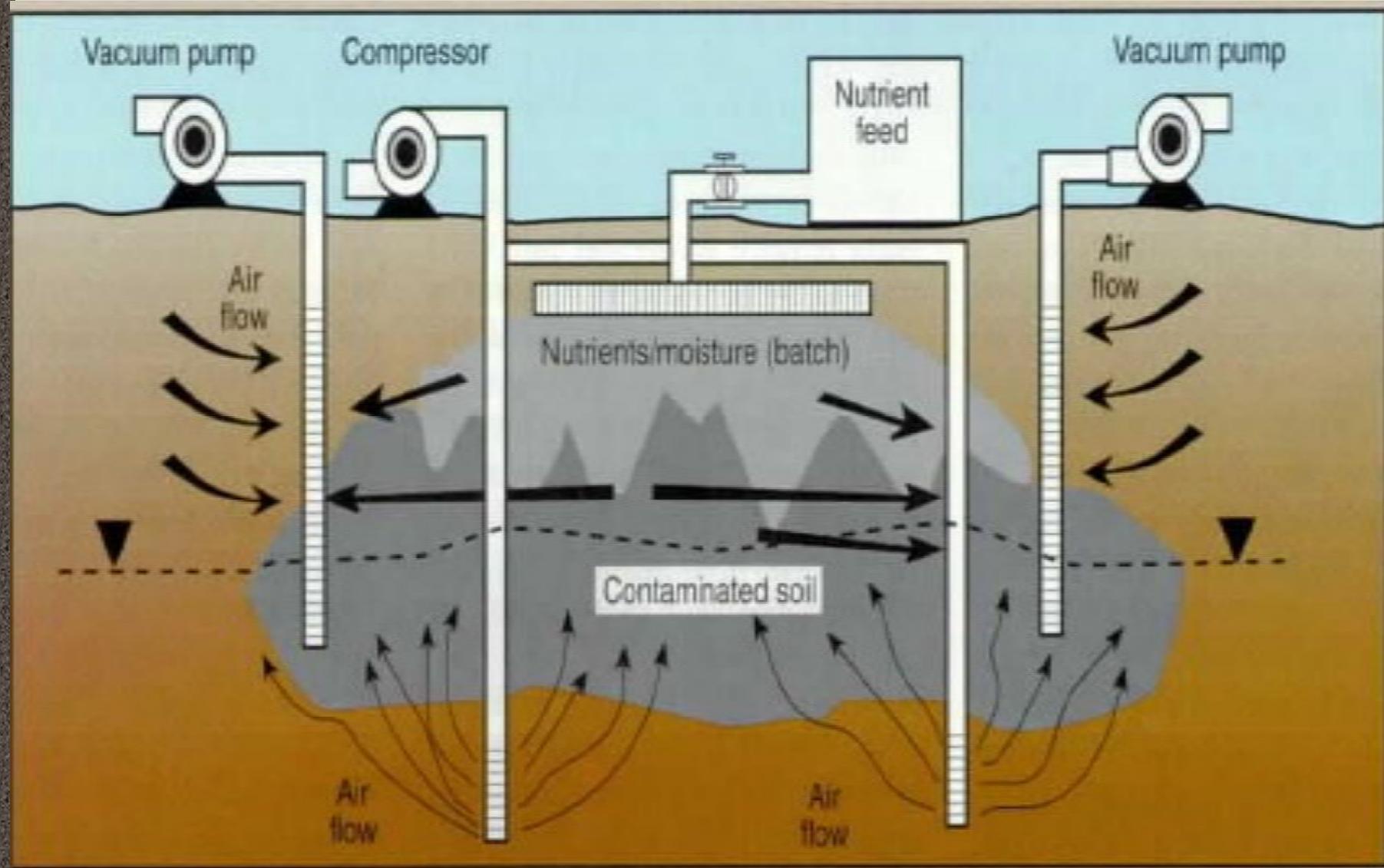
In Situ Bioremediation

- **Bioventing**
 - One of the most common approaches in soil
 - Supply air and nutrients via wells
 - Takes advantage of indigenous microorganisms
- **In situ biodegradation**
 - Supply air and nutrients by circulating aqueous solutions through contaminated soils or groundwater
- **Biosparging**
 - Injection of air below the water table ☐ increases groundwater oxygen concentrations and mixing in saturated zone
- **Bioaugmentation**
 - Addition of indigenous or exogenous microorganisms
 - Limits to use: competition and necessity
- **Biostimulation**
- **Natural Attenuation or Intrinsic Bioremediation**

Bioventing



Biosparging



Five Steps of In Situ Bioremediation

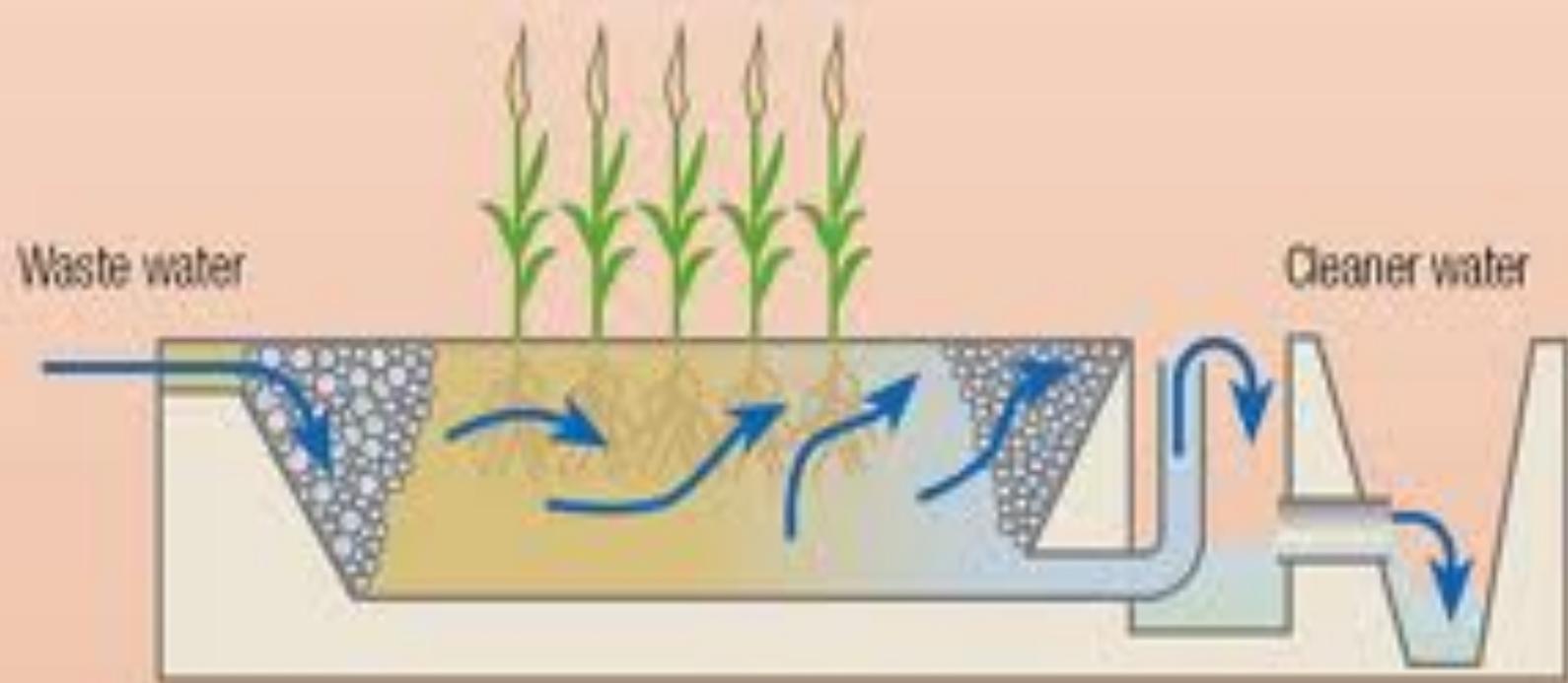
1. Site investigation
2. Treatability studies
3. Recovery of free product and removal of the contamination source
4. Design and implementation of the in situ bioremediation system
5. Monitoring and performance evaluation of the in situ bioremediation system

Ex situ Bioremediation

- **Land farming**
 - Contaminated soil is excavated and spread over land
 - Soil is periodically tilled to improve aeration
 - Remediation due to indigenous microorganisms, as well as chemical and physical processes
 - Generally limited to the superficial 10–35 cm of soil
 - Can reduce monitoring and maintenance costs
- **Composting**
 - Combines contaminated soil with nonhazardous organic amendants (e.g. manure or agricultural wastes)
- **Biopiles**
 - Combination of landfarming and composting
 - Control physical losses of contaminants
- **Bioreactors**
 - Soil and water pumped up from a contaminated plume and processed through an engineered containment system
 - Degradation in a bioreactor is generally greater than *in situ* because the contained environment is more controllable and predictable

Phytoremediation

- **Phytoextraction or phytoaccumulation**
 - Plants used to accumulate contaminants in the roots and aboveground biomass
 - Can be a relatively low cost option for a large area
 - Results in biomass that must be properly disposed of or reused
- **Phytotransformation or phytodegradation**
 - Uptake of contaminants and transformation to more stable, less toxic, or less mobile forms
 - Eg. metal chromium can be reduced from hexavalent to less mobile (and non-carcinogenic) trivalent chromium
- **Phytostabilization**
 - Mobility and migration of contaminants are reduced through sorption onto or into the plant
- **Rhizodegradation**
 - Breakdown of contaminants through activity of the rhizosphere
- **Rhizofiltration**
 - Water remediation technique
 - Used to reduce contamination in natural wetlands and estuary areas.



Overview of phytoremediation applications.

| Technique | Plant mechanism | Surface medium |
|---------------------|---|---------------------------------------|
| Phytoextraction | Uptake and concentration of metal via direct uptake into the plant tissue with subsequent removal of the plants | Soils |
| Phytotransformation | Plant uptake and degradation of organic compounds | Surface water, groundwater |
| Phytostabilization | Root exudates cause metal to precipitate and become less available | Soils, groundwater, mine tailing |
| Phytodegradation | Enhances microbial degradation in rhizosphere | Soils, groundwater within rhizosphere |
| Rhizofiltration | Uptake of metals into plant roots | Surface water and water pumped |
| Phytovolatilization | Plants evaporate selenium, mercury, and volatile hydrocarbons | Soils and groundwater |
| Vegetative cap | Rainwater is evaporated by plants to prevent leaching contaminants from disposal sites | Soils |

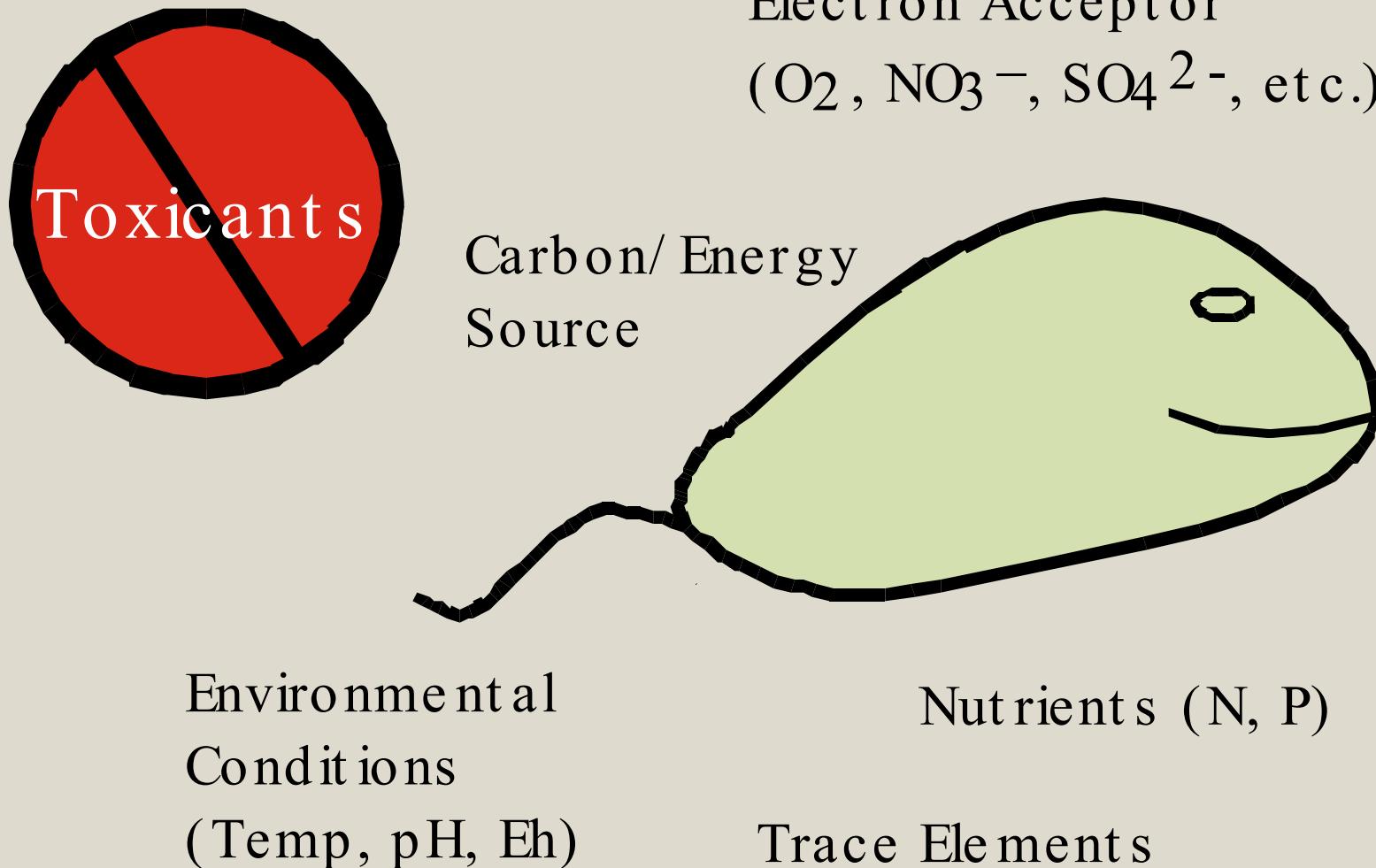
Mechanism of Bioremediation

- Conversion of contaminants to mineralized (e.g. CO_2 , H_2O , and salts) end-products via biological mechanisms
- Biotransformation refers to a biological process where the end-products are not minerals.
- Biodegradation involves the process of extracting energy from organic chemicals via oxidation of the organic chemicals

How Microbes Use the Contaminant

- Contaminants may serve as:
 - Primary substrate
 - enough available to be the sole energy source
 - Secondary substrate
 - provides energy, not available in high enough concentration
 - Cometabolic substrate
 - fortuitous transformation of a compound by a microbe relying on some other primary substrate

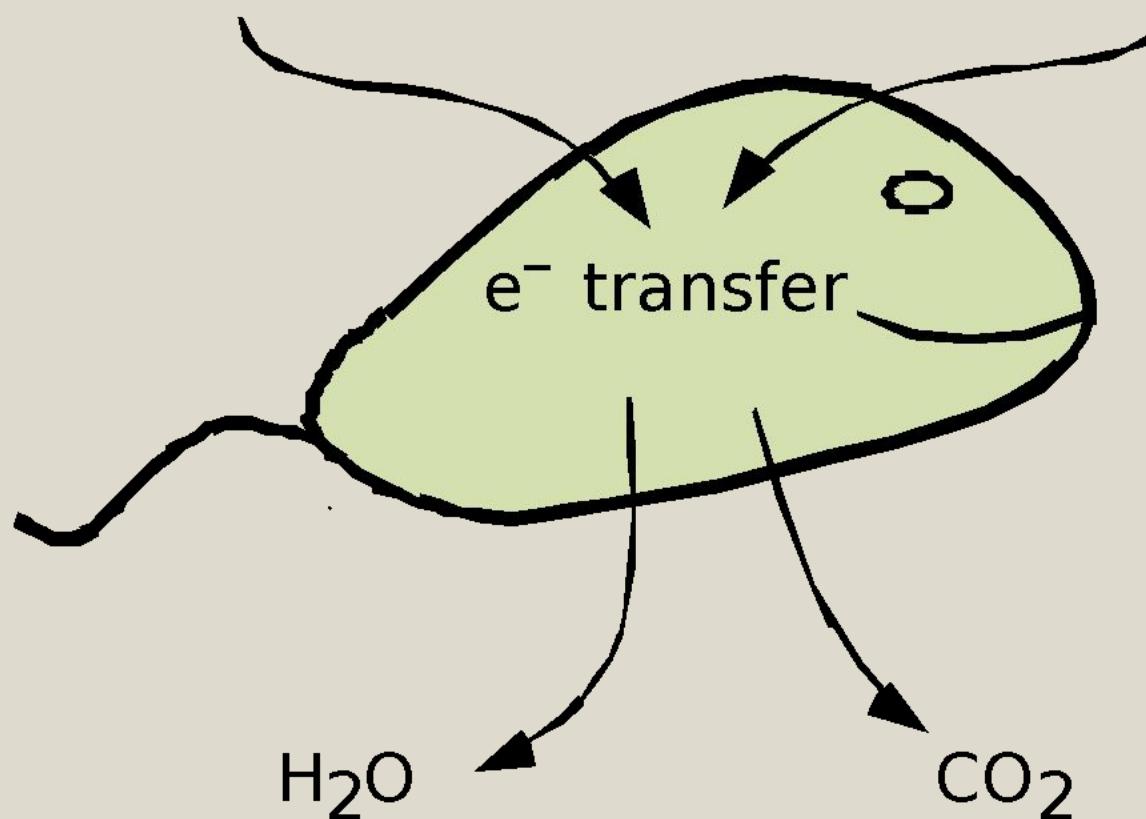
Requirements for Microbial Growth



Electron Exchange

Electron Acceptor
(O_2 , NO_3^- , SO_4^{2-} , etc.)

Carbon/Energy Source
Electron Donor



Aerobic vs Anaerobic biodegradation

Aerobic biodegradation

- If oxygen is the terminal electron acceptor, the process is called aerobic biodegradation

Anaerobic biodegradation

- All other biological degradation processes are classified as anaerobic biodegradation

Environmental Factors

Environmental conditions affecting degradation.

| Parameters | Condition required for microbial activity | Optimum value for an oil degradation |
|------------------|---|---|
| Soil moisture | 25–28% of water holding capacity | 30–90% |
| Soil pH | 5.5–8.8 | 6.5–8.0 |
| Oxygen content | Aerobic, minimum air-filled pore space of 10% | 10–40% |
| Nutrient content | N and p for microbial growth | C:N:P = 100:10:1 |
| Temperature (°C) | 15–45 | 20–30 |
| Contaminants | Not too toxic | Hydrocarbon 5–10% of dry weight of soil |
| Heavy metals | Total content 2000 ppm | 700 ppm |
| Type of soil | Low clay or silt content | |

Summary

Summary of bioremediation strategies.

| Technology | Examples | Benefits | Limitations | Factors to consider |
|----------------|---|--|--|---|
| <i>In situ</i> | <i>In situ</i> bioremediation Biosparging Bioventing Bioaugmentation | Most cost efficient Noninvasive Relatively passive Natural attenuation processes Treats soil and water | Environmental constraints Extended treatment time Monitoring difficulties | Biodegradative abilities of indigenous microorganisms Presence of metals and other inorganics Environmental parameters Biodegradability of pollutants Chemical solubility Geological factors Distribution of pollutants |
| <i>Ex situ</i> | Landfarming Composting Biopiles | Cost efficient Low cost Can be done on site | Space requirements Extended treatment time Need to control abiotic loss Mass transfer problem Bioavailability limitation | See above |

BIOSENSOR

What is a Biosensor?

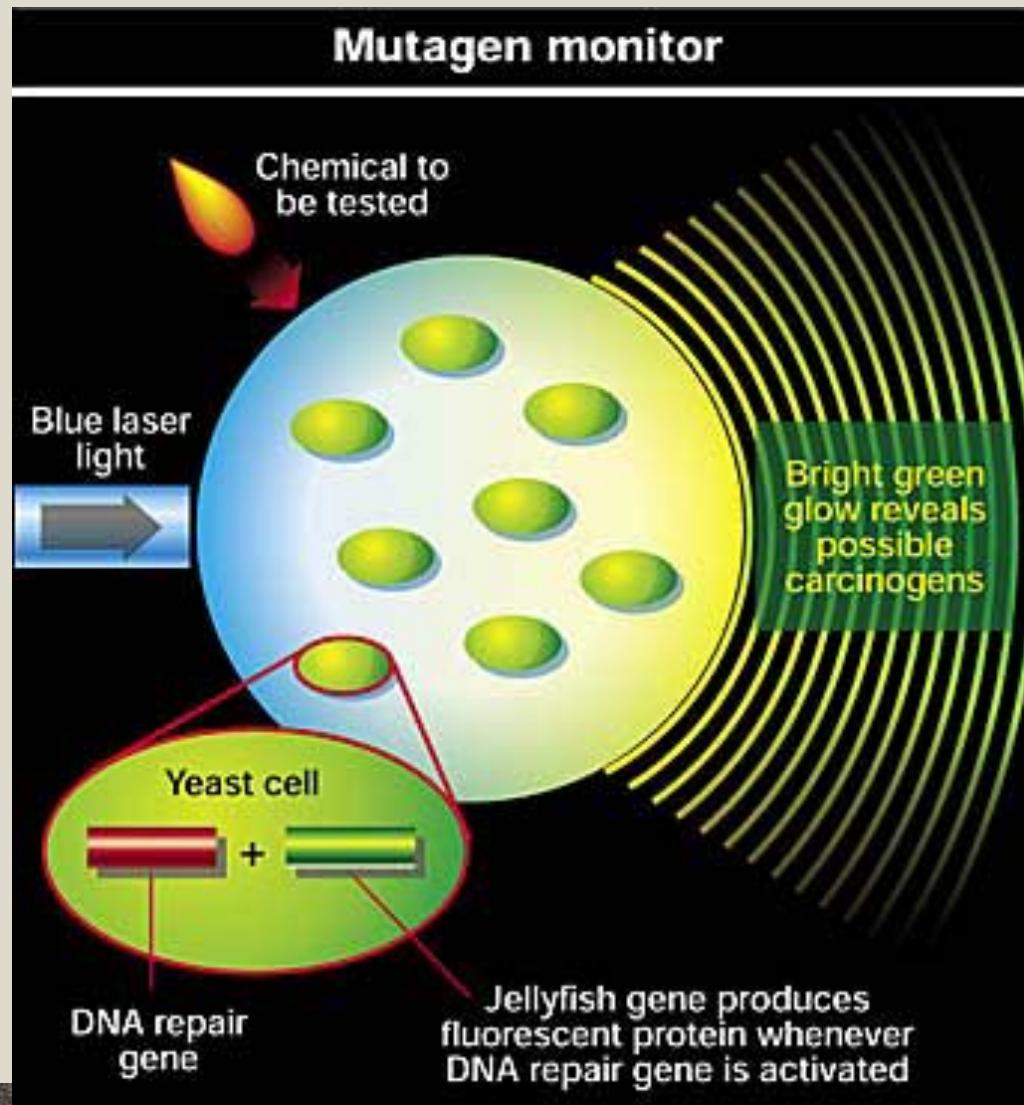
A biosensor is a self-contained integrated device that is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element which is in direct spatial contact with a transduction element (IUPAC, 1996)



1) Biosensor ≠ Bioanalytical system

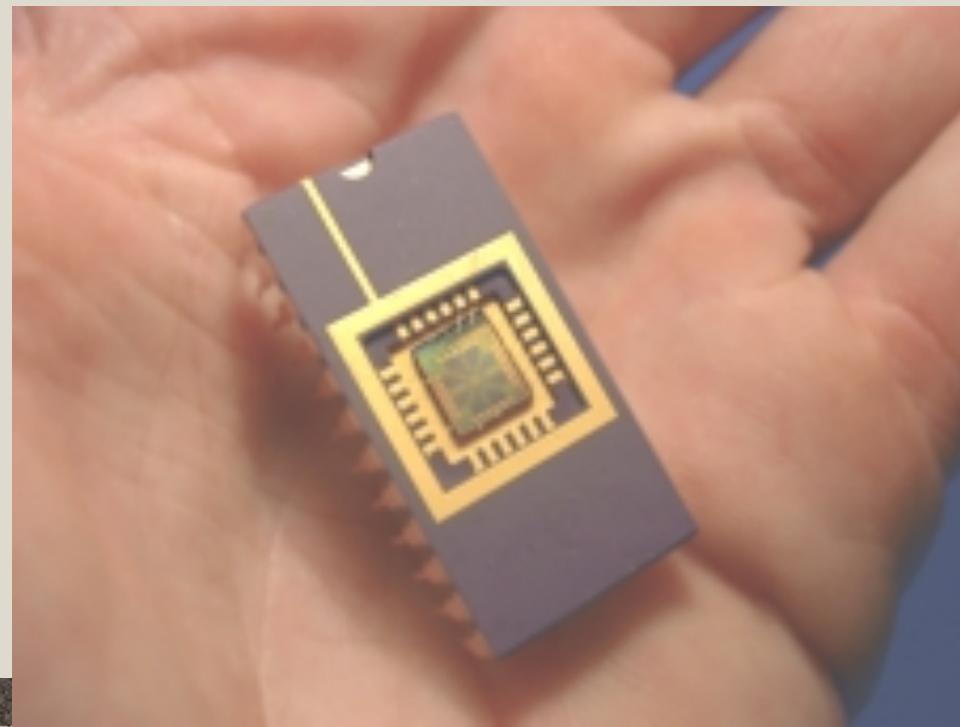
2) An enzyme electrode is a biosensor

“Biosensor” – Any device that uses specific biochemical reactions to detect chemical compounds in biological samples.

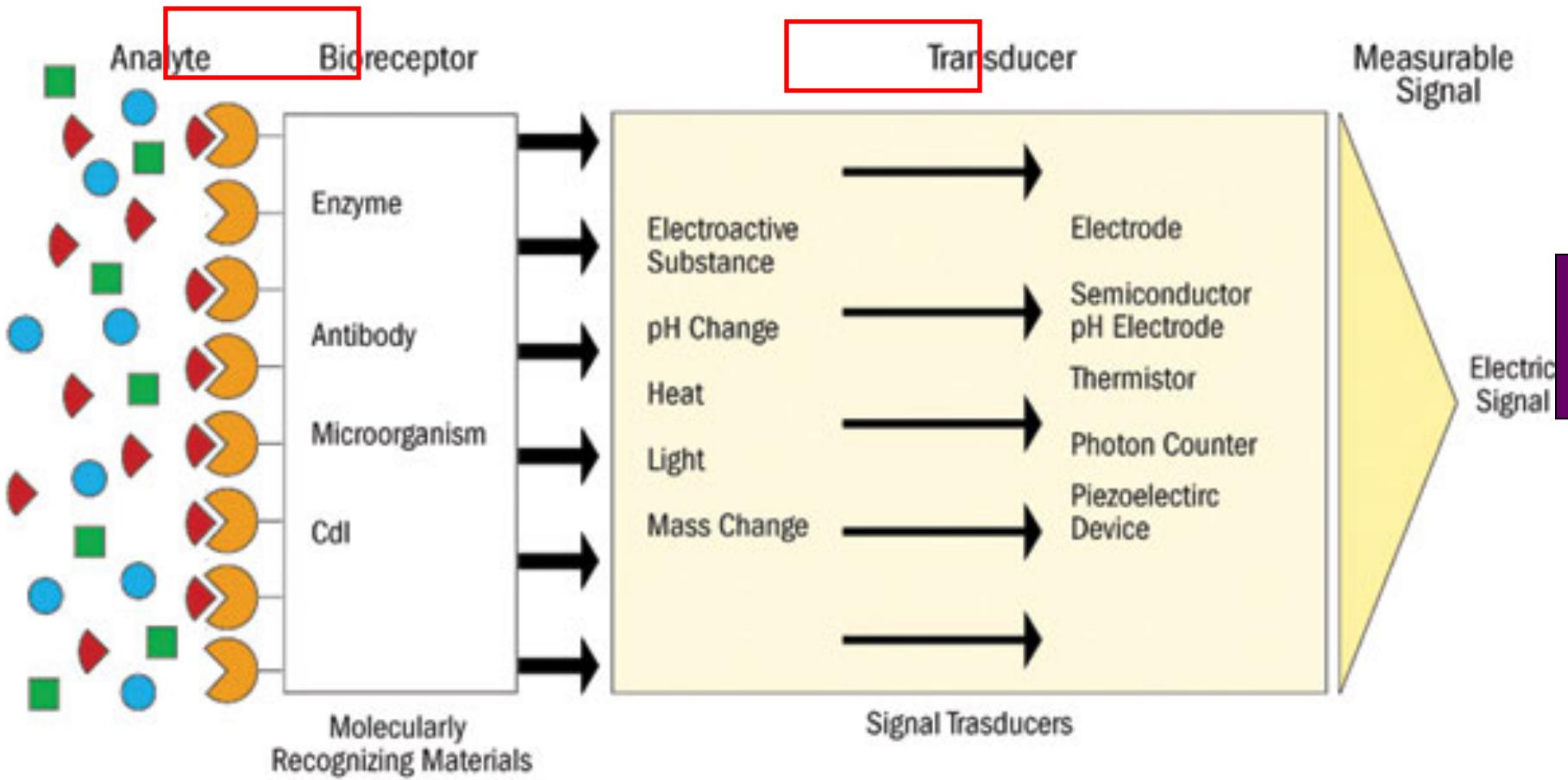


Current Definition

A sensor that integrates a biological element with a physiochemical transducer to produce an electronic signal proportional to a single analyte which is then conveyed to a detector.



Components of a Biosensor



Detector

History of Biosensors

- 1916 First report on immobilization of proteins : adsorption of invertase on activated charcoal
- 1922 First glass pH electrode
- 1956 Clark published his definitive paper on the oxygen electrode.
- 1962 First description of a biosensor: an amperometric enzyme electrode for glucose (Clark)
- 1969 Guilbault and Montalvo – First potentiometric biosensor: urease immobilized on an ammonia electrode to detect urea

History of Biosensors

- 1970 Bergveld – ion selective Field Effect Transistor (ISFET)
- 1975 Lubbers and Opitz described a fibre-optic sensor with immobilised indicator to measure carbon dioxide or oxygen.
- 1975 First commercial biosensor (Yellow springs Instruments glucose biosensor)
- 1975 First microbe based biosensor, First immunosensor
- 1976 First bedside artificial pancreas (Miles)
- 1980 First fibre optic pH sensor for in vivo blood gases (Peterson)
- 1982 First fibre optic-based biosensor for glucose
- 1983 First surface plasmon resonance (SPR) immunosensor
- 1984 First mediated amperometric biosensor: glucose oxidase for glucose detection ferrocene used with

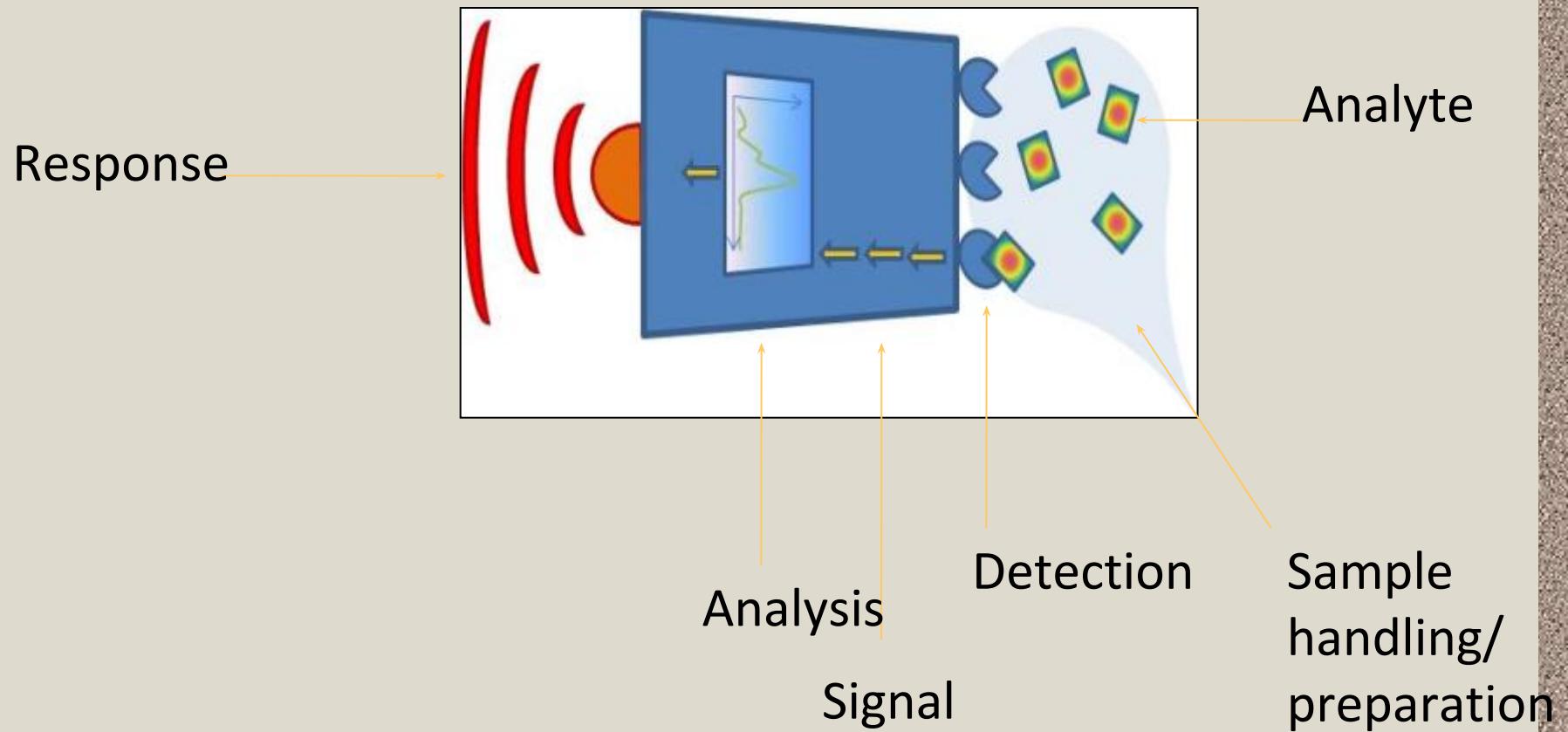
History of Biosensors

- 1987 Blood-glucose biosensor launched by ExacTech MediSense
- 1990 SPR based biosensor by Pharmacia BIACore
- 1992 Hand held blood biosensor by i-STAT
- 1996 Launching of Glucocard
- 1998 Blood glucose biosensor launch by LifeScan FastTake
- 1998 Roche Diagnostics by Merger of Roche and Boehringer mannheim
- Current etc Quantom dots, nanoparticles, nanowire, nanotube,

Basic Characteristics of a Biosensor

- 1. LINEARITY** Linearity of the sensor should be high for the detection of high substrate concentration.
- 2. SENSITIVITY** Value of the electrode response per substrate concentration.
- 3. SELECTIVITY** Chemicals Interference must be minimised for obtaining the correct result.
- 4. RESPONSE TIME** Time necessary for having 95% of the response.

Biosensor



Biosensor

1. The Analyte (What do you want to detect)

Molecule - Protein, toxin, peptide, vitamin, sugar, metal ion

2. Sample handling (How to deliver the analyte to the sensitive region?)

*(Micro) fluidics - Concentration increase/decrease),
Filtration/selection*

Biosensor

◆3. Detection/Recognition

**(How do you specifically recognize
the analyte?)**

4. Signal

(How do you know there was a detection)

Example of biosensors



Pregnancy test

Detects the hCG protein in urine.



Glucose monitoring device (for diabetes patients)

Monitors the glucose level in the blood.

Example of biosensors



**Infectious disease
biosensor from RBS**



**Old time coal miners'
biosensor**

Research Biosensors



Biacore Biosensor platform

Typical Sensing Techniques for Biosensors

- ✓ Fluorescence
- ✓ DNA Microarray
- ✓ SPR Surface plasmon resonance
- ✓ Impedance spectroscopy
- ✓ SPM (Scanning probe microscopy, AFM, STM)
- ✓ QCM (Quartz crystal microbalance)
- ✓ SERS (Surface Enhanced Raman Spectroscopy)
- ✓ Electrochemical

Types of Biosensors

- 1. Calorimetric Biosensor**
- 2. Potentiometric Biosensor**
- 3. Amperometric Biosensor**
- 4. Optical Biosensor**
- 5. Piezo-electric Biosensor**

Piezo-Electric Biosensors

Piezo-electric devices use gold to detect the specific angle at which electron waves are emitted when the substance is exposed to laser light or crystals, such as quartz, which vibrate under the influence of an electric field.

The change in frequency is proportional to the mass of absorbed material.

Electrochemical Biosensors

- **For applied current: Movement of e- in redox reactions detected when a potential is applied between two electrodes.**

Potentiometric Biosensor

- For voltage: Change in distribution of charge is detected using ion-selective electrodes, such as pH-meters.

Optical Biosensors

- Colorimetric for color

Measure change in light adsorption

- Photometric for light intensity

Photon output for a luminescent or fluorescent process can be detected with photomultiplier tubes or photodiode systems.

Calorimetric Biosensors

If the enzyme catalyzed reaction is exothermic, two thermistors may be used to measure the difference in resistance between reactant and product and, hence, the analyte concentration.

Electrochemical DNA Biosensor

- **Steps involved in electrochemical DNA hybridization biosensors:**
 - **Formation of the DNA recognition layer**
 - **Actual hybridization event**
 - **Transformation of the hybridization event into an electrical signal**

DNA biosensor

Motivated by the application to clinical diagnosis and genome mutation detection

Types DNA Biosensors

- Electrodes
- Chips
- Crystals

Wearable Biosensors



Smart Shirt

Ring Sensor



Biosensors on the Nanoscale

- Molecular sheaths around the nanotube are developed that respond to a particular chemical and modulate the nanotube's optical properties.
- A layer of olfactory proteins on a nanoelectrode react with low-concentration odorants (SPOT-NOSED Project). Doctors can use to diagnose diseases at earlier stages.
- Nanosphere lithography (NSL) derived triangular Ag nanoparticles are used to detect streptavidin down to one picomolar concentrations.
- The School of Biomedical Engineering has developed an anti- body based piezoelectric nanobiosensor to be used for anthrax/HIV/hepatitis detection

Potential Applications

- Clinical
- Diagnostic
- Research
- Environmental
- Food & Beverage
- BioDefense
- Medical

Environ- mental

- Rapid Biochemical Oxygen Demand (BOD) tests
- Water bodies & waste-water Tests: lakes, ponds, streams, rivers, bays, etc
- Rapid Toxic Pollution Monitoring

Biol Pharma Research

- Special purpose sensors: optical, electrochemical, etc.
- Protein chip based Systems
- LabChip, Microfluidic Devices
- High Throughput & Drug Discovery Systems

\$7.3 B
Market
in 2003

BioDefense

- Rapid BW detectors; Remote & Standoff BW detectors
- LabChip, microfluidic, optical devices
- Military requirements
- Civil defense, anti-terrorism

Food & Beverage

- Food Safety: Rapid tests for disease, bacteria, BSE
- Electronic Nose & Taste
- Food Decay Detection
- Process Quality

Medical

- Diabetes - Blood glucose meters; consumer, point of care, artificial pancreas, etc.
- Glucose test strips for glucose meters
- Other medical tests - blood gases, lactate, urea, creatinin, etc; central labs, point of care, etc

Application of Biosensor

- ◆ Food Analysis
- ◆ Study of biomolecules and their interaction
- ◆ Drug Development
- ◆ Crime detection
- ◆ Medical diagnosis (both clinical and laboratory use)
- ◆ Environmental field monitoring
- ◆ Quality control
- ◆ Industrial Process Control
- ◆ Detection systems for biological warfare agents
- ◆ Manufacturing of pharmaceuticals and replacement organs

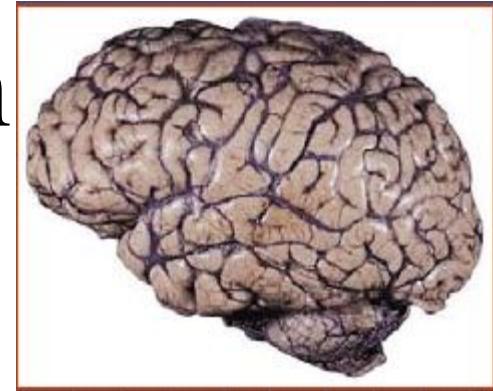
- Biosensors play a part in the field of environmental quality, medicine and industry mainly by identifying material and the degree of concentration present

UNIT 5

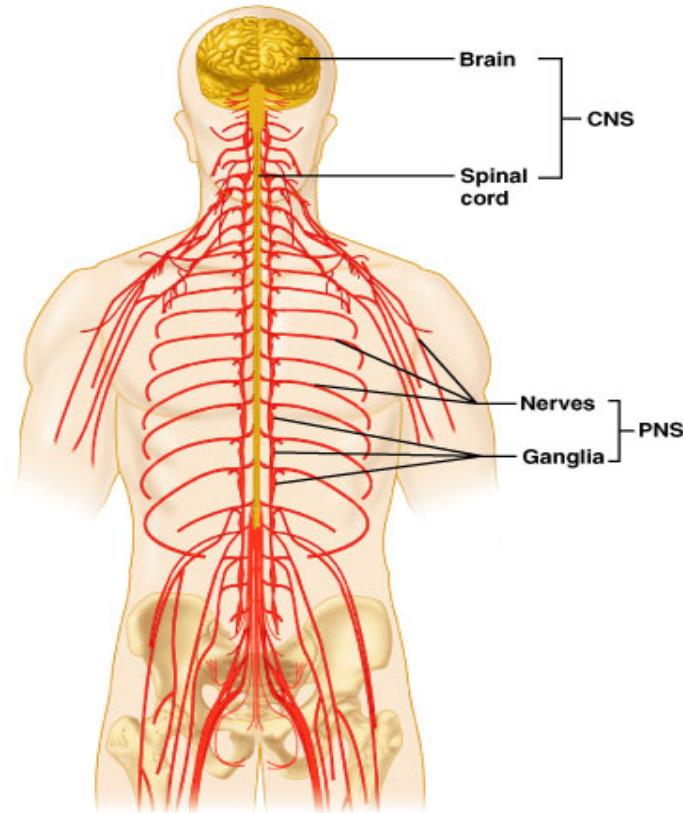
Sensory and Immune systems

Nervous system

- The task of nervous system is to coordinate the mental processes by which we perceive, act, learn and remember.
- The human brain is a network of billions of individual nerve cells interconnected in systems that construct our perceptions of the external world, fix our attention, and control the machinery of our actions.
- The nervous system has two classes of cells: *nerve cells (neurons) and glial cells (glia)*.

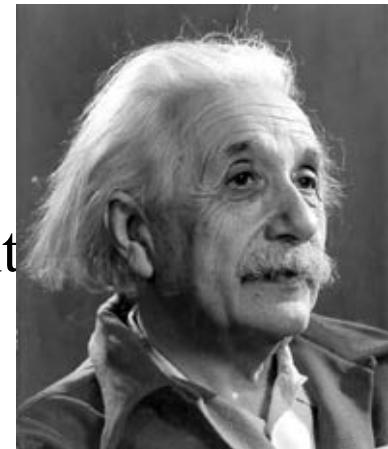


**Neurotransmitters
Neuropeptides**



Supporting Cells (Neuroglial Cells) in the CNS

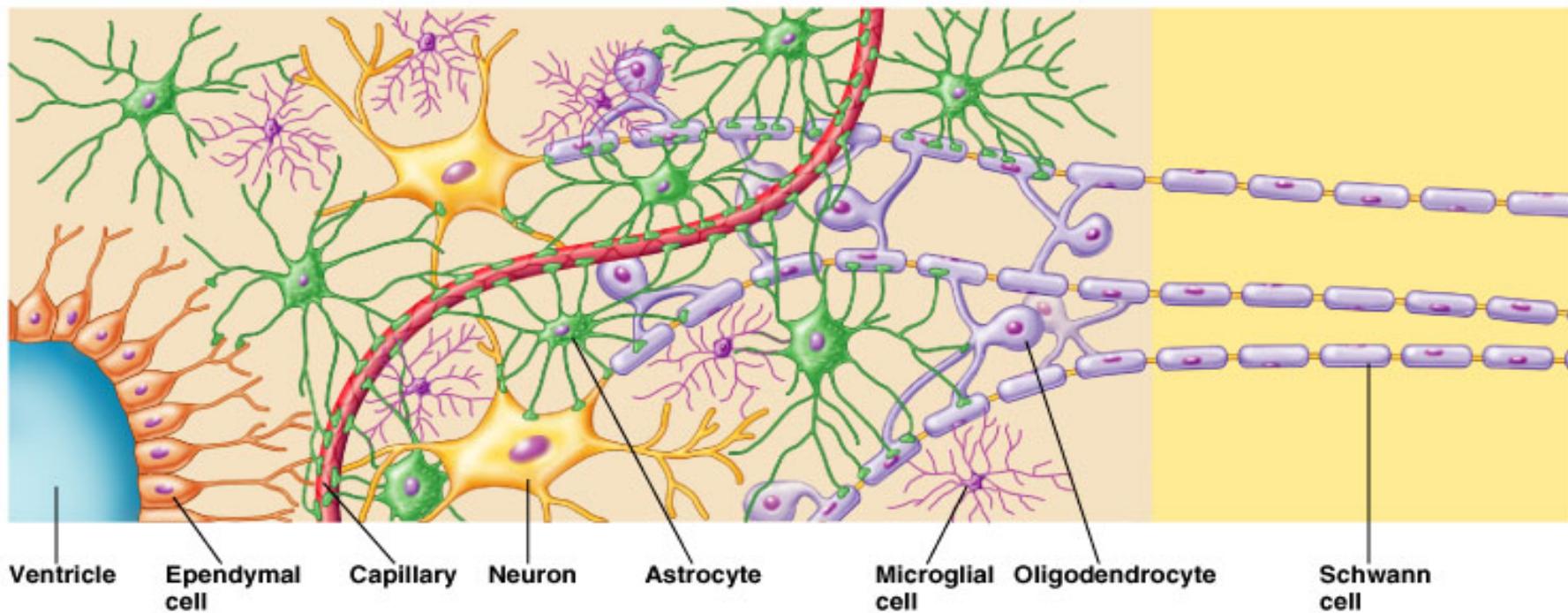
- Neuroglia – usually only refers to supporting cells in the CNS, but can be used for PNS
 - Glial cells have branching processes and a central cell body
 - Outnumber neurons 10 to 1 (the guy on the right had an inordinate amount of them).
 - Make up half the mass of the brain
 - Can divide throughout life



Glial cells

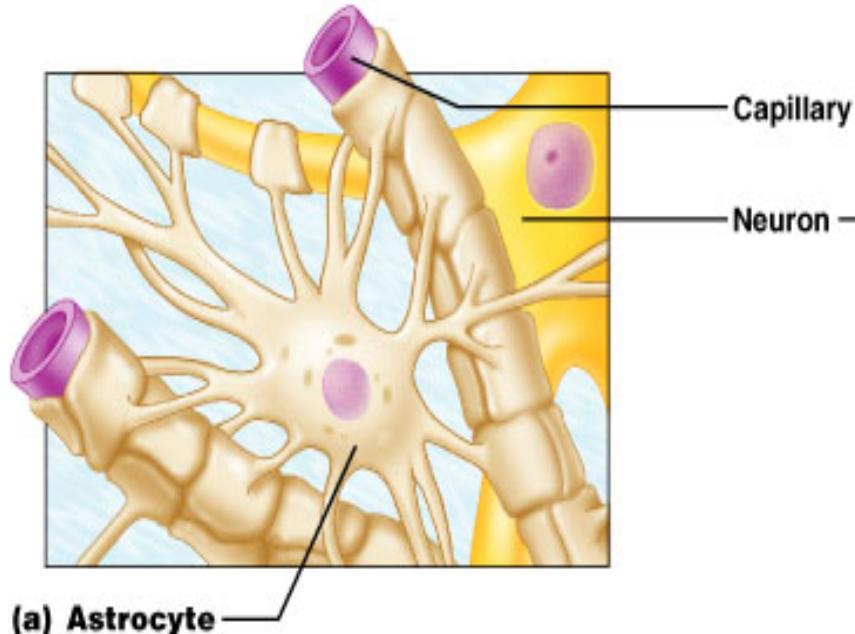
- Glial cells are support cells.
- They are more in number than neurons.
- There are between 10 and 50 times more glia than neurons in the brain of humans.
- The name for these cells derives from the Greek word for glue. In actual terms, the glia do not commonly hold nerve cells together but surround the neurons.

Central Nervous System

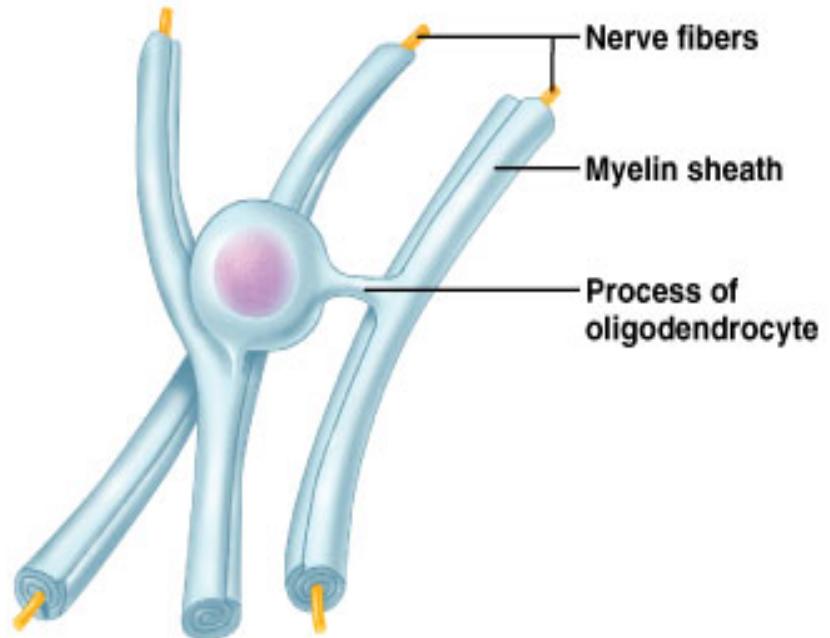


Types of Glial Cells in the CNS: Astrocytes, Oligodendrocytes, and Microglia

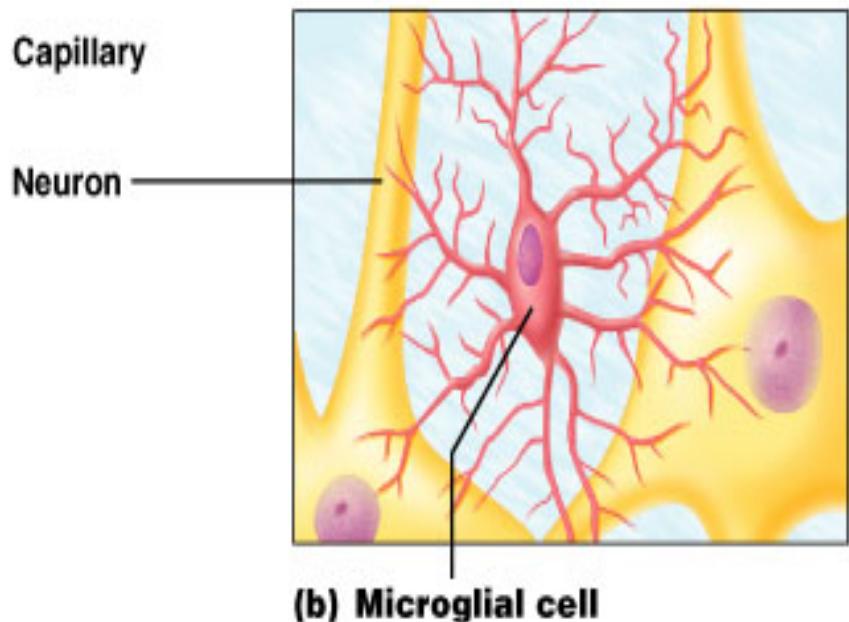
- **Astrocytes:** Most abundant glial cell type, irregular star-shaped cell bodies .
 - Take up and release ions to control the environment around neurons
 - Recapture and recycle neurotransmitters
 - Involved with synapse formation in developing neural tissue
 - Produce molecules necessary for neural growth
 - Propagate calcium signals that may be involved in memory



- **Oligodendrocytes:** Have few branches.
- Wrap their cell processes around axons in CNS
- Produce myelin sheaths for rapid conduction of nerve impulses
 - *Schwann cells* surround axons in the PNS and form myelin sheath around axons of the PNS

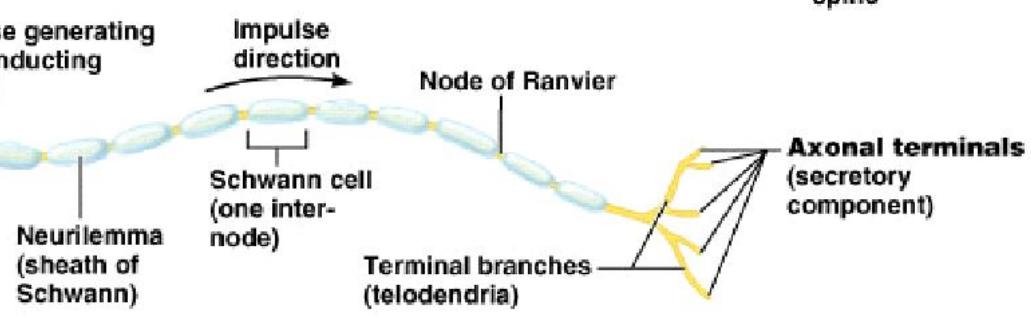
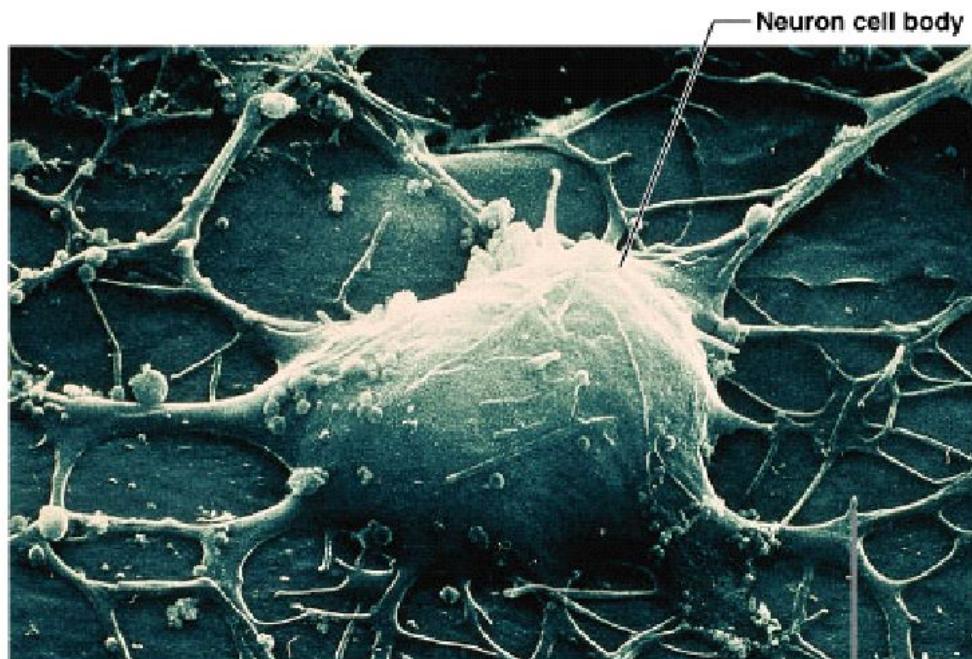
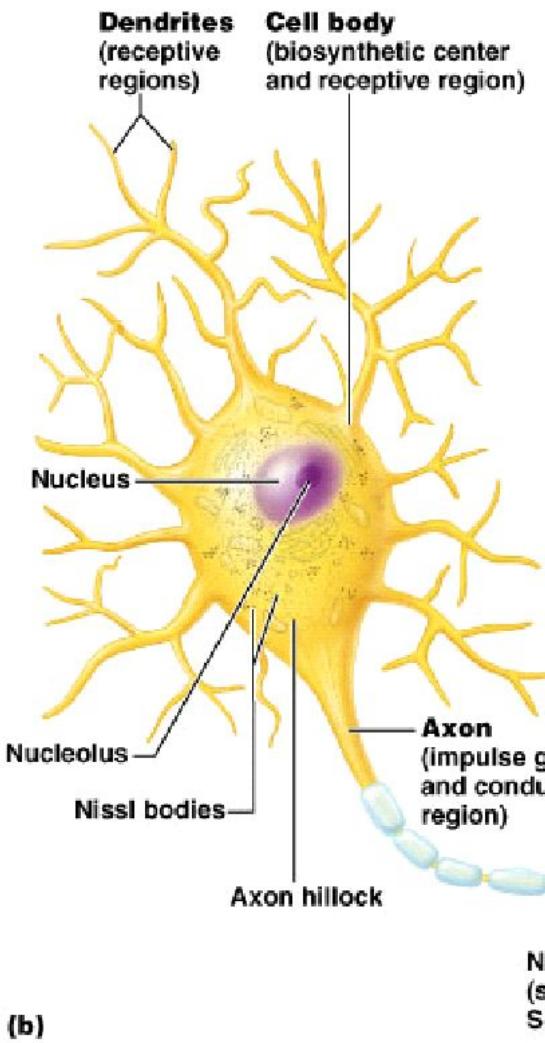


- ***Microglia:*** Smallest and least abundant.
 - Phagocytes – the macrophages of the CNS
 - Engulf invading microorganisms and dead neurons
 - Derived from blood cells called monocytes



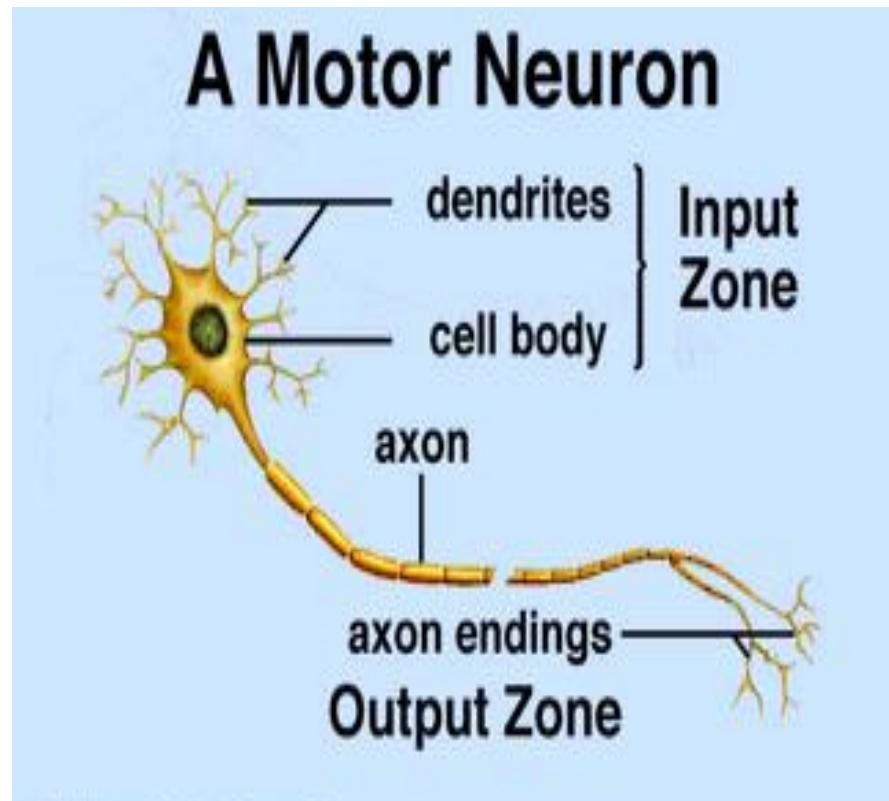
Nerve cells- Neurons

- Nerve cells are the main signaling units of the nervous system.
- A typical neuron has four morphologically defined regions: *the cell body, dendrites, the axon, and presynaptic terminal*
- The *cell body (soma)* is the metabolic center of the cell. It contains the nucleus which stores the genes of the cell, as well as the endoplasmic reticulum, an extension of the nucleus where the cell's proteins are synthesized.
- The cell body gives rise to two kinds of processes: several short *dendrites* and one, long, tubular *axon*.
- These processes vary in number & relative length but always serve to conduct impulses (with dendrites conducting impulses toward the cell body and axons conducting impulses away from the cell body as shown in the figure).



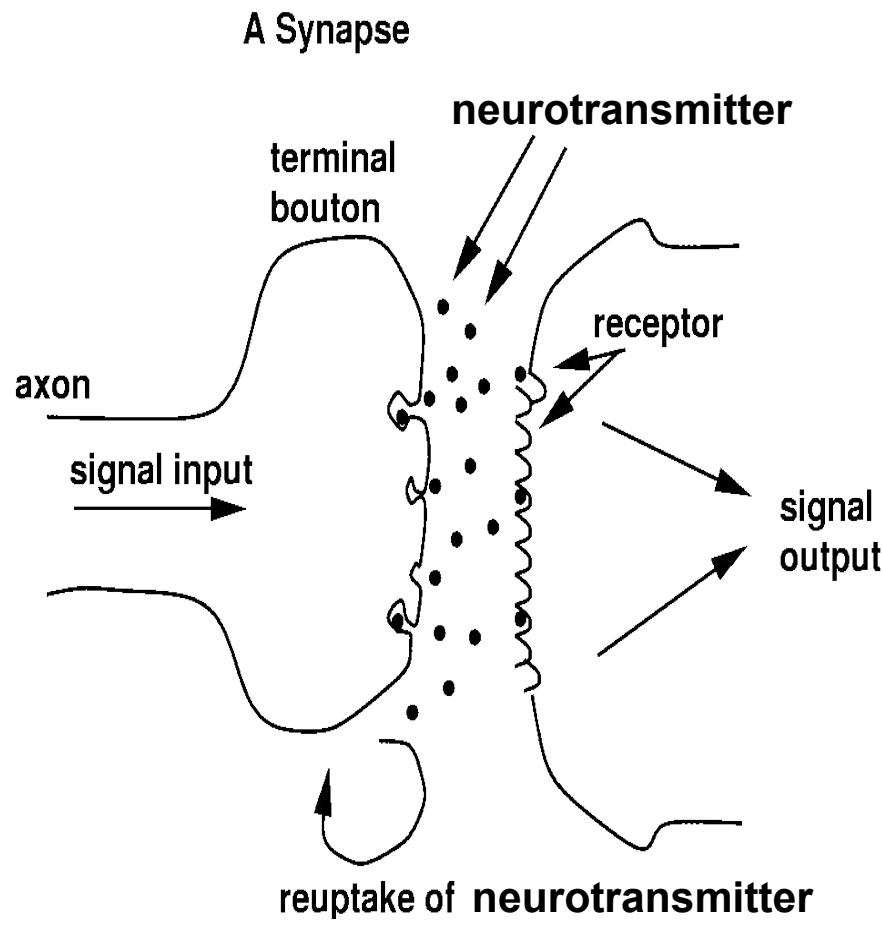
Action Potential

- A neuron receives input from other neurons (typically many thousands). All the input signals are integrated. Once input exceeds a critical level, the neuron discharges a **spike** - an electrical pulse that travels from the body, down the axon, to the next neuron(s) (or other receptors). This spiking event is also called **depolarization**, and is followed by a **refractory period**, during which the neuron is unable to fire.

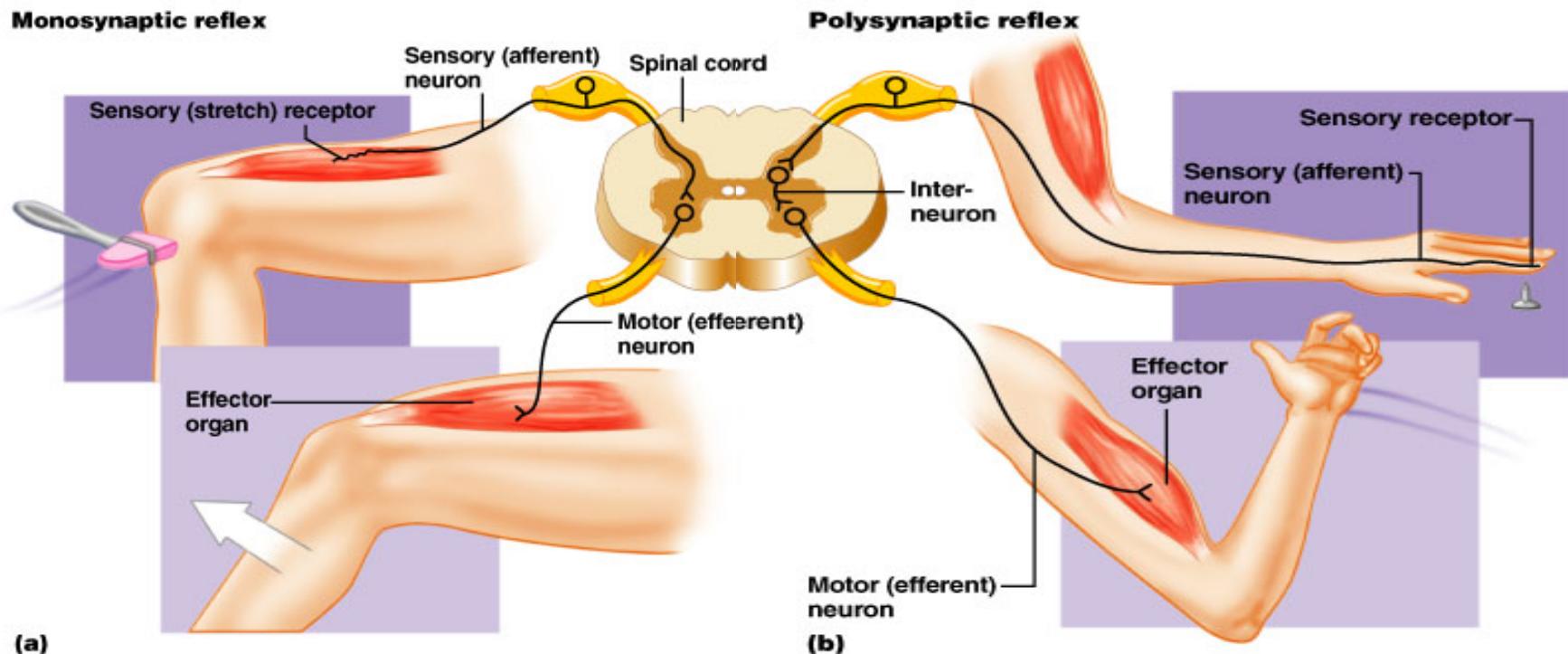


Synapse

- The axon endings (Output Zone) almost touch the dendrites or cell body of the next neuron.
- Transmission of an electrical signal from one neuron to the next is effected by neurotransmitters, chemicals which are released from the first neuron and which bind to receptors in the second. This link is called a synapse.
- The extent to which the signal from one neuron is passed on to the next depends on many factors, e.g. the amount of neurotransmitter available, the number and arrangement of receptors, amount of neurotransmitter reabsorbed, etc.



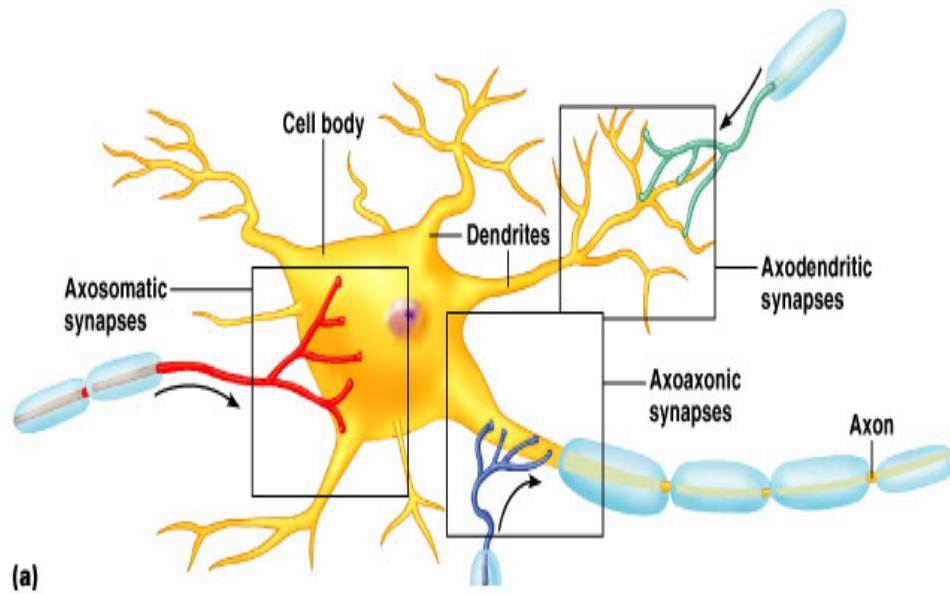
- Sensory signals picked up by sensory receptors
 - Carried by afferent nerve fibers of PNS to the CNS
- Motor signals are carried away from the CNS
 - Carried by efferent nerve fibers of PNS to effectors
 - Innervate muscles and glands



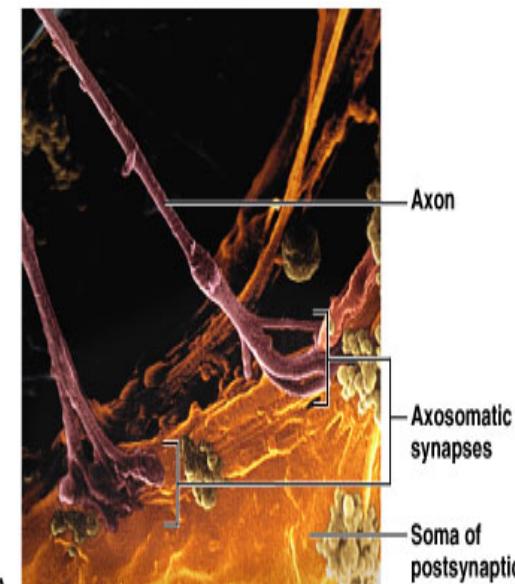
Animation on nerve cells

- <https://www.youtube.com/watch?v=NsBaPtemAjs>

- The dendrites branch out in a tree-like fashion and are the main apparatus for receiving incoming signals from other nerve cells.
- In contrast, the axon extends away from the cell body and is the main conducting unit for carrying signals for other neurons.



(a)

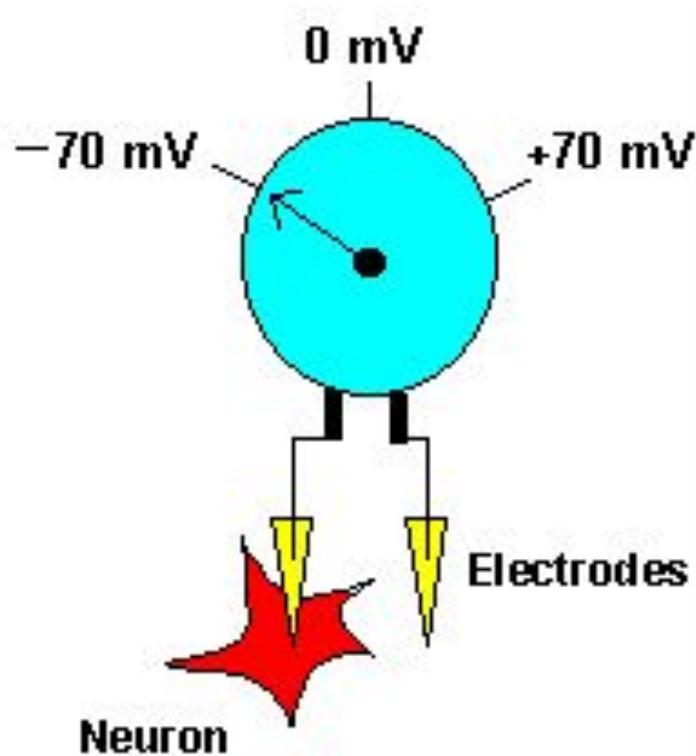


(b)

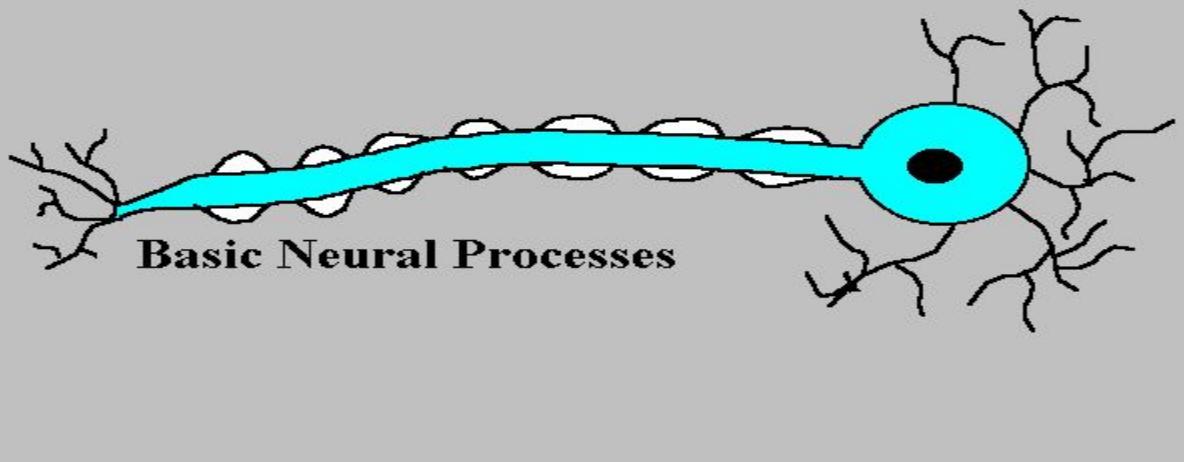
An Animation for nerve impulse

<https://www.youtube.com/watch?v=b2ctEsGEpe0>

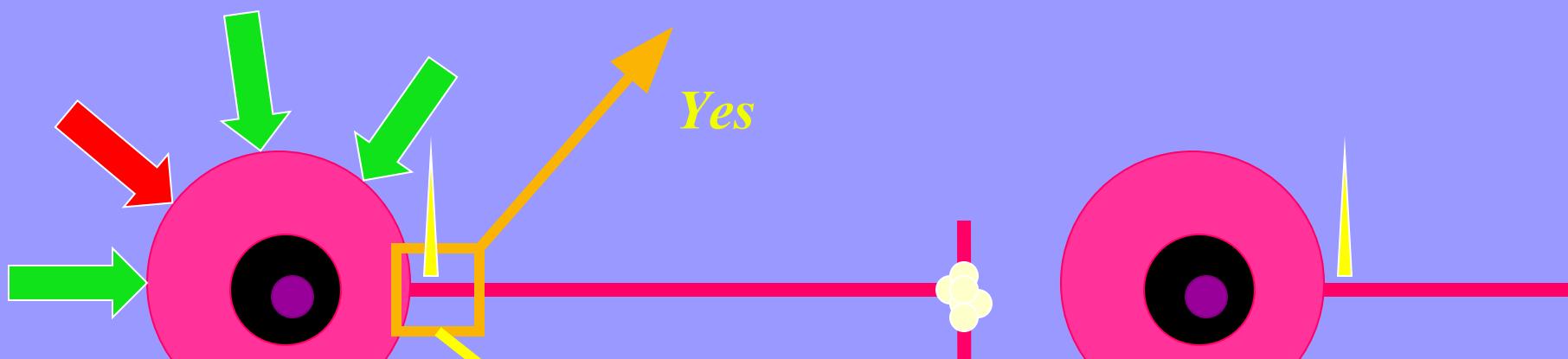
- An axon can convey electrical signals along distances ranging from 0.1 mm to 3 m. These electrical signals called *action potentials* are rapid, transient with an amplitude of 100 mV and a duration of about 1ms.
- Neurons can respond to stimuli and conduct impulses because a membrane potential is established across the cell membrane. In other words, there is an unequal distribution of ions (charged atoms) on the two sides of a nerve cell membrane.
- This can be illustrated with a voltmeter: With one electrode placed inside a neuron and the other outside, the voltmeter is 'measuring' the difference in the distribution of ions on the inside versus the outside (see the adjoining figure). And, in this example, the voltmeter reads -70 mV (mV = millivolts). In other words, the inside of the neuron is slightly negative relative to the outside. This difference is referred to as the Resting Membrane Potential. It is called a RESTING potential because it occurs when a membrane is not being stimulated or conducting impulses (in other words, it's resting).



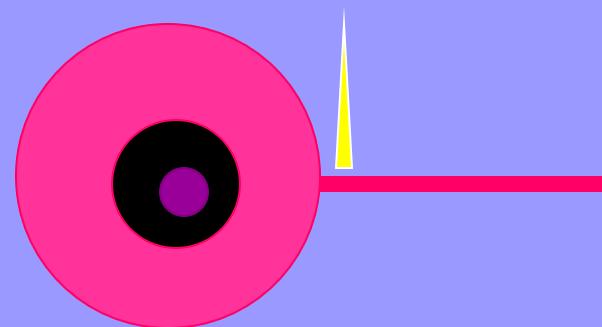
Nerve Conduction



Yes or No



*Axon hillock or
Initial segment*



Key:

- = Structure
- = Function

Central Nervous System (CNS)

- Brain and spinal cord
- Integrative and control centers



Peripheral Nervous System (PNS)

- Cranial nerves and spinal nerves
- Communication lines between the CNS and the rest of the body



Sensory (afferent) division

- Somatic and visceral sensory nerve fibers
- Conducts impulses from receptors to the CNS

Motor (efferent) division

- Motor nerve fibers
- Conducts impulses from the CNS to effectors (muscles and glands)

Autonomic nervous system (ANS)

- Visceral motor (involuntary)
- Conducts impulses from the CNS to cardiac muscles, smooth muscles, and glands

Parasympathetic division

- Conserves energy
- Promotes "housekeeping" functions during rest

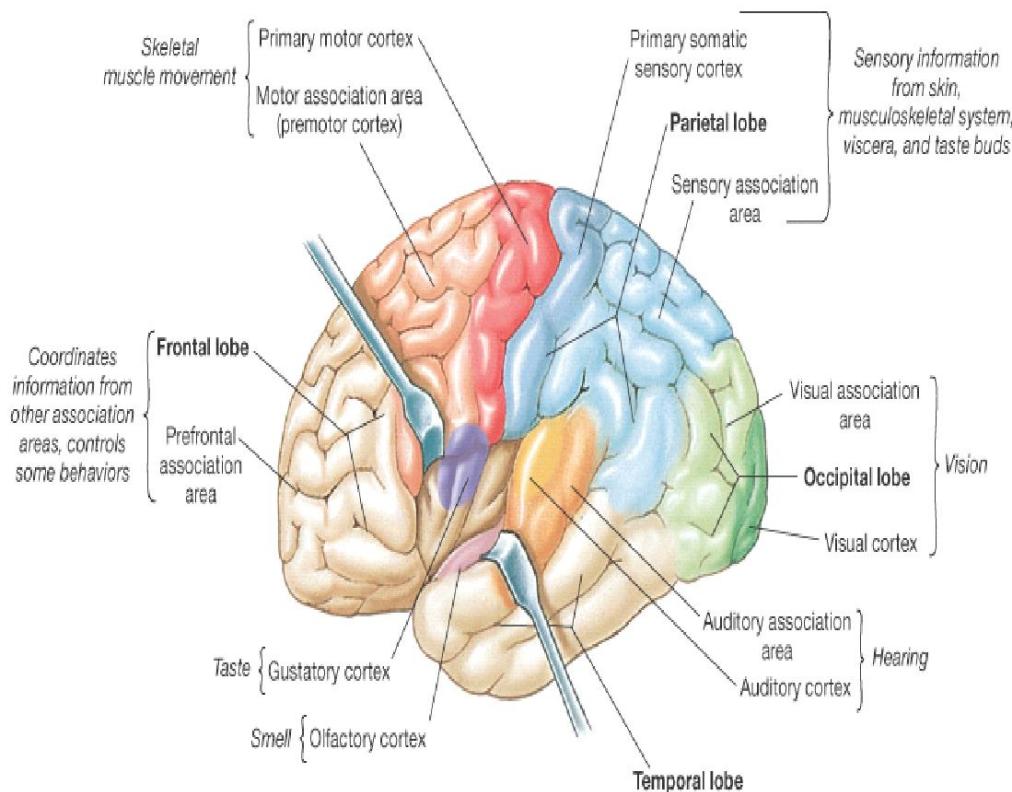
Somatic nervous system

- Somatic motor (voluntary)
- Conducts impulses from the CNS to skeletal muscles



Neural Networks in the Brain

- The **brain** is not a homogeneous organ. At the largest anatomical scale, we distinguish **cortex**, **midbrain**, **brainstem**, and **cerebellum**. Each of these can be hierarchically subdivided into many **regions**, and **areas** within each region, either according to the anatomical structure of the neural networks within it, or according to the function performed by them.
- In addition to these long-range connections, neurons also link up with many thousands of their neighbors. In this way they form very dense, complex local networks:



Computer-based Neural Networks

- The brain's network of neurons forms a massively parallel information processing system. This contrasts with conventional computers, in which a single processor executes a single series of instructions.
- Despite of being built with very slow hardware, the brain has quite remarkable capabilities:
 - its performance tends to degrade gracefully under partial damage. In contrast, most programs and engineered systems are brittle: if you remove some arbitrary parts, very likely the whole will cease to function.
 - it can learn (reorganize itself) from experience.
 - this means that partial recovery from damage is possible if healthy units can learn to take over the functions previously carried out by the damaged areas.
 - it performs massively parallel computations extremely efficiently. For example, complex visual perception occurs within less than 100 ms, that is, 10 processing steps!
 - it supports our intelligence and self-awareness. (Nobody knows yet how this occurs.)

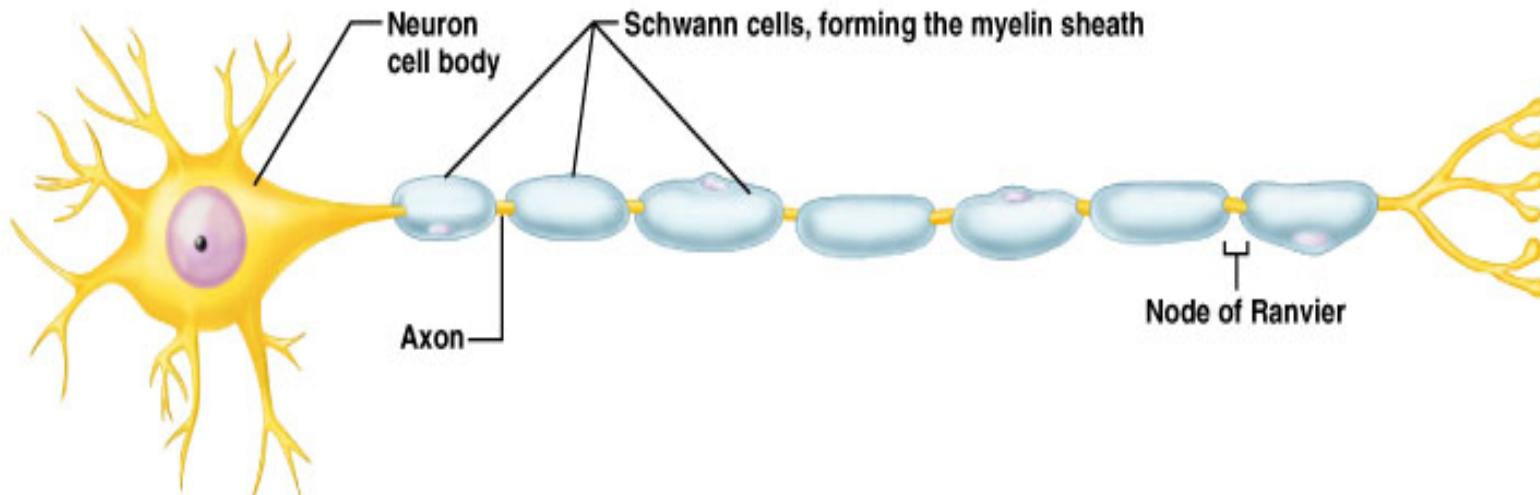
Applications of Neural Networks

- **Aerospace:** High performance aircraft autopilots, flight path simulations, aircraft control systems, autopilot enhancements, aircraft component simulations, aircraft component fault detectors
- **Automotive:** Automobile automatic guidance systems, warranty activity analyzers
- **Banking:** Check and other document readers, credit application evaluators
- **Cognitive science:** Modeling higher level reasoning, language, problem solving, Modeling lower level reasoning, vision, audition speech recognition, speech generation
- **Defense:** Weapon steering, target tracking, object discrimination, facial recognition, new kinds of sensors, sonar, radar and image signal processing including data compression, feature extraction and noise suppression, signal/image identification
- **Electronics:** Code sequence prediction, integrated circuit chip layout, process control, chip failure analysis, machine vision, voice synthesis, nonlinear modeling
- **Entertainment:** Animation, special effects, market forecasting
- **Financial:** Real estate appraisal, loan advisor, mortgage screening, corporate bond rating, credit line use analysis, portfolio trading program, corporate financial analysis, currency price prediction
- **Insurance:** Policy application evaluation, product optimization
- **Manufacturing:** Manufacturing process control, product design and analysis, process and machine diagnosis, real-time particle identification, visual quality inspection systems, beer testing, welding quality analysis, paper quality prediction, computer chip quality analysis, analysis of grinding operations, chemical product design analysis, machine maintenance analysis, project bidding, planning and management, dynamic modeling of chemical process systems

- **Mathematics:** Nonparametric statistical analysis and regression.
- **Medical:** Breast cancer cell analysis, EEG and ECG analysis, prosthesis design, optimization of transplant times, hospital expense reduction, hospital quality improvement, emergency room test advisement
- **Neurobiology:** Modeling models of how the brain works, neuron-level, higher levels: vision, hearing, etc. Overlaps with cognitive folks.
- **Oil and Gas:** Exploration
- **Philosophy:** Can human souls/behavior be explained in terms of symbols, or does it require something lower level, like a neurally based model?
- **Robotics:** Trajectory control, forklift robot, manipulator controllers, vision systems
- **Speech:** Speech recognition, speech compression, vowel classification, text to speech synthesis
- **Securities:** Market analysis, automatic bond rating, stock trading advisory systems
- **Telecommunications:** Image and data compression, automated information services, real-time translation of spoken language, customer payment processing systems
- **Transportation:** Truck brake diagnosis systems, vehicle scheduling, routing systems

Disorders of the Nervous System

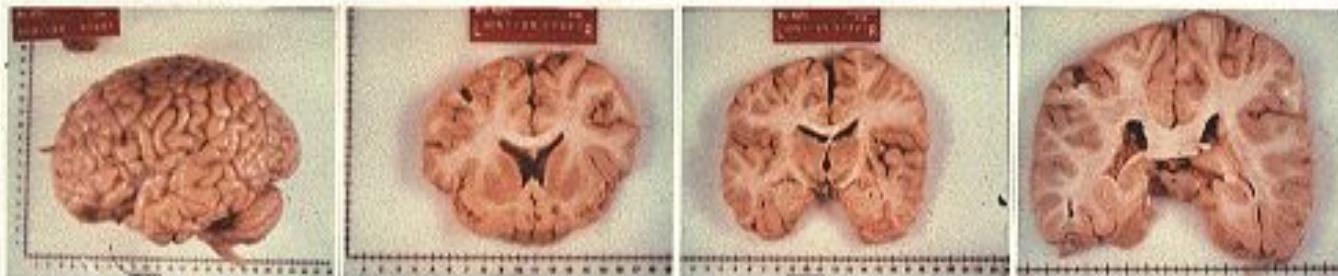
- Multiple sclerosis – common cause of neural disability
 - Varies widely in intensity among those affected
 - Cause is incompletely understood
 - An autoimmune disease
 - Immune system attacks the myelin around axons in the CNS



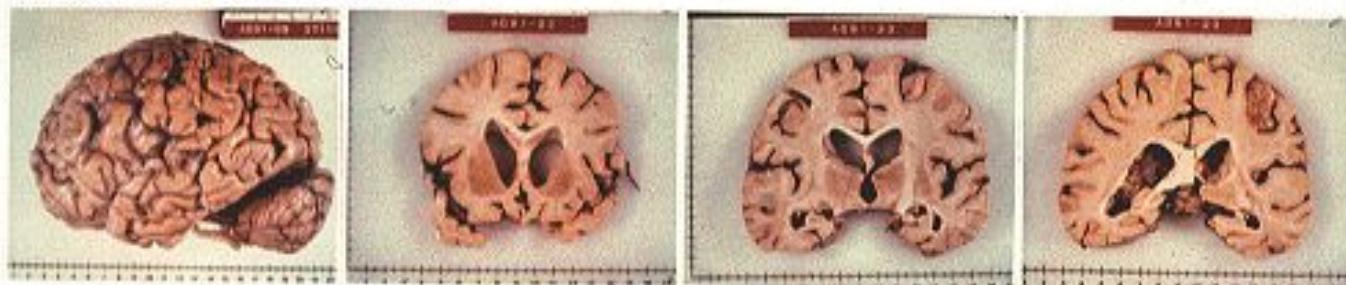
(a) PNS

BRAIN ATROPHY VISUAL STANDARDS

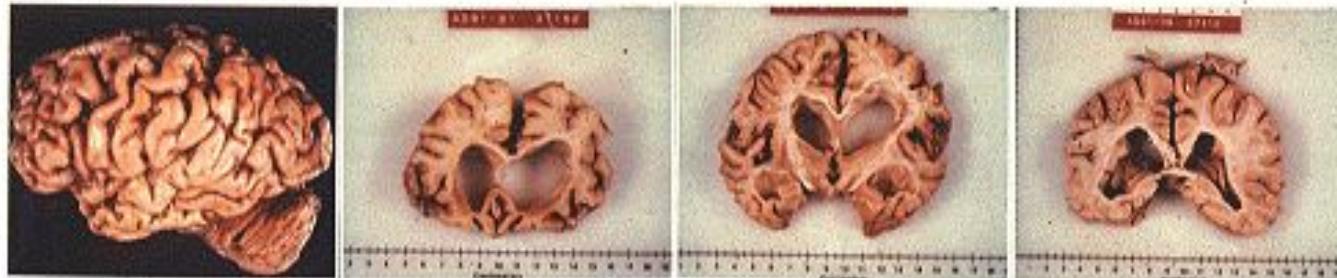
GRADE = 1 (NONE, NL FOR AGE)



GRADE = 2 (MODERATE)



GRADE = 3 (SEVERE)

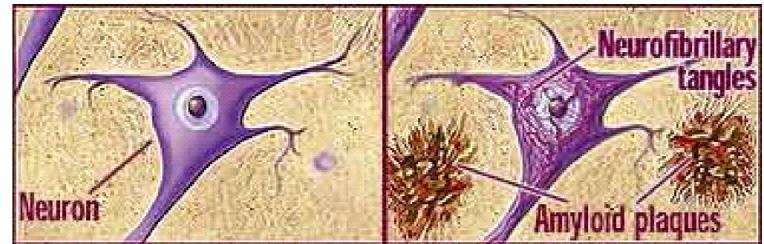


frontal horns

body/temporal horns

trigone

- **Alzheimer's Disease:**
 - Age-associated disorder
 - Loss of memory, cognition, and executive performances
 - Deposits of amyloid plaques and neurofibrillary tangles that interfere with neuronal functions
 - Loss of cholinergic neuronal functions
- **Parkinson's Disease:**
 - Age-associated disorder
 - Rigidity and incoordination interfering with mobility
 - Loss of dopaminergic neuronal functions



Alzheimer's Disease



Parkinson's Disease

List of neurodegenerative diseases

- Alexander's disease
- Alper's disease
- Alzheimer's disease
- Amyotrophic lateral sclerosis
- Ataxia telangiectasia
- Batten disease (also known as Spielmeyer-Vogt-Sjogren-Batten disease)
- Bovine spongiform encephalopathy (BSE)
- Canavan disease
- Cockayne syndrome
- Corticobasal degeneration
- Creutzfeldt-Jakob disease
- Huntington's disease
- HIV-associated dementia
- Kennedy's disease
- Krabbe's disease
- Lewy body dementia
- Machado-Joseph disease (Spinocerebellar ataxia type 3)
- Multiple sclerosis
- Multiple System Atrophy
- Narcolepsy
- Neuroborreliosis
- Parkinson's disease
- Pelizaeus-Merzbacher Disease
- Pick's disease
- Primary lateral sclerosis
- Prion diseases
- Refsum's disease
- Schilder's disease
- Subacute combined degeneration of spinal cord secondary to Pernicious Anaemia
- Schizophrenia
- Spielmeyer-Vogt-Sjogren-Batten disease (also known as Batten disease)
- Spinocerebellar ataxia (multiple types with varying characteristics)
- Spinal muscular atrophy
- Steele-Richardson-Olszewski disease
- Tabes dorsalis

Immune System

Introduction

Fluid Systems of the Body

Innate Immunity

Adaptive or Acquired Immunity

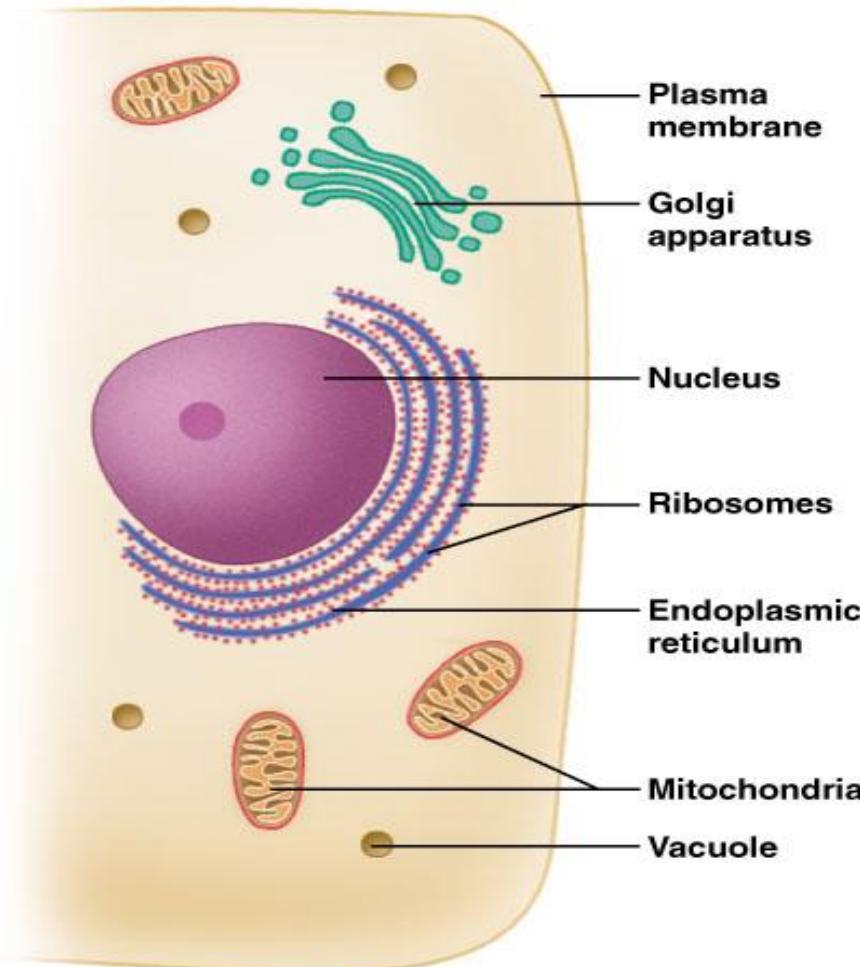
Cell-mediated immunity

Humoral immunity

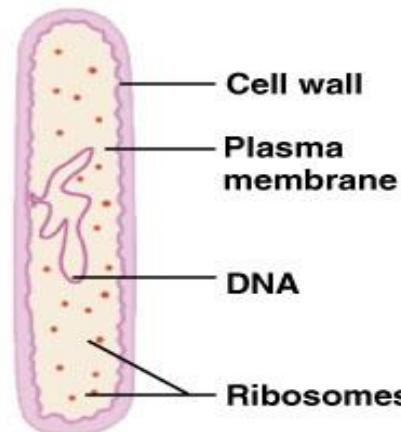
Immune Engineering

Cell Signaling

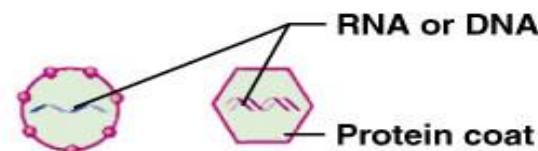
Eukaryotic Cells, Bacteria, and Viruses



(a) Eukaryotic cell



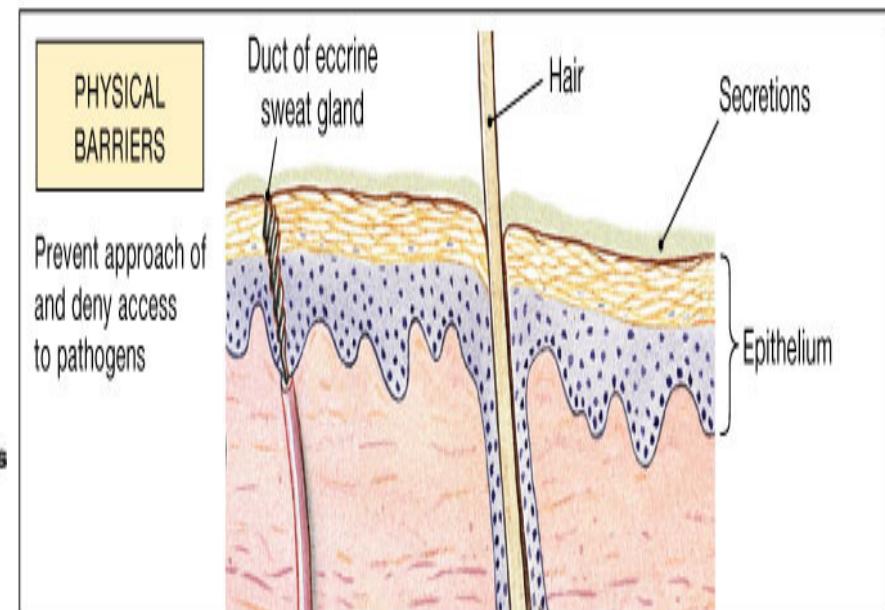
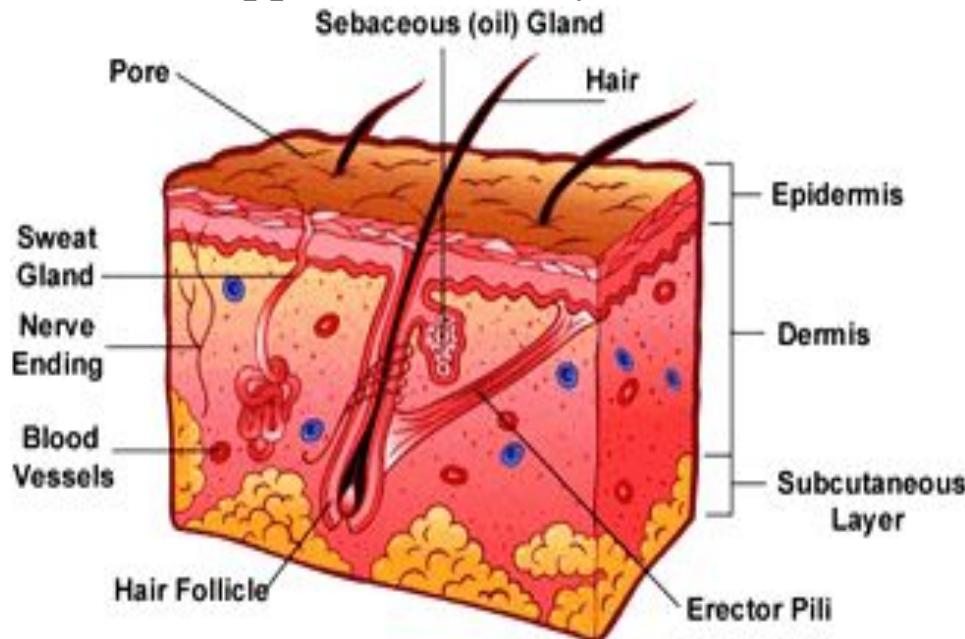
(b) Bacterium



(c) Viruses

Body Defenses: Overview

- Physical barriers: skin & epithelial linings & cilia
- Chemical: acids, mucus & lysozymes
- Immune defenses – internal
 - Innate, non-specific, immediate response (min/hrs)
 - Acquired – attack a specific pathogen (antigen)
- Steps in Immune defense
 - Detect invader/foreign cells
 - Communicate alarm & recruit immune cells
 - Suppress or destroy invader

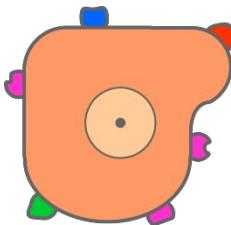


Discrimination of self from non-self

“The success of the immune system depends on its ability to discriminate between foreign (nonself) and host (self) cells. Survival requires both the **ability** to mount a destructive immune response against **nonself** and the **inability** to mount a destructive response against **self**. ”

Markers of Self

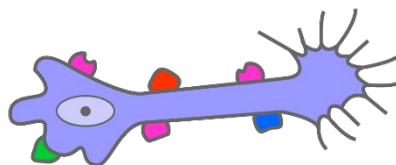
Epithelial cell



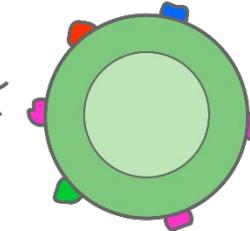
Muscle cell



Nerve cell



Leukocyte



Attributed by Joanne Kelly, RPTC, C.

At the heart of the immune response is the ability to **distinguish between “self” and “non-self.”**

- Every cell in your body carries the same set of distinctive surface proteins that distinguish you as “self.”
- Normally your immune cells do not attack your own body tissues, which all carry the same pattern of self-markers; rather, your immune system coexists peaceably with your other body cells in a state known as self-tolerance.

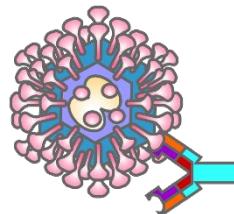
This set of unique markers on human cells is called the **major histocompatibility complex (MHC) proteins.**

Markers of Non-self

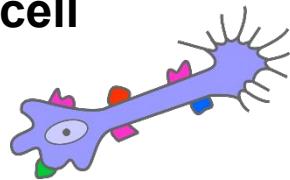
Bacteria



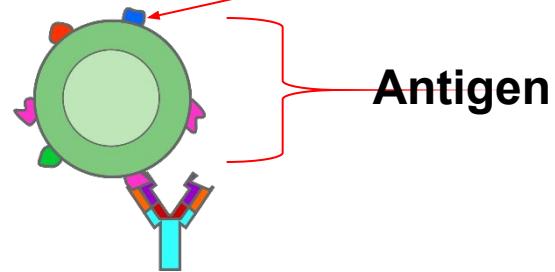
SARS virus



Non-self nerve cell



Non-self leukocyte



Epitope

Antigen

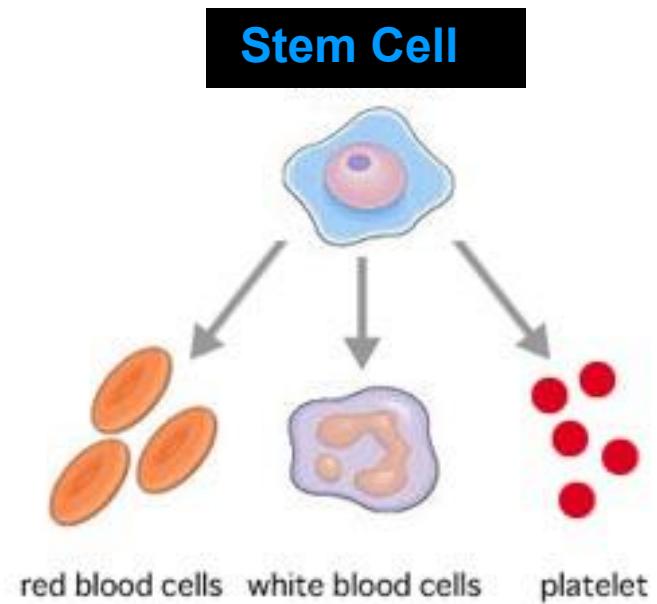
Antigen = any non-self substance

- Virus
- Bacteria
- Non-self cell (foreign cell)

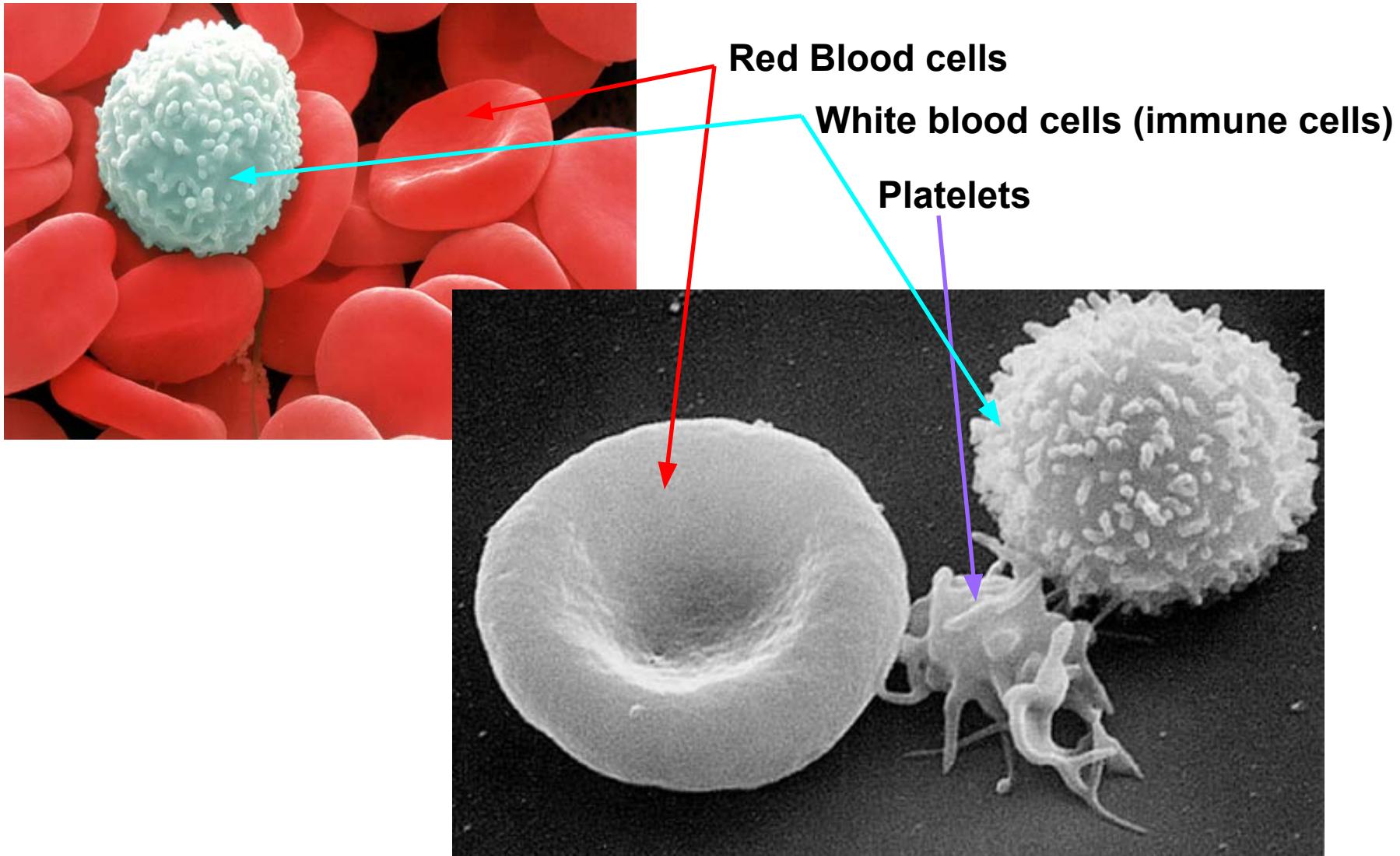
Epitope = The distinctive markers on antigens that trigger an immune response

Blood

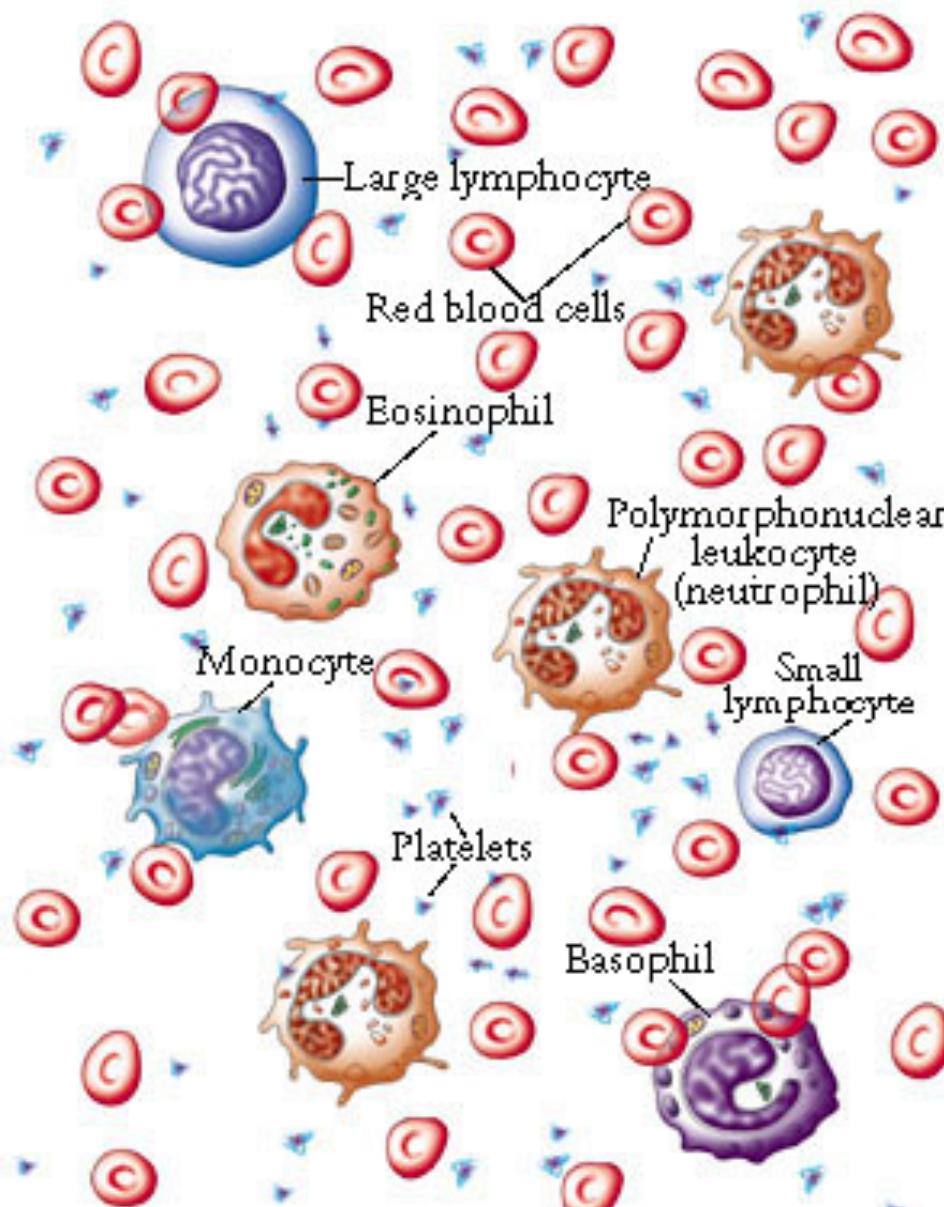
- Blood is 55% liquid (plasma) and 45% cellular
- Cellular component of blood:
 - Red blood cells = carry oxygen
 - White blood cells = immune system
 - Platelets = clot blood
- All blood cells arise from a pluri-potent stem cell found in bone marrow



Blood cells

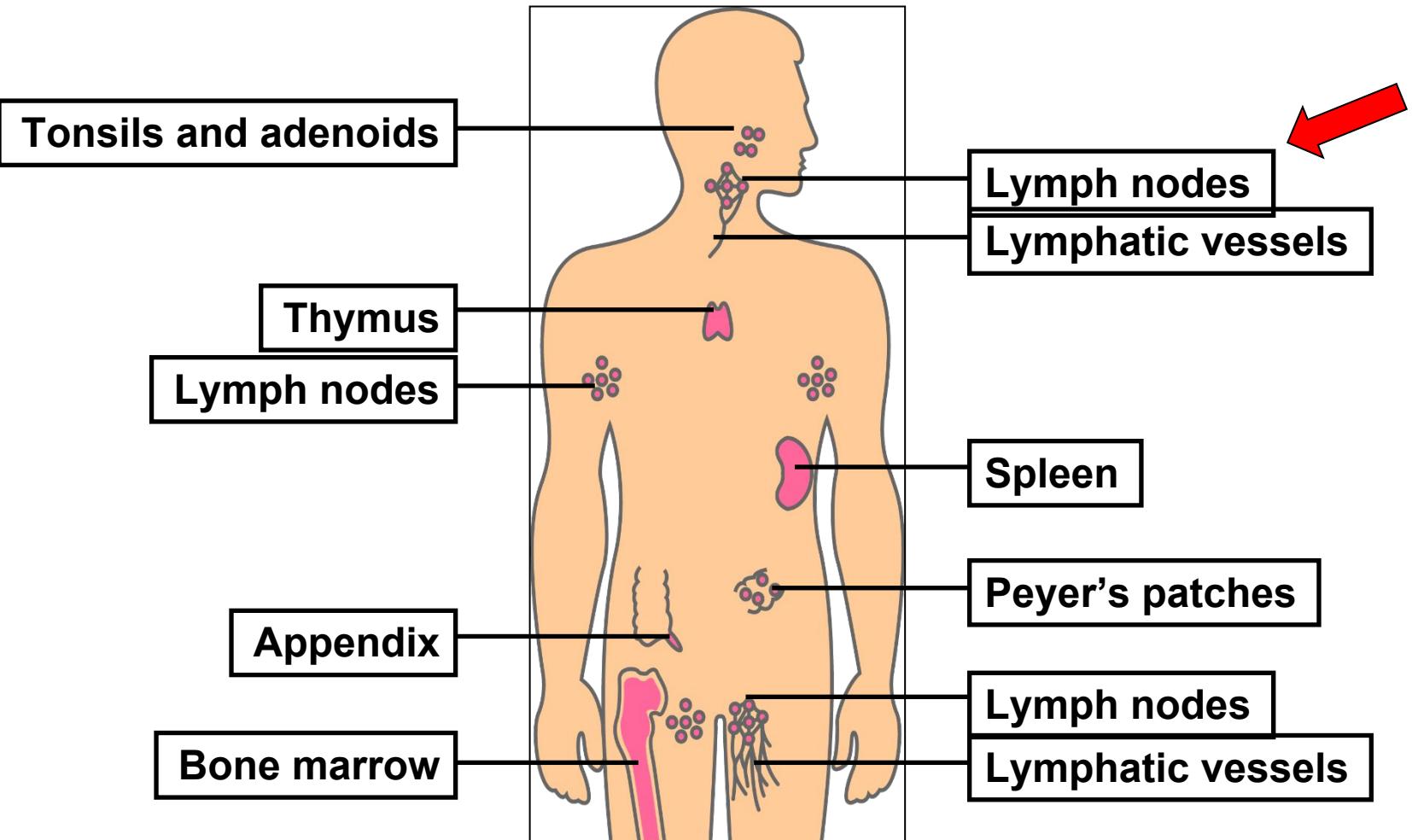


Leukocytes in the Blood



| | | | |
|------------------------|-------------------|---|---------------|
| Red Blood Cells | | $5.0 \times 10^6/\text{mm}^3$ | |
| Platelets | | $2.5 \times 10^5/\text{mm}^3$ | |
| Leukocytes | | $7.3 \times 10^3/\text{mm}^3$ | |
| 1 | Neutrophil | | 50-70% |
| 2 | Lymphocyte | | 20-40% |
| 3 | Monocyte | | 1-6% |
| 4 | Eosinophil | | 1-3% |
| 5 | Basophil | | <1% |

Organs of the Immune System



Bone marrow, the soft tissue in the hollow center of bones, is the ultimate source of all blood cells, including the immune cells.

Lymphatic System

The organs of your immune system are connected with one another and with other organs of the body by a network of lymphatic vessels.

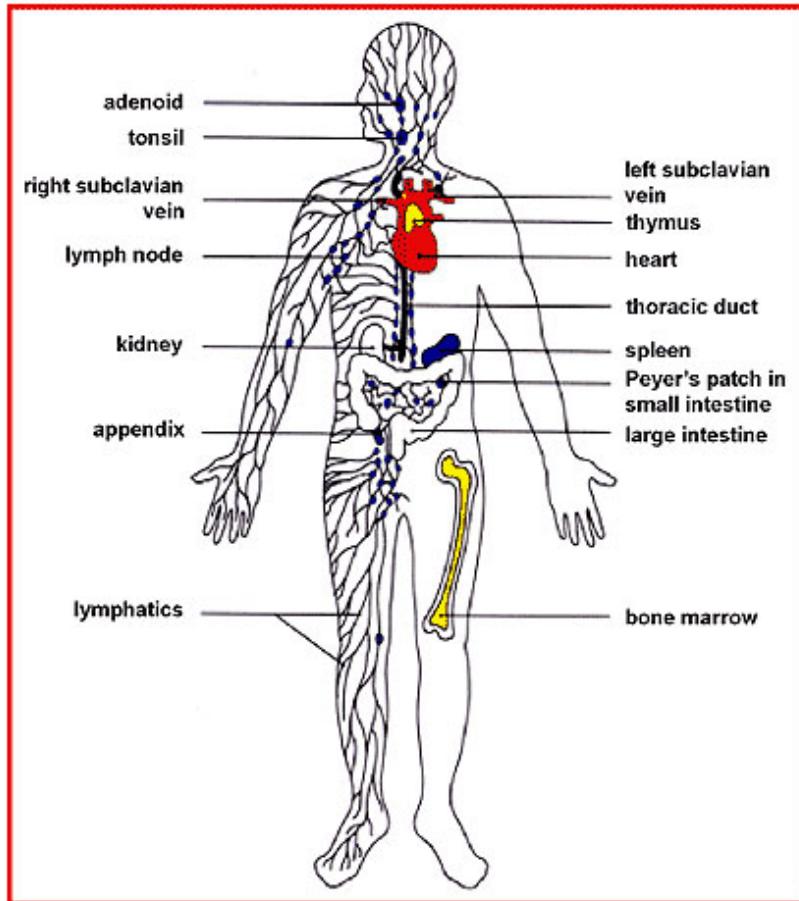
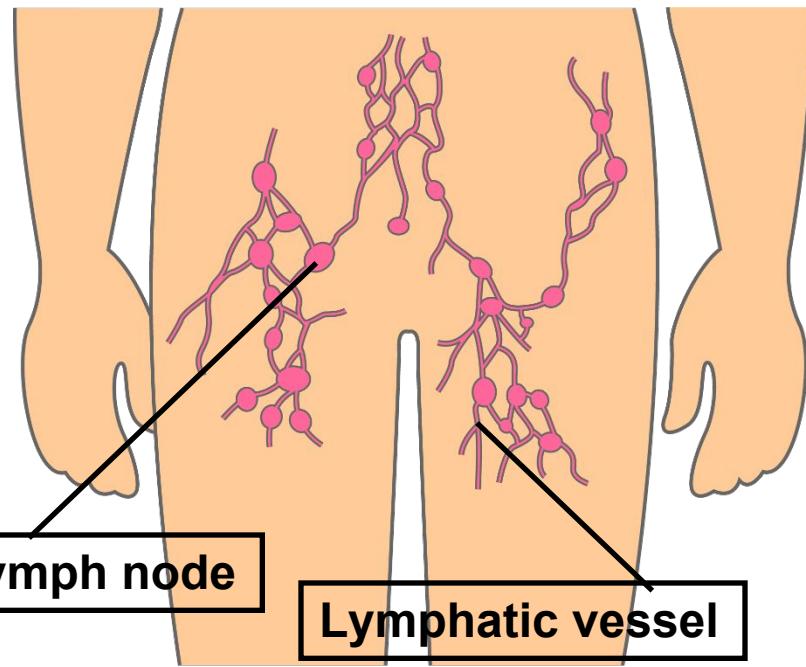


Figure 1. The immune system.

Lymphatic System

The organs of your immune system are connected with one another and with other organs of the body by a network of lymphatic vessels.

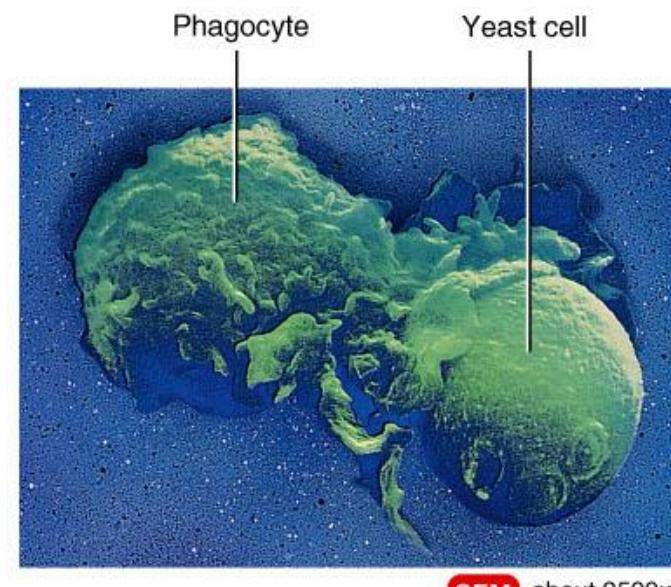


1. Lymphatic **vessels** closely parallels the body's veins and arteries
 - Lymphatic vessels carry *lymph*, a clear fluid that bathes the body's tissues
 - Cells/fluids are exchanged between blood and lymphatic vessels, enabling the lymphatic system to monitor the body for invading microbes.
2. Lymph **nodes** contain high levels of immune cells

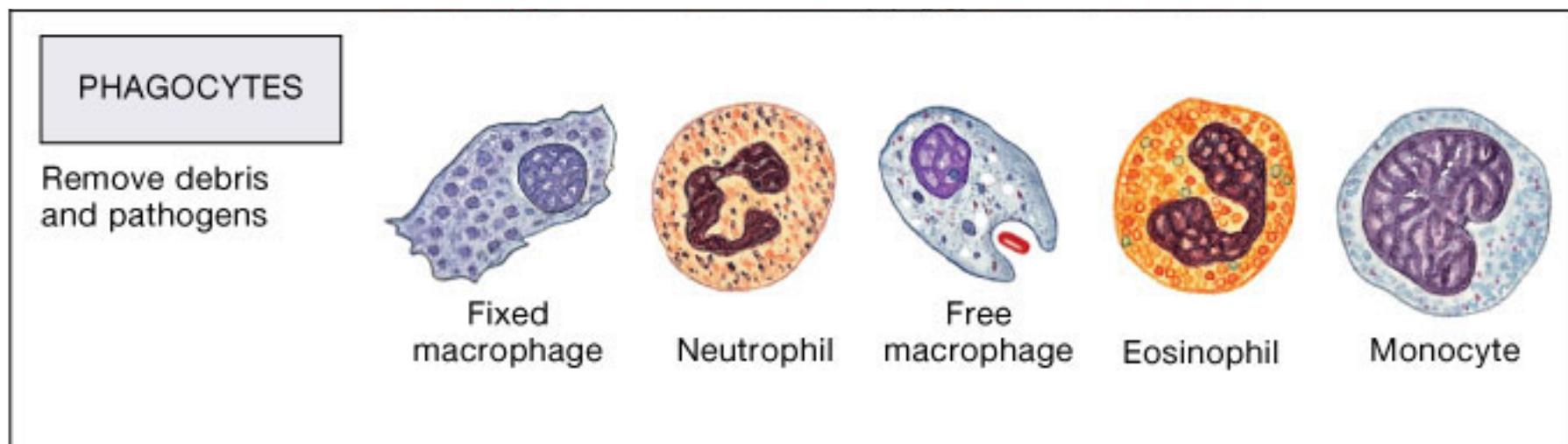
Nonspecific Defenses, Phagocytes

- Remove cellular debris and respond to invasion by foreign pathogens

- Monocyte-macrophage system - Fixed and free
- Microphages – Neutrophils and eosinophils
- Move by diapedesis
- Exhibit chemotaxis

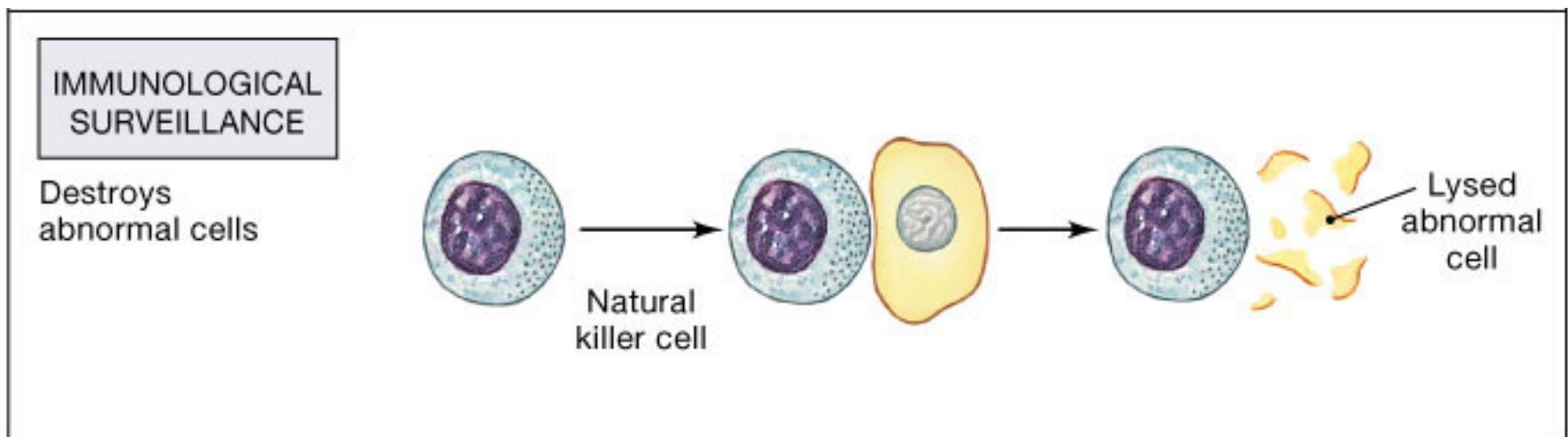


(b) Phagocyte engulfing a yeast cell

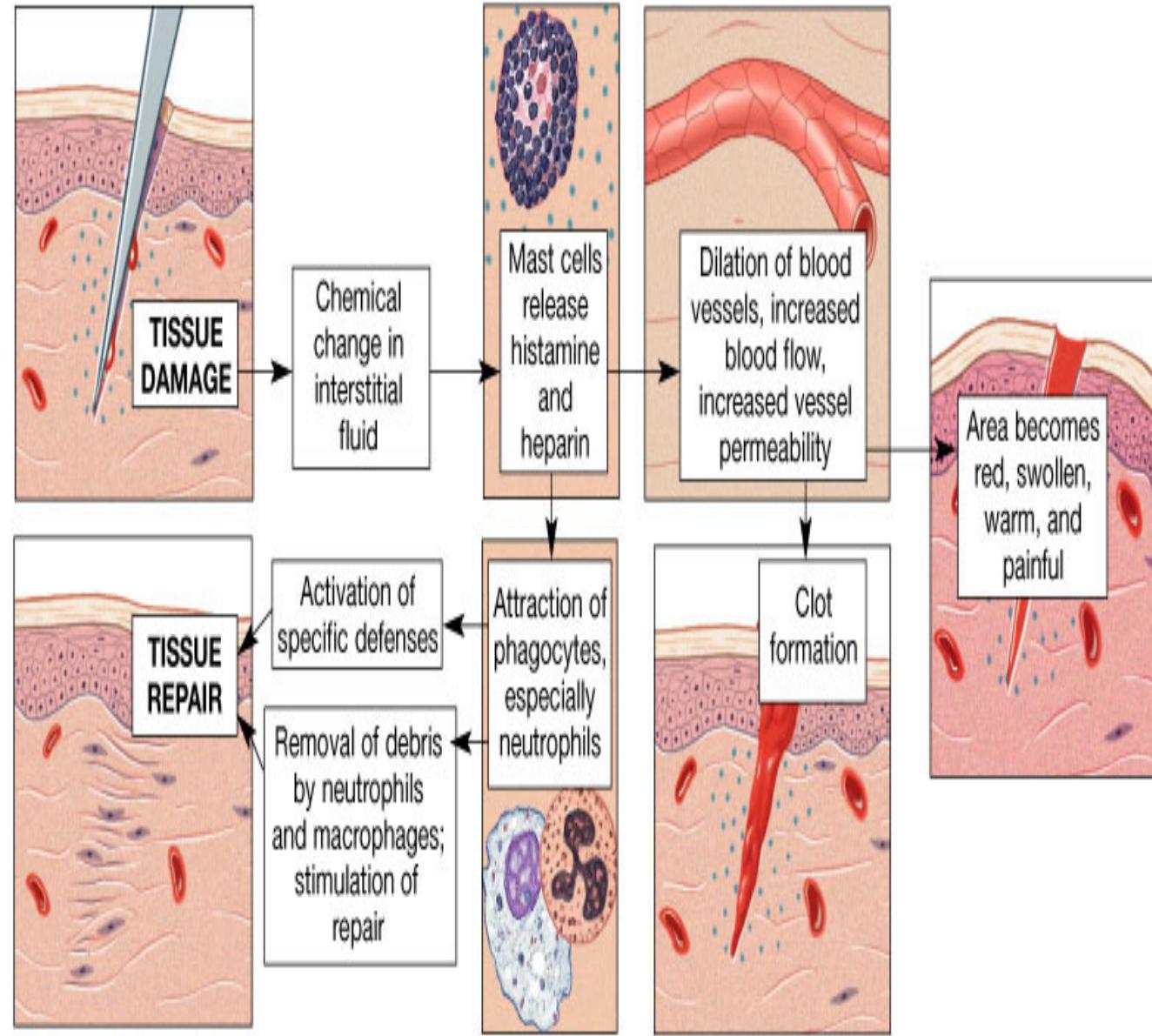


Nonspecific Defenses, Immunological surveillance

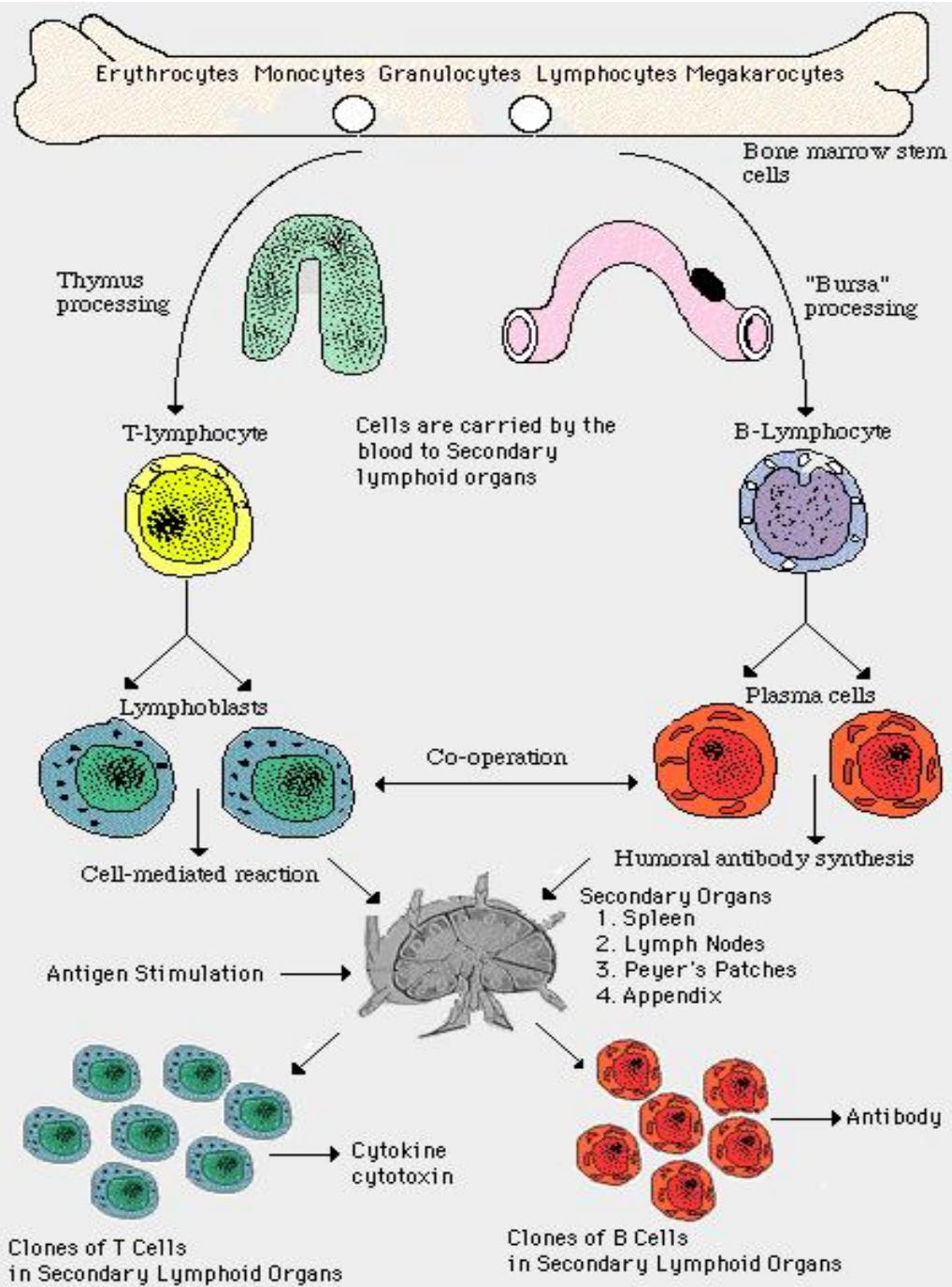
- Constant monitoring of normal tissue by NK cells
- NK cells
 - Recognize cell surface markers on foreign cells
 - Destroy cells with foreign antigens



Inflammation



Adaptive or Acquired Immunity

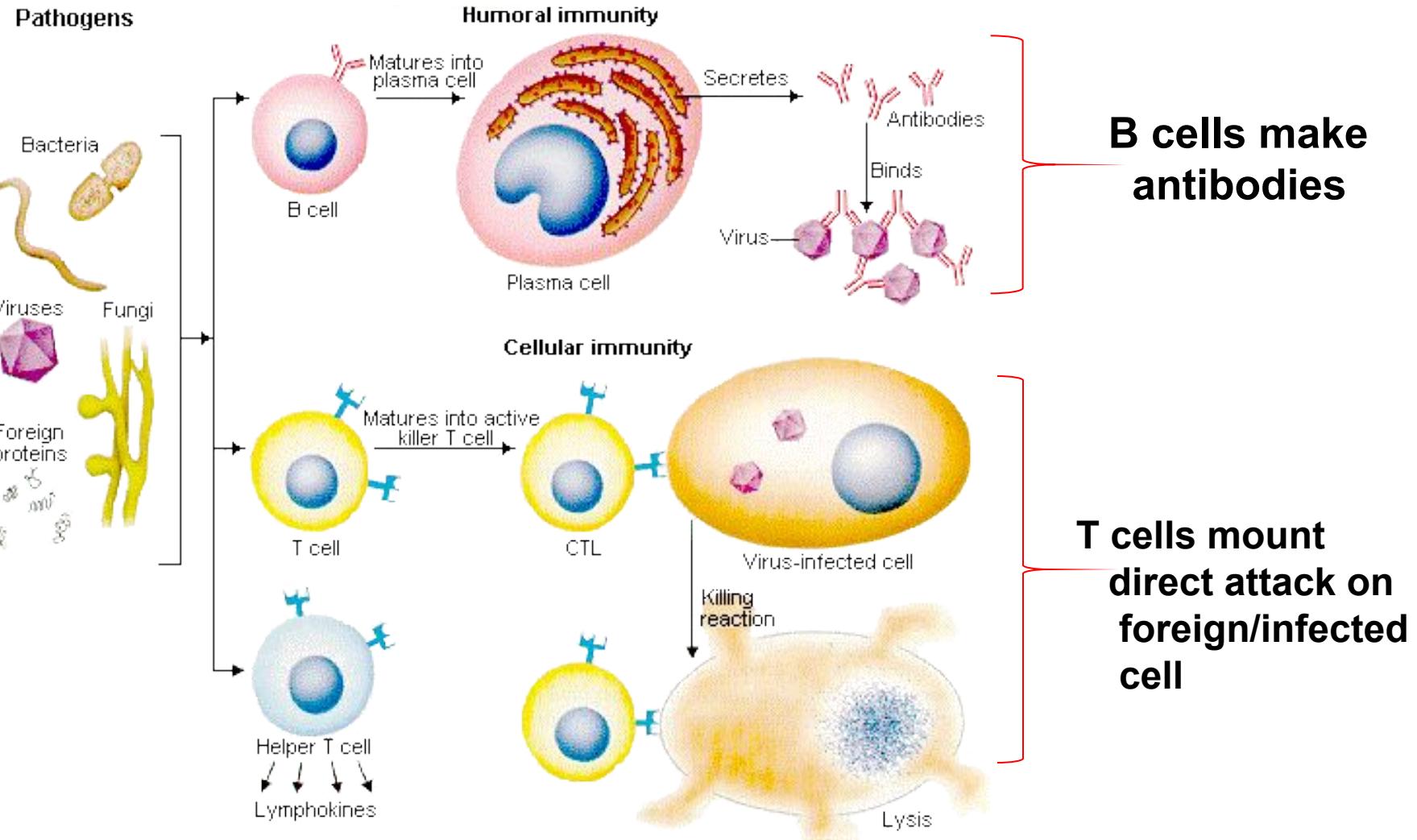


- Acquired after birth
- Seen only in vertebrates
- Characteristic features are:
 - Diversity
 - Specificity
 - Self vs non-self
 - Memory

Immune Response System

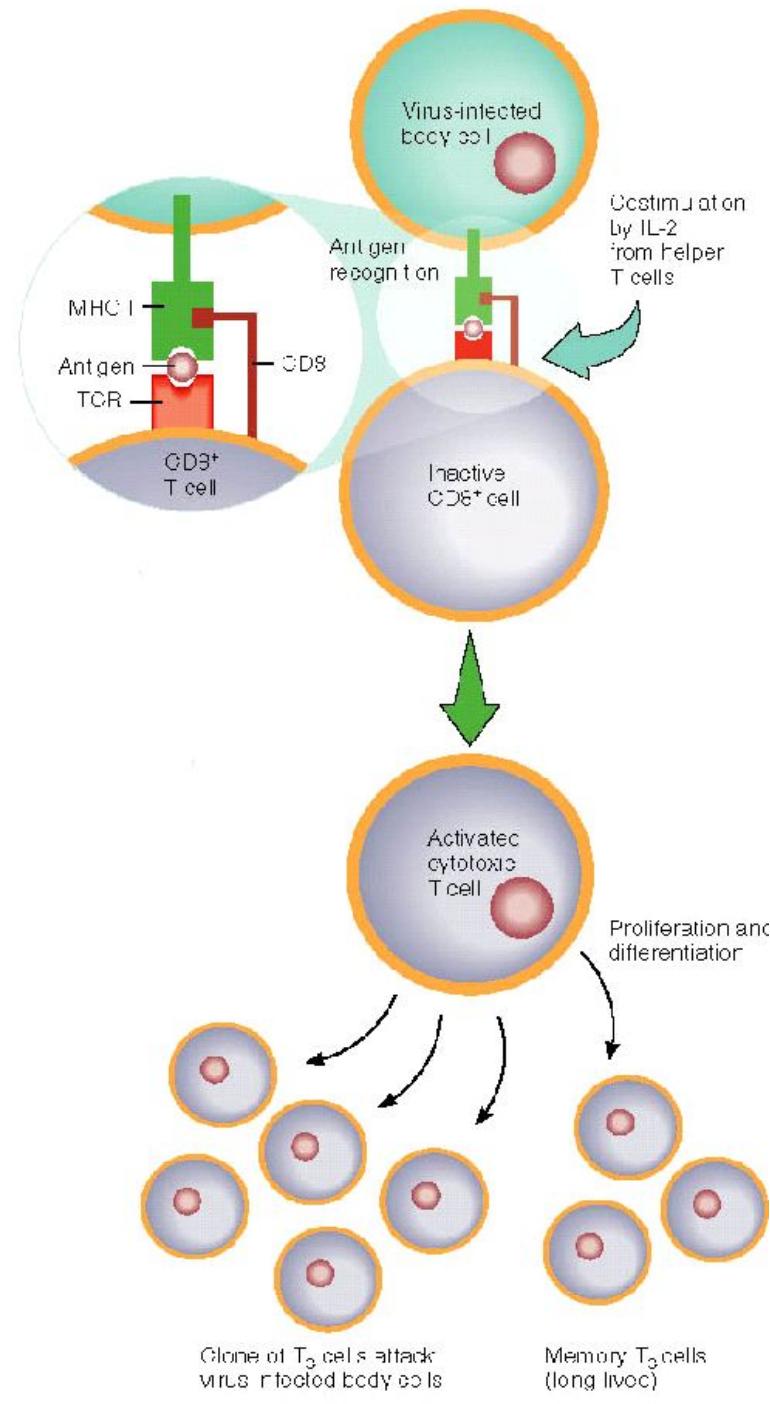
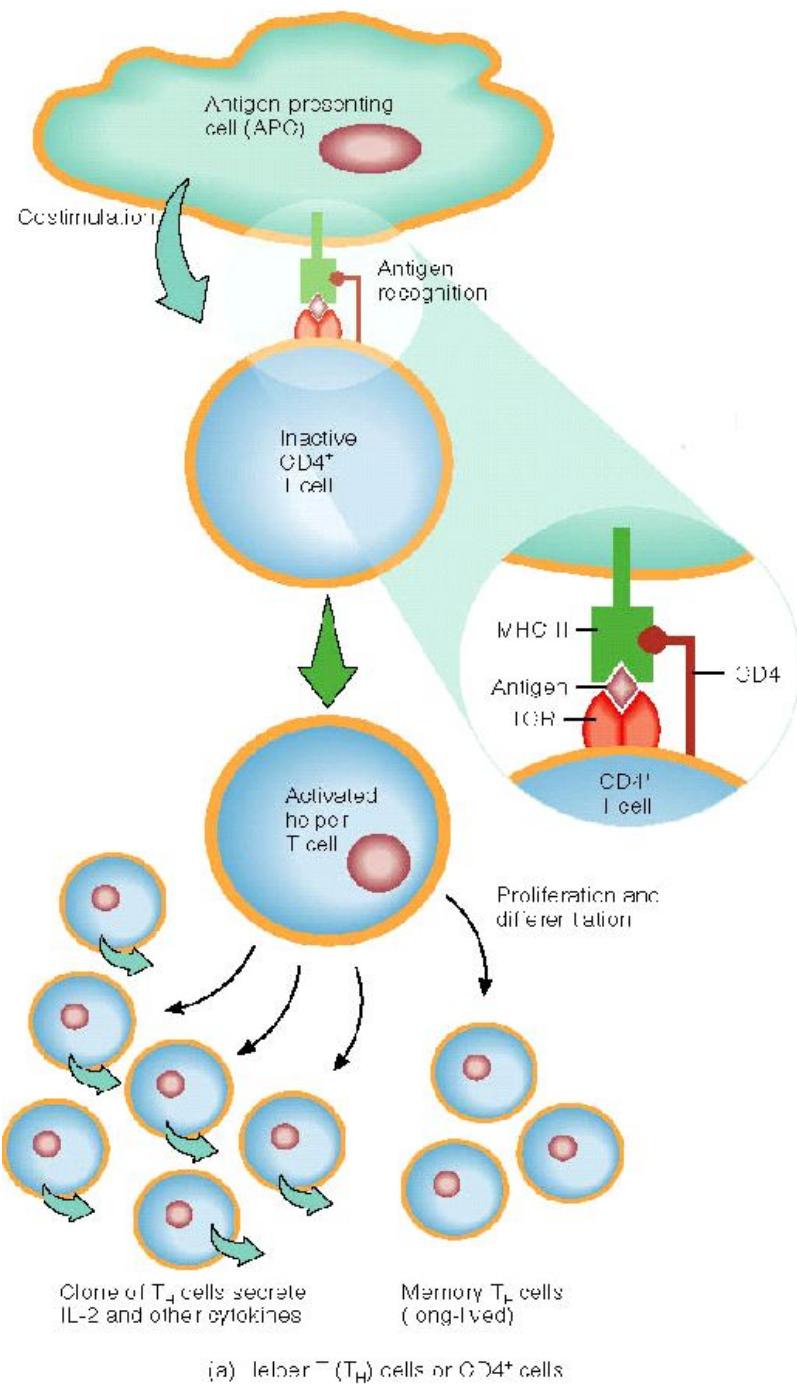
Made up of two cellular systems (lymphocytes)

1. Humoral immunity - **B cells**
2. Cell-mediated immunity - **T cells**



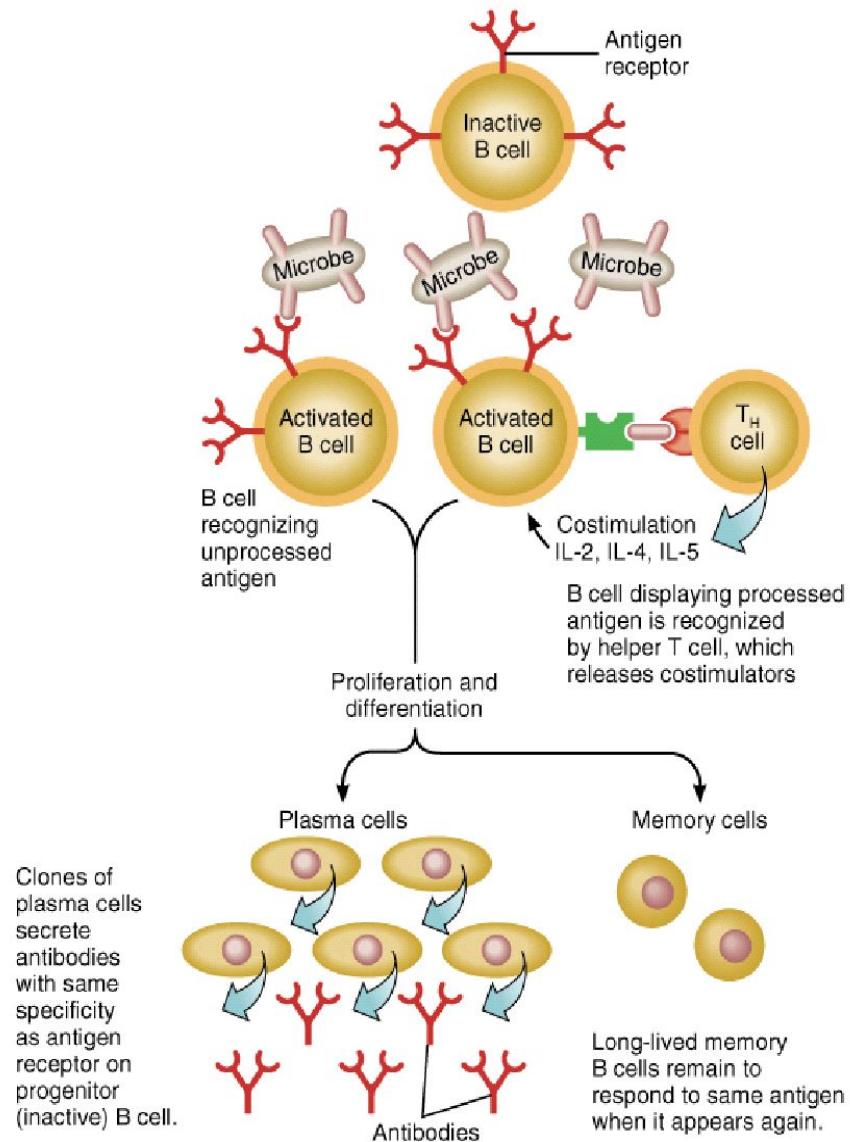
Cell mediated immunity

- T cells must be activated
- Must have both surface antigen recognition and costimulation to activate
- T cell receptors recognise and bind to specific antigen presented with MHC complexes
 - T cell only activated if binds to antigen and receives costimulation
 - Co-stimulation provided by cytokines or membrane proteins
 - Need for co-stimulation prevents immune responses occurring accidentally
 - Recognition (binding to receptor) without costimulation results in anergy (prolonged state of inactivity) in both B and T cells
- Once T cell co-stimulated it is activated
 - Proliferates
 - Differentiates (forms more highly specialised cells)
- Activation, proliferation and differentiation occurs in secondary lymphatic organs and tissues

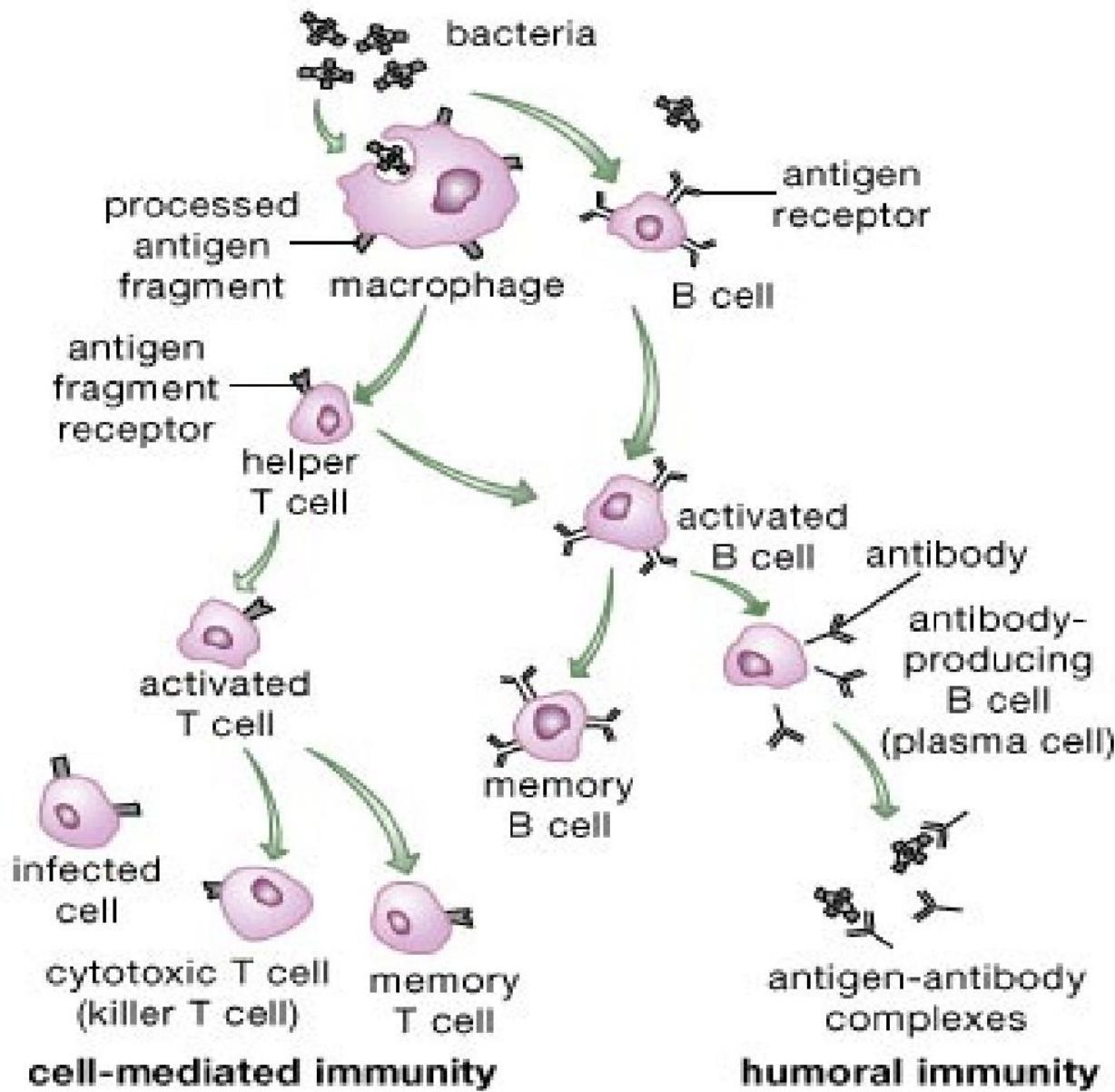


Humoral (Antibody-mediated) immunity

- Mediated by B cells
- Antigen can activate B cell in two ways:
 - direct binding
 - provokes less vigorous response
 - B cells process antigen (act as APC) and display processed antigen with MHC proteins
 - T_H cells recognise processed antigen
 - T_H cells provide co-stimulation for B cell
- Activated B cell
 - proliferates and differentiates
 - plasma cells
 - secrete antibodies with same antigen binding properties as receptors
 - memory B cells



Summary of Acquired Immunity

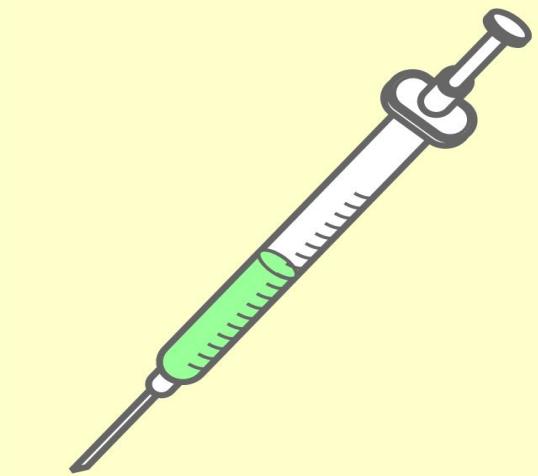


Immunity: Active and Passive

Active immunity



Naturally acquired



Artificially acquired

Passive immunity



Naturally acquired



Artificially acquired



HIV/AIDS Patient



Aging



Malaria-parasitic disease



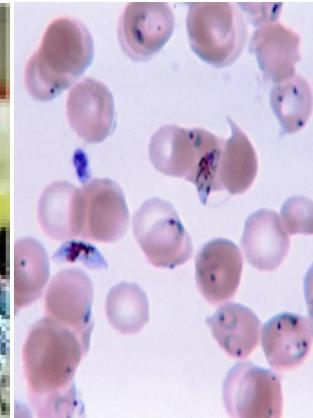
Smoking causes cancer



Diabetes

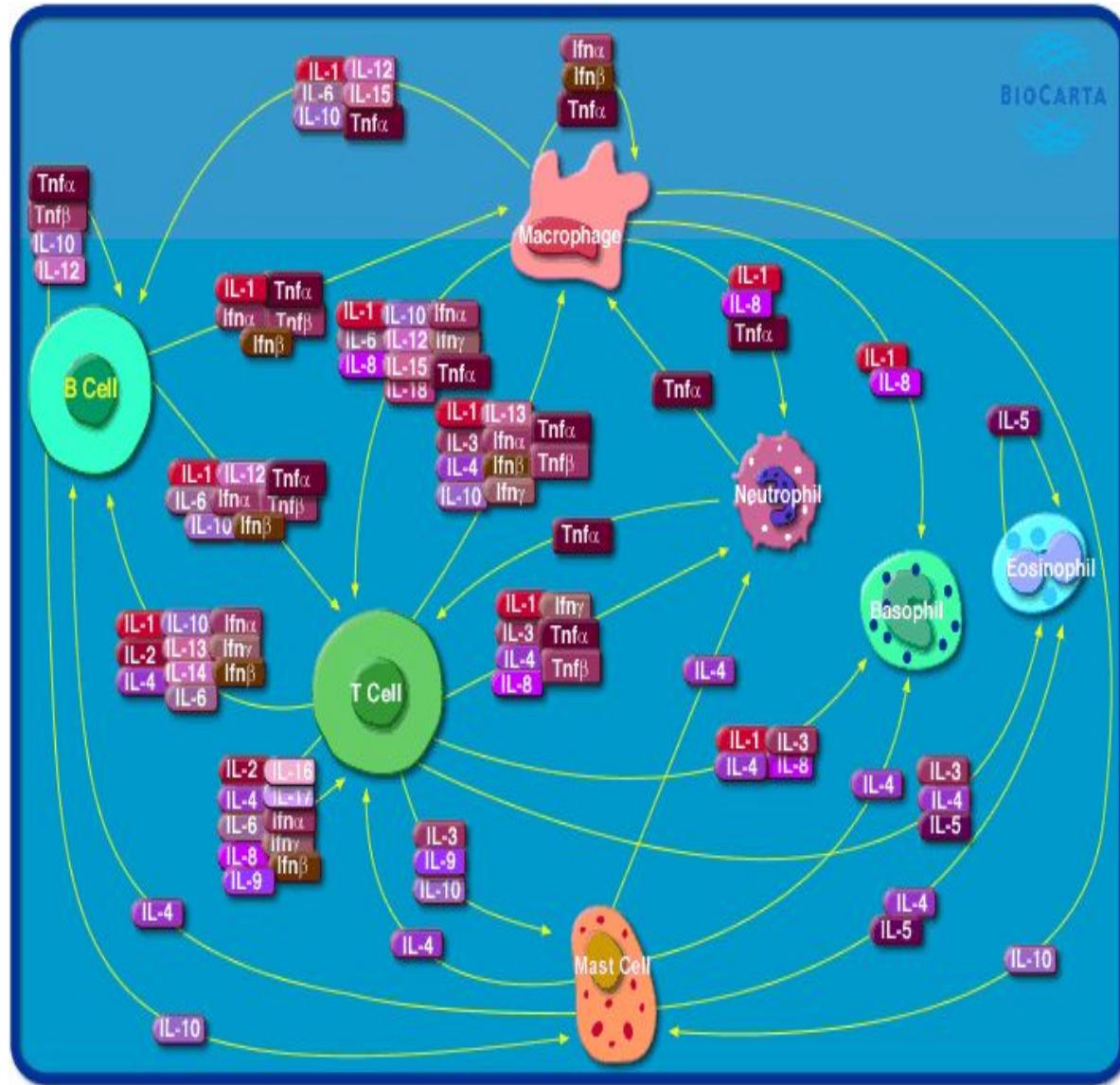


Rheumatoid arthritis



Immune Engineering

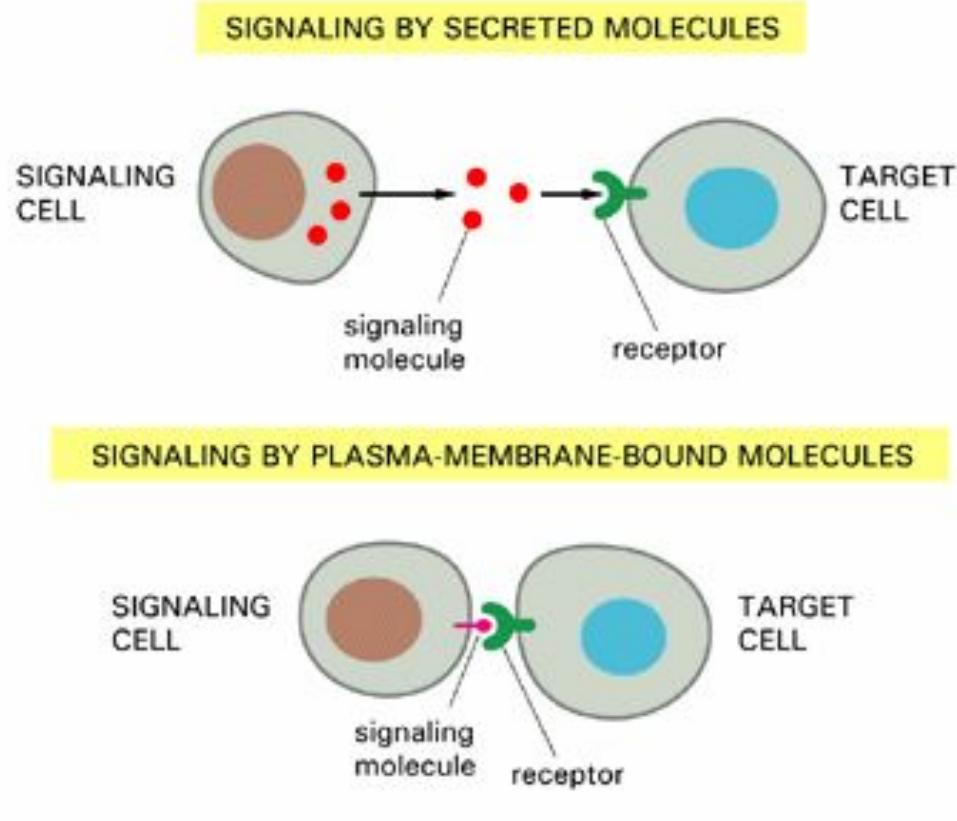
- The complexity of the immune system can be compared to that of the brain.
- There is a vast number of cells, molecules, and organs that compose the immune system, and these have to act in concert, and together with other vital systems, so as to promote and maintain life.
- Neither can the immune system act in isolation to maintain life, nor can a higher organism live without an immune system.



- **Artificial immune systems (AIS) compose a new computational intelligence approach inspired by theoretical and experimental immunology with applications to problem solving.**
- **Like all new approach, the field still lacks a more formal description and better theoretical foundations.**
- **The application of mathematical analysis and modeling to immunology may result in outcomes such as a deeper and more quantitative description of how the immune system works, a more critical analysis of hypothesis, it can assist in the prediction of behaviors and the design of experiments.**

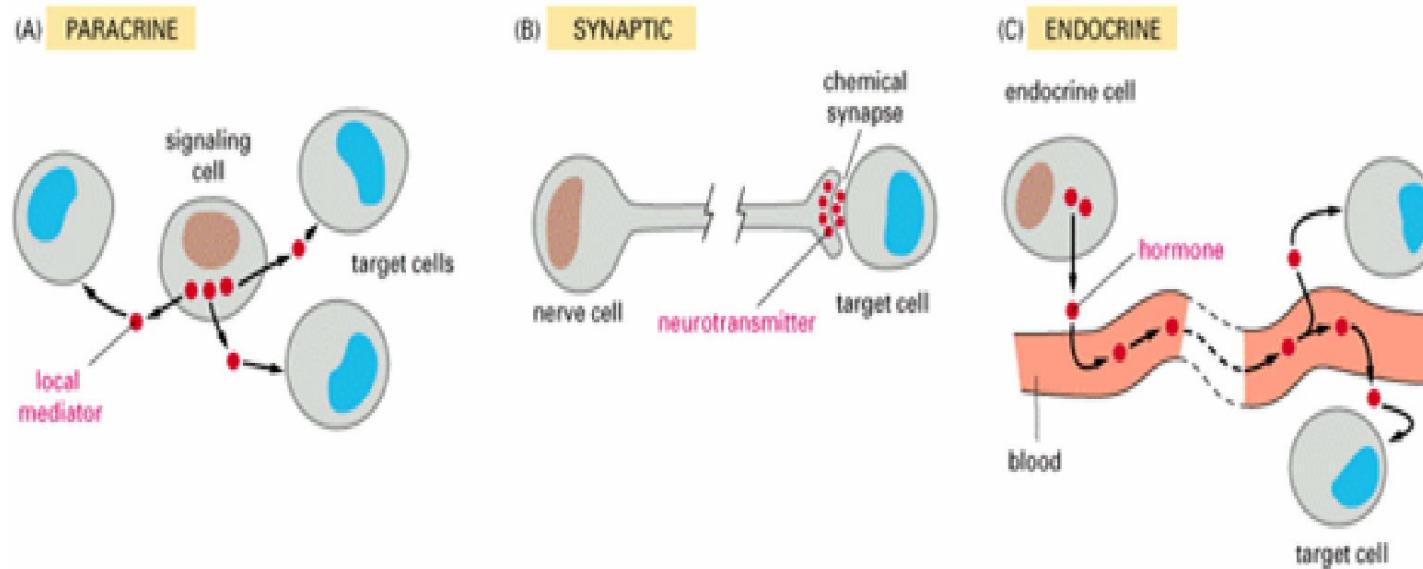
General Principles of Cell Signaling

- Unicellular organisms resembling present-day bacteria were present on Earth for about 2.5 billion years before the first multicellular organism appeared.
- One reason why multicellularity was so slow to evolve may have been related to the difficulty of developing the elaborate cell communication mechanisms that a multicellular organism needs.
- These communication mechanisms depend heavily on extracellular **signal molecules**, which are produced by the cells to signal to their neighbors or to cells further away.
- The signal molecules are mainly proteins.
- These proteins include cell-surface *receptor proteins*, which bind the signal molecule, plus a variety of *intracellular signaling proteins* that distribute the signal to appropriate parts of the cell.



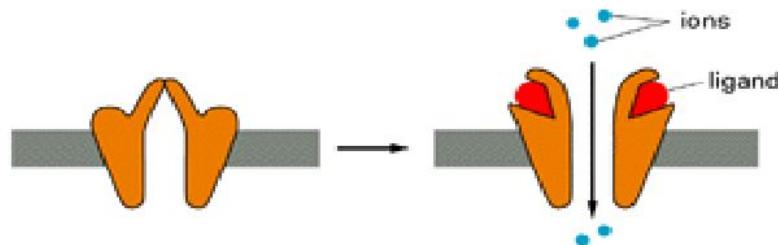
Secreted Molecules Mediate Three Forms of Signaling: Paracrine, Synaptic, and Endocrine

- Paracrine signaling depends on signals that are released into the extracellular space and act locally on neighboring cells.
- Synaptic signaling is performed by neurons that transmit signals electrically along their axons and release neurotransmitters at synapses, which are often located far away from the body.
- Endocrine signaling depends on endocrine cells which secrete hormones into the bloodstream that are then distributed widely throughout the body

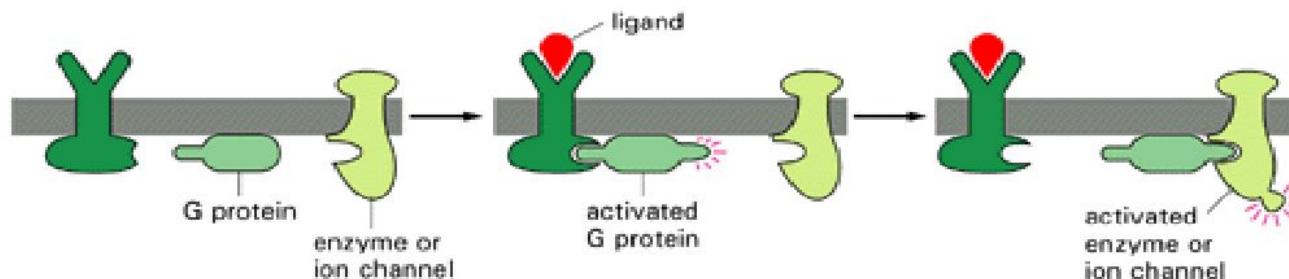


There Are Three Known Classes of Cell-Surface Receptor Proteins: Ion-Channel-linked, G-Protein-linked, and Enzyme-linked

(A) ION-CHANNEL-LINKED RECEPTOR



(B) G-PROTEIN-LINKED RECEPTOR



(C) ENZYME-LINKED RECEPTOR

