Molecular Cellular Physiology

2nd Year Biotechnology

By

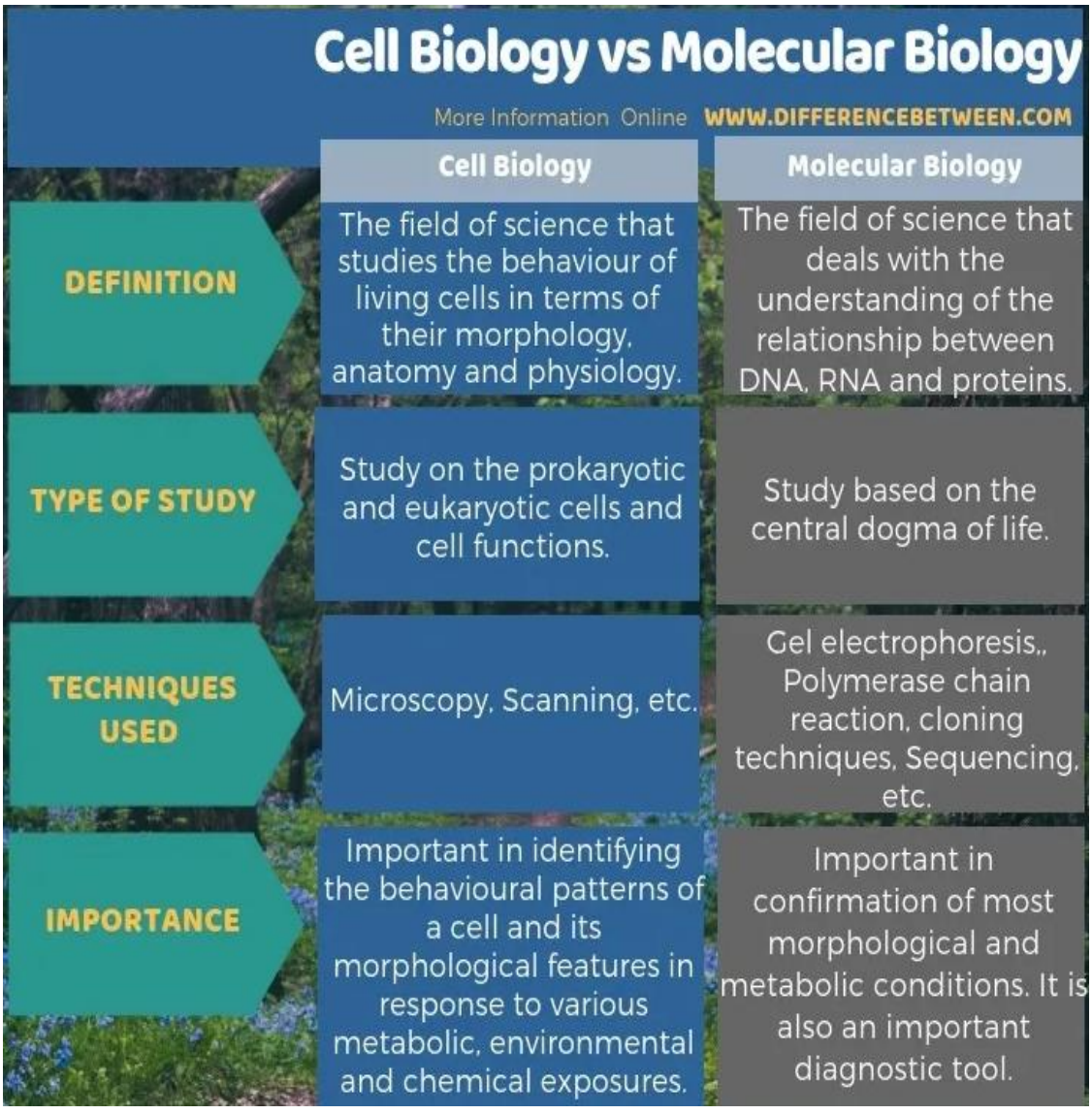
Dr\M. Bassyouni M EL-Mahdi

Associate Professor of
Molecular Genetics and Molecular Biology

Molecular Physiology

Molecular Physiology focuses on the scientific study of dynamic interactive processes and biochemical communications at the subcellular level.

The key difference between cell biology and molecular biology is that the cell biology mainly concentrates on studying cellular mechanisms of the cell while the molecular biology mainly concentrates on studying the cellular molecules especially DNA.



Cells: Structure and Function

What is the cell?

The cell is defined as the structural and functional unit of all living organisms and it has ability to carry out all the functions and features of life such as feeding, breathing, growth, reproduction and Excretion.

Cytology

The branch of life science that deals with the study of cells in terms of structure, function and chemistry.

CELLS 'Scope of Discovery :

The phrase 'cell' and the Greek word 'kyto' are synonymous for Latin word 'cella' which means a small room.

In I665, the phrase "cell" used by the English Scientist, Robert Hooke who was the first to describe cells through examining a slice of cork as he noticed that this piece of cork comprised of many tiny square boxes (small rooms) and called them cells.

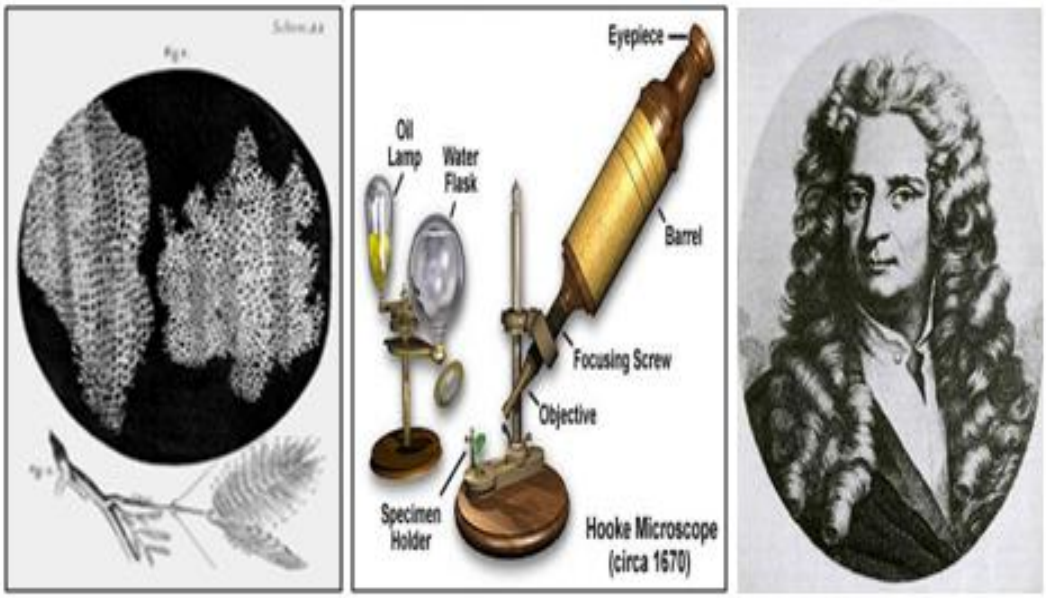
Scientists "I50 years later" were able to observe and understand more parts of **the** cell due to the work of Antonie van Leeuenhoek (1675, a Dutch lens maker) who described the first living cells.

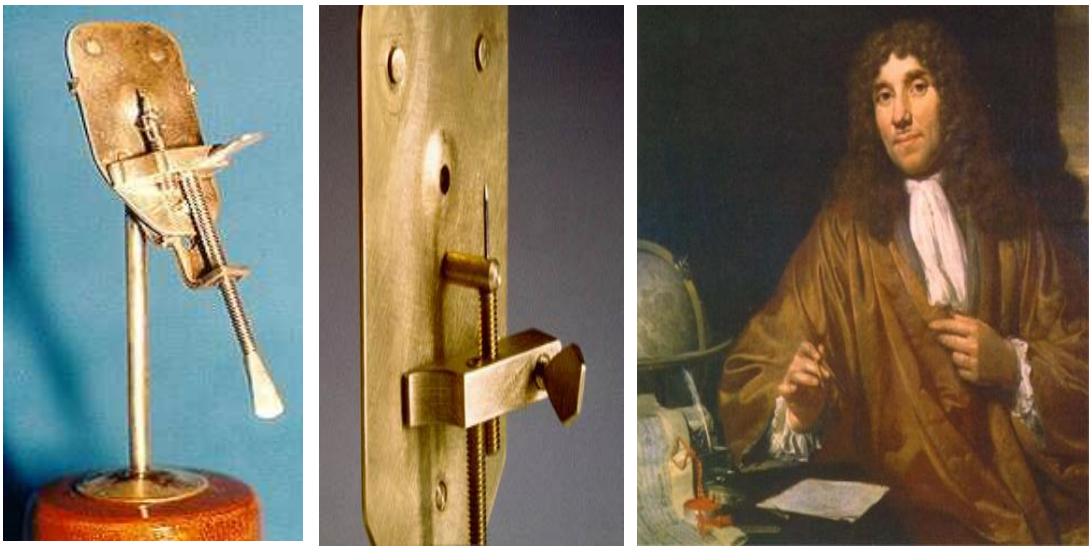
In 1838, Dutch botanist Matthias Schleiden concluded that all plants are composed of cells. A year later, a German zoologist, Theodor Schwaan postulated that animals are also composed of cells. In 1855, Rudolph Virchow, a Gennan doctor, declare that all cells must come from other cells by the process of cell division. The work carried out by the three above mentioned scientists was combined into what is now known as the Cell theory.

Cell theory.

1-All living things are made of cells.

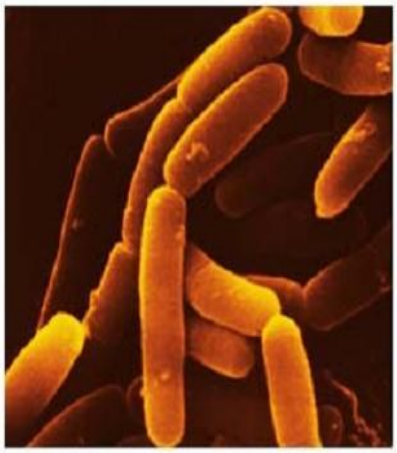
2-The cell is the smallest living thing that can perform all the biological functions of life. 3- All cells must come from pre-existing cells.





Robert Hooke (1665): observed a thin slice of cork with his microscope. He described what he observed as "little boxes" (cells). Anton van Leeuwenhoek (1675): was the first to observe and describe the living cells

Some organisms consist of a single cells = unicellular organism, others are multicellular aggregates of specialized cells. Whether multicellular or unicellular, all organisms must accomplish the same functions: uptake and processing of nutrients excretion of wastes response to environmental stimuli and reproduction among others



Bacteria

Archaea

Protista

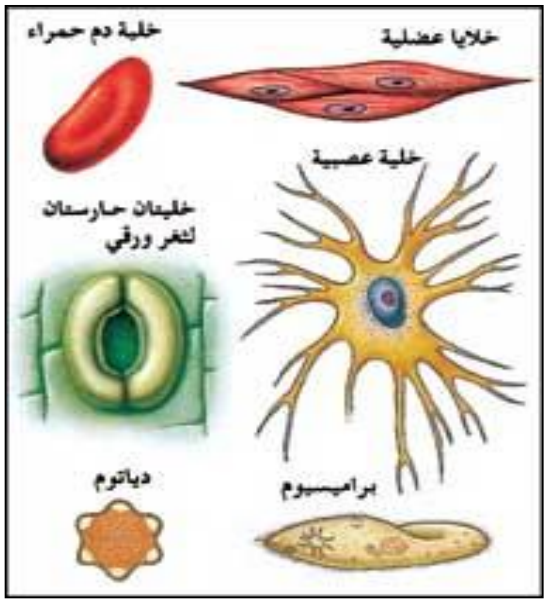
Plantae

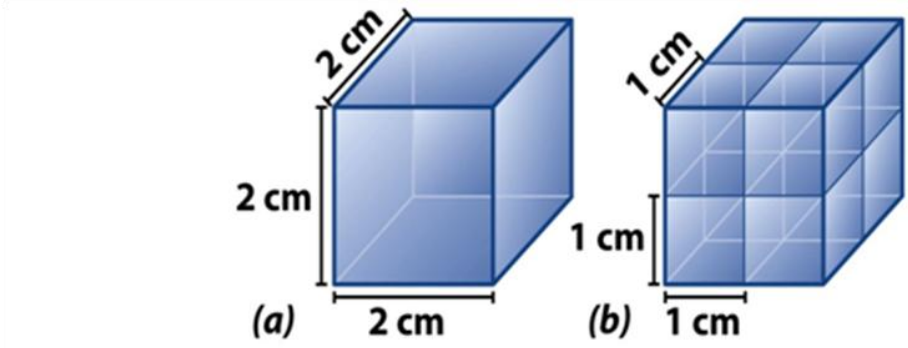
Fungi

Animalia

Cell Shapes

Cells differ in terms of form and structure depending on their locations in the body and its functions, some of them take different forms. For example, white blood cells moving amebic movement and shaped in various forms, red blood cells are round and smooth making them very flexible, moving easily through the blood vessels, while others have a fixed form, such as sperm cells and egg cells, and neurons.



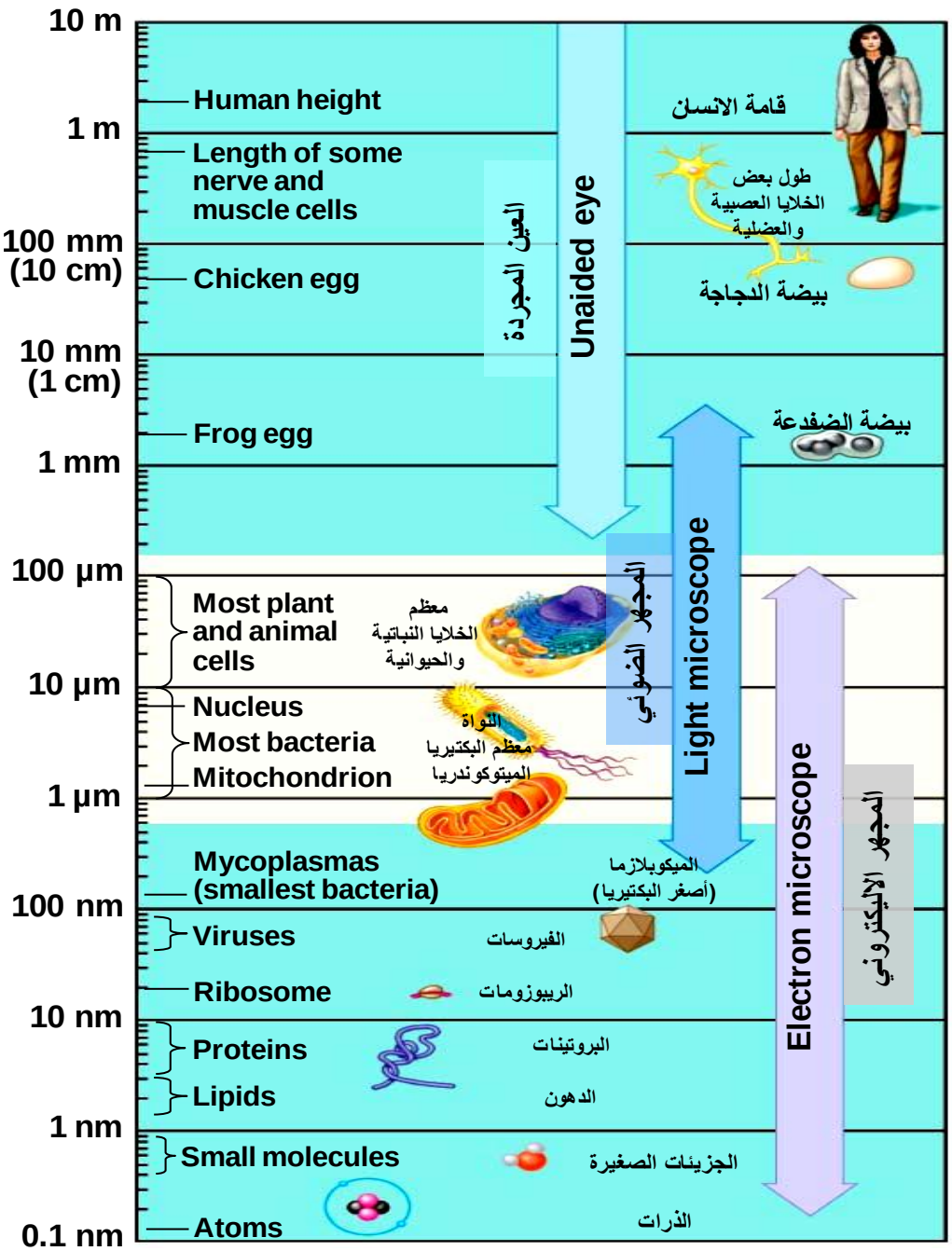


Number of cells	1	8
Total surface area	24 cm^2	48 cm^2
Total volume	8 cm^3	8 cm^3
Surface area/volume	24/8 = 3:1	48/8 = 6:1

Cells Have Large Surface Area-to-Volume Ratio

Cell Size

Cells vary in size from each other. For example, In the human body, cell size ranges between 200 and 15,000 micron (micron =0.001 millimeter.). In birds, there are cells you can seen by the naked eye (e.g an egg cells), also there are cells such as neurons to a length of several feet



The

Chemical Composition of the Cell

Chemical compounds in the cell can be divided into two major groups: Organic and Inorganic compounds

Organic compounds

They are chemical compounds that contain the element carbon. Organic compounds in the cell include carbohydrates, protein, lipids and nuclei acids.

Some of these compounds are synthesised by the cell itself.

Inorganic compounds

They include minerals, salts vitamins, and water that is an inorganic compound, polar molecule which is composed of hydrogen and oxygen.

It is an important compound in the cell.

Carbohydrates

Supply energy for cell processes.

A means of storing energy.

Give structural support to cell walls

Lipids

Store large amounts of energy, energy source. Major role in the structure of the cell membranes. Act as a source of metabolic water. Reduce the loss of water by evaporation

Proteins

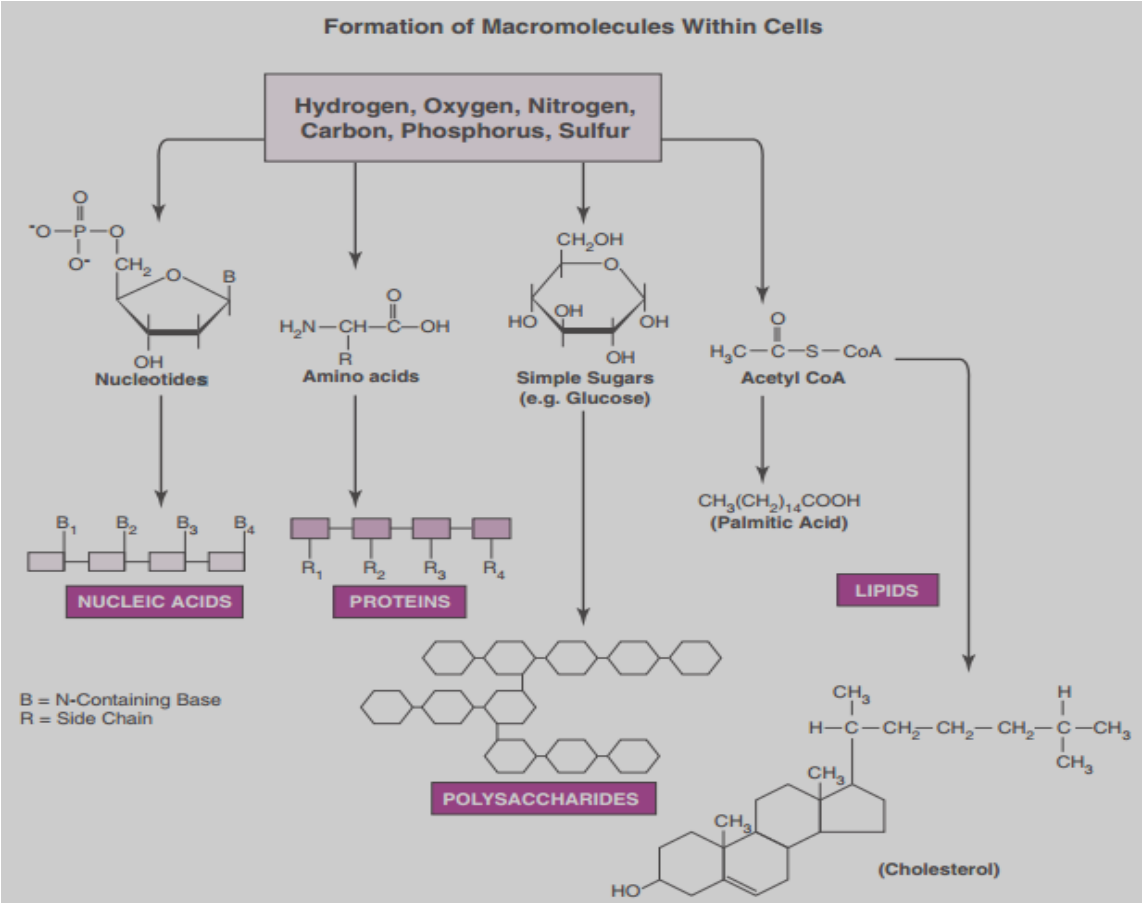
Act as building blocks of many structural components of the cell ; required for growth. Form enzymes which catalyse chemical reactions

Form hormones which control growth and metabolism

Nucleic acids

Contain the genetic information of cells

Play a vital role in protein synthesis



Cells can be Studied using the:

1-Microscopes (light or electrons microscope).

2-The Cell fractionation

Light Microscopes

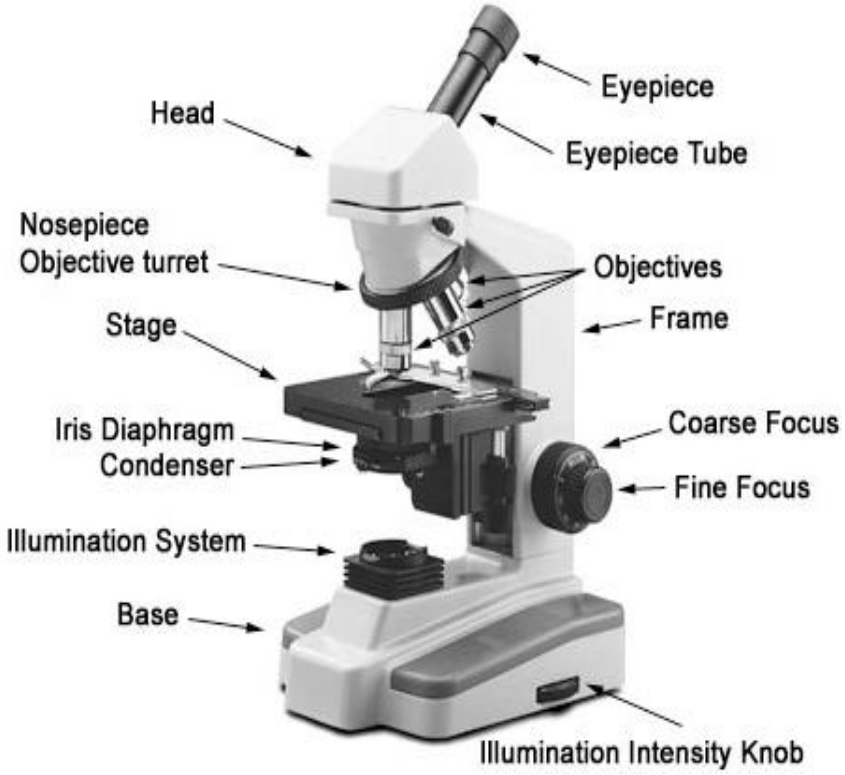
As most cells are between 1-100 µm in diameter which can be visualized by light microscope (LM) as the visible light is passed through the specimen and then through glass lenses that lenses refract light such that the image is magnified into the eyes or the video screen.

Magnification and resolving power

Magnification power = the ratio of an object's image to its

real size. Magnification of LM ~ X1,000

Resolving power = the measure of the image clarity. It is the minimum distance two points can be separated and still viewed as two separate points.



Electron microscope (EM)

Transmission electron microscope (TEM)

-study internal ultrastructure of cells,

-electron beam was aimed through the thin section of specimen

-the image was focused and magnified by electromagnet (instead of glass lenses)

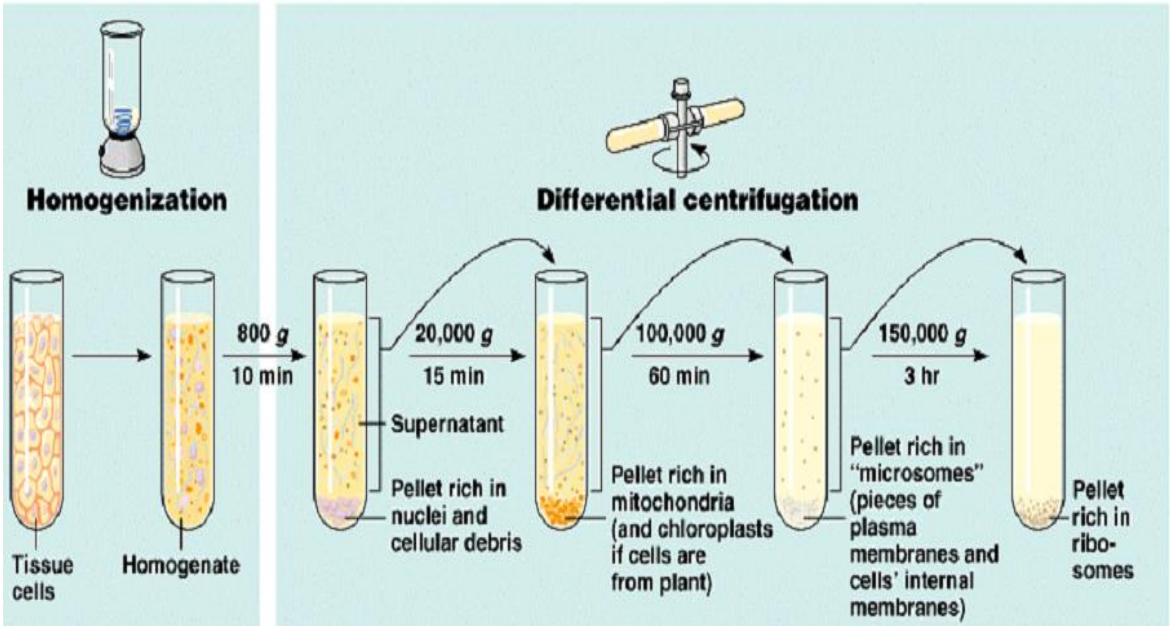
Scanning electron microscope (SEM)

-study the surface structure of the cells as sample surface is covered with the thin film of gold, then electron beam excites the electrons on the sample surface

-the secondary electrons are collected and focused on a screen and image appeared 3-dimensional

Cell Fractionation

-Separating the organelles of cells for functional study. The disrupted cells are centrifuged at different speed and duration to fractionate components of different sizes

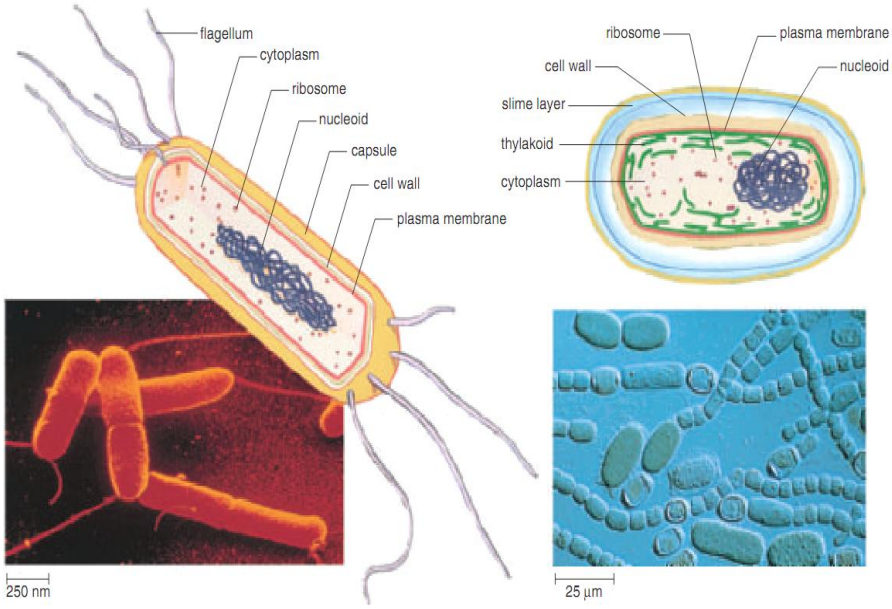


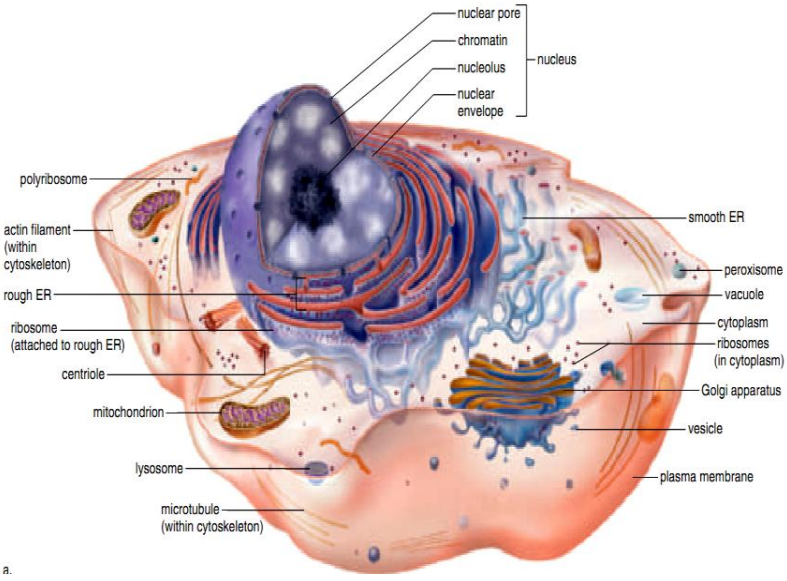
Types of cells

Cells can be subdivided into the following subcategories

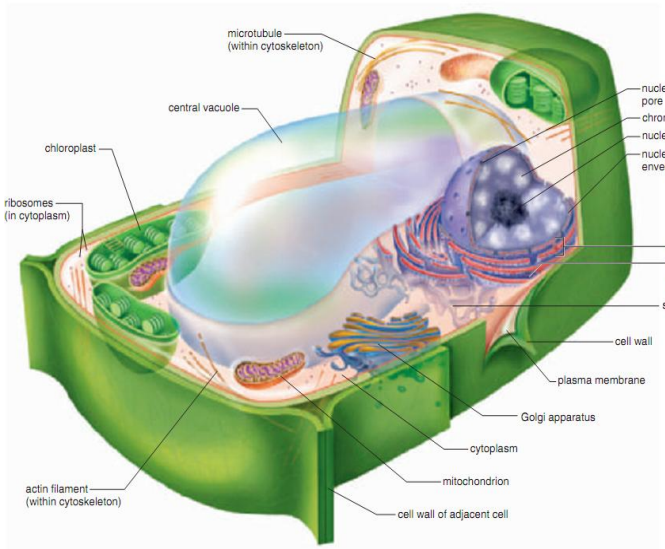
1-Prokaryotic cells: The cell does not have a nucleus as eukaryotic cells do. Archaea and bacteria are both prokaryotes, cells so small they are just visible with the light microscope. No membrane bound nucleus.

2-Eukaryotic cells: The cells, have a nucleus. A nucleus bound by membrane, It is a large structure that controls the workings of the cell because it contains the genes. It Include fungi, protists, plant, and animal cells possess many organelles. Both animals and plants have eukaryotic cells


flagellum
cytoplasm
ribosome
nucleoid
capsule
cell wall
plasma membrane
250 nm
ribosome
plasma membrane
cell wall
nucleoid
slime layer
thylakoid
cytoplasm
25 µm


nuclear pore
chromatin
nucleolus
nuclear envelope
nucleus
polyribosome
actin filament (within cytoskeleton)
rough ER
ribosome (attached to rough ER)
centriole
mitochondrion
lysosome
microtubule (within cytoskeleton)
smooth ER
peroxisome
vacuole
cytoplasm
ribosomes (in cytoplasm)
Golgi apparatus
vesicle
plasma membrane

a.


microtubule (within cytoskeleton)
central vacuole
chloroplast
ribosomes (in cytoplasm)
nuclear pore
chromatin
nucleolus
nuclear envelope
nucleus
rough ER
ribosome (attached to rough ER)
smooth ER
cell wall
plasma membrane
Golgi apparatus
cytoplasm
mitochondrion
actin filament (within cytoskeleton)
cell wall of adjacent cell

Eukaryotes vs. Prokaryotes

	Prokaryotes	Eukaryotes
nucleus?	NO (nucleoid)	YES
membrane-bound	NO	YES (Many)
size	1 - 10 mm	10 - 50 mm
when evolved?	3.5 billion years ago	1.5 billion years ago
cytoplasm?	YES	YES
cell membrane?	YES	YES
cell wall?	Some Do	Plants
ribosomes?	YES	YES
DNA?	Circular Free Floating	Chromosomes in Nucleus
examples	Bacteria	Plants, Animals, Fungi, and Protists

Cell Specialization

The cells in multicellular organisms can develop in different ways to perform different tasks.

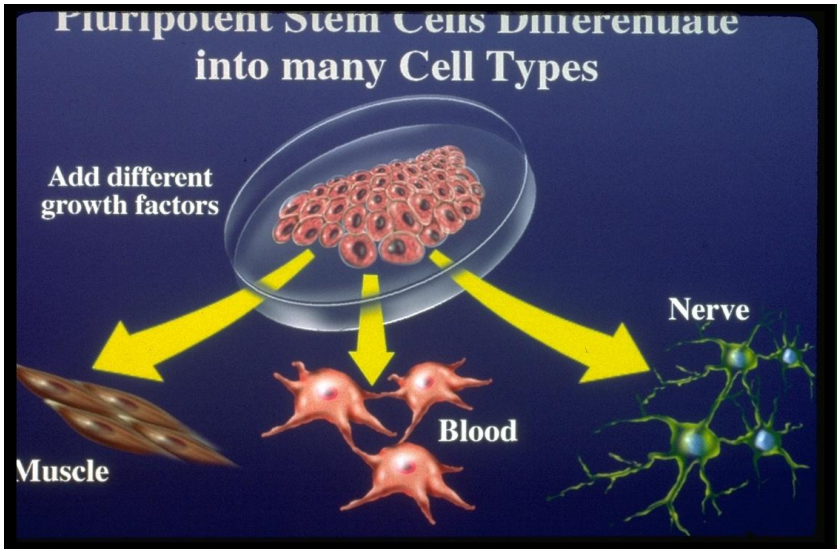
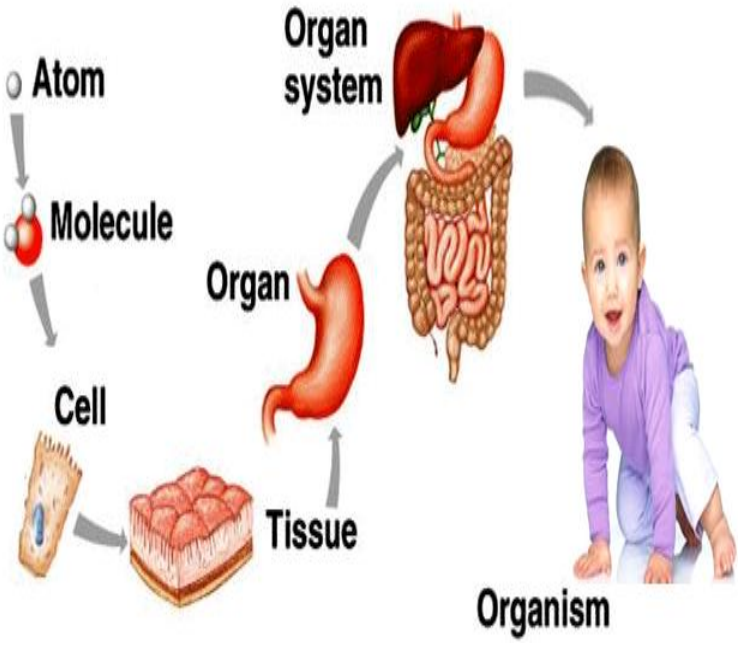


Table 1.1. Cellular functions in some specialized cells.

Function	Specialized Cell(s)
Movement	Muscle cell
Synthesis and secretion of enzymes	Pancreatic acinar cells
Synthesis and secretion of mucous substances	Mucous-gland cells
Synthesis and secretion of steroids	Some adrenal gland, testis, and ovary cells
Ion transport	Cells of the kidney and salivary gland ducts
Intracellular digestion	Macrophages and some white blood cells
Transformation of physical and chemical stimuli into nervous impulses	Sensory cells
Metabolite absorption	Cells of the intestine

Levels of Biological Organization

- **Organism**
- **Organ system**
- **Organ**
- **Tissue**
- **Cell**
- **Organelle**
- **Atoms and molecules**



CELL COMPONENTS

The cell is composed of two basic parts: cytoplasm (Gr. kytos, cell, + plasma, thing formed) and nucleus (L. nux, nut). Individual cytoplasmic components are usually not clearly distinguishable in common hematoxylin and eosin-stained preparations. The nucleus, however, appears intensely stained dark blue or black.

Cytoplasm

The outermost component of the cell, separating the cytoplasm from its extracellular environment, is the plasma membrane (plasmalemma).

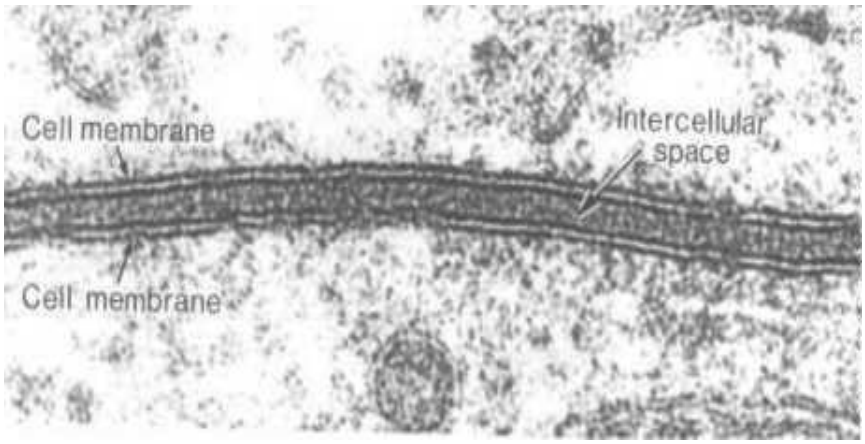
However, there is a continuum between the interior of the cell and extracellular macromolecules.

The cytoplasm of eukaryotic cells is divided into several distinct compartments by membranes that regulate the intracellular traffic of ions and molecules. These compartments concentrate enzymes and the respective substrates, thus increasing the efficiency of the cell.

Plasma Membrane

All eukaryotic cells are enveloped by a limiting membrane composed of phospholipids, cholesterol, proteins, and chains of oligosaccharides covalently linked to phospholipids and protein molecules.

The cell is highly organized with many functional units or organelles. Most of these units are limited by one or more membranes. To perform the function of the organelle, the membrane is specialized in that it contains specific proteins and lipid components that enable it to perform its unique roles for that cell or organelle. In essence membranes are essential for the integrity and function of the cell.



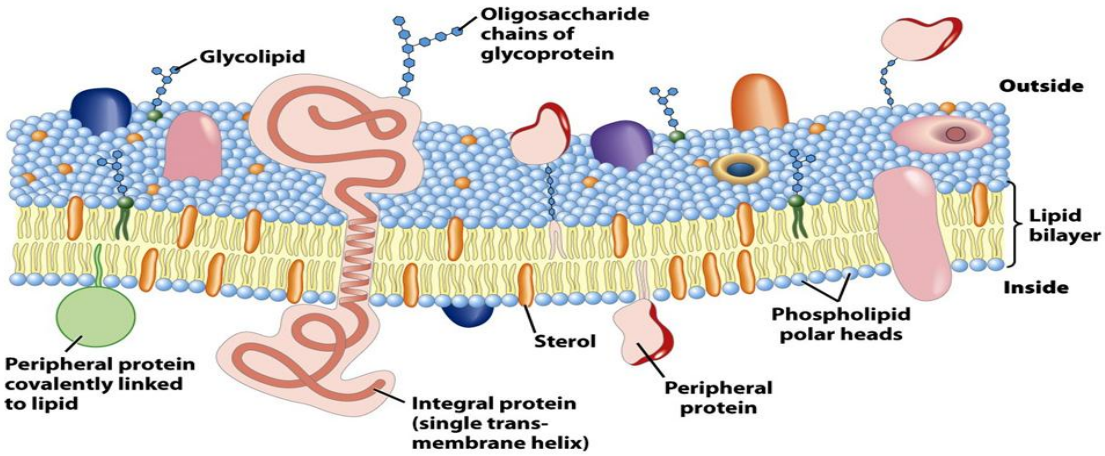
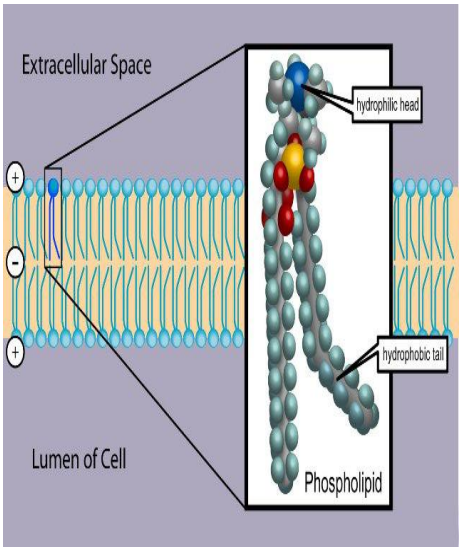


Figure 11-3
Lehninger Principles of Biochemistry, Fifth Edition



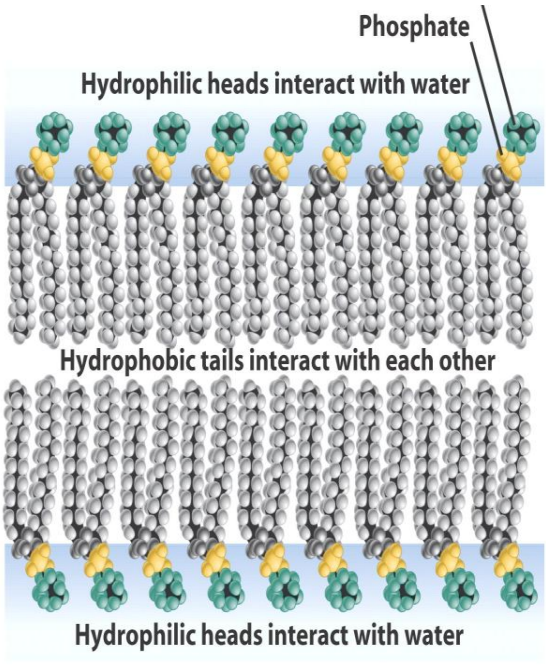


Figure 6-7 part 2 Biological Science, 2/e

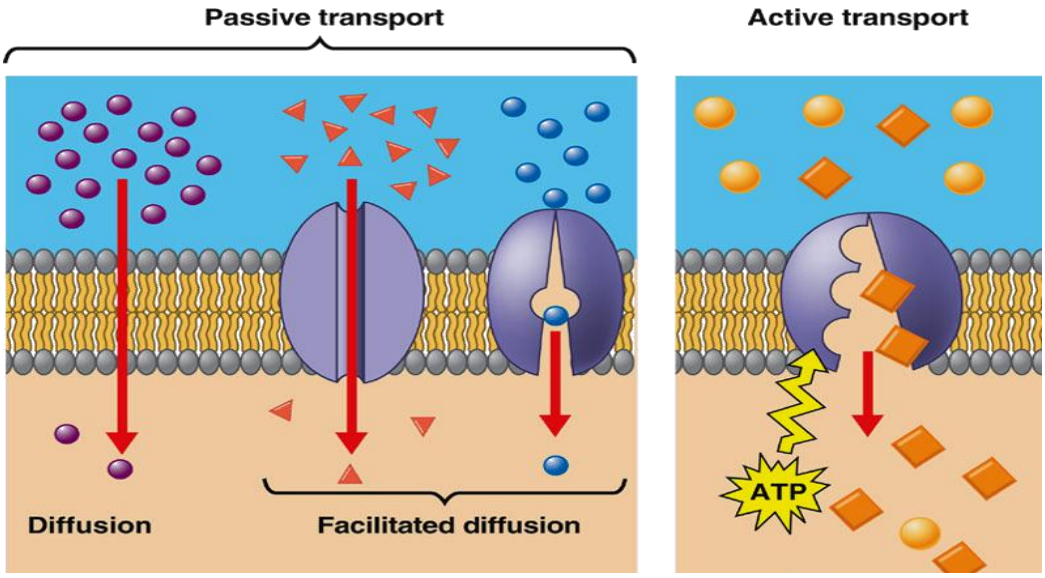
Plasma Membrane Transport Process

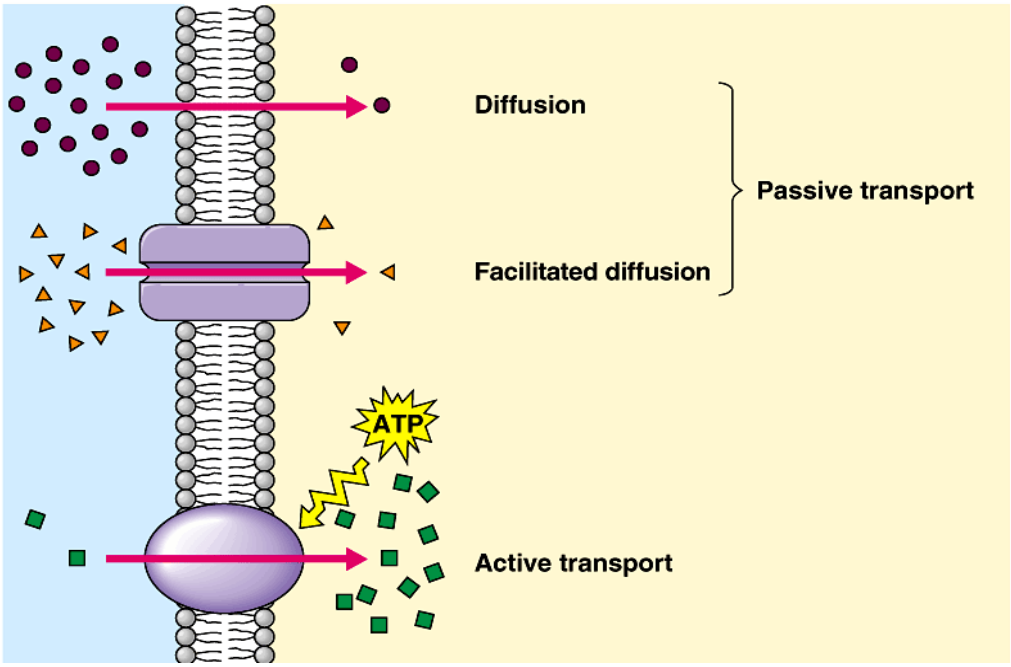
This process icludes transport of one molecule (uniport) two different molecules (symport

Types of transport:

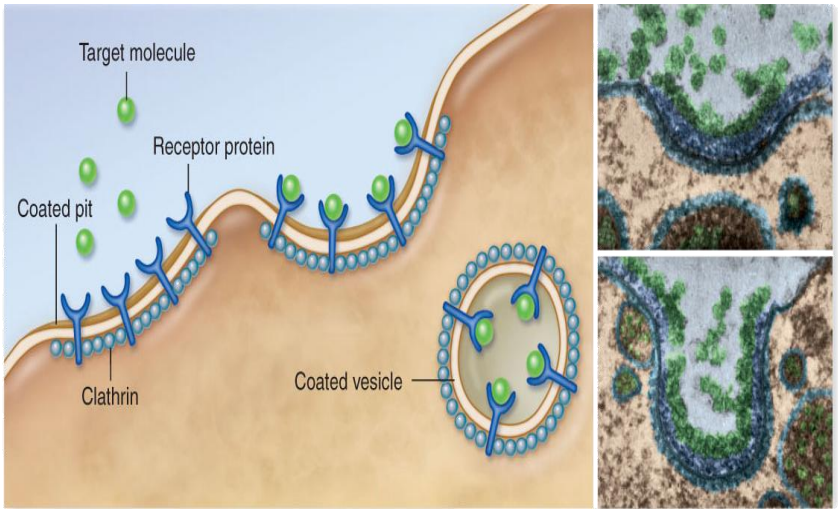
Passive transport ; Simple diffusion ; Facilitated diffusion ; Osmosis

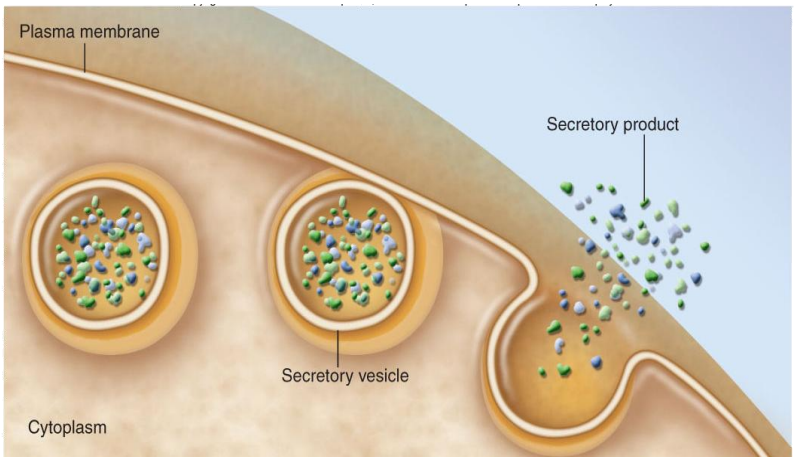
Active transport ; Bulk transport (Energy Required)





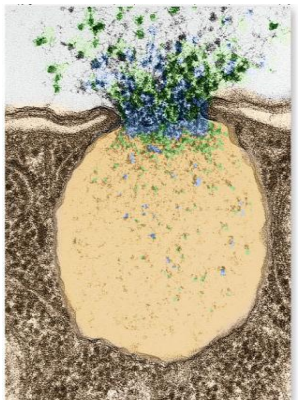
Bulk Transport



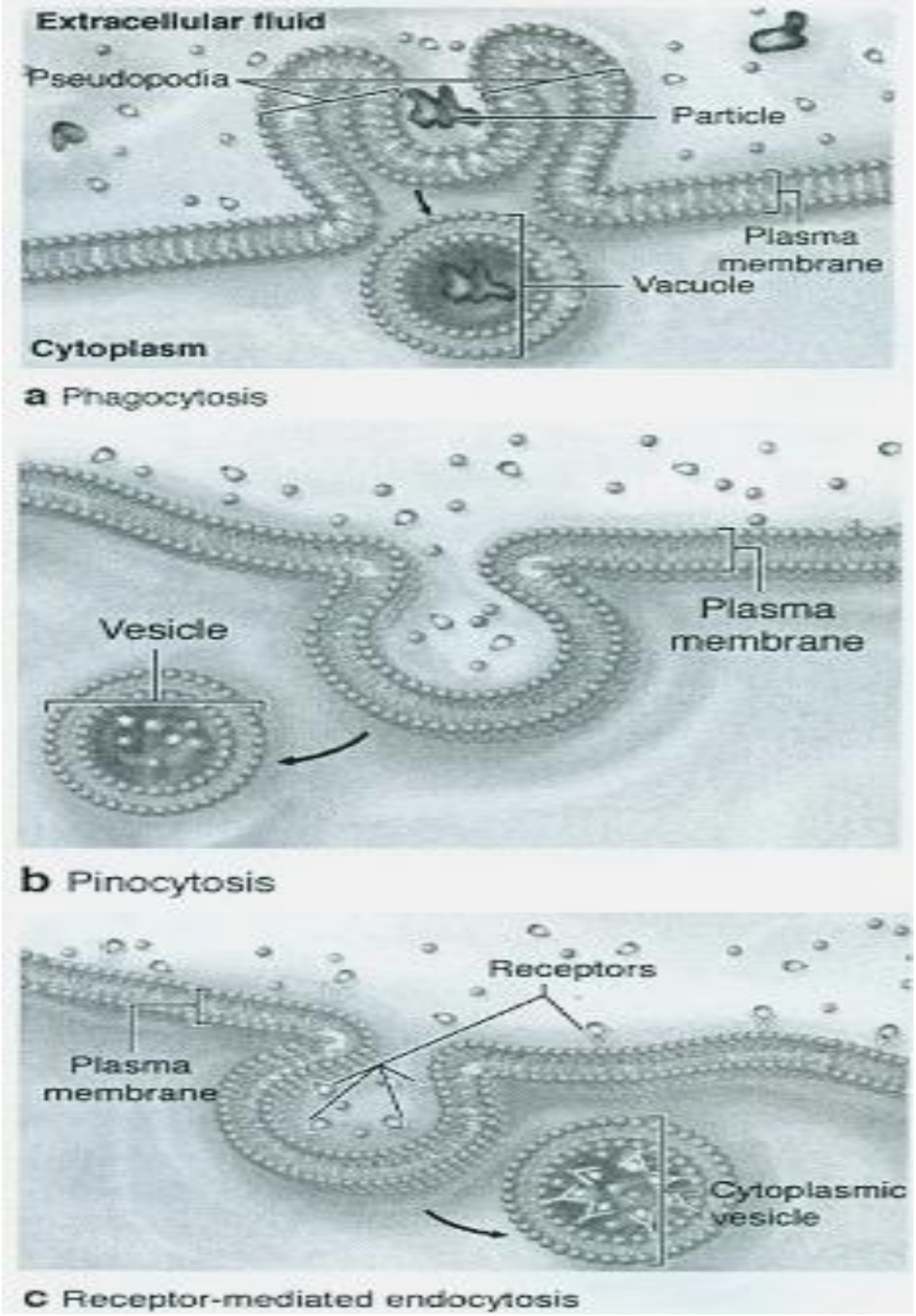


• Receptor-mediated endocytosis

proteins bind molecules, vesicles inside•



Molecules moved OUT - <u>exocytosis</u>


Extracellular fluid
Pseudopodia
Particle
Plasma membrane
Vacuole
Cytoplasm
a Phagocytosis
Vesicle
Plasma membrane
b Pinocytosis
Receptors
Plasma membrane
Cytoplasmic vesicle
c Receptor-mediated endocytosis

Signal Reception and Transduction

Cells in a multicellular organism need to communicate with one another to regulate their development into tissues, to control their growth and division, and to coordinate their functions.

Soluble extracellular signaling molecules bind receptor proteins only found on their target cells. Each cell type in the body contains a distinctive set of receptor proteins that enable it to respond to a complementary set of signaling molecules in a specific, programmed way.

Such signaling can take different routes:

** In endocrine signaling, the signal molecules (called hormones) are carried in the blood to target cells throughout the body.

** In paracrine signaling, the chemical mediators are rapidly metabolized so that they act only on local cells very close to the source.

"‘* In synaptic signaling, a special kind of paracrine interaction, neurotransmitters act only on adjacent cells through special contact areas called symapses.

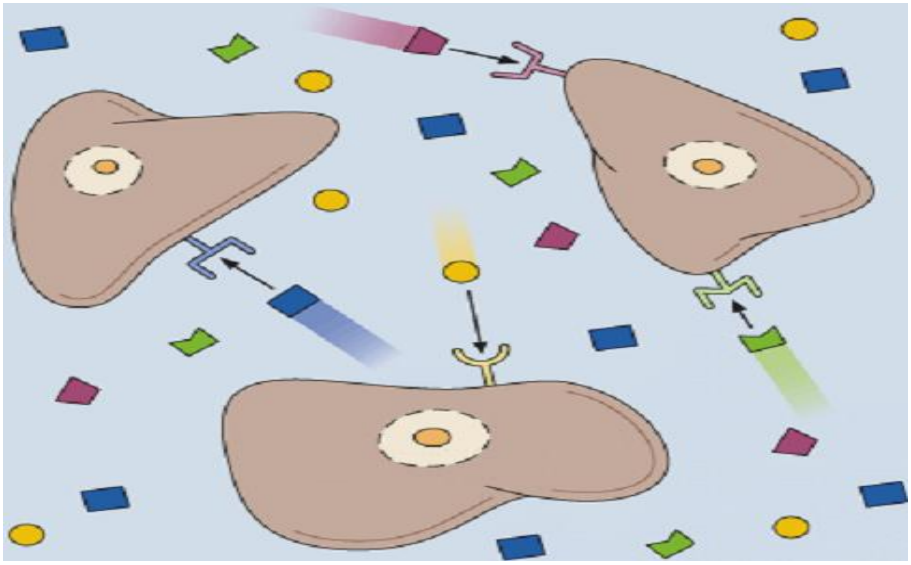
** In autocrine signaling, signals bind receptors on the same cell type that produced the messenger molecule.

** In juxtacrine signaling, important in early embryonic tissue interactions, signaling

molecules remain part of a cell's surface and bind surface receptors of the target cell. when the two cells make direct physical contact. Cells respond to chemical signals according to the library of receptors they have.

Below, three cells appear with different receptors, and the extracellular environment contains several ligands that will interact with the appropriate receptors.

Considering that the extracellular environment contains a multitude of molecules, it is important that ligands and the respective receptors exhibit complementary morphology and great affinity.



Signaling molecules differ in their water solubility. Small hydrophobic signaling molecules, such as steroid and thyroid hormones, diffuse through the plasma membrane of the target cell and activate receptor proteins inside the cell.
In contrast, hydrophilic signaling molecules, including neurotransmitters, most hormones, and local chemical mediators (paracrine signals), activate receptor proteins on the surface of target cells.

These receptors, which span the cell membrane, relay information to a series of intracellular intermediaries that ultimately passes the signal to its final destination in either the cytoplasm or the nucleus.
The numerous intercellular hydrophilic messengers rely on membrane proteins that direct the flow of information from the receptor to the rest of the cell. The best studied of these proteins are the G proteins, so named because they bind to guanine nucleotides

All Physiological Change Is Mediated by Proteins

All physiological change is mediated by a single class of polymeric macromolecules (large molecules), the proteins. Protein function can be subdivided into a number of categories: catalysis, reaction coupling, transport, structure, and signaling.

Catalysis is the ability to increase greatly the rate of a chemical reaction without altering the equilibrium of the reaction. The majority of biochemical reactions occur at a physiologically useful rate only because of protein catalysts, called enzymes. Examples of enzymatic catalysis in the synthesis of a class of physiological regulator molecules, catecholamines, are given later in this chapter.

At the molecular level, an enzyme-catalyzed reaction generally unfolds as shown in Figure 3. First, the enzyme randomly encounters the specific reactant(s), or substrate(s), in solution. Occasionally such an encounter takes place in a manner that allows the enzyme to form an enzyme-substrate complex. The enzyme binds the substrate in a groove or pocket, called the active site (or catalytic site) into which the substrate perfectly fits. The active site is a precisely tailored environment that facilitates the substrate's transformation into the transition state of the reaction. Rather than simply binding to the active site, the substrate also rearranges itself upon binding to more closely resemble the transition state. Similarly, the enzyme can also undergo slight conformational changes to better accommodate the substrate and allow more favorable interactions between the transition state and the active site. The energy released by forming these favorable interactions contributes to lowering the activation energy ($\Delta G^{\ddagger}$) as we will see in the next sections. The enzyme-stabilized transition state then undergoes additional changes to become an enzyme-bound product. Once the substrate is in the transition state, little energy is needed to push it downhill into product. The

enzyme-product complex then dissociates into free product and free enzyme. The released enzyme is then ready to catalyze the conversion of another molecule of the substrate into product. It is important to note that, as a catalyst, the enzyme is not permanently altered by the reaction; when the reaction is complete, the enzyme returns to the same state it was in before the reaction began.

In reaction coupling, two reactions are joined together with the transfer of energy. Energy from a spontaneous reaction (similar to water flowing downhill) is funneled to a nonspontaneous reaction (e.g., sawing wood) so that the sum of the two reactions is spontaneous. That is, the energy liberated by the "downhill" reaction is used to drive the "uphill" reaction. This is the basic function of a motor; the "downhill" burning of gasoline is coupled with the "uphill" movement of the car. The ability of proteins to couple spontaneous and nonspontaneous reactions allows cells to be chemical motors, using chemical energy to do various jobs of work. One such job of work, the contraction of striated muscle, is discussed later with particular emphasis on the proteins involved.

Proteins provide a pathway for the membrane transport of most molecules and all ions into and out of the cell. Transport and transport proteins are discussed more fully after a discussion of the lipid bilayer membrane, the major obstacle to transport.

Proteins that form filaments and that glue cells to each other and to their environment are responsible for the structure and organization of cells and multicellular assemblies (i.e., the tissues and organs of animals). The internal structure of the muscle cell, as well as its ability to do work, is a result of the properties of the muscle proteins discussed later.

At its most basic level, signaling requires only a controlled change or difference. Human signaling occurs by way of open and closed electrical circuits (telegraphy),

puffs of smoke in the air, and complex black marks on a contrasting background (numbers and letters). As discussed next, a fundamental property of proteins is the ability to change shape. The cell can use changes of protein shape directly to send signals, and the function of some proteins is purely informational. That is, all that some proteins do by changing shape is transmit and transduce information. Information can be defined as "any difference that makes a difference," or more simply, any difference that regulates something. Catalysis, coupling, transport, structural, and signaling functions can be combined on individual protein molecules. As will become apparent, such multifunctional proteins carry out many important physiological functions. Also important is that a change in one or more of these protein functions can be used to carry information, to serve as a signal within the cell. Thus, in addition to proteins specialized exclusively to carry information, changes in enzymatic activity or ion transport can also make a difference, transmitting information and triggering an appropriate response.

Protein Function Depends on Protein Shape and Shape Changes

Protein function is founded on two molecular characteristics: (1) proteins can bind to other molecules very specifically; and (2) proteins change shape, which in turn alters their binding properties and their function. The binding specificity of proteins is the result of their complex three-dimensional structure. Grooves or indentations on the surface of protein molecules, called binding sites, permit specific interactions with a molecule of a complementary shape, called the ligand. This complementary-shape mechanism underlying binding is similar to the shape interaction between a lock and key.

Several aspects of the lock-and-key analogy are worth noting. As with a lock, only a small part of the protein is engaged in binding. The binding is very specific; small changes in the shape of the binding site (keyhole) or the shape of the ligand (key) can cause major changes in protein (lock) behavior. Similar to the lock and

key, the complementary-shape interaction serves a recognition function; only those molecules with the right shape affect protein function. This recognition function plays a primary role in information transfer. The protein recognizes a particular signal by binding to it, thus changing the protein's shape and thus its function. Unlike the majority of locks, however, proteins frequently have multiple binding sites for multiple ligands.

Thus the three-dimensional shape of a protein, its conformation, determines protein function. A major force that stabilizes protein conformation is the hydrophobic interaction. Oily, hydrophobic (water-fearing) amino acids tend to congregate in the middle of a protein away from water, whereas hydrophilic (water-loving) amino acids tend to be found on the protein's outer surface interacting with the abundant cellular water. The hydrophobic interaction is also important in stabilizing the interaction of proteins with the lipids of biological membranes, as discussed shortly. Protein shape is also stabilized by hydrogen bonding between polar amino acid pairs in the polypeptide (protein) chain.

The same weak forces responsible for protein conformation are also used to hold the ligand in the protein-binding site. The position of the ligand in the binding site is stabilized by hydrogen bonds between the polar groups of the ligand and polar, amino acid side groups lining the binding site, just as hydrogen bonds within the polypeptide chain stabilize the shape of the polypeptide. Precisely because the same forces are responsible for the shape of the protein and for its binding properties, shape influences binding, and in turn, binding can influence protein shape. The ability of proteins to change shape is called allostery (Greek, "other shape").

Allosteric changes in protein conformation arise in four general ways. One way, just mentioned, is that most proteins change shape depending on which ligands are bound at particular binding sites (Figure 1-1, A). The sequence—specific ligand

binding → protein shape change → change in protein-binding properties and protein function → this change regulates something—is a common molecular mechanism underlying physiological control. This method involves no alteration in the covalent structure of the protein.

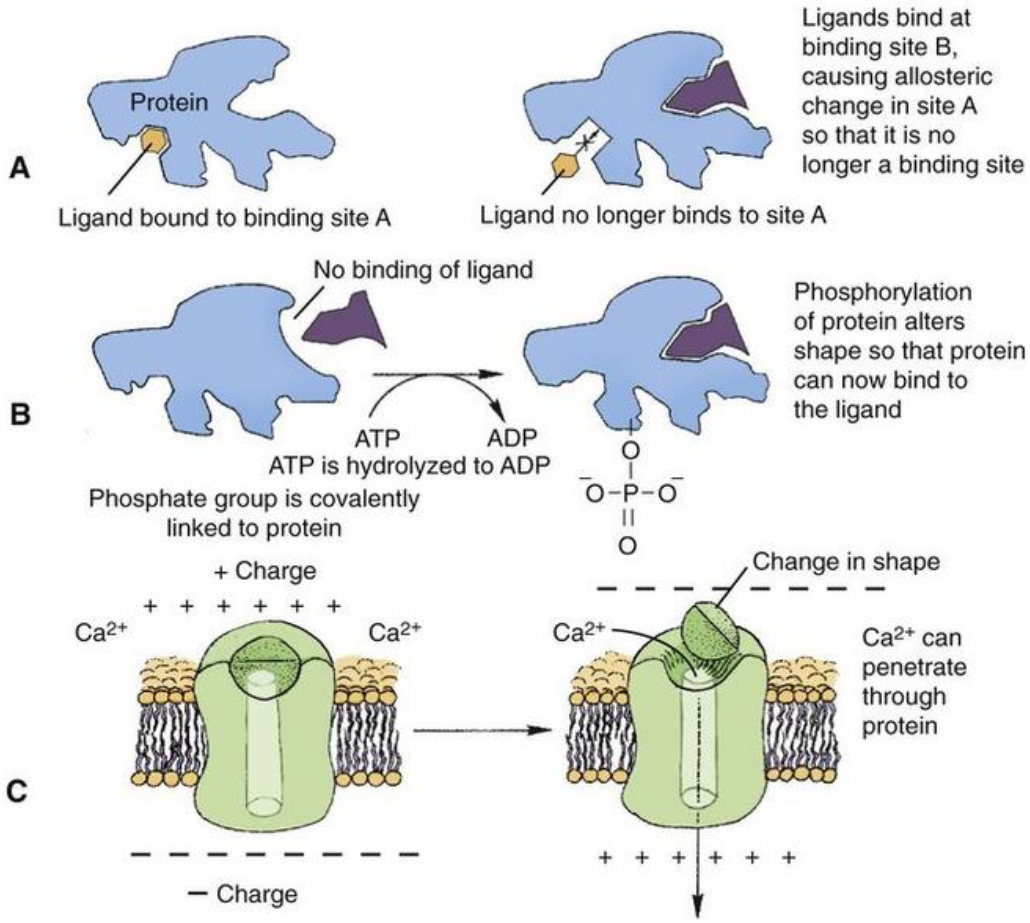


FIGURE 1-1 Three common mechanisms of allosteric shape change in proteins. A, Ligand binding. Ligand binding to an allosteric site (site B) on a protein changes the protein's conformation such that binding site A is altered; ligand no longer binds at site A because of the binding event at site B. B, Phosphorylation. Addition of a phosphate group to a serine, threonine, or tyrosine residue of a protein alters the protein's conformation, changing its binding characteristics. In this hypothetical example, phosphorylation activates an otherwise inactive protein. Some proteins inactivate by this mechanism. ATP, Adenosine triphosphate; ADP, adenosine diphosphate. C, Voltage-dependent proteins. The conformation of some

proteins, particularly ion channels, is altered by the electrical field surrounding the protein. Shown here is the opening (activation) of a voltage-dependent, gated Ca2+ channel when the membrane depolarizes.

A second method of producing conformational change, however, occurs as a result of the covalent modification of one or more of the amino acid side groups of the protein (see Figure 1-1, B). By far the most common such change is the covalent addition of a phosphate group to the hydroxyl (—OH) group on the side chain of serine, threonine, or tyrosine residues in the protein. This modification is called phosphorylation. Because the phosphate group is highly charged, phosphorylation of a protein alters hydrogen bonding and other electrostatic interactions within the protein chain, altering its conformation and functional properties.

In a third method, some physiologically important proteins change shape in response to the electrical field surrounding the protein (see Figure 1-1, C). These respond to a voltage change by altering the position of charged amino acids, thus altering protein shape.

The fourth method of protein shape change is the least well understood (not shown). Some proteins change shape in a controlled manner in response to mechanical forces. Although this is not surprising, because all solids and solidlike substances change shape at least slightly in response to force, we know relatively little about mechanosensitive proteins. The best current example is a protein involved in the very early events of hearing that changes its transport of ions in response to the mechanical stimulation by sound (small changes of air pressure in waves).

The significance of binding specificity and allostery can be better appreciated with two examples of their roles in physiological function. The first example is the role of enzymes in synthesizing three small, structurally similar, nonprotein signaling molecules. This example shows how binding specificity is important in catalytic

function and how allostery underlies the regulation of the synthesis. The second example is more complex: the role of proteins in the contraction of muscle. The contraction of muscle shows how proteins can exploit the basic properties of specific binding and allosteric shape change to do more than one job of work at the same time; muscle proteins serve a structural role, serve a catalytic function, and couple the "downhill" hydrolysis of adenosine triphosphate (ATP) to do mechanical work, the "uphill" lifting of weight.

A Series of Enzymatic Reactions Converts Tyrosine into the Signaling Molecules Dopamine, Norepinephrine, and Epinephrine

Figure 1-2 is a diagram of the series of reactions by which the amino acid tyrosine is converted into three different signaling molecules: (1) dopamine, a brain neurotransmitter; (2) norepinephrine, a neurotransmitter of the brain and peripheral autonomic nervous system; and (3) epinephrine, an autonomic neurotransmitter and hormone. Dopamine, norepinephrine, and epinephrine share a similar structure. All contain a phenyl (benzene) ring with two hydroxyl groups (i.e., catechol) and an amine group (thus catecholamines). They are among the large number of molecules that function as neurotransmitters. That is, the electrically coded information sent along nerve cells causes the release of a chemical, the neurotransmitter, at the terminal of the neuron, which is next to a target cell, such as another nerve, a muscle, or an endocrine cell. The electrically encoded information of the nerve is transmitted to the target cell by the binding of the neurotransmitter to proteins on the surface of the target cell. Proper neurotransmitter synthesis is crucial to nervous function and physiological regulation.

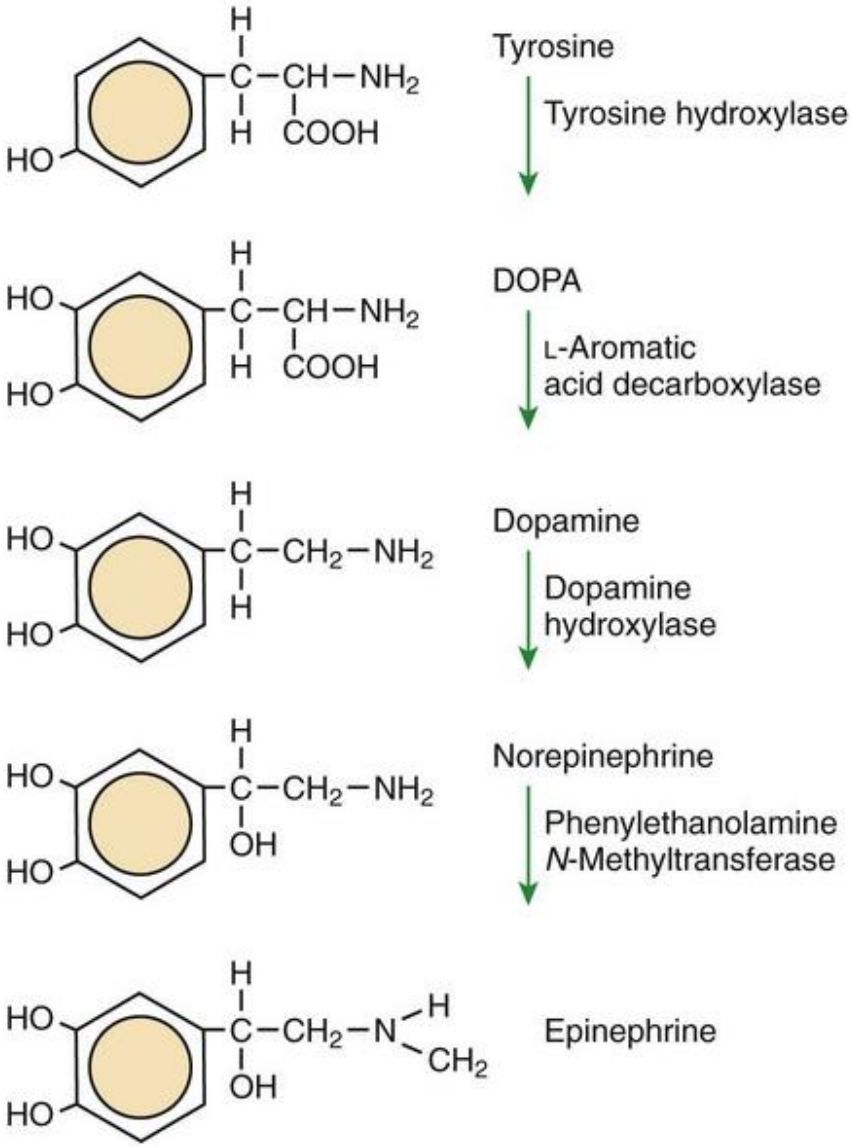


FIGURE 1-2 Epinephrine biosynthetic pathway. The amino acid tyrosine is metabolized to the neurotransmitters dopamine, norepinephrine, and epinephrine. The diagram shows the names and structural formulas for each compound in the path and the names of the enzymes that catalyze each reaction. DOPA, Dihydroxyphenylalanine.

In the first step of catecholamine biosynthesis, tyrosine binds to the enzyme tyrosine hydroxylase, which catalyzes the addition of another hydroxyl group to the phenyl group to form dihydroxyphenylalanine, almost always called DOPA. This hydroxyl group alters the enzyme-ligand interaction; the key no longer fits the keyhole. DOPA is released from the tyrosine hydroxylase and is then bound by

another enzyme, L-aromatic amino acid decarboxylase. As the name implies, this enzyme catalyzes the removal of the carboxyl group, converting DOPA to dopamine. Dopamine is converted into norepinephrine by the activity of dopamine hydroxylase, which adds yet another hydroxyl group, this time to the two-carbon tail of dopamine. Finally, addition of a methyl group to the amino nitrogen by phenylethanolamine N-methyltransferase gives rise to epinephrine (also called adrenalin). Note the binding specificity of the enzymes: whereas the catecholamine structures are all similar to one another, different enzymes bind each one (e.g., epinephrine does not bind to dopamine hydroxylase).

The allosteric properties of one enzyme in this pathway provide an example of physiological regulation. Certain hormones and neurotransmitters cause the phosphorylation of tyrosine hydroxylase, the first enzyme in the pathway, increasing its activity. That is, phosphorylation of the enzyme increases the rate at which it catalyzes the conversion of tyrosine to DOPA. Because this step is the slowest in the pathway, an increase in the activity of this protein increases the net rate of synthesis of all the catecholamines. Regulated decreases in the rate of catecholamine synthesis are achieved by a different allosteric mechanism: binding of end products to the enzyme. Dopamine, norepinephrine, and epinephrine can all bind to tyrosine hydroxylase at a site different than the site for tyrosine. These binding events inhibit the enzymatic activity. The inhibition of the pathway by its own end products makes this a classic case of allosteric control called end-product inhibition. Many substances regulate their own synthesis by inhibiting an initial enzyme in the pathway. If the cell has enough end products, these products inhibit further synthesis by allosteric changes in the enzyme. This is an example of the following sequence: specific binding → protein shape change → change in protein-binding properties and protein function → this change regulates something.

Molecular Genetic Aspects of Catecholamines

There are four known human catecholamine-synthesizing enzymes genes (**TH**, **DBH, PNMT, and AADC**).

1-In humans, **T**yrosine **H**ydroxylase is encoded by the TH gene, and the enzyme is present in the central nervous system (CNS), peripheral sympathetic neurons and the adrenal medulla. It mapped on chromosome 11, region q14-q21.

The TH gene provides instructions for making the enzyme tyrosine hydroxylase, which is important for normal functioning of the nervous system. Tyrosine hydroxylase takes part in the first step of the pathway that produces a group of hormones called catecholamines.

2-In humans, Dopamine beta (β)-hydroxylase is encoded by the DBH gene.

The DBH gene provides instructions for producing the enzyme dopamine beta (β)-hydroxylase. The DBH gene to chromosome 9q34**.**

The dopamine beta (β)-hydroxylase enzyme converts dopamine to norepinephrine, both of which are chemical messengers (neurotransmitters) that transmit signals between nerve cells.

3-In humans, Phenylethanolamine-*N*-Methyltransferase enzyme is encoded by the PNMT gene. The PNMT gene provides instructions for producing the enzyme Phenylethanolamine-*N*-Methyltransferase that catalyzes the last step—the addition of a methyl group-in the synthesis of epinephrine.

The PNMT enzyme is also present in very small amounts in the ganglion cells within the heart and in the CNS. The PNMT gene found on chromosome 17.

4-In humans, the aromatic l-amino acid decarboxylase (AADC) enzyme is encoded by the AADC/ *DDC* gene. The *DDC* gene provides instructions for making the aromatic l-amino acid decarboxylase (AADC) enzyme, which is important in the brain and nervous system.

This enzyme takes part in the pathway that produces dopamine and serotonin, which are chemical messengers that transmit signals between nerve cells (neurotransmitters).

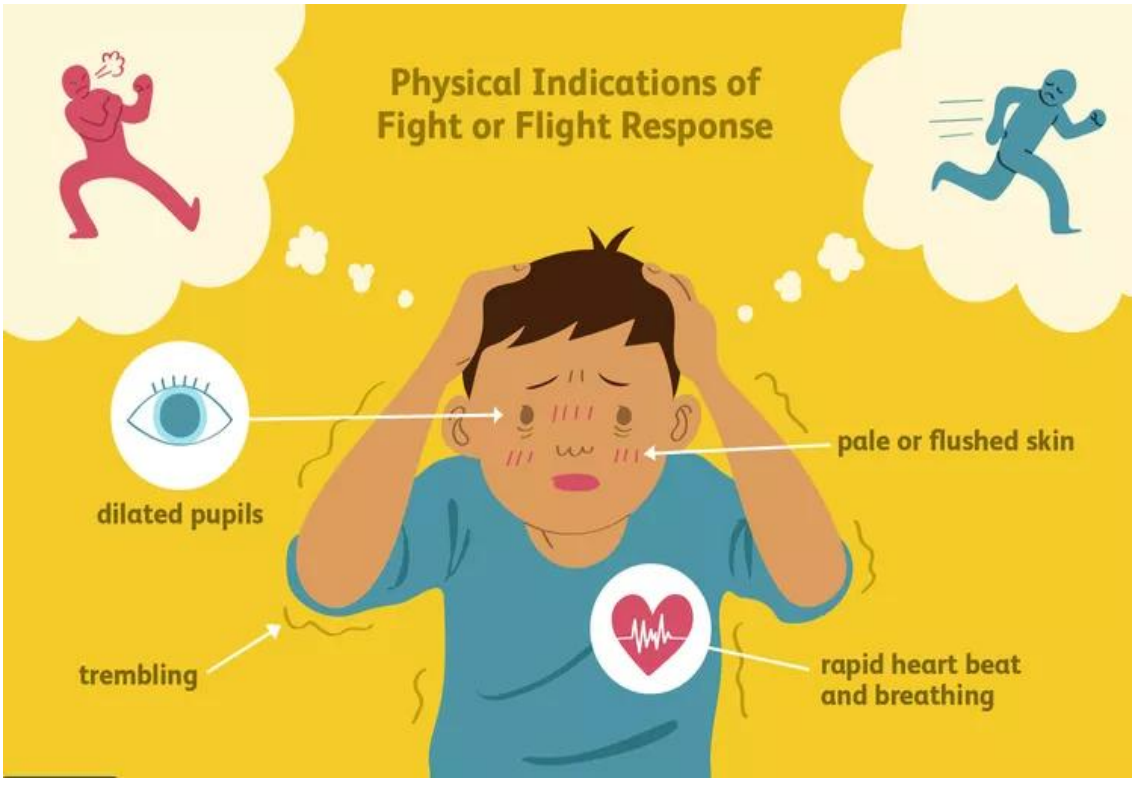
Aromatic L-amino acid decarboxylase (AADC or AAAD), also known as DOPA decarboxylase (DDC), tryptophan decarboxylase, and 5-hydroxytryptophan decarboxylase. The DDC gene located I on chromosome 17q21-q22

Why are the Catecholamines are vital?

They are play an important part of the body's stress response, which can be vital in a **fight-or-flight response** to a perceived threat.

They are produced in the adrenal glands, the brainstem, and the brain. In the brain they act as neurotransmitters.

In the blood they circulate and act as hormones and are broken down after just a few minutes and excreted in the urine



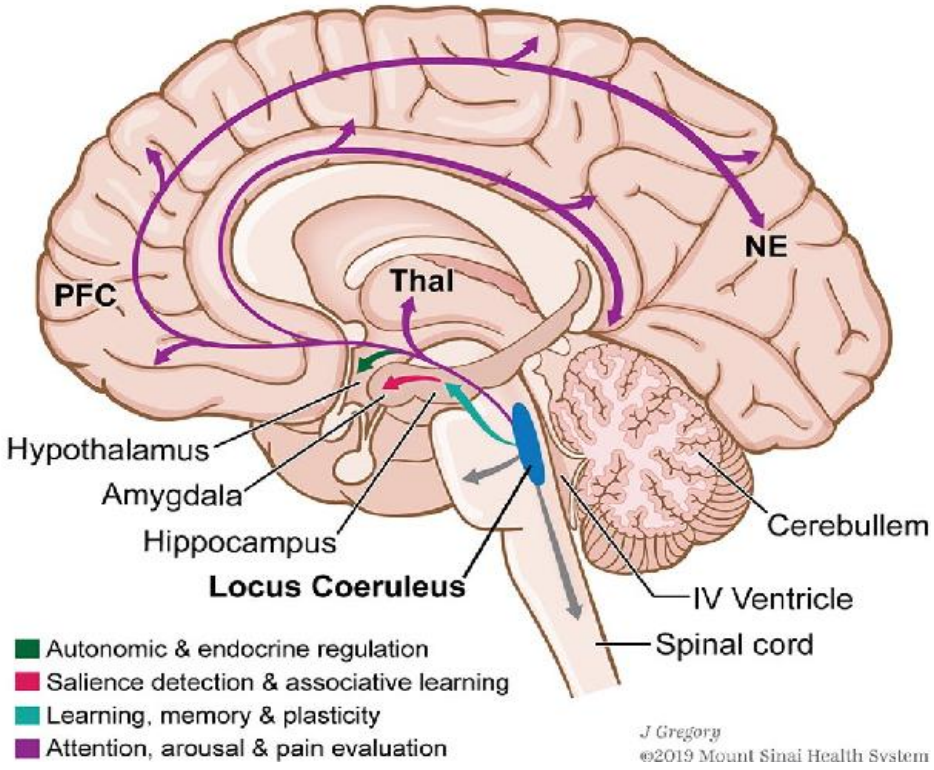
Locus coeruleus

The Locus coeruleus is the brain's main source of the neurotransmitter noradrenaline (norepinephrine).

This chemical is excitatory and is released in response to pain or stress, stimulating what is referred to as the 'fight-or-flight' mechanism.

Dysregulation of the central noradrenergic system is a core feature of post-traumatic stress disorder (PTSD).

There are molecular changes in locus coeruleus (LC) triggered by single-prolonged stress (SPS) PTSD model at a time as long as behavioral symptoms are manifested



Major locus coeruleus (LC) projections throughout the central nervous system play distinct functional roles. Ascending LC projections innervate the hypothalamus for autonomic and endocrine regulation; the amygdala for salience detection and associative learning; the hippocampus to influence learning, memory and plasticity; and the cortex, for regulation of attention, arousal and the cognitive evaluation of pain. Descending LC projections (gray) reach the periaqueductal gray and other brainstem nuclei, as well as the spinal cord.

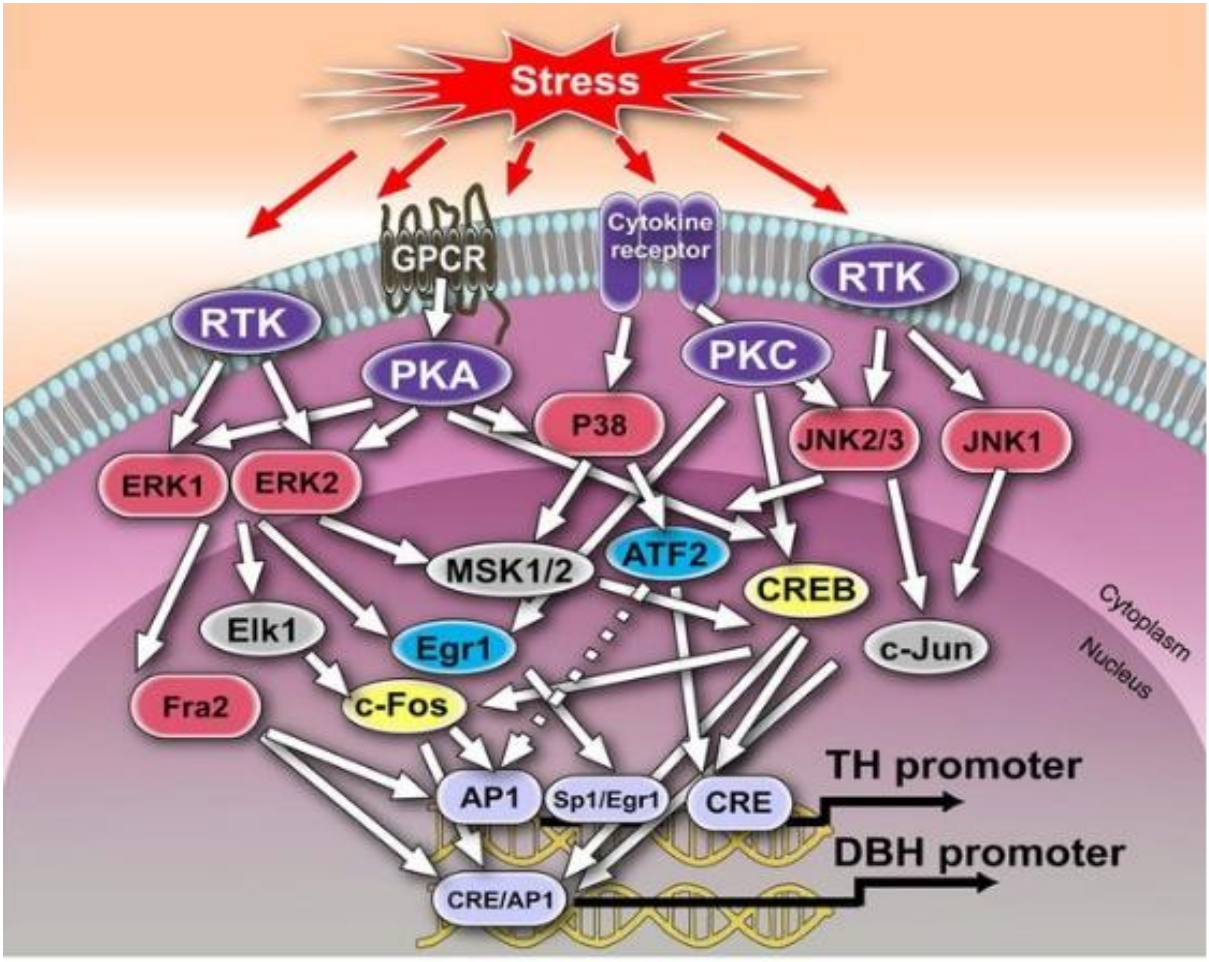


Diagram of stress-induced signaling events leading to the induction of TH and DBH gene transcription in LC (locus coeruleus).

Muscle Contraction and its Initiation and Cessation Depend on the Binding Specificity and Allosteric Properties of Proteins

There are three types of muscle tissue in vertebrates: (1) skeletal muscle, responsible for the animal's ability to move; (2) cardiac muscle, a muscle type found only in the heart but structurally similar to skeletal muscle; and (3) smooth muscle, which surrounds hollow organs such as blood vessels, gut, and uterus. All

three produce tensile force by contracting and shortening the length of the muscle. All muscle contraction occurs by the binding and the allosteric properties of two proteins, actin and myosin. Starting and stopping the contraction process depends on two additional proteins in skeletal and cardiac muscle, troponin and tropomyosin. Contraction initiation and cessation in smooth muscle depend on a different system with different proteins, and are discussed later in this chapter.

Myosin is a large protein whose shape resembles a two-headed golf club. The elongated tail of the myosin molecule corresponds to the shaft of the golf club, and there are two knobs at one end of the tail that, as with golf clubs, are called heads. Myosin tails bind specifically to other myosin tails, forming bipolar aggregates called thick filaments (Figure 1-3). Myosin heads specifically bind ATP and another muscle protein, actin. Actin binds to itself to form long, thin filaments, called thin filaments in muscle and called F-actin (filamentous actin) in other cell types. Actin filaments play an important architectural role in all animal cells. Although actin is best understood in muscle cells, all animal cells depend on actin filaments for their shape and for their capacity to migrate in their environment. Actin filaments can be “woven” in various ways to produce different structures, such as ropelike bundles and clothlike networks. These actin bundles and actin networks are used to support the cell in particular shapes, similar to ropes holding up the woven cloth of a tent.

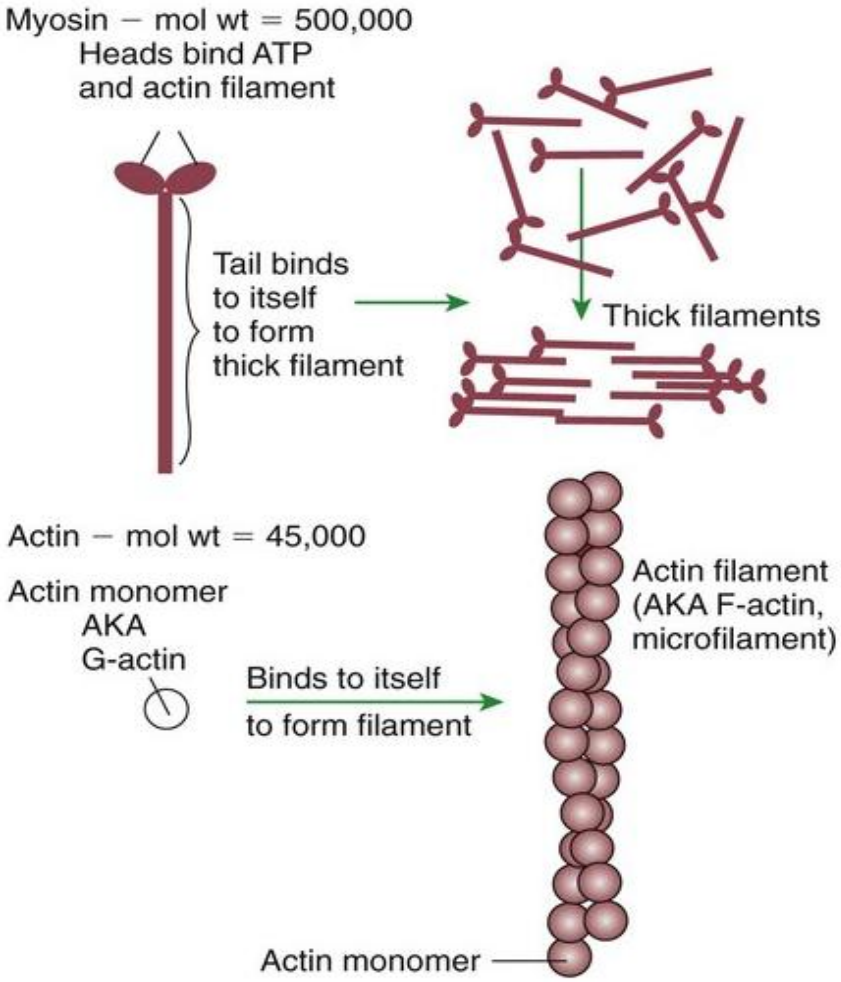


FIGURE 1-3 Assembly of myosin and actin to form filamentous structure. Myosin tails aggregate with one another to form a thick filament, a substructure of striated muscle. Actin monomers (G-actin) are a single polypeptide chain forming a globular protein that can bind to other actin monomers to form actin filament, also called microfilaments. The actin filament is the basic structure of striated muscle thin filaments; thin filaments also have troponin and tropomyosin as part of their structure

In muscle, the interaction of myosin, ATP, and actin produces contraction and force, as shown in Figure 1-4:

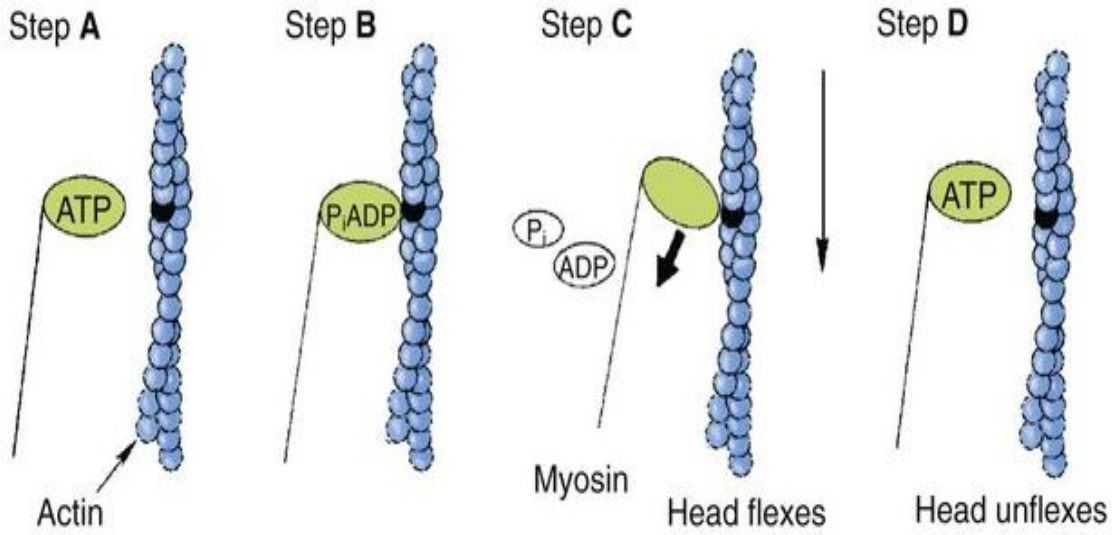


FIGURE 1-4 Power stroke of actomyosin. A, The myosin head has bound to adenosine triphosphate (ATP). In this conformation, myosin has little affinity to bind to actin. B, ATP is partially hydrolyzed to adenosine diphosphate (ADP) and inorganic phosphate (Pi); the hydrolysis is partial because the products remain bound to the myosin head. The change in what is bound to the myosin (ADP and Pi, not ATP) has the conformation of myosin so that it binds to actin with high affinity. C, Hydrolysis is complete; myosin releases ADP and Pi. This change in what is bound at the myosin head causes an allosteric change in the head; it flexes. Because the myosin head is still bound to the thin filament, the flexion causes the thin filament to slide past the thick filament. D, New ATP molecule binds to the myosin head; as for step A, myosin had little affinity for actin in this state, and the head releases from the thin filament and unflexes.

Step A: ATP binds to a myosin head; in this conformation, myosin has little ability to bind to actin.

Step B: Enzymatic activity associated with the myosin head, an adenosinetriphosphatase (ATPase), rapidly causes a partial hydrolysis of ATP to adenosine diphosphate (ADP) and inorganic phosphate (Pi), both of which stay bound to the myosin. With ADP and Pi bound, myosin has a slightly different shape that binds avidly to nearby actin filaments.

Step C: When myosin binds to actin, called cross-bridging, the myosin head couples the complete hydrolysis of ATP to a forceful flexing of the myosin head. This allosteric change causes the actin filament to slide past the thick filament. This sliding puts the actin filament under tension, which in turn causes the muscle to contract (shorten) against the load of the muscle (i.e., lifting a weight or pumping out blood). All muscle contraction depends on this sliding filament mechanism of actin and myosin-based filaments. This same allosteric change of myosin also alters myosin-binding properties so that it releases the ADP and Pi.

Step D: The binding of a new ATP molecule to the myosin head again causes myosin to change shape; the head unflexes and loses its affinity for actin, releasing the cross-bridge, and the cycle can start over. Rigor mortis of dead animals is caused by a lack of new ATP to bind to myosin heads. In the absence of ATP, myosin heads remain in Step C (i.e., bound to actin). The muscle is stiff because it is completely cross-bridged together.

This actomyosin motor uses the binding and allosteric properties of proteins to (1) create structural filaments capable of withstanding and transmitting mechanical force, (2) catalyze the hydrolysis of ATP, and (3) couple the "downhill" ATP hydrolysis to the "uphill" contraction to produce force. For just the one protein, myosin, there are a number of examples of the characteristic sequence described earlier: specific binding → protein shape change → change in protein-binding properties and protein function → this change makes a difference.

This system of contractile proteins requires some control so that, for example, the heart beats rhythmically and skeletal muscle contraction is coordinated. At the organismal level, skeletal and cardiac muscle contraction is primarily under control by electrical stimulation from nerves or other electrically active cells (see Chapter 6). The transmission of electrical excitation to the actomyosin system is called excitation-contraction coupling. Excitation-contraction coupling in all types of

muscle depends on changes in intracellular calcium ion (Ca2+) concentration. In skeletal and cardiac muscle, but not smooth muscle, two additional thin-filament proteins, troponin and tropomyosin, are required for this coupling. (Excitation-contraction coupling for smooth muscle is discussed later in this chapter.) In striated muscles, troponin binds to tropomyosin and to Ca2+. Tropomyosin is a long, thin protein that binds in the groove of the actin filament in such a way that its positions, high in the groove or snuggled down deep in the groove, allow or prevent the myosin head access to the thin filament (Figure 1-5). Excitation-contraction coupling of striated muscle works as follows:

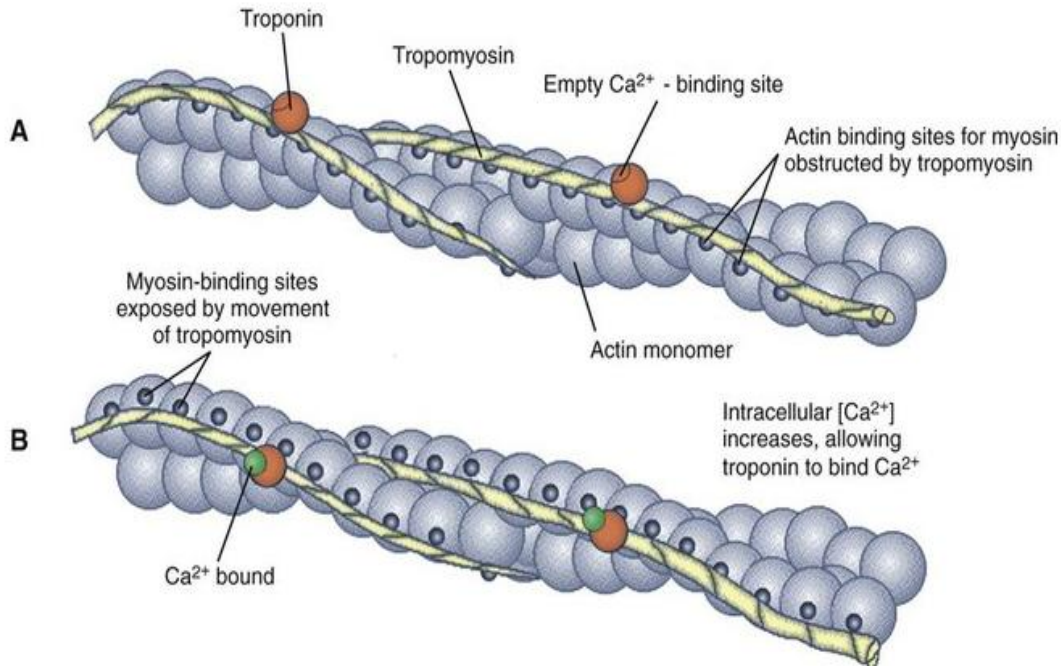


FIGURE 1-5 Regulation of the actomyosin ATPase and striated muscle contraction by Ca2+. A, In the absence of high concentrations of Ca2+, tropomyosin sits in the groove of the actin filament to obstruct the binding sites on actin for myosin. B, In the presence of higher Ca2+ concentrations, the ion binds to troponin, causing an allosteric change in the interaction of troponin with tropomyosin. This allosteric change in turn changes the interaction of tropomyosin with the actin filament to expose the myosin-binding sites on actin.

Step A: Electrical excitation of a striated muscle cell causes an increase in the intracellular concentration of Ca2+.

Step B: The additional Ca2+ binds to troponin, causing an allosteric change in troponin.

Step C: Because Ca2+ is bound to troponin, which in turn is bound to tropomyosin, the Ca2+-induced change in troponin conformation is transmitted to the tropomyosin molecule. When troponin binds Ca2+, tropomyosin changes its binding to actin in such a way that it exposes the actin site for myosin cross-bridging. (Tropomyosin snuggles down deeper in its actin groove, revealing actin to the myosin head.) As long as troponin binds Ca2+, the muscle contracts by the actomyosin cycle outlined earlier.

Step D: When the Ca2+ concentration drops to normal, however, troponin no longer binds Ca2+. This causes tropomyosin to move up in the thin filament groove so that it again blocks the myosin-binding sites on actin. Myosin heads can no longer cross-bridge, and muscle contraction stops. As with the actomyosin force generation itself, its regulation also shows many examples of the specific binding function. The specific binding of Ca2+ to troponin is a purely informational use of protein binding and shape change; that is, troponin has no catalytic, transport, or structural function, but transmits the "on" signal to the next protein. The binding of tropomyosin to actin serves not only a regulatory role but also a structural role; the actin filament is stabilized by tropomyosin, making it less likely to disassemble into actin subunits. The change in the binding geometry of tropomyosin that directly regulates myosin access to actin is a good example of the importance of allosteric change and the following sequence: specific binding (troponin to tropomyosin) → protein (tropomyosin) shape change → change in protein-binding properties (tropomyosin to actin) → a difference in the position of tropomyosin, which in turn regulates the actomyosin motor.