# SECTION V

## Biochemistry of Extracellular & Intracellular Communication

# Membranes: Structure & Function 41

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## BIOMEDICAL IMPORTANCE

Membranes are highly viscous, plastic structures. Plasma membranes form closed compartments around cellular protoplasm to separate one cell from another and thus permit cellular individuality. The plasma membrane has **selective permeabilities** and acts as a barrier, thereby maintaining differences in composition between the inside and outside of the cell. The selective permeabilities are provided mainly by **channels** and **pumps** for ions and substrates. The plasma membrane also exchanges material with the extracellular environment by exocytosis and endocytosis, and there are special areas of membrane structure—the gap junctions—through which adjacent cells exchange material. In addition, the plasma membrane plays key roles in **cell-cell interactions** and in **transmembrane signaling.**

Membranes also form **specialized compartments** within the cell. Such intracellular membranes help shape many of the morphologically distinguishable structures (organelles), eg, mitochondria, ER, sarcoplasmic reticulum, Golgi complexes, secretory granules, lysosomes, and the nuclear membrane. Membranes localize **enzymes,** function as integral elements in **excitation-response coupling,** and provide sites of **energy transduction,** such as in photosynthesis and oxidative phosphorylation.

**Changes** in membrane structure (eg caused by ischemia) can affect water balance and ion flux and therefore every process within the cell. Specific deficiencies or alterations of certain membrane components lead to a variety of **diseases** (see Table 41–5). In short, normal cellular function depends on normal membranes.

## MAINTENANCE OF A NORMAL INTRA- & EXTRACELLULAR ENVIRONMENT IS FUNDAMENTAL TO LIFE

Life originated in an aqueous environment; enzyme reactions, cellular and subcellular processes, and so forth have therefore evolved to work in this milieu. Since mammals live in a gaseous environment, how is the aqueous state maintained? Membranes accomplish this by internalizing and compartmentalizing body water.

### The Body's Internal Water Is Compartmentalized

Water makes up about 60% of the lean body mass of the human body and is distributed in two large compartments.

#### A. Intracellular Fluid (ICF)

This compartment constitutes two-thirds of total body water and provides the environment for the cell (1) to make, store, and utilize energy; (2) to repair itself; (3) to replicate; and (4) to perform special functions.

#### B. Extracellular Fluid (ECF)

This compartment contains about one-third of total body water and is distributed between the plasma and interstitial compartments. The extracellular fluid is a delivery system. It brings to the cells nutrients (eg, glucose, fatty acids, amino acids), oxygen, various ions and trace minerals, and a variety of regulatory molecules (hormones) that coordinate the functions of widely separated cells. Extracellular fluid removes $CO_2$, waste

products, and toxic or detoxified materials from the immediate cellular environment.

### The Ionic Compositions of Intracellular & Extracellular Fluids Differ Greatly

As illustrated in Table 41–1, the **internal environment** is rich in $K^+$ and $Mg^{2+}$, and phosphate is its major anion. **Extracellular fluid** is characterized by high $Na^+$ and $Ca^{2+}$ content, and $Cl^-$ is the major anion. Note also that the concentration of glucose is higher in extracellular fluid than in the cell, whereas the opposite is true for proteins. Why is there such a difference? It is thought that the primordial sea in which life originated was rich in $K^+$ and $Mg^{2+}$. It therefore follows that enzyme reactions and other biologic processes evolved to function best in that environment—hence the high concentration of these ions within cells. Cells were faced with strong selection pressure as the sea gradually changed to a composition rich in $Na^+$ and $Ca^{2+}$. Vast changes would have been required for evolution of a completely new set of biochemical and physiologic machinery; instead, as it happened, cells developed barriers—membranes with associated "pumps"—to maintain the internal microenvironment.

## MEMBRANES ARE COMPLEX STRUCTURES COMPOSED OF LIPIDS, PROTEINS, & CARBOHYDRATES

We shall mainly discuss the membranes present in eukaryotic cells, although many of the principles described also apply to the membranes of prokaryotes. The various cellular membranes have **different compositions**, as reflected in the ratio of protein to lipid (Figure 41–1). This is not surprising, given their divergent functions. Membranes are asymmetric sheet-like enclosed structures with distinct inner and outer surfaces.

***Table 41–1.*** Comparison of the mean concentrations of various molecules outside and inside a mammalian cell.

| Substance | Extracellular Fluid | Intracellular Fluid |
|---|---|---|
| $Na^+$ | 140 mmol/L | 10 mmol/L |
| $K^+$ | 4 mmol/L | 140 mmol/L |
| $Ca^{2+}$ (free) | 2.5 mmol/L | 0.1 µmol/L |
| $Mg^{2+}$ | 1.5 mmol/L | 30 mmol/L |
| $Cl^-$ | 100 mmol/L | 4 mmol/L |
| $HCO_3^-$ | 27 mmol/L | 10 mmol/L |
| $PO_4^{3-}$ | 2 mmol/L | 60 mmol/L |
| Glucose | 5.5 mmol/L | 0–1 mmol/L |
| Protein | 2 g/dL | 16 g/dL |

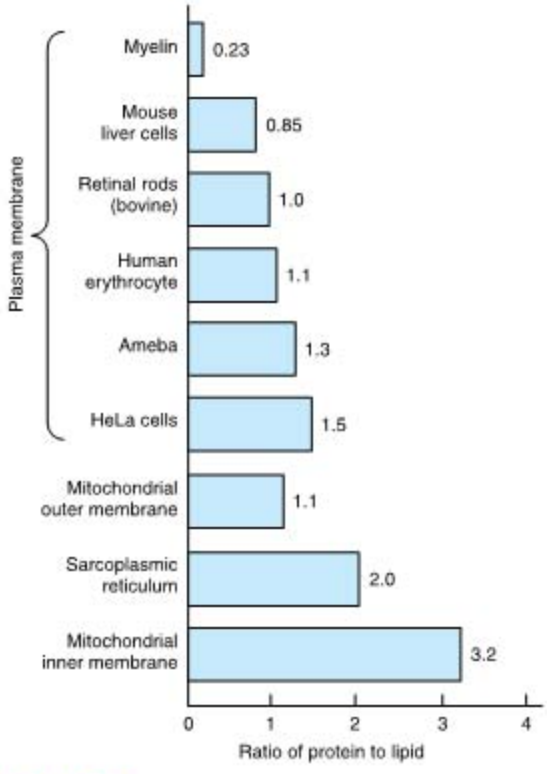


***Figure 41–1.*** Ratio of protein to lipid in different membranes. Proteins equal or exceed the quantity of lipid in nearly all membranes. The outstanding exception is myelin, an electrical insulator found on many nerve fibers.

These sheet-like structures are **noncovalent assemblies** that are thermodynamically stable and metabolically active. Numerous proteins are located in membranes, where they carry out specific functions of the organelle, the cell, or the organism.

### The Major Lipids in Mammalian Membranes Are Phospholipids, Glycosphingolipids, & Cholesterol

#### A. Phospholipids

Of the two major phospholipid classes present in membranes, **phosphoglycerides** are the more common and consist of a glycerol backbone to which are attached two fatty acids in ester linkage and a phosphorylated alcohol (Figure 41–2). The fatty acid constituents are usually even-numbered carbon molecules, most commonly containing 16 or 18 carbons. They are unbranched and can be saturated or unsaturated. The simplest phosphoglyceride is phosphatidic acid, which is

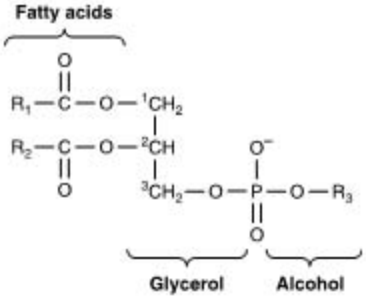


***Figure 41–2.*** A phosphoglyceride showing the fatty acids ($R_1$ and $R_2$), glycerol, and phosphorylated alcohol components. In phosphatidic acid, $R_3$ is hydrogen.

1,2-diacylglycerol 3-phosphate, a key intermediate in the formation of all other phosphoglycerides (Chapter 24). In other phosphoglycerides, the 3-phosphate is esterified to an alcohol such as ethanolamine, choline, serine, glycerol, or inositol (Chapter 14).

The second major class of phospholipids is composed of **sphingomyelin,** which contains a sphingosine backbone rather than glycerol. A fatty acid is attached by an amide linkage to the amino group of sphingosine, forming ceramide. The primary hydroxyl group of sphingosine is esterified to phosphorylcholine. Sphingomyelin, as the name implies, is prominent in myelin sheaths.

The amounts and fatty acid compositions of the various phospholipids vary among the different cellular membranes.

### B. Glycosphingolipids

The glycosphingolipids (GSLs) are sugar-containing lipids built on a backbone of ceramide; they include galactosyl- and glucosylceramide (cerebrosides) and the gangliosides. Their structures are described in Chapter 14. They are mainly located in the plasma membranes of cells.

### C. Sterols

The most common sterol in membranes is **cholesterol** (Chapter 14), which resides mainly in the plasma membranes of mammalian cells but can also be found in lesser quantities in mitochondria, Golgi complexes, and nuclear membranes. Cholesterol intercalates among the phospholipids of the membrane, with its hydroxyl group at the aqueous interface and the remainder of the molecule within the leaflet. Its effect on the fluidity of membranes is discussed subsequently.

All of the above lipids can be separated from one another by techniques such as column, thin layer, and gas-liquid chromatography and their structures established by mass spectrometry.

Each eukaryotic cell membrane has a somewhat different lipid composition, though phospholipids are the major class in all.

## Membrane Lipids Are Amphipathic

All major lipids in membranes contain both hydrophobic and hydrophilic regions and are therefore termed **"amphipathic."** Membranes themselves are thus amphipathic. If the hydrophobic regions were separated from the rest of the molecule, it would be insoluble in water but soluble in oil. Conversely, if the hydrophilic region were separated from the rest of the molecule, it would be insoluble in oil but soluble in water. The amphipathic nature of a phospholipid is represented in Figure 41–3. Thus, the polar head groups of the phospholipids and the hydroxyl group of cholesterol interface with the aqueous environment; a similar situation applies to the sugar moieties of the GSLs (see below).

**Saturated fatty acids** have straight tails, whereas **unsaturated fatty acids,** which generally exist in the cis form in membranes, make kinked tails (Figure 41–3). As more kinks are inserted in the tails, the membrane becomes less tightly packed and therefore more fluid. **Detergents** are amphipathic molecules that are important in biochemistry as well as in the household. The molecular structure of a detergent is not unlike that of a phospholipid. Certain detergents are widely used to solubilize membrane proteins as a first step in their purification. The hydrophobic end of the detergent binds to

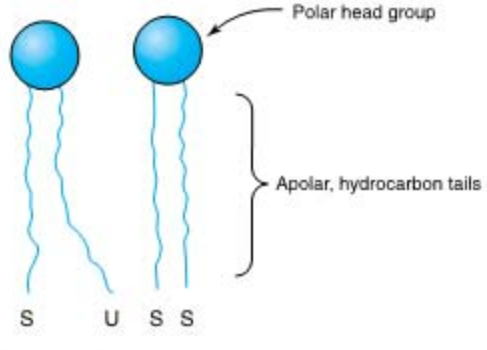


***Figure 41–3.*** Diagrammatic representation of a phospholipid or other membrane lipid. The polar head group is hydrophilic, and the hydrocarbon tails are hydrophobic or lipophilic. The fatty acids in the tails are saturated (S) or unsaturated (U); the former are usually attached to carbon 1 of glycerol and the latter to carbon 2. Note the kink in the tail of the unsaturated fatty acid (U), which is important in conferring increased membrane fluidity.

hydrophobic regions of the proteins, displacing most of their bound lipids. The polar end of the detergent is free, bringing the proteins into solution as detergent-protein complexes, usually also containing some residual lipids.

## Membrane Lipids Form Bilayers

The amphipathic character of phospholipids suggests that the two regions of the molecule have incompatible solubilities; however, in a solvent such as water, phospholipids organize themselves into a form that thermodynamically serves the solubility requirements of both regions. A **micelle** (Figure 41–4) is such a structure; the hydrophobic regions are shielded from water, while the hydrophilic polar groups are immersed in the aqueous environment. However, micelles are usually relatively small in size (eg, approximately 200 nm) and thus are limited in their potential to form membranes.

As was recognized in 1925 by Gorter and Grendel, a **bimolecular layer,** or **lipid bilayer,** can also satisfy the thermodynamic requirements of amphipathic molecules in an aqueous environment. **Bilayers,** not micelles, are indeed the key structures in biologic membranes. A bilayer exists as a sheet in which the hydrophobic regions of the phospholipids are protected from the aqueous environment, while the hydrophilic regions are immersed in water (Figure 41–5). Only the ends or edges of the bilayer sheet are exposed to an unfavorable environment, but even these exposed edges can be eliminated by folding the sheet back upon itself to form an enclosed vesicle with no edges. A bilayer can extend over relatively large distances (eg, 1 mm). The closed bilayer provides one of the most essential properties of membranes. It is impermeable to most water-soluble molecules, since they would be insoluble in the hydrophobic core of the bilayer.

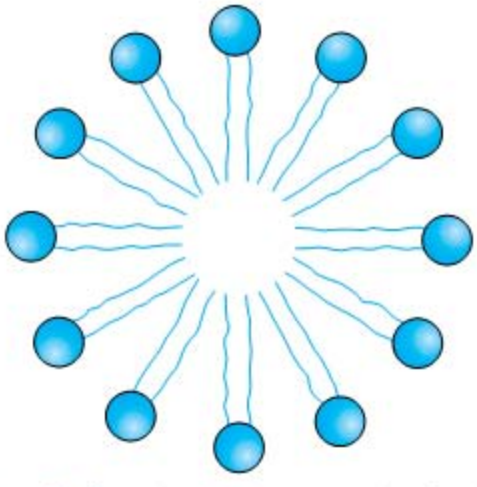

***Figure 41–4.*** Diagrammatic cross-section of a micelle. The polar head groups are bathed in water, whereas the hydrophobic hydrocarbon tails are surrounded by other hydrocarbons and thereby protected from water. Micelles are relatively small (compared with lipid bilayers) spherical structures.

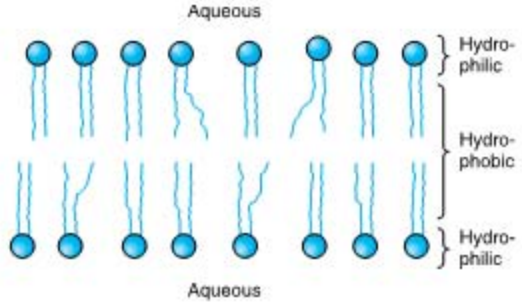


***Figure 41–5.*** Diagram of a section of a bilayer membrane formed from phospholipid molecules. The unsaturated fatty acid tails are kinked and lead to more spacing between the polar head groups, hence to more room for movement. This in turn results in increased membrane fluidity. (Slightly modified and reproduced, with permission, from Stryer L: Biochemistry, 2nd ed. Freeman, 1981.)

Lipid bilayers are formed by **self-assembly,** driven by the **hydrophobic effect.** When lipid molecules come together in a bilayer, the entropy of the surrounding solvent molecules increases.

Two questions arise from consideration of the above. First, how many biologic materials are **lipid-soluble** and can therefore readily enter the cell? Gases such as oxygen, $CO_2$, and nitrogen—small molecules with little interaction with solvents—readily diffuse through the hydrophobic regions of the membrane. The permeability coefficients of several ions and of a number of other molecules in a lipid bilayer are shown in Figure 41–6. The three electrolytes shown ($Na^+$, $K^+$, and $Cl^-$) cross the bilayer much more slowly than water. In general, the permeability coefficients of small molecules in a lipid bilayer correlate with their solubilities in nonpolar solvents. For instance, steroids more readily traverse the lipid bilayer compared with electrolytes. The high permeability coefficient of water itself is surprising but is partly explained by its small size and relative lack of charge.

The second question concerns **molecules that are not lipid-soluble:** How are the transmembrane concentration gradients for non-lipid-soluble molecules maintained? The answer is that membranes contain proteins,

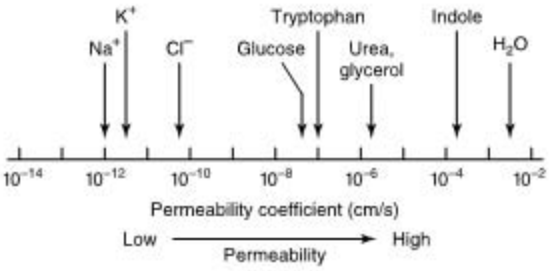


***Figure 41–6.*** Permeability coefficients of water, some ions, and other small molecules in lipid bilayer membranes. Molecules that move rapidly through a given membrane are said to have a high permeability coefficient. (Slightly modified and reproduced, with permission, from Stryer L: *Biochemistry*, 2nd ed. Freeman, 1981.)

and proteins are also amphipathic molecules that can be inserted into the correspondingly amphipathic lipid bilayer. Proteins form channels for the movement of ions and small molecules and serve as transporters for larger molecules that otherwise could not pass the bilayer. These processes are described below.

## Membrane Proteins Are Associated With the Lipid Bilayer

Membrane **phospholipids** act as a solvent for membrane proteins, creating an environment in which the latter can function. Of the 20 amino acids contributing to the primary structure of **proteins,** the functional groups attached to the α carbon are strongly hydrophobic in six, weakly hydrophobic in a few, and hydrophilic in the remainder. As described in Chapter 5, the α-helical structure of proteins minimizes the hydrophilic character of the peptide bonds themselves. Thus, proteins can be amphipathic and form an integral part of the membrane by having hydrophilic regions protruding at the inside and outside faces of the membrane but connected by a hydrophobic region traversing the hydrophobic core of the bilayer. In fact, those portions of membrane proteins that traverse membranes do contain substantial numbers of hydrophobic amino acids and almost invariably have either a high α-helical or β-pleated sheet content. For many membranes, a stretch of approximately 20 amino acids in an α helix will span the bilayer.

It is possible to calculate whether a particular sequence of amino acids present in a protein is consistent with a **transmembrane location.** This can be done by consulting a table that lists the hydrophobicities of each of the 20 common amino acids and the free energy values for their transfer from the interior of a membrane to water. Hydrophobic amino acids have positive values; polar amino acids have negative values. The total free energy values for transferring successive sequences of 20 amino acids in the protein are plotted, yielding a so-called **hydropathy plot.** Values of over 20 kcal·mol$^{-1}$ are consistent with—but do not prove—a transmembrane location.

Another aspect of the interaction of lipids and proteins is that some proteins are anchored to one leaflet or another of the bilayer by **covalent linkages to certain lipids.** Palmitate and myristate are fatty acids involved in such linkages to specific proteins. A number of other proteins (see Chapter 47) are linked to glycophosphatidylinositol (GPI) structures.

## Different Membranes Have Different Protein Compositions

The **number of different proteins** in a membrane varies from less than a dozen in the sarcoplasmic reticulum to over 100 in the plasma membrane. Most membrane proteins can be separated from one another using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), a technique that has revolutionized their study. In the absence of SDS, few membrane proteins would remain soluble during electrophoresis. Proteins are the **major functional molecules** of membranes and consist of enzymes, pumps and channels, structural components, antigens (eg, for histocompatibility), and receptors for various molecules. Because every membrane possesses a different complement of proteins, there is no such thing as a typical membrane structure. The enzymatic properties of several different membranes are shown in Table 41–2.

## Membranes Are Dynamic Structures

Membranes and their components are **dynamic structures.** The lipids and proteins in membranes undergo turnover there just as they do in other compartments of the cell. Different lipids have different turnover rates, and the turnover rates of individual species of membrane proteins may vary widely. The membrane itself can turn over even more rapidly than any of its constituents. This is discussed in more detail in the section on endocytosis.

## Membranes Are Asymmetric Structures

This asymmetry can be partially attributed to the irregular distribution of proteins within the membranes. An **inside-outside asymmetry** is also provided by the external location of the carbohydrates attached to membrane proteins. In addition, specific enzymes are lo-

***Table 41–2.*** Enzymatic markers of different membranes.[1]

| Membrane | Enzyme |
|---|---|
| Plasma | 5′-Nucleotidase<br>Adenylyl cyclase<br>$Na^+$-$K^+$ ATPase |
| Endoplasmic reticulum | Glucose-6-phosphatase |
| Golgi apparatus | |
| Cis | GlcNAc transferase I |
| Medial | Golgi mannosidase II |
| Trans | Galactosyl transferase |
| TGN | Sialyl transferase |
| Inner mitochondrial membrane | ATP synthase |

[1]Membranes contain many proteins, some of which have enzymatic activity. Some of these enzymes are located only in certain membranes and can therefore be used as markers to follow the purification of these membranes.
TGN, trans golgi network.

cated exclusively on the outside or inside of membranes, as in the mitochondrial and plasma membranes.

There are **regional asymmetries** in membranes. Some, such as occur at the villous borders of mucosal cells, are almost macroscopically visible. Others, such as those at gap junctions, tight junctions, and synapses, occupy much smaller regions of the membrane and generate correspondingly smaller local asymmetries.

There is also inside-outside (transverse) asymmetry of the **phospholipids.** The **choline-containing phospholipids** (phosphatidylcholine and sphingomyelin) are located mainly in the outer molecular layer; the **aminophospholipids** (phosphatidylserine and phosphatidylethanolamine) are preferentially located in the inner leaflet. Obviously, if this asymmetry is to exist at all, there must be limited transverse mobility (flip-flop) of the membrane phospholipids. In fact, phospholipids in synthetic bilayers exhibit an **extraordinarily slow rate of flip-flop;** the half-life of the asymmetry can be measured in several weeks. However, when certain membrane proteins such as the erythrocyte protein glycophorin are inserted artificially into synthetic bilayers, the frequency of phospholipid flip-flop may increase as much as 100-fold.

The mechanisms involved in the establishment of lipid asymmetry are not well understood. The enzymes involved in the synthesis of phospholipids are located on the cytoplasmic side of microsomal membrane vesicles. Translocases **(flippases)** exist that transfer certain phospholipids (eg, phosphatidylcholine) from the inner to the outer leaflet. Specific **proteins that preferentially bind** individual phospholipids also appear to be present in the two leaflets, contributing to the asymmetric distribution of these lipid molecules. In addition, phospholipid exchange proteins recognize specific phospholipids and transfer them from one membrane (eg, the endoplasmic reticulum [ER]) to others (eg, mitochondrial and peroxisomal). There is further asymmetry with regard to **GSLs** and also **glycoproteins;** the sugar moieties of these molecules all protrude outward from the plasma membrane and are absent from its inner face.

## Membranes Contain Integral & Peripheral Proteins (Figure 41–7)

It is useful to classify membrane proteins into two types: **integral** and **peripheral.** Most membrane proteins fall into the integral class, meaning that they interact extensively with the phospholipids and require the use of detergents for their solubilization. Also, they generally span the bilayer. Integral proteins are usually globular and are themselves amphipathic. They consist of two hydrophilic ends separated by an intervening hydrophobic region that traverses the hydrophobic core of the bilayer. As the structures of integral membrane proteins were being elucidated, it became apparent that certain ones (eg, transporter molecules, various receptors, and G proteins) span the bilayer many times (see Figure 46–5). Integral proteins are also asymmetrically distributed across the membrane bilayer. This asymmetric orientation is conferred at the time of their insertion in the lipid bilayer. The hydrophilic external region of an amphipathic protein, which is synthesized on polyribosomes, must traverse the hydrophobic core of its target membrane and eventually be found on the outside of that membrane. The molecular mechanisms involved in insertion of proteins into membranes and the topic of membrane assembly are discussed in Chapter 46.

**Peripheral proteins** do not interact directly with the phospholipids in the bilayer and thus do not require use of detergents for their release. They are weakly bound to the hydrophilic regions of specific integral proteins and can be released from them by treatment with salt solutions of high ionic strength. For example, ankyrin, a peripheral protein, is bound to the integral protein "band 3" of erythrocyte membrane. Spectrin, a cytoskeletal structure within the erythrocyte, is in turn bound to ankyrin and thereby plays an important role in maintenance of the biconcave shape of the erythrocyte. Many hormone receptor molecules are integral proteins, and the specific polypeptide hormones that bind to these receptor molecules may therefore be considered peripheral proteins. Peripheral proteins, such as polypeptide hormones, may help organize the distribu-

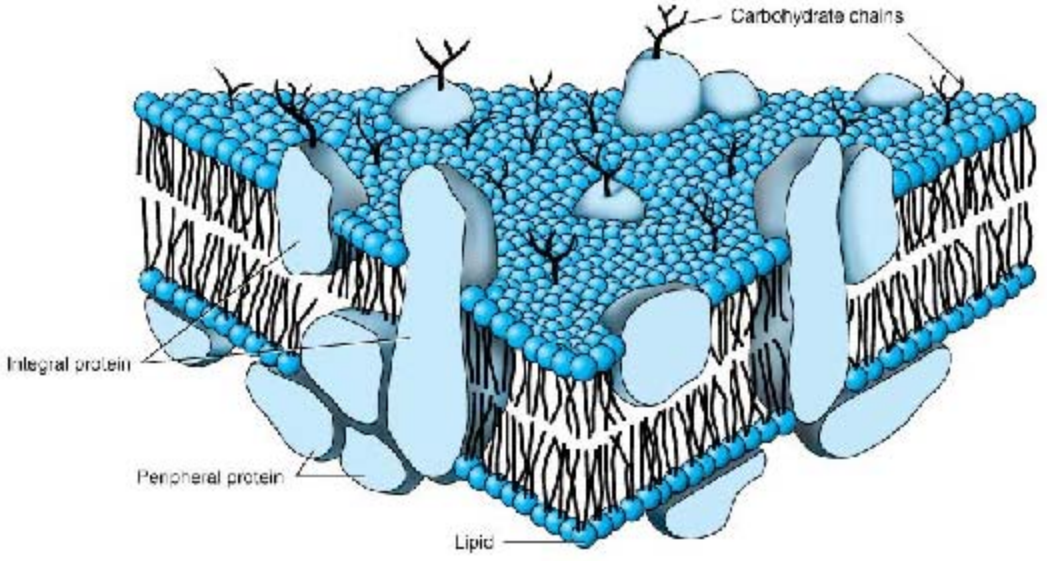


***Figure 41–7.*** The fluid mosaic model of membrane structure. The membrane consists of a bimolecular lipid layer with proteins inserted in it or bound to either surface. Integral membrane proteins are firmly embedded in the lipid layers. Some of these proteins completely span the bilayer and are called transmembrane proteins, while others are embedded in either the outer or inner leaflet of the lipid bilayer. Loosely bound to the outer or inner surface of the membrane are the peripheral proteins. Many of the proteins and lipids have externally exposed oligosaccharide chains. (Reproduced, with permission, from Junqueira LC, Carneiro J: *Basic Histology: Text & Atlas,* 10th ed. McGraw-Hill, 2003.)

tion of integral proteins, such as their receptors, within the plane of the bilayer (see below).

## ARTIFICIAL MEMBRANES MODEL MEMBRANE FUNCTION

Artificial membrane systems can be prepared by appropriate techniques. These systems generally consist of mixtures of one or more phospholipids of natural or synthetic origin that can be treated (eg, by using mild sonication) to form spherical vesicles in which the lipids form a bilayer. Such vesicles, surrounded by a lipid bilayer, are termed **liposomes.**

Some of the advantages and uses of artificial membrane systems in the study of membrane function can be briefly explained.

(1) The lipid content of the membranes can be varied, allowing systematic examination of the effects of varying lipid composition on certain functions. For instance, vesicles can be made that are composed solely of phosphatidylcholine or, alternatively, of known mixtures of different phospholipids, glycolipids, and cholesterol. The fatty acid moieties of the lipids used can also be varied by employing synthetic lipids of known composition to permit systematic examination of the effects of fatty acid composition on certain membrane functions (eg, transport).

(2) Purified membrane proteins or enzymes can be incorporated into these vesicles in order to assess what factors (eg, specific lipids or ancillary proteins) the proteins require to reconstitute their function. Investigations of purified proteins, eg, the $Ca^{2+}$ ATPase of the sarcoplasmic reticulum, have in certain cases suggested that only a single protein and a single lipid are required to reconstitute an ion pump.

(3) The environment of these systems can be rigidly controlled and systematically varied (eg, ion concentrations). The systems can also be exposed to known ligands if, for example, the liposomes contain specific receptor proteins.

(4) When liposomes are formed, they can be made to entrap certain compounds inside themselves, eg, drugs and isolated genes. There is interest in using liposomes to distribute drugs to certain tissues, and if components (eg, antibodies to certain cell surface molecules) could be incorporated into liposomes so that they would be targeted to specific tissues or tumors, the therapeutic impact would be considerable. DNA entrapped inside liposomes appears to be less sensitive to

attack by nucleases; this approach may prove useful in attempts at gene therapy.

## THE FLUID MOSAIC MODEL OF MEMBRANE STRUCTURE IS WIDELY ACCEPTED

The **fluid mosaic model** of membrane structure proposed in 1972 by Singer and Nicolson (Figure 41–7) is now widely accepted. The model is often likened to icebergs (membrane proteins) floating in a sea of predominantly phospholipid molecules. Early evidence for the model was the finding that certain species-specific integral proteins (detected by fluorescent labeling techniques) rapidly and randomly redistributed in the plasma membrane of an interspecies hybrid cell formed by the artificially induced fusion of two different parent cells. It has subsequently been demonstrated that phospholipids also undergo rapid redistribution in the plane of the membrane. This diffusion within the plane of the membrane, termed translational diffusion, can be quite rapid for a phospholipid; in fact, within the plane of the membrane, one molecule of phospholipid can move several micrometers per second.

The phase changes—and thus the **fluidity** of membranes—are largely dependent upon the lipid composition of the membrane. In a lipid bilayer, the hydrophobic chains of the fatty acids can be highly aligned or ordered to provide a rather stiff structure. As the temperature increases, the hydrophobic side chains undergo a transition from the ordered state (more gel-like or crystalline phase) to a disordered one, taking on a more liquid-like or fluid arrangement. The temperature at which the structure undergoes the transition from ordered to disordered (ie, melts) is called the **"transition temperature" ($T_m$)**. The longer and more saturated fatty acid chains interact more strongly with each other via their longer hydrocarbon chains and thus cause higher values of $T_m$—ie, higher temperatures are required to increase the fluidity of the bilayer. On the other hand, unsaturated bonds that exist in the cis configuration tend to increase the fluidity of a bilayer by decreasing the compactness of the side chain packing without diminishing hydrophobicity (Figure 41–3). The phospholipids of cellular membranes generally contain at least one unsaturated fatty acid with at least one cis double bond.

**Cholesterol** modifies the fluidity of membranes. At temperatures below the $T_m$, it interferes with the interaction of the hydrocarbon tails of fatty acids and thus increases fluidity. At temperatures above the $T_m$, it limits disorder because it is more rigid than the hydrocarbon tails of the fatty acids and cannot move in the membrane to the same extent, thus limiting fluidity. At high cholesterol:phospholipid ratios, transition temperatures are altogether indistinguishable.

The **fluidity** of a membrane significantly affects its **functions.** As membrane fluidity increases, so does its permeability to water and other small hydrophilic molecules. The lateral mobility of integral proteins increases as the fluidity of the membrane increases. If the active site of an integral protein involved in a given function is exclusively in its hydrophilic regions, changing lipid fluidity will probably have little effect on the activity of the protein; however, if the protein is involved in a transport function in which transport components span the membrane, lipid phase effects may significantly alter the transport rate. The insulin receptor is an excellent example of altered function with changes in fluidity. As the concentration of unsaturated fatty acids in the membrane is increased (by growing cultured cells in a medium rich in such molecules), fluidity increases. This alters the receptor so that it binds more insulin.

A state of fluidity and thus of translational mobility in a membrane may be confined to certain regions of membranes under certain conditions. For example, protein-protein interactions may take place within the plane of the membrane, such that the integral proteins form a rigid matrix—in contrast to the more usual situation, where the lipid acts as the matrix. Such regions of rigid protein matrix can exist side by side in the same membrane with the usual lipid matrix. Gap junctions and tight junctions are clear examples of such side-by-side coexistence of different matrices.

### Lipid Rafts & Caveolae Are Special Features of Some Membranes

While the fluid mosaic model of membrane structure has stood up well to detailed scrutiny, additional features of membrane structure and function are constantly emerging. Two structures of particular current interest, located in surface membranes, are lipid rafts and caveolae. The former are dynamic areas of the exoplasmic leaflet of the lipid bilayer enriched in cholesterol and sphingolipids; they are involved in signal transduction and possibly other processes. Caveolae may derive from lipid rafts. Many if not all of them contain the protein caveolin-1, which may be involved in their formation from rafts. Caveolae are observable by electron microscopy as flask-shaped indentations of the cell membrane. Proteins detected in caveolae include various components of the signal-transduction system (eg, the insulin receptor and some G proteins), the folate receptor, and endothelial nitric oxide synthase (eNOS). Caveolae and lipid rafts are active areas of research, and ideas concerning them and their possible roles in various diseases are rapidly evolving.

## MEMBRANE SELECTIVITY ALLOWS SPECIALIZED FUNCTIONS

If the plasma membrane is relatively impermeable, how do most molecules enter a cell? How is selectivity of this movement established? Answers to such questions are important in understanding how cells adjust to a constantly changing extracellular environment. Metazoan organisms also must have means of communicating between adjacent and distant cells, so that complex biologic processes can be coordinated. These signals must arrive at and be transmitted by the membrane, or they must be generated as a consequence of some interaction with the membrane. Some of the major mechanisms used to accomplish these different objectives are listed in Table 41–3.

### Passive Mechanisms Move Some Small Molecules Across Membranes

Molecules can passively traverse the bilayer down electrochemical gradients by **simple diffusion** or by **facilitated diffusion.** This spontaneous movement toward equilibrium contrasts with **active transport,** which requires energy because it constitutes movement against an electrochemical gradient. Figure 41–8 provides a schematic representation of these mechanisms.

As described above, some solutes such as gases can enter the cell by diffusing down an electrochemical gradient across the membrane and do not require metabolic energy. The simple **passive diffusion** of a solute across the membrane is limited by the thermal agitation of that specific molecule, by the concentration gradient across the membrane, and by the solubility of that solute (the permeability coefficient, Figure 41–6) in the hydrophobic core of the membrane bilayer. Solubility is inversely proportionate to the number of hydrogen bonds that must be broken in order for a solute in the external aqueous phase to become incorporated in the hydrophobic bilayer. Electrolytes, poorly soluble in lipid, do not form hydrogen bonds with water, but they do acquire a shell of water from hydration by electrostatic interaction. The size of the shell is directly proportionate to the charge density of the electrolyte. Electrolytes with a large charge density have a larger shell of hydration and thus a slower diffusion rate. $Na^+$, for example, has a higher charge density than $K^+$. Hydrated $Na^+$ is therefore larger than hydrated $K^+$; hence, the latter tends to move more easily through the membrane.

The following factors affect **net diffusion** of a substance: (1) Its concentration gradient across the membrane. Solutes move from high to low concentration. (2) The electrical potential across the membrane. Solutes move toward the solution that has the opposite charge. The inside of the cell usually has a negative charge. (3) The permeability coefficient of the substance for the membrane. (4) The hydrostatic pressure gradient across the membrane. Increased pressure will increase the rate and force of the collision between the molecules and the membrane. (5) Temperature. Increased temperature will increase particle motion and thus increase the frequency of collisions between external particles and the membrane. In addition, a multitude of channels exist in membranes that route the entry of ions into cells.

***Table 41–3.*** Transfer of material and information across membranes.

| |
|---|
| **Cross-membrane movement of small molecules** |
| Diffusion (passive and facilitated) |
| Active transport |
| **Cross-membrane movement of large molecules** |
| Endocytosis |
| Exocytosis |
| **Signal transmission across membranes** |
| Cell surface receptors |
| 1. Signal transduction (eg, glucagon → cAMP) |
| 2. Signal internalization (coupled with endocytosis, eg, the LDL receptor) |
| Movement to intracellular receptors (steroid hormones; a form of diffusion) |
| **Intercellular contact and communication** |

### Ion Channels Are Transmembrane Proteins That Allow the Selective Entry of Various Ions

In natural membranes, as opposed to synthetic membrane bilayers, there are transmembrane channels, pore-like structures composed of proteins that constitute selective **ion channels.** Cation-conductive channels have an average diameter of about 5–8 nm and are negatively charged within the channel. The permeability of a channel depends upon the size, the extent of hydration, and the extent of charge density on the ion. Specific channels for $Na^+$, $K^+$, $Ca^{2+}$, and $Cl^-$ have been identified; two such channels are illustrated in Figure 41–9. Both are seen to consist of four subunits. Each subunit consists of six $\alpha$-helical transmembrane domains. The amino and carboxyl terminals of both ion channels are located in the cytoplasm, with both extracellular and intracellular loops being present. The actual pores in the channels through which the ions pass are not shown in the figure. They form the center (diameter about 5–8 nm) of a structure formed by apposition of the subunits. The channels are very selective, in most cases permitting the passage of only one type of ion ($Na^+$, $Ca^{2+}$, etc). Many variations on the above structural themes are found, but

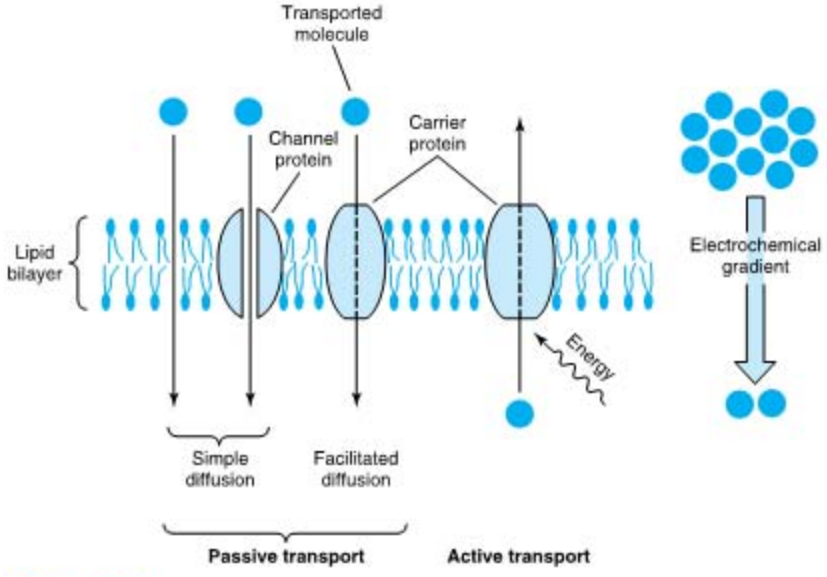


***Figure 41–8.*** Many small uncharged molecules pass freely through the lipid bilayer. Charged molecules, larger uncharged molecules, and some small uncharged molecules are transferred through channels or pores or by specific carrier proteins. Passive transport is always down an electrochemical gradient, toward equilibrium. Active transport is against an electrochemical gradient and requires an input of energy, whereas passive transport does not. (Redrawn and reproduced, with permission, from Alberts B et al: *Molecular Biology of the Cell.* Garland, 1983.)

all ion channels are basically made up of transmembrane subunits that come together to form a central pore through which ions pass selectively. The combination of x-ray crystallography (where possible) and site-directed mutagenesis affords a powerful approach to delineating the structure-function relationships of ion channels.

The membranes of **nerve cells** contain well-studied ion channels that are responsible for the action potentials generated across the membrane. The activity of some of these channels is controlled by neurotransmitters; hence, channel activity can be regulated. One ion can regulate the activity of the channel of another ion. For example, a decrease of $Ca^{2+}$ concentration in the extracellular fluid increases membrane permeability and increases the diffusion of $Na^{+}$. This depolarizes the membrane and triggers nerve discharge, which may explain the numbness, tingling, and muscle cramps symptomatic of a low level of plasma $Ca^{2+}$.

Channels are open transiently and thus are "gated." Gates can be controlled by opening or closing. In **ligand-gated channels,** a specific molecule binds to a receptor and opens the channel.**Voltage-gated channels** open (or close) in response to changes in membrane potential. Some properties of ion channels are listed in Table 41–4; other aspects of ion channels are discussed briefly in Chapter 49.

## Ionophores Are Molecules That Act as Membrane Shuttles for Various Ions

Certain microbes synthesize small organic molecules, **ionophores,** that function as shuttles for the movement of ions across membranes. These ionophores contain hydrophilic centers that bind specific ions and are surrounded by peripheral hydrophobic regions; this arrangement allows the molecules to dissolve effectively in the membrane and diffuse transversely therein. Others, like the well-studied polypeptide gramicidin, form channels.

Microbial toxins such as diphtheria toxin and activated serum complement components can produce large pores in cellular membranes and thereby provide macromolecules with direct access to the internal milieu.

## Aquaporins Are Proteins That Form Water Channels in Certain Membranes

In certain cells (eg, red cells, cells of the collecting ductules of the kidney), the movement of water by simple

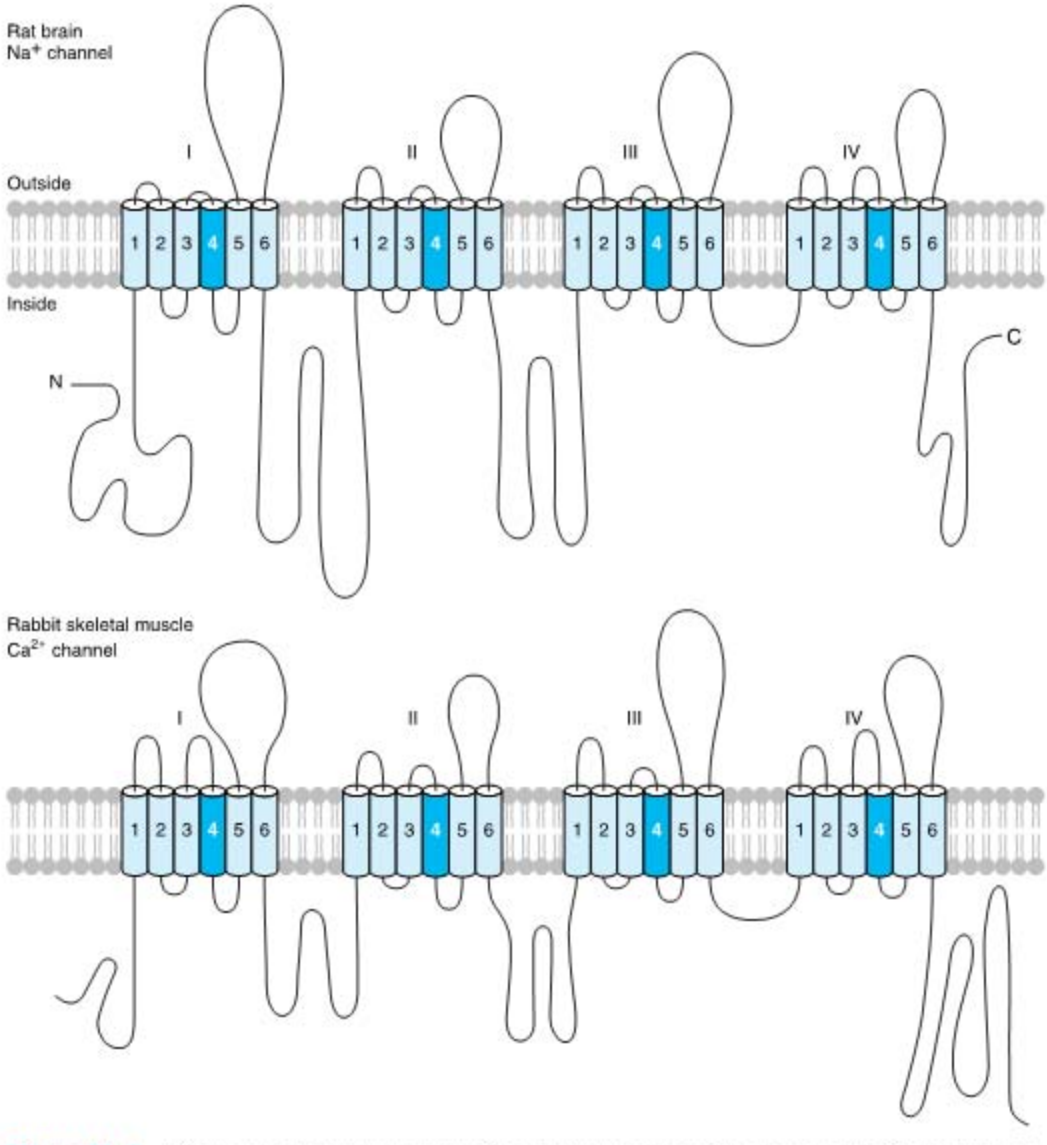


***Figure 41–9.*** Diagrammatic representation of the structures of two ion channels. The Roman numerals indicate the four subunits of each channel and the Arabic numerals the α-helical transmembrane domains of each subunit. The actual pores through which the ions pass are not shown but are formed by apposition of the various subunits. The specific areas of the subunits involved in the opening and closing of the channels are also not indicated. (After WK Catterall. Modified and reproduced from Hall ZW: *An Introduction to Molecular Neurobiology.* Sinauer, 1992.)

***Table 41–4.*** Some properties of ion channels.

- They are composed of transmembrane protein subunits.
- Most are highly selective for one ion; a few are nonselective.
- They allow impermeable ions to cross membranes at rates approaching diffusion limits.
- They can permit ion fluxes of $10^6$–$10^7$/s.
- Their activities are regulated.
- The two main types are voltage-gated and ligand-gated.
- They are usually highly conserved across species.
- Most cells have a variety of $Na^+$, $K^+$, $Ca^{2+}$, and $Cl^-$ channels.
- Mutations in genes encoding them can cause specific diseases.[1]
- Their activities are affected by certain drugs.

[1]Some diseases caused by mutations of ion channels are briefly discussed in Chapter 49.

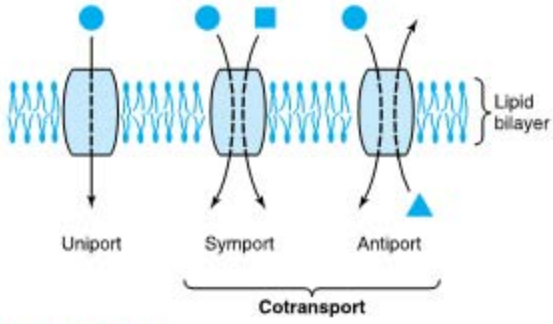


***Figure 41–10.*** Schematic representation of types of transport systems. Transporters can be classified with regard to the direction of movement and whether one or more unique molecules are moved. (Redrawn and reproduced, with permission, from Alberts B et al: *Molecular Biology of the Cell.* Garland, 1983.)

diffusion is augmented by movement through water channels. These channels are composed of tetrameric transmembrane proteins named aquaporins. At least five distinct aquaporins (AP-1 to AP-5) have been identified. Mutations in the gene encoding AP-2 have been shown to be the cause of one type of nephrogenic diabetes insipidus.

## PLASMA MEMBRANES ARE INVOLVED IN FACILITATED DIFFUSION, ACTIVE TRANSPORT, & OTHER PROCESSES

Transport systems can be described in a functional sense according to the number of molecules moved and the direction of movement (Figure 41–10) or according to whether movement is toward or away from equilibrium. A **uniport** system moves one type of molecule bidirectionally. In **cotransport** systems, the transfer of one solute depends upon the stoichiometric simultaneous or sequential transfer of another solute. A **symport** moves these solutes in the same direction. Examples are the proton-sugar transporter in bacteria and the $Na^+$ - sugar transporters (for glucose and certain other sugars) and $Na^+$-amino acid transporters in mammalian cells. **Antiport** systems move two molecules in opposite directions (eg, $Na^+$ in and $Ca^{2+}$ out).

Molecules that cannot pass freely through the lipid bilayer membrane by themselves do so in association with carrier proteins. This involves two processes—**facilitated diffusion** and **active transport**—and highly specific transport systems.

Facilitated diffusion and active transport share many features. Both appear to involve **carrier proteins,** and both show **specificity** for ions, sugars, and amino acids. Mutations in bacteria and mammalian cells (including some that result in human disease) have supported these conclusions. Facilitated diffusion and active transport **resemble a substrate-enzyme reaction** except that no covalent interaction occurs. These points of resemblance are as follows: (1) There is a specific binding site for the solute. (2) The carrier is saturable, so it has a maximum rate of transport ($V_{max}$; Figure 41–11). (3) There is a binding constant ($K_m$) for the solute, and

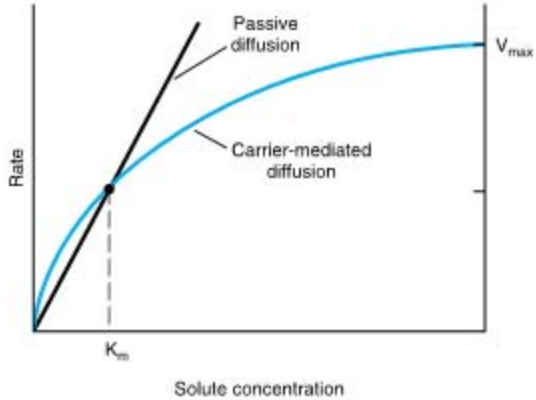


***Figure 41–11.*** A comparison of the kinetics of carrier-mediated (facilitated) diffusion with passive diffusion. The rate of movement in the latter is directly proportionate to solute concentration, whereas the process is saturable when carriers are involved. The concentration at half-maximal velocity is equal to the binding constant ($K_m$) of the carrier for the solute. ($V_{max}$ maximal rate.)

so the whole system has a $K_m$ (Figure 41–11). (4) Structurally similar competitive inhibitors block transport.

Major **differences** are the following: (1) Facilitated diffusion can operate bidirectionally, whereas active transport is usually unidirectional. (2) Active transport always occurs against an electrical or chemical gradient, and so it requires energy.

## Facilitated Diffusion

Some specific solutes diffuse down electrochemical gradients across membranes more rapidly than might be expected from their size, charge, or partition coefficients. This **facilitated diffusion** exhibits properties distinct from those of simple diffusion. The rate of facilitated diffusion, a uniport system, can be saturated; ie, the number of sites involved in diffusion of the specific solutes appears finite. Many facilitated diffusion systems are stereospecific but, like simple diffusion, require no metabolic energy.

As described earlier, the inside-outside asymmetry of membrane proteins is stable, and mobility of proteins across (rather than in) the membrane is rare; therefore, transverse mobility of specific carrier proteins is not likely to account for facilitated diffusion processes except in a few unusual cases.

A **"Ping-Pong" mechanism** (Figure 41–12) explains facilitated diffusion. In this model, the carrier protein exists in two principal conformations. In the "pong" state, it is exposed to high concentrations of solute, and molecules of the solute bind to specific sites on the carrier protein. Transport occurs when a conformational change exposes the carrier to a lower concentration of solute ("ping" state). This process is completely reversible, and net flux across the membrane depends upon the concentration gradient. The rate at which solutes enter a cell by facilitated diffusion is determined by the following factors: (1) The concentration gradient across the membrane. (2) The amount of carrier available (this is a key control step). (3) The rapidity of the solute-carrier interaction. (4) The rapidity of the conformational change for both the loaded and the unloaded carrier.

**Hormones** regulate facilitated diffusion by changing the number of transporters available. **Insulin** increases glucose transport in fat and muscle by recruiting transporters from an intracellular reservoir. Insulin also enhances amino acid transport in liver and other tissues. One of the coordinated actions of **glucocorticoid hormones** is to enhance transport of amino acids into liver, where the amino acids then serve as a substrate for gluconeogenesis. **Growth hormone** increases amino acid transport in all cells, and estrogens do this in the uterus. There are at least five different carrier systems for amino acids in animal cells. Each is specific for a group of closely related amino acids, and most operate as $Na^+$-symport systems (Figure 41–10).

## Active Transport

The process of active transport differs from diffusion in that molecules are transported away from thermodynamic equilibrium; hence, **energy is required.** This energy can come from the hydrolysis of **ATP,** from **electron movement,** or from **light.** The maintenance of electrochemical gradients in biologic systems is so important that it consumes perhaps 30–40% of the total energy expenditure in a cell.

In general, cells maintain a low intracellular $Na^+$ concentration and a high intracellular $K^+$ concentration (Table 41–1), along with a net negative electrical potential inside. The pump that maintains these gradients is an **ATPase** that is activated by $Na^+$ and $K^+$ ($Na^+$-$K^+$ ATPase; see Figure 41–13). The ATPase is an integral

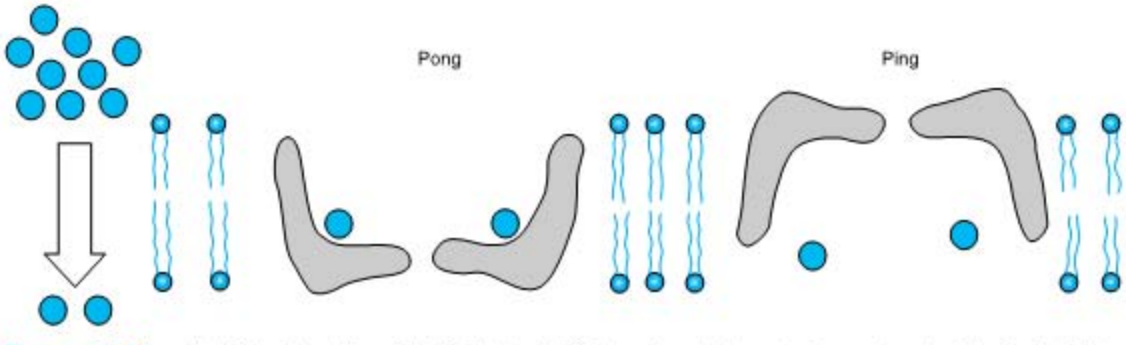


***Figure 41–12.*** The "Ping-Pong" model of facilitated diffusion. A protein carrier (gray structure) in the lipid bilayer associates with a solute in high concentration on one side of the membrane. A conformational change ensues ("pong" to "ping"), and the solute is discharged on the side favoring the new equilibrium. The empty carrier then reverts to the original conformation ("ping" to "pong") to complete the cycle.

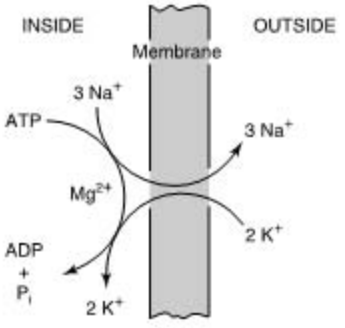


***Figure 41–13.*** Stoichiometry of the $Na^+$-$K^+$ ATPase pump. This pump moves three $Na^+$ ions from inside the cell to the outside and brings two $K^+$ ions from the outside to the inside for every molecule of ATP hydrolyzed to ADP by the membrane-associated ATPase. Ouabain and other cardiac glycosides inhibit this pump by acting on the extracellular surface of the membrane. (Courtesy of R Post.)

membrane protein and requires phospholipids for activity. The ATPase has catalytic centers for both ATP and $Na^+$ on the cytoplasmic side of the membrane, but the $K^+$ binding site is located on the extracellular side of the membrane. **Ouabain** or **digitalis** inhibits this ATPase by binding to the extracellular domain. Inhibition of the ATPase by ouabain can be antagonized by extracellular $K^+$.

## Nerve Impulses Are Transmitted Up & Down Membranes

The membrane forming the surface of **neuronal cells** maintains an asymmetry of inside-outside voltage (electrical potential) and is electrically excitable. When appropriately stimulated by a chemical signal mediated by a specific synaptic membrane receptor (see discussion of the transmission of biochemical signals, below), gates in the membrane are opened to allow the rapid influx of $Na^+$ or $Ca^{2+}$ (with or without the efflux of $K^+$), so that the voltage difference rapidly collapses and that segment of the membrane is depolarized. However, as a result of the action of the ion pumps in the membrane, the gradient is quickly restored.

When large areas of the membrane are **depolarized** in this manner, the electrochemical disturbance propagates in wave-like form down the membrane, generating a **nerve impulse. Myelin sheets,** formed by Schwann cells, wrap around nerve fibers and provide an electrical insulator that surrounds most of the nerve and greatly speeds up the propagation of the wave (signal) by allowing ions to flow in and out of the membrane only where the membrane is free of the insulation. The myelin membrane is composed of phospholipids, cholesterol, proteins, and GSLs. Relatively few proteins are found in the myelin membrane; those present appear to hold together multiple membrane bilayers to form the hydrophobic insulating structure that is impermeable to ions and water. Certain diseases, eg, multiple sclerosis and the Guillain-Barré syndrome, are characterized by demyelination and impaired nerve conduction.

## Glucose Transport Involves Several Mechanisms

A discussion of the **transport of glucose** summarizes many of the points made in this chapter. Glucose must enter cells as the first step in energy utilization. In adipocytes and muscle, glucose enters by a specific transport system that is enhanced by insulin. Changes in transport are primarily due to alterations of $V_{max}$ (presumably from more or fewer active transporters), but changes in $K_m$ may also be involved. Glucose transport involves different aspects of the principles of transport discussed above. Glucose and $Na^+$ bind to different sites on the glucose transporter. $Na^+$ moves into the cell down its electrochemical gradient and "drags" glucose with it (Figure 41–14). Therefore, the greater the $Na^+$ gradient, the more glucose enters; and if $Na^+$ in extracellular fluid is low, glucose transport stops. To maintain a steep $Na^+$ gradient, this $Na^+$-glucose symport is dependent on gradients generated by an $Na^+$-$K^+$ pump that maintains a low intracellular $Na^+$ concentration. Similar mechanisms are used to transport other sugars as well as amino acids.

The transcellular movement of sugars involves one additional component: a uniport that allows the glucose accumulated within the cell to move across a different surface toward a new equilibrium; this occurs in intestinal and renal cells, for example.

## Cells Transport Certain Macromolecules Across the Plasma Membrane

The process by which cells take up large molecules is called **"endocytosis."** Some of these molecules (eg, polysaccharides, proteins, and polynucleotides), when hydrolyzed inside the cell, yield nutrients. Endocytosis provides a mechanism for regulating the content of certain membrane components, hormone receptors being a case in point. Endocytosis can be used to learn more about how cells function. DNA from one cell type can be used to transfect a different cell and alter the latter's function or phenotype. A specific gene is often employed in these experiments, and this provides a unique way to study and analyze the regulation of that gene. DNA transfection depends upon endocytosis; endocy-

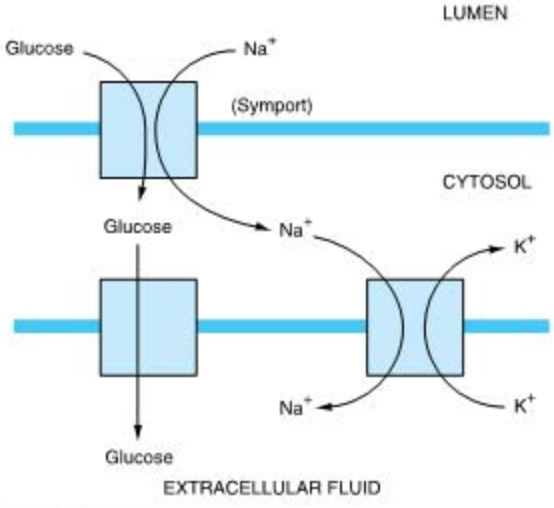


***Figure 41–14.*** The transcellular movement of glucose in an intestinal cell. Glucose follows $Na^+$ across the luminal epithelial membrane. The $Na^+$ gradient that drives this symport is established by $Na^+$ -$K^+$ exchange, which occurs at the basal membrane facing the extracellular fluid compartment. Glucose at high concentration within the cell moves "downhill" into the extracellular fluid by facilitated diffusion (a uniport mechanism).

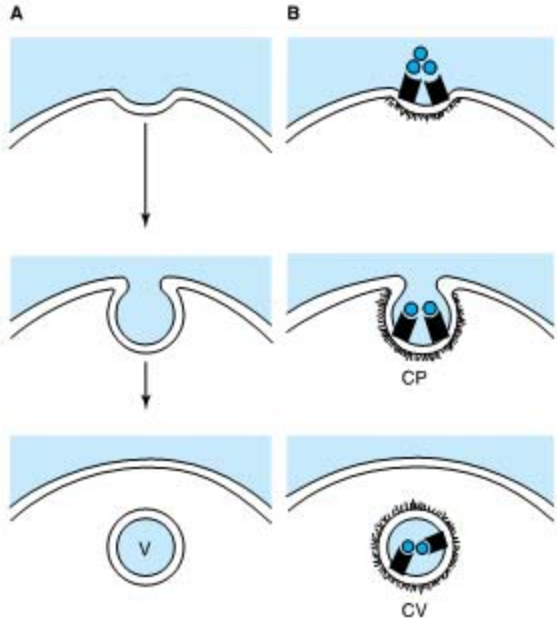


***Figure 41–15.*** Two types of endocytosis. An endocytotic vesicle (V) forms as a result of invagination of a portion of the plasma membrane. Fluid-phase endocytosis **(A)** is random and nondirected. Receptor-mediated endocytosis **(B)** is selective and occurs in coated pits (CP) lined with the protein clathrin (the fuzzy material). Targeting is provided by receptors (black symbols) specific for a variety of molecules. This results in the formation of a coated vesicle (CV).

tosis is responsible for the entry of DNA into the cell. Such experiments commonly use calcium phosphate, since $Ca^{2+}$ stimulates endocytosis and precipitates DNA, which makes the DNA a better object for endocytosis. Cells also release macromolecules by **exocytosis.** Endocytosis and exocytosis both involve vesicle formation with or from the plasma membrane.

## A. Endocytosis

All eukaryotic cells are continuously ingesting parts of their plasma membranes. Endocytotic vesicles are generated when segments of the plasma membrane invaginate, enclosing a minute volume of extracellular fluid and its contents. The vesicle then pinches off as the fusion of plasma membranes seals the neck of the vesicle at the original site of invagination (Figure 41–15). This vesicle fuses with other membrane structures and thus achieves the transport of its contents to other cellular compartments or even back to the cell exterior. Most endocytotic vesicles fuse with primary lysosomes to form secondary lysosomes, which contain hydrolytic enzymes and are therefore specialized organelles for intracellular disposal. The macromolecular contents are digested to yield amino acids, simple sugars, or nucleotides, and they diffuse out of the vesicles to be reused in the cytoplasm. Endocytosis requires (1) energy, usually from the hydrolysis of ATP; (2) $Ca^{2+}$ in extracellular fluid; and (3) contractile elements in the cell (probably the microfilament system) (Chapter 49).

There are two general types of endocytosis. **Phagocytosis** occurs only in specialized cells such as macrophages and granulocytes. Phagocytosis involves the ingestion of large particles such as viruses, bacteria, cells, or debris. Macrophages are extremely active in this regard and may ingest 25% of their volume per hour. In so doing, a macrophage may internalize 3% of its plasma membrane each minute or the entire membrane every 30 minutes.

**Pinocytosis** is a property of all cells and leads to the cellular uptake of fluid and fluid contents. There are two types. **Fluid-phase pinocytosis** is a nonselective process in which the uptake of a solute by formation of small vesicles is simply proportionate to its concentration in the surrounding extracellular fluid. The formation of these vesicles is an extremely active process. Fi-

broblasts, for example, internalize their plasma membrane at about one-third the rate of macrophages. This process occurs more rapidly than membranes are made. The surface area and volume of a cell do not change much, so membranes must be replaced by exocytosis or by being recycled as fast as they are removed by endocytosis.

The other type of pinocytosis, **absorptive pinocytosis,** is a receptor-mediated selective process primarily responsible for the uptake of macromolecules for which there are a finite number of binding sites on the plasma membrane. These high-affinity receptors permit the selective concentration of ligands from the medium, minimize the uptake of fluid or soluble unbound macromolecules, and markedly increase the rate at which specific molecules enter the cell. The vesicles formed during absorptive pinocytosis are derived from invaginations (pits) that are coated on the cytoplasmic side with a filamentous material. In many systems, the protein **clathrin** is the filamentous material. It has a three-limbed structure (called a triskelion), with each limb being made up of one light and one heavy chain of clathrin. The polymerization of clathrin into a vesicle is directed by **assembly particles**, composed of four **adapter proteins.** These interact with certain amino acid sequences in the receptors that become cargo, ensuring selectivity of uptake. The lipid **$PIP_2$** also plays an important role in vesicle assembly. In addition, the protein **dynamin**, which both binds and hydrolyzes GTP, is necessary for the pinching off of clathrin-coated vesicles from the cell surface. Coated pits may constitute as much as 2% of the surface of some cells.

As an example, the low-density lipoprotein (LDL) molecule and its receptor (Chapter 25) are internalized by means of coated pits containing the LDL receptor. These endocytotic vesicles containing LDL and its receptor fuse to lysosomes in the cell. The receptor is released and recycled back to the cell surface membrane, but the apoprotein of LDL is degraded and the cholesteryl esters metabolized. Synthesis of the LDL receptor is regulated by secondary or tertiary consequences of pinocytosis, eg, by metabolic products—such as cholesterol—released during the degradation of LDL. Disorders of the LDL receptor and its internalization are medically important and are discussed in Chapter 25.

Absorptive pinocytosis of **extracellular glycoproteins** requires that the glycoproteins carry specific carbohydrate recognition signals. These recognition signals are bound by membrane receptor molecules, which play a role analogous to that of the LDL receptor. A galactosyl receptor on the surface of hepatocytes is instrumental in the absorptive pinocytosis of asialoglycoproteins from the circulation (Chapter 47). Acid hydrolases taken up by absorptive pinocytosis in fibroblasts are recognized by their mannose 6-phosphate moieties. Interestingly, the mannose 6-phosphate moiety also seems to play an important role in the intracellular targeting of the acid hydrolases to the lysosomes of the cells in which they are synthesized (Chapter 47).

There is a **dark side** to receptor-mediated endocytosis in that viruses which cause such diseases as hepatitis (affecting liver cells), poliomyelitis (affecting motor neurons), and AIDS (affecting T cells) initiate their damage by this mechanism. Iron toxicity also begins with excessive uptake due to endocytosis.

## B. Exocytosis

Most cells release macromolecules to the exterior by exocytosis. This process is also involved in membrane remodeling, when the components synthesized in the Golgi apparatus are carried in vesicles to the plasma membrane. The signal for exocytosis is often a hormone which, when it binds to a cell-surface receptor, induces a local and transient change in $Ca^{2+}$ concentration. $Ca^{2+}$ triggers exocytosis. Figure 41–16 provides a comparison of the mechanisms of exocytosis and endocytosis.

Molecules released by exocytosis fall into three categories: (1) They can attach to the cell surface and become peripheral proteins, eg, antigens. (2) They can become part of the extracellular matrix, eg, collagen and glycosaminoglycans. (3) They can enter extracellular fluid and signal other cells. Insulin, parathyroid hormone, and the catecholamines are all packaged in gran-


***Figure 41–16.*** A comparison of the mechanisms of endocytosis and exocytosis. Exocytosis involves the contact of two inside surface (cytoplasmic side) monolayers, whereas endocytosis results from the contact of two outer surface monolayers.

ules and processed within cells, to be released upon appropriate stimulation.

## Some Signals Are Transmitted Across Membranes

Specific biochemical signals such as neurotransmitters, hormones, and immunoglobulins bind to specific **receptors** (integral proteins) exposed to the outside of cellular membranes and transmit information through these membranes to the cytoplasm. This process, called **transmembrane signaling**, involves the generation of a number of signals, including cyclic nucleotides, calcium, phosphoinositides, and diacylglycerol. It is discussed in detail in Chapter 43.

## Information Can Be Communicated by Intercellular Contact

There are many areas of intercellular contact in a metazoan organism. This necessitates contact between the plasma membranes of the individual cells. Cells have developed specialized regions on their membranes for intercellular communication in close proximity. **Gap junctions** mediate and regulate the passage of ions and small molecules (up to 1000–2000 MW) through a narrow hydrophilic core connecting the cytosol of adjacent cells. These structures are primarily composed of the protein **connexin,** which contains four membrane-spanning α helices. About a dozen genes encoding different connexins have been cloned. An assembly of 12 connexin molecules forms a structure (a connexon) with a central channel that forms bridges between adjacent cells. Ions and small molecules pass from the cytosol of one cell to that of another through the channels, which open and close in a regulated fashion.

# MUTATIONS AFFECTING MEMBRANE PROTEINS CAUSE DISEASES

In view of the fact that membranes are located in so many organelles and are involved in so many processes, it is not surprising that **mutations** affecting their protein constituents should result in many diseases or disorders. Proteins in membranes can be classified as **receptors, transporters, ion channels, enzymes,** and **structural components.** Members of all of these classes are often **glycosylated,** so that mutations affecting this process may alter their function. Examples of diseases or disorders due to abnormalities in membrane proteins are listed in Table 41–5; these mainly reflect mutations in proteins of the **plasma membrane,** with one affecting lysosomal function (I-cell disease). Over 30 genetic diseases or disorders have been ascribed to mutations affecting various proteins involved in the transport of amino acids, sugars, lipids, urate, anions, cations, water, and vitamins across the plasma membrane. Mutations in genes encoding proteins in other membranes can also have harmful consequences. For example, mutations in genes encoding **mitochondrial membrane proteins** involved in oxidative phosphorylation can cause neurologic and other problems (eg, Leber's hereditary optic neuropathy; LHON). Membrane proteins can also be affected by conditions other than mutations. Formation of **autoantibodies** to the acetylcholine receptor in skeletal muscle causes myasthenia gravis. **Ischemia** can quickly affect the integrity of various ion channels in membranes. Abnormalities of membrane constituents other than proteins can also be harmful. With regard to **lipids,** excess of cholesterol (eg, in familial hypercholesterolemia), of lysophospholipid (eg, after bites by certain snakes, whose venom contains phospholipases), or of glycosphingolipids (eg, in a sphingolipidosis) can all affect membrane function.

## Cystic Fibrosis Is Due to Mutations in the Gene Encoding a Chloride Channel

Cystic fibrosis (CF) is a recessive genetic disorder prevalent among whites in North America and certain parts of northern Europe. It is characterized by chronic bacterial infections of the airways and sinuses, fat maldigestion due to pancreatic exocrine insufficiency, infertility in males due to abnormal development of the vas deferens, and elevated levels of chloride in sweat (> 60 mmol/L).

After a Herculean landmark endeavor, the gene for CF was identified in 1989 on chromosome 7. It was found to encode a protein of 1480 amino acids, named cystic fibrosis transmembrane regulator (CFTR), a cyclic AMP-regulated $Cl^-$ channel (see Figure 41–17). An abnormality of membrane $Cl^-$ permeability is believed to result in the increased viscosity of many bodily secretions, though the precise mechanisms are still under investigation. The commonest mutation (~70% in certain Caucasian populations) is deletion of three bases, resulting in loss of residue 508, a phenylalanine ($\Delta F_{508}$). However, more than 900 other mutations have been identified. These mutations affect CFTR in at least four ways: (1) its amount is reduced; (2) depending upon the particular mutation, it may be susceptible to misfolding and retention within the ER or Golgi apparatus; (3) mutations in the nucleotide-binding domains may affect the ability of the $Cl^-$ channel to open, an event affected by ATP; (4) the mutations may also reduce the rate of ion flow through a channel, generating less of a $Cl^-$ current.

The most serious and life-threatening complication is recurrent pulmonary infections due to overgrowth of various pathogens in the viscous secretions of the respi-

***Table 41–5.*** Some diseases or pathologic states resulting from or attributed to abnormalities of membranes.[1]

| Disease | Abnormality |
|---|---|
| Achondroplasia (MIM 100800) | Mutations in the gene encoding the fibroblast growth factor receptor 3 |
| Familial hypercholesterolemia (MIM 143890) | Mutations in the gene encoding the LDL receptor |
| Cystic fibrosis (MIM 219700) | Mutations in the gene encoding the CFTR protein, a $Cl^-$ transporter |
| Congenital long QT syndrome (MIM 192500) | Mutations in genes encoding ion channels in the heart |
| Wilson disease (MIM 277900) | Mutations in the gene encoding a copper-dependent ATPase |
| I-cell disease (MIM 252500) | Mutations in the gene encoding GlcNAc phosphotransferase, resulting in absence of the Man 6-P signal for lysosomal localization of certain hydrolases |
| Hereditary spherocytosis (MIM 182900) | Mutations in the genes encoding spectrin or other structural proteins in the red cell membrane |
| Metastasis | Abnormalities in the oligosaccharide chains of membrane glycoproteins and glycolipids are thought to be of importance |
| Paroxysmal nocturnal hemoglobinuria (MIM 311770) | Mutation resulting in deficient attachment of the GPI anchor to certain proteins of the red cell membrane |

[1]The disorders listed are discussed further in other chapters. The table lists examples of mutations affecting receptors, a transporter, an ion channel, an enzyme, and a structural protein. Examples of altered or defective glycosylation of glycoproteins are also presented. Most of the conditions listed affect the plasma membrane.

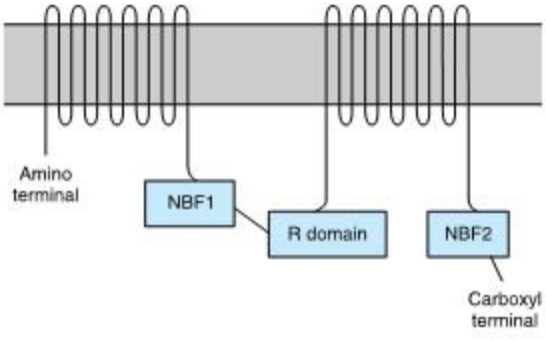


***Figure 41–17.*** Diagram of the structure of the CFTR protein (not to scale). The protein contains twelve transmembrane segments (probably helical), two nucleotide-binding folds or domains (NBF1 and NBF2), and one regulatory (R) domain. NBF1 and NBF2 probably bind ATP and couple its hydrolysis to transport of $Cl^-$. Phe 508, the major locus of mutations in cystic fibrosis, is located in NBF1.

ratory tract. Poor nutrition as a result of pancreatic insufficiency worsens the situation. The treatment of CF thus requires a comprehensive effort to maintain nutritional status, to prevent and combat pulmonary infections, and to maintain physical and psychologic health. Advances in molecular genetics mean that mutation analysis can be performed for prenatal diagnosis and for carrier testing in families in which one child already has the condition. Efforts are in progress to use gene therapy to restore the activity of CFTR. An aerosolized preparation of human DNase that digests the DNA of microorganisms in the respiratory tract has proved helpful in therapy.

## SUMMARY

- Membranes are complex structures composed of lipids, carbohydrates, and proteins.
- The basic structure of all membranes is the lipid bilayer. This bilayer is formed by two sheets of phospholipids in which the hydrophilic polar head groups

are directed away from each other and are exposed to the aqueous environment on the outer and inner surfaces of the membrane. The hydrophobic nonpolar tails of these molecules are oriented toward each other, in the direction of the center of the membrane.

- Membrane proteins are classified as integral if they are firmly embedded in the bilayer and as peripheral if they are loosely attached to the outer or inner surface.
- The 20 or so different membranes in a mammalian cell have intrinsic functions (eg, enzymatic activity), and they define compartments, or specialized environments, within the cell that have specific functions (eg, lysosomes).
- Certain molecules freely diffuse across membranes, but the movement of others is restricted because of size, charge, or solubility.
- Various passive and active mechanisms are employed to maintain gradients of such molecules across different membranes.
- Certain solutes, eg, glucose, enter cells by facilitated diffusion, along a downhill gradient from high to low concentration. Specific carrier molecules, or transporters, are involved in such processes.
- Ligand- or voltage-gated ion channels are often employed to move charged molecules ($Na^+$, $K^+$, $Ca^{2+}$, etc) across membranes.
- Large molecules can enter or leave cells through mechanisms such as endocytosis or exocytosis. These processes often require binding of the molecule to a receptor, which affords specificity to the process.
- Receptors may be integral components of membranes (particularly the plasma membrane). The interaction of a ligand with its receptor may not involve the movement of either into the cell, but the interaction results in the generation of a signal that influences intracellular processes (transmembrane signaling).
- Mutations that affect the structure of membrane proteins (receptors, transporters, ion channels, enzymes, and structural proteins) may cause diseases; examples include cystic fibrosis and familial hypercholesterolemia.

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# The Diversity of the Endocrine System

42

*Daryl K. Granner, MD*

| | |
|---|---|
| **ACTH** | Adrenocorticotropic hormone |
| **ANF** | Atrial natriuretic factor |
| **cAMP** | Cyclic adenosine monophosphate |
| **CBG** | Corticosteroid-binding globulin |
| **CG** | Chorionic gonadotropin |
| **cGMP** | Cyclic guanosine monophosphate |
| **CLIP** | Corticotropin-like intermediate lobe peptide |
| **DBH** | Dopamine β-hydroxylase |
| **DHEA** | Dehydroepiandrosterone |
| **DHT** | Dihydrotestosterone |
| **DIT** | Diiodotyrosine |
| **DOC** | Deoxycorticosterone |
| **EGF** | Epidermal growth factor |
| **FSH** | Follicle-stimulating hormone |
| **GH** | Growth hormone |
| **IGF-I** | Insulin-like growth factor-I |
| **LH** | Luteotropic hormone |
| **LPH** | Lipotropin |
| **MIT** | Monoiodotyrosine |
| **MSH** | Melanocyte-stimulating hormone |
| **OHSD** | Hydroxysteroid dehydrogenase |
| **PNMT** | Phenylethanolamine-*N*-methyltransferase |
| **POMC** | Pro-opiomelanocortin |
| **SHBG** | Sex hormone-binding globulin |
| **StAR** | Steroidogenic acute regulatory (protein) |
| **TBG** | Thyroxine-binding globulin |
| **TEBG** | Testosterone-estrogen-binding globulin |
| **TRH** | Thyrotropin-releasing hormone |
| **TSH** | Thyrotropin-stimulating hormone |

## BIOMEDICAL IMPORTANCE

The survival of multicellular organisms depends on their ability to adapt to a constantly changing environment. Intercellular communication mechanisms are necessary requirements for this adaptation. The nervous system and the endocrine system provide this intercellular, organism-wide communication. The nervous system was originally viewed as providing a fixed communication system, whereas the endocrine system supplied hormones, which are mobile messages. In fact, there is a remarkable convergence of these regulatory systems. For example, neural regulation of the endocrine system is important in the production and secretion of some hormones; many neurotransmitters resemble hormones in their synthesis, transport, and mechanism of action; and many hormones are synthesized in the nervous system. The word "hormone" is derived from a Greek term that means to arouse to activity. As classically defined, a hormone is a substance that is synthesized in one organ and transported by the circulatory system to act on another tissue. However, this original description is too restrictive because hormones can act on adjacent cells (paracrine action) and on the cell in which they were synthesized (autocrine action) without entering the systemic circulation. A diverse array of hormones—each with distinctive mechanisms of action and properties of biosynthesis, storage, secretion, transport, and metabolism—has evolved to provide homeostatic responses. This biochemical diversity is the topic of this chapter.

## THE TARGET CELL CONCEPT

There are about 200 types of differentiated cells in humans. Only a few produce hormones, but virtually all of the 75 trillion cells in a human are targets of one or more of the over 50 known hormones. The concept of the target cell is a useful way of looking at hormone action. It was thought that hormones affected a single cell type—or only a few kinds of cells—and that a hormone elicited a unique biochemical or physiologic action. We now know that a given hormone can affect several different cell types; that more than one hormone can affect a given cell type; and that hormones can exert many dif-

ferent effects in one cell or in different cells. With the discovery of specific cell-surface and intracellular hormone receptors, the definition of a target has been expanded to include any cell in which the hormone (ligand) binds to its receptor, whether or not a biochemical or physiologic response has yet been determined.

Several factors determine the response of a target cell to a hormone. These can be thought of in two general ways: (1) as factors that affect the concentration of the hormone at the target cell (see Table 42–1) and (2) as factors that affect the actual response of the target cell to the hormone (see Table 42–2).

## HORMONE RECEPTORS ARE OF CENTRAL IMPORTANCE

### Receptors Discriminate Precisely

One of the major challenges faced in making the hormone-based communication system work is illustrated in Figure 42–1. Hormones are present at very low concentrations in the extracellular fluid, generally in the range of $10^{-15}$ to $10^{-9}$ mol/L. This concentration is much lower than that of the many structurally similar molecules (sterols, amino acids, peptides, proteins) and other molecules that circulate at concentrations in the $10^{-5}$ to $10^{-3}$ mol/L range. Target cells, therefore, must distinguish not only between different hormones present in small amounts but also between a given hormone and the $10^{6}$- to $10^{9}$-fold excess of other similar molecules. This high degree of discrimination is provided by cell-associated recognition molecules called receptors. Hormones initiate their biologic effects by binding to specific receptors, and since any effective control system also must provide a means of stopping a response, hormone-induced actions generally terminate when the effector dissociates from the receptor.

A target cell is defined by its ability to selectively bind a given hormone to its cognate receptor. Several biochemical features of this interaction are important in order for hormone-receptor interactions to be physiologically relevant: (1) binding should be specific, ie, displaceable by agonist or antagonist; (2) binding should be saturable; and (3) binding should occur within the concentration range of the expected biologic response.

***Table 42–1.*** Determinants of the concentration of a hormone at the target cell.

| |
|---|
| The rate of synthesis and secretion of the hormones. |
| The proximity of the target cell to the hormone source (dilution effect). |
| The dissociation constants of the hormone with specific plasma transport proteins (if any). |
| The conversion of inactive or suboptimally active forms of the hormone into the fully active form. |
| The rate of clearance from plasma by other tissues or by digestion, metabolism, or excretion. |

***Table 42–2.*** Determinants of the target cell response.

| |
|---|
| The number, relative activity, and state of occupancy of the specific receptors on the plasma membrane or in the cytoplasm or nucleus. |
| The metabolism (activation or inactivation) of the hormone in the target cell. |
| The presence of other factors within the cell that are necessary for the hormone response. |
| Up- or down-regulation of the receptor consequent to the interaction with the ligand. |
| Postreceptor desensitzation of the cell, including down-regulation of the receptor. |

### Both Recognition & Coupling Domains Occur on Receptors

All receptors have at least two functional domains. A recognition domain binds the hormone ligand and a second region generates a signal that couples hormone recognition to some intracellular function. Coupling (signal transduction) occurs in two general ways. Polypeptide and protein hormones and the catecholamines bind to receptors located in the plasma membrane and thereby generate a signal that regulates various intracellular functions, often by changing the activity of an enzyme. In contrast, steroid, retinoid, and thyroid hormones interact with intracellular receptors, and it is this ligand-receptor complex that directly provides the signal, generally to specific genes whose rate of transcription is thereby affected.

The domains responsible for hormone recognition and signal generation have been identified in the protein polypeptide and catecholamine hormone receptors. Steroid, thyroid, and retinoid hormone receptors have several functional domains: one site binds the hormone; another binds to specific DNA regions; a third is involved in the interaction with other coregulator proteins that result in the activation (or repression) of gene transcription; and a fourth may specify binding to one or more other proteins that influence the intracellular trafficking of the receptor.

The dual functions of binding and coupling ultimately define a receptor, and it is the coupling of hormone binding to signal transduction—so-called **receptor-effector coupling**—that provides the first step in amplification of the hormonal response. This dual purpose also distinguishes the target cell receptor from the

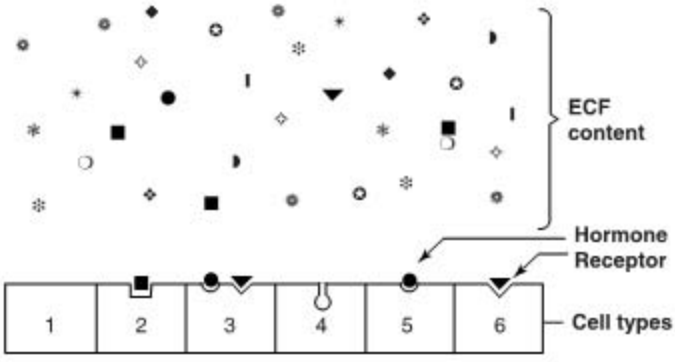


***Figure 42–1.*** Specificity and selectivity of hormone receptors. Many different molecules circulate in the extracellular fluid (ECF), but only a few are recognized by hormone receptors. Receptors must select these molecules from among high concentrations of the other molecules. This simplified drawing shows that a cell may have no hormone receptors (1), have one receptor (2+5+6), have receptors for several hormones (3), or have a receptor but no hormone in the vicinity (4).

plasma carrier proteins that bind hormone but do not generate a signal (see Table 42–6).

### Receptors Are Proteins

Several classes of peptide hormone receptors have been defined. For example, the insulin receptor is a heterotetramer ($\alpha_2\beta_2$) linked by multiple disulfide bonds in which the extracellular $\alpha$ subunit binds insulin and the membrane-spanning $\beta$ subunit transduces the signal through the tyrosine protein kinase domain located in the cytoplasmic portion of this polypeptide. The receptors for insulin-like growth factor I (IGF-I) and epidermal growth factor (EGF) are generally similar in structure to the insulin receptor. The growth hormone and prolactin receptors also span the plasma membrane of target cells but do not contain intrinsic protein kinase activity. Ligand binding to these receptors, however, results in the association and activation of a completely different protein kinase pathway, the Jak-Stat pathway. Polypeptide hormone and catecholamine receptors, which transduce signals by altering the rate of production of cAMP through G-proteins, are characterized by the presence of seven domains that span the plasma membrane. Protein kinase activation and the generation of cyclic AMP, (cAMP, 3′5′-adenylic acid; see Figure 20–5) is a downstream action of this class of receptor (see Chapter 43 for further details).

A comparison of several different steroid receptors with thyroid hormone receptors revealed a remarkable conservation of the amino acid sequence in certain regions, particularly in the DNA-binding domains. This led to the realization that receptors of the steroid or thyroid type are members of a large superfamily of nuclear receptors. Many related members of this family have no known ligand at present and thus are called orphan receptors. The nuclear receptor superfamily plays a critical role in the regulation of gene transcription by hormones, as described in Chapter 43.

## HORMONES CAN BE CLASSIFIED IN SEVERAL WAYS

Hormones can be classified according to chemical composition, solubility properties, location of receptors, and the nature of the signal used to mediate hormonal action within the cell. A classification based on the last two properties is illustrated in Table 42–3, and general features of each group are illustrated in Table 42–4.

The hormones in group I are lipophilic. After secretion, these hormones associate with plasma transport or carrier proteins, a process that circumvents the problem of solubility while prolonging the plasma half-life of the hormone. The relative percentages of bound and free hormone are determined by the binding affinity and binding capacity of the transport protein. The free hormone, which is the biologically active form, readily traverses the lipophilic plasma membrane of all cells and encounters receptors in either the cytosol or nucleus of target cells. The ligand-receptor complex is assumed to be the intracellular messenger in this group.

The second major group consists of water-soluble hormones that bind to the plasma membrane of the target cell. Hormones that bind to the surfaces of cells communicate with intracellular metabolic processes through intermediary molecules called **second messengers** (the hormone itself is the first messenger), which are generated as a consequence of the ligand-receptor interaction. The second messenger concept arose from an observation that epinephrine binds to the plasma membrane of certain cells and increases intracellular cAMP. This was followed by a series of experiments in which cAMP was found to mediate the effects of many hormones. Hormones that clearly employ this mechanism are shown in group II.A of Table 42–3. To date, only one hormone, atrial natriuretic factor (ANF), uses cGMP as its second messenger, but other hormones will probably be added to group II.B. Several hormones, many of which were previously thought to affect cAMP, appear to use ionic calcium ($Ca^{2+}$) or

***Table 42–3.*** Classification of hormones by mechanism of action.

| |
|---|
| ***I. Hormones that bind to intracellular receptors*** |
| Androgens |
| Calcitriol ($1,25[OH]_2$-$D_3$) |
| Estrogens |
| Glucocorticoids |
| Mineralocorticoids |
| Progestins |
| Retinoic acid |
| Thyroid hormones ($T_3$ and $T_4$) |
| ***II. Hormones that bind to cell surface receptors*** |
| **A. The second messenger is cAMP:** |
| $\alpha_2$-Adrenergic catecholamines |
| β-Adrenergic catecholamines |
| Adrenocorticotropic hormone |
| Antidiuretic hormone |
| Calcitonin |
| Chorionic gonadotropin, human |
| Corticotropin-releasing hormone |
| Follicle-stimulating hormone |
| Glucagon |
| Lipotropin |
| Luteinizing hormone |
| Melanocyte-stimulating hormone |
| Parathyroid hormone |
| Somatostatin |
| Thyroid-stimulating hormone |
| **B. The second messenger is cGMP:** |
| Atrial natriuretic factor |
| Nitric oxide |
| **C. The second messenger is calcium or phosphatidylinositols (or both):** |
| Acetylcholine (muscarinic) |
| $\alpha_1$-Adrenergic catecholamines |
| Angiotensin II |
| Antidiuretic hormone (vasopressin) |
| Cholecystokinin |
| Gastrin |
| Gonadotropin-releasing hormone |
| Oxytocin |
| Platelet-derived growth factor |
| Substance P |
| Thyrotropin-releasing hormone |
| **D. The second messenger is a kinase or phosphatase cascade:** |
| Chorionic somatomammotropin |
| Epidermal growth factor |
| Erythropoietin |
| Fibroblast growth factor |
| Growth hormone |
| Insulin |
| Insulin-like growth factors I and II |
| Nerve growth factor |
| Platelet-derived growth factor |
| Prolactin |

***Table 42–4.*** General features of hormone classes.

| | Group I | Group II |
|---|---|---|
| Types | Steroids, iodothyronines, calcitriol, retinoids | Polypeptides, proteins, glycoproteins, catecholamines |
| Solubility | Lipophilic | Hydrophilic |
| Transport proteins | Yes | No |
| Plasma half-life | Long (hours to days) | Short (minutes) |
| Receptor | Intracellular | Plasma membrane |
| Mediator | Receptor-hormone complex | cAMP, cGMP, $Ca^{2+}$, metabolites of complex phosphoinositols, kinase cascades |

metabolites of complex phosphoinositides (or both) as the intracellular signal. These are shown in group II.C of the table. The intracellular messenger for group II.D is a protein kinase-phosphatase cascade. Several of these have been identified, and a given hormone may use more than one kinase cascade. A few hormones fit into more than one category, and assignments change as new information is brought forward.

## DIVERSITY OF THE ENDOCRINE SYSTEM

### Hormones Are Synthesized in a Variety of Cellular Arrangements

Hormones are synthesized in discrete organs designed solely for this specific purpose, such as the thyroid (triiodothyronine), adrenal (glucocorticoids and mineralocorticoids), and the pituitary (TSH, FSH, LH, growth hormone, prolactin, ACTH). Some organs are designed to perform two distinct but closely related functions. For example, the ovaries produce mature oocytes and the reproductive hormones estradiol and progesterone. The testes produce mature spermatozoa and testosterone. Hormones are also produced in specialized cells within other organs such as the small intestine (glucagon-like peptide), thyroid (calcitonin), and kidney (angiotensin II). Finally, the synthesis of some hormones requires the parenchymal cells of more than one organ—eg, the skin, liver, and kidney are required for the production of $1,25(OH)_2$-$D_3$ (calcitriol). Examples of this diversity in the approach to hormone synthesis, each of which has evolved to fulfill a specific purpose, are discussed below.

## Hormones Are Chemically Diverse

Hormones are synthesized from a wide variety of chemical building blocks. A large series is derived from cholesterol. These include the glucocorticoids, mineralocorticoids, estrogens, progestins, and $1,25(OH)_2$-$D_3$ (see Figure 42–2). In some cases, a steroid hormone is the precursor molecule for another hormone. For example, progesterone is a hormone in its own right but is also a precursor in the formation of glucocorticoids, mineralocorticoids, testosterone, and estrogens. Testosterone is an obligatory intermediate in the biosynthesis of estradiol and in the formation of dihydrotestosterone (DHT). In these examples, described in detail below, the final product is determined by the cell type and the associated set of enzymes in which the precursor exists.

The amino acid tyrosine is the starting point in the synthesis of the catecholamines and of the thyroid hormones tetraiodothyronine (thyroxine; $T_4$) and triiodothyronine ($T_3$) (Figure 42–2). $T_3$ and $T_4$ are unique in that they require the addition of iodine (as $I^-$) for bioactivity. Because dietary iodine is very scarce in many parts of the world, an intricate mechanism for accumulating and retaining $I^-$ has evolved.

Many hormones are polypeptides or glycoproteins. These range in size from thyrotropin-releasing hormone (TRH), a tripeptide, to single-chain polypeptides like adrenocorticotropic hormone (ACTH; 39 amino acids), parathyroid hormone (PTH; 84 amino acids), and growth hormone (GH; 191 amino acids) (Figure 42–2). Insulin is an AB chain heterodimer of 21 and 30 amino acids, respectively. Follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH), and chorionic gonadotropin (CG) are glycoprotein hormones of αβ heterodimeric structure. The α chain is identical in all of these hormones, and distinct β chains impart hormone uniqueness. These hormones have a molecular mass in the range of 25–30 kDa depending on the degree of glycosylation and the length of the β chain.

## Hormones Are Synthesized & Modified For Full Activity in a Variety of Ways

Some hormones are synthesized in final form and secreted immediately. Included in this class are the hormones derived from cholesterol. Others such as the catecholamines are synthesized in final form and stored in the producing cells. Others are synthesized from precursor molecules in the producing cell, then are processed and secreted upon a physiologic cue (insulin). Finally, still others are converted to active forms from precursor molecules in the periphery ($T_3$ and DHT). All of these examples are discussed in more detail below.

# MANY HORMONES ARE MADE FROM CHOLESTEROL

## Adrenal Steroidogenesis

The adrenal steroid hormones are synthesized from cholesterol. Cholesterol is mostly derived from the plasma, but a small portion is synthesized in situ from acetyl-CoA via mevalonate and squalene. Much of the cholesterol in the adrenal is esterified and stored in cytoplasmic lipid droplets. Upon stimulation of the adrenal by ACTH, an esterase is activated, and the free cholesterol formed is transported into the mitochondrion, where a **cytochrome P450 side chain cleavage enzyme (P450scc)** converts cholesterol to pregnenolone. Cleavage of the side chain involves sequential hydroxylations, first at $C_{22}$ and then at $C_{20}$, followed by side chain cleavage (removal of the six-carbon fragment isocaproaldehyde) to give the 21-carbon steroid (Figure 42–3, top). An ACTH-dependent **steroidogenic acute regulatory (StAR) protein** is essential for the transport of cholesterol to P450scc in the inner mitochondrial membrane.

All mammalian steroid hormones are formed from cholesterol via pregnenolone through a series of reactions that occur in either the mitochondria or endoplasmic reticulum of the adrenal cell. Hydroxylases that require molecular oxygen and NADPH are essential, and dehydrogenases, an isomerase, and a lyase reaction are also necessary for certain steps. There is cellular specificity in adrenal steroidogenesis. For instance, 18-hydroxylase and 19-hydroxysteroid dehydrogenase, which are required for aldosterone synthesis, are found only in the zona glomerulosa cells (the outer region of the adrenal cortex), so that the biosynthesis of this mineralocorticoid is confined to this region. A schematic representation of the pathways involved in the synthesis of the three major classes of adrenal steroids is presented in Figure 42–4. The enzymes are shown in the rectangular boxes, and the modifications at each step are shaded.

### A. Mineralocorticoid Synthesis

Synthesis of aldosterone follows the mineralocorticoid pathway and occurs in the zona glomerulosa. Pregnenolone is converted to progesterone by the action of two smooth endoplasmic reticulum enzymes, **3β-hydroxysteroid dehydrogenase (3β-OHSD)** and **$\Delta^{5,4}$-isomerase.** Progesterone is hydroxylated at the $C_{21}$ position to form 11-deoxycorticosterone (DOC), which is an active ($Na^+$-retaining) mineralocorticoid. The next hydroxylation, at $C_{11}$, produces corticosterone, which has glucocorticoid activity and is a weak mineralocorticoid (it has less than 5% of the potency of aldosterone). In some species (eg, rodents), it is the most potent glucocorticoid.

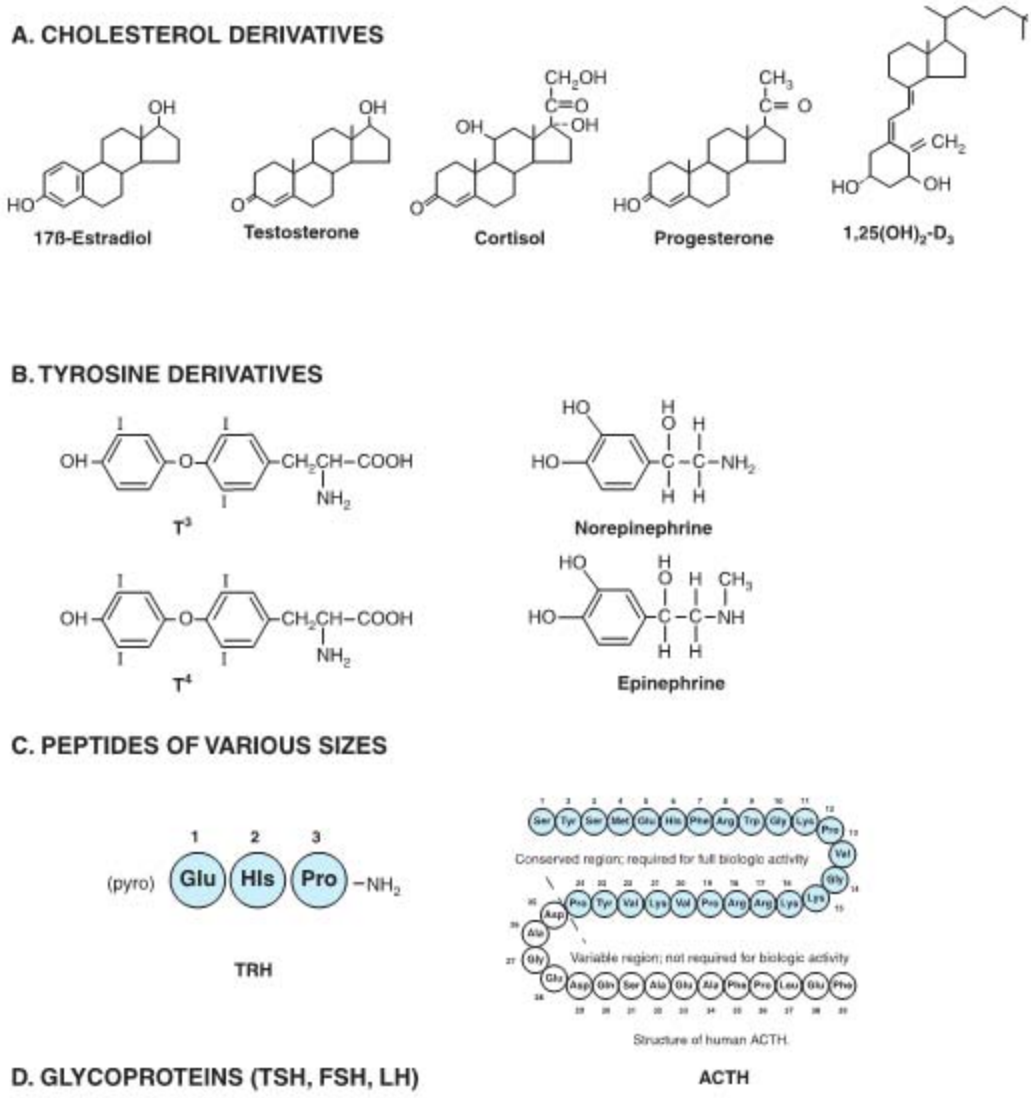


***Figure 42–2.*** Chemical diversity of hormones. **A.** Cholesterol derivatives. **B.** Tyrosine derivatives. **C.** Peptides of various sizes **D.** Glycoproteins (TSH, FSH, LH) with common α subunits and unique β subunits.

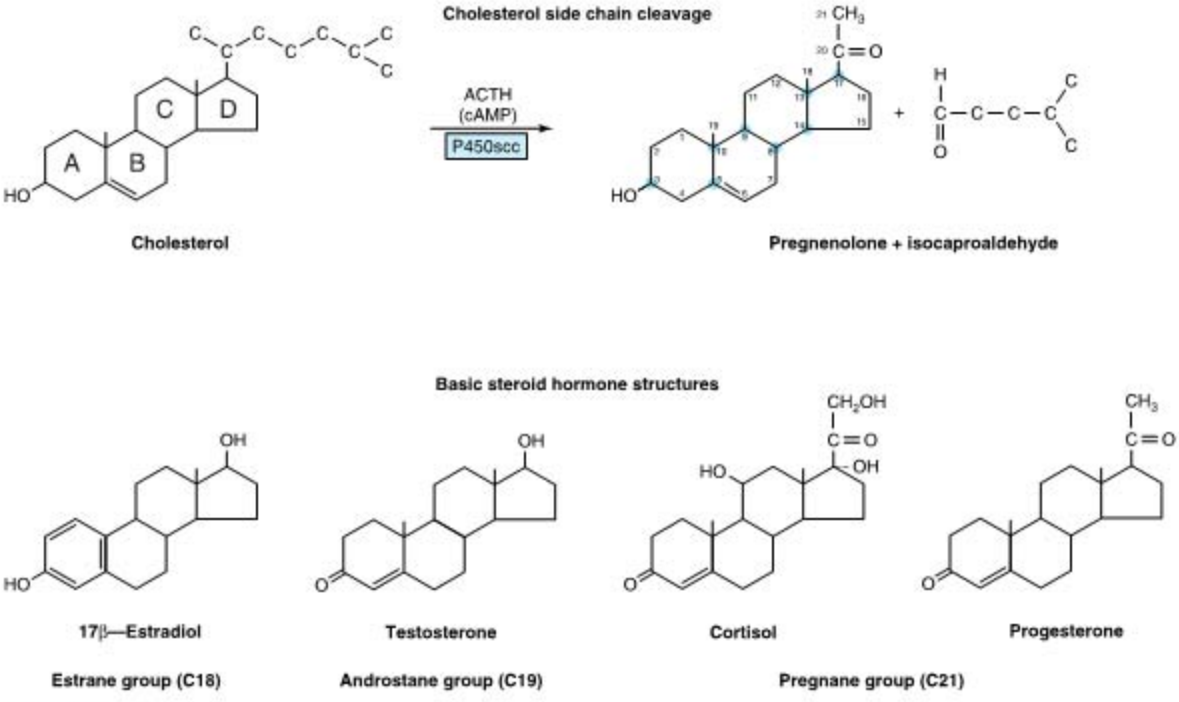


***Figure 42–3.*** Cholesterol side-chain cleavage and basic steroid hormone structures. The basic sterol rings are identified by the letters A–D. The carbon atoms are numbered 1–21 starting with the A ring. Note that the estrane group has 18 carbons (C18), etc.

$C_{21}$ hydroxylation is necessary for both mineralocorticoid and glucocorticoid activity, but most steroids with a $C_{17}$ hydroxyl group have more glucocorticoid and less mineralocorticoid action. In the zona glomerulosa, which does not have the smooth endoplasmic reticulum enzyme 17α-hydroxylase, a mitochondrial 18-hydroxylase is present. The **18-hydroxylase (aldosterone synthase)** acts on corticosterone to form 18-hydroxycorticosterone, which is changed to aldosterone by conversion of the 18-alcohol to an aldehyde. This unique distribution of enzymes and the special regulation of the zona glomerulosa by $K^+$ and angiotensin II have led some investigators to suggest that, in addition to the adrenal being two glands, the adrenal cortex is actually two separate organs.

## B. Glucocorticoid Synthesis

Cortisol synthesis requires three hydroxylases located in the fasciculata and reticularis zones of the adrenal cortex that act sequentially on the $C_{17}$, $C_{21}$, and $C_{11}$ positions. The first two reactions are rapid, while $C_{11}$ hydroxylation is relatively slow. If the $C_{11}$ position is hydroxylated first, the action of **17α-hydroxylase** is impeded and the mineralocorticoid pathway is followed (forming corticosterone or aldosterone, depending on the cell type). 17α-Hydroxylase is a smooth endoplasmic reticulum enzyme that acts upon either progesterone or, more commonly, pregnenolone. 17α-Hydroxyprogesterone is hydroxylated at $C_{21}$ to form 11-deoxycortisol, which is then hydroxylated at $C_{11}$ to form cortisol, the most potent natural glucocorticoid hormone in humans. 21-Hydroxylase is a smooth endoplasmic reticulum enzyme, whereas 11β-hydroxylase is a mitochondrial enzyme. Steroidogenesis thus involves the repeated shuttling of substrates into and out of the mitochondria.

## C. Androgen Synthesis

The major androgen or androgen precursor produced by the adrenal cortex is dehydroepiandrosterone (DHEA). Most 17-hydroxypregnenolone follows the glucocorticoid pathway, but a small fraction is subjected to oxidative fission and removal of the two-carbon side chain through the action of 17,20-lyase. The lyase activity is actually part of the same enzyme (P450c17) that catalyzes 17α-hydroxylation. This is therefore a **dual function protein.** The lyase activity is important in both the adrenals and

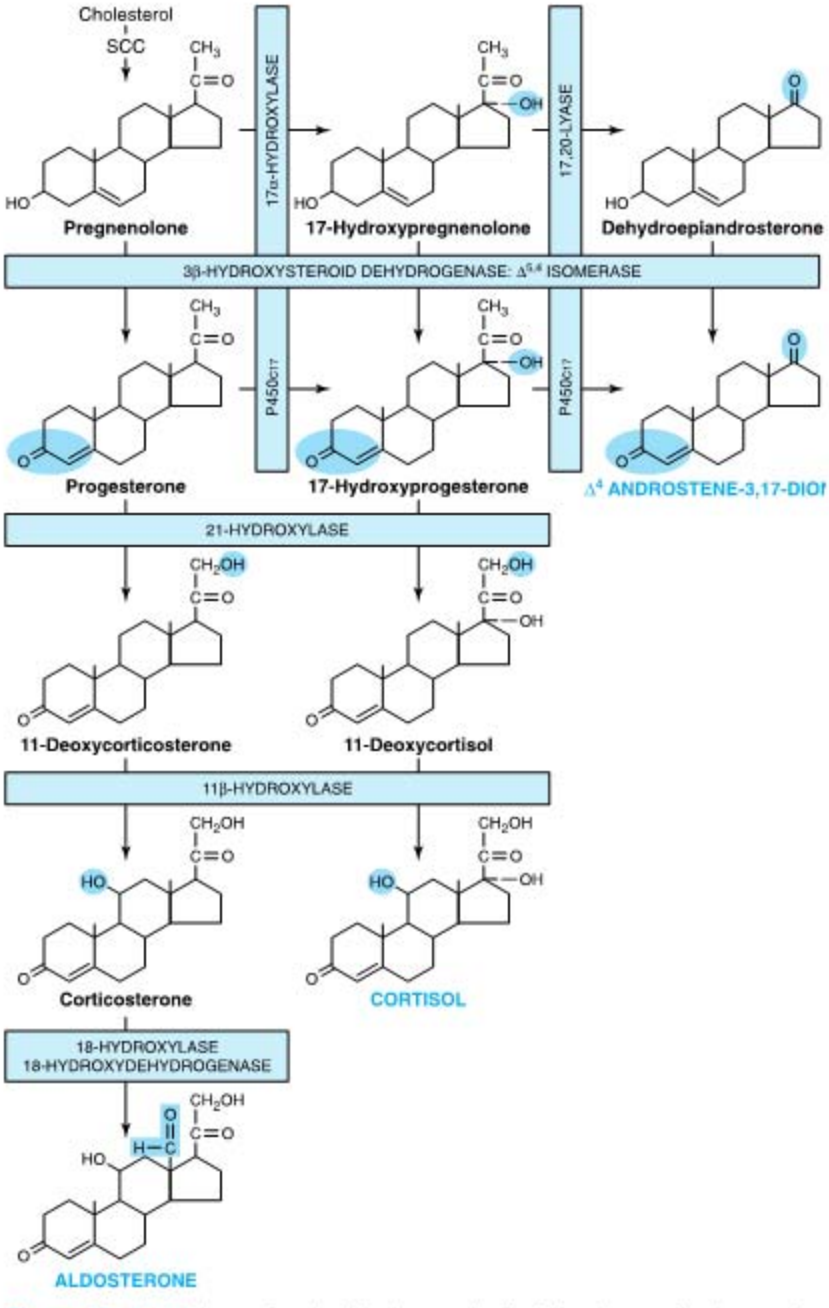


***Figure 42–4.*** Pathways involved in the synthesis of the three major classes of adrenal steroids (mineralocorticoids, glucocorticoids, and androgens). Enzymes are shown in the rectangular boxes, and the modifications at each step are shaded. Note that the 17α-hydroxylase and 17,20-lyase activities are both part of one enzyme, designated P450c17. (Slightly modified and reproduced, with permission, from Harding BW: In: *Endocrinology*, vol 2. DeGroot LJ [editor]. Grune & Stratton, 1979.)

the gonads and acts exclusively on 17α-hydroxy-containing molecules. Adrenal androgen production increases markedly if glucocorticoid biosynthesis is impeded by the lack of one of the hydroxylases **(adrenogenital syndrome).** DHEA is really a prohormone, since the actions of 3β-OHSD and $\Delta^{5,4}$-isomerase convert the weak androgen DHEA into the more potent **androstenedione.** Small amounts of androstenedione are also formed in the adrenal by the action of the lyase on 17α-hydroxyprogesterone. Reduction of androstenedione at the $C_{17}$ position results in the formation of **testosterone,** the most potent adrenal androgen. Small amounts of testosterone are produced in the adrenal by this mechanism, but most of this conversion occurs in the testes.

## Testicular Steroidogenesis

Testicular androgens are synthesized in the interstitial tissue by the Leydig cells. The immediate precursor of the gonadal steroids, as for the adrenal steroids, is cholesterol. The rate-limiting step, as in the adrenal, is delivery of cholesterol to the inner membrane of the mitochondria by the transport protein StAR. Once in the proper location, cholesterol is acted upon by the side chain cleavage enzyme P450scc. The conversion of cholesterol to pregnenolone is identical in adrenal, ovary, and testis. In the latter two tissues, however, the reaction is promoted by LH rather than ACTH.

The conversion of pregnenolone to testosterone requires the action of five enzyme activities contained in three proteins: (1) 3β-hydroxysteroid dehydrogenase (3β-OHSD) and $\Delta^{5,4}$-isomerase; (2) 17α-hydroxylase and 17,20-lyase; and (3) 17β-hydroxysteroid dehydrogenase (17β-OHSD). This sequence, referred to as the **progesterone (or $\Delta^4$) pathway,** is shown on the right side of Figure 42–5. Pregnenolone can also be converted to testosterone by the **dehydroepiandrosterone (or $\Delta^5$) pathway,** which is illustrated on the left side of Figure 42–5. The $\Delta^5$ route appears to be most used in human testes.

The five enzyme activities are localized in the microsomal fraction in rat testes, and there is a close functional association between the activities of 3β-OHSD and $\Delta^{5,4}$-isomerase and between those of a 17α-hydroxylase and 17,20-lyase. These enzyme pairs, both contained in a single protein, are shown in the general reaction sequence in Figure 42–5.

## Dihydrotestosterone Is Formed From Testosterone in Peripheral Tissues

Testosterone is metabolized by two pathways. One involves oxidation at the 17 position, and the other involves reduction of the A ring double bond and the 3-ketone. Metabolism by the first pathway occurs in many tissues, including liver, and produces 17-ketosteroids that are generally inactive or less active than the parent compound. Metabolism by the second pathway, which is less efficient, occurs primarily in target tissues and produces the potent metabolite dihydrotestosterone (DHT).

The most significant metabolic product of testosterone is DHT, since in many tissues, including prostate, external genitalia, and some areas of the skin, this is the active form of the hormone. The plasma content of DHT in the adult male is about one-tenth that of testosterone, and approximately 400 μg of DHT is produced daily as compared with about 5 mg of testosterone. About 50–100 μg of DHT are secreted by the testes. The rest is produced peripherally from testosterone in a reaction catalyzed by the NADPH-dependent **5α-reductase** (Figure 42–6). Testosterone can thus be considered a prohormone, since it is converted into a much more potent compound (dihydrotestosterone) and since most of this conversion occurs outside the testes. Some estradiol is formed from the peripheral aromatization of testosterone, particularly in males.

## Ovarian Steroidogenesis

The estrogens are a family of hormones synthesized in a variety of tissues. 17β-Estradiol is the primary estrogen of ovarian origin. In some species, estrone, synthesized in numerous tissues, is more abundant. In pregnancy, relatively more estriol is produced, and this comes from the placenta. The general pathway and the subcellular localization of the enzymes involved in the early steps of estradiol synthesis are the same as those involved in androgen biosynthesis. Features unique to the ovary are illustrated in Figure 42–7.

Estrogens are formed by the aromatization of androgens in a complex process that involves three hydroxylation steps, each of which requires $O_2$ and NADPH. The **aromatase enzyme complex** is thought to include a P450 monooxygenase. Estradiol is formed if the substrate of this enzyme complex is testosterone, whereas estrone results from the aromatization of androstenedione.

The cellular source of the various ovarian steroids has been difficult to unravel, but a transfer of substrates between two cell types is involved. Theca cells are the source of androstenedione and testosterone. These are converted by the aromatase enzyme in granulosa cells to estrone and estradiol, respectively. Progesterone, a precursor for all steroid hormones, is produced and secreted by the corpus luteum as an end-product hormone because these cells do not contain the enzymes necessary to convert progesterone to other steroid hormones (Figure 42–8).

Significant amounts of estrogens are produced by the peripheral aromatization of androgens. In human males, the peripheral aromatization of testosterone to estradiol ($E_2$) accounts for 80% of the production of the latter. In females, adrenal androgens are important

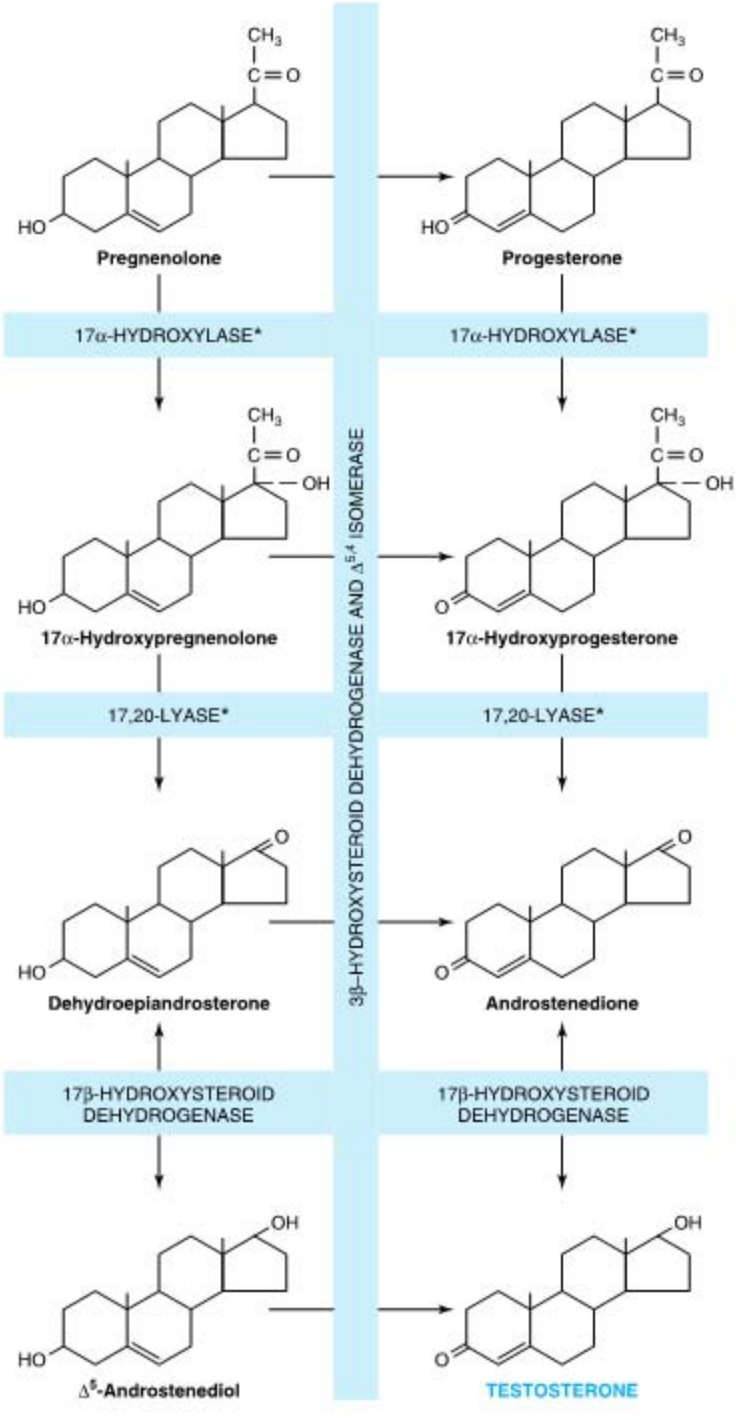


***Figure 42–5.*** Pathways of testosterone biosynthesis. The pathway on the left side of the figure is called the $\Delta^5$ or dehydroepiandrosterone pathway; the pathway on the right side is called the $\Delta^4$ or progesterone pathway. The asterisk indicates that the 17α-hydroxylase and 17,20-lyase activities reside in a single protein, P450c17.

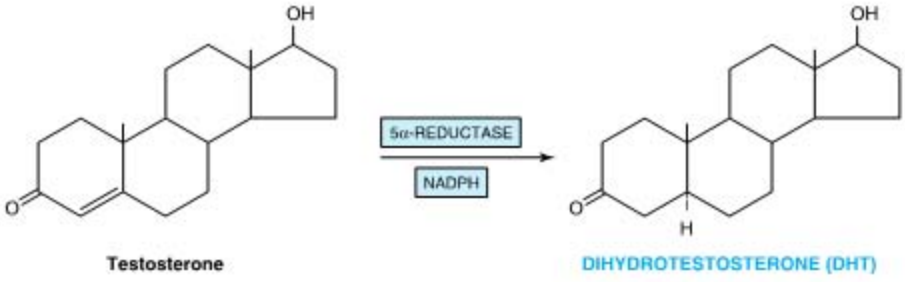


***Figure 42–6.*** Dihydrotestosterone is formed from testosterone through action of the enzyme 5α-reductase.

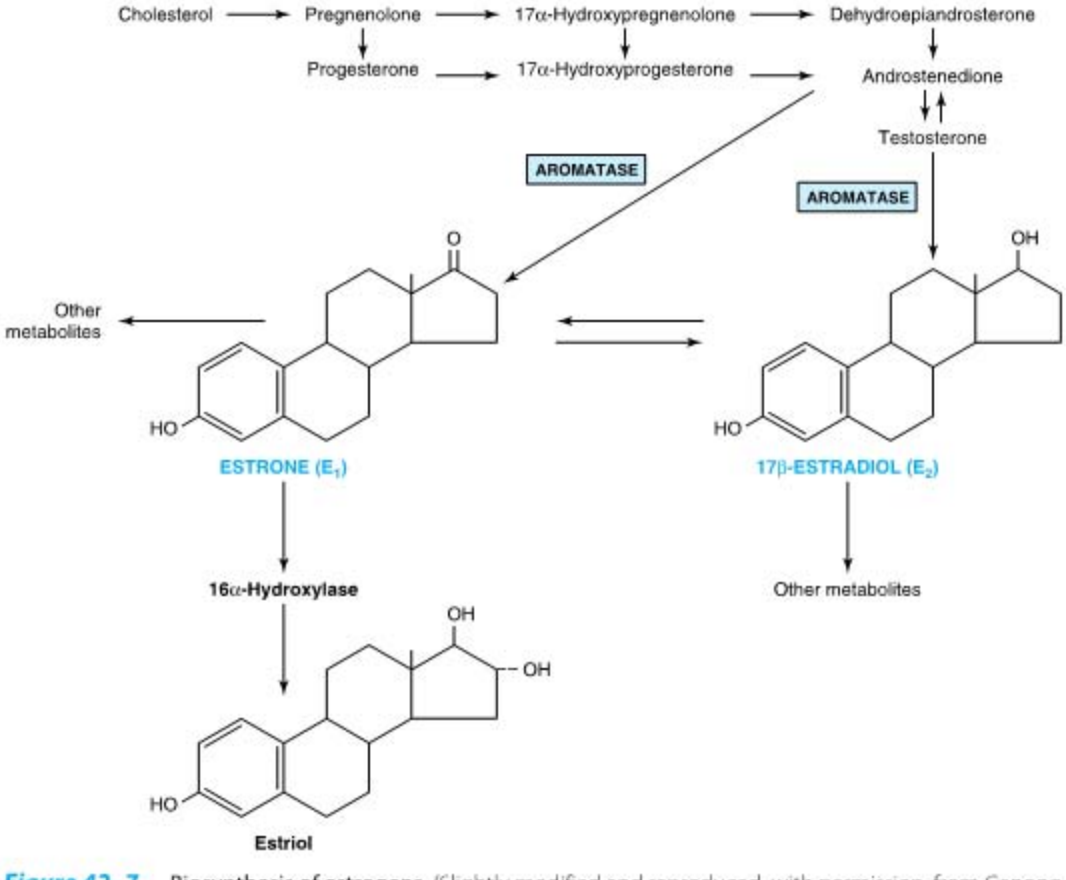


***Figure 42–7.*** **Biosynthesis of estrogens.** (Slightly modified and reproduced, with permission, from Ganong WF: *Review of Medical Physiology,* 20th ed. McGraw-Hill, 2001.)

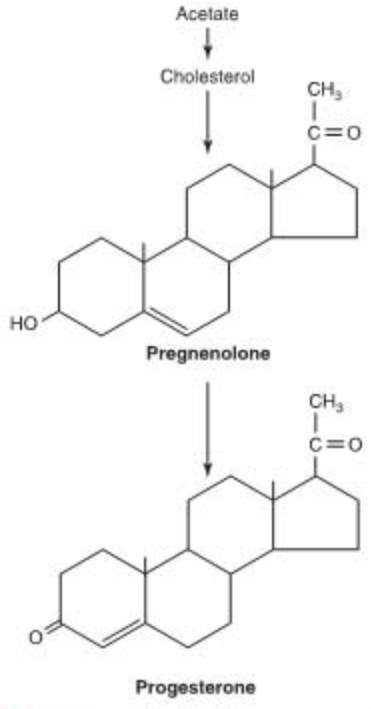


***Figure 42–8.*** Biosynthesis of progesterone in the corpus luteum.

substrates, since as much as 50% of the $E_2$ produced during pregnancy comes from the aromatization of androgens. Finally, conversion of androstenedione to estrone is the major source of estrogens in postmenopausal women. Aromatase activity is present in adipose cells and also in liver, skin, and other tissues. Increased activity of this enzyme may contribute to the "estrogenization" that characterizes such diseases as cirrhosis of the liver, hyperthyroidism, aging, and obesity.

## $1,25(OH)_2$-$D_3$ (Calcitriol) Is Synthesized From a Cholesterol Derivative

$1,25(OH)_2$-$D_3$ is produced by a complex series of enzymatic reactions that involve the plasma transport of precursor molecules to a number of different tissues (Figure 42–9). One of these precursors is vitamin D—really not a vitamin, but this common name persists. The active molecule, $1,25(OH)_2$-$D_3$, is transported to other organs where it activates biologic processes in a manner similar to that employed by the steroid hormones.

### A. Skin

Small amounts of the precursor for $1,25(OH)_2$-$D_3$ synthesis are present in food (fish liver oil, egg yolk), but most of the precursor for $1,25(OH)_2$-$D_3$ synthesis is produced in the malpighian layer of the epidermis from 7-dehydrocholesterol in an ultraviolet light-mediated, nonenzymatic **photolysis** reaction. The extent of this conversion is related directly to the intensity of the exposure and inversely to the extent of pigmentation in the skin. There is an age-related loss of 7-dehydrocholesterol in the epidermis that may be related to the negative calcium balance associated with old age.

### B. Liver

A specific transport protein called the **vitamin D-binding protein** binds vitamin $D_3$ and its metabolites and moves vitamin $D_3$ from the skin or intestine to the liver, where it undergoes 25-hydroxylation, the first obligatory reaction in the production of $1,25(OH)_2$-$D_3$. 25-Hydroxylation occurs in the endoplasmic reticulum in a reaction that requires magnesium, NADPH, molecular oxygen, and an uncharacterized cytoplasmic factor. Two enzymes are involved: an NADPH-dependent cytochrome P450 reductase and a cytochrome P450. This reaction is not regulated, and it also occurs with low efficiency in kidney and intestine. The $25(OH)_2$-$D_3$ enters the circulation, where it is the major form of vitamin D found in plasma, and is transported to the kidney by the vitamin D-binding protein.

### C. Kidney

$25(OH)_2$-$D_3$ is a weak agonist and must be modified by hydroxylation at position $C_1$ for full biologic activity. This is accomplished in mitochondria of the renal proximal convoluted tubule by a three-component monooxygenase reaction that requires NADPH, $Mg^{2+}$, molecular oxygen, and at least three enzymes: (1) a flavoprotein, renal ferredoxin reductase; (2) an iron sulfur protein, renal ferredoxin; and (3) cytochrome P450. This system produces $1,25(OH)_2$-$D_3$, which is the most potent naturally occurring metabolite of vitamin D.

# CATECHOLAMINES & THYROID HORMONES ARE MADE FROM TYROSINE

## Catecholamines Are Synthesized in Final Form & Stored in Secretion Granules

Three amines—dopamine, norepinephrine, and epinephrine—are synthesized from tyrosine in the chromaffin cells of the adrenal medulla. The major product of the adrenal medulla is epinephrine. This compound constitutes about 80% of the catecholamines in the medulla, and it is not made in extramedullary tissue. In contrast, most of the norepinephrine present in organs innervated by sympathetic nerves is made in situ (about 80% of the total), and most of the rest is made in other nerve endings and reaches the target sites via the circu-

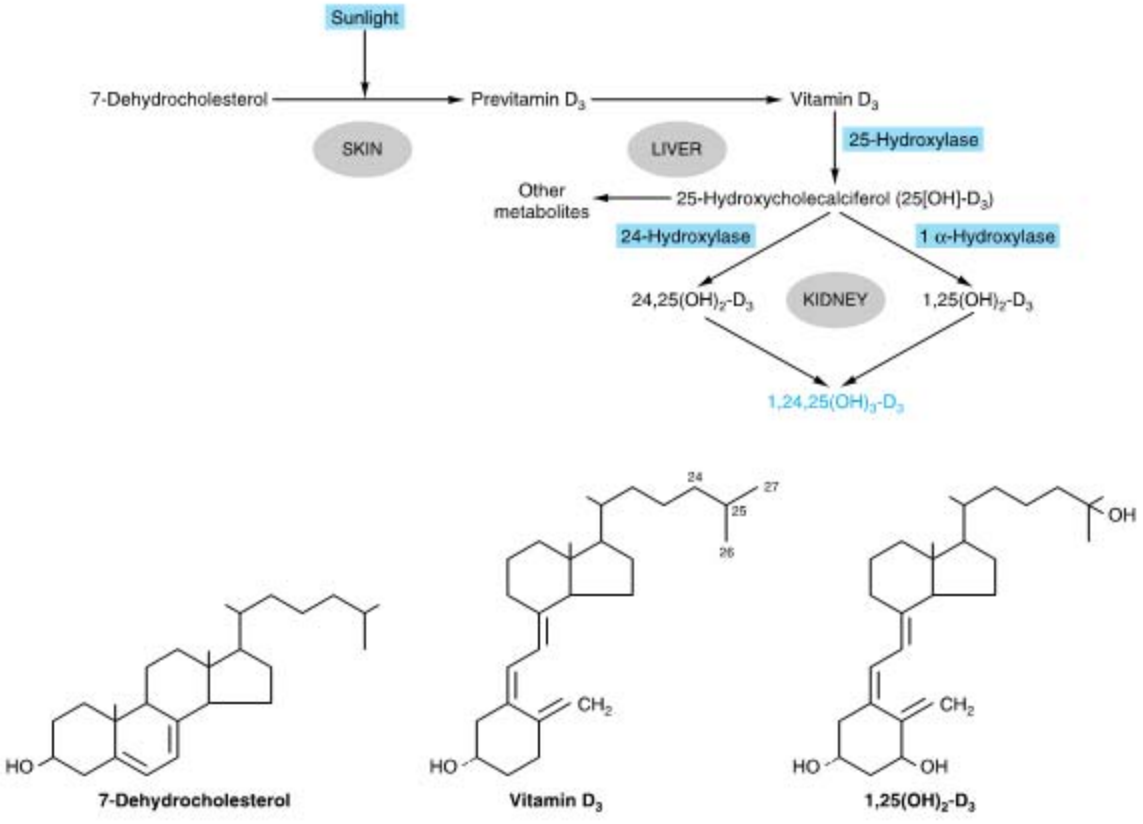


***Figure 42–9.*** Formation and hydroxylation of vitamin $D_3$. 25-Hydroxylation takes place in the liver, and the other hydroxylations occur in the kidneys. 25,26$(OH)_2$-$D_3$ and 1,25,26$(OH)_3$-$D_3$ are probably formed as well. The formulas of 7-dehydrocholesterol, vitamin $D_3$, and 1,25$(OH)_2$-$D_3$ are also shown. (Modified and reproduced, with permission, from Ganong WF: *Review of Medical Physiology*, 20th ed. McGraw-Hill, 2001.)

lation. Epinephrine and norepinephrine may be produced and stored in different cells in the adrenal medulla and other chromaffin tissues.

The conversion of tyrosine to epinephrine requires four sequential steps: (1) ring hydroxylation; (2) decarboxylation; (3) side chain hydroxylation to form norepinephrine; and (4) N-methylation to form epinephrine. The biosynthetic pathway and the enzymes involved are illustrated in Figure 42–10.

### A. Tyrosine Hydroxylase Is Rate-Limiting for Catecholamine Biosynthesis

**Tyrosine** is the immediate precursor of catecholamines, and **tyrosine hydroxylase** is the rate-limiting enzyme in catecholamine biosynthesis. Tyrosine hydroxylase is found in both soluble and particle-bound forms only in tissues that synthesize catecholamines; it functions as an oxidoreductase, with tetrahydropteridine as a cofactor, to convert L-tyrosine to L-dihydroxyphenylalanine **(L-dopa).** As the rate-limiting enzyme, tyrosine hydroxylase is regulated in a variety of ways. The most important mechanism involves feedback inhibition by the catecholamines, which compete with the enzyme for the pteridine cofactor. Catecholamines cannot cross the blood-brain barrier; hence, in the brain they must be synthesized locally. In certain central nervous system diseases (eg, Parkinson's disease), there is a local deficiency of dopamine synthesis. L-Dopa, the precursor of dopamine, readily crosses the blood-brain barrier and so is an important agent in the treatment of Parkinson's disease.

### B. Dopa Decarboxylase Is Present in All Tissues

This soluble enzyme requires pyridoxal phosphate for the conversion of L-dopa to 3,4-dihydroxyphenylethylamine **(dopamine).** Compounds that resemble L-dopa, such as α-methyldopa, are competitive inhibitors of this reaction. α-Methyldopa is effective in treating some kinds of hypertension.

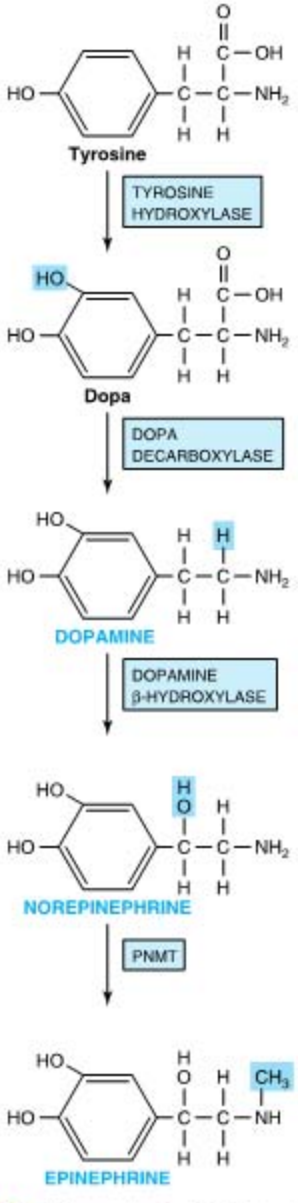


***Figure 42–10.*** Biosynthesis of catecholamines. (PNMT, phenylethanolamine-*N*-methyltransferase.)

### C. Dopamine β-Hydroxylase (DBH) Catalyzes the Conversion of Dopamine to Norepinephrine

DBH is a monooxygenase and uses ascorbate as an electron donor, copper at the active site, and fumarate as modulator. DBH is in the particulate fraction of the medullary cells, probably in the secretion granule; thus, the conversion of dopamine to **norepinephrine** occurs in this organelle.

### D. Phenylethanolamine-*N*-methyltransferase (PNMT) Catalyzes the Production of Epinephrine

PNMT catalyzes the N-methylation of norepinephrine to form **epinephrine** in the epinephrine-forming cells of the adrenal medulla. Since PNMT is soluble, it is assumed that norepinephrine-to-epinephrine conversion occurs in the cytoplasm. The synthesis of PNMT is induced by glucocorticoid hormones that reach the medulla via the intra-adrenal portal system. This special system provides for a 100-fold steroid concentration gradient over systemic arterial blood, and this high intra-adrenal concentration appears to be necessary for the induction of PNMT.

## $T_3$ & $T_4$ Illustrate the Diversity in Hormone Synthesis

The formation of **triiodothyronine ($T_3$)** and **tetraiodothyronine (thyroxine; $T_4$)** (see Figure 42–2) illustrates many of the principles of diversity discussed in this chapter. These hormones require a rare element (iodine) for bioactivity; they are synthesized as part of a very large precursor molecule (thyroglobulin); they are stored in an intracellular reservoir (colloid); and there is peripheral conversion of $T_4$ to $T_3$, which is a much more active hormone.

The thyroid hormones $T_3$ and $T_4$ are unique in that iodine (as iodide) is an essential component of both. In most parts of the world, iodine is a scarce component of soil, and for that reason there is little in food. A complex mechanism has evolved to acquire and retain this crucial element and to convert it into a form suitable for incorporation into organic compounds. At the same time, the thyroid must synthesize thyronine from tyrosine, and this synthesis takes place in thyroglobulin (Figure 42–11).

**Thyroglobulin** is the precursor of $T_4$ and $T_3$. It is a large iodinated, glycosylated protein with a molecular mass of 660 kDa. Carbohydrate accounts for 8–10% of the weight of thyroglobulin and iodide for about 0.2–1%, depending upon the iodine content in the diet. Thyroglobulin is composed of two large subunits. It contains 115 tyrosine residues, each of which is a potential site of iodination. About 70% of the iodide in thyroglobulin exists in the inactive precursors, **monoiodotyrosine (MIT)** and **diiodotyrosine (DIT),** while 30% is in the **iodothyronyl residues,** $T_4$ and $T_3$. When iodine supplies are sufficient, the $T_4$:$T_3$ ratio is about 7:1. In **iodine deficiency,** this ratio decreases, as does the DIT:MIT ratio. Thyroglobulin, a large molecule of about 5000 amino acids, provides the confor-

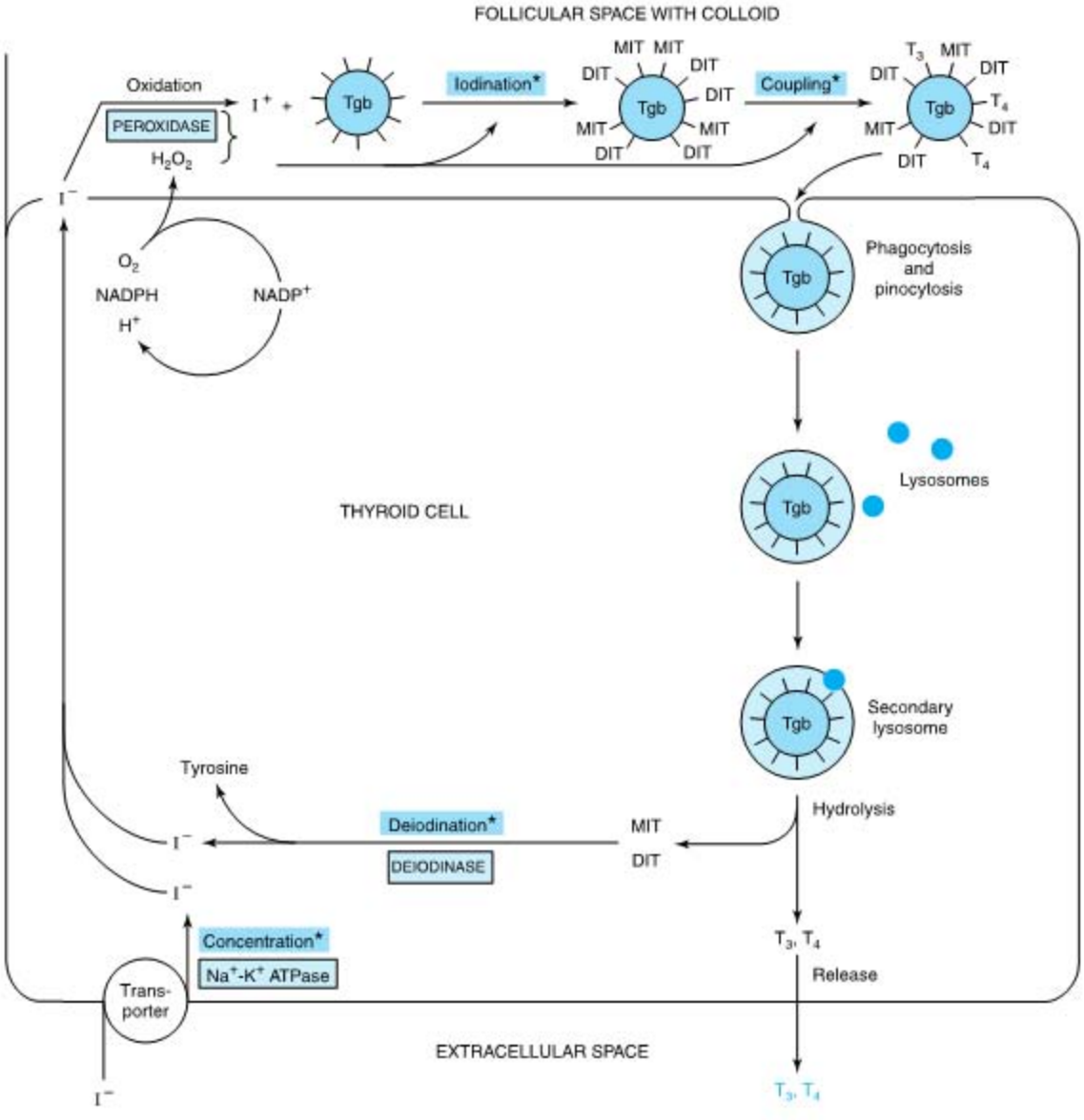


***Figure 42–11.*** Model of iodide metabolism in the thyroid follicle. A follicular cell is shown facing the follicular lumen (top) and the extracellular space (at bottom). Iodide enters the thyroid primarily through a transporter (bottom left). Thyroid hormone synthesis occurs in the follicular space through a series of reactions, many of which are peroxidase-mediated. Thyroid hormones, stored in the colloid in the follicular space, are released from thyroglobulin by hydrolysis inside the thyroid cell. (Tgb, thyroglobulin; MIT, monoiodotyrosine; DIT, diiodotyrosine; $T_3$, triiodothyronine; $T_4$, tetraiodothyronine.) Asterisks indicate steps or processes that are inherited enzyme deficiencies which cause congenital goiter and often result in hypothyroidism.

mation required for tyrosyl coupling and iodide organification necessary in the formation of the diaminoacid thyroid hormones. It is synthesized in the basal portion of the cell and moves to the lumen, where it is a storage form of $T_3$ and $T_4$ in the colloid; several weeks' supply of these hormones exist in the normal thyroid. Within minutes after stimulation of the thyroid by TSH, colloid reenters the cell and there is a marked increase of phagolysosome activity. Various acid proteases and peptidases hydrolyze the thyroglobulin into its constituent amino acids, including $T_4$ and $T_3$, which are discharged from the basal portion of the cell (see Figure 42–11). Thyroglobulin is thus a very large prohormone.

### Iodide Metabolism Involves Several Discrete Steps

The thyroid is able to concentrate $I^-$ against a strong electrochemical gradient. This is an energy-dependent process and is linked to the $Na^+$-$K^+$ ATPase-dependent thyroidal $I^-$ transporter. The ratio of iodide in thyroid to iodide in serum (T:S ratio) is a reflection of the activity of this transporter. This activity is primarily controlled by TSH and ranges from 500:1 in animals chronically stimulated with TSH to 5:1 or less in hypophysectomized animals (no TSH). The T:S ratio in humans on a normal iodine diet is about 25:1.

The thyroid is the only tissue that can oxidize $I^-$ to a higher valence state, an obligatory step in $I^-$ organification and thyroid hormone biosynthesis. This step involves a heme-containing peroxidase and occurs at the luminal surface of the follicular cell. Thyroperoxidase, a tetrameric protein with a molecular mass of 60 kDa, requires hydrogen peroxide as an oxidizing agent. The $H_2O_2$ is produced by an NADPH-dependent enzyme resembling cytochrome *c* reductase. A number of compounds inhibit $I^-$ oxidation and therefore its subsequent incorporation into MIT and DIT. The most important of these are the thiourea drugs. They are used as antithyroid drugs because of their ability to inhibit thyroid hormone biosynthesis at this step. Once iodination occurs, the iodine does not readily leave the thyroid. Free tyrosine can be iodinated, but it is not incorporated into proteins since no tRNA recognizes iodinated tyrosine.

The coupling of two DIT molecules to form $T_4$—or of an MIT and DIT to form $T_3$—occurs within the thyroglobulin molecule. A separate coupling enzyme has not been found, and since this is an oxidative process it is assumed that the same thyroperoxidase catalyzes this reaction by stimulating free radical formation of iodotyrosine. This hypothesis is supported by the observation that the same drugs which inhibit $I^-$ oxidation also inhibit coupling. The formed thyroid hormones remain as integral parts of thyroglobulin until the latter is degraded, as described above.

A deiodinase removes $I^-$ from the inactive mono- and diiodothyronine molecules in the thyroid. This mechanism provides a substantial amount of the $I^-$ used in $T_3$ and $T_4$ biosynthesis. A peripheral deiodinase in target tissues such as pituitary, kidney, and liver selectively removes $I^-$ from the 5′ position of $T_4$ to make $T_3$ (see Figure 42–2), which is a much more active molecule. In this sense, $T_4$ can be thought of as a prohormone, though it does have some intrinsic activity.

## SEVERAL HORMONES ARE MADE FROM LARGER PEPTIDE PRECURSORS

Formation of the critical disulfide bridges in insulin requires that this hormone be first synthesized as part of a larger precursor molecule, proinsulin. This is conceptually similar to the example of the thyroid hormones, which can only be formed in the context of a much larger molecule. Several other hormones are synthesized as parts of large precursor molecules, not because of some special structural requirement but rather as a mechanism for controlling the available amount of the active hormone. PTH and angiotensin II are examples of this type of regulation. Another interesting example is the POMC protein, which can be processed into many different hormones in a tissue-specific manner. These examples are discussed in detail below.

### Insulin Is Synthesized as a Preprohormone & Modified Within the β Cell

Insulin has an AB heterodimeric structure with one intrachain (A6–A11) and two interchain disulfide bridges (A7–B7 and A20–B19) (Figure 42–12). The A and B chains could be synthesized in the laboratory, but attempts at a biochemical synthesis of the mature insulin molecule yielded very poor results. The reason for this became apparent when it was discovered that insulin is synthesized as a **preprohormone** (molecular weight approximately 11,500), which is the prototype for peptides that are processed from larger precursor molecules. The hydrophobic 23-amino-acid pre-, or leader, sequence directs the molecule into the cisternae of the endoplasmic reticulum and then is removed. This results in the 9000-MW proinsulin molecule, which provides the conformation necessary for the proper and efficient formation of the disulfide bridges. As shown in Figure 42–12, the sequence of proinsulin, starting from the amino terminal, is B chain—connecting (C) peptide—A chain. The proinsulin molecule undergoes a series of site-specific peptide cleavages that result in the formation of equimolar amounts of mature insulin and C peptide. These enzymatic cleavages are summarized in Figure 42–12.

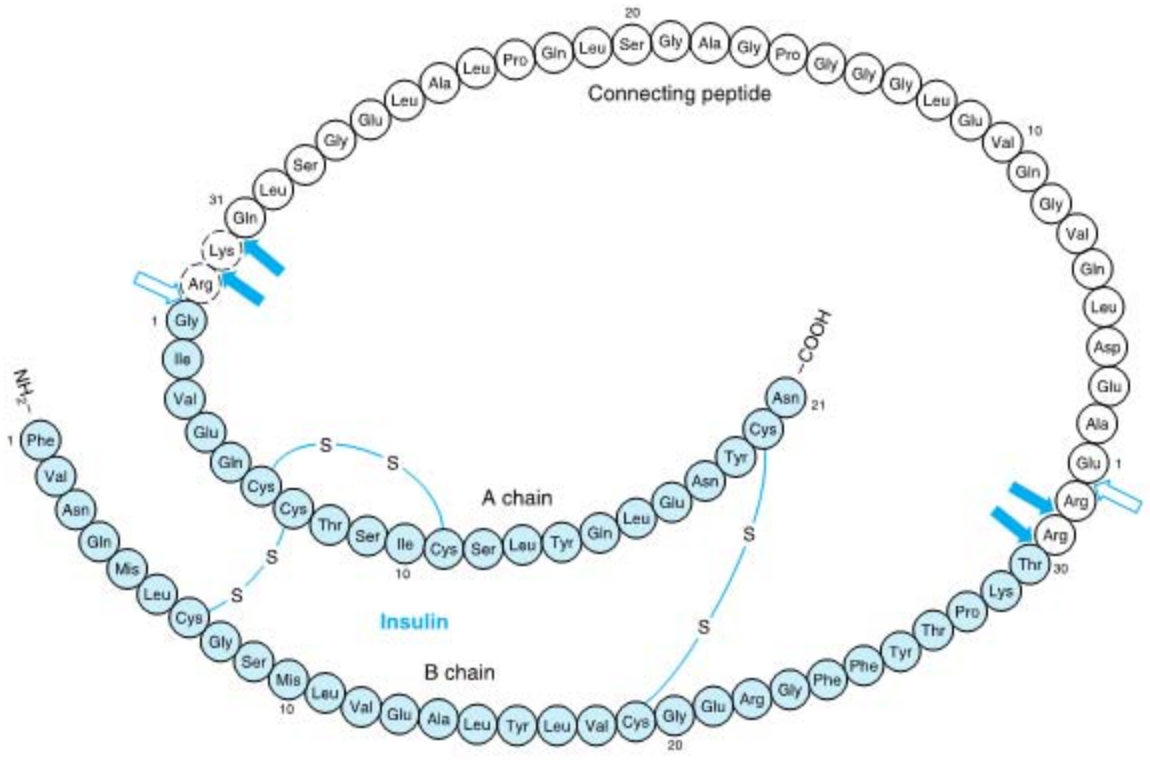


***Figure 42–12.*** Structure of human proinsulin. Insulin and C-peptide molecules are connected at two sites by dipeptide links. An initial cleavage by a trypsin-like enzyme (open arrows) followed by several cleavages by a carboxypeptidase-like enzyme (solid arrows) results in the production of the heterodimeric (AB) insulin molecule (light blue) and the C-peptide.

## Parathyroid Hormone (PTH) Is Secreted as an 84-Amino-Acid Peptide

The immediate precursor of PTH is **proPTH,** which differs from the native 84-amino-acid hormone by having a highly basic hexapeptide amino terminal extension. The primary gene product and the immediate precursor for proPTH is the 115-amino-acid **preproPTH.** This differs from proPTH by having an additional 25-amino-acid amino terminal extension that, in common with the other leader or signal sequences characteristic of secreted proteins, is hydrophobic. The complete structure of preproPTH and the sequences of proPTH and PTH are illustrated in Figure 42–13. $PTH_{1-34}$ has full biologic activity, and the region 25–34 is primarily responsible for receptor binding.

The biosynthesis of PTH and its subsequent secretion are regulated by the plasma ionized calcium ($Ca^{2+}$) concentration through a complex process. An acute decrease of $Ca^{2+}$ results in a marked increase of PTH mRNA, and this is followed by an increased rate of PTH synthesis and secretion. However, about 80–90% of the proPTH synthesized cannot be accounted for as intact PTH in cells or in the incubation medium of experimental systems. This finding led to the conclusion that most of the proPTH synthesized is quickly degraded. It was later discovered that this rate of degradation decreases when $Ca^{2+}$ concentrations are low, and it increases when $Ca^{2+}$ concentrations are high. Very specific fragments of PTH are generated during its proteolytic digestion (Figure 42–13). A number of proteolytic enzymes, including cathepsins B and D, have been identified in parathyroid tissue. Cathepsin B cleaves PTH into two fragments: $PTH_{1-36}$ and $PTH_{37-84}$. $PTH_{37-84}$ is not further degraded; however, $PTH_{1-36}$ is rapidly and progressively cleaved into di- and tripeptides. Most of the proteolysis of PTH occurs within the gland, but a number of studies confirm that PTH, once secreted, is proteolytically degraded in other tissues, especially the liver, by similar mechanisms.

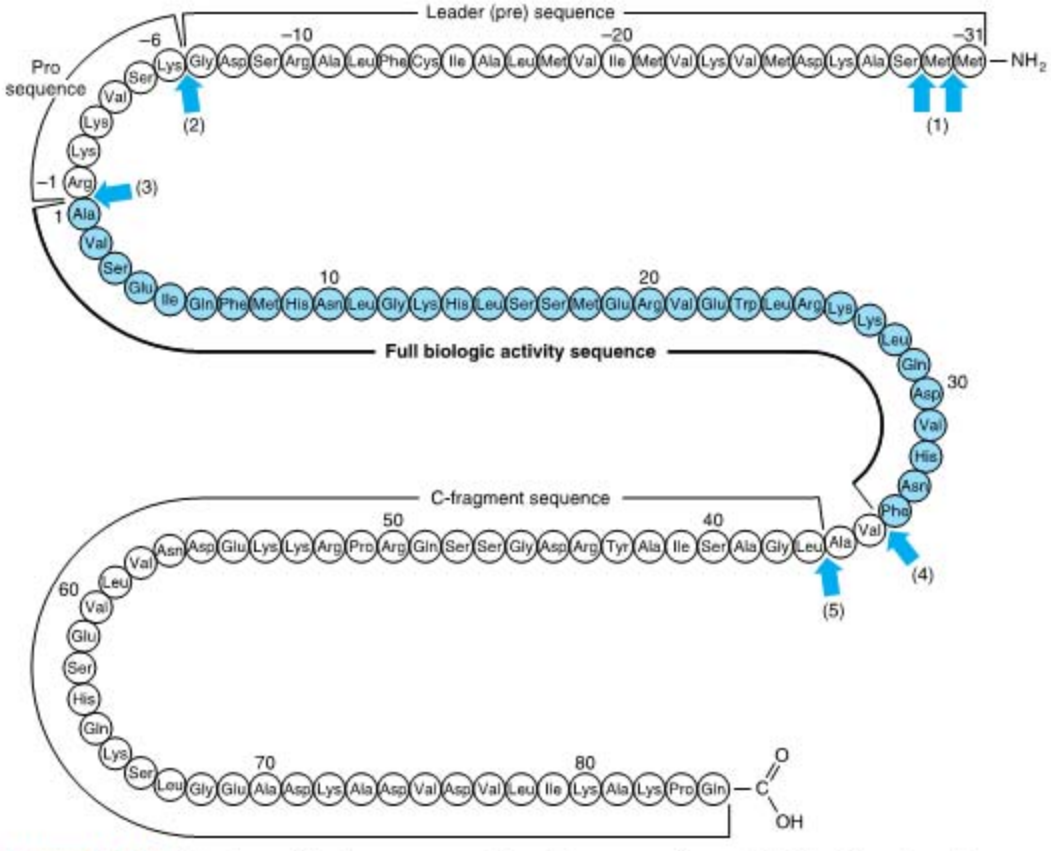


***Figure 42–13.*** **Structure of bovine preproparathyroid hormone. Arrows indicate sites cleaved by processing enzymes in the parathyroid gland (1–5) and in the liver after secretion of the hormone (4–5). The biologically active region of the molecule is flanked by sequence not required for activity on target receptors.** (Slightly modified and reproduced, with permission, from Habener JF: Recent advances in parathyroid hormone research. Clin Biochem 1981;14:223.)

## Angiotensin II Is Also Synthesized From a Large Precursor

The renin-angiotensin system is involved in the regulation of blood pressure and electrolyte metabolism (through production of aldosterone). The primary hormone involved in these processes is angiotensin II, an octapeptide made from angiotensinogen (Figure 42–14). Angiotensinogen, a large $\alpha_2$-globulin made in liver, is the substrate for renin, an enzyme produced in the juxtaglomerular cells of the renal afferent arteriole. The position of these cells makes them particularly sensitive to blood pressure changes, and many of the physiologic regulators of renin release act through renal baroreceptors. The juxtaglomerular cells are also sensitive to changes of $Na^+$ and $Cl^-$ concentration in the renal tubular fluid; therefore, any combination of factors that decreases fluid volume (dehydration, decreased blood pressure, fluid or blood loss) or decreases NaCl concentration stimulates renin release. Renal sympathetic nerves that terminate in the juxtaglomerular cells mediate the central nervous system and postural effects on renin release independently of the baroreceptor and salt effects, a mechanism that involves the β-adrenergic receptor. Renin acts upon the substrate angiotensinogen to produce the decapeptide angiotensin I.

Angiotensin-converting enzyme, a glycoprotein found in lung, endothelial cells, and plasma, removes two carboxyl terminal amino acids from the decapeptide angiotensin I to form angiotensin II in a step that is not thought to be rate-limiting. Various nonapeptide analogs of angiotensin I and other compounds act as competitive inhibitors of converting enzyme and are used to treat renin-dependent hypertension. These are

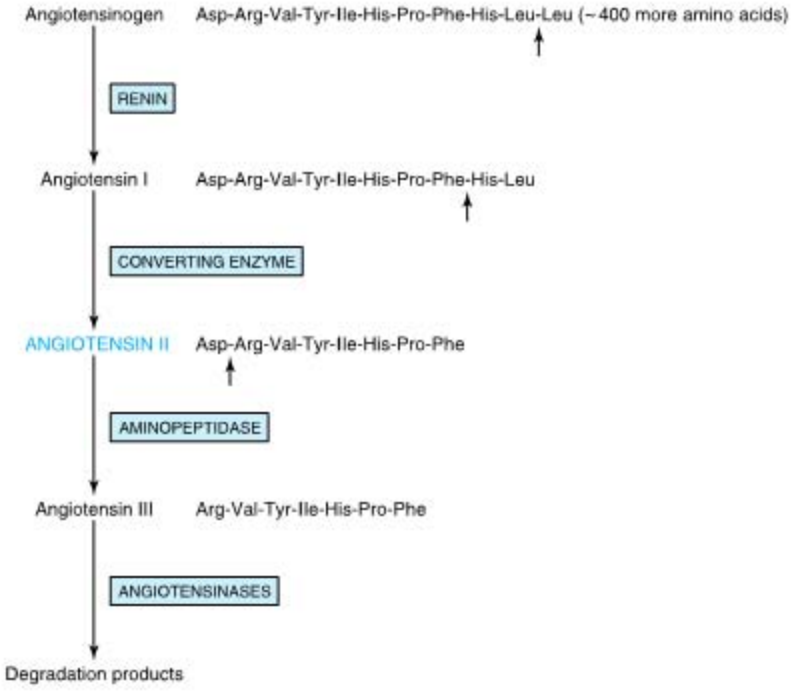


***Figure 42–14.*** Formation and metabolism of angiotensins. Small arrows indicate cleavage sites.

referred to as **angiotensin-converting enzyme (ACE) inhibitors.** Angiotensin II increases blood pressure by causing vasoconstriction of the arteriole and is a very potent vasoactive substance. It inhibits renin release from the juxtaglomerular cells and is a potent stimulator of aldosterone production. This results in $Na^+$ retention, volume expansion, and increased blood pressure.

In some species, angiotensin II is converted to the heptapeptide angiotensin III (Figure 42–14), an equally potent stimulator of aldosterone production. In humans, the plasma level of angiotensin II is four times greater than that of angiotensin III, so most effects are exerted by the octapeptide. Angiotensins II and III are rapidly inactivated by angiotensinases.

Angiotensin II binds to specific adrenal cortex glomerulosa cell receptors. The hormone-receptor interaction does not activate adenylyl cyclase, and cAMP does not appear to mediate the action of this hormone. The actions of angiotensin II, which are to stimulate the conversion of cholesterol to pregnenolone and of corticosterone to 18-hydroxycorticosterone and aldosterone, may involve changes in the concentration of intracellular calcium and of phospholipid metabolites by mechanisms similar to those described in Chapter 43.

## Complex Processing Generates the Pro-opiomelanocortin (POMC) Peptide Family

The POMC family consists of peptides that act as hormones (ACTH, LPH, MSH) and others that may serve as neurotransmitters or neuromodulators (endorphins) (see Figure 42–15). POMC is synthesized as a precursor molecule of 285 amino acids and is processed differently in various regions of the pituitary.

The POMC gene is expressed in the anterior and intermediate lobes of the pituitary. The most conserved sequences between species are within the amino terminal fragment, the ACTH region, and the β-endorphin region. POMC or related products are found in several other vertebrate tissues, including the brain, placenta, gastrointestinal tract, reproductive tract, lung, and lymphocytes.

The POMC protein is processed differently in the anterior lobe than in the intermediate lobe. The intermediate lobe of the pituitary is rudimentary in adult humans, but it is active in human fetuses and in pregnant women during late gestation and is also active in many animal species. Processing of the POMC protein in the peripheral tissues (gut, placenta, male reproductive tract) resem-

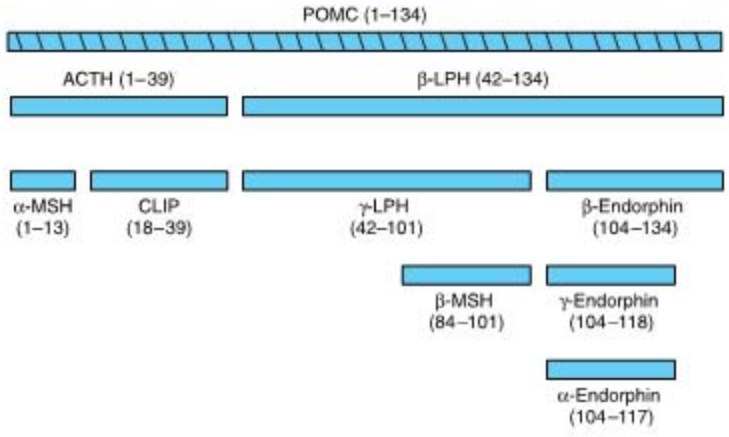


***Figure 42–15.*** Products of pro-opiomelanocortin (POMC) cleavage. (MSH, melanocyte-stimulating hormone; CLIP, corticotropin-like intermediate lobe peptide; LPH, lipotropin.)

bles that in the intermediate lobe. There are three basic peptide groups: (1) ACTH, which can give rise to α-MSH and corticotropin-like intermediate lobe peptide (CLIP); (2) β-lipotropin (β-LPH), which can yield γ-LPH, β-MSH, and β-endorphin (and thus α- and γ-endorphins); and (3) a large amino terminal peptide, which generates γ-MSH. The diversity of these products is due to the many dibasic amino acid clusters that are potential cleavage sites for trypsin-like enzymes. Each of the peptides mentioned is preceded by Lys-Arg, Arg-Lys, Arg-Arg, or Lys-Lys residues. After the prehormone segment is cleaved, the next cleavage, in both anterior and intermediate lobes, is between ACTH and β-LPH, resulting in an amino terminal peptide with ACTH and a β-LPH segment (Figure 42–15). $ACTH_{1-39}$ is subsequently cleaved from the amino terminal peptide, and in the anterior lobe essentially no further cleavages occur. In the intermediate lobe, $ACTH_{1-39}$ is cleaved into α-MSH (residues 1–13) and CLIP (18–39); β-LPH (42–134) is converted to γ-LPH (42–101) and β-endorphin (104–134). β-MSH (84–101) is derived from γ-LPH.

There are extensive additional tissue-specific modifications of these peptides that affect activity. These modifications include phosphorylation, acetylation, glycosylation, and amidation.

## THERE IS VARIATION IN THE STORAGE & SECRETION OF HORMONES

As mentioned above, the steroid hormones and $1,25(OH)_2$-$D_3$ are synthesized in their final active form. They are also secreted as they are made, and thus there is no intracellular reservoir of these hormones. The catecholamines, also synthesized in active form, are stored in granules in the chromaffin cells in the adrenal medulla. In response to appropriate neural stimulation, these granules are released from the cell through exocytosis, and the catecholamines are released into the circulation. A several-hour reserve supply of catecholamines exists in the chromaffin cells.

Parathyroid hormone also exists in storage vesicles. As much as 80–90% of the proPTH synthesized is degraded before it enters this final storage compartment, especially when $Ca^{2+}$ levels are high in the parathyroid cell (see above). PTH is secreted when $Ca^{2+}$ is low in the parathyroid cells, which contain a several-hour supply of the hormone.

The human pancreas secretes about 40–50 units of insulin daily, which represents about 15–20% of the hormone stored in the B cells. Insulin and the C-peptide (see Figure 42–12) are normally secreted in equimolar amounts. Stimuli such as glucose, which provokes insulin secretion, therefore trigger the processing of proinsulin to insulin as an essential part of the secretory response.

A several-week supply of $T_3$ and $T_4$ exists in the thyroglobulin that is stored in colloid in the lumen of the thyroid follicles. These hormones can be released upon stimulation by TSH. This is the most exaggerated example of a prohormone, as a molecule containing approximately 5000 amino acids must be first synthesized, then degraded, to supply a few molecules of the active hormones $T_4$ and $T_3$.

The diversity in storage and secretion of hormones is illustrated in Table 42–5.

**Table 42–5.** Diversity in the storage of hormones.

| Hormone | Supply Stored in Cell |
|---|---|
| Steroids and 1,25$(OH)_2$-$D_3$ | None |
| Catecholamines and PTH | Hours |
| Insulin | Days |
| $T_3$ and $T_4$ | Weeks |

## SOME HORMONES HAVE PLASMA TRANSPORT PROTEINS

The class I hormones are hydrophobic in chemical nature and thus are not very soluble in plasma. These hormones, principally the steroids and thyroid hormones, have specialized plasma transport proteins that serve several purposes. First, these proteins circumvent the solubility problem and thereby deliver the hormone to the target cell. They also provide a circulating reservoir of the hormone that can be substantial, as in the case of the thyroid hormones. Hormones, when bound to the transport proteins, cannot be metabolized, thereby prolonging their plasma half-life ($t_{1/2}$). The binding affinity of a given hormone to its transporter determines the bound versus free ratio of the hormone. This is important because only the free form of a hormone is biologically active. In general, the concentration of free hormone in plasma is very low, in the range of $10^{-15}$ to $10^{-9}$ mol/L. It is important to distinguish between plasma transport proteins and hormone receptors. Both bind hormones but with very different characteristics (Table 42–6).

The hydrophilic hormones—generally class II and of peptide structure—are freely soluble in plasma and do not require transport proteins. Hormones such as insulin, growth hormone, ACTH, and TSH circulate in the free, active form and have very short plasma half-lives. A notable exception is IGF-I, which is transported bound to members of a family of binding proteins.

**Table 42–6.** Comparison of receptors with transport proteins.

| Feature | Receptors | Transport Proteins |
|---|---|---|
| Concentration | Very low (thousands/cell) | Very high (billions/µL) |
| Binding affinity | High (pmol/L to nmol/L range) | Low (µmol/L range) |
| Binding specificity | Very high | Low |
| Saturability | Yes | No |
| Reversibility | Yes | Yes |
| Signal transduction | Yes | No |

### Thyroid Hormones Are Transported by Thyroid-Binding Globulin

Many of the principles discussed above are illustrated in a discussion of thyroid-binding proteins. One-half to two-thirds of $T_4$ and $T_3$ in the body is in an extrathyroidal reservoir. Most of this circulates in bound form, ie, bound to a specific binding protein, **thyroxine-binding globulin (TBG).** TBG, a glycoprotein with a molecular mass of 50 kDa, binds $T_4$ and $T_3$ and has the capacity to bind 20 µg/dL of plasma. Under normal circumstances, TBG binds—noncovalently—nearly all of the $T_4$ and $T_3$ in plasma, and it binds $T_4$ with greater affinity than $T_3$ (Table 42–7). The plasma half-life of $T_4$ is correspondingly four to five times that of $T_3$. The small, unbound (free) fraction is responsible for the biologic activity. Thus, in spite of the great difference in total amount, the free fraction of $T_3$ approximates that of $T_4$, and given that $T_3$ is intrinsically more active than $T_4$, most biologic activity is attributed to $T_3$. TBG does not bind any other hormones.

### Glucocorticoids Are Transported by Corticosteroid-Binding Globulin

Hydrocortisone (cortisol) also circulates in plasma in protein-bound and free forms. The main plasma binding protein is an α-globulin called **transcortin,** or **corticosteroid-binding globulin (CBG).** CBG is produced in the liver, and its synthesis, like that of TBG, is increased by estrogens. CBG binds most of the hormone when plasma cortisol levels are within the normal range; much smaller amounts of cortisol are bound to albumin. The avidity of binding helps determine the biologic half-lives of various glucocorticoids. Cortisol binds tightly to CBG and has a $t_{1/2}$ of 1.5–2 hours, while corticosterone, which binds less tightly, has a $t_{1/2}$ of less than 1 hour (Table 42–8). The unbound (free) cortisol constitutes about 8% of the total and represents the biologically active fraction. Binding to CBG is not restricted to glucocorticoids. Deoxycorticosterone and

**Table 42–7.** Comparison of $T_4$ and $T_3$ in plasma.

| | Total Hormone (µg/dL) | Free Hormone | | | $t_{1/2}$ in Blood (days) |
|---|---|---|---|---|---|
| | | Percent of Total | ng/dL | Molarity | |
| $T_4$ | 8 | 0.03 | ~2.24 | $3.0 \times 10^{-11}$ | 6.5 |
| $T_3$ | 0.15 | 0.3 | ~0.4 | $\sim 0.6 \times 10^{-11}$ | 1.5 |

***Table 42–8.*** Approximate affinities of steroids for serum-binding proteins.

| | SHBG[1] | CBG[1] |
|---|---|---|
| Dihydrotestosterone | 1 | > 100 |
| Testosterone | 2 | > 100 |
| Estradiol | 5 | > 10 |
| Estrone | > 10 | > 100 |
| Progesterone | > 100 | ~ 2 |
| Cortisol | > 100 | ~ 3 |
| Corticosterone | > 100 | ~ 5 |

[1]Affinity expressed as $K_d$ (nmol/L).

progesterone interact with CBG with sufficient affinity to compete for cortisol binding. Aldosterone, the most potent natural mineralocorticoid, does not have a specific plasma transport protein. Gonadal steroids bind very weakly to CBG (Table 42–8).

## Gonadal Steroids Are Transported by Sex Hormone-Binding Globulin

Most mammals, humans included, have a plasma β-globulin that binds testosterone with specificity, relatively high affinity, and limited capacity (Table 42–8). This protein, usually called **sex hormone-binding globulin (SHBG)** or testosterone-estrogen-binding globulin (TEBG), is produced in the liver. Its production is increased by estrogens (women have twice the serum concentration of SHBG as men), certain types of liver disease, and hyperthyroidism; it is decreased by androgens, advancing age, and hypothyroidism. Many of these conditions also affect the production of CBG and TBG. Since SHBG and albumin bind 97–99% of circulating testosterone, only a small fraction of the hormone in circulation is in the free (biologically active) form. The primary function of SHBG may be to restrict the free concentration of testosterone in the serum. Testosterone binds to SHBG with higher affinity than does estradiol (Table 42–8). Therefore, a change in the level of SHBG causes a greater change in the free testosterone level than in the free estradiol level.

Estrogens are bound to SHBG and progestins to CBG. SHBG binds estradiol about five times less avidly than it binds testosterone or DHT, while progesterone and cortisol have little affinity for this protein (Table 42–8). In contrast, progesterone and cortisol bind with nearly equal affinity to CBG, which in turn has little avidity for estradiol and even less for testosterone, DHT, or estrone.

These binding proteins also provide a circulating reservoir of hormone, and because of the relatively large binding capacity they probably buffer against sudden changes in the plasma level. Because the metabolic clearance rates of these steroids are inversely related to the affinity of their binding to SHBG, estrone is cleared more rapidly than estradiol, which in turn is cleared more rapidly than testosterone or DHT.

## SUMMARY

- The presence of a specific receptor defines the target cells for a given hormone.
- Receptors are proteins that bind specific hormones and generate an intracellular signal (receptor-effector coupling).
- Some hormones have intracellular receptors; others bind to receptors on the plasma membrane.
- Hormones are synthesized from a number of precursor molecules, including cholesterol, tyrosine per se, and all the constituent amino acids of peptides and proteins.
- A number of modification processes alter the activity of hormones. For example, many hormones are synthesized from larger precursor molecules.
- The complement of enzymes in a particular cell type allows for the production of a specific class of steroid hormone.
- Most of the lipid-soluble hormones are bound to rather specific plasma transport proteins.

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# Hormone Action & Signal Transduction

43

*Daryl K. Granner, MD*

## BIOMEDICAL IMPORTANCE

The homeostatic adaptations an organism makes to a constantly changing environment are in large part accomplished through alterations of the activity and amount of proteins. Hormones provide a major means of facilitating these changes. A hormone-receptor interaction results in generation of an intracellular signal that can either regulate the activity of a select set of genes, thereby altering the amount of certain proteins in the target cell, or affect the activity of specific proteins, including enzymes and transporter or channel proteins. The signal can influence the location of proteins in the cell and can affect general processes such as protein synthesis, cell growth, and replication, perhaps through effects on gene expression. Other signaling molecules—including cytokines, interleukins, growth factors, and metabolites—use some of the same general mechanisms and signal transduction pathways. Excessive, deficient, or inappropriate production and release of hormones and of these other regulatory molecules are major causes of disease. Many pharmacotherapeutic agents are aimed at correcting or otherwise influencing the pathways discussed in this chapter.

## HORMONES TRANSDUCE SIGNALS TO AFFECT HOMEOSTATIC MECHANISMS

The general steps involved in producing a coordinated response to a particular stimulus are illustrated in Figure 43–1. The stimulus can be a challenge or a threat to the organism, to an organ, or to the integrity of a single cell within that organism. Recognition of the stimulus is the first step in the adaptive response. At the organismic level, this generally involves the nervous system and the special senses (sight, hearing, pain, smell, touch). At the organismic or cellular level, recognition involves physicochemical factors such as pH, $O_2$ tension, temperature, nutrient supply, noxious metabolites, and osmolarity. Appropriate recognition results in the release of one or more hormones that will govern generation of the necessary adaptive response. For purposes of this discussion, the hormones are categorized as described in Chapter 42, ie, based on the location of their specific cellular receptors and the type of signals generated. Group I hormones interact with an intracellular receptor and group II hormones with receptor recognition sites located on the extracellular surface of the plasma membrane of target cells. The cytokines, interleukins, and growth factors should also be considered in this latter category. These molecules, of critical importance in homeostatic adaptation, are hormones in the sense that they are produced in specific cells, have the equivalent of autocrine, paracrine, and endocrine actions, bind to cell surface receptors, and activate many of the same signal transduction pathways employed by the more traditional group II hormones.

## SIGNAL GENERATION

### The Ligand-Receptor Complex Is the Signal for Group I Hormones

The lipophilic group I hormones diffuse through the plasma membrane of all cells but only encounter their specific, high-affinity intracellular receptors in target cells. These receptors can be located in the cytoplasm or in the nucleus of target cells. The hormone-receptor complex first undergoes an **activation reaction.** As shown in Figure 43–2, receptor activation occurs by at least two mechanisms. For example, glucocorticoids diffuse across the plasma membrane and encounter their cognate receptor in the cytoplasm of target cells. Ligand-receptor binding results in the dissociation of heat shock protein 90 (hsp90) from the receptor. This step appears to be necessary for subsequent nuclear localization of the glucocorticoid receptor. This receptor also contains nuclear localization sequences that assist in the translocation from cytoplasm to nucleus. The now activated receptor moves into the nucleus (Figure 43–2) and binds with high affinity to a specific DNA sequence called the **hormone response element (HRE).** In the case illustrated, this is a glucocorticoid response element, or GRE. Consensus sequences for HREs are shown in Table 43–1. The DNA-bound, liganded receptor serves as a high-affinity binding site for

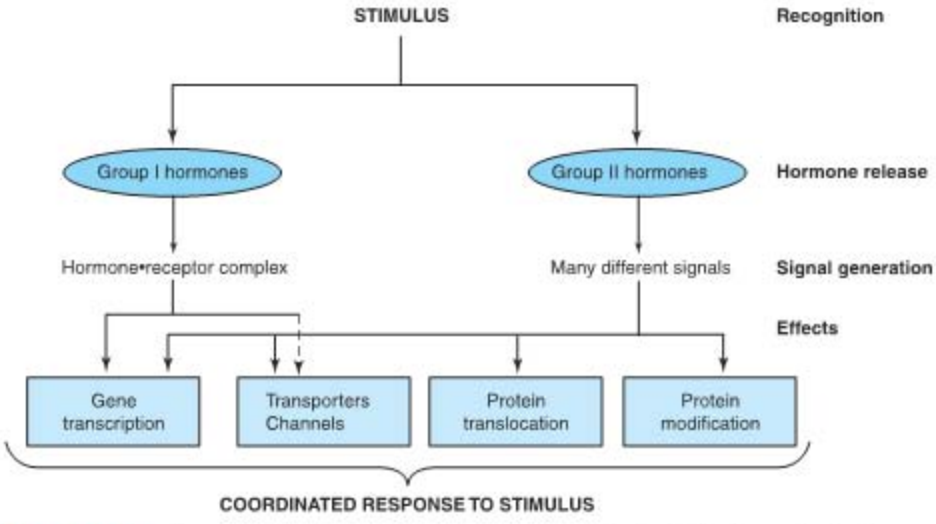


***Figure 43–1.*** Hormonal involvement in responses to a stimulus. A challenge to the integrity of the organism elicits a response that includes the release of one or more hormones. These hormones generate signals at or within target cells, and these signals regulate a variety of biologic processes which provide for a coordinated response to the stimulus or challenge. See Figure 43–8 for a specific example.

one or more coactivator proteins, and accelerated gene transcription typically ensues when this occurs. By contrast, certain hormones such as the thyroid hormones and retinoids diffuse from the extracellular fluid across the plasma membrane and go directly into the nucleus. In this case, the cognate receptor is already bound to the HRE (the thyroid hormone response element [TRE], in this example). However, this DNA-bound receptor fails to activate transcription because it is complexed with a corepressor. Indeed, this receptor-corepressor complex serves as an active repressor of gene transcription. The association of ligand with these receptors results in dissociation of the corepressor. The liganded receptor is now capable of binding one or more coactivators with high affinity, resulting in the activation of gene transcription. The relationship of hormone receptors to other nuclear receptors and to coregulators is discussed in more detail below.

By selectively affecting gene transcription and the consequent production of appropriate target mRNAs, the amounts of specific proteins are changed and metabolic processes are influenced. The influence of each of these hormones is quite specific; generally, the hormone affects less than 1% of the genes, mRNA, or proteins in a target cell; sometimes only a few are affected. The nuclear actions of steroid, thyroid, and retinoid hormones are quite well defined. Most evidence suggests that these hormones exert their dominant effect on modulating gene transcription, but they—and many of the hormones in the other classes discussed below—can act at any step of the "information pathway" illustrated in Figure 43–3. Direct actions of steroids in the cytoplasm and on various organelles and membranes have also been described.

## GROUP II (PEPTIDE & CATECHOLAMINE) HORMONES HAVE MEMBRANE RECEPTORS & USE INTRACELLULAR MESSENGERS

Many hormones are water-soluble, have no transport proteins (and therefore have a short plasma half-life), and initiate a response by binding to a receptor located in the plasma membrane (see Tables 42–3 and 42–4). The mechanism of action of this group of hormones can best be discussed in terms of the **intracellular signals** they generate. These signals include cAMP (cyclic AMP; 3′,5′-adenylic acid; see Figure 18–5), a nucleotide derived from ATP through the action of adenylyl cyclase; cGMP, a nucleotide formed by guanylyl cyclase; $Ca^{2+}$; and phosphatidylinositides. Many of these second messengers affect gene transcription, as described in the previous paragraph; but they also influ-

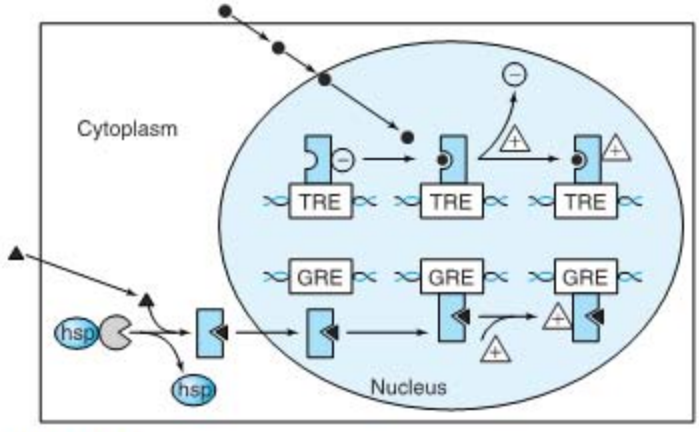


***Figure 43–2.*** Regulation of gene expression by class I hormones. Steroid hormones readily gain access to the cytoplasmic compartment of target cells. Glucocorticoid hormones (solid triangles) encounter their cognate receptor in the cytoplasm, where it exists in a complex with heat shock protein 90 (hsp). Ligand binding causes dissociation of hsp and a conformational change of the receptor. The receptor•ligand complex then traverses the nuclear membrane and binds to DNA with specificity and high affinity at a glucocorticoid response element (GRE). This event triggers the assembly of a number of transcription coregulators (△), and enhanced transcription ensues. By contrast, thyroid hormones and retinoic acid (●) directly enter the nucleus, where their cognate receptors are already bound to the appropriate response elements with an associated transcription repressor complex (⊖). This complex, which consists of molecules such as N-CoR or SMRT (see Table 43–6) in the absence of ligand, actively inhibits transcription. Ligand binding results in dissociation of the repressor complex from the receptor, allowing an activator complex to assemble. The gene is then actively transcribed.

ence a variety of other biologic processes, as shown in Figure 43–1.

## G Protein-Coupled Receptors (GPCR)

Many of the group II hormones bind to receptors that couple to effectors through a GTP-binding protein intermediary. These receptors typically have seven hydrophobic plasma membrane-spanning domains. This is illustrated by the seven interconnected cylinders extending through the lipid bilayer in Figure 43–4. Receptors of this class, which signal through guanine nucleotide-bound protein intermediates, are known as **G protein-coupled receptors,** or **GPCRs.** To date, over 130 G protein-linked receptor genes have been cloned from various mammalian species. A wide variety of responses are mediated by the GPCRs.

## cAMP Is the Intracellular Signal for Many Responses

Cyclic AMP was the first intracellular signal identified in mammalian cells. Several components comprise a system for the generation, degradation, and action of cAMP.

### A. Adenylyl Cyclase

Different peptide hormones can either stimulate (s) or inhibit (i) the production of cAMP from adenylyl cy-

***Table 43–1.*** The DNA sequences of several hormone response elements (HREs).[1]

| Hormone or Effector | HRE | DNA Sequence |
|---|---|---|
| Glucocorticoids<br>Progestins<br>Mineralocorticoids<br>Androgens | GRE<br>PRE<br>MRE<br>ARE | GGTACA NNN TGTTCT |
| Estrogens | ERE | AGGTCA --- TGA/TCCT |
| Thyroid hormone<br>Retinoic acid<br>Vitamin D | TRE<br>RARE<br>VDRE | AGGTCA N3,4,5, AGGTCA |
| cAMP | CRE | TGACGTCA |

[1]Letters indicate nucleotide; N means any one of the four can be used in that position. The arrows pointing in opposite directions illustrate the slightly imperfect inverted palindromes present in many HREs; in some cases these are called "half binding sites" because each binds one monomer of the receptor. The GRE, PRE, MRE, and ARE consist of the same DNA sequence. Specificity may be conferred by the intracellular concentration of the ligand or hormone receptor, by flanking DNA sequences not included in the consensus, or by other accessory elements. A second group of HREs includes those for thyroid hormones, estrogens, retinoic acid, and vitamin D. These HREs are similar except for the orientation and spacing between the half palindromes. Spacing determines the hormone specificity. VDRE (N=3), TRE (N=4), and RARE (N=5) bind to direct repeats rather than to inverted repeats. Another member of the steroid receptor superfamily, the retinoid X receptor (RXR), forms heterodimers with VDR, TR, and RARE, and these constitute the *trans*-acting factors. cAMP affects gene transcription through the CRE.

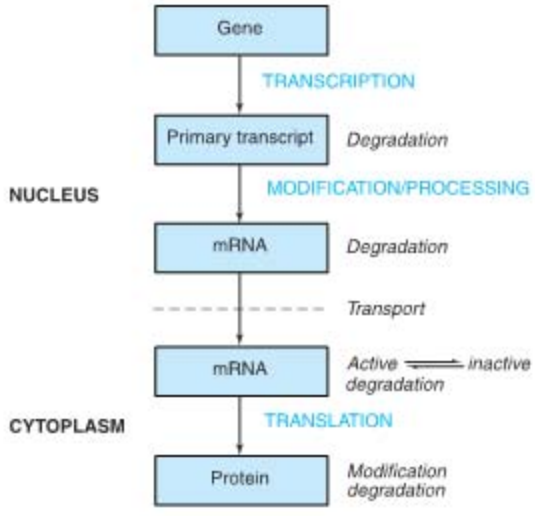


***Figure 43–3.*** The "information pathway." Information flows from the gene to the primary transcript to mRNA to protein. Hormones can affect any of the steps involved and can affect the rates of processing, degradation, or modification of the various products.

clase, which is encoded by at least nine different genes (Table 43–2). Two parallel systems, a stimulatory (s) one and an inhibitory (i) one, converge upon a single catalytic molecule (C). Each consists of a receptor, $R_s$ or $R_i$, and a regulatory complex, $G_s$ and $G_i$. $G_s$ and $G_i$ are each trimers composed of α, β, and γ subunits. Because the α subunit in $G_s$ differs from that in $G_i$, the proteins, which are distinct gene products, are designated $\alpha_s$ and $\alpha_i$. The α subunits bind guanine nucleotides. The β and γ subunits are always associated (βγ) and appear to function as a heterodimer. The binding of a hormone to $R_s$ or $R_i$ results in a receptor-mediated activation of G, which entails the exchange of GDP by GTP on α and the concomitant dissociation of βγ from α.

The $\alpha_s$ protein has intrinsic GTPase activity. The active form, $\alpha_s$•GTP, is inactivated upon hydrolysis of the GTP to GDP; the trimeric $G_s$ complex ($\alpha\beta\gamma$) is then re-formed and is ready for another cycle of activation. Cholera and pertussis toxins catalyze the ADP-ribosylation of $\alpha_s$ and $\alpha_{i\text{-}2}$ (see Table 43–3), respectively. In the case of $\alpha_s$, this modification disrupts the intrinsic GTP-ase activity; thus, $\alpha_s$ cannot reassociate with βγ and is therefore irreversibly activated. ADP-ribosylation of $\alpha_{i\text{-}2}$ prevents the dissociation of $\alpha_{i\text{-}2}$ from βγ, and free $\alpha_{i\text{-}2}$ thus cannot be formed. $\alpha_s$ activity in such cells is therefore unopposed.

There is a large family of G proteins, and these are part of the superfamily of GTPases. The G protein family is classified according to sequence homology into four subfamilies, as illustrated in Table 43–3. There are 21 α, 5 β, and 8 γ subunit genes. Various combinations of these subunits provide a large number of possible αβγ and cyclase complexes.

The α subunits and the βγ complex have actions independent of those on adenylyl cyclase (see Figure 43–4 and Table 43–3). Some forms of $\alpha_i$ stimulate $K^+$ channels and inhibit $Ca^{2+}$ channels, and some $\alpha_s$ molecules have the opposite effects. Members of the $G_q$ family activate the phospholipase C group of enzymes. The βγ complexes have been associated with $K^+$ channel stimulation and phospholipase C activation. G proteins are involved in many important biologic processes in addition to hormone action. Notable examples include olfaction ($\alpha_{OLF}$) and vision ($\alpha_t$). Some examples are listed in Table 43–3. GPCRs are implicated in a number of diseases and are major targets for pharmaceutical agents.

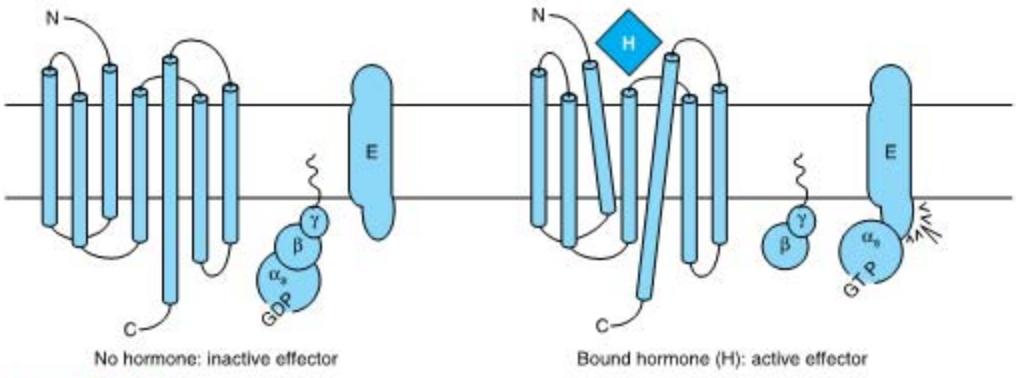


***Figure 43–4.*** Components of the hormone receptor–G protein effector system. Receptors that couple to effectors through G proteins (GPCR) typically have seven membrane-spanning domains. In the absence of hormone (left), the heterotrimeric G-protein complex (α, β, γ) is in an inactive guanosine diphosphate (GDP)-bound form and is probably not associated with the receptor. This complex is anchored to the plasma membrane through prenylated groups on the βγ subunits (wavy lines) and perhaps by myristoylated groups on α subunits (not shown). On binding of hormone (H) to the receptor, there is a presumed conformational change of the receptor—as indicated by the tilted membrane spanning domains—and activation of the G-protein complex. This results from the exchange of GDP with guanosine triphosphate (GTP) on the α subunit, after which α and βγ dissociate. The α subunit binds to and activates the effector (E). E can be adenylyl cyclase, $Ca^{2+}$, $Na^+$, or $Cl^-$ channels ($\alpha_s$), or it could be a $K^+$ channel ($\alpha_i$), phospholipase Cβ ($\alpha_q$), or cGMP phosphodiesterase ($\alpha_t$). The βγ subunit can also have direct actions on E. (Modified and reproduced, with permission, from Granner DK in: *Principles and Practice of Endocrinology and Metabolism*, 3rd ed. Becker KL [editor]. Lippincott, 2000.)

***Table 43–2.*** Subclassification of group II.A hormones.

| Hormones That Stimulate Adenylyl Cyclase ($H_s$) | Hormones That Inhibit Adenylyl Cyclase ($H_i$) |
|---|---|
| ACTH | Acetylcholine |
| ADH | $\alpha_2$-Adrenergics |
| β-Adrenergics | Angiotensin II |
| Calcitonin | Somatostatin |
| CRH | |
| FSH | |
| Glucagon | |
| hCG | |
| LH | |
| LPH | |
| MSH | |
| PTH | |
| TSH | |

## B. Protein Kinase

In prokaryotic cells, cAMP binds to a specific protein called catabolite regulatory protein (CRP) that binds directly to DNA and influences gene expression. In eukaryotic cells, cAMP binds to a protein kinase called **protein kinase A (PKA)** that is a heterotetrameric molecule consisting of two regulatory subunits (R) and two catalytic subunits (C). cAMP binding results in the following reaction:

$$4\ \text{cAMP} + R_2C_2 \rightleftharpoons R_2 \cdot (4\ \text{cAMP}) + 2C$$

The $R_2C_2$ complex has no enzymatic activity, but the binding of cAMP by R dissociates R from C, thereby activating the latter (Figure 43–5). The active C subunit catalyzes the transfer of the γ phosphate of ATP to a serine or threonine residue in a variety of proteins. The consensus phosphorylation sites are -Arg-Arg/Lys-X-Ser/Thr- and -Arg-Lys-X-X-Ser-, where X can be any amino acid.

Protein kinase activities were originally described as being "cAMP-dependent" or "cAMP-independent." This

***Table 43–3.*** Classes and functions of selected G proteins.[1,2]

| Class or Type | Stimulus | Effector | Effect |
|---|---|---|---|
| $G_s$ | | | |
| $\alpha_s$ | Glucagon, β-adrenergics | ↑ Adenylyl cyclase<br>↑ Cardiac $Ca^{2+}$, $Cl^-$, and $Na^+$ channels | Gluconeogenesis, lipolysis, glycogenolysis |
| $\alpha_{olf}$ | Odorant | ↑ Adenylyl cyclase | Olfaction |
| $G_i$ | | | |
| $\alpha_{i-1,2,3}$ | Acetylcholine,<br>$\alpha_2$-adrenergics<br>$M_2$ cholinergics | ↓ Adenylyl cyclase<br>↑ Potassium channels<br>↓ Calcium channels | Slowed heart rate |
| $\alpha_o$ | Opioids, endorphins | ↑ Potassium channels | Neuronal electrical activity |
| $\alpha_t$ | Light | ↑ cGMP phosphodiesterase | Vision |
| $G_q$ | | | |
| $\alpha_q$ | $M_1$ cholinergics<br>$\alpha_1$-Adrenergics | ↑ Phospholipase C-β1 | ↑ Muscle contraction and ↑ Blood pressure |
| $\alpha_{11}$ | $\alpha_1$-Adrenergics | ↑ Phospholipase c-β2 | |
| $G_{12}$ | | | |
| $\alpha_{12}$ | ? | $Cl^-$ channel | ? |

[1]Modified and reproduced, with permission, from Granner DK in: *Principles and Practice of Endocrinology and Metabolism*, 3rd ed. Becker KL (editor). Lippincott, 2000.

[2]The four major classes or families of mammalian G proteins ($G_s$, $G_i$, $G_q$, and $G_{12}$) are based on protein sequence homology. Representative members of each are shown, along with known stimuli, effectors, and well-defined biologic effects. Nine isoforms of adenylyl cyclase have been identified (isoforms I–IX). All isoforms are stimulated by $\alpha_s$; $\alpha_i$ isoforms inhibit types V and VI, and $\alpha_o$ inhibits types I and V. At least 16 different α subunits have been identified.

classification has changed, as protein phosphorylation is now recognized as being a major regulatory mechanism. Several hundred protein kinases have now been described. The kinases are related in sequence and structure within the catalytic domain, but each is a unique molecule with considerable variability with respect to subunit composition, molecular weight, autophosphorylation, $K_m$ for ATP, and substrate specificity.

## C. Phosphoproteins

The effects of cAMP in eukaryotic cells are all thought to be mediated by protein phosphorylation-dephosphorylation, principally on serine and threonine residues. The control of any of the effects of cAMP, including such diverse processes as steroidogenesis, secretion, ion transport, carbohydrate and fat metabolism, enzyme induction, gene regulation, synaptic transmission, and cell growth and replication, could be conferred by a specific protein kinase, by a specific phosphatase, or by specific substrates for phosphorylation. These substrates help define a target tissue and are involved in defining the extent of a particular response within a given cell. For example, the effects of cAMP on gene transcription are mediated by the protein **cyclic AMP response element binding protein (CREB).** CREB binds to a cAMP responsive element (CRE) (see Table 43–1) in its nonphosphorylated state and is a weak activator of transcription. When phosphorylated by PKA, CREB binds the coactivator **CREB-binding protein CBP/p300** (see below) and as a result is a much more potent transcription activator.

## D. Phosphodiesterases

Actions caused by hormones that increase cAMP concentration can be terminated in a number of ways, including the hydrolysis of cAMP to 5′-AMP by phosphodiesterases (see Figure 43–5). The presence of these hydrolytic enzymes ensures a rapid turnover of the signal (cAMP) and hence a rapid termination of the biologic process once the hormonal stimulus is removed. There are at least 11 known members of the phosphodiesterase family of enzymes. These are subject to regulation by their substrates, cAMP and cGMP; by hormones; and by intracellular messengers such as calcium, probably acting through calmodulin. Inhibitors of phosphodiesterase, most notably methylated xanthine derivatives such as caffeine, increase intracellular cAMP and mimic or prolong the actions of hormones through this signal.

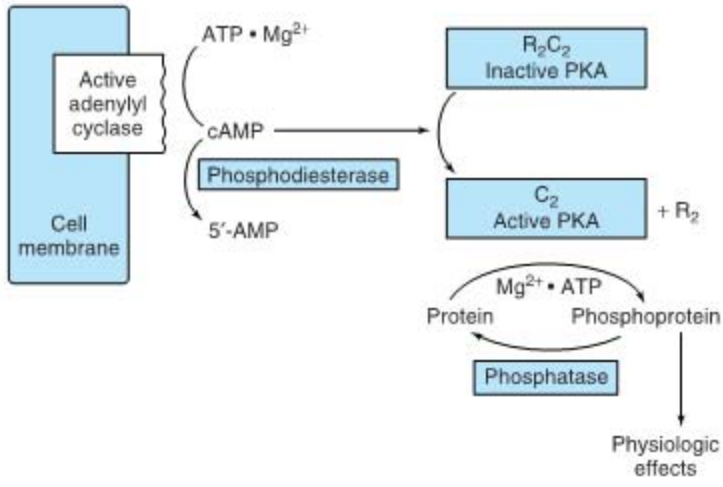


***Figure 43–5.*** Hormonal regulation of cellular processes through cAMP-dependent protein kinase (PKA). PKA exists in an inactive form as an $R_2C_2$ heterotetramer consisting of two regulatory and two catalytic subunits. The cAMP generated by the action of adenylyl cyclase (activated as shown in Figure 43–4) binds to the regulatory (R) subunit of PKA. This results in dissociation of the regulatory and catalytic subunits and activation of the latter. The active catalytic subunits phosphorylate a number of target proteins on serine and threonine residues. Phosphatases remove phosphate from these residues and thus terminate the physiologic response. A phosphodiesterase can also terminate the response by converting cAMP to 5′-AMP.

### E. Phosphoprotein Phosphatases

Given the importance of protein phosphorylation, it is not surprising that regulation of the protein dephosphorylation reaction is another important control mechanism (see Figure 43–5). The phosphoprotein phosphatases are themselves subject to regulation by phosphorylation-dephosphorylation reactions and by a variety of other mechanisms, such as protein-protein interactions. In fact, the substrate specificity of the phosphoserine-phosphothreonine phosphatases may be dictated by distinct regulatory subunits whose binding is regulated hormonally. The best-studied role of regulation by the dephosphorylation of proteins is that of glycogen metabolism in muscle. Two major types of phosphoserine-phosphothreonine phosphatases have been described. Type I preferentially dephosphorylates the β subunit of phosphorylase kinase, whereas type II dephosphorylates the α subunit. Type I phosphatase is implicated in the regulation of glycogen synthase, phosphorylase, and phosphorylase kinase. This phosphatase is itself regulated by phosphorylation of certain of its subunits, and these reactions are reversed by the action of one of the type II phosphatases. In addition, two heat-stable protein inhibitors regulate type I phosphatase activity. Inhibitor-1 is phosphorylated and activated by cAMP-dependent protein kinases; and inhibitor-2, which may be a subunit of the inactive phosphatase, is also phosphorylated, possibly by glycogen synthase kinase-3.

## cGMP Is Also an Intracellular Signal

Cyclic GMP is made from GTP by the enzyme guanylyl cyclase, which exists in soluble and membrane-bound forms. Each of these isozymes has unique physiologic properties. The atriopeptins, a family of peptides produced in cardiac atrial tissues, cause natriuresis, diuresis, vasodilation, and inhibition of aldosterone secretion. These peptides (eg, atrial natriuretic factor) bind to and activate the membrane-bound form of guanylyl cyclase. This results in an increase of cGMP by as much as 50-fold in some cases, and this is thought to mediate the effects mentioned above. Other evidence links cGMP to vasodilation. A series of compounds, including nitroprusside, nitroglycerin, nitric oxide, sodium nitrite, and sodium azide, all cause smooth muscle re-

laxation and are potent vasodilators. These agents increase cGMP by activating the soluble form of guanylyl cyclase, and inhibitors of cGMP phosphodiesterase (the drug sildenafil [Viagra], for example) enhance and prolong these responses. The increased cGMP activates cGMP-dependent protein kinase (PKG), which in turn phosphorylates a number of smooth muscle proteins. Presumably, this is involved in relaxation of smooth muscle and vasodilation.

## Several Hormones Act Through Calcium or Phosphatidylinositols

Ionized calcium is an important regulator of a variety of cellular processes, including muscle contraction, stimulus-secretion coupling, the blood clotting cascade, enzyme activity, and membrane excitability. It is also an intracellular messenger of hormone action.

### A. Calcium Metabolism

The extracellular calcium ($Ca^{2+}$) concentration is about 5 mmol/L and is very rigidly controlled. Although substantial amounts of calcium are associated with intracellular organelles such as mitochondria and the endoplasmic reticulum, the intracellular concentration of free or ionized calcium ($Ca^{2+}$) is very low: 0.05–10 µmol/L. In spite of this large concentration gradient and a favorable transmembrane electrical gradient, $Ca^{2+}$ is restrained from entering the cell. A considerable amount of energy is expended to ensure that the intracellular $Ca^{2+}$ is controlled, as a prolonged elevation of $Ca^{2+}$ in the cell is very toxic. A $Na^+/Ca^{2+}$ exchange mechanism that has a high capacity but low affinity pumps $Ca^{2+}$ out of cells. There also is a $Ca^{2+}$/proton ATPase-dependent pump that extrudes $Ca^{2+}$ in exchange for $H^+$. This has a high affinity for $Ca^{2+}$ but a low capacity and is probably responsible for fine-tuning cytosolic $Ca^{2+}$. Furthermore, $Ca^{2+}$ ATPases pump $Ca^{2+}$ from the cytosol to the lumen of the endoplasmic reticulum. There are three ways of changing cytosolic $Ca^{2+}$: (1) Certain hormones (class II.C, Table 42–3) by binding to receptors that are themselves $Ca^{2+}$ channels, enhance membrane permeability to $Ca^{2+}$ and thereby increase $Ca^{2+}$ influx. (2) Hormones also indirectly promote $Ca^{2+}$ influx by modulating the membrane potential at the plasma membrane. Membrane depolarization opens voltage-gated $Ca^{2+}$ channels and allows for $Ca^{2+}$ influx. (3) $Ca^{2+}$ can be mobilized from the endoplasmic reticulum, and possibly from mitochondrial pools.

An important observation linking $Ca^{2+}$ to hormone action involved the definition of the intracellular targets of $Ca^{2+}$ action. The discovery of a $Ca^{2+}$-dependent regulator of phosphodiesterase activity provided the basis for a broad understanding of how $Ca^{2+}$ and cAMP interact within cells.

### B. Calmodulin

The calcium-dependent regulatory protein is calmodulin, a 17-kDa protein that is homologous to the muscle protein troponin C in structure and function. Calmodulin has four $Ca^{2+}$ binding sites, and full occupancy of these sites leads to a marked conformational change, which allows calmodulin to activate enzymes and ion channels. The interaction of $Ca^{2+}$ with calmodulin (with the resultant change of activity of the latter) is conceptually similar to the binding of cAMP to PKA and the subsequent activation of this molecule. Calmodulin can be one of numerous subunits of complex proteins and is particularly involved in regulating various kinases and enzymes of cyclic nucleotide generation and degradation. A partial list of the enzymes regulated directly or indirectly by $Ca^{2+}$, probably through calmodulin, is presented in Table 43–4.

In addition to its effects on enzymes and ion transport, $Ca^{2+}$/calmodulin regulates the activity of many structural elements in cells. These include the actin-myosin complex of smooth muscle, which is under β-adrenergic control, and various microfilament-mediated processes in noncontractile cells, including cell motility, cell conformation changes, mitosis, granule release, and endocytosis.

### C. Calcium Is a Mediator of Hormone Action

A role for $Ca^{2+}$ in hormone action is suggested by the observations that the effect of many hormones is (1) blunted by $Ca^{2+}$-free media or when intracellular calcium is depleted; (2) can be mimicked by agents that increase cytosolic $Ca^{2+}$, such as the $Ca^{2+}$ ionophore A23187; and (3) influences cellular calcium flux. The regulation of glycogen metabolism in liver by vasopressin and α-adrenergic catecholamines provides a good example. This is shown schematically in Figures 18–6 and 18–7.

***Table 43–4.*** Enzymes and proteins regulated by calcium or calmodulin.

| |
|---|
| Adenylyl cyclase |
| $Ca^{2+}$-dependent protein kinases |
| $Ca^{2+}$-$Mg^{2+}$ ATPase |
| $Ca^{2+}$-phospholipid-dependent protein kinase |
| Cyclic nucleotide phosphodiesterase |
| Some cytoskeletal proteins |
| Some ion channels (eg, L-type calcium channels) |
| Nitric oxide synthase |
| Phosphorylase kinase |
| Phosphoprotein phosphatase 2B |
| Some receptors (eg, NMDA-type glutamate receptor) |

A number of critical metabolic enzymes are regulated by $Ca^{2+}$, phosphorylation, or both, including glycogen synthase, pyruvate kinase, pyruvate carboxylase, glycerol-3-phosphate dehydrogenase, and pyruvate dehydrogenase.

### D. Phosphatidylinositide Metabolism Affects $Ca^{2+}$-Dependent Hormone Action

Some signal must provide communication between the hormone receptor on the plasma membrane and the intracellular $Ca^{2+}$ reservoirs. This is accomplished by products of phosphatidylinositol metabolism. Cell surface receptors such as those for acetylcholine, antidiuretic hormone, and $\alpha_1$-type catecholamines are, when occupied by their respective ligands, potent activators of phospholipase C. Receptor binding and activation of phospholipase C are coupled by the $G_q$ isoforms (Table 43–3 and Figure 43–6). Phospholipase C catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate to inositol trisphosphate ($IP_3$) and 1,2-diacylglycerol (Figure 43–7). Diacylglycerol is itself capable of activating **protein kinase C (PKC),** the activity of which also depends upon $Ca^{2+}$. $IP_3$, by interacting with a specific intracellular receptor, is an effective releaser of $Ca^{2+}$ from

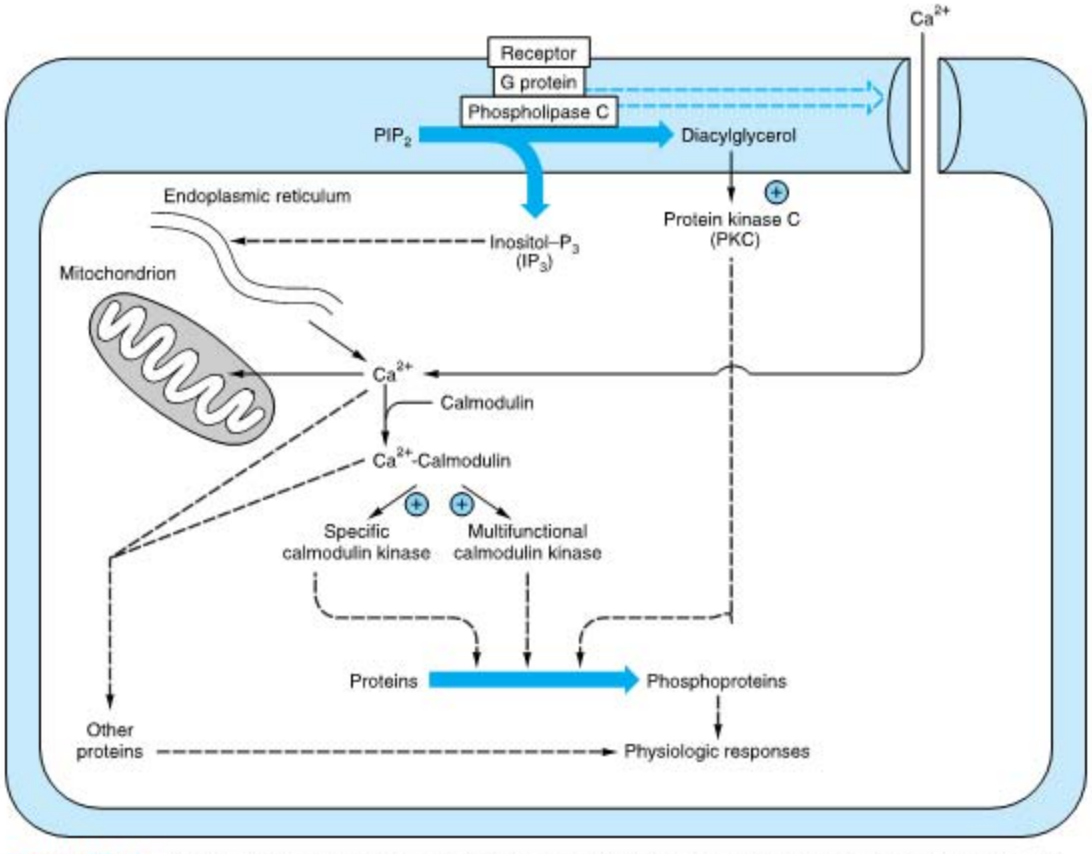


***Figure 43–6.*** Certain hormone-receptor interactions result in the activation of phospholipase C. This appears to involve a specific G protein, which also may activate a calcium channel. Phospholipase C results in generation of inositol trisphosphate ($IP_3$), which liberates stored intracellular $Ca^{2+}$, and diacylglycerol (DAG), a potent activator of protein kinase C (PKC). In this scheme, the activated PKC phosphorylates specific substrates, which then alter physiologic processes. Likewise, the $Ca^{2+}$-calmodulin complex can activate specific kinases, two of which are shown here. These actions result in phosphorylation of substrates, and this leads to altered physiologic responses. This figure also shows that $Ca^{2+}$ can enter cells through voltage- or ligand-gated $Ca^{2+}$ channels. The intracellular $Ca^{2+}$ is also regulated through storage and release by the mitochondria and endoplasmic reticulum. (Courtesy of JH Exton.)

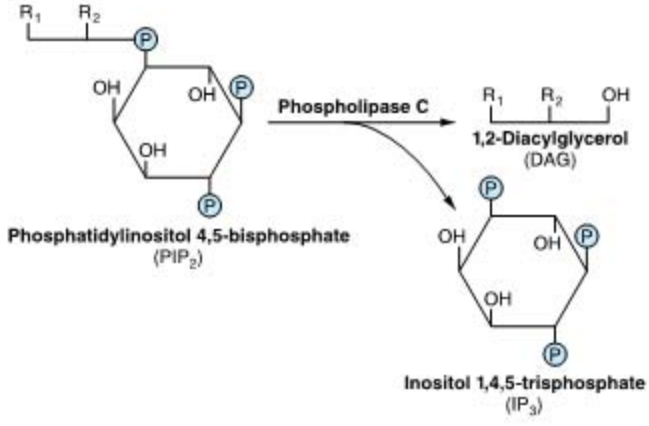


***Figure 43–7.*** Phospholipase C cleaves $PIP_2$ into diacylglycerol and inositol trisphosphate. $R_1$ generally is stearate, and $R_2$ is usually arachidonate. $IP_3$ can be dephosphorylated (to the inactive I-1,4-$P_2$) or phosphorylated (to the potentially active I-1,3,4,5-$P_4$).

intracellular storage sites in the endoplasmic reticulum. Thus, the hydrolysis of phosphatidylinositol 4,5-bisphosphate leads to activation of PKC and promotes an increase of cytoplasmic $Ca^{2+}$. As shown in Figure 43–4, the activation of G proteins can also have a direct action on $Ca^{2+}$ channels. The resulting elevations of cytosolic $Ca^{2+}$ activate $Ca^{2+}$–calmodulin-dependent kinases and many other $Ca^{2+}$–calmodulin-dependent enzymes.

Steroidogenic agents—including ACTH and cAMP in the adrenal cortex; angiotensin II, $K^+$, serotonin, ACTH, and cAMP in the zona glomerulosa of the adrenal; LH in the ovary; and LH and cAMP in the Leydig cells of the testes—have been associated with increased amounts of phosphatidic acid, phosphatidylinositol, and polyphosphoinositides (see Chapter 14) in the respective target tissues. Several other examples could be cited.

The roles that $Ca^{2+}$ and polyphosphoinositide breakdown products might play in hormone action are presented in Figure 43–6. In this scheme the activated protein kinase C can phosphorylate specific substrates, which then alter physiologic processes. Likewise, the $Ca^{2+}$-calmodulin complex can activate specific kinases. These then modify substrates and thereby alter physiologic responses.

## Some Hormones Act Through a Protein Kinase Cascade

Single protein kinases such as PKA, PKC, and $Ca^{2+}$-calmodulin (CaM)-kinases, which result in the phosphorylation of serine and threonine residues in target proteins, play a very important role in hormone action. The discovery that the EGF receptor contains an intrinsic tyrosine kinase activity that is activated by the binding of the ligand EGF was an important breakthrough. The insulin and IGF-I receptors also contain intrinsic ligand-activated tyrosine kinase activity. Several receptors—generally those involved in binding ligands involved in growth control, differentiation, and the inflammatory response—either have intrinsic tyrosine kinase activity or are associated with proteins that are tyrosine kinases. Another distinguishing feature of this class of hormone action is that these kinases preferentially phosphorylate tyrosine residues, and tyrosine phosphorylation is infrequent (< 0.03% of total amino acid phosphorylation) in mammalian cells. A third distinguishing feature is that the ligand-receptor interaction that results in a tyrosine phosphorylation event initiates a cascade that may involve several protein kinases, phosphatases, and other regulatory proteins.

### A. Insulin Transmits Signals by Several Kinase Cascades

The insulin, epidermal growth factor (EGF), and IGF-I receptors have intrinsic protein tyrosine kinase activities located in their cytoplasmic domains. These activities are stimulated when the receptor binds ligand. The receptors are then autophosphorylated on tyrosine residues, and this initiates a complex series of events (summarized in simplified fashion in Figure 43–8). The phosphorylated insulin receptor next phosphorylates insulin receptor substrates (there are at least four of these molecules, called IRS 1–4) on tyrosine residues. Phosphorylated IRS binds to the Src homology 2 (SH2) domains of a variety of proteins that are directly involved in mediating different effects of insulin. One of these proteins, PI-3 kinase, links insulin receptor activation to insulin action through activation of a number of molecules, including the kinase PDK1 (phosphoinositide-dependent kinase-1). This enzyme propagates the signal through several other kinases, including PKB (akt), SKG, and aPKC (see legend to Figure 43–8 for definitions and expanded abbreviations). An alternative

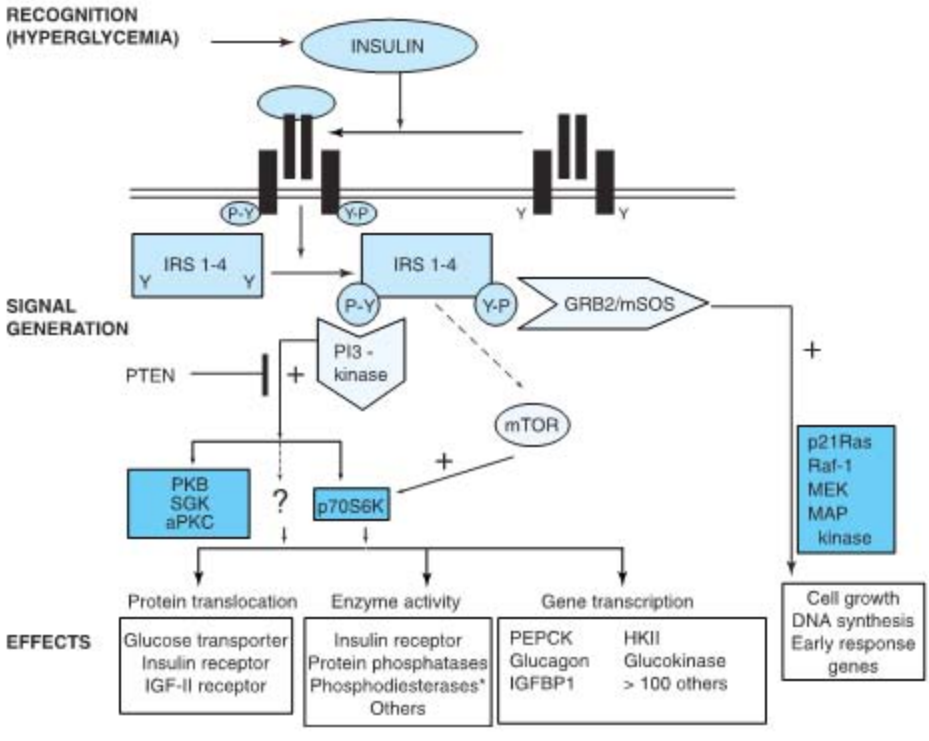


***Figure 43–8.*** Insulin signaling pathways. The insulin signaling pathways provide an excellent example of the "recognition → hormone release → signal generation → effects" paradigm outlined in Figure 43–1. Insulin is released in response to hyperglycemia. Binding of insulin to a target cell-specific plasma membrane receptor results in a cascade of intracellular events. Stimulation of the intrinsic tyrosine kinase activity of the insulin receptor marks the initial event, resulting in increased tyrosine (Y) phosphorylation (Y → Y-P) of the receptor and then one or more of the insulin receptor substrate molecules (IRS 1–4). This increase in phosphotyrosine stimulates the activity of many intracellular molecules such as GTPases, protein kinases, and lipid kinases, all of which play a role in certain metabolic actions of insulin. The two best-described pathways are shown. First, phosphorylation of an IRS molecule (probably IRS-2) results in docking and activation of the lipid kinase, PI-3 kinase, which generates novel inositol lipids that may act as "second messenger" molecules. These, in turn, activate PDK1 and then a variety of downstream signaling molecules, including protein kinase B (PKB or akt), SGK, and aPKC. An alternative pathway involves the activation of p70S6K and perhaps other as yet unidentified kinases. Second, phosphorylation of IRS (probably IRS-1) results in docking of GRB2/mSOS and activation of the small GTPase, p21RAS, which initiates a protein kinase cascade that activates Raf-1, MEK, and the p42/p44 MAP kinase isoforms. These protein kinases are important in the regulation of proliferation and differentiation of several cell types. The mTOR pathway provides an alternative way of activating p70S6K and appears to be involved in nutrient signaling as well as insulin action. Each of these cascades may influence different physiologic processes, as shown. Each of the phosphorylation events is reversible through the action of specific phosphatases. For example, the lipid phosphatase PTEN dephosphorylates the product of the PI-3 kinase reaction, thereby antagonizing the pathway and terminating the signal. Representative effects of major actions of insulin are shown in each of the boxes. The asterisk after phosphodiesterase indicates that insulin indirectly affects the activity of many enzymes by activating phosphodiesterases and reducing intracellular cAMP levels. (IGFBP, insulin-like growth factor binding protein; IRS 1–4, insulin receptor substrate isoforms 1–4); PI-3 kinase, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog deleted on chromosome 10; PKD1, phosphoinositide-dependent kinase; PKB, protein kinase B; SGK, serum and glucocorticoid-regulated kinase; aPKC, atypical protein kinase C; p70S6K, p70 ribosomal protein S6 kinase; mTOR, mammalian target of rapamycin; GRB2, growth factor receptor binding protein 2; mSOS, mammalian son of sevenless; MEK, MAP kinase kinase and ERK kinase; MAP kinase, mitogen-activated protein kinase.)

pathway downstream from PKD1 involves p70S6K and perhaps other as yet unidentified kinases. A second major pathway involves mTOR. This enzyme is directly regulated by amino acids and insulin and is essential for p70S6K activity. This pathway provides a distinction between the PKB and p70S6K branches downstream from PKD1. These pathways are involved in protein translocation, enzyme activity, and the regulation, by insulin, of genes involved in metabolism (Figure 43–8). Another SH2 domain-containing protein is GRB2, which binds to IRS-1 and links tyrosine phosphorylation to several proteins, the result of which is activation of a cascade of threonine and serine kinases. A pathway showing how this insulin-receptor interaction activates the mitogen-activated protein (MAP) kinase pathway and the anabolic effects of insulin is illustrated in Figure 43–8. The exact roles of many of these docking proteins, kinases, and phosphatases remain to be established.

### B. The Jak/STAT Pathway Is Used by Hormones and Cytokines

Tyrosine kinase activation can also initiate a phosphorylation and dephosphorylation cascade that involves the action of several other protein kinases and the counterbalancing actions of phosphatases. Two mechanisms are employed to initiate this cascade. Some hormones, such as growth hormone, prolactin, erythropoietin, and the cytokines, initiate their action by activating a tyrosine kinase, but this activity is not an integral part of the hormone receptor. The hormone-receptor interaction promotes binding and activation of **cytoplasmic protein tyrosine kinases,** such as **Tyk-2, Jak1,** or **Jak2.** These kinases phosphorylate one or more cytoplasmic proteins, which then associate with other docking proteins through binding to SH2 domains. One such interaction results in the activation of a family of cytosolic proteins called **signal transducers and activators of transcription (STATs).** The phosphorylated STAT protein dimerizes and translocates into the nucleus, binds to a specific DNA element such as the interferon response element, and activates transcription. This is illustrated in Figure 43–9. Other SH2 docking events may result in the activation of PI 3-kinase, the MAP kinase pathway (through SHC or GRB2), or G protein-mediated activation of phospholipase C (PLCγ) with the attendant production of diacylglycerol and activation of protein kinase C. It is apparent that there is a potential for "cross-talk" when different hormones activate these various signal transduction pathways.

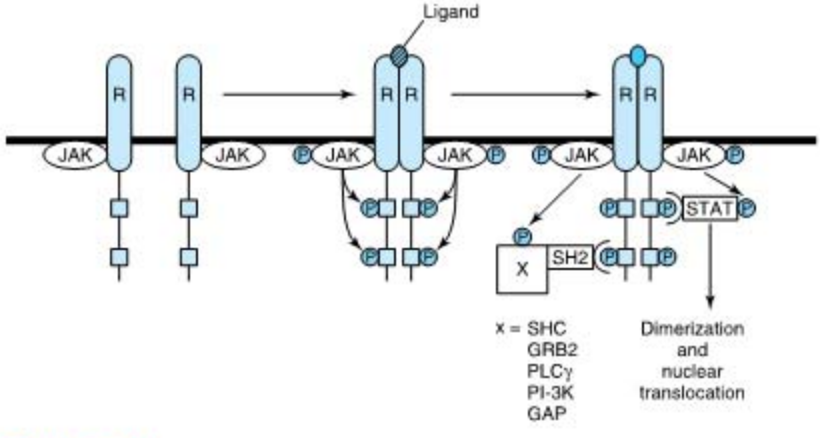


***Figure 43–9.*** Initiation of signal transduction by receptors linked to Jak kinases. The receptors (R) that bind prolactin, growth hormone, interferons, and cytokines lack endogenous tyrosine kinase. Upon ligand binding, these receptors dimerize and an associated protein (Jak1, Jak2, or TYK) is phosphorylated. Jak-P, an active kinase, phosphorylates the receptor on tyrosine residues. The STAT proteins associate with the phosphorylated receptor and then are themselves phosphorylated by Jak-P. STAT℗ dimerizes, translocates to the nucleus, binds to specific DNA elements, and regulates transcription. The phosphotyrosine residues of the receptor also bind to several SH2 domain-containing proteins. This results in activation of the MAP kinase pathway (through SHC or GRB2), PLCγ, or PI-3 kinase.

### C. The NF-κB Pathway Is Regulated by Glucocorticoids

The transcription factor NF-κB is a heterodimeric complex typically composed of two subunits termed p50 and p65 (Figure 43–10). Normally, NF-κB is kept sequestered in the cytoplasm in a transcriptionally inactive form by members of the inhibitor of NF-κB (IκB) family. Extracellular stimuli such as proinflammatory cytokines, reactive oxygen species, and mitogens lead to activation of the IκB kinase complex, IKK, which is a heterohexameric structure consisting of α, β, and γ subunits. IKK phosphorylates IκB on two serine residues, and this targets IκB for ubiquitination and subsequent degradation by the proteasome. Following IκB degradation, free NF-κB can now translocate to the nucleus, where it binds to a number of gene promoters and activates transcription, particularly of genes involved in the **inflammatory response.** Transcriptional regulation by NF-κB is mediated by a variety of coactivators such as CREB binding protein (CBP), as described below (Figure 43–13).

**Glucocorticoid hormones** are therapeutically useful agents for the treatment of a variety of inflammatory and immune diseases. Their anti-inflammatory and immunomodulatory actions are explained in part by the inhibition of NF-κB and its subsequent actions. Evidence for three mechanisms for the inhibition of NF-κB by glucocorticoids has been presented: (1) Glucocorticoids increase IκB mRNA, which leads to an increase of IκB protein and more efficient sequestration of NF-κB in the cytoplasm. (2) The glucocorticoid receptor competes with NF-κB for binding to coactivators. (3) The glucocorticoid receptor directly binds to the p65 subunit of NF-κB and inhibits its activation (Figure 43–10).

## HORMONES CAN INFLUENCE SPECIFIC BIOLOGIC EFFECTS BY MODULATING TRANSCRIPTION

The signals generated as described above have to be translated into an action that allows the cell to effectively adapt to a challenge (Figure 43–1). Much of this

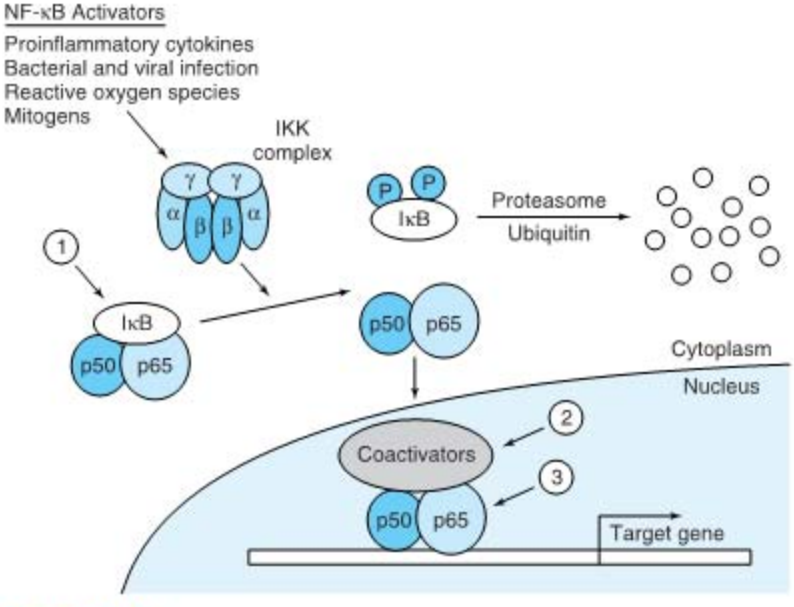


***Figure 43–10.*** Regulation of the NF-κB pathway. NF-κB consists of two subunits, p50 and p65, which regulate transcription of many genes when in the nucleus. NF-κB is restricted from entering the nucleus by IκB, an inhibitor of NF-κB. IκB binds to—and masks—the nuclear localization signal of NF-κB. This cytoplasmic protein is phosphorylated by an IKK complex which is activated by cytokines, reactive oxygen species, and mitogens. Phosphorylated IκB can be ubiquitinylated and degraded, thus releasing its hold on NF-κB. Glucocorticoids affect many steps in this process, as described in the text.

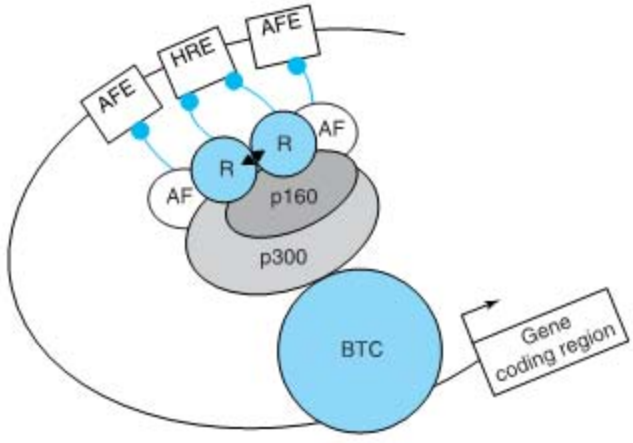


***Figure 43–11.*** The hormone response transcription unit. The hormone response transcription unit is an assembly of DNA elements and bound proteins that interact, through protein-protein interactions, with a number of coactivator or corepressor molecules. An essential component is the hormone response element which binds the ligand (▲)-bound receptor (R). Also important are the accessory factor elements (AFEs) with bound transcription factors. More than two dozen of these accessory factors (AFs), which are often members of the nuclear receptor superfamily, have been linked to hormone effects on transcription. The AFs can interact with each other, with the liganded nuclear receptors, or with coregulators. These components communicate with the basal transcription complex through a coregulator complex that can consist of one or more members of the p160, corepressor, mediator-related, or CBP/p300 families (see Table 43–6).

adaptation is accomplished through alterations in the rates of transcription of specific genes. Many different observations have led to the current view of how hormones affect transcription. Some of these are as follows: (1) Actively transcribed genes are in regions of "open" chromatin (defined by a susceptibility to the enzyme DNase I), which allows for the access of transcription factors to DNA. (2) Genes have regulatory regions, and transcription factors bind to these to modulate the frequency of transcription initiation. (3) The hormone-receptor complex can be one of these transcription factors. The DNA sequence to which this binds is called a hormone response element (HRE; see Table 43–1 for examples). (4) Alternatively, other hormone-generated signals can modify the location, amount, or activity of transcription factors and thereby influence binding to the regulatory or response element. (5) Members of a large superfamily of nuclear receptors act with—or in a manner analogous to—hormone receptors. (6) These nuclear receptors interact with another large group of coregulatory molecules to effect changes in the transcription of specific genes.

## Several Hormone Response Elements (HREs) Have Been Defined

Hormone response elements resemble enhancer elements in that they are not strictly dependent on position and location. They generally are found within a few hundred nucleotides upstream (5′) of the transcription initiation site, but they may be located within the coding region of the gene, in introns. HREs were defined by the strategy illustrated in Figure 39–11. The consensus sequences illustrated in Table 43–1 were arrived at through analysis of several genes regulated by a given hormone using simple, heterologous reporter systems (see Figure 39–10). Although these simple HREs bind the hormone-receptor complex more avidly than surrounding DNA—or DNA from an unrelated source—and confer hormone responsiveness to a reporter gene, it soon became apparent that the regulatory circuitry of natural genes must be much more complicated. Glucocorticoids, progestins, mineralocorticoids, and androgens have vastly different physiologic actions. How could the specificity required for these effects be achieved through regulation of gene expression by the same HRE (Table 43–1)? Questions like this have led to experiments which have allowed for elaboration of a very complex model of transcription regulation. For example, the HRE must associate with other DNA elements (and associated binding proteins) to function optimally. The extensive sequence similarity noted between steroid hormone receptors, particularly in their DNA-binding domains, led to discovery of the **nuclear receptor superfamily** of proteins. These—and a large number of **coregulator proteins**—allow for a wide variety of DNA-protein and protein-protein interactions and the specificity necessary for highly regulated physiologic control. A schematic of such an assembly is illustrated in Figure 43–11.

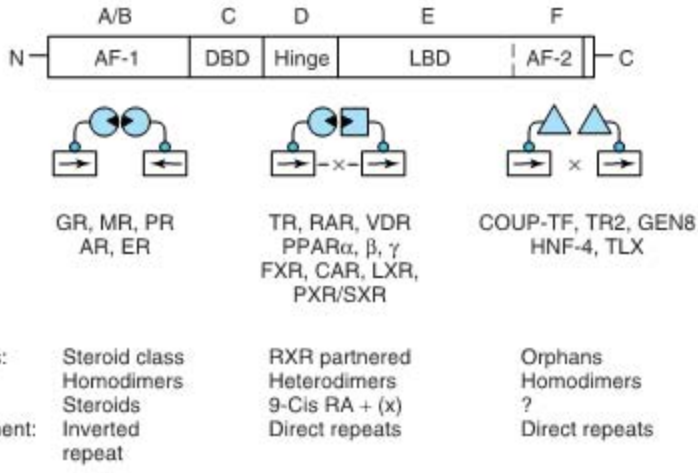


***Figure 43–12.*** The nuclear receptor superfamily. Members of this family are divided into six structural domains (A–F). Domain A/B is also called AF-1, or the modulator region, because it is involved in activating transcription. The C domain consists of the DNA-binding domain (DBD). The D region contains the hinge, which provides flexibility between the DBD and the ligand-binding domain (LBD, region E). The amino (N) terminal part of region E contains AF-2, another domain important for transactivation. The F region is poorly defined. The functions of these domains are discussed in more detail in the text. Receptors with known ligands, such as the steroid hormones, bind as homodimers on inverted repeat half-sites. Other receptors form heterodimers with the partner RXR on direct repeat elements. There can be nucleotide spacers of one to five bases between these direct repeats (DR1–5). Another class of receptors for which ligands have not been determined (orphan receptors) bind as homodimers to direct repeats and occasionally as monomers to a single half-site.

## There Is a Large Family of Nuclear Receptor Proteins

The nuclear receptor superfamily consists of a diverse set of transcription factors that were discovered because of a sequence similarity in their DNA-binding domains. This family, now with more than 50 members, includes the nuclear hormone receptors discussed above, a number of other receptors whose ligands were discovered after the receptors were identified, and many putative or orphan receptors for which a ligand has yet to be discovered.

These nuclear receptors have several common structural features (Figure 43–12). All have a centrally located **DNA-binding domain (DBD)** that allows the receptor to bind with high affinity to a response element. The DBD contains two zinc finger binding motifs (see Figure 39–14) that direct binding either as homodimers, as heterodimers (usually with a retinoid X receptor [RXR] partner), or as monomers. The target response element consists of one or two half-site consensus sequences arranged as an inverted or direct repeat. The spacing between the latter helps determine binding specificity. Thus, a direct repeat with three, four, or five nucleotide spacer regions specifies the binding of the vitamin D, thyroid, and retinoic acid receptors, respectively, to the same consensus response element (Table 43–1). A multifunctional **ligand-binding domain (LBD)** is located in the carboxyl terminal half of the receptor. The LBD binds hormones or metabolites with selectivity and thus specifies a particular biologic response. The LBD also contains domains that mediate the binding of heat shock proteins, dimerization, nuclear localization, and transactivation. The latter function is facilitated by the carboxyl terminal transcription activation function **(AF-2 domain),** which forms a surface required for the interaction with

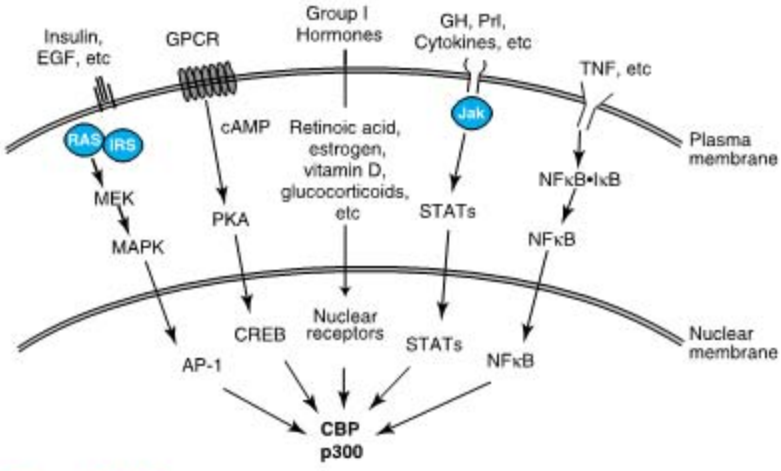


***Figure 43–13.*** Several signal transduction pathways converge on CBP/p300. Ligands that associate with membrane or nuclear receptors eventually converge on CBP/p300. Several different signal transduction pathways are employed. EGF, epidermal growth factor; GH, growth hormone; Prl, prolactin; TNF, tumor necrosis factor; other abbreviations are expanded in the text.

coactivators. A highly variable **hinge region** separates the DBD from the LBD. This region provides flexibility to the receptor, so it can assume different DNA-binding conformations. Finally, there is a highly variable amino terminal region that contains another transactivation domain referred to as **AF-1.** Less well defined, the AF-1 domain may provide for distinct physiologic functions through the binding of different coregulator proteins. This region of the receptor, through the use of different promoters, alternative splice sites, and multiple translation initiation sites, provides for receptor isoforms that share DBD and LBD identity but exert different physiologic responses because of the association of various coregulators with this variable amino terminal AF-1 domain.

It is possible to sort this large number of receptors into groups in a variety of ways. Here they are discussed according to the way they bind to their respective DNA elements (Figure 43–12). Classic hormone receptors for glucocorticoids (GR), mineralocorticoids (MR), estrogens (ER), androgens (AR), and progestins (PR) bind as homodimers to inverted repeat sequences. Other hormone receptors such as thyroid (TR), retinoic acid (RAR), and vitamin D (VDR) and receptors that bind various metabolite ligands such as PPAR α β, and γ, FXR, LXR, PXR/SXR, and CAR bind as heterodimers, with retinoid X receptor (RXR) as a partner, to direct repeat sequences (see Figure 43–12 and Table 43–5). Another group of orphan receptors that as yet have no known ligand bind as homodimers or monomers to direct repeat sequences.

As illustrated in Table 43–5, the discovery of the nuclear receptor superfamily has led to an important understanding of how a variety of metabolites and xenobiotics regulate gene expression and thus the metabolism, detoxification, and elimination of normal body products and exogenous agents such as pharmaceuticals. Not surprisingly, this area is a fertile field for investigation of new therapeutic interventions.

## A Large Number of Nuclear Receptor Coregulators Also Participate in Regulating Transcription

Chromatin remodeling, transcription factor modification by various enzyme activities, and the communication between the nuclear receptors and the basal transcription apparatus are accomplished by protein-protein interactions with one or more of a class of coregulator molecules. The number of these coregulator molecules now exceeds 100, not counting species variations and splice variants. The first of these to be described was the **CREB-binding protein, CBP.** CBP, through an amino terminal domain, binds to phosphorylated serine 137 of CREB and mediates transactivation in response to cAMP. It thus is described as a coactivator. CBP and

***Table 43–5.*** Nuclear receptors with special ligands.[1]

| Receptor | | Partner | Ligand | Process Affected |
|---|---|---|---|---|
| Peroxisome Proliferator-activated | PPARα | RXR (DR1) | Fatty acids | Peroxisome proliferation |
| | PPARβ | | Fatty acids | |
| | PPARγ | | Fatty acids | Lipid and carbohydrate metabolism |
| | | | Eicosanoids, thiazolidinediones | |
| Farnesoid X | FXR | RXR (DR4) | Farnesol, bile acids | Bile acid metabolism |
| Liver X | LXR | RXR (DR4) | Oxysterols | Cholesterol metabolism |
| Xenobiotic X | CAR | RXR (DR5) | Androstanes | |
| | | | Phenobarbital | Protection against certain drugs, toxic metabolites, and xenobiotics |
| | | | Xenobiotics | |
| | PXR | RXR (DR3) | Pregnanes | |
| | | | Xenobiotics | |

[1]Many members of the nuclear receptor superfamily were discovered by cloning, and the corresponding ligands were then identified. These ligands are not hormones in the classic sense, but they do have a similar function in that they activate specific members of the nuclear receptor superfamily. The receptors described here form heterodimers with RXR and have variable nucleotide sequences separating the direct repeat binding elements (DR1–5). These receptors regulate a variety of genes encoding cytochrome p450s (CYP), cytosolic binding proteins, and ATP-binding cassette (ABC) transporters to influence metabolism and protect cells against drugs and noxious agents.

its close relative, p300, interact directly or indirectly with a number of signaling molecules, including activator protein-1 (AP-1), signal transducers and activators of transcription (STATs), nuclear receptors, and CREB (Figure 39–11). **CBP/p300** also binds to the p160 family of coactivators described below and to a number of other proteins, including viral transcription factor Ela, the $p90^{rsk}$ protein kinase, and RNA helicase A. It is important to note that **CBP/p300 also has intrinsic histone acetyltransferase (HAT) activity.** The importance of this is described below. Some of the many actions of CBP/p300, which appear to depend on intrinsic enzyme activities and its ability to serve as a scaffold for the binding of other proteins, are illustrated in Figure 43–11. Other coregulators may serve similar functions.

Several other families of coactivator molecules have been described. Members of the **p160 family of coactivators,** all of about 160 kDa, include (1) SRC-1 and NCoA-1; (2) GRIP 1, TIF2, and NCoA-2; and (3) p/CIP, ACTR, AIB1, RAC3, and TRAM-1 (Table 43–6). The different names for members within a subfamily often represent species variations or minor splice variants. There is about 35% amino acid identity between members of the different subfamilies. The p160 coactivators share several properties. They (1) bind nuclear receptors in an agonist and AF-2 transactivation domain-dependent manner; (2) have a conserved amino terminal basic helix-loop-helix (bHLH) motif (see Chapter 39); (3) have a weak carboxyl terminal transactivation domain and a stronger amino terminal

***Table 43–6.*** Some mammalian coregulator proteins.

| | | |
|---|---|---|
| **I.** | **300-kDa family of coactivators** | |
| | CBP | CREB-binding protein |
| | p300 | Protein of 300 kDa |
| **II.** | **160-kDa family of coactivators** | |
| | A. SRC-1 | Steroid receptor coactivator 1 |
| | NCoA-1 | Nuclear receptor coactivator 1 |
| | B. TIF2 | Transcriptional intermediary factor 2 |
| | GRIP1 | Glucocorticoid receptor-interacting protein |
| | NCoA-2 | Nuclear receptor coactivator 2 |
| | C. p/CIP | p300/CBP cointegrator-associated protein 1 |
| | ACTR | Activator of the thyroid and retinoic acid receptors |
| | AIB | Amplified in breast cancer |
| | RAC3 | Receptor-associated coactivator 3 |
| | TRAM-1 | TR activator molecule 1 |
| **III.** | **Corepressors** | |
| | NCoR | Nuclear receptor corepressor |
| | SMRT | Silencing mediator for RXR and TR |
| **IV.** | **Mediator-related proteins** | |
| | TRAPs | Thyroid hormone receptor-associated proteins |
| | DRIPs | Vitamin D receptor-interacting proteins |
| | ARC | Activator-recruited cofactor |

transactivation domain in a region that is required for the CBP/p160 interaction; (4) contain at least three of the **LXXLL motifs** required for protein-protein interaction with other coactivators; and (5) often have HAT activity. The role of HAT is particularly interesting, as mutations of the HAT domain disable many of these transcription factors. Current thinking holds that these HAT activities acetylate histones and result in remodeling of chromatin into a transcription-efficient environment; however, other protein substrates for HAT-mediated acetylation have been reported. Histone acetylation/deacetylation is proposed to play a critical role in gene expression.

A small number of proteins, including NCoR and SMRT, comprise the **corepressor family.** They function, at least in part, as described in Figure 43–2. Another family includes the TRAPs, DRIPs, and ARC (Table 43–6). These so-called **mediator-related proteins** range in size from 80 kDa to 240 kDa and are thought to be involved in linking the nuclear receptor-coactivator complex to RNA polymerase II and the other components of the basal transcription apparatus.

The exact role of these coactivators is presently under intensive investigation. Many of these proteins have intrinsic enzymatic activities. This is particularly interesting in view of the fact that acetylation, phosphorylation, methylation, and ubiquitination—as well as proteolysis and cellular translocation—have been proposed to alter the activity of some of these coregulators and their targets.

It appears that certain combinations of coregulators—and thus different combinations of activators and inhibitors—are responsible for specific ligand-induced actions through various receptors.

## SUMMARY

- Hormones, cytokines, interleukins, and growth factors use a variety of signaling mechanisms to facilitate cellular adaptive responses.
- The ligand-receptor complex serves as the initial signal for members of the nuclear receptor family.
- Class II hormones, which bind to cell surface receptors, generate a variety of intracellular signals. These include cAMP, cGMP, $Ca^{2+}$, phosphatidylinositides, and protein kinase cascades.
- Many hormone responses are accomplished through alterations in the rate of transcription of specific genes.
- The nuclear receptor superfamily of proteins plays a central role in the regulation of gene transcription.
- These receptors, which may have hormones, metabolites, or drugs as ligands, bind to specific DNA elements as homodimers or as heterodimers with RXR. Some—orphan receptors—have no known ligand but bind DNA and influence transcription.
- Another large family of coregulator proteins remodel chromatin, modify other transcription factors, and bridge the nuclear receptors to the basal transcription apparatus.

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