Chapter 21— Biochemistry of Hormones II: Steroid Hormones

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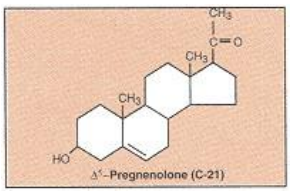


21.1— Overview

Steroid hormones in the human include cortisol as the major glucocorticoid or anti-stress hormone, aldosterone as an important regulator of Na^+ uptake, and the sex and progestational hormones. Sex hormones are 17β-estradiol in females and testosterone in males. Progesterone is the major progestational hormone. Testosterone is reduced in some target tissues to dihydrotestosterone, a higher affinity ligand for the androgen receptor. Vitamin D_3 is converted to the steroid hormone, dihydroxy vitamin D_3. Genes in the steroid receptor supergene family include retinoic acid receptors and thyroid hormone receptor, although the ligands for these additional receptors are not derivatives of cholesterol. Retinoic acid and thyroid hormone, however, have six-membered ring structures that could be considered to resemble the A ring of a steroid.

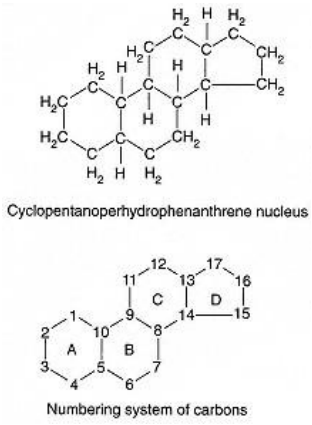


Figure 21.1
The steroid nucleus.

Steroidal structure will be reviewed with the synthesis and inactivation of steroid hormones. Regulation of synthesis of steroid hormones is reviewed with respect to the renin–angiotensin system for aldosterone, the gonadotropes, especially follicle-stimulating hormone for 17β-estradiol, and the vitamin D_3 mechanism. Steroid hormone transport is reviewed with respect to the transporting proteins in blood. A general model for steroid hormone action at the cellular level is presented with information on receptor activation and regulation of receptor levels. Specific examples of steroid hormone action for programmed cell death and for stress are presented. Finally, the roles of steroid hormone receptors as transcriptional transactivators and repressors are reviewed.

21.2— Structures of Steroid Hormones

Steroid hormones are derived in specific tissues in the body and are divided into two classes: the **sex** and **progestational hormones,** and the **adrenal hormones.** They are synthesized from cholesterol and all of these hormones pass through the required intermediate, ^{5}-pregnenolone. The structure of steroid hormones is related to the **cyclopentanoperhydrophenanthrene** nucleus. The numbering of the cyclopentanoperhydrophenanthrene ring system and the lettering of the rings is presented in Figure 21.1. The ring system of the steroid hormones is stable and not catabolized by mammalian cells. Conversion of active hormones to less active or inactive forms involves alteration of ring substituents rather than the ring structure itself. The parental precursor of the steroid is **cholesterol,** shown in Figure 21.2. The biosynthesis of cholesterol is given on p. 410.

The major steroid hormones of humans and their actions are shown in Table 21.1. Many of these hormones are similar in gross structure, although the specific receptor for each hormone is able to distinguish the cognate ligand. In the cases of cortisol and aldosterone, however, there is overlap in the ability of each specific receptor to bind both ligands. Thus the availability and concen-

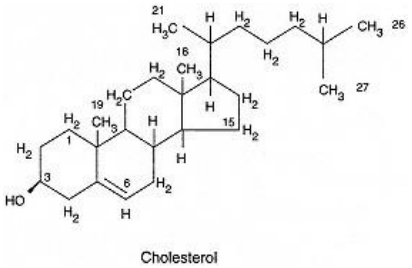
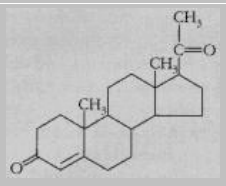
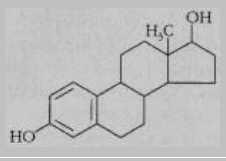
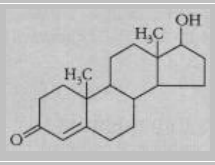
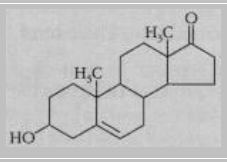
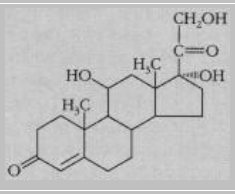
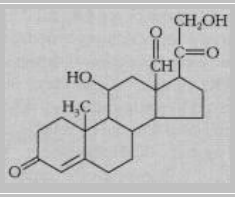
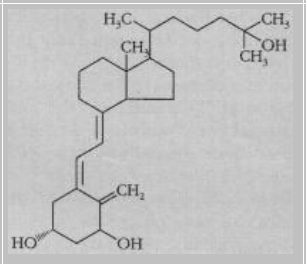


Figure 21.2
Structure of cholesterol.

TABLE 21.1 Major Steroid Hormones of Humans

Hormone	*Structure*	*Secretion from*	*Secretion Signal*[a]	*Functions*
Progesterone		Corpus luteum	LH	Maintains (with estradiol) the uterine endometrium for implantation; differentiation factor for mammary glands
17β-Estradiol		Ovarian follicle; corpus luteum; (Sertoli cell)	FSH	Female: regulates gondotropin secretion in ovarian cycle (see Chapter 20); maintains (with progesterone) uterine endometrium; differentiation of mammary gland. Male: negative feedback inhibitor of Leydig cell synthesis of testosterone
Testosterone		Leydig cells of testis; (adrenal gland); ovary	LH	Male: after conversion to dihydrotestosterone, production of sperm proteins in Sertoli cells; secondary sex characteristics (in some tissues testosterone is active hormone)
Dehydroepian-drosterone		Reticularis cells	ACTH	Various protective effects; weak androgen; can be converted to estrogen; no receptor yet found; inhibitor of G6-PDH: regulates NAD^+ coenzymes
Cortisol		Fasciculata cells	ACTH	Stress adaptation through various cellular phenotypic expressions; slight elevation of liver glycogen; killing effect on certain T cells in high doses; elevates blood pressure; sodium uptake in luminal epithelia
Aldosterone		Glomerulosa cells of adrenal cortex	Angiotensin II/III	Causes sodium ion uptake via conductance channel; occurs in high levels during stress; raises blood pressure; fluid volume increased
1,25-Dihydroxy-vitamin D_3		Vitamin D arises in skin cells after irradiation and then successive hydroxylations occur in liver and kidney to yield active form of hormone	PTH (stimulates kidney proximal tubule hydroxylation system)	Facilitates Ca^{2+} and phosphate absorption by intestinal epithelial cells; induces intracellular calcium-binding protein

[a] LH, luteinizing hormone; FSH, follicle-stimulating hormone; ACTH, adrenocorticotropic hormone; PTH, parathyroid hormone.

trations of each receptor and the relative amounts of each hormone in a given cell become paramount considerations. The steroid hormones listed in Table 21.1 can be described as classes based on the carbon number in their structures. Thus a C-27 steroid is $1,25(OH)_2D_3$; C-21 steroids are **progesterone, cortisol,** and **aldosterone;** C-19 steroids are **testosterone** and **dehydroepiandrosterone;** and a C-18 steroid is **17 β-estradiol.** Classes, such as sex hormones, can be distinguished easily by the carbon number, C-19 being androgens, C-18 being estrogens, and C-21 being progestational or adrenal steroids. Aside from the number of carbon atoms in a class structure, certain substituents in the ring system are characteristic. For example, glucocorticoids and mineralocorticoids (typically aldosterone) possess a C-11 OH or oxygen function. In rare exceptions, certain synthetic compounds can elicit a response without a C-11 OH group but they require a new functional group in proximity within the A-B ring system. Estrogens do not have a C-19 methyl group and the A ring is contracted by the content of three double bonds. Many receptors recognize the ligand A ring primarily, the estrogen receptor can distinguish the A ring of estradiol stretched out of the plane of the B-C-D rings compared to other steroids in which the A ring is coplanar with the B-C-D rings. These relationships are shown in Figure 21.3.

21.3— Biosynthesis of Steroid Hormones

Steroid Hormones Are Synthesized from Cholesterol

Hormonal regulation of steroid hormone biosynthesis is generally believed to be mediated by an elevation of intracellular **cAMP** and **Ca^{2+},** although generation of **inositol triphosphate** may also be involved, as shown in Figure 21.4. The stimulatory response of cAMP is mediated via acute (occurring within seconds to minutes) and chronic (requiring hours) effects on steroid synthesis. The acute effect is to mobilize and deliver cholesterol, the precursor for all steroid hormones, to the mitochondrial inner membrane, where it is metabolized to pregnenolone by the cytochrome P450 cholesterol side chain cleavage enzyme (see Chapter 22 for discussion of P450 enzymes). In contrast, the chronic effects of cAMP are mediated via increased transcription of the genes that encode the steroidogenic enzymes and are thus responsible for maintaining optimal long-term steroid production. Data demonstrate that a protein is induced and that this newly synthesized regulatory protein actually facilitates the translocation of cholesterol from outer to inner mitochondrial membrane where the P450 enzyme is located. This 30-kDa phosphoprotein is designated as the **steroidogenic acute regulatory (StAR)** protein. In humans, StAR mRNA has been shown to be specifically expressed in testis and ovary, known sites of steroidogenesis. Patients with lipoid congenital adrenal hyperplasia (LCAH), an inherited disease in which both adrenal and gonadal steroidogenesis is significantly impaired and lipoidal deposits occur in these tissues, express truncated and non-functional StAR proteins. These biochemical and genetic data strongly suggest that StAR protein is the hormone-induced protein factor that mediates acute regulation of steroid hormone biosynthesis.

Pathways for conversion of cholesterol to the adrenal cortical steroid hormones are presented in Figure 21.5. Cholesterol is the major precursor and undergoes side chain cleavage to form ^{5}-pregnenolone releasing a C_6 aldehyde, isocaproaldehyde. **Δ^5-Pregnenolone** is mandatory in the synthesis of all steroid hormones. As shown in Figure 21.5, pregnenolone can be converted directly to progesterone, which requires two cytoplasmic enzymes, **3 β-ol dehydrogenase** and **$\Delta^{4,5}$-isomerase.** The dehydrogenase converts the 3-OH group of pregnenolone to a 3-keto group and the isomerase moves the double bond from the B ring to the A ring to produce progesterone. In the *corpus luteum* the bulk

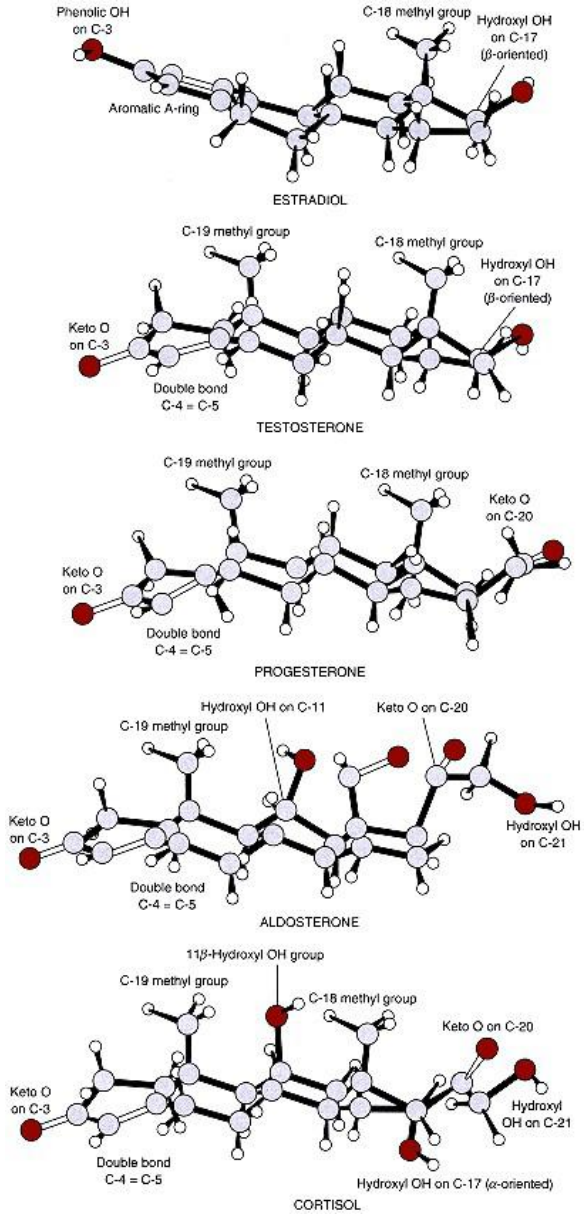


Figure 21.3
"Ball-and-stick" representations of the structures of some steroid hormones determined by X-ray crystallographic methods.
Details of each structure are labeled. In aldosterone the acetal grouping

is where R_1, R_2, R_3 refer to different substituents.

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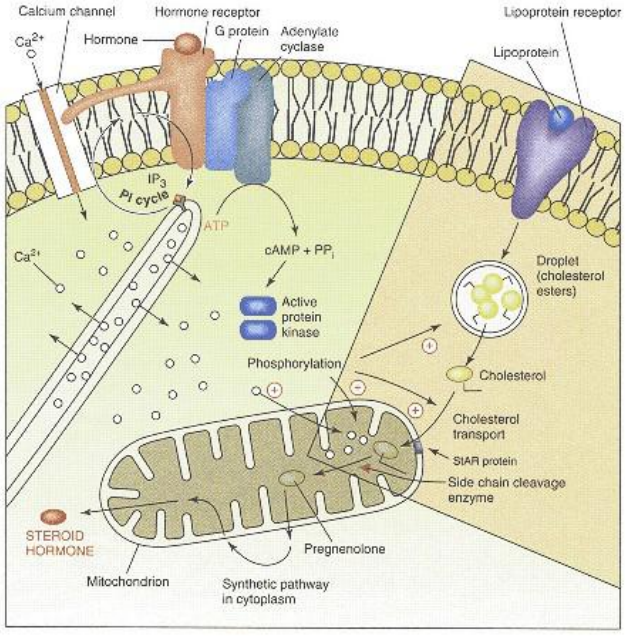


Figure 21.4
Overview of hormonal stimulation of steroid hormone biosynthesis.
Nature of the hormone (top of figure) depends on the cell type and receptor (ACTH for cortisol synthesis; FSH for estradiol synthesis; LH for testosterone synthesis, etc., as given in Table 20.1). It binds to cell membrane receptor and activates adenylate cyclase mediated by a stimulatory G-protein. Receptor, activated by hormone, may directly stimulate a calcium channel or indirectly stimulate it by activating the phosphatidylinositol cycle (PI cycle) as shown in Figure 20.25. If the PI cycle is concurrently stimulated, IP_3 could augment cytosol Ca^{2+} levels from the intracellular calcium store. The increase in cAMP activates protein kinase A (Figure 21.21) whose phosphorylations cause increased hydrolysis of cholesteryl esters from the droplet to free cholesterol and increase cholesterol transport into the mitochondrion. The combination of elevated Ca^{2+} levels and protein phosphorylation, as well as induction of the StAR protein, result in increased side chain cleavage and steroid biosynthesis. These combined reactions overcome the rate-limiting steps in steroid biosynthesis and more steroid is produced, which is secreted into the extracellular space and circulated to the target tissues in the bloodstream.

of steroid synthesis stops at this point. Progesterone is further converted to aldosterone or cortisol. Conversion of pregnenolone to **aldosterone,** which occurs in the adrenal *zona glomerulosa* cells, requires endoplasmic reticulum 21-hydroxylase, and mitochondrial 11β-hydroxylase and 18-hydroxylase. To form cortisol, primarily in adrenal *zona fasciculata* cells, endoplasmic reticulum **17-hydroxylase** and **21-hydroxylase** are required together with mitochondrial **11 β-hydroxylase.** The endoplasmic reticulum (ER) hydroxylases are all cytochrome P450-linked enzymes (see Chapter 22). ^{5}-Pregnenolone is converted to **dehydroepiandrosterone** in the adrenal *zona reticularis* cells by the action of 17α-hydroxylase of the endoplasmic reticulum to form 17α-hydroxypregnenolone and then by the action of a carbon side chain-cleavage system to form dehydroepiandrosterone.

Cholesterol is also converted to the sex hormones by way of ^{5}-pregnenolone (Figure 21.6). **Progesterone** can be formed as described above and further converted to testosterone by the action of the endoplasmic reticulum enzymes and 17-dehydrogenase. **Testosterone,** so formed, is a major secretory product in the Leydig cells of the testis and undergoes conversion to dihydrotestosterone in some androgen target cells before binding to the androgen receptor. This conversion requires the activity of **5α-reductase** located in the ER and nuclear fractions. Pregnenolone can enter an alternative pathway to form dehydroepiandrosterone as described above. This compound can be converted to 17β-estradiol via the aromatase enzyme system and the action of 17-reductase. Also, estradiol can be formed from testosterone by the action of the aromatase system.

The hydroxylases of endoplasmic reticulum involved in steroid hormone synthesis are cytochrome P450 enzymes (Chapter 22). Molecular oxygen (O_2) is a substrate with one oxygen atom incorporated into the steroidal substrate (as an OH) and the second atom incorporated into a water molecule. Electrons

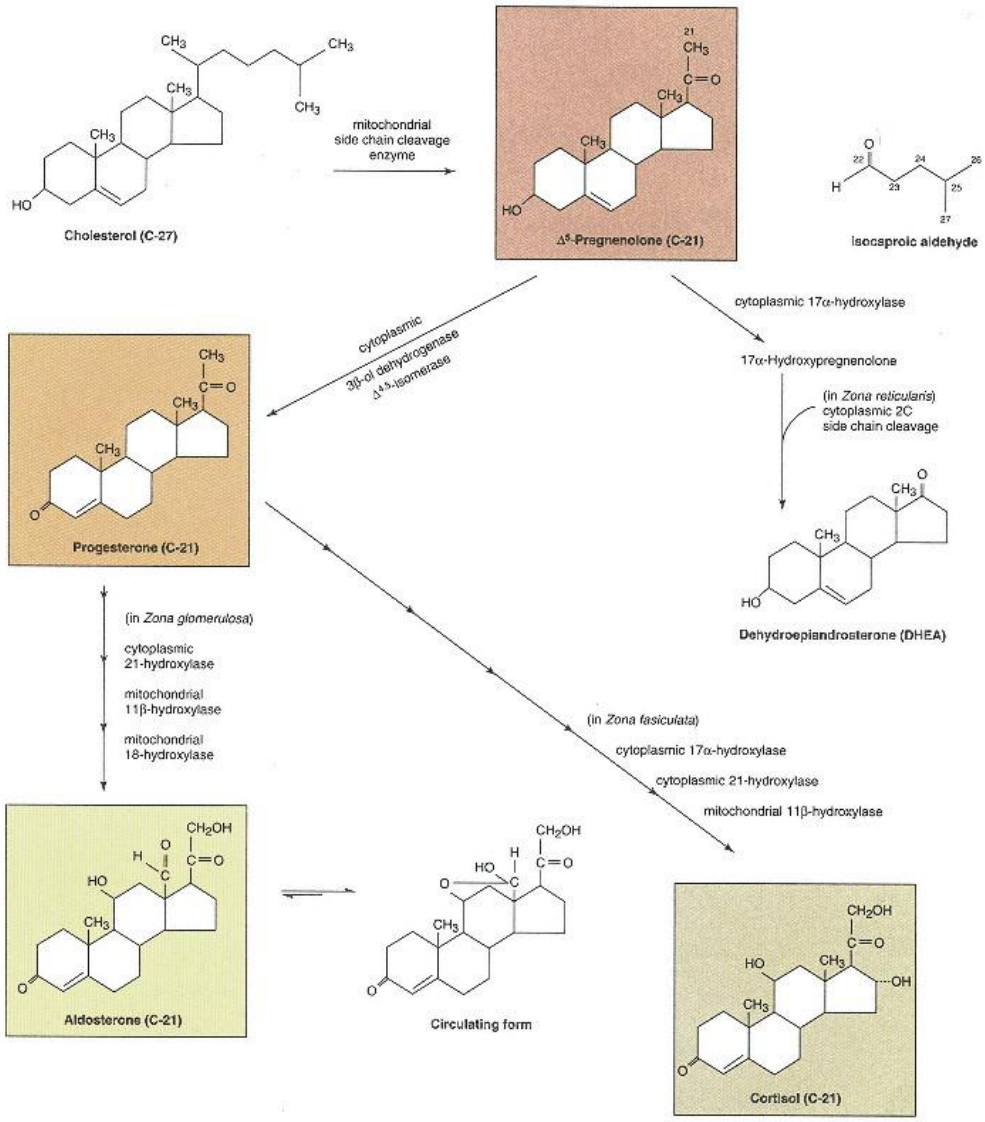


Figure 21.5
Conversion of cholesterol to adrenal cortical hormones.

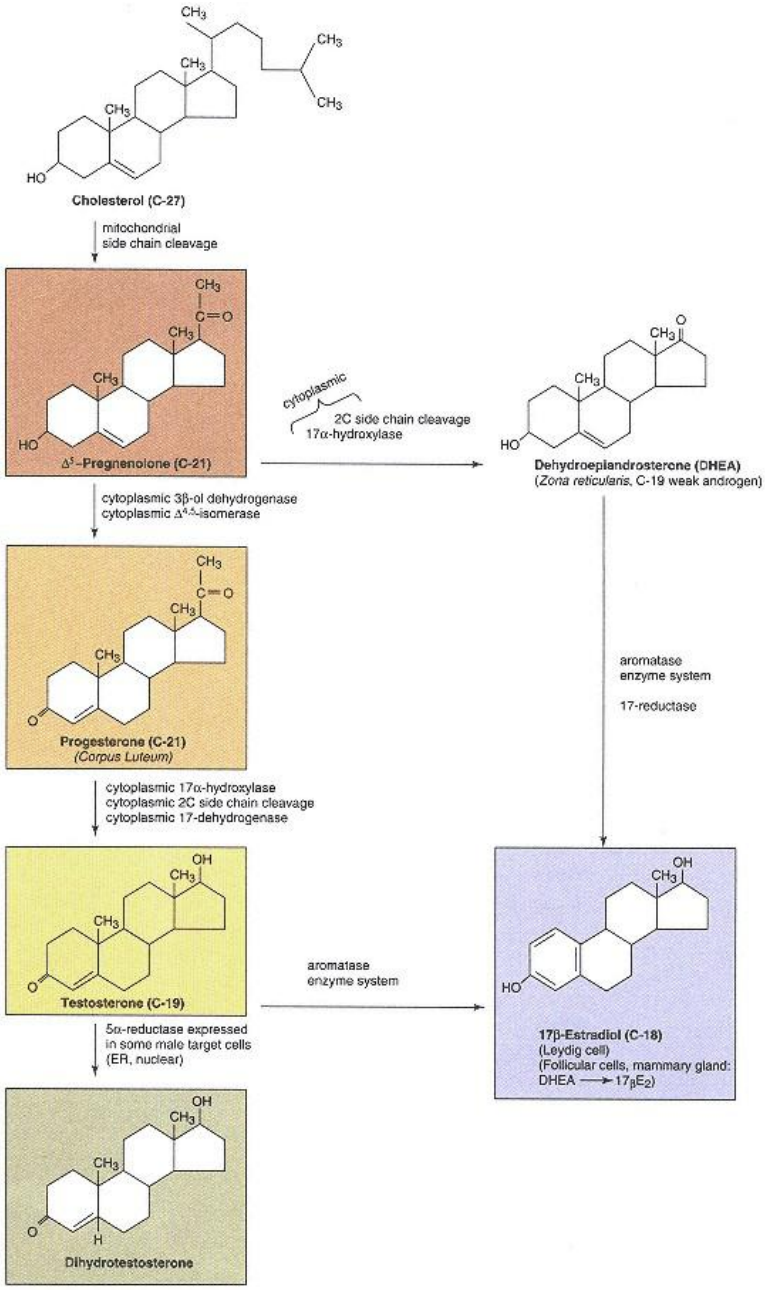


Figure 21.6
Conversion of cholesterol to sex hormones.
Mt, mitochondrial, cyto, cytoplasmic; and ER, endoplasmic reticulum.

are generated from NADH or NADPH through a flavoprotein to ferredoxin or similar nonheme protein. Various agents can induce the levels of cytochrome P450.

Note that there is movement of intermediates in and out of the mitochondrial compartment during the steroid synthetic process.

21.4— Metabolic Inactivation of Steroid Hormones

A feature of the steroid ring system is its great stability. For the most part, inactivation of steroid hormones involves reduction. Testosterone is initially reduced to a more active form by the enzyme 5α-reductase to form **dihydrotestosterone,** the preferred ligand for the androgen receptor. However, further reduction similar to the other steroid hormones results in inactivation. The inactivation reactions predominate in liver and generally render the steroids more water soluble, as marked by subsequent conjugation with glucuronides or sulfates (see Chapter 22) that are excreted in the urine. Table 21.2 summarizes reactions leading to inactivation and excretory forms of the steroid hormones.

21.5— Cell–Cell Communication and Control of Synthesis and Release of Steroid Hormones

Secretion of steroid hormones from cells where they are synthesized is elicited by other hormones. Many, but not all, such systems are described in Chapter 20, Figures 20.2 and 20.3. The hormones that directly stimulate the biosynthesis and secretion of the steroid hormones are summarized in Table 21.3. The signals for stimulation of biosynthesis and secretion of steroid hormones are polypeptide hormones operating through cognate cell membrane receptors. In some systems where both cAMP and the phosphatidylinositol (PI) cycle are involved, it is not clear whether one second messenger predominates. In many such systems, for example, aldosterone synthesis and secretion, probably several components (i.e., acetylcholine muscarinic receptor, atriopeptin receptor, and their second messengers) are involved in addition to the signal listed in Table 21.3.

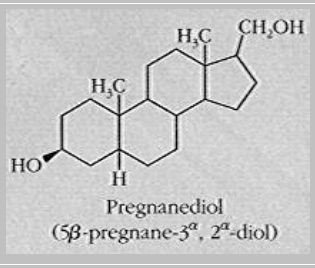
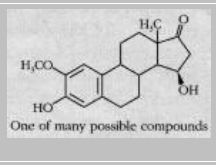
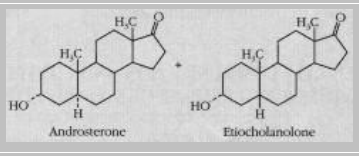
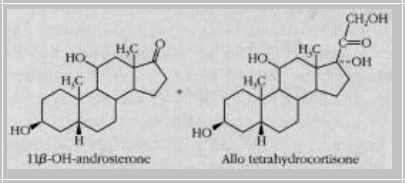
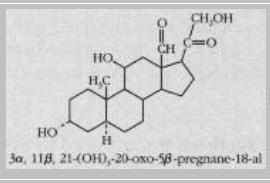
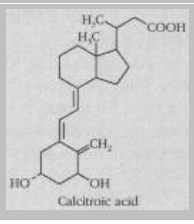
Steroid Hormone Synthesis Is Controlled by Specific Hormones

The general mechanism for hormonal stimulation of steroid hormone synthesis is presented in Figure 21.4. Figure 21.7 (p. 903) presents the system for stimulation of cortisol biosynthesis and release. The role of Ca^{2+} in steroid synthesis and/or secretion is unclear. Rate-limiting steps in the biosynthetic process involve the availability of cholesterol from cholesteryl esters in the droplet, the transport of cholesterol to the inner mitochondrial membrane (StAR protein), and the upregulation of the otherwise rate-limiting side chain cleavage reaction.

Aldosterone

Figure 21.8 (p. 904) shows the overall reactions leading to the secretion of aldosterone in the adrenal zona glomerulosa cell. This set of regulatory controls on aldosterone synthesis and secretion is complicated. The main driving force is **angiotensin II** generated from the signaling to the **renin–angiotensin system** shown in Figure 21.9 (p. 905). Essentially, the signal is generated under conditions when blood [Na^+] and blood pressure (blood volume) are required to be increased. The N-terminal decapeptide of circulating **α_2-globulin (angiotensinogen)** is cleaved by **renin,** a protease. This decapeptide is the hormonally inactive precursor, angiotensin I. It is converted to the octapeptide hormone, angiotensin II, by the action of converting enzyme. Angiotensin II is converted to the heptapeptide, angiotensin III, by an aminopeptidase. Both angiotensins

TABLE 21.2 Excretion Pathways for Steroid Hormones

Steroid Class	*Starting Steroid*	*Inactivation Steps*	*A:B Ring Junction*	*Steroid Structure Representations of Excreted Product*	*Principal Conjugate Present*[a]
Progestins	Progesterone	1. Reduction of C-20 2. Reduction of 4-ene-3-one	(cis)	 Pregnanediol (5β-pregnane-3^{α}, 2^{α}-diol)	G
Estrogens	Estradiol	1. Oxidation of 17β-OH 2. Hydroxylation at C-2 with subsequent methylation 3. Further hydroxylation or ketone formation at a variety of positions (e.g., C-6, C-7, C-14, C-15, C-16, C-18)		 One of many possible compounds	G
Androgens	Testosterone	1. Reduction of 4-ene-3-one 2. Oxidation of C-17 hydroxyl	(cis and trans)		G, S
Glucocorticoids	Cortisol	1. Reduction of 4-ene-3-one 2. Reduction of 20-oxo group 3. Side chain cleavage	(trans)		G
Mineralocorticoids	Aldosterone	1. Reduction of 4-ene-3-one	(trans)		G
Vitamin D metabolites	$1,25(OH)_2D_3$	1. Side chain cleavage between C-23 and C-24		 Calcitroic acid	?

Source: From Norman, A. W., and Litwack, G. *Hormones*. Orlando, FL: Academic Press, 1987.

[a] G, Glucuronide; S, sulfate.

II and III can bind to the angiotensin receptor (Figure 21.8), which activates the phosphatidylinositol cycle to generate IP_3 and DAG. IP_3 stimulates release of calcium ions from the intracellular calcium storage vesicles. In addition, the activity of the Ca^{2+} channel is stimulated by the angiotensin–receptor complex. K^+ ions are also required to stimulate the Ca^{2+} channel and these events lead to a greatly increased level of cytoplasmic Ca^{2+}. The enhanced cytoplasmic Ca^{2+}

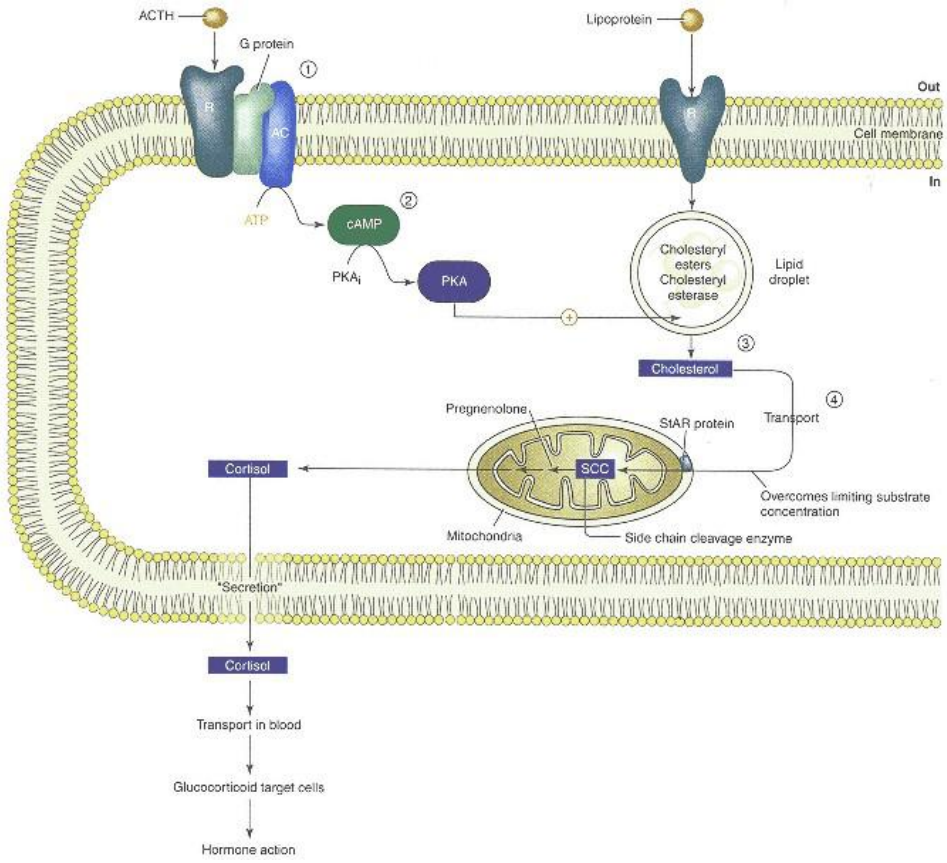


Figure 21.7
Action of ACTH on adrenal fasciculata cells to enhance production and secretion of cortisol.
AC, adenylate cyclase; cAMP, cyclic AMP; PKA, protein kinase A; SCC, side chain cleavage system of enzymes. StAR (steroidogenic acute regulatory)
protein is a cholesterol transporter functioning between the outer and inner mitochondrial membranes.

TABLE 21.3 Hormones that Directly Stimulate Synthesis and Release of Steroid Hormones

Steroid Hormone	*Steroid-Producing Cell or Structure*	*Signal*[a]	*Second Messenger*	*Signal System*
Cortisol	Adrenal zona fasciculata	ACTH	cAMP, PI cycle, Ca^{2+}	Hypothalamic–pituitary cascade
Aldosterone	Adrenal zona glomerulosa	Angiotensin II/III	PI cycle, Ca^{2+}	Renin–angiotensin system
Testosterone	Leydig cell	LH	cAMP	Hypothalamic–pituitary cascade
17β-Estradiol	Ovarian follicle	FSH	cAMP	Hypothalamic–pituitary–ovarian cycle
Progesterone	Corpus luteum	LH	cAMP	Hypothalamic–pituitary–ovarian cycle
1,25 $(OH)_2$ Vitamin D_3	Kidney	PTH	cAMP	Sunlight, parathyroid glands, plasma Ca^{2+} level

[a] ACTH, adrenocorticotropic hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; PI, phosphatidylinositol; PTH, parathyroid hormone.

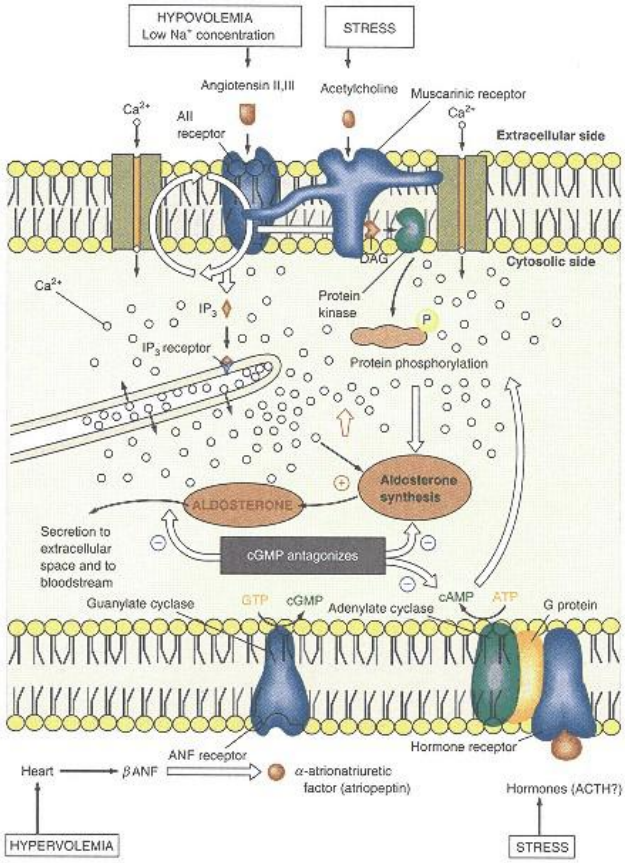


Figure 21.8
Reactions leading to the secretion of aldosterone in the adrenal *zona glomerulosa* cell.
cGMP, cyclic GMP; ANF, atrial natriuretic factor; see Figure 21.7 for additional abbreviations.

has a role in aldosterone secretion and together with diacylglycerol stimulates protein kinase C. **Acetylcholine** released through the neuronal stress signals has similar effects mediated by the muscarinic acetylcholine receptor to further reinforce Ca^{2+} uptake by the cell and stimulation of protein kinase C. Enhanced protein kinase C activity leads to protein phosphorylations that stimulate the rate-limiting steps of aldosterone synthesis leading to elevated levels of aldosterone, which are then secreted into the extracellular space and finally into the blood. Once in the blood aldosterone enters the distal kidney cell, binds to its receptor, which initially may be cytoplasmic, and ultimately stimulates expression of proteins that increase the transport of Na^+ from the glomerular filtrate to the blood (see p. 1043).

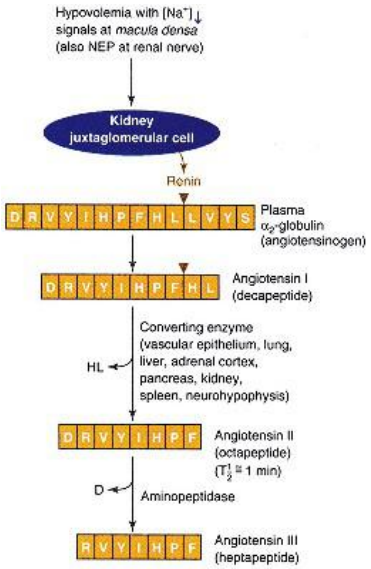


Figure 21.9
Renin–angiotensin system.
Amino acid abbreviations are found on p. 27.
NEP, norepinephrine.

Signals opposite to those that activate the formation of angiotensin generate **atrial natriuretic factor** (ANF) or atriopeptin from the heart atria (Figure 21.8; see also Figure 20.45). ANF binds to a specific *zona glomerulosa* cell membrane receptor and activates guanylate cyclase, which is part of the same receptor polypeptide so that the cytosolic level of cGMP increases. Cyclic GMP antagonizes the synthesis and secretion of aldosterone as well as the formation of cAMP by adenylate cyclase. Involvement of ACTH in aldosterone synthesis and release may involve adenylate cyclase but may be of secondary importance.

Aldosterone should be regarded as a stress hormone since its presence in elevated levels in blood occurs as a result of stressful situations. In contrast, cortisol, also released in stress has an additional biorhythmic release (possibly under control of serotonin and vasopressin), which accounts for a substantial reabsorption of Na^+ probably through glucocorticoid stimulation of the Na^+–H^+ antiport in luminal epithelial cells in addition to the many other activities of cortisol (e.g., anti-inflammatory action, control of T-cell growth factors, synthesis of glycogen, and effects on carbohydrate metabolism).

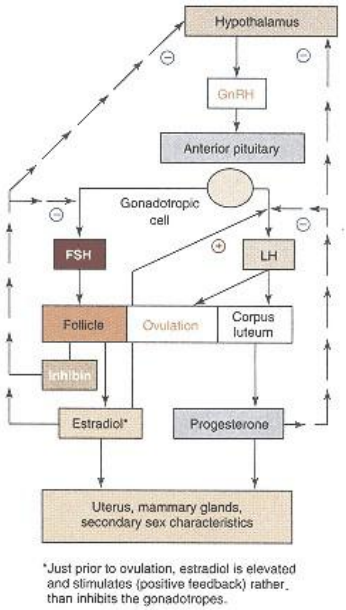


Figure 21.10
Formation and secretion of 17 β-estradiol and progesterone.

Estradiol

Control of formation and secretion of **17 β-estradiol**, the female sex hormone, is shown in Figure 21.10. During development, control centers for the steady-state and cycling levels arise in the CNS. Their functions are required to initiate the ovarian cycle at puberty. These centers must harmonize with the firing of other neurons, such as those producing a clock-like mechanism via release of catecholamines or other amines to generate the pulsatile release of gonadotropin-releasing hormone (GnRH), probably at hourly intervals. Details of these reactions are presented on page 867, Chapter 20. The FSH circulates and binds to, its cognate receptor on the cell membrane of the ovarian follicle cell and

through its second messengers, primarily cAMP and the activation of cAMP-dependent protein kinase, there is stimulation of the synthesis and secretion of the female sex hormone, 17β-estradiol. At normal stimulated levels of 17β-estradiol, there is a negative feedback on the **gonadotrope** (anterior pituitary), suppressing further secretion of FSH. Near ovarian midcycle, however, there is a superstimulated level of 17β-estradiol produced that has a positive rather than a negative feedback effect on the gonadotrope. This causes very high levels of LH to be released, referred to as the LH spike, and elevated levels of FSH. The level of FSH released is substantially lower than LH because the follicle produces **inhibin,** a polypeptide hormone that specifically inhibits FSH release without affecting LH release. The elevation of LH in the LH spike participates in the process of ovulation. After ovulation, the remnant of the follicle is differentiated into the functional *corpus luteum*, which now synthesizes progesterone (and also some estradiol), under the influence of elevated LH levels. Progesterone, however, is a feedback inhibitor of LH synthesis and release (operating through a progesterone receptor in the gonadotropic cell) and eventually the *corpus luteum* dies, owing to a fall in the level of available LH and the production of oxytocin, a luteolytic agent, by the *corpus luteum*. Prostaglandin F_{2_α} may also be involved. With the death of the *corpus luteum*, the blood levels of progesterone and estradiol fall, causing menstruation as well as a decline in the negative feedback effects of these steroids on the anterior pituitary and hypothalamus, and the cycle begins again. Clinical Correlation 21.1 describes how oral contraceptives interrupt this sequence.

The situation is similar in males with respect to regulation of gonadotropin secretion, but LH acts principally on the Leydig cell for the stimulated production of testosterone, and FSH acts on the Sertoli cells to stimulate production of inhibin and sperm proteins. Production of testosterone is subject to the negative feedback effect of 17β-estradiol synthesized in the Sertoli cell. The 17β-estradiol so produced operates through a nuclear estrogen receptor in the Leydig cell to produce inhibition of testosterone synthesis at the transcriptional level. In all cases of steroid hormone production, the synthetic system resembles that shown in Figure 21.4.

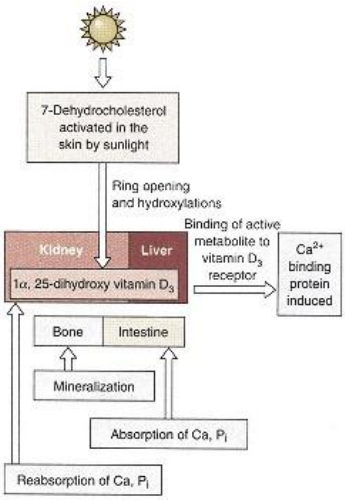


Figure 21.11
The vitamin D endocrine system.
P_i, inorganic phosphate.
Adapted from Norman, A. W. and Litwack, G. *Hormones*. Orlando, FL: Academic Press, 1987, f. 379.

CLINICAL CORRELATION 21.1

Oral Contraception

Oral contraceptives usually contain an estrogen and a progestin. Taken orally, the levels of these steroids increase in blood to a level where secretion of FSH and LH is repressed. Consequently, the gonadotropic hormone levels in blood fall and there is insufficient FSH to drive development of the ovarian follicle. As a result, the follicle does not mature and ovulation cannot occur. In addition, any *corpora lutea* cannot survive because of low LH levels. In sum, the ovarian cycle ceases. The uterine endometrium thickens and remains in this state, however, because of elevated levels of estrogen and progestin. Pills without the steroids (placebos) are usually inserted in the regimen at about the 28th day and, as a result, blood levels of steroids fall dramatically and menstruation occurs. When oral contraceptive steroids are resumed, the blood levels of estrogen and progestin increase again and the uterine endometrium thickens. This sequence creates a false "cycling" because of the occurrence of menstruation at the expected time in the cycle. The ovarian cycle and ovulation are suppressed by the oral contraceptive based on the negative feedback effects of estrogen and progestin on the secretion of the anterior pituitary gonadotropes. It is also possible to provide contraception by implanting in the skin silicone tubes containing progestins. The steroid is slowly released, providing contraception for up to 3–5 years.

Zatuchni, G. I. Female contraception. In: K. L. Becker (Ed.), *Principles and Practice of Endocrinology and Metabolism*. New York: Lippincott, 1990, p. 861; and Shoupe, D., and Mishell, D. R. Norplant: subdermal implant system for long term contraception. *Am. J. Obstet. Gynecol.* 160:1286, 1988.

Vitamin D_3

Activation of vitamin D to dihydroxy vitamin D_3 produces a hormone that has the general features of a steroid hormone. The active form of vitamin D stimulates intestinal absorption of dietary calcium and phosphorus, the mineralization of bone matrix, bone resorption, and reabsorption of calcium and phosphate in the renal tubule. The **vitamin D endocrine system** is diagrammed in Figure 21.11.7-Dehydrocholesterol is activated in the skin by sunlight to form **vitamin D_3 (cholecalciferol).** This form is hydroxylated first in the liver to **25-hydroxy vitamin D_3 (25-hydroxycholecalciferol)** and subsequently in the kidney to form the **1α,25-vitamin D_3 (1,25$(OH)_2D_3$)(1α,25-dihydroxycholecalciferol).** The hormone can bind to nuclear 1,25$(OH)_2D_3$ receptors in intestine, bone, and kidney and then transcriptionally activate genes encoding calcium-binding proteins whose actions may lead to the absorption and reabsorption of Ca^{2+} (as well as phosphorus). The subcellular mode of action is presented in Figure 21.12. In this scheme the active form of vitamin D_3 enters the intestinal cell from the blood side and migrates to the nucleus. Once inside it binds to the high-affinity vitamin D_3 receptor, which probably undergoes an activation event, and associates with a vitamin D_3-responsive element to activate genes responsive to the hormone. Messenger RNA is produced and translated in the cytoplasm; these RNAs encode calcium-binding proteins, Ca^{2+}-ATPase, other ATPases, membrane components, and facilitators of vesicle formation. Increased levels of calcium-binding proteins may cause increased uptake of Ca^{2+} from the intestine or may simply buffer the cytoplasm against high free Ca^{2+} levels.

With each of the steroid-producing systems discussed, feedback controls are operative whereby sufficient amounts of the circulating steroid hormone inhibit the further production and release of intermediate hormones in the pathway at the levels of the pituitary and hypothalamus, as viewed in Figure 20.3. In the case of the vitamin D systems, the controls are different since the steroid production is not stimulated by the cascade process applicable to estra-

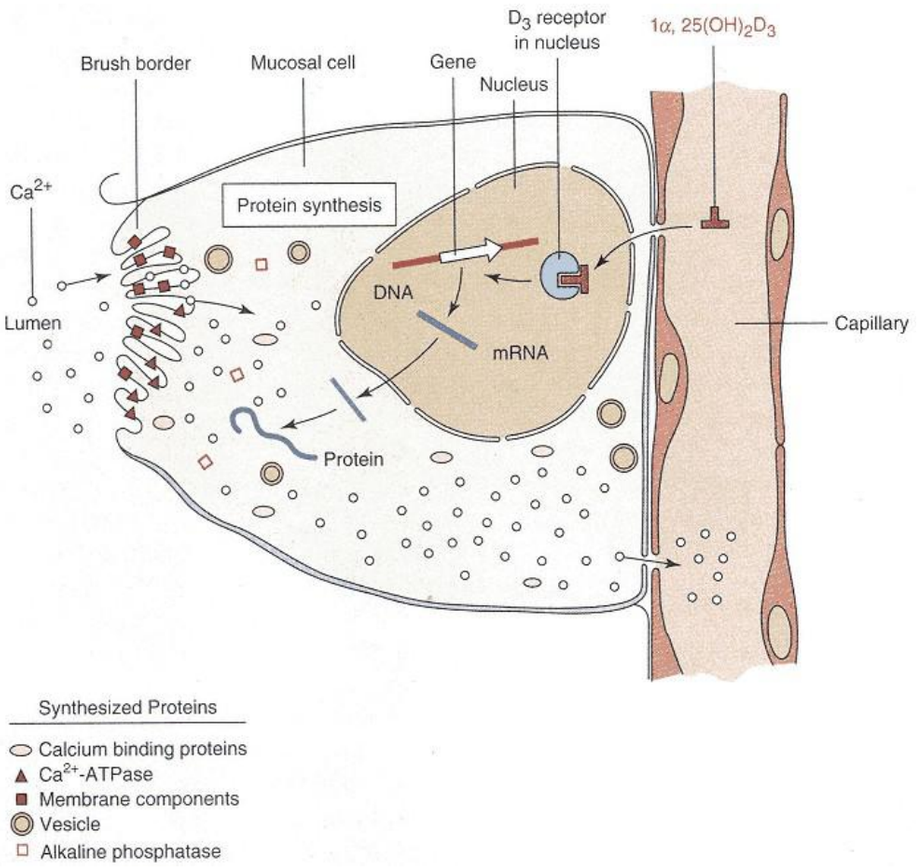


Figure 21.12
Schematic model to describe the action of 1,25$(OH)_2D_3$ in the intestine in stimulating intestinal calcium transport.
Redrawn from Nemere, I., and Norman, A. W. *Biochim. Biophys. Acta* 694:307, 1982.

diol. When the circulating levels of the active form of vitamin D (1, 25$(OH)_2D_3$) are high, hydroxylations at the 24 and 25 positions are favored and the inactive 24,25 $(OH)_2$-vitamin D_3 compound is generated.

21.6— Transport of Steroid Hormones in Blood

Steroid Hormones Are Bound to Specific Proteins or Albumin in Blood

There are four major proteins in the circulation that account for much of the steroid hormones bound in the blood. They assist in maintaining a level of these hormones in the circulation and protect the hormone from metabolism and inactivation. The binding proteins of importance are corticosteroid-binding globulin protein, sex hormone-binding protein, androgen-binding protein, and albumin.

Corticosteroid-binding globulin (CBG) or **transcortin** is about 52 kDa, is 3–4 mg% in human plasma, and binds about 80% of the total 17-hydroxysteroids in the blood. In the case of cortisol, which is the principal antistress corticosteroid in humans, about 75% is bound by CBG, 22% is bound in a loose manner to albumin, and 8% is in free form. The unbound cortisol is the form that can permeate cells and bind to intracellular receptors to produce biological effects. The CBG has a high affinity for cortisol with a binding constant (K_a) of 2.4×10^7 M^{-1}. Critical structural determinants for steroid binding to CBG are the ^{4}-3-ketone and 20-ketone structures. Aldosterone binds weakly to CBG but is also bound by albumin and other plasma proteins. Normally, 60% of aldosterone is bound to albumin and 10% is bound to CBG. In human serum, albumin is 1000-fold the concentration of CBG and binds cortisol with an affinity of 10^3 M^{-1}, much lower than the affinity of CBG for cortisol. Thus cortisol will always fill CBG-binding sites first. During stress, when secretion of cortisol is very high, CBG sites will be filled but there will be sufficient albumin to accommodate excess cortisol.

Sex hormone binding globulin (SHBG) (40 kDa) binds androgens with an affinity constant of about 10^9 M^{-1}, which is much tighter than albumin binding of androgens. One to three percent of testosterone is unbound in the circulation and 10% is bound to SHBG, with the remainder bound to albumin. The level of SHBG is probably important in controlling the balance between circulating androgens and estrogens along with the actual amounts of these hormones produced in given situations. About 97–99% of bound testosterone is bound reversibly to SHBG but much less estrogen is bound to this protein in the female. As mentioned above, only the unbound steroid hormone can permeate cells and bind to intracellular receptors, thus expressing its activity. The level of SHBG before puberty is about the same in males and females, but, at puberty, when the functioning of the sex hormones becomes important, there is a small decrease in the level of circulating SHBG in females and a larger decrease in males, ensuring a relatively greater amount of the unbound, biologically active sex hormones—testosterone and 17β-estradiol. In adults, males have about one- half as much circulating SHBG as females, so that the unbound testosterone in males is about 20 times greater than in females. In addition, the total (bound plus unbound) concentration of testosterone is about 40 times greater in males. Testosterone itself lowers SHBG levels in blood, whereas 17 β-estradiol raises SHBG levels in blood. These effects have important ramifications in pregnancy and in other conditions.

Androgen binding protein (ABP) is produced by Sertoli cells in response to testosterone and FSH, both of which stimulate protein synthesis in these cells. Androgen-binding protein is doubtless not of great importance in the entire blood circulation but is important because it maintains a ready supply of testosterone for the production of protein constituents of spermatozoa. Its

role may be to maintain a high local concentration of testosterone in the vicinity of the developing germ cells within the tubules.

From a variety of studies it is clear that these, as well as other transport proteins, protect the circulating pool of steroid hormones. They supply free steroids that can enter cellular targets after dissociation from the bound forms as more free hormone is utilized, thus serving the needs of target cells by a mass action effect.

21.7— Steroid Hormone Receptors

Steroid Hormones Bind to Specific Intracellular Protein Receptors

The general model for steroid hormone action presented in Figure 21.13 takes into account the differences among steroid receptors in terms of their location within the cell. In contrast to polypeptide hormone receptors that are generally located on/in the cell surface, steroid hormone receptors, as well as other related receptors for nonsteroids (i.e. thyroid hormone, retinoic acid, vitamin D_3), are located in the cell interior. Among the steroid receptors there appear to be some differences as to the subcellular location of the **non-DNA-binding forms** of these **receptors.** The glucocorticoid receptor and possibly the aldosterone receptor appear to reside in the cytoplasm, whereas the other receptors, for which suitable data have been collected, may be located within the nucleus, presumably in association with DNA, although not necessarily at productive acceptor sites on the DNA. Figure 21.13, Step 1, shows a bound and a free form of a steroid hormone(s). The free form may enter the cell by a process of diffusion. In the case of glucocorticoids, like cortisol, the steroid would bind

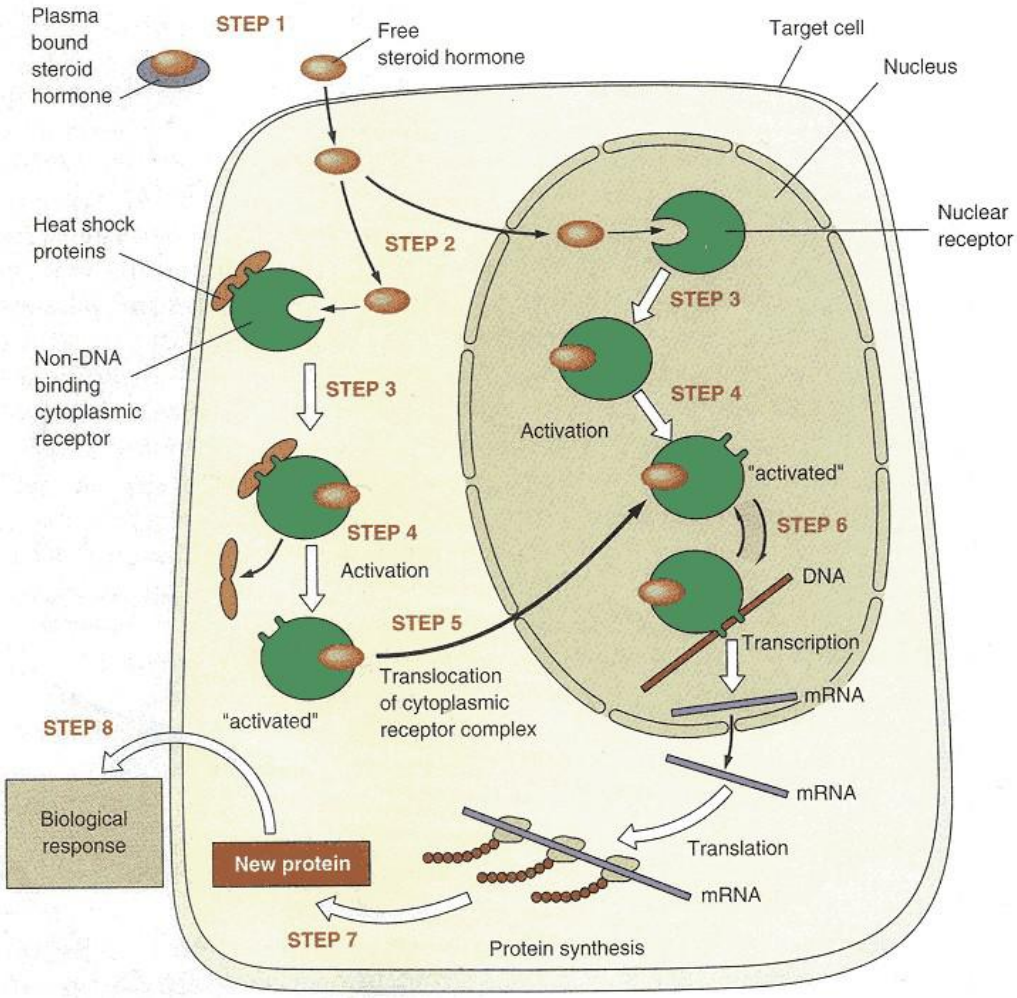


Figure 21.13
Model of steroid hormone action.
Step 1—Dissociation of free hormone (biologically active) from circulating transport protein;
Step 2—diffusion of free ligand into cytosol or nucleus;
Step 3—binding of ligand to unactivated cytoplasmic or nuclear receptor;
Step 4—activation of cytosolic or nuclear hormone–receptor complex to activated, DNA-binding form;
Step 5—translocation of activated cytosolic hormone–receptor complex into nucleus;
Step 6—binding of activated hormone–receptor complexes to specific response elements within the DNA;
Step 7—synthesis of new proteins encoded by hormone-responsive genes; and
Step 8—alteration in phenotype or metabolic activity of target cell mediated by specifically induced proteins.

to an unactivated receptor with an open ligand binding site (Step 3). The binding constant for this reaction is on the order of 10^9 M^{-1}, compared to about 10^7 M^{-1} for the binding to CBG (see above). The non-DNA-binding form also referred to as the unactivated or nontransformed receptor is about 300 kDa, because other proteins may be associated in the complex. Many investigators believe that a dimer of the 90-kDa protein, which is a heat shock protein that is induced when cells are stressed **(heat shock proteins),** is associated with the receptor in this form and occludes its DNA-binding domain, accounting for its non-DNA-binding activity. Associated with this dimer of hsp90 is another heat shock protein designated as hsp56, which interestingly also functions as an immunophilin and, as such, can bind to a number of potent immunosuppressive drugs. The dimer of the 90-kDa heat shock protein is depicted by the pair of red ovals attached to the cytoplasmic receptor that block the DNA-binding domain pictured as a pair of "fingers" in the subsequently activated form. Activation or transformation to the **DNA-binding form** is accomplished by release of the 90-kDa heat shock proteins (Step 4). It is not clear what actually drives the activation step(s). Clearly, the binding of the steroidal ligand is important but other factors may be involved. A low molecular weight component has been proposed to be part of the cross-linking between the nonhomologous proteins and the receptor in the DNA-binding complex. In the case of glucocorticoid receptor, only the non-DNA-binding form has a high affinity for binding steroidal ligand. Following activation and exposure of the DNA-binding domain, the receptor translocates to the nucleus (Step 5), binds to DNA, and "searches" the DNA for a high-affinity acceptor site. At this site the bound receptor complex, frequently a homodimer, acts as a transactivation factor, which together with other transactivators allows for the starting of RNA polymerase and stimulation of transcription. In some cases the binding of the receptor may lead to repression of transcription and this effect is less well understood. New mRNAs are translocated to the cytoplasm and assembled into translation complexes for the synthesis of proteins (Step 7) that alter metabolism and functioning of the target cell (Step 8).

When the unoccupied (nonliganded) steroid hormone receptor is located in the nucleus, as may be the case with the estradiol, progesterone, androgen, and vitamin D_3 receptors (see Figure 21.12), the steroid must travel through the cytoplasm and cross the perinuclear membrane. It is not clear whether this transport through the cytoplasm (aqueous environment) requires a transport protein for the hydrophobic steroid molecules. Once inside the nucleus the steroid can bind to the high-affinity, unoccupied receptor, presumably already on DNA, and cause it to be "activated" to a form bound to the acceptor site. The ligand might promote a conformation that decreases the off-rate of the receptor from its acceptor, if it is located on or near its acceptor site, or might cause the receptor to initiate searching if the unoccupied receptor associates with DNA at a locus remote from the acceptor site. Consequently, the mechanism underlying activation of nuclear receptors is less well understood as compared to activation of cytoplasmic receptors. After binding of activated receptor complexes to DNA acceptor sites, enhancement or repression of transcription occurs.

Consensus DNA sequences defining specific **hormone response elements (HREs)** for the binding of various activated steroid hormone–receptor complexes are summarized in Table 21.4. Receptors for glucocorticoids, mineralocorticoids, progesterone, and androgen all bind to the same HRE on the DNA. Thus, in a given cell type, the extent and type of receptor expressed will determine the hormone sensitivity. For example, sex hormone receptors are expressed in only a few cell types and the progesterone receptor is likewise restricted to certain cells, whereas the glucocorticoid receptor is expressed in a large number of cell types. In cases where aldosterone and cortisol receptors are coexpressed, only one form may predominate depending on the cell type. Some tissues, such as the kidney and colon, are known targets for aldosterone

CLINICAL CORRELATION 21.2

Apparent Mineralocorticoid Excess Syndrome

Some patients (usually children) exhibit symptoms, including hypertension, hypokalemia, and suppression of the reninangiotensin–aldosterone system, that would be expected if they were hypersecreting aldosterone. Since bioassays of plasma and urine sometimes fail to identify any excess of mineralocorticoids, these patients are said to suffer from the apparent mineralocorticoid excess (AME) syndrome. This syndrome results as a consequence of the failure of cortisol inactivation by the 11β-hydroxy-steroid dehydrogenase enzyme. Inactivity of this key enzyme gives cortisol direct access to the renal mineralocorticoid receptor. Since cortisol circulates at much higher concentrations than aldosterone, this glucocorticoid saturates these mineralocorticoid receptors and functions as an agonist, causing sodium retention and suppression of the renin–angiotensin–aldosterone axis. Although this AME syndrome can result from a congenital defect in the distal nephron 11β-hydroxysteroid dehydrogenase isoform, which renders the enzyme incapable of converting cortisol to cortisone (binds poorly to mineralocorticoid receptors), it can also be acquired by ingesting excessive amounts of licorice. The major component of licorice is glycyrrhizic acid and its hydrolytic product, glycyrrhetinic acid (GE). This active ingredient (GE) acts as a potent inhibitor of 11β-hydroxysteroid dehydrogenase. By blocking activity of this inactivating enzyme, GE facilitates the binding of cortisol to renal mineralocorticoid receptors and hence induces AME syndrome.

Edwards, C. R. W. Primary mineralocorticoid excess syndromes. In: L. J. DeGroot (Ed.), *Endocrinology*. Philadelphia: Saunders, pp. 1775–1803, 1995; and Shackleton, C. H. L., and Stewart, P. M. The hypertension of apparent mineralocorticoid excess syndrome. In: E. G. Biglieri and J. C. Melby (Eds.), *Endocrine Hypertension*. New York: Raven Press, 1990, pp. 155–173.

TABLE 21.4 Steroid Hormone Receptor Responsive DNA Elements: Consensus Acceptor Site

Element	*DNA Sequence*[a]
POSITIVE	
Glucocorticoid responsive element (GRE)	5 -GGTACAnnnTGTTCT-3
Mineralocorticoid responsive element (MRE)	
Progesterone responsive element (PRE)	
Androgen responsive element (ARE)	
Estrogen responsive element (ERE)	5 AGGTCAnnnTCACT-3
NEGATIVE	
Glucocorticoid responsive element	5 -ATYACNnnnTGATCW-3

Source: Data are summarized from work of Beato, M. *Cell* 56:355, 1989.

[a] n, any nucleotide; Y, a purine; W, a pyrimidine.

and express relatively high levels of mineralocorticoid receptors as well as glucocorticoid receptors. These mineralocorticoid target tissues express the enzyme **11 β-hydroxysteroid dehydrogenase** (see Clin. Corr. 21.2). This enzyme converts cortisol and corticosterone, both of which can bind to the mineralocorticoid receptor with high affinity, to their 11-keto analogs, which bind poorly to the mineralocorticoid receptor. This inactivation of corticosterone and cortisol, which circulate at much higher concentrations than aldosterone, facilitates the binding of aldosterone to the mineralocorticoid receptors in these classical target tissues. In tissues that express mineralocorticoid receptors but are not considered target tissues, this enzyme may not be expressed, and in these situations the mineralocorticoid receptors may simply function as pseudo-glucocorticoid receptors and mediate the effects of low circulating levels of cortisol (predominant glucocorticoid in humans). Thus the mineralocorticoid and glucocorticoid receptors may regulate the expression of an overlapping gene network in various target tissues. As also indicated in Table 21.4, the activated estrogen–receptor complex recognizes a distinct or unique response element. All of the response elements listed at the top of Table 21.4 function as positive elements, since binding of the indicated steroid receptors results in an increase in the rate of transcription of the associated gene.

Glucocorticoid hormones also repress transcription of specific genes. For example, glucocorticoids are known to repress transcription of the **proopio-melanocortin gene** (POMC) (see p. 849), which contains the ACTH sequences. Glucocorticoid-mediated repression of *POMC* gene expression thus plays a key role in the negative feedback loop regulating the rate of secretion of ACTH and ultimately cortisol. Negative glucocorticoid response elements (nGREs) mediate this repression of the *POMC* gene as well as other important genes. A general model of positive as well as negative transcriptional effects mediated by steroid receptors is shown in Figure 21.14: In (*a*) binding of a steroid receptor (R) homodimer to its response element allows it to interact synergistically with a positive transcription factor (TF) and hence induce gene transcription; in (*b*) binding of a receptor dimer to its response element displaces a positive transcription factor (TF) but has no or weak transactivation potential because no synergizing factor is nearby; and in (*c*) the DNA-AP1 (positive factor) may interact in a protein–protein fashion in such a way that the transactivating functions of both proteins are inhibited and gene transcription is repressed.

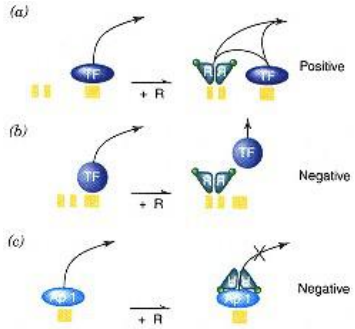


Figure 21.14
Positive and negative transcriptional effects of steroid receptors.
Redrawn from Renkowitz, R. *Ann. N.Y. Acad. Sci*. 684:1, 1993.

Some members of this receptor supergene family can mediate gene silencing. Silencer elements, in analogy to enhancer elements, function independently of their position and orientation. The silencer for a particular gene consists of modules that independently repress gene activity. In the absence of their specific ligands, the **thyroid hormone receptor** (T_3R) and **retinoic acid receptor** (RAR) appear to bind to specific silencer elements and repress gene transcription. This silencing activity may occur via destabilization of the transcription initiation complex or via direct or indirect effects on the carboxy-terminal domain of RNA polymerase II. After binding of their respective ligands, these two receptors lose this silencing activity and are converted into transactivators of gene transcription.

As indicated in Figure 21.14, **dimerization** of receptor monomers is a prerequisite for efficient DNA binding and transcriptional activation by most steroid receptors. Strong interactions between these monomers are mediated by the ligand-binding domains of several steroid receptors. The dimerization domain of the ligand-binding domain has been proposed to form a helical structure containing a succession of hydrophobic sequences that would generate a leucine zipper-like structure or a helix–turn–zipper motif (see p. 110), which are known to be necessary for the dimerization of other transcription factors. Although the majority of receptors in this superfamily form homodimers, heterodimers have also been detected. More specifically, a distinct class of retinoic acid receptors, classified as retinoid X receptors (RXRs), regulate gene expression via heterodimerization with the other distinct form of the retinoic acid receptor (RAR), the thyroid hormone receptor, and other members of this receptor superfamily. A model for the stabilization of the transcriptional preinitiation complex by an RXR/RAR heterodimer is presented in Figure 21.15.

Thus the changes produced in different cells by the activation of steroid hormone receptors may be different in different cells that contain the relevant receptor in suitable concentration. The whole process is triggered by the entry of the steroidal ligand in amounts that supersede the dissociation constant of the receptor. The different phenotypic changes in different cell types in response to a specific hormone then summate to give the systemic or organismic response to the hormone.

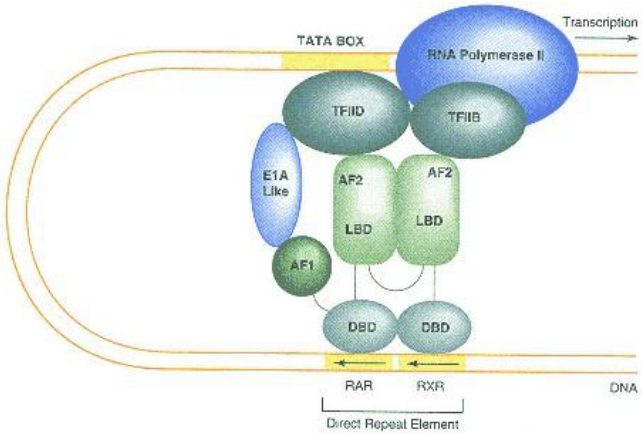


Figure 21.15
Model for stabilization of preinitiation complex by an RXR/RAR heterodimer.
TF, transcription factor; LBD, ligand-binding domain; DBD, DNA-binding domain; AF1, activation function located in amino-terminal region of receptor, which may provide contact with cell-specific proteins; AF2, activation function located within ligand-binding domain, which interacts directly with transcriptional machinery.

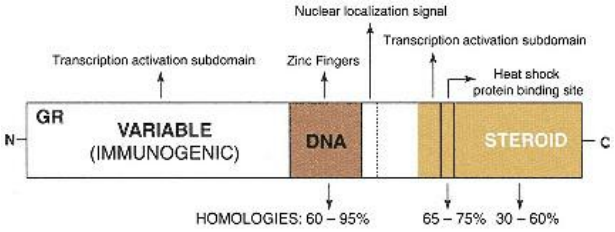


Figure 21.16
Model of a typical steroid hormone receptor.
The results are derived from studies on cDNA in various laboratories, especially those of R. Evans and K. Yamamoto.

Some Steroid Receptors Are Part of the cErbA Family of Proto-oncogenes

The glucocorticoid receptor is conveniently divided into three major **functional domains** (Figure 21.16). Starting at the C terminus, the steroid-binding domain is indicated and has 30–60% homology with the **ligand-binding domains** of other receptors in the steroid receptor family. The more alike two steroids that bind different receptors are, the greater the extent of homology to be anticipated in this domain. The steroid-binding domain contains a sequence that may be involved in the binding of molybdate and a dimer of the 90-kDa heat shock protein whose function would theoretically result in the assembly of the high molecular weight unactivated–nontransformed steroid–receptor complex. To the left of that domain is a region that modifies transcription. In the center of the molecule is the **DNA-binding domain.** Among the steroid receptors there is 60–95% homology in this domain. Two zinc fingers (see p. 108) interact with DNA. The structure of the zinc finger DNA-binding motif is shown in Figure 21.17. The N-terminal domain contains the principal **antigenic domains** and a site that modulates transcriptional activation. The amino acid sequences in this site are highly variable among the steroid receptors. These features are common to all steroid receptors. The family of steroid receptors is diagrammed in Figure 21.17. The ancestor to which these receptor genes are related is *v-erbA* or *c-erbA* (see p. 889). *v-ErbA* is an oncogene that binds to DNA but has no ligand-binding domain. In some cases the DNA-binding domains are homologous enough that more than one receptor will bind to a common responsive element (consensus sequence on DNA) as shown in Table 21.4. In addition to those genes pictured in Figure 21.18, the **aryl hydrocarbon receptor** (Ah) may also be a member of this family. The Ah receptor binds carcinogens with increasing affinity paralleling increasing carcinogenic potency and translocates the carcinogen to the cellular nucleus unless the receptor is already located in the nucleus. The N-terminal portions of the receptors usually contain major antigenic sites and may also contain a site that is active in modulating binding of the receptor to DNA.

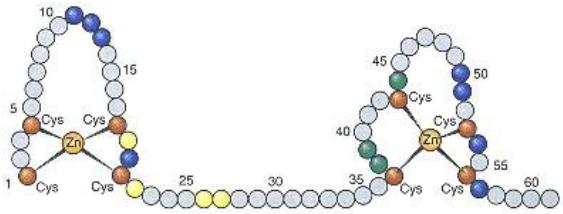


Figure 21.17
Structure of the zinc finger located within the glucocorticoid receptor DNA-binding domain as determined by X-ray crystallography.
Yellow circles indicate amino acid residues (located in GR monomer) that interact with base pairs. Blue circles are those making phosphate backbone contacts. Green circles are those participating in dimerization.
Redrawn from Luisi, B. F., Schwabe, J. W. R., and Freedman, L. P. In: G. Litwack (Ed.), *Vitamins and Hormones*, Vol. 49. San Diego, Academic Press, 1994, pp. 1–47.

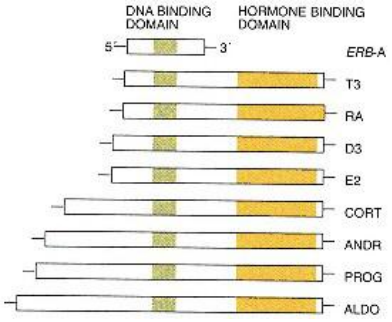


Figure 21.18
Steroid receptor gene superfamily.
T3, triiodothyronine; RA, retinoic acid; D3, dihydroxy vitamin D_3; E2, estradiol; CORT, cortisol; ANDR, androgen; PROG, progesterone; ALDO, aldosterone. Figure shows roughly the relative sizes of the genes for these receptors. Information derived from the laboratories of R. Evans, K. Yamamoto, P. Chambon, and others. In some cases there is high homology in DNA-binding domains and lower homology in ligand-binding domains.

Thyroid hormone and retinoic acid receptors are also members of the same superfamily of receptors although their ligands are not steroids. They do contain six-membered rings as shown in Figure 21.19. For some steroid receptors the A ring is the prominent site of recognition by the receptor, presenting the likelihood that the A ring inserts into the binding pocket of the receptor. In some cases, derivatives of the structures with a six-membered ring bind to the estradiol and glucocorticoid receptors. Thus the ring structures of thyroid hormone and retinoic acid have structural similarities not unlike many of the steroidal ligands involved in binding.

The receptors in this large gene family may act as transcriptional activators that together with other transcriptional regulators bring about gene activation.

21.8— Receptor Activation: Upregulation and Downregulation

Little is known about activation of steroid receptors. Activation converts a non-DNA-binding form (unactivated–nontransformed) of the receptor to a form (activated–transformed) that is able to bind nonspecific DNA or specific DNA (hormone-responsive element). The likelihood that certain receptors are cytoplasmic (glucocorticoid receptor and possibly the mineralocorticoid receptor) while others seem to be nuclear (progesterone, estradiol, vitamin D_3, and androgen receptors) may have a bearing on the significance of the activation phenomena. Most information is available for cytoplasmic receptors. The current view is that the non-DNA-binding form is a heteromeric trimer consisting of one molecule of receptor and a dimer of 90-kDa heat shock protein, as shown in Figure 21.20. The DNA-binding site of the receptor is blocked by the heteromeric proteins or by some other factor or by a combination of both. Upon activation–transformation a stepwise disaggregation of this complex could occur, leading to the activated receptor having its DNA-binding site fully exposed. The reaction may be initiated by the binding of steroid to the ligand-binding site that produces a conformational change in the receptor protein.

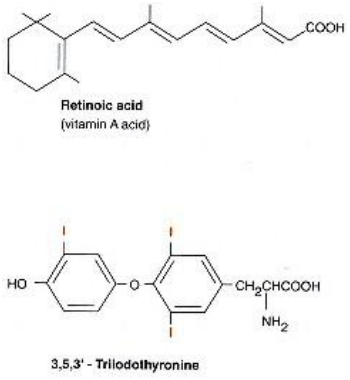


Figure 21.19
Structures of retinoic acid (vitamin A acid) and 3,5,3 -triiodothyronine.

Although the conditions required to induce activation *in vitro* are well known, the primary signal within the cell is not. Many believe that the binding of ligand alone is not sufficient to cause the activation process. Clearly, elevated temperature is a requirement for this conformational change, since incubation of target cells with appropriate steroids at low temperatures fails to result in *in vivo* activation and subsequent translocation. Once the liberated receptor is free in the cytoplasm it crosses the perinuclear membrane, perhaps through a nucleopore, to enter the nucleus. It binds nonspecifically and specifically to

chromatin, probably as a dimer, presumably in search of the specific response element (Table 21.4). Thus these receptors are transacting factors and may act in concert with other transacting factors to provide the appropriate structure to initiate transcription. Most steroid receptors have in their DNA-binding domains an SV40-like sequence (i.e., Pro-Lys-Lys-Lys-Arg-Lys-Val) known to code for nuclear translocation. Steroid receptors have variants of this sequence; some degeneracy is permitted but probably a specific lysine residue cannot be altered. This signal may provide recognition for the nucleopore.

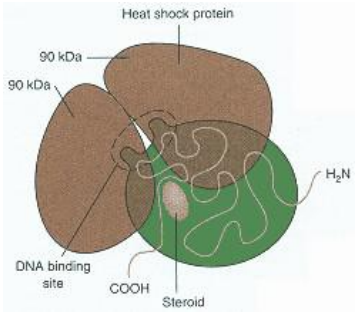


Figure 21.20
Hypothetical minimal model of a non-DNA-binding form of a steroid receptor. This form of the receptor cannot bind to DNA because the DNA-binding site is blocked by the 90-kDa hsp proteins or by some other constituent. Mass of this complex is approximately 300 kDa.

Steroid Receptors Can Be Upregulated or Downregulated Depending on Exposure to the Hormone

In general, many membrane or intracellular receptors are downregulated when the cell has been exposed to a certain amount of the hormonal ligand. In some cases, the downregulation is called "desensitization." **Downregulation** can take many forms. For membrane receptors the mechanism may be internalization by endocytosis of the receptors after exposure to hormone (see p.876). Internalization reduces the number of receptors on the cell surface and renders the cell less responsive to hormone; that is, desensitizes the cell. In the case of intracellular steroid receptors, downregulation generally takes the form of reducing the level of receptor mRNA, which decreases the concentration of receptor molecules. The receptor gene may have a specific responsive element on its promoter whose action results in an inhibition of transcription of receptor mRNA or the receptor may stimulate transcription of a gene that codes for a protein that degrades the mRNA of the receptor. Sequences are now being recognized on receptor gene promoters that may bind activated steroid–receptor complexes and result in inhibition of transcription (Table 21.4). Downregulation of receptors by their own ligands plays an important physiological role because it prevents overstimulation of target cells when circulating hormone levels are elevated.

Although downregulation of steroid receptor levels by their cognate hormones appears to be the most frequently detected form of autoregulation, it is not common to all target cells. In fact, glucocorticoid-mediated upregulation has been reported in a number of responsive cells. Since all of these cells are growth inhibited by these hormones, it was initially suggested that hormone-mediated upregulation may be required for subsequent growth inhibition. However, the fact that glucocorticoid-mediated upregulation also occurs in human lymphoid cells, which express glucocorticoid receptors but are not growth inhibited by these steroids, demonstrates that this positive **autoregulation** is neither the result nor cause of hormone-mediated growth arrest.

21.9— Specific Example of Steroid Hormone Action at Cell Level: Programmed Death

Programmed cell death or **apoptosis** is a suicide process by which cells die according to a program that may be beneficial for the organism. It can result from the rise or fall in the level of a specific hormone(s). Uterine endometrial cells at the beginning of menstruation are an example where programmed cell death is initiated by the fall in levels of progesterone and estradiol in the blood (see Clin. Corr. 21.3). Another case is **apoptosis** of thymus cells during development when the adrenal cortex becomes functional and begins to synthesize and secrete relatively large amounts of cortisol. A newborn has a large thymus but when cortisol is synthesized and released the thymus cortical cells begin to die until a resistant core of cells is reached and the gland achieves its adult size. Thus programmed cell death is a mechanism used in development for the maturation of certain organs as well as in cyclic systems where cells

CLINICAL CORRELATION 21.3

Programmed Cell Death in the Ovarian Cycle

During the ovarian cycle, the ovarian follicle expels the mature ovum at day 14 and the remaining cells of the follicle are differentiated into a functional *corpus luteum*. The *corpus luteum* produces some estradiol to partially replace that provided earlier by the maturing follicle. However, its principal product is progesterone. Estradiol and progesterone are the main stimulators of uterine endometrial wall thickening in preparation for implantation. One of the proteins induced by estradiol action in the endometrium is the progesterone receptor. Thus the uterine endometrial cells become exquisitely sensitive to estradiol as well as progesterone. The corpus luteum supplies the latter, but in the absence of fertilization and development of an embryo, the corpus luteum lives only for a short while and then atrophies because of lack of LH or chorionic gonadotropin, a hormone produced by the early embryo. The production of oxytocin and $PGF_{2\alpha}$ in the ovary may bring about the destruction of the corpus luteum (luteolysis). Blood levels of estradiol and progesterone fall dramatically after luteolysis and the stimulators of uterine endometrial cells disappear, causing degeneration of this thickened, vascularized layer of tissue and precipitating menstruation. These cells die by programmed cell death (apoptosis) due to the withdrawal of steroids. The hallmark of programmed cell death is internucleosomal cleavage of DNA. Thus programmed cell death appears to play specific roles in development and in tissue cycling either due to a specific hormonal stimulus or to withdrawal of hormone(s) as described here.

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proliferate and then regress until another cycle is initiated to begin the proliferation all over again, as is the case with the ovarian cycle.

Glucocorticoid-induced apoptosis in thymocytes is mediated by the intracellular glucocorticoid receptor. There are two phases to this complex process: inhibition of cell proliferation (cytostatic phase) followed by a cytolytic phase characterized by internucleosomal DNA cleavage and ultimate cell death (cytolytic phase). These two phases are not necessarily linked, since some cells are growth inhibited, but not lysed, by glucocorticoid hormones. The precise mechanism by which glucocorticoid–receptor complexes induce cell death is not fully understood. Exposure to hormone may result in a conformational change in chromatin with the unmasking of internucleosomal linker DNA regions, which are substrates for a nuclease. Treatment of thymocytes with glucocorticoids results in the activation of a constitutive, endogenous Ca^{2+}/Mg^{2+}-dependent endonuclease, while similar treatment of human leukemic T cells results in the activation of Ca^{2+}/Mg^{2+}-independent nuclease. Recent studies have demonstrated that the Ca^{2+}/Mg^{2+}-dependent nuclease that is activated by glucocorticoids in rat thymocytes is homologous with a cyclophilin. These proteins are high-affinity binding proteins for the immunosuppressive drug, cyclosporin A, and have Ca^{2+}/Mg^{2+}-dependent nuclease activity. The mechanism(s) by which glucocorticoid hormones induce lysis of thymocytes versus leukemic T cells appears to differ in several other respects. Treatment of sensitive T cells with these hormones results in upregulation of glucocorticoid receptor mRNA levels, while identical treatment of thymocytes appears to result in down-regulation of mRNA levels. Also, the mRNA levels for an important growth factor, *c-myc*, are repressed in glucocorticoid-treated T cells but induced in thymocytes. Thus the cytostatic and cytolytic phases of apoptosis may be mediated by slightly different pathways in these two different cell types.

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Questions

J. Baggott and C. N. Angstadt

1. The C-21 steroid hormones include:

   A. aldosterone.

   B. dehydroepiandrosterone.

   C. estradiol.

   D. testosterone.

   E. vitamin D_3.

2. Side chain cleavage enzyme complex activity may be stimulated by all of the following EXCEPT:

   A. cAMP.

   B. Ca^{2+} released via stimulation of the IP_3 pathway.

   C. Ca^{2+} entering the cell through a channel.

   D. 5 -AMP.

   E. induction of the StAR protein.

3. ^{5}-Pregnenolone is a precursor of all of the following EXCEPT:

   A. aldosterone.

   B. cortisol.

   C. 17β-estradiol.

   D. progesterone.

   E. vitamin D_3.

4. Major steps in the inactivation and excretion of ALL classes of steroid hormones (except vitamin D_3) include:

   A. conjugation to glucuronic acid.

   B. conjugation to sulfuric acid.

   C. hydroxylation.

   D. oxidation.

   E. side chain cleavage.

5. All of the following may be involved in the action of steroid hormone receptors EXCEPT:

   A. binding of the hormone to an intracellular receptor.

   B. activation of a G-protein.

   C. association with a heat shock protein (hsp90) with a cytoplasmic receptor.

   D. binding to a receptor in the nucleus.

   E. translocation of a cytoplasmic hormone–receptor complex into the nucleus.

6. Retinoic acid and its derivatives:

   A. may activate genes by preventing the binding of receptor proteins to silencer elements.

   B. bind to homodimeric proteins, which in turn bind to DNA.

   C. bind to DNA via leucine zipper motifs.

   D. are vitamin derivatives and hence have no effect on regulation of gene expression.

   E. may substitute for thyroid hormones in binding to the thyroid hormone receptor.

7. Reactions in the pathway of synthesis of active vitamin D involve all of the following organs EXCEPT:

   A. skin.

   B. kidney.

   C. liver.

   D. intestine.

Refer to the following for Questions 8–11:

   A. corticosteroid binding globulin

   B. serum albumin

   C. sex hormone-binding globulin

   D. androgen-binding protein

   E. transferrin

8. Major aldosterone carrier in blood.

9. Produced by the Sertoli cells.

10. Binds about 20% of the cortisol in the plasma.

11. At puberty decreases more in males than in females.

12. Receptors for steroid hormones are found in:

   A. cell membranes.

   B. cytoplasm.

   C. ribosomes.

   D. mitochondria.

   E. Golgi apparatus.

13. Which of the following involve(s) a response element of DNA that differs from all of the other listed hormones?

A. estrogen

B. glucocorticoid

C. mineralocorticoid

D. progesterone

14. All of the following receptors may belong to the steroid receptor gene superfamily EXCEPT:

A. aryl hydrocarbon receptor.

B. erbA protein.

C. retinoic acid receptor.

D. thyroid hormone receptor.

E. α-tocopherol receptor.

Refer to the following for Questions 15 and 16:

A. programmed cell death

B. stress response

C. downregulation of steroid receptors

D. upregulation of steroid receptors

E. silencing

15. Mechanism for the maturation of certain organs.

16. Receptor mRNA is reduced.

Answers

1. A B and D: These are C-19 androgens. C: Estradiol is a C-18 estrogen. E: Vitamin D_3 is a C-27 compound (pp. 899–900).

2. D See Figure 21.4, p. 898.

3. E See Figure 21.5 (p. 899) and Figure 21.6 (p. 900) for the synthesis of A–D. The synthesis of vitamin D_3 is summarized in Figure 21.11 (pp. 906–907).

4. A Oxidation (including hydroxylation) and reduction are common in steroid hormone degradation. Glucocorticoids undergo side chain cleavage. Conjugation to sulfate is important in the excretion of androgens. But conjugation to glucuronide is significant for all steroid hormones except vitamin D_3 (Table 21.2, p. 902).

5. B G-proteins are generally associated with signal transduction for receptors on the membrane surface. See Figure 21.13 for the roles of the other choices.

6. A The retinoic acid receptor (RAR) binds to specific silencer elements in the absence of the ligand, retinoic acid. When bound, transcription of the gene is repressed. In addition, there are retinoid X receptors (RXR), which also affect gene expression, via heterodimerization with RAR (p. 912, Figure 21.14*b*).

7. D Intestine is a target organ of the active hormone, but is not involved in synthesis. See Figure 21.11, p. 906. A: Light-induced cleavage of 7-dehydrocholesterol occurs in the skin. B: Hydroxylation of 25-(OH)D_3 occurs in the kidney. C: Hydroxylation of D_3 occurs in the liver.

8. B See p. 908.

9. D See p. 908.

10. B Cortisol-binding globulin carries most of the cortisol. Serum albumin, however, nonspecifically binds a large number of hydrophobic substances, including cortisol (p. 908).

11. C As a result, there is more unbound testosterone circulating in the blood of males (p. 908).

12. B B: In addition, the nucleus contains steroid hormone receptors. See Figure 21.13, p. 909. A: Membrane receptors are generally associated with nonhydrophobic hormones, such as epinephrine and peptide hormones (Figure 21.4, p. 898).

13. A The *positive* glucocorticoid response element is the same as the mineralocorticoid response element and the progesterone response element. The estrogen response element differs (Table 21.4, p. 911).

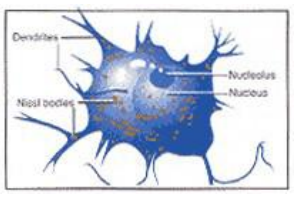
14. E Note that *c-erbA* is a protooncogene. See p. 913, Figure 21.17.

15. A Thymus cortical cells are killed by cortisol (p. 915).

16. C This contrasts with cell membrane receptors, which can be internalized to render the cell less responsive (p. 915).

Chapter 22—
Molecular Cell Biology

Thomas E. Smith



22.1— Overview

Animals sense their environment through the responses of certain organs to stimuli: touch, pain, heat, cold, intensity (light or noise), color, shape, position, pitch, quality, acid, sweet, bitter, salt, alkaline, fragrance, and so on. Externally, these generally reflect responses of the skin, eye, ear, tongue, and nose to stimuli. Some of these signals are localized to the point at which they occur; others—sound and sight—are projected in space, that is, the environment outside and distant to the animal.

Discrimination of these signals occurs at the point of reception, but acknowledgment of what they are occurs as a result of secondary stimulation of the nervous system and transmission of the signals to the brain. In many instances, a physical response is indicated, which results in muscular activity, either voluntary or involuntary. Common to these events is electrical activity associated with signal transmission along neurons and chemical activity associated with signal transmission across synaptic junctions. In all cases, stimuli received from the environment in the form of pressure (skin, feeling), light (eye, sight), noise (ear, hearing), taste (tongue), or smell (nose) are converted (transduced) into electrical impulses and to some other form of energy in order to effect the desired terminal response dictated by the brain. A biochemical component is associated with each of these events.

General biochemical mechanisms of signal transduction and amplification will be discussed as they relate to biochemical events involved in nerve transmission, vision, and muscular contraction. Finally, a specialized case of biochemical signal amplification will be discussed, namely, blood coagulation. This process is initiated on membrane surfaces as a result of the exposure of specific proteins that act as receptors and form nucleation sites for formation of multienzyme complexes. These multienzyme complexes lead to the amplification of blood coagulation through a cascade mechanism.

22.2— Nervous Tissue: Metabolism and Function

Knowledge of the chemical composition of the **brain** began with the work of J. L. W. Thudichum in 1884 and the publication of his monogram, "A Treatise on the Chemical Composition of the Brain, Based Throughout on Original Research" (cited in West and Todd, *Textbook of Biochemistry,* MacMillan, 1957). Thudichum's research was supplemented with the work of others during those earlier years. There have been almost explosive advances during more recent years, through the use of molecular biological techniques, not only in our knowledge of the composition of the brain but also of molecular mechanisms involved in many brain/neuronal functions.

About 2.4% of an individual's body weight is nervous tissue, of which approximately 83% is the brain. The **nervous system** provides the communications network between the senses, the environment, and all parts of the body. The brain is the command center. This system is always functioning and requires a large amount of energy to keep it operational. Under normal conditions, the brain derives its **energy** from **glucose metabolism. Ketone bodies** can cross the **blood–brain barrier** and be metabolized by brain tissue. Their metabolism becomes more prominent during **starvation,** but even then they cannot replace the need for glucose. The human brain uses approximately 103 g of glucose per day. For a 1.4-kg brain, this corresponds to a rate of utilization of approximately 0.3 μmol min^{-1} g^{-1} of tissue. This rate of glucose utilization represents a capacity for **ATP production** through the **tricarboxylic acid (TCA) cycle** alone of approximately 6.8 μmol min^{-1} g^{-1} of tissue. Of course, the TCA cycle is not 100% efficient for ATP production, nor is all of the glucose metabolized through it. Most of the ATP used by the brain and other nervous tissue is

generated aerobically through the TCA cycle, which functions at near maximum capacity. **Glycolysis** functions at approximately 20% capacity. Much of the energy used by the brain is to maintain ionic gradients across the plasma membranes, to effect various storage and transport processes, and for the synthesis of neurotransmitters and other cellular components.

Two features of brain composition are worth noting. It contains specialized and **complex lipids,** but they appear to function to maintain membrane integrity (see Chapter 5) rather than to have metabolic roles. There is generally a rapid **turnover rate** of **brain proteins** relative to other body proteins in spite of the fact that the cells do not divide after they have differentiated.

Cells of the nervous system responsible for collecting and transmitting messages are the **neurons.** They are very highly specialized (Figure 22.1). Each neuron consists of a cell body, **dendrites** that are short antenna-like protrusions that receive signals from other cells, and an **axon** that extends from the cell body and transmits signals to other cells. The central nervous system (CNS) is a highly integrated system where individual neurons can receive signals from a variety of different sources, including both inhibitory and excitatory stimuli.

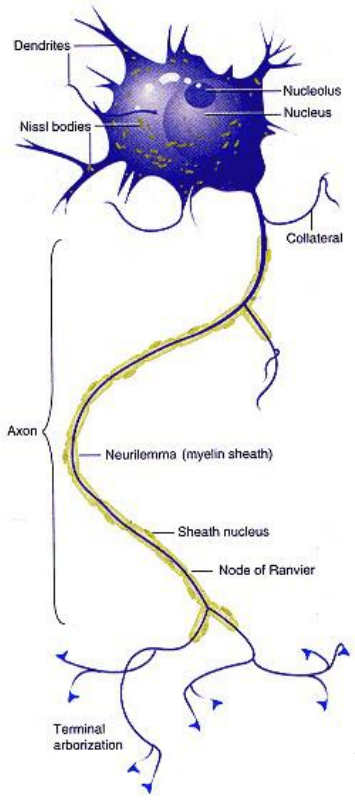


Figure 22.1
A motor nerve cell and investing membranes.

Cells other than neurons exist in the CNS. In the brain, there are about 10 times more glial cells than there are neurons. Glial cells occupy spaces between neurons and provide some electrical insulation. Glial cells are generally not electrically active, and they are capable of division. There are basically five types of glial cells: Schwann cells, oligodendrocytes, microglia, ependymal cells, and astrocytes. Each type of glial cell has a specialized function, but only astrocytes appear to be directly associated with biochemical functions related to neuronal activity. One is metabolic (see discussion below on GABA) and the other anatomical.

Astrocytes send out processes at the external surfaces of the CNS. These processes are linked to form anatomical complexes that provide sealed barriers and isolate the CNS from the external environment. Astrocytes also send out similar processes to the circulatory system, inducing the endothelial cells of the capillaries to become sealed by forming tight junctions that prevent the passive entry into the brain of water-soluble molecules. These tight junctions form what is commonly known as the **blood–brain barrier.** Water-soluble compounds enter the brain only if there are specific membrane transport systems for them.

The normal individual has between 10^{11} and 10^{13} neurons, and communication between them is by electrical and chemical signals. Electrical signals transmit nerve impulses down the axon and chemicals transmit signals across the gap between cells. Some of the biochemical events that give the cell its electrical properties and are involved in the propagation of an impulse will be discussed.

ATP and Transmembrane Electrical Potential in Neurons

Adenosine triphosphate generated from the metabolism of glucose is used to help maintain an **equilibrium electrical potential** across the membrane of the neuron of approximately –70 mV, with the inside being more negative than the outside. This potential is maintained by the action of the **Na^+, K^+ ion pump** (see pp. 206–207), the energy for which is derived from the hydrolysis of ATP to give ADP and inorganic phosphate. This system pumps Na^+ out of the cell by an antiport mechanism, whereas K^+ is moved into the cell. The channels through which Na^+ enters the cell are **voltage gated;** that is, the proteins of the channel undergo a charge-dependent conformation change and open when the electrical potential across the membrane decreases (specifically, becomes less negative) by a value greater than some threshold value. When the membrane becomes depolarized, Na^+, whose concentration is higher outside the cell than inside, flows into the cell and K^+, whose concentration is greater inside the cell, flows out of the cell, both going down their respective concentration gradients. The channels are open in a particular geographical

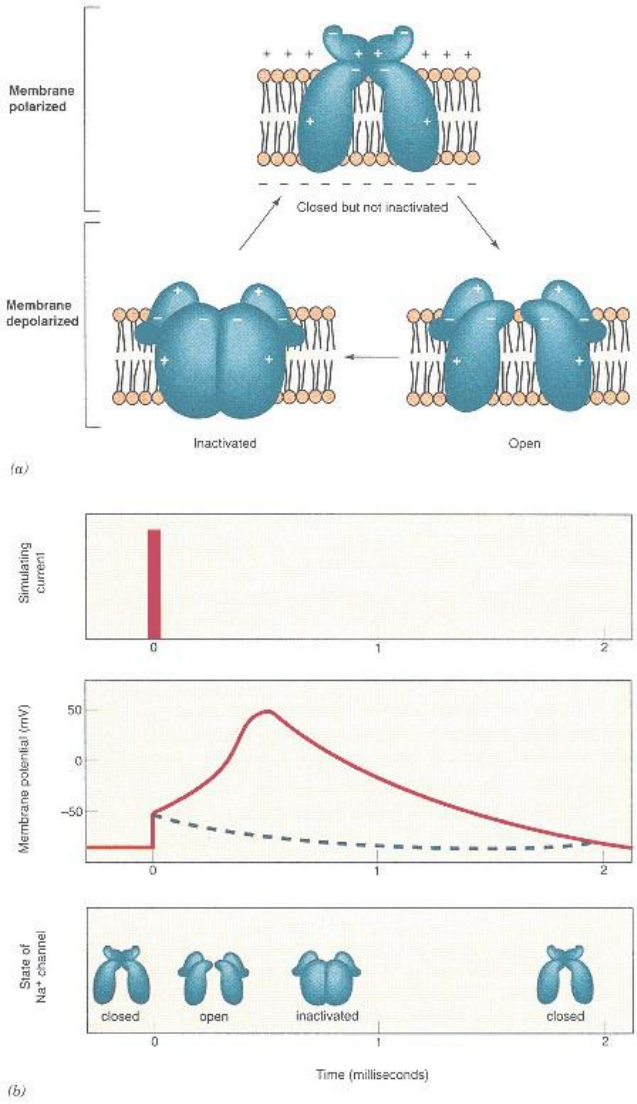


Figure 22.2

Schematic of Na^+ channels opening and closing during nerve impulse transmission.
Redrawn from Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., and Watson, J. *Molecular Biology of the Cell*, 2d ed. New York: Garland Publishing, 1989, p. 1071.

region of the cell for fractions of a millisecond (Figure 22.2). The localized depolarization (voltage change) causes a conformation change in the neighboring proteins that make up the **voltage-gated ion channels.** These channels open momentarily to allow more ions in and, thus, by affecting adjacent channel proteins, allow the process to continue down the axon. There is a finite recovery

time. During this time, the proteins that form the channels cannot repeat the process of opening. Thus charge propagation proceeds in one direction. It is the progressive depolarization and repolarization along the length of the axon that allow electrical impulses to be propagated undiminished in amplitude. Electrical impulse transmission is a continuous process in nervous tissue, and it is the ATP generated primarily from the metabolism of glucose that keeps the system operational.

A current area of active research in biochemistry involves the use of gene cloning and engineering techniques to isolate ion channel proteins and to determine their structures and elucidate their mechanisms of action. A considerable amount of information has been obtained in recent years on how mutations in voltage-gated ion channels may affect muscle function. Considerably less is known, however, about the relationship between structural disorders of ion channels in neurons and clinical disorders.

TABLE 22.1 Some of the Neurotransmitters Found in Nervous Tissue

EXCITATORY
Acetylcholine
Aspartate
Dopamine
Histamine
Norepinephrine
Epinephrine
ATP
Glutamate
5-Hydroxytryptamine
INHIBITORY
4-Aminobutyrate
Glycine
Taurine

Neuron–Neuron Interaction Occurs through Synapses

There are generally two mechanisms for **neuron–neuron interaction**: through **electrical synapses** or through **chemical synapses.** Electrical synapses permit the more rapid transfer of signals from cell to cell. Chemical synapses allow for various levels of versatility in cell–cell communication. T. R. Elliot, in a paper published in 1904, was one of the first scientists to clearly express the idea that signaling between nerves could be chemical. Needless to say, considerably more information is now known about this mode of neuron–neuron communication. Chemical synapses are of two types: those that bind directly to an ion channel and cause it to open or to close, and those that bind to a receptor that releases a second messenger that reacts with the ion channel to cause it to open or to close. Primary emphasis here is on chemical synapses.

Chemical neurotransmitters fit the following criteria: they are found in the presynaptic axon terminal; enzymes necessary for their syntheses are present in the presynaptic neuron; stimulation under physiological conditions results in their release; mechanisms exist (within the synaptic junction) for rapid termination of their action; and their direct application to the postsynaptic terminal mimics the action of nervous stimulation. A sixth criterion, as a corollary of the five criteria listed above, is that drugs that modify the metabolism of the neurotransmitter should have predictable physiological effects *in vivo*, assuming that the drug is transported to the site where the neurotransmitter acts.

Chemical neurotransmitters may be excitatory or inhibitory. **Excitatory neurotransmitters** include acetylcholine and the catecholamines. **Inhibitory neurotransmitters** include γ-aminobutyric acid (also referred to as GABA or 4-aminobutyric acid), glycine, and taurine (Table 22.1).

The two major inhibitory neurotransmitters in the central nervous system are glycine and GABA. Glycine acts predominantly in the spinal cord and the brain stem, and γ-aminobutyric acid (GABA) acts predominantly in all other parts of the brain. **Strychnine** (Figure 22.3), a highly poisonous alkaloid obtained from *Nux vomica* and related plants of the genus *Strychnos*, binds to **glycine receptors** of the CNS. It has been used in very small amounts as a CNS stimulant. Can you propose how it works? The **GABA receptor** also reacts with a variety of pharmacologically significant agents such as benzodiazepines (Figure 22.4) and barbiturates. As with strychnine and glycine, there is little structural similarity between GABA and benzodiazepines.

Figure 22.3
Structures of glycine and strychnine.

The genes for the **acetylcholine receptor,** which also binds nicotinic acid, the glycine receptor, and the GABA receptor have been cloned and their amino acid sequences inferred. There is a relatively high degree of homology in their primary amino acid sequences.

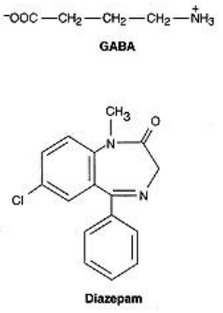


Figure 22.4
Structures of GABA and diazepam.

A model of one-half of the GABA receptor is shown in Figure 22.5. This receptor has an $_{2}$ $_{2}$ composition. The polypeptides are synthesized with "signal

peptides" that direct their transport to the membrane. The α subunit has 456 amino acid residues and the β subunit has 474. The signal peptides are cleaved, leaving α and β subunits of 429 and 449 amino acid residues, respectively. Interestingly, the pharmaceutical agents bind to the α subunit, whereas GABA, the natural inhibitory neurotransmitter, binds to the β subunit. The protrusion of an extended length of the amino-terminal end of each polypeptide to the extracellular side of the membrane suggests that the residues to which the channel regulators bind are at the N terminal. A smaller C-terminal segment is also on the extracellular side of the membrane. The four subunits of the receptor form a channel through which small negative ions (Cl^-) can flow, depending on what is bound to the receptor end of the molecule.

All neurotransmitters are made and stored in **presynaptic neurons.** They are released after stimulation of the neuron, traverse the synapse, and bind to a specific receptor on the postsynaptic junction to elicit a response in the next cell. If the neurotransmitter is an excitatory one, it causes depolarization of the membrane as described above. If it is an inhibitory neurotransmitter, it binds to a channel-linked receptor and causes a conformation change that opens the pores and permits small negatively charged ions, specifically Cl^-, to enter. The net effect of this is to increase the chloride conductance of the postsynaptic membrane, making it more difficult for it to become depolarized—that is, effectively causing **hyperpolarization.**

Synthesis, Storage, and Release of Neurotransmitters

Nonpeptide neurotransmitters may be synthesized in almost any part of the neuron, in the cytoplasm near the nucleus, or in the axon. Most nonpeptide neurotransmitters are amino acids, derivatives of amino acids, or other intermediary metabolites. Synthesis and degradation of many of them have been discussed elsewhere, but some aspects of their metabolism relative to nerve transmission will be discussed later in this chapter.

Neurotransmitters travel rapidly across the **synaptic junction** (which is about 20 nm across), bind to receptors on the postsynaptic side, induce

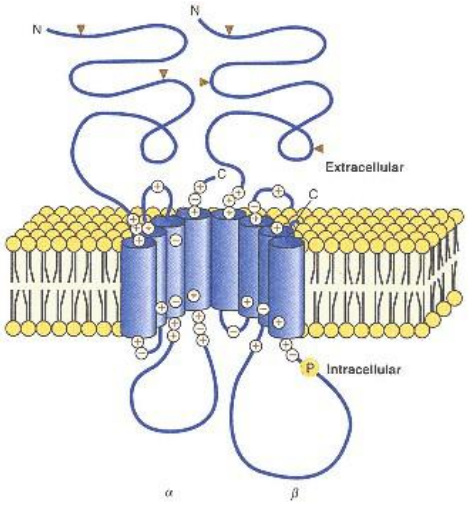


Figure 22.5
Schematic model of one-half of the GABA receptor embedded in the cell membrane.
The complete receptor has an $_{2}$ $_{2}$ structure and forms an ion channel. The site labeled P is a serine residue that may be phosp- horylated by a cAMP-dependent protein kinase. Redrawn from Schofield, P. R., Darlison, M. G., Fujita, N. et al. *Nature* 328:221, 1987.

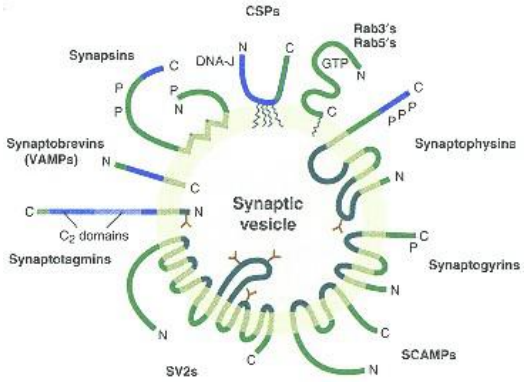


Figure 22.6
Schematic drawing of the relative arrangement of proteins of the synaptic vesicle (SV).
Rab proteins are attached by isoprenyl groups and cysteine string proteins by palmitoyl chains to SVs. The N and C termini of proteins are marked by N and C, respectively. Phosphorylation sites are indicated by P.
Redrawn from Sudhof, T. C. *Nature* 375:645, 1995.

conformational changes in receptors and/or that membrane, and start the process of electrical impulse propagation in the postsynaptic neuron. Storage and release of neurotransmitters are intricate processes, but many details of the mechanism of these processes have begun to unfold. It has been shown by conventional techniques that some neurons contain more than one chemical type of neurotransmitter. The significance of this observation is unclear. Release of neurotransmitter is a **quantal event;** that is, a nerve impulse reaching the presynaptic terminal results in the release of transmitters from a fixed number of **synaptic vesicles.** Release of neurotransmitters involves attachment of the synaptic vesicle to the membrane and **exocytosis** of their content into the synaptic cleft.

Storage of neurotransmitters occurs in large or small vesicles in the presynaptic terminal. Small vesicles are the predominant type and exist in two pools: free and attached to cytoskeletal proteins, mainly actin. Small vesicles contain only "classical" small molecule type transmitters, whereas large vesicles may contain "classical" small molecule neurotransmitters and neuropeptides. Some may also contain enzymes for synthesis of norepinephrine from dopamine. A schematic diagram of a small synaptic vesicle is shown in Figure 22.6. The genes for many of the proteins attached to the synaptic vesicle have been cloned and significant amounts of information about their functions are known. Table 22.2 contains a list of some of those proteins. Some of their properties are briefly described. Figure 22.7 shows schematically how some of them may be arranged on the synaptic vesicle and how they may interact with the plasma membrane of the presynaptic neuron.

1. **Synapsin** exists as a family of proteins encoded by two genes. The proteins differ primarily in the C-terminal end (Figure 22.8). They constitute about 9% of the total protein of the synaptic vesicle membrane. All can be phosphorylated near their N termini by either **cAMP-dependent protein kinase** and/or **calcium–calmodulin (CaM) kinase I,** which is considered to be the physiologically important one relative to nerve transmission. Synapsins Ia and Ib can also be phosphorylated by **CaM kinase II** near their C termini, a region that is missing in synapsin IIa and IIb.

Synapsin has a major role in determining whether the synaptic vesicles are in the free pool and available for binding to the presynaptic membrane. Nerve stimulation leads to the entry of Ca^{2+} into the presynaptic vesicle (see Clin. Corr. 22.1). CaM kinase I (II also) is activated and phosphorylates synapsin. This either prevents binding of synaptic vesicles to the cytoskeletal proteins or

TABLE 22.2 List of Synaptic Vesicle Proteins

Synapsin	Ia
	Ib
	IIa
	IIb
Synaptophysin	
Synaptotagmin (p65)	
Syntaxin (p35)	
Synaptobrevin/VAMP	
Rab3 and rabphilin	
SV-2	
Vacuolar proton pump	

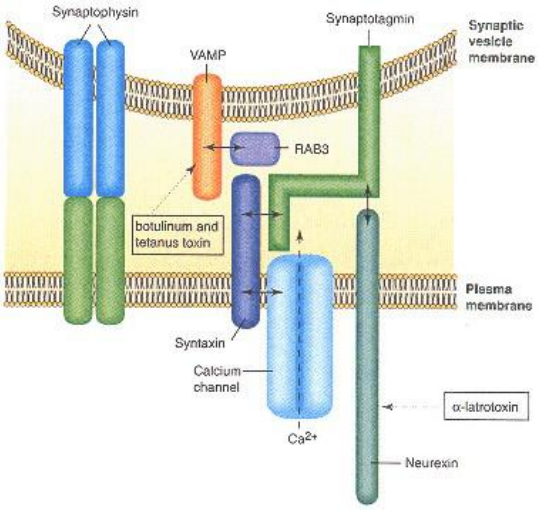


Figure 22.7
Schematic diagram showing how some of the synaptic vesicle proteins may interact with plasma membrane proteins to effect exocytosis.
Redrawn from Bennett, M. K., and Scheller, R. H.
Proc. Natl. Acad. Sci. USA, 90:2559, 1993.

releases them from those binding sites. The result is an increase in the free pool of synaptic vesicles. It has also been observed that **calcium–calmodulin** itself can bind synapsin and competitively block its interaction with actin. Calcium–calmodulin therefore regulates the number of free synaptic vesicles in the two pools by two mechanisms.

2. **Synaptophysin** is an integral membrane protein of synaptic vesicles that is structurally similar to gap junction proteins. It may be involved in the **formation of a channel** from the synaptic vesicle through the presynaptic membrane to permit the passage of neurotransmitters into the synaptic cleft.

3. **Synaptotagmin** is also an integral membrane protein of synaptic vesicles that interacts in a Ca^{2+}-dependent manner with specific proteins localized

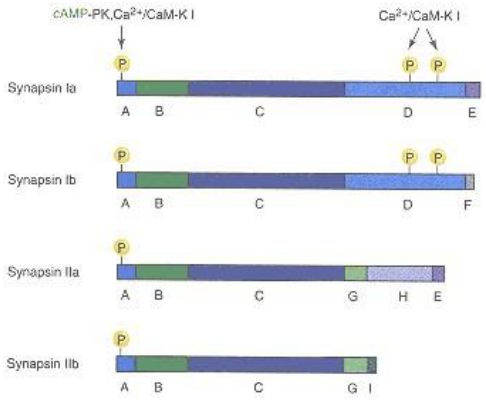


Figure 22.8
Structural arrangement of the synapsin family of proteins.
Redrawn from Chilcote, T. J., Siow, Y. L., Schaeffer, E., et al.
J. Neurochem. 63:1568, 1994.

CLINICAL CORRELATION 22.1

Lambert–Eaton Myasthenic Syndrome

Lambert–Eaton myasthenic syndrome (LEMS) is an autoimmune disease in which the body raises antibodies against voltage-gated calcium channels (VGCC) located on presynaptic nerve termini. Upon depolarization of presynaptic neurons, calcium channels at presynaptic nerve termini open, permitting the influx of calcium ions. This increase in calcium ion concentration initiates events of the synapsin cycle and leads to release of neurotransmitters into synaptic junctions. When autoantibodies against VGCC react with neurons at neuromuscular junctions, calcium ions cannot enter and the amount of acetylcholine released into synaptic junctions is diminished. Since action potentials to muscles may not be induced, the effect mimics that of classic myasthenia gravis.

LEMS has been observed in conjunction with other conditions such as small cell lung cancer. Some patients have shown a neurological disorder manifesting itself as subacute cerebellar degeneration (SCD). Plasma exchange (removal of antibodies) and immunosuppressive treatments have been effective for LEMS, but the latter treatment is less effective on SCD.

Diagnostic assays for LEMS depend on the detection of antibodies in patients' sera against VGCC. There are at least four subtypes of VGCC: T, L, N, and P. It has been found that the P subtype may be the one responsible for initiating neurotransmitter release at the neuromuscular junction in mammals. A peptide toxin produced by a cone snail (*Conus magnus*) binds to P-type VGCC in cerebella extracts. This small peptide has been labeled with ^{125}I, bound to VGCC in cerebella extracts, and the radiolabeled complex was precipitated by sera of patients who have been clinically and electrophysiologically defined as LEMS positive. This assay may prove useful not only in detecting LEMS but also in providing a means of finding out more about the antigenicity of the area(s) on the VGCCs to which antibodies are raised.

Goldstein, J. M., Waxman, S. G., Vollmer, T. L., et al. Subacute cerebellar degeneration and Lambert–Eaton myasthenic syndrome associated with antibodies to voltage-gated calcium channels: differential effect of immunosuppressive therapy on central and peripheral defects. *J. Neurol. Neurosurg. Psychiatry* 57:1138, 1994; and Motomura, M., Johnston, I., Lang, B., et al. An improved diagnostic assay for Lambert–Eaton myasthenic syndrome. *J. Neurol. Neurosurg. Psycbiatry* 58:85, 1995.

on the presynaptic plasma membrane. It is probably involved in the process of **docking** of synaptic vesicles to the membrane.

4. **Syntaxin** is an integral membrane protein of the plasma membrane of the presynaptic neuron. Syntaxin binds synaptotagmin and mediates its interaction with Ca^{2+} channels at the site of release of the neurotransmitters. It also appears to have a role in **exocytosis.**

5. **Synaptobrevin/VAMP** (or vesicle-associated membrane protein) exists as a family of two small proteins of 18 and 17 kDa. They are anchored in the cytoplasmic side of the synaptic vesicle membrane through a single C-terminal domain and appear to be involved in **vesicle transport** and/or exocytosis. VAMPs appear to be involved in the release of synaptic vesicles from the plasma membrane of the presynaptic neuron. **Tetanus** and **botulinum toxins** bind VAMPs, causing slow and irreversible inhibition of transmitter release.

6. **Rab3** is one among a large rab family of **GTP-binding proteins**. Rab3 is specific for synaptic vesicles and is involved in the docking and **fusion process of exocytosis.** Rab3 is anchored to the membrane through a **polyprenyl side chain** near its C-terminal end. Elimination by genetic engineering of the polyprenyl side chain binding site did not alter its function *in vitro*, but it is not clear whether this is also true *in vivo*.

7. **SV-2** is a large **glycoprotein** with 12 transmembrane domains. No function has yet been assigned to it.

8. **Vacuolar proton pump** is an **ATPase** found in the vesicle membrane that is responsible for the **transport of neurotransmitters** into the synaptic vesicle.

Termination of Signals at Synaptic Junctions

Neurotransmitter action may be terminated by metabolism, reuptake, and/or diffusion into other cell types. Neurotransmitters responsible for fast responses are generally inactivated by one or both of the first two mechanisms. The following sections will outline some biochemical pathways involved in the synthesis and the degradation of representative fast-acting neurotransmitters—

specifically, acetylcholine, catecholamines, 5-hydroxytryptamine, and 4-aminobutyrate (GABA).

Acetylcholine

Reactions involving **acetylcholine** at the synapse are summarized in Figure 22.9. Acetylcholine is synthesized by the condensation of choline and acetyl CoA in a reaction catalyzed by **choline acetyltransferase** found in the cytosol of the neuron. The reaction is

$$\underset{\text{Choline}}{(CH_3)_3\overset{+}{N}CH_2CH_2OH} + CH_3CO{-}SCoA \longrightarrow \underset{\text{Acetylcholine}}{(CH_3)_3\overset{+}{N}CH_2CH_2OCOCH_3} + CoASH$$

Choline is derived mainly from the diet; however, some may come from reabsorption from the synaptic junction or from other metabolic sources (see p. 460). The major source of acetyl CoA is the decarboxylation of pyruvate by the **pyruvate dehydrogenase complex** in mitochondria. Since choline acetyltransferase is present in the cytosol, acetyl CoA must get into the cytosol for the reaction to occur. The same mechanism discussed previously (see p. 371) for getting acetyl CoA across the inner mitochondrial membrane (as citrate) operates in presynaptic neurons.

Acetylcholine is released and reacts with the **nicotinic–acetylcholine receptor** located in the postsynaptic membrane (see Clin. Corr. 22.2). The action of acetylcholine at the postsynaptic membrane is terminated by the action of the enzyme **acetylcholinesterase,** which hydrolyzes the acetylcholine to acetate and choline:

$$\text{Acetylcholine} + H_2O \rightleftharpoons \text{acetate} + \text{choline}$$

Choline is taken up by the presynaptic membrane and reutilized for synthesis of more acetylcholine. Acetate probably gets reabsorbed into the blood and is metabolized by tissues other than nervous tissue.

An X-ray crystallographic structure of acetylcholinesterase is shown in Figure 22.10. Its mechanism of action is similar to that of serine proteases (see p. 97). It too has a **catalytic triad,** but the amino acids in that triad, from N to C

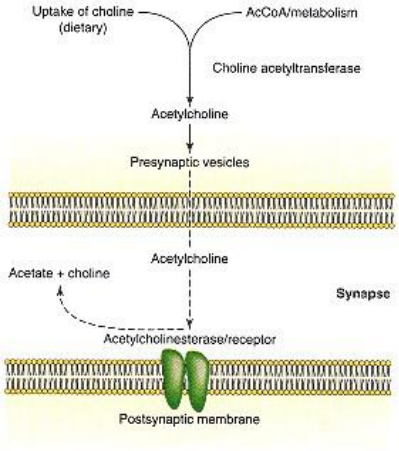


Figure 22.9
Summary of the reactions of acetylcholine at the synapse.
AcCoA, acetyl coenzyme A.

CLINICAL CORRELATION 22.2

Myasthenia Gravis: A Neuromuscular Disorder

Myasthenia gravis is an acquired autoimmune disease characterized by muscle weakness due to decreased neuromuscular signal transmission. The neurotransmitter involved is acetylcholine. The sera of more than 90% of patients with myasthenia gravis have antibodies to the nicotinic–acetylcholine receptor (AChR) located on the postsynaptic membrane of the neuromuscular junction. Antibodies against the AChR interact with it and inhibit its function, either its ability to bind acetylcholine or its ability to undergo conformation changes necessary to effect ion transport. Evidence in support of myasthenia gravis as an autoimmune disease affecting the AChR is the finding that the number of AChRs is reduced in patients with the disease, and experimental models of myasthenia gravis have been generated by either immunizing animals with the AChR or by injecting them with antibodies against it.

It is not known what events trigger the onset of the disease. There are a number of environmental antigens that have epitopes resembling those on the AChR. A rat monoclonal antibody of the IgM type prepared against AChRs reacts with two proteins obtained from the intestinal bacterium *Escherichia coli*. Both of the proteins are membrane proteins of 38 and 55 kDa, the smaller of which is located in the outer membrane. This does not suggest that exposure to *E. coli* proteins is likely to trigger the disease. The sera of both normal individuals and myasthenia gravis patients have antibodies against a large number of *E. coli* proteins. Some environmental antigens from other sources also react with antibodies against AChRs.

The thymus gland, which is involved in antibody production, is also implicated in this disease. Antibodies have been found in thymus glands of myasthenia gravis patients that react with AChRs and with environmental antigens. The relationship between environmental antigens, thymus antibodies against AChRs, and onset of myasthenia gravis is unclear.

Myasthenia gravis patients may receive one or a combination of several therapies. Pyridostigmine bromide, a reversible inhibitor of acetylcholine esterase (AChE) that does not cross the blood–brain barrier, has been used. The inhibition of AChE within the synapse by drugs of this type increases the half-time for acetylcholine hydrolysis. This leads to an increase in the concentration of acetylcholine, stimulation of more AChR, and increased signal transmission. Other treatments include use of immunosuppressant drugs, steroids, and surgical removal of the thymus gland to decrease the rate of production of antibodies. Future treatment may include the use of anti-idiotype antibodies to the AChR antibodies, and/or the use of small nonantigenic peptides that compete with AChR epitopes for binding to the AChR antibodies.

Stefansson, K., Dieperink, M. E., Richman, D. P., Gomez, C. M., and Marton, L. S. *N. Engl. J. Med.* 312:221, 1985; Drachman, D. B. (Ed.). Myasthenia gravis: biology and treatment. *Ann. N.Y. Acad. Sci.* 505:1, 1987; and Steinman, L., and Mantegazza, R. *FASEB J.* 4:2726, 1990.

termini, are in reverse order to those of the serine proteases, and glutamate instead of aspartate is involved.

Catecholamines

The **catecholamine neurotransmitters** are **dopamine** (3,4-dihydroxyphenylethylamine), **norepinephrine,** and **epinephrine** (Figure 22.11). Their biosynthesis has been discussed (see p. 466).

The action of catecholamine neurotransmitters is terminated by reuptake into the presynaptic neuron by specific transporter proteins. Cocaine, for example, binds to the **dopamine transporter** and blocks its reuptake. Dopamine remains within the synapse for a prolonged period of time and continues to stimulate the receptors of the postsynaptic neuron. Once inside the neuron,

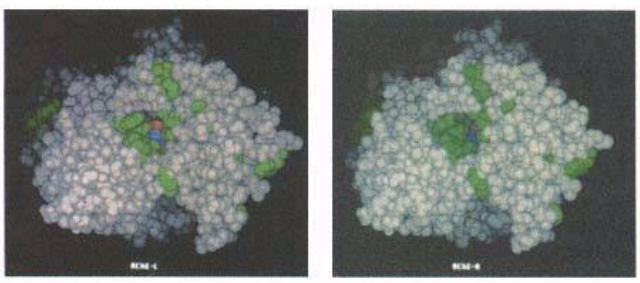


Figure 22.10
Space-filling stereo view of acetylcholinesterase looking down into the active site.
Aromatic residues are in green, Ser-200 is red, Glu-199 is cyan, and other residues are gray.

these neurotransmitters may be either repackaged into synaptic vesicles or metabolized. The two enzymes primarily involved in their metabolism are **catechol-*O*-methyltransferase** and **monoamine oxidase.** The metabolic reactions are shown in Figure 22.12. Catechol-*O*-methyltransferase catalyzes the transfer of a methyl group from *S*-adenosylmethionine to one of the phenolic OH groups. Monoamine oxidase catalyzes the oxidative deamination of these amines to aldehydes and ammonium ions. Monoamine oxidase can use them as substrates whether or not they have been altered by the methyltransferase. The end product of dopamine metabolism is homovanillic acid, and that of epinephrine and norepinephrine is 3-methoxy-4-hydroxymandelic acid.

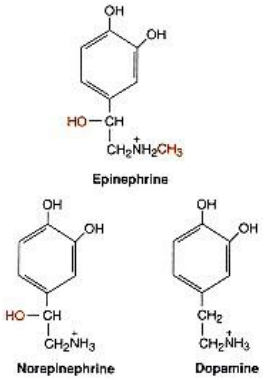


Figure 22.11
Catecholamine neurotransmitters.

5-Hydroxytryptamine (Serotonin)

Serotonin, 5-hydroxytryptamine, is derived from tryptophan (see p. 476). Like dopamine, the action of serotonin is terminated by its reuptake into the presynaptic neuron by a specific transporter. Some types of depression are associated with low brain levels of serotonin. The action of some **antidepressants** such as Paxil (paroxetine hydrochloride), Prozac (fluoxetine hydrochloride), and Zoloft (sertraline hydrochloride) is linked to their ability to inhibit

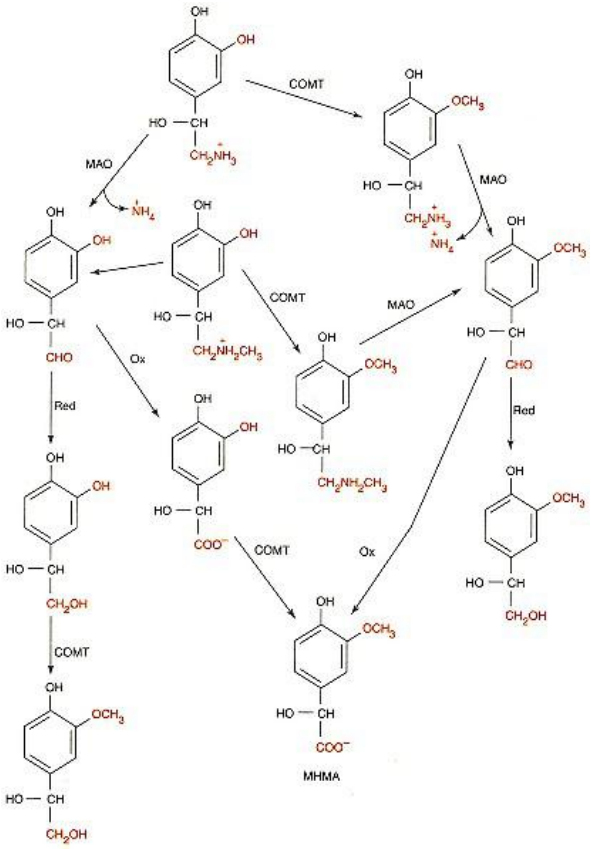


Figure 22.12
Pathways of catecholamine degradation.
COMT, catechol-O-methyltransferase (requires S-adenosylmethionine); MAO, monoamine oxidase; Ox, oxidation; Red, reduction. The end product of epinephrine and norepinephrine metabolism is 3-methoxy-4-hydroxymandelic acid.

serotonin reuptake. Once inside the presynaptic neuron, serotonin may be either repackaged in synaptic vesicles or metabolized. The primary route for its **degradation** is **oxidative deamination** to the corresponding acetaldehyde catalyzed by the enzyme monoamine oxidase (Figure 22.13). The aldehyde is further oxidized to 5-hydroxyindole-3-acetate by an aldehyde dehydrogenase.

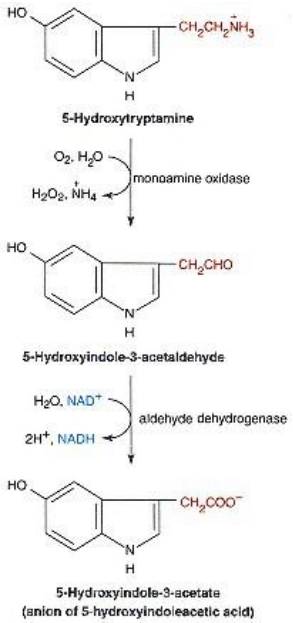


Figure 22.13
Degradation of
5-hydroxytryptamine (serotonin).

4-Aminobutyrate (γ-Aminobutyrate)

γ-Aminobutyrate (GABA), an inhibitory neurotransmitter, is synthesized and degraded through a series of reactions commonly known as the **GABA shunt.** In brain tissue, it appears that GABA and glutamate, an excitatory neurotransmitter, may share some common routes of metabolism in astrocytes (Figure 22.14). Both are taken up by astrocytes and converted to glutamine, which is then transported back into presynaptic neurons. In excitatory neurons, glutamine is converted to glutamate and repackaged in synaptic vesicles. In inhibitory neurons, glutamine is converted to glutamate and then to GABA, which is repackaged in synaptic vesicles.

It has been suggested that brain levels of GABA in some epileptic patients may be low. **Valproic acid** (2-propylpentanoic acid) apparently increases brain levels of GABA. The mechanism by which it does so is not clear. Valproic acid is metabolized primarily in the liver by glucuronidation and urinary excretion of the glucuronides, or by mitochondrial β-oxidation and microsomal oxidation.

Neuropeptides Are Derived from Precursor Proteins

Peptide neurotransmitters are generally synthesized as larger proteins and are cleaved by proteolysis to produce the neuropeptide molecules. Their synthesis requires the same biochemical machinery as does any protein synthesis and takes place in the cell body, not the axon. They travel down the axon to the presynaptic region by one of two generic mechanisms: **fast axonal transport** at a rate of about 400 millimeters per day and slow axonal transport at a rate of 1–5 millimeters per day. Since axons may vary in length from 1 millimeter to 1 meter, theoretically the total transit time could vary from 150 milliseconds to 200 days. It is highly unlikely that the latter transit time occurs under normal

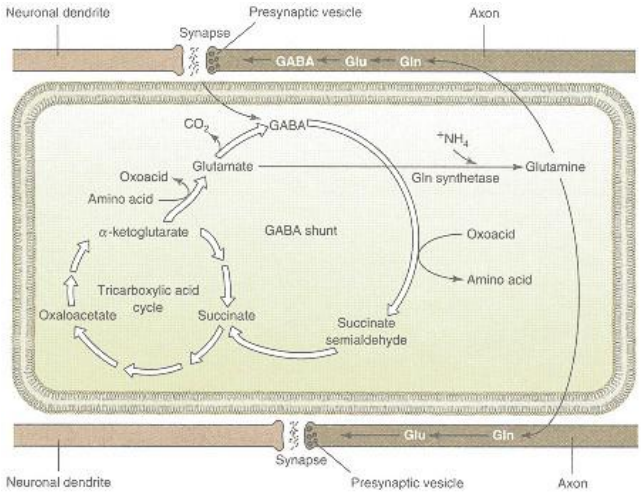


Figure 22.14
Involvement of the astrocytes in the metabolism of GABA and glutamate.

TABLE 22.3 Peptides Found in Brain Tissue[a]

Peptide	*Structure*
β-endorphin	Y G G F M T S E K S Q T P L V T
	L F K N A I I K N A Y K K G E
Met-enkephalin	Y G G F M
Leu-enkephalin	Y G G F L
Somatostatin	A G C K N F F W | | C S T F T K
Luteinizing hormone-releasing hormone	p-E H W S Y G L R P G-NH_2
Thyrotropin-releasing hormone	p-E H P-NH_2
Substance P	R P K P E E F F G L M-NH_2
Neurotensin	p-E L Y E N K P R R P Y I L
Angiotensin I	D R V Y I H P F H L
Angiotensin II	D R V Y I H P F
Vasoactive	H S D A V F T D N Y T R L R
intestinal peptide	K E M A V K K Y L N S I L N-NH_2

[a] Peptides with p preceding the structure indicate that the N terminal is pyroglutamate. Those with NH_2 at the end indicate that the C terminal is an amide.

physiological conditions, and the upper limit is probably hours rather than days. Recent experiments suggest that the faster transit times prevail.

Neuropeptides mediate **sensory and emotional responses** such as those associated with hunger, thirst, sex, pleasure, and pain. Included in this category are **enkephalins, endorphins,** and **substance P.** Substance P is an excitatory neurotransmitter that has a role in pain transmission, whereas endorphins have roles in eliminating the sensation of pain. Some of the peptides found in brain tissue are shown in Table 22.3. Note that Met-enkephalin is derived from the N-terminal region of β-endorphin. The N-terminal or both the N- and C-terminal amino acids of many of the neuropeptide transmitters are modified. For a further discussion of these peptides, see Chapter 20.

22.3— The Eye: Metabolism and Vision

The eye, our window to the outside world, allows us to view the beauties of nature, the beauties of life, and, *vide* this textbook, the beauties of biochemistry. What are the features of this organ that permit this view? A view through any window, through any camera lens, is clearest when unobstructed. The eye has evolved in such a way that a similar objective has been achieved. It is composed of live tissues that require continuous nourishment for survival. Energy and metabolites for growth and maintenance are derived from nutrients by conventional biochemical mechanisms, but the structures responsible for these processes are arranged and distributed such that they do not interfere with the visual process. Also, the brain has devised an enormously efficient filtering system that makes invisible objects within the eye that may appear to lead to visual distortion. In addition, different tissues use specific metabolic pathways to accommodate their unique needs. A schematic diagram of a cross section of the eye is shown in Figure 22.15.

Light entering the eye passes progressively through the **cornea;** the anterior chamber, which consists of the **aqueous humor;** the lens; the vitreous body, which consists of the **vitreous humor;** and finally focuses on the **retina,** which contains the visual sensing apparatus. The exterior of the cornea is bathed by

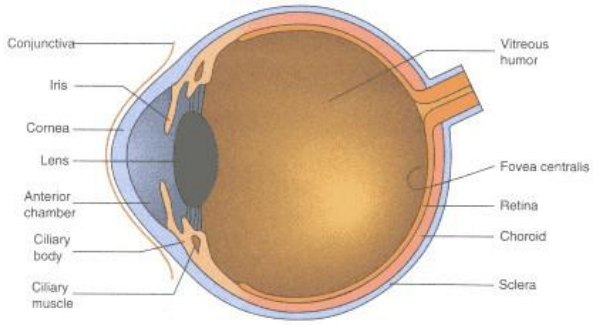


Figure 22.15
Schematic of a horizontal section of the left eye.

tears, while the interior is bathed by the aqueous humor, an iso-osmotic fluid containing salts, albumin, globulin, glucose, and other constituents. The aqueous humor brings nutrients to the cornea and to the lens, and it removes end products of metabolism from them. The vitreous humor is a collagenous or gelatinous mass that helps maintain the shape of the eye while allowing it to remain somewhat pliable.

The Cornea Derives ATP from Aerobic Metabolism

The eye is an extension of the nervous system, and like other tissues of the central nervous system, the major metabolic fuel is glucose. The cornea, which is not a homogeneous tissue, obtains a relatively large percentage of its ATP from aerobic metabolism. About 30% of glucose used by the cornea is metabolized by glycolysis and about 65% by the **hexose monophosphate pathway.** On a relative weight basis, the cornea has the highest activity of the hexose monophosphate pathway of any other mammalian tissue. It also has a high activity of **glutathione reductase,** an activity that requires NADPH, a product of the hexose monophosphate pathway. Corneal epithelium is permeable to atmospheric oxygen, that is necessary for various oxidative reactions. The reactions of oxygen can result in the formation of various **active oxygen species** that are harmful to the tissues, perhaps in some cases by oxidizing protein sulfhydryl groups to disulfides. Reduced glutathione (GSH) is used to reduce those disulfide bonds back to their original native states while GSH itself is converted to oxidized glutathione (GSSG). Furthermore, oxidized glutathione (GSSG) may also be formed by auto-oxidation. Glutathione reductase uses NADPH to reduce GSSG to 2GSH.

$$GSSG + NADPH + H^+ \xrightleftharpoons{\text{GSH reductase}} 2GSH + NADP^+$$

The activities of the hexose monophosphate pathway and the glutathione reductase maintain this tissue in an appropriately reduced state by effectively neutralizing the active oxygen species.

Lens Consists Mostly of Water and Protein

The **lens** is bathed on one side by the aqueous humor and supported on the other side by the vitreous humor. The lens has no blood supply, but it is metabolically active. It gets nutrients from the aqueous humor and eliminates waste into the aqueous humor. The lens is mostly water and proteins. The majority of the proteins are the **α-, β-, and γ-crystallins.** There are also albuminoids, enzymes, and membrane proteins that are synthesized in an epi-

TABLE 22.4 Eye Lens Crystallins and Their Relationships with Other Proteins

Crystallin	*Distribution*	[*Related*] *or Identical*
α	All vertebrates	**Small heat shock proteins** (αB)
		[*Schistosoma mansoni* antigen]
β	All vertebrates	[*Myxococcus xanthus* protein S]
γ	(embryonic γ not in birds)	[*Physarum polycephalum* spherulin 3a]
Taxon-specific enzyme crystallins		
	Most birds, reptiles	**Argininosuccinate lyase** (2)
	Crocodiles, some birds	**Lactate dehydrogenase B**
	Guinea pig, camel, llama	**NADPH: quinone oxidoreductase**
	Elephant shrew	**Aldehyde dehydrogenase I**

Source: Wistow, G. *TIBS* 18:301, 1993.

thelial layer around the edge of the lens. Some other types of proteins that are found in lens, including the lens of species other than vertebrates, are shown in Table 22.4. This shows that lens proteins may have different genetic origins and functions in other tissues. The most important physical requirement of these proteins is that they maintain a clear crystalline state. The center area of the lens, the core, consists of the lens cells that were present at birth. The lens grows from the periphery (Figure 22.16). The human lens increases in weight and thickness with age and becomes less elastic. This is accompanied by a loss of near vision (Table 22.5); a condition referred to as **presbyopia.** On average the lens may increase threefold in size and approximately 1 1/2-fold in thickness from birth to about age 80.

Lens proteins must be maintained in a native unaggregated state. They are sensitive to various insults such as changes in oxidation–reduction state, osmolarity, excessively increased concentrations of metabolites, and physical insults such as UV irradiation. Reactions that help maintain structural integrity of the lens are the Na^+, K^+–ATPase for osmotic balance, glutathione reductase for redox state balance, and protein synthesis for growth and maintenance. Energy for these processes comes from the metabolism of glucose. About 85% of the glucose metabolized by the lens is by glycolysis, 10% by the hexose monophosphate pathway, and 3% by the tricarboxylic acid cycle, presumably by the cells located at the periphery.

Cataract is the only known disease of the lens. Cataracts are opacities of lenses brought about by a loss of osmolarity and a change in solubility of some

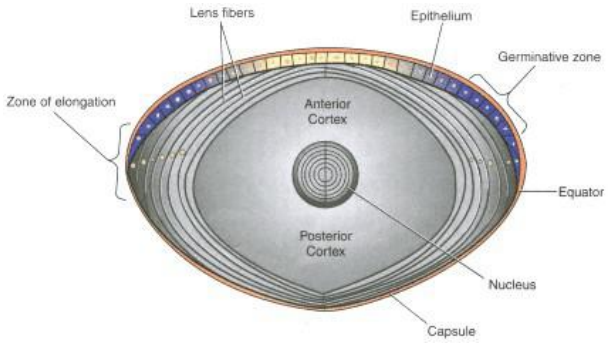


Figure 22.16
Schematic representation of a meridional section of a mammalian lens.

of the proteins, resulting in regions of high light scatter. Cataracts affect about 1 million people per year in the United States, and there are no known cures or preventative measures. The remedy is lens replacement, a very common operation in the United States. There are basically two types of cataracts: **senile cataracts** and **diabetic cataracts.** Both are the result of changes in the solubility and aggregation state of the lens crystallins. In senile cataracts, changes in the architectural arrangement of the lens crystallins are age-related and due to such changes as breakdown of the protein molecules starting at the C-terminal ends, deamidation, and racemization of aspartyl residues. Diabetic cataracts result from loss in osmolarity of the lens due to the activity of **aldose reductase** and **polyol (aldose) dehydrogenase** of the polyol metabolic pathway. When the glucose concentration in the lens is high, aldose reductase reduces some of it to **sorbitol** (Figure 22.17), which may be converted to **fructose** by polyol dehydrogenase. In human lens, the ratio of activities of these two enzymes favors sorbitol accumulation, especially since sorbitol is not used otherwise, and it diffuses out of the lens rather slowly. Accumulation of sorbitol in the lens increases osmolarity of the lens, affects the structural organization of the crystalline proteins within the lens, and enhances the rate of protein aggregation and denaturation. The areas where this occurs will have increased light scattering properties—which is the definition of cataracts. Normally, sorbitol formation is not a problem because the K_m of aldose reductase for glucose is about 200 mM and very little sorbitol would be formed. In diabetics, where the circulating concentration of glucose is high, activity of this enzyme can be significant.

TABLE 22.5 Changes in Focal Distance with Age

Age	*Focal Distance (in.)*
10	2.8
20	4.4
35	9.8
45	26.2
70	240.0

Source: Adapted from Koretz, J. F., and Handelman, G. H. *Sci. Am.*, 92, July 1988.

The Retina Derives ATP from Anaerobic Glycolysis

The **retina,** like the lens, depends heavily on anaerobic glycolysis for ATP production. Unlike the lens, the retina is a vascular tissue, but there are essentially no blood vessels in the area where visual acuity is greatest, the **fovea centralis** (see Clin. Corr. 22.3). Mitochondria are present in the retina, including in the rods and in the cones. There are no mitochondria in the outer segments of the rods and cones where the visual pigments are located.

NADH produced during glycolysis can be used to reduce pyruvate to lactate. The lactate dehydrogenase of the retina can use either NADH or NADPH, the

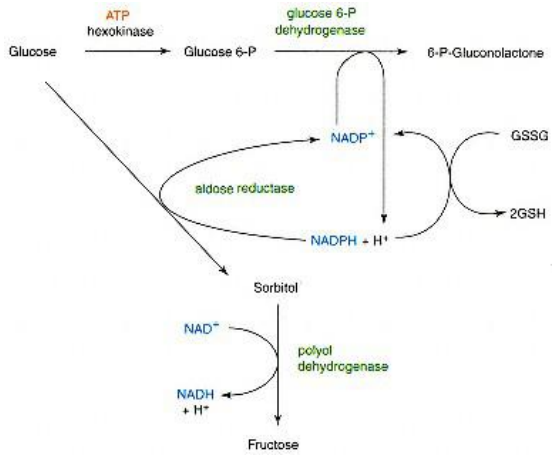


Figure 22.17
Metabolic interrelationships of lens metabolism.

CLINICAL CORRELATION 22.3

Macula Degeneration: Other Causes of Vision Loss

Many diseases of the eye affect vision, not all of which have clear, direct biochemical origins. The most serious eye diseases are those that result in blindness. Glaucoma is the most common and there is a direct causal relationship with diabetes, the biochemistry of which is fairly well known. Glaucoma can be treated and blindness does not have to be a result.

Macula degeneration leads to blindness and there is no cure. The macula is a circular area of the retina, the center of which is the fovea centralis, the area containing the greater concentration of cones and the one of greatest visual acuity. Macula degeneration may be among the leading causes of blindness in people over the age of 50. Macula degeneration is of two types: dry and wet. The dry form develops gradually over time, whereas the wet form develops rapidly and can lead to blindness within days. Macula degeneration occurs when blood vessels rupture under the macula, leading to a loss of the nutrient supply and a rapid loss of vision. Experimental procedures are in progress to surgically remove scar tissue that develops and to transplant tissue from the rear of the eye to restore nourishment to the photoreceptor cells.

Rupture of blood vessels that obscure macula details and result in rapid onset of blindness may be temporary in some cases. Six cases of sudden visual loss associated with sexual activity have been reported that are not associated with a sexually transmitted disease. Vision was lost in one eye apparently during, but most often reported a few days after engaging in, "highly stimulatory" sexual activity. Blindness was due to rupture of blood vessels in the macula area. When patients did see an ophthalmologist, most were reluctant to discuss what they were doing when sight loss was first observed. Four of the patients recovered with restoration of vision upon reabsorption of blood. In one case, where blood was trapped between the vitreous gel and the retinal surface directly in front of the fovea, the hemorrhage cleared only slightly during the next month, but visual acuity did not improve. The patient did not return for a follow-up examination, but there was no indication that the condition was permanent. Since most of the persons affected by this phenomenon were over the age of 39, it may be a worry more to professors than to students. It also may give a new meaning to the phrase "love is blind."

Friberg, T. R., Braunstein, R. A., and Bressler, N. M. *Arch. Ophthalmol.* 113:738, 1995.

latter being formed from the hexose monophosphate pathway. It is not clear whether lactate dehydrogenase of the retina plays any substantial role in mediating the regulation of glucose metabolism through either of these pathways by its selective use of NADH or NADPH.

Visual Transduction Involves Photochemical, Biochemical, and Electrical Events

Figure 22.18 shows an electron micrograph and schematic of the retinal membrane. Light entering the eye through the lens passes the optic nerve fibers, the ganglion neurons, the bipolar neurons, and the nuclei of the rods and cones before it reaches the outer segment of the rods and cones where the **signal transduction** process begins. The **pigmented epithelial** layer of the eye, the choroid, lies behind the retina, absorbs the excess light, and prevents reflections back into the rods and cones where it may cause distortion or blurring of the image (see Clin. Corr. 22.4).

The eye may be compared with a video camera. The camera collects images, converts them into electrical pulses, records them on magnetic tape, and allows their visualization by decoding the taped information. The eye focuses on an image by projecting that image onto the retina. A series of events begins, the first of which is photochemical, followed by biochemical events that amplify the signal, and finally electrical impulses are sent to the brain where the image is reconstructed in "the mind's eye." During this process, the initial event has been transformed from a physical event to a chemical reaction, through a series of biochemical reactions, to an electrical event, to a conscious acknowledgment of the presence of an object in the environment outside the body.

When photons of light enter the eye and are absorbed by photoreceptors in the **outer segments** of **rods** or **cones,** they cause isomerization of the visual pigment, **retinal,** from the 11-*cis* form to the all-*trans* form. This isomerization causes a conformation change in the protein moiety of the complex and affects the resting membrane potential of the cell, resulting in an electrical signal being

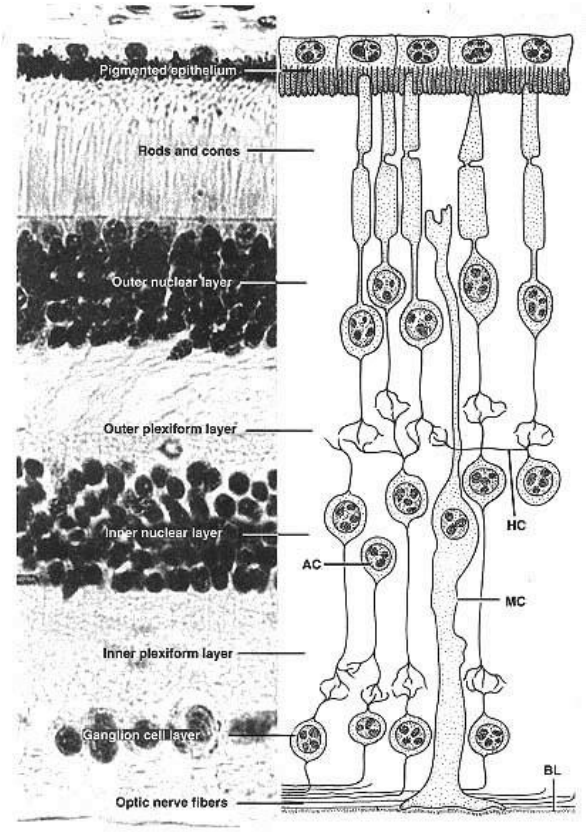


Figure 22.18
Electron micrograph and schematic representation of cells of the human retina.
Tips of rods and cones are buried in the pigmented epithelium of the outermost layer. Rods and cones form synaptic junctions with many bipolar neurons, which in turn form synapses with cells in the ganglion layer that send axons through the optic nerve to the brain. The synapse of a rod or cone with many cells is important for the integration of information. HC, horizontal cells; AC, amacrine cell; MC, Müller cell; BL, basal lamina.
Reprinted with permission from Kessel, R. G., and Kardon, R. H., *Tissues and Organs: A Text-Atlas of Scanning Electron Microscopy*. New York: W. H. Freeman, 1979, p. 87.

transmitted by way of the optic nerve to the brain. These processes will be discussed later in more detail.

Photoreceptor Cells Are Rods and Cones

The **photoreceptor cells** of the eye are the **rods** and the **cones** (Figure 22.18). Each type has flattened disks that contain a photoreceptor pigment. This pigment is **rhodopsin** in the rod cells, and **red, green,** or **blue pigment** in the cone cells. Rhodopsin is a transmembrane protein to which is bound a prosthetic group, **11-*cis*-retinal.** Rhodopsin minus its prosthetic group is **opsin.** The three proteins that form the red, green, and blue pigments of cone cells are different from each other and from opsin.

Rhodopsin, an approximately 40-kDa protein, contains seven transmembrane α-helices. The 11-*cis*-retinal is attached through a protonated Schiff base to the -amino group of lysine-296 on the seventh helix. Lysine-296 lies about midway between the two faces of the membrane (Figure 22.19*a*). A 9-Å resolution three-dimensional (3-D) model for rhodopsin, obtained by cryomicroscopy, shows that most of the helices are perpendicular to the surface of the membrane

CLINICAL CORRELATION 22.4

Niemann–Pick Disease and Retinitis Pigmentosa

There are central nervous system disorders associated with the Niemann–Pick group of diseases that can become evident by ocular changes. Some of these are observed as abnormal macula with gray discoloration and granular pigmentation or granule opacities about the fovea.

Acute type I Niemann–Pick disease, lipidosis with sphingomyelinase deficiency and primary sphingomyelin storage, may show a cherry red spot in the retina in as many as 50% of patients. Macula halo syndrome applies to the crystalloid opacities seen in some patients with subacute type I disease. They form a halo approximately one-half the disk diameter at their outer edge and are scattered throughout the various layers of the retina. They do not interfere with vision.

In an 11-year-old girl who had type II disease, more extensive ocular involvement was observed. There was sphingomyelin storage in the keratocytes of the cornea, the lens, the retinal ganglion cells, the pigmented epithelium, the corneal tract, and the fibrous astrocytes of the optic nerve.

Retinitis pigmentosa is a secondary effect of the abnormal biochemistry associated with Niemann–Pick disease.

Spence, M. W., and Callahan, J. W. In: C. R. Schriver, A. L. Beaudet, W. Sly, and D. Volle (Eds.), *The Metabolic Basis of Inherited Disease*, New York: McGraw-Hill, 1989, pp. 1656–1676.

(Figure 22.19*b*). Some, however, are distorted from this perpendicular arrangement. It is not known whether the orientation of those distorted helices is associated with binding of 11-*cis*-retinal since this low-resolution structure will not permit tracing of the carbon backbone structure of rhodopsin. See also Clin. Corr. 22.5.

Reactions involved in the formation of 11-*cis*-retinal from **β-carotene** and rhodopsin from opsin and 11-*cis*-retinal are shown in Figure 22.20. The 11-*cis*-retinal is derived from **vitamin A** and/or β-carotene of the diet. These are

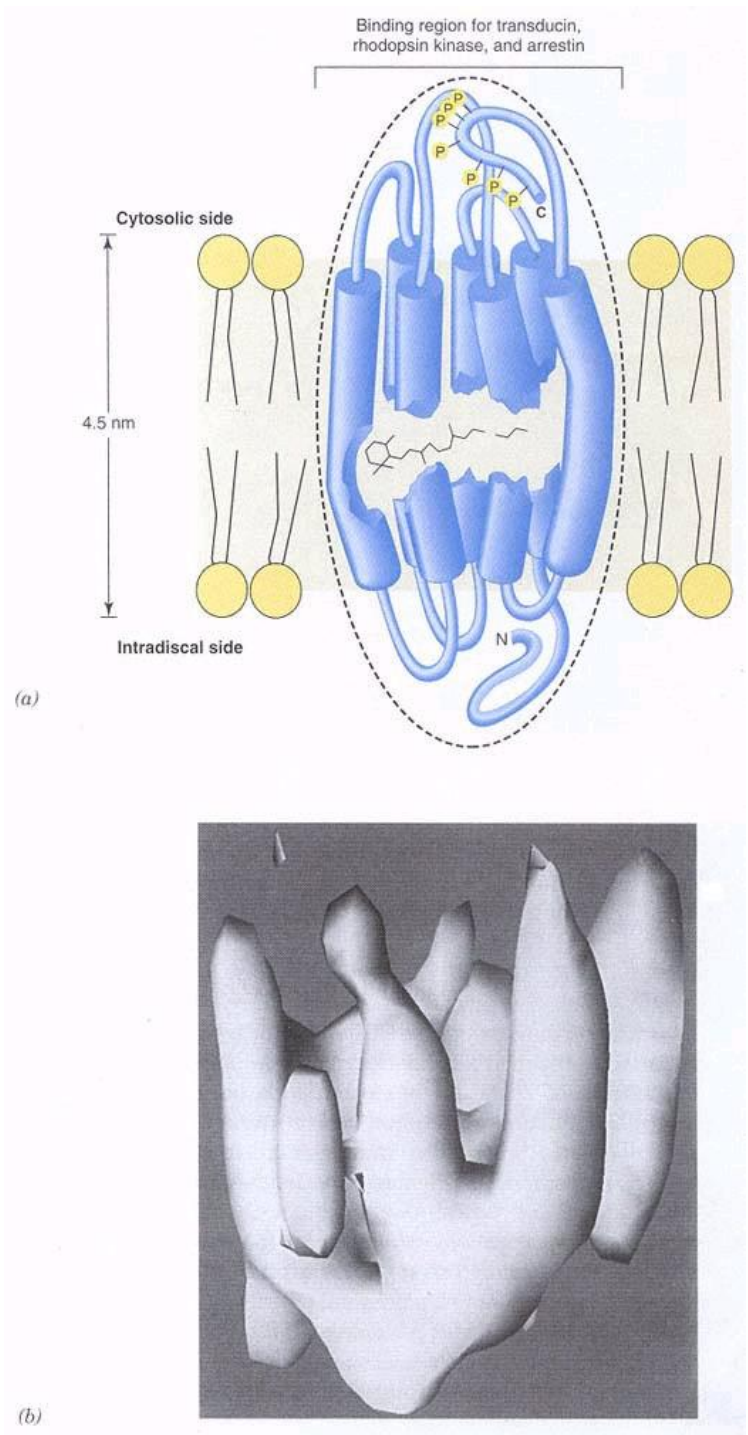


Figure 22.19
Rhodopsin.
(a) A model of the structure of vertebrate rhodopsin.
(b) A 9-Å resolution 3-D model for rhodopsin obtained by cryomicroscopy.
(a) Redrawn from Stryer, L. *Annu. Rev. Neurosci*. 9:87, 1986 (based on Dratz and Hargrave, 1983).
(b) Reproduced with permission from Unger, V. M. and Schertler, G. F. X. *Biophys. J. J*. 68:1776, 1995. Photograph generously supplied by Dr. G. F. X. Schertler.

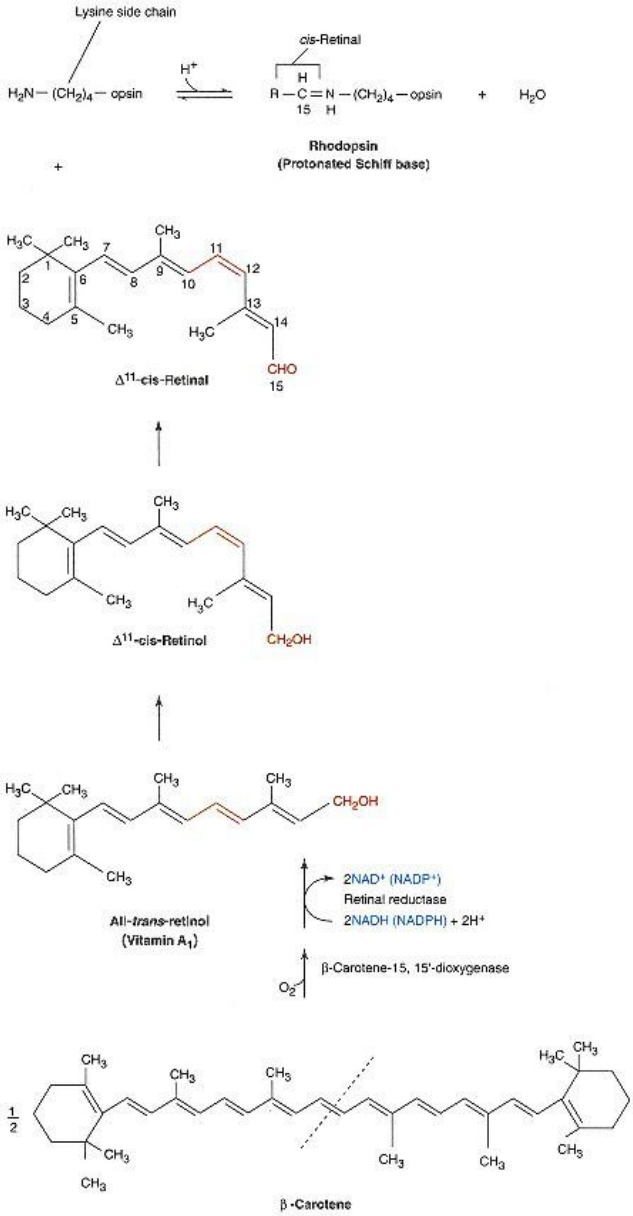


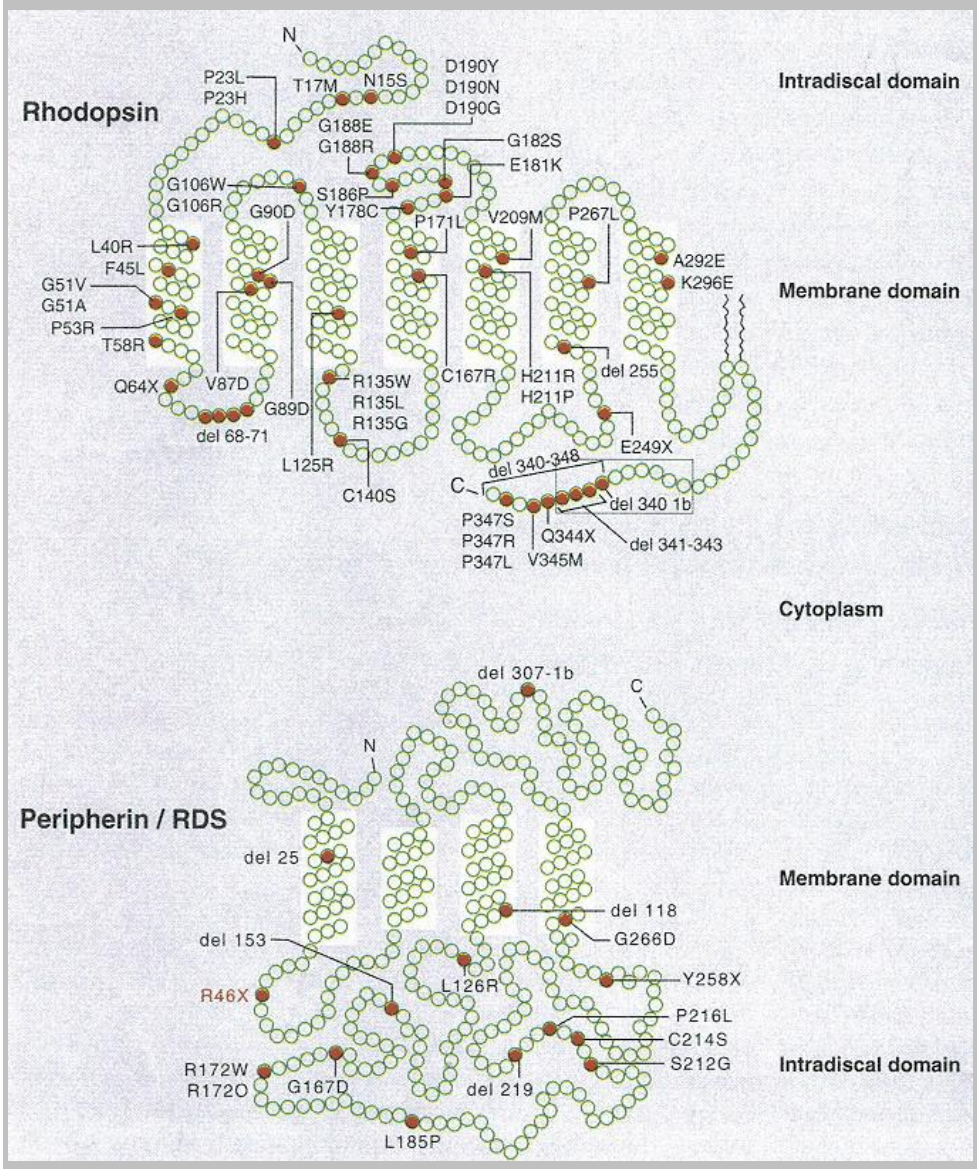
Figure 22.20
Formation of 11-cis-retinal and rhodopsin from β-carotene.

CLINICAL CORRELATION 22.5

Retinitis Pigmentosa Resulting from a *De Novo* Mutation in the Gene Coding for Peripherin

A group of heterogeneous diseases of variable clinical and genetic origins have been placed under the category of retinitis pigmentosa (RP). Several of these have origins in abnormal lipid metabolism. Approximately 1.5 million people throughout the world are affected by this disease. It is a slowly progressive condition associated with loss of night and peripheral vision. It can be inherited through an autosomal dominant, recessive, or X-linked mode. RP has been associated with mutations in the protein moiety of rhodopsin and in a related protein, peripherin/RDS, both of which are integral membrane proteins. Peripherin is a 344 amino acid residue protein located in the rim region of the disk membrane. Structural models of these two proteins are shown in the figure below. Filled circles and other notations in the figure mark residues or regions that have been correlated with RP or other retinal degenerations.

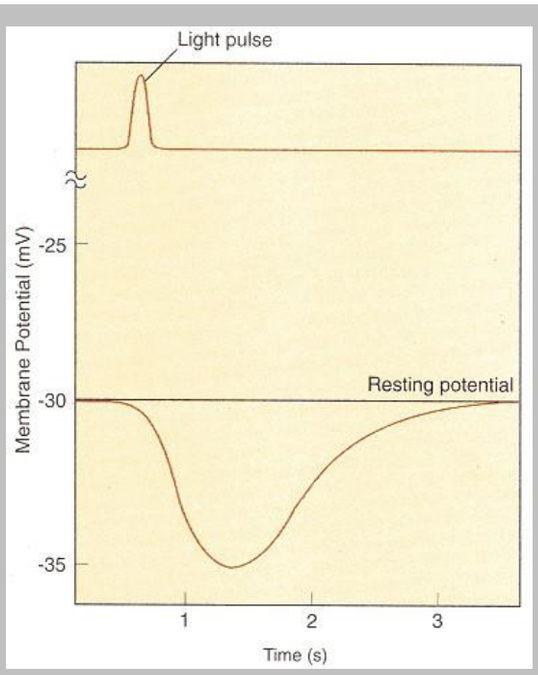
A case has been described where a *de novo* mutation in exon 1 of the gene coding for peripherin resulted in the onset of RP. Using molecular biological techniques, Lam et al. (1995) found the specific change in peripherin to be a C-to-T transition in the first nucleotide of codon 46. This resulted in changing an arginine to a stop codon (R46X). The pedigree of this family is shown in the figure on next page. Neither parent had the mutation and genetic typing analysis (20 different short tandem repeat polymorphisms) showed that the probability that the proband's parents are not his actual biological parents is less than 1 in 10 billion. This establishes with near certainty that the mutation is *de novo*.



Schematic representation of structural models for rhodopsin (top) and peripherin/RDS (bottom). The location of mutations in amino acid residues that segregate with RP or other retinal degenerations are shown as solid red circles.

(Table continued on next page)

Clin. Corr. 22.5 (continued)



Pedigree of family.
Males are squares, females are circles.
Solid squareindicates the proband.
A slash through a symbol indicates deceased.
From Lam et al. (1995).

This R46X mutation has been observed in another unrelated patient. These observations demonstrate the importance of the use of DNA analysis to establish the genetic basis for RP, especially considering that RP symptoms have been associated with a variety of other diseases, such as those related to abnormal lipid metabolism.

Shastry, B. S. *Am. J. Med. Genet.* 52:467, 1994; and Lam, B. L., Vandenburgh, K., Sheffield, V. C., and Stone, E. M. *Am. J Ophthalmol.* 119:65, 1995.

transported to specific sites in the body while attached to specific carrier proteins. Cleavage of β-carotene yields two molecules of **all-*trans*-retinol.** There is an enzyme in the pigmented epithelial cell layer of the retina that catalyzes the isomerization of all-*trans*-retinol to **11-*cis*-retinol.** Oxidation of the 11-*cis*-retinol to 11-cis-retinal and its binding to opsin occur in the rod outer segment.

The absorption spectra of 11-*cis*-retinal and the four visual pigments are shown in Figure 22.21. There is a shift in the wavelength of maximum absorption of 11-*cis*-retinal upon binding to opsin and the protein components of the other visual pigments. Absorption bands for the pigments are coincident with their light sensitivity.

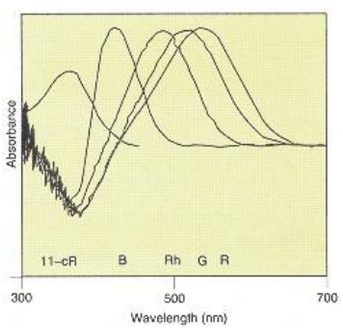


Figure 22.21
Absorption spectra of
11-cis-retinal and the four visual pigments.
Absorbance is relative and was obtained for pigments as difference spectra from reconstituted recombinant apoproteins. The spectrum for 11-cis-retinal (11-cR) is in the absence of protein. B, blue pigment; Rh, rhodopsin; G, green; R, red.
Adapted from Nathans, J. *Cell*, 78:357, 1994.

The magnitude of change in the electrical potential of photoreceptor cells following exposure to a light pulse is different in magnitude from that of neurons during depolarization. The **resting potential** of rod cell membrane is approximately **–30 mV** instead of the –70 mV observed with neurons. Excitation of rod cells causes **hyperpolarization** of the membrane, from about –30 mV to about –35 mV (Figure 22.22). It takes hundreds of milliseconds for the potential to reach its maximum state of hyperpolarization. A number of biochemical events take place during this time interval and before the potential returns to its resting state.

The initial events, absorption of photons of light and the subsequent isomerization of 11-*cis*-retinal, are rapid, requiring only picoseconds. Following this, a series of changes take place in rhodopsin, leading to various short-lived conformational states (Figure 22.23), each of which has specific absorption characteristics. Finally, rhodopsin dissociates, giving opsin and all-*trans*-retinal.

At 37°C, activated rhodopsin has decayed in slightly more than 1 millisecond through several intermediates to **metarhodopsin II.** Metarhodopsin II has a half-life of approximately 1 minute. It is the **active rhodopsin** species, R*, that is involved in the biochemical reactions of interest. Metarhodopsin II will have begun to form within hundredths of microseconds of the initial event. All of the first series of reactions shown in Figure 22.23 take place in the disk of the

rod outer segment. Upon dissociation of metarhodopsin into opsin and all-*trans*-retinal, the all-*trans*-retinal is enzymatically converted to all-*trans*-retinol by **all-*trans*-retinol dehydrogenase** that is located in the rod outer segment. All-*trans*-retinol is transported (or diffuses) into the pigmented epithelium where a specific isomerase converts it to 11-*cis*-retinol. The 11-*cis*-retinol is then transported (or diffuses) back into the rod outer segment and is reoxidized to 11-*cis*-retinal. Since the all-*trans*-retinol dehydrogenase appears to have only about 6% as much activity with 11-*cis*-retinal, it appears that another enzyme may be responsible for its oxidation. Once the aldehyde is formed, it can recombine with opsin to form rhodopsin. Rhodopsin is now in a state to begin the cycle again. The same events take place in the cones with the three proteins of the red, green, and blue pigments.

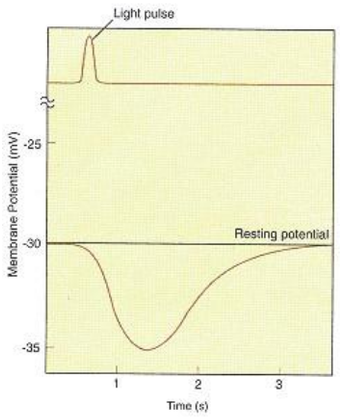


Figure 22.22
Changes in the potential of a rod cell membrane after a light pulse.
Redrawn from Darnell, J., Lodish, H., and Baltimore, D. *Molecular Cell Biology*. New York: Scientific American Books, 1986, p. 763.

There are three interconnecting "mini" biochemical cycles involved in the conversion of light energy to nerve impulses (Figure 22.24). These cycles describe the reactions of rhodopsin, **transducin,** and **phosphodiesterase,** respectively. The net result of their operation is to cause a hyperpolarization of the plasma membrane of the rod (or cone) cells, that is, from –30 mV to approximately –35 mV. It is important to understand first what the biochemical mechanism is for maintaining the plasma membrane at –30 mV.

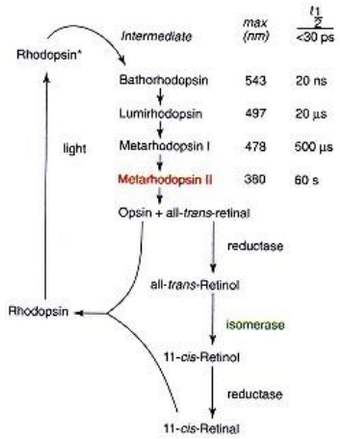


Figure 22.23
Light activation of rhodopsin.

Rod cells of a fully dark-adapted human can detect a flash of light that emits as few as 50 photons. The rod is a specialized type of neuron in that the signal generated does not depend on an all-or-none event. The signal may be graded in intensity, reflected by the extent that the millivolt potential of the plasma membrane changes from its steady-state value of –30 mV. This **steady-state potential** is maintained at a more positive value because **Na^+ channels** of the photoreceptor cells are **ligand gated** and are maintained in a partially opened state. The ligand responsible for keeping some of the Na^+ channels open is **cyclic GMP (cGMP).** cGMP binds to them in a concentration-dependent, kinetically dynamic manner. Biochemical events that affect the concentration of cGMP within rod and cone cells also affect the number of Na^+ channels that are open and, hence, the membrane potential (Figure 22.24).

Active rhodopsin (R*, namely, metarhodopsin II) forms a complex with transducin. Transducin is a classical type of **G-protein** and functions in a manner very similar to that described on page 859 in relation to the action of some hormones. In the R*–transducin complex (R*–$T_{\alpha,\beta,\gamma}$ complex), transducin undergoes a conformation change that facilitates an exchange of its bound **GDP** with **GTP.** When this occurs, the α subunit (T_α) of the trimeric molecule dissociates from its β, γ subunits. T_α interacts with and activates **phosphodiesterase (PDE),** which hydrolyzes cGMP to 5′-GMP, resulting in a decreased concentration of cGMP and a decrease in the number of channels held open. The membrane potential becomes more negative, that is, hyperpolarized.

The diagram of Figure 22.24 shows in cartoon form two such channels embedded in the plasma membrane, one of which has cGMP bound to it and is open. The other does not have cGMP bound to it and it is closed. By this mechanism, the concentration of Na^+ in the cell is directly linked to the concentration of cGMP and, thus, also to the membrane potential.

PDE in rod cells is a **heterotetrameric protein** consisting of one each α and β catalytic subunits and two γ regulatory subunits. T_α–GTP forms a complex with the γ subunits of PDE, resulting in their dissociation from the catalytic subunits, freeing the catalytically active α,β-dimeric PDE subunit complex. T_α has GTPase activity. Hydrolysis of bound GTP to GDP and inorganic phosphate (P_i) results in dissociation of T_α from the regulatory γ subunits of PDE, permitting them to reassociate with the catalytic subunits and to inhibit the PDE activity. The same reactions occur in cone cells, but the catalytic subunit of cone cell PDE is composed of two α catalytic subunits instead of α,β subunits as are present in rod cells.

cGMP concentration is regulated by intracellular Ca^{2+} concentration. Calcium enters rod cells in the dark through sodium channels, increasing its concen-

tration to the 500-nM range. At these concentrations, activity of **guanylate cyclase** is low. When sodium channels are closed, Ca^{2+} entry is inhibited, but efflux mediated by the sodium/calcium–potassium exchanger is unchanged (top complex of the plasma membrane in Figure 22.24). This results in a decrease in the intracellular Ca^{2+} concentration, which in turn leads to activation of guanylate cyclase and increased production of cGMP from GTP.

Both the resynthesis of cGMP and the hydrolysis of T_α–GTP play important roles in stopping the reactions of the visual cycle. The inactivation of activated rhodopsin, R*, is also very important.

Activated rhodopsin, R*, is phosphorylated by **rhodopsin kinase** in the presence of ATP (Figure 22.24). The R*–P_i has high binding affinity for the cytosolic protein, **arrestin.** The arrestin–R*–P_i complex is no longer capable of interacting with transducin. The kinetics of arrestin binding to the activated-phosphorylated rhodopsin is sufficiently rapid *in vivo* to stop the cascade of reactions.

Rhodopsin is regenerated through another series of reactions and the cycle can be initiated again by photons of light. Figure 22.23 shows that the series of reactions leading to the regeneration of rhodopsin includes the dissociation of all-*trans*-retinal from metarhodopsin. The regeneration of 11-*cis*-retinal from all-*trans*-retinal occurs by reactions previously described and occurs before it is used again to form rhodopsin.

Major proteins involved in the visual cycle are listed in Table 22.6.

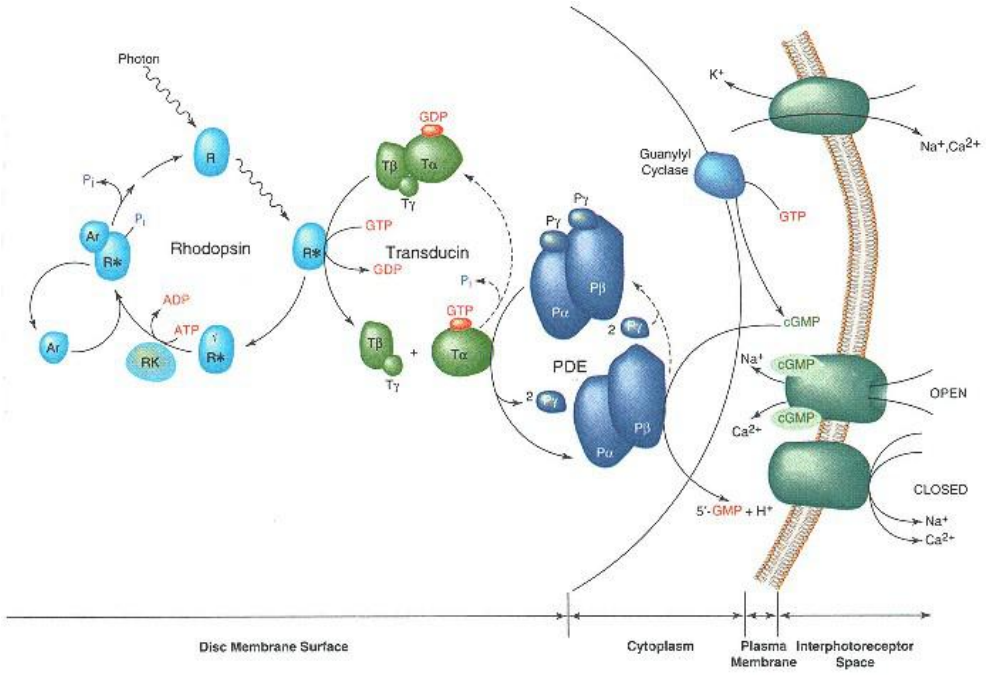


Figure 22.24
Cascade of biochemical reactions involved in the visual cycle.
Redrawn from Farber, D. B. *Invest. Ophthalmol. Vis. Sci*. 36:263, 1995.

TABLE 22.6 Major Proteins Involved in the Phototransduction Cascade

Protein	*Relation to Membrane*	*Molecular Mass (kDa)*	*Concentration in Cytoplasm (μM)*
Rhodopsin	Intrinsic	39	—
Transducin ($\alpha + \beta + \gamma$)	Peripheral or soluble	80	500
Phosphodiesterase	Peripheral	200	150
Rhodopsin kinase	Soluble	65	5
Arrestin	Soluble	48	500
Guanylate cyclase	Attached to cytoskeleton	?	?
cGMP-activated channel	Intrinsic	66	?

Color Vision Originates in the Cones

Even though there are photographic artists, such as the late Ansel Adams, who make the world look beautiful in black and white, the intervention of colors in the spectrum of life's pictures brings another degree of beauty to the wonders of nature and the beauty of life . . . even the ability to make a distinction between tissues from histological staining. The ability of humans to distinguish colors resides within a relatively small portion of the visual system, the cones. The number of cones within the human eye are few compared with the number of rods. Some animals like dogs have even fewer cones, and other animals, like birds, have many more.

The general mechanism by which light stimulates cone cells is exactly the same as it is for rod cells. There are **three types of cone cells**, defined by the visual pigments they contain, which are either blue, green, or red. Normally, only one type of visual pigment occurs in a single cell. The blue pigment has optimum absorbance at 420 nm, the green pigment at 535 nm, and the red pigment at 565 nm (Figure 22.21). Each of these pigments has 11-*cis*-retinal as the prosthetic group, and, when activated by light, the 11-*cis*-retinal isomerizes to all-*trans*-retinal in exactly the same manner as it does in the rod cells. Colors other than those of the visual pigments are distinguished by graded stimulation of the different cones and comparative analysis by the brain. Color vision is **trichromatic.**

The characteristic of color discrimination by cone cells is an inherent property of the proteins of the visual pigments to which the 11-*cis*-retinal is attached. The 11-*cis*-retinal is attached to each of the proteins through a protonated Schiff base. The conjugated double-bond system of 11-*cis*-retinal influences the absorption spectrum of the pigment (Figure 22.21). When 11-*cis*-retinal is bound to different visual proteins, amino acid residues in the local areas around the protonated base and the conjugated -bond system influence the energy level and give different absorption spectra with absorption maxima that are different for the different color pigments.

Genes for the color pigments have been cloned and their amino acid sequences inferred from the gene sequences. A structural comparison of the sequences of the visual pigments is shown in Figure 22.25. Open circles represent amino acids that are the same, and closed circles represent amino acids that are different. A string of closed circles at either end may represent an extension of the chain of one protein relative to the other. The red and green pigments show the greatest degree of homology, about 96% identity, whereas the degree of homology between different pairs of the others is between 40% and 45%.

Genes encoding the **visual pigments** have been mapped to specific chromosomes (see Clin. Corr. 22.6). The rhodopsin gene resides on the third chromo-

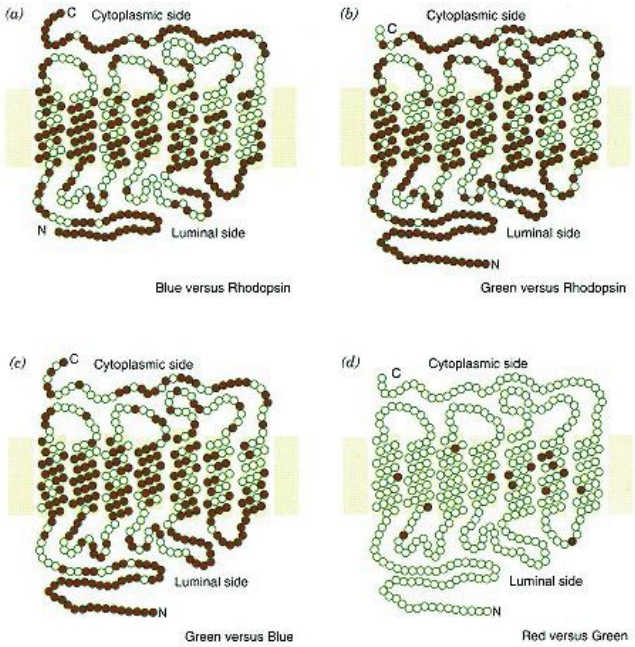


Figure 22.25
Comparisons of the amino acid sequences of the human visual pigments.
Each red dot indicates an amino acid difference.
Adapted from Nathans, J. *Annu. Rev. Neurosci*. 10:163, 1987.

some, the gene encoding the blue pigment resides on the seventh chromosome, and the two genes for the red and green pigments reside on the X chromosome. Abnormal color vision results from mutations in one or more of these genes (see Clin. Corr. 22.6). In spite of their great similarity, the red and green pigments are distinctly different proteins. Individuals have been identified with inherited variations that affect one but not both pigments simultaneously. In addition, there may be more than one gene for the green pigment, but it appears that only one is expressed.

The person who developed the atomic theory of chemistry, John Dalton (1766–1844), was color blind. He thought his color blindness was due to the vitreous humor being tinted blue, selectively absorbing longer wavelengths of light. He instructed that after his death his eyes be examined to determine whether his theory was correct. An autopsy revealed that the vitreous humor was "perfectly pellucid," normal. Using DNA analysis on his preserved eyes obtained from the British Museum, it has now been demonstrated that Dalton was missing the blue pigment. Thus, instead of having trichromatic vision, he was dichromatic with a vision type referred to as **deuteranopia.** The type of color blindness of one who is missing the green pigment is **protanopia.**

Other Physical and Chemical Differences between Rods and Cones

The sensitivity and the response time of the rods are different from that of the cones. Absorption of a single photon by photoreceptors in rod cells generates a current of approximately 1–3 picoamperes ($1–3 \times 10^{-12}$ pA), whereas the same event in the cones generates a current of approximately 10 femtoamperes (10×10^{-15} fA), about 1/100th of the rod response. The response time of cone cells, however, is about four times faster than that of rod cells. Thus the cones

CLINICAL CORRELATION 22.6

Abnormalities in Color Perception

The chromosomal arrangement of genes for vision precludes inheritance of a single defective gene from one parent that would render recipients sightless. Genes that code for visual pigments occur on chromosomes that exist in pairs except in males where there is a single X chromosome containing the genes for red and green pigments. In females, there is a pair of X chromosomes and, therefore, color vision abnormalities in females are rare, affecting only about 0.5% of the population. By contrast, about 8% of males have abnormal color vision that affects red or green perception and, on rare occasions, both. For the sake of simplicity, the proteins coded for by the different genes will be referred to as pigments in spite of the fact that they become visual pigments only when they form complexes with 11-*cis*-retinal.

The gene that codes for the protein moiety of rhodopsin, the rod pigment, is located on the third chromosome. Genes that code for the three pigment proteins of cone cells are located on two different chromosomes. The gene for the blue pigment is on the seventh chromosome. The genes for the red and green pigments are tightly linked and are on the X chromosome, which normally contains one gene for the green pigment and from one to three genes for the green pigment. In a given set of cones, only one of these gene types is expressed, either the gene for the red pigment or one of the genes for the green pigment.

Genetic mutations may cause structural abnormalities in the proteins that influence the binding of retinal or the environment in which retinal resides. In addition, the gene for the protein of a specific pigment may not be expressed. If 11-cis-retinal does not bind or one of the proteins is not expressed, the individual will have dichromatic color vision and be color blind for the color of the missing pigment. If the mutation changes the environment around the 11-*cis*-retinal, shifting the absorption spectrum of the pigment, the individual will have abnormal trichromatic color vision; that is, the degree of stimulation of one or more of the three cone pigments will be abnormal. This will result in a different integration of the signal and hence a different interpretation of color.

Vollrath, D., Nathans, J., and Davis, R. W. *Science* 240:1669, 1988; and Nathans, J. *Cell* 78:357, 1994.

are better suited for discerning rapidly changing events and the rods are better suited for low-light visual sensitivity.

22.4— Muscle Contraction

On the basis of an extensive evaluation of electron micrographs of skeletal muscle tissue, the **sliding filament model** for muscle contraction was proposed. This simple but eloquent model has weathered the test of time. Genes for many of the proteins found in muscle tissue have been cloned, and the amino acid sequences of the proteins they encode inferred from their cDNA sequences. Three-dimensional structures of some of these proteins have also been published. Although the detailed picture of muscle contraction has not been completed, a clearer understanding of the process is emerging. In this section, some biochemical aspects of the mechanism of muscle contraction will be discussed. Primary emphasis will be on skeletal muscle rather than cardiac and smooth muscles.

Skeletal Muscle Contraction Follows an Electrical to Chemical to Mechanical Path

The signal for skeletal muscle contraction begins with an electrical impulse from a nerve. This is followed by a chemical change occurring within the unit cell of the muscle, and is followed by contraction, a mechanical process. Thus the **signal transduction** process goes from **electrical** to **chemical** to **mechanical.**

Figure 22.26 is a schematic diagram showing the structural organization of skeletal muscle. Muscle consists of bundles of fibers (diagram *c*). Each bundle is called a **fasciculus** (diagram *b*). The fibers are made up of **myofibrils** (diagram *d*), and each myofibril is a continuous series of muscle cells or units called **sarcomeres.** The muscle cell is multinucleated and is no longer capable of division. Most muscle cells survive for the life of the animal, but they can be replaced when lost or lengthened by fusion of **myoblast cells.**

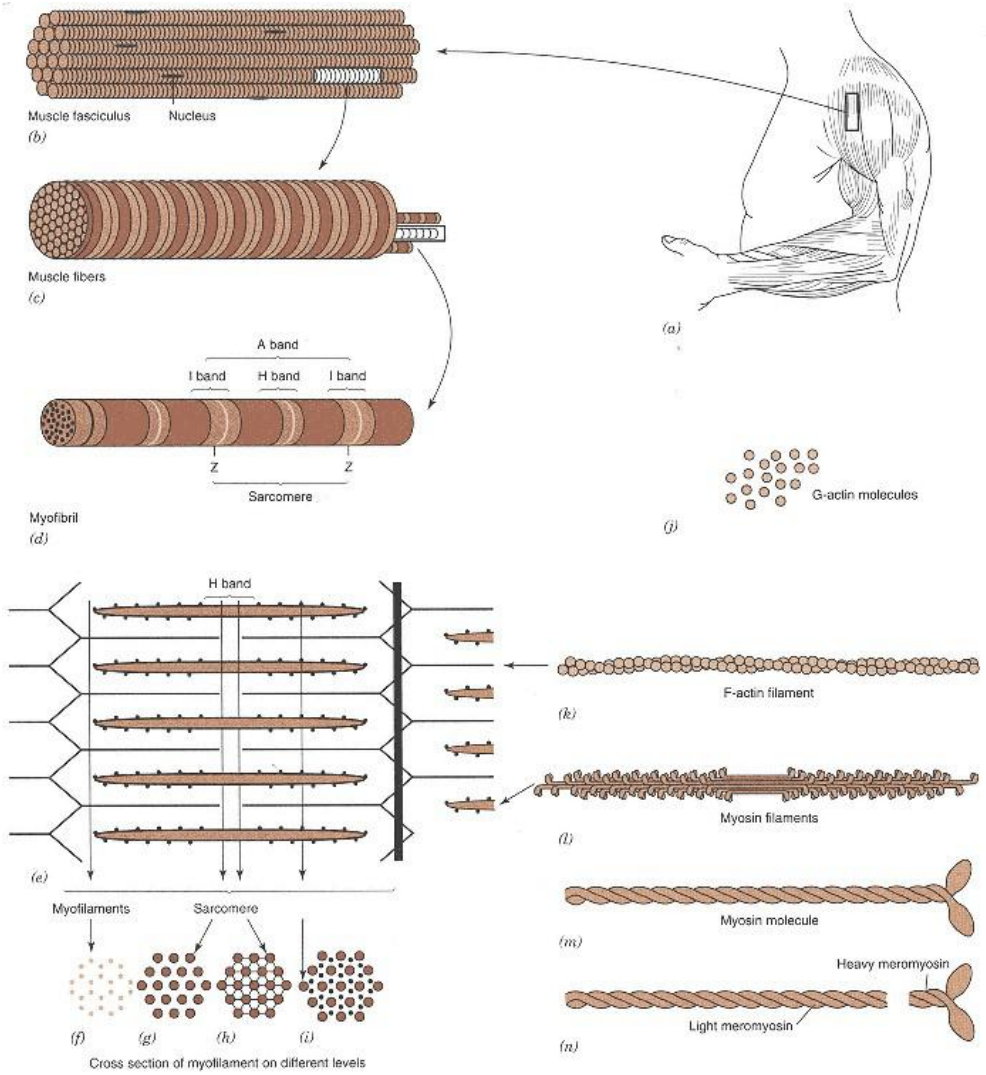


Figure 22.26
Structural organization of skeletal muscle.
Redrawn from Bloom, W. D., and Fawcett, D. W. *Textbook of Histology*, 10th ed. Philadelphia: Saunders, 1975.

A muscle cell is shown diagrammatically in Figure 22.27. Note that the myofibrils are surrounded by a membranous structure called the **sarcoplasmic reticulum.** At discrete intervals along the fasciculi and connected to the terminal cisterna of the sarcoplasmic reticulum are **transverse tubules.** The transverse tubules are connected to the external plasma membrane that surrounds the

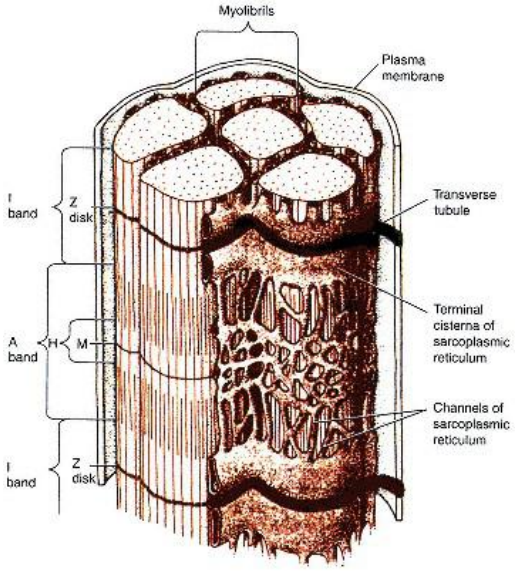


Figure 22.27
Schematic representation of a bundle of six myofibrils.
The lumen of the transverse tubules connects with the extracellular medium and enters the fibers at the Z disk. Reprinted with permission from Darnell, J., Lodish, H., and Baltimore, D. *Molecular Cell Biology*. New York: Scientific American Books, 1986, p. 827.

entire structure. The nuclei and the mitochondria lie just inside the plasma membrane.

The single contractile unit, the sarcomere, extends from Z line to Z line (Figures 22.26*d* and 22.27). Bands seen in the sarcomere are due to the arrangement of specific proteins (Figure 22.26*e*). Two types of fibers are apparent: long thick ones with protrusions on both ends lie near the center of the sarcomere, and long thin ones are attached to the **Z line.** The **I band (isotropic)** extends for a short distance on both sides of the Z line. This region contains only **thin filaments** that are attached to a protein band within the Z line. The **H band** is in the center of the sarcomere. There are no thin filaments within this region. In the middle of the H band, there is a somewhat diffuse band due to the presence of other proteins that assist in cross-linking the fibers of the **heavy filaments** (Figure 22.26, pattern h). The **A band (anisotropic)** is located between the inner edges of the I bands. When the muscle contracts, the H and I bands shorten, but the distance between the Z line and the near edge of the H band remains constant. The distance between the innermost edges of the I bands on both ends of the sarcomere also remains constant. This occurs because the length of the thin filaments and the thick filaments does not change during contraction. Contraction therefore results when these filaments "slide" past each other.

TABLE 22.7 Molecular Weights of Skeletal Muscle Contractile Proteins

Myosin	500,000
Heavy chain	200,000
Light chain	20,000
Actin monomer (G-actin)	42,000
Tropomyosin	70,000
Troponin	76,000
Tn-C subunit	18,000
Tn-I subunit	23,000
Tn-T subunit	37,000
α-Actinin	200,000
C-protein	150,000
β-Actinin	60,000
M-protein	100,000

The contractile elements, sarcomeres, consist of many different proteins, eight of which are listed in Table 22.7. The two most abundant proteins in the sarcomere are **myosin** and **actin.** About 60–70% of the muscle protein is myosin and about 20–25% is actin. The thick filament is mostly myosin and the thin filament is mostly actin. Three other proteins listed in Table 22.7 are associated with thin filaments, and two are associated with thick filaments.

Myosin Forms the Thick Filament of Muscle

The schematic drawing of the myosin molecule in Figure 22.28*a* is a representation of the electron micrographs in Figure 22.28*b*. Myosin, a long molecule with two globular heads on one end, is composed of two heavy chains of about 230 kDa each. Bound to each heavy chain in the vicinity of the head group is a dissimilar pair of **light chains,** each of which is approximately 20 kDa. The

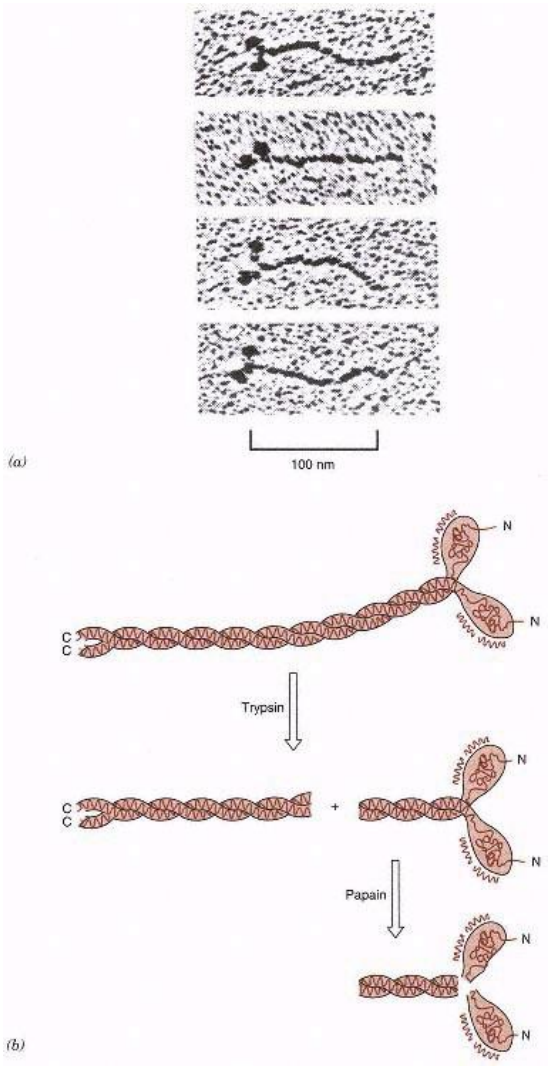


Figure 22.28
Myosin.
(a) Electron micrographs of the myosin molecule.
(b) Schematic drawing of a myosin molecule. Diagram shows the two heavy chains and the two light chains of myosin. Also shown are the approximate positions of cleavage by trypsin and papain.
Reprinted with permission from Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., and Watson, J. *Molecular Biology of the Cell*, 2nd ed. New York: Garland Publishing, 1983.

light chains are **"calmodulin-like"** proteins that bind calcium. One from each myosin can be removed easily without affecting *in vitro* function.

The carboxyl end of myosin is located in the tail section. The tail section of the two heavy chains are coiled around each other in an α-helical arrangement (Figure 22.28*a*). Trypsin cleaves the tail section at about one-third of its length from the head to produce **heavy meromyosin** (the head group and a short tail) and **light meromyosin** (the remainder of the tail section). Only light meromyosin has the ability to aggregate under physiological conditions, suggesting that aggregation is one of its roles in heavy chain formation. The head section can be separated from the remainder of the tail section by treatment with **papain.** The myosin head group resulting from this cleavage is referred to as **subfragment 1** or **S-1.** Action of these proteases also demonstrates that the molecule has at least two hinge points in the vicinity of the head–tail junction (Figure 22.28*a*).

cDNAs for myosin from many different species and from different types of muscle have been cloned and amino acid sequences for these myosin molecules inferred. Myosin has evolved very slowly, and there is a very high degree of homology among them, particularly within the head, or globular, region. There is somewhat less sequence homology within the tail region, but functional homology exists to an extraordinarily high degree regardless of length, which ranges from about 86 to about 150 nm for different species. The myosin head group contains nearly one-half of the total number of amino acid residues of the entire molecule in mammals, and it varies in the number of residues from only about 839 to about 850.

Myosin forms a **symmetrical tail-to-tail aggregate** around the M line of the H zone in the sarcomere. Its tail sections are aligned in a parallel manner on both sides of the M line with the head groups pointing towards the Z line. Each thick filament contains about 400 molecules of myosin. The C-protein (Table 22.7) is involved in their assembly. The M-protein is also involved, presumably to hold the tail sections together as well as to anchor them to the M line of the H zone.

The globular head section of myosin contains the **ATPase** activity that provides energy for contraction and the **actin binding site.** The S-1 fragment also contains the binding sites for the **essential light chain** and the **regulatory light chain.** A space-filling model of the three-dimensional structure of the myosin S-1 fragment is shown in Figure 22.29. The actin binding region is located at the lower right-hand corner and the cleft, visible in that region of the molecule, points toward the active site region where ATP binds. The 25-, 50-, and 20-kDa domains of the heavy chain are colored green, red, and blue, respectively. The essential light chain (ELC) and the regulatory light chain (RLC) are shown in yellow and magenta, respectively.

The active (ATP binding) site is also an open cleft about 13 Å deep and 13 Å wide. It is separated from the actin binding site by approximately 35 Å.

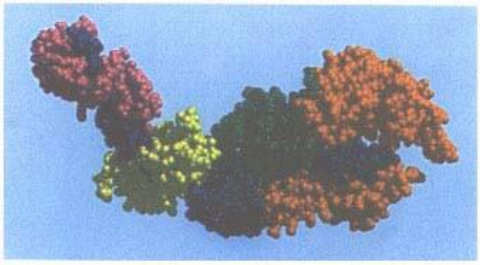


Figure 22.29
Space-filling model of the amino acid residues in myosin S-1 fragment.
The 25-, 50-, and 20-kDa domains of the heavy chain are green, red, and blue, respectively. The essential and regulatory light chains are yellow and magenta, respectively.

Myosin binding to actin shows stereo specificity. The ELC and RLC are associated with a single long helix that connects the head region with the tail section. There is room for flexibility, which requires only a low energy expenditure, between the ELC and the connecting single helix. The conformation of myosin that has ATP bound to it has an affinity for actin that is 1/10,000 that of the conformation of myosin that does not have ATP bound to it! Thus the process of chemical energy transduction to mechanical work depends on the primary event of protein conformation changes that occur upon binding of ATP, its hydrolysis, and product dissociation.

Actin, Tropomyosin, and Troponin Are Thin Filament Proteins

Actin is a major protein of the thin filament and makes up about 20–25% of muscle protein. It is synthesized as a 42-kDa globular protein. It has a better than 90% conserved amino acid sequence among a variety of species. This is shown in Table 22.8 for skeletal muscle, smooth muscle, and cardiac muscle actin in three different species of animals. Differences are observed at most in about seven different positions. In fact, the primary amino acid sequences of more than 30 different actin isotypes, with the longest containing 375 amino acid residues, reveal that a maximum of only 32 residues in any of them had been substituted. A significant number of them occurred at the N terminal, which may be predicted considering that all actin molecules are posttranscriptionally modified at the N terminal. The N-terminal methionine is acetylated and removed, and the next amino acid is acetylated. The process may end at this stage or it may be repeated one or two additional times. In all cases, the N-terminal amino acid will be acetylated.

As first synthesized, actin is called **G-actin** for globular actin. The structure in Figure 22.30 shows that it is not strictly globular. Actin has two distinct domains of approximately equal size that, historically, have been designated as large (left) and small (right) domains. Each of these domains consists of two subdomains. Both the N-terminal and C-terminal amino acid residues are located within subdomain 1 of the small domain. The molecule has polarity, and when it aggregates to form **F-actin**, or fibrous actin, it does so with a specific directionality. This is important for the "stick and pull" processes involved in sarcomere shortening during muscular contraction.

G-actin contains a specific binding site, located between the two major domains, for ATP and a divalent metal ion. Mg^{2+} ion is most likely the physiologically important cation, but Ca^{2+} also binds tightly and competes with Mg^{2+} for the same tight binding site. It is the **G-actin–ATP–Mg^{2+} complex** that aggregates to form the **F-actin polymer** (see Figure 22.34). Aggregation can occur from either direction, but kinetic data indicate that the preferred direction of aggregation is

TABLE 22.8 Summary of the Amino Acid Differences Between Chicken Gizzard Smooth Muscle Actin, Skeletal Muscle Actin, and Bovine Cardiac Actin

	Residue Number						
Actin Type	***1***	***2***	***3***	***17***	***89***	***298***	***357***
Skeletal muscle[a]	Asp	Glu	Asp	Val	Thr	Met	Thr
Cardiac muscle[b]		Asp	Glu			Leu	Ser
Smooth muscle[c]	Absent		Glu	Cys	Ser	Leu	Ser

Source: Adapted from Vandekerckhove, J., and Weber K. *FEBS Lett.* 102:219, 1979.

[a] From rabbit, bovine, and chicken skeletal muscle.

[b] From bovine heart.

[c] From chicken gizzard.

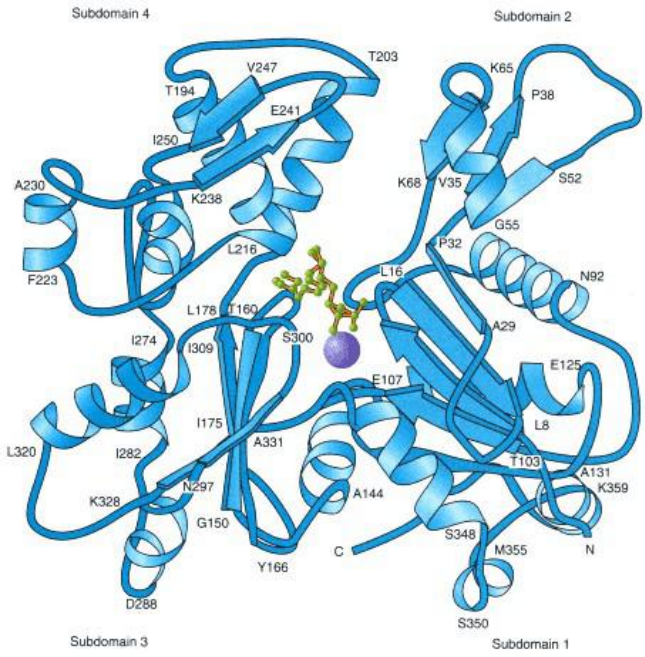


Figure 22.30
Secondary structural elements of G-actin crystal structure.
ADP and the metal ion are shown in the cleft between the two large domains.
Redrawn with permission from Lorenz, M., Popp, D., and Holmes, K. C. *J. Mol. Biol.* 234:826, 1993. By permission of the publisher, Academic Press Limited, London.

by extension from the large end of the molecule where the rate is diffusion controlled. ATP hydrolysis occurs by orders of magnitude faster in the aggregated actin than it does in the monomer. G-actin–ADP–Mg^{2+} also aggregates to form F-actin but at a slower rate. Orientation of G-actin molecules in F-actin is such that subdomains 1 and 2 are to the outside where myosin binding sites are located. F-actin may be viewed as either (1) a single-start, left-handed helix with rotation of the monomers through an approximate 166° with a rise of 27.5 Å or (2) a two-start, right-handed helix with a half pitch of 350–380 Å.

There are a number of proteins in the cytosol that bind actin. **β-Actinin** binds to F-actin and plays a major role in limiting the length of the thin filament. **α-Actinin**, a homodimeric protein with a subunit molecular weight of 90–110 kDa, binds adjacent actin monomers of F-actin at positions 86–117 and 350–375 and strengthens the fiber. It also helps to anchor the actin filament to the Z line of the sarcomere. There are two other major proteins associated with the thin filament, **tropomyosin** and **troponin**.

Tropomyosin is a rod-shaped protein consisting of two dissimilar subunits, each of about 35 kDa. It forms aggregates in a head-to-tail configuration. This polymerized protein interacts in a flexible manner with the thin filament throughout its entire length. It fits within the groove of the helical assembly of the actin monomers of F-actin. Each of the single tropomyosin molecules interacts with about seven monomers of actin. The site on actin with which tropomyosin interacts is between subdomains 1 and 3. Tropomyosin helps to stabilize the thin filament and to transmit signals for conformation change to other components of the thin filament upon Ca^{2+} binding. Bound to each individual tropomyosin molecule is one molecule of troponin.

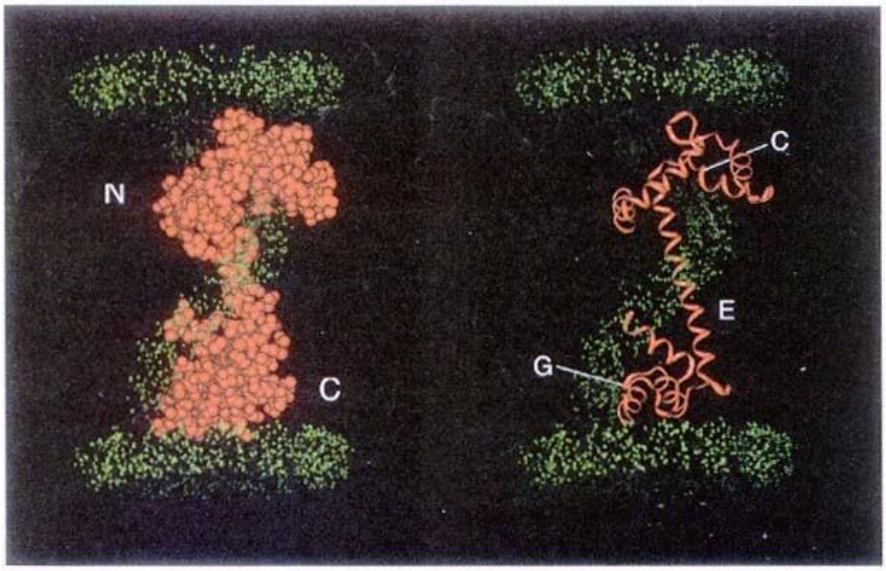


Figure 22.31
Best fit model for the 4 Ca^{2+} · Tn-C · Tn-I complex.
A model for the complex of 4 Ca^{2+} · troponin C · troponin I based on neutron scattering studies with deuterium labeling and contrast variation (Olah, C. A., and Trewhella, J., Biochemistry 33:12800, 1994). (Right) A view showing the spiral path of troponin I (green crosses) winding around the 4 Ca^{2+} · troponin C that is represented by an α-carbon backbone trace (red ribbon) with the C, E, and G helices labeled. (Left) The same view with 4 Ca^{2+} · troponin C represented as a CPK model. Photograph generously supplied by Dr. J. Trewhella.

Troponin has three dissimilar subunits designated Tn-C, Tn-I, and Tn-T with molecular weights of about 18 kDa, 21 kDa, and 37 kDa, respectively. The Tn-T subunit binds to tropomyosin. The Tn-I subunit is involved in the inhibition of the binding of actin to myosin in the absence of Ca^{2+}. The Tn-C subunit, a calmodulin-like protein, binds Ca^{2+} and induces a conformation change that alters the conformation of Tn-I and tropomyosin, resulting in exposure of the actin–myosin binding sites.

A three-dimensional structure of Tn-C shows it to be a dumbbell-shaped molecule with much similarity to calmodulin. A structural model of the calcium saturated Tn-C–Tn-I complex is shown in Figure 22.31. The Tn-I subunit fits around the central region of Tn-C in a helical coil conformation and forms caps over it at each end. The cap regions of Tn-I are in close contact with Tn-C when Tn-C is fully saturated with calcium ions. Tn-C has four divalent metal ion binding sites. Two are in the N-terminal region, are high affinity (K_{dissoc} of about 10^{-7} M), and are presumed to be always occupied since this is about the concentration of calcium ions in resting cells. Under these conditions, Tn-I has a conformation that permits its interaction with binding sites on actin, inhibiting myosin binding and preventing contraction. Upon excitation, the calcium ion concentration increases to about 10^{-5} M, high enough to effect calcium binding to sites within the N-terminal region of Tn-C. Tn-I now binds preferentially to Tn-C in a capped structural conformation as shown in Figure 22.31. Myosin binding sites on actin are now exposed. The relatively loose interaction of tropomyosin with actin gives it the flexibility to alter its conformation as a function of calcium ion concentration and to assist in blockage of the myosin binding sites on actin. (See Clin. Corr. 22.7 for additional information about troponin.)

Figure 22.26*i* shows schematically a cross section of the sarcomere and the relative arrangement of the thin and thick filaments. There are six thin filaments surrounding each thick filament. The arrangement of myosin head groups around the thick filaments and the flexibility of those head groups make it possible for each thick filament to interact with multiple thin filaments. When **cross-bridges** are formed between the thick and thin filaments, they do so in patterns consistent with that shown in the electron micrograph of Figure 22.32. This figure shows a two-dimensional view of the myosin of the thick filament interacting with the actin of the thin filaments lying on either side of it. Similar interactions of myosin occur with the actin of the other four thin filaments that surround it.

CLINICAL CORRELATION 22.7

Troponin Subunits as Markers for Myocardial Infarction

Troponin has three subunits (Tn-T, Tn-I, and Tn-C) each of which is expressed by more than one gene. Two genes code for skeletal muscle Tn-I, one in fast- and one in slow-skeletal muscle; and one gene codes for cardiac muscle Tn-I. The genes that code for Tn-T have the same distribution pattern. They differ in that the slow-skeletal muscle gene for Tn-I is also expressed in fetal heart tissue. The gene for the cardiac form of Tn-I appears to be specific for heart tissue. Tn-C is encoded by two genes, but neither gene appears to be expressed only in cardiac tissue.

The cardiac form of Tn-I in humans is about 31 amino acids longer than the skeletal muscle form, which makes it easy to differentiate from others. Serum levels of Tn-I increase within four hours of an acute myocardial infarction and remain high for about seven days in about 68% of patients tested. Almost 25% of one group of patients tested also showed a slight increase in the cardiac-form of Tn-I after acute skeletal muscle injury. This would be a good but not a very sensitive test for myocardial infarction.

Two isoforms of cardiac Tn-T, Tn-T_1, and Tn-T_2, are present in adult human cardiac tissue. Two additional isoforms are also present in fetal heart tissue. Speculation is that the isoforms are the result of alternative splicing of mRNA. Serum levels of Tn-T_2 increase within four hours of acute myocardial infarction and remain high for up to 14 days. The appearance of Tn-T_2 in serum is 100% sensitive and 95% specific for detection of myocardial infarction. In the United States, the Food and Drug Administration has given approval for marketing of the first Tn-T assay for acute myocardial infarction. Myocardial infarcts are either undiagnosed or misdiagnosed in hospital patients admitted for other causes, or in 5 million or more people who go to doctors for episodes of chest pain. It is believed that this test will be sufficiently specific to diagnose myocardial incidents and to help direct doctors to proper treatment of these individuals.

Anderson, P. A. W., Malouf, N. N., Oakeley, A. E., Pagani, E. D., and Allen, P. D. *Circ. Res.* 69:1226, 1991; and Ottlinger, M. E., and Sacks, D. B. *Clin. Lab. News*, 33, 1994.

Muscle Contraction Requires Ca^{2+} Interaction

Contraction of skeletal muscle is initiated by transmission of **nerve impulses** across the **neuromuscular junction** mediated by release into the synaptic cleft of the neurotransmitter **acetylcholine**. The **acetylcholine receptors** are associated with the plasma membrane and are **ligand gated**. Binding of acetylcholine causes them to open and to permit Ca^{2+}/Na^{+} to enter the sarcomere. The electron micrograph and accompanying diagrams of Figure 22.33 provide a picture of the anatomical relationship between the presynaptic nerve and the sarcomere. There are transverse tubules along the membrane in the vicinity of the Z lines that are connected to the terminal cisternae of the sarcoplasmic reticulum. Nerve impulses result in a depolarization of the plasma membrane and the transverse tubules, and an influx of Ca^{2+} into the sarcomere. As indicated above, Ca^{2+} concentration increases about 100-fold, permitting it to bind to the low-affinity sites of Tn-C and to initiate the contraction process. (See Clin. Corr. 22.8.)

Energy for Muscle Contraction Is Supplied by ATP Hydrolysis

ATP is an absolute requirement for muscular contraction. ATP hydrolysis by the **myosin–ATPase** to give the myosin–ADP complex and inorganic phos-

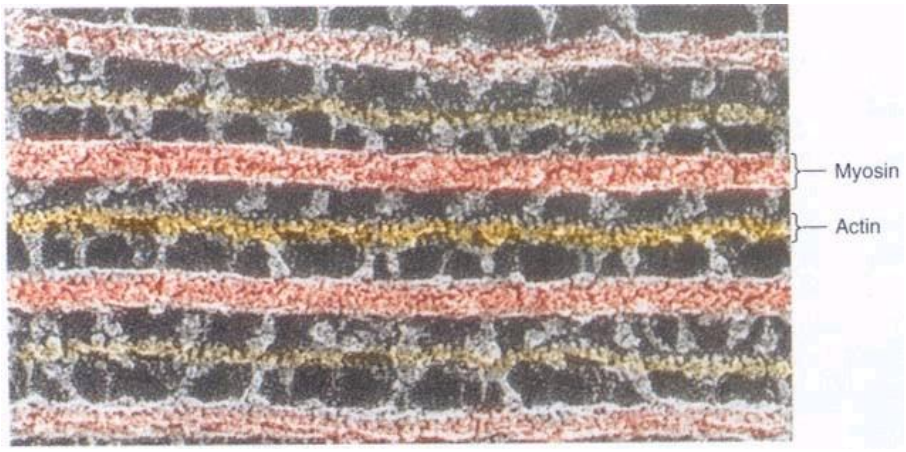


Figure 22.32
Electron micrograph of actin–myosin cross-bridges in a striated insect flight muscle.
Reproduced with permission from Darnell, J., Lodish, H., and Baltimore, D. *Molecular Cell Biology*. New York: Scientific American Books, 1986.

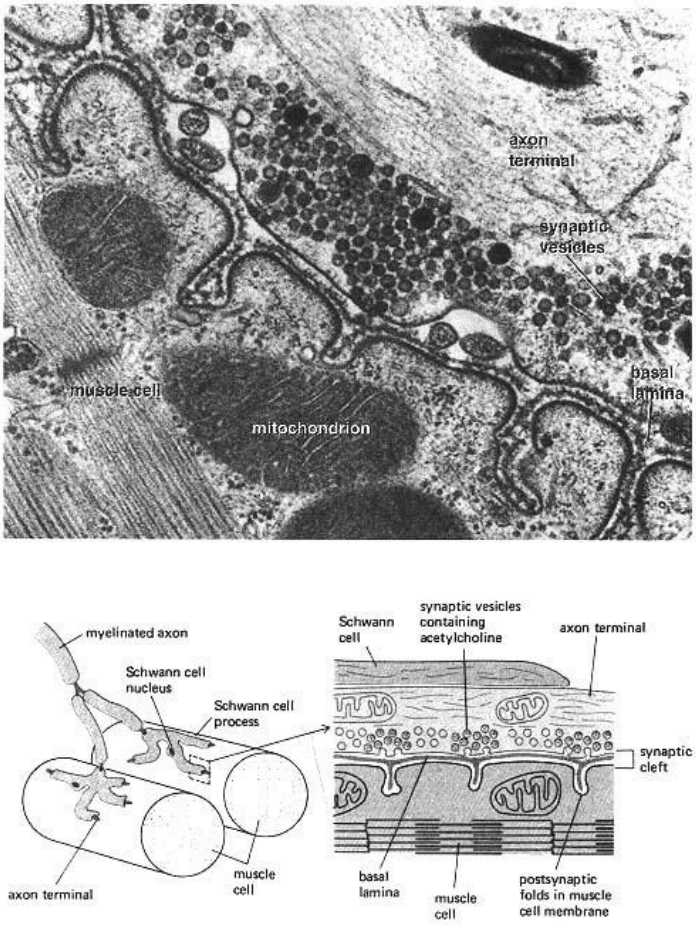


Figure 22.33
Neuromuscular junction.
(*a*) Electron micrograph of a neuromuscular junction.
(*b*) Schematic diagram of the neuromuscular junction shown in (*a*).
Reproduced with permission from Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., and Watson, J. *Molecular Biology of the Cell.* New York: Garland Publishing, 1983.

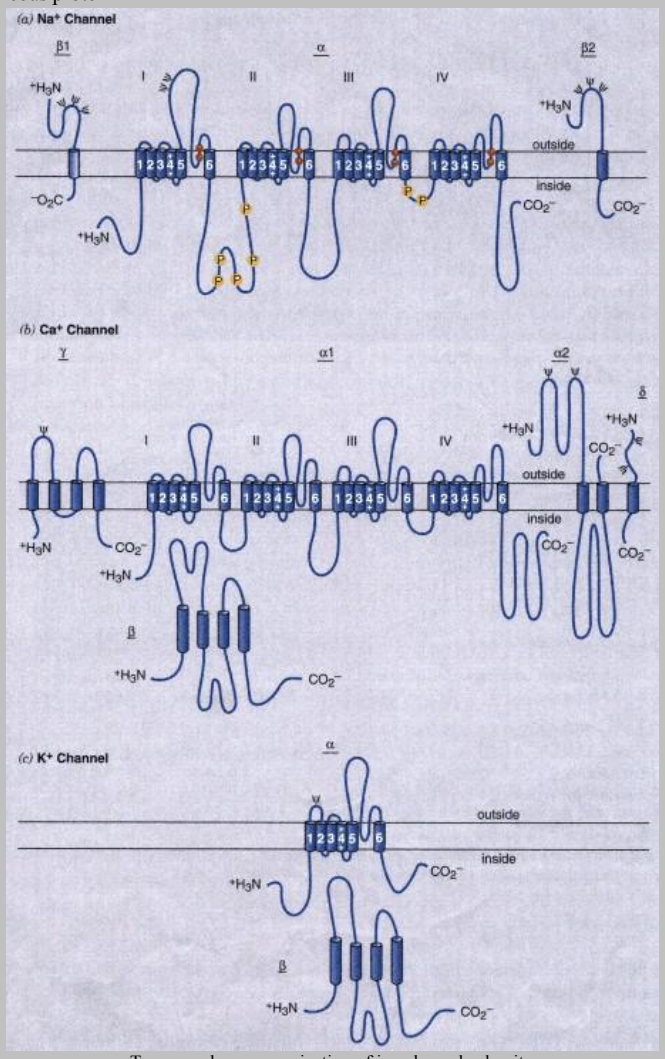
phate leads to a myosin conformation that has an increased binding affinity for actin. Additional ATP is required for the dissociation of the myosin–actin complex.

The concentration of ATP in the sarcomere remains fairly constant even during strenuous muscle activity, because of increased metabolic activity and of the action of two enzymes: **creatine phosphokinase** and **adenylate kinase.** Creatine phosphokinase catalyzes the transfer of phosphate from phosphocre-

CLINICAL CORRELATION 22.8

Voltage-Gated Ion Channelopathies

Action potentials in nerve and muscle are propagated by the operation of voltage-gated ion channels. Generally, there are three recognized types of voltage-gated cation channels: Na^+, Ca^{2+}, and K^+. Each of these has been cloned, primary sequence inferred from the DNA sequence, and a model constructed of how each may be assembled in the membrane. Each is a heterogeneous protein



Transmembrane organization of ion channel subunits.
Glycosylation and phosphorylation sites are marked. From Catterall (1995).

consisting of various numbers of α and β subunits. A linear model of the arrangement of each of these is shown in the figure above. In actual fact, they are arranged in a more-or-less circular manner with a channel formed through the middle of the α subunits. Roles of the β subunits are still being elucidated, but they appear to help stabilize and/or regulate activity of α subunits.

Toxins are being used to study subunit function. Tetrodotoxin and saxitoxin block Na^+ channel pores of the α subunit. Scorpion toxins also bind to the α subunit and appear to affect activation and inactivation gating. Experiments of this type suggest that the α subunit is involved in both conductance and gating.

Even though the Na^+ channel was first cloned from nerve tissue, the electroplax of the eel, more is known about how mutations affect its function in muscle. Voltage-gated channels from nerve and muscle tissue show high homology in many of the transmembrane domains but are less conserved in the intracellular connecting loops. A common effect of mutations in Na^+ channels is muscle weakness or paralysis. Some inherited sodium voltagegated ion channelopathies are listed below. Each of these is reported to result from a single amino acid change in the α subunit. The inheritance pattern generally is dominant.

Disorder	***Unique Clinical Feature***
Hyperkalemic periodic paralysis	Induced by rest after exercise, or the intake of K^+
Paramyotonia congenita	Cold-induced myotonia
Sodium channel myotonia	Constant myotonia

It has been surmised (by Hoffman, 1995; see Catterall, 1995) that if the membrane potential is slightly more positive (i.e., changes from –70 to –60 mV), the myofiber can reach the threshold more easily and the muscle becomes hyperexcitable. If the membrane potential becomes even more positive (i.e., up to –40 mV) the fiber cannot fire an action potential. This inability to generate an action potential is synonymous with paralysis. The fundamental biochemical defect in each case is a mutation in the channel protein.

Catterall, W. A. *Annu. Rev. Biochem*. 64:493, 1995; and Hoffmann, E. P. *Annu. Rev. Med*. 46:431, 1995.

atine to ADP in an energetically favored manner:

$$\text{Phosphocreatine} + \text{ADP} \rightleftharpoons \text{ATP} + \text{creatine}$$

If the metabolic process is insufficient to keep up with the energy demand, the creatine phosphokinase system serves as a "buffer" to maintain cellular levels of ATP. The second enzyme is adenylate kinase that catalyzes the reaction

$$2\ \text{ADP} \rightleftharpoons \text{ATP} + \text{AMP}$$

ATP depletion brings about rather rigid consequences to muscle cells. When the ATP supply of the muscle is exhausted and the intracellular Ca^{2+} concentration is no longer controlled, myosin will exist exclusively bound to actin, a condition called **rigor mortis.** The function of ATP binding in muscular contraction is to promote dissociation of the actin–myosin complex, not to promote its association.

Model for Skeletal Muscle Contraction

A model of the **actin–myosin complex** is shown in Figure 22.34. The myosin head undergoes conformation changes upon binding of ATP, hydrolysis of ATP, and release of products. ATP binding leads to closure of the active site cleft and opening of the cleft in the region of the actin binding site. Hydrolysis of ATP and release of inorganic phosphate result in closure of the cleft in the actin binding region. The conformation change that occurs is evident by the movement of two cysteine-containing helices. The distance between the two cysteine residues (697 and 707) changes from about 19 A to about 2 A. If further conformation change is prevented by cross-linking these two cysteines, ADP is trapped within its binding site. A stereo view of myosin showing the reactive cysteine pocket is shown in Figure 22.34*b*.

The sequence of events leading to muscle contraction from its resting state, following Ca^{2+} entry into the cell, probably begins with the hydrolysis of bound ATP. Myosin–ATP complex has a very low affinity for actin. Thus, even with exposed actin binding sites, any interaction between myosin and actin would be weak. The first significant interaction between myosin and actin probably

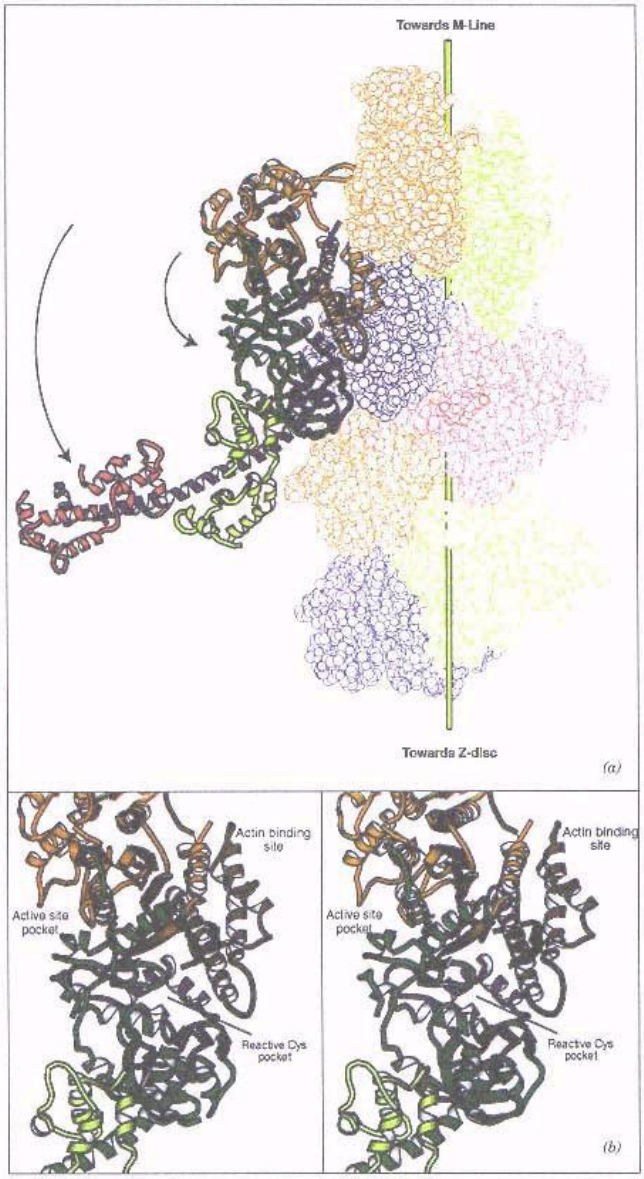


Figure 22.34
Model of actin–myosin interaction.
(a) Myosin is shown as a ribbon structure and actin as space-filling. Each G-actin monomer is represented by different colors.
(b) Stereo view of myosin showing the pocket that contains the mobile "reactive" cysteine residues.
Reproduced with permission from Rayment, I., and Holden, H. M. *TIBS* 19:129, 1994.
Photograph generously supplied by Dr. I. Rayment.

occurs upon release of inorganic phosphate. Release of ADP leads to tight binding (approximately a 10,000-fold increase) and another conformation change that results in opening of the reactive cysteine pocket. The conformation change results in a movement of the upper portion of the myosin head in the direction of the arrows in Figure 22.34*a* and movement of the thin filament in a direction away from the Z line, the **power stroke.** The thick filament is anchored in the center of the sarcomere and the **myosin head groups are polarized** in opposite directions on each side of the M line. Each thick filament contains hundreds of S-1 or myosin head units surrounded by six actin-containing thin filaments. Individual myosin units function in an asynchronous manner—possibly like changes in the position of hands on a rope in the game of tug-of-war. Thus when some myosin head groups bind with high affinity, others have low affinity.

Calcium Regulates Smooth Muscle Contraction

Calcium ions play an important role in **smooth muscle contraction** also, but there are some important differences in the mechanism by which it acts. A mechanism for calcium regulation of smooth muscle contraction is shown in Figure 22.35. Key elements of this mechanism are as follows. (1) A phosphorylated form of **myosin light chain** stimulates **Mg-ATPase,** which supplies energy for the contractile process. (2) Myosin light chain is phosphorylated by a **myosin light chain kinase (MLCK).** (3) MLCK is activated by a Ca^{2+}–calmodulin (CaM) complex. (4) Formation of the Ca^{2+}–CM complex is dependent on the concentration of intracellular Ca^{2+}. Release of Ca^{2+} from its intracellular stores or an increase in its flux across the plasma membrane is important for control. (5) Contraction is stopped by the action of a **myosin phosphatase** or the transport of Ca^{2+} out of the cell. It is apparent that, in smooth muscle, many more biochemical steps are involved in the regulation of contraction, steps that can be regulated in a progressive manner by hormones and other agents. These serve the function of smooth muscles well, namely, giving them the ability to develop various degrees of tension and to retain it for prolonged periods of time.

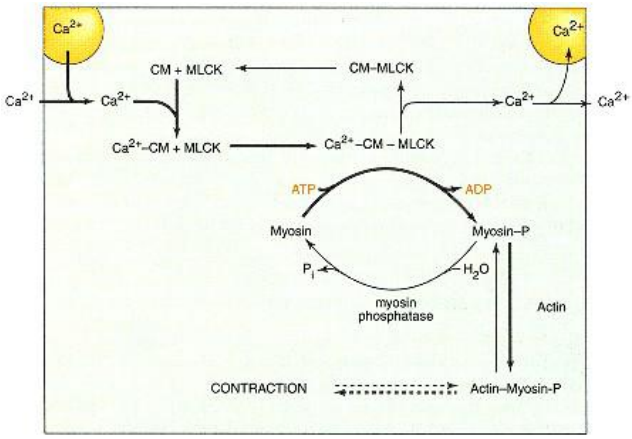


Figure 22.35
Schematic representation of the mechanism of regulation of smooth muscle contraction.
Heavy arrows show the pathway for tension development and light arrows show the pathway for release of tension. The Mg^{2+}-ATPase activity is highest in the actin–myosin-P complex. CaM, calmodulin; MLCK, myosin light chain kinase.
Adapted from Kramm, K. E., and Stull, J. T. *Annu. Rev. Pharmacol. Toxicol.* 25:593, 1985.

22.5— Mechanism of Blood Coagulation

The circulation of blood is essential for life, and the integrity of the process must be maintained. Some aspects of the importance of blood circulation in the maintenance of pH, in the transport of oxygen and nutrients to cells, and in the transport of carbon dioxide and waste products from cells are well known. This section deals primarily with a description of the system responsible for clot formation and dissolution.

Blood circulation occurs in a very specialized type of closed system in which the volume of circulating fluid is maintained fairly constant. This system is also one in which the transfer of solutes across its boundaries is a necessary function. Like any system of pipes and tubes, leaks can occur and must be repaired. The process of **blood clotting** primarily addresses the question of stopping the leaks. Secondarily, small clots may form due to disease and other abnormalities that are independent of total rupture of vesicles. Discussion of the function of the process must therefore extend beyond the primary one of leak prevention to include **clot dissolution.**

The purpose of this section is to give a general picture of the mechanism of blood clotting from a biochemical viewpoint. To this end, this section will focus on the relationship between blood clot formation, blood clot dissolution, and the enzymes and other proteins involved—their activation, regulation, inhibition, and synthesis. Blood clotting is not a process of signal transduction in the same sense as are the other topics of this chapter. Instead, it is a dynamic process of signal amplification and modulation. Some of the primary questions to be addressed are: (1) What initiates the clotting process? (2) What substances, reactions, and mechanisms are responsible for forming the clot? (3) What factors and mechanisms are involved in inhibiting the clotting process once it is initiated? (4) How is the clot dissolved?

It is important for the body to maintain **hemostasis,** that is, no bleeding. Thus the process of blood clotting is designed to stop as rapidly as possible the loss of blood following vascular injury. When such an injury occurs, three major events take place: (1) **aggregation** of a protein, **fibrin,** into an insoluble network, or clot, to cover the ruptured area to prevent the loss of blood; (2) **clumping** of **blood platelets** at the site of injury in an effort to form a physical plug to stop the leak; and (3) **vasoconstriction** in an effort to reduce the blood flow through the area. Equally important is regulation of the process to prevent excessive clot formation.

The processes mentioned above are emergency mechanisms for stopping the loss of blood. The process is not complete, however, until the ruptured vessel itself is repaired and the clot dissolved. Many of the proteins involved in blood coagulation contain **epidermal growth factor (EGF)-like domains.** Whether these EGF-like domains act directly to facilitate the regrowth of blood vesicles is not clear.

Some of the major proteins (players) involved in this process (silent drama) are listed in Table 22.9, not necessarily in order of appearance. All are important and, as time goes on, others are sure to be added. In fact, protein Z that occurs to a larger extent in children could be added but its role and function are not clear.

Clot Formation Is a Membrane-Mediated Process

Clot formation initially follows two separate pathways: **intrinsic** or **contact factor pathway** and **extrinsic** or **tissue factor pathway** (see Figures 22.36 and 22.38). These pathways merge with the formation of factor Xa, the proteinase component of the multienzyme complex that catalyzes the formation of thrombin from prothrombin. From this point on, there is a single pathway for clot formation. Historically, the term intrinsic pathway came from the observa-

TABLE 22.9 Some of the Factors Involved in Blood Coagulation, Control, and Clot Dissolution

Factor	*Name*	*Pathway*	*Characteristic*	*Concentration*[a]
I	Fibrinogen	Both		9.1
II	Prothrombin	Both	Contains N-terminal Gla residues	1.4
III	Tissue factor	Extrinsic	Transmembrane protein	—
IV	Calcium	Both		
V	Proaccelerin	Both	Protein cofactor	0.03[b]
VII	Proconvertin	Extrinsic	Endopeptidase with Gla residues	0.010[c]
VIII	Antihemophilic	Intrinsic	Protein cofactor	0.0003[b]
IX	Christmas factor	Intrinsic	Endopeptidase with Gla residues	0.089
X	Stuart factor	Both	Endopeptidase with Gla residues	0.136
XI	Thromboplastin antecedent	Intrinsic	Endopeptidase	0.031
XII	Hageman factor	Intrinsic	Endopeptidase	0.375
XIII	Proglutamidase	Both	Transpeptidase	0.031[b]
	Protein C	(*Both*)	Endopeptidase with Gla residues	0.065
	Protein S	(*Both*)	Cofactor with Gla residues	0.30
	Prekallikrein	Intrinsic	Zymogen/activator factor-XII	0.581
	HMWK[d]	Intrinsic	Receptor protein	0.636
	Antithrombin III	Both	Thrombin inhibitor	3.0
	Plasminogen		Zymogen/clot dissolution	2.4
	Heparin Co-II	Both	Thrombin inhibitor	1.364
	α_2-Antiplasmin		Plasmin inhibitor	0.952
	Protein C inhibitor		Protein C inhibitor	0.070
	α_2-Macroglobulin		Proteinase inhibitor	2.9
	LACI[e]		Extrinsic pathway inhibitor	0.003

[a] Concentrations are approximate and shown in micromolar.

[b] These values approximate solution concentrations since some are complexed with other proteins in platelets.

[c] This factor probably circulates as both VII and VIIa.

[d] HMWK is high molecular weight kininogen.

[e] LACI is lipoprotein-associated coagulation factor.

tion that blood clotting would occur spontaneously when blood was placed in clean glass test tubes, leading to the idea that all components for the clotting process were intrinsic to the circulating blood. Glass contains **anionic surfaces** that formed the nucleation points that initiate the process. In mammals, anionic surfaces are exposed upon rupture of the **endothelial lining** of the blood vessels and are the binding sites for specific factors that initiate clotting in the intrinsic pathway. Similarly, the term extrinsic came from the observation that there was another factor extrinsic to circulating blood that facilitates blood clotting. This factor was identified as **factor III, tissue factor** (see Figure 22.39*a*). Whether intrinsic or extrinsic, the process of blood coagulation is initiated on the membrane and is continued on the membrane surface at the site of injury.

Reactions of the Intrinsic Pathway

Reactions of the intrinsic pathway are shown in Figure 22.36. Upon injury to the endothelial lining of blood vessels and exposure of external membrane surfaces, the proteinase zymogen **factor XII** binds directly to anionic surfaces and undergoes a conformation change that increases its catalytic activity 10^4-to 10^5-fold. **Prekallikrein** and **factor XI,** also zymogens, circulate in blood as separate complexes with **high molecular weight kininogen (HMWK):** either a factor XI–HMWK complex or a prekallikrein–HMWK complex. In Figure 22.37 is a schematic diagram showing the functional regions of HMWK. The binding site on HMWK for prekallikrein consists of approximately 31 amino acid residues. Factor XI binds to approximately 58 amino acid residues that include the

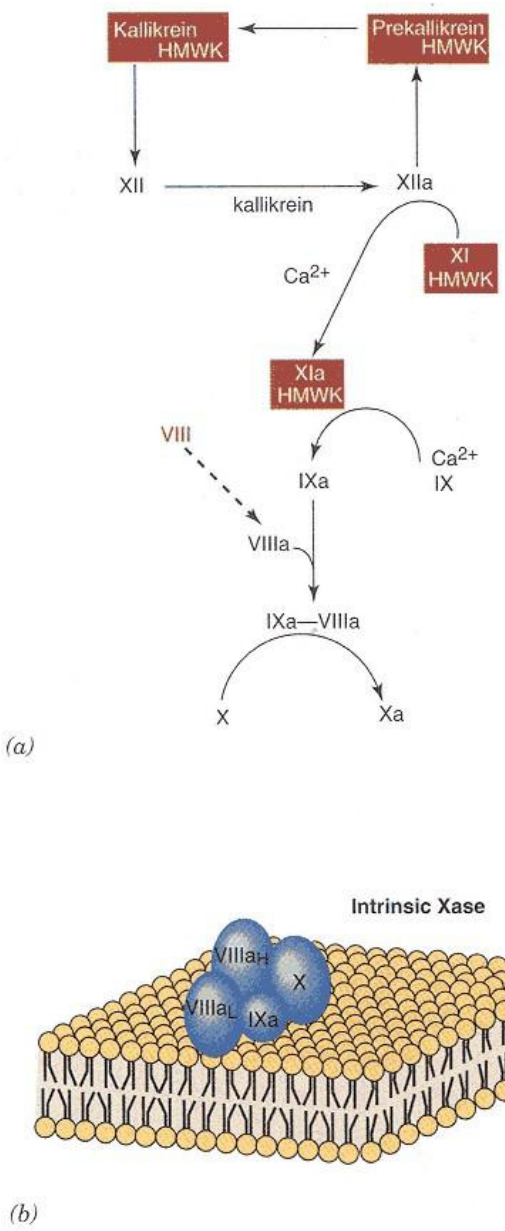


Figure 22.36
Intrinsic pathway of blood coagulation.
HMWK, high molecular weight kininogen. Activated factors are designated with an "a."
Adapted from Kalafatis, M., Swords, N. A., Rand M. D., and Mann, K. G. *Biochim. Biophys. Acta* 1227:113, 1994.

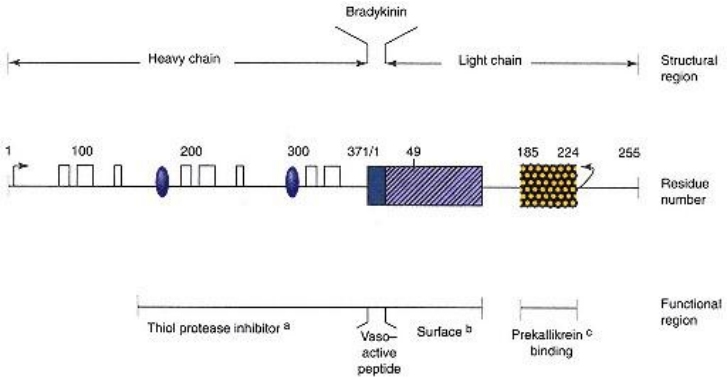


Figure 22.37
Schematic diagram of the functional regions of human high molecular weight kininogen (HMWK).
Bradykinin is derived from near the middle of HMWK by proteolysis. The resulting two chains are held together by disulfide bonds (horizontal arrows).
Redrawn from Tait. J. F., and Fujikawa, K. J. *Biol. Chem.* 261:15396, 1986.

31 to which prekallikrein binds. Factor XI and prekallikrein are attached to anionic sites of exposed membrane surfaces through their interactions with HMWK. This brings those zymogens to the site of injury and in direct proximity to factor XII. The membrane-bound "activated" form of factor XII activates prekallikrein, a 619 amino acid protein, by cleavage at Arg^{371}–Ile^{372}, to yield **kallikrein.** Kallikrein contains two chains covalently linked by a single disulfide bond. Kallikrein, whose C-terminal domain (248 amino acid residues) contains the catalytic site, further activates factor XII to give XIIa. Factor XI, which is membrane bound through its noncovalent attachment to HMWK, is activated by XIIa through proteolytic cleavage to XIa. Factor XIa activates **factor IX** to IXa. Factor IXa in the presence of **factor VIIIa,** a protein cofactor, forms the **intrinsic factor ten'ase (intrinsic Xase)** that can now activate **factor X** to Xa. Factor Xa is the catalytic moiety of the proteinase complex responsible for the activation of prothrombin to thrombin (see Clin. Corr. 22.9). This is essentially a four-step cascade started by the "contact" activation of factor XII and the **autocatalytic** action between factor XII and kallikrein to give XIIa (step 1). Factor XIIa activates factor XI (step 2); factor XIa activates factor IX (step 3); and factor IXa, in the presence of VIIIa, activates factor X (step 4). If each enzyme molecule activated also catalyzed the formation of 100 others before it is inactivated, the **amplification factor** would be 1×10^6.

Reactions of the Extrinsic Pathway

A diagram of the extrinsic pathway is shown in Figure 22.38. The membrane receptor that initiates this process is factor III or tissue factor. Tissue factor (Figure 22.39*a*) is a transmembrane protein of 263 amino acids. Residues 243–263 are located on the cytosolic side of the membrane. Residues 220–242 are hydrophobic residues and represent the transmembrane sequence. Residues 1–219 are on the outside of the membrane, are exposed after injury, and form the receptor for formation of the initial complex of the extrinsic pathway. This domain is glycosylated and contains four cysteine residues. A stereo representation of a section of it highlighting some of the amino acid residues involved in factor VII binding is shown in Figure 22.39*b*.

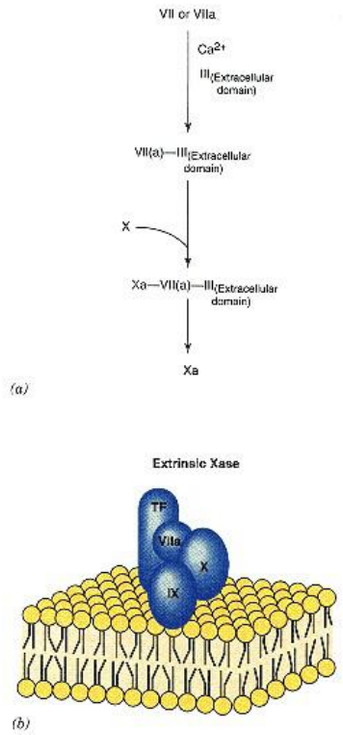


Figure 22.38
Extrinsic pathway of blood coagulation.

Tissue factor (factor III or TF) and factor VII are unique to the **extrinsic pathway** and are essentially all of its major components. Factor VII is a ***γ*-carboxyglutamyl** or Gla-containing protein that binds to tissue factor only

CLINICAL CORRELATION 22.9

Intrinsic Pathway Defects: Prekallikrein Deficiency

Components of the intrinsic pathway include factor XII (Hageman factor), factor XI, prekallikrein (Fletcher factor), and high molecular weight kininogen. Clinical disorders have been associated with defects in each of these components. Inherited disorders in each appear to be autosomal recessive. Each appears to be associated with an increase in activated partial thromboplastin time (APTT). The only one of these components directly associated with a clinical bleeding disorder is factor XI deficiency.

In some cases where there is a prekallikrein (Fletcher factor) deficiency, autocorrection after prolongation of the preincubation phase of the APTT test occurs. This phenomenon is explained by the ability of factor XII to be activated by an autocatalytic mechanism. The reaction is very slow in prekallikrein deficiency since the rapid reciprocal autoactivation between factor XII and prekallikrein cannot take place. Prekallikrein deficiency may be due to a decrease in the amount of the protein synthesized, to a genetic alteration in the protein itself that interferes with its ability to be activated, or its ability to activate factor XII. A lack of knowledge of the structure of the gene for prekallikrein precludes definitive explanations of the mechanisms operational in patients with prekallikrein deficiency. Specific deficiencies of the intrinsic pathway, however, can be localized to a specific factor if the appropriate number of tests are performed. These may include a direct measurement of the amount of each of the factors present in the patient's plasma in addition to APTT test performed with and without prolonged preincubation time. Use of these direct measurements helped diagnose a prekallikrein deficiency in a 9-year-old girl who had a prolonged APTT. The functional level of prekallikrein in this patient was less than 1/50th of the minimum normal value. Immunological test (ELISA) showed an antigen level of 20–25%, suggesting that she was synthesizing a dysfunctional molecule.

Coleman, R. W., Rao, A.K., and Rubin, R. N. *Am. J. Hematol*. 48:273, 1995.

in the presence of Ca^{2+}. The resulting TF–VII–Ca^{2+} complex is the catalytically active species. It catalyzes the formation of factor Xa from X.

The zymogen form of factor VII is initially **activated through protein–protein interaction** as a result of its binding to tissue factor. Additional factor VII is **activated by Xa** of the complex through proteolytic cleavage. Unlike other proteinases of the blood coagulation scheme, factor VIIa has a long half-life in circulating blood. Once dissociated from tissue factor, VIIa is not catalytically active, and its presence in blood would be harmless. Formation of the initial complex with TF could involve some of the already preformed factor VIIa, making it difficult to state with absolute certainty whether the zymogen form of VII in complex with tissue factor is totally responsible for the initial activation of factor X. A 3-D ribbon structural representation of factor VIIa is shown in Figure 22.40. The region for tissue factor interaction, Ca^{2+} binding, and the substrate binding pocket are highlighted.

Thrombin Converts Fibrinogen to Fibrin

The final phase in the formation of the fibrin clot (Figure 22.41) begins with action of the complex, **factor Xa–Va,** on **prothrombin.** A stereo view of factor

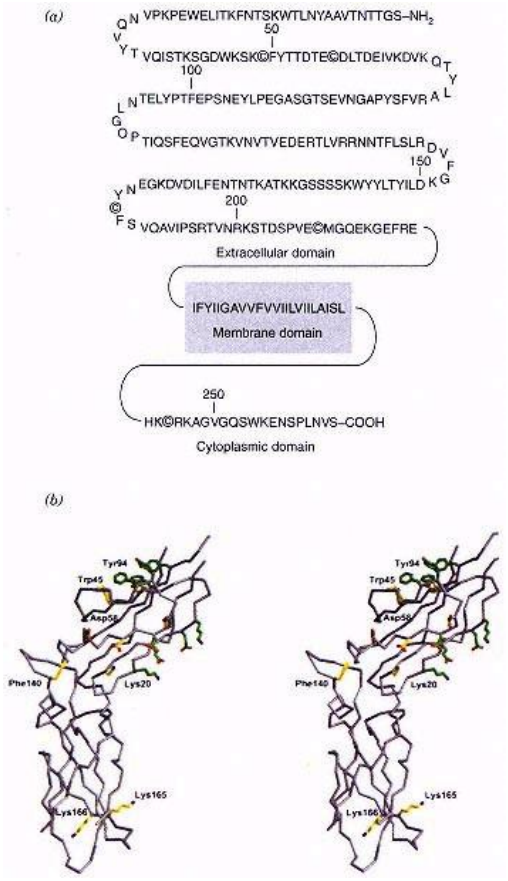


Figure 22.39
Tissue factor.
(*a*) Amino acid sequence of human tissue factor derived from its cDNA sequence.
(*b*) A stereo representation of the carbon chain of the extracellular domain of tissue factor. Residues important for binding of factor VII are shown in yellow. Clusters of aromatic and charged residues are shown in light blue.
(a) Redrawn from Spicer, E. K., Horton, R., Bloem, L., et al. Proc. Natl. Acad. Sci. USA 84:5148, 1987.
(b) Reproduced with permission from Muller, Y. A., Ultsch, M. H., Kelley, R. F., and deVos, A. M. *Biochemistry* 33:10864, 1994. Copyright 1994 American Chemical Society. Photograph generously supplied by Dr. A. de Vos.

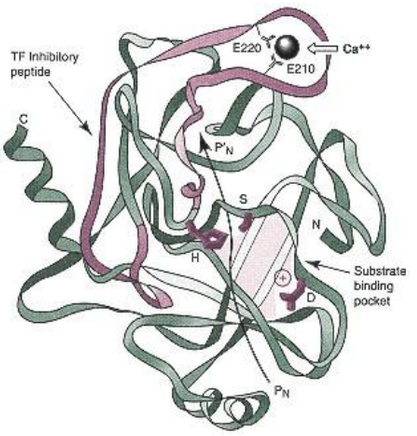


Figure 22.40
Ribbon structural representation of the protease domain of factor VIIa.
The dark ribbon labeled "TF inhibitory peptide" represents a section involved in binding to tissue factor. The catalytic triad is shown in the substrate binding pocket as H, S, and D for His^{193}, Ser^{344}, and Asp^{338}, respectively. The arrow labeled P_N–P'_N lies in the putative extended substrate binding region.
Redrawn with permission from Sabharwal, A. K., Birktoft, J. J., Gorka, J., et al. *J. Biol. Chem.* 270:1553, 1995.

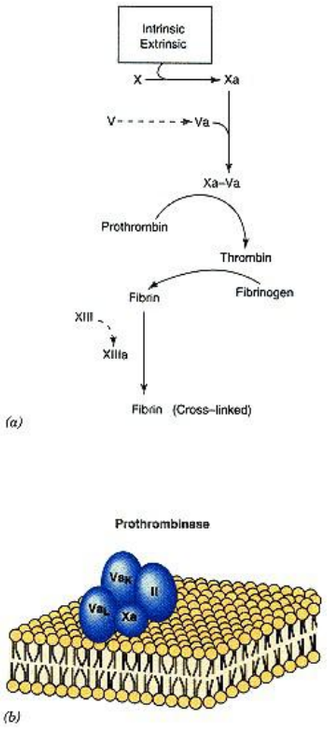


Figure 22.41
Clot forming pathway.
Adapted from Kalafatis, M., Swords, N. A., Rand, M. D., and Mann, K. G. *Biochim.* Biophys. Acta 1227:113, 1994.

Xa is shown in Figure 22.42. Factor Xa is formed by both the extrinsic and the intrinsic pathways by cleavage of factor X at positions 145 and 151 with elimination of a six amino acid peptide. Although the enzyme primarily responsible for activation of **factor V** is **thrombin,** factor Xa also catalyzes formation of Va. Thus the **prothrombinase complex, Xa–Va,** appears early in the process.

Thrombin, which circulates in plasma as prothrombin, catalyzes the conversion of **fibrinogen** to **fibrin.** Prothrombin, a 72-kDa protein (Figure 22.43), contains ten γ-carboxyglutamate (Gla) residues in its N-terminal region. Binding of calcium ions to these residues facilitates binding of prothrombin to membrane surfaces and to the Xa–Va complex at the site of injury. The prothrombinase complex (Xa–Va) activates prothrombin by making two proteolytic cleavages on the carboxyl side of arginine residues, first at position 320 and then at position 284. The active thrombin molecule (α-thrombin) consists of two chains, one of 6 kDa and the other of 31 kDa, that are covalently linked by a disulfide

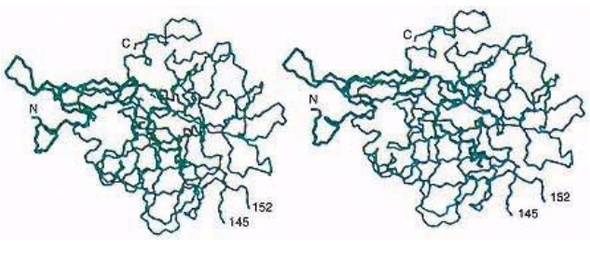


Figure 22.42
Stereo view of the CN-backbone structure of factor Xa.
The EGF-like domain is in bold.
Redrawn from Padmanabhan, K., Padmanabhan, K. P., Tulinsky, A., et al. *J. Mol. Biol* 232:947, 1993.

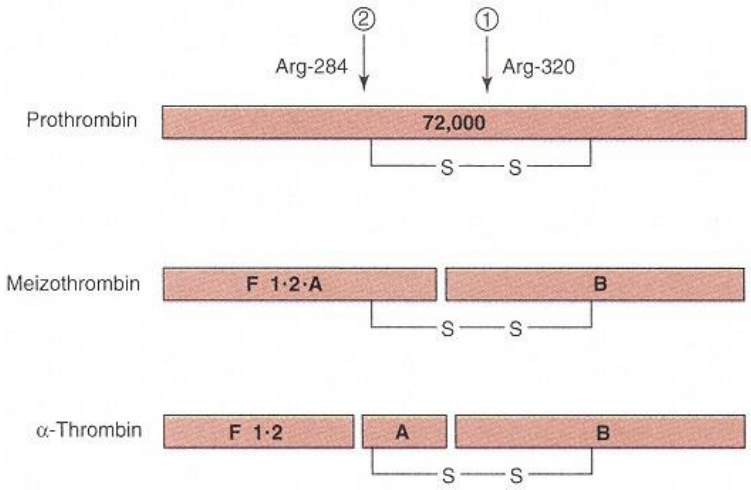


Figure 22.43
Schematic diagram of prothrombin activation.

bond. A stereo view of the active α-thrombin molecule is shown in Figure 22.44. Regions involved in some of its functions are highlighted. The substrate for thrombin is fibrinogen.

Fibrinogen is a large molecule of approximately 340 kDa consisting of two tripeptide units with α,β,γ structure (Figure 22.45). The subunits are "tied" together at their N-terminal regions by a group of disulfide bonds. Fibrinogen has three globular domains, one on each end and one in the middle where the chains are joined. The globular domains are separated by rod-like domains. A short segment of the free N-terminal regions projects out from the central globular domain. The N-terminal region of the and the subunits, through charge–charge repulsion, prevent aggregation of fibrinogen. Thrombin cleaves these N-terminal peptides and allows the resulting fibrin molecules to aggregate and to form the **"soft" clot.** The soft clot is stabilized and strengthened by the action of **factor XIIIa, transglutamidase.** This enzyme catalyzes the formation of an **isopeptide linkage** by replacing the -amide group of glutamine residues of one chain with the -amino group of lysine residues of another chain (Figure 22.46) with the release of ammonia. This cross-linking of fibrin completes the steps involved in the formation of the hard clot.

Major Roles of Thrombin

α-Thrombin activates the protein cofactors V and VIII and it is also involved in **platelet aggregation.** Factor V is a 330,000 molecular weight protein. Activation of factor V by thrombin occurs through proteolytic cleavage at Arg^{709} and Arg^{1545}. Factor Va is a heterodimer consisting of an N-terminal domain of 105

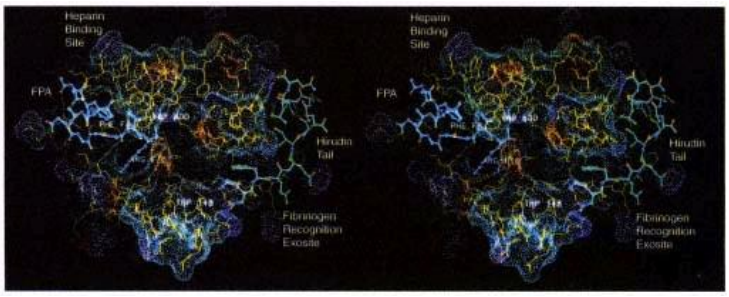


Figure 22.44
Stereo view of the active site cleft of human α-thrombin.
Dark blue, basic amino acids, red, acid; light blue, neutral. The active site goes from left to right. Figure courtesy of Dr. M. T. Stubbs II, Max-Planck Institut für Biochemie, Martinsreid, Germany.

kDa and a C-terminal domain of 74 kDa. These two subunits are noncovalently held together by a calcium ion (Figure 22.47).

Factor VIII circulates in plasma attached to another protein, **von Willebrand's factor (vWF).** Factor VIII is a 285-kDa protein that is activated by thrombin cleavage at Arg^{372}, Arg^{740}, Arg^{1648}, and Arg^{1689}. The latter cleavage releases VIIIa from vWF. Factor VIIIa is a heterotrimer (Figure 23.47) composed of N-terminal peptides of 40 kDa (A_2) and 50 kDa (A_1), and a C-terminal peptide of 74 kDa (A_3). Factor VIIIa also contains a Ca^{2+} bridge between the N- and C-terminal domains. Classic hemophilia results from a deficiency in factor VIII (see Clin. Corr. 22.10).

Thrombin also activates factor XIII, transglutamidase (Figure 22.48). **Protransglutamidase** exists in both plasma and platelets. The structural form of the platelet enzyme is $_2$, whereas that of the plasma form is $_2\ _2$. Thrombin cleaves the α subunit of both the platelet and the plasma forms of transglutaminase. Cleavage of the α subunit of the plasma form of the enzyme leads to dissociation of the β subunit, which is not catalytically active. The platelet form of the enzyme is released at the site of fibrin aggregation and is activated just by cleavage of the α subunit.

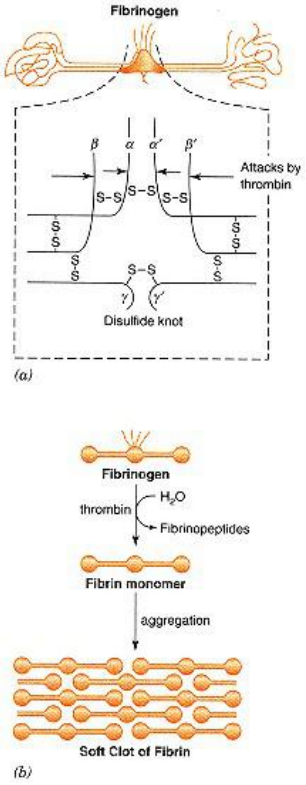


Figure 22.45
Diagrammatic representation of the fibrinogen molecule and its conversion to the soft clot of fibrin.

Formation of a Platelet Plug

The clumping of platelets at the site of injury is mediated by the presence of thrombin. There is a **thrombin receptor**, a member of the seven-transmembrane-domain family of receptors, on the outside of endothelial cells. This receptor is exposed upon injury and is activated by α-thrombin. **Aggregation of platelets** is facilitated by their initial binding to this activated receptor. In addition to the formation of a physical plug, platelets undergo a morphological change and release other chemicals that elicit other actions (Figure 22.49): ADP, serotonin, some types of phospholipids, and proteins that aid in coagulation and tissue repair. A glycoprotein, von Willebrand's factor (vWF) is released, concentrates in the area of the injury, and also forms a link between the exposed receptor and the platelets. von Willebrand's factor also serves as a carrier for factor VIII. Activation and release of factor VIII from vWF have been discussed.

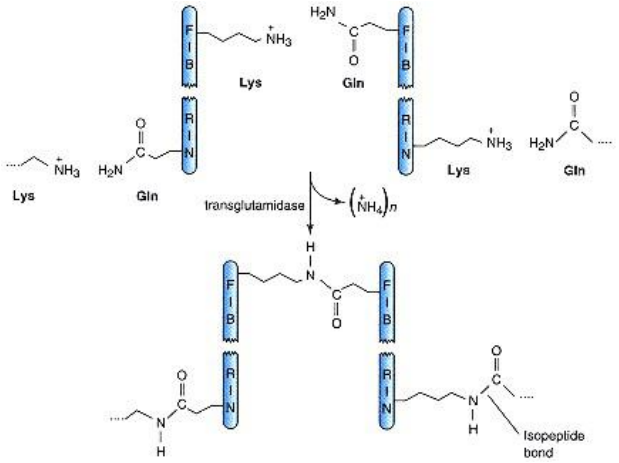


Figure 22.46
Reactions catalyzed by transglutamidase.

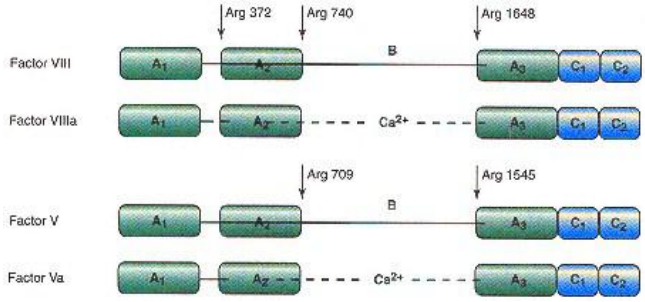


Figure 22.47
Organizational structure of cofactor proteins, factors VIII and V.
Positions for thrombin cleavage are shown. A's and C's represent structural domains.
Redrawn from Kalafatis, M., Swords, N. A., Rand, M. D., and Mann, K. G.
Biochim. Biophys. Acta 1227:113, 1994.

Platelet aggregation becomes autocatalytic with the release of ADP and **thromboxane A_2.** Platelet factor IV, **heparin binding protein,** prevents heparin–antithrombin III complexes from inhibiting serine proteinase coagulation factors, and it attracts cells with anti-inflammatory activity to the site of injury. About 20% of factor V exists in platelets as does one form of factor XIII, the transglutamidase.

Intact vascular endothelium does not normally initiate platelet aggregation since receptors and other elements are not exposed and activators such as ADP are rapidly degraded or are not in blood in sufficient concentration to be effective. The endothelium also secretes **prostacyclin(PGI_2),** a potent inhibitor of platelet aggregation.

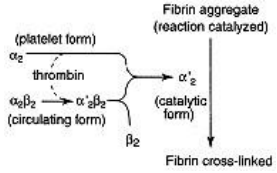


Figure 22.48
Activation of transglutamidase
by thrombin.

Properties of Some of the Proteins Involved in Coagulation

Calcium ions have at least two important functions in blood coagulation. They form complexes with factors that contain γ-carboxyglutamyl (Gla) residues and

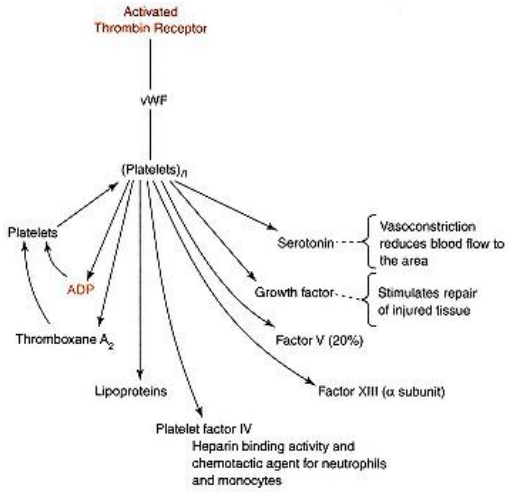


Figure 22.49
Action of platelets in blood coagulation.

CLINICAL CORRELATION 22.10

Classic Hemophilia

Hemophilia is an inherited disorder characterized by a permanent tendency for hemorrhages, spontaneous or traumatic, due to a defective blood clotting system. Classic hemophilia, hemophilia A, is an X-linked recessive disorder characterized by a deficiency of factor VIII. About 1 in 10,000 males is born with a deficiency of factor VIII. Of the approximate 25,000 hemophiliacs in the United States, more than 80% are of the A type. Hemophilia B is due to a dysfunction in factor IX.

Some hemophilia A patients may have a normal prothrombin time if the concentration of tissue factor is high. One possible explanation for this is that factor V in human plasma is much lower in concentration than factor X. Activation of an amount of factor X to Xa in excess of that required to bind all of factor Va would initiate blood clotting by the extrinsic pathway and give a normal prothrombin time. The intrinsic pathway would not function normally due to the deficiency in factor VIII. Without the two pathways operating in concert, the overall process of blood clotting would be impaired. Both factor Xa and thrombin activate factor V and are involved in a number of other reactions. If the overall process is not accelerated at its onset by intervention of the intrinsic pathway, due to kinetics of the interaction of thrombin and factor Xa with the normally low concentration of factor V, the clotting disorder is expressed. The blood levels of factor VII in severe hemophilia A patients are less than 5% of normal. These patients have generally been treated by blood transfusion with its associated dangers: the possibility of contraction of hepatitis or HIV, and the 6% possibility of patients making autoantibodies. Treatment of hemophiliacs has been made much safer as a result of cloning and expression of the gene for factor VIII. The pure protein can be administered to patients with none of the associated dangers mentioned above.

Nemerson, Y. *Blood* 71:1, 1988.

induce conformational and electronic states that facilitate their interaction with membrane "receptors" for initiation and localization of their reactions. Calcium ions also bind at sites other than Gla residues, producing protein conformational changes that enhance catalytic activity. Evidence for this second role for calcium ions comes from the observation that activation of at least one of the enzymes leads to both the cleavage and elimination of the N-terminal region containing the Gla residues, but calcium ions are still required for its effective participation in blood coagulation.

A schematic representation of the structural arrangement of five of the **Gla-containing proteins** listed in Table 22.9 is shown in Figure 22.50. Gla-containing residues are located in the N-terminal region of the molecules followed by a structural component that resembles epidermal growth factor. The position of proteolytic cleavage by activation proteinases is generally at an amino acid residue located between cysteine residues that form a disulfide bond. Activation may or may not result in loss of a small peptide. *Prothrombin is the only one whose activation is by cleavage outside the bridging disulfide bond and results in elimination of the Gla peptide*. Factor VII is activated by cleavage of a single Arg^{152}–Ile^{153} bond. Factor IX is activated by cleavages at Arg^{145} and Arg^{180} with the release of an approximately 11-kDa peptide. Factor X consists of two chains connected by a disulfide bridge. It is activated by cleavage of its heavy chain at Arg^{194}–Ile^{195}. The Gla residues are located in the light chain. Protein C also consists of a heavy and a light chain connected by a disulfide bond. Cleavage of an Arg–Ile bond at position 169 results in its activation.

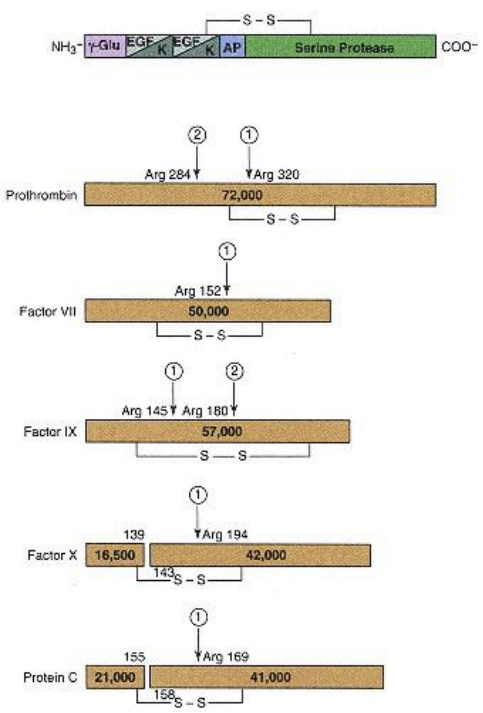


Figure 22.50
Gla-containing proteins.
(*a*) General structure of the γ-carboxyglutamyl-containing proteins.
(*b*) Structural organization of the zymogens and their cleavage sites for activation.

Role of Vitamin K in Protein Carboxylase Reactions

Modification of prothrombin, **protein C, protein S,** and factors VII, IX, and X to form Gla residues occurs during **synthesis** by a **carboxylase** located on the luminal side of the rough endoplasmic reticulum. **Vitamin K** (phytonadione, the "koagulation" vitamin) is an essential **cofactor** for this carboxylase. During the reaction, the dihydroquinone or reduced form of vitamin K (Figure 22.51), vit K(H_2), is oxidized to the epoxide form, vit K(O), using molecular oxygen. A plausible mechanism involves the addition of molecular oxygen to the C-1 position of dihydro-vitamin K and its subsequent rearrangement to an alkoxide with a pK_a of ~20. This intermediate serves as a strong base and abstracts a

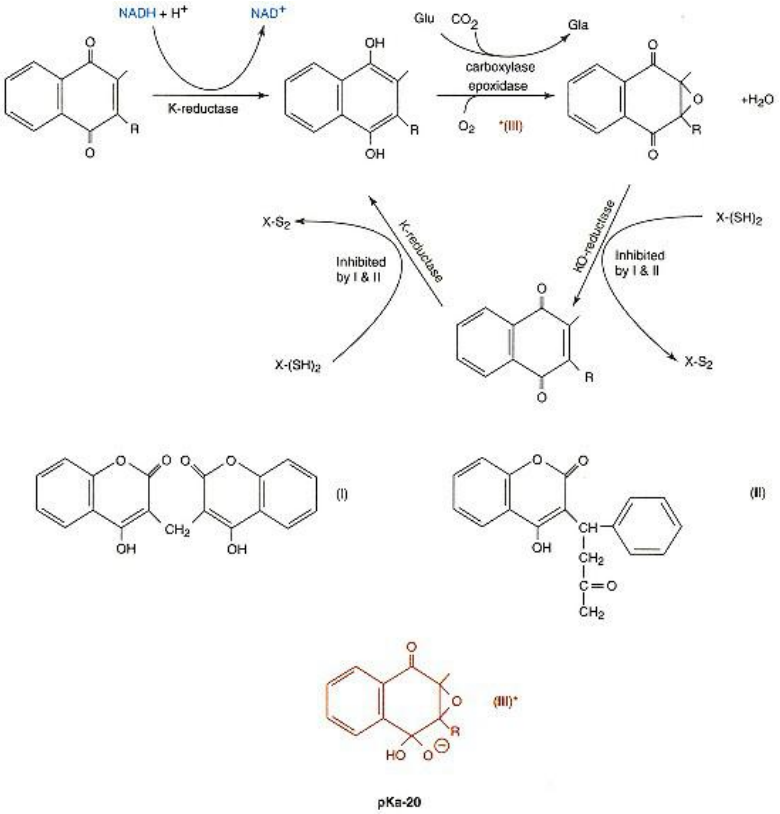
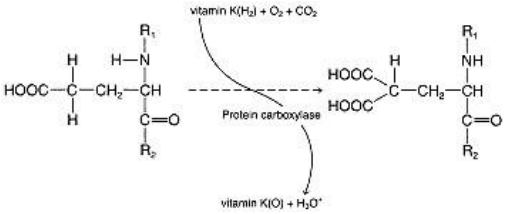


Figure 22.51
The vitamin K cycle as it functions in protein glutamyl carboxylation reaction.
X-$(SH)_2$ and X-S_2 represent the reduced and oxidized forms, respectively, of a thioredoxin. The NADH-dependent and the dithiol-dependent vitamin K reductases are different enzymes. The dithiol-dependent K and KO reductases are inhibited by dicumarol (I) and warfarin (II). *Possible alkoxide intermediate (III).
Redrawn and modified from Vermeer, C. *Biochem. J.* 266:625, 1990.

proton from the γ-methylene carbon of glutamate, yielding a carbanion that can add to CO_2 by a nucleophilic mechanism (Figure 22.51). The **vitamin K epoxide** formed is converted back to the **dihydroquinone** by enzymes that require dithiols like **thioredoxin** as cofactors. Analogs of vitamin K inhibit dithiol-requiring vitamin K reductases and result in conversion of all available vitamin K to the epoxide form that is not functional in this reaction. The overall carboxylation reaction is



The structure of two analogs, **dicumarol** and **warfarin,** that interfere with the action of vitamin K are shown in Figure 22.51. In animals treated with these compounds, prothrombin, protein C, protein S, and factors VII, IX, and X are not posttranslationally modified, are deficient in Ca^{2+} binding, and cannot participate in blood coagulation. Dicumarol and warfarin have no effect on blood coagulation in the test tube.

Control of the Synthesis of Gla-Proteins

Gla-peptides that are released from prothrombin upon activation are removed from circulation by the liver. These N-terminal Gla-containing peptides stimulate the *de novo* synthesis of Gla-requiring proteins of the blood coagulation scheme (Figure 22.52). The proteins are synthesized even in the absence of vitamin K

CLINICAL CORRELATION 22.11

Thrombosis and Defects of the Protein C Pathway

Four major proteins are involved in the action of protein C in regulating blood coagulation: protein C itself; protein S, a cofactor for protein C action; factor Va; and factor VIIIa. The latter two are substrates for catalytic action of the protein C–protein S complex. Mutations, generally inherited, in any of them can result in venous thrombosis with various degrees of severity.

De novo mutations have also been identified in patients showing type I protein C deficiency. One was the result of a missense mutation, a transition of T to C, resulting in the change of a codon for amino acid residue 270 from TCG to CCG. This gave Pro instead of Ser at that position, resulting in a conformational change that affected activity. The gene for protein C is on chromosome 2 and has 9 exons and 8 introns. In another patient, a *de novo* mutation located at the exon VI–intron f junction was detected. A 5-bp deletion (underlined below) occurred, resulting in a "read through" of sections of the intron.

Exon VI <> Intron f

Normal sequence: **CAC CCC GCA** G<>GTGAGAAGCCCCCAATAT
His Pro Ala

Mutated sequence: **CAC CCC GCA** GGA GCC CCC AAT AT
His Pro Ala Gly Ala Pro Asn

The normally translated sequence is in bold type. The degree of severity of thrombotic events depends on the extent to which the gene inherited from the other parent is normal and the extent to which it is expressed.

Resistance to the action of activated protein C as a result of single point mutations in its substrates, factor Va and factor VIIIa, can occur. This prevents or retards their inactivation through the proteolytic action of protein C. The most commonly identified cause of inherited resistance to the action of activated protein C is single point mutations in the gene for factor V.

A third cause of protein C-related thrombosis is a defect in protein S. Fewer specific details are available that permit a definition of the mechanism of the interaction between protein C and protein S, and likewise of the mutations that affect its function. It is quite clear, however, that protein S deficiency leads to thrombotic events. Venous thrombosis occurs in almost one-half of patients at some stage of their lives if they have deficiencies in functional amounts of protein S.

Gandrille, S., Jude, B., Alhenc-gelas, M., et al. *Blood* 84:2566, 1994; Zoller, B., Berntsdotter, A., Garcia de Frutos, P., et al. *Blood* 85:3518, 1995; and Reistma, P. H., Bernardi, F., Doig, R. G., et al. *Thromb. Haemost.* 73:876, 1995.

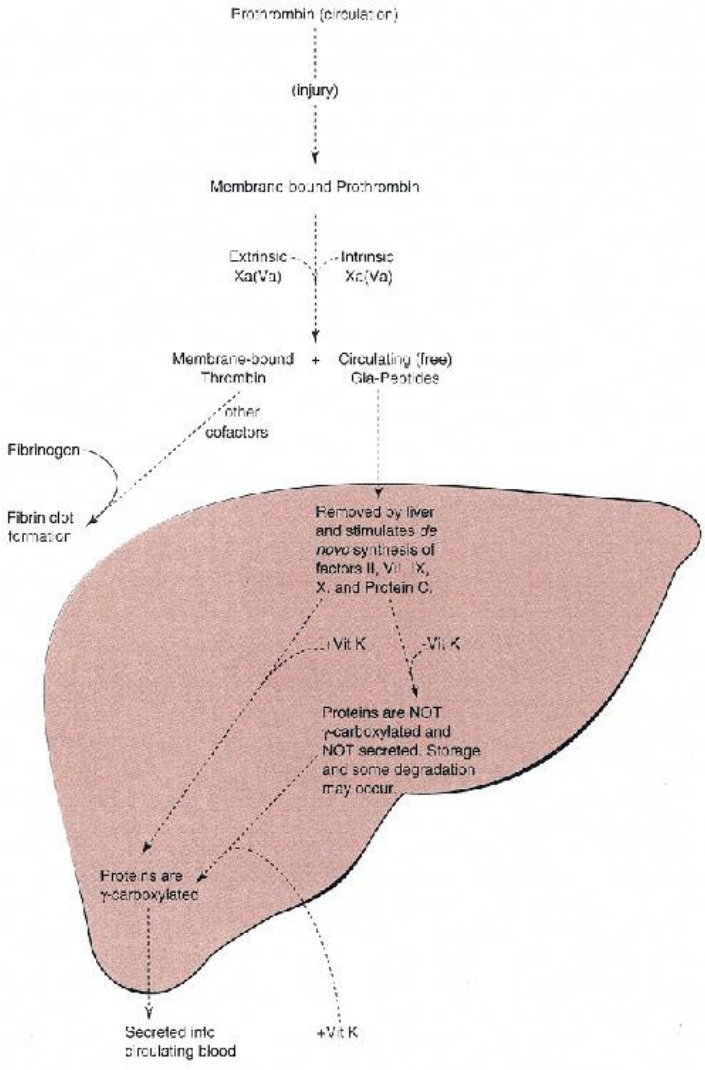


Figure 22.52
Role of Gla peptides in the regulation of *de novo* synthesis of coagulation factors.

or in the presence of antagonists of vitamin K. They are not secreted into the circulation, however. When vitamin K is restored, or is added in high enough concentrations to overcome the effects of antagonists, the preformed proteins are carboxylated and secreted into the circulation.

Activation of blood coagulation is a one-way process. The use of the activation peptides released from prothrombin to signal the liver to synthesize more of these proteins is an efficient mechanism for maintaining their concentrations in blood at effective levels. Monitoring of patients on long-term therapy with vitamin K antagonists is necessary to assure that posttranslational modification to produce the Gla-containing proteins is not shut down completely.

Dual Role of Thrombin in Promoting Coagulation and Clot Dissolution

The process of blood coagulation is self-controlling. One protein involved is **protein C.** Protein C, a Gla-containing protein, is activated in a membrane-

bound complex of thrombin, **thrombomodulin,** and calcium. Thrombomodulin is an integral glycoprotein of the endothelial cell membrane that contains 560 amino acid residues. Thrombomodulin shows amino acid **sequence homology** with the **low-density lipoprotein receptor** but very little with tissue factor. There is, however, a great deal of similarity in functional domains between tissue factor and thrombomodulin, each of which functions as a receptor and activator for a proteinase. Thrombomodulin carries out this function for thrombin for activation of the proteinase, protein C. Binding of thrombin to thrombomodulin reduces its catalytic specificity for fibrinogen and enhances its specificity for protein C. Protein C inhibits coagulation by

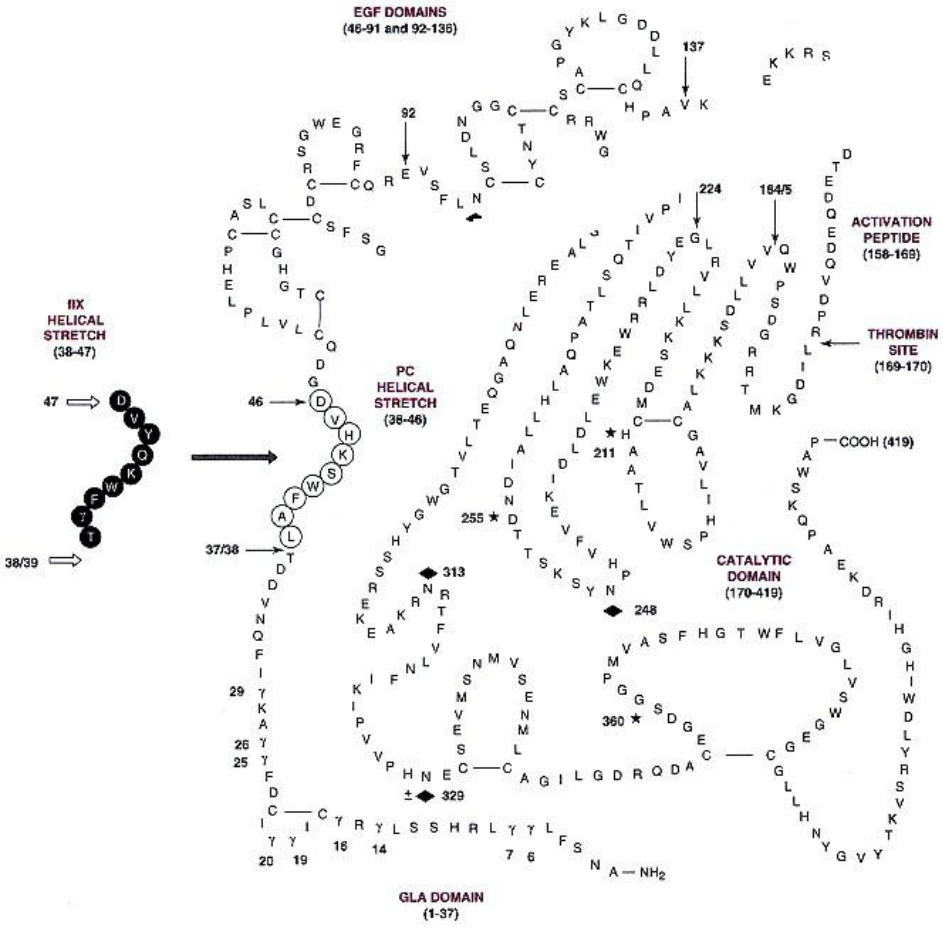


Figure 22.53
Primary structure of recombinant protein C.
Redrawn with permission from Christiansen, W. T., Geng, J. P.,
and Castillino, F. J. *Biochemistry* 34:8082, 1995. Copyright 1995 American Chemical Society.

inactivating factors Va and VIIIa. Another Gla-containing protein, **protein S** (a 75-kDa protein), is a cofactor for protein C. Deficiency in protein S and/or protein C, leads to **thrombotic diseases** (see Clin. Corr. 22.11). A schematic representation of protein C showing some of its reactive regions is depicted in Figure 22.53.

The Allosteric Role of Thrombin in Controlling Coagulation

Important reactions of thrombin relative to its dual role in the processes of promoting and stopping coagulation are summarized in Figure 22.54. Thrombin exists in two conformational forms: one is stabilized by Na^+ and has high

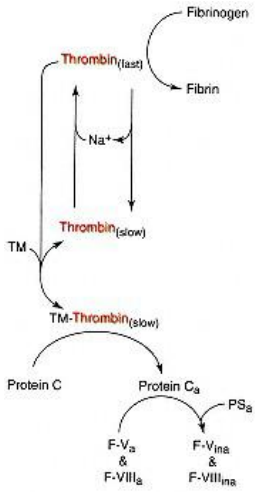


Figure 22.54
Allosteric reactions of thrombin and its actions on fibrinogen and protein C.

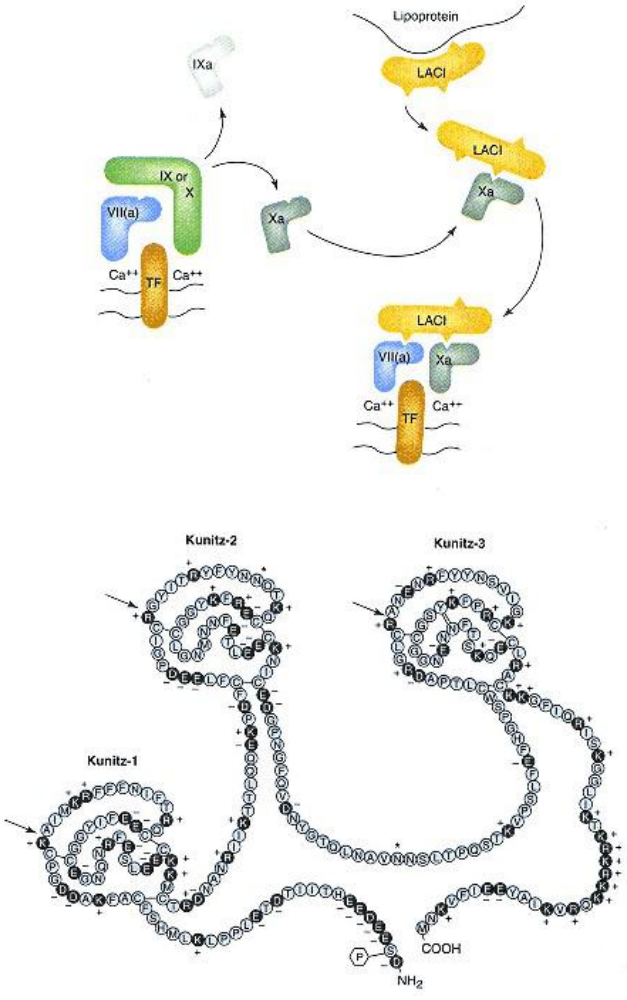


Figure 22.55
Proposed mechanism of inhibition of the extrinsic pathway.
LACI is lipoprotein-associated coagulation factor whose structure is shown in (*b*). Kunitz domain 1 inhibits factor VIIa and Kunitz domain 2 inhibits factor Xa. Arrows indicate the presumed location of the active-site inhibitor region for each domain. Redrawn with permission from Broze, G. J., Girard, T. J., and Novotny, W. F. *Biochemistry* 29:7539, 1990. Copyright 1990 American Chemical Society.

specificity for catalyzing the conversion of fibrinogen to fibrin; the other conformational form predominates in the absence of sodium, has low specificity for fibrinogen conversion, but high specificity for thrombomodulin binding and activity on protein C. These forms are referred to as "fast" and "slow," respectively. This dynamic **"feedback" mechanism** is important for stopping the clotting process at its point of origin. Many thrombotic diseases are associated with mutations in protein C that affect its activation by thrombin.

Inhibitors of the Plasma Serine Proteinases

Proteinase inhibitors in blood interact with enzymes of the blood coagulation system. Most of these fit into the serpin family of inhibitors. The term **serpin** was coined by Carrell and Travis and stands for **ser**ine **p**roteinase **in**hibitor. There is a tertiary structural similarity between them with a common core domain of about 350 amino acids. **Antithrombin III** is one of the major serpins and inhibits most of the serine proteinases of coagulation. Inhibition of the proteinases is a **kinetic process** that can begin almost as soon as coagulation itself begins. Initially, formation of inhibitor complexes is slow because the concentrations of the enzymes with which the inhibitors interact are low. As activation of the enzymes proceeds, inhibition increases and becomes more prominent. These reactions, and destruction of protein cofactors, eventually stop the coagulation process completely. In general, **proteinase–inhibitor complexes** do not dissociate readily and are removed intact from blood by the liver.

Inhibition of the extrinsic pathway, that is, the TF–VIIa–Ca^{2+}–Xa complex, is unique and involves specific interaction with a **lipoprotein-associated coagulation inhibitor (LACI),** formerly known as **anticonvertin.** LACI is a 32-kDa protein that contains three tandem domains (Figure 22.55, p. 974). Each domain is a functionally homologous protease inhibitor that resembles other individual protease inhibitors such as the bovine **pancreas trypsin inhibitor.** LACI inhibits the extrinsic pathway by interacting specifically with the TF–VIIa–Ca^{2+}–Xa complex. Domain 1 binds to factor Xa and domain 2 binds to factor VIIa of the complex. Binding of LACI to VIIa does not occur unless Xa is present. The uniqueness of this reaction is that LACI is a multi-enzyme inhibitor in which each of its separate domains inhibits the action of one of the enzymes of the multi-enzyme complex of the extrinsic pathway.

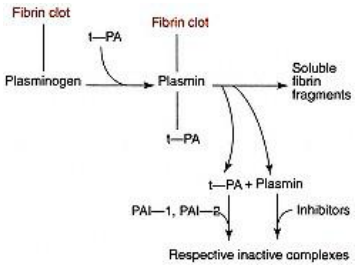


Figure 22.56
Reactions involved in clot dissolution.

Fibrinolysis Requires Plasminogen and Tissue Plasminogen Activator (t-PA) to Produce Plasmin

Reactions of **fibrinolysis** are shown in Figure 22.56. Lysis of the fibrin clot occurs through action of the enzyme **plasmin,** which is formed from **plasminogen** through the action of **tissue plasminogen activator (t-PA** or **TPA).** Plasminogen has high affinity for fibrin clots and forms complexes with fibrin throughout various regions of the fibrin network. t-PA also binds to fibrin clots and activates plasminogen to plasmin by specific bond cleavage. The clot is then solubilized by the action of plasmin.

t-PA is a 72-kDa protein with several functional domains. It has a growth factor domain near its N terminus, two adjacent **Kringle domains** that interact with fibrin, and a domain with protease activity that is close to its C terminus. Kringle domains are conserved sequences that fold into large loops stabilized by disulfide bonds. These domains are important structural features for protein–protein interactions that occur with several blood coagulation factors. t-PA is activated by cleavage between an Arg–Ile bond, resulting in a molecule with a heavy and a light chain. The serine protease activity is located within the light chain.

Activity of t-PA is regulated by protein inhibitors. Four immunologically distinct types of inhibitors have been identified, two of which are of greater physiological significance because they react rapidly with t-PA and are specific for it. They are **plasminogen activator-inhibitor type 1 (PAI-1)** and **plasminogen activator-inhibitor type 2 (PAI-2).** The human PAI-2 is a 415 amino acid protein.

Starting and stopping blood coagulation follow essentially the same type process, binding and proteolysis. Both are one-way processes and the only mechanism for replenishing the proteins once they are used is by resynthesis.

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Questions

C. N. Angstadt and J. Baggott

1. In the propagation of a nerve impulse by an electrical signal:

A. the electrical potential across the membrane maintained by the ATP-driven Na^+,K^+ ion pump becomes more negative.

B. local depolarization of the membrane causes protein conformational changes that allow Na^+ and K^+ to move down their concentration gradients.

C. charge propagation is bidirectional along the axon.

D. "voltage-gated" ion channels have a finite recovery time so the amplitude of the impulse changes as it moves along the axon.

E. astrocytes are the antenna-like protrusions that receive signals from other cells.

2. All of the following are characteristics of nonpeptide neurotransmitters EXCEPT:

A. they transmit the signal across the synapse between cells.

B. they must be made in the cell body and then travel down the axon to the presynaptic terminal.

C. electrical stimulation increasing Ca^{2+} in the presynaptic terminal fosters their release from storage vesicles.

D. binding to receptors on the postsynaptic terminal induces a conformational change in proteins of that membrane.

E. their actions are terminated by specific mechanisms within the synaptic junction.

Refer to the following for Questions 3–5.

A. acetylcholine

B. 4-aminobutyrate (GABA)

C. catecholamines

D. 5-hydroxytryptamine (serotonin)

3. Binding to its receptor opens a channel for Cl^-, causing hyperpolarization of the cell.

4. Termination of the signal typically involves the actions of both methyltransferase and monoamine oxidase, as well as reuptake into the presynaptic neuron.

5. Action is terminated by an esterase.

6. Which of the following is a correct statement about biochemical events occurring in the eye is (are) true?

A. Glucose in the lens is metabolized by the TCA cycle in order to provide ATP for the Na^+, K^+–ATPase.

B. Controlling the blood glucose level might reduce the incidence of diabetic cataracts by allowing the production of sorbitol.

C. The high rate of the hexose monophosphate pathway in the cornea is necessary to provide NADPH as a substrate for glutathione reductase.

D. The retina contains mitochondria so it depends on the TCA cycle for its production of ATP.

E. Cataracts are the result of increasing blood flow in the lens leading to disaggregation of lens proteins.

7. Which of the following statements about rhodopsin is true?

A. Rhodopsin is the primary photoreceptor of both rods and cones.

B. The prosthetic group of rhodopsin is all-*trans*-retinol derived from cleavage of β-carotene.

C. Conversion of rhodopsin to activated rhodopsin, R*, by a light pulse requires depolarization of the cell.

D. Rhodopsin is located in the cytosol of the cell.

E. Absorption of a photon of light by rhodopsin causes an isomerization of 11-*cis*-retinal to all-*trans*-retinal.

8. All of the following statements about the transduction of the light signal on rhodopsin are true EXCEPT:

A. cGMP is involved in the transmission of the signal between the disk membrane and the plasma membrane.

B. it involves the G-protein, transducin.

C. cGMP concentration is increased in the presence of an activated rhodopsin–transducin–GTP complex.

D. the signal is turned off, in part, by the GTPase activity of the α subunit of transducin.

E. both guanylate cyclase and phosphodiesterase are regulated by calcium concentration.

9. The cones of the retina:

A. are responsible for color vision.

B. are much more numerous than the rods.

C. have red, blue, and green light-sensitive pigments that differ because of small differences in the retinal prosthetic group.

D. do not use transducin in signal transduction.

E. are better suited for discerning rapidly changing visual events because a single photon of light generates a stronger current than it does in the rods.

10. When a muscle contracts, the:

A. transverse tubules shorten, drawing the myofibrils and sarcoplasmic reticulum closer together.

B. thin filaments and the thick filaments of the sarcomere shorten.

C. light chains dissociate from the heavy chains of myosin.

D. H bands and I bands of the sarcomere shorten because the thin filaments and thick filaments slide past each other.

E. cross-linking of proteins in the heavy filaments increases.

11. All of the following statements about actin and myosin are true EXCEPT:

A. the globular head section of myosin has domains for binding ATP and actin.

B. actin is the major protein of the thick filament.

C. the binding of ATP to the actin–myosin complex promotes dissociation of actin and myosin.

D. F-actin, formed by aggregation of G-actin–ATP–Mg^{2+} complex, is stabilized when tropomyosin is bound to it.

E. binding of calcium to the calmodulin-like subunit of troponin induces conformational changes that permit myosin to bind to actin.

12. ATP concentration is maintained relatively constant during muscle contraction by:

A. increasing the metabolic activity.

B. the action of adenylate kinase.

C. the action of creatine phosphokinase.

D. all of the above.

E. none of the above.

13. The nerve impulse that initiates muscular contraction:

A. begins with the binding of acetylcholine to receptors in the sarcoplasmic reticulum.

B. causes both the plasma membrane and the transverse tubules to undergo hyperpolarization.

C. causes opening of calcium channels, which leads to an increase in calcium concentration within the sarcomere.

D. prevents Na^+ from entering the sarcomere.

E. prevents Ca^{2+} from binding to troponin C.

14. Platelet aggregation:

A. is initiated at the site of an injury by conversion of fibrinogen to fibrin.

B. is inhibited in uninjured blood vessels by the secretion of prostacyclin by intact vascular endothelium.

C. causes morphological changes and a release of the vasodilator, serotonin.

D. is inhibited by the release of ADP and thromboxane A_2.

E. is inhibited by von Willebrand factor (vWF).

15. In the formation of a blood clot:

A. proteolysis of γ-carboxyglutamate residues from fibrinogen to form fibrin is required.

B. the clot is stabilized by the cross-linking of fibrin molecules by the action of factor XIII, transglutamidase.

C. antagonists of vitamin K inhibit the formation of γ-carboxyglutamate residues in various proteins, thus facilitating the clotting process.

D. tissue factor, factor III, must be inactivated for the clotting process to begin.

E. the role of calcium is primarily to bind fibrin molecules together to form the clot.

16. Factor Xa, necessary for conversion of prothrombin to thrombin, is formed by the action of the TF–VII–Ca^{2+} complex on factor X:

A. only in the extrinsic pathway for blood clotting.

B. only in the intrinsic pathway for blood clotting.

C. as part of both the extrinsic and intrinsic pathways.

D. only if the normal blood clotting cascade is inhibited.

17. Lysis of a fibrin clot:

A. is in equilibrium with formation of the clot.

B. begins when plasmin binds to the clot.

C. requires the hydrolysis of plasminogen into heavy and light chains.

D. is regulated by the action of protein inhibitors on plasminogen.

E. requires the conversion of plasminogen to plasmin by t-PA (tissue plasminogen activator).

Answers

1. B This is the mechanism for impulse propagation. A: The potential becomes less negative. C: It is unidirectional. D: "Voltagegated" channels do have a finite recovery time so the amplitude remains constant. E: This describes dendrites. Astrocytes are glial cells that are involved in processes isolating the CNS from the external environment (pp. 921–923).

2. B This is true for neuropeptides, but many nonpeptide neurotransmitters are synthesized in the presynaptic terminal (pp. 923–924). A: This is a difference between electrical and chemical signals (p. 921). C: What is the role of synapsin I in this process (p. 925)? E: Make sure you know the three types of processes involved (p. 927).

3. B GABA is an inhibitory neurotransmitter. All the others are excitatory ones that cause depolarization of the cells (p. 931).

4. C Methylation by catecholamine-*O*-methyltransferase is an important part of the metabolism of the catecholamines. A: Acetylcholinesterase terminates the action of this (p. 928). B: GABA is converted into an intermediate of the TCA cycle (p. 931). D: Monoamine oxidase is the primary enzyme responsible for terminating serotonin's action (p. 930).

5. A The enzyme is acetylcholinesterase (p. 928).

6. C Make sure you understand the role of glutathione in protecting against harmful by-products from atmospheric oxygen (p. 933). A: Most of the ATP (85%) in the lens is generated by glycolysis (p. 934). B: Controlling glucose reduces sorbitol formation (p. 935). D: Its metabolism is similar to that of other eye tissues directly involved in the visual process. Thus its major source of energy is from glycolysis (p. 935). E: Lens has no blood supply. In diabetic cataracts there is increased aggregation of lens proteins because of increasing sorbitol (p. 935).

7. E This causes the conformational change of the protein that affects the resting membrane potential and initiates the rest of the events. A: Cones have the same prosthetic group but different proteins, so rhodopsin is in rods only (p. 937). B: This is the precursor of the prosthetic group 11-*cis*-retinal (p. 938). C: Isomerization of the prosthetic group leads to hyperpolarization (p. 939 Figure 22.20). D: Rhodopsin is a transmembrane protein (p. 937).

8. C The transducin complex activates the phosphodiesterase, thus lowering [cGMP] (p. 942). A: This is an example of a second messenger type chemical synapse. B and D: Transducin meets the criteria for a typical G-protein. E: The enzymes are regulated in opposite directions by Ca^{2+}, thus controlling [cGMP] (p. 943).

9. A Rods are responsible for low light vision. C: All three pigments have 11-*cis*-retinal; the proteins differ and are responsible for the slightly different spectra (pp. 937 and 944). D: The biochemical events are believed to be the same in rods and cones (p. 944). E: Cones are better suited for rapid events because their response rate is about four times faster than rods, even though their sensitivity to light is much less (p. 945).

10. D This occurs because of association–dissociation of actin and myosin (pp. 948 and 957). A: Depolarization in the transverse tubules may be involved in transmission of the signal but not directly in the contractile process (p. 954). B: The filaments do not change in length, but slide past each other (p. 948). C: This is not physiological. E: Cross-linking occurs in the H band of the sarcomere but does not change during the contractile process (p. 953).

11. B A: See Figure 22.28. C: Note that the role of ATP in contraction is to favor dissociation, not formation, of the actin–myosin

complex (p. 957). D. and E: Tropomyosin, troponin, and actin are the three major proteins of the filament. Their actions are closely interconnected (pp. 951–953).

12. D Make sure you know the reactions catalyzed by these two enzymes (pp. 954–957).

13. C A: Acetylcholine receptors are on the plasma membrane. B: The impulse results in depolarization of both of these structures. D: Both Ca^{2+} and Na^{2+} enter the sarcomere when the channels open. E: Binding of Ca^{+2} to Tn-C initiates contraction (p. 954).

14. B The "ying–yang" nature of PGI_2 and TXA_2 help to control platelet aggregation until there is a need for it. A: Initiation is by contact with an activated receptor at the site of injury. Clot formation requires activation of various enzymes (pp. 960–961). C: Serotonin is a vasoconstrictor. Vasodilation would be contraindicated in this situation (p. 967). D: TXA_2 facilitates aggregation. E: vWF forms a link between the receptor and platelets, promoting aggregation (p. 967).

15. B The cross-linking occurs between a glutamine and a lysine (Figure 22.46). A and E: γ-Carboxyglutamate residues are on various enzymes; they bind calcium and facilitate the interaction of these proteins with membranes that form the sites for initiation of reaction (pp. 968–969). C: Vitamin K is an activator for the γ-carboxylation reaction, which is a necessary posttranslational modification of some of the enzymes involved in clot formation (p. 970). D: TF, factor III, is the primary receptor for initiation of the clotting process (p. 963).

16. A Tissue factor and factor VII are unique to the extrinsic pathway. B,C: The membrane interaction with the intrinsic pathway is with high-molecular-weight kininogen and pre-kallikrein (p. 961).

17. E The clot is solubilized by plasmin. A: Both formation and lysis of clots are unidirectional. B: Both plasminogen and t-PA bind to the clot. C and D: Both of these refer to t-PA (pp. 975–976).