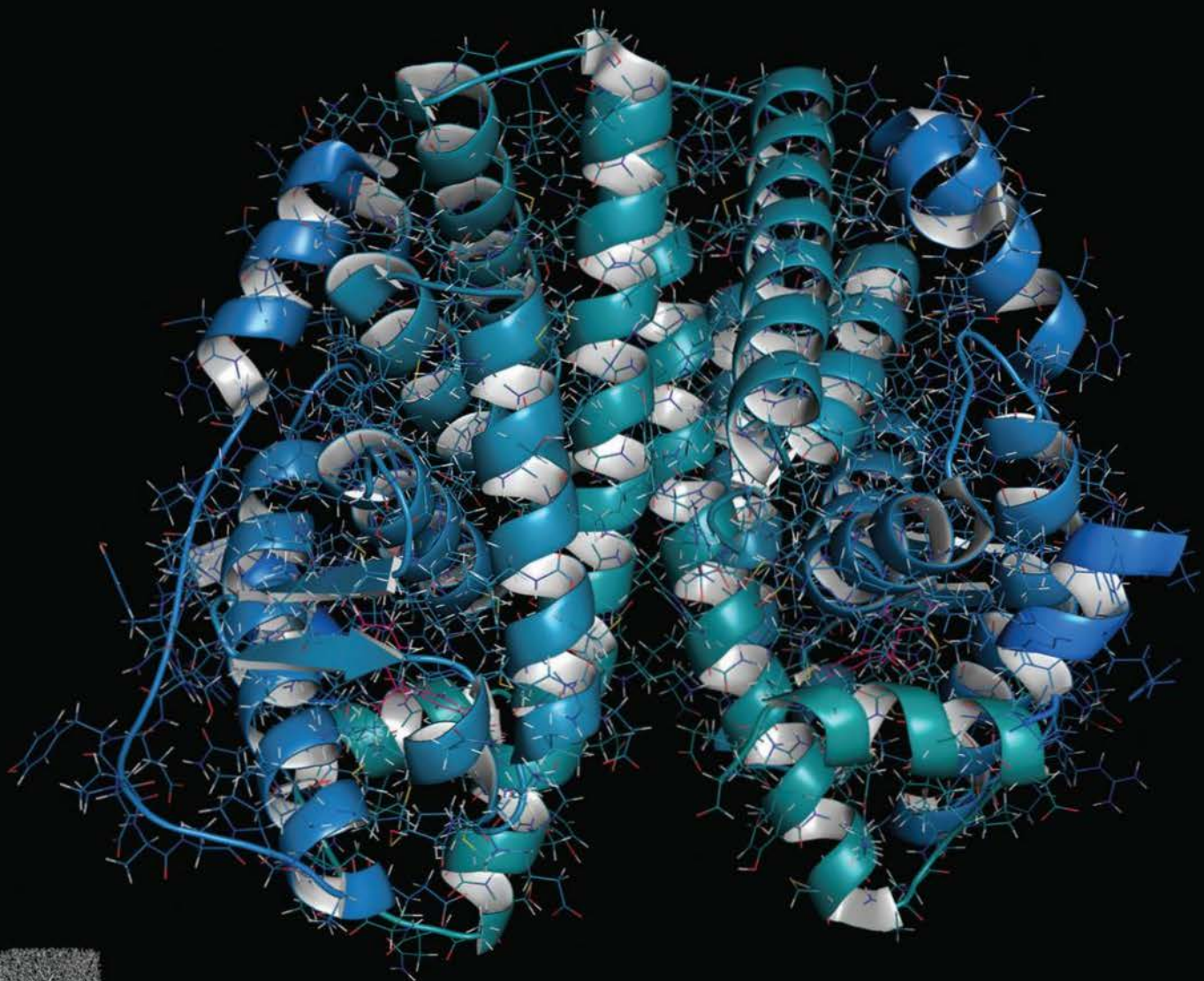
GOODMAN'S

BASIC MEDICAL ENDOCRINOLOGY

Elizabeth H. Holt • Beatrice Lupsa
Grace S. Lee • Hanan Bassyouni
Harry E. Peery



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FIFTH EDITION

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ELIZABETH H. HOLT
Associate Professor of Medicine, Endocrinology, Yale School of Medicine, New Haven, CT, United States

BEATRICE LUPSA
Assistant Professor of Medicine, Endocrinology, Yale School of Medicine, New Haven, CT, United States

GRACE S. LEE
Assistant Professor of Medicine, Endocrinology, Yale School of Medicine, New Haven, CT, United States

Endocrinologist, VA CT Healthcare System, West Haven, CT, United States

HANAN BASSYOUNI
Clinical Associate Professor, Cumming School of Medicine; Faculty of Medicine, Department of Medicine; Section of Endocrinology and Metabolism, University of Calgary, Calgary, AB, Canada

HARRY E. PEERY
The Karl Riabowol Laboratory, Arnie Charbonneau Cancer Research Institute and Department of Biochemistry and Molecular Biology, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada

Program in Pharmaceutical Bioengineering, Department of Bioengineering, School of Medicine and College of Engineering, University of Washington, Seattle, WA, United States

Founding Director and Research Director, The Anti-NMDA Receptor Encephalitis Foundation, Inc., Ottawa, Ontario, Canada

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Radarweg 29, PO Box 211, 1000 AE Amsterdam, Netherlands
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Dedications

We dedicate this book to patients with endocrine disorders and to our colleagues in the clinics and laboratories who are working to understand and treat those disorders.

Elizabeth H. Holt:
To my patients

Grace S. Lee:
To my loving husband and children.

Beatrice Lupsa:
To my father, Mircea who inspired me to be a physician.

Hanan Bassyouni:
To my Parents, husband and boys for always supporting and loving me. And to my friends, patients and students as I would not be who I am today without you.

Harry E. Peery:
To my wife, Susan B. Peery, and to our children, grandchildren, friends, students and colleagues who have supported me over the years and to the memory of Betty Noling who taught me how to write at Far Hills Country Day School.

Advice to Future Colleagues:

"Plan ahead.
Always be concerned about the dangers of mural dyslexia: The inability to read the writing on the wall."

- Lloyd H.(Holly) Smith, late renowned endocrinologist, internal medicine educator, administrator and author.

Contents

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Preface

It has been 33 years since Maurice Goodman put pen to paper and wrote his Basic Medical Endocrinology in 1988, mainly for the medical students he had been teaching for 25 years but also for graduate students and upper division undergraduates. At that time, almost all medical schools still had the basic sciences taught by PhDs as part of the first 2 years (preclerkship years) of medical school.

Fast forward to 2008, when the fourth edition introduced color to the book. By that time—20 years after the first edition—interest in 2 years of basic sciences for medical students was waning. More and more, it was the clinical specialist who was teaching those courses with an emphasis on clinical examples rather than on the biochemical basis of the subject matter. This approach was accelerated even more when the basic sciences became more interested in molecular biology, genetics, and epigenetics rather than clinical applications. Yet, Dr. Goodman's text was still being appreciated at the graduate and upper division undergraduate levels.

Moving forward another 13 years, and we find that in 2021 this is still true, but now many medical schools are eliminating the basic sciences altogether and focusing on clinicians teaching only background information that is clinically relevant. As a result, these new medical programs are usually shortened by a year with an emphasis on educating family physicians rather than research physicians.

During those intervening years since the fourth edition, Dr. Goodman retired from both teaching and writing. We were invited by the publisher to take over the fifth edition of this venerable work. It was a challenge for two reasons. First, to fill Dr. Goodman's shoes and retain and expand on the molecular aspects of endocrinology and, second, to recapture some of the medical markets that evaporated when basic sciences stopped being taught by nonclinicians.

To this end, board-certified endocrinologists at leading universities in the United States and Canada were invited to provide case studies and to add some clinically related material throughout the book. At the same time, the molecular aspects of the field were updated by a respected professional in the basic sciences who teaches in the United States and Canada.

While much of the book retains the original work of Dr. Goodman—for whom we are deeply indebted—there has also been extensive updating. To begin with, we added many illustrations to depict the molecular aspects of endocrinology. These were mainly taken from other Elsevier medical publications, although some were drawn specially for this text. We also added clinical illustrations—again from Elsevier's extensive medical library of journals and textbooks.

We have also added new information—such as the putative role of oxytocin in males—and condensed other areas so that the text could still fit in a relatively small volume. While we hope these changes will meet with approval, we are always open to suggestions from the users of our book. Feel free to contact any one of the authors.

The cover image is the molecular structure of the estrogen receptor, present in both males and females. We chose this because it is the same structure in both genders, which helps underscore the promotion of equality and respect in the sciences and in medicine—and in life.

Last but not least, we want to thank our acquisition editor, Tari Broderick, our development editor, Kristi Anderson and production editor, Kiruthika Govindaraju, at Elsevier, all of whom patiently and expertly helped us in every aspect of this project. We also want to thank our spouses, who had to live a very lonely existence while we crafted our manuscript. We are deeply indebted to them for their understanding.

Elizabeth H. Holt[1], Beatrice Lupsa[1], Grace S. Lee[1], Hanan Bassyouni[2] and Harry E. Peery[2]

[1]Yale School of Medicine, Yale University, New Haven, CT, United States [2]Cumming School of Medicine, University of Calgary, Calgary, AB, Canada

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CHAPTER

1

Introduction

Case Study: Giantism

Normally, cells communicate with other cells in an orderly fashion. However, in 1918 this did not happen with growth hormone in Robert Wadlow (Fig. 1.1). Did he have a tumor or did something happen to the cells that stimulated the release of growth hormone? We will never know. We do know that he began to grow very fast as a child (Table 1.1).

FIGURE 1.1 From a postcard showing Robert Wadlow—over 8 ft. 9 in. in this picture—with his father, who is 5′11″. Source: Wikipedia.

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(*cont'd*)

TABLE 1.1 Growth chart of Robert Wadlow.

Age	Height	Weight	Notes	Size of	Year
Birth	1 ft. 8 in. (0.51 m)	8 lb 6 oz (3.8 kg)	Normal height of weight.	Average	February 22, 1918
6 months	2 ft. 10.5 in. (0.88 m)	30 lb (14 kg)		2 year old	August 22, 1918
1 year	3 ft. 6 in. (1.07 m)	45 lb (20 kg)	When he began to walk at 11 months, he was 3 ft. 3.5 in. (1.00 m) tall and weighed 40 lbs.	5 year old	February 22, 1919
18 months	4 ft. 3 in. (1.30 m)	67 lb (30 kg)		8 year old	August 22, 1919
2 years	4 ft. 6 in. (1.37 m)	75 lb (34 kg)		10 year old	1920
3 years	4 ft. 11 in. (1.50 m)	89 lb (40 kg)		12 year old	1921
4 years	5 ft. 3 in. (1.60 m)	105 lb (48 kg)		14 year old	1922
5 years	5 ft. 6.5 in. (1.69 m)	140 lb (64 kg)	At 5 years of age, attending kindergarten, Robert was 5′ 6 1/2" tall. He wore clothes that would fit a 17-year-old boy.	15 year old	1923
6 years	5 ft. 7 in. (1.70 m)	146 lb (66 kg)		15 year old	1924
7 years	5 ft. 10 in. (1.78 m)	159 lb (72 kg)		Height of adult male	1925
8 years	6 ft. 0 in. (1.83 m)	169 lb (77 kg)			1926
9 years	6 ft. 2 in. (1.88 m)	180 lb (82 kg)	Weighing 180 lb, he was strong enough to carry his father (who was sitting in a living room chair) up the stairs to the second floor.		1927
10 years	6 ft. 5 in. (1.96 m)	210 lb (95 kg)			1928
11 years	6 ft. 11 in. (2.11 m)	241 lb (109 kg)			1929
12 years	7 ft. 2 in. (2.18 m)	287 lb (130 kg)			1930
13 years	7 ft. 4 in. (2.24 m)	301 lb (137 kg)	World's tallest Boy Scout, averaging a growth of 4 in. (10 cm) per year since birth and wearing size 19 (US) shoes.		1931
14 years	7 ft. 5 in. (2.26 m)	331 lb (150 kg)			1932
15 years	7 ft. 10 in. (2.39 m)	354 lb (161 kg)			1933
16 years	8 ft. 1.5 in. (2.48 m)	374 lb (170 kg)			1934
17 years	8 ft. 3 in. (2.51 m)	382 lb (173 kg)	Graduated from high school on January 8, 1936 and was 8 ft. 3 in. (2.51 m).	Height of Sultan Kosen, the tallest living man	1935
18 years	8 ft. 4 in. (2.54 m)	391 lb (177 kg)			1936
19 years	8 ft. 6 in. (2.59 m)	480 lb (220 kg)			1937
20 years	8 ft. 7 in. (2.62 m)	488 lb (221 kg)			1938
21 years	8 ft. 8 in. (2.64 m)	492 lb (223 kg)			1939

(*Continued*)

(*cont'd*)

TABLE 1.1 (Continued)

Age	Height	Weight	Notes	Size of	Year
22.4 years	8 ft. 11.1 in. (2.72 m)	439 lb (199 kg)	At death, he was the world's tallest man according to the Guinness World Records.		June 27, 1940

His height would increase until he died. He was not well during the last year of life and so lost weight. No medical cause for his ill-health was ever given.

His condition is called giantism and is due to the overproduction of growth hormone. He was producing so much growth hormone that it competed with the receptors for insulin, causing him to develop diabetes mellitus (type II diabetes). Through processes that are not yet understood, he developed peripheral neuropathy that is the loss of sensation in his feet, legs, hands, and arms because of the diabetes.

Growth was so fast that his bones could not develop quickly enough to hold his weight. This was especially true of his legs. He had to walk with braces on his legs, use a cane, and lean on objects when standing still (Fig. 1.2).

In 1940 he developed a blister on his leg from a new brace. It became infected and, since this was before the era of antibiotics, he died of septicemia at the age of 22. He was still growing at the time of his death.

FIGURE 1.2 RobertWadlow leaning on a 1932 Ford.

Types of chemical messengers

Chemical messengers, such as growth hormone in Robert Wadlow, are one-way by which cells communicate with each other in a multicellular organism. This type of communication is slower but more sustained than the lightening-like response of the nervous system to potentially life-threatening stimuli from a body surface or viscera.

Evolutionarily, cells needed to communicate with other cells in a multicellular organism. Many of these chemical messengers have been conserved so that the mating factor receptor in primitive plants such as baker's yeast (*Saccharomyces cerevisiae*) is also to be found in animals although slightly transformed.

Types of intercellular chemical messengers include (1) autocrines—released by the cell as a self-regulator; (2) paracrines—released by neighboring cells to regulate a larger group of cells; (3) endocrines (hormones)—substances released from a distance and which strictly speaking travel part of the way in blood to reach their target tissues (however, the term is often used to mean any substance traveling in extracellular fluid); and, (4), pherocrines—substances released from one organism and passing through the environment (air or water) to affect other organisms. (Perfumes have as their base a pherocrine-like substance from either plants or animals.) Fig. 1.3 summarizes these concepts.

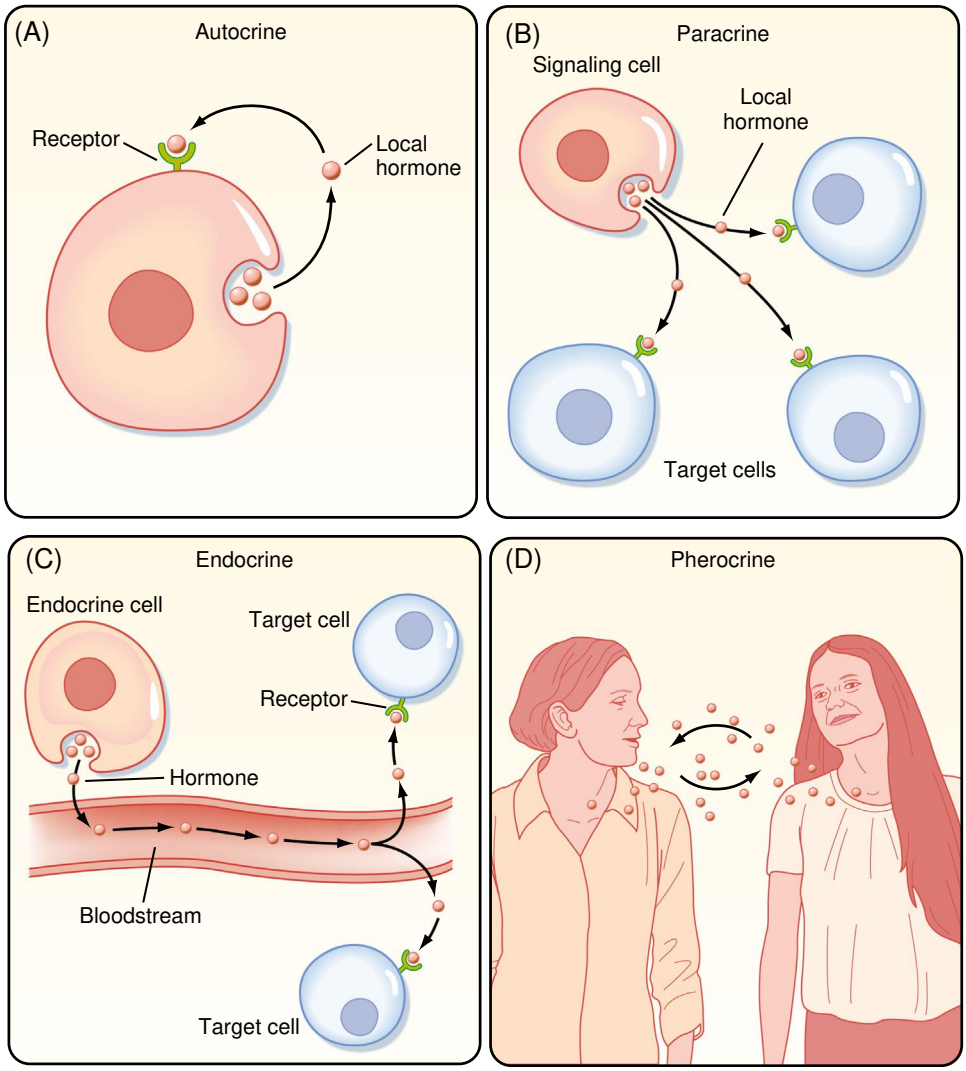
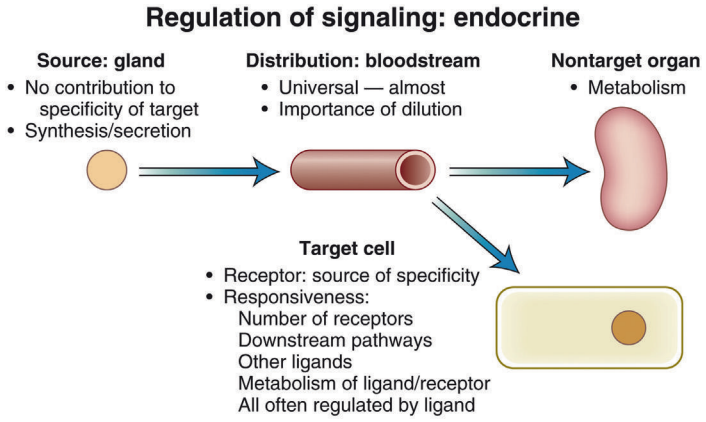


FIGURE 1.3 Types of chemical communication. (The fourth type needs to be illustrated.) Source: *From Figure 3.3 in Koeppen B. M., & Stanton B. W. (2018).* Berne and Levy's physiology *(7th ed.). Elsevier, p. 38.*

Paracrines and hormones are the most common form of intercellular communication, and while there are similarities, there are also differences as seen in Fig. 1.4.

Endocrine substances from specific glands are traditionally called hormones because they travel in the blood for at least part of their journey. Most tissues in the body produce endocrine substances and these substances are, by virtue of their traveling in the blood, also called hormones. This text will primarily be concerned with hormones of clinical importance that are produced by specialized glands. However, it will also discuss those produced by the brain, heart, and gastrointestinal tract.

Hormones are either modified proteins or steroid structures derived from cholesterol. Those that are protein usually interact with cell surface receptors and do not penetrate the cell (an exception is thyroid hormone) while those that are steroid enter the cell and combine with receptors in the nucleus as is illustrated by the effect of progesterone (a steroid), insulin-like growth factor-1, and luteinizing hormone (a protein) (Fig. 1.5).



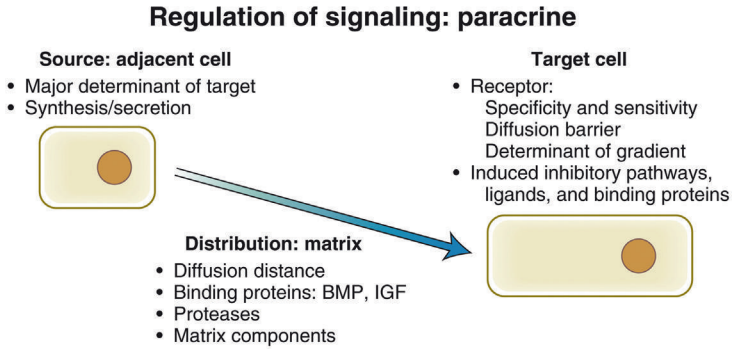


FIGURE 1.4 Hormone and paracrine signaling. Source: *From Figure 1.1, Melmed, S., Polonsky, K. S., Larson, P. R., & Kronenberg, H. M. (2016).* William's textbook of endocrinology *(13th ed.). Elsevier, p. 2.*

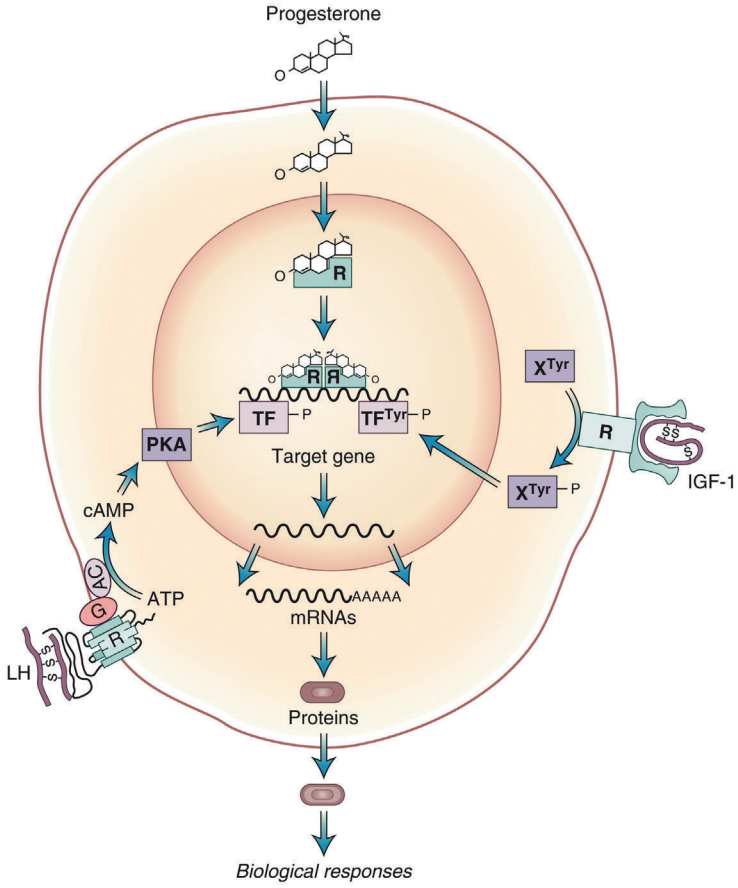


FIGURE 1.5 Showing proteins interacting with surface receptors and steroids interacting with receptors in nucleus. *AC*, Adenylate cyclase; *G*, heterotrimeric G-protein; *mRNAs*, messenger RNAs; *PKA*, protein kinase A; *R*, receptor molecule; *TF*, transcription factor; *Tyr*, tyrosine found in protein X; *X*, unknown protein substrate. Source: *From Figure 1.2, Melmed, S., Polonsky, K. S., Larson, P. R., & Kronenberg, H. M. (2016).* William's textbook of endocrinology *(13th ed.). Elsevier, p. 6.*

At the molecular level (boxed material for additional information)

Biosynthesis of hormones

Many small molecules, including nitric oxide and derivatives of amino acids and fatty acids, function as neurotransmitters or paracrine signals, but usually are not considered to be hormones, and so are discussed only when pertinent to the actions of hormones. Hormone synthesis and storage, particularly for the amino acid and steroid hormones, are presented later. A brief review of these steps also provides an opportunity for a general consideration of gene expression and protein synthesis and provides some background for understanding hormone actions.

Protein and peptide hormones are encoded in genes on the DNA (deoxyribonucleic acid) (Figs. 1.6–1.10) with each hormone usually represented only once in the genome. Information determining the amino acid

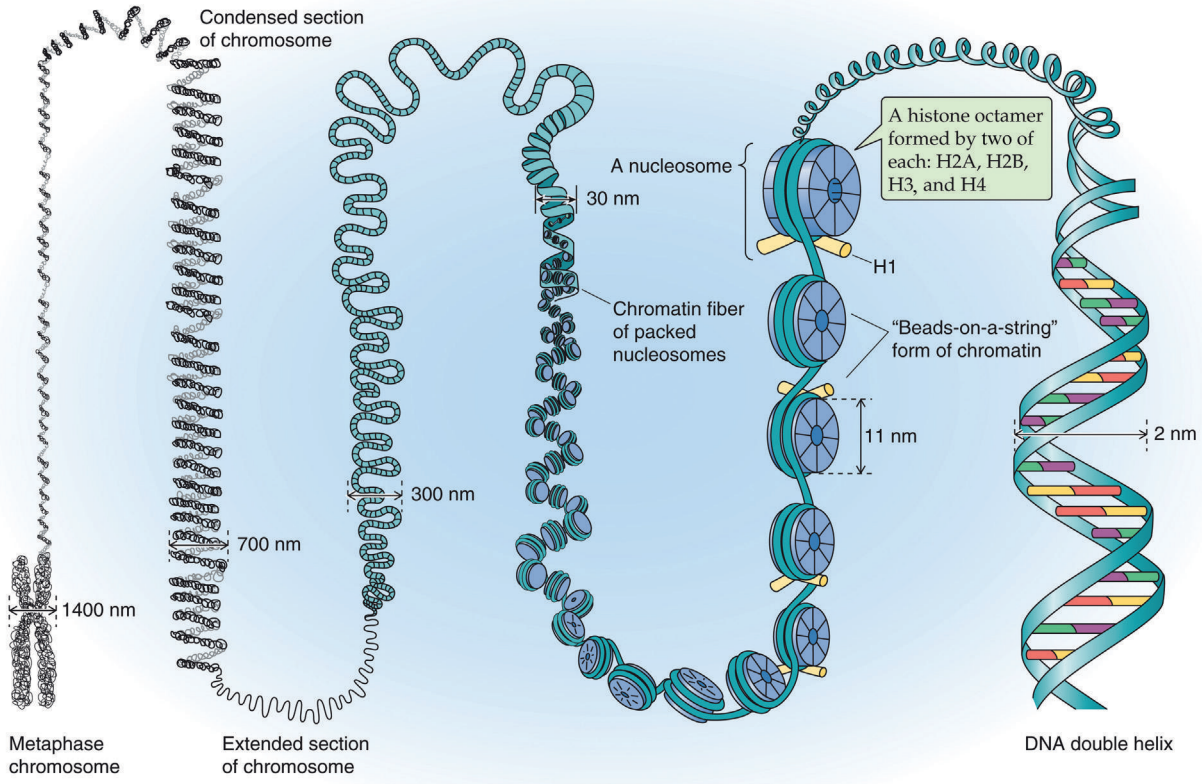


FIGURE 1.6 The organization of nuclear DNA. At the light microscopic level, the nuclear genetic material is organized into dispersed, transcriptionally active euchromatin or densely packed, transcriptionally inactive heterochromatin. Chromatin can also be mechanically connected with the nuclear membrane, and nuclear membrane perturbation can, thus, influence transcription. Chromosomes (as shown) can only be visualized by light microscopy during cell division. During mitosis, they are organized into paired chromatids connected at centromeres; the centromeres act as the locus for the formation of a kinetochore protein complex that regulates chromosome segregation at metaphase. The telomeres are repetitive nucleotide sequences that cap the termini of chromatids and permit repeated chromosomal replication without the loss of DNA at the chromosome ends. The chromatids are organized into short "P" (petite) and long "Q" (next letter in the alphabet) arms. The characteristic banding pattern of chromatids has been attributed to relative GC content (less GC content in bands relative to interbands), with genes tending to localize to interband regions. Individual chromatin fibers comprise a string of nucleosomes—DNA wound around octameric histone cores—with the nucleosomes connected via DNA linkers. Promoters are noncoding regions of DNA that initiate gene transcription; they are on the same strand and upstream of their associated gene. Enhancers are regulatory elements that can modulate gene expression over distances of 100kB or more by looping back onto promoters and recruiting additional factors that are needed to drive the expression of pre-mRNA species. The intronic sequences are subsequently spliced out of the pre-mRNA to produce the definitive message that is translated into protein—without the 3′- and 5′-UTR. In addition to the enhancer, promoter, and UTR sequences, noncoding elements are found throughout the genome; these include short repeats, regulatory factor binding regions, noncoding regulatory RNAs, and transposons. *UTR*, Untranslated regions. Source*: From Figure 4-3 in Kumar V., Abbas A.K., & Aster J.C. (2015).* Robbins and Cotran pathologic basis of disease *(9th ed.). Elsevier, p. 2.*

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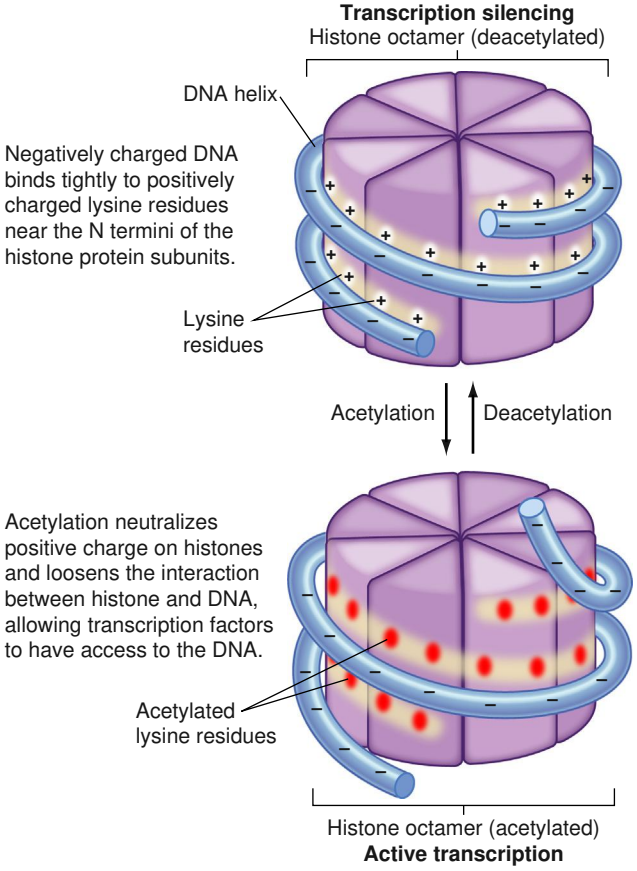

FIGURE 1.7 The ability to transcribe a region of DNA depends on whether the histone is acetylated or not. The reverse can also take place: a gene may be silenced if the histone is not acetylated. *DNA*, Deoxyribonucleic acid. Source*: Redrawn from Figure 4-17 in Boron, W. F., & Boulpaep, E. L. (2017).* Medical physiology *(3rd ed.). Elsevier, p. 95.*

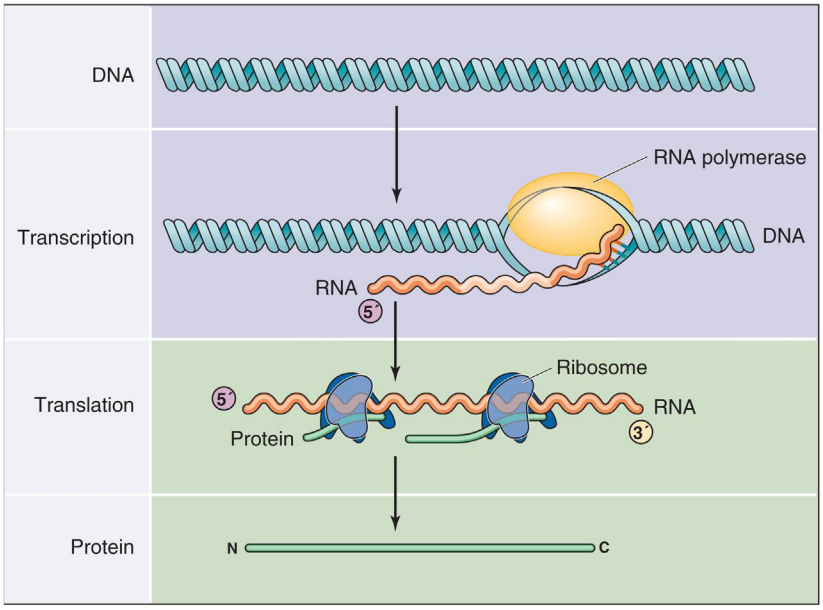

FIGURE 1.8 Summary of steps from DNA to protein. *DNA*, Deoxyribonucleic acid. Source*: From Figure 4-1 in Boron, W. F., & Boulpaep, E. L. (2017).* Medical physiology *(3rd ed.). Elsevier, p. 74.*

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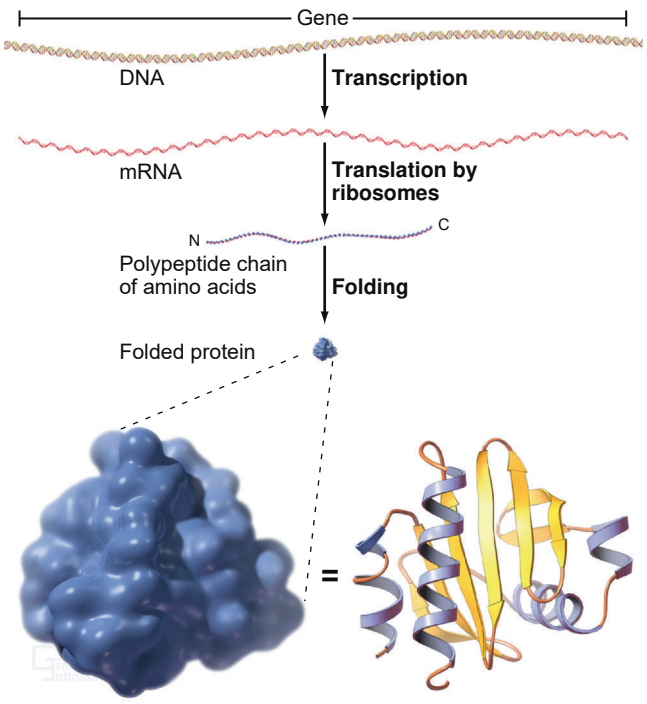

FIGURE 1.9 The steps in the synthesis of a protein. Source: *From Figure 1-4 in Pollard et al. (2017). Cell Biology (3rd ed.), Elsevier, p. 5.*

sequence of proteins is encoded in the nucleotide sequence of DNA (Fig. 1.11).

Nucleotides in DNA consist of a five-carbon sugar, deoxyribose, in ester linkage with a phosphate group, and attached in *N*-glycosidic linkage to one of four organic bases: adenine (A), guanine (G), thymidine (T), or cytidine (C). The ability of the purine bases (A) and (G) to form complementary pairs with the pyrimidine bases (T) and (C), respectively, on an adjacent strand of DNA is the fundamental property that permits accurate replication of DNA and transmission of stored information.

A single strand of DNA is a chain of millions of nucleotides linked by phosphate groups that form ester bonds with hydroxyl groups at carbon 3 of one deoxyribose and carbon 5 of the next deoxyribose. The DNA in each chromosome is present as a pair of long strands oriented in opposite directions and is organized into nucleosomes, each of which consists of a stretch of about 180 nucleotides tightly wound around a complex of eight histone molecules. The nucleosomes are linked by stretches of about 30 nucleotides, and the whole double strand of nucleoproteins is tightly coiled in a higher order of organization to form the chromosomes (Fig. 1.6).

The coiled DNA can be silenced or activated depending on whether the histone is acetylated (see Fig. 1.7).

Instructions for protein structure are transmitted from the DNA to ribosomes, the cytoplasmic sites of protein synthesis by using a messenger ribonucleic acid (mRNA) template, are summarized in Fig. 1.8.

All RNAs differ in structure from DNA only in having ribose instead of deoxyribose as its sugar and uridine (U) instead of thymidine as one of its pyrimidine bases. The nucleotide sequence of the mRNA precursor is complementary to the nucleotide sequence of DNA. mRNA synthesis proceeds linearly from an upstream "start site" designated by a particular sequence of nucleotides in DNA in a process called transcription. The start site is located downstream from the promoter region, which contains sequences to which regulatory proteins can bind, and a short sequence where RNA polymerase II and a large aggregate of proteins, the general transcription complex, binds. The DNA that is transcribed comprises segments that encode structural and regulatory information called exons separated by intervening sequences of

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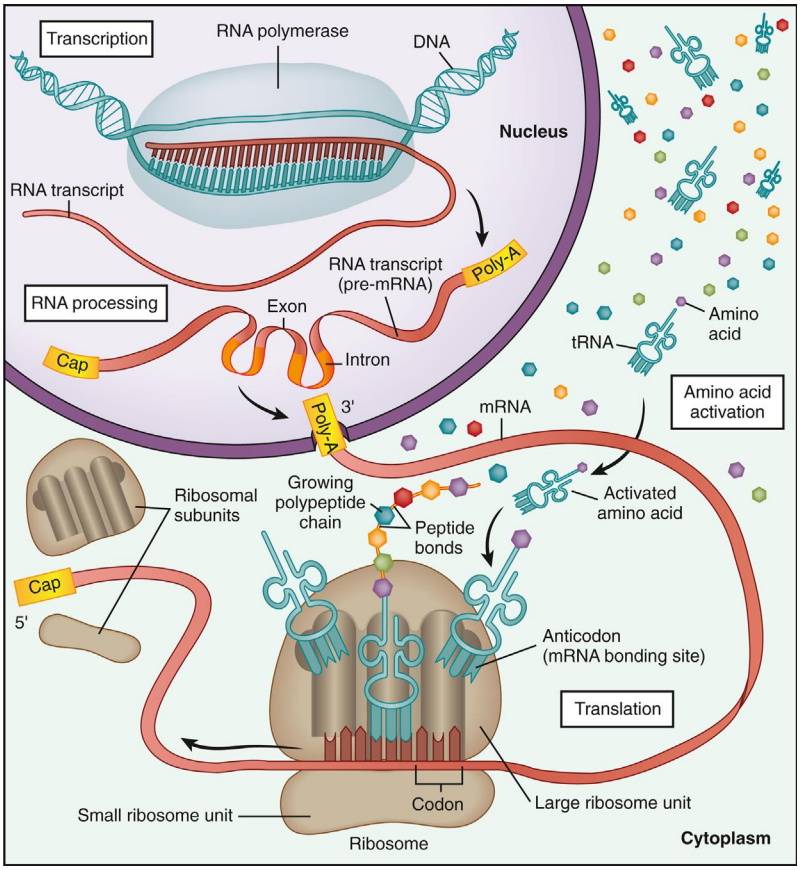

FIGURE 1.10 Summary of transcription. Source*: From Figure 4-9 in McCance and Huether, Mosby (2019). Pathophysiology (8th ed.), p. 141.*

DNA with no coding function, called introns (Fig. 1.9) and the process is summarized in Fig. 1.10.

Transcription is regulated by nuclear proteins called transcription factors or transactivating factors, which bind to regulatory sites that are usually upstream from the promoter and stimulate or repress gene transcription. These proteins form complexes with multiple other transcription factors and proteins called coactivators or corepressors, which not only govern attachment and activity of the general transcription complex but also control the "tightness" of the DNA coil and hence the accessibility of genes to the transcription apparatus. Transcription proceeds from the start site through the introns and exons and a downstream flanking sequence where a long polyadenine tail is added. A special "cap" structure containing methylated guanosine added to the opposite end of the RNA transcript permits its export from the nucleus after it is modified further by removal of the introns and attachment of the exons to each other in a process called splicing. Under some circumstances the splicing reactions may bypass some exons or parts of exons, which are then omitted from the final mRNA transcript. Because of such alternate splicing, a single gene can give rise to more than one mRNA transcript, and hence more than one protein product (Figs. 1.11 and 1.12).

Multiple mRNA transcripts may also be produced from some genes that have more than one site at which transcription can start.

Upon export from the nucleus, the mRNA transcripts attach to ribosomes where they are translated into protein. Ribosomes are large complexes of RNA and enzymes that "read" the mRNA code in triplets of nucleotides called

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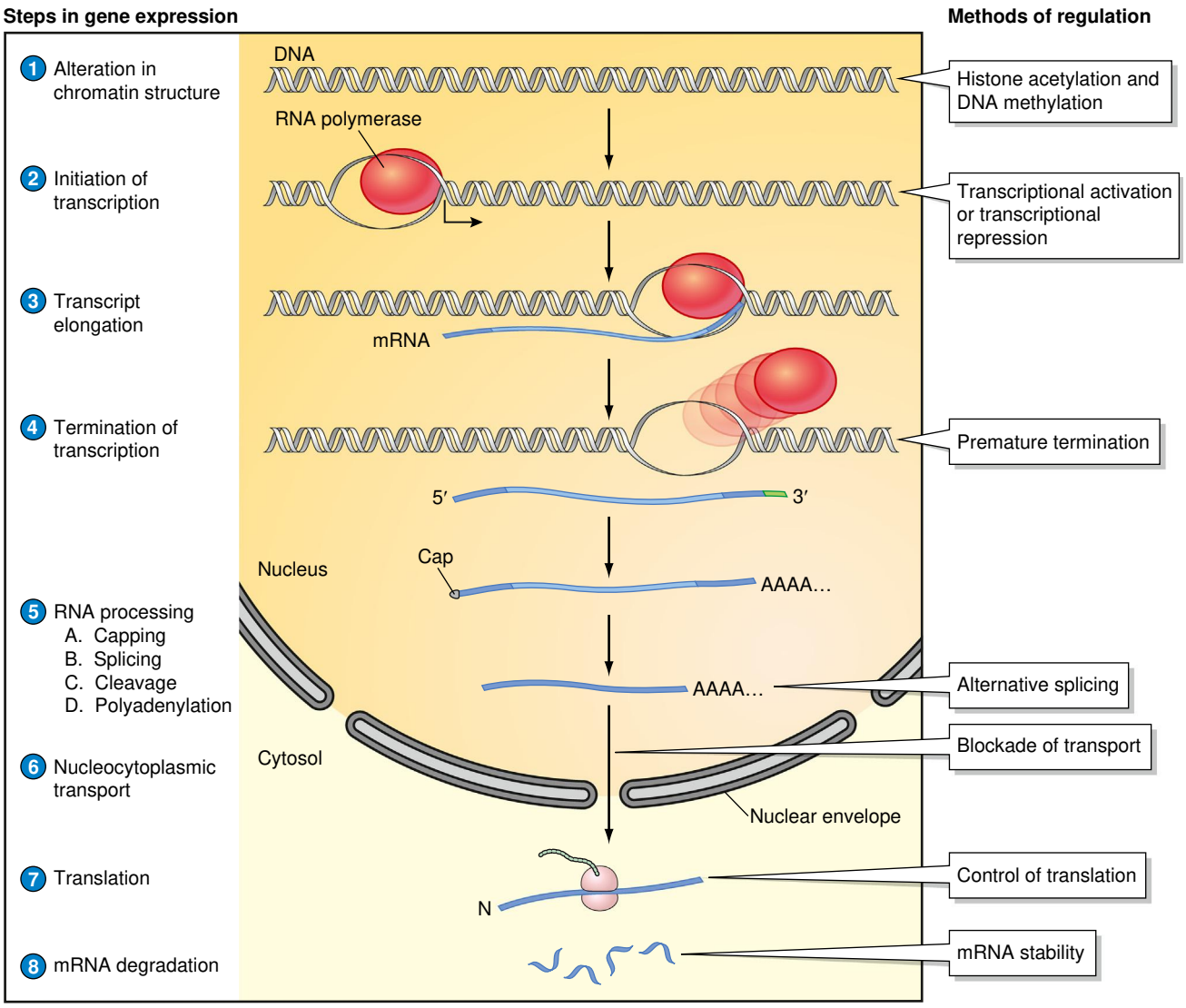

FIGURE 1.11 Steps in gene expression. Each of these steps can be altered leading to an endocrine imbalance or disorder. Source: *Redrawn from Figure 4-4 in Boron, W. F., & Boulpaep, E. L. (2017).* Medical physiology *(3rd ed.). Elsevier, p. 77.*

codons. The translation initiation site begins with the codon for methionine. Each codon designates a specific amino acid. Triplets of complementary nucleotides (anticodons) are found in small RNA molecules called transfer RNA (tRNA) each of which binds a particular amino acid and delivers it to the ribosome. Alignment of amino acids in the proper sequence is achieved by the complementary pairing of anticodons in the tRNA with codons in the mRNA (Fig. 1.12).

The tRNA thus delivers the correct amino acid to the carboxyl terminus of the growing peptide chain and holds it in position so that ribosomal enzymes can release it from the tRNA and link it to the peptide. Once the peptide bond is formed, the empty tRNA is released and the ribosome moves down the mRNA to the next codon where the next tRNA molecule charged with its amino acid waits to bind to its complementary codon. Elongation of the chain continues until the ribosome reaches a "stop" codon at which time it dissociates from the mRNA. As each ribosome moves down the mRNA, other ribosomes attach behind it to repeat the process. In this way a single mRNA molecule may be translated many times before it is degraded.

(*cont'd*)

Protein and peptide hormones are synthesized as larger molecules (prohormones and preprohormones) than the final secretory product. Proteins destined for secretion have a hydrophobic sequence of 12–30 amino acids at their amino terminals. This signal sequence is recognized by a special structure that directs the growing peptide chain through a protein channel in the endoplasmic reticular membrane (see Fig. 1.13) and into the cisternae of the endoplasmic reticulum.

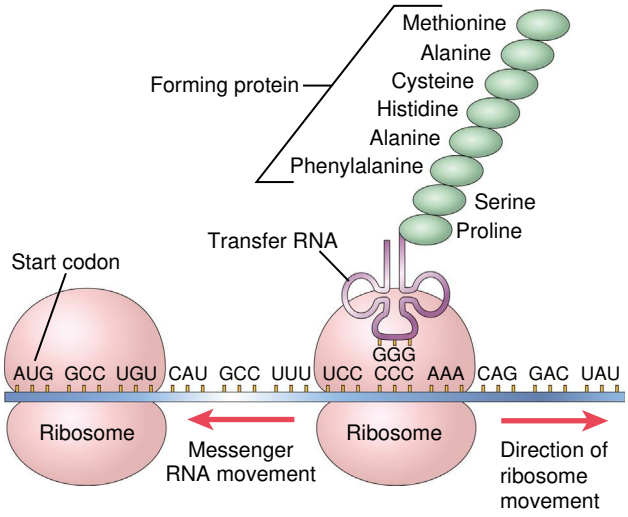

FIGURE 1.12 Translation. AUG codes for methionine, which always starts the translation of any protein. Source: *Modified from Figure 3.9 in J. E. Hall. (2016).* Guyton and Hall's textbook of medical physiology *(13th ed.). Elsevier, p. 32.*

Postsynthetic processing begins in the endoplasmic reticulum and continues as hormone precursors are translocated to the Golgi apparatus for final processing and packagingfor export. Processing begins even as the peptide chain is still elongating and includes cleavage to remove the signal peptide (Fig. 1.13). Interactions with intrinsic endoplasmic reticulum proteins facilitate proper folding and catalyze the formation of disulfide bonds linking cysteine residues. Other processing of peptide hormones may include glycosylation (addition of carbohydrate chains to asparagine residues) or coupling of subunits that are products of different genes, as seen with the pituitary glycoproteins (see Chapter 2: Pituitary Gland). Glycosylation begins in the endoplasmic reticulum and is completed in the Golgi complex, but final processing of peptide chains takes place in the secretory granules (Fig. 1.14).

The hormones, along with trypsin-like peptidases called hormone convertases, carboxypeptidase, amidating enzymes, and other peptide processing enzymes, are packaged into immature secretory vesicles that bud off from the *trans* face of the Golgi stacks (see Figs. 1.15 and 1.16).

Other proteins that are incorporated into secretory vesicles include one or more proteins of the family of

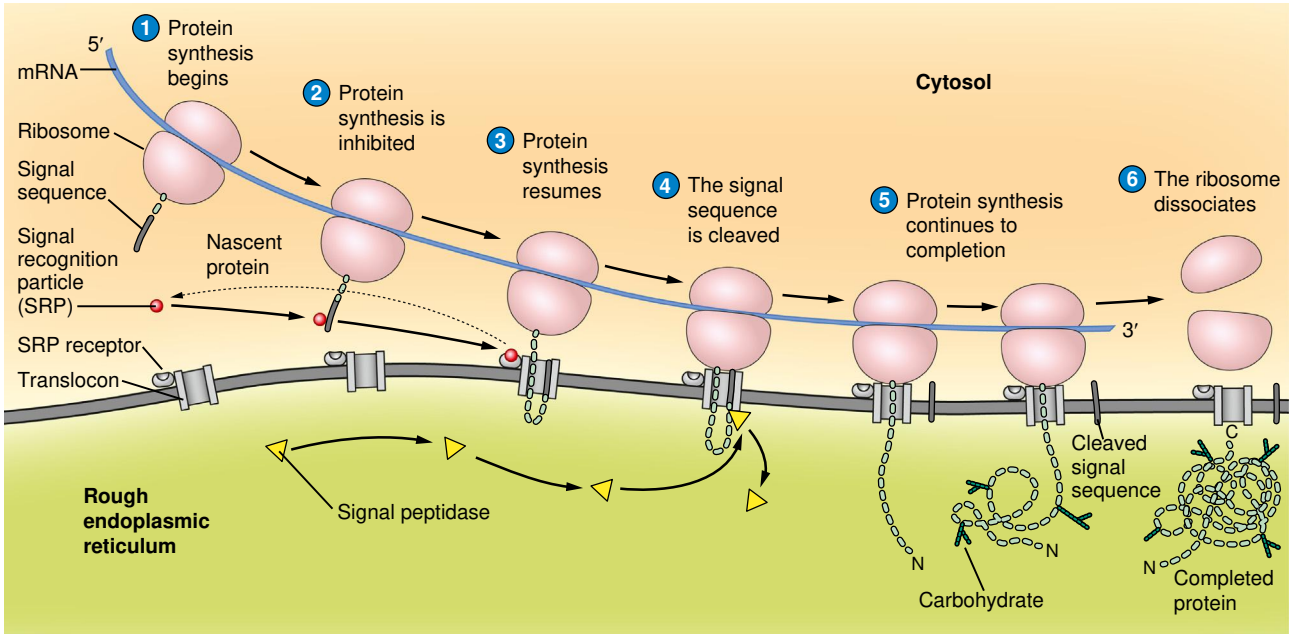

FIGURE 1.13 Synthesis and translocation into the rough endoplasmic reticulum cisterna of a protein hormone. Source: *Redrawn from Figure 2-15 in Boron, W. F., & Boulpaep, E. L. (2017).* Medical physiology *(3rd ed.). Elsevier, p. 29.*

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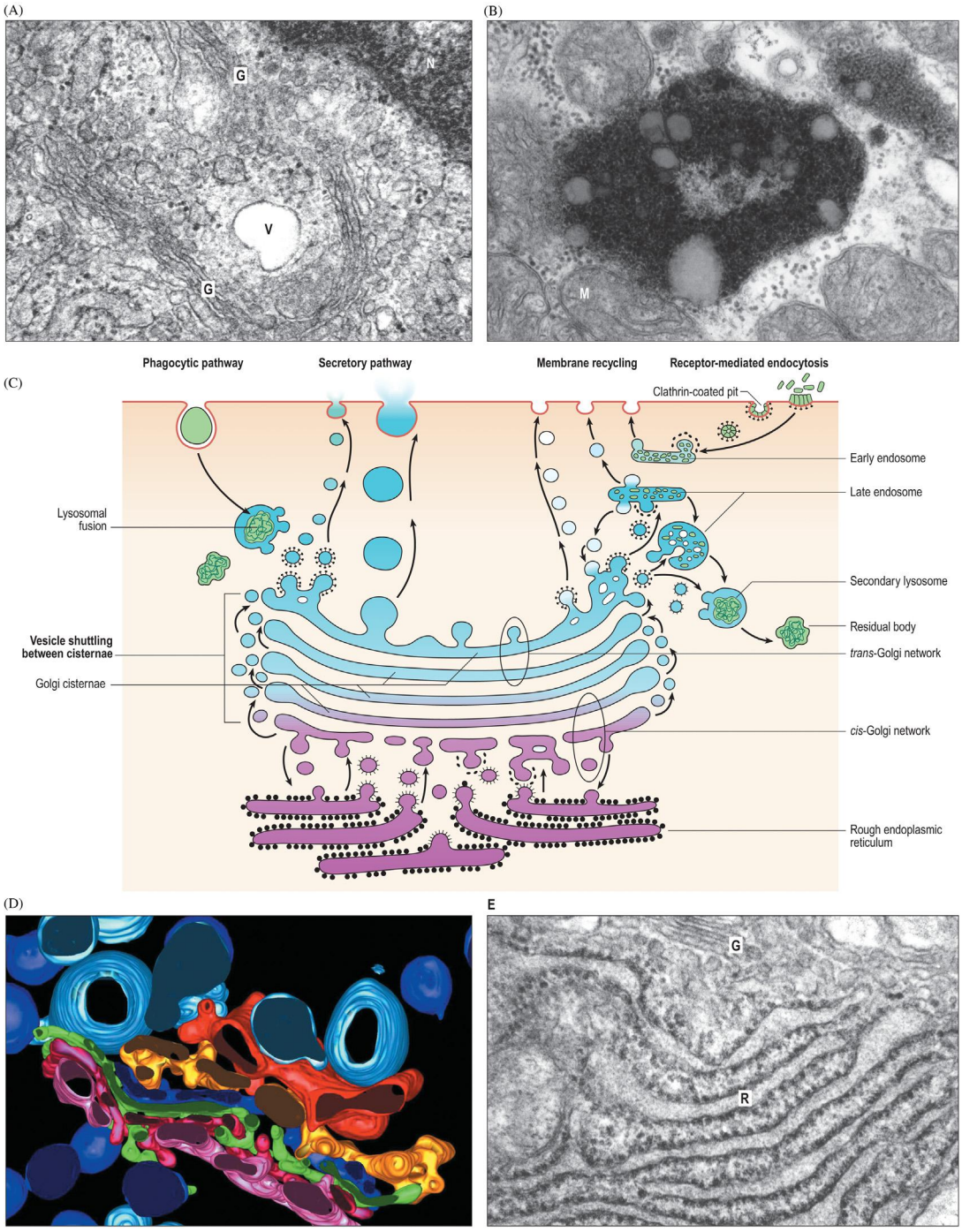

FIGURE 1.14 Posttranslational processing. Many different proteins go through the Golgi apparatus. The *cis* and *trans* Golgi networks refers to the beginning and end of the Golgi, respectively. As a protein moves through the Golgi, it is modified further. Source: *From Figure 1.5 S. Standring. (2016).* Gray's anatomy *(41st ed.). Elsevier, p. 6.*

secretogranins. These large acidic proteins contribute to the sorting of hormone into the immature vesicles, facilitate cleavage reactions at appropriate sites in the prohormone, and organize condensation of the hormone and associated proteins into dense granules. Proton pumps in the vesicle membrane acidify vesicular fluid, which

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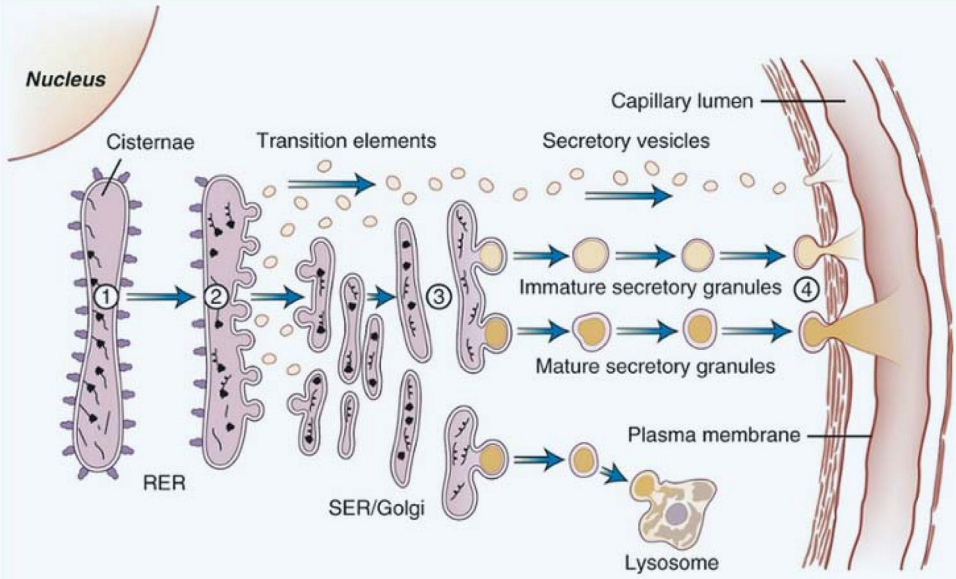

FIGURE 1.15 Steps in the posttranslational production of protein hormones. *RER*, Rough endoplasmic reticulum; *SER/Golgi*, smooth endoplasmic reticulum/Golgi apparatus. Source*: From Figure 3-1 in Melmed, S., Polonsky, K. S., Larson, P. R., & Kronenberg, H. M. (2016).* William's textbook of endocrinology *(13th ed.). Elsevier, p. 20.*

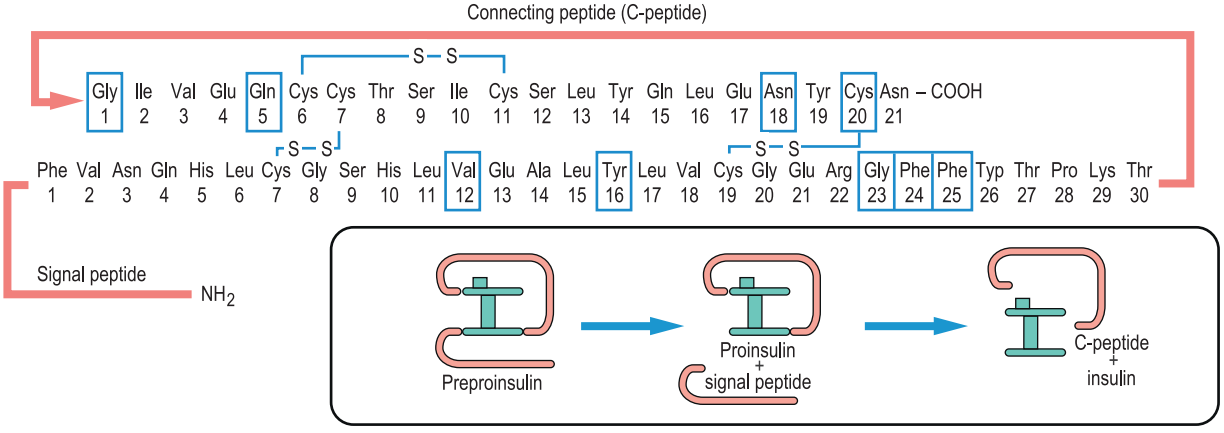

FIGURE 1.16 Showing posttranslational modification of preproinsulin. Boxes around amino acids indicate those involved in binding to the insulin receptor. Source*: From Figure 21.2. J. W. Baynes, & M. H. Dominiczak. (2019).* Medical biochemistry *(5th ed.). Elsevier, p. 445*

activates convertases and promotes extrusion of water. Cleavage of the prohormones removes those amino acid sequences that may have functioned to target the peptides to the secretory granules or to orient folding of the molecule so that disulfide bridges form in the right places. Cleavage of the prohormones by hormone convertases may yield more than one biologically active peptide from a single precursor, as seen with the adrenal corticotropic hormone and glucagon families of hormones (see Chapter 2: Pituitary Gland,

(*cont'd*)

and Chapter 6: Hormones of the Gastrointestinal Tract). Amidation of the carboxyl terminus using glycine as the amino donor is a common feature of the final maturation of many hormones. Because these processing reactions take place in secretory granules, peptide fragments, enzymes and fragments of the secretogranins, and other molecules are secreted along with the hormone. Incompletely processed or intact prohormones also escape into the circulation, sometimes in large amounts. This situation may be indicative of hyperactivity of endocrine cells or even aberrant production of hormone by nonendocrine tumor cells. Some prohormones have biological activity, and their effects may be the first manifestation of neoplasia. Some rare inherited diseases are attributable to defects in processing of normal precursor molecules. Fig. 1.16 shows the posttranslational modification of insulin.

Postsynthetic processing to the final biologically active form is not limited to the peptide hormones. Other hormones may be formed from their precursors after secretion. Postsecretory transformations to more active forms may occur in liver, kidney, fat, or blood, as well as in the target tissues themselves. For example, thyroxine, the major secretory product of the thyroid gland, is converted extrathyroidally to triiodothyronine, which is the biologically active form of the hormone (see Chapter 3: Thyroid Gland). Testosterone, the male hormone, is converted to the more active dihydrotestosterone within some target tissues and may even be converted to the female hormone, estrogen, in other tissues (see Chapter 12: Hormonal Control of Reproduction in the Male). These peripheral transformations, besides confounding the student of endocrinology, are additional sites that are vulnerable to derangement and hence must be considered as possible causes of endocrine disease.

Storage and release of hormones

Protein-derived hormones are stored in large amounts in their gland of origin so they can be released in large quantities in emergencies. While slower to have an effect than a purely nervous system response, their effect is more sustained so will continue after the immediate emergency has passed. This provides insurance against resurgence of the emergency need. Some of these protein hormone-secreting glands are neuron-derived and so the hormones are often thought of as modified neurotransmitters. Oxytocin, vasopressin, epinephrine, and norepinephrine are examples. Norepinephrine is a common neurotransmitter in the nervous system. But as a product of the adrenal medulla, it is released into the extracellular space and diffuses from there into the capillaries and so is considered a hormone. Epinephrine, too, can be found as a neurotransmitter in some parts of the brain but is considered a hormone when released from the adrenal medulla.

Some protein-derived hormones, such as thyroxin and triiodothyronine, collectively called thyroid hormone, are transported in the blood bound to proteins in the albumin fraction. Others, such as epinephrine and norepinephrine, are water-soluble and are not bound to a carrier protein.

Steroid hormones are derived from cholesterol that is either taken up from the blood or synthesized de novo within the gland or tissue. Once formed, the steroid hormones are released instead of being stored. Being insoluble in water, steroid hormones are transported in blood proteins. Hormones of the adrenal cortex, ovary, and testes fall into this category.

An exception to single endocrine gland synthesis is calcitriol, also known as vitamin D hormone, which is synthesized in a series of steps involving multiple organs (Fig. 1.17).

The first step is in the skin in the presence of a band of the ultraviolet spectrum of sunlight, in which cholecalciferol is produced from 7-dehydrocholesterol. This is transported in the blood to the liver where a hydroxy (−OH) group is added to form 25-hydroxycholecalciferol. This travels in the blood to the kidneys where, in the presence of parathormone from the parathyroid glands, a second hydroxy group is enzymatically added to form 1,25-dihydroxycholecalciferol, better known as calcitriol or vitamin D hormone. This acts on intestinal epithelial cells to take up calcium and on osteocytes to cause the reabsorption of noncrystallized (labile) bone, resulting in calcium being released into the blood. If parathormone is not present in the kidney, then 24,25-dihydroxycholecalciferol is formed. Just placing the −OH group at the 24 instead of the 1 position makes the steroid virtually inactive.

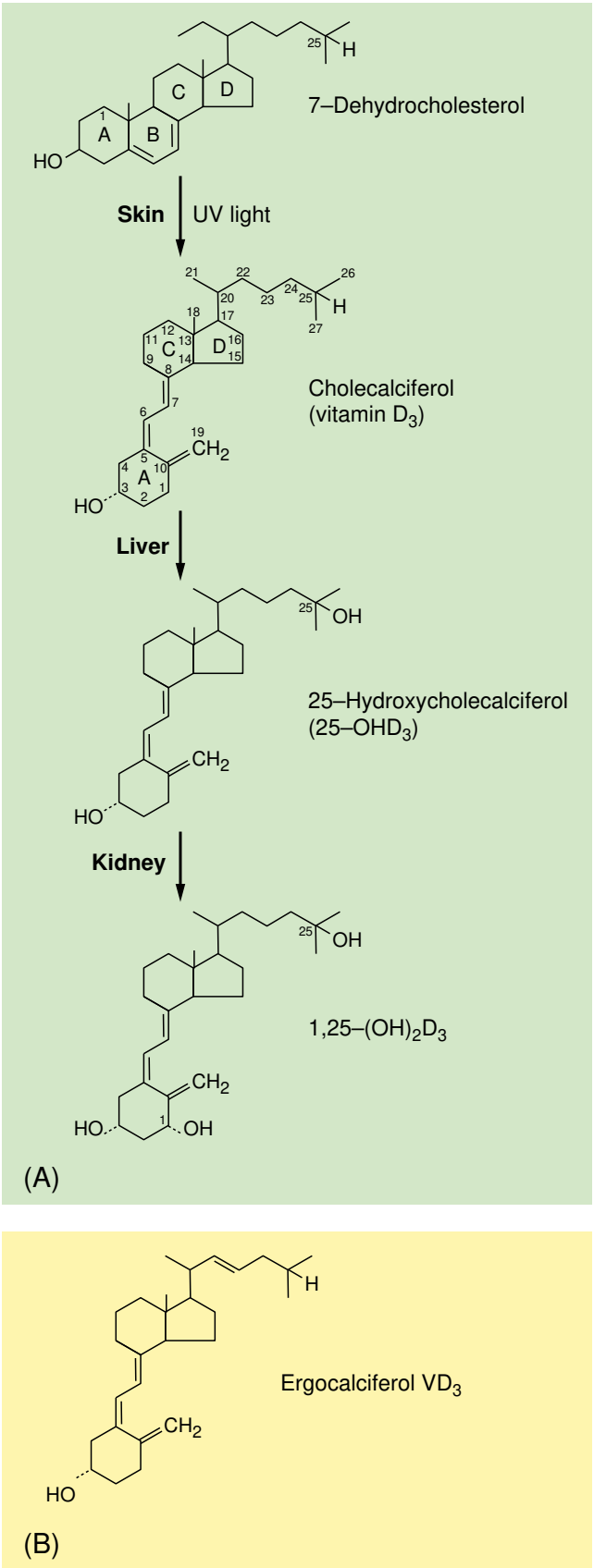

FIGURE 1.17 The stepwise synthesis of calcitriol (vitamin D hormone) from 7-dehyrocholesterol. Source: *Redrawn from Figure 52-9 in Boron, W. F., & Boulpaep, E. L. (2017).* Medical physiology *(3rd ed.). Elsevier, p. 1064.*

At the molecular level (boxed material for additional information)

Release of hormones

Protein and peptide hormones and the tyrosine derivatives, epinephrine and norepinephrine, are stored as dense granules in membrane-bound vesicles and are secreted in response to an external stimulus by the process of exocytosis (Fig. 1.18). Exocytosis can be considered to occur in several stages: (1) recruitment of vesicles to the plasma membrane, (2) tethering or docking to

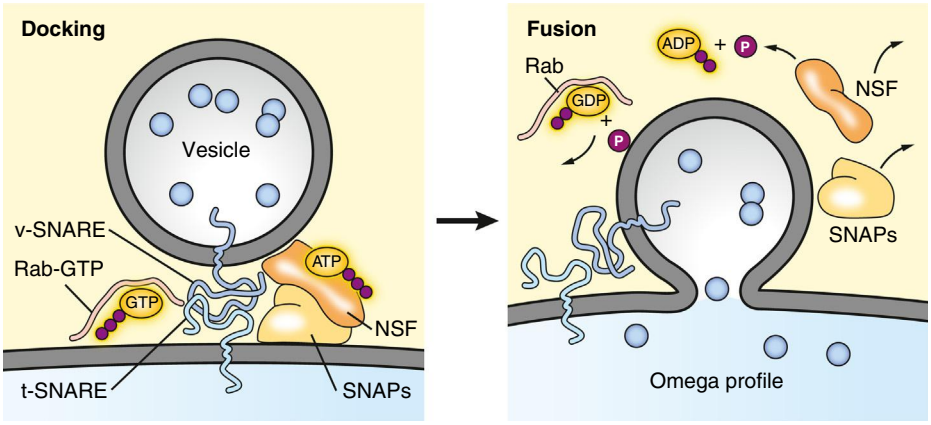

FIGURE 1.18 Exocytosis. Source: *From Figure 2-19 in Boron, W. F., & Boulpaep, E. L. (2017). Medical physiology (3rd ed.). Elsevier, p. 36.*

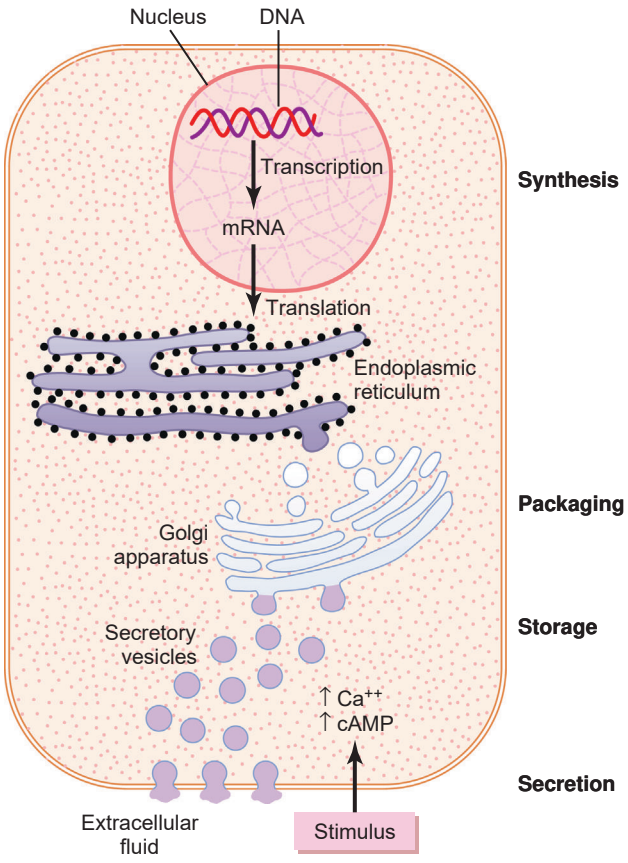

FIGURE 1.19 Exocytosis in detail. Source: *From Figure 75-2 in John E. Hall, Saunders (2016). Guyton and Hall Textbook of Medical Physiology (13th ed.), p. 926.*

(*cont'd*)

appropriate membrane loci, (3) priming in preparation for (4) fusion of the vesicular membrane with the plasma membrane to form a secretory pore that dilates to allow the vesicular contents to escape into the extracellular fluid, and (5) retrieval of the vesicular membrane by endocytosis to membrane by endocytosis to prevent an unsustainable increase in membrane surface that would otherwise occur.

Intrinsic proteins in the membranes of the vesicles and the plasma membranes called SNAREs (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) govern all stages. The human genome encodes 36 SNARE proteins, which are found in all secretory cells and neurons. SNARE proteins in vesicular membranes help to target vesicles to appropriate loci in the plasma membrane. Most secretory vesicles reside deep in the cytosol and are recruited to the plasma membrane by calcium-dependent translocation along microtubules and microfilaments. A subset of secretory vesicles, called the readily releasable pool, is docked just below the plasma membranes tethered to the submembranous cytoskeleton by proteins (see Fig. 1.19). Vesicles are primed for secretion by an energy-dependent mechanism in which SNAREs in the vesicular membrane form loose attachments to partnering SNAREs in the cell membrane. A surge in intracellular free calcium, produced by the activation of calcium channels, triggers a conformational change in the SNARE complex that pulls the vesicular and plasma membranes into such close apposition that fusion occurs. As the fusion pore enlarges, the vesicle ultimately everts and unloads its cargo into the extracellular space.

Hormone transportation and fate

Most hormones circulate in blood in free solution at low, nanomolar (10^{-9} M) or even picomolar (10^{-12} M), concentrations. Steroid and thyroid hormones have limited solubility in water and so are bound to carrier proteins synthesized by the liver. Some protein and peptide hormones also circulate complexed with specific binding proteins (see Chapter 11: Hormonal Control of Growth, and Chapter 14: Hormonal Control of Pregnancy and Lactation). Bound hormones are in equilibrium with a small fraction, sometimes less than 1%, of free hormone in plasma. Only free hormones cross the capillary endothelium to reach their sites of action. Protein binding prevents renal loss of hormone by filtration and slows the rate of hormone biotransformation by decreasing cellular uptake. Bound proteins may affect hormonal responses by facilitating or impeding delivery of hormones to cells. Biological responses are related to the concentration of hormone reaching target cells, rather than the total amount present in blood. Increases in blood binding proteins during pregnancy or decreases due to liver or kidney disease can produce changes in total amounts of hormones circulating in blood even though free concentrations may be normal.

Most hormones are destroyed rapidly after secretion and have a half-life in blood of less than 10 minutes. The half-life is the time needed for its concentration to be reduced by half and depends on its rate of biotransformation (metabolism) and on the rapidity with which it can equilibrate with fluids in extravascular compartments. Epinephrine has a half-life of seconds, whereas thyroid hormone has a half-life of days. The half-life of a hormone is different from its duration of action. Some hormonal effects are produced instantaneously and may disappear as rapidly. Other hormonal effects occur after a lag time of minutes or hours, and the time of maximum response may have no relation to the hormone concentration in the blood.

The time it takes for a hormonal response to decrease may be from a few seconds to several days. Some responses persist after hormonal concentrations have returned to basal levels. Understanding a hormone dose–response curve over time and the onset and duration of action are important for an understanding of normal physiology, endocrine disease, and the limitations of hormone therapy.

In any regulatory system involving hormones or any other signal is the necessity for the signal to disappear once the appropriate information has been conveyed. Only a small amount of hormone is degraded as an aftermath to the process of signaling its biological effects. The remainder must be inactivated and excreted. Inactivation of hormones occurs enzymatically in cells, blood or intercellular spaces in the liver, kidney, and target cells. Degradation of peptide and protein hormones often involves uptake into cells by endocytosis that delivers them to cellular sites of degradation, the lysosomes and proteasomes (see Fig. 1.20). Inactivation may involve complete metabolism of the hormone so that no recognizable product appears in urine, or it

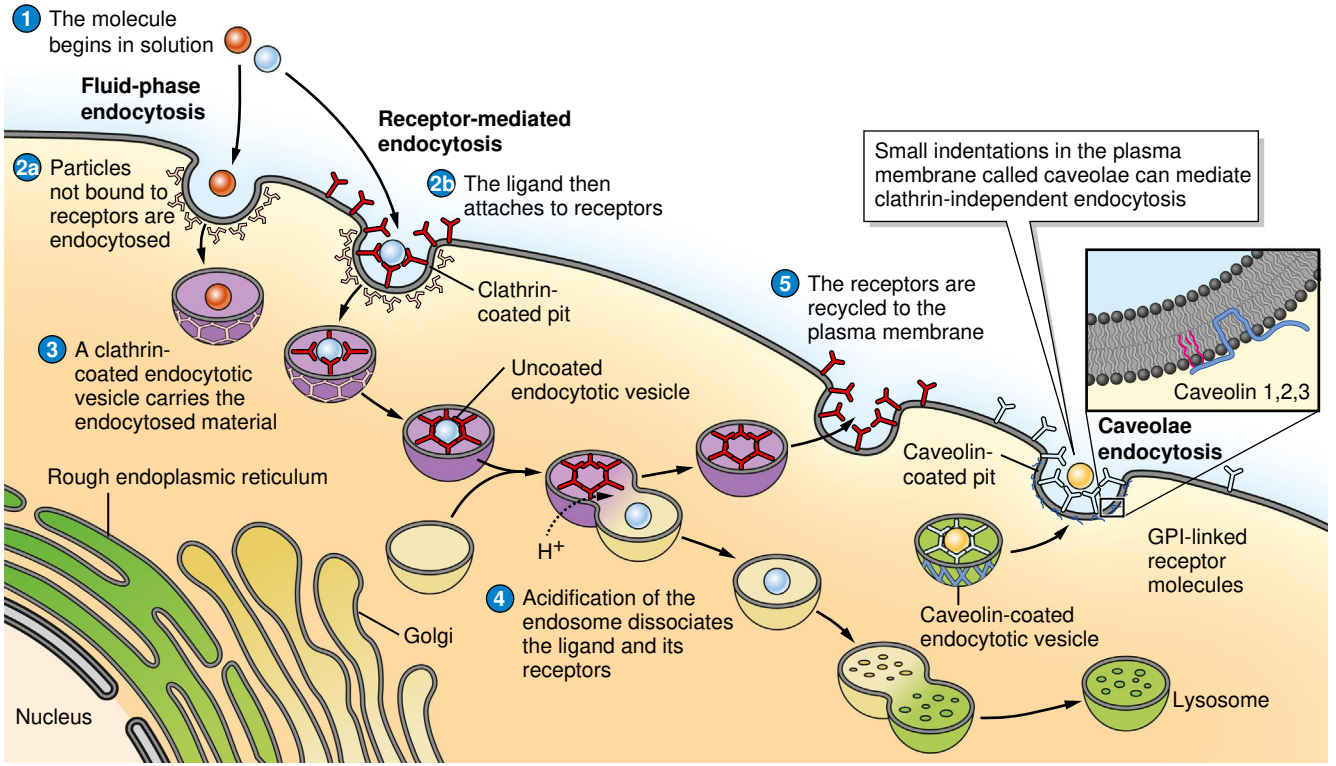

FIGURE 1.20 Endocytosis of hormones. Source: *Redrawn from Figure 2-22 in Boron, W. F., & Boulpaep, E. L. (2017).* Medical physiology *(3rd ed.). Elsevier, p. 41.*

may be limited to some simple one- or two-step process such as addition of a methyl group or glucuronic acid. In the latter cases, recognizable degradation products are found in urine and can be measured to obtain a crude index of the rate of hormone production.

The ultimate mission of a hormone is to change the activity of target cells. Cellular activity is determined by the genes that are expressed to carry out cellular functions, including cellular structure. Hormonal messages must be converted to biochemical events that influence gene expression, biochemical reaction rates, and structural changes. Conversion of a hormonal message to cellular responses is called signal transduction and the series of biochemical changes that are set in motion are described as signaling pathways, although a signaling network might be a more accurate descriptor, as pathways branch and converge only to branch again. Signal transduction is a complex topic and the focus of intense investigation in many laboratories around the world. Detailed consideration is beyond the scope of this text. Instead, only general patterns of signal transduction are considered in the following section, but the topic will be revisited where appropriate in subsequent chapters in discussing individual hormones.

Hormone specificity

Because all hormones travel in blood from their glands of origin to their target tissues, all cells are exposed to all kinds of hormones. Yet under normal circumstances, cells respond only to hormones for which the cell has a receptor. A hormone receptor is a molecule or complex of molecules inside of or on the surface of a cell that binds its hormone resulting in a molecular change, which initiates a characteristic response or group of responses. The mechanisms by which receptors operate and are regulated are not unique to endocrinology.

Hormone receptors

Characteristics of receptors

Hormone receptors are proteins or glycoproteins that are able to

- distinguish a hormone from other molecules that may have very similar structures,
- bind to the hormone (sometimes called a ligand) even when its concentration is exceedingly low (10^{-8}–10^{-12} M),
- undergo a conformational change when bound to the hormone, and
- catalyze biochemical events or transmit changes in molecular conformation to adjacent molecules that produce a biochemical change.

The receptor may be a single molecule or in a subunit of a receptor complex. A hormone activates a receptor by binding to it. Any biochemical change initiated by the activated receptor is due to the properties of the receptor and not of the hormone. Under some pathophysiological conditions an aberrant antibody may react with a receptor and produce a disease state that is indistinguishable from the disease that results from the overproduction of the hormone. Hormone receptors are found on the surface, in the cytosol, or in the nucleus of target cells. Transmembrane receptors span the thickness of the cell membrane, with the hormone-binding molecule on the outer surface. Receptor molecules on the cytosolic face of the membrane communicate with other membrane or cytosolic proteins. *Trans* membrane receptors may be distributed over the entire surface or they may be confined to some microinvaginations called caveolae (Fig. 1.20).

Usually, only a few thousand receptor molecules are present in a target cell. Cells can change the number of receptors, and their responsiveness to hormones as physiological events warrant (see Chapter 5: Principles of Hormone Integration). Some receptors may be expressed only at a certain stage in the life of a cell or as a consequence of stimulation by other hormones. Often cells adjust the number of receptors they express with the amount of signal that activates them. Frequent or intense stimulation may cause a cell to downregulate (decrease) the number of receptors expressed. Conversely, cells may upregulate receptors in the face of rare or absent stimulation or in response to other signals.

Membrane receptors are internalized either alone or bound to their hormones (receptor-mediated endocytosis) and, like other cellular proteins, are broken down and replaced many times over during the lifetime of a cell. Adjustments in the relative rates of receptor synthesis or degradation may result in either up- or downregulation of receptors. Cells can also up- or downregulate receptor function through reversible covalent modifications such as adding or removing phosphate groups. Membrane-associated receptors cycle between the plasma membrane and internal membranes, and their relative abundance on the cell surface can be adjusted by reversibly sequestering them in intracellular vesicles.

Although the mammalian organism expresses literally thousands of different receptor molecules that subserve a wide variety of functions in addition to endocrine signaling, there are relatively few general patterns of signaling. Based upon the nucleotide sequence and organization of their genes and the

structure of their proteins, receptors, like other proteins, can be organized into families or superfamilies that presumably arose from the same ancient progenitor gene. Even for distantly related receptors, the general features of signal transduction follow common broad outlines that are seen with families of molecules that receive and transduce signals in eukaryotic cells of species ranging from yeast to humans.

Hormonal actions mediated by intracellular receptors

The cholesterol derivatives (steroid and vitamin D hormones) are lipid-soluble and enter cells by diffusion through the lipid bilayer of the plasma membrane. Thyroid hormones, which are α-amino acids, have large nonpolar constituents and may penetrate cell membranes by diffusion, but carrier-mediated transport appears to be the primary means of entry (see Fig. 1.21).

These hormones bind to receptors that are located in the cell nucleus or cytoplasm and produce most of their effects by altering rates of gene expression. Receptors bound to steroid hormones, in turn, bind to specific nucleotide sequences in DNA (deoxyribonucleic acid), called hormone response elements (HREs), located upstream of the transcription start sites of the genes they regulate. The end result of stimulation with these hormones is a change in genomic readout, which may be expressed in the formation of new proteins or modification of the rates of synthesis of proteins already in production.

Intracellular hormone receptors belong to a very large family of transcription factors found throughout the animal kingdom. Genes for 48 members of this family have been identified in the human genome. Many of these are called orphan receptors because their ligands have not yet been identified. The most highly conserved region of nuclear receptors is a stretch of about 65–70 amino acid residues that constitutes the DNA-binding domain. This region contains one or two molecules of zinc, each coordinated with four or six cysteine residues, respectively, so that two loops of about 12 amino acids each are formed. These so-called zinc fingers can insert in a half-turn of the DNA helix and grasp the DNA at the site of the HRE (Fig. 1.22).

The hormone-binding domain, which is near the carboxyl terminus, also contains amino acid sequences that are necessary for the activation of transcription. Between the DNA-binding domain and the amino terminus is the so-called hypervariable region, which, as

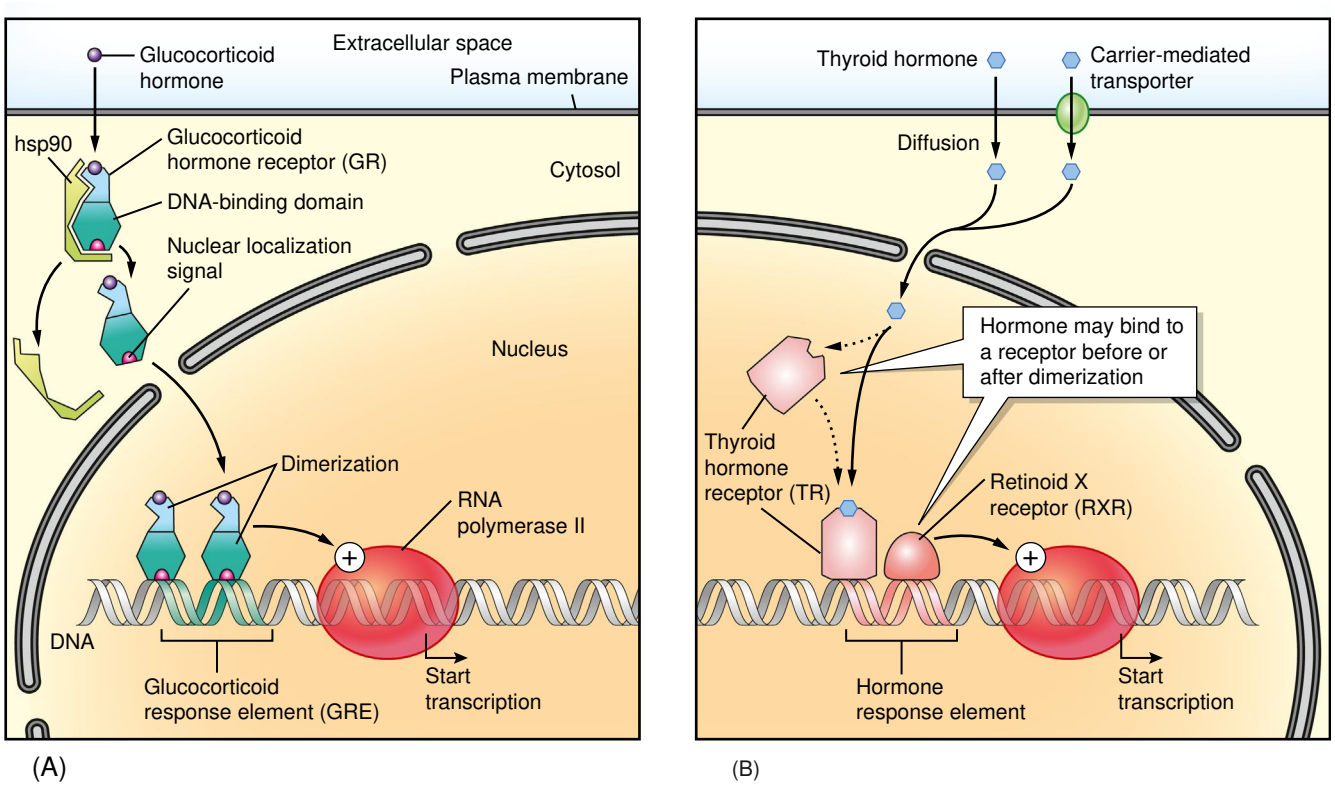

FIGURE 1.21 Transcriptional activation by a steroid and by thyroid hormone. Source: *Redrawn from Figure 4-15 in Boron, W. F., & Boulpaep, E. L. (2017).* Medical physiology *(3rd ed.). Elsevier, p. 92.*

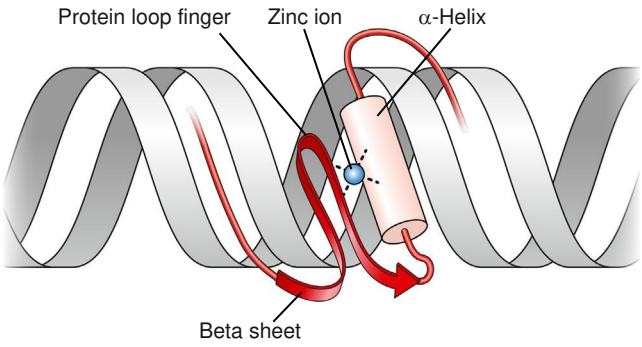

FIGURE 1.22 In this image of a zinc finger in an HRE, one zinc ion coordinates with two residues on the α-helix and two residues on the beta sheet to form the protein loop known as a finger. The finger is composed of 30 amino acids. *HRE*, Hormone response element. Source*: Redrawn from Figure 4-9 in Boron, W. F., & Boulpaep, E. L. (2017).* Medical physiology *(3rd ed.). Elsevier, p. 83.*

its name implies, differs both in size and amino acid sequence for each receptor.

The steroid hormone receptors constitute a closely related group within the family. In the unstimulated state, steroid hormone receptors are noncovalently complexed with other proteins (Fig. 1.23), including a dimer of the 90,000 Da heat shock protein (Hsp 90) that attaches adjacent to the hormone-binding domain (Fig. 1.23).

Heat shock proteins are abundant cellular proteins that are found in prokaryotes and all eukaryotic cells and are so named because their synthesis abruptly increases when cells are exposed to high temperature or other stressful conditions. These proteins are thought to keep the receptor in a configuration that is favorable for binding the hormone and incapable of binding to DNA. Binding to its hormone causes the receptor to dissociate from Hsp 90 and the other proteins. The bound receptor then forms a dimer with another liganded receptor molecule and undergoes a conformational change that increases its affinity for binding to DNA. After binding to the DNA the receptor dimers recruit other nuclear regulatory proteins, including coactivators, that facilitate uncoiling of the DNA to make it accessible to the ribonucleic acid (RNA) polymerase complex. Receptors for at least four different steroid hormones bind to the identical HRE, and yet each governs expression of a unique complement of genes. Expression of genes that are specific for each hormone is determined by which receptor is present in a particular cell, by the cohort of nuclear transcription factors, coactivators, and corepressors that are available to complex with the receptor in that cell, and by the characteristics of the regulatory components in the DNA (Fig. 1.24).

Receptors for thyroid hormone and vitamin D and compounds related to vitamin A (retinoic acid) belong to another closely related group within the same

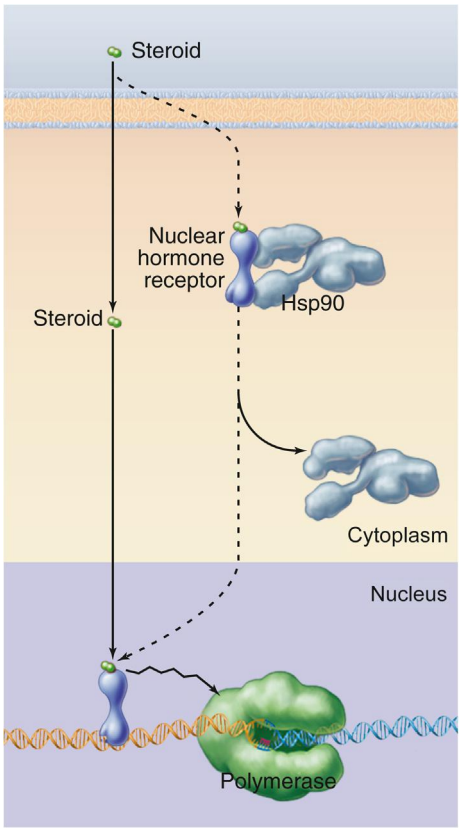

FIGURE 1.23 Steroid hormone receptors. Estrogens bind to Hsp 90, while other steroids go directly to the nucleus. Source*: From Figure 10.21 A in T. D. Pollard, et al. (2017).* Cell biology *(3rd ed.). Elsevier, p. 185.*

family of proteins as the steroid hormone receptors. Unlike the steroid hormone receptors, these receptors are bound to their HREs in DNA even in the absence of hormone and do not form complexes with Hsp 90. In further distinction from the receptors for steroid hormones, receptors for thyroid hormone and vitamin D may bind to DNA either as homodimers or as heterodimers formed with a receptor for 9-*cis* retinoic acid, often called the RXR receptor. In the absence of ligand, these DNA-bound receptors form complexes with other nuclear proteins that may promote or inhibit transcription. Upon binding its hormone, the receptor undergoes a conformational change that displaces the associated proteins and allows others to bind with the result that transcription is either activated or suppressed (Fig. 1.24).

Many steps lie between the activation of transcription and changes in cellular behavior. These include synthesis and processing of RNA, exporting it to cytosolic sites of protein synthesis, protein synthesis itself, protein processing, and delivery of the newly formed proteins to appropriate loci within the cells. These reactions necessarily occur sequentially and each takes

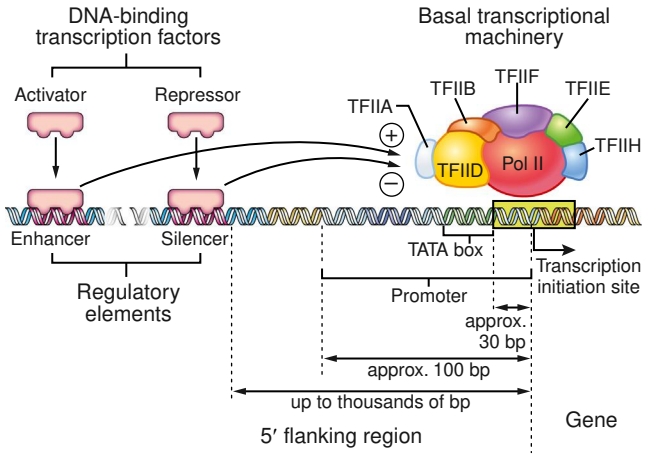

FIGURE 1.24 The basal transcriptional machinery regulates DNA transcription, showing the regulatory elements, the promotor region, and the gene itself. Pol II, RNA polymerase II. There are six general transcription factors, named TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH; transcription factors IIA, IIB, IIE, etc.; TATA, thymine/adenine box that is part of the promotor region. *DNA*, Deoxyribonucleic acid; *RNA*, ribonucleic acid. Source: *Redrawn from Figure 4-6 in Boron, W. F., & Boulpaep, E. L. (2017).* Medical physiology *(3rd ed.). Elsevier, p. 79.*

time. Transcription proceeds at a rate of about 40 nucleotides per second, so that transcribing a gene that contains 10,000 nucleotides takes almost 5 minutes. Processing the pre-RNA to mature messenger RNA (mRNA) is even slower, so that nearly 20 minutes elapse from the time RNA synthesis is initiated to the time the mRNA exits the nucleus. Protein synthesis is much faster. About 15 amino acids per second are added to the growing peptide chain. All factors considered, changes in cellular behavior that result from steroid hormone action usually are not seen for at least 30 minutes after entry of the hormone into the cells. The final protein makeup of the cell at any time thereafter is also determined by rates of RNA and protein degradation. A complete catalog of which proteins are formed in any particular cell type because of hormone action should become available soon thanks to the successful completion of the Human Genome Project and the technology that permits screening of the entire library of mRNA expressed within a cell. Gaining an understanding of the physiological role of each of these proteins will take a bit longer.

As blood levels decline, intracellular concentrations of hormone decline. Because binding is reversible, hormones dissociate from receptors and are cleared from the cell by diffusion into the extracellular fluid, usually after metabolic conversion to an inactive form. Unloaded steroid receptors dissociate from their DNA-binding sites and regulatory proteins and either recycle into new complexes with Hsp 90 and other proteins through some energy-dependent process or are degraded and replaced by new synthesis. RNA transcripts of hormone-sensitive genes are degraded usually within minutes to hours of their formation. Without continued hormonal stimulation of their synthesis, RNA templates for hormone-dependent proteins disappear, and the proteins they encode can no longer be formed. The proteins are degraded with half-lives that may range from seconds to days. Thus, just as there is delay in onset, effects of the hormones that act through nuclear receptors may persist after the hormone has been cleared from the cell.

Accumulating evidence indicates that hormones that once were thought to act only through nuclear receptors produce some rapid effects that are independent of changes in gene expression. For the most part the rapid responses that are produced are complementary to the delayed genomically mediated responses. It is likely that other, yet to be identified, receptors for these hormones are present on the cell surface or that some nuclear receptors are expressed on the cell surface as well as internally.

Hormonal actions mediated by surface receptors

The protein and peptide hormones and the amine derivatives of tyrosine cannot readily diffuse across the plasma membranes of their target cells. These hormones produce their effects by binding to receptors on the cell surface and rely on molecules on the cytosolic side of the membrane to convey the signal to the appropriate intracellular effector sites that bring about the hormonal response. There are two receptor types that involve cytoplasmic changes: G-protein-coupled receptors and catalytic receptors.

The G-protein-coupled receptors

The most frequently encountered cell surface receptors belong to a very large superfamily of proteins that couple with guanosine nucleotide-binding

proteins (G-proteins) to communicate with intracellular effector molecules. This ancient superfamily of receptor molecules is widely expressed throughout eukaryotic phyla. G-protein-coupled receptors are crucial for sensing signals in the external environment such as light, taste, and odor as well as signals transmitted by hormones. G-protein-coupled receptors receive signals carried by a wide range of neurotransmitters, immune modulators, and paracrine factors. More than 1000 different G-protein-coupled receptors may be expressed in humans. About 30% of all effective pharmaceutical agents are said to target actions mediated by receptors in this superfamily and account for about two-thirds of the prescriptions written by physicians.

All G-protein-coupled receptors are composed of single strands of protein and contain seven stretches of about 25 amino acids that each form membrane-spanning α-helices (Fig. 1.25).

The single long peptide chain that constitutes the receptor thus threads through the membrane seven times creating three extracellular and three intracellular loops. For this reason, these receptors are sometimes called heptahelical receptors. The amino terminal tail is extracellular and along with the external loops may contain covalently bound carbohydrate. The carboxyl tail lies within the cytoplasm. The lengths of the loops and the carboxyl and amino terminal tails vary in characteristic ways among the different families and subfamilies of these receptors. Outward-facing components of the receptor, including parts of the α-helices, contribute to the hormone recognition and binding site. The cytosolic loops and carboxyl tail bind to specific G-proteins near the interface of the membrane and the cytosol.

G-proteins are heterotrimers that comprise α-, β-, and γ-subunits. Lipid moieties covalently attached to the α- and γ-subunits insert into the inner leaflet of the plasma membrane bilayer and tether the G-proteins to the membrane. The α-subunits are enzymes (GTPases) that catalyze the conversion of guanosine triphosphate (GTP) to guanosine diphosphate (GDP) (Fig. 1.26).

In the inactivated or resting state, the catalytic site in the α-subunit is occupied by GDP. When the receptor binds to its hormone, a conformational change transmitted across the membrane allows its cytosolic domain to interact with the α-subunit of the G-protein in a way that causes the α-subunit to release the GDP in exchange for a molecule of GTP, and to dissociate from the βγ-subunits, which remain tightly bound to each other (Fig. 1.27).

Though tethered to the membrane, the dissociated subunits can move laterally along the inner surface of the membrane. In its GTP-bound state the α-subunit interacts

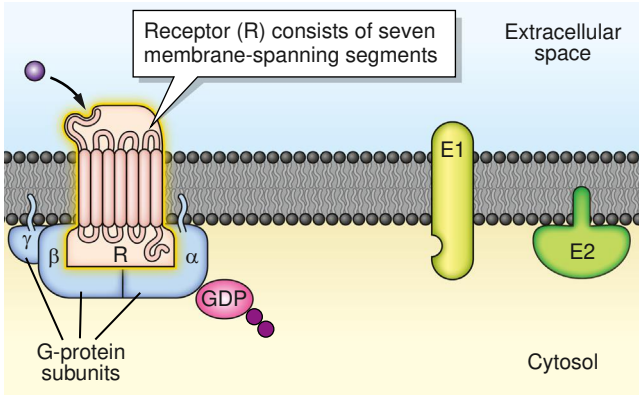

FIGURE 1.25 In step 1, this is the G-protein receptor showing the seven membrane-spanning segments and the hormone interacting with the receptor. Source: *Redrawn from Figure 3-4 in Boron, W. F., & Boulpaep, E. L. (2017).* Medical physiology *(3rd ed.). Elsevier, p. 54.*

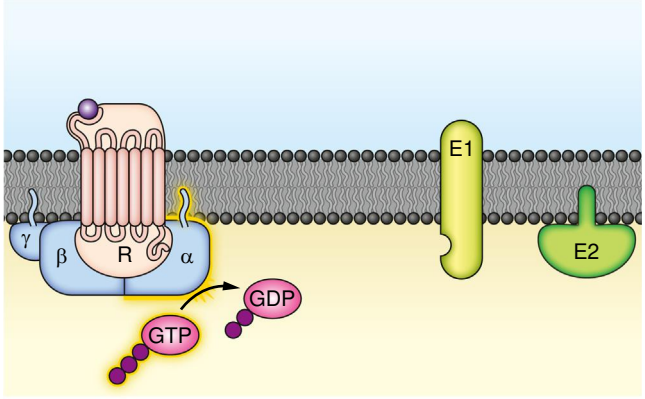

FIGURE 1.26 In step 2 the GTP is converted by GTPase (in the α-subunit) to GDP. *GDP*, Guanosine diphosphate; *GTP*, guanosine triphosphate. Source: *Redrawn from Figure 3-4 in Boron, W. F., & Boulpaep, E. L. (2017).* Medical physiology *(3rd ed.). Elsevier, p. 54.*

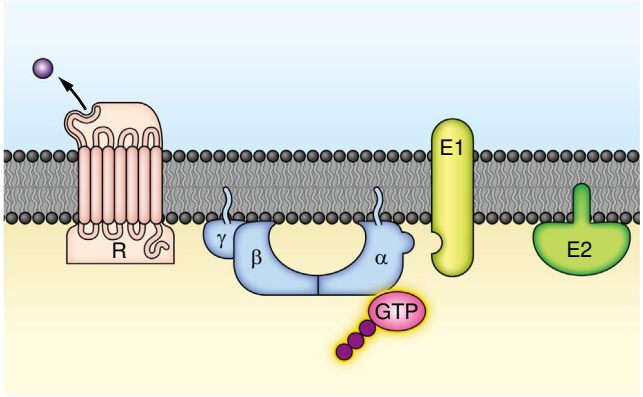

FIGURE 1.27 In step 3, the energy in GTP dissociates the G-protein from the receptor and changes its configuration. The hormone also leaves the receptor. *GTP*, Guanosine triphosphate. Source: *Redrawn from Figure 3-4 in Boron, W. F., & Boulpaep, E. L. (2017).* Medical physiology *(3rd ed.). Elsevier, p. 54.*

with and modifies the activity of membrane-associated enzymes that initiate the hormonal response (Fig. 1.28).

The liberated βγ-complex can also bind to cellular proteins and modify their activities, and both the free α-subunit and the βγ-subunits can bind to ion channel proteins and cause the channels to open or close (see Fig. 1.29).

Hydrolysis of GTP to GDP restores the resting state of the α-subunit allowing it to reassociate with the βγ-subunits to reconstitute the heterotrimer (see Fig. 1.30).

GTPase activity of the α-subunit is relatively slow. Consequently, the α-subunit may interact multiple times with effector enzymes before it returns to its resting state. In addition, because some G-proteins may be as much as one hundred times as abundant as the receptors they associate with, a single hormone-bound receptor may interact sequentially with multiple G-proteins before the hormone dissociates from the receptor. These characteristics provide mechanisms for amplification of the signal.

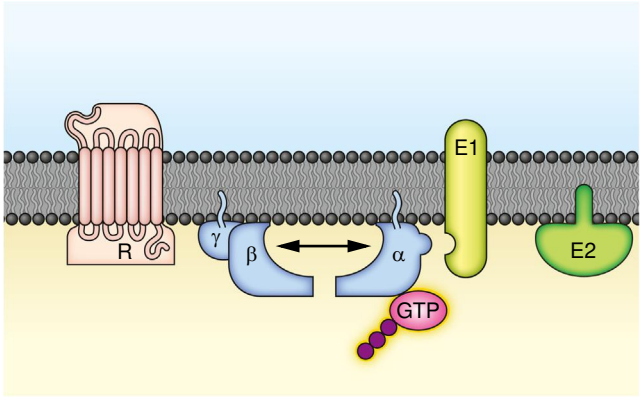

FIGURE 1.28 Instep 4, the α-GTP and the β- and γ-subunits dissociate. *GTP*, Guanosine triphosphate. Source: *Redrawn from Figure 3-4 in Boron, W. F., & Boulpaep, E. L. (2017).* Medical physiology *(3rd ed.). Elsevier, p. 54.*

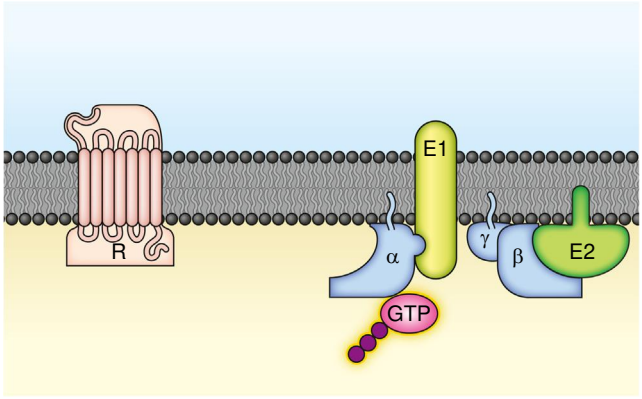

FIGURE 1.29 Step 5. Source: *Redrawn from Figure 3-4 in Boron, W. F., & Boulpaep, E. L. (2017).* Medical physiology *(3rd ed.). Elsevier, p. 54.*

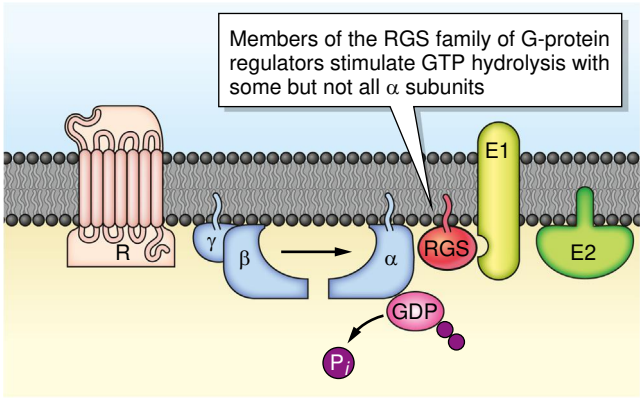

FIGURE 1.30 In step 6 (final step), inorganic phosphate is removed from GTP and the molecule becomes once again GDP. *GDP*, Guanosine diphosphate; *GTP*, guanosine triphosphate. Source: *Redrawn from Figure 3-4 in Boron, W. F., & Boulpaep, E. L. (2017).* Medical physiology *(3rd ed.). Elsevier, p. 54.*

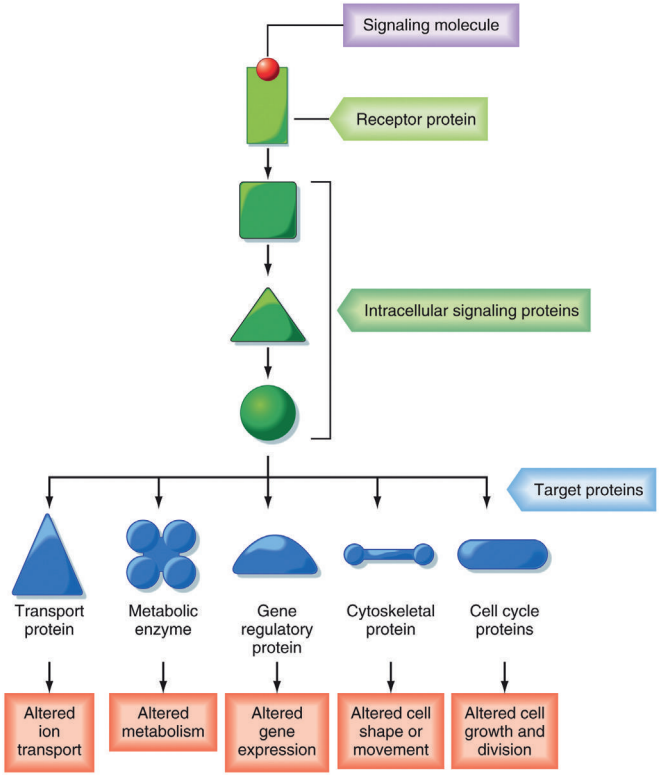

FIGURE 1.31 Diversification of the signal from a single hormone (signal molecule). Source: *From Figure 3.1 in Koeppen B. M., & Stanton B. W. (2018).* Berne and Levy's physiology *(7th ed.). Elsevier, p. 38.*

The interaction of a single hormone molecule with a single receptor molecule may result in multiple signal-generating events within a cell (Fig. 1.31).

Once a signal combines with a receptor, it can be amplified and diversified within a cell so that a stronger signal with different effect can occur (Fig. 1.32).

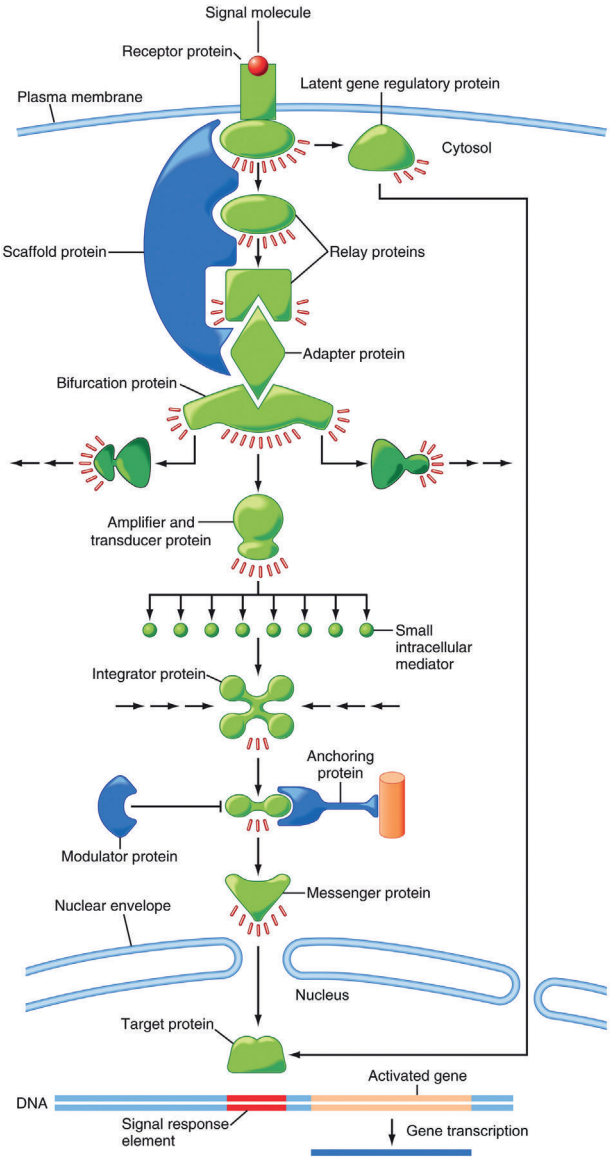

FIGURE 1.32 Illustrating different effects, with the pathway for one of those effects as a result of a hormone (signal molecule) interacting with a receptor. Source: *From Figure 3.7 in Koeppen B. M., & Stanton B. W. (2018).* Berne and Levy's physiology *(7th ed.). Elsevier, p. 43.*

Desensitization and downregulation of G-protein-coupled receptors

In addition to simple dissociation of hormone from its receptor, signaling often is terminated and receptors are desensitized to further stimulation by active cellular processes. G-protein-coupled receptors may be inactivated by phosphorylation of one of their intracellular loops catalyzed by G-protein receptor kinases. Phosphorylation uncouples the receptor from the α-subunit and promotes binding to a cytoplasmic protein of the β-arrestin family. Binding to β-arrestin may lead to receptor internalization and downregulation by sequestration in intracellular vesicles (Fig. 1.35). Sequestered receptors may recycle to the cell surface, or when cellular stimulation is prolonged, they may be degraded in lysosomes. β-Arrestins may have the additional function of serving as scaffolds for binding to a variety of other proteins, including the mitogen-activated protein (MAP) kinases (see later), and thereby provide a pathway for signaling between G-protein-coupled receptors and the nucleus (Fig. 1.35).

The second messenger concept

For a cell surface hormonal signal to be effective, it must be transmitted to the intracellular organelles and enzymes that produce the cellular response. To reach intracellular effectors, the G-protein-coupled receptors rely on intermediate molecules called second messengers, which are formed and/or released into the cytosol in response to hormonal stimulation (the first message). Second messengers activate intracellular enzymes and also amplify signals. A single hormone molecule interacting with a single receptor may result in the formation of tens or hundreds of second messenger molecules, each of which might activate an enzyme that in turn catalyzes the

At the molecular level (boxed material for additional information)

Classes of G-protein

At least three different classes of G-protein α-subunits are involved in transduction of hormonal signals. Each class includes the products of several closely related genes. α-Subunits of the "s" (stimulatory) class (Gαs) stimulate the transmembrane enzyme, adenylyl cyclase, to catalyze the synthesis of cyclic 3′,5′ adenosine monophosphate (cyclic AMP) from adenosine triphosphate, whereas α-subunits of the "i" (inhibitory) class (Gαi) inhibit the activity of adenylyl cyclase (Fig. 1.33).

α-Subunits belonging to the "q" class stimulate the activity of the membrane-bound enzyme phospholipase C-β, which catalyzes the hydrolysis of the membrane phospholipid, phosphatidylinositol 4,5-bisphosphate, to liberate inositol 1,4,5 trisphosphate (IP3) and diacylglycerol (DAG) (Fig. 1.34).

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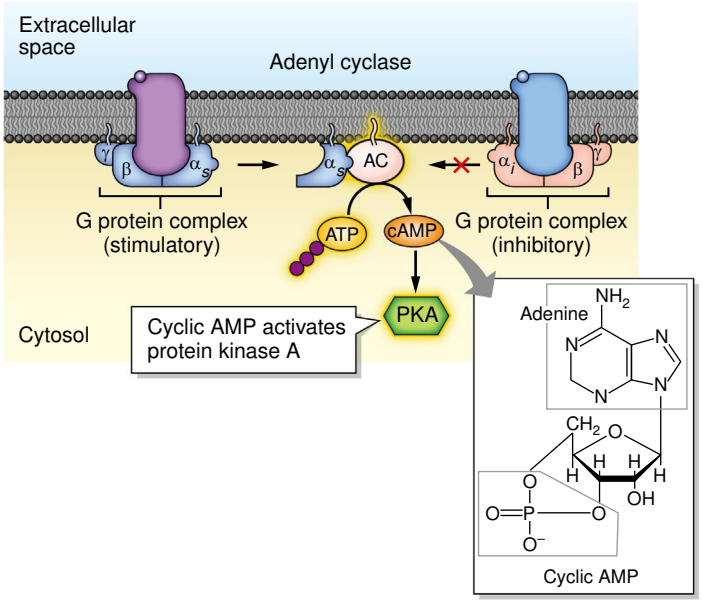

FIGURE 1.33 When α-s combines with adenyl cyclase, the effect is stimulatory; when αi combines with adenyl cyclase, the result is inhibitory. Source: *Redrawn from Figure 3-5 in Boron, W. F., & Boulpaep, E. L. (2017).* Medical physiology *(3rd ed.). Elsevier, p. 55.*

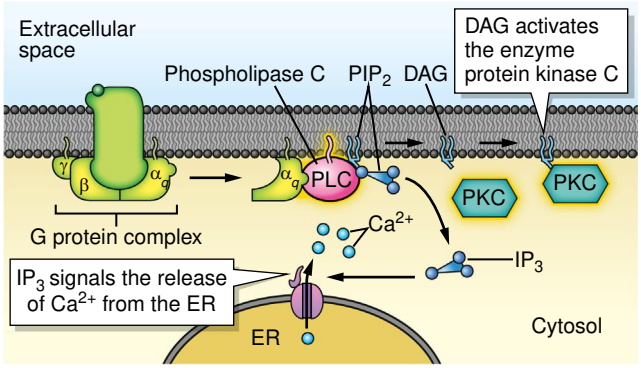

FIGURE 1.34 When αq combines with PLC, the PLC converts PIP2 to IP3 and DAG. Each of these, in turn, has different effects as illustrated, as for example, the IP3 release of calcium from the ER, the ER being the major storehouse for intracellular calcium. *DAG*, Diacylglycerol; *PIP2*, phosphatidyl inositol 4,5-bisphosphate; *PLC*, phospholipase C; *IP3*, inositol 1,4,5 triphosphate. Source: *Redrawn from Figure 3-5C in Boron, W. F., & Boulpaep, E. L. (2017).* Medical physiology *(3rd ed.). Elsevier, p. 55.*

The α-subunits of the heterotrimeric G-proteins are closely related to the small G-proteins that function as biochemical switches to regulate such processes as entry of proteins into the nucleus, sorting and trafficking of intracellular vesicles, and cytoskeletal rearrangements. There are 6 superfamilies of these small G-proteins, the most common is RAS (with over 100 members), which regulates cell proliferation. Like the α-subunits, the small G-proteins are GTPases that are in their active state when bound to GTP and in their inactive state when bound to GDP. Unlike the α-subunits, the small G-proteins do not interact directly with hormone receptors or associate with βγ-subunits. Instead of liganded receptors, the small G-proteins are activated by proteins called nucleotide exchange factors that cause them to dissociate from GDP and bind GTP. They remain activated as they slowly convert GTP to GDP, but inactivation can be accelerated by interaction with GTPase-activating proteins.

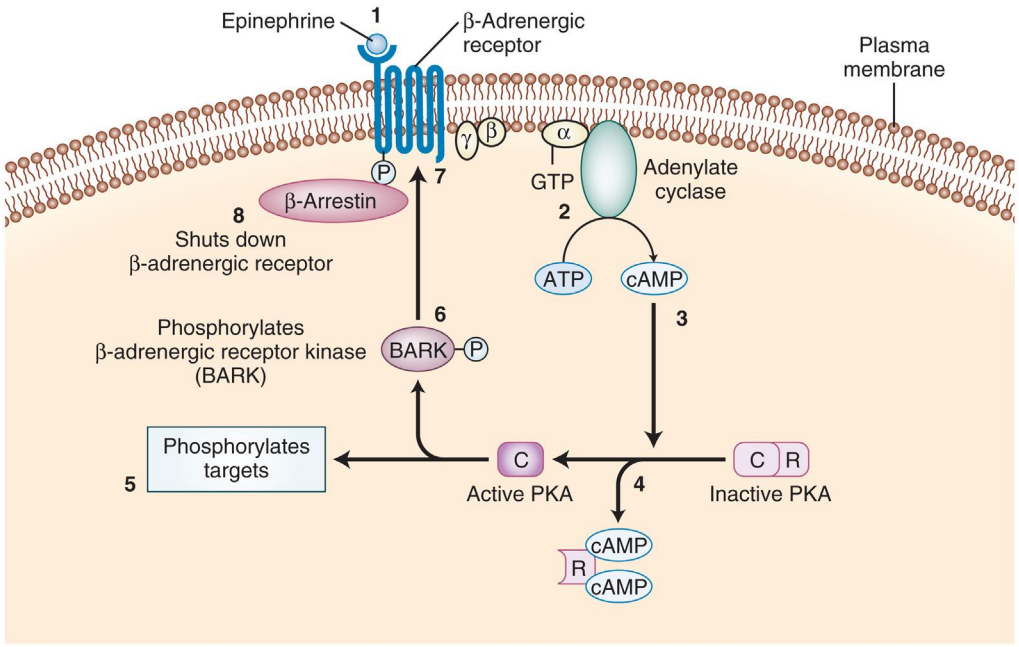

FIGURE 1.35 β-Arrestin combines with the G-protein cell receptor, inactivating it. Source: *From Figure 3-13 in Melmed, S., Polonsky, K. S., Larson, P. R., & Kronenberg, H. M. (2016).* William's textbook of endocrinology *(13th ed.). Elsevier, p. 33.*

formation of hundreds of thousands of molecules of product. Most of the responses that are mediated by second messengers are achieved by regulating the activity of enzymes in target cells, usually by adding a phosphate group. The resulting conformational change increases or decreases enzymatic activity. Therefore second messengers are responsible for specificity, diversity, and expansion or reduction in the hormone signal.

Protein kinases catalyze the transfer of the terminal phosphate from adenosine triphosphate (ATP) to a hydroxyl group in serine or threonine residues in proteins. Tyrosine kinases phosphorylate the hydroxyl groups of tyrosine residues in this way. The human genome contains about a thousand genes that encode protein kinases, but only a few are activated by second messengers. Many protein kinases are themselves activated by phosphorylation catalyzed by other protein kinases. Protein phosphatases remove phosphate groups from these residues and thus restore them to their unstimulated state. For the most part, protein phosphatases are constitutively active (i.e., are always present and active), but some specific protein phosphatases are inducible (i.e., they are directly or indirectly activated in response to hormonal stimulation).

Unlike responses that require the synthesis of new cellular proteins, responses that result from phosphorylation–dephosphorylation reactions occur very quickly, and therefore most second messenger-mediated responses are turned on and off without appreciable latency. However, second messengers can also promote the phosphorylation of transcription factors and thus regulate transcription of specific genes in much the same way as discussed for the nuclear receptors. These responses require the same time-consuming processes as are needed for nuclear receptor–mediated changes and are seen only after a delay.

Although a very large number of hormones and other first messengers act through surface receptors, only a comparatively few substances have been identified as second messengers. This is because receptors for many different extracellular signals utilize the same second messenger. When originally proposed, the hypothesis that the same second messenger might mediate different actions of many different hormones, each of which produces a unique pattern of cellular responses, was met with skepticism. The idea did not gain widespread acceptance until it was recognized that the special nature of a cellular response is determined by the particular enzymatic machinery with which a cell is endowed rather than by the signal that turns on that machinery. Thus, when activated, a hepatic cell makes glucose, and a smooth muscle cell contracts or relaxes.

Second messengers: the cyclic 3′,5′ adenosine monophosphate system

The first of the second messengers to be recognized was cyclic 3′,5′ adenosine monophosphate (cyclic AMP). Cyclic AMP transmits the hormonal signal mainly by activating the enzyme protein kinase A (PKA) (Figs. 1.36 and 1.37).

When cellular concentrations of cyclic AMP are low, two catalytic subunits of PKA are firmly bound to a dimer of regulatory subunits that keeps the tetrameric holoenzyme in the inactive state. The catalytic and regulatory subunits of PKA are products of separate genes. Reversible binding of two molecules of cyclic AMP to each regulatory subunit liberates the catalytic subunits. Cyclic AMP is degraded to 5′-AMP by a family of enzymes called cyclic AMP phosphodiesterases, which cleave the bond that links the phosphate to ribose carbon 3 to form 5′ AMP (Fig. 1.38).

As cyclic AMP concentrations fall, bound cyclic AMP separates from the regulatory subunits, which then reassociates with the catalytic subunits restoring basal activity.

Regulatory regions of many genes contain a cyclic AMP response element (CRE) analogous to the HREs that bind nuclear hormone receptors as discussed earlier. One or more forms of CRE binding (CREB) proteins are found in the nuclei of most cells and are substrates for PKA. Dimers of phosphorylated CREB bind to the CREs of regulated genes and recruit other nuclear proteins to form complexes that regulate gene transcription in the same manner as described for the nuclear hormone receptors.

Not all the effects of cyclic AMP are mediated by PKA. Cyclic AMP can also bind to membrane channels and directly activate or inactivate them. Other actions of cyclic AMP that are independent of PKA are mediated by EPACs (exchange proteins activated directly by cyclic AMP). EPACs 1 and 2 are intracellular proteins that, upon binding cyclic AMP, interact with a small G-protein called RAP and cause it to exchange its bound GDP for GTP. Activated RAP participates in a variety of cellular functions, including ion channel and membrane transporter activity, cell–cell interactions, intracellular calcium signaling (see later), and exocytosis.

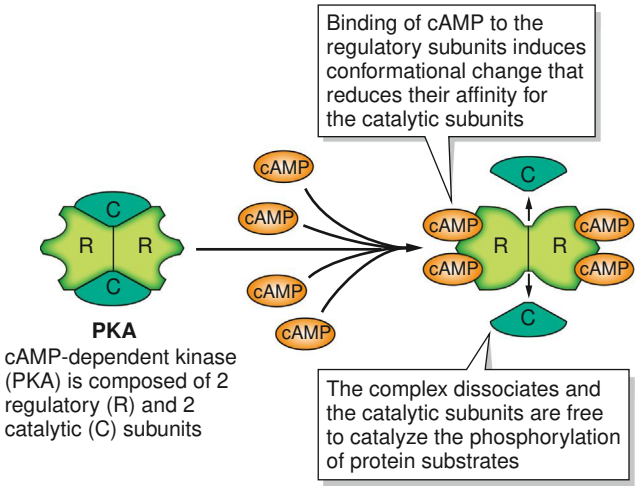

FIGURE 1.36 cAMP activates phosphate kinase. *cAMP*, Cyclic 3′,5′ adenosine monophosphate. Source: *Redrawn from Figure 3-6 in Boron, W. F., & Boulpaep, E. L. (2017).* Medical physiology *(3rd ed.). Elsevier, p. 57.*

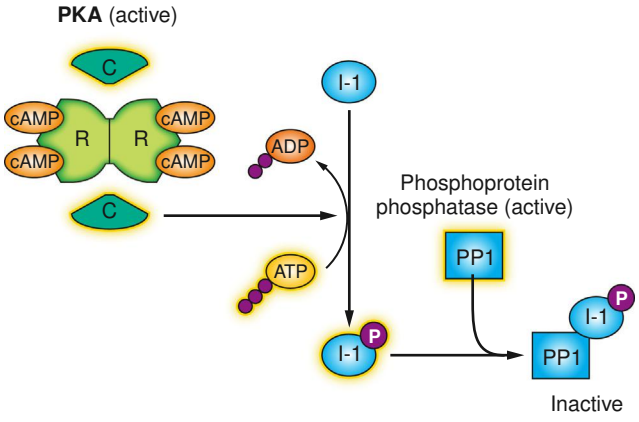

FIGURE 1.37 The activated phosphokinase phosphorylates PPI to inactivate it. *PPI*, Proton pump inhibitor. Source: *Redrawn from Figure 3-7 in Boron, W. F., & Boulpaep, E. L. (2017).* Medical physiology *(3rd ed.). Elsevier, p. 58.*

Second messengers: the calcium–calmodulin system

Calcium has long been recognized as a regulator of cellular processes and triggers such events as muscular contraction, secretion, polymerization of microtubules, and activation of various enzymes. The concentration of free calcium in cytoplasm of resting cells is very low, about one ten-thousandth of its concentration in extracellular fluid. This steep concentration gradient is maintained primarily by the actions of calcium ATPases that transfer calcium out of the cell or into storage sites within the endoplasmic reticulum and by sodium–calcium exchangers that extrude one calcium ion in exchange for three sodium ions. When cells are stimulated by some hormones, their cytosolic calcium concentration rises abruptly, increasing perhaps 10-fold or more within seconds. This is accomplished by the

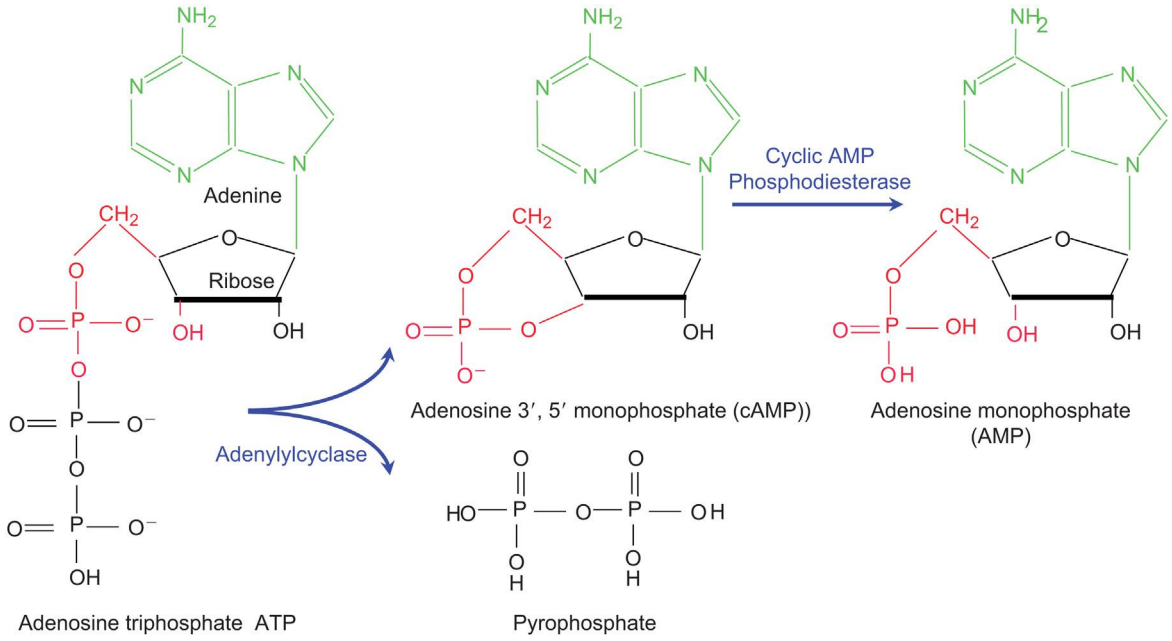

FIGURE 1.38 The conversion of ATP to cyclic AMP and degradation. *AMP*, 3′,5′ Adenosine monophosphate; *ATP*, adenosine triphosphate.

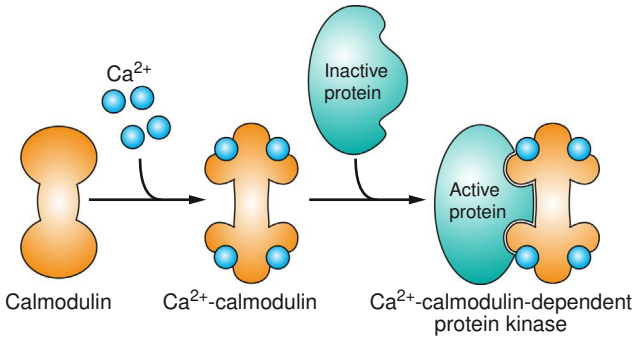

FIGURE 1.39 Calmodulin activation in the presence of calcium. Source*: Redrawn from Figure 3-9 in Boron, W. F., & Boulpaep, E. L. (2017).* Medical physiology *(3rd ed.). Elsevier, p. 57.*

release of calcium from intracellular storage sites primarily in the endoplasmic reticulum and by influx of calcium through activated calcium channels in the plasma membrane. Although calcium can directly affect the activity of some proteins, it generally does not act alone. Virtually all cells are endowed with a protein called calmodulin, which reversibly binds four calcium ions (Fig. 1.39).

When complexed with calcium, the configuration of calmodulin is modified in a way that enables it to bind to protein kinases and other enzymes and activate them. The behavior of calmodulin-dependent protein kinases is quite similar to that of PKA. Calmodulin kinase II may catalyze the phosphorylation of many of the same substrates as PKA, including CREB, and other nuclear transcription factors. Upon cessation of hormonal stimulation, calcium channels in the endoplasmic reticular and plasma membranes close, and constitutively active calcium pumps (ATPases) in these membranes restore cytoplasmic concentrations to low resting levels. A low cytosolic concentration favors the release of calcium from calmodulin, which then dissociates from the various enzymes it has activated.

Second messengers: the diacylglycerol and IP3 system

Both products of phospholipase C–catalyzed hydrolysis of phosphatidylinositol 4,5 bisphosphate,

diacylglycerols (DAG) and IP3 behave as second messengers (Fig. 1.40).

IP3 diffuses through the cytosol to reach its receptors in the membranes of the endoplasmic reticulum, the Golgi apparatus, and the nucleus. Activated IP3 receptors are calcium ion channels through which these calcium-storing organelles release calcium into the cytoplasm. Operation of these channels is modulated by phosphorylation and by calcium, which is stimulatory at low concentrations and inhibitory at high concentrations.

Because of its lipid solubility DAG remains associated with the plasma membrane to which it recruits and activates another protein kinase, protein kinase C (PKC), by increasing its affinity for phosphatidylserine in the membrane. The simultaneous increase in cytosolic calcium concentration resulting from the action of IP3 complements DAG in stimulating the catalytic activity of some members of the PKC family, and conversely phosphorylation of IP3 receptors by PKC augments their calcium-releasing activity. Some members of the PKC family are stimulated by DAG even when cytosolic calcium remains at resting levels (Fig. 1.41).

IP3 is cleared from cells by stepwise dephosphorylation to inositol. DAG is cleared by addition of a phosphate group to form phosphatidic acid, which

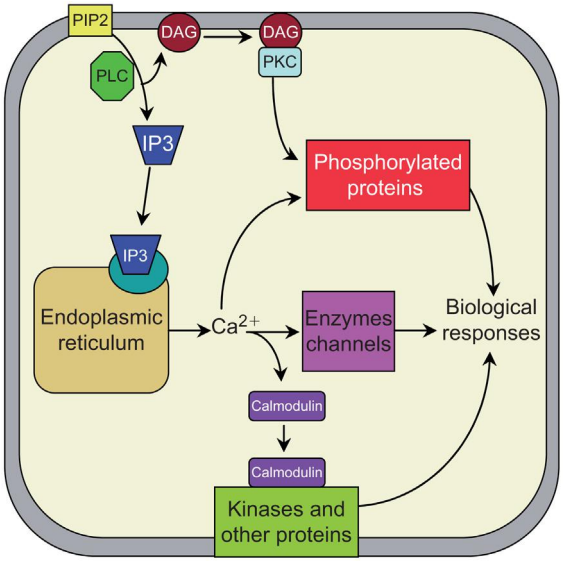

FIGURE 1.41 Signal transduction through the *IP3 DAG* second messenger system. *PIP2* is cleaved into IP3 and DAG by the action of a *PLC*. DAG activates *PKC*, which then phosphorylates a variety of proteins to produce various cell-specific effects. IP3 binds to its receptor in the membrane of the endoplasmic reticulum causing the release of Ca^{2+}, which further activates PKC, directly activates or inhibits enzymes or ion channels, or binds to calmodulin, which then binds to and activates protein kinases and other proteins. *DAG*, Diacylglycerols; *IP3*, inositol trisphosphate; *PIP2*, phosphatidyl inositol 4,5 bisphosphate; *PKC*, protein kinase C; *PLC*, phospholipase C.

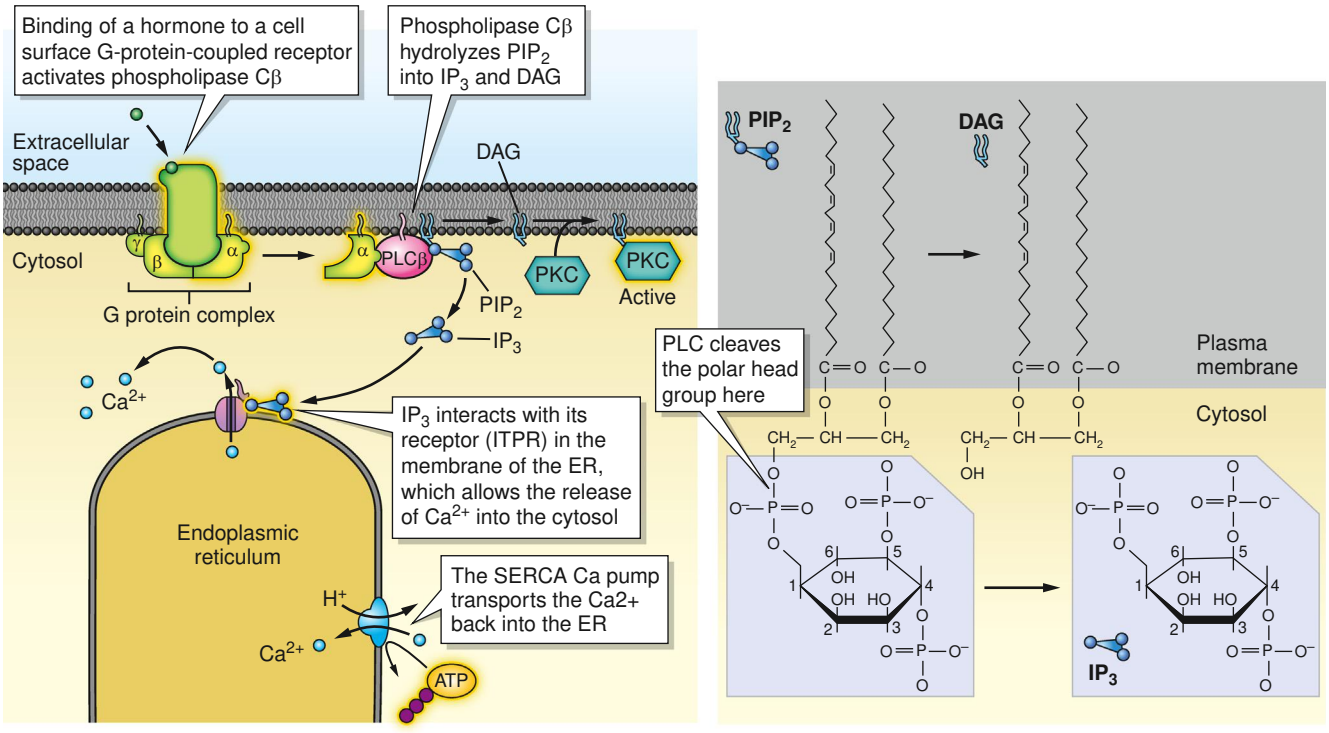

FIGURE 1.40 The production of IP3 and DAG. *DAG*, Diacylglycerol. Source: *Redrawn from Figure 3-8 A in Boron, W. F., & Boulpaep, E. L. (2017).* Medical physiology *(3rd ed.). Elsevier, p. 59.*

may then be converted to a triglyceride or resynthesized into a phospholipid. Phosphatidylinositides of the plasma membrane are regenerated by combining inositol with phosphatidic acid, which may then undergo stepwise phosphorylation of the inositol.

The phosphatidylinositol precursor of IP3 and DAG also contains a 20-carbon polyunsaturated fatty acid called arachidonic acid (Fig. 1.42).

This fatty acid typically is found in ester linkage with carbon 2 of the glycerol backbone of phospholipids and may be liberated by the action of a diacylglyceride lipase from the DAG formed in the breakdown of phosphatidylinositol. Liberation of arachidonic acid is the rate-determining step in the formation of thromboxanes, prostaglandins, and leukotrienes (see Chapter 4: The Adrenal Glands). These compounds, which are produced in virtually all cells, diffuse across the plasma membrane and behave as local regulators of nearby cells. The same hormone–receptor interaction that produces DAG and IP3 as second messages to communicate with cellular organelles frequently also results in the formation of arachidonate derivatives that inform neighboring cells that a response has been initiated. Arachidonic acid is also released from other, more abundant membrane phospholipids by the actions of the phospholipase A2 class of enzymes that can be activated by calcium, phosphorylation by PKC, and by βγ-subunits of G-proteins (Fig. 1.43).

Second messengers: cyclic guanosine 3′,5′-monophosphate

Though considerably less versatile than cyclic AMP, cyclic guanosine 3′,5′-monophosphate (cyclic GMP) plays an analogous role in many cells. Its formation from GTP is catalyzed by the enzyme guanylyl cyclase. Guanylyl cyclase and cyclic GMP–dependent protein kinase activities are present in many cells, but the activation of guanylyl cyclase is quite different from that of adenylyl cyclase. Guanylyl cyclase activity is an intrinsic property of the transmembrane receptor for atrial natriuretic hormone and is activated without the intercession of a G-protein. Guanylyl cyclase is also present in a soluble form within the cytoplasm of many cells and is activated by nitric oxide (NO). Increased formation of cyclic GMP in vascular smooth muscle is associated with relaxation and may account for vasodilator responses to the atrial natriuretic hormone (see Chapter 9: Regulation of Salt and Water Balance).

Receptors that signal through tyrosine kinase

Some hormones transmit their messages from the cell surface to intracellular effectors without the agency of second messengers. Receptors for these hormones rely on physical association between proteins (protein–protein interactions) to activate enzymes that phosphorylate transcription factors and other cytosolic proteins in much the same way as already discussed. The tyrosine kinase–dependent receptors have a single membrane-spanning region and either have intrinsic protein tyrosine kinase enzymatic activity in their intracellular domains or are associated with cytosolic protein tyrosine kinases. Receptors for insulin, the insulin-like growth factors, and the epidermal growth factor have intrinsic protein kinase activity; receptors for growth hormone, prolactin, erythropoietin, and some cytokines rely on an associated cytosolic tyrosine kinase called JAK2. Generally, the tyrosine kinase–dependent receptors are synthesized as dimers or form dimers when activated by their ligands. When a hormone binds to the extracellular domain, a conformational change in the receptor activates protein tyrosine kinases that catalyze the phosphorylation of hydroxyl groups of tyrosine residues in the cytosolic portion of the receptor itself, in the associated kinase (autophosphorylation), and in other cytosolic proteins that complex with the phosphorylated receptor.

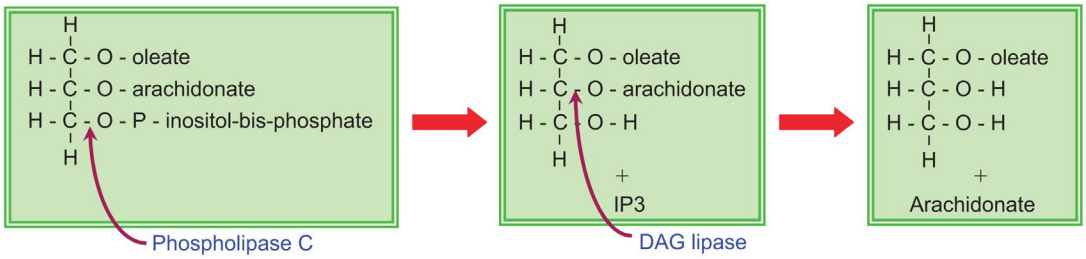

FIGURE 1.42 Arachidonate formation and the action of phospholipase C and DAG. *DAG*, Diacylglycerols.

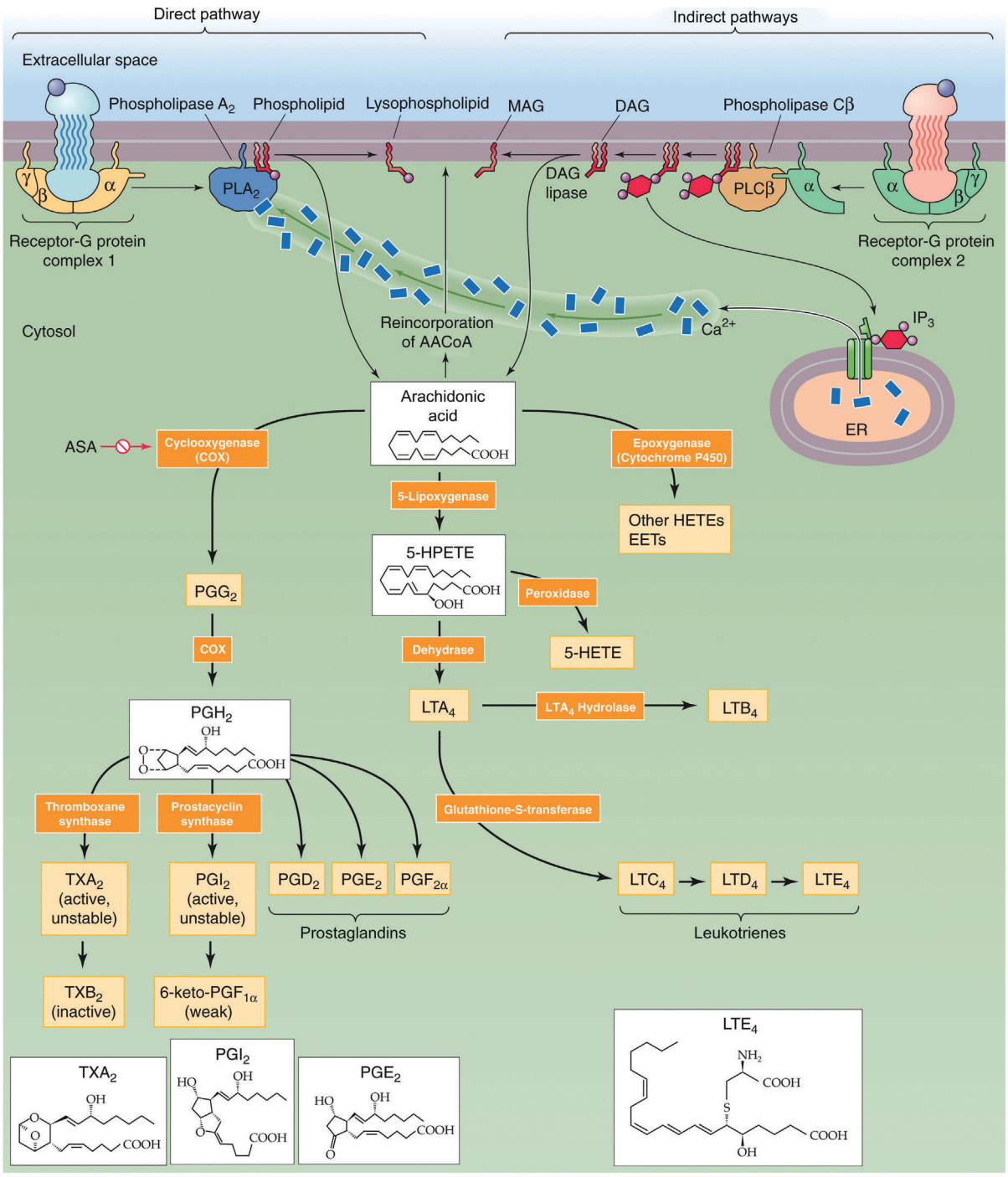

FIGURE 1.43 Formation of arachidonic acid derivatives. Source: *From Figure 3-11 in Boron, W. F., & Boulpaep, E. L. (2017).* Medical physiology *(3rd ed.). Elsevier, p. 63.*

One of the remarkable features of signaling that is particularly evident in considering the tyrosine kinase–dependent receptors is that virtually no transduction mechanism or signaling pathway is uniquely associated with expression of the actions of any one hormone. Rather, actions of the various hormones are produced through the use of many of the same pathways. For example, tyrosine phosphorylation of the insulin receptor substrates followed by the activation of phosphatidylinositol-3 kinase is a common feature of signaling by insulin, growth hormone, prolactin, leptin, several cytokines, and erythropoietin, although each hormone produces unique effects. As noted for the actions of

At the molecular level (boxed material for additional information)

Tyrosine kinases

The protein substrates for receptor-activated tyrosine kinases may have catalytic activity or may act as scaffolds to which other proteins are recruited and positioned so that enzymatic modifications are facilitated. As a result, large multiprotein signaling complexes are formed. Phosphorylated tyrosines act as docking sites for proteins that contain *src* homology 2 (SH2) domains. SH2 domains are named for the particular configuration of the tyrosine phosphate–binding region originally discovered in v-src, the cancer-inducing protein tyrosine kinase of the Rous sarcoma virus. SH2 domains represent one type of a growing list of modules within a protein that recognize and bind to specific complementary motifs in another protein. A typical SH2 domain consists of about 100 amino acid residues and recognizes a phosphorylated tyrosine in the context of the three or four amino acid residues that are downstream from the tyrosine. There are multiple SH2 groups that recognize phosphorylated tyrosines in different contexts. Typically, multiple tyrosines are phosphorylated so that several different SH2-containing proteins are recruited and initiate multiple signaling pathways. Although some responses to the activation of tyrosine kinases include modifications of cellular metabolism without nuclear participation, they often involve a change in genomic readout that promotes cell division (mitogenesis) or differentiation. One way that these receptors communicate with the genome is through the activation of the MAP kinase pathway. MAP kinase is a cytosolic enzyme that is activated by phosphorylation of both serine and tyrosine residues and then enters the nucleus where it phosphorylates and activates certain transcription factors. Activation of MAP kinase follows an indirect route that involves a small G-protein called Ras, which was originally discovered as a constitutively activated protein present in many tumors. One of the proteins that docks with phosphorylated tyrosine residues is the growth factor–binding protein 2 (Grb2). Grb2 is an adaptor protein that has an SH2 group at one end and other binding motifs at its opposite end, which enable it to bind other proteins, including a nucleotide exchange factor called SOS (Son of Sevenless[1]). By means of these protein–protein interactions, the activated receptor can thus communicate with an activate SOS, which causes Ras to exchange GTP for GDP. The effector for the Ras that is thus activated is the enzyme Raf kinase, which phosphorylates and activates the first of a cascade of MAP kinases that ultimately result in phosphorylation of nuclear transcription factors (Fig. 1.44).

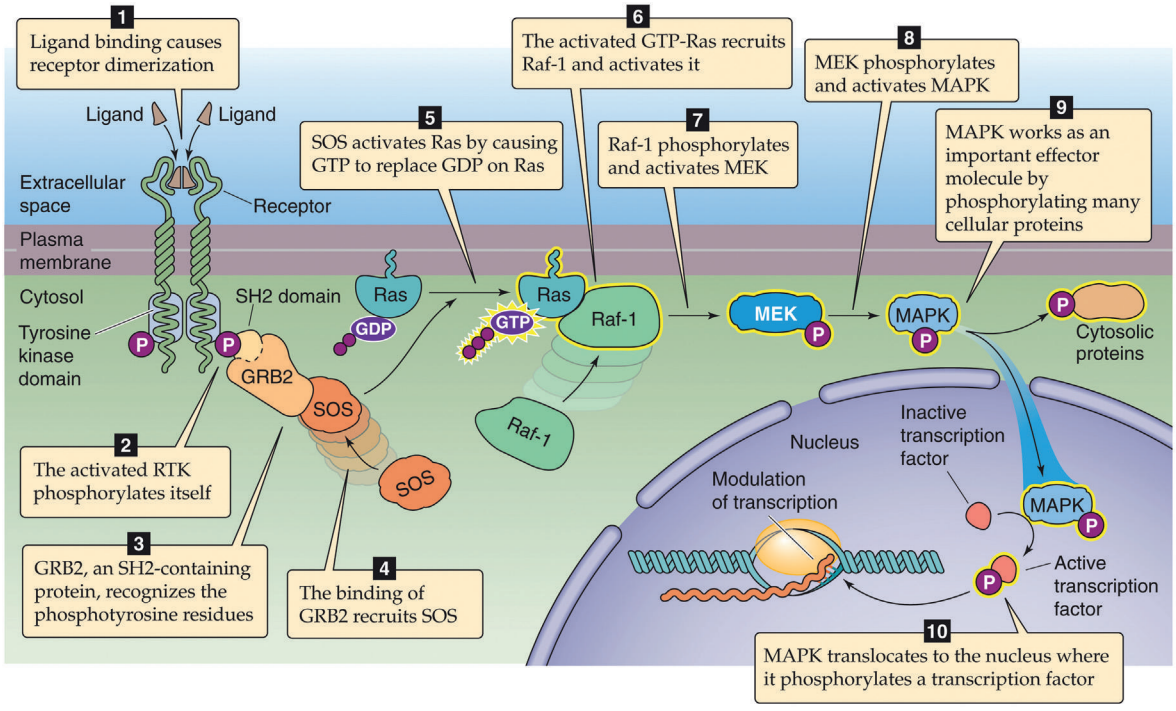

FIGURE 1.44 The Ras pathway. For details, see text. Source: *From Figure 3-13 in Boron, W. F., & Boulpaep, E. L. (2017).* Medical physiology *(3rd ed.). Elsevier, p. 69.*

(*cont'd*)

The gamma form of phospholipase C is another effector protein that is recruited to tyrosine phosphorylated receptors by way of its SH2 group. It is also a substrate for tyrosine kinases and is activated by tyrosine phosphorylation. Activation of this member of the phospholipase C family of proteins results in hydrolysis of phosphatidylinositol bisphosphate to produce DAG and IP3 in the same manner as already discussed for the beta forms of the enzyme associated with G-protein-coupled receptors. In this manner, tyrosine kinase–dependent receptors can stimulate cellular changes that are mediated by PKC and the calcium–calmodulin second messenger system, including phosphorylation of nuclear transcription factors by calmodulin kinase (Fig. 1.45).

Another mechanism for modifying gene expression involves the activation of a family of proteins called Stat (signal transducer and activator of transcription) proteins. The Stat proteins are transcription factors that reside in the cytosol in their inactive state. They have an SH2 group that enables them to bind to tyrosine phosphorylated proteins. When bound to the receptor/kinase complex, Stat proteins become tyrosine phosphorylated, whereupon they dissociate from their docking sites, form homodimers, and enter the nucleus where they activate transcription of specific genes (Figs. 1.46 and 1.47).

Although they were discovered as the substrates for the insulin receptor tyrosine kinase, the insulin receptor

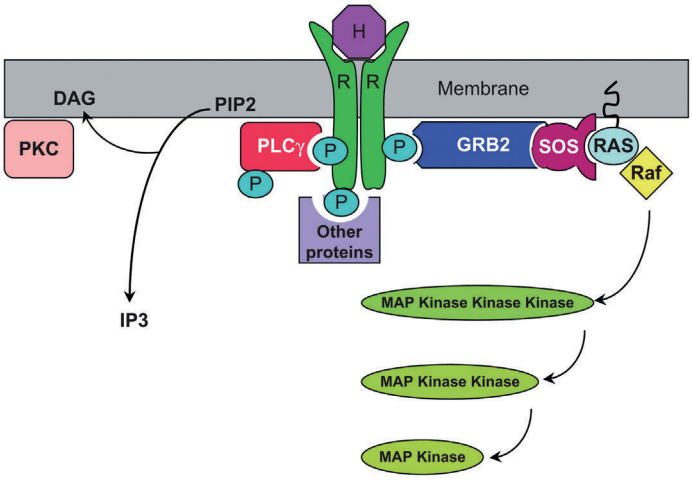

FIGURE 1.45 Phosphorylation of tyrosines on dimerized *R* following *H* binding provides docking sites for the attachment of proteins that transduce the hormonal signal. The *GRB2* binds to a phosphorylated tyrosine in the receptor and binds at its other end to the nucleotide exchange factor *SOS*, which stimulates the small G-protein *Ras* to exchange its GDP for GTP. Thus activated, Ras in turn activates the protein kinase *Raf*, which phosphorylates *MAP* kinase and initiates the *MAP* kinase cascade that ultimately phosphorylates nuclear transcription factors. The *PLC* γ docks on the phosphorylated receptor and is then tyrosine phosphorylated and activated to cleave phosphatidyl inositol 4,5 bisphosphate (*PIP2*) releasing DAG and inositol trisphosphate (*IP3*) and activating protein kinase C (*PKC*) as shown in Fig. 1.19. *DAG*, Diacylglycerols; *GDP*, guanosine diphosphate; *GRB2*, growth factor–binding protein 2; *GTP*, guanosine triphosphate; *H*, hormone; *MAP*, mitogen-activated protein; *PLC* γ, γ isoform of phospholipase C; *R*, receptors.

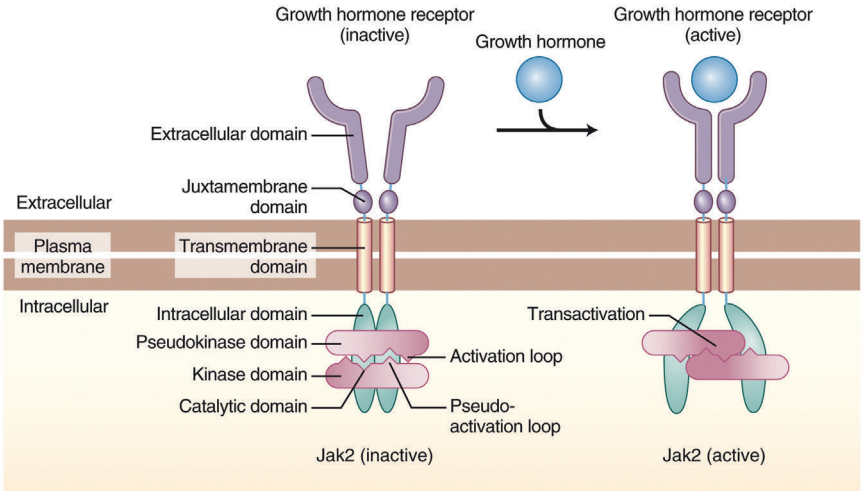

FIGURE 1.46 Scissor model for the activation of human growth hormone. Source: *From Figure 3-11 in Melmed, S., Polonsky, K. S., Larson, P. R., & Kronenberg, H. M. (2016).* William's textbook of endocrinology *(13th ed.). Elsevier, p. 31.*

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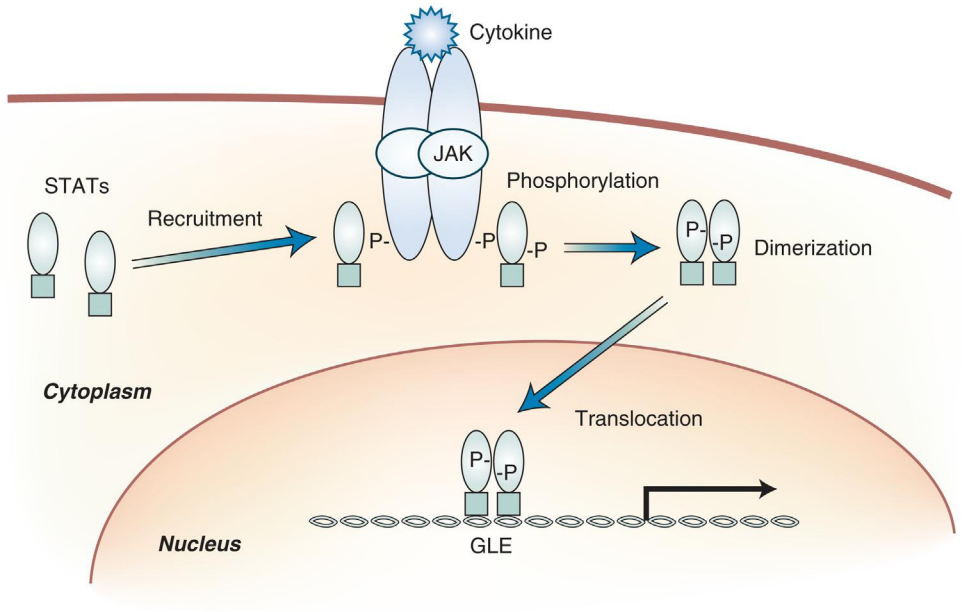

FIGURE 1.47 A continuation of Fig. 1.46 showing the effect on the nucleus. Source: *From Figure 3-12 in Melmed, S., Polonsky, K. S., Larson, P. R., & Kronenberg, H. M. (2016).* William's textbook of endocrinology *(13th ed.). Elsevier, p. 31.*

substrates (1–4) play an important role in the signaling pathways of many of the hormones and cytokines that act by way of tyrosine phosphorylation. These large proteins are phosphorylated at multiple tyrosine residues and recruit proteins that anchor other kinase-dependent signaling pathways. One of the most important of these proteins is phosphatidylinositol-3 (PI-3) kinase, which catalyzes the phosphorylation of carbon 3 of the inositol of phosphatidylinositol bisphosphate in cell membranes to form phosphatidylinositol 3,4,5 trisphosphate (PIP3). PI-3 kinase consists of a regulatory subunit that contains an SH2 domain and a catalytic subunit. Binding of the regulatory subunit to phosphorylated tyrosines of the receptor-associated complex activates the catalytic subunit. The enzymes activated by associating with PIP3 are protein kinases that regulate cellular metabolism, vesicle trafficking, cytoskeletal changes, and other responses. These responses are considered further in Chapter 7, The Pancreatic Islets, and illustrated in Fig. 7.19.

[1](In smaller case) This seemingly unusual name refers to a gene (*sev*) in the embryo of the fruit fly for the development of the R7 retina of its compound eye. If it is not present (=less), the fly cannot detect a certain band of ultraviolet light, hence R7-less or "sevenless." Proteins interacting with this gene were given whimsical names such as "son" "bride" depending on their function. These same proteins were found to be in signaling molecules in higher organisms.

the cyclic AMP-dependent hormones, the nature of the final result is a function of the particular target cell and its unique complement of enzymes and transcriptional machinery, and not the signaling pathway as shown in Fig. 1.48.

Regulation of hormone secretion

For hormones to function as carriers of critical information, their secretion must be turned on and off at precisely the right times. The organism must have some way of knowing when there is a need for a hormone to be secreted, how much is needed, and when that need has passed. As hormonal control is discussed in this and subsequent chapters, it is important to identify and understand the components of the regulation of each hormonal secretion because (1) derangements in any of the components are the bases of endocrine disease and (2) manipulation of any

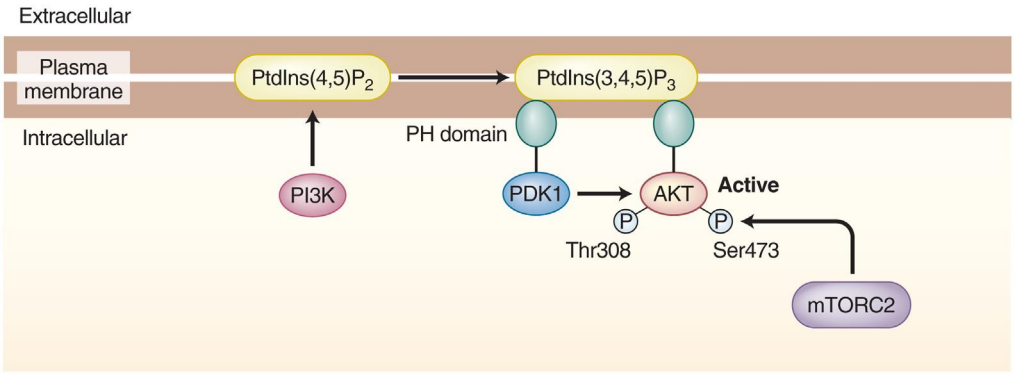

FIGURE 1.48 Activation of AKT one of the pathways in the activation of hormones. Source: *From Figure 3-15 in Melmed, S., Polonsky, K. S., Larson, P. R., & Kronenberg, H. M. (2016).* William's textbook of endocrinology *(13th ed.). Elsevier, p. 35.*

component provides an opportunity for therapeutic intervention.

Negative feedback

Secretion of most hormones is regulated by negative feedback. By negative feedback, we mean that some consequence of hormone secretion acts directly or indirectly on the secretory cell in a negative way to inhibit further secretion. A simple example from everyday experience is the thermostat. When the temperature in a room falls below some preset level, the thermostat signals the furnace to produce heat. When room temperature rises to the preset level, the signal from the thermostat to the furnace is shut off, and heat production ceases until the temperature again falls. This is a simple closed-loop feedback system and is analogous to the regulation of glucagon secretion. A fall in blood glucose detected by the alpha cells of the islets of Langerhans causes them to release glucagon, which stimulates the liver to release glucose and thereby increase blood glucose concentrations (Fig. 1.49).

With restoration of blood glucose to some predetermined level or set point, further secretion of glucagon is inhibited. This simple example involves only secreting and responding cells. Other systems may be considerably more complex and involve one or more intermediary events, but the essence of negative feedback regulation remains the same: hormones produce biological effects that directly or indirectly inhibit their further secretion.

A problem that emerges with this system of control is that the thermostat maintains room temperature constant only if the natural tendency of the temperature is to fall. If the temperature were to rise, it could not be controlled by simply turning off the furnace. This problem is at least partially resolved in hormonal systems, because at physiological set points the basal rate of secretion usually is not zero. In this example, when there is a rise in blood glucose concentration, glucagon secretion can be diminished and therefore diminish the impetus on the liver to release glucose. Some regulation above and below the set point can therefore be accomplished with just one feedback loop; this mechanism is seen in some endocrine control systems. Regulation is more efficient and precise, however, with a second, opposing loop, which is activated when the controlled variable deviates in the opposite direction. For the example with regulation of blood glucose, that second loop is provided by insulin. Insulin inhibits glucose production by the liver and is secreted in response to an elevated blood glucose level. Protection against deviation in either direction often is achieved in biological systems by the opposing actions of antagonistic control systems (Fig. 1.50).

Closed-loop negative feedback control as just described can maintain conditions only in a state of constancy. Such systems are effective in guarding against upward or downward deviations from some predetermined set point, but changing environmental demands often require temporary deviation from constancy. This can be accomplished in some cases by adjusting the set point and in other cases by a signal that overrides the set point. For example, epinephrine secreted by the adrenal medulla in response to some emergency inhibits insulin secretion and increases glucagon secretion even though the concentration of glucose in the blood may already be high. Whether the set point is changed or overridden, deviation from constancy is achieved by the intervention of some additional signal from outside the negative feedback system. In most cases, that additional signal originates with the nervous system.

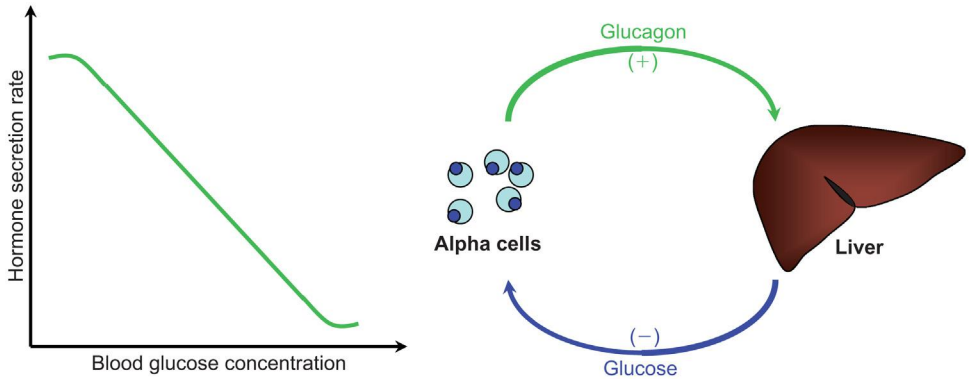

FIGURE 1.49 Negative feedback.

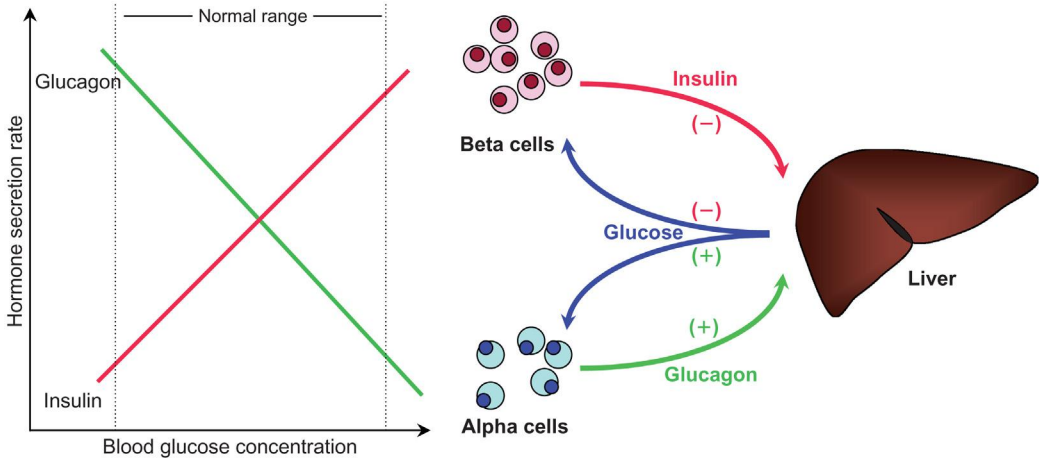

FIGURE 1.50 Negative feedback regulation of blood glucose concentration by insulin and glucagon. (–) = Inhibits, (+) = stimulates.

Hormones also initiate or regulate processes that are not limited to steady or constant conditions. Virtually all these processes are self-limiting, and their control resembles negative feedback, but of the open-loop type. For example, oxytocin is a hormone that is secreted by hypothalamic nerve cells the axons of which terminate in the posterior pituitary gland. Its secretion is necessary for the extrusion of milk from the lumen of the mammary alveoli into secretory ducts so that the infant suckling at the nipple can receive milk. In this case, sensory nerve endings in the nipple detect the signal and convey afferent information to the central nervous system, which in turn signals the release of oxytocin from axon terminals in the pituitary gland. Oxytocin causes myoepithelial cells in the breast to contract, resulting in delivery of milk to the infant. When the infant is satisfied, the suckling stimulus at the nipple ceases.

Positive feedback

By positive feedback, it is meant that some consequence of hormonal secretion acts on the secretory cells to provide an augmented drive for secretion. Rather than being self-limiting, as with negative feedback, the drive for secretion becomes progressively more intense. Positive feedback systems are unusual in biology, as they terminate with some cataclysmic, explosive event. A good example of a positive feedback system involves oxytocin and its other effect: causing contraction of uterine muscle during childbirth (Fig. 1.51). In this case the stimulus for oxytocin secretion is dilation of the uterine cervix. Upon receipt of this information through sensory nerves, the brain signals the release of oxytocin from nerve endings in the posterior pituitary gland. Enhanced uterine contraction in response to

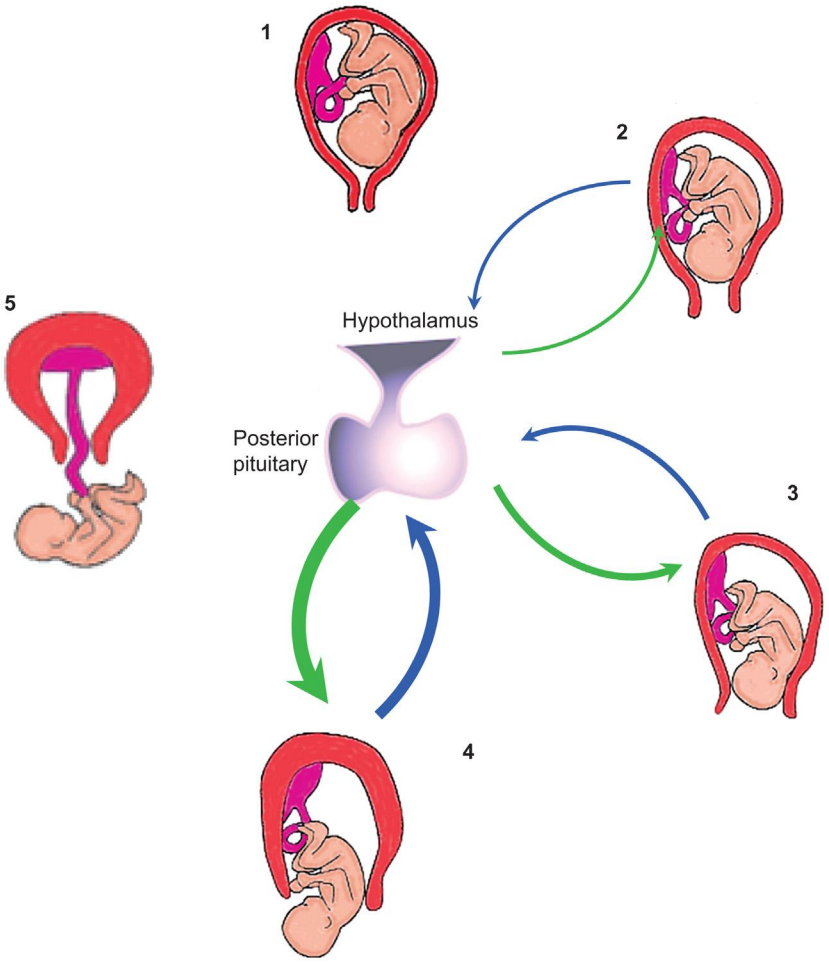

FIGURE 1.51 Positive feedback during labor and delivery.

oxytocin results in greater dilation of the cervix, which strengthens the signal for oxytocin release and so on until the infant is expelled from the uterine cavity.

Feed forward

Feed-forward controls can be considered as anticipatory or preemptive and prepare the body for an impending change or demand. For example, following a meal rich in glucose, secretory cells in the mucosa of the gastrointestinal tract secrete hormones that signal the pancreas to secrete insulin (see Chapter 6: Hormones of the Gastrointestinal Tract, and Chapter 7: The Pancreatic Islets). Having increased insulin already in the blood by the time, the glucose is absorbed thus moderates the change in blood glucose that might otherwise occur if insulin were secreted after the blood glucose concentrations started to increase. Unlike feedback systems, feed-forward systems are unaffected by the consequences of the changes they evoke and simply are shut off when the stimulus disappears.

Measurement of hormones

Whether it is for the purpose of diagnosing a patient's disease or research to gain understanding of normal physiology, it is often necessary to measure how much hormone is present in some biological fluid. Chemical detection of hormones in blood is difficult. Except for the thyroid hormones, which contain large amounts of iodine, there is no unique chemistry that sets hormones apart from other bodily constituents. Furthermore, hormones circulate in blood at minute concentrations, which further complicates the problem of their detection. Consequently, the earliest methods developed for measuring hormones were bioassays and depend on the ability of a hormone to produce a characteristic biological response. For example, induction of ovulation in the rabbit in response to an injection of urine from a pregnant woman is an indication of the presence of the placental hormone chorionic gonadotropin and is the basis for the rabbit test that

was used for many years as an indicator of early pregnancy (see Chapter 14: Hormonal Control of Pregnancy and Lactation). Before hormones were identified chemically they were quantitated in units of the biological responses they produced. For example, a unit of insulin is defined as one-third of the amount needed to lower blood sugar in a 2-kg rabbit to convulsive levels within 3 hours. Although bioassays are now seldom used, some hormones, including insulin, are still standardized in terms of biological units. Terms such as milliunits and microunits are still in use.

Immunoassays

As knowledge of hormone structure increased, it became evident that peptide hormones are not identical in all species. Small differences in amino acid sequence, which may not affect the biological activity of a hormone, were found to produce antibody reactions after prolonged administration. Hormones isolated from one species were recognized as foreign substances in recipient animals of another species, which often produced antibodies to the foreign hormone. Antibodies are exquisitely sensitive and can recognize and react with tiny amounts of the foreign material (antigens) that evoked their production, even in the presence of large amounts of other substances that may be similar or different. Techniques have been devised to exploit this characteristic of antibodies for the measurement of hormones, and to detect antibody–antigen reactions even when minute quantities of antigen (hormone) are involved.

Radioimmunoassay

Reaction of a hormone with an antibody results in a complex with altered properties such that it is precipitated out of solution or behaves differently when subjected to electrophoresis or adsorption to charcoal or other substances. A typical radioimmunoassay takes advantage of the fact that iodine of high specific radioactivity can be incorporated readily into tyrosine residues of peptides and proteins and thereby permits detection and quantitation of tiny amounts of hormone. Hormones present in biological fluids are not radioactive but can compete with radioactive hormone for a limited number of antibody-binding sites. To perform a radioimmunoassay, a sample of plasma containing an unknown amount of hormone is mixed in a test tube with a known amount of antibody and a known amount of radioactive iodinated hormone. The unlabeled hormone present in the plasma competes with the iodine-labeled hormone for binding to the antibody. The more hormone present in the plasma sample, the less iodinated hormone can bind to the antibody. Antibody-bound radioactive iodine then is separated from unbound iodinated hormone by any of a variety of physicochemical means, and the ratio of bound to unbound radioactivity is determined. The amount of hormone present in plasma can be estimated by comparison with a standard curve constructed using known amounts of unlabeled hormone instead of the biological fluid samples (Fig. 1.52).

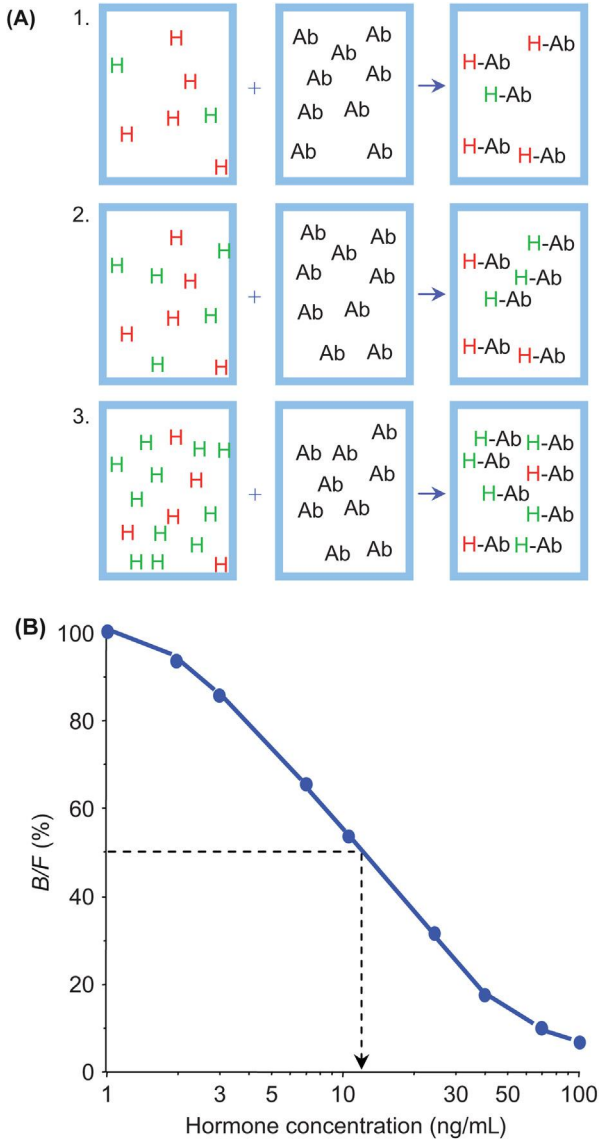

FIGURE 1.52 (A) Competing reactions that form the basis of the radioimmunoassay. Labeled hormone (*H*, shown in *red*) competes with the hormone in a biological sample (*green* H) for a limited amount of *Ab*. As the concentration of hormone in the biological sample rises (rows 1, 2, and 3), decreasing amounts of the labeled hormone appear in the *H-Ab* complex and the ratio of *B*/*F* labeled hormone decreases. (B) A typical standard curve used to estimate the amount of hormone in the biological sample. A *B*/*F* ratio of 50% corresponds to 12 ng/mL in this example. *Ab*, antibodies; *B*/*F*, bound/free; *H-Ab*, hormone–antibody.