CHAPTER 41

The Diversity of the Endocrine System

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

BIOMEDICAL IMPORTANCE

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

THE TARGET CELL CONCEPT

There are about 200 types of differentiated cells in humans. Only a few produce hormones, but virtually all of the 75 trillion cells in a human are targets of one or more of the over 50 known hormones. The concept of the target cell is a useful way of looking at hormone action. It was thought that hormones affected a single cell type—or only a few kinds of cells—and that a hormone elicited a unique biochemical or physiologic action. We now know that a given hormone can affect several different cell types; that more than one hormone can affect a given cell type; and that hormones can exert many different effects in one cell or in different cells. With the discovery of specific cell-surface and intracellular hormone receptors, the definition of a target has been expanded to include any cell in which the hormone (ligand) binds to its receptor, whether or not a biochemical or physiologic response has yet been determined.

Several factors determine the response of a target cell to a hormone. These can be thought of in two general ways: (1) as

TABLE 41–1 Determinants of the Concentration of a Hormone at the Target Cell

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

TABLE 41–2 Determinants of the Target Cell Response

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

factors that affect the concentration of the hormone at the target cell (Table 41–1) and (2) as factors that affect the actual response of the target cell to the hormone (Table 41–2).

HORMONE RECEPTORS ARE OF CENTRAL IMPORTANCE

Receptors Discriminate Precisely

One of the major challenges faced in making the hormone-based communication system work is illustrated in Figure 41–1. Hormones are present at very low concentrations in the extracellular fluid, generally in the atto- to nanomolar range (10^{-15} to 10^{-9} mol/L). This concentration is much lower than that of the many structurally similar molecules (sterols, amino acids, peptides, proteins) and other molecules that circulate at concentrations in the micro- to millimolar (10^{-6} to 10^{-3}) mol/L range. Target cells, therefore, must distinguish not only between different hormones present in small amounts but also between a given hormone and the 10^6- to 10^9-fold excess of other similar molecules. This high degree of discrimination is provided by cell-associated recognition molecules called receptors. Hormones initiate their biologic effects by binding to specific receptors, and since any effective control system also must provide a means of stopping a response, hormone-induced actions generally but not always terminate when the effector dissociates from the receptor (Figure 38–1).

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

Both Recognition & Coupling Domains Occur on Receptors

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

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

The dual functions of binding and coupling ultimately define a receptor, and it is the coupling of hormone binding to signal transduction—so-called **receptor-effector coupling**—that provides the first step in amplification of the hormonal response. This dual purpose also distinguishes the target cell receptor from the plasma carrier proteins that bind hormone but do not generate a signal (see Table 41–6).

Receptors Are Proteins

Several classes of peptide hormone receptors have been defined. For example, the insulin receptor is a heterotetramer composed of two copies of two different protein subunits ($\alpha_2\beta_2$) linked by multiple disulfide bonds in which the extracellular α subunit binds insulin and the membrane-spanning β subunit transduces the signal through the tyrosine protein kinase domain located in the cytoplasmic portion of this polypeptide. The receptors for insulin-like growth factor I (IGF-I) and epidermal growth factor (EGF) are generally similar in structure to the insulin receptor. The growth hormone and prolactin receptors also span the plasma membrane of target cells but do not con-

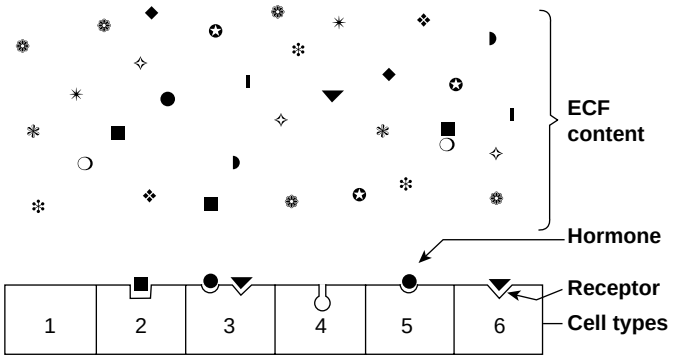

FIGURE 41–1 Specificity and selectivity of hormone receptors. Many different molecules circulate in the extracellular fluid (ECF), but only a few are recognized by hormone receptors. Receptors must select these molecules from among high concentrations of the other molecules. This simplified drawing shows that a cell may have no hormone receptors (1), have one receptor (2+5+6), have receptors for several hormones (3), or have a receptor but no hormone in the vicinity (4).

tain intrinsic protein kinase activity. Ligand binding to these receptors, however, results in the association and activation of a completely different protein kinase signaling pathway, the Jak-Stat pathway. Polypeptide hormone and catecholamine receptors, which transduce signals by altering the rate of production of cAMP through G-proteins, are characterized by the presence of seven domains that span the plasma membrane. Protein kinase activation and the generation of cyclic AMP (cAMP, 3′5′-adenylic acid; see Figure 19–5) is a downstream action of this class of receptor (see Chapter 42 for further details).

A comparison of several different steroid receptors with thyroid hormone receptors revealed a remarkable conservation of the amino acid sequence in certain regions, particularly in the DNA-binding domains. This led to the realization that receptors of the steroid or thyroid type are members of a large superfamily of nuclear receptors. Many related members of this family currently have no known ligand and thus are called orphan receptors. The nuclear receptor superfamily plays a critical role in the regulation of gene transcription by hormones, as described in Chapter 42.

HORMONES CAN BE CLASSIFIED IN SEVERAL WAYS

Hormones can be classified according to chemical composition, solubility properties, location of receptors, and the nature of the signal used to mediate hormonal action within the cell. A classification based on the last two properties is illustrated in Table 41–3, and general features of each group are illustrated in Table 41–4.

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

The second major group consists of water-soluble hormones that bind to the plasma membrane of the target cell. Hormones that bind to the surfaces of cells communicate with intracellular metabolic processes through intermediary molecules called **second messengers** (the hormone itself is the first messenger), which are generated as a consequence of the ligand–receptor interaction. The second messenger concept arose from an observation that epinephrine binds to the plasma membrane of certain cells and increases intracellular cAMP. This was followed by a series of experiments in which cAMP was found to mediate the effects of many hormones. Hormones that clearly employ this mechanism are shown in group II.A of Table 41–3. Atrial natriuretic factor (ANF), uses cGMP as its second messenger (group II.B). Several hormones, many of which were previously thought to affect cAMP, appear to use ionic calcium (Ca^{2+}) or metabolites of complex phosphoinositides (or both) as the intracellular signal. These are shown in group II.C of the table. The intracellular messenger for group II.D is a protein kinase-phosphatase cascade. Several of these have been identified, and a given hormone may use more than one kinase cascade. A few hormones fit into more than one category, and assignments change as new information is brought forward.

DIVERSITY OF THE ENDOCRINE SYSTEM

Hormones Are Synthesized in a Variety of Cellular Arrangements

Hormones are synthesized in discrete organs designed solely for this specific purpose, such as the thyroid (triiodothyronine), adrenal (glucocorticoids and mineralocorticoids), and the pituitary (TSH, FSH, LH, growth hormone, prolactin, ACTH). Some organs are designed to perform two distinct but closely related functions. For example, the ovaries produce mature oocytes and the reproductive hormones estradiol and progesterone. The testes produce mature spermatozoa and testosterone. Hormones are also produced in specialized cells within other organs such as the small intestine (glucagon-like peptide), thyroid (calcitonin), and kidney (angiotensin

TABLE 41–3 Classification of Hormones by Mechanism of Action

I. Hormones that bind to intracellular receptors
Androgens
Calcitriol (1,25[OH]$_2$-D$_3$)
Estrogens
Glucocorticoids
Mineralocorticoids
Progestins
Retinoic acid
Thyroid hormones (T$_3$ and T$_4$)
II. Hormones that bind to cell surface receptors
A. The second messenger is cAMP
α$_2$-Adrenergic catecholamines
β-Adrenergic catecholamines
Adrenocorticotropic hormone (ACTH)
Antidiuretic hormone
Calcitonin
Chorionic gonadotropin, human (CG)
Corticotropin-releasing hormone
Follicle-stimulating hormone (FSH)
Glucagon
Lipotropin (LPH)
Luteinizing hormone (LH)
Melanocyte-stimulating hormone (MSH)
Parathyroid hormone (PTH)
Somatostatin
Thyroid-stimulating hormone (TSH)
B. The second messenger is cGMP
Atrial natriuretic factor
Nitric oxide
C. The second messenger is calcium or phosphatidylinositols (or both)
Acetylcholine (muscarinic)
α$_1$-Adrenergic catecholamines
Angiotensin II
Antidiuretic hormone (vasopressin)
Cholecystokinin
Gastrin
Gonadotropin-releasing hormone
Oxytocin
Platelet-derived growth factor (PDGF)
Substance P
Thyrotropin-releasing hormone (TRH)
D. The second messenger is a kinase or phosphatase cascade
Adiponectin
Chorionic somatomammotropin
Epidermal growth factor
Erythropoietin
Fibroblast growth factor (FGF)
Growth hormone (GH)
Insulin
Insulin-like growth factors I and II
Leptin
Nerve growth factor (NGF)
Platelet-derived growth factor
Prolactin

TABLE 41–4 General Features of Hormone Classes

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

II). Finally, the synthesis of some hormones requires the parenchymal cells of more than one organ—eg, the skin, liver, and kidney are required for the production of 1,25(OH)$_2$-D$_3$ (calcitriol). Examples of this diversity in the approach to hormone synthesis, each of which has evolved to fulfill a specific purpose, are discussed below.

Hormones Are Chemically Diverse

Hormones are synthesized from a wide variety of chemical building blocks. A large series is derived from cholesterol. These include the glucocorticoids, mineralocorticoids, estrogens, progestins, and 1,25(OH)$_2$-D$_3$ (Figure 41–2). In some cases, a steroid hormone is the precursor molecule for another hormone. For example, progesterone is a hormone in its own right but is also a precursor in the formation of glucocorticoids, mineralocorticoids, testosterone, and estrogens. Testosterone is an obligatory intermediate in the biosynthesis of estradiol and in the formation of dihydrotestosterone (DHT). In these examples, described in detail below, the final product is determined by the cell type and the associated set of enzymes in which the precursor exists.

The amino acid tyrosine is the starting point in the synthesis of the catecholamines and of the thyroid hormones tetraiodothyronine (thyroxine; T$_4$) and triiodothyronine (T$_3$) (Figure 41–2). T$_3$ and T$_4$ are unique in that they require the addition of iodine (as I^-) for bioactivity. Because dietary iodine is very scarce in many parts of the world, an intricate mechanism for accumulating and retaining I^- has evolved.

Many hormones are polypeptides or glycoproteins. These range in size from thyrotropin-releasing hormone (TRH), a tripeptide, to single-chain polypeptides like adrenocorticotropic hormone (ACTH; 39 amino acids), parathyroid hormone (PTH; 84 amino acids), and growth hormone (GH; 191 amino acids) (Figure 41–2). Insulin is an AB chain heterodimer of 21 and 30 amino acids, respectively. Follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH), and chorionic gonadotropin (CG) are glycoprotein hormones of αβ heterodimeric structure. The α chain is identical in all of these hormones,

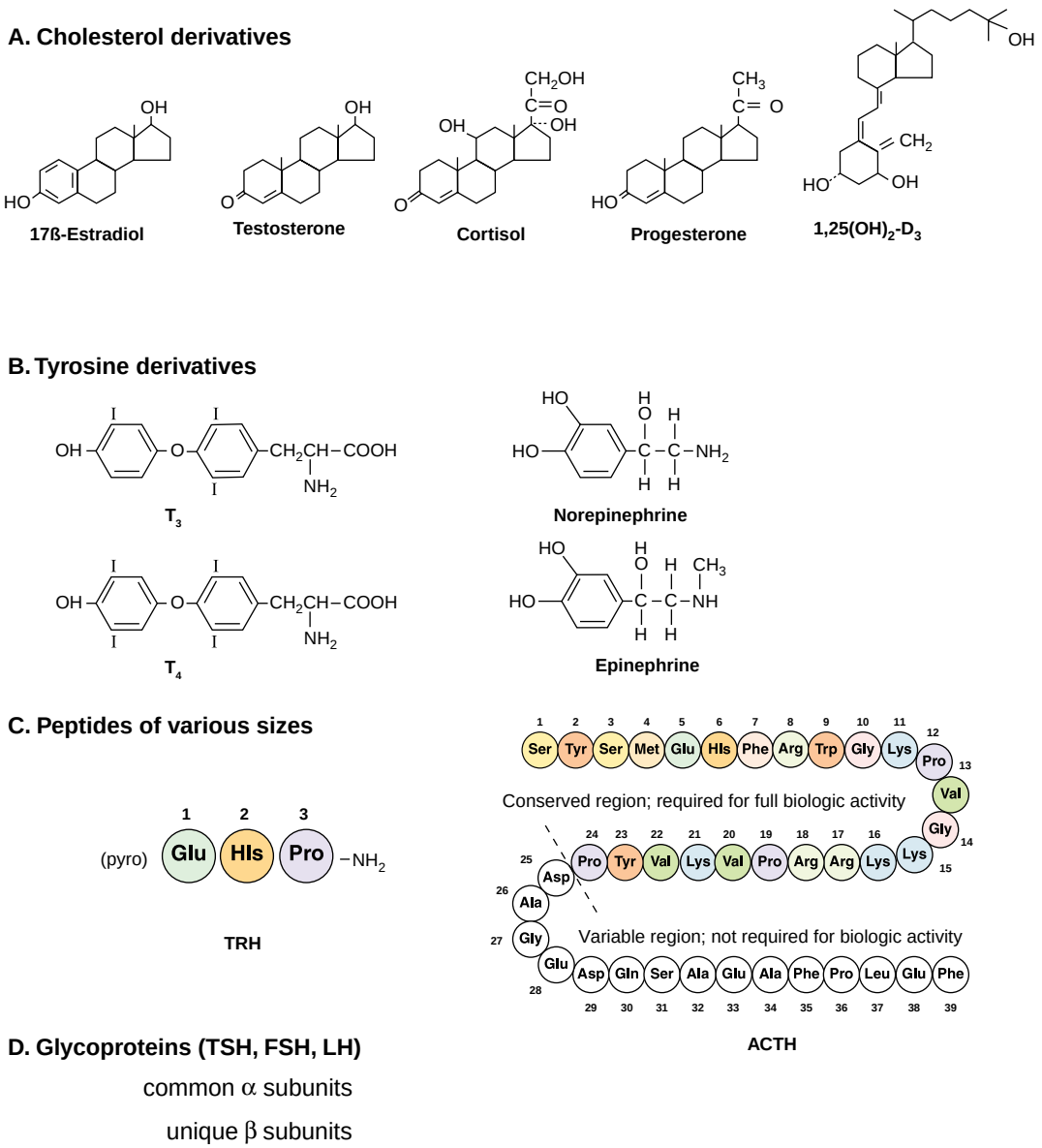

FIGURE 41–2 Chemical diversity of hormones. **(A)** Cholesterol derivatives. **(B)** Tyrosine derivatives. **(C)** Peptides of various sizes. **(D)** Glycoproteins (TSH, FSH, LH) with common α subunits and unique β subunits.

and distinct β chains impart hormone uniqueness. These hormones have a molecular mass in the range of 25–30 kDa depending on the degree of glycosylation and the length of the β chain.

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

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

MANY HORMONES ARE MADE FROM CHOLESTEROL

Adrenal Steroidogenesis

The adrenal steroid hormones are synthesized from cholesterol. Cholesterol is mostly derived from the plasma, but a small portion is synthesized in situ from acetyl-CoA via mevalonate and squalene. Much of the cholesterol in the adrenal is esterified and stored in cytoplasmic lipid droplets. Upon stimulation of the adrenal by ACTH, an esterase is activated,

and the free cholesterol formed is transported into the mitochondrion, where a **cytochrome P450 side chain cleavage enzyme (P450scc)** converts cholesterol to pregnenolone. Cleavage of the side chain involves sequential hydroxylations, first at C_{22} and then at C_{20}, followed by side chain cleavage (removal of the six-carbon fragment isocaproaldehyde) to give the 21-carbon steroid (Figure 41–3, top). An ACTH-dependent **steroidogenic acute regulatory (StAR) protein** is essential for the transport of cholesterol to P450scc in the inner mitochondrial membrane.

All mammalian steroid hormones are formed from cholesterol via pregnenolone through a series of reactions that occur in either the mitochondria or endoplasmic reticulum of the producing cell. Hydroxylases that require molecular oxygen and NADPH are essential, and dehydrogenases, an isomerase, and a lyase reaction are also necessary for certain steps. There is cellular specificity in adrenal steroidogenesis. For instance, 18-hydroxylase and 19-hydroxysteroid dehydrogenase, which are required for aldosterone synthesis, are found only in the zona glomerulosa cells (the outer region of the adrenal cortex), so that the biosynthesis of this mineralocorticoid is confined to this region. A schematic representation of the pathways involved in the synthesis of the three major classes of adrenal steroids is presented in Figure 41–4. The enzymes are shown in the rectangular boxes, and the modifications at each step are shaded.

Mineralocorticoid Synthesis

Synthesis of aldosterone follows the mineralocorticoid pathway and occurs in the zona glomerulosa. Pregnenolone is converted to progesterone by the action of two smooth endoplasmic reticulum enzymes, **3β-hydroxysteroid dehydrogenase (3β-OHSD)** and **$\Delta^{5,4}$-isomerase.** Progesterone is hydroxylated at the C_{21} position to form 11-deoxycorticosterone (DOC), which is an active (Na^+-retaining) mineralocorticoid. The next hydroxylation, at C_{11}, produces corticosterone, which has glucocorticoid activity and is a weak mineralocorticoid (it has less than 5% of the potency of aldosterone). In some species (eg, rodents), it is the most potent glucocorticoid. C_{21} hydroxylation is necessary for both mineralocorticoid and glucocorticoid activity, but most steroids with a C_{17} hydroxyl group have more glucocorticoid and less mineralocorticoid action. In the zona glomerulosa, which does not have the smooth endoplasmic reticulum enzyme 17α-hydroxylase, a mitochondrial 18-hydroxylase is present. The **18-hydroxylase (aldosterone synthase)** acts on corticosterone to form 18-hydroxycorticosterone, which is changed to aldosterone by conversion of the 18-alcohol to an aldehyde. This unique distribution of enzymes and the special regulation of the zona glomerulosa by K^+ and angiotensin II have led some investigators to suggest that, in addition to the adrenal being two glands, the adrenal cortex is actually two separate organs.

Glucocorticoid Synthesis

Cortisol synthesis requires three hydroxylases located in the fasciculata and reticularis zones of the adrenal cortex that act sequentially on the C_{17}, C_{21}, and C_{11} positions. The first two reactions are rapid, while C_{11} hydroxylation is relatively slow. If the C_{11} position is hydroxylated first, the action of **17α-hydroxylase** is impeded and the mineralocorticoid pathway is followed

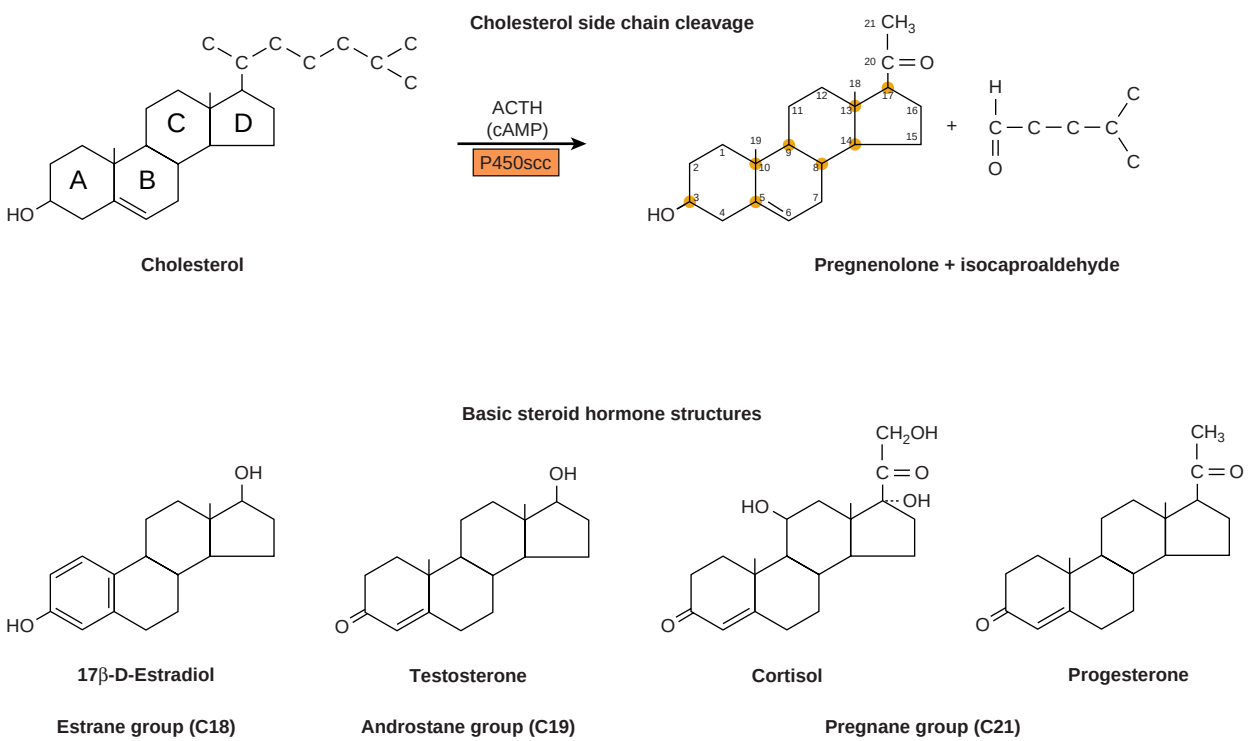

FIGURE 41–3 Cholesterol side-chain cleavage and basic steroid hormone structures. The basic sterol rings are identified by the letters A–D. The carbon atoms are numbered 1–21, starting with the A ring.

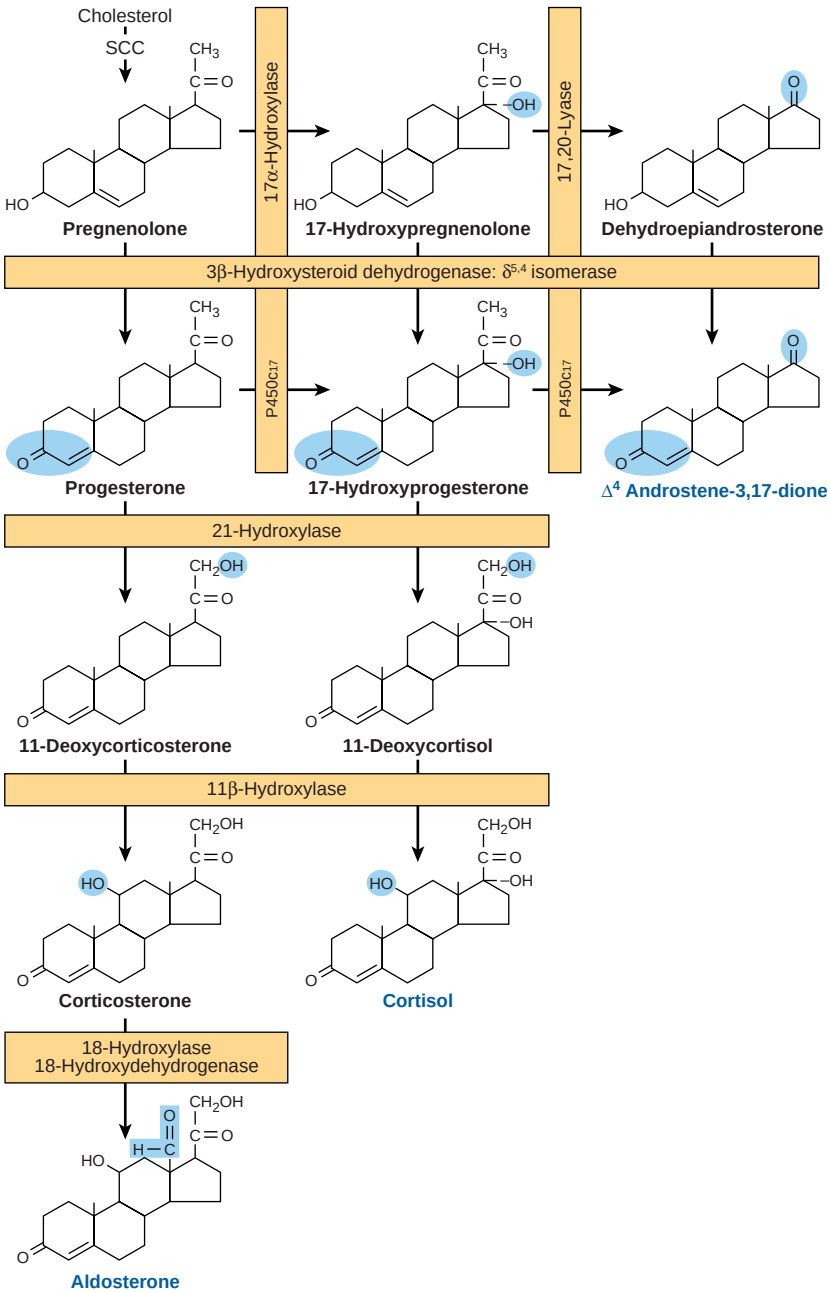

FIGURE 41–4 Pathways involved in the synthesis of the three major classes of adrenal steroids (mineralocorticoids, glucocorticoids, and androgens). Enzymes are shown in the rectangular boxes, and the modifications at each step are shaded. Note that the 17α-hydroxylase and 17,20-lyase activities are both part of one enzyme, designated P450c17. (Slightly modified and reproduced, with permission, from Harding BW: In: *Endocrinology*, vol 2. DeGroot LJ [editor]. Grune & Stratton, 1979. Copyright © 1979 Elsevier Inc. Reprinted with permission from Elsevier.)

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(forming corticosterone or aldosterone, depending on the cell type). 17α-Hydroxylase is a smooth endoplasmic reticulum enzyme that acts upon either progesterone or, more commonly, pregnenolone. 17α-Hydroxyprogesterone is hydroxylated at C_{21} to form 11-deoxycortisol, which is then hydroxylated at C_{11} to form cortisol, the most potent natural glucocorticoid hormone in humans. 21-Hydroxylase is a smooth endoplasmic reticulum enzyme, whereas 11β-hydroxylase is a mitochondrial enzyme. Steroidogenesis thus involves the repeated shuttling of substrates into and out of the mitochondria.

Androgen Synthesis

The major androgen or androgen precursor produced by the adrenal cortex is dehydroepiandrosterone (DHEA). Most 17-hydroxypregnenolone follows the glucocorticoid pathway, but a small fraction is subjected to oxidative fission and removal of the two-carbon side chain through the action of 17,20-lyase. The lyase activity is actually part of the same enzyme (P450c17) that catalyzes 17α-hydroxylation. This is therefore a **dual-function protein.** The lyase activity is important in both the adrenals and the gonads and acts exclusively on 17α-hydroxy-containing molecules. Adrenal androgen production increases markedly if glucocorticoid biosynthesis is impeded by the lack of one of the hydroxylases **(adrenogenital syndrome).** DHEA is really a prohormone, since the actions of 3β-OHSD and $\Delta^{5,4}$-isomerase convert the weak androgen DHEA into the more potent **androstenedione.** Small amounts of androstenedione are also formed in the adrenal by the action of the lyase on 17α-hydroxyprogesterone.

Reduction of androstenedione at the C_{17} position results in the formation of **testosterone,** the most potent adrenal androgen. Small amounts of testosterone are produced in the adrenal by this mechanism, but most of this conversion occurs in the testes.

Testicular Steroidogenesis

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

The conversion of pregnenolone to testosterone requires the action of five enzyme activities contained in three proteins: (1) 3β-hydroxysteroid dehydrogenase (3β-OHSD) and $\Delta^{5,4}$-isomerase; (2) 17α-hydroxylase and 17,20-lyase; and (3) 17β-hydroxysteroid dehydrogenase (17β-OHSD). This sequence, referred to as the **progesterone (or Δ^4)** pathway, is shown on the right side of Figure 41–5. Pregnenolone can also be converted to testosterone by the **dehydroepiandrosterone (or Δ^5)** pathway, which is illustrated on the left side of Figure 41–5. The Δ^5 route appears to be most used in human testes.

The five enzyme activities are localized in the microsomal fraction in rat testes, and there is a close functional association between the activities of 3β-OHSD and $\Delta^{5,4}$-isomerase and between those of a 17α-hydroxylase and 17,20-lyase. These enzyme pairs, both contained in a single protein, are shown in the general reaction sequence in Figure 41–5.

Dihydrotestosterone Is Formed from Testosterone in Peripheral Tissues

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

The most significant metabolic product of testosterone is DHT, since in many tissues, including prostate, external genitalia, and some areas of the skin, this is the active form of the hormone. The plasma content of DHT in the adult male is about one-tenth that of testosterone, and approximately 400 μg of DHT is produced daily as compared with about 5 mg of testosterone. About 50–100 μg of DHT are secreted by the testes. The rest is produced peripherally from testosterone in a reaction catalyzed by the NADPH-dependent **5α-reductase** (Figure 41–6). Testosterone can thus be considered a prohormone, since it is converted into a much more potent compound (dihydrotestosterone) and since most of this conversion occurs outside the testes. Some estradiol is formed from the peripheral aromatization of testosterone, particularly in males.

Ovarian Steroidogenesis

The estrogens are a family of hormones synthesized in a variety of tissues. 17β-Estradiol is the primary estrogen of ovarian origin. In some species, estrone, synthesized in numerous tissues, is more abundant. In pregnancy, relatively more estriol is produced, and this comes from the placenta. The general pathway and the subcellular localization of the enzymes involved in the early steps of estradiol synthesis are the same as those involved in androgen biosynthesis. Features unique to the ovary are illustrated in Figure 41–7.

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

The cellular source of the various ovarian steroids has been difficult to unravel, but a transfer of substrates between two cell types is involved. Theca cells are the source of androstenedione and testosterone. These are converted by the aromatase enzyme in granulosa cells to estrone and estradiol, respectively. Progesterone, a precursor for all steroid hormones, is produced and secreted by the corpus luteum as an end-product hormone because these cells do not contain the enzymes necessary to convert progesterone to other steroid hormones (Figure 41–8).

Significant amounts of estrogens are produced by the peripheral aromatization of androgens. In human males, the peripheral aromatization of testosterone to estradiol (E_2) accounts for 80% of the production of the latter. In females, adrenal androgens are important substrates, since as much as 50% of the E_2 produced during pregnancy comes from the aromatization of androgens. Finally, conversion of androstenedione to estrone is the major source of estrogens in postmenopausal women. Aromatase activity is present in adipose cells and also in liver, skin, and other tissues. Increased activity of this enzyme may contribute to the "estrogenization" that characterizes such diseases as cirrhosis of the liver, hyperthyroidism, aging, and obesity. Aromatase inhibitors show promise as therapeutic agents in breast cancer and possibly in other female reproductive tract malignancies.

1,25(OH)$_2$-D$_3$ (Calcitriol) Is Synthesized from a Cholesterol Derivative

1,25(OH)$_2$-D$_3$ is produced by a complex series of enzymatic reactions that involve the plasma transport of precursor molecules to a number of different tissues (Figure 41–9). One of these precursors is vitamin D—really not a vitamin, but this common name persists. The active molecule, 1,25(OH)$_2$-D$_3$, is transported to other organs where it activates biologic processes in a manner similar to that employed by the steroid hormones.

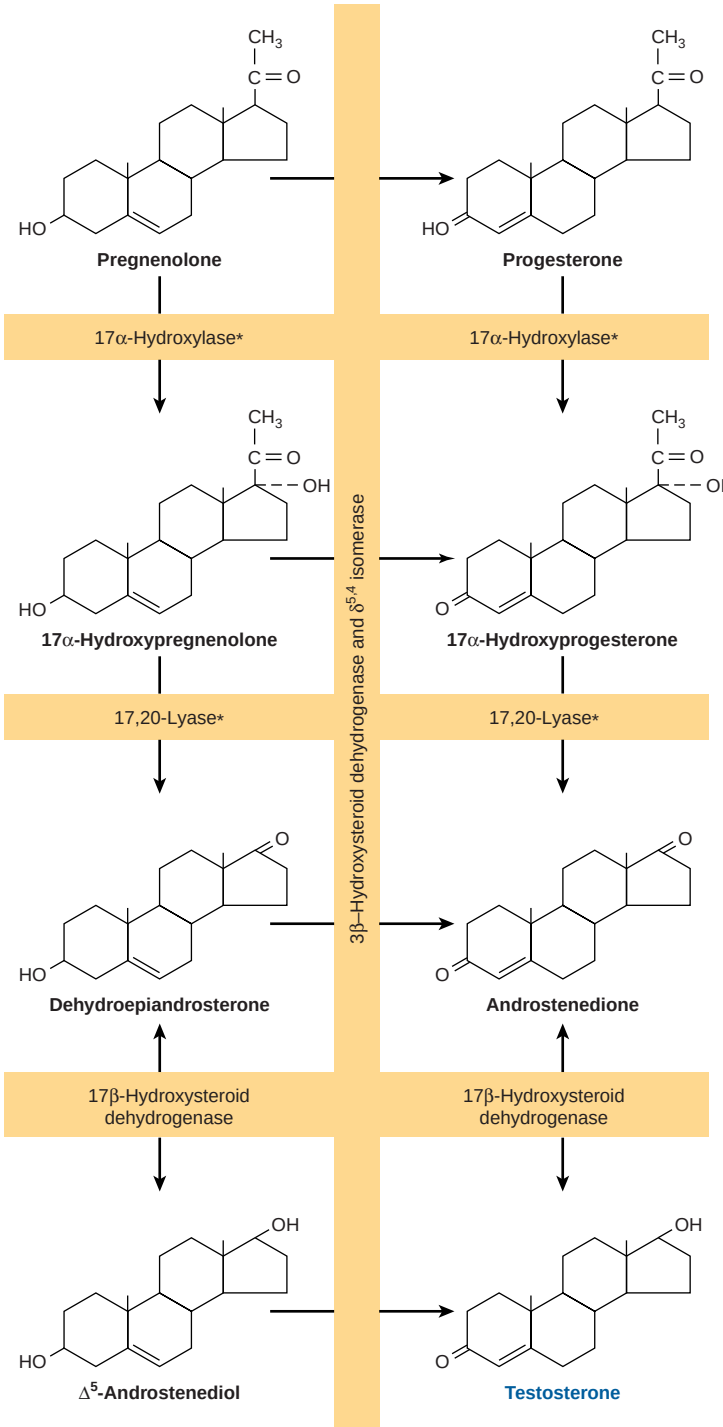

FIGURE 41–5 Pathways of testosterone biosynthesis. The pathway on the left side of the figure is called the Δ^5 or dehydroepiandrosterone pathway; the pathway on the right side is called the Δ^4 or progesterone pathway. The asterisk indicates that the 17α-hydroxylase and 17,20-lyase activities reside in a single protein, P450c17.

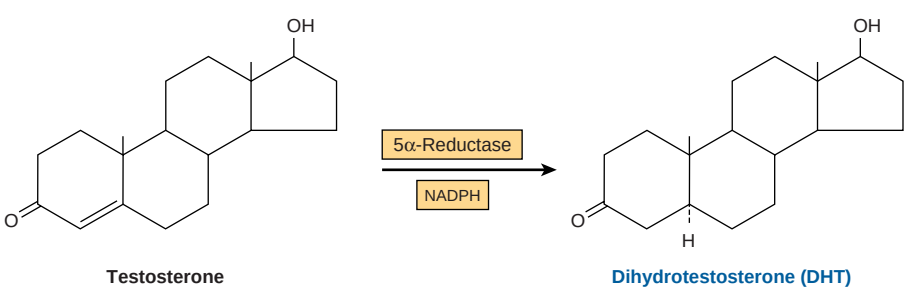

FIGURE 41–6 Dihydrotestosterone is formed from testosterone through action of the enzyme 5α-reductase.

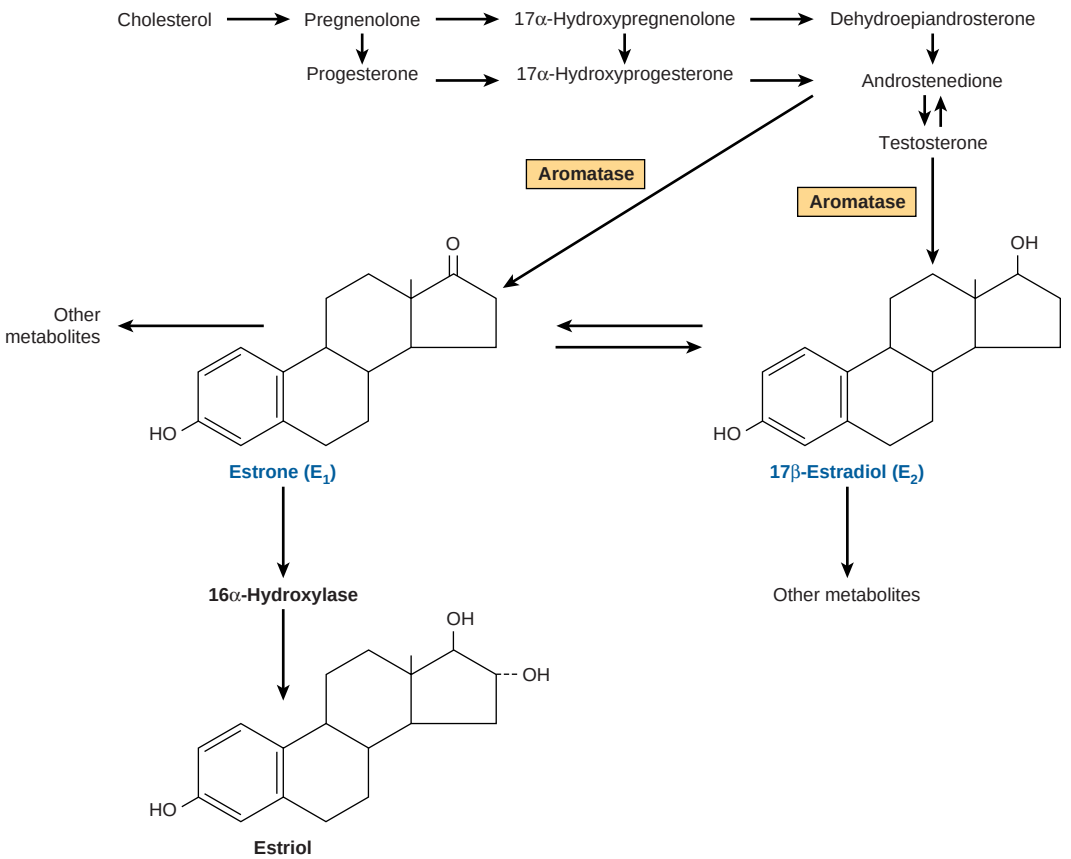

FIGURE 41–7 Biosynthesis of estrogens. (Slightly modified and reproduced, with permission, from Ganong WF: *Review of Medical Physiology,* 21st ed. McGraw-Hill, 2005.)

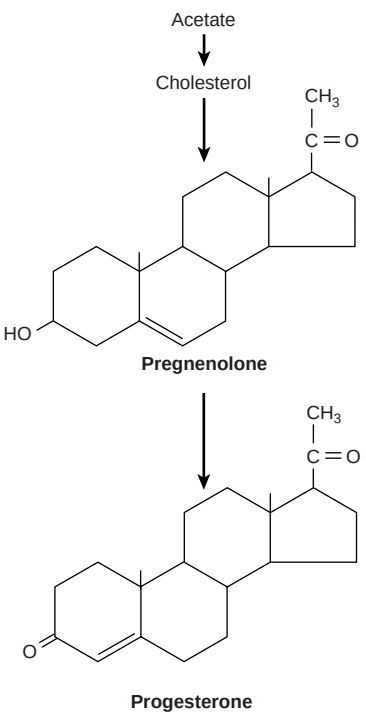

FIGURE 41–8 Biosynthesis of progesterone in the corpus luteum.

Skin

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

Liver

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

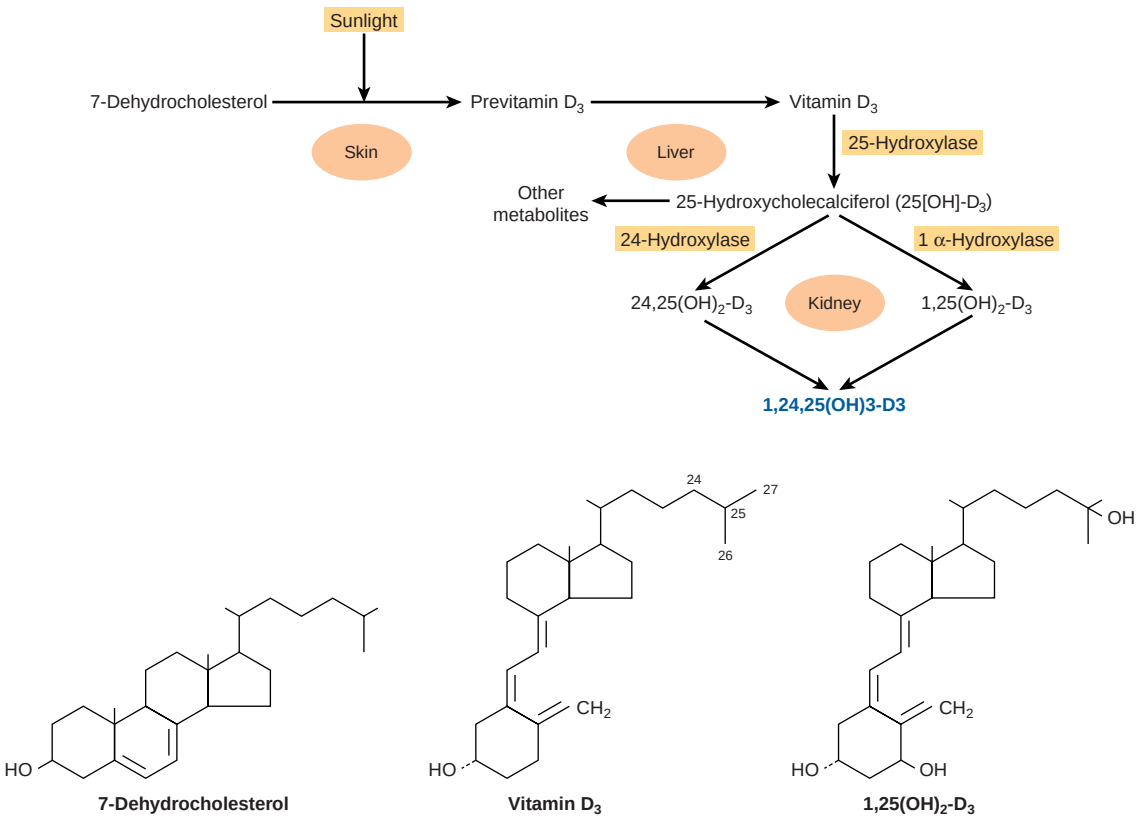

FIGURE 41–9 Formation and hydroxylation of vitamin D_3. 25-Hydroxylation takes place in the liver, and the other hydroxylations occur in the kidneys. $25,26(OH)_2$-D_3 and $1,25,26(OH)_3$-D_3 are probably formed as well. The formulas of 7-dehydrocholesterol, vitamin D_3, and $1,25(OH)_2$-D_3 are also shown. (Modified and reproduced, with permission, from Ganong WF: *Review of Medical Physiology*, 21st ed. McGraw-Hill, 2005.)

Kidney

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

CATECHOLAMINES & THYROID HORMONES ARE MADE FROM TYROSINE

Catecholamines Are Synthesized in Final Form & Stored in Secretion Granules

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

The conversion of tyrosine to epinephrine requires four sequential steps: (1) ring hydroxylation; (2) decarboxylation; (3) side-chain hydroxylation to form norepinephrine; and (4) *N*-methylation to form epinephrine. The biosynthetic pathway and the enzymes involved are illustrated in Figure 41–10.

Tyrosine Hydroxylase Is Rate-Limiting for Catecholamine Biosynthesis

Tyrosine is the immediate precursor of catecholamines, and **tyrosine hydroxylase** is the rate-limiting enzyme in catecholamine biosynthesis. Tyrosine hydroxylase is found in both soluble and particle-bound forms only in tissues that synthesize catecholamines; it functions as an oxidoreductase, with tetrahydropteridine as a cofactor, to convert L-tyrosine to L-dihydroxyphenylalanine **(L-dopa).** As the rate-limiting enzyme, tyrosine hydroxylase is regulated in a variety of ways. The most important mechanism involves feedback inhibi-

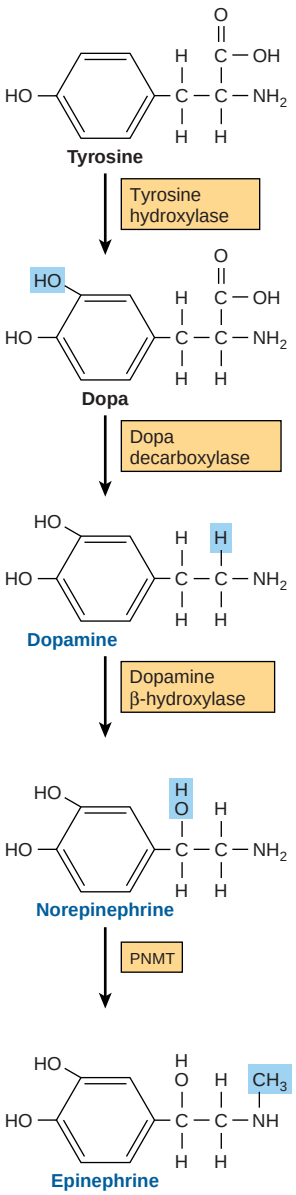

FIGURE 41–10 Biosynthesis of catecholamines. (PNMT, phenylethanolamine-*N*-methyltransferase.)

tion by the catecholamines, which compete with the enzyme for the pteridine cofactor. Catecholamines cannot cross the blood–brain barrier; hence, in the brain they must be synthesized locally. In certain central nervous system diseases (eg, Parkinson's disease), there is a local deficiency of dopamine synthesis. L-Dopa, the precursor of dopamine, readily crosses the blood–brain barrier and so is an important agent in the treatment of Parkinson disease.

Dopa Decarboxylase Is Present in All Tissues

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

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

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

Phenylethanolamine-*N*-Methyltransferase (PNMT) Catalyzes the Production of Epinephrine

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

T_3 & T_4 Illustrate the Diversity in Hormone Synthesis

The formation of **triiodothyronine (T_3)** and **tetraiodothyronine (thyroxine; T_4)** (see Figure 41–2) illustrates many of the principles of diversity discussed in this chapter. These hormones require a rare element (iodine) for bioactivity; they are synthesized as part of a very large precursor molecule (thyroglobulin); they are stored in an intracellular reservoir (colloid); and there is peripheral conversion of T_4 to T_3, which is a much more active hormone.

The thyroid hormones T_3 and T_4 are unique in that iodine (as iodide) is an essential component of both. In most parts of the world, iodine is a scarce component of soil, and for that reason there is little in food. A complex mechanism has evolved to acquire and retain this crucial element and to convert it into a form suitable for incorporation into organic compounds. At the same time, the thyroid must synthesize thyronine from tyrosine, and this synthesis takes place in thyroglobulin (Figure 41–11).

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

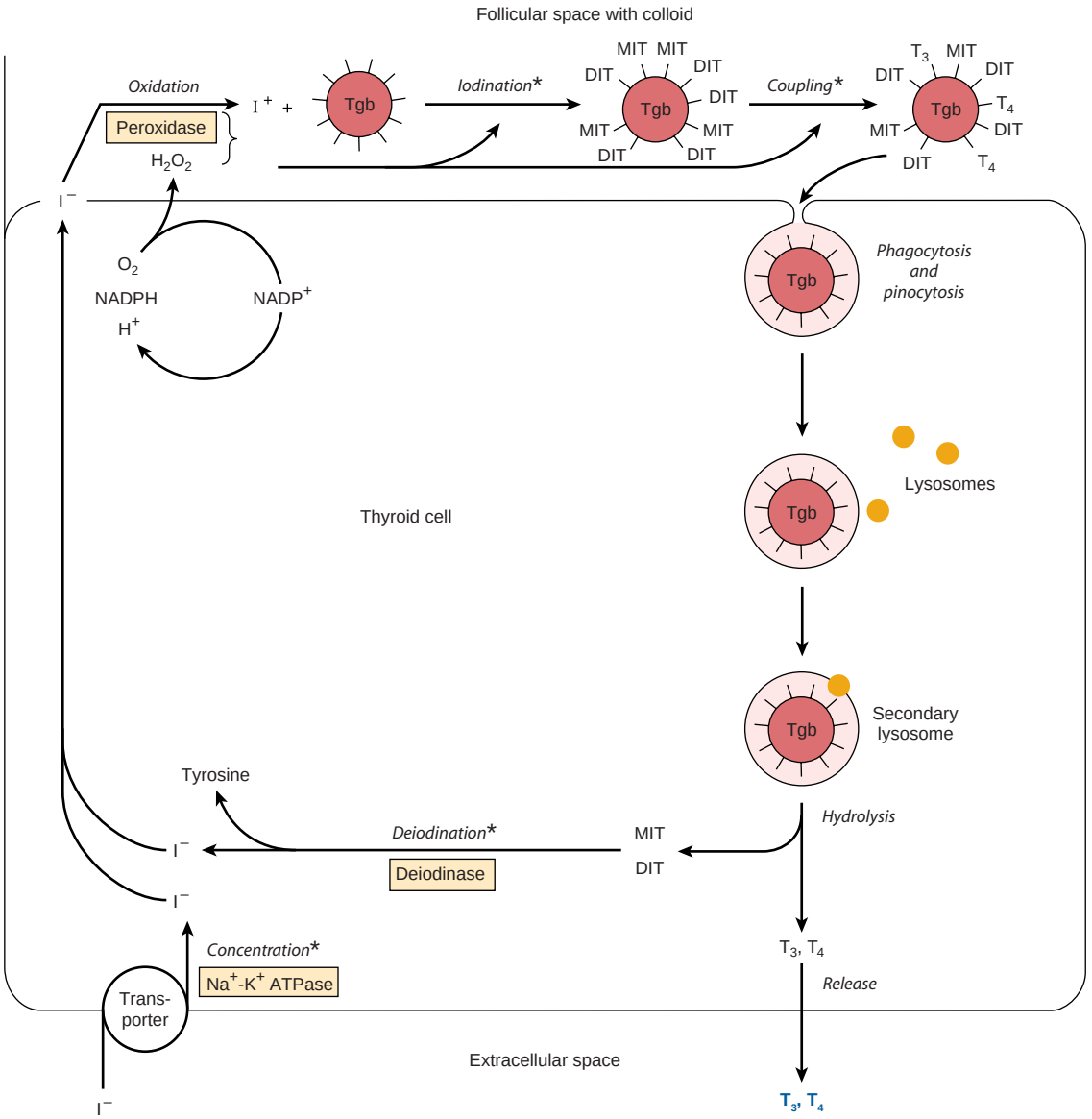

FIGURE 41–11 Model of iodide metabolism in the thyroid follicle. A follicular cell is shown facing the follicular lumen (**top**) and the extracellular space (**bottom**). Iodide enters the thyroid primarily through a transporter (**bottom left**). Thyroid hormone synthesis occurs in the follicular space through a series of reactions, many of which are peroxidase-mediated. Thyroid hormones, stored in the colloid in the follicular space, are released from thyroglobulin by hydrolysis inside the thyroid cell. (Tgb, thyroglobulin; MIT, monoiodotyrosine; DIT, diiodotyrosine; T_3, triiodothyronine; T_4, tetraiodothyronine.) Asterisks indicate steps or processes where inherited enzyme deficiencies cause congenital goiter and often result in hypothyroidism.

formation of the diaminoacid thyroid hormones. It is synthesized in the basal portion of the cell and moves to the lumen, where it is a storage form of T_3 and T_4 in the colloid; several weeks' supply of these hormones exist in the normal thyroid. Within minutes after stimulation of the thyroid by TSH, colloid reenters the cell and there is a marked increase of phagolysosome activity. Various acid proteases and peptidases hydrolyze the thyroglobulin into its constituent amino acids, including T_4 and T_3, which are discharged from the basal portion of the cell (see Figure 41–11). Thyroglobulin is thus a very large prohormone.

Iodide Metabolism Involves Several Discrete Steps

The thyroid is able to concentrate I^- against a strong electrochemical gradient. This is an energy-dependent process and is linked to the Na^+-K^+ ATPase-dependent thyroidal I^- transporter. The ratio of iodide in thyroid to iodide in serum (T:S ratio) is a reflection of the activity of this transporter. This activity is primarily controlled by TSH and ranges from 500:1 in animals chronically stimulated with TSH to 5:1 or less in hy-

pophysectomized animals (no TSH). The T:S ratio in humans on a normal iodine diet is about 25:1.

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

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

A deiodinase removes I^- from the inactive mono and diiodothyronine molecules in the thyroid. This mechanism provides a substantial amount of the I^- used in T_3 and T_4 biosynthesis. A peripheral deiodinase in target tissues such as pituitary, kidney, and liver selectively removes I^- from the 5′ position of T_4 to make T_3 (see Figure 41–2), which is a much more active molecule. In this sense, T_4 can be thought of as a prohormone, though it does have some intrinsic activity.

Several Hormones Are Made from Larger Peptide Precursors

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

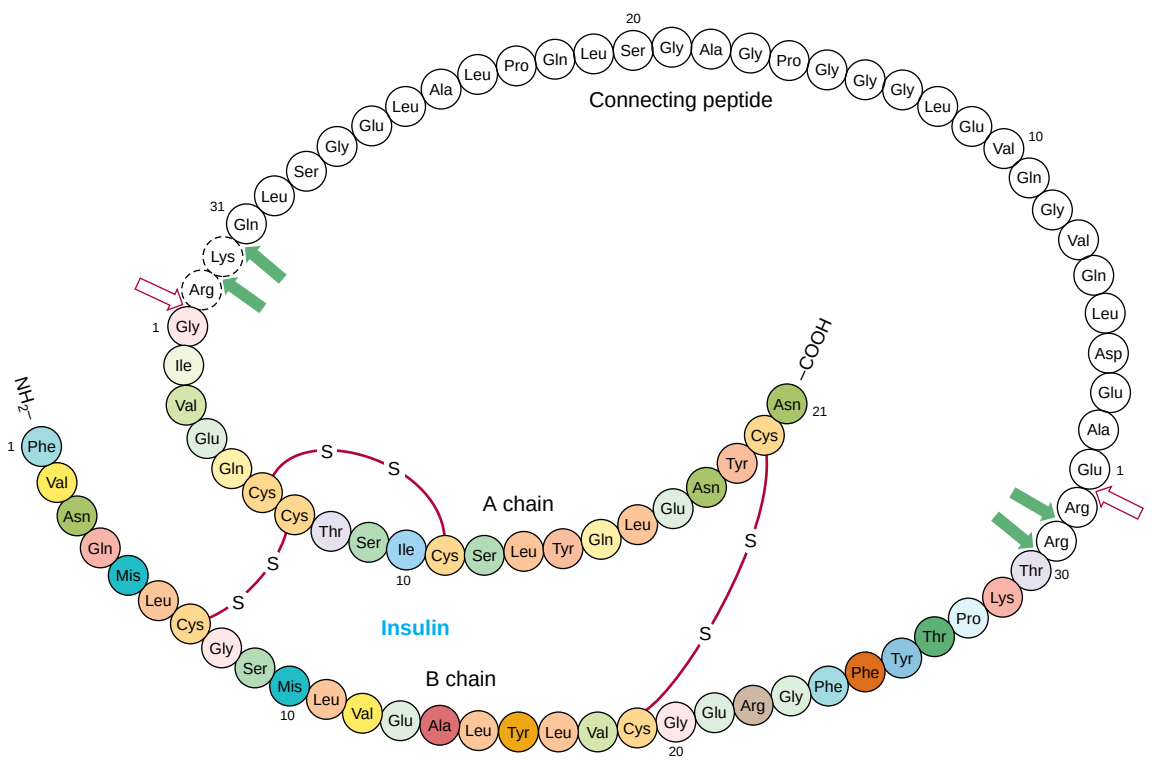

FIGURE 41–12 Structure of human proinsulin. Insulin and C-peptide molecules are connected at two sites by dipeptide links. An initial cleavage by a trypsin-like enzyme (open arrows) followed by several cleavages by a carboxypeptidase-like enzyme (**solid arrows**) results in the production of the heterodimeric (AB) insulin molecule (**colored**) and the C-peptide (**white**).

protein, which can be processed into many different hormones in a tissue-specific manner. These examples are discussed in detail below.

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

Insulin has an AB heterodimeric structure with one intrachain (A6–A11) and two interchain disulfide bridges (A7–B7 and A20–B19) (Figure 41–12). The A and B chains could be synthesized in the laboratory, but attempts at a biochemical synthesis of the mature insulin molecule yielded very poor results. The reason for this became apparent when it was discovered that insulin is synthesized as a **preprohormone** (molecular weight approximately 11,500), which is the prototype for peptides that are processed from larger precursor molecules. The hydrophobic 23-amino-acid pre-, or leader, sequence directs the molecule into the cisternae of the endoplasmic reticulum and then is removed. This results in the 9000-MW proinsulin molecule, which provides the conformation necessary for the proper and efficient formation of the disulfide bridges. As shown in Figure 41–12, the sequence of proinsulin, starting from the amino terminal, is B chain—connecting (C) peptide—A chain. The proinsulin molecule undergoes a series of site-specific peptide cleavages that result in the formation of equimolar amounts of mature insulin and C-peptide. These enzymatic cleavages are summarized in Figure 41–12.

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

The immediate precursor of PTH is **proPTH,** which differs from the native 84-amino-acid hormone by having a highly basic hexapeptide amino terminal extension. The primary gene product and the immediate precursor for proPTH is the 115-amino-acid **preproPTH.** This differs from proPTH by having an additional 25-amino-acid amino terminal extension that, in common with the other leader or signal sequences characteristic of secreted proteins, is hydrophobic. The complete structure of preproPTH and the sequences of proPTH and PTH are illustrated in Figure 41–13. $PTH_{1–34}$ has full biologic activity, and the region 25–34 is primarily responsible for receptor binding.

The biosynthesis of PTH and its subsequent secretion are regulated by the plasma ionized calcium (Ca^{2+}) concentration through a complex process. An acute decrease of Ca^{2+} results in a marked increase of PTH mRNA, and this is followed by an increased rate of PTH synthesis and secretion. However, about 80–90% of the proPTH synthesized cannot be accounted for as intact PTH in cells or in the incubation medium of experimental systems. This finding led to the conclusion that most of the proPTH synthesized is quickly degraded. It was later discovered that this rate of degradation decreases when Ca^{2+} concentrations are low, and it increases when Ca^{2+} concentrations are high. A Ca^{2+} receptor on the surface of the parathyroid cell mediates these effects. Very specific fragments of PTH are

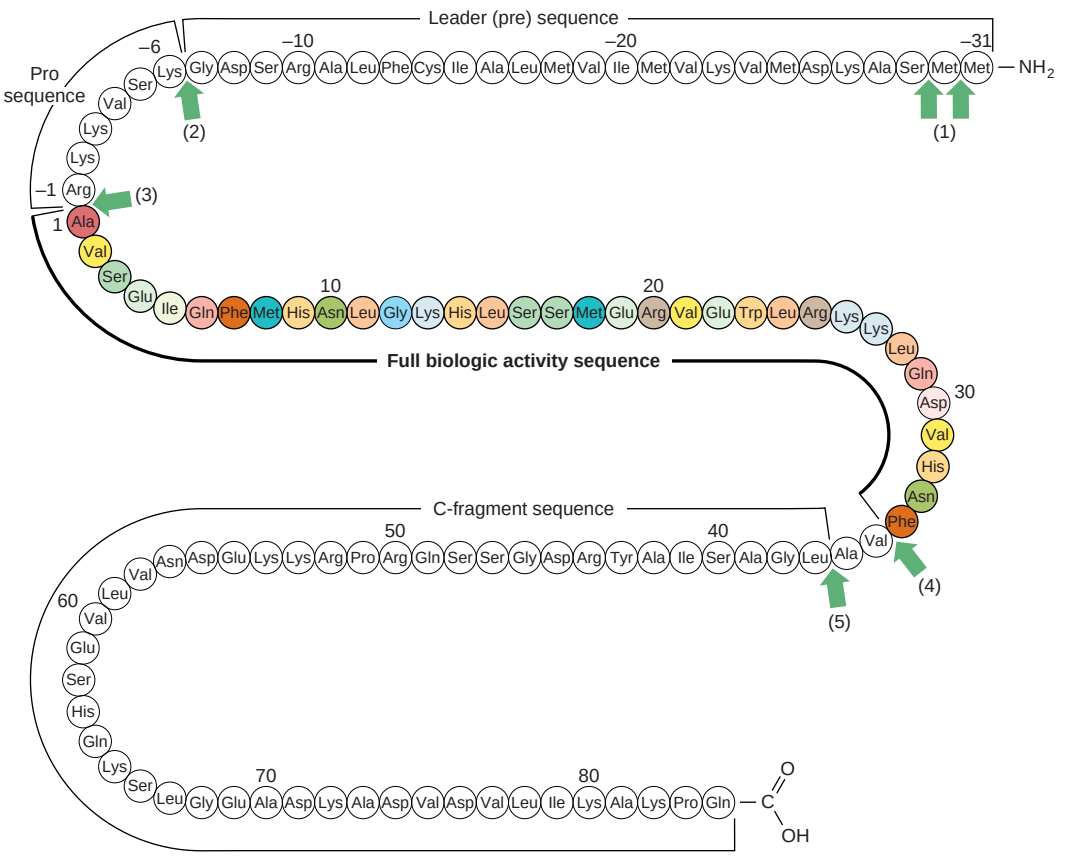

FIGURE 41–13
Structure of bovine preproparathyroid hormone. Arrows indicate sites cleaved by processing enzymes in the parathyroid gland (1–5) and in the liver after secretion of the hormone (4–5). The biologically active region of the molecule (**colored**) is flanked by sequence not required for activity on target receptors. (Slightly modified and reproduced, with permission, from Habener JF: Recent advances in parathyroid hormone research. Clin Biochem 1981;14:223. Copyright © 1981. Reprinted with permission from Elsevier.)

generated during its proteolytic digestion (Figure 41–13). A number of proteolytic enzymes, including cathepsins B and D, have been identified in parathyroid tissue. Cathepsin B cleaves PTH into two fragments: $PTH_{1–36}$ and $PTH_{37–84}$. $PTH_{37–84}$ is not further degraded; however, $PTH_{1–36}$ is rapidly and progressively cleaved into di- and tripeptides. Most of the proteolysis of PTH occurs within the gland, but a number of studies confirm that PTH, once secreted, is proteolytically degraded in other tissues, especially the liver, by similar mechanisms.

Angiotensin II Is Also Synthesized from a Large Precursor

The renin-angiotensin system is involved in the regulation of blood pressure and electrolyte metabolism (through production of aldosterone). The primary hormone involved in these processes is angiotensin II, an octapeptide made from angiotensinogen (Figure 41–14). Angiotensinogen, a large α_2-globulin made in liver, is the substrate for renin, an enzyme produced in the juxtaglomerular cells of the renal afferent arteriole. The position of these cells makes them particularly sensitive to blood pressure changes, and many of the physiologic regulators of renin release act through renal baroreceptors. The juxtaglomerular cells are also sensitive to changes of Na^+ and Cl^- concentration in the renal tubular fluid; therefore, any combination of factors that decreases fluid volume (dehydration, decreased blood pressure, fluid or blood loss) or decreases NaCl concentration stimulates renin release. Renal sympathetic nerves that terminate in the juxtaglomerular cells mediate the central nervous system and postural effects on renin release independently of the baroreceptor and salt effects, a mechanism that involves the β-adrenergic receptor. Renin acts upon the substrate angiotensinogen to produce the decapeptide angiotensin I.

Angiotensin-converting enzyme, a glycoprotein found in lung, endothelial cells, and plasma, removes two carboxyl terminal amino acids from the decapeptide angiotensin I to form angiotensin II in a step that is not thought to be rate-limiting. Various nonapeptide analogs of angiotensin I and other compounds act as competitive inhibitors of converting enzyme and are used to treat renin-dependent hypertension. These are referred to as **angiotensin-converting enzyme (ACE) inhibitors.** Angiotensin II increases blood pressure by causing vasoconstriction of the arteriole and is a very potent vasoactive substance. It inhibits renin release from the juxtaglomerular cells and is a potent stimulator of aldosterone production. This results in Na^+ retention, volume expansion, and increased blood pressure.

In some species, angiotensin II is converted to the heptapeptide angiotensin III (Figure 41–14), an equally potent stimulator of aldosterone production. In humans, the plasma level of angiotensin II is four times greater than that of angiotensin III, so most effects are exerted by the octapeptide. Angiotensins II and III are rapidly inactivated by angiotensinases.

Angiotensin II binds to specific adrenal cortex glomerulosa cell receptors. The hormone-receptor interaction does not activate adenylyl cyclase, and cAMP does not appear to mediate the action of this hormone. The actions of angiotensin II, which are to stimulate the conversion of cholesterol to pregnenolone and of corticosterone to 18-hydroxycorticosterone and aldosterone, may involve changes in the concentration of intracellular calcium and of phospholipid metabolites by mechanisms similar to those described in Chapter 42.

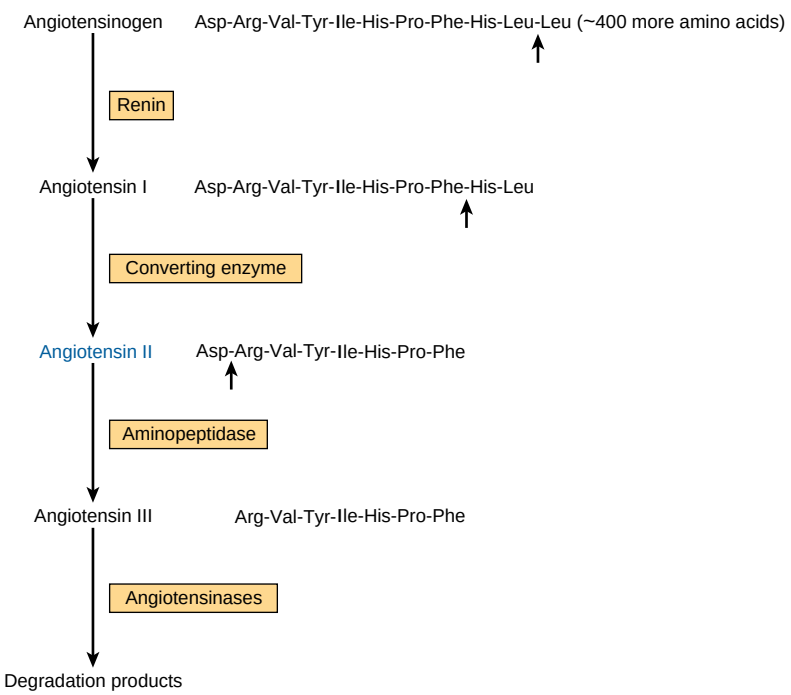

FIGURE 41–14 Formation and metabolism of angiotensins. Small arrows indicate cleavage sites.

Complex Processing Generates the Pro-Opiomelanocortin (POMC) Peptide Family

The POMC family consists of peptides that act as hormones (ACTH, LPH, MSH) and others that may serve as neurotransmitters or neuromodulators (endorphins) (Figure 41–15). POMC is synthesized as a precursor molecule of 285 amino acids and is processed differently in various regions of the pituitary.

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

The POMC protein is processed differently in the anterior lobe than in the intermediate lobe. The intermediate lobe of the pituitary is rudimentary in adult humans, but it is active in human fetuses and in pregnant women during late gestation and is also active in many animal species. Processing of the POMC protein in the peripheral tissues (gut, placenta, male reproductive tract) resembles that in the intermediate lobe. There are three basic peptide groups: (1) ACTH, which can give rise to α-MSH and corticotropin-like intermediate lobe peptide (CLIP); (2) β-lipotropin (β-LPH), which can yield γ-LPH, β-MSH, and β-endorphin (and thus α- and γ-endorphins); and (3) a large amino terminal peptide, which generates γ-MSH (not shown). The diversity of these products is due to the many dibasic amino acid clusters that are potential cleavage sites for trypsin-like enzymes. Each of the peptides mentioned is preceded by Lys-Arg, Arg-Lys, Arg-Arg, or Lys-Lys residues. After the prehormone segment is cleaved, the next cleavage, in both anterior and intermediate lobes, is between ACTH and β-LPH, resulting in an amino terminal peptide with ACTH and a β-LPH segment (Figure 41–15). $ACTH_{1–39}$ is subsequently cleaved from the amino terminal peptide, and in the anterior lobe essentially no further cleavages occur. In the intermediate lobe, $ACTH_{1–39}$ is cleaved into α-MSH (residues 1–13) and CLIP (18–39); β-LPH (42–134) is converted to γ-LPH (42–101) and β-endorphin (104–134). β-MSH (84–101) is derived from γ-LPH, while γ-MSH (50–74) is derived from a POMC N-terminal fragment (1–74).

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

Mutations of the α-MSH receptor are linked to a common, early-onset form of obesity. This observation has redirected attention to the POMC peptide hormones.

THERE IS VARIATION IN THE STORAGE & SECRETION OF HORMONES

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

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

The human pancreas secretes about 40–50 units of insulin daily; this represents about 15–20% of the hormone stored in the β cells. Insulin and the C-peptide (see Figure 41–12) are normally secreted in equimolar amounts. Stimuli such as glucose, which provokes insulin secretion, therefore trigger the processing of proinsulin to insulin as an essential part of the secretory response.

A several-week supply of T_3 and T_4 exists in the thyroglobulin that is stored in colloid in the lumen of the thyroid follicles. These hormones can be released upon stimulation by

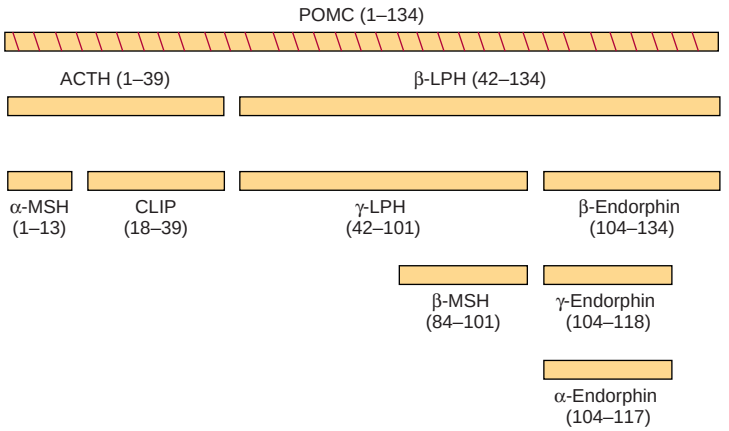

FIGURE 41–15 Products of pro-opiomelanocortin (POMC) cleavage. (MSH, melanocyte-stimulating hormone; CLIP, corticotropin-like intermediate lobe peptide; LPH, lipotropin.)

TABLE 41–5 Diversity in the Storage of Hormones

Hormone	Supply Stored in Cell
Steroids and 1,25(OH)$_2$-D$_3$	None
Catecholamines and PTH	Hours
Insulin	Days
T_3 and T_4	Weeks

TSH. This is the most exaggerated example of a prohormone, as a molecule containing approximately 5000 amino acids must be first synthesized, then degraded, to supply a few molecules of the active hormones T_4 and T_3.

The diversity in storage and secretion of hormones is illustrated in Table 41–5.

SOME HORMONES HAVE PLASMA TRANSPORT PROTEINS

The class I hormones are hydrophobic in chemical nature and thus are not very soluble in plasma. These hormones, principally the steroids and thyroid hormones, have specialized plasma transport proteins that serve several purposes. First, these proteins circumvent the solubility problem and thereby deliver the hormone to the target cell. They also provide a circulating reservoir of the hormone that can be substantial, as in the case of the thyroid hormones. Hormones, when bound to the transport proteins, cannot be metabolized, thereby prolonging their plasma half-life ($t_{1/2}$). The binding affinity of a given hormone to its transporter determines the bound versus free ratio of the hormone. This is important because only the free form of a hormone is biologically active. In general, the concentration of free hormone in plasma is very low, in the range of 10^{-15} to 10^{-9} mol/L. It is important to distinguish between plasma transport proteins and hormone receptors. Both bind hormones but with very different characteristics (Table 41–6).

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

TABLE 41–6 Comparison of Receptors with Transport Proteins

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

TABLE 41–7 Comparison of T_4 and T_3 in Plasma

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

Thyroid Hormones Are Transported by Thyroid-Binding Globulin

Many of the principles discussed above are illustrated in a discussion of thyroid-binding proteins. One-half to two-thirds of T_4 and T_3 in the body is in an extrathyroidal reservoir. Most of this circulates in bound form, ie, bound to a specific binding protein, **thyroxine-binding globulin (TBG).** TBG, a glycoprotein with a molecular mass of 50 kDa, binds T_4 and T_3 and has the capacity to bind 20 µg/dL of plasma. Under normal circumstances, TBG binds—noncovalently—nearly all of the T_4 and T_3 in plasma, and it binds T_4 with greater affinity than T_3 (Table 41–7). The plasma half-life of T_4 is correspondingly four to five times that of T_3. The small, unbound (free) fraction is responsible for the biologic activity. Thus, in spite of the great difference in total amount, the free fraction of T_3 approximates that of T_4, and given that T_3 is intrinsically more active than T_4, most biologic activity is attributed to T_3. TBG does not bind any other hormones.

Glucocorticoids Are Transported by Corticosteroid-Binding Globulin

Hydrocortisone (cortisol) also circulates in plasma in protein-bound and free forms. The main plasma binding protein is

TABLE 41–8 Approximate Affinities of Steroids for Serum-Binding Proteins

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

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

an α-globulin called **transcortin,** or **corticosteroid-binding globulin (CBG).** CBG is produced in the liver, and its synthesis, like that of TBG, is increased by estrogens. CBG binds most of the hormone when plasma cortisol levels are within the normal range; much smaller amounts of cortisol are bound to albumin. The avidity of binding helps determine the biologic half-lives of various glucocorticoids. Cortisol binds tightly to CBG and has a $t_{1/2}$ of 1.5–2 h, while corticosterone, which binds less tightly, has a $t_{1/2}$ of less than 1 h (Table 41–8). The unbound (free) cortisol constitutes about 8% of the total and represents the biologically active fraction. Binding to CBG is not restricted to glucocorticoids. Deoxycorticosterone and progesterone interact with CBG with sufficient affinity to compete for cortisol binding. Aldosterone, the most potent natural mineralocorticoid, does not have a specific plasma transport protein. Gonadal steroids bind very weakly to CBG (Table 41–8).

Gonadal Steroids Are Transported by Sex-Hormone-Binding Globulin

Most mammals, humans included, have a plasma β-globulin that binds testosterone with specificity, relatively high affinity, and limited capacity (Table 41–8). This protein, usually called **sex-hormone-binding globulin (SHBG)** or testosterone-estrogen-binding globulin (TEBG), is produced in the liver. Its production is increased by estrogens (women have twice the serum concentration of SHBG as men), certain types of liver disease, and hyperthyroidism; it is decreased by androgens, advancing age, and hypothyroidism. Many of these conditions also affect the production of CBG and TBG. Since SHBG and albumin bind 97–99% of circulating testosterone, only a small fraction of the hormone in circulation is in the free (biologically active) form. The primary function of SHBG may be to restrict the free concentration of testosterone in the serum. Testosterone binds to SHBG with higher affinity than does estradiol (Table 41–8). Therefore, a change in the level of SHBG causes a greater change in the free testosterone level than in the free estradiol level.

Estrogens are bound to SHBG and progestins to CBG. SHBG binds estradiol about five times less avidly than it binds testosterone or DHT, while progesterone and cortisol have little affinity for this protein (Table 41–8). In contrast, progesterone and cortisol bind with nearly equal affinity to CBG, which in turn has little avidity for estradiol and even less for testosterone, DHT, or estrone.

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

SUMMARY

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

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CHAPTER 42

Hormone Action & Signal Transduction

P. Anthony Weil, PhD

BIOMEDICAL IMPORTANCE

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

HORMONES TRANSDUCE SIGNALS TO AFFECT HOMEOSTATIC MECHANISMS

The general steps involved in producing a coordinated response to a particular stimulus are illustrated in Figure 42–1. The stimulus can be a challenge or a threat to the organism, to an organ, or to the integrity of a single cell within that organism. Recognition of the stimulus is the first step in the adaptive response. At the organismic level, this generally involves the nervous system and the special senses (sight, hearing, pain, smell, touch). At the organismic or cellular level, recognition involves physicochemical factors such as pH, O_2 tension, temperature, nutrient supply, noxious metabolites, and osmolarity. Appropriate recognition results in the release of one or more hormones that will govern generation of the necessary adaptive response. For purposes of this discussion, the hormones are categorized as described in Chapter 41, ie, based on the location of their specific cellular receptors and the type of signals generated. Group I hormones interact with an intracellular receptor and group II hormones with receptor recognition sites located on the extracellular surface of the plasma membrane of target cells. The cytokines, interleukins, and growth factors should also be considered in this latter category. These molecules, of critical importance in homeostatic adaptation, are hormones in the sense that they are produced in specific cells, have the equivalent of autocrine, paracrine, and endocrine actions, bind to cell surface receptors, and activate many of the same signal transduction pathways employed by the more traditional group II hormones.

SIGNAL GENERATION

The Ligand–Receptor Complex Is the Signal for Group I Hormones

The lipophilic group I hormones diffuse through the plasma membrane of all cells but only encounter their specific, high-affinity intracellular receptors in target cells. These receptors can be located in the cytoplasm or in the nucleus of target cells. The hormone–receptor complex first undergoes an **activation reaction.** As shown in Figure 42–2, receptor activation occurs by at least two mechanisms. For example, glucocorticoids diffuse across the plasma membrane and encounter their cognate receptor in the cytoplasm of target cells. Ligand–receptor binding results in a conformational change in the receptor leading to the dissociation of heat shock protein 90 (hsp90). This step appears to be necessary for subsequent nuclear localization of the glucocorticoid receptor. This receptor also contains a nuclear localization sequence that is now free to assist in the translocation from cytoplasm to nucleus. The activated receptor moves into the nucleus (Figure 42–2) and binds with high affinity to a specific DNA sequence called the **hormone response element (HRE).** In the case illustrated, this is a glucocorticoid response element, or GRE. Consensus sequences for HREs are shown in Table 42–1. The DNA-bound, liganded receptor serves as a high-affinity binding site for one or more coactivator proteins, and accelerated gene transcription typically ensues when this occurs. By contrast, certain hormones such as the thyroid hormones and retinoids diffuse from the extracellular fluid across the plasma membrane and go directly into the nucleus. In this case, the cognate receptor is already bound to the HRE (the thyroid hormone response element [TRE], in this

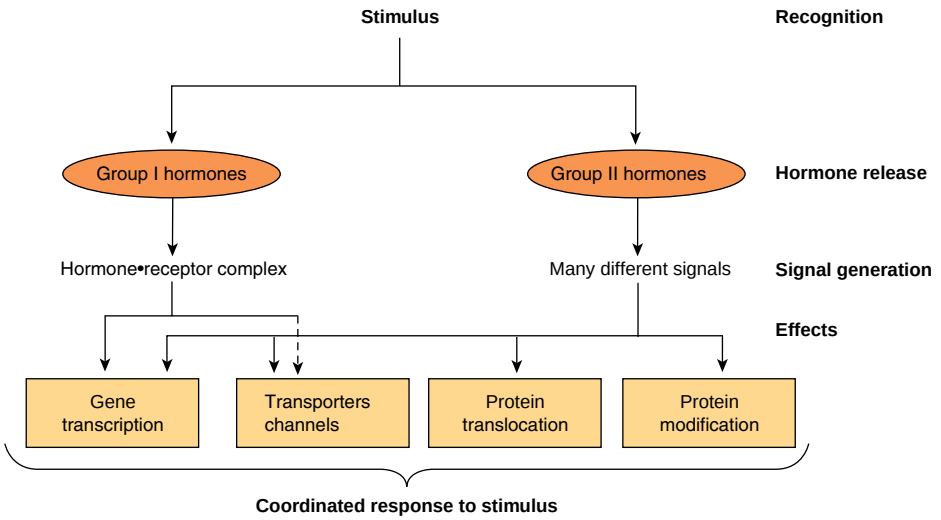

FIGURE 42–1 Hormonal involvement in responses to a stimulus. A challenge to the integrity of the organism elicits a response that includes the release of one or more hormones. These hormones generate signals at or within target cells, and these signals regulate a variety of biologic processes that provide for a coordinated response to the stimulus or challenge. See Figure 42–8 for a specific example.

example). However, this DNA-bound receptor fails to activate transcription because it exists in complex with a corepressor. Indeed, this receptor-corepressor complex serves as an active repressor of gene transcription. The association of ligand with these receptors results in dissociation of the corepressor(s). The liganded receptor is now capable of binding one or more co-activators with high affinity, resulting in the recruitment of pol II + GTFs and activation of gene transcription. The relationship of hormone receptors to other nuclear receptors and to coregulators is discussed in more detail below.

By selectively affecting gene transcription and the consequent production of appropriate target mRNAs, the amounts of specific proteins are changed and metabolic processes are influenced. The influence of each of these hormones is quite specific; generally, a given hormone affects less than 1% of the genes, mRNA, or proteins in a target cell; sometimes only a few are affected. The nuclear actions of steroid, thyroid, and retinoid hormones are quite well defined. Most evidence suggests

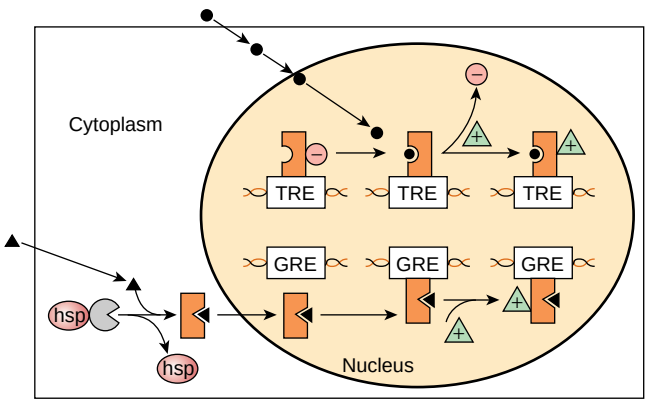

FIGURE 42–2 Regulation of gene expression by two different class I hormones, thyroid hormone and glucocorticoids. Steroid hormones readily gain access to the cytoplasmic compartment of target cells. Glucocorticoid hormones (solid triangles) encounter their cognate receptor (GR) in the cytoplasm, where GR exists in a complex with heat shock protein 90 (hsp). Ligand binding causes dissociation of hsp and a conformational change of the receptor. The receptor-ligand complex then traverses the nuclear membrane and binds to DNA with specificity and high affinity at a glucocorticoid response element (GRE). This event affects the architecture of a number of transcription coregulators (green triangles), and enhanced transcription ensues. By contrast, thyroid hormones and retinoic acid (•) directly enter the nucleus, where their cognate heterodimeric (TR-RXR; see Figure 42–12) receptors are already bound to the appropriate response elements with an associated transcription repressor complex (red circles). Hormone–receptor binds, which again induces conformational changes leading to a reorganization of receptor (TR)-coregulator interactions (ie, molecules such as N-CoR or SMRT [see Table 42–6]). Ligand binding results in dissociation of the repressor complex from the receptor, allowing an activator complex to assemble. The gene is then actively transcribed.

TABLE 42–1 The DNA Sequences of Several Hormone Response Elements (HREs)[1]

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

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

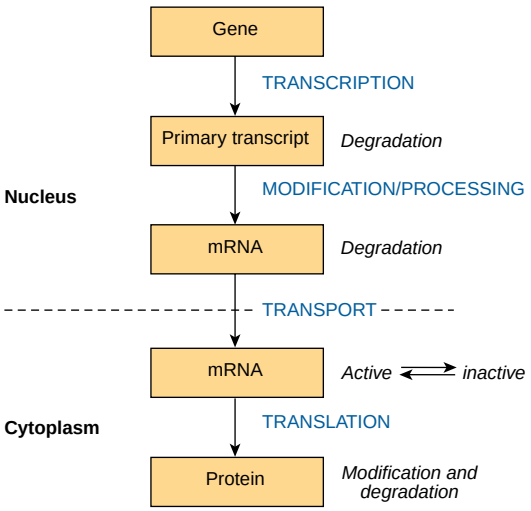

FIGURE 42–3 The "information pathway." Information flows from the gene to the primary transcript to mRNA to protein. Hormones can affect any of the steps involved and can affect the rates of processing, degradation, or modification of the various products.

that these hormones exert their dominant effect on modulating gene transcription, but they—and many of the hormones in the other classes discussed below—can act at any step of the "information pathway," as illustrated in Figure 42–3, to control specific gene expression and, ultimately, a biological response. Direct actions of steroids in the cytoplasm and on various organelles and membranes have also been described. Recently microRNAs have been implicated in mediating some of the diverse actions of the peptide hormone insulin.

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

Many hormones are water-soluble, have no transport proteins (and therefore have a short plasma half-life), and initiate a response by binding to a receptor located in the plasma membrane (Tables 41–3 & 41–4). The mechanism of action of this group of hormones can best be discussed in terms of the **intracellular signals** they generate. These signals include cAMP (cyclic AMP; 3′,5′-adenylic acid; see Figure 19–5), a nucleotide derived from ATP through the action of adenylyl cyclase; cGMP, a nucleotide formed by guanylyl cyclase; Ca^{2+}; and phosphatidylinositides; such molecules are termed second messengers as their synthesis is triggered by the presence of the primary hormone (molecule) binding its receptor. Many of these second messengers affect gene transcription, as described in the previous paragraph; but they also influence a variety of other biologic processes, as shown in Figure 42–3.

G Protein–Coupled Receptors (GPCR)

Many of the group II hormones bind to receptors that couple to effectors through a GTP-binding protein intermediary. These receptors typically have seven hydrophobic plasma membrane-spanning domains. This is illustrated by the seven interconnected cylinders extending through the lipid bilayer in Figure 42–4. Receptors of this class, which signal through guanine nucleotide-bound protein intermediates, are known as **G**

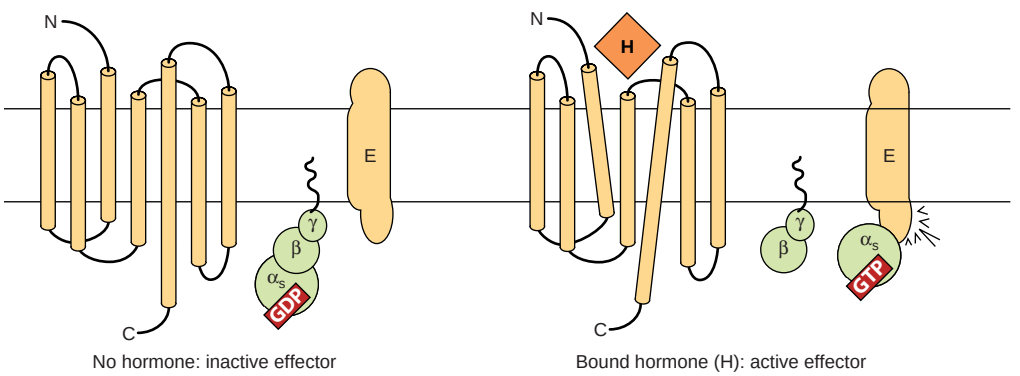

FIGURE 42–4 Components of the hormone receptor–G protein effector system. Receptors that couple to effectors through G proteins (GPCR) typically have seven membrane-spanning domains. In the absence of hormone (**left**), the heterotrimeric G-protein complex (α,β,γ) is in an inactive guanosine diphosphate (GDP)-bound form and is probably not associated with the receptor. This complex is anchored to the plasma membrane through prenylated groups on the βγ subunits (**wavy lines**) and perhaps by myristoylated groups on α subunits (not shown). On binding of hormone (H) to the receptor, there is a presumed conformational change of the receptor—as indicated by the tilted membrane spanning domains—and activation of the G-protein complex. This results from the exchange of GDP with guanosine triphosphate (GTP) on the α subunit, after which α and βγ dissociate. The α subunit binds to and activates the effector (E). E can be adenylyl cyclase, Ca^{2+}, Na^{+}, or Cl^{-} channels (α_s), or it could be a K^{+} channel (α_i), phospholipase Cβ (α_q), or cGMP phosphodiesterase (α_t). The βγ subunit can also have direct actions on E. (Modified and reproduced, with permission, from Granner DK in: *Principles and Practice of Endocrinology and Metabolism,* 2nd ed. Becker KL [editor]. Lippincott, 1995.)

protein–coupled receptors, or **GPCRs.** To date, hundreds of G protein–linked receptor genes have been identified; this represents the largest family of cell surface receptors in humans. A wide variety of responses are mediated by the GPCRs.

cAMP Is the Intracellular Signal for Many Responses

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

Adenylyl Cyclase

Different peptide hormones can either stimulate (s) or inhibit (i) the production of cAMP from adenylyl cyclase, which is encoded by at least nine different genes (Table 42–2). Two parallel systems, a stimulatory (s) one and an inhibitory (i) one, converge upon a catalytic molecule (C). Each consists of a receptor, R_s or R_i, and a regulatory complex, G_s and G_i. G_s and G_i are each trimers composed of α, β, and γ subunits. Because the α subunit in G_s differs from that in G_i, the proteins, which are distinct gene products, are designated α_s and α_i. The α subunits bind guanine nucleotides. The β and γ subunits are always associated (βγ) and appear to function as a heterodimer. The binding of a hormone to R_s or R_i results in a receptor-mediated activation of G, which entails the exchange of GDP by GTP on α and the concomitant dissociation of βγ from α.

The α_s protein has intrinsic GTPase activity. The active form, α_s·GTP, is inactivated upon hydrolysis of the GTP to GDP; the trimeric G_s complex (αβγ) is then re-formed and is ready for another cycle of activation. Cholera and pertussis toxins catalyze the ADPribosylation of α_s and $\alpha_{i\text{-}2}$ (see Table 42–3), respectively. In the case of α_s, this modification disrupts the intrinsic GTPase activity; thus, α_s cannot reassociate with βγ and is therefore irreversibly activated. ADP ribosylation of $\alpha_{i\text{-}2}$ prevents the dissociation of $\alpha_{i\text{-}2}$ from βγ, and free $\alpha_{i\text{-}2}$ thus cannot be formed. α_s activity in such cells is therefore unopposed.

There is a large family of G proteins, and these are part of the superfamily of GTPases. The G protein family is classified according to sequence homology into four subfamilies, as illustrated in Table 42–3. There are 21 α, 5 β, and 8 γ subunit genes. Various combinations of these subunits provide a large number of possible αβγ and cyclase complexes.

The α subunits and the βγ complex have actions independent of those on adenylyl cyclase (see Figure 42–4 & Table 42–3). Some forms of α_i stimulate K^+ channels and inhibit Ca^{2+} channels, and some α_s molecules have the opposite effects. Members of the G_q family activate the phospholipase C group of enzymes. The βγ complexes have been associated with K^+ channel stimulation and phospholipase C activation. G proteins are involved in many important biologic processes in addition to hormone action. Notable examples include olfaction (α_{OLF}) and vision (α_t). Some examples are listed in Table 42–3. GPCRs are implicated in a number of diseases and are major targets for pharmaceutical agents.

Protein Kinase

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

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

The R_2C_2 complex has no enzymatic activity, but the binding of cAMP by R induces dissociation of the R-C complex, thereby activating the latter (Figure 42–5). The active C subunit catalyzes the transfer of the γ phosphate of ATP to a serine or threonine residue in a variety of proteins. The consensus phosphorylation sites are -ArgArg/Lys-X-Ser/Thr- and -Arg-Lys-X-X-Ser-, where X can be any amino acid.

Protein kinase activities were originally described as being "cAMP-dependent" or "cAMP-independent." This classification has changed, as protein phosphorylation is now recognized as being a major regulatory mechanism. Several hundred protein kinases have now been described. The kinases are related in sequence and structure within the catalytic domain, but each is a unique molecule with considerable variability with respect to subunit composition, molecular weight, autophosphorylation, K_m for ATP, and substrate specificity. Both kinase and protein phosphatase activities can be targeted by interaction with specific kinase binding proteins. In the case of PKA, such targeting proteins are termed **AKAPs (A kinase anchoring proteins)**, they serve as scaffolds, which localize PKA near to substrates thereby focusing PKA activity toward

TABLE 42–2 Subclassification of Group II.A Hormones

Hormones That Stimulate Adenylyl Cyclase (H_s)	Hormones That Inhibit Adenylyl Cyclase (H_i)
ACTH	Acetylcholine
ADH	α_2-Adrenergics
β-Adrenergics	Angiotensin II
Calcitonin	Somatostatin
CRH	
FSH	
Glucagon	
hCG	
LH	
LPH	
MSH	
PTH	
TSH	

TABLE 42–3 Classes and Functions of Selected G Proteins[1]

Class or Type	Stimulus	Effector	Effect
G_s			
α_s	Glucagon, β-adrenergics	↑Adenylyl cyclase ↑Cardiac Ca^{2+}, Cl^-, and Na^+ channels	Glyconeogenesis, lipolysis, glycogenolysis Olfaction
α_{olf}	Odorant	↑Adenylyl cyclase	
G_i			
$\alpha_{i\text{-}1,2,3}$	Acetylcholine, α_2-adrenergics	↓Adenylyl cyclase ↑Potassium channels	Slowed heart rate
	M_2 cholinergics	↓Calcium channels	
α_0	Opioids, endorphins	↑Potassium channels	Neuronal electrical activity
α_t	Light	↑cGMP phosphodiesterase	Vision
G_q			
α_q	M_1 cholinergics α_1-Adrenergics	↑Phospholipase C-β1	↓Muscle contraction and
α_{11}	α_1-Adrenergics	↑Phospholipase C-β2	↓Blood pressure
G_{12}			
α_{12}	?	Cl^- channel	?

Source: Modified and reproduced, with permission, from Granner DK in: *Principles and Practice of Endocrinology and Metabolism,* 2nd ed. Becker KL (editor). Lippincott, 1995.
[1]The four major classes or families of mammalian G proteins (G_s, G_i, G_q, and G_{12}) are based on protein sequence homology. Representative members of each are shown, along with known stimuli, effectors, and well-defined biologic effects. Nine isoforms of adenylyl cyclase have been identified (isoforms I–IX). All isoforms are stimulated by α_s; α_i isoforms inhibit types V and VI, and α_0 inhibits types I and V. At least 16 different α subunits have been identified.

physiological substrates and facilitating spatiotemporal biological regulation while also allowing for common, shared proteins to elicit specific physiological responses. Multiple AKAPs have been described; they can bind PKA and other kinases as well as phosphatases, phosophodiestases (which hydrolyze cAMP) and protein kinase substrates.

Phosphoproteins

The effects of cAMP in eukaryotic cells are all thought to be mediated by protein phosphorylation-dephosphorylation, principally on serine and threonine residues. The control of any of the effects of cAMP, including such diverse processes as steroidogenesis, secretion, ion transport, carbohydrate and fat metabolism, enzyme induction, gene regulation, synaptic transmission, and cell growth and replication, could be conferred by a specific protein kinase, by a specific phosphatase, or by specific substrates for phosphorylation. These substrates help define a target tissue and are involved in defining the extent of a particular response within a given cell. For example, the effects of cAMP on gene transcription are mediated by the protein **cyclic AMP response element binding protein (CREB).** CREB binds to a cAMP responsive element (CRE) (see Table 42–1) in its nonphosphorylated state and is a weak activator of transcription. When phosphorylated by PKA, CREB binds the coactivator **CREB-binding protein CBP/p300** (see below) and as a result is a much more potent transcription activator. CBP and the related p300 contain histone acetyltransferase

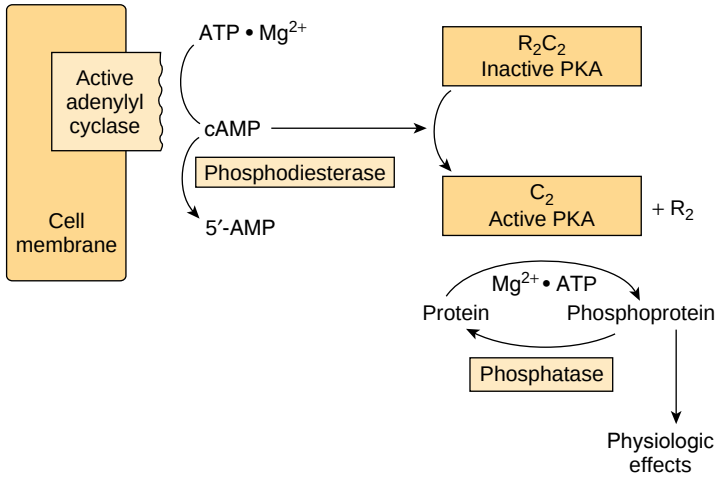

FIGURE 42–5 Hormonal regulation of cellular processes through cAMP-dependent protein kinase (PKA). PKA exists in an inactive form as an R_2C_2 heterotetramer consisting of two regulatory and two catalytic subunits. The cAMP generated by the action of adenylyl cyclase (activated as shown in Figure 42–4) binds to the regulatory (R) subunit of PKA. This results in dissociation of the regulatory and catalytic subunits and activation of the latter. The active catalytic subunits phosphorylate a number of target proteins on serine and threonine residues. Phosphatases remove phosphate from these residues and thus terminate the physiologic response. A phosphodiesterase can also terminate the response by converting cAMP to 5′-AMP.

activities, and hence serve as chromatin-active transcriptional coregulators (Chapters 36, 38). Interestingly, CBP/p300 can also acetylate certain transcription factors thereby stimulating their ability to bind DNA and modulate transcription.

Phosphodiesterases

Actions caused by hormones that increase cAMP concentration can be terminated in a number of ways, including the hydrolysis of cAMP to 5′-AMP by phosphodiesterases (see Figure 42–5). The presence of these hydrolytic enzymes ensures a rapid turnover of the signal (cAMP) and hence a rapid termination of the biologic process once the hormonal stimulus is removed. There are at least 11 known members of the phosphodiesterase family of enzymes. These are subject to regulation by their substrates, cAMP and cGMP; by hormones; and by intracellular messengers such as calcium, probably acting through calmodulin. Inhibitors of phosphodiesterase, most notably methylated xanthine derivatives such as caffeine, increase intracellular cAMP and mimic or prolong the actions of hormones through this signal.

Phosphoprotein Phosphatases

Given the importance of protein phosphorylation, it is not surprising that regulation of the protein dephosphorylation reaction is another important control mechanism (see Figure 42–5). The phosphoprotein phosphatases are themselves subject to regulation by phosphorylation-dephosphorylation reactions and by a variety of other mechanisms, such as protein-protein interactions. In fact, the substrate specificity of the phosphoserine-phosphothreonine phosphatases may be dictated by distinct regulatory subunits whose binding is regulated hormonally. One of the best-studied roles of regulation by the dephosphorylation of proteins is that of glycogen metabolism in muscle. Two major types of phosphoserine-phosphothreonine phosphatases have been described. Type I preferentially dephosphorylates the β subunit of phosphorylase kinase, whereas type II dephosphorylates the α subunit. Type I phosphatase is implicated in the regulation of glycogen synthase, phosphorylase, and phosphorylase kinase. This phosphatase is itself regulated by phosphorylation of certain of its subunits, and these reactions are reversed by the action of one of the type II phosphatases. In addition, two heat-stable protein inhibitors regulate type I phosphatase activity. Inhibitor-1 is phosphorylated and activated by cAMP-dependent protein kinases; and inhibitor-2, which may be a subunit of the inactive phosphatase, is also phosphorylated, possibly by glycogen synthase kinase-3. Phosphatases that attack phosphotyrosine are also important in signal transduction (see Figure 42–8).

cGMP Is Also an Intracellular Signal

Cyclic GMP is made from GTP by the enzyme guanylyl cyclase, which exists in soluble and membrane-bound forms. Each of these isozymes has unique physiologic properties. The atriopeptins, a family of peptides produced in cardiac atrial tissues, cause natriuresis, diuresis, vasodilation, and inhibition of aldosterone secretion. These peptides (eg, atrial natriuretic factor) bind to and activate the membrane-bound form of guanylyl cyclase. This results in an increase of cGMP by as much as 50-fold in some cases, and this is thought to mediate the effects mentioned above. Other evidence links cGMP to vasodilation. A series of compounds, including nitroprusside, nitroglycerin, nitric oxide, sodium nitrite, and sodium azide, all cause smooth muscle relaxation and are potent vasodilators. These agents increase cGMP by activating the soluble form of guanylyl cyclase, and inhibitors of cGMP phosphodiesterase (the drug sildenafil [Viagra], for example) enhance and prolong these responses. The increased cGMP activates cGMP-dependent protein kinase (PKG), which in turn phosphorylates a number of smooth muscle proteins. Presumably, this is involved in relaxation of smooth muscle and vasodilation.

Several Hormones Act Through Calcium or Phosphatidylinositols

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

Calcium Metabolism

The extracellular calcium (Ca^{2+}) concentration is about 5 mmol/L and is very rigidly controlled. Although substantial amounts of calcium are associated with intracellular organelles such as mitochondria and the endoplasmic reticulum, the intracellular concentration of free or ionized calcium (Ca^{2+}) is very low: 0.05–10 μmol/L. In spite of this large concentration gradient and a favorable trans-membrane electrical gradient, Ca^{2+} is restrained from entering the cell. A considerable amount of energy is expended to ensure that the intracellular Ca^{2+} is controlled, as a prolonged elevation of Ca^{2+} in the cell is very toxic. A Na^{+}/Ca^{2+} exchange mechanism that has a high-capacity but low-affinity pumps Ca^{2+} out of cells. There also is a Ca^{2+}/proton ATPase-dependent pump that extrudes Ca^{2+} in exchange for H^{+}. This has a high affinity for Ca^{2+} but a low capacity and is probably responsible for fine-tuning cytosolic Ca^{2+}. Furthermore, Ca^{2+}-ATPases pump Ca^{2+} from the cytosol to the lumen of the endoplasmic reticulum. There are three ways of changing cytosolic Ca^{2+}: (1) Certain hormones (class II.C, Table 41–3) by binding to receptors that are themselves Ca^{2+} channels, enhance membrane permeability to Ca^{2+} and thereby increase Ca^{2+} influx. (2) Hormones also indirectly promote Ca^{2+} influx by modulating the membrane potential at the plasma membrane. Membrane depolarization opens voltage-gated Ca^{2+} channels and allows for Ca^{2+} influx. (3) Ca^{2+} can be mobilized from the endoplasmic reticulum, and possibly from mitochondrial pools.

An important observation linking Ca^{2+} to hormone action involved the definition of the intracellular targets of Ca^{2+} action. The discovery of a Ca^{2+}-dependent regulator of phos-

phodiesterase activity provided the basis for a broad understanding of how Ca^{2+} and cAMP interact within cells.

Calmodulin

The calcium-dependent regulatory protein is calmodulin, a 17-kDa protein that is homologous to the muscle protein troponin C in structure and function. Calmodulin has four Ca^{2+} binding sites, and full occupancy of these sites leads to a marked conformational change, which allows calmodulin to activate enzymes and ion channels. The interaction of Ca^{2+} with calmodulin (with the resultant change of activity of the latter) is conceptually similar to the binding of cAMP to PKA and the subsequent activation of this molecule. Calmodulin can be one of numerous subunits of complex proteins and is particularly involved in regulating various kinases and enzymes of cyclic nucleotide generation and degradation. A partial list of the enzymes regulated directly or indirectly by Ca^{2+}, probably through calmodulin, is presented in Table 42–4.

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

Calcium Is a Mediator of Hormone Action

A role for Ca^{2+} in hormone action is suggested by the observations that the effect of many hormones is (1) blunted by Ca^{2+}-free media or when intracellular calcium is depleted; (2) can be mimicked by agents that increase cytosolic Ca^{2+}, such as the Ca^{2+} ionophore A23187; and (3) influences cellular calcium flux. The regulation of glycogen metabolism in liver by vasopressin and β-adrenergic catecholamines provides a good example. This is shown schematically in Figures 19–6 and 19–7.

TABLE 42–4 Enzymes and Proteins Regulated by Calcium or Calmodulin

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

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

Phosphatidylinositide Metabolism Affects Ca^{2+}-Dependent Hormone Action

Some signal must provide communication between the hormone receptor on the plasma membrane and the intracellular Ca^{2+} reservoirs. This is accomplished by products of phosphatidylinositol metabolism. Cell surface receptors such as those for acetylcholine, antidiuretic hormone, and α_1-type catecholamines are, when occupied by their respective ligands, potent activators of phospholipase C. Receptor binding and activation of phospholipase C are coupled by the G_q isoforms (Table 42–3 & Figure 42–6). Phospholipase C catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate to inositol trisphosphate (IP_3) and 1,2-diacylglycerol (Figure 42–7). Diacylglycerol is itself capable of activating **protein kinase C (PKC),** the activity of which also depends upon Ca^{2+}. IP_3, by interacting with a specific intracellular receptor, is an effective releaser of Ca^{2+} from intracellular storage sites in the endoplasmic reticulum. Thus, the hydrolysis of phosphatidylinositol 4,5-bisphosphate leads to activation of PKC and promotes an increase of cytoplasmic Ca^{2+}. As shown in Figure 42–4, the activation of G proteins can also have a direct action on Ca^{2+} channels. The resulting elevations of cytosolic Ca^{2+} activate Ca^{2+}–calmodulin-dependent kinases and many other Ca^{2+}–calmodulin-dependent enzymes.

Steroidogenic agents—including ACTH and cAMP in the adrenal cortex; angiotensin II, K^+, serotonin, ACTH, and cAMP in the zona glomerulosa of the adrenal; LH in the ovary; and LH and cAMP in the Leydig cells of the testes—have been associated with increased amounts of phosphatidic acid, phosphatidylinositol, and polyphosphoinositides (see Chapter 15) in the respective target tissues. Several other examples could be cited.

The roles that Ca^{2+} and polyphosphoinositide breakdown products might play in hormone action are presented in Figure 42–6. In this scheme the activated protein kinase C can phosphorylate specific substrates, which then alter physiologic processes. Likewise, the Ca^{2+}-calmodulin complex can activate specific kinases. These then modify substrates and thereby alter physiologic responses.

Some Hormones Act Through a Protein Kinase Cascade

Single protein kinases such as PKA, PKC, and Ca^{2+}-calmodulin (CaM)-kinases, which result in the phosphorylation of serine and threonine residues in target proteins, play a very important role in hormone action. The discovery that the EGF receptor contains an intrinsic tyrosine kinase activity that is activated by the binding of the ligand EGF was an important breakthrough. The insulin and IGF-I receptors also contain intrinsic ligand-activated tyrosine kinase activity. Several

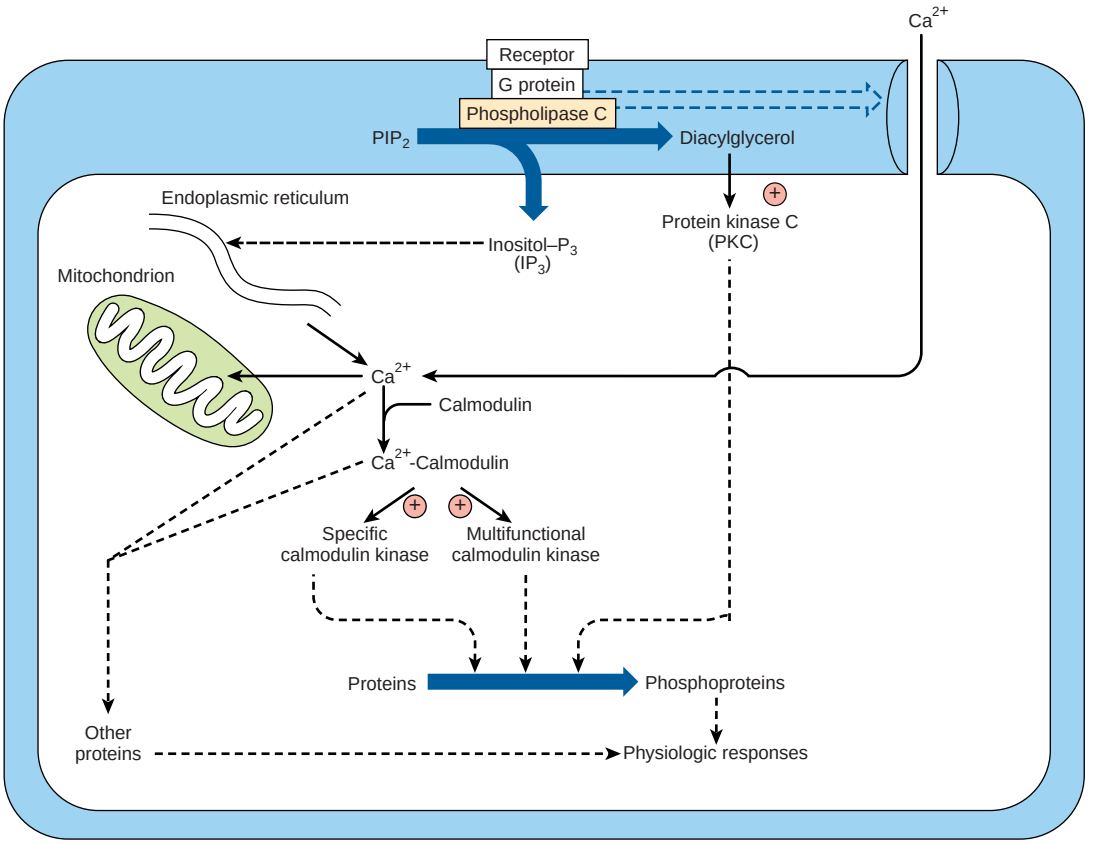

FIGURE 42–6 Certain hormone–receptor interactions result in the activation of phospholipase C. This appears to involve a specific G protein, which also may activate a calcium channel. Phospholipase C results in generation of inositol trisphosphate (IP_3), which liberates stored intracellular Ca^{2+}, and diacylglycerol (DAG), a potent activator of protein kinase C (PKC). In this scheme, the activated PKC phosphorylates specific substrates, which then alter physiologic processes. Likewise, the Ca^{2+}-calmodulin complex can activate specific kinases, two of which are shown here. These actions result in phosphorylation of substrates, and this leads to altered physiologic responses. This figure also shows that Ca^{2+} can enter cells through voltage- or ligand-gated Ca^{2+} channels. The intracellular Ca^{2+} is also regulated through storage and release by the mitochondria and endoplasmic reticulum. (Courtesy of JH Exton.)

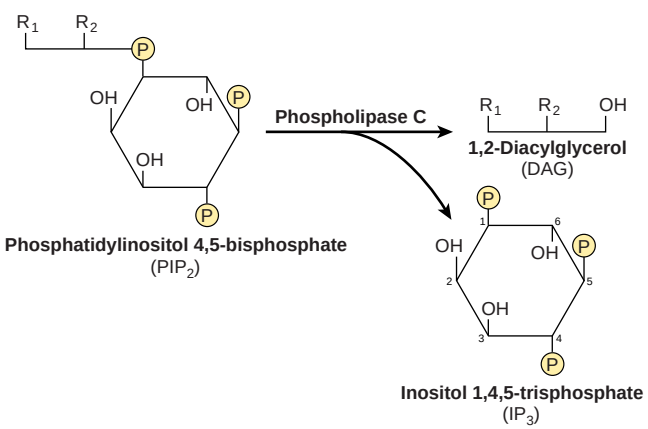

FIGURE 42–7 Phospholipase C cleaves PIP_2 into diacylglycerol and inositol trisphosphate. R_1 generally is stearate, and R_2 is usually arachidonate. IP_3 can be dephosphorylated (to the inactive I-1,4-P_2) or phosphorylated (to the potentially active I-1,3,4,5-P_4).

receptors—generally those involved in binding ligands involved in growth control, differentiation, and the inflammatory response—either have intrinsic tyrosine kinase activity or are associated with proteins that are tyrosine kinases. Another distinguishing feature of this class of hormone action is that these kinases preferentially phosphorylate tyrosine residues, and tyrosine phosphorylation is infrequent (<0.03% of total amino acid phosphorylation) in mammalian cells. A third distinguishing feature is that the ligand–receptor interaction that results in a tyrosine phosphorylation event initiates a cascade that may involve several protein kinases, phosphatases, and other regulatory proteins.

Insulin Transmits Signals by Several Kinase Cascades

The insulin, epidermal growth factor (EGF), and IGF-I receptors have intrinsic protein tyrosine kinase activities located

in their cytoplasmic domains. These activities are stimulated when the receptor binds ligand. The receptors are then autophosphorylated on tyrosine residues, and this initiates a complex series of events (summarized in simplified fashion in Figure 42–8). The phosphorylated insulin receptor next phosphorylates insulin receptor substrates (there are at least four of these molecules, called IRS 1–4) on tyrosine residues. Phosphorylated IRS binds to the Src homology 2 (SH2) domains of a variety of proteins that are directly involved in mediating different effects of insulin. One of these proteins, PI-3 kinase, links insulin receptor activation to insulin action through activation of a number of molecules, including the kinase PDK1 (phosphoinositide-dependent kinase-1). This enzyme propagates the signal through several other kinases, including PKB (akt), SKG, and aPKC (see legend to Figure 42–8 for definitions and expanded abbreviations). An alternative pathway downstream from PKD1 involves p70S6K and perhaps other as yet unidentified kinases. A second major pathway involves mTOR. This enzyme is directly regulated by amino acid levels and insulin, and is essential for p70S6K activity. This pathway

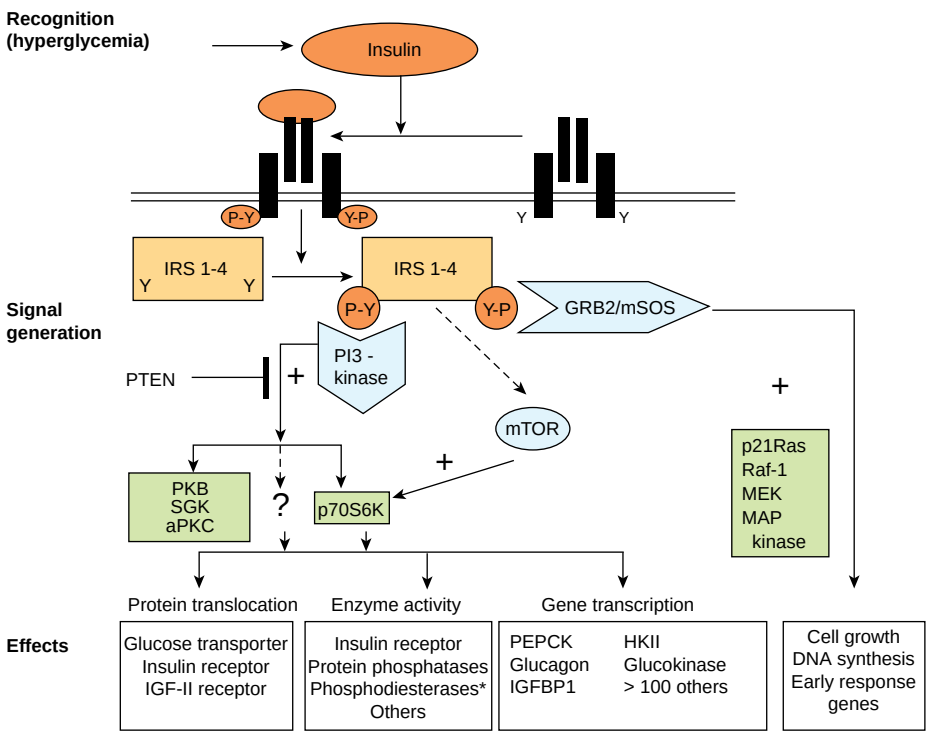

FIGURE 42–8 Insulin signaling pathways. The insulin signaling pathways provide an excellent example of the "recognition → hormone release → signal generation → effects" paradigm outlined in Figure 42–1. Insulin is released in response to hyperglycemia. Binding of insulin to a target cell-specific plasma membrane receptor results in a cascade of intracellular events. Stimulation of the intrinsic tyrosine kinase activity of the insulin receptor marks the initial event, resulting in increased tyrosine (Y) phosphorylation (Y → Y-P) of the receptor and then one or more of the insulin receptor substrate molecules (IRS 1–4). This increase in phosphotyrosine stimulates the activity of many intracellular molecules such as GTPases, protein kinases, and lipid kinases, all of which play a role in certain metabolic actions of insulin. The two best-described pathways are shown. First, phosphorylation of an IRS molecule (probably IRS-2) results in docking and activation of the lipid kinase, PI-3 kinase, which generates novel inositol lipids that may act as "second messenger" molecules. These, in turn, activate PDK1 and then a variety of downstream signaling molecules, including protein kinase B (PKB or akt), SGK, and aPKC. An alternative pathway involves the activation of p70S6K and perhaps other as yet unidentified kinases. Second, phosphorylation of IRS (probably IRS-1) results in docking of GRB2/mSOS and activation of the small GTPase, p21RAS, which initiates a protein kinase cascade that activates Raf-1, MEK, and the p42/p44 MAP kinase isoforms. These protein kinases are important in the regulation of proliferation and differentiation of several cell types. The mTOR pathway provides an alternative way of activating p70S6K and appears to be involved in nutrient signaling as well as insulin action. Each of these cascades may influence different physiologic processes, as shown. All of the phosphorylation events are reversible through the action of specific phosphatases. For example, the lipid phosphatase PTEN dephosphorylates the product of the PI-3 kinase reaction, thereby antagonizing the pathway and terminating the signal. Representative effects of major actions of insulin are shown in each of the boxes. The asterisk after phosphodiesterase indicates that insulin indirectly affects the activity of many enzymes by activating phosphodiesterases and reducing intracellular cAMP levels. (IGFBP, insulin-like growth factor binding protein; IRS 1–4, insulin receptor substrate isoforms 1–4; PI-3 kinase, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog deleted on chromosome 10; PKD1, phosphoinositide-dependent kinase; PKB, protein kinase B; SGK, serum and glucocorticoid-regulated kinase; aPKC, atypical protein kinase C; p70S6K, p70 ribosomal protein S6 kinase; mTOR, mammalian target of rapamycin; GRB2, growth factor receptor binding protein 2; mSOS, mammalian son of sevenless; MEK, MAP kinase kinase and ERK kinase; MAP kinase, mitogen-activated protein kinase.)

provides a distinction between the PKB and p70S6K branches downstream from PKD1. These pathways are involved in protein translocation, enzyme activity, and the regulation, by insulin, of genes involved in metabolism (Figure 42–8). Another SH2 domain-containing protein is GRB2, which binds to IRS-1 and links tyrosine phosphorylation to several proteins, the result of which is activation of a cascade of threonine and serine kinases. A pathway showing how this insulin–receptor interaction activates the mitogen-activated protein (MAP) kinase pathway and the anabolic effects of insulin is illustrated in Figure 42–8. The exact roles of many of these docking proteins, kinases, and phosphatases remain to be established.

The Jak/STAT Pathway is Used by Hormones and Cytokines

Tyrosine kinase activation can also initiate a phosphorylation and dephosphorylation cascade that involves the action of several other protein kinases and the counterbalancing actions of phosphatases. Two mechanisms are employed to initiate this cascade. Some hormones, such as growth hormone, prolactin, erythropoietin, and the cytokines, initiate their action by activating a tyrosine kinase, but this activity is not an integral part of the hormone receptor. The hormone–receptor interaction promotes binding and activation of **cytoplasmic protein tyrosine kinases,** such as **Tyk-2, Jak1,** or **Jak2.**

These kinases phosphorylate one or more cytoplasmic proteins, which then associate with other docking proteins through binding to SH2 domains. One such interaction results in the activation of a family of cytosolic proteins called **signal transducers and activators of transcription (STATs).** The phosphorylated STAT protein dimerizes and translocates into the nucleus, binds to a specific DNA element such as the interferon response element, and activates transcription. This is illustrated in Figure 42–9. Other SH2 docking events may result in the activation of PI-3 kinase, the MAP kinase pathway (through SHC or GRB2), or G protein–mediated activation of phospholipase C (PLCγ) with the attendant production of diacylglycerol and activation of protein kinase C. It is apparent that there is a potential for "cross-talk" when different hormones activate these various signal transduction pathways.

The NF-κB Pathway Is Regulated by Glucocorticoids

The transcription factor **NF-κB is a heterodimeric complex** typically composed of two **subunits termed p50 and p65** (Figure 42–10). Normally, NF-κB is kept sequestered in the cytoplasm in a transcriptionally inactive form by members of the **Inhibitor of NF-κB (IκB) family**. Extracellular stimuli such as proinflammatory cytokines, reactive oxygen species, and mitogens lead to activation of the IκB kinase complex, IKK, which is a heterohexameric structure consisting of α, β, and γ subunits. IKK phosphorylates IκB on two serine residues, and this targets IκB for ubiquitination and subsequent degradation by the proteasome. Following IκB degradation, free NF-κB translocates to the nucleus, where it binds to a number of gene promoters and activates transcription, particularly of genes involved in the **inflammatory response.** Transcriptional regulation by NF-κB is mediated by a variety of coactivators such as CREB binding protein (CBP), as described below (see Figure 42–13).

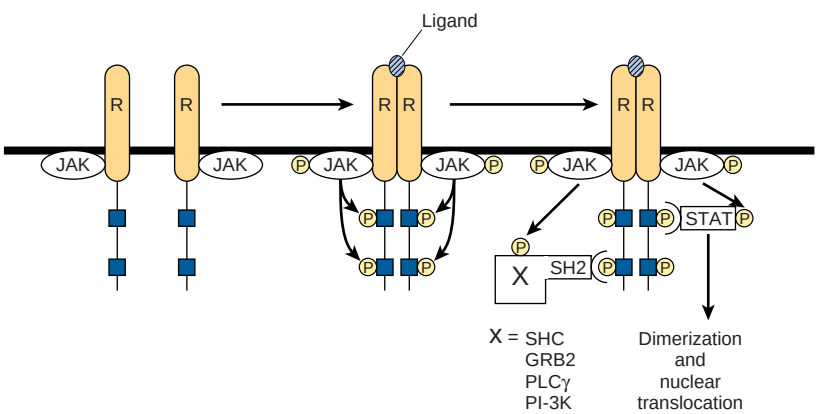

FIGURE 42–9 Initiation of signal transduction by receptors linked to Jak kinases. The receptors (R) that bind prolactin, growth hormone, interferons, and cytokines lack endogenous tyrosine kinase. Upon ligand binding, these receptors dimerize and an associated protein (Jak1, Jak2, or TYK) is phosphorylated. Jak-P, an active kinase, phosphorylates the receptor on tyrosine residues. The STAT proteins associate with the phosphorylated receptor and then are themselves phosphorylated by Jak-P. STAT Ⓟ dimerizes, translocates to the nucleus, binds to specific DNA elements, and regulates transcription. The phosphotyrosine residues of the receptor also bind to several SH2 domain-containing proteins (X-SH2). This results in activation of the MAP kinase pathway (through SHC or GRB2), PLCγ, or PI-3 kinase.

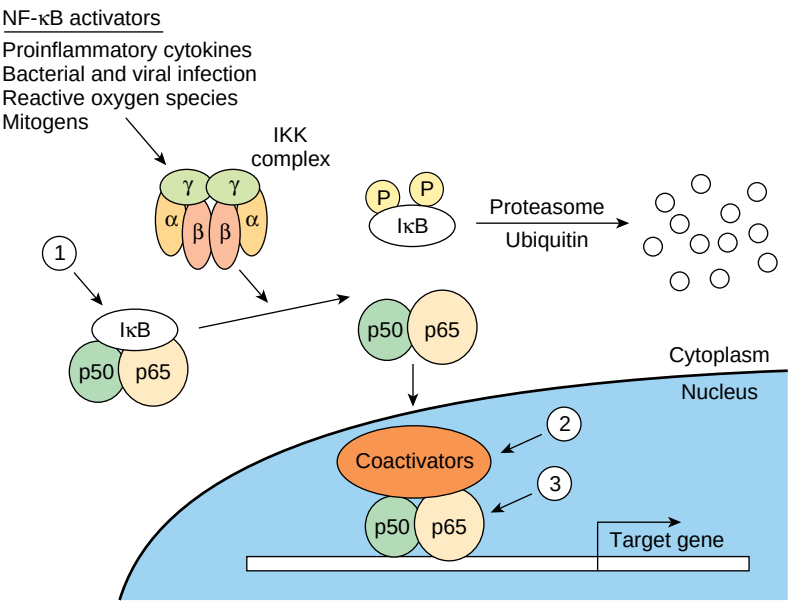

FIGURE 42–10 Regulation of the NF-κB pathway. NF-κB consists of two subunits, p50 and p65, which when present in the nucleus regulate transcription of the multitude of genes important for the inflammatory response. NF-κB is restricted from entering the nucleus by IκB, an inhibitor of NF-κB. IκB binds to—and masks—the nuclear localization signal of NF-κB. This cytoplasmic protein is phosphorylated by an IKK complex which is activated by cytokines, reactive oxygen species, and mitogens. Phosphorylated IκB can be ubiquitinylated and degraded, thus releasing its hold on NF-κB. Glucocorticoids, potent anti-inflammatory agents, are thought to affect at least three steps in this process (1, 2, 3), as described in the text.

Glucocorticoid hormones are therapeutically useful agents for the treatment of a variety of inflammatory and immune diseases. Their anti-inflammatory and immunomodulatory actions are explained in part by the inhibition of NF-κB and its subsequent actions. Evidence for three mechanisms for the inhibition of NF-κB by glucocorticoids has been presented: (1) Glucocorticoids increase IκB mRNA, which leads to an increase of IκB protein and more efficient sequestration of NF-κB in the cytoplasm. (2) The glucocorticoid receptor competes with NF-κB for binding to coactivators. (3) The glucocorticoid receptor directly binds to the p65 subunit of NF-κB and inhibits its activation (Figure 42–10).

HORMONES CAN INFLUENCE SPECIFIC BIOLOGIC EFFECTS BY MODULATING TRANSCRIPTION

The signals generated as described above have to be translated into an action that allows the cell to effectively adapt to a challenge (Figure 42–1). Much of this adaptation is accomplished through alterations in the rates of transcription of specific genes. Many different observations have led to the current view of how hormones affect transcription. Some of these are as follows: (1) Actively transcribed genes are in regions of "open" chromatin (defined by a susceptibility to the enzyme DNase I), which allows for the access of transcription factors to DNA. (2) Genes have regulatory regions, and transcription factors bind to these to modulate the frequency of transcription initiation. (3) The hormone–receptor complex can be one of these transcription factors. The DNA sequence to which this binds is called a hormone response element (HRE; see Table 42–1 for examples). (4) Alternatively, other hormone-generated signals can modify the location, amount, or activity of transcription factors and thereby influence binding to the regulatory or response element. (5) Members of a large superfamily of nuclear receptors act with—or in a manner analogous to—hormone receptors. (6) These nuclear receptors interact with another large group of coregulatory molecules to effect changes in the transcription of specific genes.

Several Hormone Response Elements (HREs) Have Been Defined

Hormone response elements resemble enhancer elements in that they are not strictly dependent on position and location or orientation. They generally are found within a few hundred nucleotides upstream (5′) of the transcription initiation site, but they may be located within the coding region of the gene, in introns. HREs were defined by the strategy illustrated in Figure 38–11. The consensus sequences illustrated in Table 42–1 were arrived at through analysis of many genes regulated by a given hormone using simple, heterologous reporter systems (see Figure 38–10). Although these simple HREs bind the hormone–receptor complex more avidly than surrounding DNA—or DNA from an unrelated source—and confer hormone responsiveness to a reporter gene, it soon became apparent that the regulatory circuitry of natural genes must be much more complicated. Glucocorticoids, progestins, mineralocorticoids, and androgens have vastly different physiologic actions. How could the specificity required for these effects be achieved through regulation of gene expression by the same HRE (Table 42–1)? Questions like this have led to experiments which have allowed for elaboration of a very complex model of transcription regulation. For example, the HRE must associate with other DNA elements (and associated binding proteins) to function optimally. The extensive sequence similarity noted between steroid hormone receptors, particularly in their DNA-binding domains, led to discovery of the **nuclear receptor superfamily** of proteins. These—and a large number of **coregulator proteins**—allow for a wide variety of DNA–protein and protein–protein interactions and the specificity

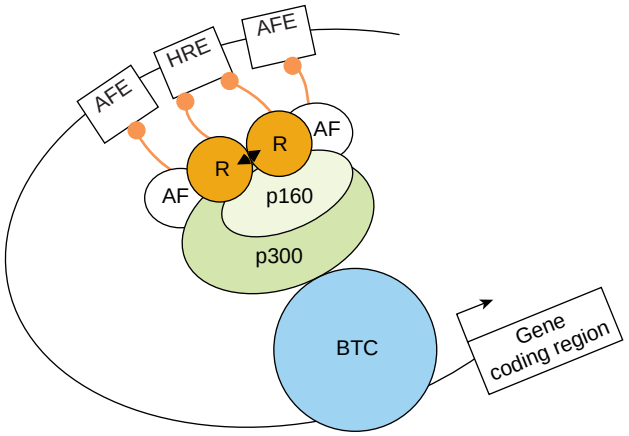

FIGURE 42–11 The hormone response transcription unit. The hormone response transcription unit is an assembly of DNA elements and bound proteins that interact, through protein-protein interactions, with a number of coactivator or corepressor molecules. An essential component is the hormone response element which binds the ligand (▲)-bound receptor (R). Also important are the accessory factor elements (AFEs) with bound transcription factors. More than two dozen of these accessory factors (AFs), which are often members of the nuclear receptor superfamily, have been linked to hormone effects on transcription. The AFs can interact with each other, with the liganded nuclear receptors, or with coregulators. These components communicate with the basal transcription complex (BTC) through a coregulator complex that can consist of one or more members of the p160, corepressor, mediator-related, or CBP/p300 families (see Table 42–6). Recall (Chapters 36, 38) that many of the transcription coregulators carry intrinsic enzymatic activities, which covalently modify the DNA, transcription proteins, and the histones present in the nucleosomes (not shown here) in and around the enhancer (HRE, AFE) and promoter. Collectively the hormone, hormone receptor, chromatin, DNA and transcription machinery integrate and process hormone signals to regulate transcription in a physiological fashion.

necessary for highly regulated physiologic control. A schematic of such an assembly is illustrated in Figure 42–11.

There Is a Large Family of Nuclear Receptor Proteins

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

These nuclear receptors have several common structural features (Figure 42–12). All have a centrally located **DNA-binding domain (DBD)** that allows the receptor to bind with high affinity to a response element. The DBD contains two zinc finger binding motifs (see Figure 38–14) that direct binding either as homodimers, as heterodimers (usually with a retinoid X receptor [RXR] partner), or as monomers. The target response element consists of one or two DNA half-site consensus sequences arranged as an inverted or direct repeat. The spacing between the latter helps determine binding specificity. Thus, in general, a direct repeat with three, four, or five nucleotide spacer regions specifies the binding of the vitamin D, thyroid, and retinoic acid receptors, respectively, to the same consensus response element (Table 42–1). A multifunctional **ligand-binding domain (LBD)** is located in the carboxyl terminal half of the receptor. The LBD binds hormones or metabolites with selectivity and thus specifies a particular biologic response. The LBD also contains domains that mediate the binding of heat shock proteins, dimerization, nuclear localization, and transactivation. The latter function is facilitated by the carboxyl terminal transcription activation function **(AF-2 domain),** which forms a surface required for the interaction with coactivators. A highly variable **hinge region** separates the DBD from the LBD. This region provides flexibility to the receptor, so it can assume different DNA-binding conformations. Finally, there is a highly variable amino terminal region that contains another transactivation domain referred to as **AF-1.** The AF-1 domain likely provides for distinct physiologic functions through the binding of different coregulator proteins. This region of the receptor, through the use of different promoters, alternative splice sites, and multiple translation initiation sites, provides for receptor isoforms that share DBD and LBD identity but exert different physiologic responses because of the association of various coregulators with this variable amino terminal AF-1 domain.

It is possible to sort this large number of receptors into groups in a variety of ways. Here they are discussed according to the way they bind to their respective DNA elements (Figure 42–12). Classic hormone receptors for glucocorticoids (GR), mineralocorticoids (MR), estrogens (ER), androgens (AR), and progestins (PR) bind as homodimers to inverted repeat sequences. Other hormone receptors such as thyroid (TR), retinoic acid (RAR), and vitamin D (VDR) and receptors that bind various metabolite ligands such as PPAR α, β, and γ, FXR, LXR, PXR/SXR, and CAR bind as heterodimers, with retinoid X receptor (RXR) as a partner, to direct repeat sequences (see Figure 42–12 & Table 42–5). Another group of orphan receptors that as yet have no known ligand bind as homodimers or monomers to direct repeat sequences.

As illustrated in Table 42–5, the discovery of the nuclear receptor superfamily has led to an important understanding of how a variety of metabolites and xenobiotics regulate gene expression and thus the metabolism, detoxification, and elimination of normal body products and exogenous agents such as pharmaceuticals. Not surprisingly, this area is a fertile field for investigation of new therapeutic interventions.

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

Chromatin remodeling (histone modifications, DNA methylation), transcription factor modification by various enzyme

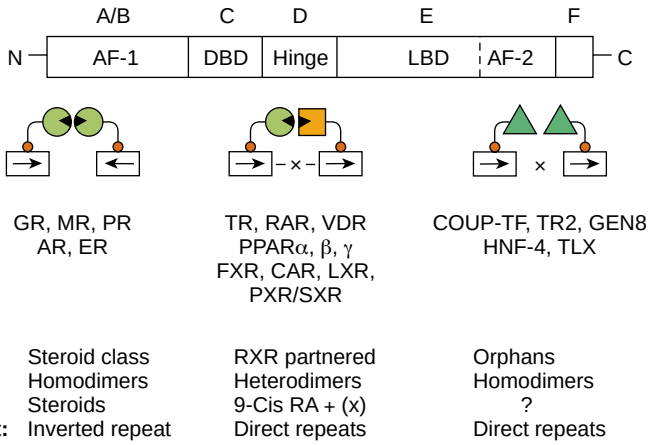

FIGURE 42–12 The nuclear receptor superfamily. Members of this family are divided into six structural domains (A–F). Domain A/B is also called AF-1, or the modulator region, because it is involved in activating transcription. The C domain consists of the DNA-binding domain (DBD). The D region contains the hinge, which provides flexibility between the DBD and the ligand-binding domain (LBD, region E). The C terminal part of region E contains AF-2, another domain important for transactivation. The F region is poorly defined. The functions of these domains are discussed in more detail in the text. Receptors with known ligands, such as the steroid hormones, bind as homodimers on inverted repeat half-sites. Other receptors form heterodimers with the partner RXR on direct repeat elements. There can be nucleotide spacers of one to five bases between these direct repeats (DR1–5). Another class of receptors for which ligands have not been determined (orphan receptors) bind as homodimers to direct repeats and occasionally as monomers to a single half-site.

activities, and the communication between the nuclear receptors and the basal transcription apparatus are accomplished by protein-protein interactions with one or more of a class of coregulator molecules. The number of these coregulator molecules now exceeds 100, not counting species variations and splice variants. The first of these to be described was the **CREB-binding protein, CBP.** CBP, through an amino terminal domain, binds to phosphorylated serine 137 of CREB and mediates transactivation in response to cAMP. It thus is described as a coactivator. CBP and its close relative, p300, interact directly or indirectly with a number of signaling molecules, including activator protein-1 (AP-1), signal transducers and activators of transcription (STATs), nuclear receptors, and CREB (Figure 42–13). **CBP/p300** also binds to the p160 family of coactivators described below and to a number of other proteins, including viral transcription factor Ela, the $p90^{rsk}$

TABLE 42–5 Nuclear Receptors with Special Ligands[1]

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

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

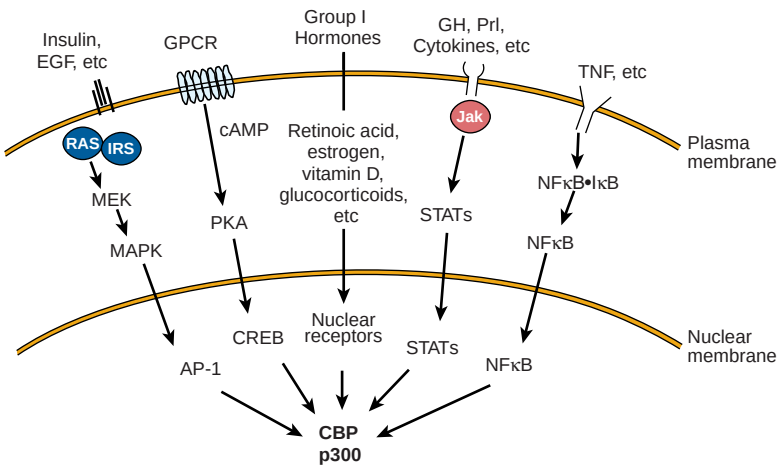

FIGURE 42–13 Several signal transduction pathways converge on CBP/p300. Many ligands that associate with membrane or nuclear receptors eventually converge on CBP/p300. Several different signal transduction pathways are employed. (EGF, epidermal growth factor; GH, growth hormone; Prl, prolactin; TNF, tumor necrosis factor; other abbreviations are expanded in the text.)

protein kinase, and RNA helicase A. It is important to note, as mentioned above, that **CBP/p300 also has intrinsic histone acetyltransferase (HAT) activity.** Some of the many actions of CBP/p300, which appear to depend on intrinsic enzyme activities and its ability to serve as a scaffold for the binding of other proteins, are illustrated in Figure 42–11. Other coregulators serve similar functions.

Several other families of coactivator molecules have been described. Members of the **p160 family of coactivators**, all of about 160 kDa, include (1) SRC-1 and NCoA-1; (2) GRIP 1, TIF2, and NCoA-2; and (3) p/CIP, ACTR, AIB1, RAC3, and TRAM-1 (Table 42–6). The different names for members within a subfamily often represent species variations or minor splice variants. There is about 35% amino acid identity between members of the different subfamilies. The p160 coactivators share several properties. They (1) bind nuclear receptors in an agonist- and AF-2 transactivation domain-dependent manner; (2) have a conserved amino terminal basic helix-loop-helix (bHLH) motif (see Chapter 38); (3) have a weak carboxyl terminal transactivation domain and a stronger amino terminal transactivation domain in a region that is required for the CBP/p160 interaction; (4) contain at least three of the **LXXLL motifs** required for protein-protein interaction with other coactivators; and (5) often have HAT activity. The role of HAT is particularly interesting, as mutations of the HAT domain disable many of these transcription factors. Current thinking holds that these HAT activities acetylate histones and result in remodeling of chromatin into a transcription-efficient environment; however, other protein substrates for HAT-mediated acetylation have been reported. Histone acetylation/deacetylation thus plays a critical role in gene expression.

A small number of proteins, including NCoR and SMRT, comprise the **corepressor family.** They function, at least in part, as described in Figure 42–2. Another family includes the TRAPs, DRIPs, and ARC (Table 42–6). These proteins represent subunits of the Mediator (Chapter 36) and range in size from 80 kDa to 240 kDa and are thought to link the nuclear receptor-coactivator complex to RNA polymerase II and the other components of the basal transcription apparatus.

TABLE 42–6 Some Mammalian Coregulator Proteins

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

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

It appears that certain combinations of coregulators—and thus different combinations of activators and inhibitors—are responsible for specific ligand-induced actions through various receptors. Furthermore, these interactions on a given promoter

are dynamic. In some cases, complexes consisting of as many as 47 transcription factors have been observed on a single gene.

SUMMARY

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

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SECTION VI SPECIAL TOPICS

CHAPTER 43

Nutrition, Digestion, & Absorption

David A. Bender, PhD

BIOMEDICAL IMPORTANCE

In addition to water, the diet must provide metabolic fuels (mainly carbohydrates and lipids), protein (for growth and turnover of tissue proteins), fiber (for bulk in the intestinal lumen), minerals (containing elements with specific metabolic functions), and vitamins and essential fatty acids (organic compounds needed in smaller amounts for other metabolic and physiologic functions). The polysaccharides, triacylglycerols, and proteins that make up the bulk of the diet must be hydrolyzed to their constituent monosaccharides, fatty acids, and amino acids, respectively, before absorption and utilization. Minerals and vitamins must be released from the complex matrix of food before they can be absorbed and utilized.

Globally, **undernutrition** is widespread, leading to impaired growth, defective immune systems, and reduced work capacity. By contrast, in developed countries, there is excessive food consumption (especially of fat), leading to obesity, and the development of cardiovascular disease and some forms of cancer. Deficiencies of vitamin A, iron, and iodine pose major health concerns in many countries, and deficiencies of other vitamins and minerals are a major cause of ill health. In developed countries nutrient deficiency is rare, although there are vulnerable sections of the population at risk. Intakes of minerals and vitamins that are adequate to prevent deficiency may be inadequate to promote optimum health and longevity.

Excessive secretion of gastric acid, associated with *Helicobacter pylori* infection, can result in the development of gastric and duodenal **ulcers;** small changes in the composition of bile can result in crystallization of cholesterol as **gallstones;** failure of exocrine pancreatic secretion (as in **cystic fibrosis**) leads to undernutrition and steatorrhea. **Lactose intolerance** is the result of lactase deficiency, leading to diarrhea and intestinal discomfort. Absorption of intact peptides that stimulate antibody responses cause **allergic reactions** and **celiac disease** is an allergic reaction to wheat gluten.

DIGESTION & ABSORPTION OF CARBOHYDRATES

The digestion of carbohydrates is by hydrolysis to liberate oligosaccharides, then free mono- and disaccharides. The increase in blood glucose after a test dose of a carbohydrate compared with that after an equivalent amount of glucose (as glucose or from a reference starchy food) is known as the **glycemic index.** Glucose and galactose have an index of 1, as do lactose, maltose, isomaltose, and trehalose, which give rise to these monosaccharides on hydrolysis. Fructose and the sugar alcohols are absorbed less rapidly and have a lower glycemic index, as does sucrose. The glycemic index of starch varies between near 1 to near 0 as a result of variable rates of hydrolysis, and that of nonstarch polysaccharides is 0. Foods that have a low glycemic index are considered to be more beneficial since they cause less fluctuation in insulin secretion. Resistant starch and nonstarch polysaccharides provide substrates for bacterial fermentation in the large intestine, and the resultant butyrate and other short chain fatty acids provide a significant source of fuel for intestinal enterocytes. There is some evidence that butyrate also has antiproliferative activity, and so provides protection against colorectal cancer.

Amylases Catalyze the Hydrolysis of Starch

The hydrolysis of starch is catalyzed by salivary and pancreatic amylases, which catalyze random hydrolysis of α(1→4) glycoside bonds, yielding dextrins, then a mixture of glucose,

maltose, and maltotriose and small branched dextrins (from the branchpoints in amylopectin).

Disaccharidases Are Brush Border Enzymes

The disaccharidases, maltase, sucrase-isomaltase (a bifunctional enzyme catalyzing hydrolysis of sucrose and isomaltose), lactase, and trehalase are located on the brush border of the intestinal mucosal cells, where the resultant monosaccharides and those arising from the diet are absorbed. Congenital deficiency of lactase occurs rarely in infants, leading to lactose intolerance and failure to thrive when fed on breast milk or normal infant formula. Congenital deficiency of sucrase-isomaltase occurs among the Inuit, leading to sucrose intolerance, with persistent diarrhea and failure to thrive when the diet contains sucrose.

In most mammals, and most human beings, lactase activity begins to fall after weaning, and is almost completely lost by late adolescence, leading to **lactose intolerance.** Lactose remains in the intestinal lumen, where it is a substrate for bacterial fermentation to lactate, resulting in abdominal discomfort and diarrhea after consumption of relatively large amounts. In two population groups, people of north European origin and nomadic tribes of sub-Saharan Africa and Arabia, lactase persists after weaning and into adult life. Marine mammals secrete a high-fat milk that contains no carbohydrate, and their pups lack lactase.

There Are Two Separate Mechanisms for the Absorption of Monosaccharides in the Small Intestine

Glucose and galactose are absorbed by a sodium-dependent process. They are carried by the same transport protein (SGLT 1), and compete with each other for intestinal absorption (Figure 43–1). Other monosaccharides are absorbed by carrier-mediated diffusion. Because they are not actively transported, fructose and sugar alcohols are only absorbed down their concentration gradient, and after a moderately high intake, some may remain in the intestinal lumen, acting as a substrate for bacterial fermentation.

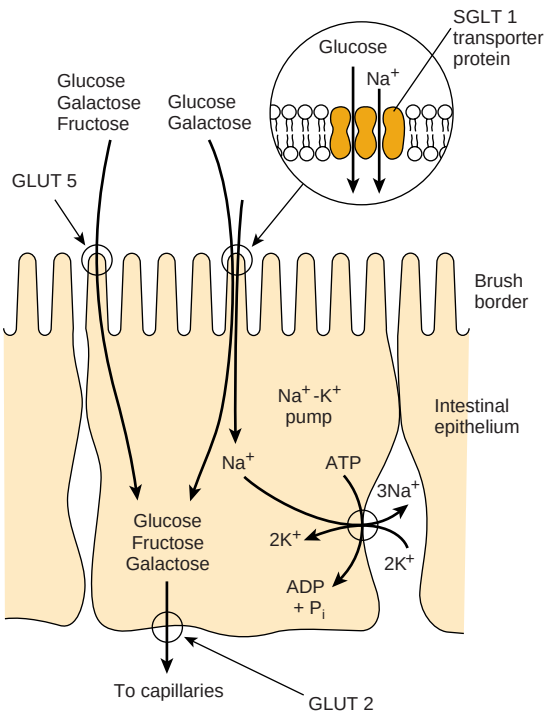

FIGURE 43–1 Transport of glucose, fructose, and galactose across the intestinal epithelium. The SGLT 1 transporter is coupled to the Na^+-K^+ pump, allowing glucose and galactose to be transported against their concentration gradients. The GLUT 5 Na^+-independent facilitative transporter allows fructose, as well as glucose and galactose, to be transported down their concentration gradients. Exit from the cell for all sugars is via the GLUT 2 facilitative transporter.

DIGESTION & ABSORPTION OF LIPIDS

The major lipids in the diet are triacylglycerols and, to a lesser extent, phospholipids. These are hydrophobic molecules, and have to be hydrolyzed and emulsified to very small droplets (micelles) before they can be absorbed. The fat-soluble vitamins, A, D, E, and K, and a variety of other lipids (including cholesterol) are absorbed dissolved in the lipid micelles. Absorption of the fat-soluble vitamins is impaired on a very low fat diet.

Hydrolysis of triacylglycerols is initiated by lingual and gastric lipases, which attack the *sn*-3 ester bond forming 1,2-diacylglycerols and free fatty acids, aiding emulsification. Pancreatic lipase is secreted into the small intestine, and requires a further pancreatic protein, colipase, for activity. It is specific for the primary ester links—ie, positions 1 and 3 in triacylglycerols—resulting in 2-monoacylglycerols and free fatty acids as the major end products of luminal triacylglycerol digestion. Monoacylglycerols are poor substrates for hydrolysis, so that less than 25% of ingested triacylglycerol is completely hydrolyzed to glycerol and fatty acids (Figure 43–2). Bile salts, formed in the liver and secreted in the bile, permit emulsification of the products of lipid digestion into micelles together with phospholipids and cholesterol from the bile. Because the micelles are soluble, they allow the products of digestion, including the fat-soluble vitamins, to be transported through the aqueous environment of the intestinal lumen and permit close contact with the brush border of the mucosal cells, allowing uptake into the epithelium. The bile salts pass on to the ileum, where most are absorbed into the **enterohepatic circulation** (Chapter 26). Within the intestinal epithelium, 1-monoacyglycerols are hydrolyzed to fatty acids and glycerol and 2-monoacylglycerols are reacylated to triacylglycerols via the **monoacylglycerol pathway.** Glycerol released in the intestinal lumen is not reutilized but passes into the portal vein; glycerol released within the epithelium is reutilized for triacylglycerol synthesis via the normal phosphatidic acid pathway (Chapter 24). Long-chain fatty acids are esterified to yield to triacylglycerol in the mucosal cells and together with the other products

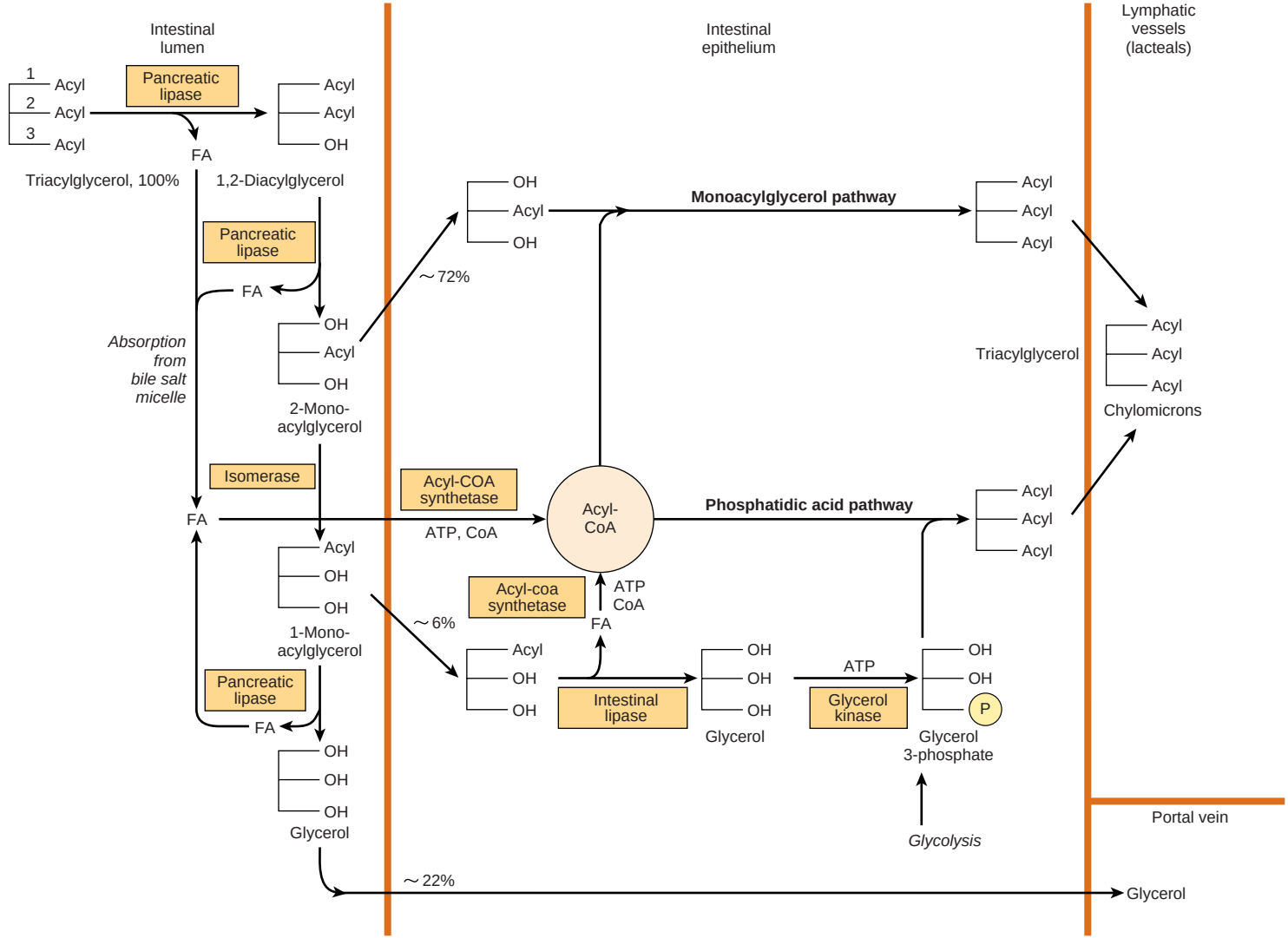

FIGURE 43–2 Digestion and absorption of triacylglycerols. The values given for percentage uptake may vary widely but indicate the relative importance of the three routes shown.

of lipid digestion, secreted as chylomicrons into the lymphatics, entering the bloodstream via the thoracic duct (Chapter 25). Short- and medium-chain fatty acids are mainly absorbed into the hepatic portal vein as free fatty acids.

Cholesterol is absorbed dissolved in lipid micelles, and is mainly esterified in the intestinal mucosa before being incorporated into chylomicrons. Unesterified cholesterol and other sterols are actively transported out of the mucosal cells into the intestinal lumen. Plant sterols and stanols (in which the B ring is saturated) compete with cholesterol for esterification, but are poor substrates. They therefore reduce the absorption of cholesterol, and act to lower serum cholesterol.

DIGESTION & ABSORPTION OF PROTEINS

Few bonds are accessible to the proteolytic enzymes that catalyze hydrolysis of peptide bonds, without prior denaturation of dietary proteins (by heat in cooking and by the action of gastric acid).

Several Groups of Enzymes Catalyze the Digestion of Proteins

There are two main classes of proteolytic digestive enzymes (**proteases**), with different specificities for the amino acids forming the peptide bond to be hydrolyzed. **Endopeptidases** hydrolyze peptide bonds between specific amino acids throughout the molecule. They are the first enzymes to act, yielding a larger number of smaller fragments. Pepsin in the gastric juice catalyzes hydrolysis of peptide bonds adjacent to aromatic and branched-chain amino acids and methionine. Trypsin, chymotrypsin, and elastase are secreted into the small intestine by the pancreas. Trypsin catalyzes hydrolysis of lysine and arginine esters, chymotrypsin esters of aromatic amino acids, and elastase esters of small neutral aliphatic amino acids. **Exopeptidases** catalyze the hydrolysis of peptide bonds, one at a time, from the ends of peptides. **Carboxypeptidase**s, secreted in the pancreatic juice, release amino acids from the free carboxyl terminal; **aminopeptidases,** secreted by the intestinal mucosal cells, release amino acids from the amino terminal. **Dipeptidases** and **tripeptidases** in the brush border of intestinal mucosal cells catalyze the hydrolysis of di- and tripeptides, which are not substrates for amino- and carboxypeptidases.

The proteases are secreted as inactive **zymogens;** the active site of the enzyme is masked by a small region of the peptide chain that is removed by hydrolysis of a specific peptide bond. Pepsinogen is activated to pepsin by gastric acid and by activated pepsin (autocatalysis). In the small intestine, trypsinogen, the precursor of trypsin, is activated by enteropeptidase, which is secreted by the duodenal epithelial cells; trypsin can then activate chymotrypsinogen to chymotrypsin, proelastase to elastase, procarboxypeptidase to carboxypeptidase, and proaminopeptidase to aminopeptidase.

Free Amino Acids & Small Peptides Are Absorbed by Different Mechanisms

The end product of the action of endopeptidases and exopeptidases is a mixture of free amino acids, di- and tripeptides, and oligopeptides, all of which are absorbed. Free amino acids are absorbed across the intestinal mucosa by sodium-dependent active transport. There are several different amino acid transporters, with specificity for the nature of the amino acid side-chain (large or small, neutral, acidic or basic). The various amino acids carried by any one transporter compete with each other for absorption and tissue uptake. Dipeptides and tripeptides enter the brush border of the intestinal mucosal cells, where they are hydrolyzed to free amino acids, which are then transported into the hepatic portal vein. Relatively large peptides may be absorbed intact, either by uptake into mucosal epithelial cells (transcellular) or by passing between epithelial cells (paracellular). Many such peptides are large enough to stimulate antibody formation—this is the basis of **allergic reactions** to foods.

DIGESTION & ABSORPTION OF VITAMINS & MINERALS

Vitamins and minerals are released from food during digestion, although this is not complete, and the availability of vitamins and minerals depends on the type of food and, especially for minerals, the presence of chelating compounds. The fat-soluble vitamins are absorbed in the lipid micelles that are the result of fat digestion; water-soluble vitamins and most mineral salts are absorbed from the small intestine either by active transport or by carrier-mediated diffusion followed by binding to intracellular proteins to achieve concentrative uptake. Vitamin B_{12} absorption requires a specific transport protein, intrinsic factor (Chapter 44); calcium absorption is dependent on vitamin D; zinc absorption probably requires a zinc-binding ligand secreted by the exocrine pancreas, and the absorption of iron is limited (see below).

Calcium Absorption Is Dependent on Vitamin D

In addition to its role in regulating calcium homeostasis, vitamin D is required for the intestinal absorption of calcium. Synthesis of the intracellular calcium-binding protein, **calbindin,** required for calcium absorption, is induced by vitamin D, which also affects the permeability of the mucosal cells to calcium, an effect that is rapid and independent of protein synthesis.

Phytic acid (inositol hexaphosphate) in cereals binds calcium in the intestinal lumen, preventing its absorption. Other minerals, including zinc, are also chelated by phytate. This is mainly a problem among people who consume large amounts of unleavened whole-wheat products; yeast contains an enzyme, **phytase**, that dephosphorylates phytate, so rendering it inactive. High concentrations of fatty acids in the intestinal lumen, as a result of impaired fat absorption, can also reduce

calcium absorption, by forming insoluble calcium salts; a high intake of oxalate can sometimes cause deficiency, since calcium oxalate is insoluble.

Iron Absorption Is Limited and Strictly Controlled, but Enhanced by Vitamin C and Alcohol

Although iron deficiency is a common problem, about 10% of the population are genetically at risk of iron overload **(hemochromatosis),** and in order to reduce the risk of adverse effects of nonenzymic generation of free radicals by iron salts, absorption is strictly regulated. Inorganic iron is transported into the mucosal cell by a proton-linked divalent metal ion transporter, and accumulated intracellularly by binding to **ferritin.** Iron leaves the mucosal cell via a transport protein ferroportin, but only if there is free **transferrin** in plasma to bind to. Once transferrin is saturated with iron, any that has accumulated in the mucosal cells is lost when the cells are shed. Expression of the ferroportin gene (and possibly also that for the divalent metal ion transporter) is downregulated by hepcidin, a peptide secreted by the liver when body iron reserves are adequate. In response to hypoxia, anemia or hemorrhage, the synthesis of hepcidin is reduced, leading to increased synthesis of ferroportin and increased iron absorption (Figure 43–3). As a result of this mucosal barrier, only about 10% of dietary iron is absorbed, and only 1–5% from many plant foods (Chapter 50).

Inorganic iron is absorbed in the Fe^{2+} (reduced) state, hence the presence of reducing agents enhances absorption. The most effective compound is **vitamin C,** and while intakes of 40–80 mg of vitamin C/day are more than adequate to meet requirements, an intake of 25–50 mg per meal enhances iron absorption, especially when iron salts are used to treat iron deficiency anemia. Alcohol and fructose also enhance iron absorption. Heme iron from meat is absorbed separately, and is considerably more available than inorganic iron. However, the absorption of both inorganic and heme iron is impaired by calcium—a glass of milk with a meal significantly reduces iron availability.

ENERGY BALANCE: OVER- & UNDERNUTRITION

After the provision of water, the body's first requirement is for metabolic fuels—fats, carbohydrates, amino acids from proteins (Table 16–1). Food intake in excess of energy expenditure leads to **obesity,** while intake less than expenditure leads to emaciation and wasting, **marasmus,** and **kwashiorkor.** Both obesity and severe undernutrition are associated with increased mortality. The body mass index = weight (in kg)/height^2 (in m) is commonly used as a way of expressing relative obesity; a desirable range is between 20 and 25.

Energy Requirements Are Estimated by Measurement of Energy Expenditure

Energy expenditure can be determined directly by measuring heat output from the body, but is normally estimated indirectly from the consumption of oxygen. There is an energy expenditure of 20 kJ/liter of oxygen consumed, regardless of whether the fuel being metabolized is carbohydrate, fat, or protein (Table 16–1).

Measurement of the ratio of the volume of carbon dioxide produced: volume of oxygen consumed **(respiratory quotient, RQ)** is an indication of the mixture of metabolic fuels being oxidized (Table 16–1).

A more recent technique permits estimation of total energy expenditure over a period of 1–2 weeks, using dual isotopically labeled water, $^2H_2{}^{18}O$. ^2H is lost from the body only in water, while ^{18}O is lost in both water and carbon dioxide; the difference in the rate of loss of the two labels permits estimation of total carbon dioxide production, and hence oxygen consumption and energy expenditure.

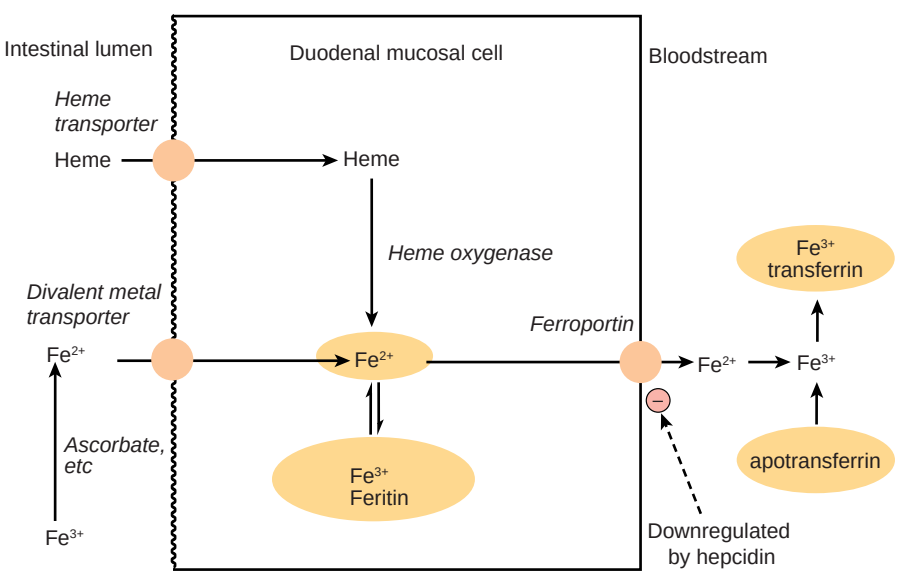

FIGURE 43–3 Absorption of iron. Hepcidin secreted by the liver downregulates synthesis of ferroportin and limits iron absorption.

Basal metabolic rate (BMR) is the energy expenditure by the body when at rest, but not asleep, under controlled conditions of thermal neutrality, measured about 12 h after the last meal, and depends on weight, age, and gender. Total energy expenditure depends on the basal metabolic rate, the energy required for physical activity and the energy cost of synthesizing reserves in the fed state. It is therefore possible to calculate an individual's energy requirement from body weight, age, gender, and level of physical activity. Body weight affects BMR because there is a greater amount of active tissue in a larger body. The decrease in BMR with increasing age, even when body weight remains constant, is the result of muscle tissue replacement by adipose tissue, which is metabolically less active. Similarly, women have a significantly lower BMR than do men of the same body weight because women's bodies contain proportionally more adipose tissue.

Energy Requirements Increase with Activity

The most useful way of expressing the energy cost of physical activities is as a multiple of BMR. Sedentary activities use only about 1.1–1.2 × BMR. By contrast, vigorous exertion, such as climbing stairs, cross-country walking uphill, etc., may use 6–8 × BMR.

Ten Percent of the Energy Yield of a Meal May Be Expended in Forming Reserves

There is a considerable increase in metabolic rate after a meal **(diet-induced thermogenesis)**. A small part of this is the energy cost of secreting digestive enzymes and of active transport of the products of digestion; the major part is the result of synthesizing reserves of glycogen, triacylglycerol, and protein.

There Are Two Extreme Forms of Undernutrition

Marasmus can occur in both adults and children, and occurs in vulnerable groups of all populations. **Kwashiorkor** affects only children, and has been reported only in developing countries. The distinguishing feature of kwashiorkor is that there is fluid retention, leading to edema, and fatty infiltration of the liver. Marasmus is a state of extreme emaciation; it is the outcome of prolonged negative energy balance. Not only have the body's fat reserves been exhausted, but there is wastage of muscle as well, and as the condition progresses there is loss of protein from the heart, liver, and kidneys. The amino acids released by the catabolism of tissue proteins are used as a source of metabolic fuel and as substrates for gluconeogenesis to maintain a supply of glucose for the brain and red blood cells (Chapter 20). As a result of the reduced synthesis of proteins, there is impaired immune response and more risk from infections. Impairment of cell proliferation in the intestinal mucosa occurs, resulting in reduction in surface area of the intestinal mucosa, and reduction in absorption of such nutrients as are available.

Patients with Advanced Cancer and AIDS Are Malnourished

Patients with advanced cancer, HIV infection and AIDS, and a number of other chronic diseases are frequently undernourished, a condition called **cachexia.** Physically, they show all the signs of marasmus, but there is considerably more loss of body protein than occurs in starvation. The secretion of cytokines in response to infection and cancer increases the catabolism of tissue protein by the ATP-dependent ubiquitin-proteasome pathway (Figure 45–8), so increasing energy expenditure. This differs from marasmus, in which protein synthesis is reduced, but catabolism in unaffected. Patients are **hypermetabolic,** ie, a considerable increase in basal metabolic rate. In addition to activation of the ubiquitin-proteasome pathway of protein catabolism, three other factors are involved. Many tumors metabolize glucose anaerobically to release lactate. This is then used for gluconeogenesis in the liver, which is energy consuming with a net cost of 6 ATP for each mol of glucose cycled (see Figure 20–4). There is increased stimulation of **uncoupling proteins** by **cytokines** leading to thermogenesis and increased oxidation of metabolic fuels. **Futile cycling of lipids** occurs because hormone sensitive lipase is activated by a proteoglycan secreted by tumors resulting in liberation of fatty acids from adipose tissue and ATP-expensive re-esterification to triacylglycerols in the liver, which are exported in VLDL.

Kwashiorkor Affects Undernourished Children

In addition to the wasting of muscle tissue, loss of intestinal mucosa and impaired immune responses seen in marasmus, children with **kwashiorkor** show a number of characteristic features. The defining characteristic is **edema,** associated with a decreased concentration of plasma proteins. In addition, there is enlargement of the liver as a result of accumulation of fat. It was formerly believed that the cause of kwashiorkor was a lack of protein, with a more or less adequate energy intake, however, analysis of the diets of affected children shows that this is not so. Children with kwashiorkor are less stunted than those with marasmus and the edema begins to improve early in treatment, when the child is still receiving a low protein diet.

Very commonly, an infection precipitates kwashiorkor. Superimposed on general food deficiency, there is probably a deficiency of the antioxidant nutrients such as zinc, copper, carotene, and vitamins C and E. The **respiratory burst** in response to infection leads to the production of oxygen and halogen **free radicals** as part of the cytotoxic action of stimulated macrophages. This added oxidant stress may well trigger the development of kwashiorkor (see Chapter 54).

PROTEIN & AMINO ACID REQUIREMENTS

Protein Requirements Can Be Determined by Measuring Nitrogen Balance

The state of protein nutrition can be determined by measuring the dietary intake and output of nitrogenous compounds from the body. Although nucleic acids also contain nitrogen, protein is the major dietary source of nitrogen and measurement of total nitrogen intake gives a good estimate of protein intake (mg N × 6.25 = mg protein, as N is 16% of most proteins). The output of N from the body is mainly in urea and smaller quantities of other compounds in urine, undigested protein in feces; significant amounts may also be lost in sweat and shed skin. The difference between intake and output of nitrogenous compounds is known as **nitrogen balance.** Three states can be defined. In a healthy adult, nitrogen balance is in **equilibrium,** when intake equals output, and there is no change in the total body content of protein. In a growing child, a pregnant woman, or a person in recovery from protein loss, the excretion of nitrogenous compounds is less than the dietary intake and there is net retention of nitrogen in the body as protein—**positive nitrogen balance.** In response to trauma or infection, or if the intake of protein is inadequate to meet requirements, there is net loss of protein nitrogen from the body—**negative nitrogen balance.** Except when replacing protein losses, nitrogen equilibrium can be maintained at any level of protein intake above requirements. A high intake of protein does not lead to positive nitrogen balance; although it increases the rate of protein synthesis, it also increases the rate of protein catabolism, so that nitrogen equilibrium is maintained, albeit with a higher rate of protein turnover.

The continual catabolism of tissue proteins creates the requirement for dietary protein, even in an adult who is not growing; although some of the amino acids released can be reutilized, much is used for gluconeogenesis in the fasting state. Nitrogen balance studies show that the average daily requirement is 0.66 g of protein/kg body weight (0.825 allowing for individual variation), approximately 55 g/day, or 0.825% of energy intake. Average intakes of protein in developed countries are of the order of 80–100 g/day, ie, 14–15% of energy intake. Because growing children are increasing the protein in the body, they have a proportionally greater requirement than adults and should be in positive nitrogen balance. Even so, the need is relatively small compared with the requirement for protein turnover. In some countries, protein intake is inadequate to meet these requirements, resulting in stunting of growth.

There Is a Loss of Body Protein in Response to Trauma & Infection

One of the metabolic reactions to a major trauma, such as a burn, a broken limb, or surgery, is an increase in the net catabolism of tissue proteins, both in response to cytokines and glucocorticoid hormones, and as a result of excessive utilization of threonine and cysteine in the synthesis of **acute-phase proteins**. As much as 6–7% of the total body protein may be lost over 10 days. Prolonged bed rest results in considerable loss of protein because of atrophy of muscles. Protein catabolism may be increased in response to cytokines, and without the stimulus of exercise it is not completely replaced. Lost protein is replaced during **convalescence,** when there is positive nitrogen balance. A normal diet is adequate to permit this replacement.

The Requirement Is Not Just for Protein, but for Specific Amino Acids

Not all proteins are nutritionally equivalent. More of some is needed to maintain nitrogen balance than others because different proteins contain different amounts of the various amino acids. The body's requirement is for amino acids in the correct proportions to replace tissue proteins. The amino acids can be divided into two groups: **essential** and **nonessential**. There are nine essential or indispensable amino acids, which cannot be synthesized in the body: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. If one of these is lacking or inadequate, then regardless of the total intake of protein, it will not be possible to maintain nitrogen balance, since there will not be enough of that amino acid for protein synthesis.

Two amino acids, cysteine and tyrosine, can be synthesized in the body, but only from essential amino acid precursors—cysteine from methionine and tyrosine from phenylalanine. The dietary intakes of cysteine and tyrosine thus affect the requirements for methionine and phenylalanine. The remaining 11 amino acids in proteins are considered to be nonessential or dispensable, since they can be synthesized as long as there is enough total protein in the diet. If one of these amino acids is omitted from the diet, nitrogen balance can still be maintained. However, only three amino acids, alanine, aspartate, and glutamate, can be considered to be truly dispensable; they are synthesized from common metabolic intermediates (pyruvate, oxaloacetate, and ketoglutarate, respectively). The remaining amino acids are considered as nonessential, but under some circumstances the requirement may outstrip the capacity for synthesis.

SUMMARY

- Digestion involves hydrolyzing food molecules into smaller molecules for absorption through the gastrointestinal epithelium. Polysaccharides are absorbed as monosaccharides, triacylglycerols as 2-monoacylglycerols, fatty acids and glycerol, and proteins as amino acids.
- Digestive disorders arise as a result of (1) enzyme deficiency, eg, lactase and sucrase; (2) malabsorption, eg, of glucose and galactose as a result of defects in the Na^+-glucose cotransporter (SGLT 1); (3) absorption of unhydrolyzed

polypeptides leading to immune responses, eg, as in celiac disease; and (4) precipitation of cholesterol from bile as gallstones.

- In addition to water, the diet must provide metabolic fuels (carbohydrate and fat) for body growth and activity, protein for synthesis of tissue proteins, fiber for roughage, minerals for specific metabolic functions, certain polyunsaturated fatty acids of the n-3 and n-6 families, and vitamins, organic compounds needed in small amounts for other essential functions.
- Twenty different amino acids are required for protein synthesis, of which nine are essential in the human diet. The quantity of protein required is affected by protein quality, energy intake, and physical activity.
- Undernutrition occurs in two extreme forms: marasmus, in adults and children, and kwashiorkor in children. Overnutrition leads to excess energy intake, and is associated with diseases such as obesity, noninsulin-dependent diabetes mellitus, atherosclerosis, cancer, and hypertension.

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CHAPTER 44

Micronutrients: Vitamins & Minerals

David A. Bender, PhD

BIOMEDICAL IMPORTANCE

Vitamins are a group of organic nutrients, required in small quantities for a variety of biochemical functions that, generally, cannot be synthesized by the body and must therefore be supplied in the diet.

The lipid-soluble vitamins are hydrophobic compounds that can be absorbed efficiently only when there is normal fat absorption. Like other lipids, they are transported in the blood in lipoproteins or attached to specific binding proteins. They have diverse functions—eg, vitamin A, vision and cell differentiation; vitamin D, calcium and phosphate metabolism, and cell differentiation; vitamin E, anti-oxidant; and vitamin K, blood clotting. As well as dietary inadequacy, conditions affecting the digestion and absorption of the lipid-soluble vitamins, such as steatorrhea and disorders of the biliary system, can all lead to deficiency syndromes, including night blindness and xerophthalmia (vitamin A); rickets in young children and osteomalacia in adults (vitamin D); neurologic disorders and hemolytic anemia of the newborn (vitamin E); and hemorrhagic disease of the newborn (vitamin K). Toxicity can result from excessive intake of vitamins A and D. Vitamin A and the carotenes (many of which are precursors of vitamin A), and vitamin E are antioxidants (Chapter 45) and have possible roles in prevention of atherosclerosis and cancer.

The water-soluble vitamins are composed of the B vitamins and vitamin C; they function mainly as enzyme cofactors. Folic acid acts as a carrier of one-carbon units. Deficiency of a single vitamin of the B complex is rare, since poor diets are most often associated with **multiple deficiency states.** Nevertheless, specific syndromes are characteristic of deficiencies of individual vitamins, eg, beriberi (thiamin); cheilosis, glossitis, seborrhea (riboflavin); pellagra (niacin); megaloblastic anemia, methylmalonic aciduria, and pernicious anemia (vitamin B_{12}); megaloblastic anemia (folic acid); and scurvy (vitamin C).

Inorganic mineral elements that have a function in the body must be provided in the diet. When the intake is insufficient, deficiency signs may arise, eg, anemia (iron), and cretinism and goiter (iodine). Excessive intakes may be toxic.

The Determination of Micronutrient Requirements Depends on the Criteria of Adequacy Chosen

For any nutrient, there is a range of intakes between that which is clearly inadequate, leading to **clinical deficiency disease,** and that which is so much in excess of the body's metabolic capacity that there may be signs of **toxicity.** Between these two extremes is a level of intake that is adequate for normal health and the maintenance of metabolic integrity. Individuals do not all have the same requirement for nutrients, even when calculated on the basis of body size or energy expenditure. There is a range of individual requirements of up to 25% around the mean. Therefore, in order to assess the adequacy of diets, it is necessary to set a reference level of intake high enough to ensure that no one either suffers from deficiency or is at risk of toxicity. If it is assumed that individual requirements are distributed in a statistically normal fashion around the observed mean requirement, then a range of ±2 × the standard deviation (SD) around the mean includes the requirements of 95% of the population. Reference or recommended intakes are therefore set at the average requirement plus 2 × SD, and so meet or exceed the requirements of 97.5% of the population.

THE VITAMINS ARE A DISPARATE GROUP OF COMPOUNDS WITH A VARIETY OF METABOLIC FUNCTIONS

A vitamin is defined as an organic compound that is required in the diet in small amounts for the maintenance of normal metabolic integrity. Deficiency causes a specific disease, which is cured or prevented only by restoring the vitamin to the diet (Table 44–1). However, **vitamin D,** which is formed in the skin from 7-dehydrocholesterol on exposure to sunlight, and **niacin,** which can be formed from the essential amino acid tryptophan, do not strictly comply with this definition.

TABLE 44–1 The Vitamins

Vitamin		Functions	Deficiency disease
Lipid-Soluable			
A	Retinol, β-carotene	Visual pigments in the retina; regulation of gene expression and cell differentiation (β-carotene is an antioxidant)	Night blindness, xerophthalmia; keratinization of skin
D	Calciferol	Maintenance of calcium balance; enhances intestinal absorption of Ca^{2+} and mobilizes bone mineral; regulation of gene expression and cell differentiation	Rickets = poor mineralization of bone; osteomalacia = bone demineralization
E	Tocopherols, tocotrienols	Antioxidant, especially in cell membranes; roles in cell signaling	Extremely rare—serious neurologic dysfunction
K	Phylloquinone: menaquinones	Coenzyme in formation of γ-carboxy-glutamate in enzymes of blood clotting and bone matrix	Impaired blood clotting, hemorrhagic disease
Water-Soluable			
B_1	Thiamin	Coenzyme in pyruvate and α-ketoglutarate dehydrogenases, and transketolase; regulates Cl^- channel in nerve conduction	Peripheral nerve damage (beriberi) or central nervous system lesions (Wernicke-Korsakoff syndrome)
B_2	Riboflavin	Coenzyme in oxidation and reduction reactions (FAD and FMN); prosthetic group of flavoproteins	Lesions of corner of mouth, lips, and tongue, seborrheic dermatitis
Niacin	Nicotinic acid, nicotinamide	Coenzyme in oxidation and reduction reactions, functional part of NAD and NADP; role in intracellular calcium regulation and cell signaling	Pellagra—photosensitive dermatitis, depressive psychosis
B_6	Pyridoxine, pyridoxal, pyridoxamine	Coenzyme in transamination and decarboxylation of amino acids and glycogen phosphorylase; modulation of steroid hormone action	Disorders of amino acid metabolism, convulsions
	Folic acid	Coenzyme in transfer of one-carbon fragments	Megaloblastic anemia
B_{12}	Cobalamin	Coenzyme in transfer of one-carbon fragments and metabolism of folic acid	Pernicious anemia = megaloblastic anemia with degeneration of the spinal cord
	Pantothenic acid	Functional part of CoA and acyl carrier protein: fatty acid synthesis and metabolism	Peripheral nerve damage (nutritional melalgia or "burning foot syndrome")
H	Biotin	Coenzyme in carboxylation reactions in gluconeogenesis and fatty acid synthesis; role in regulation of cell cycle	Impaired fat and carbohydrate metabolism, dermatitis
C	Ascorbic acid	Coenzyme in hydroxylation of proline and lysine in collagen synthesis; antioxidant; enhances absorption of iron	Scurvy—impaired wound healing, loss of dental cement, subcutaneous hemorrhage

LIPID-SOLUBLE VITAMINS

TWO GROUPS OF COMPOUNDS HAVE VITAMIN A ACTIVITY

Retinoids comprise **retinol, retinaldehyde,** and **retinoic acid** (prefovrmed vitamin A, found only in foods of animal origin); carotenoids, found in plants, are composed of carotenes and related compounds; many are precursors of vitamin A, as they can be cleaved to yield retinaldehyde, then retinol and retinoic acid (Figure 44–1). The α-, β-, and γ-carotenes and cryptoxanthin are quantitatively the most important provitamin A carotenoids. Although it would appear that one molecule of β-carotene should yield two of retinol, this is not so in practice; 6 μg of β-carotene is equivalent to 1 μg of preformed retinol. The total amount of vitamin A in foods is therefore expressed as micrograms of retinol equivalents. β-Carotene and other provitamin A carotenoids are cleaved in the intestinal mucosa by carotene dioxygenase, yielding retinaldehyde, which is reduced to retinol, esterified and secreted in chylomicrons together with esters formed from dietary retinol. The intestinal activity of carotene dioxygenase is low, so that a relatively large proportion of ingested β-carotene may appear in the circulation unchanged. While the principal site of carotene dioxy-

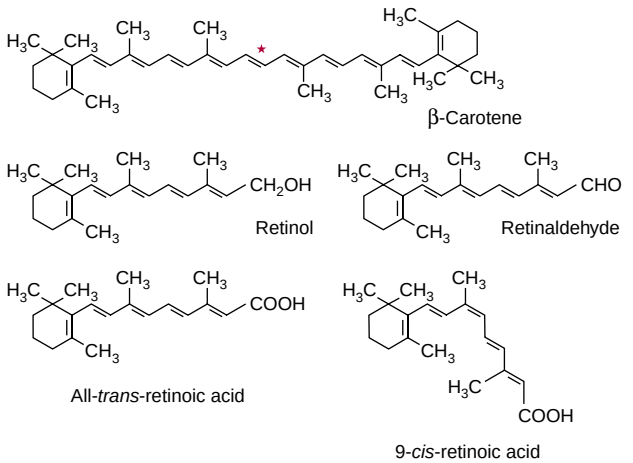

FIGURE 44–1 β-Carotene and the major vitamin A vitamers. Asterisk shows the site of cleavage of β-carotene by carotene dioxygenase, to yield retinaldehyde.

genase attack is the central bond of β-carotene, asymmetric cleavage may also occur, leading to the formation of 8′-, 10′-, and 12′-apo-carotenals, which are oxidized to retinoic acid, but cannot be used as sources of retinol or retinaldehyde.

Vitamin A Has a Function in Vision

In the retina, retinaldehyde functions as the prosthetic group of the light-sensitive opsin proteins, forming **rhodopsin** (in rods) and **iodopsin** (in cones). Any one cone cell contains only one type of opsin, and is sensitive to only one color. In the pigment epithelium of the retina, all-*trans*-retinol is isomerized to 11-*cis*-retinol and oxidized to 11-*cis*-retinaldehyde. This reacts with a lysine residue in opsin, forming the holo-protein rhodopsin. As shown in Figure 44–2, the absorption of light by rhodopsin causes isomerization of the retinaldehyde from 11-*cis* to all-*trans*, and a conformational change in opsin. This results in the release of retinaldehyde from the protein, and the initiation of a nerve impulse. The formation of the initial excited form of rhodopsin, bathorhodopsin, occurs within picoseconds of illumination. There is then a series of conformational changes leading to the formation of metarhodopsin II, which initiates a guanine nucleotide amplification cascade and then a nerve impulse. The final step is hydrolysis to release all-*trans*-retinaldehyde and opsin. The key to initiation of the visual cycle is the availability of 11-*cis*-retinaldehyde, and hence vitamin A. In deficiency, both the time taken to adapt to darkness and the ability to see in poor light are impaired.

Retinoic Acid Has a Role in the Regulation of Gene Expression and Tissue Differentiation

A major role of vitamin A is in the control of cell differentiation and turnover. All-*trans*-retinoic acid and 9-*cis*-retinoic acid (Figure 44–1) regulate growth, development, and tissue differentiation; they have different actions in different tissues. Like the thyroid and steroid hormones and vitamin D, retinoic acid binds to nuclear receptors that bind to response elements of DNA and regulate the transcription of specific genes. There are two families of nuclear retinoid receptors: the retinoic acid receptors (RAR) bind all-*trans*-retinoic acid or 9-*cis*-retinoic acid, and the retinoid X receptors (RXR) bind 9-*cis*-retinoic acid. Retinoid X receptors also form dimers with vitamin D, thyroid, and other a nuclear acting hormone receptors. Deficiency of vitamin A impairs vitamin D function because of lack of 9-*cis*-retinoic acid to form receptor dimers, while excessive vitamin A also impairs vitamin D function, because of formation of RXR-homodimers, meaning that there are not enough RXR available to form heterodimers with the vitamin D rceptor.

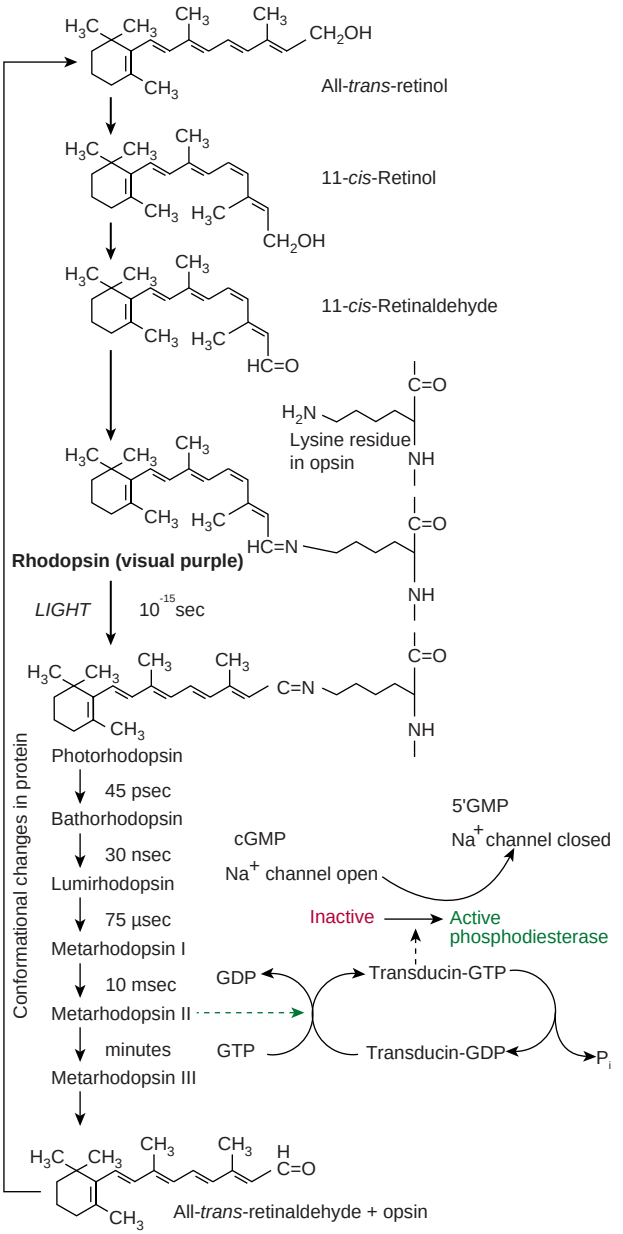

FIGURE 44–2 The role of retinaldehyde in the visual cycle.

Vitamin A Deficiency Is a Major Public Health Problem Worldwide

Vitamin A deficiency is the most important preventable cause of blindness. The earliest sign of deficiency is a loss of sensitivity to green light, followed by impairment to adapt to dim light, followed by night blindness. More prolonged deficiency leads to **xerophthalmia:** keratinization of the cornea and blindness. Vitamin A also has an important role in differentiation of immune system cells, and even mild deficiency leads to increased susceptibility to infectious diseases. Also, the synthesis of retinol binding protein is reduced in response to infection (it is a negative **acute phase protein**), decreasing the circulating concentration of the vitamin, and further impairing immune responses.

Vitamin A Is Toxic in Excess

There is only a limited capacity to metabolize vitamin A, and excessive intakes lead to accumulation beyond the capacity of binding proteins, so that unbound vitamin A causes tissue damage. Symptoms of toxicity affect the central nervous system (headache, nausea, ataxia, and anorexia, all associated with increased cerebrospinal fluid pressure); the liver (hepatomegaly with histologic changes and hyperlipidemia); calcium homeostasis (thickening of the long bones, hypercalcemia, and calcification of soft tissues); and the skin (excessive dryness, desquamation, and alopecia).

VITAMIN D IS REALLY A HORMONE

Vitamin D is not strictly a vitamin, since it can be synthesized in the skin, and under most conditions that is the major source of the vitamin. Only when sunlight exposure is inadequate is a dietary source required. Its main function is in the regulation of calcium absorption and homeostasis; most of its actions are mediated by way of nuclear receptors that regulate gene expression. It also has a role in regulating cell proliferation and differentiation. There is evidence that intakes considerably higher than are required to maintain calcium homeostasis reduce the risk of insulin resistance, obesity and the metabolic syndrome, as well as various cancers. Deficiency, leading to rickets in children and osteomalacia in adults, continues to be a problem in northern latitudes, where sunlight exposure is inadequate.

Vitamin D Is Synthesized in the Skin

7-Dehydrocholesterol (an intermediate in the synthesis of cholesterol that accumulates in the skin) undergoes a nonenzymic reaction on exposure to ultraviolet light, yielding previtamin D (Figure 44–3). This undergoes a further reaction over a period of hours to form cholecalciferol, which is absorbed into the bloodstream. In temperate climates, the plasma concentration of vitamin D is highest at the end of summer and lowest at the end of winter. Beyond latitudes about 40° north or south there is very little ultraviolet radiation of the appropriate wavelength in winter.

Vitamin D Is Metabolized to the Active Metabolite, Calcitriol, in Liver & Kidney

Cholecalciferol, either synthesized in the skin or from food, undergoes two hydroxylations to yield the active metabolite, 1,25-dihydroxyvitamin D or calcitriol (Figure 44–4). Ergocalciferol from fortified foods undergoes similar hydroxylation to yield ercalcitriol. In the liver, cholecalciferol is hydroxylated to form the 25-hydroxy-derivative, calcidiol. This is released into the circulation bound to a vitamin D binding globulin, which is the main storage form of the vitamin. In the kidney, calcidiol undergoes either 1-hydroxylation to yield the active metabolite 1,25-dihydroxy-vitamin D (calcitriol), or 24-hydroxylation to yield a probably inactive metabolite, 24,25-dihydroxyvitamin D (24-hydroxycalcidiol).

Vitamin D Metabolism Is Both Regulated by and Regulates Calcium Homeostasis

The main function of vitamin D is in the control of calcium homeostasis, and in turn, vitamin D metabolism is regulated by factors that respond to plasma concentrations of calcium and phosphate. Calcitriol acts to reduce its own synthesis by inducing the 24-hydroxylase and repressing the 1-hydroxylase in the kidney. The principal function of vitamin D is to maintain the plasma calcium concentration. Calcitriol achieves this in three ways: it increases intestinal absorption of calcium; it reduces excretion of calcium (by stimulating resorption in the distal renal tubules); and it mobilizes bone mineral. In addition, calcitriol is involved in insulin secretion, synthesis and secretion of parathyroid and thyroid hormones, inhibition of production of interleukin by activated T-lymphocytes and of immunoglobulin by activated B-lymphocytes, differentiation of monocyte precursor cells, and modulation of cell proliferation. In most of these actions, it acts like a steroid hormone, binding to nuclear receptors and enhancing gene expression, although it also has rapid effects on calcium transporters in the intestinal mucosa. For further details of the role of calcitriol in calcium homeostasis, see Chapter 47.

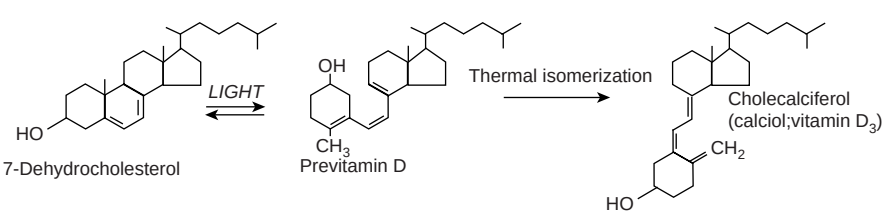

FIGURE 44–3 The synthesis of vitamin D in the skin.

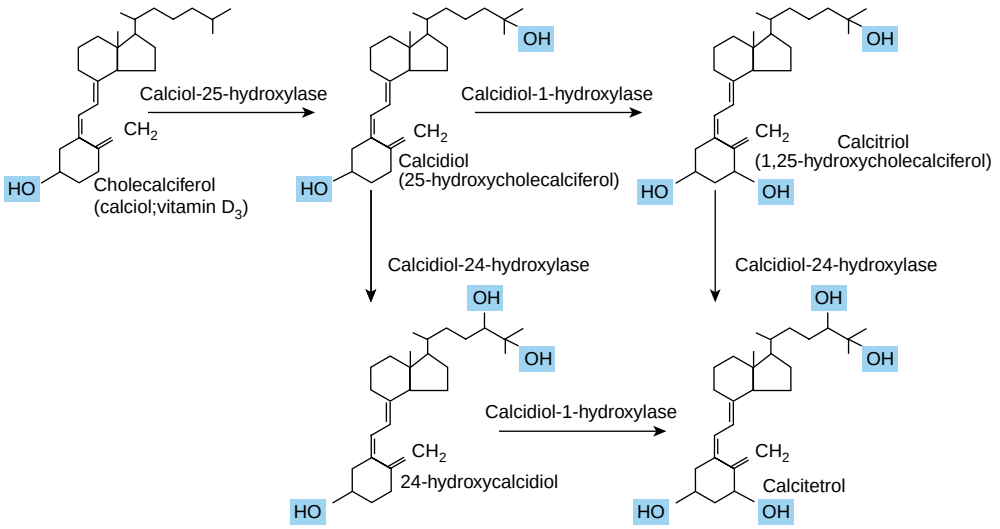

FIGURE 44–4 Metabolism of vitamin D.

Vitamin D Deficiency Affects Children & Adults

In the vitamin D deficiency disease **rickets,** the bones of children are undermineralized as a result of poor absorption of calcium. Similar problems occur as a result of deficiency during the adolescent growth spurt. **Osteomalacia** in adults results from the demineralization of bone, especially in women who have little exposure to sunlight, especially after several pregnancies. Although vitamin D is essential for prevention and treatment of osteomalacia in the elderly, there is little evidence that it is beneficial in treating **osteoporosis.**

Vitamin D Is Toxic in Excess

Some infants are sensitive to intakes of vitamin D as low as 50 μg/day, resulting in an elevated plasma concentration of calcium. This can lead to contraction of blood vessels, high blood pressure, and **calcinosis**—the calcification of soft tissues. Although excess dietary vitamin D is toxic, excessive exposure to sunlight does not lead to vitamin D poisoning, because there is a limited capacity to form the precursor, 7-dehydrocholesterol, and prolonged exposure of previtamin D to sunlight leads to formation of inactive compounds.

VITAMIN E DOES NOT HAVE A PRECISELY DEFINED METABOLIC FUNCTION

No unequivocal unique function for vitamin E has been defined. It acts as a lipid-soluble antioxidant in cell membranes, where many of its functions can be provided by synthetic antioxidants, and is important in maintaining the fluidity of cell membranes. It also has a (relatively poorly defined) role in cell signaling. Vitamin E is the generic descriptor for two families

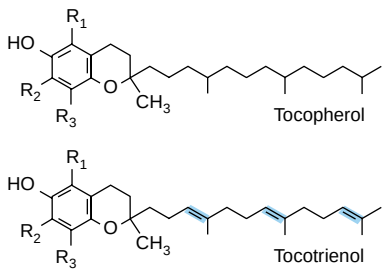

FIGURE 44–5 Vitamin E vitamers. In α-tocopherol and tocotrienol R_1, R_2, and R_3 are all —CH_3 groups. In the β-vitamers R_2 is H, in the γ-vitamers R_1 is H, and in the Δ-vitamers R_1 and R_2 are both H.

of compounds, the **tocopherols** and the **tocotrienols** (Figure 44–5). The different vitamers have different biologic potency; the most active is D-α-tocopherol, and it is usual to express vitamin E intake in terms of milligrams D-α-tocopherol equivalents. Synthetic DL-α-tocopherol does not have the same biologic potency as the naturally occurring compound.

Vitamin E Is the Major Lipid-Soluble Antioxidant in Cell Membranes and Plasma Lipoproteins

The main function of vitamin E is as a chain-breaking, free-radical-trapping antioxidant in cell membranes and plasma lipoproteins by reacting with the lipid peroxide radicals formed by peroxidation of polyunsaturated fatty acids (Chapter 45). The tocopheroxyl radical product is relatively unreactive, and ultimately forms nonradical compounds. Commonly, the tocopheroxyl radical is reduced back to tocopherol by reaction with vitamin C from plasma (Figure 44–6). The resultant monodehydroascorbate radical then undergoes enzymic or nonenzymic reaction to yield ascorbate and dehydroascorbate, neither of which is a radical.

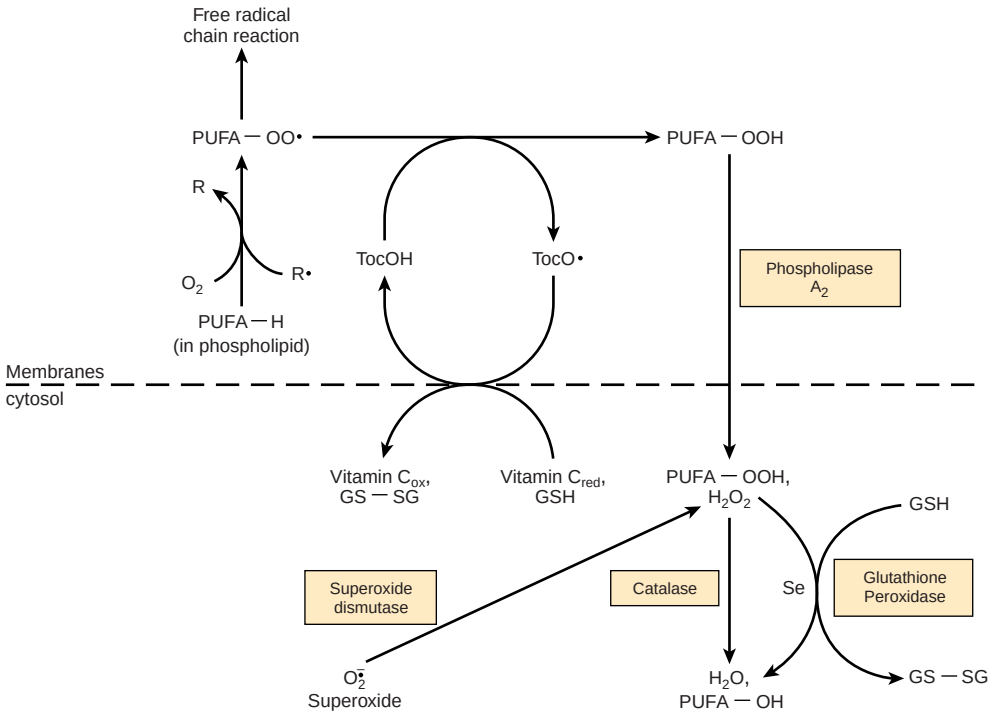

FIGURE 44–6 Interaction between antioxidants in the lipid phase (cell membranes) and the aqueous phase (cytosol). (R•, free radical; PUFA-OO•, peroxyl radical of polyunsaturated fatty acid in membrane phospholipid; PUFA-OOH, hydroxyperoxy polyunsaturated fatty acid in membrane phospholipid, released into the cytosol as hydroxyperoxy polyunsaturated fatty acid by the action of phospholipase A_2; PUFA-OH, hydroxy polyunsaturated fatty acid; Toc-OH vitamin E [α-tocopherol]; TocO•, tocopheroxyl radical; Se, selenium; SSH, reduced glutathione; GS-SG, oxidized glutathione, which is reduced to GSH after reaction with NADPH, catalyzed by glutathione reductase; PUFA-H, polyunsaturated fatty acid.)

Vitamin E Deficiency

In experimental animals, vitamin E deficiency results in resorption of fetuses and testicular atrophy. Dietary deficiency of vitamin E in humans is unknown, although patients with severe fat malabsorption, cystic fibrosis, and some forms of chronic liver disease suffer deficiency because they are unable to absorb the vitamin or transport it, exhibiting nerve and muscle membrane damage. Premature infants are born with inadequate reserves of the vitamin. The erythrocyte membranes are abnormally fragile as a result of peroxidation, leading to hemolytic anemia.

VITAMIN K IS REQUIRED FOR SYNTHESIS OF BLOOD CLOTTING PROTEINS

Vitamin K was discovered as a result of investigations into the cause of a bleeding disorder, hemorrhagic (sweet clover) disease of cattle and of chickens fed on a fat-free diet. The missing factor in the diet of the chickens was vitamin K, while the cattle feed contained **dicumarol,** an antagonist of the vitamin. Antagonists of vitamin K are used to reduce blood coagulation in patients at risk of thrombosis; the most widely used is **warfarin.**

Three compounds have the biological activity of vitamin K (Figure 44–7): **phylloquinone,** the normal dietary source, found in green vegetables; **menaquinones,** synthesized by intestinal bacteria, with differing lengths of side-chain; and **menadione** and menadiol diacetate, synthetic compounds that can be metabolized to phylloquinone. Menaquinones are absorbed to some extent, but it is not clear to what extent they are biologically active as it is possible to induce signs of vitamin K deficiency simply by feeding a phylloquinone-deficient diet, without inhibiting intestinal bacterial action.

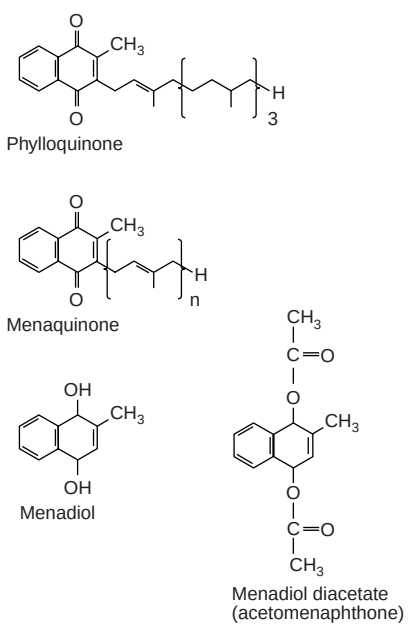

FIGURE 44–7 The vitamin K vitamers. Menadiol (or menadione) and menadiol diacetate are synthetic compounds that are converted to menaquinone in the liver.

Vitamin K Is the Coenzyme for Carboxylation of Glutamate in Postsynthetic Modification of Calcium-Binding Proteins

Vitamin K is the cofactor for the carboxylation of glutamate residues in the post-synthetic modification of proteins to form the unusual amino acid γ-carboxyglutamate (Gla) (Figure 44–8). Initially, vitamin K hydroquinone is oxidized to the epoxide, which activates a glutamate residue in the protein substrate to a carbanion, which reacts nonenzymically with carbon dioxide to form γ-carboxyglutamate. Vitamin K epoxide is reduced to the quinone by a warfarin-sensitive reductase, and the quinone is reduced to the active hydroquinone by either the same warfarin-sensitive reductase or a warfarin-insensitive quinone reductase. In the presence of warfarin, vitamin K epoxide cannot be reduced, but accumulates and is excreted. If enough vitamin K (as the quinone) is provided in the diet, it can be reduced to the active hydroquinone by the warfarin-insensitive enzyme, and carboxylation can continue, with stoichiometric utilization of vitamin K and excretion of the epoxide. A high dose of vitamin K is the antidote to an overdose of warfarin.

Prothrombin and several other proteins of the blood clotting system (Factors VII, IX, and X, and proteins C and S, Chapter 50) each contain 4–6 γ-carboxyglutamate residues. γ-Carboxyglutamate chelates calcium ions, and so permits the binding of the blood clotting proteins to membranes. In vitamin K deficiency, or in the presence of warfarin, an abnormal precursor of prothrombin (preprothrombin) containing little or no γ-carboxyglutamate, and incapable of chelating calcium, is released into the circulation.

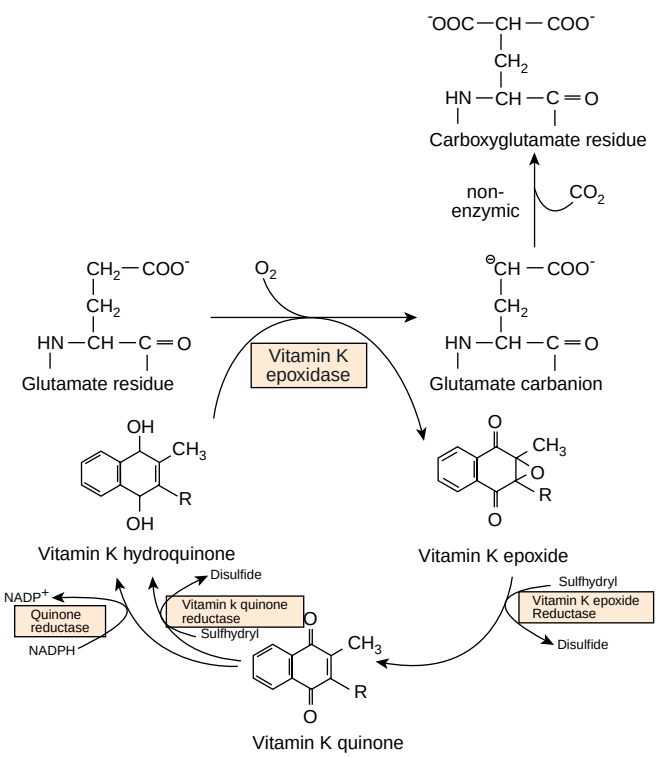

FIGURE 44–8 The role of vitamin K in the synthesis of γ-carboxyglutamate.

Vitamin K Is Also Important in Synthesis of Bone Calcium-Binding Proteins

Treatment of pregnant women with warfarin can lead to fetal bone abnormalities (fetal warfarin syndrome). Two proteins that contain γ-carboxyglutamate are present in bone, osteocalcin, and bone matrix Gla protein. Osteocalcin also contains hydroxyproline, so its synthesis is dependent on both vitamins K and C; in addition, its synthesis is induced by vitamin D. The release into the circulation of osteocalcin provides an index of vitamin D status.

WATER-SOLUBLE VITAMINS

VITAMIN B_1 (THIAMIN) HAS A KEY ROLE IN CARBOHYDRATE METABOLISM

Thiamin has a central role in energy-yielding metabolism, and especially the metabolism of carbohydrates (Figure 44–9). **Thiamin diphosphate** is the coenzyme for three multi-enzyme complexes that catalyze oxidative decarboxylation reactions: pyruvate dehydrogenase in carbohydrate metabolism (Chapter 17); α-ketoglutarate dehydrogenase in the citric acid cycle (Chapter 17); and the branched-chain keto-acid dehydrogenase involved in the metabolism of leucine, isoleucine, and valine (Chapter 29). In each case, the thiamin diphosphate provides a reactive carbon on the thiazole moiety that forms a carbanion, which then adds to the carbonyl group, eg, pyruvate. The addition compound is then decarboxylated, eliminating CO_2. Thiamin diphosphate is also the coenzyme for transketolase, in the pentose phosphate pathway (Chapter 21).

Thiamin triphosphate has a role in nerve conduction; it phosphorylates, and so activates, a chloride channel in the nerve membrane.

Thiamin Deficiency Affects the Nervous System & the Heart

Thiamin deficiency can result in three distinct syndromes: a chronic peripheral neuritis, **beriberi,** which may or may not be associated with **heart failure** and **edema;** acute pernicious (fulminating) beriberi (shoshin beriberi), in which heart failure and metabolic abnormalities predominate, without peripheral neuritis; and **Wernicke encephalopathy** with **Korsakoff psychosis,** which is associated especially with alcohol and narcotic abuse. The role of thiamin diphosphate in pyruvate dehydrogenase means that in deficiency there is impaired conversion of pyruvate to acetyl CoA. In subjects on a relatively high carbohydrate diet, this results in increased plasma

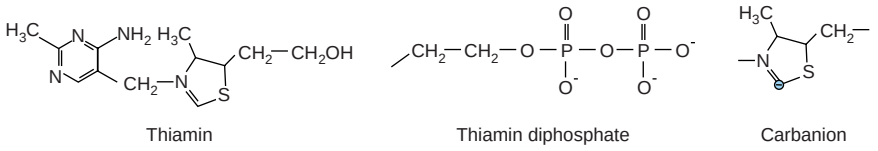

FIGURE 44–9 Thiamin, thiamin diphosphate, and the carbanion form.

concentrations of lactate and pyruvate, which may cause life-threatening **lactic acidosis.**

Thiamin Nutritional Status Can Be Assessed by Erythrocyte Transketolase Activation

The activation of apo-transketolase (the enzyme protein) in erythrocyte lysate by thiamin diphosphate added in vitro has become the accepted index of thiamin nutritional status.

VITAMIN B_2 (RIBOFLAVIN) HAS A CENTRAL ROLE IN ENERGY-YIELDING METABOLISM

Riboflavin provides the reactive moieties of the coenzymes **flavin mononucleotide (FMN)** and **flavin adenine dinucleotide (FAD)** (Figure 44–10). FMN is formed by ATP-dependent phosphorylation of riboflavin, whereas FAD is synthesized by further reaction with ATP in which its AMP moiety is transferred to FMN. The main dietary sources of riboflavin are milk and dairy products. In addition, because of its intense yellow color, riboflavin is widely used as a food additive.

Flavin Coenzymes Are Electron Carriers in Oxidoreduction Reactions

These include the mitochondrial respiratory chain, key enzymes in fatty acid and amino acid oxidation, and the citric acid cycle. Reoxidation of the reduced flavin in oxygenases and mixed-function oxidases proceeds by way of formation of the flavin radical and flavin hydroperoxide, with the intermediate generation of superoxide and perhydroxyl radicals and hydrogen peroxide. Because of this, flavin oxidases make a significant contribution to the total oxidant stress in the body (Chapter 45).

Riboflavin Deficiency Is Widespread but Not Fatal

Although riboflavin is centrally involved in lipid and carbohydrate metabolism, and deficiency occurs in many countries, it is not fatal, because there is very efficient conservation of tissue riboflavin. Riboflavin released by the catabolism of enzymes is rapidly incorporated into newly synthesized enzymes. Deficiency is characterized by cheilosis, desquamation and inflammation of the tongue, and a seborrheic dermatitis. Riboflavin nutritional status is assessed by measurement of the activation of erythrocyte glutathione reductase by FAD added in vitro.

NIACIN IS NOT STRICTLY A VITAMIN

Niacin was discovered as a nutrient during studies of **pellagra.** It is not strictly a vitamin since it can be synthesized in the body from the essential amino acid tryptophan. Two compounds, **nicotinic acid** and **nicotinamide,** have the biologic activity of niacin; its metabolic function is as the nicotinamide ring of the coenzymes **NAD** and **NADP** in oxidation/reduction reactions (Figure 44–11). Some 60 mg of tryptophan is equivalent to 1 mg of dietary niacin. The niacin content of foods is expressed as

$$\text{mg Niacin equivalents} = \text{mg Preformed niacin} + 1/60 \times \text{mg Tryptophan}$$

Because most of the niacin in cereals is biologically unavailable, this is discounted.

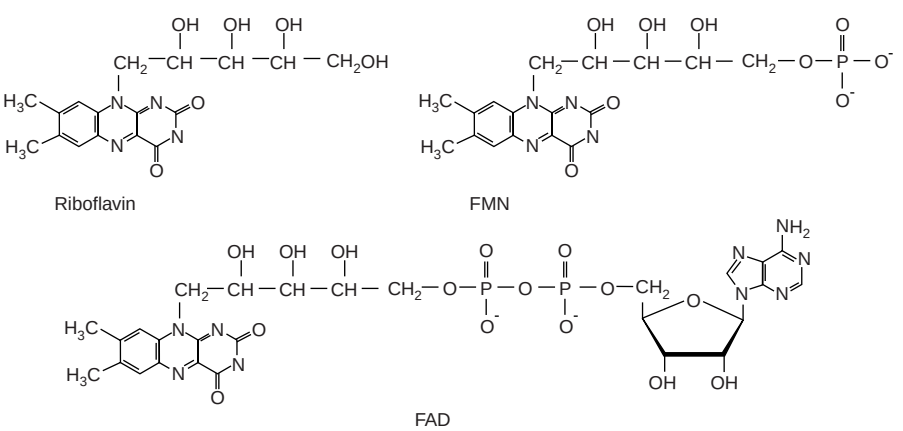

FIGURE 44–10 Riboflavin and the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD).

Niacin (nicotinic acid and nicotinamide) See also figure 7-2

FIGURE 44–11 Niacin (nicotinic acid and nicotinamide).

NAD Is the Source of ADP-Ribose

In addition to its coenzyme role, NAD is the source of ADP-ribose for the **ADP-ribosylation** of proteins and polyADP-ribosylation of nucleoproteins involved in the **DNA repair mechanism.** Cyclic ADP-ribose and nicotinic acid adenine dinucleotide, formed from NAD, act to increase intracellular calcium in response to neurotransmitters and hormones.

Pellagra Is Caused by Deficiency of Tryptophan & Niacin

Pellagra is characterized by a photosensitive dermatitis. As the condition progresses, there is dementia and possibly diarrhea. Untreated pellagra is fatal. Although the nutritional etiology of pellagra is well established, and tryptophan or niacin prevents or cures the disease, additional factors, including deficiency of riboflavin or vitamin B_6, both of which are required for synthesis of nicotinamide from tryptophan, may be important. In most outbreaks of pellagra, twice as many women as men are affected, probably the result of inhibition of tryptophan metabolism by estrogen metabolites.

Pellagra Can Occur as a Result of Disease Despite an Adequate Intake of Tryptophan & Niacin

A number of genetic diseases that result in defects of tryptophan metabolism are associated with the development of pellagra, despite an apparently adequate intake of both tryptophan and niacin. **Hartnup disease** is a rare genetic condition in which there is a defect of the membrane transport mechanism for tryptophan, resulting in large losses as a result of intestinal malabsorption and failure of the renal reabsorption mechanism. In **carcinoid syndrome,** there is metastasis of a primary liver tumor of enterochromaffin cells, which synthesize 5-hydroxytryptamine. Overproduction of 5-hydroxytryptamine may account for as much as 60% of the body's tryptophan metabolism, causing pellagra because of the diversion away from NAD synthesis.

Niacin Is Toxic in Excess

Nicotinic acid has been used to treat hyperlipidemia when of the order of 1–6 g/day are required, causing dilatation of blood vessels and flushing, along with skin irritation. Intakes of both nicotinic acid and nicotinamide in excess of 500 mg/day also cause liver damage.

VITAMIN B_6 IS IMPORTANT IN AMINO ACID & GLYCOGEN METABOLISM & IN STEROID HORMONE ACTION

Six compounds have vitamin B_6 activity (Figure 44–12): **pyridoxine, pyridoxal, pyridoxamine,** and their 5′-phosphates. The active coenzyme is pyridoxal 5′-phosphate. Some 80% of the body's total vitamin B_6 is pyridoxal phosphate in muscle, mostly associated with glycogen phosphorylase. This is not available in deficiency, but is released in starvation, when glycogen reserves become depleted, and is then available, especially in liver and kidney, to meet increased requirement for gluconeogenesis from amino acids.

Vitamin B_6 Has Several Roles in Metabolism

Pyridoxal phosphate is a coenzyme for many enzymes involved in amino acid metabolism, especially transamination and decarboxylation. It is also the cofactor of glycogen phosphorylase, where the phosphate group is catalytically important. In addition, B_6 is important in steroid hormone action. Pyridoxal phosphate removes the hormone-receptor complex from DNA binding, terminating the action of the hormones. In vitamin B_6 deficiency, there is increased sensitivity to the actions of low concentrations of estrogens, androgens, cortisol, and vitamin D.

Vitamin B_6 Deficiency Is Rare

Although clinical deficiency disease is rare, there is evidence that a significant proportion of the population have marginal vitamin B_6 status. Moderate deficiency results in abnormalities of tryptophan and methionine metabolism. Increased sensitiv-

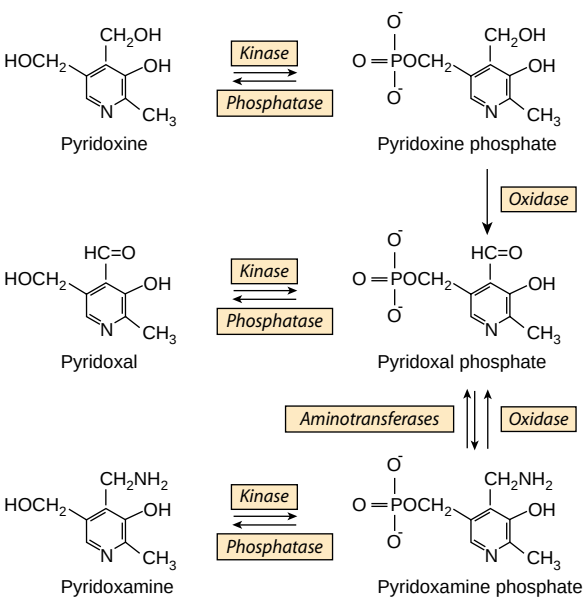

FIGURE 44–12 Interconversion of the vitamin B_6 vitamers.

ity to steroid hormone action may be important in the development of **hormone-dependent cancer** of the breast, uterus, and prostate, and vitamin B_6 status may affect the prognosis.

Vitamin B_6 Status Is Assessed by Assaying Erythrocyte Transaminases

The most widely used method of assessing vitamin B_6 status is by the activation of erythrocyte transaminases by pyridoxal phosphate added in vitro, expressed as the activation coefficient.

In Excess, Vitamin B_6 Causes Sensory Neuropathy

The development of sensory neuropathy has been reported in patients taking 2–7 g of pyridoxine per day for a variety of reasons (there is some slight evidence that it is effective in treating **premenstrual syndrome**). There was some residual damage after withdrawal of these high doses; other reports suggest that intakes in excess of 200 mg/d are associated with neurologic damage.

VITAMIN B_{12} IS FOUND ONLY IN FOODS OF ANIMAL ORIGIN

The term "vitamin B_{12}" is used as a generic descriptor for the **cobalamins**—those **corrinoids** (cobalt-containing compounds possessing the corrin ring) having the biologic activity of the vitamin (Figure 44–13). Some corrinoids that are growth factors for microorganisms not only have no vitamin B_{12} activity, but may also be anti-metabolites of the vitamin. Although it is synthesized exclusively by microorganisms, for practical purposes vitamin B_{12} is found only in foods of animal origin, there being no plant sources of this vitamin. This means that strict vegetarians (vegans) are at risk of developing B_{12} deficiency. The small amounts of the vitamin formed by bacteria on the surface of fruits may be adequate to meet requirements, but preparations of vitamin B_{12} made by bacterial fermentation are available.

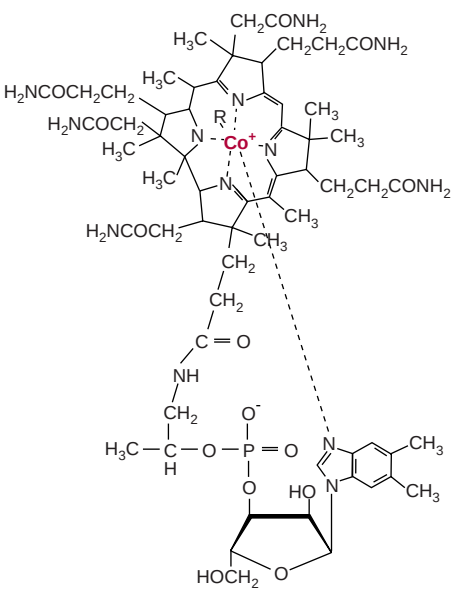

FIGURE 44–13 Vitamin B_{12}. Four coordination sites on the central cobalt atom are chelated by the nitrogen atoms of the corrin ring, and one by the nitrogen of the dimethylbenzimidazole nucleotide. The sixth coordination site may be occupied by: CN^- (cyanocobalamin), OH^- (hydroxocobalamin), H_2O (aquocobalamin, —CH_3 (methyl cobalamin) or 5′-deoxyadenosine (adenosylcobalamin).

Vitamin B_{12} Absorption Requires Two Binding Proteins

Vitamin B_{12} is absorbed bound to **intrinsic factor,** a small glycoprotein secreted by the parietal cells of the gastric mucosa. Gastric acid and pepsin release the vitamin from protein binding in food and make it available to bind to **cobalophilin,** a binding protein secreted in the saliva. In the duodenum, cobalophilin is hydrolyzed, releasing the vitamin for binding to intrinsic factor. **Pancreatic insufficiency** can therefore be a factor in the development of vitamin B_{12} deficiency, resulting in the excretion of cobalophilin-bound vitamin B_{12}. Intrinsic factor binds only the active vitamin B_{12} vitamers and not other corrinoids. Vitamin B_{12} is absorbed from the distal third of the ileum via receptors that bind the intrinsic factor-vitamin B_{12} complex, but not free intrinsic factor or free vitamin.

There Are Three Vitamin B_{12}–Dependent Enzymes

Methylmalonyl CoA mutase, leucine aminomutase, and **methionine synthase** (Figure 44–14) are vitamin B_{12}–dependent enzymes. Methylmalonyl CoA is formed as an intermediate in the catabolism of valine and by the carboxylation of propionyl CoA arising in the catabolism of isoleucine, cholesterol, and, rarely, fatty acids with an odd number of carbon atoms or directly from propionate, a major product of microbial fermentation in the rumen. It undergoes a vitamin B_{12}–dependent rearrangement to succinyl CoA, catalyzed by methylmalonyl CoA

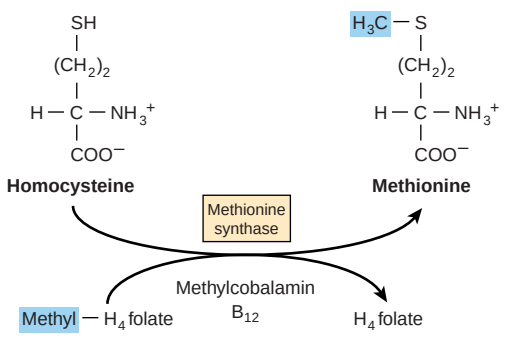

FIGURE 44–14 Homocysteine and the "folate trap." Vitamin B_{12} deficiency leads to impairment of methionine synthase, resulting in accumulation of homocysteine and trapping folate as methyltetrahydrofolate.

mutase (Figure 20–2). The activity of this enzyme is greatly reduced in vitamin B_{12} deficiency, leading to an accumulation of methylmalonyl CoA and urinary excretion of methylmalonic acid, which provides a means of assessing vitamin B_{12} nutritional status.

Vitamin B_{12} Deficiency Causes Pernicious Anemia

Pernicious anemia arises when vitamin B_{12} deficiency impairs the metabolism of folic acid, leading to functional folate deficiency that disturbs erythropoiesis, causing immature precursors of erythrocytes to be released into the circulation (megaloblastic anemia). The most common cause of pernicious anemia is failure of the absorption of vitamin B_{12} rather than dietary deficiency. This can be the result of failure of intrinsic factor secretion caused by autoimmune disease affecting parietal cells or from production of anti-intrinsic factor antibodies. There is irreversible degeneration of the spinal cord in pernicious anemia, as a result of failure of methylation of one arginine residue on myelin basic protein. This is the result of methionine deficiency in the central nervous system, rather than secondary folate deficiency.

THERE ARE MULTIPLE FORMS OF FOLATE IN THE DIET

The active form of folic acid (pteroyl glutamate) is tetrahydrofolate (Figure 44–15). The folates in foods may have up to seven additional glutamate residues linked by γ-peptide bonds. In addition, all of the one-carbon substituted folates in Figure 44–15 may also be present in foods. The extent to which the different forms of folate can be absorbed varies, and folate intakes are calculated as dietary folate equivalents – the sum of μg food folates + 1.7 × μg of folic acid (used in food enrichment).

Tetrahydrofolate Is a Carrier of One-Carbon Units

Tetrahydrofolate can carry one-carbon fragments attached to *N*-5 (formyl, formimino, or methyl groups), *N*-10 (formyl) or bridging *N*-5–*N*-10 (methylene or methenyl groups). 5-Formyl-tetrahydrofolate is more stable than folate, and is therefore used pharmaceutically (known as **folinic acid**), and the synthetic (racemic) compound **(leucovorin).** The major point of entry for one-carbon fragments into substituted folates is methylene-tetrahydrofolate (Figure 44–16), which is formed by the reaction of glycine, serine, and choline with tetrahydrofolate. Serine is the most important source of substituted folates for biosynthetic reactions, and the activity of serine hydroxymethyltransferase is regulated by the state of folate substitution and the availability of folate. The reaction is reversible, and in liver it can form serine from glycine as a substrate for gluconeogenesis. Methylene-, methenyl-, and

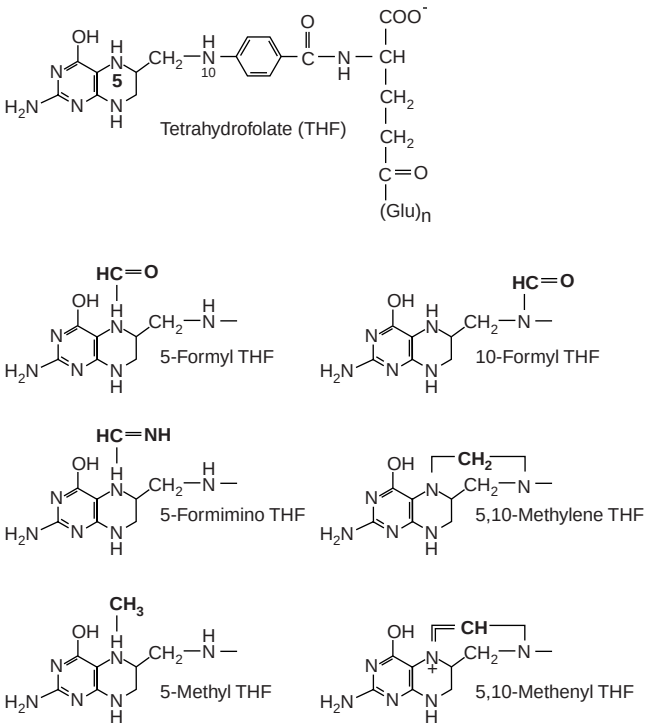

FIGURE 44–15 Tetrahydrofolic acid and the one-carbon substituted folates.

10-formyl-tetrahydrofolates are interconvertible. When one-carbon folates are not required, the oxidation of formyl-tetrahydrofolate to yield carbon dioxide provides a means of maintaining a pool of free folate.

Inhibitors of Folate Metabolism Provide Cancer Chemotherapy, Antibacterial, & Antimalarial Drugs

The methylation of deoxyuridine monophosphate (dUMP) to thymidine monophosphate (TMP), catalyzed by thymidylate synthase, is essential for the synthesis of DNA. The one-carbon fragment of methylene-tetrahydrofolate is reduced to a methyl group with release of dihydrofolate, which is then reduced back to tetrahydrofolate by **dihydrofolate reductase.** Thymidylate synthase and dihydrofolate reductase are especially active in tissues with a high rate of cell division. **Methotrexate,** an analog of 10-methyl-tetrahydrofolate, inhibits dihydrofolate reductase and has been exploited as an anti-cancer drug. The dihydrofolate reductases of some bacteria and parasites differ from the human enzyme; inhibitors of these enzymes can be used as antibacterial drugs (eg, **trimethoprim**) and antimalarial drugs (eg, **pyrimethamine**).

Vitamin B_{12} Deficiency Causes Functional Folate Deficiency—the "Folate Trap"

When acting as a methyl donor, *S*-adenosyl methionine forms homocysteine, which may be remethylated by methyl-tetrahy-

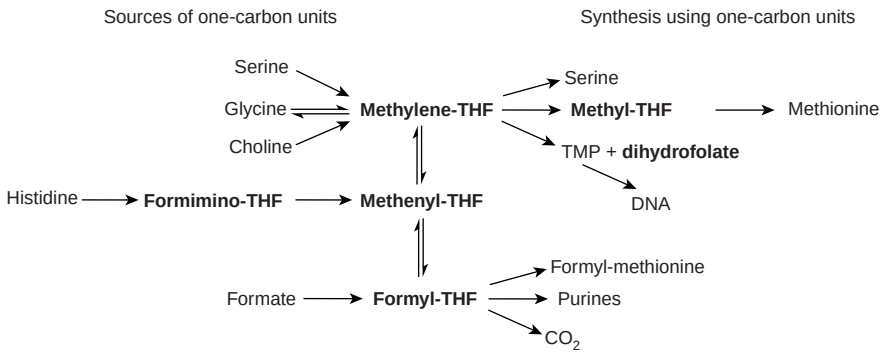

FIGURE 44–16 Sources and utilization of one-carbon substituted folates.

drofolate catalyzed by methionine synthase, a vitamin B_{12}–dependent enzyme (Figure 44–14). As the reduction of methylene-tetrahydrofolate to methyl-tetrahydrofolate is irreversible and the major source of tetrahydrofolate for tissues is methyltetrahydrofolate, the role of methionine synthase is vital, and provides a link between the functions of folate and vitamin B_{12}. Impairment of methionine synthase in vitamin B_{12} deficiency results in the accumulation of methyltetrahydrofolate—the "folate trap." There is therefore functional deficiency of folate, secondary to the deficiency of vitamin B_{12}.

Folate Deficiency Causes Megaloblastic Anemia

Deficiency of folic acid itself or deficiency of vitamin B_{12}, which leads to functional folic acid deficiency, affects cells that are dividing rapidly because they have a large requirement for thymidine for DNA synthesis. Clinically, this affects the bone marrow, leading to megaloblastic anemia.

Folic Acid Supplements Reduce the Risk of Neural Tube Defects & Hyperhomocysteinemia, & May Reduce the Incidence of Cardiovascular Disease & Some Cancers

Supplements of 400 μg/day of folate begun before conception result in a significant reduction in the incidence of **spina bifida** and other **neural tube defects.** Because of this, there is mandatory enrichment of flour with folic acid in many countries. Elevated blood homocysteine is a significant risk factor for **atherosclerosis, thrombosis,** and **hypertension.** The condition is the result of an impaired ability to form methyltetrahydrofolate by methylene-tetrahydrofolate reductase, causing functional folate deficiency, resulting in failure to remethylate homocysteine to methionine. People with an abnormal variant of methylene-tetrahydrofolate reductase that occurs in 5–10% of the population do not develop hyperhomocysteinemia if they have a relatively high intake of folate, but it is not yet known whether this affects the incidence of cardiovascular disease.

There is also evidence that low folate status results in impaired methylation of CpG islands in DNA, which is a factor in the development of colorectal and other cancers. A number of studies suggest that folate supplementation or food enrichment may reduce the risk of developing some cancers.

Folate Enrichment of Foods May Put Some People at Risk

Folate supplements will rectify the megaloblastic anemia of vitamin B_{12} deficiency but may hasten the development of the (irreversible) nerve damage found in vitamin B_{12} deficiency. There is also antagonism between folic acid and the anticonvulsants used in the treatment of epilepsy.

DIETARY BIOTIN DEFICIENCY IS UNKNOWN

The structures of biotin, biocytin, and carboxybiotin (the active metabolic intermediate) are shown in Figure 44–17. Biotin is widely distributed in many foods as biocytin (ε-aminobiotinyllysine), which is released on proteolysis. It is synthe-

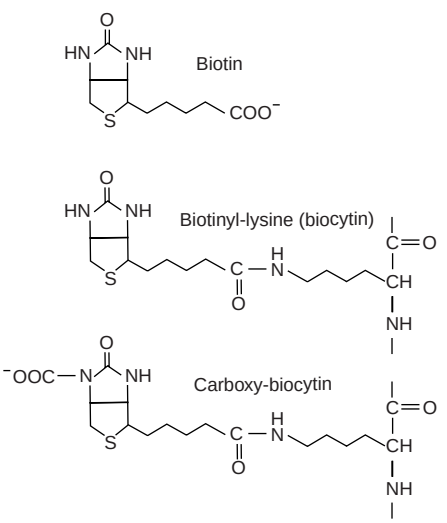

FIGURE 44–17 Biotin, biocytin, and carboxy-biocytin.

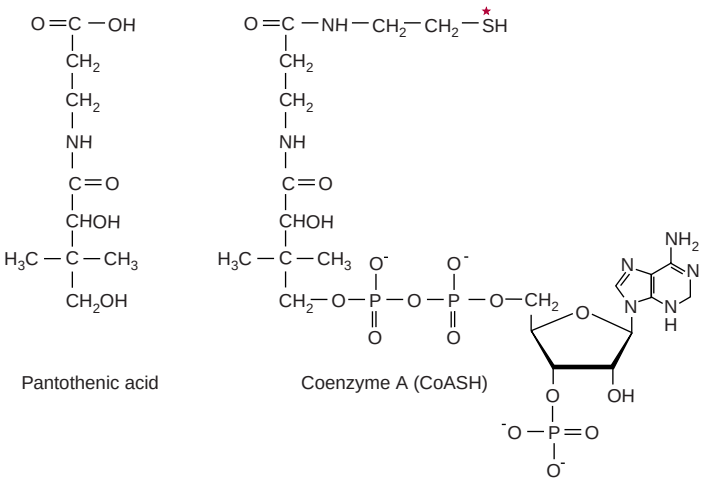

FIGURE 44–18 Pantothenic acid and coenzyme A. Asterisk shows site of acylation by fatty acids.

sized by intestinal flora in excess of requirements. Deficiency is unknown, except among people maintained for many months on total parenteral nutrition, and a very small number who eat abnormally large amounts of uncooked egg white, which contains avidin, a protein that binds biotin and renders it unavailable for absorption.

Biotin Is a Coenzyme of Carboxylase Enzymes

Biotin functions to transfer carbon dioxide in a small number of reactions: acetyl-CoA carboxylase (Figure 23–1), pyruvate carboxylase (Figure 20–1), propionyl-CoA carboxylase (Figure 20–2), and methylcrotonyl-CoA carboxylase. A holocarboxylase synthetase catalyzes the transfer of biotin onto a lysine residue of the apo-enzyme to form the biocytin residue of the holoenzyme. The reactive intermediate is 1-*N*-carboxy-biocytin, formed from bicarbonate in an ATP-dependent reaction. The carboxy group is then transferred to the substrate for carboxylation.

Biotin also has a role in regulation of the cell cycle, acting to biotinylate key nuclear proteins.

AS PART OF CoA & ACP, PANTOTHENIC ACID ACTS AS A CARRIER OF ACYL GROUPS

Pantothenic acid has a central role in acyl group metabolism when acting as the pantetheine functional moiety of coenzyme A or acyl carrier protein (ACP) (Figure 44–18). The pantetheine moiety is formed after combination of pantothenate with cysteine, which provides the–SH prosthetic group of CoA and ACP. CoA takes part in reactions of the citric acid cycle (Chapter 17), fatty acid oxidation (Chapter 22), acetylations and cholesterol synthesis (Chapter 26). ACP participates in fatty acid synthesis (Chapter 23). The vitamin is widely distributed in all food-stuffs, and deficiency has not been unequivocally reported in humans except in specific depletion studies.

ASCORBIC ACID IS A VITAMIN FOR ONLY SOME SPECIES

Vitamin C (Figure 44–19) is a vitamin for humans and other primates, the guinea pig, bats, passeriform birds, and most fishes and invertebrates; other animals synthesize it as an intermediate in the uronic acid pathway of glucose metabolism (Figure 21-4). In those species for which it is a vitamin, there is a block in the pathway as a result of absence of gulonolactone oxidase. Both ascorbic acid and dehydroascorbic acid have vitamin activity.

Vitamin C Is the Coenzyme for Two Groups of Hydroxylases

Ascorbic acid has specific roles in the copper-containing hydroxylases and the α-ketoglutarate-linked iron-containing hydroxylases. It also increases the activity of a number of other enzymes in vitro, although this is a nonspecific reducing action. In addition, it has a number of nonenzymic effects as a result of its action as a reducing agent and oxygen radical quencher (Chapter 45).

Dopamine β-hydroxylase is a copper-containing enzyme involved in the synthesis of the catecholamines (norepinephrine and epinephrine), from tyrosine in the adrenal medulla and central nervous system. During hydroxylation the Cu^{+} is oxidized to Cu^{2+}; reduction back to Cu^{+} specifically requires ascorbate, which is oxidized to monodehydroascorbate.

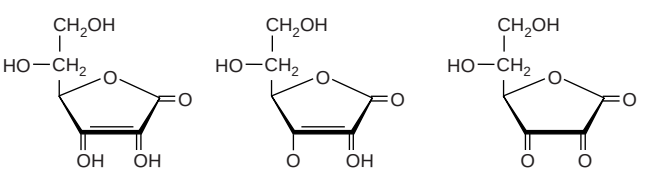

FIGURE 44–19 Vitamin C.

A number of peptide hormones have a carboxy terminal amide that is derived from a terminal glycine residue. This glycine is hydroxylated on the α-carbon by a copper-containing enzyme, **peptidylglycine hydroxylase,** which, again, requires ascorbate for reduction of Cu^{2+}.

A number of iron-containing, ascorbate-requiring hydroxylases share a common reaction mechanism, in which hydroxylation of the substrate is linked to decarboxylation of α-ketoglutarate. Many of these enzymes are involved in the modification of precursor proteins. **Proline** and **lysine hydroxylases** are required for the postsynthetic modification of **procollagen** to **collagen,** and proline hydroxylase is also required in formation of **osteocalcin** and the C1q component of **complement.** Aspartate β-hydroxylase is required for the postsynthetic modification of the precursor of protein C, the vitamin K–dependent protease that hydrolyzes activated factor V in the blood-clotting cascade (Chapter 50). Trimethyllysine and γ-butyrobetaine hydroxylases are required for the synthesis of carnitine.

Vitamin C Deficiency Causes Scurvy

Signs of vitamin C deficiency include skin changes, fragility of blood capillaries, gum decay, tooth loss, and bone fracture, many of which can be attributed to deficient collagen synthesis.

There May Be Benefits from Higher Intakes of Vitamin C

At intakes above about 100 mg/day, the body's capacity to metabolize vitamin C is saturated, and any further intake is excreted in the urine. However, in addition to its other roles, vitamin C enhances the absorption of iron, and this depends on the presence of the vitamin in the gut. Therefore, increased intakes may be beneficial. There is very little good evidence that high doses of vitamin C prevent the common cold, although they may reduce the duration and severity of symptoms.

MINERALS ARE REQUIRED FOR BOTH PHYSIOLOGIC & BIOCHEMICAL FUNCTIONS

Many of the essential minerals (Table 44–2) are widely distributed in foods, and most people eating a mixed diet are likely to receive adequate intakes. The amounts required vary from grams per day for sodium and calcium, through milligrams per day (eg, iron, zinc), to micrograms per day for the trace elements. In general, mineral deficiencies occur when foods come from one region where the soil may be deficient in some minerals (eg, iodine and selenium, deficiencies of both of which occur in many areas of the world); when foods come from a variety of regions, mineral deficiency is less likely to occur. However, iron deficiency is a general problem, because if iron losses from the body are relatively high (eg, from heavy menstrual blood loss), it is difficult to achieve an adequate intake to replace losses. Foods grown on soil containing high levels of selenium cause toxicity, and excessive intakes of sodium cause hypertension in susceptible people.

TABLE 44–2 Classification of Minerals According to Their Function

Function	Mineral
Structural function	Calcium, magnesium, phosphate
Involved in membrane function	Sodium, potassium
Function as prosthetic groups in enzymes	Cobalt, copper, iron, molybdenum, selenium, zinc
Regulatory role or role in hormone action	Calcium, chromium, iodine, magnesium, manganese, sodium, potassium
Known to be essential, but function unknown	Silicon, vanadium, nickel, tin
Have effects in the body, but essentiality is not established	Fluoride, lithium
May occur in foods and known to be toxic in excess	Aluminum, arsenic, antimony, boron, bromine, cadmium, cesium, germanium, lead, mercury, silver, strontium

SUMMARY

- Vitamins are organic nutrients with essential metabolic functions, generally required in small amounts in the diet because they cannot be synthesized by the body. The lipid-soluble vitamins (A, D, E, and K) are hydrophobic molecules requiring normal fat absorption for their absorption and the avoidance of deficiency.
- Vitamin A (retinol), present in meat, and the provitamin (β-carotene), found in plants, form retinaldehyde, utilized in vision, and retinoic acid, which acts in the control of gene expression.
- Vitamin D is a steroid prohormone yielding the active hormone calcitriol, which regulates calcium and phosphate metabolism; deficiency leads to rickets and osteomalacia.
- Vitamin E (tocopherol) is the most important antioxidant in the body, acting in the lipid phase of membranes protecting against the effects of free radicals.
- Vitamin K functions as cofactor of a carboxylase that acts on glutamate residues of precursor proteins of clotting factors and bone proteins to enable them to chelate calcium.
- The water-soluble vitamins of the B complex act as enzyme cofactors. Thiamin is a cofactor in oxidative decarboxylation of α-keto acids and of transketolase in the pentose phosphate pathway. Riboflavin and niacin are important cofactors in oxidoreduction reactions, present in flavoprotein enzymes and in NAD and NADP, respectively.
- Pantothenic acid is present in coenzyme A and acyl carrier protein, which act as carriers for acyl groups in metabolic reactions.

- Vitamin B_6 as pyridoxal phosphate, is the coenzyme for several enzymes of amino acid metabolism, including the transaminases, and of glycogen phosphorylase. Biotin is the coenzyme for several carboxylase enzymes.
- Vitamin B_{12} and folate provide one-carbon residues for DNA synthesis and other reactions; deficiency results in megaloblastic anemia.
- Vitamin C is a water-soluble antioxidant that maintains vitamin E and many metal cofactors in the reduced state.
- Inorganic mineral elements that have a function in the body must be provided in the diet. When intake is insufficient, deficiency may develop, and excessive intakes may be toxic.

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CHAPTER 45

Free Radicals and Antioxidant Nutrients

David A. Bender, PhD

BIOMEDICAL IMPORTANCE

Free radicals are formed in the body under normal conditions. They cause damage to nucleic acids, proteins, and lipids in cell membranes and plasma lipoproteins. This can cause **cancer**, **atherosclerosis** and **coronary artery disease**, and **autoimmune diseases**. Epidemiological and laboratory studies have identified a number of protective **antioxidant nutrients**: selenium, vitamins C and E, β-carotene, and a variety of polyphenolic compounds derived from plant foods. Many people take supplements of one or more antioxidant nutrients. However, intervention trials show little benefit of antioxidant supplements except among people who were initially deficient, and many trials of β-carotene and vitamin E have shown increased mortality among those taking the supplements.

Free Radical Reactions Are Self-Perpetuating Chain Reactions

Free radicals are highly reactive molecular species with an unpaired electron; they persist for only a very short time (of the order of 10^{-9}–10^{-12} sec) before they collide with another molecule and either abstract or donate an electron in order to achieve stability. In so doing, they generate a new radical from the molecule with which they collided. The only way in which a free radical can be quenched, so terminating this chain reaction, is if two radicals react together, when the unpaired electrons can become paired in one or other of the parent molecules. This is a rare occurrence, because of the very short half-life of an individual radical and the very low concentrations of radicals in tissues.

The most damaging radicals in biological systems are oxygen radicals (sometimes called reactive oxygen species)—especially superoxide, $^{\bullet}O_2^-$, hydroxyl, $^{\bullet}OH$, and perhydroxyl, $^{\bullet}O_2H$. Tissue damage caused by oxygen radicals is often called oxidative damage, and factors that protect against oxygen radical damage are known as antioxidants.

Radicals Can Damage DNA, Lipids, and Proteins

Interaction of radicals with bases in DNA can lead to chemical changes that, if not repaired (Chapter 35) may be inherited in daughter cells. Radical damage to unsaturated fatty acids in cell membranes and plasma lipoproteins leads to the formation of lipid peroxides, then highly reactive dialdehydes that can chemically modify proteins and nucleic acid bases. Proteins are also subject to direct chemical modification by interaction with radicals. Oxidative damage to tyrosine residues in proteins can lead to the formation of dihydroxyphenylalanine that can undergo non-enzymic reactions leading to further formation of oxygen radicals.

The total body radical burden can be estimated by measuring the products of lipid peroxidation. Lipid peroxides can be measured by the ferrous oxidation in xylenol orange (FOX) assay. Under acidic conditions, they oxidize Fe^{2+} to Fe^{3+}, which forms a chromophore with xylenol orange. The dialdehydes formed from lipid peroxides can be measured by reaction with thiobarbituric acid, when they form a red fluorescent adduct—the results of this assay are generally reported as total thiobarbituric acid reactive substances, TBARS. Peroxidation of n-6 polyunsaturated fatty acids leads to the formation of pentane, and of n-3 polyunsaturated fatty acids to ethane, both of which can be measured in exhaled air.

Radical Damage May Cause Mutations, Cancer, Autoimmune Disease, and Atherosclerosis

Radical damage to DNA in germ-line cells in ovaries and testes can lead to heritable mutations; in somatic cells the result may be initiation of cancer. The dialdehydes formed as a result of radical-induced lipid peroxidation in cell membranes can also modify bases in DNA.

Chemical modification of amino acids in proteins, either by direct radical action or as a result of reaction with the products of radical-induced lipid peroxidation, leads to proteins that are recognized as non-self by the immune system. The resultant antibodies will also cross-react with normal tissue proteins, so initiating autoimmune disease.

Chemical modification of the proteins or lipids in plasma low density lipoprotein (LDL) leads to abnormal LDL that is not recognized by the liver LDL receptors, and so is not cleared by the liver. The modified LDL is taken up by macrophage scavenger receptors. Lipid engorged macrophages in-

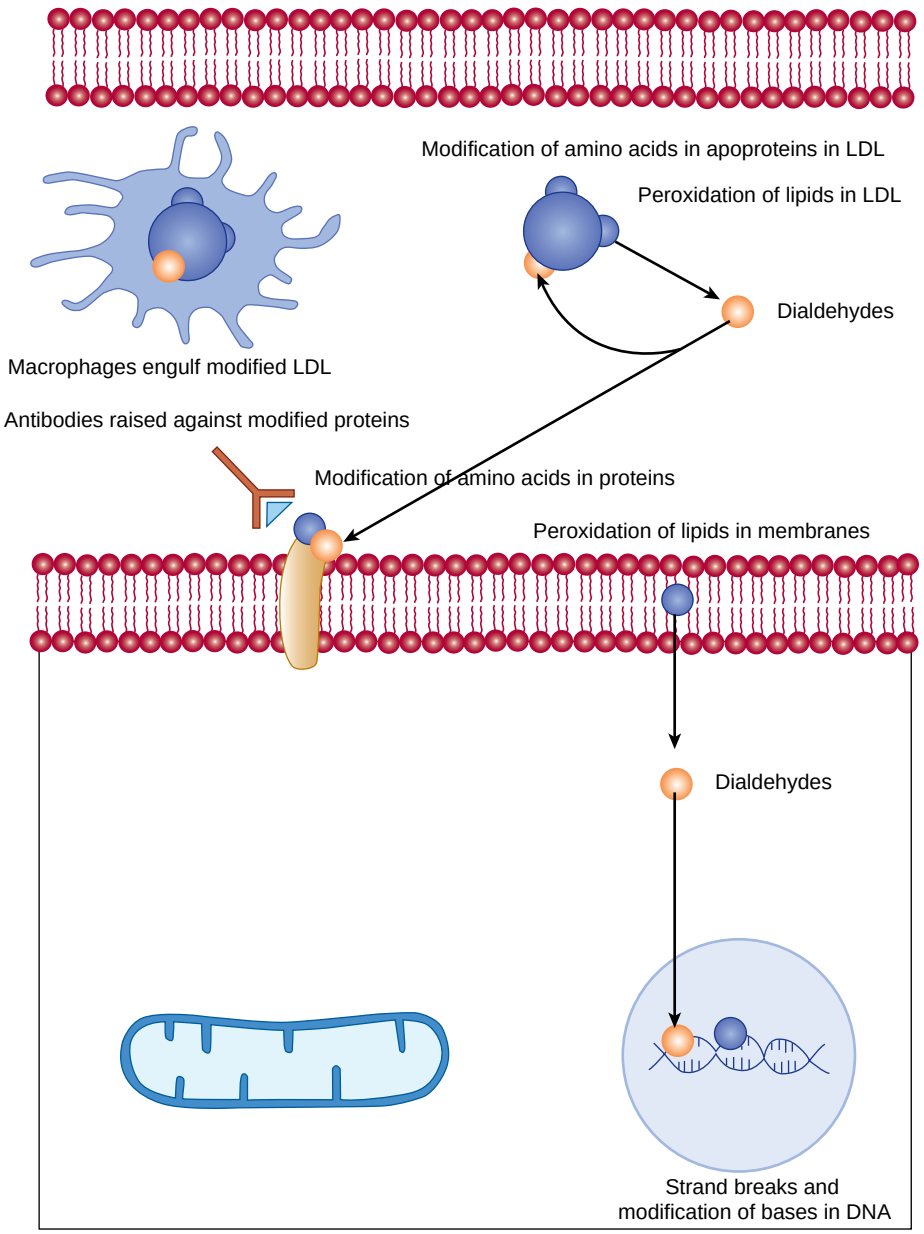

FIGURE 45–1 Tissue damage by radicals.

filtrate under blood vessel endothelium (especially when there is already some damage to the endothelium), and are killed by the high content of unesterified cholesterol they have accumulated. This occurs in the development of atherosclerotic plaques which, in extreme cases, can more or less completely occlude a blood vessel.

There Are Multiple Sources of Oxygen Radicals in the Body

Ionizing radiation (x-rays and UV) can lyse water, leading to the formation of hydroxyl radicals. Transition metal ions, including Cu^{+}, Co^{2+}, Ni^{2+}, and Fe^{2+} can react non-enzymically with oxygen or hydrogen peroxide, again leading to the formation of hydroxyl radicals. Nitric oxide (the endothelium-derived relaxation factor) is itself a radical, and, more importantly, can react with superoxide to yield peroxynitrite, which decays to form hydroxyl radicals.

The respiratory burst of activated macrophages (Chapter 52) is increased utilisation of glucose via the pentose phosphate pathway (Chapter 21) to reduce $NADP^{+}$ to NADPH, and increased utilisation of oxygen to oxidise NADPH to produce oxygen (and halogen) radicals as cytotoxic agents to kill phagocytosed microorganisms. The respiratory burst oxidase (NADPH oxidase) is a flavoprotein that reduces oxygen to superoxide: $NADPH + 2O_2 \rightarrow NADP^{+} + 2O_2^{\cdot-} + 2H^{+}$. Plasma markers of radical damage to lipids increase considerably in response to even a mild infection.

The oxidation of reduced flavin coenzymes in the mitochondrial (Chapter 13) and microsomal electron transport

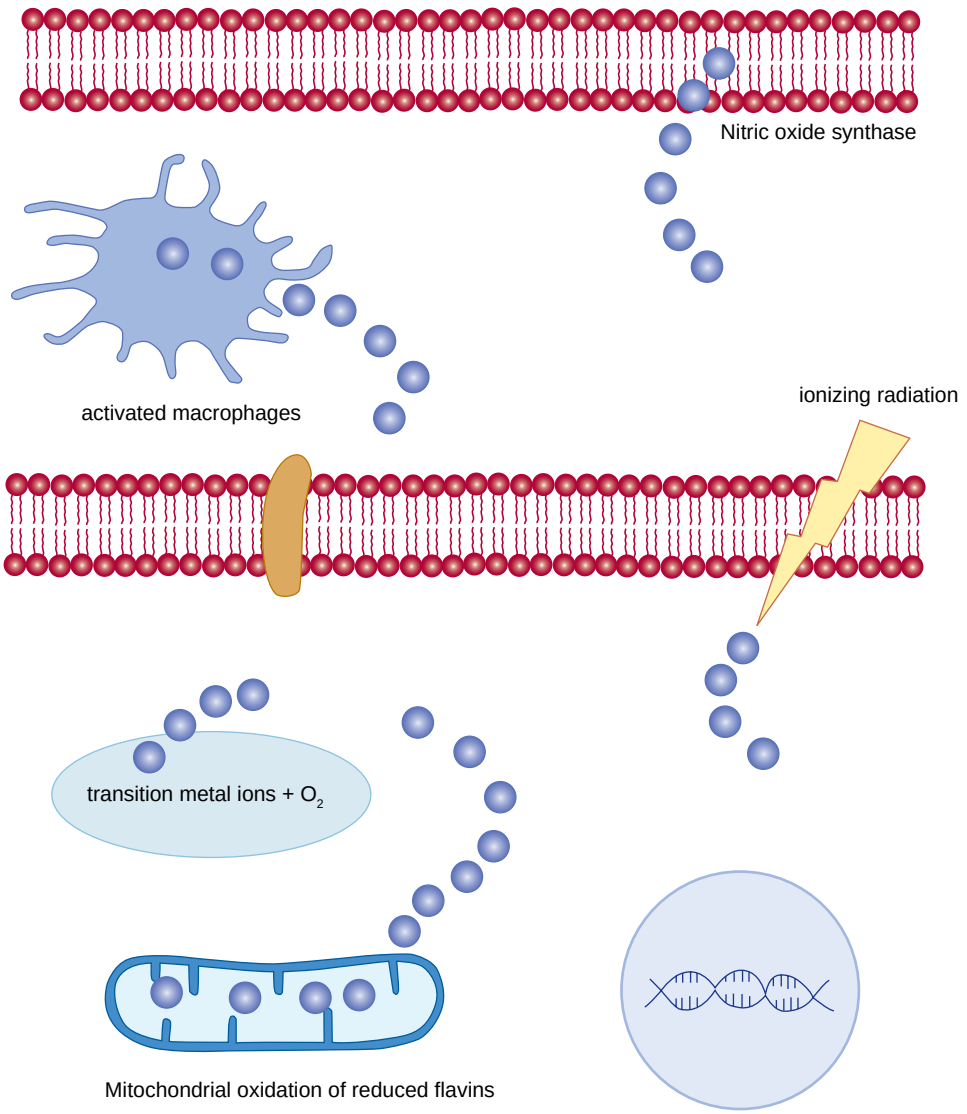

FIGURE 45–2 Sources of radicals.

chains proceeds through a series of steps in which the flavin semiquinone radical is stabilized by the protein to which it is bound, and forms oxygen radicals as transient intermediates. Although the final products are not radicals, because of the unpredictable nature of radicals there is considerable "leakage" of radicals, and some 3–5% of the daily consumption of 30 mol of oxygen by an adult human being is converted to singlet oxygen, hydrogen peroxide, and superoxide, perhydroxyl and hydroxyl radicals, rather then undergoing complete reduction to water. This results in daily production of about 1.5 mol of reactive oxygen species.

There Are Various Mechanisms of Protection Against Radical Damage

The metal ions that undergo non-enzymic reaction to form oxygen radicals are not normally free in solution, but are bound to either the proteins for which they provide the prosthetic group, or to specific transport and storage proteins, so that they are unreactive. Iron is bound to transferrin, ferritin and hemosiderin, copper to ceruloplasmin, and other metal ions are bound to metallothionein. This binding to transport proteins that are too large to be filtered in the kidneys also prevents loss of metal ions in the urine.

Superoxide is produced both accidentally and also as the reactive oxygen species required for a number of enzyme-catalyzed reactions. A family of superoxide dismutases catalyze the reaction between superoxide and water to yield oxygen and hydrogen peroxide: $O_2^{\cdot-} + H_2O \rightarrow O_2 + H_2O_2$. The hydrogen peroxide is then removed by catalase and various peroxidases: $2H_2O_2 \rightarrow 2H_2O + O_2$. Most enzymes that produce and require superoxide are in the peroxisomes, together with superoxide dismutase, catalase, and peroxidases.

The peroxides that are formed by radical damage to lipids in membranes and plasma lipoproteins are reduced to fatty acids by glutathione peroxidase, a selenium-dependent

enzyme (hence the importance of adequate selenium intake to maximize antioxidant activity), and the oxidized glutathione is reduced by NADPH-dependent glutathione reductase (Figure 21–3). Lipid peroxides are also reduced to fatty acids by reaction with vitamin E, forming the relatively stable tocopheroxyl radical, which persist long enough to undergo reduction back to tocopherol by reaction with vitamin C at the surface of the cell or lipoprotein (Figure 44–6). The resultant monodehydroascorbate radical then undergoes enzymic reduction back to ascorbate or a non-enzymic reaction of 2 mol of monodehydroascorbate to yield 1 mol each of ascorbate and dehydroascorbate.

Ascorbate, uric acid and a variety of polyphenols derived from plant foods act as water-soluble radical trapping antioxidants, forming relatively stable radicals that persist long enough to undergo reaction to non-radical products. Ubiquinone and carotenes similarly act as lipid-soluble radical-trapping antioxidants in membranes and plasma lipoproteins.

Antioxidants Can Also Be Pro-Oxidants

Although ascorbate is an anti-oxidant, reacting with superoxide and hydroxyl to yield monodehydroascorbate and hydrogen peroxide or water, it can also be a source of superoxide radicals by reaction with oxygen, and hydroxyl radicals by reaction with Cu^{2+} ions (Table 45-1). However, these pro-oxidant actions require relatively high concentrations of ascorbate that are unlikely to be reached in tissues, since once the plasma concentration of ascorbate reaches about 30 mmol/L, the renal threshold is reached, and at intakes above about 100–120 mg/day the vitamin is excreted in the urine quantitatively with intake.

A considerable body of epidemiological evidence suggested that carotene is protective against lung and other cancers. However, two major intervention trials in the 1990s showed an increase in death from lung (and other) cancer among people given supplements of β-carotene. The problem is that although β-carotene is indeed a radical trapping antioxidant under conditions of low partial pressure of oxygen, as in most tissues, at high partial pressures of oxygen (as in the lungs) and especially in high concentrations, β-carotene is an autocatalytic pro-oxidant, and hence can initiate radical damage to lipids and proteins.

Epidemiological evidence also suggests that vitamin E is protective against atherosclerosis and cardiovascular disease. However, meta-analysis of intervention trials with vitamin E shows increased mortality among those taking (high dose) supplements. These trials have all used α-tocopherol, and it is possible that the other vitamers of vitamin E that are present in foods, but not the supplements, may be important. In vitro, plasma lipoproteins form less cholesterol ester hydroperoxide when incubated with sources of low concentrations of perhydroxyl radicals when vitamin E has been removed than when it is present. The problem seems to be that vitamin E acts as an antioxidant by forming a stable radical that persists long enough to undergo metabolism to non-radical products. This means that the radical also persists long enough to penetrate deeper in to the lipoprotein, causing further radical damage, rather than interacting with a water-soluble antioxidant at the surface of the lipoprotein.

TABLE 45–1 Antioxidant and Pro-Oxidant Roles of Vitamin C

Antioxidant roles:
Ascorbate + $^{\cdot}O_2^-$ → H_2O_2 + monodehydroascorbate; catalase and peroxidases catalyze the reaction: $2H_2O_2 \rightarrow 2H_2O + O_2$
Ascorbate + $^{\cdot}OH$ → H_2O + monodehydroascorbate
Pro-oxidant roles:
Ascorbate + O_2 → $^{\cdot}O_2^-$ + monodehydroascorbate
Ascorbate + Cu^{2+} → Cu^+ + monodehydroasacorbate
$Cu^+ + H_2O_2 \rightarrow Cu^{2+} + OH^- + {}^{\cdot}OH$

SUMMARY

- Free radicals are highly reactive molecular species with an unpaired electron. They can react with, and modify, proteins, nucleic acids and fatty acids in cell membranes and plasma lipoproteins.
- Radical damage to lipids and proteins in plasma lipoproteins is a factor in the development of atherosclerosis and coronary artery disease; radical damage to nucleic acids may induce heritable mutations and cancer; radical damage to proteins may lead to the development of auto-immune diseases.
- Oxygen radicals as a result of exposure to ionising radiation, non-enzymic reactions of transition metal ions, the respiratory burst of activated macrophages, and the normal oxidation of reduced flavin coenzymes.
- Protection against radical damage is afforded by enzymes that remove superoxide ions and hydrogen peroxide, enzymic reduction of lipid peroxides linked to oxidation of glutathione, non-enzymic reaction of lipid peroxides with vitamin E, and reaction of radicals with compounds such as vitamins C and E, carotene, ubiquinone, uric acid, and dietary polyphenols that form relatively stable radicals that persist long enough to undergo reaction to non-radical products.
- Except in people who were initially deficient, intervention trials of vitamin E and β-carotene have generally shown increased mortality among those taking the supplements. β-Carotene is only an antioxidant at low concentrations of oxygen; at higher concentrations of oxygen it is an autocatalytic pro-oxidant. Vitamin E forms a stable radical that is capable of either undergoing reaction with water-soluble antioxidants or penetrating further into lipoproteins and tissues, so increasing radical damage.

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CHAPTER

46

Intracellular Traffic & Sorting of Proteins

Robert K. Murray, MD, PhD

BIOMEDICAL IMPORTANCE

Proteins must travel from polyribosomes, where they are synthesized, to many different sites in the cell to perform their particular functions. Some are destined to be components of specific organelles, others for the cytosol or for export, and yet others will be located in the various cellular membranes. Thus, there is considerable **intracellular traffic of proteins.** A major insight was the recognition by Blobel and others that for proteins to attain their proper locations, they generally contain **information** (a signal or coding sequence) that **targets** them appropriately. Once a number of the signals were defined (see Table 46–1), it became apparent that **certain diseases** result from mutations that affect these signals. In this chapter we discuss the intracellular traffic of proteins and their sorting and briefly consider some of the disorders that result when abnormalities occur.

MANY PROTEINS ARE TARGETED BY SIGNAL SEQUENCES TO THEIR CORRECT DESTINATIONS

The protein biosynthetic pathways in cells can be considered to be **one large sorting system.** Many proteins carry **signals** (usually but not always specific sequences of amino acids) that direct them to their destination, thus ensuring that they will end up in the appropriate membrane or cell compartment; these signals are a fundamental component of the sorting system. Usually the signal sequences are recognized and interact with complementary areas of other proteins that serve as receptors for those containing the signals.

A major sorting decision is made early in protein biosynthesis, when specific proteins are synthesized either on **free** or on **membrane-bound polyribosomes**. This results in two sorting branches, called the **cytosolic branch** and the **rough endoplasmic reticulum (RER) branch** (Figure 46–1). This sorting occurs because proteins synthesized on membrane-bound polyribosomes contain a **signal peptide** that mediates their attachment to the membrane of the ER. Further details on the signal peptide are given below. Proteins synthesized on **free polyribosomes** lack this particular signal peptide and are delivered into the cytosol. There they are directed to mitochondria, nuclei, and peroxisomes by specific signals—or remain in the cytosol if they lack a signal. Any protein that contains a targeting sequence that is subsequently removed is designated as a **preprotein.** In some cases a second peptide is also removed, and in that event the original protein is known as a **preproprotein** (eg, preproalbumin; Chapter 50).

Proteins synthesized and sorted in the **rough ER branch** (Figure 46–1) include many destined for various membranes (eg, of the ER, Golgi apparatus [GA], plasma membrane [PM]) and for secretion. Lysosomal enzymes are also included. These various proteins may thus reside in the membranes or lumen of the ER, or follow the major transport route of intracellular proteins to the GA. The entire pathway of ER → GA→ plasma membrane is often called the **secretory** or **exocytotic pathway.** Events along this route will be given special attention. Proteins destined for the GA, the PM, certain other sites, or for secretion are carried in **transport vesicles** (Figure 46–2)**;** a brief description of the formation of these important particles will be given subsequently. Certain other proteins destined for secretion are carried in **secretory vesicles** (Figure 46–2). These are prominent in the pancreas and certain other glands. Their mobilization and discharge are regulated and often referred to as **"regulated secretion,"** whereas the secretory pathway involving transport vesicles is called **"constitutive."** Passage of enzymes to the lysosomes using the mannose 6-phosphate signal is described in Chapter 47.

The Golgi Apparatus Is Involved in Glycosylation & Sorting of Proteins

The **GA** plays two major roles in membrane synthesis. First, it is involved in the **processing of the oligosaccharide chains** of membrane and other N-linked glycoproteins and also contains enzymes involved in O-glycosylation (see Chapter 47). Second, it is involved in the **sorting** of various proteins prior to their delivery to their appropriate intracellular destinations. All parts of the GA participate in the first role, whereas the ***trans*** **Golgi network** (TGN) is particularly involved in the second and is very rich in vesicles.

TABLE 46–1 Some Sequences or Molecules That Direct Proteins to Specific Organelles

Targeting Sequence or Compound	Organelle Targeted
Signal peptide sequence	Membrane of ER
Amino terminal KDEL sequence (Lys-Asp-Glu-Leu) in ER-resident proteins in COPI vesicles	Luminal surface of ER
Di-acidic sequences (eg, Asp-X-Glu) in membrane proteins in COPII vesicles	Golgi membranes
Amino terminal sequence (20–80 residues)	Mitochondrial matrix
NLS (eg, Pro_2-Lys_3-Arg-Lys-Val)	Nucleus
PTS (eg, Ser-Lys-Leu)	Peroxisome
Mannose 6-phosphate	Lysosome

Abbreviations: NLS, nuclear localization signal; PTS, peroxisomal-matrix targeting sequence.

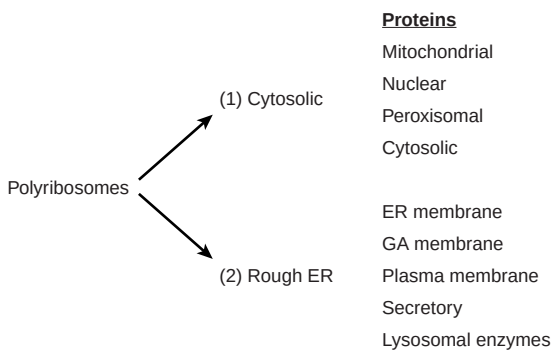

FIGURE 46–1 Diagrammatic representation of the two branches of protein sorting occurring by synthesis on (1) cytosolic and (2) membrane-bound polyribosomes. The mitochondrial proteins listed are encoded by nuclear genes; one of the signals used in further sorting of mitochondrial matrix proteins is listed in Table 46–1. (ER, endoplasmic reticulum; GA, Golgi apparatus.)

A Wide Variety of Experimental Techniques Have Been Used to Investigate Trafficking and Sorting

Approaches that have afforded major insights to the processes described in this chapter include (1) electron microscopy; (2) use of yeast mutants; (3) subcellular fractionation; (4) application of recombinant DNA techniques (eg, mutating or eliminating particular sequences in proteins, or fusing new sequences onto them); and (5) development of in vitro systems (eg, to study translocation in the ER and mechanisms of vesicle formation); (6) use of fluorescent tags to follow the movement of proteins; and (7) structural studies on certain proteins, particularly by x-ray crystallography.

The sorting of proteins belonging to **the cytosolic branch** referred to above is described next, starting with mitochondrial proteins.

THE MITOCHONDRION BOTH IMPORTS & SYNTHESIZES PROTEINS

Mitochondria contain many proteins. Thirteen polypeptides (mostly membrane components of the electron transport chain) are encoded by the **mitochondrial (mt) genome** and synthesized in that organelle using its own protein synthesizing system. However, the majority (at least several hundred) are encoded by **nuclear genes,** are synthesized outside the mitochondria on cytosolic **polyribosomes,** and must be imported. **Yeast cells** have proved to be a particularly useful system for analyzing the mechanisms of import of mitochondrial proteins, partly because it has proved possible to generate a variety of **mutants** that have illuminated the fundamental processes involved. Most progress has been made in the study of proteins present in **the mitochondrial matrix**, such as the F_1 ATPase subunits. Only the pathway of import of matrix proteins will be discussed in any detail here.

Matrix proteins must pass from cytosolic polyribosomes through the **outer** and **inner mitochondrial membranes** to reach their destination. Passage through the two membranes is called **translocation.** They have an amino terminal leader sequence **(presequence),** about 20–50 amino acids in length (see Table 46–1), which is not highly conserved but is amphipathic and contains many hydrophobic and positively charged amino acids (eg, Lys or Arg). The presequence is equivalent to a signal peptide mediating attachment of polyribosomes to membranes of the ER (see below), but in this instance **targeting proteins to the matrix**; if the leader sequence is cleaved off, potential matrix proteins will not reach their destination. Some general features of the passage of a protein from the cytosol to the mt matrix are shown in Figure 46–3.

Translocation occurs **posttranslationally,** after the matrix proteins are released from the cytosolic polyribosomes. Interactions with a number of cytosolic proteins that act as **chaperones** (see below) and as **targeting factors** occur prior to translocation.

Two distinct **translocation complexes** are situated in the outer and inner mitochondrial membranes, referred to (respectively) as TOM (translocase-of-the-outer membrane) and TIM (translocase-of-the-inner membrane). Each complex has been analyzed and found to be composed of a number of proteins, some of which act as **receptors (eg, Tom20/22)** for the incoming proteins and others as **components (eg, Tom40) of the transmembrane pores** through which these proteins must pass. Proteins must be in the **unfolded state** to pass through the complexes, and this is made possible by **ATP-dependent binding to several chaperone proteins.** The roles of chaperone proteins in protein folding are discussed later in this chapter. In mitochondria, they are involved in translocation, sorting, folding, assembly, and degradation of imported proteins. A **proton-motive force** across the inner membrane is required for import; it is made up of the **electric potential** across the membrane (inside negative) and the **pH gradient**

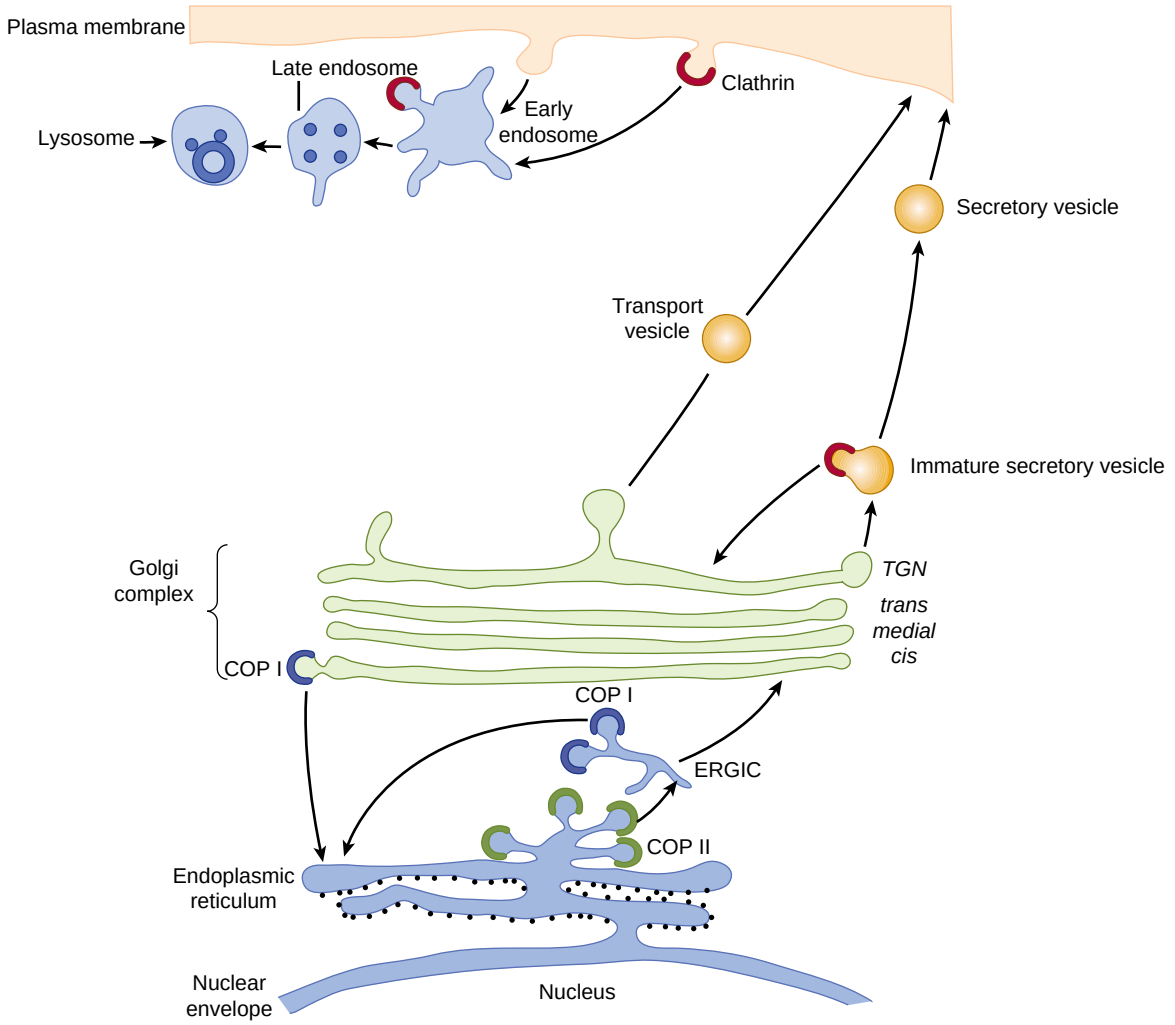

FIGURE 46–2 Diagrammatic representation of the rough ER branch of protein sorting. Newly synthesized proteins are inserted into the ER membrane or lumen from membrane-bound polyribosomes (small black circles studding the cytosolic face of the ER). Proteins that are transported out of the ER are carried in COPII vesicles to the *cis*-Golgi (anterograde transport). Movement of proteins through the Golgi appears to be mainly by cisternal maturation. In the TGN, the exit side of the Golgi, proteins are segregated and sorted. Secretory proteins accumulate in secretory vesicles (regulated secretion), from which they are expelled at the plasma membrane. Proteins destined for the plasma membrane or those that are secreted in a constitutive manner are carried out to the cell surface in as yet to be characterized transport vesicles (constitutive secretion). Clathrin-coated vesicles are involved in endocytosis, carrying cargo to late endosomes and to lysosomes. Mannose 6-phosphate (not shown; see Chapter 47) acts as a signal for transporting enzymes to lysosomes. COPI vesicles are involved in retrieving proteins from the Golgi to the ER (retrograde transport) and may be involved in some intra-Golgi. transport. The ERGIC/VTR compartment appears to be a site mainly for concentrating cargo destined for retrograde transport into COPI vesicles. (TGN, *trans*-Golgi network; ERGIC/VTR, ER-Golgi intermediate complex or vesicular tubule clusters.) (Courtesy of E Degen.)

(see Chapter 13). The positively charged leader sequence may be helped through the membrane by the negative charge in the matrix. The presequence is split off in the matrix by a **matrix-processing protease (MPP).** Contact with **other chaperones** present in the matrix is essential to complete the overall process of import. Interaction with mt-Hsp70 (mt = mitochondrial; Hsp = heat shock protein; 70 = ~70 kDa) ensures proper import into the matrix and prevents misfolding or aggregation, while interaction with the mt-Hsp60-Hsp10 system ensures proper folding. The interactions of imported proteins with the above chaperones require **hydrolysis of ATP** to drive them.

The details of how preproteins are translocated have not been fully elucidated. It is possible that the electric potential associated with the inner mitochondrial membrane causes a conformational change in the unfolded preprotein being trans-

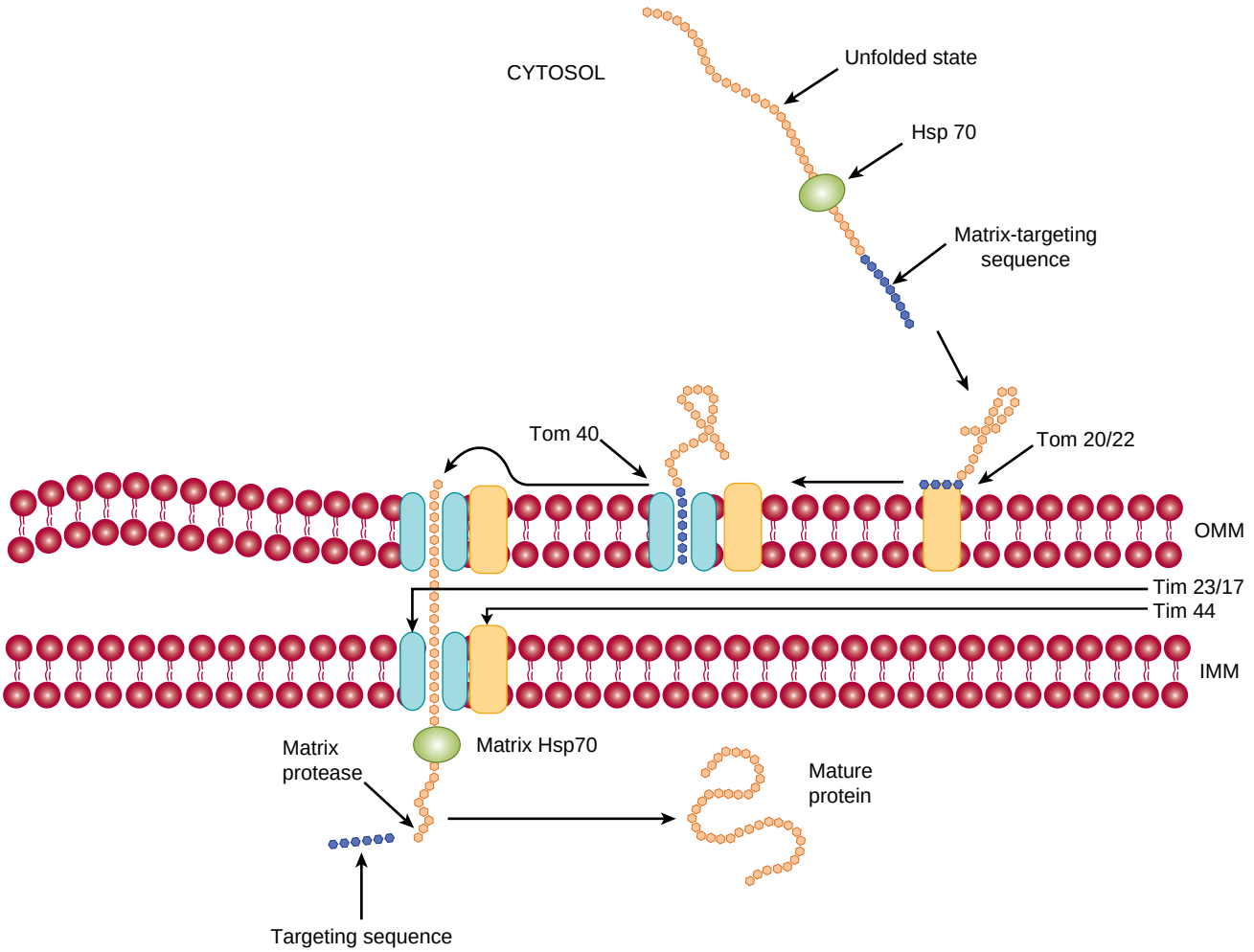

FIGURE 46–3 Schematic representation of the entry of a protein into the mitochondrial matrix. The unfolded protein synthesized on cytosolic poyribosomes and containing a matrix-targeting sequence interacts with the cytosolic chaperone Hsp 70. The protein next interacts with the mt outer membrane receptor Tom 20/22, and is transferred to the neighboring import channel Tom 40 (Tom, translocon of the outer membrane). The protein is then translocated across the channel; the channel on the inner mt membrane is largely composed of Tim 23 and Tim 17 proteins (Tim, translocon of the inner membrane). On the inside of the inner mt membrane, it interacts with the matrix chaperone Hsp 70, which in turn interacts with membrane protein Tim 44. The hydrolysis of ATP by mt Hsp70 probably helps drive the translocation, as does the electronegative interior of the matrix. The targeting sequence is subsequently cleaved by the matrix processing enzyme, and the imported protein assumes its final shape, or may interact with an mt chaperonin prior to this. At the site of translocation, the outer and inner mt membranes are in close contact. (Modified, with permission, from Lodish H, et al: *Molecular Cell Biology,* 6th ed. W.H. Freeman & Co., 2008.)

located and that this helps to pull it across. Furthermore, the fact that the matrix is more negative than the intermembrane space may "attract" the positively charged amino terminal of the preprotein to enter the matrix. Close apposition at **contact sites** between the outer and inner membranes is necessary for translocation to occur.

The above describes the major pathway of proteins destined for the mitochondrial matrix. However, certain proteins insert into the **outer mitochondrial membrane** facilitated by the TOM complex. Others stop in the **intermembrane space,** and some insert into the **inner membrane.** Yet others proceed into the matrix and then return to the inner membrane or intermembrane space. A number of proteins contain two signaling sequences—one to enter the mitochondrial matrix and the other to mediate subsequent relocation (eg, into the inner membrane). Certain mitochondrial proteins do not contain presequences (eg, cytochrome *c,* which locates in the inter membrane space), and others contain **internal presequences.** Overall, proteins employ a variety of mechanisms and routes to attain their final destinations in mitochondria.

General features that apply to the import of proteins into organelles, including mitochondria and some of the other organelles to be discussed below, are summarized in Table 46–2.

TABLE 46–2 Some General Features of Protein Import to Organelles

• Import of a protein into an organelle usually occurs in three stages: recognition, translocation, and maturation.
• Targeting sequences on the protein are recognized in the cytoplasm or on the surface of the organelle.
• The protein is generally unfolded for translocation, a state maintained in the cytoplasm by chaperones.
• Threading of the protein through a membrane requires energy and organellar chaperones on the *trans* side of the membrane.
• Cycles of binding and release of the protein to the chaperone result in pulling of its polypeptide chain through the membrane.
• Other proteins within the organelle catalyze folding of the protein, often attaching cofactors or oligosaccharides and assembling them into active monomers or oligomers.

Source: Data from McNew JA, Goodman JM: The targeting and assembly of peroxisomal proteins: some old rules do not apply. Trends Biochem Sci 1998;21:54. Reprinted with permission from Elsevier.

LOCALIZATION SIGNALS, IMPORTINS, & EXPORTINS ARE INVOLVED IN TRANSPORT OF MACROMOLECULES IN & OUT OF THE NUCLEUS

It has been estimated that more than a million macromolecules per minute are transported between the nucleus and the cytoplasm in an active eukaryotic cell. These macromolecules include histones, ribosomal proteins and ribosomal subunits, transcription factors, and mRNA molecules. The transport is bidirectional and occurs through the **nuclear pore complexes** (NPCs). These are complex structures with a mass approximately 15 times that of a ribosome and are composed of aggregates of about 30 different proteins. The minimal diameter of an NPC is approximately 9 nm. Molecules smaller than about 40 kDa can pass through the channel of the NPC by **diffusion**, but **special translocation mechanisms** exist for larger molecules. These mechanisms are under intensive investigation, but some important features have already emerged.

Here we shall mainly describe **nuclear import** of certain macromolecules. The general picture that has emerged is that proteins to be imported (cargo molecules) carry a **nuclear localization signal (NLS).** One example of an NLS is the amino acid sequence $(Pro)_2$-$(Lys)_3$-Arg-Lys-Val (see Table 46–1), which is markedly rich in basic lysine residues. Depending on which NLS it contains, a cargo molecule interacts with one of a family of soluble proteins called **importins,** and the complex **docks** transiently at the NPC. Another family of proteins called **Ran** plays a critical regulatory role in the interaction of the complex with the NPC and in its translocation through the NPC. Ran proteins are small monomeric nuclear **GTPases** and, like other GTPases, exist in either GTP-bound or GDP-bound states. They are themselves regulated by **guanine nucleotide exchange factors** (GEFs), which are located in the nucleus, and Ran **guanine-activating proteins** (GAPs), which are predominantly cytoplasmic. The GTP-bound state of Ran is favored in the nucleus and the GDP-bound state in the cytoplasm. The conformations and activities of Ran molecules vary depending on whether GTP or GDP is bound to them (the GTP-bound state is active; see discussion of G proteins in Chapter 42). The **asymmetry** between nucleus and cytoplasm—with respect to which of these two nucleotides is bound to Ran molecules—is thought to be crucial in understanding the roles of Ran in transferring complexes unidirectionally across the NPC. When **cargo molecules** are **released inside the nucleus**, the **importins recirculate to the cytoplasm** to be used again. Figure 46–4 summarizes some of the principal features in the above process.

Proteins similar to importins, referred to as **exportins,** are involved in the export of many macromolecules (various protein, tRNA molecules, ribosomal subunits and certain mRNA molecules) from the nucleus. Cargo molecules for export carry **nuclear export signals (NESs).** Ran proteins are involved in this process also, and it is now established that the processes of import and export share a number of common features. The family of importins and exportins are referred to as **karyopherins**.

Another system is involved in the translocation of the majority of **mRNA molecules**. These are exported from the nucleus to the cytoplasm as ribonucleoprotein (RNP) complexes attached to a protein named **mRNP exporter**. This is a heterodimeric molecule (ie, composed of 2 different subunits, TAP and Nxt-1) which carries RNP molecules through the NPC. Ran is not involved. This system appears to use the hydrolysis of **ATP** by an RNA helicase (Dbp5) to drive translocation.

Other **small monomeric GTPases** (eg, ARF, Rab, Ras, and Rho) are important in various cellular processes such as vesicle formation and transport (ARF and Rab; see below), certain growth and differentiation processes (Ras), and formation of the actin cytoskeleton. A process involving GTP and GDP is also crucial in the transport of proteins across the membrane of the ER (see below).

PROTEINS IMPORTED INTO PEROXISOMES CARRY UNIQUE TARGETING SEQUENCES

The **peroxisome** is an important organelle involved in aspects of the metabolism of many molecules, including fatty acids and other lipids (eg, plasmalogens, cholesterol, bile acids), purines, amino acids, and hydrogen peroxide. The peroxisome is bounded by a single membrane and contains more than 50 enzymes; catalase and urate oxidase are marker enzymes for this organelle. Its proteins are **synthesized on cytosolic polyribosomes** and fold prior to import. The pathways of import of a number of its proteins and enzymes have been studied, some being **matrix components** (see Figure 46–5) and others **mem-**

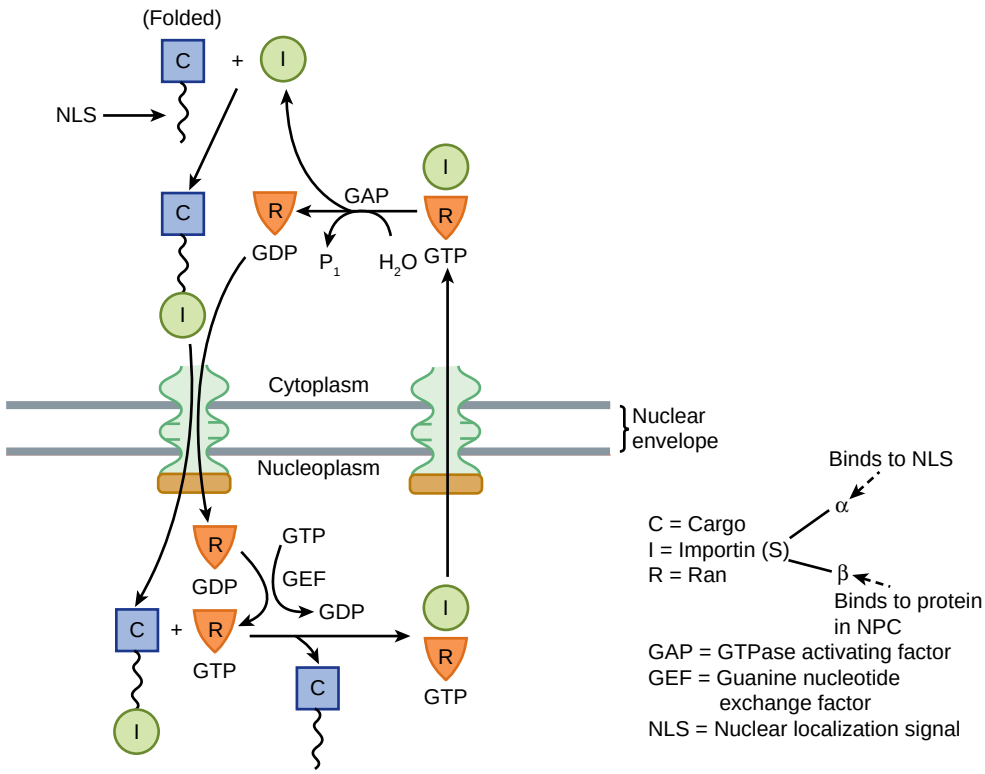

FIGURE 46–4 Simplified representation of the entry of a protein into the nucleoplasm. As shown in the top left-hand side of the figure, a cargo molecule in the cytoplasm via its NLS interacts to form a complex with an importin. (This may be either importin α or both importin α and importin β.) This complex next interacts with Ran·GDP and traverses the NPC into the nucleoplasm. In the nucleoplasm, Ran·GDP is converted to Ran·GTP by GEF, causing a conformational change in Ran resulting in the cargo molecule being released. The importin-Ran·GTP complex then leaves the nucleoplasm via the NPC to return to the cytoplasm. In the cytoplasm, due to the action of GTP-activating protein (GAP), which converts GTP to GDP, the importin is released to participate in another import cycle. The Ran·GTP is the active form of the complex, with the Ran·GDP form being considered inactive. Directionality is believed to be conferred on the overall process by the dissociation of Ran·GTP in the nucleoplasm. (C, cargo molecule; I, importin; NLS, nuclear localizing signal; NPC, nuclear pore complex; GEF, guanine nucleotide exchange factor; GAP, GTPase activating factor.) (Modified, with permission, from Lodish H, et al: *Molecular Cell Biology,* 6th ed. W.H. Freeman & Co., 2008.)

brane components. At least two **peroxisomal-matrix targeting sequences (PTSs)** have been discovered. One, **PTS1**, is a tripeptide (ie, Ser-Lys-Leu [SKL], but variations of this sequence have been detected) located at the carboxyl terminal of a number of matrix proteins, including catalase. Another, **PTS2**, is at the N-terminus and has been found in at least four matrix proteins (eg, thiolase). Neither of these two sequences is cleaved after entry into the matrix. Proteins containing PTS1 sequences **form complexes** with a cytosolic receptor protein **(Pex5)** and proteins containing PTS2 sequences complex with another receptor protein. The resulting complexes then interact with a membrane receptor complex, **Pex2/10/12**, which translocates them into the matrix. Proteins involved in further transport of proteins into the matrix are also present. Pex5 is re-cycled to the cytosol. Most peroxisomal **membrane proteins** have been found to contain neither of the above two targeting sequences, but apparently contain others. The import system can handle **intact oligomers** (eg, tetrameric catalase). Import of **matrix proteins** requires **ATP**, whereas import of **membrane proteins** does **not**.

Most Cases of Zellweger Syndrome Are Due to Mutations in Genes Involved in the Biogenesis of Peroxisomes

Interest in import of proteins into peroxisomes has been stimulated by studies on **Zellweger syndrome.** This condition is apparent at birth and is characterized by **profound neurologic impairment**, victims often dying within a year. The number of peroxisomes can vary from being almost normal to being virtually absent in some patients. Biochemical findings include an accumulation of very-long-chain fatty acids, abnormalities of the synthesis of bile acids, and a marked reduction of plasmalogens. The condition is believed to be due to **mutations**

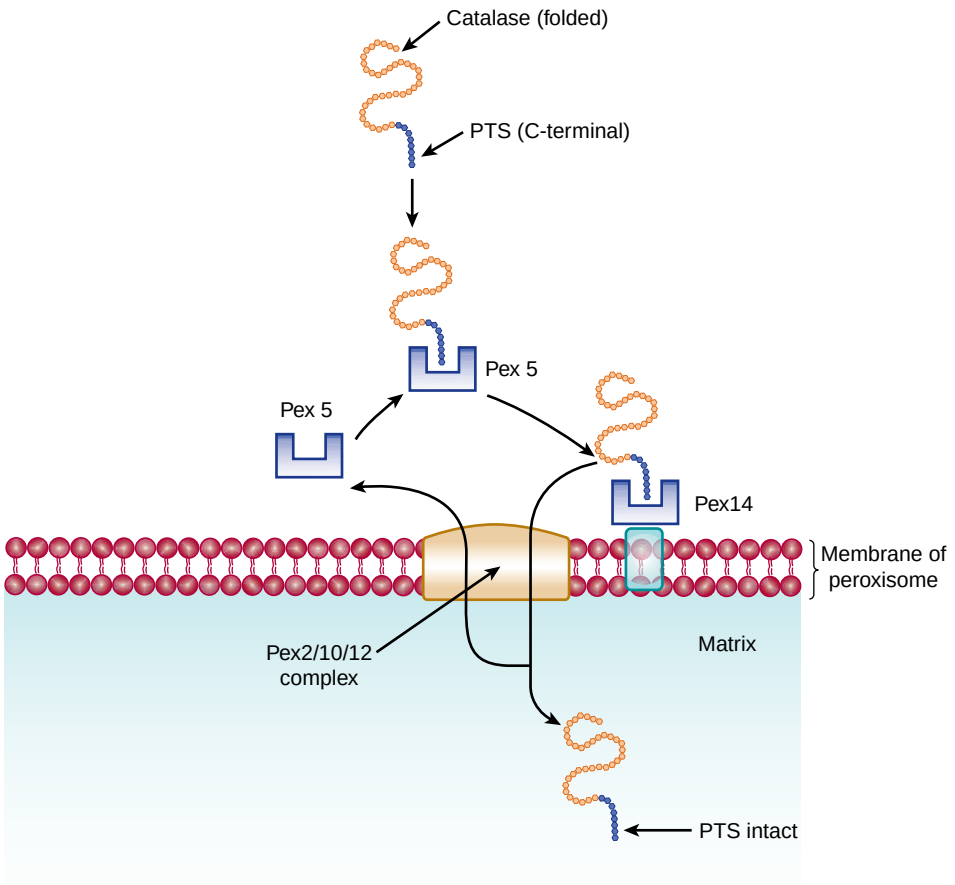

FIGURE 46–5 Schematic representation of the entry of a protein into the peroxisomal matrix. The protein to be imported into the matrix is synthesized on cytosolic polyribosomes, assumes its folded shape prior to import, and contains a C-terminal peroxisomal targeting sequence (PTS). It interacts with cytosolic receptor protein Pex5, and the complex then interacts with a receptor on the peroxisomal membrane, Pex14. In turn, the protein-Pex 14 complex passes to the Pex 2/10/12 complex on the peroxisomal membrane and is translocated. Pex 5 is returned to the cytosol. The protein retains its PTS in the matrix. (Modified, with permission, from Lodish H, et al: *Molecular Cell Biology,* 6th ed. W.H. Freeman & Co., 2008.)

in genes encoding certain proteins—so called **peroxins**—involved in various steps of **peroxisome biogenesis** (such as the import of proteins described above), or in genes encoding **certain peroxisomal enzymes** themselves. Two closely related conditions are **neonatal adrenoleukodystrophy** and **infantile Refsum disease.** Zellweger syndrome and these two conditions represent a **spectrum** of overlapping features, with Zellweger syndrome being the **most severe** (many proteins affected) and infantile Refsum disease the least severe (only one or a few proteins affected). Table 46–3 lists these and related conditions.

THE SIGNAL HYPOTHESIS EXPLAINS HOW POLYRIBOSOMES BIND TO THE ENDOPLASMIC RETICULUM

As indicated above, the **rough ER branch** is the second of the two branches involved in the synthesis and sorting of proteins. In this branch, proteins are synthesized on **membrane-bound polyribosomes** and **translocated into the lumen** of the rough ER prior to further sorting (Figure 46–2).

The **signal hypothesis** was proposed by Blobel and Sabatini partly to explain the distinction between free and membrane-bound polyribosomes. They found that proteins synthesized on membrane-bound polyribosomes contained a peptide extension **(signal peptide)** at their amino terminals which mediated their attachment to the membranes of the ER. As noted above, proteins whose entire synthesis occurs on free polyribosomes

TABLE 46–3 Disorders Due to Peroxisomal Abnormalities

	OMIM Number[1]
Zellweger syndrome	214100
Neonatal adrenoleukodystrophy	202370
Infantile Refsum disease	266510
Hyperpipecolic academia	239400
Rhizomelic chondrodysplasia punctata	215100
Adrenoleukodystrophy	300100
Pseudoneonatal adrenoleukodystrophy	264470
Pseudo-Zellweger syndrome	261515
Hyperoxaluria type 1	259900
Acatalasemia	115500
Glutaryl-CoA oxidase deficiency	231690

Source: Reproduced, with permission, from Seashore MR, Wappner RS: *Genetics in Primary Care & Clinical Medicine.* Appleton & Lange, 1996.
[1]OMIM = *Online Mendelian Inheritance in Man.* Each number specifies a reference in which information regarding each of the above conditions can be found.

TABLE 46–4 Some Properties of Signal Peptides

• Usually, but not always, located at the amino terminal
• Contain approximately 12–35 amino acids
• Methionine is usually the amino terminal amino acid
• Contain a central cluster of hydrophobic amino acids
• Contain at least one positively charged amino acid near their amino terminal
• Usually cleaved off at the carboxyl terminal end of an Ala residue by signal peptidase

lack this signal peptide. An important aspect of the signal hypothesis was that it suggested—as turns out to be the case—that **all ribosomes have the same structure** and that the distinction between membrane-bound and free ribosomes depends solely on the former carrying proteins that have signal peptides. Much evidence has confirmed the original hypothesis. Because many membrane proteins are synthesized on membrane-bound polyribosomes, the signal hypothesis plays an important role in **concepts of membrane assembly**. Some **characteristics of signal peptides** are summarized in Table 46–4.

Figure 46–6 illustrates the principal features in relation to the passage of **a secreted protein** through the membrane of the ER. It incorporates features from the original signal hypothesis and from subsequent work. The mRNA for such a protein encodes an amino terminal **signal peptide** (also variously called a leader sequence, a transient insertion signal, a signal sequence, or a presequence). The signal hypothesis proposed that the protein is inserted into the ER membrane at the same time as its mRNA is being translated on polyribosomes, so-called **cotranslational insertion.** As the signal peptide emerges from the large subunit of the ribosome, it is recognized by a **signal recognition particle (SRP)** that blocks further translation after about 70 amino acids have been polymerized (40 buried in the large ribosomal subunit and 30 exposed). The block is referred to as **elongation arrest.** The SRP contains **six proteins** and has a **7S RNA** associated with it that is closely related to the Alu family of highly repeated DNA sequences (Chapter 35). The SRP-imposed block is not released until the SRP-signal peptide-polyribosome complex has bound to the so-called **docking protein** (SRP-R, a receptor for the SRP) on the ER membrane; the SRP thus guides the signal peptide to the SRP-R and prevents premature folding and expulsion of the protein being synthesized into the cytosol.

The **SRP-R** is an integral membrane protein composed of **α** and **β subunits**. The α subunit **binds GDP** and the β subunit spans the membrane. When the SRP-signal peptide complex interacts with the receptor, the **exchange of GDP for GTP** is

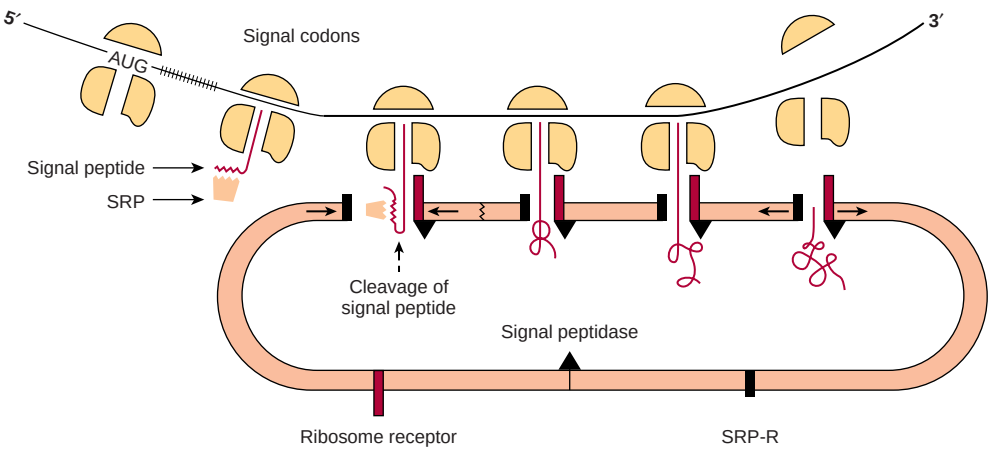

FIGURE 46–6 Diagram of the signal hypothesis for the transport of secreted proteins across the ER membrane. The ribosomes synthesizing a protein move along the messenger RNA specifying the amino acid sequence of the protein. (The messenger is represented by the line between 5′ and 3′.) The codon AUG marks the start of the message for the protein; the hatched lines that follow AUG represent the codons for the signal sequence. As the protein grows out from the larger ribosomal subunit, the signal sequence is exposed and bound by the signal recognition particle (SRP). Translation is blocked until the complex binds to the "docking protein," also designated SRP-R (represented by the black bar) on the ER membrane. There is also a receptor (red bar) for the ribosome itself. The interaction of the ribosome and growing peptide chain with the ER membrane results in the opening of a channel through which the protein is transported to the interior space of the ER. During translocation, the signal sequence of most proteins is removed by an enzyme called the "signal peptidase," located at the luminal surface of the ER membrane. The completed protein is eventually released by the ribosome, which then separates into its two components, the large and small ribosomal subunits. The protein ends up inside the ER. See text for further details. (Slightly modified and reproduced, with permission, from Marx JL: Newly made proteins zip through the cell. Science 1980;207:164. Copyright ©1980 by the American Association for the Advancement of Science.)

stimulated. This form of the receptor (with GTP bound) has a **high affinity for the SRP** and thus **releases the signal peptide**, which binds to the translocation machinery (translocon) also present in the ER membrane. The **α subunit then hydrolyzes its bound GTP**, restoring GDP and completing a GTP-GDP cycle. The **unidirectionality** of this cycle helps drive the interaction of the polyribosome and its signal peptide with the ER membrane in the forward direction.

The **translocon** consists of three membrane proteins (the **Sec61 complex**) that form a **protein-conducting channel** in the ER membrane through which the newly synthesized protein may pass. The channel appears to be **open only when a signal peptide is present**, preserving conductance across the ER membrane when it closes. The conductance of the channel has been measured experimentally.

The insertion of the signal peptide into the conducting channel, while the other end of the parent protein is still attached to ribosomes, is termed **"cotranslational insertion."** The process of elongation of the remaining portion of the protein probably facilitates passage of the nascent protein across the lipid bilayer as the ribosomes remain attached to the membrane of the ER. Thus, the rough (or ribosome-studded) ER is formed. It is important that the protein be kept in an **unfolded state** prior to entering the conducting channel—otherwise, it may not be able to gain access to the channel.

Ribosomes remain attached to the ER during synthesis of signal peptide-containing proteins but are **released** and **dissociated** into their two types of subunits when the process is completed. The signal peptide is hydrolyzed by **signal peptidase,** located on the luminal side of the ER membrane (Figure 46–6), and then is apparently rapidly degraded by proteases.

Cytochrome P450 (Chapter 53), an integral ER membrane protein, does not completely cross the membrane. Instead, it resides in the membrane with its signal peptide intact. Its passage through the membrane is prevented by a sequence of amino acids called a **halt-** or **stop-transfer signal.**

Secretory proteins and **soluble proteins destined for organelles distal to the ER** completely traverse the membrane bilayer and are discharged into the lumen of the ER. ***N*-Glycan chains,** if present, are added (Chapter 47) as these proteins traverse the inner part of the ER membrane—a process called **"cotranslational glycosylation."** Subsequently, the proteins are found in the **lumen of the Golgi apparatus,** where further changes in glycan chains occur (Figure 47–9) prior to intracellular distribution or secretion. There is strong evidence that the **signal peptide** is involved in the process of protein **insertion into ER membranes.** Mutant proteins, containing altered signal peptides in which a hydrophobic amino acid is replaced by a hydrophilic one, are not inserted into ER membranes. Nonmembrane proteins (eg, α-globin) to which signal peptides have been attached by genetic engineering can be inserted into the lumen of the ER or even secreted.

There is evidence that the ER membrane is involved in **retrograde transport** of various molecules from the ER lumen **to the cytosol**. These molecules include unfolded or misfolded glycoproteins, glycopeptides, and oligosaccharides. At least some of these molecules are **degraded in proteasomes** (see below). Whether the translocon is involved in retrotranslocation is not clear; one or more other channels may be involved. Whatever the case, there is **two-way traffic** across the ER membrane.

PROTEINS FOLLOW SEVERAL ROUTES TO BE INSERTED INTO OR ATTACHED TO THE MEMBRANES OF THE ENDOPLASMIC RETICULUM

The routes that proteins follow to be inserted into the membranes of the ER include the following.

Cotranslational Insertion

Figure 46–7 shows a variety of ways in which proteins are distributed in the plasma membrane. In particular, the **amino terminals** of certain proteins (eg, the LDL receptor) can be seen to be on the extracytoplasmic face, whereas for other proteins (eg, the asialoglycoprotein receptor) the **carboxyl terminals** are on this face. To explain these dispositions, one must consider the initial biosynthetic events at the ER membrane. The **LDL receptor** enters the ER membrane in a manner analogous to a secretory protein (Figure 46–6); it partly traverses the ER membrane, its signal peptide is cleaved, and its amino terminal protrudes into the lumen. However, it is **retained in the membrane** because it contains a highly hydrophobic segment, the **halt- or stop-transfer signal.** This sequence forms the single transmembrane segment of the protein and is its membrane-anchoring domain. The small patch of ER membrane in which the newly synthesized LDL receptor is located subsequently **buds off** as a component of a **transport vesicle.** As described below in the discussion of asymmetry of proteins and lipids in membrane assembly, the disposition of the receptor in the ER membrane is preserved in the vesicle, which eventually fuses with the plasma membrane. In contrast, the **asialoglycoprotein receptor** possesses an **internal insertion sequence,** which inserts into the membrane but is **not cleaved.** This acts as an **anchor**, and its carboxyl terminal is extruded through the membrane. The **more complex disposition** of the **transporters** (eg, for glucose) can be explained by the fact that **alternating** transmembrane α-helices act as **uncleaved insertion sequences** and as **halt-transfer signals**, respectively. Each pair of helical segments is inserted as a **hairpin**. Sequences that determine the structure of a protein in a membrane are called **topogenic sequences.** As explained in the legend to Figure 46–7, the above three proteins are examples of **type I, type II,** and **type IV** transmembrane proteins.

Synthesis on Free Polyribosomes & Subsequent Attachment to the Endoplasmic Reticulum Membrane

An example is **cytochrome b_5,** which enters the ER membrane spontaneously.

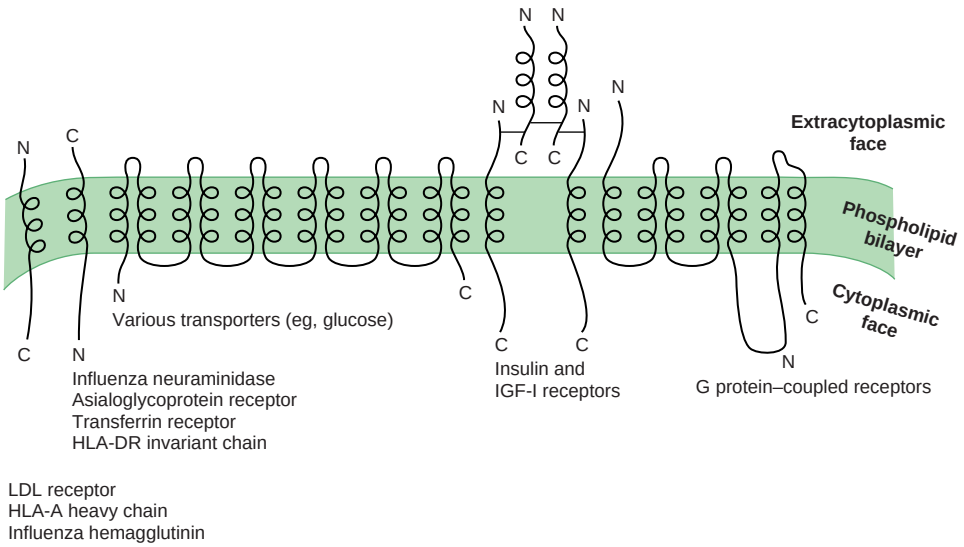

FIGURE 46–7 Variations in the way in which proteins are inserted into membranes. This schematic representation, which illustrates a number of possible orientations, shows the segments of the proteins within the membrane as α helices and the other segments as lines. The LDL receptor, which crosses the membrane once and has its amino terminal on the exterior, is called a type I transmembrane protein. The asialoglycoprotein receptor, which also crosses the membrane once but has its carboxyl terminal on the exterior, is called a type II transmembrane protein. Cytochrome P450 (not shown) is an example of a type III transmembrane protein; its disposition is similar to type I proteins, but does not contain a cleavable signal sequence. The various transporters indicated (eg, glucose) cross the membrane a number of times and are called type IV transmembrane proteins; they are also referred to as polytopic membrane proteins. (N, amino terminal; C, carboxyl terminal.) (Adapted, with permission, from Wickner WT, Lodish HF: Multiple mechanisms of protein insertion into and across membranes. Science 1985;230:400. Copyright ©1985 by the American Association for the Advancement of Science.)

Retention at the Luminal Aspect of the Endoplasmic Reticulum by Specific Amino Acid Sequences

A number of proteins possess the amino acid sequence **KDEL** (Lys-Asp-Glu-Leu) at their carboxyl terminal (see Table 46–1). KDEL-containing proteins first travel to the **GA** in COPII transport vesicles (see below), interact there with a specific KDEL receptor protein, and then **return in COPI transport vesicles to the ER**, where they dissociate from the receptor.

Retrograde Transport from the Golgi Apparatus

Certain other **non-KDEL-containing proteins** destined for the membranes of the ER also pass to the Golgi and then return, by **retrograde vesicular transport,** to the ER to be inserted therein (see below).

The foregoing paragraphs demonstrate that a **variety of routes** are involved in assembly of the proteins of the ER membranes; a similar situation probably holds for other membranes (eg, the mitochondrial membranes and the plasma membrane). Precise targeting sequences have been identified in some instances (eg, KDEL sequences).

The topic of membrane biogenesis is discussed further later in this chapter.

CHAPERONES ARE PROTEINS THAT PREVENT FAULTY FOLDING & UNPRODUCTIVE INTERACTIONS OF OTHER PROTEINS

Molecular chaperones have been referred to previously in this Chapter. A number of important properties of these proteins are listed in Table 46–5, and the names of some of particular importance in the ER are listed in Table 46–6. Basically, they **stabilize unfolded or partially folded intermediates**, allowing them time to fold properly, and prevent inappropriate interactions, thus combating the formation of nonfunctional structures. Most chaperones exhibit **ATPase activity** and bind ADP and ATP. This activity is important for their effect on protein folding. The ADP-chaperone complex often has a high affinity for the unfolded protein, which, when bound, stimulates release of ADP with replacement by ATP. The ATP-chaperone complex, in turn, releases segments of the protein that have folded properly, and the **cycle** involving ADP and ATP binding is repeated until the protein is released.

Chaperonins are the second major class of chaperones. They form complex **barrel-like structures** in which an unfolded protein is retained, giving it time and suitable conditions in which to fold properly. The mtGroEL chaperonin has been much studied. It is polymeric, has two ring-like struc-

TABLE 46–5 Some Properties of Chaperone Proteins

• Present in a wide range of species from bacteria to humans
• Many are so-called heat shock proteins (Hsp)
• Some are inducible by conditions that cause unfolding of newly synthesized proteins (eg, elevated temperature and various chemicals)
• They bind to predominantly hydrophobic regions of unfolded proteins and prevent their aggregation
• They act in part as a quality control or editing mechanism for detecting misfolded or otherwise defective proteins
• Most chaperones show associated ATPase activity, with ATP or ADP being involved in the protein–chaperone interaction
• Found in various cellular compartments such as cytosol, mitochondria, and the lumen of the endoplasmic reticulum

TABLE 46–6 Some Chaperones and Enzymes Involved in Folding That Are Located in the Rough Endoplasmic Reticulum

• BiP (immunoglobulin heavy chain binding protein)
• GRP94 (glucose-regulated protein)
• Calnexin
• Calreticulin
• PDI (protein disulfide isomerase)
• PPI (peptidyl prolyl *cis-trans* isomerase)

tures, each composed of seven identical subunits, and again ATP is involved in its action.

Several examples of chaperones were introduced above when the sorting of mitochondrial proteins was discussed. The **immunoglobulin heavy chain binding protein (BiP)** is located in the lumen of the ER. This protein **promotes proper folding by preventing aggregation** and will bind abnormally folded immunoglobulin heavy chains and certain other proteins and prevent them from leaving the ER. Another important chaperone is **calnexin,** a calcium-binding protein located in the ER membrane. This protein binds a wide variety of proteins, including major histocompatibility complex (MHC) antigens and a variety of plasma proteins. As described in Chapter 47, calnexin binds the monoglycosylated species of glycoproteins that occur during processing of glycoproteins, retaining them in the ER until the glycoprotein has folded properly. **Calreticulin,** which is also a calcium-binding protein, has properties similar to those of calnexin; it is not membrane-bound. Chaperones are not the only proteins in the ER lumen that are concerned with proper folding of proteins. Two **enzymes** are present that play an active role in folding. **Protein disulfide isomerase (PDI)** promotes rapid **formation and** reshuffling of disulfide bonds until the correct set is achieved. **Peptidyl prolyl isomerase (PPI)** accelerates folding of proline-containing proteins by catalyzing the cis-trans isomerization of X-Pro bonds, where X is any amino acid residue.

ACCUMULATION OF MISFOLDED PROTEINS IN THE ENDOPLASMIC RETICULUM CAN INDUCE THE UNFOLDED PROTEIN RESPONSE (UPR)

Maintenance of **homeostasis in the ER** is important for normal cell function. When the unique environment within the lumen of the ER is perturbed (eg, changes in ER Ca^{2+}, alterations of redox status, exposure to various toxins or some viruses), this can lead to reduced protein folding capacity and the accumulation of misfolded proteins. The accumulation of misfolded proteins in the ER is referred to as ER stress. The cell has evolved a mechanism termed the unfolded protein response (UPR) to sense the levels of misfolded proteins and initiate intracellular signaling mechanisms to compensate for the stress conditions and restore ER homeostasis. The UPR is initiated by ER stress sensors which are transmembrane proteins embedded in the ER membrane. Activation of these stress sensors causes three principal effects: transient inhibition of translation to reduce the amount of newly synthesized proteins and induction of a transcriptional response that leads to increased expression of ER chaperones and of proteins involved in degradation of misfolded ER proteins (discussed below). Therefore, the UPR increases the ER folding capacity and prevents a buildup of unproductive and potentially toxic protein products, in addition to other responses to restore cellular homeostasis. However, if impairment of folding persists, cell death pathways (apoptosis) are activated. A more complete understanding of the UPR is likely to provide new approaches to treating diseases in which ER stress and defective protein folding occur (see Table 46–7).

MISFOLDED PROTEINS UNDERGO ENDOPLASMIC RETICULUM–ASSOCIATED DEGRADATION (ERAD)

Misfolded proteins occur in many genetic diseases (eg, see Table 46–7). Proteins that misfold in the ER are selectively **transported back across the ER (retrotranslocation or dislocation)** to enter **proteasomes** present in the cytosol. The precise route by which the misfolded proteins pass back across the ER membrane is still under investigation. If a channel is involved, it does not appear to be the translocon (Sec61 complex) described earlier, although it may contain some of its components. The energy for translocation appears to be at least partly supplied by **p97**, an AAA-ATPase (one of a family of **A**TPases **A**ssociated with various cellular **A**ctivities). **Chaperones** present in the lumen of the ER (eg, BiP) and in the cytosol help target misfolded proteins to proteasomes. Prior to entering proteasomes, most proteins are **ubiquitinated** (see the next paragraph) and are escorted to proteasomes by polyubiquitin-binding proteins. Ubiquitin ligases are present in the ER membrane. The above process is referred to as ERAD and is outlined in Figure 46–8.

TABLE 46–7 Some Conformational Diseases That Are Caused by Abnormalities in Intracellular Transport of Specific Proteins and Enzymes Due to Mutations[1]

Disease	Affected Protein
α_1-Antitrypsin deficiency with liver disease (OMIM 107400)	α_1-Antitrypsin
Chediak-Higashi syndrome (OMIM 214500)	Lysosomal trafficking regulator
Combined deficiency of factors V and VIII (OMIM 227300)	ERGIC53, a mannose-binding lectin
Cystic fibrosis (OMIM 219700)	CFTR
Diabetes mellitus [some cases] (OMIM 147670)	Insulin receptor (α-subunit)
Familial hypercholesterolemia, autosomal dominant (OMIM 143890)	LDL receptor
Gaucher disease (OMIM 230800)	β-Glucosidase
Hemophilia A (OMIM 306700) and B (OMIM 306900)	Factors VIII and IX
Hereditary hemochromatosis (OMIM 235200)	HFE
Hermansky-Pudlak syndrome (OMIM 203300)	AP-3 adaptor complex β3A subunit
I-cell disease (OMIM 252500)	N-acetylglucosamine 1-phospho-transferase
Lowe oculocerebrorenal syndrome (OMIM 309000)	PIP_2 5-phosphatase
Tay-Sachs disease (OMIM 272800)	β-Hexosaminidase
von Willebrand disease (OMIM 193400)	von Willebrand factor

Abbreviation: PIP_2, phosphatidylinositol 4,5-bisphosphate.
Note: Readers should consult textbooks of medicine or pediatrics for information on the clinical manifestations of the conditions listed.
[1]See Schroder M, Kaufman RJ: The Mammalian Unfolded Protein Response. Annu Rev Biochem 2005;74, 739 and OlkonnenV, Ikonen E: Genetic defects of intracellular membrane transport. N Eng J Med 2000;343: 10095.

UBIQUITIN IS A KEY MOLECULE IN PROTEIN DEGRADATION

There are two major pathways of protein degradation in eukaryotes. One involves **lysosomal proteases** and does not require ATP. The other pathway involves **ubiquitin** and is ATP-dependent. It plays the major role in the degradation of proteins, and is particularly associated with **disposal of misfolded proteins and regulatory enzymes that have short half-lives.** Research on ubiquitin has expanded rapidly, and it is known to be involved in **cell cycle regulation** (degradation of cyclins), **DNA repair**, **activation of NFκB** (see Chapter 50), **muscle wasting**, **viral infections**, and **many other** important physiologic and pathologic processes. Ubiquitin is a **small** (76 amino acids), **highly conserved protein** that plays a key role in **marking** various proteins for subsequent **degradation in proteasomes**. The mechanism of attachment of ubiquitin to a target protein (eg, a misfolded form of CFTR, the protein involved in the causation of cystic fibrosis; see Chapters 40 & 54) is shown in Figure 46–9 and involves **three enzymes**: an **activating enzyme**, a **conjugating enzyme**, and a **ligase**. There are a number of types of conjugating enzymes, and, surprisingly, some hundreds of different ligases. It is the latter enzyme that confers substrate specificity. Once the molecule of ubiquitin is attached to the protein, a number of others are also attached, resulting in a **polyubiquitinated target protein**. It has been estimated that a **minimum of four ubiquitin molecules** must be attached to commit a target molecule to degradation in a proteasome. Ubiquitin can be **cleaved** from a target protein by deubiquitinating **enzymes** and the liberated ubiquitin can be reused.

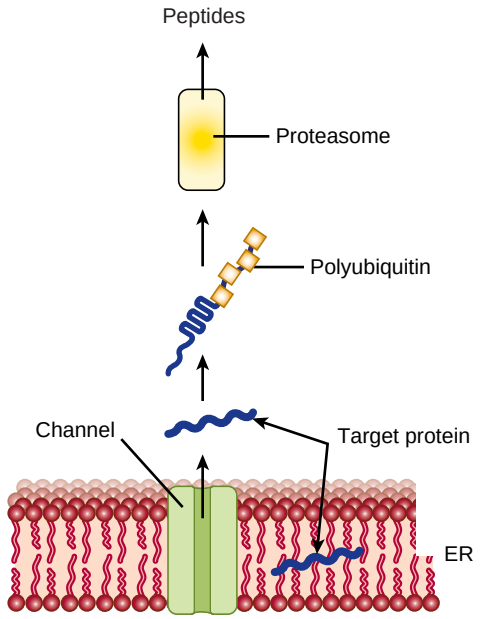

FIGURE 46–8 Schematic diagram of the events in ERAD. A target protein (which may be misfolded or normally folded) undergoes retrograde transport through the ER membrane into the cytosol, where it is subjected to polyubiquitination. Following polyubiquitination, it enters a proteasome, inside which it is degraded to small peptides that exit and may have several fates. Liberated ubiquitin molecules are recycled. The precise route by which misfolded proteins pass back through the ER membrane is not as yet known; a channel may exist (as shown in the figure), but that has not apparently been established.

Ubiquitinated Proteins Are Degraded in Proteasomes

Polyubiquitinated target proteins enter the proteasomes, located in the cytosol. The proteasome is a relatively **large cylindrical structure** and is composed of some **50 subunits.** The proteasome has a hollow **core**, and one or two **caps** that play a regulatory role. Target proteins are **unfolded** by ATPases present in the proteasome caps. Proteasomes can hydrolyze a very wide variety of peptide bonds. Target proteins pass into the core to be degraded to small peptides, which then exit the proteasome (Figure 46–8) to be further degraded by cytosolic peptidases. Both normally and abnormally folded proteins

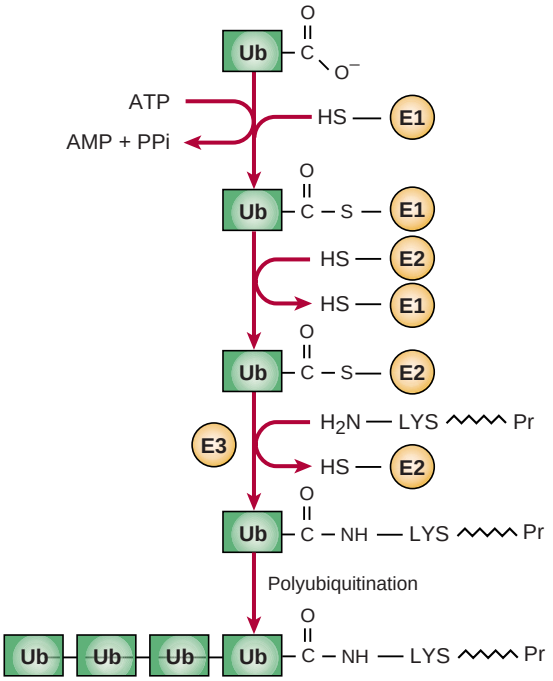

FIGURE 46–9 Sequence of reactions in addition of ubiquitin to a target protein. In the reaction catalyzed by E1, the C-terminal COO^- group of ubiquitin is linked in a thioester bond to an SH group of E1. In the reaction catalyzed by E2, the activated ubiquitin is transferred to an SH group of E2. In the reaction catalyzed by E3, ubiquitin is transferred from E2 to an ε-amino group on a lysine of the target protein. Additional rounds of ubiquitination then build up the polyubiquitin chain. (Ub, ubiquitin; E1, activating enzyme; E2, conjugating enzyme; E3, ligase; LYS ^^^^ Pr, target protein.)

are substrates for the proteasome. Liberated ubiquitin molecules are recycled. The proteasome plays an important role in **presenting small peptides** produced by **degradation of various viruses** and other molecules to **major histocompatibility class I molecules**, a key step in antigen presentation to T lymphocytes.

TRANSPORT VESICLES ARE KEY PLAYERS IN INTRACELLULAR PROTEIN TRAFFIC

Proteins that are synthesized on membrane-bound polyribosomes and are destined for the GA or PM reach these sites inside **transport vesicles.** Those vesicles involved in **anterograde transport** (COPII) from the ER to the GA and in **retrograde transport** (COPI) from the GA to the ER are clathrin-free. Transport and secretory vesicles carrying cargo from the GA to the PM are also clathrin-free. The vesicles involved in endocytosis (see discussions of the LDL receptor in Chapters 25 & 26) are coated with clathrin, as are certain vesicles carrying cargo to lysosomes. For the sake of clarity, the non-clathrin-coated vesicles are referred to in this text as **transport vesicles.** Table 46–8 summarizes **the types** and **functions** of the major vesicles identified to date.

TABLE 46–8 Some Types of Vesicles and Their Functions

Vesicle	Function
COPI	Involved in intra-GA transport and retrograde transport from the GA to the ER
COPII	Involved in export from the ER to either ERGIC or the GA
Clathrin	Involved in transport in post-GA locations including the PM, TGN and endosomes
Secretory vesicles	Involved in regulated secretion from organs such as the pancreas (eg, secretion of insulin)
Vesicles from the TGN to the PM	They carry proteins to the PM and are also involved in constitutive secretion

Abbreviations: GA, Golgi apparatus; ER, endoplasmic reticulum; ERGIC, ER-GA intermediate compartment; PM, plasma membrane; TGN, *trans*-Golgi network.
Note: Each vesicle has its own set of coat proteins. Clathrin is associated with various adapter proteins (APs), eg, AP-1, AP-2 and AP-3, forming different types of clathrin vesicles. These various clathrin vesicles have different intracellular targets. The proteins of secretory vesicles and vesicles involved in transport from the GA to the PM are not well characterized, nor are the mechanisms involved in their formations and fates.

Model of Transport Vesicles Involves SNAREs & Other Factors

Vesicles lie at the heart of intracellular transport of many proteins. Significant progress has been made in understanding the events involved in vesicle formation and transport. This has transpired because of the use of a number of approaches. In particular, the use by Schekman and colleagues of **genetic approaches for studying vesicles in yeast** and the development by Rothman and colleagues of **cell-free systems** to study vesicle formation have been crucial. For instance, it is possible to observe, by electron microscopy, budding of vesicles from Golgi preparations incubated with cytosol and ATP. The overall mechanism is complex, with its own **nomenclature** (Table 46–9), and involves a variety of cytosolic and membrane proteins, GTP, ATP, and accessory factors. **Budding**, **tethering**, **docking,** and **membrane fusion** are key steps in the life cycles of vesicles with Sar, ARF, and the Rab GTPases (see below) acting as **molecular switches.**

There are common general steps in transport vesicle formation, vesicle targeting and fusion with a target membrane, irrespective of the membrane the vesicle forms from or its intracellular destination. The nature of the coat proteins, GTPases and targeting factors differ depending on where the vesicle forms from and its eventual destination. Transport from the ER to the Golgi is the best studied example and will be used to illustrate these steps. **Anterograde vesicular transport** from the ER to the Golgi involves **COPII vesicles** and the process can be considered to occur in eight steps (Figure 46–10). The basic concept is that each transport vesicle is loaded with specific cargo and also one or more **v-SNARE**

TABLE 46–9 Some Factors Involved in the Formation of Non-Clathrin-Coated Vesicles and Their Transport

- ARF: ADP-ribosylation factor, a GTPase involved in formation of COPI and also clathrin-coated vesicles.
- Coat proteins: A family of proteins found in coated vesicles. Different transport vesicles have different complements of coat proteins.
- GTP-γ-S: A nonhydrolyzable analog of GTP, used to test the involvement of GTP in biochemical processes.
- NEM: *N*-Ethylmaleimide, a chemical that alkylates sulfhydryl groups and inactivates NSF.
- NSF: NEM-sensitive factor, an ATPase.
- Sar1: A GTPase that plays a key role in assembly of COPII vesicles.
- Sec 12: A guanine nucleotide exchange factor (GERF) that interconverts Sar1.GDP and Sar1.GTP.
- α-SNAP: Soluble NSF attachment protein. Along with NSF, this protein is involved in dissociation of SNARE complexes.
- SNARE: SNAP receptor. SNAREs are key molecules in the fusion of vesicles with acceptor membranes.
- t-SNARE: Target SNARE.
- v-SNARE: Vesicle SNARE.
- Rab proteins: A family of Ras-related proteins (monomeric GTPases) first observed in rat brain. They are active when GTP is bound. Different Rab molecules dock different vesicles to acceptor membranes.
- Rab effector proteins: A family of proteins that interact with Rab molecules; some act to tether vesicles to acceptor membranes.

proteins that direct targeting. Each target membrane bears one or more **complementary t-SNARE proteins** with which the former interact, mediating SNARE protein-dependent vesicle-membrane fusion. In addition, **Rab proteins** also help direct the vesicles to specific membranes and are involved in tethering, prior to vesicle docking at a target membrane.

Step 1: **Budding** is initiated when **Sar1** is activated by binding **GTP**, which is exchanged for GDP via the action of **Sec12**. This causes a conformational change in Sar1·GTP, embedding it in the ER membrane to form a focal point for vesicle assembly.

Step 2: Various **coat proteins** bind to **Sar1**·GTP. In turn, membrane cargo proteins bind to the coat proteins and soluble cargo proteins inside vesicles bind to receptor regions of the former. Additional coat proteins are assembled to **complete bud formation**. Coat proteins promote budding, contribute to the curvature of buds and also help sort proteins.

Step 3: The **bud pinches off**, completing formation of the coated vesicle. The curvature of the ER membrane and protein–protein and protein–lipid interactions in the bud facilitate pinching off from ER exit sites.

Step 4: **Coat disassembly** (involving **dissociation** of **Sar1** and the **shell** of coat proteins) follows **hydrolysis of bound GTP to GDP** by Sar1, promoted by a specific coat protein. Sar1 thus plays key roles in both assembly and dissociation of the coat proteins. Uncoating is necessary for fusion to occur.

Step 5: **Vesicle targeting** is achieved by attachment of **Rab** molecules to vesicles. Rab·GDP molecules in the cytosol are converted to Rab·GTP molecules by a specific guanine nucleotide exchange factor and these attach to the vesicles. The Rab.GTP molecules subsequently interact with **Rab effector proteins** on membranes to **tether** the vesicle to the membranes.

Step 6: **v-SNAREs pair with cognate t-SNAREs** in the target membrane to **dock** the vesicles and initiate fusion. Generally one v-SNARE in the vesicle pairs with three t-SNAREs on the acceptor membrane to form a tight **four-helix bundle**.

Step 7: **Fusion** of the vesicle with the acceptor membrane occurs once the v- and t-SNARES are closely aligned. After vesicle fusion and release of contents occurs, GTP is hydrolyzed to GDP, and the Rab·GDP molecules are released into the cytosol. When a SNARE on one membrane interacts with a SNARE on another membrane, linking the two membranes, this is referred to as a trans-SNARE complex or a SNARE pin. Interactions of SNARES on the same membrane form a cis-SNARE complex. In order to **dissociate the four-helix bundle** between the v- and t-SNARES so that they can be re-used, two additional proteins are required. These are an **ATPase** (NSF) and **α-SNAP**. NSF hydrolyzes ATP and the energy released dissociates the four-helix bundle making the SNARE proteins available for another round of membrane fusion.

Step 8: Certain components are **recycled** (eg, Rab, possibly v-SNAREs).

During the above cycle, SNARES, tethering proteins, Rab and other proteins all **collaborate** to deliver a vesicle and its contents to the appropriate site.

COPI, COPII, and Clathrin-Coated Vesicles Have Been Most Studied

The following points clarify and expand on the previous section.

1. As indicated in Table 46–8, there are **a number of different types of vesicles**. Other types of vesicles may remain to be discovered. Here we focus mainly on COPII, COPI and clathrin-coated vesicles. Each of these types has a different complement of proteins in its coat. The details of assembly for COPI and clathrin-coated vesicles are somewhat different from those described above. For example, **Sar1** is the protein involved in step 1 of formation of COPII vesicles, whereas **ARF** is involved in the formation of COPI and clathrin-coated vesicles. However, the principles concerning assembly of these different types are generally similar.

2. Regarding **selection** of cargo molecules by vesicles, this appears to be primarily **a function of the coat proteins** of vesicles. **Cargo molecules** via their sorting signals may interact with coat proteins either **directly** or via **intermediary proteins** that attach to coat proteins, and they then become enclosed in their appropriate vesicles. A number of **signal se-**

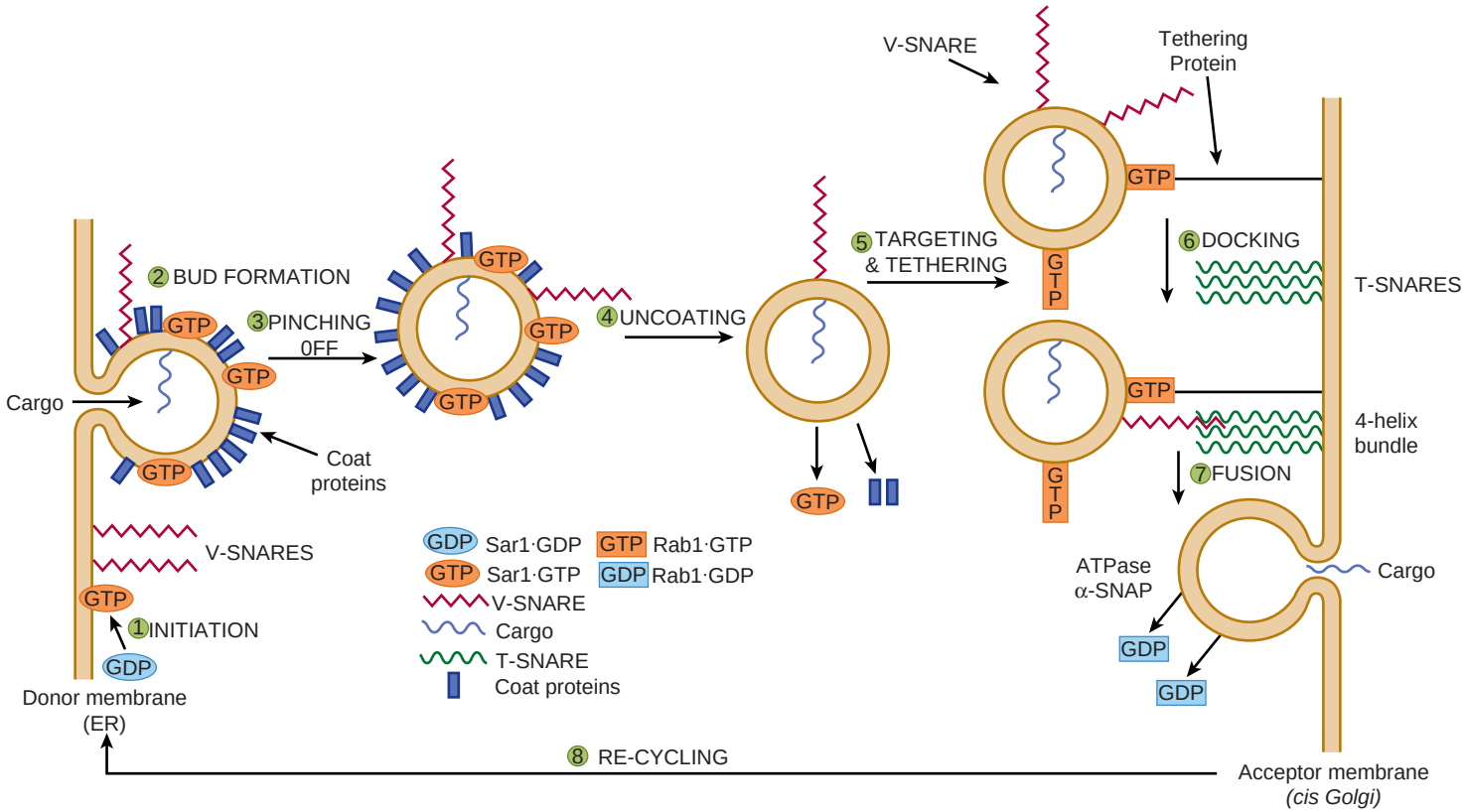

FIGURE 46–10 Model of the steps in a round of anterograde transport involving COPII vesicles. The cycle starts in the bottom left-hand side of the figure, where two molecules of Sar1 are represented as small ovals containing GDP. The steps in the cycle are described in the text. The various components are briefly described in Table 46–7. The roles of Rab and Rab effector proteins (see text) in the overall process are not dealt with in this figure. (Adapted, with permission, from Rothman JE: Mechanisms of intracellular protein transport. Nature 1994;372:55. Courtesy of E Degen.)

quences on cargo molecules have been identified (see Table 46–1). For example KDEL sequences direct certain ER resident proteins in retrograde flow to the ER in COPI vesicles. Di-acidic sequences (eg, Asp-X-Glu) and short hydrophobic sequences on membrane proteins are involved in interactions with coat proteins of COPII vesicles.

Proteins in the **apical** or **basolateral** areas of the plasma membranes of polarized epithelial cells can be transported to these sites in **transport vesicles** budding from the TGN. Different Rab proteins likely direct some vesicles to apical regions and others to basolateral regions. In certain cells, proteins are first directed to the basolateral membrane, then endocytosed and transported across the cell by **transcytosis** to the apical region. Yet another mechanism for sorting proteins to the apical region (or in some cases to the basolateral region) involves the **glycosylphosphatidylinositol (GPI) anchor** described in Chapter 47. This structure is also often present in **lipid rafts** (see Chapter 40).

Not all cargo molecules may have a sorting signal. Some highly abundant secretory proteins travel to various cellular destinations in transport vesicles by **bulk flow**; ie, they enter into transport vesicles at the same concentration that they occur in the organelle. The precise extent of bulk flow is not clearly known, although it appears that most proteins are actively sorted (concentrated) into transport vesicles and bulk flow is used by only a select group of cargo proteins.

3. Once proteins in the secretory pathway reach the *cis*-Golgi from the ER in vesicles, they can travel through the GA to the *trans*-Golgi **in vesicles,** or by a process called **cisternal maturation,** or perhaps in some cases by **simple diffusion**. A former view was that the GA is essentially a static organelle, allowing vesicular flow from one static cisterna to the next. There is now, however, evidence to support the view that the cisternae move and transform into one another (ie, cisternal maturation). In this model, vesicular elements from the ER fuse with one another to help form the *cis*-Golgi, which in turn can move forward to become the medial Golgi, etc. COPI vesicles return Golgi enzymes (eg, glycosyltransferases) back from distal cisternae of the GA to more proximal (eg, *cis*) cisternae.

4. Vesicles move through cells along **microtubules** or along **actin filaments**.

5. The fungal metabolite **brefeldin A prevents GTP from binding to ARF**, and thus inhibits formation of COPI vesicles. In its presence, the Golgi apparatus appears to **collapse into the ER**. It may do this by inhibiting the guanine nucleotide exchanger involved in formation of COPI vesicles. Brefeldin A has thus proven to be a useful tool for examining some aspects of Golgi structure and function.

6. **GTP-γ-S** (a nonhydrolyzable analog of GTP often used in investigations of the role of GTP in biochemical processes) **blocks disassembly of the coat** from coated vesicles, leading to a build-up of coated vesicles, facilitating their study.

7. As mentioned above, a family of Ras-like proteins, called the **Rab protein family,** are required in several steps of intracellular protein transport and also in regulated secretion and endocytosis. (Ras proteins are involved in cell signaling via receptor tyrosine kinases). Like Ras, Rab proteins are **small monomeric GTPases** that attach to the cytosolic faces of membranes (via **geranylgeranyl** lipid anchors). They **attach** in the **GTP-bound state** to the budding vesicle and are also present on acceptor membranes. Rab proteins interact with **Rab effector proteins**, that have various roles, such as involvement in tethering and in membrane fusion.

8. The fusion of **synaptic vesicles** with the plasma membrane of **neurons** involves a series of events similar to that described above. For example, one v-SNARE is designated **synaptobrevin** and two t-SNAREs are designated **syntaxin** and **SNAP 25** (synaptosome-associated protein of 25 kDa). **Botulinum B toxin** is one of the most lethal toxins known and the most serious cause of food poisoning. One component of this toxin is a **protease** that appears to **cleave only synaptobrevin**, thus **inhibiting release of acetylcholine** at the neuromuscular junction and possibly proving fatal, depending on the dose taken.

9. Although the above model refers to **non-clathrin-coated vesicles,** it appears likely that many of the events described above apply, at least in principle, to clathrin-coated vesicles.

10. Some proteins are further subjected to **further processing by proteolysis** while inside either transport or secretory vesicles. For example, **albumin** is synthesized by hepatocytes as **preproalbumin** (see Chapter 50). Its signal peptide is removed, converting it to **proalbumin**. In turn, proalbumin, while inside transport vesicles, is converted to **albumin** by action of **furin** (Figure 46–11). This enzyme cleaves a hexapeptide from proalbumin immediately C-terminal to a dibasic amino acid site (ArgArg). The resulting mature albumin is secreted into the plasma. Hormones such as **insulin** (see Chapter 41) are subjected to similar proteolytic cleavages while inside secretory vesicles.

THE ASSEMBLY OF MEMBRANES IS COMPLEX

There are many cellular membranes, each with its own specific features. No satisfactory scheme describing the assembly of

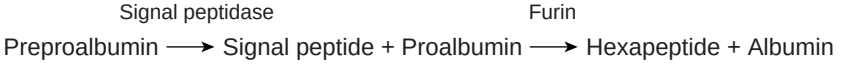

FIGURE 46–11 Cleavage of preproalbumin to proalbumin and of the latter to albumin. Furin cleaves proalbumin at the C-terminal end of a basic dipeptide (ArgArg).

any one of these membranes is available. How various proteins are initially inserted into the membrane of the ER has been discussed above. The transport of proteins, including membrane proteins, to various parts of the cell inside vesicles has also been described. Some general points about membrane assembly remain to be addressed.

Asymmetry of Both Proteins & Lipids Is Maintained During Membrane Assembly

Vesicles formed from membranes of the ER and Golgi apparatus, either naturally or pinched off by homogenization, exhibit **transverse asymmetries** of both lipid and protein. These **asymmetries are maintained** during fusion of transport vesicles with the plasma membrane. The **inside** of the vesicles after fusion becomes the **outside of the plasma membrane**, and the cytoplasmic side of the vesicles remains the cytoplasmic side of the membrane (Figure 46–12). Since the transverse asymmetry of the membranes already exists in the vesicles of the ER well before they are fused to the plasma membrane, a major problem of membrane assembly becomes understanding how the integral proteins are inserted into the lipid bilayer of the ER. This problem was addressed earlier in this chapter.

Phospholipids are the major class of lipid in membranes. The enzymes responsible for the synthesis of phospholipids reside in the cytoplasmic surface of the cisternae of the ER. As phospholipids are synthesized at that site, they probably self-assemble into thermodynamically stable bimolecular layers, thereby expanding the membrane and perhaps promoting the detachment of so-called lipid vesicles from it. It has been proposed that these vesicles travel to other sites, donating their lipids to other membranes; however, little is known about this matter. As indicated above, cytosolic proteins that take up phospholipids from one membrane and release them to another (ie, **phospholipid exchange proteins**) have been demonstrated; they probably play a role in contributing to the specific lipid composition of various membranes.

It should be noted that the **lipid compositions** of the ER, Golgi and plasma membrane differ, the latter two membranes containing **higher amounts of cholesterol**, **sphingomyelin and glycosphingolipids**, and **less phosphoglycerides** than does the ER. Sphingolipids pack more densely in membranes than do phosphoglycerides. These differences affect the structures and functions of membranes. For example, the **thickness of the bilayer** of the GA and PM is greater than that of the ER, which affects what particular transmembrane proteins are found in these organelles. Also, **lipid rafts** (see earlier discussion) are believed to be formed in the GA

Lipids & Proteins Undergo Turnover at Different Rates in Different Membranes

It has been shown that the half-lives of the lipids of the ER membranes of rat liver are generally shorter than those of its proteins, so that the **turnover rates of lipids and proteins are independent.** Indeed, different lipids have been found to have different half-lives. Furthermore, the half-lives of the proteins of these membranes vary quite widely, some exhibiting short (hours) and others long (days) half-lives. Thus, individual lipids and proteins of the ER membranes appear to be inserted into it relatively independently; this is the case for many other membranes.

The biogenesis of membranes is thus a complex process about which much remains to be learned. One indication of

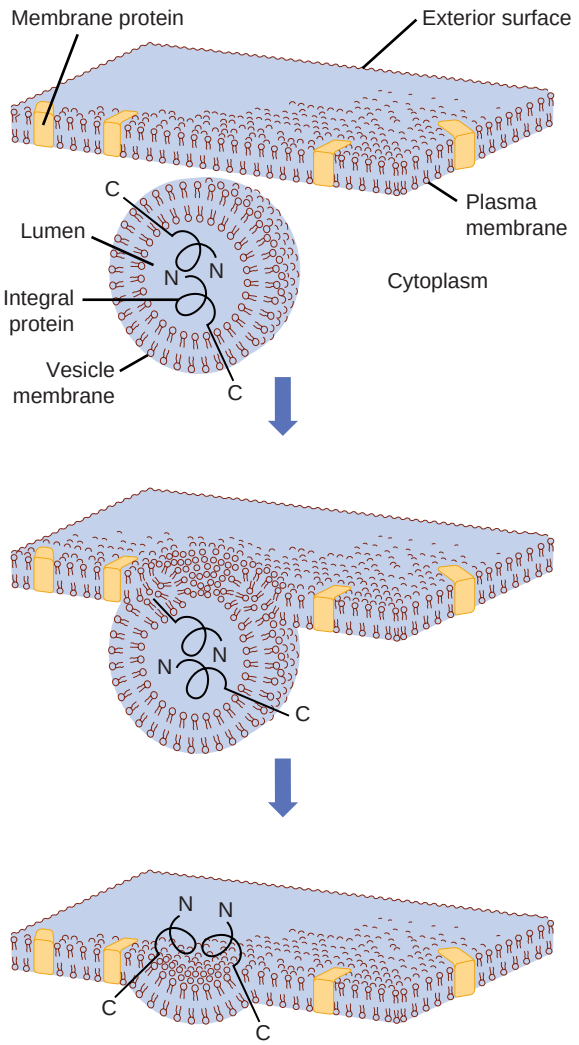

FIGURE 46–12 Fusion of a vesicle with the plasma membrane preserves the orientation of any integral proteins embedded in the vesicle bilayer. Initially, the amino terminal of the protein faces the lumen, or inner cavity, of such a vesicle. After fusion, the amino terminal is on the exterior surface of the plasma membrane. That the orientation of the protein has not been reversed can be perceived by noting that the other end of the molecule, the carboxyl terminal, is always immersed in the cytoplasm. The lumen of a vesicle and the outside of the cell are topologically equivalent. (Redrawn and modified, with permission, from Lodish HF, Rothman JE: The assembly of cell membranes. Sci Am [Jan] 1979;240:43.)

TABLE 46–10 Some Major Features of Membrane Assembly

• Lipids and proteins are inserted independently into membranes.
• Individual membrane lipids and proteins turn over independently and at different rates.
• Topogenic sequences (eg, signal [amino terminal or internal] and stop-transfer) are important in determining the insertion and disposition of proteins in membranes.
• Membrane proteins inside transport vesicles bud off the endoplasmic reticulum on their way to the Golgi; final sorting of many membrane proteins occurs in the trans-Golgi network.
• Specific sorting sequences guide proteins to particular organelles such as lysosomes, peroxisomes, and mitochondria.

the complexity involved is to consider the number of **post-translational modifications** that membrane proteins may be subjected to prior to attaining their mature state. These include disulfide formation, proteolysis, assembly into multimers, glycosylation, addition of a glycophosphatidylinositol (GPI) anchor, sulfation on tyrosine or carbohydrate moieties, phosphorylation, acylation, and prenylation—a list that is not complete. Nevertheless, significant progress has been made; Table 46–10 summarizes some of the major features of membrane assembly that have emerged to date.

Various Disorders Result from Mutations in Genes Encoding Proteins Involved in Intracellular Transport

Some disorders reflecting abnormal **peroxisomal** function and abnormalities of protein synthesis in the **ER** and of the synthesis of **lysosomal proteins** have been listed earlier in this Chapter (see Table 46–3 and Table 46–7, respectively). Many other mutations affecting intracellular protein transport to various organelles have been reported, but are not included here. The elucidation of the causes of these various **conformational disorders** has contributed significantly to our understanding of **molecular pathology**. Apart from the possibility of **gene therapy**, it is hoped that attempts to restore at least a degree of normal folding to misfolded proteins by **administration to affected individuals of small molecules** that interact specifically with such proteins will be of therapeutic benefit. This is an active area of research.

SUMMARY

- Many proteins are targeted to their destinations by signal sequences. A major sorting decision is made when proteins are partitioned between cytosolic and membrane-bound polyribosomes by virtue of the absence or presence of a signal peptide.
- Pathways of protein import into mitochondria, nuclei, peroxisomes, and the endoplasmic reticulum are described.
- Numerous proteins synthesized on membrane-bound polyribosomes proceed to the Golgi apparatus and the plasma membrane in transport vesicles.
- Many glycosylation reactions occur in compartments of the Golgi, and proteins are further sorted in the *trans*-Golgi network.
- The role of chaperone proteins in the folding of proteins is presented and the unfolded protein response is described.
- Endoplasmic reticulum–associated degradation (ERAD) is briefly described and the key role of ubiquitin in protein degradation is shown.
- A model describing budding and attachment of transport vesicles to a target membrane is summarized.
- Certain proteins (eg, precursors of albumin and insulin) are subjected to proteolysis while inside transport vesicles, producing the mature proteins.
- Small GTPases (eg, Ran, Rab) and guanine nucleotide-exchange factors play key roles in many aspects of intracellular trafficking.
- The complex process of membrane assembly is discussed briefly. Asymmetry of both lipids and proteins is maintained during membrane assembly.
- Many disorders have been shown to be due to mutations in genes that affect the folding of various proteins. These conditions are often referred to as conformational diseases. Apart from gene therapy, the development of small molecules that interact with misfolded proteins and help restore at least some of their function is an important area of research.

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CHAPTER

47

Glycoproteins

Robert K. Murray, MD, PhD

BIOMEDICAL IMPORTANCE

Glycobiology is the study of the roles of sugars in health and disease. The **glycome** is the entire complement of sugars, whether free or present in more complex molecules, of an organism. **Glycomics,** an analogous term to genomics and proteomics, is the comprehensive study of glycomes, including genetic, physiologic, pathologic, and other aspects.

One major class of molecules included in the glycome is **glycoproteins.** These are proteins that contain oligosaccharide chains (glycans) covalently attached to their polypeptide backbones. It has been estimated that approximately 50% of eukaryotic proteins have sugars attached, so that **glycosylation** (enzymic attachment of sugars) is the most frequent post-translational modification of proteins. Nonenzymic attachment of sugars to proteins can also occur, and is referred to as **glycation.** This process can have serious pathologic consequences (eg, in poorly controlled diabetes mellitus). Glycoproteins are one class of **glycoconjugate** or **complex carbohydrate**—equivalent terms used to denote molecules containing one or more carbohydrate chains covalently linked to protein (to form glycoproteins or proteoglycans) or lipid (to form glycolipids). (**Proteoglycans** are discussed in Chapter 48 and **glycolipids** in Chapter 15.) Almost all the **plasma proteins** of humans—with the notable exception of albumin—are glycoproteins. Many **proteins of cellular membranes** (Chapter 40) contain substantial amounts of carbohydrate. A number of the **blood group substances** are glycoproteins, whereas others are glycosphingolipids. Certain **hormones** (eg, chorionic gonadotropin) are glycoproteins. A major problem in cancer is **metastasis,** the phenomenon whereby cancer cells leave their tissue of origin (eg, the breast), migrate through the bloodstream to some distant site in the body (eg, the brain), and grow there in an unregulated manner, with catastrophic results for the affected individual. Many cancer researchers think that alterations in the structures of glycoproteins and other glycoconjugates on the surfaces of cancer cells are important in the phenomenon of metastasis.

GLYCOPROTEINS OCCUR WIDELY & PERFORM NUMEROUS FUNCTIONS

Glycoproteins occur in most organisms, from bacteria to humans. Many viruses also contain glycoproteins, some of which have been much investigated, in part because they often play key roles in viral attachment to cells (eg, HIV-1 and influenza A virus). Numerous proteins with diverse functions are glycoproteins (Table 47–1); their carbohydrate content ranges from 1% to over 85% by weight.

Many studies have been conducted in an attempt to define the precise roles oligosaccharide chains play in the functions of glycoproteins. Table 47–2 summarizes results from such studies. Some of the functions listed are firmly established; others are still under investigation.

OLIGOSACCHARIDE CHAINS ENCODE BIOLOGIC INFORMATION

An enormous number of glycosidic linkages can be generated between sugars. For example, three different hexoses may be linked to each other to form over 1000 different trisaccharides. The conformations of the sugars in oligosaccharide chains vary depending on their linkages and proximity to other molecules with which the oligosaccharides may interact. It is now established that certain oligosaccharide chains encode **biologic information** and that this depends upon their constituent sugars, their sequences, and their linkages. For instance, mannose 6-phosphate residues target newly synthesized lysosomal enzymes to that organelle (see later). The biologic information that sugars contain is expressed via interactions between specific sugars, either free or in glycoconjugates, and proteins (such as lectins; see below) or other molecules. These interactions lead to changes of cellular activity. Thus, deciphering the so-called "**sugar code of life"** (one of the principal aims of glycomics) entails elucidating all of the interactions that sugars and sugar-containing molecules participate in, and also the results of these interactions on cellular behavior. This will not be an easy task, considering the diversity of glycans found in cells.

TECHNIQUES ARE AVAILABLE FOR DETECTION, PURIFICATION, STRUCTURAL ANALYSIS, & SYNTHESIS OF GLYCOPROTEINS

A variety of methods used in the detection, purification, and structural analysis of glycoproteins are listed in Table 47–3.

TABLE 47–1 Some Functions Served by Glycoproteins

Function	Glycoproteins
Structural molecule	Collagens
Lubricant and protective agent	Mucins
Transport molecule	Transferrin, ceruloplasmin
Immunologic molecule	Immunoglobulins, histocompatibility antigens
Hormone	Chorionic gonadotropin, thyroid-stimulating hormone (TSH)
Enzyme	Various, eg, alkaline phosphatase
Cell attachment-recognition site	Various proteins involved in cell-cell (eg, sperm-oocyte), virus-cell, bacterium-cell, and hormone-cell interactions
Antifreeze	Certain plasma proteins of cold-water fish
Interact with specific carbohydrates	Lectins, selectins (cell adhesion lectins), antibodies
Receptor	Various proteins involved in hormone and drug action
Affect folding of certain proteins	Calnexin, calreticulin
Regulation of development	Notch and its analogs, key proteins in development
Hemostasis (and thrombosis)	Specific glycoproteins on the surface membranes of platelets

TABLE 47–2 Some Functions of the Oligosaccharide Chains of Glycoproteins

- Modulate physicochemical properties, eg, solubility, viscosity, charge, conformation, denaturation, and binding sites for various molecules, bacteria viruses and some parasites
- Protect against proteolysis, from inside and outside of cell
- Affect proteolytic processing of precursor proteins to smaller products
- Are involved in biologic activity, eg, of human chorionic gonadotropin (hCG)
- Affect insertion into membranes, intracellular migration, sorting and secretion
- Affect embryonic development and differentiation
- May affect sites of metastases selected by cancer cells

Source: Adapted from Schachter H: Biosynthetic controls that determine the branching and heterogeneity of protein-bound oligosaccharides. Biochem Cell Biol 1986;64:163.

TABLE 47–3 Some Important Methods Used to Study Glycoproteins

Method	Use
Periodic acid–Schiff reagent	Detects glycoproteins as pink bands after electrophoretic separation.
Incubation of cultured cells with a radioactive sugar	Leads to detection of glycoproteins as radioactive bands after electrophoretic separation.
Treatment with appropriate endo- or exoglycosidase or phospholipases	Resultant shifts in electrophoretic migration help distinguish among proteins with N-glycan, O-glycan, or GPI linkages and also between high mannose and complex N-glycans.
Sepharose-lectin column chromatography	To purify glycoproteins or glycopeptides that bind the particular lectin used.
Compositional analysis following acid hydrolysis	Identifies sugars that the glycoprotein contains and their stoichiometry.
Mass spectrometry	Provides information on molecular mass, composition, sequence, and sometimes branching of a glycan chain.
NMR spectroscopy	To identify specific sugars, their sequence, linkages, and the anomeric nature of glycosidic linkages.
Methylation (linkage) analysis	To determine linkages between sugars.
Amino acid or cDNA sequencing	Determination of amino acid sequence.

The conventional methods used to purify proteins and enzymes are also applicable to the purification of glycoproteins. Once a glycoprotein has been purified, the use of **mass spectrometry** and **high-resolution NMR spectroscopy** can often identify the structures of its glycan chains. Analysis of glycoproteins can be complicated by the fact that they often exist as **glycoforms;** these are proteins with identical amino acid sequences but somewhat different oligosaccharide compositions. Although linkage details are not stressed in this chapter, it is critical to appreciate that the precise natures of the linkages between the sugars of glycoproteins are of fundamental importance in determining the structures and functions of these molecules.

Impressive advances are also being made in **synthetic chemistry,** allowing synthesis of complex glycans that can be tested for biologic and pharmacologic activity. In addition, methods have been developed that use simple organisms, such as yeasts, to secrete human glycoproteins of therapeutic value (eg, erythropoietin) into their surrounding medium.

EIGHT SUGARS PREDOMINATE IN HUMAN GLYCOPROTEINS

About 200 monosaccharides are found in nature; however, only eight are commonly found in the oligosaccharide chains of glycoproteins (Table 47–4). Most of these sugars were described in Chapter 14. *N*-Acetylneuraminic acid (NeuAc) is usually found at the termini of oligosaccharide chains, attached

TABLE 47–4 The Principal Sugars Found in Human Glycoproteins[1]

Sugar	Type	Abbreviation	Nucleotide Sugar	Comments
Galactose	Hexose	Gal	UDP-Gal	Often found subterminal to NeuAc in N-linked glycoproteins. Also found in the core trisaccharide of proteoglycans.
Glucose	Hexose	Glc	UDP-Glc	Present during the biosynthesis of N-linked glycoproteins but not usually present in mature glycoproteins. Present in some clotting factors.
Mannose	Hexose	Man	GDP-Man	Common sugar in N-linked glycoproteins.
N-Acetylneuraminic acid	Sialic acid (nine C atoms)	NeuAc	CMP-NeuAc	Often the terminal sugar in both N- and O-linked glycoproteins. Other types of sialic acid are also found, but NeuAc is the major species found in humans. Acetyl groups may also occur as O-acetyl species as well as N-acetyl.
Fucose	Deoxyhexose	Fuc	GDP-Fuc	May be external in both N- and O-linked glycoproteins or internal, linked to the GlcNAc residue attached to Asn in N-linked species. Can also occur internally attached to the OH of Ser (eg, in t-PA and certain clotting factors).
N-Acetylgalactosamine	Aminohexose	GalNAc	UDP-GalNAc	Present in both N- and O-linked glycoproteins.
N-Acetylglucosamine	Aminohexose	GlcNAc	UDP-GlcNAc	The sugar attached to the polypeptide chain via Asn in N-linked glycoproteins; also found at other sites in the oligosaccharides of these proteins. Many nuclear proteins have GlcNAc attached to the OH of Ser or Thr as a single sugar.
Xylose	Pentose	Xyl	UDP-Xyl	Xyl is attached to the OH of Ser in many proteoglycans. Xyl in turn is attached to two Gal residues, forming a link trisaccharide. Xyl is also found in t-PA and certain clotting factors.

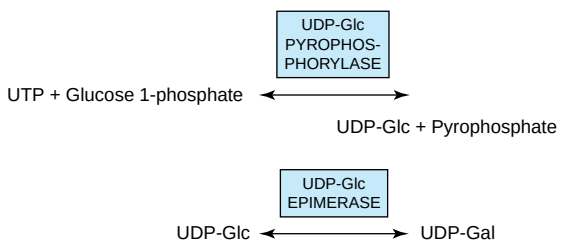
[1]Structures of glycoproteins are illustrated in Chapter 14.

to subterminal galactose (Gal) or *N*-acetylgalactosamine (GalNAc) residues. The other sugars listed are generally found in more internal positions. **Sulfate** is often found in glycoproteins, usually attached to Gal, GalNAc, or GlcNAc.

NUCLEOTIDE SUGARS ACT AS SUGAR DONORS IN MANY BIOSYNTHETIC REACTIONS

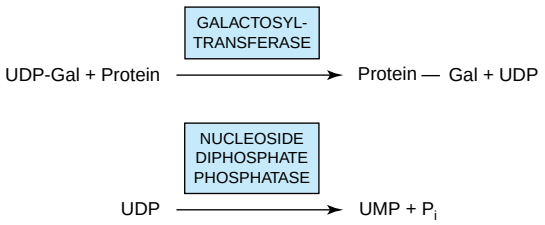
It is important to understand that in most biosynthetic reactions, it is not the free sugar or phosphorylated sugar that is involved in such reactions, but rather the corresponding **nucleotide sugar**. The first nucleotide sugar to be reported was uridine diphosphate glucose (UDP-Glc); its structure is shown in Figure 19–2. The common nucleotide sugars involved in the biosynthesis of glycoproteins are listed in Table 47–4; the reasons some contain UDP and others guanosine diphosphate (GDP) or cytidine monophosphate (CMP) are not clear. Many of the glycosylation reactions involved in the biosynthesis of glycoproteins utilize these compounds (see below). The **anhydro nature** of the linkage between the phosphate group and the sugars is of the high-energy, high-group-transfer-potential type (Chapter 11). The sugars of these compounds are thus **"activated"** and can be transferred to suitable acceptors provided appropriate transferases are available.

Most nucleotide sugars are formed in the cytosol, generally from reactions involving the corresponding nucleoside triphosphate. CMP-sialic acids are formed in the nucleus. Formation of uridine diphosphate galactose (UDP-Gal) requires the following two reactions in mammalian tissues:



Because many glycosylation reactions occur within the lumen of the Golgi apparatus, **carrier systems** (permeases, transporters) are necessary to transport nucleotide sugars across the Golgi membrane. Systems transporting UDP-Gal, GDP-Man, and CMP-NeuAc into the cisternae of the Golgi apparatus have been described. They are **antiport** systems; ie, the influx of one molecule of nucleotide sugar is balanced by the efflux of one molecule of the corresponding nucleotide (eg,

UMP, GMP, or CMP) formed from the nucleotide sugars. This mechanism ensures an adequate concentration of each nucleotide sugar inside the Golgi apparatus. UMP is formed from UDP-Gal in the above process as follows:



EXO- & ENDOGLYCOSIDASES FACILITATE STUDY OF GLYCOPROTEINS

A number of **glycosidases** of defined specificity have proved useful in examining structural and functional aspects of glycoproteins (Table 47–5). These enzymes act at either external (exoglycosidases) or internal (endoglycosidases) positions of oligosaccharide chains. Examples of exoglycosidases are **neuraminidases** and **galactosidases;** their sequential use removes terminal NeuAc and subterminal Gal residues from most glycoproteins. **Endoglycosidases F** and **H** are examples of the latter class; these enzymes cleave the oligosaccharide chains at specific GlcNAc residues close to the polypeptide backbone (ie, at internal sites; Figure 47–5) and are thus useful in releasing large oligosaccharide chains for structural analyses. A glycoprotein can be treated with one or more of the above glycosidases to analyze the effects on its biologic behavior of removal of specific sugars.

THE MAMMALIAN ASIALOGLYCOPROTEIN RECEPTOR IS INVOLVED IN CLEARANCE OF CERTAIN GLYCOPROTEINS FROM PLASMA BY HEPATOCYTES

Experiments performed by Ashwell and his colleagues in the early 1970s played an important role in focusing attention on the functional significance of the oligosaccharide chains of glycoproteins. They treated rabbit ceruloplasmin (a plasma protein; see Chapter 50) with neuraminidase in vitro. This procedure exposed subterminal Gal residues that were normally masked by terminal NeuAc residues. Neuraminidase-treated radioactive ceruloplasmin was found to disappear rapidly from the circulation, in contrast to the slow clearance of the untreated protein. Very significantly, when the Gal residues exposed to treatment with neuraminidase were removed by treatment with a galactosidase, the clearance rate of the protein returned to normal. Further studies demonstrated that liver cells contain a **mammalian asialoglycoprotein receptor** that recognizes the Gal moiety of many desialylated plasma proteins and leads to their endocytosis. This work indicated that an individual sugar, such as Gal, could play an important role in governing at least one of the biologic properties (ie, time of residence in the circulation) of certain glycoproteins. This greatly strengthened the concept that oligosaccharide chains could contain biologic information.

TABLE 47–5 Some Glycosidases Used to Study the Structure and Function of Glycoproteins[1]

Enzymes	Type
Neuraminidases	Exoglycosidase
Galactosidases	Exo-or endoglycosidase
Endoglycosidase F	Endoglycosidase
Endoglycosidase H	Endoglycosidase

[1]The enzymes are available from a variety of sources and are often specific for certain types of glycosidic linkages and also for their anomeric natures. The sites of action of endoglycosidases F and H are shown in Figure 47–5. F acts on both high-mannose and complex oligosaccharides, whereas H acts on the former.

LECTINS CAN BE USED TO PURIFY GLYCOPROTEINS & TO PROBE THEIR FUNCTIONS

Lectins are **carbohydrate-binding proteins** that agglutinate cells or precipitate glycoconjugates; a number of lectins are themselves glycoproteins. Immunoglobulins that react with sugars are not considered lectins. Lectins contain at least two sugar-binding sites; proteins with a single sugar-binding site will not agglutinate cells or precipitate glycoconjugates. The specificity of a lectin is usually defined by the sugars that are best at inhibiting its ability to cause agglutination or precipitation. Enzymes, toxins, and transport proteins can be classified as lectins if they bind carbohydrate. Lectins were first discovered in plants and microbes, but many lectins of animal origin are now known. The mammalian asialoglycoprotein receptor described above is an important example of an animal lectin. Some important lectins are listed in Table 47–6. Much current research is centered on the roles of various animal lectins in the mechanisms of action of glycoproteins, some of which are discussed below (eg, with regard to the selectins).

Numerous lectins have been purified and are commercially available; three plant lectins that have been widely used experimentally are listed in Table 47–7. Among many uses, lectins have been employed to purify specific glycoproteins, as tools for probing the glycoprotein profiles of cell surfaces, and as reagents for generating mutant cells deficient in certain enzymes involved in the biosynthesis of oligosaccharide chains.

THERE ARE THREE MAJOR CLASSES OF GLYCOPROTEINS

Based on the nature of the linkage between their polypeptide chains and their oligosaccharide chains, glycoproteins can be divided into three major classes (Figure 47–1): (1) those containing an **O-glycosidic linkage** (ie, O-linked), involv-

TABLE 47–6 Some Important Lectins

Lectins	Examples or Comments
Legume lectins	Concanavalin A, pea lectin
Wheat germ agglutinin	Widely used in studies of surfaces of normal cells and cancer cells
Ricin	Cytotoxic glycoprotein derived from seeds of the castor plant
Bacterial toxins	Heat-labile enterotoxin of *E coli* and cholera toxin
Influenza virus hemagglutinin	Responsible for host-cell attachment and membrane fusion
C-type lectins	Characterized by a Ca^{2+}-dependent carbohydrate recognition domain (CRD); includes the mammalian asialoglycoprotein receptor, the selectins, and the mannose-binding protein
S-type lectins	β-Galactoside-binding animal lectins with roles in cell-cell and cell-matrix interactions
P-type lectins	Mannose 6-P receptor
I-type lectins	Members of the immunoglobulin super-family, eg, sialoadhesin mediating adhesion of macrophages to various cells

TABLE 47–7 Three Plant Lectins and the Sugars with Which They Interact[1]

Lectin	Abbreviation	Sugars
Concanavalin A	ConA	Man and Glc
Soybean lectin		Gal and GalNAc
Wheat germ agglutinin	WGA	Glc and NeuAc

[1]In most cases, lectins show specificity for the anomeric nature of the glycosidic linkage (α or β); this is not indicated in the table.

ing the hydroxyl side chain of serine or threonine and a sugar such as *N*-acetylgalactosamine (GalNAc-Ser[Thr]); (2) those containing an **N-glycosidic linkage** (ie, N-linked), involving the amide nitrogen of asparagine and *N*-acetylglucosamine (GlcNAc-Asn); and (3) those linked to the carboxyl terminal amino acid of a protein via a phosphoryl-ethanolamine moiety joined to an oligosaccharide (glycan), which in turn is linked via glucosamine to phosphatidylinositol (PI). This latter class is referred to as **glycosylphosphatidylinositol-anchored** (**GPI-anchored,** or **GPI-linked**) glycoproteins. It is involved in directing certain glycoproteins to the apical or basolateral areas of the plasma membrane of certain polarized epithelial cells (see Chapter 46 and below). Other minor classes of glycoproteins also exist.

The number of oligosaccharide chains attached to one protein can vary from one to 30 or more, with the sugar chains ranging from one or two residues in length to much larger structures. Many proteins contain more than one type of sugar chain; for instance, **glycophorin,** an important red cell membrane glycoprotein (Chapter 52), contains both O- and N-linked oligosaccharides.

GLYCOPROTEINS CONTAIN SEVERAL TYPES OF O-GLYCOSIDIC LINKAGES

At least four subclasses of O-glycosidic linkages are found in human glycoproteins: (1) The **GalNAcSer(Thr)** linkage shown in Figure 47–1 is the predominant linkage. Two typical oligosaccharide chains found in members of this subclass are shown in Figure 47–2. Usually a Gal or a NeuAc residue is attached to the GalNAc, but many variations in the sugar compositions and lengths of such oligosaccharide chains are found. This type of linkage is found in **mucins** (see below). (2) **Proteoglycans** contain a **Gal-Gal-Xyl-Ser** trisaccharide (the so-called link trisaccharide). (3) **Collagens** contain a **Gal-hydroxylysine (Hyl)** linkage. (Subclasses [2] and [3] are discussed further in Chapter 48.) (4) Many **nuclear proteins** (eg, certain transcription factors) and **cytosolic proteins** contain side chains consisting of a single GlcNAc attached to a serine or threonine residue **(GlcNAc-Ser[Thr]).**

Mucins Have a High Content of O-Linked Oligosaccharides & Exhibit Repeating Amino Acid Sequences

Mucins are glycoproteins with two major characteristics: (1) a high content of **O-linked oligosaccharides** (the carbohydrate content of mucins is generally more than 50%); and (2) the presence of **repeating amino acid sequences** (tandem repeats) in the center of their polypeptide backbones, to which the O-glycan chains are attached in clusters (Figure 47–3). These sequences are rich in serine, threonine, and proline. Although O-glycans predominate, mucins often contain a number of N-glycan chains. Both **secretory** and **membrane-bound** mucins occur. The former are found in the mucus present in the secretions of the gastrointestinal, respiratory, and reproductive tracts. **Mucus** consists of about 94% water and 5% mucins, with the remainder being a mixture of various cell molecules, electrolytes, and remnants of cells. Secretory mucins generally have an oligomeric structure and thus often have a very high molecular mass. The oligomers are composed of monomers linked by disulfide bonds. Mucus exhibits a high **viscosity** and often forms a **gel.** These qualities are functions of its content of mucins. The high content of O-glycans confers an extended structure on mucins. This is in part explained by steric interactions between their GalNAc moieties and adjacent amino acids, resulting in a chain-stiffening effect so that the conformations of mucins often become those of rigid rods. Intermolecular noncovalent interactions between various sugars on neighboring glycan chains contribute to gel formation. The high content of **NeuAc** and **sulfate** residues found in many mucins confers a negative charge on them. With regard to function, mucins help **lubricate** and form a **protective physical barrier** on epithelial surfaces. Membrane-bound mucins participate in various **cell-**

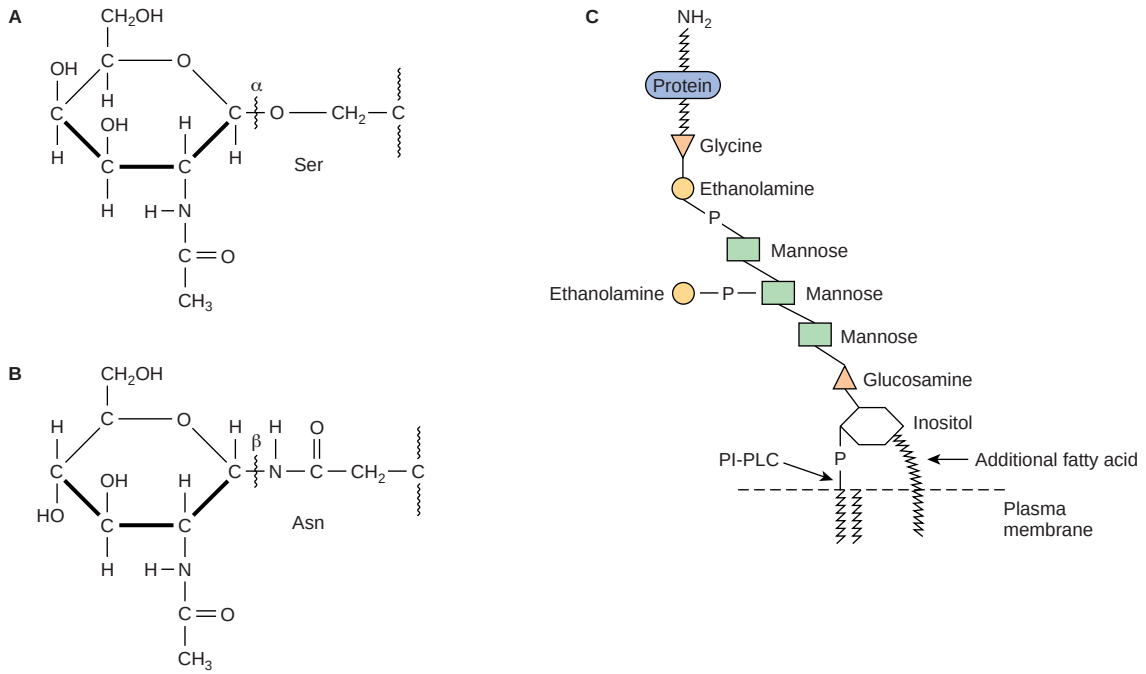


FIGURE 47–1 Depictions of **(A)** an O-linkage (*N*-acetylgalactosamine to serine), **(B)** an N-linkage (*N*-acetylglucosamine to asparagine), and **(C)** a glycosylphosphatidylinositol (GPI) linkage. The GPI structure shown is that linking acetylcholinesterase to the plasma membrane of the human red blood cell. The carboxyl terminal amino acid is glycine joined in amide linkage via its COOH group to the NH_2 group of phosphorylethanolamine, which in turn is joined to a mannose residue. The core glycan contains three mannose and one glucosamine residues. The glucosamine is linked to inositol, which is attached to phosphatidic acid. The site of action of PI-phospholipase C (PI-PLC) is indicated. The structure of the core glycan is shown in the text. This particular GPI contains an extra fatty acid attached to inositol and also an extra phosphorylethanolamine moiety attached to the middle of the three mannose residues. Variations found among different GPI structures include the identity of the carboxyl terminal amino acid, the molecules attached to the mannose residues, and the precise nature of the lipid moiety.

cell interactions (eg, involving selectins; see below). The density of oligosaccharide chains makes it difficult for **proteases** to approach their polypeptide backbones, so that mucins are often resistant to their action. Mucins also tend to "mask" certain surface antigens. Many cancer cells form excessive amounts of mucins; perhaps the mucins may mask certain surface antigens on such cells and thus protect the cells from immune surveillance. Mucins also carry cancer-specific peptide and carbohydrate epitopes (an epitope is a site on an antigen recognized by an antibody, also called an antigenic determinant). Some of these epitopes have been used to stimulate an immune response against cancer cells.

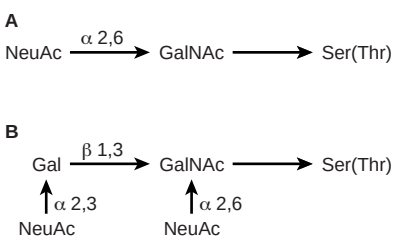


FIGURE 47–2 Structures of two O-linked oligosaccharides found in **(A)** submaxillary mucins and **(B)** fetuin and in the sialoglycoprotein of the membrane of human red blood cells. (Modified and reproduced, with permission, from Lennarz WJ: *The Biochemistry of Glycoproteins and Proteoglycans.* Plenum Press, 1980. Reproduced with kind permission from Springer Science and Business Media.)

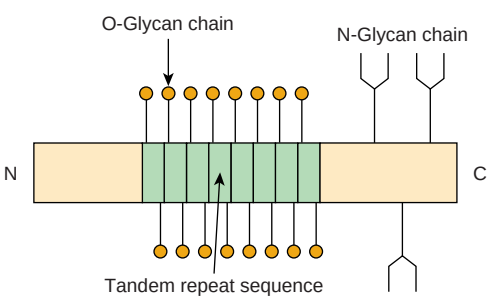


FIGURE 47–3 Schematic diagram of a mucin. O-glycan chains are shown attached to the central region of the extended polypeptide chain and N-glycan chains to the carboxyl terminal region. The narrow rectangles represent a series of tandem repeat amino acid sequences. Many mucins contain cysteine residues whose SH groups form interchain linkages; these are not shown in the figure. (Adapted, with permission, from Strous GJ, Dekker J: Mucin-type glycoproteins. Crit Rev Biochem Mol Biol 1992;27:57. Copyright ©1992. Reproduced by permission of Taylor & Francis Group, LLC.)

The **genes** encoding the polypeptide backbones of a number of mucins derived from various tissues (eg, pancreas, small intestine, trachea and bronchi, stomach, and salivary glands) have been cloned and sequenced. These studies have revealed new information about the polypeptide backbones of mucins (size of tandem repeats, potential sites of N-glycosylation, etc.) and ultimately should reveal aspects of their genetic control. Some important properties of mucins are summarized in Table 47–8.

The Biosynthesis of O-Linked Glycoproteins Uses Nucleotide Sugars

The polypeptide chains of O-linked and other glycoproteins are encoded by mRNA species; because most glycoproteins are membrane-bound or secreted, they are generally translated on membrane-bound polyribosomes (Chapter 37). Hundreds of different oligosaccharide chains of the O-glycosidic type exist. These glycoproteins are built up by the **stepwise donation of sugars from nucleotide sugars,** such as UDP-GalNAc, UDPGal, and CMP-NeuAc. The enzymes catalyzing this type of reaction are membrane-bound **glycoprotein glycosyltransferases.** Generally, synthesis of one specific type of linkage requires the activity of a correspondingly specific transferase. The factors that determine which specific serine and threonine residues are glycosylated have not been identified but are probably found in the peptide structure surrounding the glycosylation site. The enzymes assembling O-linked chains are located in the Golgi apparatus, sequentially arranged in an assembly line with terminal reactions occurring in the *trans*-Golgi compartments.

The major features of the biosynthesis of O-linked glycoproteins are summarized in Table 47–9.

TABLE 47–8 Some Properties of Mucins

• Found in secretions of the gastrointestinal, respiratory, and reproductive tracts and also in membranes of various cells.
• Exhibit high content of O-glycan chains, usually containing NeuAc.
• Contain repeating amino acid sequences rich in serine, threonine, and proline.
• Extended structure contributes to their high viscoelasticity.
• Form protective physical barrier on epithelial surfaces, are involved in cell–cell interactions, and may contain or mask certain surface antigens.

TABLE 47–9 Summary of Main Features of O-Glycosylation

• Involves a battery of membrane-bound glycoprotein glycosyltransferases acting in a stepwise manner; each transferase is generally specific for a particular type of linkage.
• The enzymes involved are located in various subcompartments of the Golgi apparatus.
• Each glycosylation reaction involves the appropriate nucleotide sugar.
• Dolichol-P-P-oligosaccharide is not involved, nor are glycosidases; and the reactions are not inhibited by tunicamycin.
• O-Glycosylation occurs posttranslationally at certain Ser and Thr residues.

N-LINKED GLYCOPROTEINS CONTAIN AN Asn-GLcNAc LINKAGE

N-Linked glycoproteins are distinguished by the presence of the Asn-GlcNAc linkage (Figure 47–1). It is the major class of glycoproteins and has been much studied, since the most readily accessible glycoproteins (eg, plasma proteins) mainly belong to this group. It includes both **membrane-bound** and **circulating** glycoproteins. The principal difference between this and the previous class, apart from the nature of the amino acid to which the oligosaccharide chain is attached (Asn vs Ser or Thr), concerns their biosynthesis.

Complex, Hybrid, & High-Mannose Are the Three Major Classes of N-Linked Oligosaccharides

There are three major classes of N-linked oligosaccharides: **complex, hybrid**, and **high-mannose** (Figure 47–4). Each type shares a common pentasaccharide, $Man_3GlcNAc_2$—shown within the boxed area in Figure 47–4 and depicted also in Figure 47–5—but they differ in their outer branches. The presence of the **common pentasaccharide** is explained by the fact that all three classes share an initial common mechanism of biosynthesis. Glycoproteins of the complex type generally contain terminal NeuAc residues and underlying Gal and GlcNAc residues, the latter often constituting the disaccharide *N*-acetyllactosamine. Repeating ***N*-acetyllactosamine units**—[Galβ1–3/4GlcNAcβ 1–3]$_n$ (poly-*N*-acetyllactosaminoglycans)—are often found on N-linked glycan chains. I/i blood group substances belong to this class. The majority of complex-type oligosaccharides contain two, three, or four outer branches (Figure 47–4), but structures containing five branches have also been described. The oligosaccharide branches are often referred to as **antennae,** so that bi-, tri-, tetra-, and penta-antennary structures may all be found. A bewildering number of chains of the complex type exist, and that indicated in Figure 47–4 is only one of many. Other complex chains may terminate in Gal or Fuc. High-mannose oligosaccharides typically have two to six additional Man residues linked to the pentasaccharide core. Hybrid molecules contain features of both of the two other classes.

The Biosynthesis of N-Linked Glycoproteins Involves Dolichol-P-P-Oligosaccharide

Leloir and his colleagues described the occurrence of a **dolichol-pyrophosphate-oligosaccharide (Dol-P-Poligosaccharide),** which subsequent research showed to play a key role in the biosynthesis of N-linked glycoproteins. The oligosaccharide chain of this compound generally has the structure

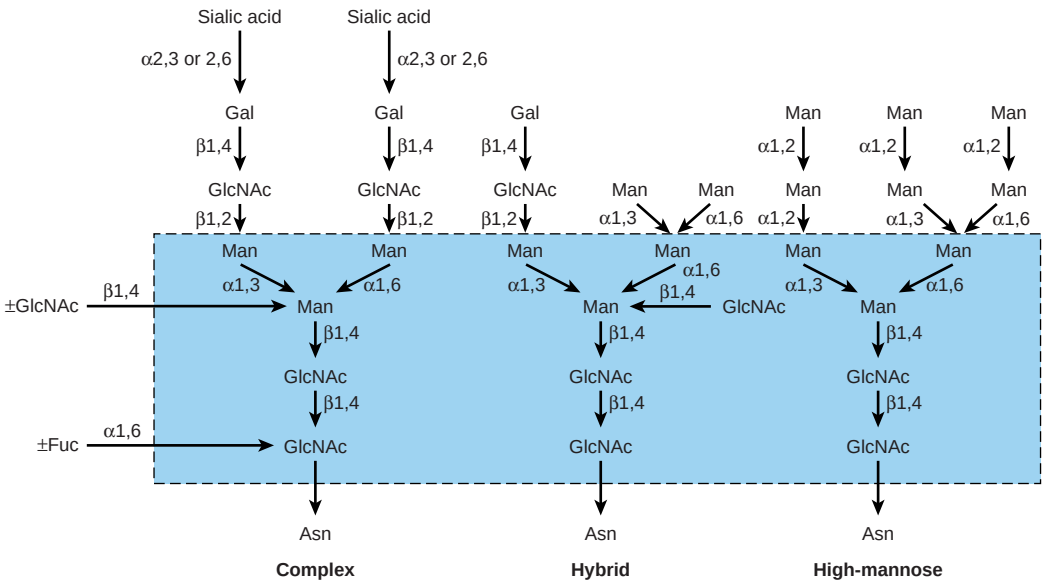


FIGURE 47–4 Structures of the major types of asparagine-linked oligosaccharides. The boxed area encloses the pentasaccharide core common to all N-linked glycoproteins. (Reproduced, with permission, from Kornfeld R, Kornfeld S: Assembly of asparagine-linked oligosaccharides. Annu Rev Biochem 1985;54:631. Copyright © 1985 by Annual Reviews. Reprinted with permission.)

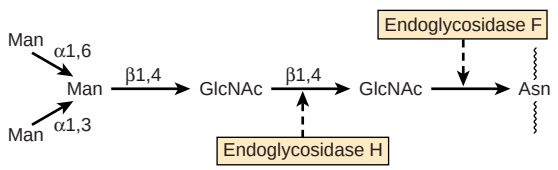


FIGURE 47–5 Schematic diagram of the pentasaccharide core common to all N-linked glycoproteins and to which various outer chains of oligosaccharides may be attached. The sites of action of endoglycosidases F and H are also indicated.

$R\text{-}GlcNAc_2Man_9Glc_3$ (R = DolP-P). The sugars of this compound are first assembled on the Dol-P-P backbone, and the oligosaccharide chain is then transferred en bloc to suitable Asn residues of acceptor apoglycoproteins during their synthesis on membrane-bound polyribosomes. All N-glycans have a common pentasaccharide core structure (Figure 47–5).

To form **high-mannose** chains, only the Glc residues plus certain of the peripheral Man residues are removed. To form an oligosaccharide chain of the **complex type,** the Glc residues and four of the Man residues are removed by glycosidases in the endoplasmic reticulum and Golgi. The sugars characteristic of complex chains (GlcNAc, Gal, NeuAc) are added by the action of individual glycosyltransferases located in the Golgi apparatus. The phenomenon whereby the glycan chains of N-linked glycoproteins are first partially degraded and then in some cases rebuilt is referred to as **oligosaccharide processing. Hybrid chains** are formed by partial processing, forming complex chains on one arm and Man structures on the other arm.

Thus, the initial steps involved in the biosynthesis of the N-linked glycoproteins differ markedly from those involved in the biosynthesis of the O-linked glycoproteins. The former involves Dol-P-P-oligosaccharide; the latter, as described earlier, does not.

The process of N-glycosylation can be broken down into two stages: (1) assembly of Dol-P-P-oligosaccharide and transfer of the oligosaccharide; and (2) processing of the oligosaccharide chain.

Assembly & Transfer of Dolichol-P-P-Oligosaccharide

Polyisoprenol compounds exist in both bacteria and eukaryotic cells. They participate in the synthesis of bacterial polysaccharides and in the biosynthesis of N-linked glycoproteins and GPI anchors. The polyisoprenol used in eukaryotic tissues is **dolichol,** which is, next to rubber, the longest naturally occurring hydrocarbon made up of a single repeating unit. Dolichol is composed of 17–20 repeating isoprenoid units (Figure 47–6).

Before it participates in the biosynthesis of Dol-PP-oligosaccharide, dolichol must first be phosphorylated to form dolichol phosphate (Dol-P) in a reaction catalyzed by **dolichol kinase** and using ATP as the phosphate donor.

Dolichol-P-P-GlcNAc (Dol-P-P-GlcNAc) is the key lipid that acts as an acceptor for other sugars in the assembly of Dol-P-P-oligosaccharide. It is synthesized in the membranes of the endoplasmic reticulum from Dol-P and UDP-GlcNAc in the following reaction, catalyzed by GlcNAc-P transferase:

$$\text{Dol-P} + \text{UDP-GlcNAc} \rightarrow \text{Dol-P-P-GlcNAc} + \text{UMP}$$

The above reaction—which is the first step in the assembly of Dol-P-P-oligosaccharide—and the other later reactions are

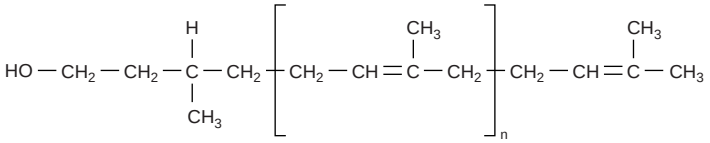


FIGURE 47–6 The structure of dolichol. The phosphate in dolichol phosphate is attached to the primary alcohol group at the left-hand end of the molecule. The group within the brackets is an isoprene unit (n = 17–20 isoprenoid units).

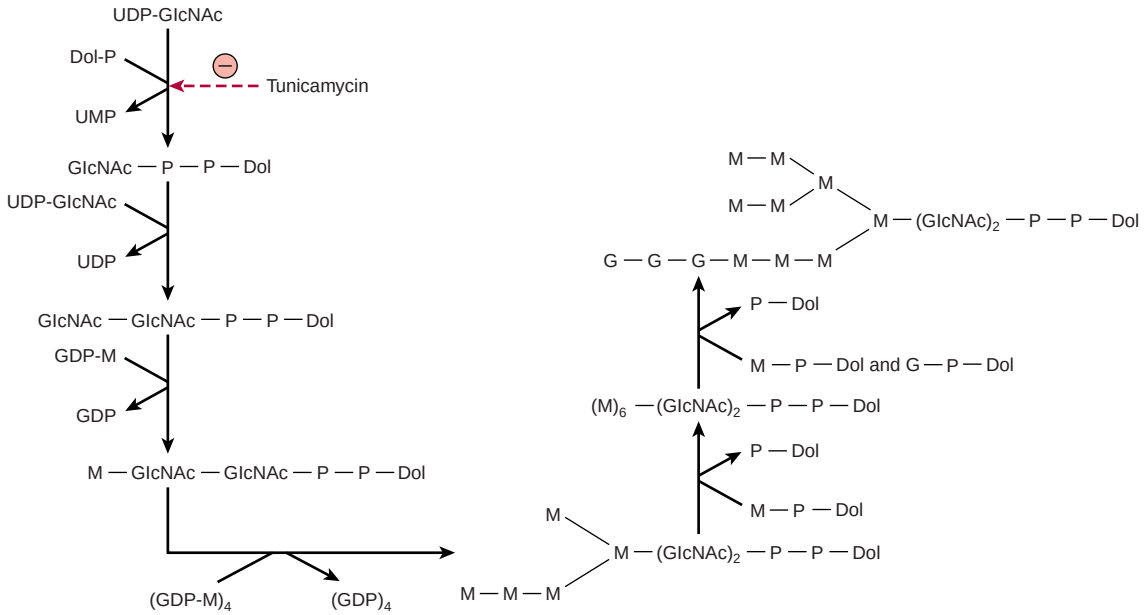


FIGURE 47–7 Pathway of biosynthesis of dolichol-P-P-oligosaccharide. The specific linkages formed are indicated in Figure 47–8. Note that the first five internal mannose residues are donated by GDP-mannose, whereas the more external mannose residues and the glucose residues are donated by dolichol-P-mannose and dolichol-P-glucose. (UDP, uridine diphosphate; Dol, dolichol; P, phosphate; UMP, uridine monophosphate; GDP, guanosine diphosphate; M, mannose; G, glucose.)

summarized in Figure 47–7. The essential features of the subsequent steps in the assembly of Dol-P-P-oligosaccharide are as follows:

1. A second GlcNAc residue is added to the first, again using UDP-GlcNAc as the donor.
2. Five Man residues are added, using GDP-mannose as the donor.
3. Four additional Man residues are next added, using Dol-P-Man as the donor. Dol-P-Man is formed by the following reaction:

$$\text{Dol-P} + \text{GDP-Man} \rightarrow \text{Dol-P-Man} + \text{GDP}$$

4. Finally, the three peripheral glucose residues are donated by Dol-P-Glc, which is formed in a reaction analogous to that just presented except that Dol-P and UDP-Glc are the substrates.

It should be noted that the first seven sugars (two GlcNAc and five Man residues) are donated by nucleotide sugars, whereas the last seven sugars (four Man and three Glc residues) added are donated by dolichol-sugars. The net result is assembly of the compound illustrated in Figure 47–8 and referred to in shorthand as Dol-P-P-$GlcNAc_2Man_9Glc_3$.

The oligosaccharide linked to dolichol-P-P is transferred en bloc to form an N-glycosidic bond with one or more specific Asn residues of an acceptor protein emerging from the luminal surface of the membrane of the endoplasmic reticulum. The reaction is catalyzed by **oligosaccharide: protein transferase,** a membrane-associated enzyme complex. The transferase will recognize and transfer any substrate with the general structure Dol-P-P-$(GlcNAc)_2$-R, but it has a strong preference for the Dol-P-P-$GlcNAc_2Man_9Glc_3$ structure. Glycosylation occurs at the Asn residue of an Asn-XSer/Thr tripeptide

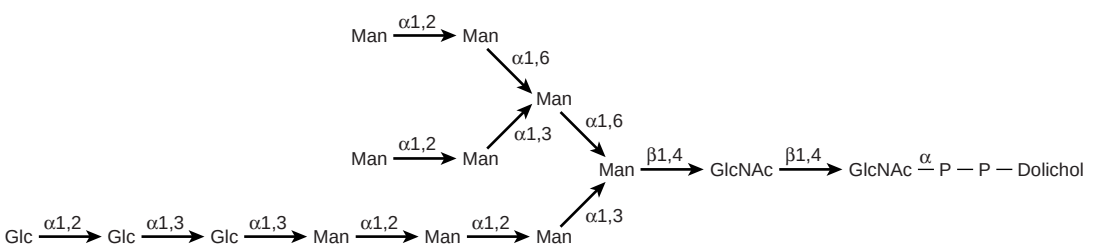


FIGURE 47–8 Structure of dolichol-P-P-oligosaccharide. (From Li E, et al: Structure of the lipid-linked oligosaccharide precursor of the complex-type oligosaccharides of the vesicular stomatitis virus G protein. J Biol Chem 1978;253:7762.)

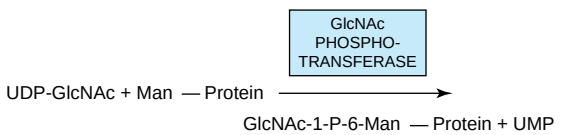
sequence, where X is any amino acid except proline, aspartic acid, or glutamic acid. A tripeptide site contained within a β turn is favored. Only about one-third of the Asn residues that are potential acceptor sites are actually glycosylated, suggesting that factors other than the tripeptide are also important.

The acceptor proteins are of both the secretory and integral membrane class. Cytosolic proteins are rarely glycosylated. The transfer reaction and subsequent processes in the glycosylation of N-linked glycoproteins, along with their subcellular locations, are depicted in Figure 47–9. The other product of the oligosaccharide: protein transferase reaction is dolichol-PP, which is subsequently converted to dolichol-P by a phosphatase. The dolichol-P can serve again as an acceptor for the synthesis of another molecule of Dol-P-P-oligosaccharide.

Processing of the Oligosaccharide Chain

1. Early Phase. The various reactions involved are indicated in Figure 47–9. The oligosaccharide: protein transferase catalyzes reaction 1 (see above). Reactions 2 and 3 involve the removal of the terminal Glc residue by glucosidase I and of the next two Glc residues by glucosidase II, respectively. In the case of **high-mannose** glycoproteins, the process may stop here, or up to four Man residues may also be removed. However, to form **complex** chains, additional steps are necessary, as follows. Four external Man residues are removed in reactions 4 and 5 by at least two different mannosidases. In reaction 6, a GlcNAc residue is added to the Man residue of the Man 1–3 arm by GlcNAc transferase I. The action of this latter enzyme permits the occurrence of reaction 7, a reaction catalyzed by yet another mannosidase (Golgi α-mannosidase II) and which results in a reduction of the Man residues to the core number of three (Figure 47–5).

An important additional pathway is indicated in reactions I and II of Figure 47–9. This involves enzymes destined for **lysosomes.** Such enzymes are targeted to the lysosomes by a specific chemical marker. In reaction I, a residue of GlcNAc-1-P is added to carbon 6 of one or more specific Man residues of these enzymes. The reaction is catalyzed by a GlcNAc phosphotransferase, which uses UDPGlcNAc as the donor and generates UMP as the other product:



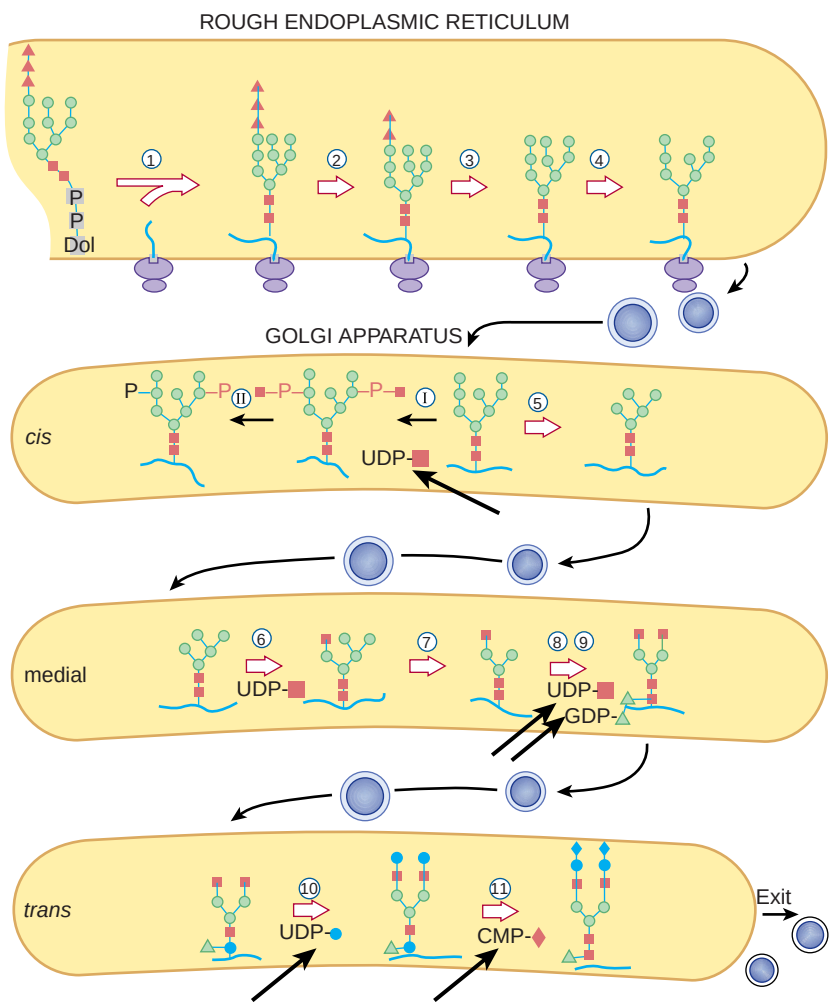
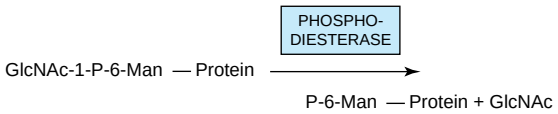


FIGURE 47–9 Schematic pathway of oligosaccharide processing. The reactions are catalyzed by the following enzymes: ① oligosaccharide: protein transferase; ② α-glucosidase I; ③ α-glucosidase II; ④ endoplasmic reticulum α 1,2-mannosidase; I *N*-acetylglucosaminylphosphotransferase; II *N*-acetylglucosamine-1-phosphodiester α-*N*-acetylglucosaminidase; ⑤ Golgi apparatus α-mannosidase I; ⑥ *N*-acetylglucosaminyltransferase I; ⑦ Golgi apparatus α-mannosidase II; ⑧ *N*-acetylglucosaminyltransferase II; ⑨ fucosyltransferase; ⑩ galactosyltransferase; ⑪ sialyltransferase. The thick arrows indicate various nucleotide sugars involved in the overall scheme. (Solid square, *N*-acetylglucosamine; open circle, mannose; solid triangle, glucose; open triangle, fucose; solid circle, galactose; solid diamond, sialic acid.) (Reproduced, with permission, from Kornfeld R, Kornfeld S: Assembly of asparagine-linked oligosaccharides. Annu Rev Biochem 1985;54:631. Copyright © 1985 by Annual Reviews. Reprinted with permission.)

In reaction II, the GlcNAc is removed by the action of a phosphodiesterase, leaving the Man residues phosphorylated in the 6 position:



Man 6-P receptors, located in the Golgi apparatus, bind the Man 6-P residues of these enzymes and direct them to the lysosomes. Fibroblasts from patients with **I-cell disease** (see below) are severely deficient in the activity of the GlcNAc phosphotransferase.

2. Late Phase. To assemble a typical complex oligosaccharide chain, additional sugars must be added to the structure formed in reaction 7. Hence, in reaction 8, a second GlcNAc is added to the peripheral Man residue of the other arm of the bi-antennary structure shown in Figure 47–9; the enzyme catalyzing this step is GlcNAc transferase II. Reactions 9, 10, and 11 involve the addition of Fuc, Gal, and NeuAc residues at the sites indicated, in reactions catalyzed by fucosyl, galactosyl, and sialyl transferases, respectively. The assembly of poly-*N*-acetyllactosamine chains requires additional GlcNAc transferases.

The Endoplasmic Reticulum & Golgi Apparatus Are the Major Sites of Glycosylation

As indicated in Figure 47–9, the endoplasmic reticulum and the Golgi apparatus are the major sites involved in glycosylation processes. The assembly of Dol-P-P-oligosaccharide occurs on both the cytoplasmic and luminal surfaces of the ER membranes. Addition of the oligosaccharide to protein occurs in the rough endoplasmic reticulum during or after translation. Removal of the Glc and some of the peripheral Man residues also occurs in the endoplasmic reticulum. The Golgi apparatus is composed of *cis*, medial, and *trans* cisternae; these can be separated by appropriate centrifugation procedures. Vesicles containing glycoproteins bud off in the endoplasmic reticulum and are transported to the *cis*-Golgi. Various studies have shown that the enzymes involved in glycoprotein processing show differential locations in the cisternae of the Golgi. As indicated in Figure 47–9, Golgi α-mannosidase I (catalyzing reaction 5) is located mainly in the *cis*-Golgi, whereas GlcNAc transferase I (catalyzing reaction 6) appears to be located in the medial Golgi, and the fucosyl, galactosyl, and sialyl transferases (catalyzing reactions 9, 10, and 11) are located mainly in the *trans*-Golgi. The major features of the biosynthesis of N-linked glycoproteins are summarized in Table 47–10 and should be contrasted with those previously listed (Table 47–9) for O-linked glycoproteins.

Some Glycan Intermediates Formed During N-Glycosylation Have Specific Functions

The following are a number of specific functions of N-glycan chains that have been established or are under investigation:

TABLE 47–10 Summary of Main Features of N-Glycosylation

- The oligosaccharide $Glc_3Man_9(GlcNAc)_2$ is transferred from dolichol-P-P-oligosaccharide in a reaction catalyzed by oligosaccharide:protein transferase, which is inhibited by tunicamycin.
- Transfer occurs to specific Asn residues in the sequence AsnX-Ser/Thr, where X is any residue except Pro, Asp, or Glu.
- Transfer can occur cotranslationally in the endoplasmic reticulum.
- The protein-bound oligosaccharide is then partially processed by glucosidases and mannosidases; if no additional sugars are added, this results in a high-mannose chain.
- If processing occurs down to the core heptasaccharide ($Man_5[GlcNAc]_2$), complex chains are synthesized by the addition of GlcNAc, removal of two Man, and the stepwise addition of individual sugars in reactions catalyzed by specific transferases (eg, GlcNAc, Gal, NeuAc transferases) that employ appropriate nucleotide sugars.

(**1**) The involvement of the **mannose 6-P signal** in targeting of certain lysosomal enzymes is clear (see above and discussion of I-cell disease, below). (**2**) It is likely that the large N-glycan chains present on newly synthesized glycoproteins may assist in keeping these proteins in a **soluble state** inside the lumen of the endoplasmic reticulum. (**3**) One species of N-glycan chains has been shown to play a role in the folding and retention of certain glycoproteins in the lumen of the endoplasmic reticulum. **Calnexin** is a protein present in the endoplasmic reticulum membrane that acts as a chaperone (Chapter 46) and lectin. Binding to calnexin prevents a glycoprotein from aggregating. It has been found that calnexin will bind specifically to a number of glycoproteins (eg, the influenza virus hemagglutinin [HA]) that possess the **monoglycosylated core structure.** This species is the product of reaction 2 shown in Figure 47–9, but from which the terminal glucose residue has been removed, leaving only the innermost glucose attached. Calnexin and the bound glycoprotein form a complex with **ERp57**, a homolog of protein disulfide isomerase (PDI), which catalyzes disulfide bond interchange, facilitating proper folding. The bound glycoprotein is released from its complex with calnexin-ERp57 when the sole remaining glucose is hydrolyzed by glucosidase II and **leaves the ER if properly folded**. If **not properly folded**, an ER **glucosyltransferase** recognizes this and **re-glucosylates** the glycoprotein, which **re-binds** to the calnexin-Erp57 complex. If now properly folded, the glycoprotein is again de-glucosylated and leaves the ER. If not capable of proper folding, it is **translocated out of the ER** into the cytoplasm, where it is **degraded** (compare Figure 46–8). This so-called **calnexin cycle** is illustrated in Figure 47–10. In this way, calnexin retains certain partly folded (or misfolded) glycoproteins and releases them when further folding has occurred. The glucosyltransferase, by **sensing** the folding of the glycoprotein and only re-glucosylating misfolded proteins, is a key component of the cycle. The calnexin cycle is an important component of the **quality control systems** operating

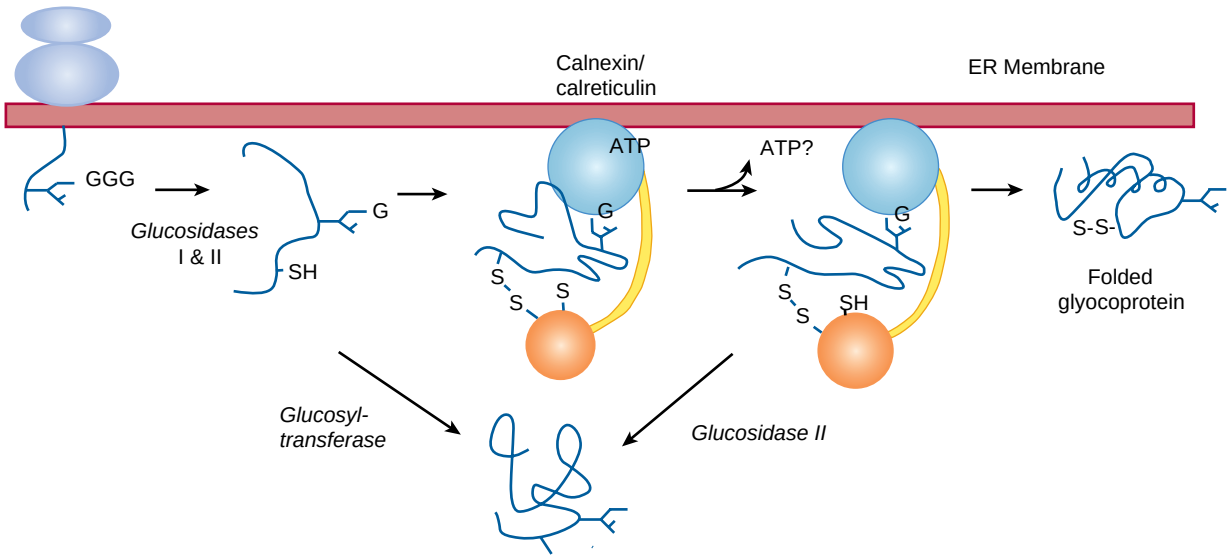


FIGURE 47–10 Model of the calnexin cycle. As a nascent (growing) polypeptide chain enters the ER, certain Asn residues are glycosylated by addition of $Glc_3Man_9GlcNAc_2$ (see text). The outermost two molecules of glucose are removed via the actions of glucosidases I and II. This exposes the innermost molecule of glucose, which is recognized by the lectin sites of calnexin and calreticulin. In their ATP-bound state, calnexin and calreticulin bind to the monoglucosylated oligosaccharide (via their lectin sites) as well as to hydrophobic segments of the unfolded glycoprotein (via their polypeptide binding or chaperone sites). Glycoprotein dissociation involves the action of glucosidase II to remove the terminal glucose and also a change in affinity of the polypeptide binding site. After dissociation, if folding does not occur rapidly, the glycoprotein is re-glucosylated by an ER glucosyltransferase, which acts only on non-native protein conformers (conformer = a protein in one of several possible conformations). The re-glucosylated glycoprotein can then re-bind to the ATP form of calnexin/calreticulin. Thus, both the glucosyltransferase and calnexin/calreticulin act as folding sensors. This cycle of binding and release has three functions: it prevents glycoprotein aggregation; it retains non-native conformers in the ER until a native structure is acquired (quality control); and binding to calnexin/calreticulin brings ERp57 into proximity with the non-native glycoprotein. ERp57 catalyzes disulfide bond formation and isomerization within the glycoprotein substrate, assisting it to assume its native conformation. If the glycoprotein is not capable of proper folding, it is translocated out of the ER into the cytoplasm for proteosomal degradation (compare Figure 46–8). Calreticulin, a soluble ER protein, plays a similar role to calnexin. (G, glucose.) (Figure and legend generously supplied by Dr D B Williams, and modified slightly with his permission.)

in the lumen of the ER. The soluble ER protein **calreticulin** performs a similar function.

Several Factors Regulate the Glycosylation of Glycoproteins

It is evident that glycosylation of glycoproteins is a complex process involving a large number of enzymes. It has been estimated that some 1% of the human genome may be involved with glycosylation events. Another index of its complexity is that more than ten distinct GlcNAc transferases involved in glycoprotein biosynthesis have been reported, and others are theoretically possible. Multiple species of the other glycosyltransferases (eg, sialyltransferases) also exist. Controlling factors of the first stage of N-linked glycoprotein biosynthesis (ie, **oligosaccharide assembly and transfer**) include (1) the presence of suitable acceptor sites in proteins, (2) the tissue level of Dol-P, and (3) the activity of the oligosaccharide: protein transferase.

Some factors known to be involved in the regulation of **oligosaccharide processing** are listed in Table 47–11. Two of the points listed merit further comment. First, **species variations** among processing enzymes have assumed importance in relation to production of glycoproteins of therapeutic use by means of recombinant DNA technology. For instance, **recombinant erythropoietin** (epoetin alfa; EPO) is sometimes administered to patients with certain types of chronic anemia in order to stimulate erythropoiesis. The half-life of EPO in plasma is influenced by the nature of its glycosylation pattern, with certain patterns being associated with a short half-life, appreciably limiting its period of therapeutic effectiveness. It is thus important to harvest EPO from host cells that confer a pattern of glycosylation consistent with a normal half-life in plasma. Second, there is great interest in analysis of the activities of glycoprotein-processing enzymes in various types of **cancer cells.** These cells have often been found to synthesize different oligosaccharide chains (eg, they often exhibit greater branching) from those made in control cells. This could be due to cancer cells containing different patterns of glycosyltransferases from those exhibited by corresponding normal cells, due to specific gene activation or repression. The differences in oligosaccharide chains could affect adhesive interactions between cancer cells and their normal parent tissue cells, contributing to metastasis. If a correlation could be found be-

TABLE 47–11 Some Factors Affecting the Activities of Glycoprotein Processing Enzymes

Factor	Comment
Cell type	Different cell types contain different profiles of processing enzymes.
Previous enzyme	Certain glycosyltransferases act only on an oligosaccharide chain if it has already been acted upon by another processing enzyme.[1]
Development	The cellular profile of processing enzymes may change during development if their genes are turned on or off.
Intracellular location	For instance, if an enzyme is destined for insertion into the membrane of the ER (eg, HMG-CoA reductase), it may never encounter Golgi-located processing enzymes.
Protein conformation	Differences in conformation of different proteins may facilitate or hinder access of processing enzymes to identical oligosaccharide chains.
Species	Same cells (eg, fibroblasts) from different species may exhibit different patterns of processing enzymes.
Cancer	Cancer cells may exhibit processing enzymes different from those of corresponding normal cells.

[1]For example, prior action of GlcNAc transferase I is necessary for the action of Golgi α-mannosidase II.

tween the activity of particular processing enzymes and the **metastatic properties** of cancer cells, this could be important as it might permit synthesis of drugs to inhibit these enzymes and, secondarily, metastasis.

The genes encoding many glycosyltransferases have already been **cloned**, and others are under study. Cloning has revealed new information on both protein and gene structures. The latter should also cast light on the mechanisms involved in their **transcriptional control**, and **gene knockout studies** are being used to evaluate the biologic importance of various glycosyltransferases.

Tunicamycin Inhibits N- But Not O-Glycosylation

A number of compounds are known to inhibit various reactions involved in glycoprotein processing. **Tunicamycin, deoxynojirimycin,** and **swainsonine** are three such agents. The reactions they inhibit are indicated in Table 47–12. These agents can be used experimentally to inhibit various stages of glycoprotein biosynthesis and to study the effects of specific alterations upon the process. For instance, if cells are grown in the presence of tunicamycin, no glycosylation of their normally N-linked glycoproteins will occur. In certain cases, lack of glycosylation has been shown to increase the susceptibility of these proteins to proteolysis. Inhibition of glycosylation does not appear to have a consistent effect upon the secretion

TABLE 47–12 Three Inhibitors of Enzymes Involved in the *N*-Glycosylation of Glycoproteins and Their Sites of Action

Inhibitor	Site of Action
Tunicamycin	Inhibits GlcNAc-P transferase, the enzyme catalyzing addition of GlcNAc to dolichol-P, the first step in the biosynthesis of oligosaccharide-P-P-dolichol
Deoxynojirimycin	Inhibitor of glucosidases I and II
Swainsonine	Inhibitor of mannosidase II

of glycoproteins that are normally secreted. The inhibitors of glycoprotein processing listed in Table 47–12 do not affect the biosynthesis of O-linked glycoproteins. The extension of O-linked chains can be prevented by GalNAc-benzyl. This compound competes with natural glycoprotein substrates and thus prevents chain growth beyond GalNAc.

SOME PROTEINS ARE ANCHORED TO THE PLASMA MEMBRANE BY GLYCOSYLPHOSPHATIDYLINOSITOL STRUCTURES

Glycosylphosphatidylinositol (GPI)-linked glycoproteins comprise the third major class of glycoprotein. The GPI structure (sometimes called a "sticky foot") involved in linkage of the enzyme acetylcholinesterase (ACh esterase) to the plasma membrane of the red blood cell is shown in Figure 47–1. GPI-linked proteins are anchored to the outer leaflet of the plasma membrane by the fatty acids of phosphatidylinositol (PI). The PI is linked via a GlcN moiety to a glycan chain that contains various sugars (eg, Man, GlcN). In turn, the oligosaccharide chain is linked via phosphorylethanolamine in an amide linkage to the carboxyl terminal amino acid of the attached protein. The core of most GPI structures contains one molecule of phosphorylethanolamine, three Man residues, one molecule of GlcN, and one molecule of phosphatidylinositol, as follows:

Ethanolamine-phospho → 6Manα1 →
2Manα1 → 6Manα1 → GlNα1 →
6 — *myo* – inositol – 1 – phospholipid

Additional constituents are found in many GPI structures; for example, that shown in Figure 47–1 contains an extra phosphorylethanolamine attached to the middle of the three Man moieties of the glycan and an extra fatty acid attached to GlcN. The functional significance of these variations among structures is not understood. This type of linkage was first detected by the use of bacterial PI-specific phospholipase C (PI-PLC), which was found to release certain proteins from the plasma membrane of cells by splitting the bond indicated in Figure 47–1. Examples of some proteins that are anchored by this type of linkage are given in Table 47–13. At least three possible functions of this type of linkage have been suggested: (1) The GPI anchor may allow greatly enhanced **mobility** of a protein

TABLE 47–13 *Some* GPI-Linked Proteins

• Acetylcholinesterase (red cell membrane)
• Alkaline phosphatase (intestinal, placental)
• Decay-accelerating factor (red cell membrane)
• 5′-Nucleotidase (T lymphocytes, other cells)
• Thy-1 antigen (brain, T lymphocytes)
• Variable surface glycoprotein (*Trypanosoma brucei*)

in the plasma membrane compared with that observed for a protein that contains transmembrane sequences. This is perhaps not surprising, as the GPI anchor is attached only to the outer leaflet of the lipid bilayer, so that it is freer to diffuse than a protein anchored via both leaflets of the bilayer. Increased mobility may be important in facilitating rapid responses to appropriate stimuli. (2) Some GPI anchors may connect with **signal transduction** pathways. (3) It has been shown that GPI structures can **target** certain proteins to apical domains and also basolateral domains of the plasma membrane of certain polarized epithelial cells. The biosynthesis of GPI anchors is complex and begins in the endoplasmic reticulum. The GPI anchor is assembled independently by a series of enzyme-catalyzed reactions and then transferred to the carboxyl terminal end of its acceptor protein, accompanied by cleavage of the preexisting carboxyl terminal hydrophobic peptide from that protein. This process is sometimes called **glypiation.** An acquired defect in an early stage of the biosynthesis of the GPI structure has been implicated in the causation of **paroxysmal nocturnal hemoglobinuria** (see later).

ADVANCED GLYCATION END-PRODUCTS (AGEs) ARE THOUGHT TO BE IMPORTANT IN THE CAUSATION OF TISSUE DAMAGE IN DIABETES MELLITUS

Glycation refers to non-enzymic attachment of sugars (mainly glucose) to amino groups of proteins and also to other molecules (eg, DNA, lipids). Glycation is distinguished from **glycosylation** because the latter involves enzyme-catalyzed attachment of sugars. When glucose attaches to a protein, intermediate products formed include **Schiff bases**. These can further re-arrange by the **Amadori rearrangement** to **ketoamines** (see Figure 47–11). The overall series of reactions is known as the **Maillard reaction**. These reactions are involved in the **browning** of certain foodstuffs that occurs on storage or processing (eg, heating). The end-products of glycation reactions are termed **advanced glycation end-products (AGEs)**.

The major medical interest in AGEs has been in relation to them causing **tissue damage in diabetes mellitus**, in which the level of blood glucose is often consistently elevated, promoting increased glycation. At constant time intervals, the extent of glycation is more or less proportional to the blood glucose level. It has also been suggested that AGEs are involved in other processes, such as **aging**.

Glycation of collagen and other proteins in the ECM alters their properties (eg, increasing the **cross-linking of collagen**). Cross-linking can lead to accumulation of various plasma proteins in the walls of blood vessels; in particular, accumulation of **LDL** can contribute to **atherogenesis**. AGEs appear to be involved in both **microvascular** and **macrovascular** damage in diabetes mellitus. Also endothelial cells and macrophages have AGE receptors on their surfaces. Uptake of glycated proteins by these receptors can activate the transcription factor **NF-kB** (see Chapter 50), generating a variety of **cytokines** and **pro-inflammatory molecules**. It is thus believed that AGEs are one significant contributor to some of the pathologic finding found in diabetes mellitus (see Figure 47–12). **Aminoguanidine**, an inhibitor of the formation of AGEs, may be of benefit in reducing the organ and tissue complications of diabetes.

Non-enzymic attachment of glucose to **hemoglobin A** present in red blood cells (ie, formation of **HbA_{1c}**) occurs in normal individuals and is increased in patients with diabetes mellitus whose blood sugar levels are elevated. As discussed in Chapter 6, measurement of HbA_{1c} has become a very important part of the **management of patients with diabetes mellitus**.

GLYCOPROTEINS ARE INVOLVED IN MANY BIOLOGIC PROCESSES & IN MANY DISEASES

As listed in Table 47–1, glycoproteins have many different functions; some have already been addressed in this chapter and

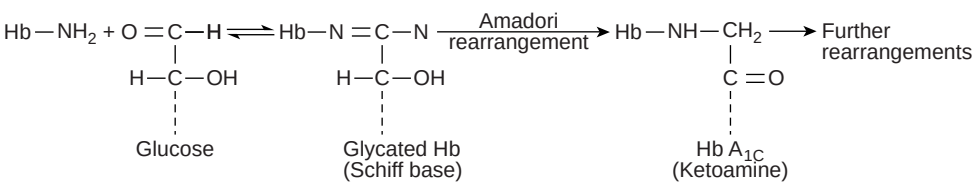


FIGURE 47–11 Formation of AGEs from glucose. Glucose is shown interacting with the amino group of hemoglobin (Hb) forming a Schiff base. This is subject to the Amadori rearrangement, forming a ketoamine. Further rearrangements can occur, leading to other AGEs.

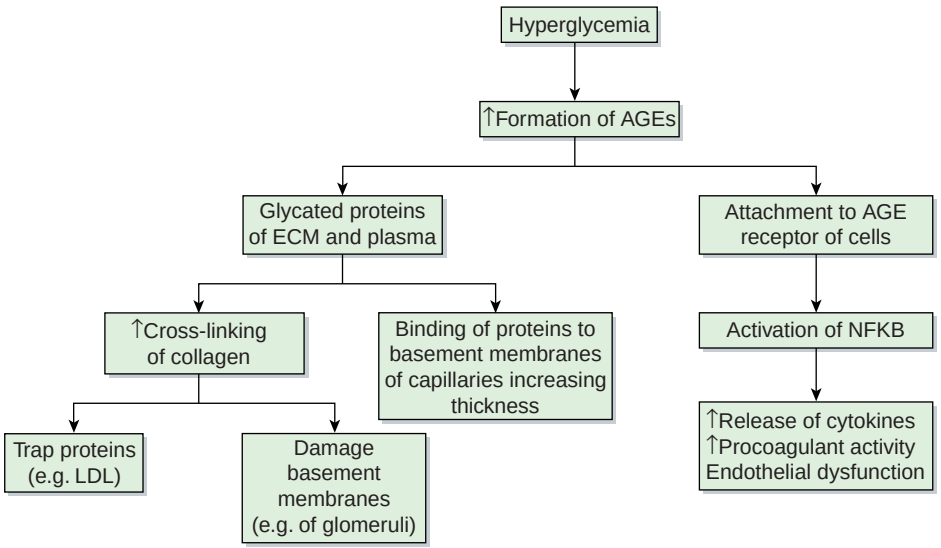


FIGURE 47–12 Some consequences of the formation of AGEs. Hyperglycemia (eg, occurring in poorly controlled diabetes) leads to the formation of AGEs. These can occur in proteins of the ECM or plasma. In the ECM, they can cause increased cross-linking of collagen, which can trap proteins such as LDL (contributing to atherogenesis) and damage basement membranes in the kidneys and other sites. Thickening of basement membranes can also occur by binding of glycated proteins to them. AGEs can attach to AGE receptors on cells, activating NFkB (see Chapter 50), which has several consequences (as shown). Damage to renal basement membranes, thickening of these membranes in capillaries and endothelial dysfunction are found in ongoing uncontrolled diabetes mellitus.

others are described elsewhere in this text (eg, transport molecules, immunologic molecules, and hormones). Here, their involvement in two specific processes—**fertilization** and **inflammation**—will be briefly described. In addition, the **bases of a number of diseases** that are due to abnormalities in the synthesis and degradation of glycoproteins will be summarized.

Glycoproteins Are Important in Fertilization

To reach the plasma membrane of an oocyte, a sperm has to traverse the **zona pellucida (ZP),** a thick, transparent, noncellular envelope that surrounds the oocyte. The zona pellucida contains three glycoproteins of interest, ZP1–3. Of particular note is ZP3, an O-linked glycoprotein that functions as a receptor for the sperm. A protein on the sperm surface, possibly galactosyl transferase, interacts specifically with oligosaccharide chains of ZP3; in at least certain species (eg, the mouse), this interaction, by transmembrane signaling, induces the **acrosomal reaction,** in which enzymes such as proteases and hyaluronidase and other contents of the acrosome of the sperm are released. Liberation of these enzymes helps the sperm to pass through the zona pellucida and reach the plasma membrane (PM) of the oocyte. In hamsters, it has been shown that another glycoprotein, PH-30, is important in both the binding of the PM of the sperm to the PM of the oocyte and also in the subsequent fusion of the two membranes. These interactions enable the sperm to enter and thus fertilize the oocyte. It may be possible to **inhibit fertilization** by developing drugs or antibodies that interfere with the normal functions of ZP3 and PH-30 and which would thus act as contraceptive agents.

Selectins Play Key Roles in Inflammation & in Lymphocyte Homing

Leukocytes play important roles in many inflammatory and immunologic phenomena. The first steps in many of these phenomena are interactions between circulating leukocytes and **endothelial cells** prior to passage of the former out of the circulation. Work done to identify specific molecules on the surfaces of the cells involved in such interactions has revealed that leukocytes and endothelial cells contain on their surfaces specific lectins, called **selectins,** that participate in their intercellular adhesion. Features of the three major classes of selectins are summarized in Table 47–14. Selectins are single-chain Ca^{2+}-binding transmembrane proteins that contain a number of domains (Figure 47–13). Their amino terminal ends contain the lectin domain, which is involved in binding to specific carbohydrate ligands.

The adhesion of neutrophils to endothelial cells of postcapillary venules can be considered to occur in four stages, as shown in Figure 47–14. The initial baseline stage is succeeded by **slowing or rolling** of the neutrophils, mediated by selectins. Interactions between L-selectin on the neutrophil surface and CD34 and GlyCAM-1 or other glycoproteins on the endothelial surface are involved. These particular interactions are initially

TABLE 47–14 Some Molecules Involved in Leukocyte-Endothelial Cell Interactions

Molecule	Cell	Ligands
Selectins		
L-selectin	PMN, lymphs	CD34, Gly-CAM-[1], sialyl-Lewis^x, and others
P-selectin	EC, platelets	P-selectin glycoprotein ligand-1 (PSGL-1), sialyl-Lewis^x, and others
E-selectin	EC	Sialyl-Lewis^x and others
Integrins		
LFA-1	PMN, lymphs	ICAM-1, ICAM-2
(CD11a/CD18)		
Mac-1	PMN	ICAM-1 and others
(CD11b/CD18)		
Immunoglobulin superfamily		
ICAM-1	Lymphs, EC	LFA-1, Mac-1
ICAM-2	Lymphs, EC	LFA-1
PECAM-1	EC, PMN, lymphs	Various platelets

Source: Modified, with permission, from Albelda SM, Smith CW, Ward PA: Adhesion molecules and inflammatory injury. FASEB J 1994;8:504.
Abbreviations: PMN, polymorphonuclear leukocytes; EC, endothelial cell; lymphs, lymphocytes; CD, cluster of differentiation; ICAM, intercellular adhesion molecule; LFA-1, lymphocyte function-associated antigen-1; PECAM-1, platelet endothelial cell adhesion cell molecule-1.

[1]These are ligands for lymphocyte L-selectin; the ligands for neutrophil L-selectin have not apparently been identified.

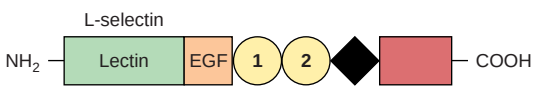


FIGURE 47–13 Schematic diagram of the structure of human L-selectin. The extracellular portion contains an amino terminal domain homologous to C-type lectins and an adjacent epidermal growth factor-like domain. These are followed by a variable number of complement regulatory-like modules (numbered circles) and a trans-membrane sequence (black diamond). A short cytoplasmic sequence (red rectangle) is at the carboxyl terminal. The structures of P- and E-selectin are similar to that shown except that they contain more complement-regulatory modules. The numbers of amino acids in L-, P-, and E- selectins, as deduced from the cDNA sequences, are 385, 789, and 589, respectively. (Reproduced, with permission, from Bevilacqua MP, Nelson RM: Selectins. J Clin Invest 1993;91:370.)

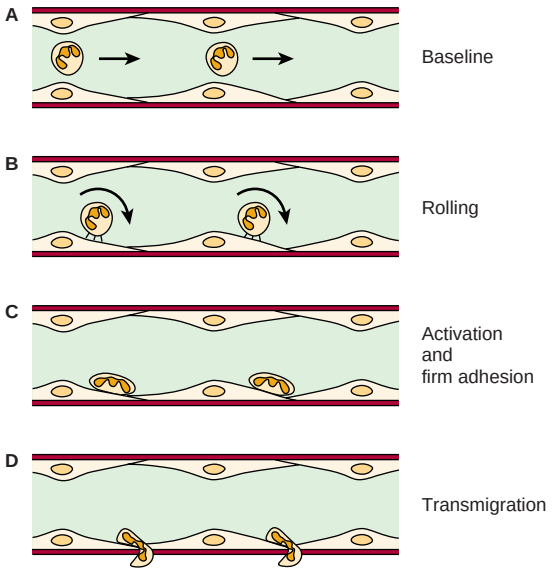


FIGURE 47–14 Schematic diagram of neutrophil-endothelial cell interactions. **(A)** Baseline conditions: Neutrophils do not adhere to the vessel wall. **(B)** The first event is the slowing or rolling of the neutrophils within the vessel (venule) mediated by selectins. **(C)** Activation occurs, resulting in neutrophils firmly adhering to the surfaces of endothelial cells and also assuming a flattened shape. This requires interaction of activated CD18 integrins on neutrophils with ICAM-1 on the endothelium. **(D)** The neutrophils then migrate through the junctions of endothelial cells into the interstitial tissue; this requires involvement of PECAM-1. Chemotaxis is also involved in this latter stage. (Reproduced, with permission, from Albelda SM, Smith CW, Ward PA: Adhesion molecules and inflammatory injury. FASEB J 1994;8;504.)

short-lived, and the overall binding is of relatively low affinity, permitting rolling. However, during this stage, **activation** of the neutrophils by various chemical mediators (discussed below) occurs, resulting in a change of shape of the neutrophils and firm adhesion of these cells to the endothelium. An additional set of **adhesion molecules** is involved in firm adhesion, namely, LFA-1 and Mac-1 on the neutrophils and ICAM-1 and ICAM-2 on endothelial cells. LFA-1 and Mac-1 are CD11/CD18 integrins (see Chapter 52 for a discussion of integrins), whereas ICAM-1 and ICAM-2 are members of the immunoglobulin superfamily. The fourth stage is **transmigration** of the neutrophils across the endothelial wall. For this to occur, the neutrophils insert pseudopods into the junctions between endothelial cells, squeeze through these junctions, cross the basement membrane, and then are free to migrate in the extravascular space. Platelet-endothelial cell adhesion molecule-1 (PECAM-1) has been found to be localized at the junctions of endothelial cells and thus may have a role in transmigration. A variety of biomolecules have been found to be involved in **activation** of neutrophil and endothelial cells, including tumor necrosis factor, various interleukins, platelet activating factor (PAF), leukotriene B_4, and certain complement fragments. These compounds stimulate various signaling pathways, resulting in changes in cell shape and function, and some are also chemotactic. One important functional change is **recruitment of selectins** to the cell surface, as in some cases selectins are stored in granules (eg, in endothelial cells and platelets).

The precise chemical nature of some of the ligands involved in selectin-ligand interactions has been determined. All three selectins bind **sialylated and fucosylated oligosaccharides,** and in particular all three bind **sialyl-Lewis^x**

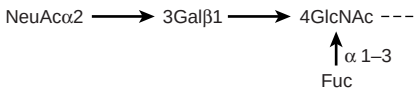


FIGURE 47–15 Schematic representation of the structure of sialyl-Lewis^x.

(Figure 47–15), a structure present on both glycoproteins and glycolipids. Whether this compound is the actual ligand involved in vivo is not established. Sulfated molecules, such as the sulfatides (Chapter 15), may be ligands in certain instances. This basic knowledge is being used in attempts to synthesize compounds that block selectin-ligand interactions and thus may inhibit the inflammatory response. Approaches include administration of specific monoclonal antibodies or of chemically synthesized analogs of sialyl-Lewis^x, both of which bind selectins. **Cancer cells** often exhibit sialyl-Lewis^x and other selectin ligands on their surfaces. It is thought that these ligands play a role in the invasion and metastasis of cancer cells.

Abnormalities in the Synthesis of Glycoproteins Underlie Certain Diseases

Table 47–15 lists a number of conditions in which abnormalities in the synthesis of glycoproteins are of importance. As mentioned above, many **cancer cells** exhibit different profiles of oligosaccharide chains on their surfaces, some of which may contribute to metastasis.

The **congenital disorders of glycosylation (CDG)** are a group of disorders of considerable current interest. The major features of these conditions are summarized in Table 47–16.

Leukocyte adhesion deficiency (LAD) II is a rare condition probably due to mutations affecting the activity of a Golgi-located GDP-fucose transporter. It can be considered a congenital disorder of glycosylation. The absence of fucosylated ligands for selectins leads to a marked decrease in neutrophil rolling. Subjects suffer life-threatening, recurrent bacterial infections and also psychomotor and mental retardation. The condition appears to respond to oral fucose.

Hereditary erythroblastic multinuclearity with a positive acidified lysis test (HEMPAS)—congenital dyserythropoietic anemia type II—is another disorder in which abnormalities in the processing of N-glycans are thought to be involved. Some cases have been claimed to be due to defects in alpha–mannosidase II.

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired mild anemia characterized by the presence of hemoglobin in urine due to hemolysis of red cells, particularly during sleep. This latter phenomenon may reflect a slight drop in plasma pH during sleep, which increases susceptibility to lysis by the complement system (Chapter 50). The basic defect in paroxysmal nocturnal hemoglobinuria is the acquisition of somatic mutations in the *PIG-A* (for phosphatidylinositol glycan class A) gene of certain hematopoietic cells. The product of this gene appears to be the enzyme that links glucosamine to phosphatidylinositol in the GPI structure (Figure 47–1). Thus, proteins that are anchored by a GPI linkage are deficient in the red cell membrane. Two proteins are of particular interest:

TABLE 47–15 Some Diseases Due to or Involving Abnormalities in the Biosynthesis of Glycoproteins

Disease	Abnormality
Cancer	Increased branching of cell surface glycans or presentation of selectin ligands may be important in metastasis.
Congenital disorders of glycosylation[1]	See Table 47–16.
HEMPAS[2] (OMIM 224100)	Abnormalities in certain enzymes (eg, mannosidase II and others) involved in the biosynthesis of N-glycans, particularly affecting the red blood cell membrane.
Leukocyte adhesion deficiency, type II (OMIM 266265)	Probably mutations affecting a Golgi-located GDP-fucose transporter, resulting in defective fucosylation.
Paroxysmal nocturnal hemoglobinuria (OMIM 311770)	Acquired defect in biosynthesis of the GPI[3] structures of decay accelerating factor (DAF) and CD59.
I-cell disease (OMIM 252500)	Deficiency of GlcNAc phosphotransferase, resulting in abnormal targeting of certain lysosomal enzymes.

[1]The OMIM number for congenital disorder of glycosylation type Ia is 212065.
[2]Hereditary erythroblastic multinuclearity with a positive acidified serum lysis test (congenital dyserythropoietic anemia type II). This is a relatively mild form of anemia. It reflects at least in part the presence in the red cell membranes of various glycoproteins with abnormal N-glycan chains, which contribute to the susceptibility to lysis.
[3]Glycosylphosphatidylinositol.

TABLE 47–16 Major Features of the Congenital Disorders of Glycosylation

- Autosomal recessive disorders
- Multisystem disorders that have probably not been recognized in the past
- Generally affect the central nervous system, resulting in psychomotor retardation and other features
- Type I disorders are due to mutations in genes encoding enzymes (eg, phosphomannomutase-2 [PMM-2], causing CDG Ia) involved in the synthesis of dolichol-P-P-oligosaccharide
- Type II disorders are due to mutations in genes encoding enzymes (eg, GlcNAc transferase-2, causing CDG IIa) involved in the processing of N-glycan chains
- At least 15 distinct disorders have been recognized
- Isoelectric focusing of transferrin is a useful biochemical test for assisting in the diagnosis of these conditions; truncation of the oligosaccharide chains of this protein alters its iso-electric focusing pattern
- Oral mannose has proved of benefit in the treatment of CDG Ia

Abbreviation: CDG, congenital disorder of glycosylation.

decay accelerating factor (DAF) and another protein designated CD59. They normally interact with certain components of the complement system (Chapter 50) to prevent the hemolytic actions of the latter. However, when they are deficient, the complement system can act on the red cell membrane to cause hemolysis. A monoclonal antibody to C5, a terminal component of the complement system, has proven useful in the management of PNH. by inhibiting the complement cascade. PNH can be diagnosed relatively simply, as the red cells are much more sensitive to hemolysis in normal serum acidified to pH 6.2 (Ham's test); the complement system is activated under these conditions, but normal cells are not affected. Figure 47–16 summarizes the etiology of PNH.

Study of the **congenital muscular dystrophies (CMDs)** has revealed that certain of them (eg, the Walker-Warburg syndrome, muscle-eye-brain disease, Fukuyama CMD) are the result of defects in the synthesis of glycans in the protein α-dystroglycan (α-DG). This protein protrudes from the surface membrane of muscle cells and interacts with laminin-2 (merosin) in the basal lamina (see Figure 49–11). If the glycans of α-DG are not correctly formed (as a result of mutations in genes encoding certain glycosyltransferases), this results in defective interaction of α-DG with laminin, which in turn leads to the development of a CMD.

Rheumatoid arthritis is associated with an alteration in the glycosylation of circulating immunoglobulin G (IgG) molecules (Chapter 50), such that they lack galactose in their Fc regions and terminate in GlcNAc. **Mannose-binding protein** (MBP, not to be confused with the mannose 6-P receptor), a C-lectin synthesized by liver cells and secreted into the circulation, binds mannose, GlcNAc, and certain other sugars. It can thus bind agalactosyl IgG molecules, which subsequently activate the complement system (see Chapter 50), contributing to chronic inflammation in the synovial membranes of joints.

MBP can also bind the above sugars when they are present on the surfaces of certain bacteria, fungi, and viruses, preparing these pathogens for opsonization or for destruction by the complement system. This is an example of **innate immunity,** not involving immunoglobulins or T lymphocytes. Deficiency of this protein in young infants as a result of mutation renders them very susceptible to **recurrent infections**.

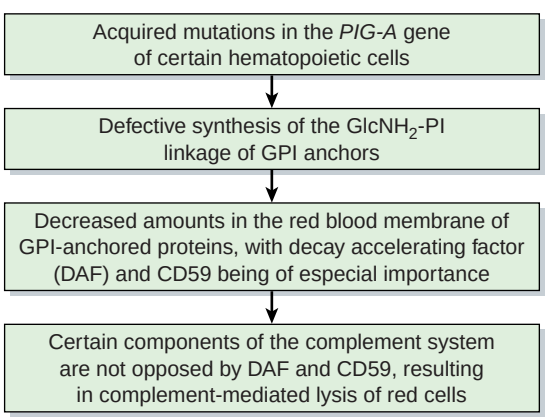


FIGURE 47–16 Scheme of causation of paroxysmal nocturnal hemoglobinuria (OMIM 311770).

I-Cell Disease Results from Faulty Targeting of Lysosomal Enzymes

As indicated above, Man 6-P serves as a chemical marker to target certain lysosomal enzymes to that organelle. Analysis of cultured fibroblasts derived from patients with I-cell (inclusion cell) disease played a large part in revealing the above role of Man 6-P. I-cell disease is an uncommon condition characterized by severe progressive psychomotor retardation and a variety of physical signs, with death often occurring in the first decade. Cultured cells from patients with I-cell disease were found to lack almost all of the normal lysosomal enzymes; the lysosomes thus accumulate many different types of undegraded molecules, forming inclusion bodies. Samples of plasma from patients with the disease were observed to contain very high activities of lysosomal enzymes; this suggested that the enzymes were being synthesized but were failing to reach their proper intracellular destination and were instead being secreted. Cultured cells from patients with the disease were noted to take up exogenously added lysosomal enzymes obtained from normal subjects, indicating that the cells contained a normal receptor on their surfaces for endocytic uptake of lysosomal enzymes. In addition, this finding suggested that lysosomal enzymes from patients with I-cell disease **might lack a recognition marker**. Further studies revealed that lysosomal enzymes from normal individuals carried the Man 6-P recognition marker described above, which interacted with a specific intracellular protein, the Man 6-P receptor. Cultured cells from patients with I-cell disease were then found to be **deficient** in the activity of the *cis*-Golgi-located **GlcNAc phosphotransferase**, explaining how their lysosomal enzymes failed to acquire the Man 6-P marker. It is now known that there are **two Man 6-P receptor proteins**, one of high (275 kDa) and one of low (46 kDa) molecular mass. These proteins are **lectins**, **recognizing Man 6-P**. The former is cation-independent and also binds IGF-II (hence it is named the Man 6-P–IGF-II receptor), whereas the latter is cation-dependent in some species and does not bind IGF-II. It appears that both receptors function in the intracellular sorting of lysosomal enzymes into clathrin-coated vesicles, which occurs in the *trans*-Golgi subsequent to synthesis of Man 6-P in the *cis*-Golgi. These vesicles then leave the Golgi and fuse with a prelysosomal compartment. The **low pH** in this compartment causes the lysosomal enzymes to **dissociate** from their receptors and subsequently enter into lysosomes. The receptors are recycled and reused. Only the smaller receptor functions in the endocytosis of **extracellular** lysosomal enzymes, which is a minor pathway for lysosomal location. Not all cells employ the Man 6-P receptor to target their lysosomal enzymes (eg, hepatocytes use a different but undefined pathway); furthermore, not all lysosomal enzymes are targeted by this mechanism. Thus, biochemical

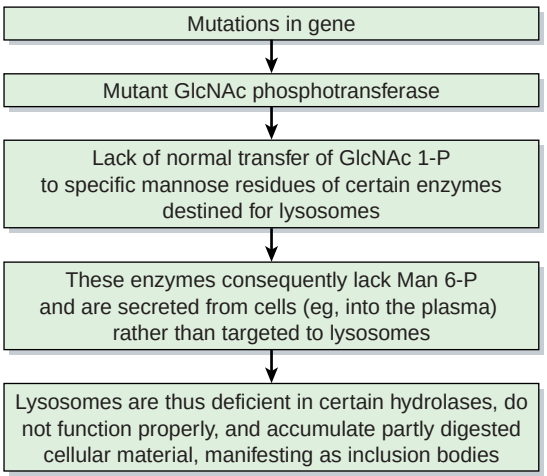


FIGURE 47–17 Summary of the causation of I-cell disease (OMIM 252500).

investigations of I-cell disease not only led to elucidation of its basis but also contributed significantly to knowledge of how newly synthesized proteins are targeted to specific organelles, in this case the lysosome. Figure 47–17 summarizes the causation of I-cell disease.

Pseudo-Hurler polydystrophy is another genetic disease closely related to I-cell disease. It is a **milder condition**, and patients may survive to adulthood. Studies have revealed that the **GlcNAc phosphotransferase** involved in I-cell disease has several domains, including a catalytic domain and a domain that specifically recognizes and interacts with lysosomal enzymes. It has been proposed that the defect in pseudo-Hurler polydystrophy lies in the latter domain, and the retention of some catalytic activity results in a milder condition.

Genetic Deficiencies of Glycoprotein Lysosomal Hydrolases Cause Diseases Such as α-Mannosidosis

Glycoproteins, like most other biomolecules, undergo both synthesis and degradation (ie, turnover). Degradation of the oligosaccharide chains of glycoproteins involves a battery of lysosomal hydrolases, including α-neuraminidase, β-galactosidase, β-hexosaminidase, α- and β-mannosidases, α-*N*-acetylgalactosaminidase, α-fucosidase, endo- β-*N*-acetylglucosaminidase, and aspartylglucosaminidase. The sites of action of the last two enzymes are indicated in the legend to Figure 47–5. Genetically determined defects of the activities of these enzymes can occur, resulting in abnormal degradation of glycoproteins. The accumulation in tissues of such degraded glycoproteins can lead to various diseases. Among the best-recognized of these diseases are mannosidosis, fucosidosis, sialidosis, aspartylglycosaminuria, and Schindler disease, due respectively to deficiencies of α-mannosidase, α-fucosidase, α-neuraminidase, aspartylglucosaminidase, and α-*N*-acetylgalactosaminidase. These diseases, which are relatively uncommon, have a variety of manifestations; some of their major features are listed in Table 47–17. The fact that patients affected by these disorders all show signs referable to the **central nervous system** reflects the importance of glycoproteins in the development and normal function of that system.

TABLE 47–17 Major Features of Some Diseases[1] Due to Deficiencies of Glycoprotein Hydrolases[2]

• Usually exhibit mental retardation or other neurologic abnormalities, and in some disorders coarse features or visceromegaly (or both)
• Variations in severity from mild to rapidly progressive
• Autosomal recessive inheritance
• May show ethnic distribution (eg, aspartylglycosaminuria is common in Finland)
• Vacuolization of cells observed by microscopy in some disorders
• Presence of abnormal degradation products (eg, oligosaccharides that accumulate because of the enzyme deficiency) in urine, detectable by TLC and characterizable by GLC-MS
• Definitive diagnosis made by assay of appropriate enzyme, often using leukocytes
• Possibility of prenatal diagnosis by appropriate enzyme assays
• No definitive treatment at present

[1]α-Mannosidosis, β-mannosidosis, fucosidosis, sialidosis, aspartylglycosaminuria, and Schindler disease.
[2]OMIM numbers: α-mannosidosis, 248500; β-mannosidosis, 248510; fucosidosis, 230000; sialidosis, 256550; aspartylglycosaminuria, 208400; Schindler disease, 609241.

THE GLYCANS OF GLYCOCONJUGATES ARE INVOLVED IN THE BINDING OF VIRUSES, BACTERIA, & CERTAIN PARASITES TO HUMAN CELLS

A principal feature of glycans, and one that explains many of their biologic actions, is that they **bind** specifically to a variety of molecules such as proteins or other glycans. One reflection of this is their ability to bind certain viruses, many bacteria and some parasites.

Influenza virus A binds to cell surface glycoprotein receptor molecules containing NeuAc via a protein named **hemagglutinin (H).** It also possesses a **neuraminidase (N)** that plays a key role in allowing elution of newly synthesized progeny from infected cells. If this process is inhibited, spread of the viruses is markedly diminished. Inhibitors of this enzyme (eg, zanamivir, oseltamivir) are now available for use in treating patients with influenza. Influenza viruses are classified according to the type of hemagglutinin and neuraminidase that they possess. There are at least 16 types of hemagglutinin and 9 types of neuraminidase. Thus, **avian influenza virus** is classified as **H5N1**. There is great interest in how this virus attaches to human cells, in view of the possibility of a pandemic

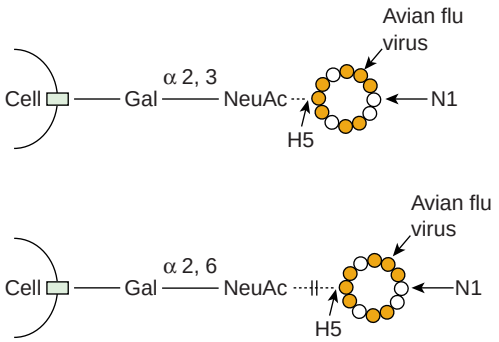


FIGURE 47–18 Schematic representation of binding of the avian influenza virus (H5N1) to a respiratory epithelial cell. The viral hemagglutin (HA) mediates its entry to cells by binding to a glycan on the cell surface that is terminated by the disaccharide galactose → α 2,3-NeuAc. It will not bind to a glycan terminated by galactose → α 2,6-NeuAc, which is the type predominantly found in the human respiratory tract. If the viral HA alters via mutation to be able to bind to the latter disaccharide, this could greatly increase its pathogenicity for humans. (H5, hemagglutinin type 5; N1, neuraminidase type 1.)

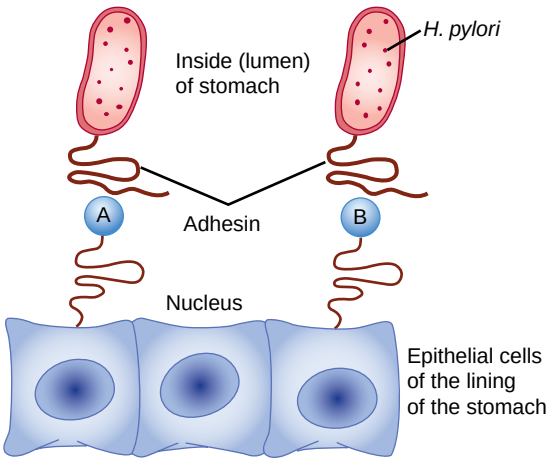


FIGURE 47–19 Attachment of *Helicobacter pylori* to epithelial cells of the stomach. Adhesin, a protein present in the tail of *H pylori,* interacts with two different glycans (structures shown below) present in glycoproteins on the surface of gastric epithelial cells. This provides an attachment site for the bacterium. Subsequently it liberates molecules, such as ammonia, that contribute to initiating peptic ulceration. (A) NeuAcα2,3Galβ1,4—Protein (Neuraminyl-galactose); (B) Fucα1,2Galβ1,3GlcNAc—Protein (Lewis^{B} substance).

occurring. It has been found that the virus preferentially attaches to glycans terminated by the disaccharide **galactose → α 2,3-NeuAc** (Figure 47–18). However, the predominant disaccharide terminating glycans in cells of the human respiratory tract is **galactose → α 2,6-NeuAc**. If a change in the structure of the viral hemagglutinin (due to mutation) occurs that allows it to bind to the latter disaccharide, this could greatly increase the potential infectivity of the virus, possibly resulting in very serious consequences.

Human immunodeficiency virus type 1 (HIV-1), thought by most to be the cause of AIDS, attaches to cells via one of its surface glycoproteins (gp120) and uses another surface glycoprotein (gp 41) to fuse with the host cell membrane. **Antibodies** to gp 120 develop during infection by HIV-1, and there has been interest in using the protein as a vaccine. One major problem with this approach is that the structure of gp 120 can change relatively rapidly, allowing the virus to escape from the neutralizing activity of antibodies directed against it.

Helicobacter pylori is believed to be the major cause of **peptic ulcers**. Studies have shown that this bacterium binds to at least two different glycans present on the surfaces of epithelial cells in the stomach (see Figure 47–19). This allows it to establish a stable attachment site to the stomach lining, and subsequent secretion of ammonia and other molecules by the bacterium are believed to initiate ulceration.

Similarly, many **bacteria that cause diarrhea** are also known to attach to surface cells of the intestine via glycans present in glycoproteins or glycolipids.

The basic cause of **cystic fibrosis** (CF) is mutations in the gene encoding CFTR (see Chapters 40 & 54). A major problem in this disease is recurring lung infections by bacteria such as *Pseudomonas aeruginosa.* In CF, a relative dehydration of respiratory secretions occurs secondary to changes in electrolyte composition in the airway as a result of mutations in CFTR. Bacteria such as *P aeruginosa* attach to the sugar chains of mucins and find the dehydrated environment in the bronchioles a favorable location in which to multiply.

The attachment of ***Plasmodium falciparum***—one of the types of plasmodia causing **malaria**—to human cells is mediated by a GPI present on the surface of the parasite.

Various researchers are **analyzing the surfaces of viruses, bacteria, parasites** and **human cells** to determine which molecules are involved in attachment. It is important to define the precise nature of the interactions between invading organisms and host cells, as this will hopefully lead to the development of drugs or other agents that will specifically inhibit attachment.

THE PACE OF RESEARCH IN GLYCOMICS IS ACCELERATING

Research on glycoconjugates in the past has been hampered by the lack of availability of suitable technics to determine the structures of glycans. However, appropriate analytical technics are now available (some of which are listed in Table 47–3), as are powerful new genetic technics (eg, knock-outs and knock-downs using RNAi molecules). It is certain that research in glycomics will not only provide a wealth of structural information on glyconconjugates, helping to disclose "the sugar code of life," but will also uncover many new important biologic interactions that are sugar-dependent and will provide targets for drug and other therapies.

SUMMARY

- Glycoproteins are widely distributed proteins—with diverse functions—that contain one or more covalently linked carbohydrate chains.
- The carbohydrate components of a glycoprotein range from 1% to more than 85% of its weight and may be simple or very complex in structure. Eight sugars are mainly found in the sugar chains of human glycoproteins: xylose, fucose, galactose, glucose, mannose, N-acetylgalactosamine, N-acetylglucosamine and N-acetylneuraminic acid.
- At least certain of the oligosaccharide chains of glycoproteins encode biologic information; they are also important to glycoproteins in modulating their solubility and viscosity, in protecting them against proteolysis, and in their biologic actions.
- The structures of oligosaccharide chains can be elucidated by gas-liquid chromatography, mass spectrometry, and high-resolution NMR spectrometry.
- Glycosidases hydrolyze specific linkages in oligosaccharides and are used to explore both the structures and functions of glycoproteins.
- Lectins are carbohydrate-binding proteins involved in cell adhesion and many other biologic processes.
- The major classes of glycoproteins are O-linked (involving an OH of serine or threonine), N-linked (involving the N of the amide group of asparagine), and glycosylphosphatidylinositol (GPI)-linked.
- Mucins are a class of O-linked glycoproteins that are distributed on the surfaces of epithelial cells of the respiratory, gastrointestinal, and reproductive tracts.
- The endoplasmic reticulum and Golgi apparatus play a major role in glycosylation reactions involved in the biosynthesis of glycoproteins.
- The oligosaccharide chains of O-linked glycoproteins are synthesized by the stepwise addition of sugars donated by nucleotide sugars in reactions catalyzed by individual specific glycoprotein glycosyltransferases.
- In contrast, the synthesis of N-linked glycoproteins involves a specific dolichol-P-P-oligosaccharide and various glycotransferases and glycosidases. Depending on the enzymes and precursor proteins in a tissue, it can synthesize complex, hybrid, or high-mannose types of N-linked oligosacccharides.
- Glycoproteins are implicated in many biologic processes. For instance, they have been found to play key roles in fertilization and inflammation.
- A number of diseases involving abnormalities in the synthesis and degradation of glycoproteins have been recognized. Glycoproteins are also involved in many other diseases, including influenza, AIDS, rheumatoid arthritis, cystic fibrosis and peptic ulcer.
- Developments in the new field of glycomics are likely to provide much new information on the roles of sugars in health and disease and also indicate targets for drug and other types of therapies.

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