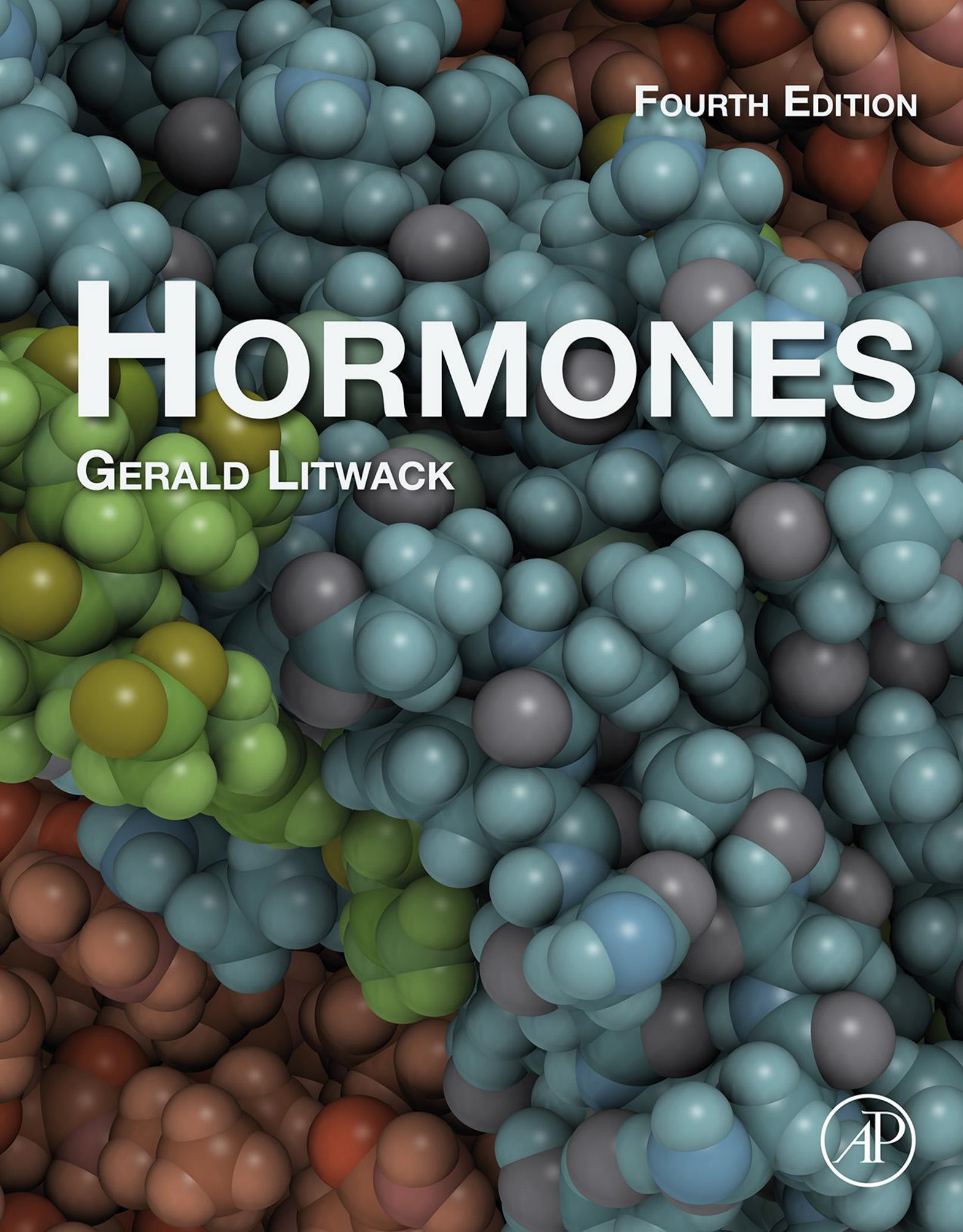
FOURTH EDITION
HORMONES
GERALD LITWACK

Hormones

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Hormones

Fourth Edition

Gerald Litwack, PhD

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Dedication

**For my family: Ellie, Geoffrey and Susanna, Kate and Andrew,
Claudia, Debbie, David and my very young granddaughter,
Olivia, who seems to like books.**

**Also, in remembrance of Anthony (Tony) Norman and his wife,
Helen Henry, both of whom contributed to previous editions of this book.**

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Contents

About the author

Gerald (Gerry) Litwack was educated at Hobart College (BA) and graduated from the University of Wisconsin, Madison (MS and PhD in biochemistry). After spending a summer at the University of Wisconsin as an instructor in a course on enzymes, he sailed to Paris (Liberte) where he spent a year as a postdoctoral fellow of the National Foundation for Infantile Paralysis at the Laboratoire de Chimie Biologique of the Sorbonne. He then became an Assistant Professor of Biochemistry at Rutgers University, later becoming an Associate Professor at the Graduate School of Medicine of the University of Pennsylvania. Future posts took him to the Fels Institute for Cancer Research and Molecular Biology at the Temple University School of Medicine where he was the Laura H. Carnell Professor of Biochemistry and the Deputy Director of the Institute. Later, he was the Chairman of the Department of Biochemistry and Molecular Pharmacology, a Professor of Biochemistry, Vice Dean for Research, and the Director of the Institute for Apoptosis at Thomas Jefferson University Kimmel Medical College with future appointments as a Visiting Scholar at the Geffen School of Medicine at UCLA, the Founding Chair of the Department of Basic Science of the Geisinger Commonwealth School of Medicine, and, as his final position, a Professor of Molecular and Cellular Medicine and the Associate Director of the Institute for Regenerative Medicine at Texas A&M University School of Medicine. Dr. Litwack was a Sabbatical Visitor at the University of California, Berkeley, the University of California, San Francisco, the Courtauld Institute of Biochemistry (London), and the Wistar Institute. He was appointed as the Emeritus Professor and/or Chairman at Rutgers University, Thomas Jefferson University, and Geisinger Commonwealth School of Medicine. He has published more than 350 papers in scientific journals, is named on more than 20 patents, and held numerous editorial positions on biochemical and cancer journals. He has authored 3 textbooks in the areas of biochemistry or endocrinology and has edited more than 65 books. Following his retirement, he lives with his family in Los Angeles where he continues his work as an author and editor and paints in watercolor.

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Preface to the fourth edition

The untimely death of Dr. Helen Henry in 2018 followed by the death of her husband, Dr. Anthony (Tony) Norman in 2019, the authors of the third edition of *Hormones*, left vacant an author for the fourth edition. Tony Norman and I wrote the first and second editions of this book. We would take turns in working together, sometimes on the East Coast and sometimes on the West Coast. I could not participate in the preparation of the third edition, published in 2014, because I was involved heavily in authoring the first edition of *Human Biochemistry*, a large textbook that was published by Academic Press late in 2018. This book, *Hormones*, originated from a series of lectures that I presented on the subject as part of the biochemistry course for medical students at the Temple University School of Medicine in the 1980s. Originally, I was to be the first author; however, as the book progressed, it was clear that Tony had much more secondary support (secretarial and other) than I had. For example, his people were instrumental in researching data and preparing the extensive Appendices. For this reason, I decided that it was only reasonable that Tony should be the first author.

After I learned, in April of 2020, about the death of Helen and Tony, I volunteered to be the author of the fourth edition of the book, unless Academic Press/Elsevier had other ideas. They professed their interest in my participation and decided that it was time for the preparation of the fourth edition since nearly 6 years had elapsed from the publication of the third edition.

After reviewing the third edition, it was clear that a great deal of the information and many of the figures were still timely. Accordingly, I have retained many portions of the third edition. I have updated references in all chapters and have elaborated on many subjects, either missing from the third edition or mentioned only briefly. In total, I believe that the fourth edition of the book is up-to-date and more informative on several subjects and stronger than previous editions.

There are a number of changes and additions in this edition. Some of these changes involve up-to-date revisions, enlarged discussions, and, in a few cases, corrections. The most major of these additions concerns a much greater emphasis on hormone receptors and their signaling pathways. A great deal of this information is conveyed in new figures; therefore this edition is longer than previous ones.

I am greatly indebted to Pat Gonzalez, Senior Project Manager at Elsevier, for her ability to cope with my rudimentary method of adding new information to the existing text. She, in addition to Peter B. Linsley of Elsevier, also facilitated the second edition of *Human Biochemistry* (Academic Press) published in 2021. The first edition of *Human Biochemistry* was published late in 2018.

The cover of this edition is graced by a background of molecular structures extending to its entirety. These are the structures of a molecule of follicle-stimulating hormone (FSH) bound to its receptor. This hormone is one of two gonadotrophs (FSH and LH) important in the regulation of development, growth, and reproduction.

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May 26, 2021

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Chapter 1

Hormones: an introduction

I Overview of hormones

A Introduction

The term "hormone" is derived from the Greek *hormon*, the present participle of *impel*, or *set in motion*, an apt characterization of these potent molecules. "Endocrine" is also derived from the Greek: *endo-* for *internal* or *within* and *krinein* meaning *separate*. This term conveys the distance of the site of secretion from the site of action that characterized the systems, such as the pancreas, the thyroid, and the reproductive glands that were studied in the early days of endocrinology.

The cellular constituent that is the immediate recipient of the hormone is the receptor, an entity importance of which is now so dominant in the study of hormones that it is hard to imagine that the existence and nature of these molecules were not appreciated until the early 1970s. The biochemical organization of receptors is diverse but each receptor is structurally organized so that it can specifically recognize and interact with its hormone. Because of the low circulating concentrations of the hormones, the receptor must have a very efficient "capture" mechanism for its hormone. As a consequence of the receptor−hormone interaction (however, transient it may be), signal transduction occurs and a specific biological response(s) is generated within and, in some instances, around the target cell—that is, the cell responds to the presence of the hormone.

The domain of endocrinology includes the study of how, in a higher organism, cell A communicates with cell B by sending a chemical messenger or hormone. A detailed understanding of a particular endocrine system includes an understanding of the following: (1) the anatomical description of cells A and B and their immediate environment, as well as the distance between A and B; (2) the chemical structure of the hormone (H); (3) the details of the biosynthesis of the hormone by cell A; (4) the mode of transfer of H from cell A to cell B; (5) the detailed mechanism by which cell B uses receptors to detect the presence of H; (6) how cell B transduces the presence of H to initiate and sustain a biological response; and (7) how cell B communicates via a feedback loop with cell A to indicate the adequate presence of the hormone.

The study of endocrinology over the past century has been dependent upon the scientific methodologies available to probe the various endocrine systems. Thus in the interval 1900−60, endocrinology was largely pursued at the physiological level. This resulted in the discovery of approximately 25 hormones. The time it took to achieve structural understanding of a hormone was usually inversely proportional to the size of the hormone. For example, the complete structure of thyroxine (molecular weight 770) was defined in 1926, while the sequence and structure of the small protein hormone insulin was not obtained until 1953 (amino acid sequence) and 1969 (three-dimensional structure).

The biochemical era of endocrinology began in approximately 1955−60 and extends to the present time. The availability of radioactive isotopes of carbon (^{14}C), hydrogen (^{3}H), phosphorus (^{32}P), among others, coupled with advances in chemical methodology (chromatography, mass spectrometry, nuclear magnetic resonance spectroscopy, and X-ray crystallography) has led to the detection and chemical characterization of minute quantities (nanograms or picograms) of new hormones and the characterization of many receptors. Now we are experiencing the cellular and molecular biological era of endocrinology. We have an increased ability to visualize how molecules behave in cells, through fluorescent dye tagging, confocal microscopy, and other imaging advances. The sequencing and continuing analysis of the human genome has expanded our knowledge of the molecular players (hormones and receptors) and their evolutionary relationship to each other. The ability to generate mouse models with specific genetic attributes that can be expressed as a function of development stage or other variables helps to fine-tune our functional understanding of hormonal processes. As is always the case in scientific inquiry, new information results in new questions, which leads to new techniques to answer them.

The objective of this book is to provide a status report on the field of human hormones, viewed in the light of our current understanding of cellular and subcellular architecture, as well as the molecular details of their mode of action. In this chapter, some of the first principles of hormone action are presented as a foundation for

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the more specific considerations of individual hormonal systems in the chapters to follow.

B Review of animal cell structure

To describe the details of the synthesis of hormones and their interactions with target cells in the chapters to follow, it is appropriate to present a brief review of cellular organization. A typical animal cell is shown in Fig. 1.1.

1 Nuclear organization

In eukaryotes the nucleus, containing the chromatin, and the cytoplasm are separated, except during interphase of mitosis. The nuclear envelope, consisting of two membranes separated by a small space, is perforated by nuclear pores through which the transport of macromolecules, proteins, and RNA, between the two major compartments of the cell, takes place. For example, messenger and transfer RNA as well as ribosomal subunits must move from the nucleus to the cytoplasm (Fig. 1.2) and proteins that participate in the synthesis, repair, and transcription of DNA must move into the nucleus from the cytoplasm. The latter include the steroid hormone receptors and other proteins that regulate gene transcription that will be discussed in the following chapters.

Fig. 1.3A shows the fundamental organization of DNA, beginning with the structure of the double helix in the top panel. This is the form in which DNA is found except when it is being transcribed or replicated, at which times the two strands of the double helix are separated. Inside the nucleus is the nucleolus where the DNA-encoding ribosomal RNA is continually being transcribed. The remainder of the DNA in the eukaryotic nucleus is present in a more tightly packed form, arising from an association between the DNA (with its negatively charged sugar–phosphate backbone) and basic, positively charged proteins called histones; the final step in making DNA

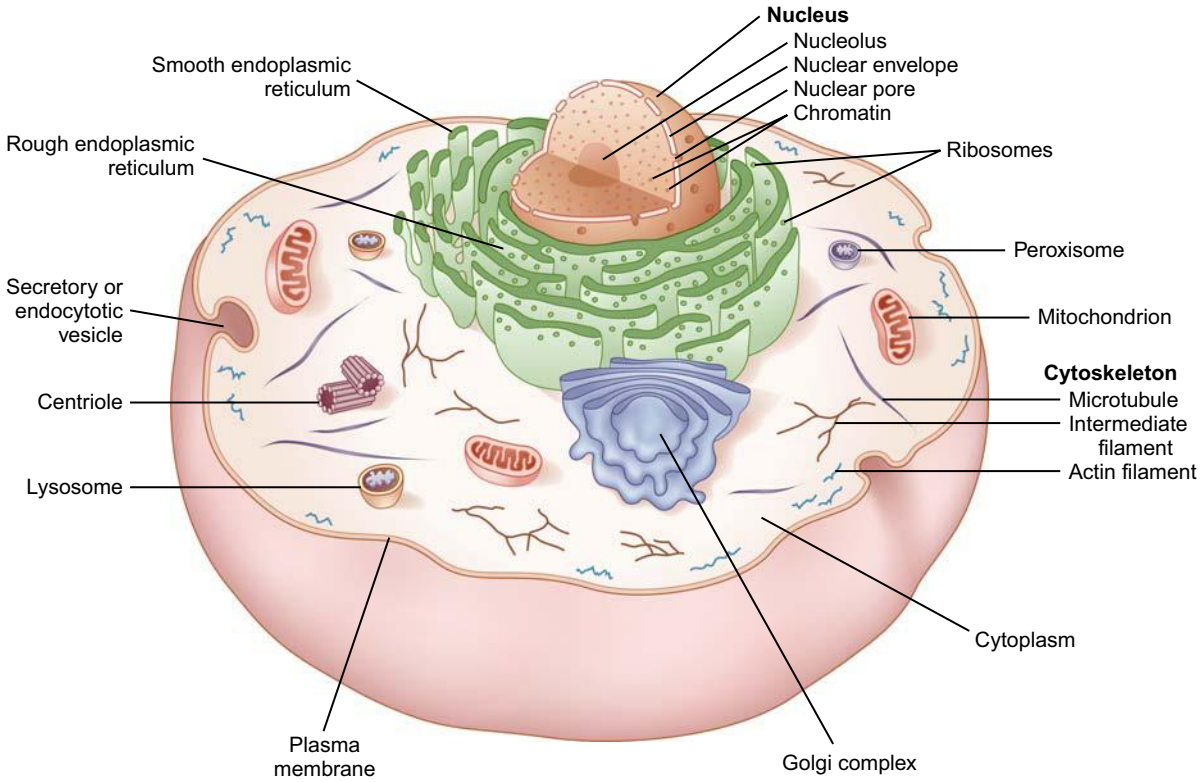

FIGURE 1.1 Structural elements of animal cells. The major features shared by animal cells are shown. In eukaryotes the nuclear membrane separates the genetic material, the chromatin, from the cytoplasm. Molecules move between the two compartments through nuclear pores and a portion of the chromatin (see Fig. 1.3), the nucleolus, is dedicated to the continual production of ribosomes. The cytoplasmic organelles depicted include the smooth endoplasmic reticulum (the microsomes of fractionated cells), which carries out metabolic conversions of carbohydrates and lipids, and the rough endoplasmic reticulum, associated with ribosomes that are synthesizing proteins to be secreted by the cell. These proteins are collected and processed in the Golgi apparatus. Mitochondria generate energy for the cell's function from the products of the metabolism of carbohydrates, fats, and proteins. Peroxisomes and lysosomes degrade molecules that are no longer needed or are potentially harmful. Elements of the cell's cytoskeleton are shown, including the centriole, part of the organizing center for microtubules. The plasma membrane is described in Fig. 1.5.

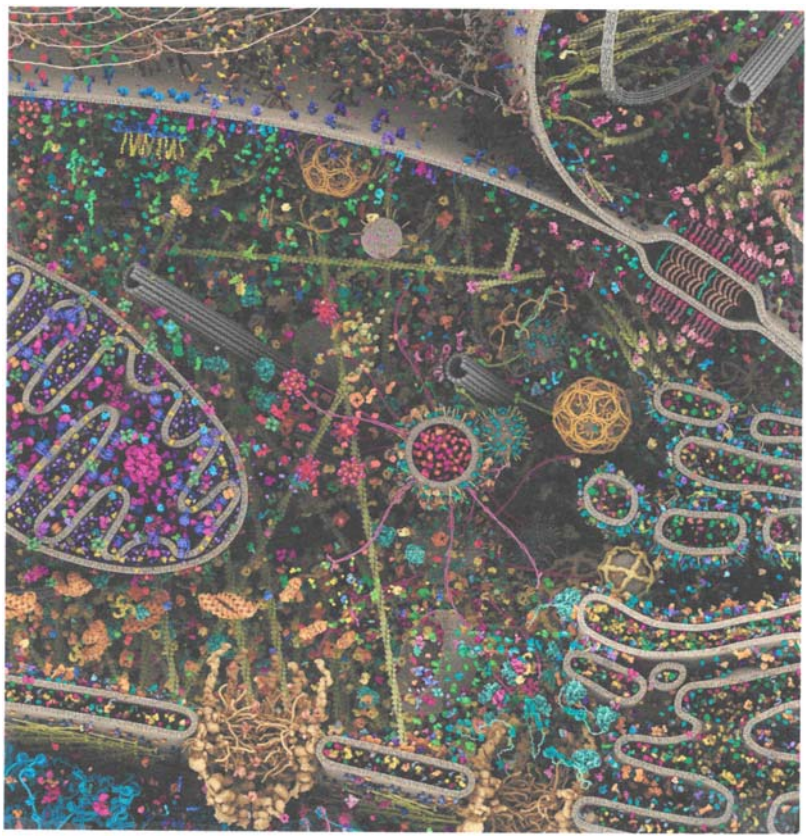

FIGURE 1.2 This is the most detailed image of a human cell. It was achieved by the combined usage of three methodologies: X-ray, nuclear magnetic resonance, and cryo-electron microscopy. The image is described as cellular landscape cross-section through a eukaryotic cell and is the work of Evan Ingersoll and Gael McGill. *Reproduced from https://gaelmcgill.artstation.com/projects/Pm0.Jl.1.*

accessible for transcription involves modification of histone proteins (acetylation) to loosen their association with the DNA. Further steps in the coiling and compaction of DNA are illustrated in Fig. 1.3B. The result of this process is the packing of a linear molecule of DNA that is about 10^5 μm (micrometer) long into a nucleus with a diameter of about 10 μm (Fig. 1.3B).

Fig. 1.4 illustrates details of right-handed double-stranded DNA.

2 The plasma membrane

Although the precise content of substances that comprise the cell membrane differs in different cell types, many components are common to all membranes. These are lipids (including phospholipids, cholesterol, and glycolipids), proteins, and glycoproteins. The cell membrane that encloses the cell resembles the internal membranes, such as those associated with the nucleus, mitochondria, and microsomes.

The primary lipid component of cellular membranes consists of the amphipathic phospholipids containing a polar head group, such as choline, ethanolamine, or serine and a hydrophobic tail consisting of two long-chain fatty acids, usually one saturated and one unsaturated (see Fig. 8.5B). In an aqueous environment, these molecules form a bilayer with the hydrophobic tails on the inside and the polar head groups on the outside, as shown in Fig. 1.5. Cholesterol molecules are interspersed among the fatty acid side chains; the amount of cholesterol, which can have effects on local membrane fluidity, varies with the type of membrane.

Many different types of proteins, seen in Fig. 1.5, are found in or associated with the plasma membrane. In many chapters in this book, we will be considering the actions of membrane hormone receptors that have both extracellular and intracellular domains, and we will look at the structure and function of these integral proteins in some detail. We will also be looking at intracellular proteins that are partially embedded in the lipids of the inner face of the plasma membrane as well as some that are more loosely associated with it.

Complex oligosaccharides may appear on the outer surface as derivatives of sphingosine or other lipids (glycolipids). Proteins may have complex polysaccharides attached to them. Complex polysaccharides that may appear on the surface of the plasma membrane are pictured in Fig. 1.6A. These carbohydrate moieties may play important recognition functions or in the case of

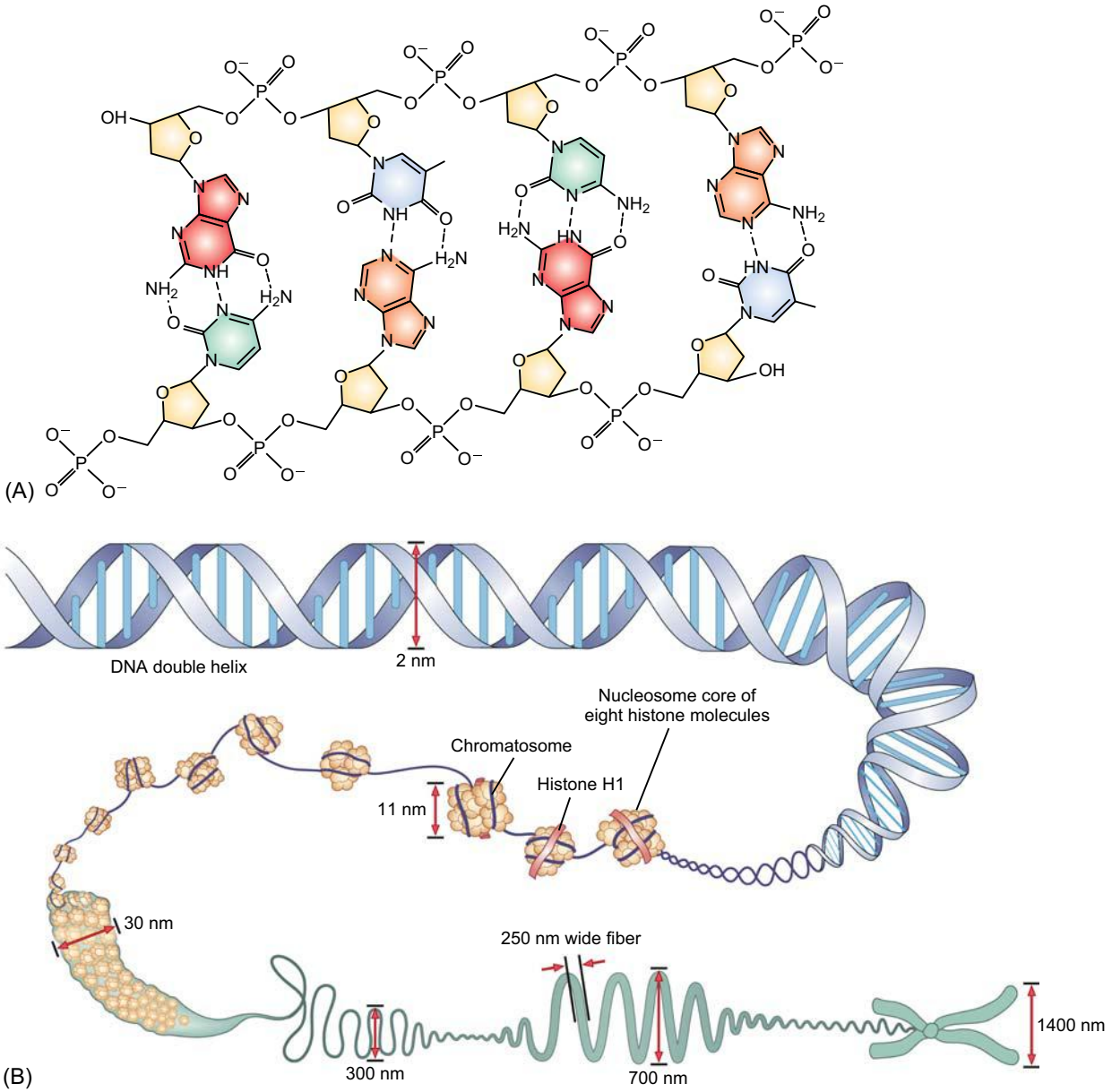

FIGURE 1.3 Organization of DNA. (A) *Double-helical DNA*. The chemical nature of the DNA double helix is shown for a stretch of four base pairs. The negatively charged sugar–phosphate backbone is shown in yellow. Each purine, adenine (A, *orange*), or guanine (G, *red*) pairs with a pyrimidine, thymine (T, *blue*) or cytosine (C, *green*), respectively. The two DNA strands are complementary and antiparallel to one another. The double-helical structure of DNA is stabilized by the hydrogen bonds between the bases on each strand, two for each AT base pair and three for each GC base pair as well as by interactions between the stacked bases in the interior of the helix. (B) *Organization of DNA in chromosomes*. The compaction of double-helical DNA into a chromatid of a chromosome is shown. The first step is the coiling of the double helix of DNA around a core of histone proteins to form the core nucleosome. Histone H1 joins these "beads on a string," 11 nm across, to promote their coiling upon themselves to form a 30-nm fiber. Further structural details are not completely understood, but include 300-nm loops and further coiling of these into the 700-nm chromatid.

membrane receptors for hormones may influence the accessibility of receptors for their ligands.

3 Intracellular organelles

Some of the other intracellular organelles that will be encountered in the cells that make or respond to hormones are illustrated in Figs. 1.1 and 1.2. For example, the synthesis and secretion of protein and peptide hormones depend on the rough endoplasmic reticulum and Golgi apparatus and the specific processing enzymes therein. Lysosomes play an important role in the secretion of thyroid hormones (THs), and both the mitochondria and the smooth endoplasmic reticulum (the microsomal fraction of the cell) are the sites of steroid hormone synthesis in the adrenal gland, gonads, and placenta.

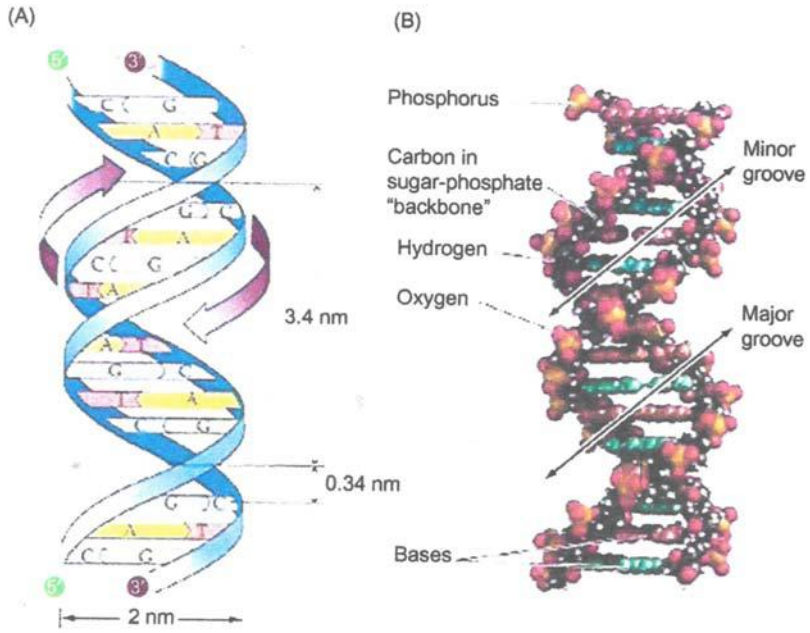

FIGURE 1.4 (A) A right-handed DNA double helix. Down the center of the structure is the axis of symmetry. The diameter of the helix is about 2 nm. One 360-degree turn of the helix takes about 8–10 base pairs and occupies a space of 3.4 nm. The antiparallel character of the two strands is shown by the 5′ end of one strand opposing the 3′ end of the antiparallel strand at the top and the bottom. In (B) the various groupings are indicated. There is a major groove and a minor groove. Many of the transactivating proteins (DNA-binding proteins with a specific function) bind in one of these grooves of DNA to the promoter of a specific gene. The deoxyribose phosphate backbone occurs on the outside of the helix and the hydrophobic bases are located in the interior. The negative charges on the phosphate groups in the backbone attract positively charged motifs on DNA-binding proteins. *Reproduced from Fig. 10.15 of G. Litwack (Ed.), Human Biochemistry, Academic Press, 2018.*

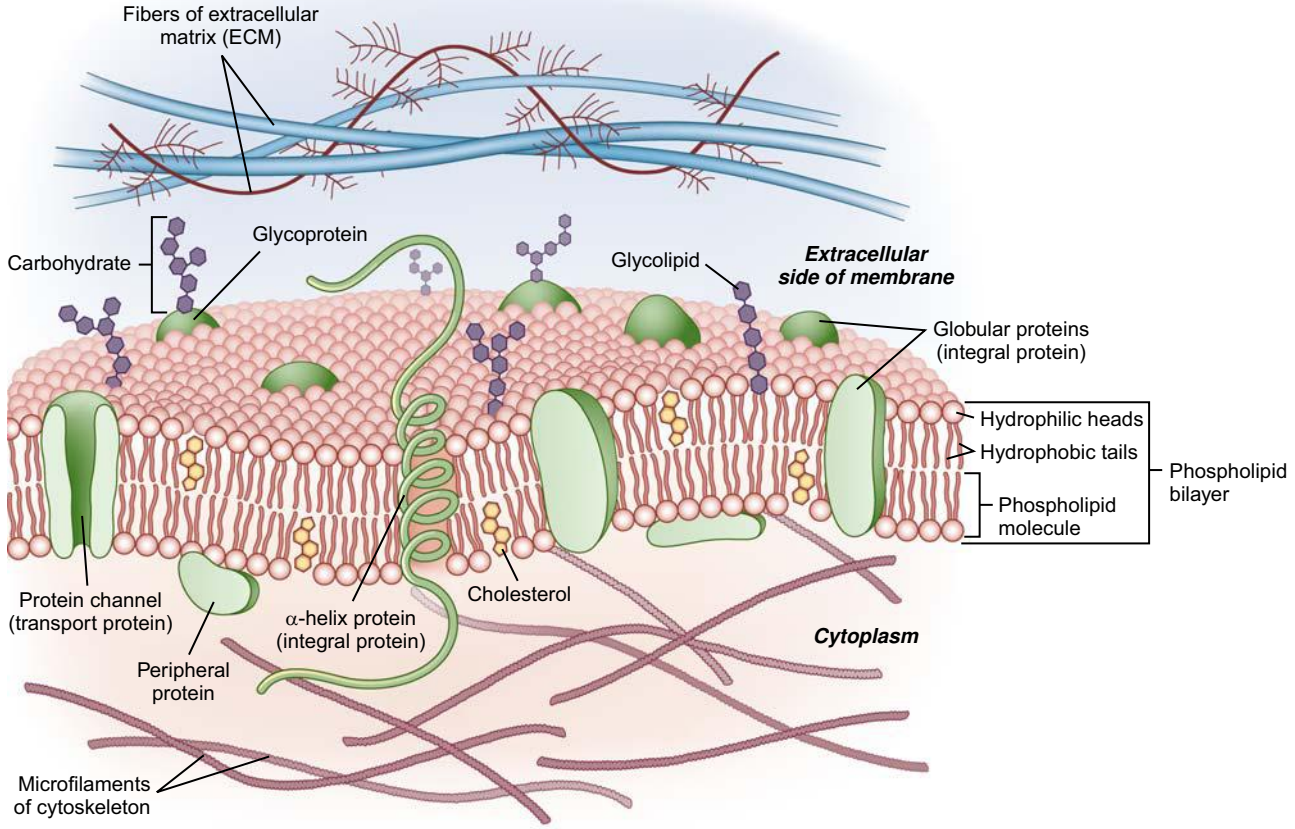

FIGURE 1.5 The plasma membrane. The phospholipids that make up all cellular membranes are oriented with their polar head groups (usually choline, ethanolamine, or serine) facing the aqueous environments inside and outside the cell and their hydrophobic fatty acid side chains toward the interior of the bilayer. Cholesterol molecules (*yellow*) are interspersed among the lipids in both layers. Integral membrane proteins traverse the bilayer as illustrated by the transport protein/channel on the left or the helical protein in the middle; peripheral proteins are embedded in only one side of the bilayer; surface proteins are associated with one face of the membrane but not embedded in it. The exterior surface of the cell displays, in addition to proteins, carbohydrate moieties attached to membrane lipids (glycolipids) and to proteins (glycoproteins).

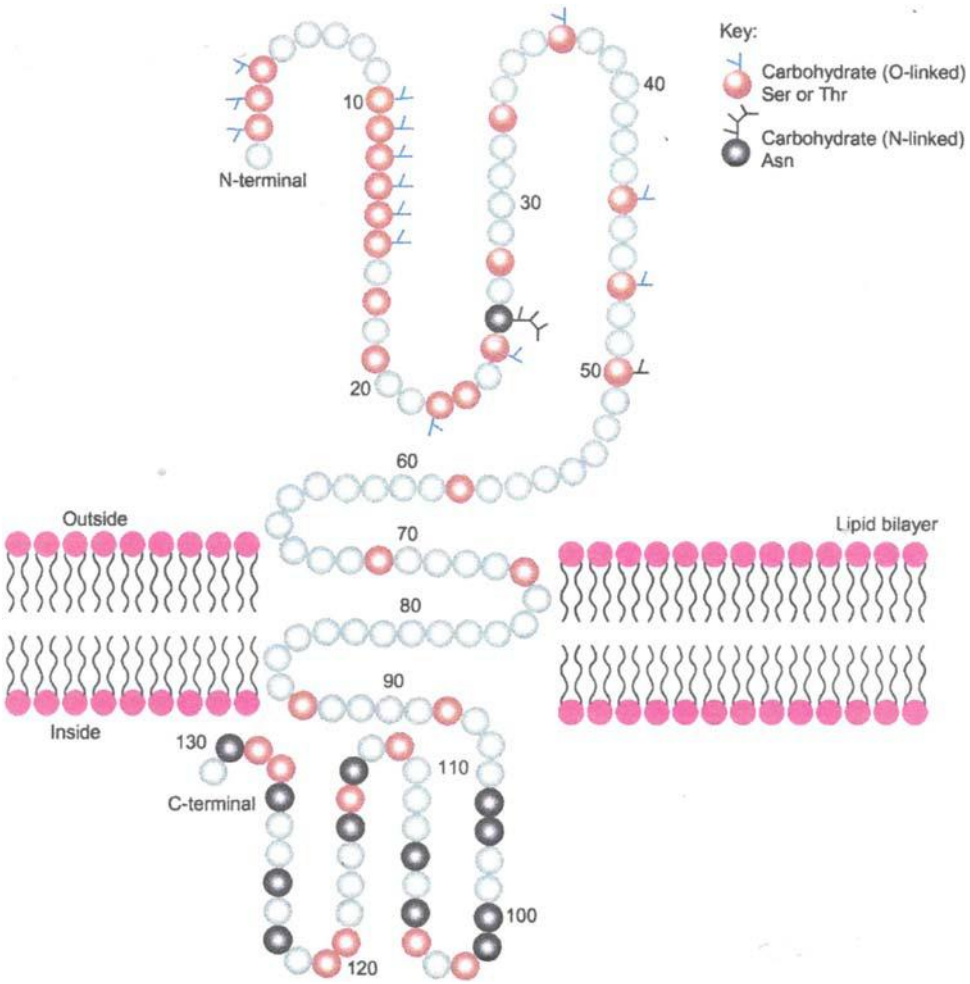

FIGURE 1.6 Diagram of a transmembrane protein with sugar substitutions in the extracellular domain. Sugars that frequently appear in glycoproteins are glucose, galactose, mannose, fucose, and acetylated amino sugars, such as *N*-acetylgalactosamine, *N*-acetylglucosamine, and *N*-acetylneuraminic acid (NANA or sialic acid). Sugars can be O-linked through Ser or Thr or N-linked through Asn. *Reproduced from Fig. 6.29 of G. Litwack, Human Biochemistry, Academic Press, 2018.*

C Hormones and their communication systems

1 Types of hormone molecules

Hormones are heterogeneous in their molecular size, chemical properties, and pathways of synthesis. Nitric oxide (NO; see Chapter 15: Hormones Related to the Kidney and Cardiovascular System) is at one extreme of the size range; the pituitary gonadotropins (Chapter 3: The Hypothalamus and Anterior Pituitary) consisting of two subunits are among the largest of the protein hormones with molecular weights ranging between 25 and 36 kDa, depending on the extent of added carbohydrates (glycosylation). Peptide or protein hormones range from 3 amino acids [thyrotropin releasing hormone (TRH), Chapter 3: The Hypothalamus and Anterior Pituitary] to over 100 per subunit. TH (Chapter 5: Thyroid Hormones) and epinephrine (Chapter 11: Hormones of the Adrenal Medulla) are derived from the amino acid tyrosine. Steroid hormones, and vitamin D and its metabolites are derived from cholesterol or 7-dehydrocholesterol, respectively (Chapter 2: Steroid Hormones: Chemistry, Biosynthesis, and Metabolism). Arachidonic acid, cleaved from membrane phospholipids, is the main precursor of the prostaglandins and other eicosanoids (Chapter 8: Eicosanoids).

The initial step in the action of a hormone, the interaction with its receptor, depends to some extent on its chemical nature. Peptide and protein hormones have receptors that are membrane-spanning proteins so that the molecule does not have to enter the cell but can deliver its message on the outside where it will be conveyed to the interior of the cell by structural changes in the receptor protein. Steroid hormones, considered to be soluble in the phospholipid bilayer, can enter the cell so that the receptors for these hormones are located either in the cytoplasm or the nucleus of the cell. The actions of these hormones are propagated by interaction of the receptor with nuclear proteins and DNA. The amino acid–derived hormones differ from one another: TH has an intracellular receptor similar to those for the steroid hormones and epinephrine interacts with its membrane receptor.

Thus the hormonal messaging systems have evolved using a variety of types of molecules and mechanisms of

actions. Understanding these in settings of particular systems is a major focus of this book.

2 Types of hormonal communication systems

Hormones are chemical messengers that send a signal within a physiological system from point A (secretion) to point B (biological action). Three variations on the anatomical and, therefore, functional relationship between point A and point B of these systems are illustrated in Fig. 1.7.

The classic systemic endocrine system is shown in the top panel. The hormone is biosynthesized (and perhaps, but not necessarily, stored) within specific cells associated with an anatomically defined endocrine gland. Upon the receipt of an appropriate physiological signal, which may take the form of either a change in the concentration of some component in the blood (e.g., another hormone, Ca^{2+}, glucose) or a neural signal, the hormone is released into the circulation. It is transported in the bloodstream to one or more target cells, which are defined as targets by the presence of a specific high-affinity receptor, either on the membrane or within the cell, for the hormone. It is what the receptor does after interacting with the hormone that determines the biological response. As will be seen in several of the chapters in this book that many, if not most, hormones have numerous and diverse target cells and the response to the hormone may vary with cell type, indicating that other players in or around the target cell may affect the outcome of hormone–receptor interactions.

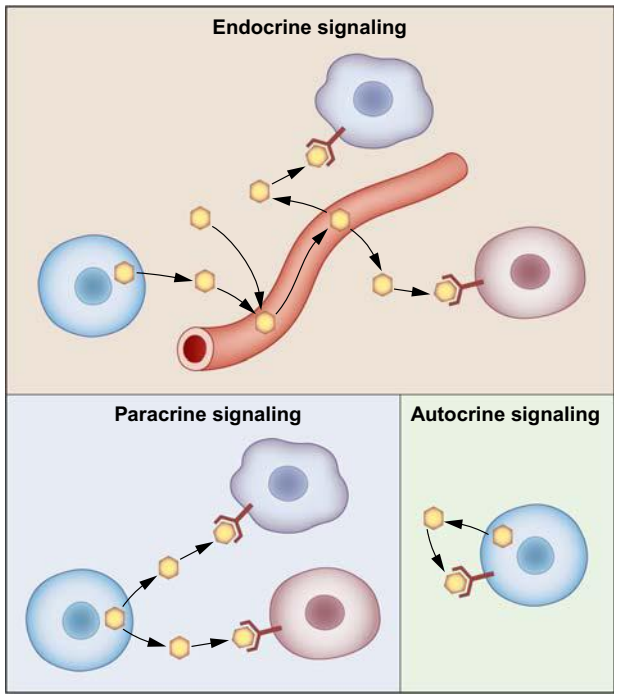

FIGURE 1.7 Types of hormonal signaling. Three of the ways that hormones secreted by one cell can carry out its signaling function are illustrated. The top panel shows the classical endocrine system with a specialized hormone-synthesizing cell secreting its product into the bloodstream. It is carried throughout the body and may interact with one or many distant target cells, which are distinguishable by the presence of a specific receptor for the hormone on its surface (shown) or within the cell (not illustrated). In paracrine signaling (lower left), the signaling cell, which also has many other functions, releases the hormone into the intracellular space and it moves small distances to nearby cells. These target cells also have a specific receptor for the hormone (membrane or intracellular). In paracrine signaling the cells reached by the hormone may be of the same or different types. Finally, cells that secrete the hormone and also have receptors that bind and respond to it are displaying autocrine signaling, as shown on the lower right of the figure.

In some or portions of some endocrine systems, the hormone-secreting cell releases its product not into the general circulation but into a closed system, such as the hypothalamic–pituitary portal system. In this case the hypothalamic-releasing hormones are released into and diluted by a limited volume, ensuring that most of the hormone molecules will be delivered to the anterior pituitary, which contains their target cells (Chapter 3: The Hypothalamus and Anterior Pituitary; Fig. 3.5).

The lower left panel of Fig. 1.7 shows a type of hormonal communication system that does not involve the circulatory system at all. In paracrine systems, hormones secreted from the signaling cell interact with specific high-affinity receptors in neighboring cells that are reached by diffusion: that is, the distance from point A to point B is decreased and dilution in the bloodstream is avoided. As with endocrine systems, the nearby target cells may be all the same type or may differ from each other as illustrated. Most prostaglandins (Chapter 8: Eicosanoids) act through paracrine mechanisms. Several, if not all, of the steroid hormones act by paracrine in addition to endocrine mechanisms. For example, in the testis testosterone is not only released into the blood from the interstitial cells in which it is produced but also diffuses to nearby seminiferous tubules to support the production of sperm (Chapter 12: Androgens). IGF-1 (Chapter 3: The Hypothalamus and Anterior Pituitary and Chapter 17: Growth Factors) is a protein hormone secreted into the bloodstream by the liver in response to growth hormone but is also secreted by other cells to control the growth and differentiation of neighboring cells.

Finally, some cells both produce and respond to the same hormone. This type of system is referred to as autocrine. Examples of these systems involve growth factors and the control (or lack thereof in malignancy) of cellular proliferation.

D Biosynthesis of peptide and protein hormones

The biosynthesis of hormones occurs in specialized cells, usually present in endocrine glands, which express the enzymes that catalyze the steps of their formation and have any other necessary molecules required. Chapter 2,

Steroid Hormones: Chemistry, Biosynthesis, and Metabolism, describes the production of the steroid hormones, while Chapter 5, Thyroid Hormones, Chapter 8, Eicosanoids, and Chapter 11, Hormones of the Adrenal Medulla, describe the biosynthesis of the THs, eicosanoids, and epinephrine, respectively.

Protein and peptide hormones are biosynthesized in specific cells, through the well-known processes of transcription of a specific message encoded in the DNA of the gene for the protein and the translation of the RNA message (mRNA) into a protein. As with other proteins, variations in modifications to the initially produced mRNA and/or protein leads to deviation from the original "one gene, one protein" concept. The biosynthesis of peptide and protein hormones yields many examples of such deviations.

It is now quite well recognized that not only does one gene not lead to a single protein, but also one gene does not lead to a single RNA; that is, two or more RNA transcripts can arise through alternative processing of a single primary transcript. Fig. 1.8 shows schematically how this happens. Exons are joined by splicing them together at very specific sites. Splice site recognition can vary from one cell to another, causing the primary transcript to differ between two cell types. The production of either calcitonin (CT) or CT gene–related peptide was one of the first examples of alternative splicing to be elucidated (see Fig. 9.10). Alternative splicing is by no means an unusual method of generating multiple products of the same gene. While the exact percentage of protein coding genes subject to alternative splicing is not yet known, recent genomic analyses suggest that this number may be as high as 90%.

Another layer of variability in the final product of a gene is the posttranslational processing of the initial protein product. Broadly speaking, this term includes the myriad modifications of the side chains of the amino acids as well as the addition of sugar or lipid moieties to the protein backbone. For this discussion, however, we will confine our attention to alteration of the initially translated protein by proteolytic cleavage, yielding smaller protein or peptide products. These cleavages are catalyzed by one of a family of proprotein convertases (PC1–PC7), serine endoproteases at cleavage sites in the precursor protein that are designated by two basic amino acids (Lys–Lys, Arg–Arg, or Lys–Arg). The reactions take place largely in the rough endoplasmic reticulum and in the Golgi apparatus as the hormone is being prepared for movement into secretory vesicles.

Fig. 1.9 illustrates some examples of the posttranslational cleavage events that yield active hormones. Most simply, virtually all hormones (and other secreted proteins) are synthesized as pre- or pre-pro-hormones, that is, with one or two sequences to be removed, usually prior to secretion. The first of these is generally a signal for the initial intracellular localization of the new protein molecule. The mature form of parathyroid hormone (PTH, Fig. 9.9) contains 84 amino acids from which pre- and prosequences of 25 and 6 amino acids, respectively, have been removed.

The second example in Fig. 1.9 shows a precursor protein that contains within its sequence several biologically

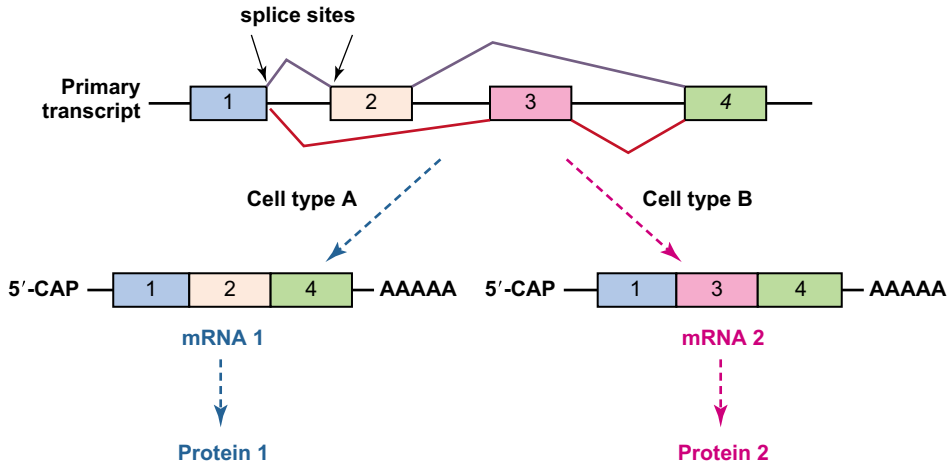

FIGURE 1.8 Alternative splicing of mRNA for hormones. In eukaryotes almost all genes for proteins consist of regions of DNA that carry the code for the protein (exons; *colored boxes*) interrupted by noncoding sequences (*gray line*) in the primary mRNA transcript. Maturation of the primary transcript involves the splicing of these coding regions together as well as the addition of the 5′ cap and the poly A tail typical of eukaryotic mRNA. The splicing of the exons takes place in the nucleus and is carried out by large RNA/protein complexes called spliceosomes. It is the spliceosomes that are responsible for splice site (specific DNA sequences) selection. In the example shown the spliceosomes of one cell type use the splice sites between exons 1, 2, and 4, while those in cell type 2 use exons 1, 3, and 4. The two mature RNAs, thus, encode different proteins. See Fig. 9.10 for the example of calcitonin and calcitonin gene–related peptide. *mRNA*, RNA message.

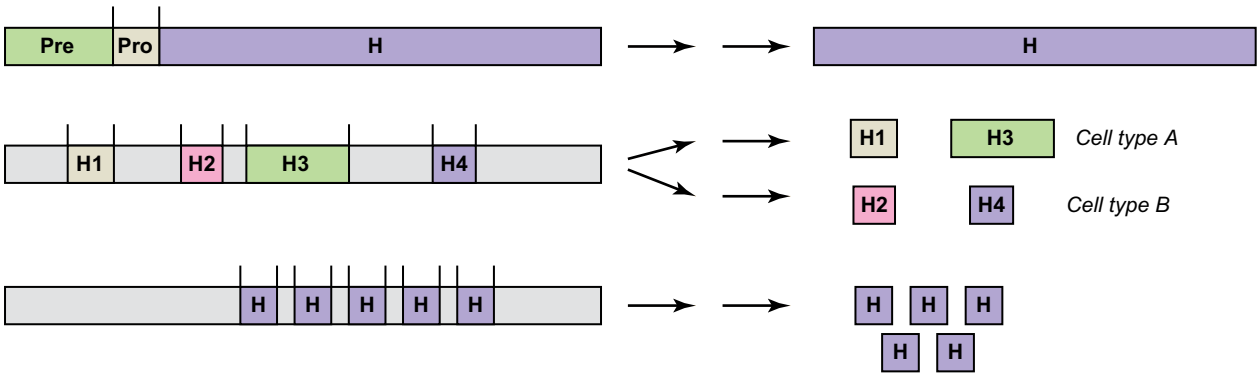

FIGURE 1.9 Processing of pre- and prohormones. Many protein and peptide hormones (right) are synthesized within a larger precursor protein (left), of which three examples are shown here. The process is catalyzed by specific proteases that cleave the protein at specific sites (vertical lines), usually preceded by two basic (Lys, Arg) amino acids. Much of this processing takes place in the endoplasmic reticulum and Golgi apparatus and in secretory vesicles prior to the secretion of the hormones. As illustrated in the top example, many hormones are synthesized with one or two N-terminal portions that are sequentially removed to form the active hormone; see parathyroid hormone, Fig. 9.9. In the second example, several different active peptides are within a single precursor protein, which is processed differently in different cell types; see POMC, Fig. 3.15. Third, a precursor protein can contain several copies of the hormone, each of which is excised at a pair of specific proteolytic sites, as in the case of the tri-peptide, TRH (Fig. 3.9). *POMC*, Proopiomelanocortin; *TRH*, thyrotropin releasing hormone.

active peptides, and which can be differentially processed in different cell types. Such a situation is exemplified by proopiomelanocortin (Fig. 3.15). Adrenocorticotrophic hormone and other hormones are the processing products in pituitary corticotrophs, whereas a different of set of peptides, including β-endorphins, result from the processing of the same precursor in the cells of the intermediate lobe of the brain.

Finally, the precursor protein can contain several copies of a single peptide hormone, as is the case for TRH (Fig. 3.9). This example, as well as that of insulin (not shown in Fig. 1.9 but see Fig. 6.5), played important roles in the establishment of the idea that precursor protein molecules harbor active peptides within their sequences. In the case of insulin, this theory, based on the increasing availability of information from protein sequencing followed by that of DNA sequencing, solved the long-standing question of the origin of the two subunits of insulin. As shown in Fig. 6.5, it is now understood that insulin is synthesized as a single molecule. Disulfide bonds are formed to join two portions of the molecule and proteolytic cleavages release the two joined subunits from the proprotein.

E Regulation of hormone synthesis, secretion, and serum levels

1 Control of synthesis and secretion

The production and/or secretion of most hormones are regulated by the homeostatic mechanisms operative in that particular endocrine system. The secretion or release of the hormone is normally (in the absence of an endocrine disease related to hormone secretion) related to the requirement for the biological response(s) generated by the hormone in question. Once this requirement has been met, the secretion of the hormone is curtailed to prevent an overresponse. Thus a characteristic feature of most endocrine systems is the existence of a feedback loop that limits or regulates the secretion of the hormonal messenger.

Two general categories of endocrine feedback systems are illustrated in Fig. 1.10: those in which the function achieved by the hormone (e.g., elevated serum Ca^{2+} or elevated blood glucose) directly feeds back upon the endocrine gland that secretes the hormone; and those involving the central nervous system (CNS) and hypothalamus. On the left is shown the first case, a simple but effective system, in which changes in the circulating amount of something of physiological importance, in this case serum Ca^{2+}, is both the biological response and the agent that exerts negative feedback inhibition on the gland producing the hormone that caused its increase. Although the actual control of PTH is considerably more complex than shown in this figure (see Chapter 9: Calcium-Regulating Hormones: Vitamin D, Parathyroid Hormone, Calcitonin & Fibroblast Growth Factor-23), the secretion of the hormone in response to low serum Ca^{2+} and its cessation when this cation returns to normal levels is at the heart of the regulation of PTH. Another example of this type of control is the stimulation of insulin by elevated levels of blood glucose and the fall of the hormone when glucose levels fall in response to its actions.

On the right side of Fig. 1.10 is shown a generalized version of a hypothalamic–pituitary–peripheral gland axis, of which several will be encountered in the following chapters. Under the control of numerous areas in the

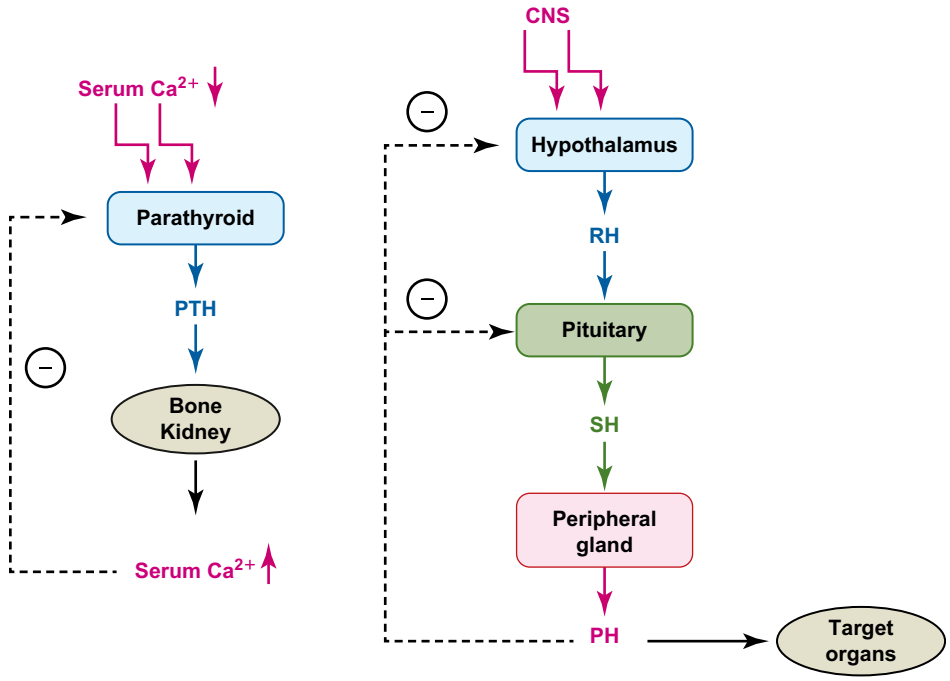

FIGURE 1.10 Models of the regulation of hormone secretion. Two general models of the physiological homeostatic control of hormone secretion are shown. On the left is the type of negative feedback exemplified by the secretion of PTH by the parathyroid gland. The stimulus for the secretion of PTH is a drop in serum Ca^{2+} below the threshold of normality. A calcium sensor in the parathyroid gland cell detects this drop and sets into motion events leading to increased synthesis of PTH and its secretion into the bloodstream. At its target cells, PTH stimulates the movement of Ca^{2+} into the blood and the negative feedback effect of normal circulating levels of the cation result in reduced production and secretion of PTH. See Chapter 9, Calcium-Regulating Hormones: Vitamin D, Parathyroid Hormone, Calcitonin & Fibroblast Growth Factor-23, and especially Fig. 9.12 for more details regarding this system. On the right is a generic version of the hypothalamic–pituitary–peripheral organ axis seen for the hormones of the thyroid gland, gonads, adrenal cortex, and other pituitary hormones. In these systems the hypothalamus receives input from many different areas of the CNS and responds by secreting a hormone (RH, or, in some cases a release-inhibiting hormone) that stimulates specific cells of the pituitary to secrete a peptide hormone that stimulates a peripheral gland (SH). This peripheral gland secretes another hormone, PH, which acts on its target cells to bring about the appropriate biological response and at the same time exerts negative feedback effects on the hypothalamus and/or the pituitary to turn off the system. As will be seen in the chapters devoted to these systems, the actual controls are considerably more complex than depicted here, but the underlying blueprint for them is constant. *CNS*, Central nervous system; *PH,* peripheral hormone; *PTH*, parathyroid hormone; *RH*, releasing hormone; *SH,* secreted hormone.

CNS, specific neurons of the hypothalamus secrete a given hormone (e.g., TRH) that, rather than entering the general circulation, enters the hypothalamic–pituitary portal system and stimulates the secretion of a particular peptide hormone (e.g., thyroid stimulating hormone). This hormone is released into the circulation and travels to its target peripheral endocrine gland (e.g., the thyroid) where it stimulates the release of that gland's hormone (e.g., TH). TH has many target tissues in which it brings about biological responses, but most important in the context of the current discussion are its feedback effects on the hypothalamus and pituitary to shut off the stimulatory hormones from these glands. Again, there are many variations on this basic theme which will be encountered in the consideration of the thyroid gland (Chapter 5: Thyroid Hormones), the gonads (Chapter 12: Androgens and Chapter 13: Estrogens and Progestins), and the adrenal cortex (Chapter 10: Adrenal Corticoids).

The cellular and molecular details of how the synthesis and secretion of hormones are regulated by the players described earlier and others will be covered in the relevant chapters. Here it is important to note that while usually the emphasis is on the increased synthesis of hormones as a point of regulation, there are many other possible regulatory points and these vary with the type of hormone. For example, the steroid hormones (excluding vitamin D metabolites) are regulated primarily at the first step in their synthesis (the cleavage of the side chain of cholesterol; see Chapter 2: Steroid Hormones: Chemistry, Biosynthesis, and Metabolism) and are released as synthesized, not stored in the gland. TH, on the other hand, is stored in large quantities within the thyroid gland. The short-term regulation of its secretion is on the secretory process, while the synthetic process takes place over a longer time frame. Peptide hormones, such as insulin, PTH, and the trophic hormones of the pituitary, are stored in varying amounts in the glands, so the relative roles of synthesis and secretion in the regulatory processes also vary among these hormones.

Two other contributors to the biological availability of hormones deserve mention here. One is the conversion of

a relative inactive hormone to an active one in its target glands as occurs with TH and, in some cases, testosterone. Second, the removal of active hormone from the blood must occur as part of the attenuation of its effect (in addition to shutting off the flow of new hormone into the blood). Thus the half-life of an active hormone in the blood, which can vary from seconds to days, is important in understanding its regulatory dynamics.

2 Binding proteins

As discussed in Chapter 2, Steroid Hormones: Chemistry, Biosynthesis, and Metabolism, most steroid hormones have limited solubility in plasma due to their intrinsic hydrophobic character; accordingly, steroid hormones (see Table 2.5) as well as TH (see Table 5.2) are largely (99%) bound to specific plasma transport proteins (PTP), which are synthesized in the liver. Each transport protein has a specific ligand-binding domain (LBD) for its cognate hormone. These ligand domains display little amino acid sequence homology with the ligand binding of the cognate receptors. Nevertheless, the PTP LBD also displays a high affinity (see Section II.D) for its ligand: usually the K_d for the PTP ligand is 10−100 × lower than the K_d of the hormone's receptor.

The current view is that it is the "free" form of steroid hormones and not the complex of the hormone with its PTP that interacts with receptors in or on the target cells to begin the sequence of steps, which results in the generation of a biological response. For some endocrine systems, the concentration of the plasma transport protein can be subject to physiological regulation; that is, the concentration of PTP can be either increased or decreased. Thus changes in the amount of PTP can alter the amount of free hormone in the blood, as well as affect the total amount of hormone in the blood. This role of the binding proteins in the availability of steroid and THs can be of considerable physiological relevance in clinical situations.

II Hormone receptors

A Introduction

When a hormone arrives at a target cell, the first step in delivering its message is interaction with a specific protein receptor. It is the presence of this receptor in the cell that renders it a target for the hormone. All receptors have two key components: (1) a ligand-binding domain that noncovalently but stereospecifically binds the correct hormone for that receptor and (2) an effector domain that responds to the presence of the hormone bound to the ligand domain and initiates the generation of the biological response(s). The interaction between the ligand-binding domain and the effector domain is most likely achieved by a conformational change in the receptor protein so that the effector site may interact with other cellular constituents to initiate the next steps in the signal transduction process (see Section III). In general, steroid hormones and TH interact with receptors that are within the cell, either the nucleus or the cytoplasm (although some form of steroid receptors may reside in the plasma membrane and may show a preference for steroid analogs that differs from the classic cytoplasmic receptor), whereas protein hormones, prostaglandins, and the catecholamines interact with the extracellular ligand-binding domains of plasma membrane−spanning receptors. Exceptions to this generalization exist and will be pointed out when they are encountered. Since mechanisms exist to inactivate and remove both hormone and receptor molecules to curtail their signaling, there is a continuing need for the renewal (biosynthesis and secretion) of the hormone by the endocrine gland and of the receptor by the population of target cells.

B Membrane receptors

Membrane receptors for hormones and other extracellular signals have three clearly identifiable domains: the extracellular component, the membrane-spanning component, and the intracellular component. Each domain has biochemical properties reflecting its location and function. Frequently the membrane receptor comprises a single polypeptide chain where the N-terminus lies outside the cell and the C-terminus lies inside the cell. Others, such as ion channel receptors, are composed of subunits. The diameter of a typical cell membrane is 100 Å, requiring 20−25 amino acid residues organized into an α-helix to cross the membrane once. Since the membrane is hydrophobic, it is not surprising that a receptor's membrane-spanning region consists largely of hydrophobic and noncharged amino acids. Membrane receptors are broadly classified by the number of membrane-spanning regions (for our purposes, one or seven) and by what the cytoplasmic portion of the receptor does when an activating ligand binds. In this section the structures of one type of seven- and one type of single-membrane spanning membrane receptor will be considered. The signaling by these and other receptors will be considered in Section III.A.

1 Protein-coupled receptors

The most frequently encountered class of receptors in the context of hormones is the diverse group of G protein (for guanine nucleotide−binding protein)−coupled receptors for which about 800 genes exist in the human genome. Each of these is specific for ligand and response. G protein−coupled receptors (GPCRs) are found in virtually all eukaryotes and participate in many different cellular functions. About half of the GPCR genes encode receptors

that have olfactory functions. About 350 GPCRs have hormones, growth factors, and other small molecules as ligands. Fig. 1.11 shows the fundamentals of the structure of these receptors. The receptor itself has seven α-helical membrane spanning regions. This folding generates three extracellular and three intracellular loops. In some GPCRs, palmitoylation of a cysteine residue in the carboxy region results in another loop. The membrane-spanning helices have been shown by X-ray crystallography to cross at angles to one another as depicted on the right side of panel (A) of Fig. 1.11. The N-terminus of GPCRs is highly variable, as expected from the variety of signals to which these proteins respond. On the right side of panel (A) are shown three examples of types of ligand binding. Small molecules and small peptides have access to a cleft within the helices for binding, whereas larger proteins, such as the

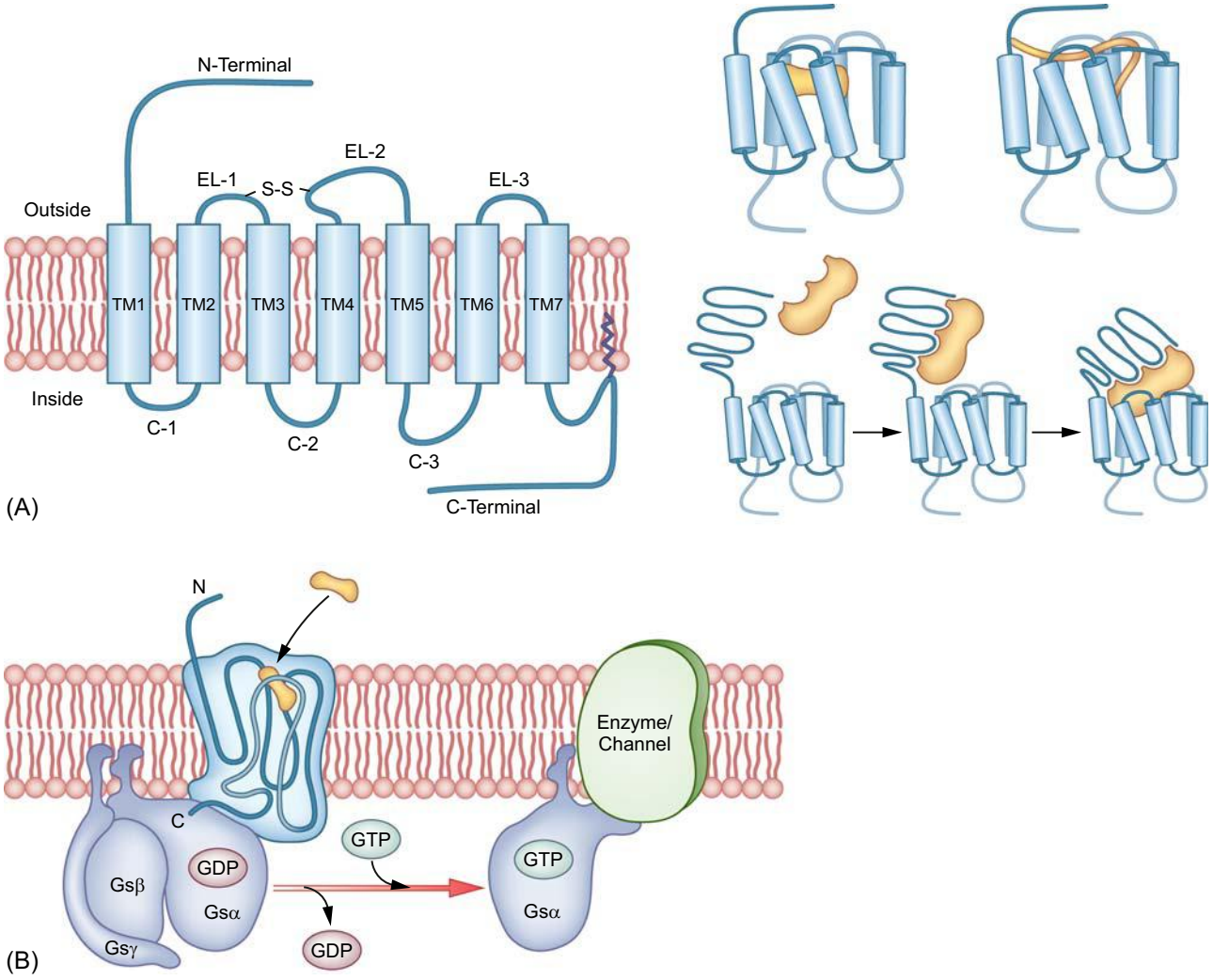

FIGURE 1.11 GPCRs. (A) *General structure of GPCRs*. The GPCRs comprise a large family of proteins that share the main structural features shown in the left side of panel (A). The predominant characteristic of these proteins is the arrangement of their single polypeptide chains into seven membrane spanning regions, creating three extracellular and three intracellular loops. One or more sites on the intracellular C-terminal portion of the cell may be palmitoylated, which plays a role in the receptor's position in the membrane. The right-hand side of panel (A) shows examples of the heterogeneity of the N-terminal portion of GPCRs, reflecting the diversity of ligands for these proteins. Top left: small molecules such as catecholamines or eicosanoids bind to a pocket within the membrane spanning helices; top right, small peptides are partially within a binding pocket but also interact with the extracellular portion of the receptor; bottom, large glycoproteins such as the gonadotrophins or growth factors have binding sites created by the structure of the extracellular portion of the receptor. (B) *Receptor interaction with G-protein*. Inactive G-proteins (left) consist of three subunits in a heterotrimer, α, β, and γ. Two of the subunits, α and γ, have lipid moieties binding them to the membrane and GDP is bound to the α-subunit. When a ligand binds to the receptor and activates it, GDP is replaced with GTP; the α-subunit dissociates from the trimer and moves through the membrane to a nearby protein, an enzyme or ion channel, for example, and activates it, initiating the biological response (see Fig. 1.20). *GDP*, Guanosine diphosphate; *GPCRs*, G protein–coupled receptors; *GTP*, guanosine triphosphate.

glycoprotein gonadotrophins, bind to a site within a longer, more elaborate N-terminus.

The coupling of a GPCR to a G-protein is illustrated in panel (B) of Fig. 1.11. G-proteins are composed of three subunits, α, β, and γ—that is, they are heterotrimeric. The complex is anchored to the membrane by lipid moieties on the α and γ subunits. The contact with the receptor occurs between the cytoplasmic carboxy terminal of the receptor and the α subunit. The α subunit also has a guanine nucleotide−binding site. In the absence of ligand activation, guanosine diphosphate (GDP) occupies this site, and the complex is inactive. When ligand is bound, guanosine triphosphate (GTP) replaces GDP, Gα dissociates from Gβγ and moves through the membrane to a nearby effector protein, such as an enzyme that produces a second messenger or an ion channel, which itself becomes activated upon binding of the α subunit. Gα has intrinsic GTPase activity, which may be aided by nearby GAP (GTPase acceleratory protein) proteins. Gα−GDP quickly finds and binds to a free Gβγ dimer and the inactive heterotrimer is reformed.

As will be discussed in more detail in Section III.A, the biological response that results from a ligand binding to a GPCR depends upon the G-protein attached to the receptor. The human genome encodes about 200 different G-proteins (proteins that bind guanosine nucleotides), a subclass of which are the heterotrimeric proteins ("large G-proteins") described previously.

2 Receptor tyrosine kinases

Receptor tyrosine kinases, or RTKs, are single membrane−spanning receptors and are defined by the presence of tyrosine kinase activity as the main cytoplasmic constituent and initiator of signal transduction. There are 58 receptor tyrosine kinases encoded in the human genome, several of which are important in hormone signaling. Fig. 1.12 illustrates some of the differences in structure seen in this type of receptor. In their monomeric forms, RTKs are single membrane−spanning receptors. However, these receptors dimerize upon the binding of one-ligand molecule, two-ligand molecules, or one-ligand dimer (see Chapter 17: Growth Factors for more details). In some cases, closely related receptors [e.g., epidermal growth factor (EGF) receptor and HER2] in the same cell may heterodimerize. The insulin and IGF-1 receptors, members of the same family, are an exception to this pattern. They exist as dimers of two hemireceptors, each consisting of two subunits, the extracellular α-subunit and the intracellular β-subunit, joined by disulfide bonds. A further set of disulfide bonds joins the two hemireceptors to form the dimerized receptor that then binds one molecule of ligand.

The N-terminal extracellular ligand-binding domains of RTKs consist of one or a few of about 20 structural motifs. In the examples of RTKs in Fig. 1.12, cysteine-rich regions appear in the EGF and insulin/IGF-1 families, whereas the FGFR (fibroblast growth factor receptor) family, along with several others not shown here, consists of a group of immunogammaglobulin-like domains. The carboxy terminals differ primarily in whether the tyrosine kinase catalytic domain is present as a contiguous sequence of amino acids or whether it is interrupted by a stretch of up to 100 non-TK amino acids, as in the insulin/IGF-1 receptors and FGFR. This is referred to as a split tyrosine kinase domain. The insertion has autophosphorylation sites, which suggests that it may be important in interacting with signal-transducing molecules (see Fig. 17.9).

C The nuclear receptor family

The nuclear receptors are a group of ancient evolutionarily related transcription factors. Sequencing of the human genome has revealed 48 members of this class, of which about half appear to be orphans, that is, no activity-modulating ligand has yet been identified for them. We will focus our attention on the 10 separately encoded proteins, activity of which is modulated by the hormones discussed in the following chapters.

The structural organization of the nuclear receptors for the classical steroid hormones, $1\alpha,25(OH)_2$-vitamin D_3, TH, and retinoic acid is shown in Fig. 1.13A. Each of these proteins functions as a DNA-binding protein, regulating, in a ligand-dependent (and sometimes ligand-independent) way, the expression of genes related to the biological response of the hormone (described in Section III.C).

The receptors for TH (TR) and $1\alpha,25(OH)_2$-vitamin D_3 (VDR) are typically found in the nucleus of target cells where they (especially TR) may be bound to corepressor molecules which suppress DNA transcription (see Chapter 5: Thyroid Hormones). These receptors form heterodimers with retinoic X receptor (RXR) (also in the nucleus) to bind to specific DNA sequences. The receptors for cortisol, the glucocorticoid receptor (GR) and aldosterone, the mineralocorticoid receptor (MR) are in the cytoplasm prior to ligand binding, where they are bound to chaperone proteins (heat-shock proteins) that maintain them in an inactive state. Upon ligand binding, they undergo nuclear translocation and homodimerization prior to binding to specific DNA sequences. The receptors for progesterone (PR), androgens (AR), and estrogens (ER) also form homodimers and may either be in the nucleus prior to ligand binding or travel between the two compartments.

As shown in Fig. 1.13A, the nuclear receptors consist of a single polypeptide chain divided into six domains. The N-terminal sequence is highly variable in both

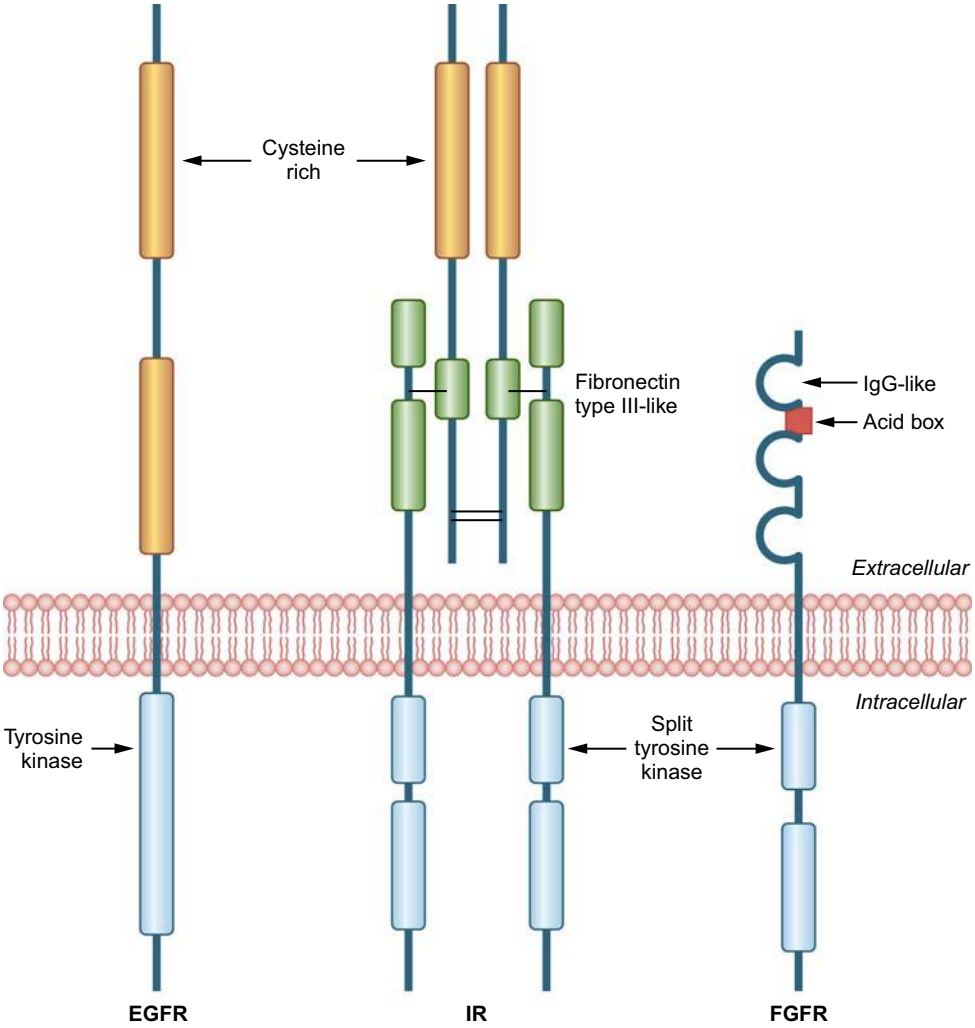

FIGURE 1.12 General structure of receptor tyrosine kinases. The structural features of the RTK are illustrated in three examples: the EGFR; the Ins/IGF-1 receptor; and the FGFR. The RTKs are single membrane spanning proteins with a variable extracellular N-terminal region and a cytoplasmic carboxyl portion that contains the catalytic activity to phosphorylate tyrosines (tyrosine kinase; *blue*) in itself (autophosphorylation) or in nearby proteins. In some receptors (e.g., insulin, FGF), the catalytic domain is split by a nontyrosine kinase sequence. Examples of N-terminal region motifs include cysteine-rich sequences (*gold*), fibronectin type III-like regions (*green*), a series of IgG (*blue*) regions, and the acid box (*red*) as seen between the first and second IgG sequences in the FGF receptor. Most RTKs are monomers that dimerize upon ligand binding. The members of the insulin/IGF-1 family, however, exist as a dimer of disulfide-linked monomers, each consisting of two subunits. See Chapter 17, Growth Factors, for more details on the structures of these and related receptors and their interactions with their ligands. *EGFR*, Epidermal growth factor receptor; *FGF*, fibroblast growth factor; *FGFR*, fibroblast growth factor receptor; *IgG*, immunogammaglobulin; *RTK*, receptor tyrosine kinases.

sequence and length, accounting for the overall difference in size of the receptor proteins. It contains one DNA-binding sequence, termed the AF-1 domain, which not only can regulate gene transcription independently of ligand binding but can also be controlled by ligand binding. This section of the protein can undergo posttranslational modification such as phosphorylation and may also interact with the C-terminal to affect the three-dimensional structure of the protein.

Functionally, the two most critical portions of the receptors are the DNA-binding and ligand-binding (E/F) domains. The three-dimensional organization of both is shown in Fig. 1.13B. The C-domain, on the left, is a highly conserved sequence encoding a two zinc finger motif which is widely used in transcription factors. As seen in Fig. 1.14, the "fingers" resulting from the coordination of a Zn^{2+} atom by four cysteine residues are the contact sites for DNA-binding, one of which carries the amino acid sequence for recognizing the correct specific DNA site (hormone response element, HRE; see Section III.B) for binding.

The E/F domain is composed of 12 α-helices (Fig. 1.13) arranged in approximately the same three-dimensional structure for all the nuclear receptors. It is less highly conserved than the C-domain as it differs

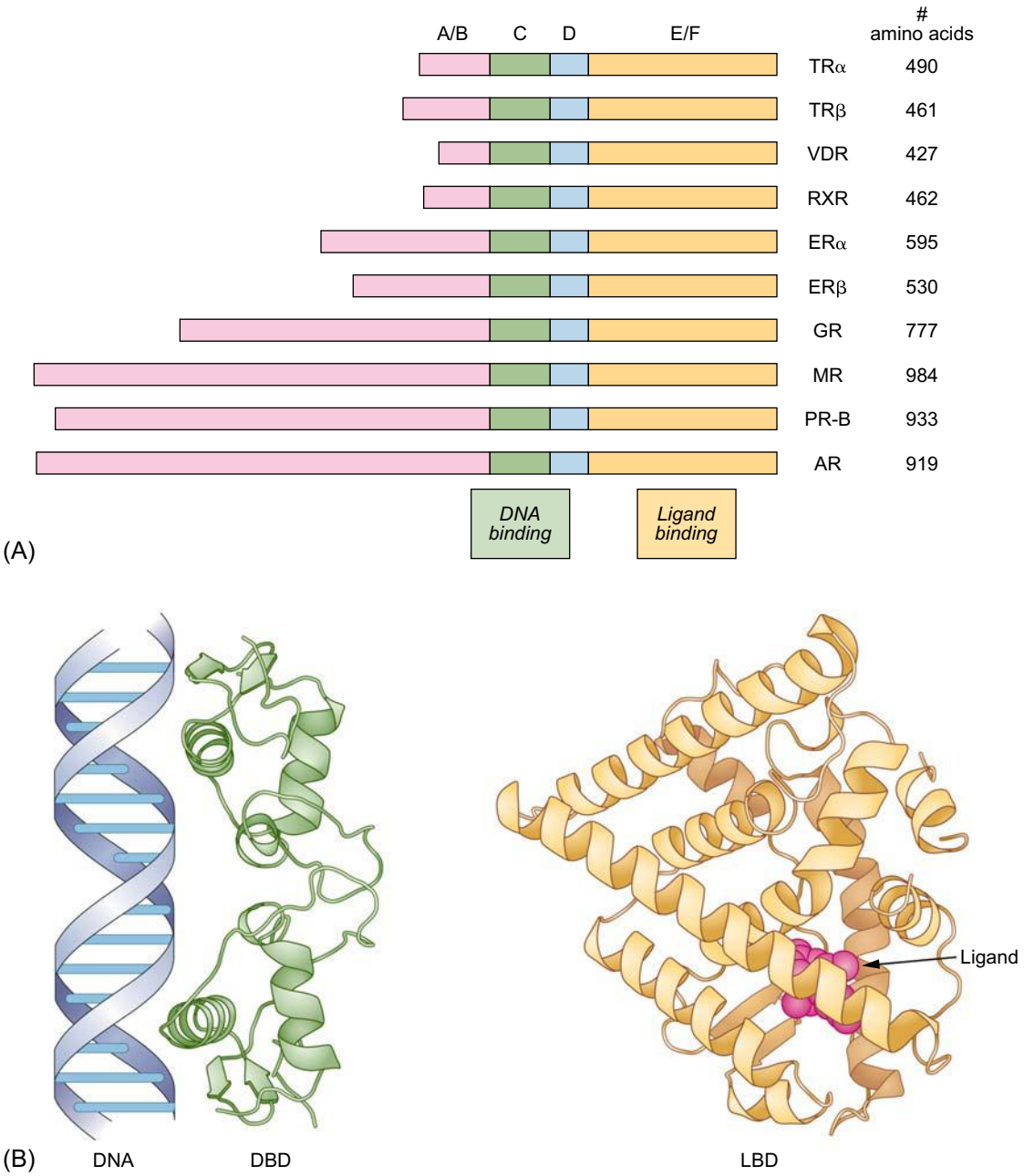

FIGURE 1.13 Nuclear receptor structure. (A) *Primary structural organization*. Shown are the structural features of nuclear receptors for thyroid hormone (TRα and TRβ), 1,25-dihydroxyvitamin D_3 (VDR), the retinoic acid (RXR), estrogen (ERα and ERβ), cortisol (GR), aldosterone (MR), progesterone (PR B), and testosterone/dihydrotestosterone (AR). These receptors share a highly conserved DNA-binding domain (C, *green*) and a short nonconserved region (D, *blue*), which serve as a hinge between the N-terminal and C-terminal portions of the molecule. The ligand-binding domain (*gold*) is less conserved than the DNA-binding domain but is approximately the same size and adopts approximately the same three-dimensional structure in all the receptors. The difference in size between the receptor proteins is the highly variable N-terminal A/B domain (*pink*). Two elements that are necessary for the control of gene transcription, termed activation functions, exist, AF-1 in the A/B domain and AF-2 in the E/F domain. (B) *Three-dimensional structure of the DNA- and ligand-binding domains of the nuclear receptors*. For the DNA-binding domain (left), the interaction of the ER homodimer with DNA is shown. Each DNA-binding site consists of two loops of DNA known as zinc fingers and described more fully in Fig. 1.14. Recognition of the specific DNA sequence to be bound lies within the CI zinc finger (closest to the DNA), whereas CII is involved with receptor dimerization. The ligand-binding domains of the nuclear receptors are less conserved than the DNA-binding domain, but they share many common features. There are 12 α-helices arranged in three layers. The ligand-binding pocket is within the more variable region. In addition to ligand binding, there are sites for a dimerization surface, a coregulator-binding surface, and ligand-dependent transcriptional activation moiety, AF-2. *RXR*, retinoic X receptor; *VDR*, vitamin D_3 receptor.

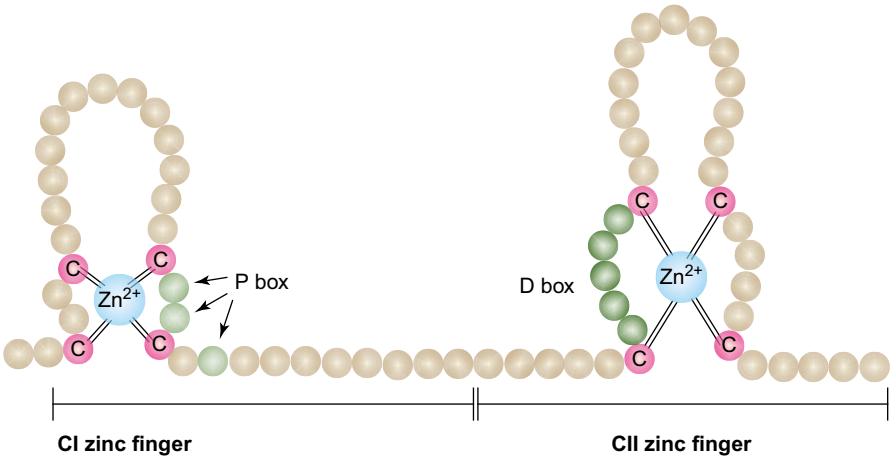

FIGURE 1.14 Steroid hormone receptor zinc fingers. The amino acids in the DNA-binding domain (see Fig. 1.13) of a steroid hormone nuclear receptor are represented by circles. The coordination of a Zn^{2+} atom (*blue*) by four cysteines (*pink*) causes the formation of a loop. One of these, CI, which contains the P-box (*light green*), is involved in binding to the specific DNA-binding site (hormone response element, see Fig. 1.25) and discriminating between closely related sites for different hormones. The D-box (*dark green*) in the second zinc finger, CII, plays a role in receptor dimerization.

among receptors to accommodate different ligands in the ligand-binding pocket. This region also contains the dimerization interface for the formation of both homodimers and heterodimers. Finally, the ligand-dependent transcription function, AF-2, resides in the E/F domain, specifically the C-terminal helix 12. This small helix has a great deal of ligand-dependent flexibility and its position determines the access of nuclear coregulators to the receptor protein (see Section III.C).

D Measurement of hormone–receptor interactions

For every category of hormone (steroid, peptide, or protein) to produce a biological response, it is essential that the hormone physically binds to its partner (cognate) receptor. The receptor is always a protein that has a specific binding domain with high affinity for its ligand. The protein receptor either folds itself around the ligand or the protein's most stable structure/shape is one that is formed as a ligand attempts to bind to its receptor. The binding of a hormone to its receptor is never a covalent linkage; such a bond would be equivalent to turning a light on in a room and forgetting to ever turn it off. The interactions between the receptor and its cognate hormone are facilitated by the formation of a cluster of noncovalent interactions. They can be electrostatic (+ vs. −) or hydrogen bonding (between a hydrogen acceptor and a hydrogen donor) and/or hydrophobic interactions.

$$\text{Hormone} + \text{Receptor} \rightleftharpoons \text{Receptor} - \text{Ligand complex}$$

$$[H] + [R] \underset{k_{-1}}{\overset{k_{+1}}{\rightleftarrows}} [\text{HR}]$$

This reaction can be converted into an equation that mathematically describes the dynamic movement of the forward reaction and the reverse reaction. This is the heart of the Scatchard analysis.

Historically, the principal technique for studying the quantitative interaction of hormones with their receptors in vitro is Scatchard saturation analysis. The technique is dependent upon access to high specific activity radioactive preparations of the hormone under study. Steroid hormones and proteins or peptides can be chemically synthesized to incorporate radioactive carbon (^{14}C; half-life = 5730 years), and/or radioactive hydrogen (^{3}H; half-life of 12.3 years) to make the hormones radioactive for long intervals of time. Neither ^{14}C nor ^{3}H is hazardous to health. There is no radioactive form of oxygen for carboxyl and hydroxyl groups.

Since the cognate receptor has a highly specific ligand-binding domain, under usual incubation conditions, the hormone–receptor complex is formed rapidly, within several minutes at room temperature. The association of H and R to yield HR and the dissociation of HR into H and R are readily reversible processes; that is, it is a dynamic equilibrium since the hormone does not become covalently bound to the receptor. Thus the equilibrium can be expressed in terms of the association constant, K_a, which is mathematically equivalent to 1/dissociation constant (K_d). The individual rate constants k_{+1} and k_{-1} numerically describe the rates of the forward (on-rate) and backward (off-rate) reactions, respectively, as written in the next equation:

$$K_a = \frac{[\text{RH}]}{[\text{H}][\text{R}]} = \frac{k_{+1}}{k_{-1}} = \frac{1}{K_d} \tag{1.1}$$

As shown in Fig. 1.15, hormone bound to receptor is corrected for nonspecific binding of the hormone to other components in the assay. This can be measured conveniently, if the hormone is radiolabeled, by measuring the radioactive receptor without (*brown line* in Fig. 1.15) and with the addition of an excess (100–1000 times) of unlabeled hormone (*blue line*). The excess unlabeled hormone displaces the high-affinity hormone-binding sites but not the low-affinity nonspecific binding sites. Thus when the "radioactive plus

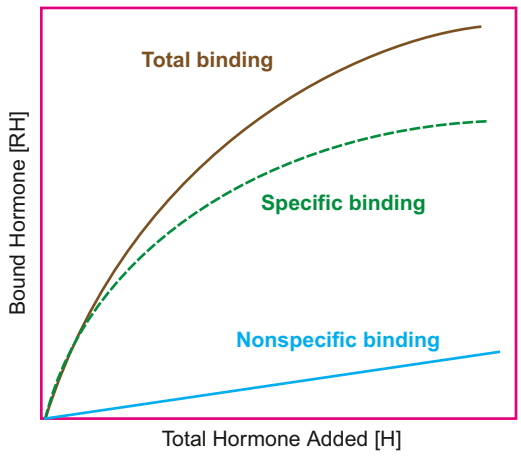

FIGURE 1.15 A typical plot showing the concentration dependency for the hormone [H] under experimental evaluation. The horizontal axis shows increasing concentrations of [H] that generate the data shown on the vertical axis of the increasing amounts of bound hormone [RH]. There are two categories of bound hormone; the brown solid line shows "total binding" and the green dashed line shows the "specific binding." "Total binding" represents the sum of "nonspecific binding" (*bottom solid blue line*) plus "specific binding." The "specific binding" value comes to a horizontal asymptote because there is only a limited amount of receptor. It is crucial to the success of the experiment that there is precisely the same amount of unoccupied receptor in all samples at the start of the experiment. The bottom line labeled "nonspecific binding" increases linearly with the amount of H that was added.

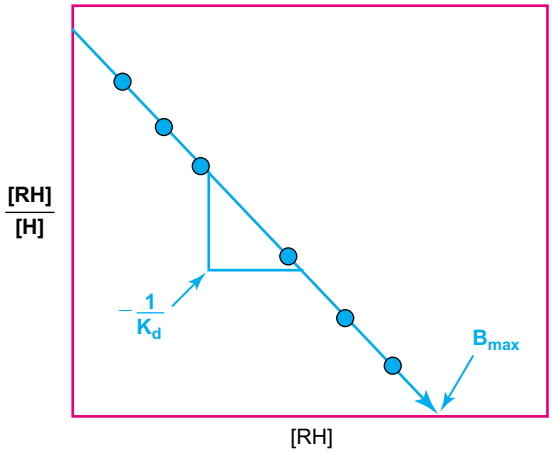

FIGURE 1.16 Typical plot of a Scatchard analysis of specific binding of hormone to its receptor. The vertical axis shows the ratio of bound ligand [RH] to free hormone [H].

nonradioactive" curve is subtracted from the "radioactive" curve, the resulting curve (*green dashed line*) represents specific binding to receptor. This is of critical importance when the receptor is measured in a system containing other proteins. As an approximation, 20 times the K_d value of hormone is usually enough to saturate the receptor.

The Scatchard plot of bound/free = [RH]/[H] on the ordinate versus bound [RH] on the abscissa yields a straight line, as shown in Fig. 1.16. The goal is to determine accurately the numerical value of the dissociation constant, K_d. Most measurements of K_d are made by using the Scatchard analysis, which is a manipulation of the equilibrium equation. The equation can be developed by a number of routes but can be envisioned from mass action analysis of the preceding equation. At equilibrium the total possible number of binding sites (B_{max}) is equal to the unbound plus the bound sites, so that $B_{max} = [R] + [RH]$, and the unbound sites (R) will be equal to $[R] = B_{max} - [RH]$. To consider the sites left unbound in the reaction, the equilibrium equation becomes

$$K_a = \frac{[RH]}{[H](B_{max} - [RH])} \tag{1.2}$$

Thus

$$\begin{aligned} \frac{\text{Bound}}{\text{Free}} &= \frac{[RH]}{[H]} = K_a(B_{max} - [RH]) \\ &= \frac{1}{K_d}(B_{max} - [RH]) \end{aligned} \tag{1.3}$$

When the line in Fig. 1.16 is extrapolated to the abscissa, the intercept gives the value of B_{max} (the total number of specific receptor-binding sites). The slope of the straight line is $-K_a$ or $-1/K_d$.

The K_d values for steroid receptors typically fall in the range of 10^{-10}–10^{-8} M. This very low number is a reflection of how far to the right the reaction between H and R to form RH lies. That is to say, in a mixture of H and R, there is virtually no free hormone and most exists as HR. Also, the low K_d value is a testament to the three-dimensional organization of the ligand-binding domain, which very effectively captures the ligand. These interactions are generally marked by a high degree of specificity so that both parameters describe interactions of a high order, indicating the uniqueness of receptors and the selectivity of signal reception.

From these analyses, information is obtained about the K_d and the maximal number of high-affinity receptor sites (receptor number) in the system. Fig. 1.17 illustrates the regulation of steroid receptor performance for binding the same ligand to three related receptors that have similar but not identical amino acid sequences. The three receptors' K_ds for binding curves *K*, *L*, and *M* are 10^{-10}, 10^{-9} and 10^{-8} M, respectively. If the prevailing hormone concentration bathing the target cell is 10^{-9} M, then depending upon the affinity of the receptor for its ligand, the level of receptor occupancy can vary from 80% (curve *K*) to 50% (curve *L*) to 20% (curve *M*). These differences in receptor performance very likely reflect differences in the amino acid sequence in the interior of the ligand binding domain.

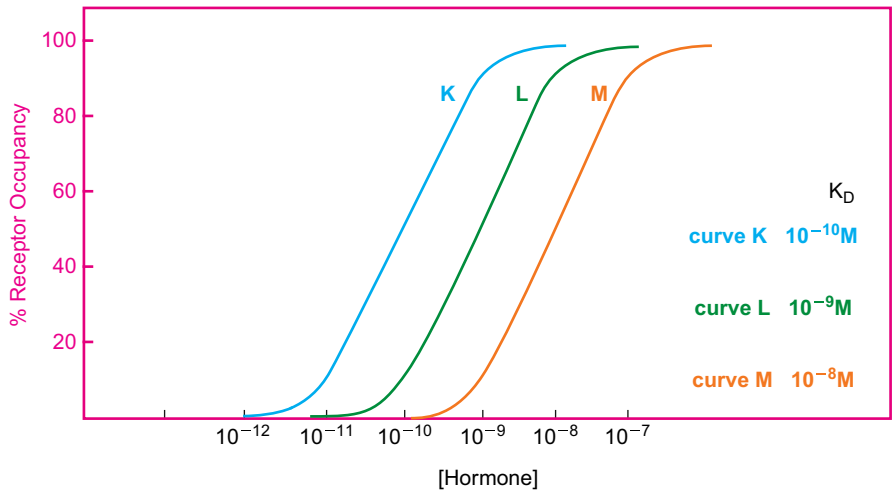

FIGURE 1.17 Regulation of receptor performance, by determination of the K_d. The horizontal line shows the concentration dependency of three different hormones (K, L, and M), which have different affinities for binding to the receptor from 10^{-11} to 10^{-8} M. The specific K_d values for K, L, and M are listed in the right side of the box. It is clear that hormone K has the highest affinity for the binding to the receptor and that hormone M has the lowest affinity for the receptor. This result was obtained by using the Scatchard analysis approach.

III Mechanisms of hormone action

A Cell signaling by membrane receptors

In Section II the structures of some membrane receptors were described, along with the fact that to deliver its message to the cell, the hormone has to cause the receptor to change so that it generates a change within the cell. In this section, we will consider what some of those intracellular changes are and how they initiate a series of events that will bring about a change in the cell's activity, the biological response to the hormone. The realm of intracellular signaling is vast and it will be necessary for us to focus our attention on the portions of systems that are encountered most frequently in the study of hormones.

1 G protein–coupled receptors

As discussed in Section II.B, a heterotrimeric G-protein that has GDP bound to its α-subunit is inactive. As illustrated in Fig. 1.11, when a ligand binds to a GPCR, the receptor changes conformation and interacts with an adjacent G-protein in such a way that the latter exchanges its GDP for a GTP, thus activating the α-subunit. That is, the receptor acts as the guanine nucleotide exchange factor (GEF) that activates this particular G-protein. Different activated α-subunits have different activities. The human genome encodes 16 different α-subunits, along with 5β- and 14γ-subunits. β/γ-subunits appear to be mostly interchangeable with regard to their interactions with α-subunits, but it is now also recognized that these two proteins have some activities of their own, either as a dimer or individually. We will only be concerned with the activities of the α-subunits in the following discussion. Events downstream of the signaling pathways are not presented in detail here but do appear in the chapters dealing with specific hormonal systems.

Fig. 1.19 shows the outcome of receptor-initiated G-protein activation in the case of the three types of α-subunits that will be encountered most frequently in this book. On the left are $G_s\alpha$ and $G_i\alpha$. These proteins interact with the membrane enzyme adenylyl cyclase, which converts one molecule of ATP into one of cyclic AMP (cAMP) (adenosine-3′-5′-cyclic monophosphate) the first intracellular second message to be discovered in the 1970s. For example, Fig. 1.18A shows the interaction of adrenaline binding with the adrenergic receptor (containing G_α, G_β, and G_γ) and its interaction with adenylyl (adenylate) cyclase. Cyclic AMP binds to the regulatory subunits of protein kinase A (PKA) (cyclic AMP-dependent protein kinase) causing the catalytic subunits to become active. At this point the pathway can go in one of several directions, depending on the cell type. All outcomes are dependent on the phosphorylation of protein substrates at specific serine or threonine residues by the activated PKA. Four of these are shown in Fig. 1.19, illustrating the diversity of possible responses to this second messenger, including changes in ion transport, in gene transcription, and in the activity of existing enzyme proteins. The activity of target proteins might be either increased or decreased by phosphorylation. An extracellular signaling agent that triggers the activation of a $G_i\alpha$ protein will have the opposite effect on a pathway that is stimulated by $G_s\alpha$.

On the right side of Fig. 1.19, $G_q\alpha$ is shown interacting with phospholipase C. This enzyme catalyzes the reaction shown in Fig. 1.20, the hydrolysis of the membrane lipid phosphatidylinositol 4,5-bis-phosphate into IP_3 (inositol 1,4,5-triphosphate) and diacylglycerol (DAG), each of which are second messengers. DAG is necessary for the activation of protein kinase C and IP_3 activates the release of Ca^{2+} from intracellular stores in the endoplasmic reticulum. This divalent cation acts as yet another

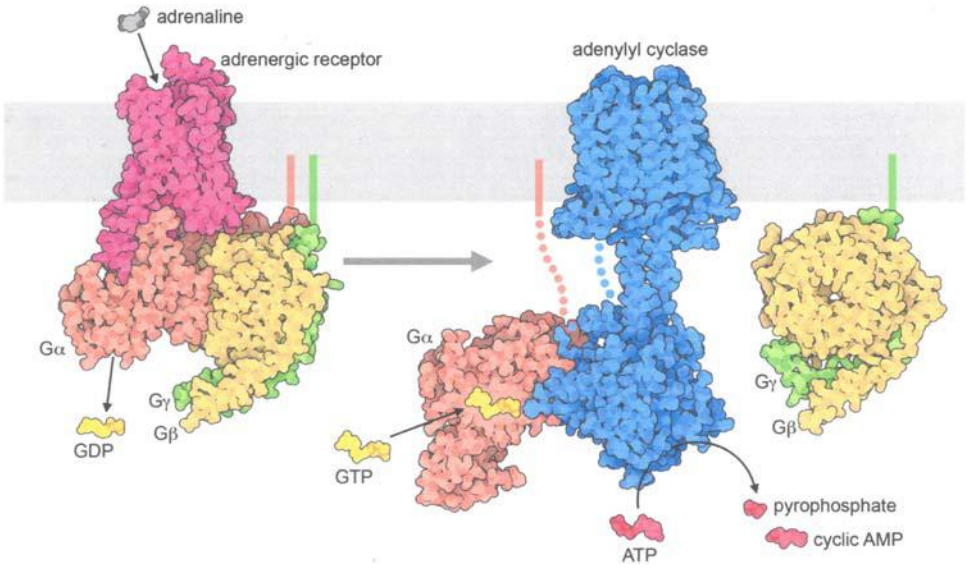

FIGURE 1.18 Signaling with G-proteins. Hormones like adrenaline bind to a GPCR receptor (*left*) which binds to a heterotrimeric G-protein and releases GDP. Then the G-protein separates into two pieces and the G_α-subunit binds to GTP and activates adenylyl cyclase (*right*). *GDP*, Guanosine diphosphate; *GPCR*, G protein–containing receptor; *GTP*, guanosine triphosphate. *Reproduced from https://pdb101.rcsb.org/motm/58.*

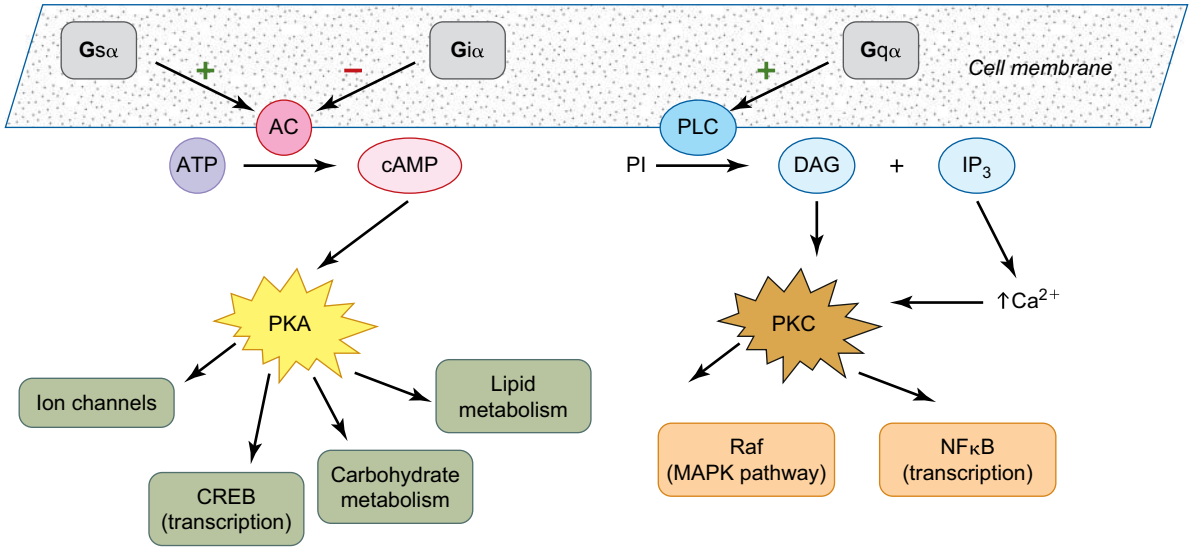

FIGURE 1.19 Hormonal signaling by GPCRs. Two of the most common signaling pathways used by GPCRs are illustrated, those initiated by $G_s\alpha$, $G_i\alpha$, and $G_q\alpha$. These three G-proteins are each the most widely distributed members of the three subfamilies of G-proteins which bear the names $G_s\alpha$, $G_{i/o}\alpha$, and $G_{q/11}\alpha$, respectively. Sometimes the abbreviations of the proteins will have a different order of the α and the subfamily, s, i, or q. In Fig. 1.11 the G-protein molecules are shown after being released from their βγ partners through interaction with a GPCR (see Fig. 1.11). As shown in this figure, $G_s\alpha$ and $G_i\alpha$ stimulate or inhibit, respectively, adenylyl cyclase in the plasma membrane, bringing about an increase or decrease in this second messenger within the cell. When levels of cAMP rise, PKA (cyclic AMP-dependent protein kinase) is activated. Depending on the cell type, one or more steps of activation (or in some cases inactivation) ensue, some of which may involve additional phosphorylation events. Examples of these include the opening of ion channels in the cell membrane, phosphorylation of the transcription factor CREB protein, activation or inhibition of enzymatic steps in the metabolism of glycolysis or lipids. On the right side of the figure, Gqα, also released from a receptor–G protein complex, activates phospholipase C, which catalyzes the release of two second messengers, inositol triphosphate (IP_3) and DAG. See Fig. 1.20 for the details of this reaction. DAG activates protein kinase C, which can activate one of several targets, in this example Raf, which allows entry to the MAPK pathway (see Fig. 1.22) or the transcription factor NFκB to affect gene transcription. *cAMP*, Cyclic AMP; *CREB*, cyclic AMP response element binding protein; *DAG*, diacylglycerol; *MAPK*, mitogen-activated protein kinase; *NFκB*, nuclear factor-κB; *PKA*, protein kinase A.

second messenger with many possible actions in the cell, including the stimulation, along with DAG, of protein kinase C. Targets for protein kinase C include the phosphorylation and augmentation of enzymes in the mitogen-activated protein (MAP) kinase pathway (see Fig. 1.23 in Section III.B) and the phosphorylation of the nuclear transcription factor nuclear factor-κB. The increased intracellular Ca^{2+} may activate exocytosis,

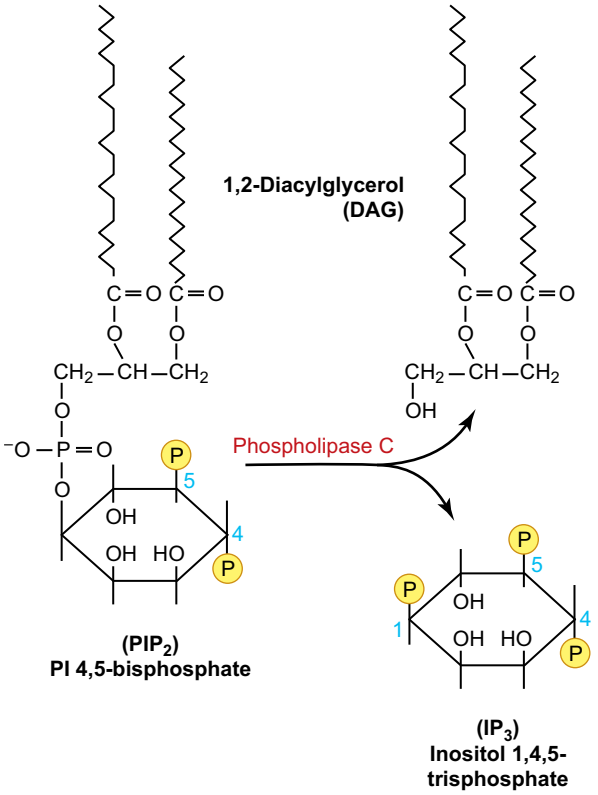

FIGURE 1.20 Phospholipase C reaction. The cleavage by phospholipase C of phosphatidylinositol 4,5-bisphosphonate into DAG and inositol 1,4,5-triphosphate (IP_3) is shown. *DAG*, Diacylglycerol.

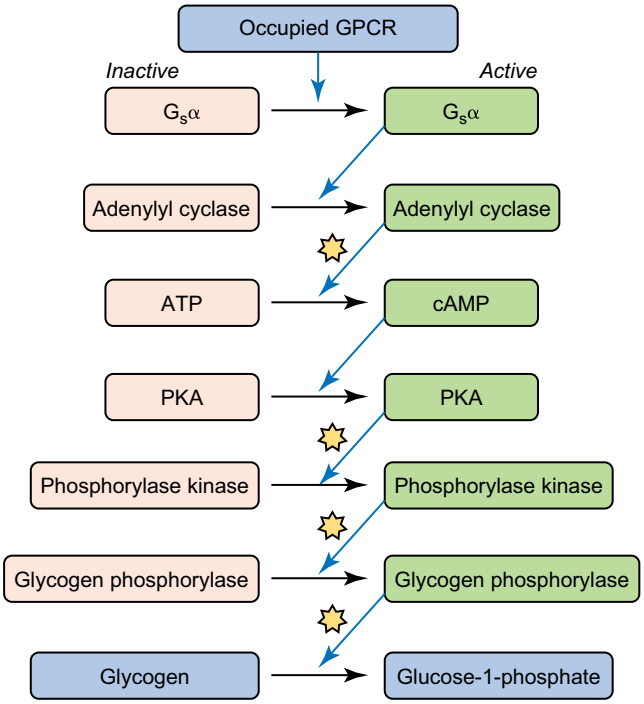

FIGURE 1.21 Amplification of hormonal signaling. The cascade of events from the binding of epinephrine to its G protein–coupled receptor in skeletal muscle to the breakdown of glycogen is shown as one example of the amplification of hormonal signaling. On the left are molecules that are inactive in the cascade prior to the initial binding event and on the right are the active forms of the molecules. As shown in Figs. 1.11 and 1.19, $G_s\alpha$ is activated by hormone binding and, in turn, activates adenylyl cyclase in the membrane, generating the second messenger cAMP. PKA (cyclic AMP-dependent protein kinase) phosphorylates and activates glycogen phosphorylase kinase (phosphorylase kinase), which phosphorylates and activates glycogen phosphorylase. The phosphorylation of glycogen initiates its breakdown into, ultimately, glucose, which is used for the energy needs of the muscle cell. At the steps with a gold star, one activated molecule may generate 10–100 (or more) active molecules. Thus the signal from one occupied GPCR can be amplified several fold, allowing for a large rapid response to a small signal. *cAMP*, Cyclic AMP; *GPCR*, G protein–coupled receptor; *PKA*, protein kinase A.

the basis of the Ca^{2+} secretion coupling mechanism often associated with the secretion of peptide hormones by their secretory glands, for example, pituitary hormones and insulin.

Ca^{2+} may also bind to calmodulin, a 17-kDa calcium-sensing protein. Calmodulin is present in the cytoplasmic compartment of virtually all cells of higher organisms. It binds four Ca^{2+} ions tightly ($K_d \cong 10^{-8}$ M) and then undergoes a conformational change so that it interacts with a number of Ca^{2+}-regulated proteins. These include Ca^{2+}–calmodulin-dependent protein kinases, Ca^{2+}-ATPase (a Ca^{2+} pump), myosin light chain kinase, and phosphatidylinositol-3-kinase, to name a few.

Regardless of the type of activated G-protein α-subunit, the GTP bound to the α-subunit is soon hydrolyzed to GDP. The α-subunit is inactivated and binds again to the βγ-subunits, turning off the signal. The speed with which the signal is turned off may be modulated by nearby proteins called GTPase activating (or accelerating) proteins, or GAPs. These proteins can, in turn, be regulated both positively and negatively by other signaling systems. This is an example of one type of cross talk between signaling pathways.

One important feature of signaling pathways initiated by membrane receptors, their amplification, or cascade property is exemplified by one of the first of the pathways to be elucidated, the cAMP-mediated control of glycogen breakdown in the skeletal muscle. As depicted in Fig. 1.21, several steps in the cascade (indicated by the stars) allow for, at the very minimum, amplification of 1–2 orders of magnitude because of the catalytic nature of the event. Thus a rapid robust response can be obtained from a fairly small change in the concentration of circulating hormone. This is a general feature of membrane-initiated signaling events that involve one or more catalytic events. A higher order cascade is seen in hormonal systems that involve the CNS, hypothalamus, pituitary, and peripheral target organ, as described in Fig. 3.1.

2 Receptor tyrosine kinases

Fig. 1.22 illustrates three of the main signaling pathways used by receptor tyrosine kinases. On the right is the MAP kinase cascade with three tiers of activation

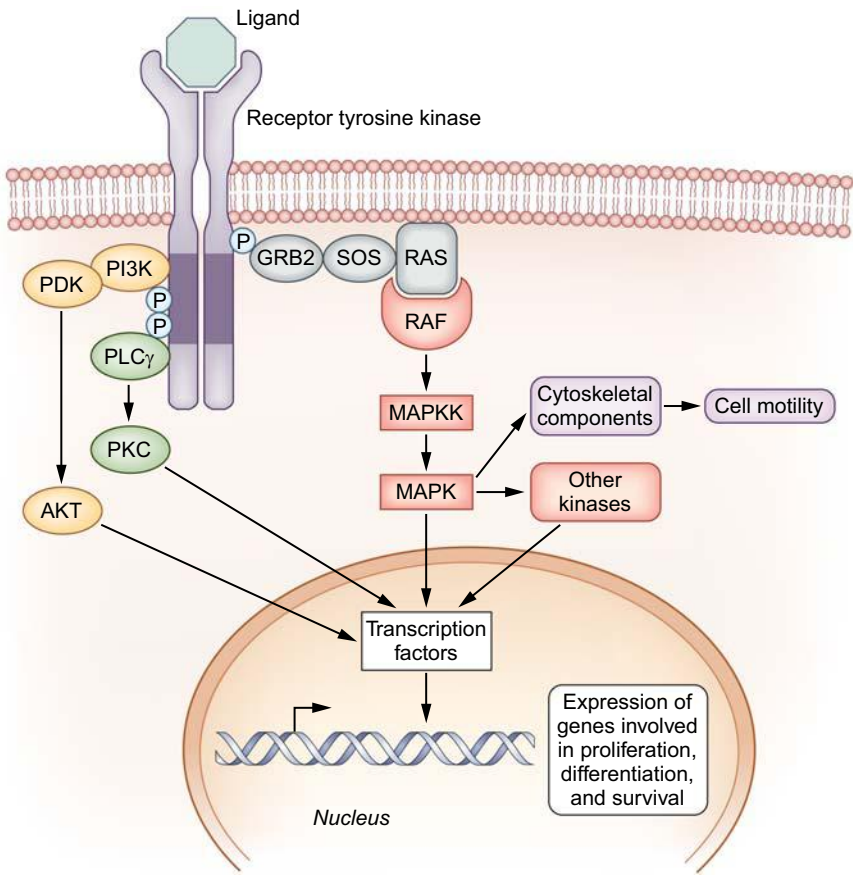

FIGURE 1.22 RTK signaling. The active form of an RTK is a dimer that is formed either before or as the ligand binds. Such an activated form is depicted here (*purple*; see Fig. 1.12 for some specific RTK structures). The extracellular ligand binding/dimerization brings about a conformational change in the receptor protein that conveys the message to the intracellular portion of the receptor. Tyrosine kinase (*dark purple*) is activated and autophosphorylates the cytoplasmic portion of the receptor (*blue circles*). This leads to the interaction of the receptor with other intracellular transducing enzymes such as phospholipase C, leading to the activation of PKC (see Fig. 1.19) or PI3 kinase which phosphorylates and activates AKT. Protein kinases B and C phosphorylate and activate proteins involved in the downstream effects of the hormone, including transcription factors. On the right is shown the activation of the MAP kinase pathway through the adaptor protein GRB2, the guanine nucleotide exchange factor SOS, and the activation of the small G-protein, Ras. This leads to the cascade of the activation of Raf (MAPKKK), MAPKK, and MAP kinase (ERK1/2), which can phosphorylate cytoplasmic proteins and nuclear transcription factors. *AKT*, Protein kinase B; *MAP*, mitogen-activated protein; *MAPKK*, MAP kinase kinase; *PDK*, phosphoinositide-dependent kinase; *PI3K*, phosphatidylinositol-3-kinase; *PKC*, protein kinase C; *SOS*, son of seven less.

beginning with that of the kinase Raf (MAPKKK) by a small G-protein, Ras. Ras, in turn, is activated by the GEF, SOS, recruited to the phosphorylated RTK by the adaptor protein GRB2. MAP kinase has many possible target proteins to alter both events in the cytoplasm and transcription in the nucleus. The exact signaling pathway used will depend on the ligand and the availability of pathway components in the cell being stimulated.

The phosphorylation of the cytoplasmic domain of the RTKs can also activate the PLCγ/protein kinase C pathway described in Fig. 1.19. In addition, phosphatidylinositol (PI) 3-kinase adds a phosphate group to the 3-position of phosphatidylinositol-4,5-bis-phosphonate, resulting in the triphosphorylated polar head group PI-3,4,5-triphosphate, which activates phosphoinositide-dependent kinase. This protein kinase activates protein kinase B, also known as protein kinase B, which phosphorylates nuclear transcription factors.

Note that these pathways share the same cascade property as that described in Fig. 1.21, with amplification at each catalytic phosphorylation step. Also as with the GPCR pathways described earlier, the RTK pathways are subject to termination to limit the timing and size of the signal. Protein phosphatases, often subject to their own regulatory systems, are available to remove the activating phosphate groups to provide this termination function.

Although the GPCR and RTK pathways are responsible for transmitting many of the hormonal signals to be encountered in this book, there are others that are less frequently encountered, but no less important to the system in which they play a role. These include the JAK/STAT pathway, which is described in connection with growth hormone in Chapter 3, The Hypothalamus and Anterior Pituitary; the guanylyl cyclase pathway that mediates the actions of atrial natriuretic factor as described in Chapter 10, Adrenal Corticoids; and the Smad pathway of TGFβ in Chapter 17, Growth Factors.

B Regulation of gene transcription by steroid hormones

The nuclear receptors for the steroid hormones, including $1,25(OH)_2D_3$, and for TH are highly regulated gene transcription factors. As with membrane hormone receptors, the regulation of the activity of these proteins occurs through the binding of ligand and a consequent conformational change that alters the activity of the receptor. Stimulation

(activation) or repression of the transcription of specific genes ensues, altering the biological activities of the cell.

One way in which members of the group of hormone-regulated nuclear receptors differ is their location in the cell in the absence of ligand. Originally it appeared that the receptors for the cholesterol-derived classic steroid hormones were localized in the cytoplasm of the cell in the absence of ligand where they are bound to molecular chaperone proteins, HSP90 (heat-shock protein 90) and in some cases HSP70. Fig. 1.23 illustrates this difference between intracellular localization of the two types of receptors. It is now understood that this is not a rigid classification scheme; both forms of the estrogen receptor (ERα and ERβ), the androgen receptor, and one form of the progesterone receptor (see Chapter 13: Estrogens and Progestins) are found in the nucleus and/or travel freely between cytoplasm and nucleus in the unliganded state. Receptors for TH and $1,25(OH)_2D_3$ localize in the nucleus upon completion of synthesis where they bind to other proteins and to DNA. What all these receptors have in common is that, regardless of intracellular localization, in the unoccupied state they are maintained in an inactive state and binding of the ligand activates them and, if necessary, causes or permits their translocation to the nucleus.

The other feature that distinguishes receptors for TH and $1,25(OH)_2D_3$ from the other receptors is that these two form heterodimers with RXR, a general nuclear receptor heterodimerization partner, while the others form homodimers. In either case, dimerization is a necessary step of the activation process. The dimerization step of the glucocorticoid receptor may occur in the cytoplasm (not specified in Fig. 1.23) and be transported into the nucleus as the homodimer. This is followed by binding to a specific DNA sequence, often but not always, in the 5′ regulatory (promoter) region of the gene, transcription of which is being regulated.

The activated DNA-bound receptor dimer recruits other proteins to the region of the transcriptional start site. These

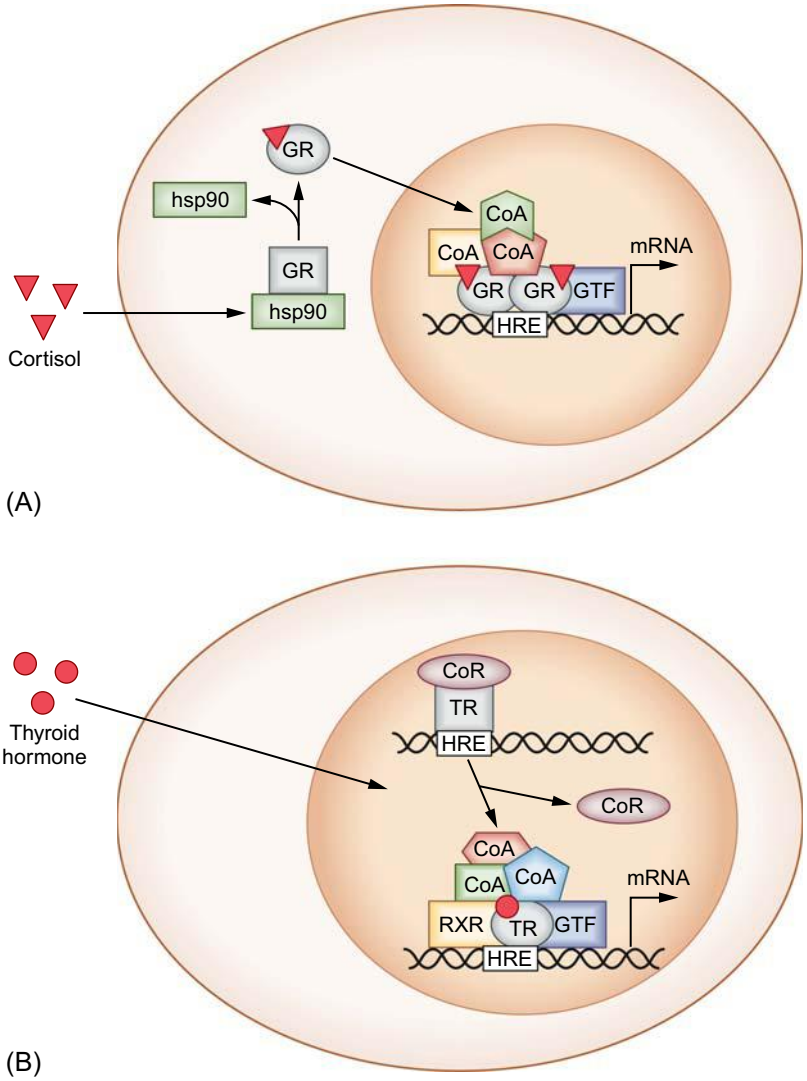

FIGURE 1.23 Transcriptional activation by nuclear receptors. Nuclear receptors for some of the classical steroid hormones are typified in this figure by the glucocorticoid receptor, GR, and its interaction with cortisol (panel A). In the absence of ligand, these receptors are in the cytosol complexed with heat shock proteins (*green*; HSP) that maintain them in an inactive state. The conformational change brought about by ligand binding causes the HSP to dissociate and the receptor translocates to the nucleus. GR, MR, ER, AR, and PR all forms heterodimers prior to DNA binding. Nuclear receptors for $1,25(OH)_2D_3$ (VDR) and TR are in the nucleus (panel B) prior to ligand binding and form heterodimers with the RXR. These are usually maintained in an inactive state by forming a complex with a corepressor (*purple*; CoR), which can bind to DNA and repress its transcription. In this case, ligand binding results in a conformational change that causes the corepressor to dissociate, activating the VDR/RXR heterodimer. The ligand-activated dimer of either type of nuclear receptor (hetero- or homodimer) binds to a specific sequence of DNA, a HRE (see Fig. 1.24). A variety of proteins, termed coactivators, are recruited to the complex to modify chromatin structure and recruit and stabilize the basal transcriptional machinery. This includes the GTF and DNA-dependent RNA polymerase. *CoR*, Corepressor proteins; *GTF*, general transcriptional factors; *HRE*, hormone response element; *HSP*, heat shock protein; *RXR*, retinoic X receptor; *TR*, thyroid hormone receptor; *VDR*, vitamin D_3 receptor.

proteins are termed coactivators. Among the first of these to be recognized were the steroid receptor coactivators, SRC-1, SRC-2, and SRC-3. These proteins bind to the receptor dimer and recruit other coactivator proteins, some of which have the enzymatic activity to modify the structure of chromatin. For example, histone acetylases (p300/CBP) neutralize the positively charged histones forming the nucleosomes (see Fig. 1.3), rendering the gene to be regulated more available for the binding of transcriptional proteins. Other histone modifications catalyzed by coactivators, such as methylation by arginine methyltransferases (PRMT1/CARM) and phosphorylation, play a role in chromatin modification. Still other coactivators recruited by SRC interact with and stabilize the basal transcriptional machinery, including basal transcriptional factors, and RNA polymerase II. Not all coactivators are present in the regulatory complex at the same time, but join, carry out their function, and are released to make room for the next set of coactivators. The combined result of these sequential activities is an increased rate of transcription of the gene that is being regulated.

Fig. 1.24 shows two typical DNA sequences of HREs for ER and VDR. HREs are composed of two hexameric half-site motifs. As can be seen in the figure, the directionality of both the relationship of the monomers to each other and the DNA half sites to each other differ according to homo- versus heterodimerization. Recall from Fig. 1.13 that it is the two zinc fingers of the receptor that are making contact with the DNA.

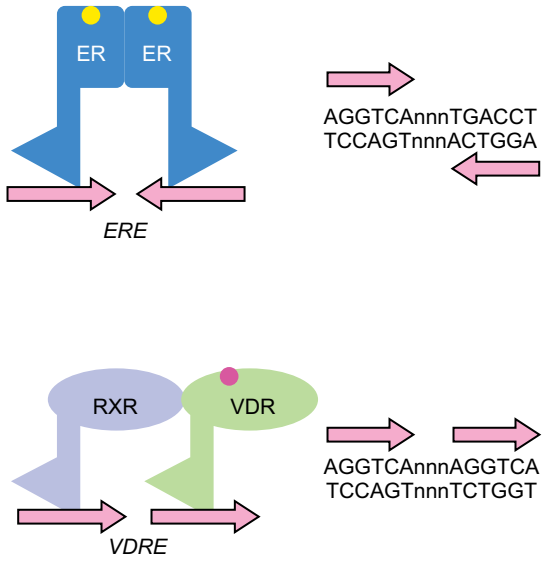

FIGURE 1.24 Nuclear receptor binding to DNA. The binding of nuclear hormone receptors to HRE within DNA is depicted. Monomers in homodimeric receptors are arranged as mirror images (head-to-head) and bind to hormone response elements that are inverted repeats, as shown in the example of an ERE at the right. The heterodimers that the receptors for $1,25(OH)_2D_3$ (VDR) and TR form with RXR are arranged sequentially (head-to-tail) and bind to response elements that are direct repeats as shown for VDR and the VDRE in the lower part of the figure. In response elements for homodimeric receptors, the number of nucleotides between the two repeats (*n*) is 3, while those for the heterodimeric receptors may vary from 3 to 5 (represented by *n*) with the response element. *ERE*, Estrogen response element; *HRE*, hormone response elements; *RXR*, retinoic X receptor; *TR*, thyroid hormone receptor; *VDR*, vitamin D_3 receptor; *VDRE*, vitamin D receptor element.

Nuclear receptors can also bring about repression of gene transcription. In some cases, notably that of the TH receptor (see Fig. 5.15), the unliganded receptor is complexed with the corepressor, silencing mediator for retinoid/TH receptors, which recruits proteins with histone deacetylase activity. The removal of acetyl groups from histones leads to their reassociation with DNA, reformation of the nucleosome, and inaccessibility of the DNA to other binding and activating proteins.

C Membrane-initiated actions by steroid hormones

It has become accepted that a fundamental activity of a steroid receptor with its bound ligand hormone working in a target cell is to either stimulate or repress the expression of genes. The steroid hormone $1\alpha,25(OH)_2D_3$, bound to its receptor, VDR, can activate as many 3000 genes (microarray data) from the ~total 22,000 human genes. It is likely that these numbers are similar for the other steroid hormones as well as TH.

It is also now understood that the hormones that bind nuclear receptors and initiate changes in gene expression also have receptor-mediated effects on processes independent of these nuclear actions. Table 1.1 compares the nuclear and nonnuclear (i.e., membrane initiated) actions of these hormones. Frequently, these rapid nongenomic responses are generated within 1−2 minutes to 15−45 minutes. This contrasts with genomic responses, which generally take several hours to days to be fully functional and apparent and which can be blocked by inhibitors of transcription and translation.

As one example, the steroid hormone $1\alpha,25(OH)_2D_3$ has been shown to initiate many biological responses via rapid response pathways that are independent of parallel genomic responses. Like the other steroid receptors, the VDR has a LBD and a DNA-binding domain that are essential to create a functional nuclear heterodimer with the retinoid X receptor (see Fig. 1.13). However, $1\alpha,25(OH)_2D_3$ is distinguished from the other steroid hormones in its conformational flexibility, as illustrated in Fig. 1.25.

Two characteristic shapes of $1\alpha,25(OH)_2D_3$, a bowl shape and a linear shape, are illustrated. It has been shown experimentally that one of these shapes, the bowl shape, generates genomic responses and the planar shape leads to nongenomic responses. These types of studies clearly support the conclusion that rapid nongenomic responses are functionally unique and different from genomic responses. Several examples of

TABLE 1.1 Nuclear receptors and involvement in genomic and/or rapid biological responses.

(Steroid receptor) and ligand	No. of aa receptor	Steroid nuclear receptors and gene transcription generate GR	Steroid membrane receptor generates RR
(Thyroid β) T_3	461	T_3 binding to its nuclear receptor, in target cells, stimulates dissociation of corepressors, recruitment of coactivators, etc. to complete a GR	T_3 activates PI3 kinases and MAP kinase *RR* pathways which can result in glucose uptake, Ca^{2+}-ATPase, Na^+/H^+ antiporter
(Vitamin D receptor) $1\alpha,25(OH)_2$-vitamin D_3	427	Both intestinal Ca^{2+} absorption and kidney Ca^{2+} reabsorption requires *GR* for the production of new CaBP	*RR* opening of Cl^- channels in osteoblasts and keratinocytes in 20 min; insulin secretion from β cells, MAP kinase activation in NB4 cells
(Estrogen receptor α) Estradiol	595	ERα *GR* are required for normal ovarian function	ERα activates PI3K and then AKt *RR* stimulates nitric oxide (NO)
(Estrogen receptor β) Estradiol	530	ERβ *GR* are required for ovulation and pregnancy	The cell membrane ERβ bound to caveola has been implicated in RR
(Glucocorticoid receptor) Cortisol	777	KO of the mouse GC receptor is lethal at time of birth	Cortisol stimulates PI3-kinase/Akt to activate in seconds NO release
(Mineralocorticoid receptor) Aldosterone	919	Mr *KO* mice die of Na^+ and H_2O deprivation	Aldosterone activates in 3–15 min the *RR* of Na^+/H^+ exchange in renal cells
(Progesterone receptor) Progesterone	933	The progesterone receptor participates in *GR* sexual differentiation determination	Progesterone stimulates *RR* within seconds to minutes, the acrosome reaction in spermatozoa
(Androgen receptor) Testosterone	919	*KO* of the AR male mouse causes the development of female genitalia	Activation of MAP kinase then activates the ERK pathway via *RR*

AKt, Protein kinase B; *CaBP*, calcium-binding proteins; *ERK*, extracellular signal-regulated kinase; *GR*, genomic responses; *MAP*, mitogen-activated protein; *PI3K*, phosphatidylinositol-3 kinase; *RR*, rapid responses. RR are not dependent on genomic responses. *KO*, knockout renders–affected genes inactive.

VDR-mediated rapid responses include the following: the rapid stimulation of intestinal Ca^{2+} absorption, in vivo; insulin secretion from rat pancreatic β cells; mouse osteoblastic cells in tissue culture that were patch clamped resulted in the rapid opening of voltage-gated chloride channels and also stimulation of exocytosis. And in Sertoli cells, located in seminiferous tubules, $1\alpha,25(OH)_2D_3$ stimulated the rapid opening of Ca^{2+} and Cl^- channels.

The subcellular location of VDR capable of producing both genomic and rapid responses is shown in Fig. 1.26. The majority of the VDR is in the nucleus (80%–85%) with the rest divided between the cytoplasm/ER (10%–15%) and caveoli (5%). It is the ~5% of caveolae-bound VDR that has been shown to be involved in voltage-gated opening of chloride channels and exocytosis.

The rapid membrane responses are not unique to the VDR receptors. Table 1.1 compares for the following receptors (thyroid β, estrogen α and β, glucocorticoid, cortisol, mineralocorticoid aldosterone, progesterone, androgen receptors, and the VDR). All the listed steroids have been shown to repeatedly participate in both activation and repression of genomic responses.

It has also been established that both the ERα and androgen receptors, in addition to the cell membrane caveolae, specifically bind to the cell's mitochondria. In summary, steroid hormones clearly can stand alone with their receptors and initiate rapid nongenomic responses, genomic effects in the cell nucleus that are mediated by second messenger pathway, and also genomic effects where the steroid receptors function as transcription factors.

IV Clinical aspects

In this chapter, some of the shared features of hormones, their receptors, and the cellular context in which they are found have been presented. In the following chapters, dealing with specific hormones, aspects of these subjects will be developed in more specific detail. In each of these chapters the final section will focus on one or more clinical aspects of the hormone(s) discussed in the chapter. It is this connection between the basic science underlying our knowledge of the structures, synthesis, and actions of the hormones and their receptors and the clinical outcomes of derangements in any of these that makes the study of this area so engaging.

Given the broad range of hormones that utilize GPCRs, it is perhaps not surprising that there are a number of mutations in them that result in either loss (hypo-) or gain (hyper-) of function phenotypes. A few examples

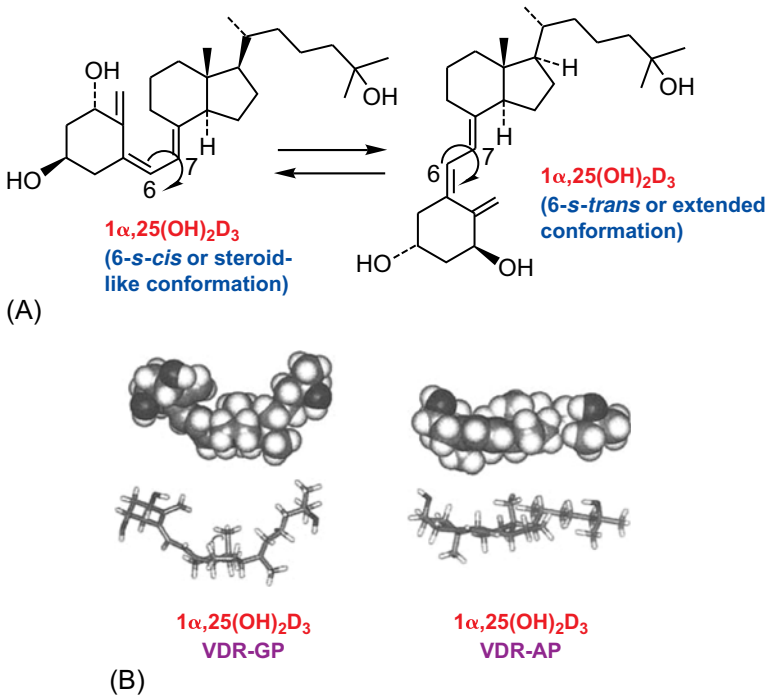

FIGURE 1.25 The steroid hormone $1\alpha,25(OH)_2D_3$ is conformationally flexible and presents different 3D shapes for binding to its receptor, the VDR. Panel (A) presents the structure of two shapes of $1\alpha,25(OH)_2D_3$. The 6-s-*cis* presentation shape on the left side is in rapid exchange with the 6-s-*trans* or extended shape. The change from *cis* to *trans* occurs by a 360-degree rotation around the 6−7 single bond (see the two arrows). This interchange process occurs at the rate of several million times per second. This extreme rotation allows the molecule to assume a very large family of three-dimensional shapes. Panel (B) has two extreme presentations of $1\alpha,25(OH)_2D_3$. The top row is a space-filling three-dimensional representation of 6-s-*cis*-shaped $1\alpha,25(OH)_2D_3$. The two dark images on the left end of the bowl shape represent the space-filling of the 1α-OH group (bottom surface) and the 3β-OH group (upper surface). At the right-hand end of the bowl shape is the space filling view of carbon-25 OH group. On the right side is the three-dimensional planar shape of $1\alpha,25(OH)_2D_3$. The right-hand three-dimensional ligand shows a 6-s-*trans* presentation; overall it has a planar shape. The left dark image is the 1α-OH group, while the 3β-OH group is hidden on the "back" surface. At the right end of the planar shape is the dark image of the 25-OH group. The bottom labels for panel (B) are the VDR-GP (for the VDR genomic pocket engaged in gene transcription; left side) and the VDR-AP (for the VDR alternative pocket engaged in nongenomic responses; right side). In order for the VDR to generate genomic responses, it has been experimentally established that the VDR must have a bound $1\alpha,25(OH)_2D_3$ ligand that is bowl shaped. This can function to turn selected genes either on or off. For the VDR that is localized to the membrane caveolae where it produces rapid responses, it must have a bound $1\alpha,25(OH)_2D_3$ that is in the planar conformational shape. The different shapes of the VDR-GP and the VDR-AP ligands were evaluated by separate determination of the X-ray structure of the VDR-GP with a bound bowl-shape ligand and computer modeling of the VDR-AP with an AP ligand with a planar shape. Panel (B) has two extreme presentations of $1\alpha,25(OH)_2D_3$. The top row is a space-filling three-dimensional representation of 6-s-*cis*-shaped $1\alpha,25(OH)_2D_3$; it has a bowl-like shape. The right-hand three-dimensional ligand shows a 6-s-*trans* presentation; it has a planar shape. The second row is a ball-and-stick presentation of $1\alpha,25(OH)_2D_3$ in the bowl (left) or planar (right) shape. In order for the VDR to generate genomic responses, that is, affect the rate of gene transcription, it has been experimentally established that the VDR must have a bound $1\alpha,25(OH)_2D_3$ ligand in the bowl shape. This is referred to as the VDR-GP. The VDR that is localized to the membrane caveolae and produces "rapid" responses must have a bound $1\alpha,25(OH)_2D_3$ that is in the planar conformational shape. This binding site is referred to as the VDR-AP. The different shapes of the VDR-GP and VDR-AP ligand were evaluated by separate determination of the X-ray structure of each and computer modeling of the AP ligand. *VDR*, Vitamin D receptor.

of each of these types of defects in specific receptors are presented in Tables 1.2 and 1.3. Mutations may render the GPCR unable to bind its hormone with appropriate strength; affect its ability to undergo the appropriate conformational change; or otherwise block its ability to bind to and activate its G-protein. Since most of the losses of function diseases are recessive, it appears that one undamaged allele can compensate for the mutated one.

Loss of function mutations can be nonsense, missense, or frameshift mutations but gain of function mutations are generally missense. The result might be a protein with an incorrect amino acid at a certain position leading, for example, to a receptor that is either overly or constitutively activated. The study of such mutations has yielded important insights into the role of three-dimensional structure and function of GPCRs.

Because of their wide distribution in the regulation of physiological processes, GPCRs make up an important portion of targeted drug development by pharmaceutical companies. It has been estimated that GPCRs constitute as much as

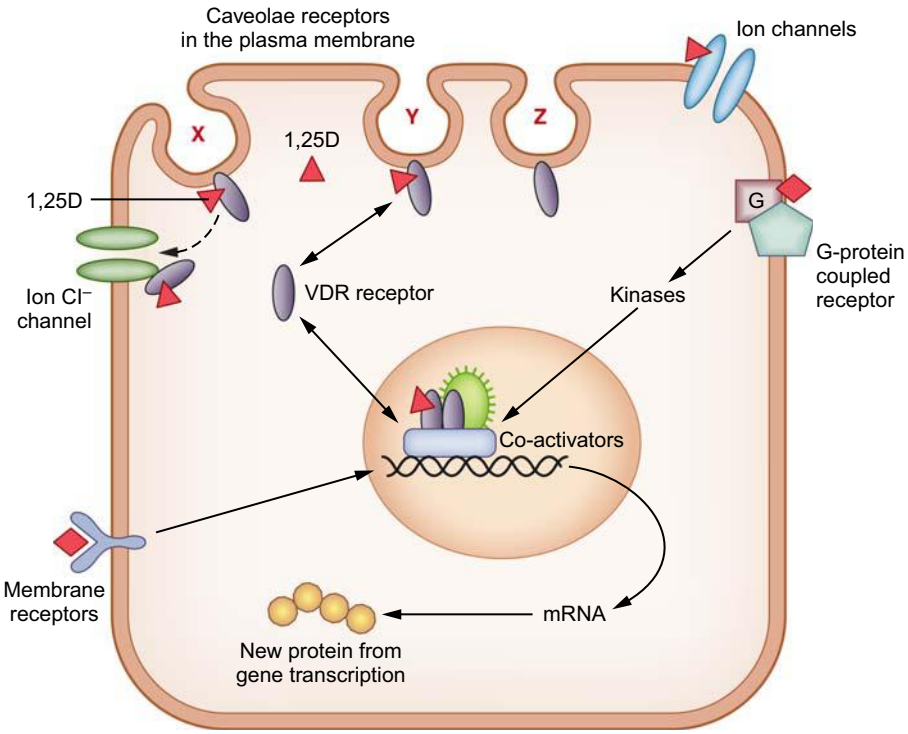

FIGURE 1.26 Distribution of VDR in a target cell. Target cells for the steroid hormone $1\alpha,25(OH)_2D_3$ have VDR distributed between the nucleus (~80%), the cytosolic compartment (~15%), and the interior surface of the plasma membrane caveolae (~5%). The VDR can move by diffusion to these three compartments. The VDR monomer has hydrophobic bonds with the intracellular surface of the caveolae membrane. The X, Y, and Z red letters are outside the cell. The VDR, arriving from the nucleus and cytosol, docks with the surface of the caveolae facing the cytosol of the cell. When the caveolae becomes associated with a monomeric VDR with a bound $1\alpha,25(OH)_2D_3$ (*red triangle*), it moves (in this example) to the nearest chloride channel to activate rapid responses. However, the caveolae-associated VDR must have the $1\alpha,25(OH)_2D_3$ bound to the VDR-AP, and not the VDR-GP for this to happen. This VDR/planar ligand is competent to initiate a variety of rapid responses (see Table 1.1). Illustrated in this figure is the VDR/$1\alpha,25(OH)_2D_3$-initiated opening of chloride channels in the plasma membrane. In the nucleus of the cell, VDR with a bound $1\alpha,25(OH)_2D_3$ forms a heterodimer with the retinoid X receptor to activate gene transcription (see Figs. 1.23 and 1.24). Gene KO of the VDR in mice results in the loss of both $1\alpha,25(OH)_2D_3$-stimulated gene activation and the activation of rapid responses, including the opening of chloride channels, and even exocytosis from the cell. *KO*, Knockout; *VDR*, vitamin D receptor; *VDR-AP*, vitamin D receptor alternate pocket; *VDR-GP*, vitamin D receptor genomic pocket.

TABLE 1.2 Loss of function endocrine diseases resulting from G protein–coupled receptor mutations.

Receptor	Disease	Inheritance
AVP receptor 2	Nephrogenic diabetes insipidus	X-linked R
LH	Leydig cell hypoplasia (males), primary amenorrhea (females)	AR
FSH	Sperm-related hypofertility (males)	AR
GnRH	Central hypogonadotropic hypogonadism	AR
TRH	Central hypothyroidism	AR
TSH	Congenital hypothyroidism	AD
GHRH	Short stature (growth hormone deficiency)	AR
Melanocortin 4	Extreme obesity	AR/codominant

AD, Autosomal dominant; *AR*, autosomal recessive; *AVP*, arginine vasopressin; *FSH*, follicle stimulating hormone; *GHRH*, growth hormone–releasing hormone; *GnRH*, gonadotropin releasing hormone; *LH*, luteinizing hormone; *TRH*, thyrotropin releasing hormone; *TSH*, thyrotropin.

TABLE 1.3 Gain of function endocrine diseases resulting from G protein–coupled receptor mutations.

Receptor	Disease	Inheritance
AVP receptor 2	Nephrogenic syndrome of inappropriate antidiuresis	X-linked D
LH	Precocious puberty (male only)	AD
TSH	Nonautoimmune familial hyperthyroidism	AD
PTH and PTHrP	Jansen metaphyseal hypercalciuria	AD
Ca^{2+}-sensing	Familial hypocalcemic hypercalciuria	AD

AD, Autosomal dominant; *AVP*, arginine vasopressin; *LH*, luteinizing hormone; *PTH*, parathyroid hormone; *PTHrP*, parathyroid hormone–like peptide; *TSH*, thyrotropin stimulating hormone.

65% of prescription drugs. Given that 3%–4% of the human genome codes for these proteins, they will surely continue to be a rich source of information about cellular regulation and of development of drugs to treat GPCR-related diseases.

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Chapter 2

Steroid hormones: chemistry, biosynthesis, and metabolism

I Introduction

A General comments

This chapter deals with the structural chemistry and biosynthetic pathways of the major classes of steroid hormones. All have a complicated structure of fused rings which can be modified by functional group substitution at many points. Furthermore, the presence of asymmetric carbon atoms introduces steric modifications and isomeric possibilities. The reader will find it prudent to first grasp the essential features of the steroid structures and relationships before attempting to delve into a consideration of their specific hormonal activities in later chapters. Then, when so doing, it may be helpful to turn back to the appropriate portion of this chapter to further heighten the understanding of the structures of the hormones under review.

B Historical perspective

The first steroid hormone, estrone, was isolated in 1929 at a time before the characteristic ring structure of the steroid nucleus had been elucidated. Today well over 230 naturally occurring steroids have been isolated and chemically characterized. In addition, an uncountable number of steroids and steroid analogs have been chemically synthesized and evaluated for their drug properties.

The development of our modern understanding of hormones and the science of endocrinology has closely paralleled studies on the isolation, chemical characterization, and synthesis of steroids and the subsequent elucidation of their pathways of biosynthesis and catabolism. The foundation of many of these developments with steroid hormones is to be found in a lengthy series of papers authored by Professor Adolf O. R. Windaus' chemistry laboratory in Gottingen, Germany (1925−35[1]) that led to the structural determination of cholesterol. This was an extraordinarily challenging problem given the limitation that the techniques of nuclear magnetic resonance spectroscopy, mass spectrometry, and ultraviolet and infrared spectroscopy were not available at that time. Instead, the structure was determined through elaborate classical organic chemistry manipulations, which involved the conversion of the compound under study to known reference compounds. At the present time the application of the powerful separation techniques of high-performance liquid chromatography or gas chromatography, combined with the use of continuous online monitoring by mass spectrometry with computer-assisted data storage and analysis, frequently permits unequivocal structural determinations on impure samples that contain less than 1 ng of the steroid of interest.

C Radioactive steroids

An equally important contribution to our present understanding of the biochemical properties and structure of steroids was the introduction and general availability of radioactively labeled compounds in the 1960s. Radioactive steroids offer two major advantages: first, the presence of the radioactive label provides a significant increase in the sensitivity of detection of the steroid under study in living animals or cells. Prior to the advent of radioactive steroids, investigators relied upon colorimetric or bioassay procedures to quantitate the steroid of interest. Second, the availability of radioactive compounds permitted the investigator to detect, from either in vivo whole animals or in vitro experiments with perfused organs, tissue slices, cell suspensions, cell homogenates, or purified enzyme preparations, the presence of new compounds that in the absence of a radioactive label would otherwise not have been discovered. Thus it was through the application of radioisotope techniques, modern procedures of

1. The Nobel Prize in Chemistry in 1928 was awarded to Adolf Windaus "for the services rendered through his research into the constitution of the sterols and their connection with the vitamins."

Hormones. DOI: https://doi.org/10.1016/B978-0-323-90262-5.00018-4

chromatography, and structure determination that whole categories of new steroid hormones were discovered. For example, research on vitamin D metabolites (Chapter 9: Calcium-Regulating Hormones: Vitamin D, Parathyroid Hormone, Calcitonin, and Fibroblast Growth Factor-23) and catechol estrogens (Chapter 13: Estrogens and Progestins) benefited from preparations of radioactive vitamin D_3 and catechol estrogens.

D Molecular biology contributions

Initially the premise was one step in steroid metabolism was handled by one enzyme. However, the advent of cloning of the cDNAs of each enzymatic step resulted in the discovery that there were fewer steroid proteins than the number of separate enzyme steps. That is to say, one steroid enzyme could carry out two to three quite different catalytic steps. Also it was learned that certain enzymatic steps that occurred in several different tissues were, in fact, carried out by the same enzyme.

II Chemistry of steroids

A Basic ring structure

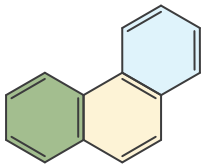
Steroids are derived from a phenanthrene ring structure to which a pentano ring has been attached; this yields in the completely hydrogenated form, cyclopentanoperhydrophenanthrene, or the sterane ring structure (see Fig. 2.1).

Steroid structures are not normally written with all the carbon and hydrogen atoms as illustrated in the middle panel of Fig. 2.1; instead, the shorthand notation as presented in the bottom panel for sterane of Fig. 2.1 is usually employed. In this representation the hydrogen atoms are not indicated, and unless specified otherwise it is assumed that the cyclohexane A, B, and C rings and the cyclopentane D ring are fully reduced; that is, each carbon has its full complement of carbon and/or hydrogen bonds. Also, indicated for the bottom sterane structure (Fig. 2.1) is the standard numbering system for each of the 17-carbon atoms in the four rings. The three six-carbon cyclohexane rings are designated A, B, and C rings and the five-carbon cyclopentane ring is denoted as the D ring.

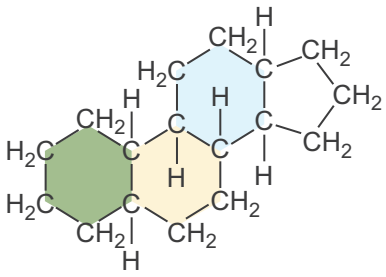
B Classes of steroids

In mammalian systems, there are six families of steroid hormones that can be classified on both a structural and a biological (hormonal) basis (see Fig. 2.2). They are the estrogens and progestins (female sex steroids), androgens (male sex steroids), mineralocorticoids (aldosterone), glucocorticoids (cortisol), and vitamin D [$1\alpha,25(OH)_2D_3$]. Also, the bile acids are structurally related to cholesterol and, thus, constitute a seventh member of the steroid family. All of these steroids are biologically derived from cholesterol. Table 2.1 summarizes some fundamental relationships of these principal mammalian classes of steroids.

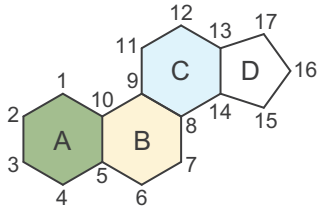
The parent ring structure for cholesterol is the fully saturated ring structure cholestane (see top row of Fig. 2.3). Cholestane, which has 27 carbons, differs from sterane (Fig. 2.2) by the addition of an 8-carbon side chain on carbon-17 of ring D and the presence of two angular methyl groups at the junctures of the A:B (carbon-10) and C:D rings (carbon-13). The cholestane ring structure also gives rise to the parent ring structures



Phenanthrene



Cyclopentanoperhydrophenanthrene (sterane)



Sterane

FIGURE 2.1 Parent ring structures of steroids. The creation of the 5-carbon (pentano) ring on phenanthrene followed by the reduction of all the aromatic double bonds creates the foundational, completely hydrogenated, cyclopentanoperhydrophenathrene, also known as sterane (the middle structure). The bottom structure is a different presentation of sterane that illustrates the numbering system or zip code for each of its 17 carbons and the convention for the A, B, C, and D labels for the 4 rings.

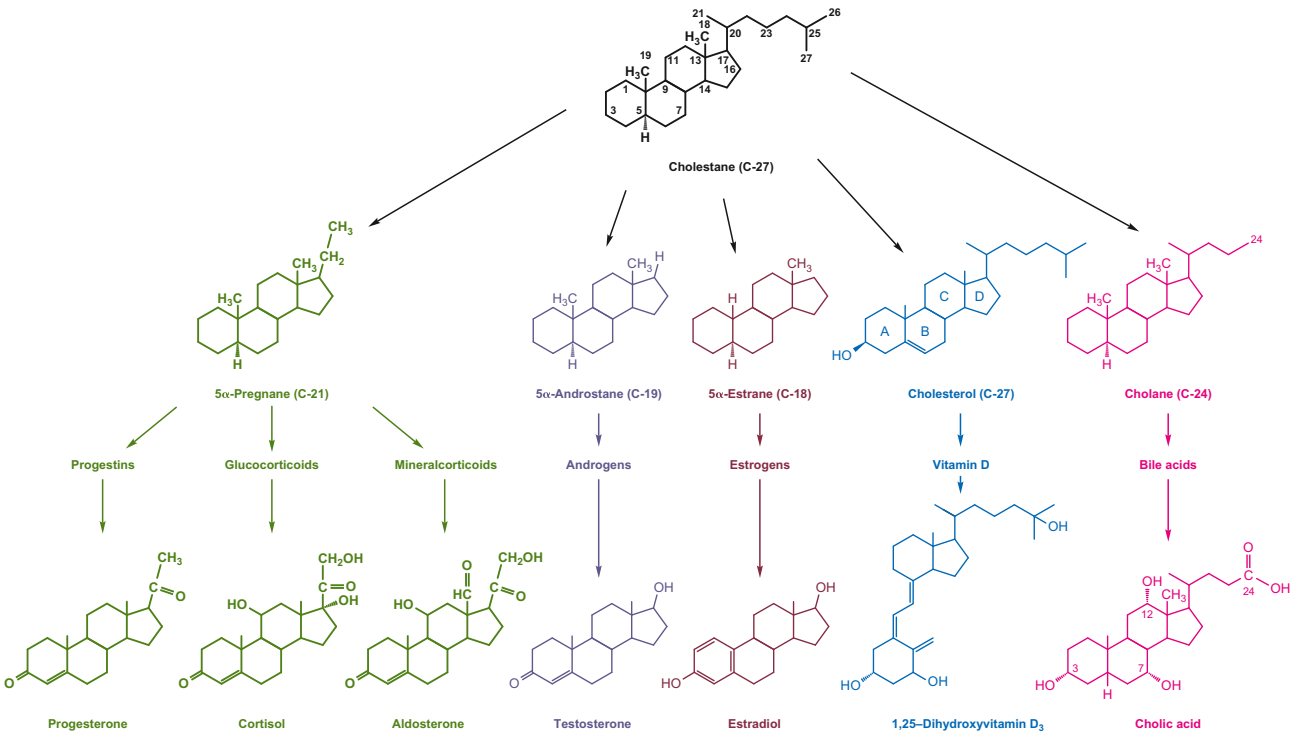


FIGURE 2.2 Family tree of the seven principal classes of steroids (bottom row) that are structurally derived from the parent cholestane (top row). Cholestane has 10 additional carbons added to sterane (see Fig. 2.1); these include two methyl groups, C-18 and C-19, added, respectively, to C-13 and C-10 and an eight-carbon side chain (C-20 to C-27) attached to C-17 of the D-ring.

TABLE 2.1 Classes of steroids.

Steroid class	Principal active steroid in humans[a]	Number of carbon atoms	Parent ring structure[a]
Estrogens	Estradiol	18	Estrane
Androgens	Testosterone	19	Androstane
Progestins	Progesterone	21	Pregnane
Glucocorticoids	Cortisol	21	Pregnane
Mineralocorticoids	Aldosterone	21	Pregnane
Vitamin D steroids	1,25-Dihydroxyvitamin D_3	27	Cholestane
Bile acids	Cholic acid	24	Cholane

[a]*The parent ring steroid structures and active steroid hormone are given in Fig. 2.2.*

for the six classes of mammalian steroids and the bile acids, as summarized in Table 2.1. The parent ring compounds are the completely saturated ring structures pregnane, androstane, estrane, and cholane, which is structurally related to cholestane. These relationships are depicted in Fig. 2.2. The parent ring structures are used as the stem term in constructing the formal nomenclature of any of these steroids.

The biosynthetic pathway of production of each of these general steroid classes will be presented separately later in this chapter. A discussion of their hormonal and biochemical aspects will appear later in individual chapters.

C Structural modification

The basic ring structures presented in Fig. 2.2 can be subjected to a wide array of modifications by the introduction of hydroxyl or carbonyl substituents and unsaturation (double or triple bonds). In addition, heteroatoms such as nitrogen or sulfur can replace the ring carbons, and halogens and sulfhydryl or amino groups may replace steroid

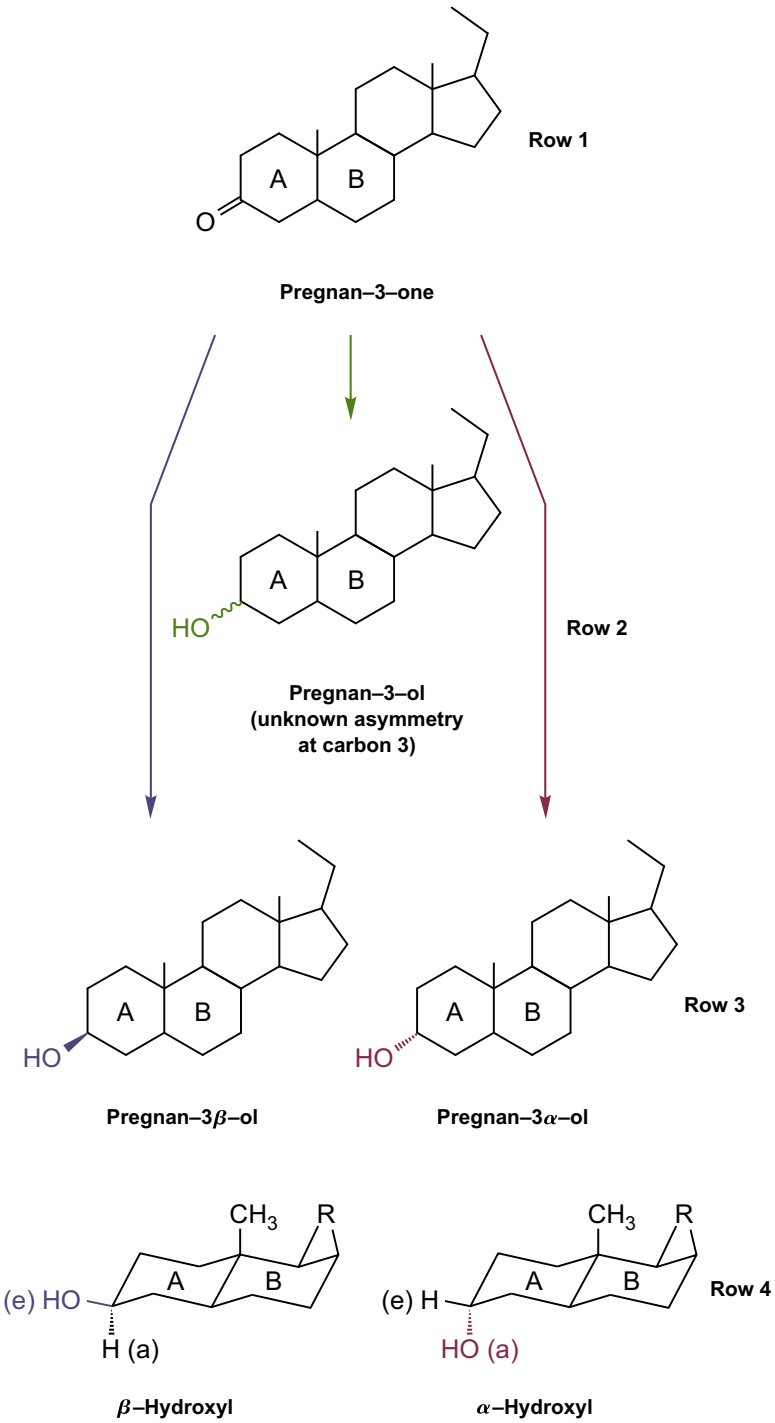


FIGURE 2.3 Structural consequences resulting from the reduction of the 3-keto group of pregnan-3-one to a hydroxyl group (see Row 1). As shown in Row 2, the orientation of the hydroxyl group is not designated (see the green wavy bond of the C3 hydroxyl group). As shown in Row 3, there are two structural options for the orientation of the hydroxyl group on C-3. The hydroxyl can be either "above" the plane of the page (see the *solid* blue hydroxyl group) or below the plane of the page (see the *dashed* red hydroxyl group). Thus in Row 3, application of steroid nomenclature rules designates that the red hydroxyl *below* the plane of the page is a 3α-hydroxyl while the blue hydroxy *above* the plane of the page is a 3β-hydroxyl. Row 4 presents the chair presentation for the A/B rings of the two pregnane isomers; this further emphasizes that the alpha and beta 3-hydroxyl groups each have a distinctly different orientation in space.

hydroxyl moieties. Furthermore, the ring size can be expanded or contracted by the addition or removal of carbon atoms. The consequences of these structural modifications are designated by the application of the standard organic nomenclature conventions for steroids. The pertinent examples of this system are summarized in Table 2.2. Prefixes and suffixes are used to indicate the type of structural modification. Any number of prefixes may be employed (each with its own appropriate carbon number and specified in the order of decreasing preference of acid, lactone, ester, aldehyde, ketone, alcohol, amine, and ether); however, only one suffix is permitted.

Table 2.3 tabulates the systematic and trivial names of many common steroids. All of these formal names are

TABLE 2.2 Steroid nomenclature conventions.

Modification	Prefix	Suffix
Hydroxyl group (OH)	Hydroxy-	-ol
Hydroxyl above plane of ring	β-Hydroxy	–
Hydroxyl below plane of ring	α-Hydroxy	–
Keto or carbonyl group (C=O)	Oxo-	-one
Aldehyde (CHO)	–	-al
Carboxylic acid (COOH)	Carboxy-	-oic acid
Double bond (C=C)	–	-ene
Triple bond (C≡C)	–	-yne
Saturated ring system	–	-ane
One less carbon atom	Nor-	–
One additional carbon atom	Homo-	–
One additional oxygenation	Oxo-	–
One less oxygen atom	Deoxy-	–
Two additional hydrogen atoms	Dihydro-	–
Two less hydrogen atoms	Dehydro-	–
Two groups on same sides of plane	*cis*-	–
Two groups on opposites sides of plane	*trans*-	–
Other ring forms (rings A and B *trans*, as in allopregnane)	Allo-	–
Opening of a steroid ring (as in vitamin D's B ring)	Seco-	–
Conversion at a numbered carbon from conventional orientation (as in epicholesterol or 3α-cholesterol)	Epi-	–

TABLE 2.3 Trivial and systematic names of some common steroids.

Trivial name	Systematic name
Aldosterone	18,11-Hemiacetal of 11β,21-dihydroxy-3,20-dioxopregn-4-en-18-al
Androstenedione	Androst-4-ene-3,17-dione
Androsterone	3α-Hydroxy-5α-androstan-l7-one
Cholesterol	Cholest-5-ene-3β-ol
Cholic acid	3α,7α,12α-Trihydroxy-5β-cholan-24-oic acid
Corticosterone	11β,21-Dihydroxypregn-4-ene-3,20-dione
Cortisol	11β,17,21-Trihydroxypregn-4-ene-3,20-dione
Cortisone	17,21-Dihydroxypregn-4-ene-3,11,20-trione
Dehydroepiandrosterone	3β-Hydroxy-5-androstene-17-one
Deoxycorticosterone	21-Hydroxypregn-4-ene-3,20-dione
Ergosterol	5,7,22-Ergostatriene-3β-ol
Estrone	3-Hydroxyestra-1,3,5(10)-triene-17-one
Progesterone	Pregn-4-ene-3,20-dione
Testosterone	17β-Hydroxyandrost-4-en-3-one
Vitamin D_3 (cholecalciferol)	9,10-Seco-5,7,10(19)-cholesta-triene-3β-ol
Vitamin D_2 (ergocalciferol)	9,10-Seco-5,7,10(19),22-ergosta-tetraene-3β-ol

devised in accordance with the official nomenclature rules for steroids laid down by the International Union of Pure and Applied Chemistry (IUPAC).[2]

D Asymmetric carbons

An important structural feature of any steroid is recognition of the presence of asymmetric carbon atoms and designation in the formal nomenclature of the structural isomer that is present. Thus the reduction of pregnan-3-one to the corresponding 3-alcohol will produce two epimeric steroids (see Fig. 2.3). The resulting hydroxyl may be above the plane of the A ring and is so designated on the structure by a bold solid line; it is referred to as a 3β-ol. The epimer, or 3α-ol, has the hydroxyl below the plane of the A ring and is so designated by a dotted line for the C · OH bond. If the α- or β-orientation of a substituent group is not known, it is designated with a wavy line (C ∼ OH).

Another locus where asymmetric carbon atoms play an important role in steroid structure determination is the junction between each of the A, B, C, and D rings. Fig. 2.4 illustrates these relationships for cholestanol and coprostanol. Thus in the 5α-form, the 19-methyl and α-hydrogen on carbon-5 are on opposite sides of the plane of the A:B ring; this is referred to as a *trans* fusion. When the 19-methyl and β-hydrogen on carbon-5 are on the same side of the A:B ring fusion, this is denoted *cis* fusion. In the instance of *cis* fusion of the A:B rings, the

2. The IUPAC definitive rules of steroid nomenclature are presented in full in *Pure & Applied Chemistry* 31 (1972), 285–322 or *Biochemistry* 10 (1971), 4994–4995.

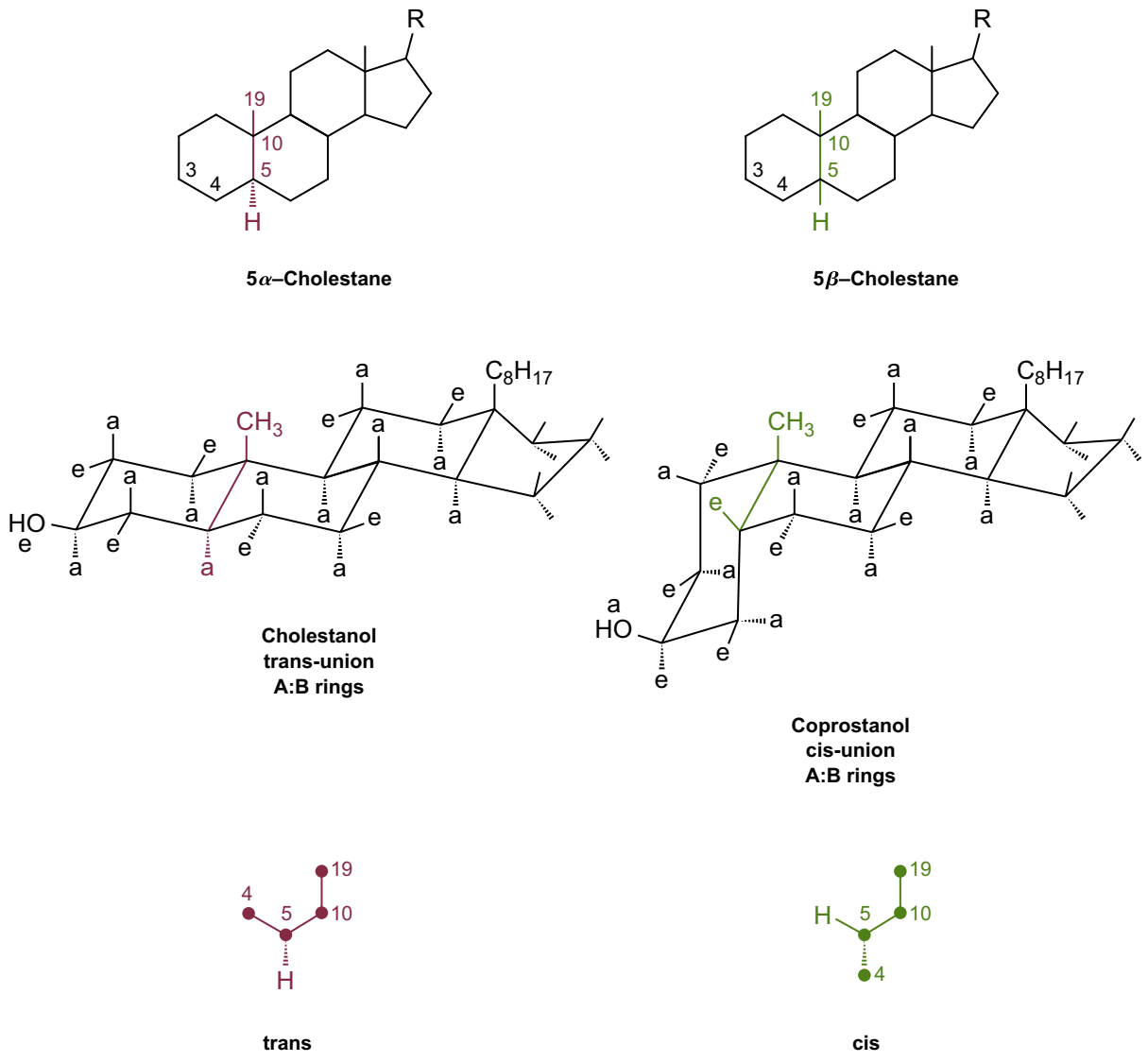
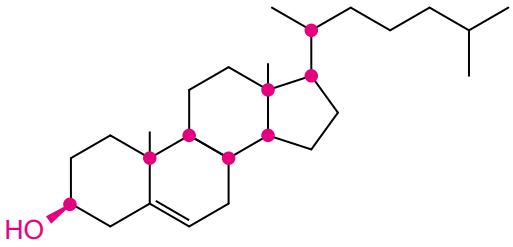


FIGURE 2.4 Comparison of structural relationships resulting from *cis* or *trans* A:B ring fusion in 5α-cholestane versus 5β-cholestane (top row) and cholestanol versus coprostanol (second row). In 5α-cholestane and cholestanol (left side of rows 1 and 2) the A:B ring fusion is *trans* (C-19-methyl-C-5H), while in 5β-cholestane and coprostanol (right side of rows 1 and 2) the A:B ring fusion is *cis* (C-19-methyl-C-5H). The orientation of substituents around carbon-5 for the *cis* and *trans* circumstances are illustrated in the bottom row (●–● indicates carbon–carbon bonds). Finally, with respect to the hydroxyl on C-3, its *beta* orientation is maintained for both cholestanol and coprostanol in spite of their different A/B ring fusions.

steroid structure can no longer be drawn in one plane. Thus in all 5β-steroid structures that have *cis* fusion between rings A and B, the A ring is bent into a second plane that is at approximately a right angle to the B:C:D rings (see Fig. 2.4).

Thus each of the ring junction carbons is potentially asymmetric, and the naturally occurring steroid will have only one of the two possible orientations at each ring junction. Although there are two families of naturally occurring steroids with either *cis* or *trans* fusion of the A:B rings, it is known that the ring fusions of B:C and C:D in virtually all naturally occurring steroids are *trans*. Consideration of the estrogen steroid series (see Fig. 2.2) is a special case in which the A ring is aromatic; thus there is no *cis–trans* isomerism possible at carbons-5 and -10.

The side chain is a third domain of the steroid structure where asymmetry considerations are important. Historically, interest first centered on carbon-20 of the cholesterol side chain, although side chain asymmetry is also now known to be crucial for the insect steroid hormone ecdysterone, for a number of vitamin D metabolites (see Fig. 2.2 and Chapter 9: Calcium-Regulating Hormones: Vitamin D, Parathyroid Hormone, Calcitonin, and Fibroblast Growth Factor-23), and in the production of many bile acids (see cholic acid in Fig. 2.2). While the



Cholesterol

FIGURE 2.5 Asymmetric carbons of cholesterol. The eight asymmetric carbons of cholesterol are indicated by the magenta dots (●). There are 2^8 or 256 structural isomers of cholesterol. Only one structural isomer is produced in higher animals.

α or β notation is satisfactory for the designation of substituents of the A, B, C, and D ring structures, this terminology is not applicable to the side chain. This is because there is free rotation of the side chain at the carbon-17−carbon-20 bond; thus the side chain may assume a number of orientations in relation to the ring structure.

Fig. 2.5 identifies for cholesterol the eight asymmetric carbon atoms. Introduction of the $\Delta^{5,6}$-double bond in cholesterol deletes one asymmetric center, whereas addition of the eight-carbon side chain adds one asymmetric carbon at position 17. Carbon-20 of the side chain is also asymmetric. Finally, the introduction of a hydroxyl group on carbon-3 creates still another asymmetric center. Thus there are a total of eight asymmetric carbons or 2^8=256 possible structural isomers. Considering that cholesterol is the most prevalent naturally occurring steroid, it is an impressive testament to the precision of evolutionary events and to the specificity of the many enzymes involved in the biosynthesis of cholesterol that only one major sterol product is present in mammalian systems.

E Conformational flexibility of steroids

The steroid nucleus, sterane, is composed of three cyclohexane rings and one cyclopentane ring. The six-carbon atoms of a cyclohexane ring are not fixed rigidly in space but are capable of interchanging through turning and twisting between several structural arrangements in space called conformations.

The two principal conformations of a cyclohexane ring are the *chair* and *boat* forms (see Fig. 2.6). Each of the two substituent groups on the six-carbon atoms of the cyclohexane ring may exist in either the general plane of the ring and are designated as equatorial (*e*) or a plane perpendicular to the ring plane and are designated as axial (*a*). For the equatorial bonds, it is possible to superimpose on the equatorial notation an indication of whether they are below (α) or above (β) the general plane of the ring. Cyclohexane is highly conformationally mobile, interchanging between the

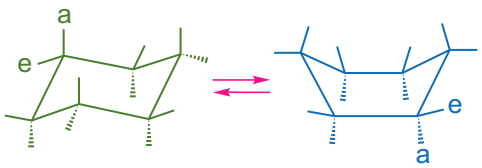


FIGURE 2.6 Principal conformational representations of cyclohexane. The left conformer of cyclohexane is a chair, while the right conformer is a boat. They interchange about a million times per second.

boat and chair forms many thousands of times per second. The most stable form of the cyclohexane ring is the chair form; in this conformer, there is a greater interatomic distance between the equatorial and axial hydrogens than in the boat form. Fig. 2.4 illustrates the nature of all of the equatorial (*e*) and axial (*a*) hydrogens on the cholestane and coprostane ring structures.

As indicated, the B and C rings of both cholestane and coprostane are locked into chair conformations (see Fig. 2.4). Although, in principle, the A ring of both these steroids is free to interchange between the boat and chair representations, the chair form is believed to be much more favored. In the case of vitamin D steroids, which do not have an intact B ring due to the breakage of the carbon-9−carbon-10 bond (thus they are termed secosteroids), the A ring is much more conformationally mobile than that of the usual cholesterol-derived steroids (see Fig. 9.6).

An important point for the reader to consider is that the usual structural representation given for steroids (e.g., see Figs. 2.2−2.4) provides no clear designation of either the three-dimensional geometry or the space-filling aspects of the electron orbitals associated with each atom involved in the formation of the requisite bonds required for the full molecular structure. A comparison for cholestanol is given in Fig. 2.7 of the planar representation (A), the planar conformational model (B), a Dreiding three-dimensional model emphasizing bond lengths and angles (C), and a Corey−Pauling three-dimensional space-filling model (D). Certainly the space-filling molecular representation most closely approximates the reality of the three-dimensional shape of the steroid and, thus, provides an insight into the overall molecular shape required to produce a biological response.

The approach of steroid conformational analysis has been of great value to the organic chemist as a tool to predict or understand the course of synthetic organic chemical reactions. It is also known that conformational considerations play an increasingly useful role in the understanding of steroid hormone−receptor interactions. Steroid receptors are known to have very precise ligand specificities; see, for example, Table 2.4, which illustrates the structural preferences of the nuclear receptor for the

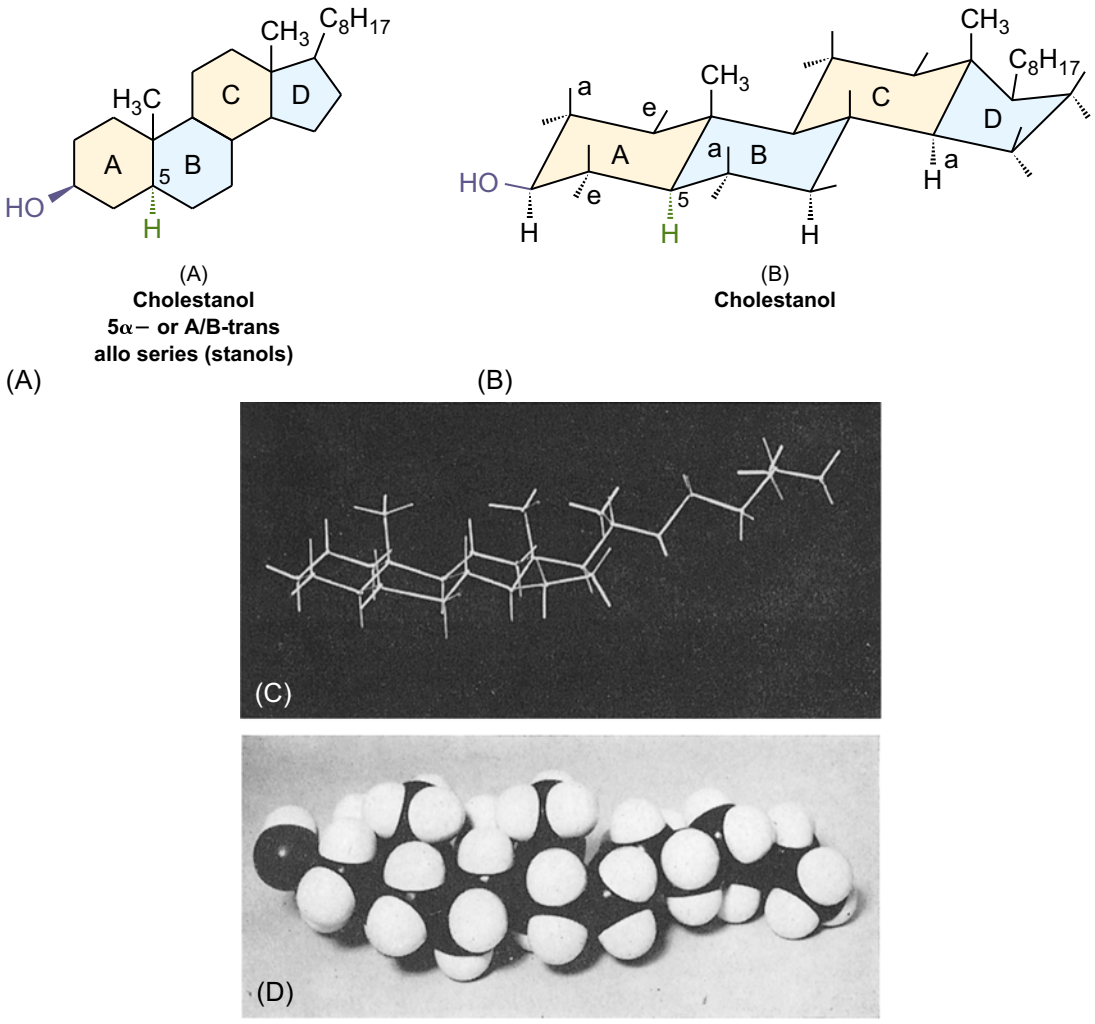


FIGURE 2.7 Four structural representations of cholestanol. (A) A typical two-dimensional structure of cholestanol drawn on a page. (B) A planar conformational model, also drawn on a page. (C) A Dreiding three-dimensional "stick" model of cholestanol emphasizing bond angles and interatomic distances. (D) A Corey–Pauling three-dimensional space-filling model of cholestanol. The C and D models can either be manually assembled from a kit of all possible parts or can be modeled in three dimensions on a computer using a highly sophisticated program.

TABLE 2.4 Ligand specificity of the nuclear $1\alpha,25(OH)_2D_3$ receptor.

Ligand	Structural modification	RCI[a]
$1\alpha,25(OH)_2D_3$ See structure in Fig. 2.2	The naturally occurring steroid hormone (see Chapter 9: Calcium-Regulating Hormones: Vitamin D, Parathyroid Hormone, Calcitonin, and Fibroblast Growth Factor-23, for further details)	100%
$1\alpha,25(OH)_2$ – 24-nor-D_3	Shorten side chain by one carbon	67
$1\alpha,25(OH)_2$ – 3-epi-D_3	Orientation of 3β-OH changed	24
$1\alpha,25(OH)_2$ – 24a-dihomo-D_3	Lengthen side chain by two carbons	24
$1\beta,25(OH)_2D_3$	Orientation of 1α-OH changed to 1β	0.8
1α $(OH)D_3$	Lacks 25-OH	0.15
$25(OH)D_3$	Lacks 1α-OH	0.15
$1\alpha,25(OH)_2$ – 7-dehydrocholesterol	Lacks a broken B ring; is not a secosteroid	0.10
Vitamin D_3	Lacks both lα, and 25-OH groups	0.0001

RCI, Relative competitive index; *VDR*, vitamin D receptor.
[a]*The RCI is a measure of the ability of a nonradioactive ligand to compete, under in vitro conditions, with radioactive $1\alpha,25(OH)_2D_3$ for binding to the nuclear $1\alpha,25(OH)_2D_3$ receptor (VDR). The RCI is expressed as a percent. Thus the binding of $1\alpha,25(OH)_2D_3$ to its VDR is 100%. When one of the three hydroxyls on $1\alpha,25(OH)_2D_3$ are missing as in 1α $(OH)D_3$, $25(OH)D_3$, or 3-epi,$1\alpha,25(OH)_2D_3$, the RCI values fall to 24% (for the 3βOH) and fall dramatically to 0.15% (for the separate loss of the 1α-OH or the 25-OH).*
Source: Data taken from R. Bouillon, W.H. Okamura, A.W. Norman, Structure–function relationships in the vitamin D endocrine system. Endocr. Rev. 16 (1995) 200–257.

steroid hormone 1α,25(OH)$_2$_vitamin D$_3$. It would be surprising, therefore, if the intimate local structure of the receptor's ligand-binding site did not have the capability of distinguishing and discriminating between the presence or absence of a key hydroxyl or the various conformational forms of the same steroid hormone.

Fig. 2.8 presents a schematic view of the vitamin D receptor (VDR) as it interacts with the three key hydroxyls on carbons 1α, 3β, and 25 of the steroid hormone 1α,25(OH)$_2$D$_3$ to create three anchoring hydrogen bonds (X, Y, and Z) which collectively create a functional ligand−receptor complex (see Fig. 2.8A). The importance of these three hydrogen bonds has been deduced via the evaluation of the three-dimensional structure of the VDR as determined via X-ray crystallographic analysis with its bound ligand the 1α,25(OH)$_2$D$_3$ steroid hormone. As shown in panel B of Fig. 2.8, a modified ligand [25(OH)D$_3$], which is missing the key 1α-hydroxyl group on the

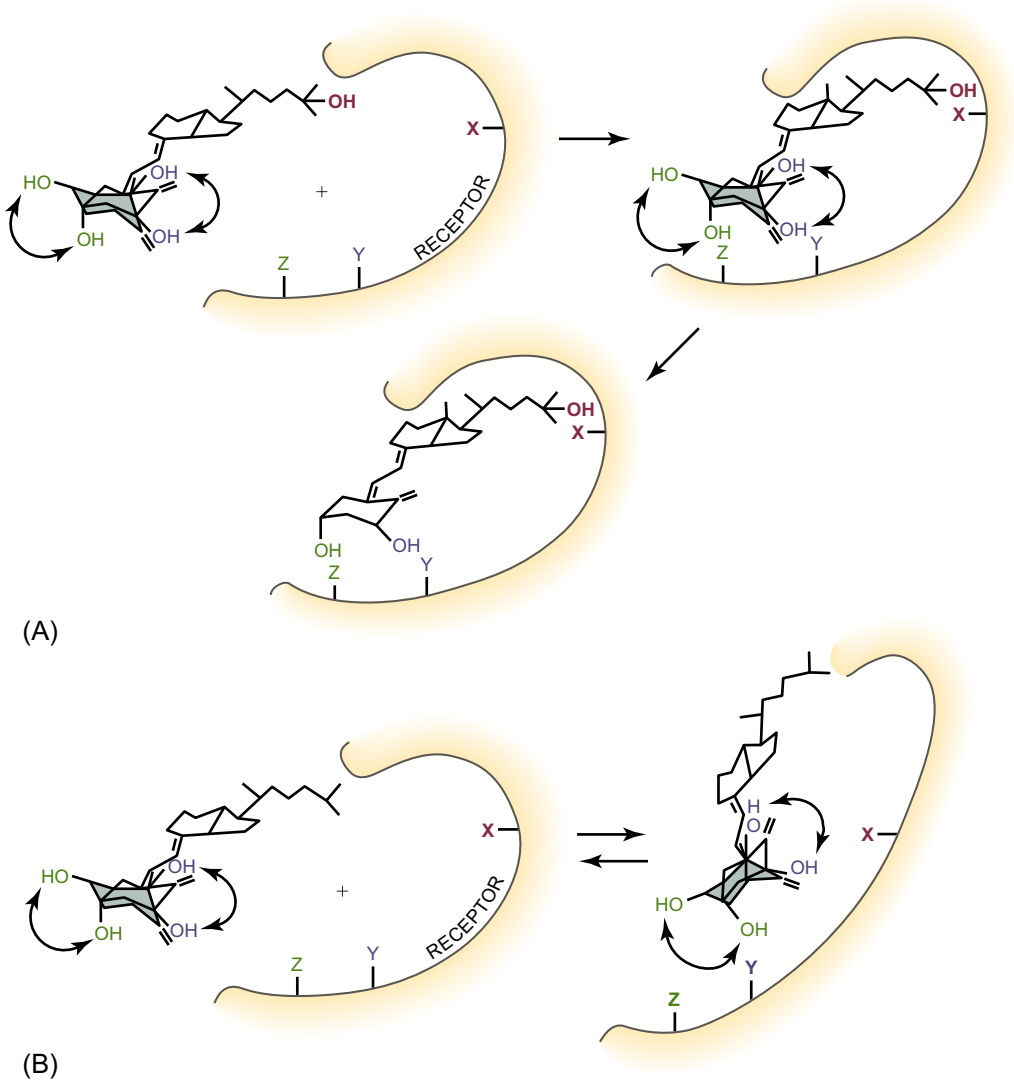


FIGURE 2.8 The absence of the 25-hydroxyl of the steroid hormone 1α,25(OH)$_2$D$_3$ reduces the affinity of 1α (OH)D$_3$ to bind to the VDR by ~660 fold. The schematic model of the VDR illustrates how the receptor for 1α,25(OH)$_2$D$_3$ first "captures" and then forms a stable hydrogen bond (with X) with the 25-hydroxyl group on the end of the conformationally mobile 8-carbon side chain. This is followed by the conformationally mobile A ring's two hydroxyls of 1α,25(OH)$_2$D$_3$ (the 1β and the 3β) to form a stable (using Y and Z) receptor−ligand complex. The X, Y, and Z labels indicate known binding domains on the receptor's interior which each form a stabilizing hydrogen bond with the three hydroxyls of 1α,25(OH)$_2$D$_3$ when the ligand is docked inside the VDR. Panel (A) illustrates the proposed steering effects of the 25-hydroxyl group docking with X which then permits capture of the conformationally active A ring's two hydroxyls by Y and Z. Panel (B) illustrates the consequences of the absence of the side chain 25-hydroxyl group on the poor ability of 1α (OH)D$_3$'s 1α- and 3β-hydroxyls to be captured by the VDR. This is reflected by the fact that 1α (OH)D$_3$ binds to the VDR only 0.15% as well as 1α,25(OH)$_2$D$_3$ (100%; see Table 2.4 and its legend). Thus the following calculation can be made: [RCI for 1α,25(OH)$_2$D$_3$]/[RCI for 1α (OH)D$_3$]=[100]/[0.15]=666-fold difference in relative binding in favor of 1α,25(OH)$_2$D$_3$. *VDR*, Vitamin D receptor.

A-ring, can only bind to the VDR 0.15% as well as the natural hormone, $1\alpha,25(OH)_2D_3$ (see Table 2.4); thus it is unable to form a stable ligand–receptor complex and, therefore, it is not able to produce significant biological responses. Comparable structure–function studies for all the steroid hormones have been carried out by many pharmaceutical companies to identify analogs of the natural steroid hormone(s) that are able to produce useful selective biological responses.

F Other steroid structures

Fig. 2.9 illustrates the diversity of the steroid structures in a wide variety of biological settings ranging from insect

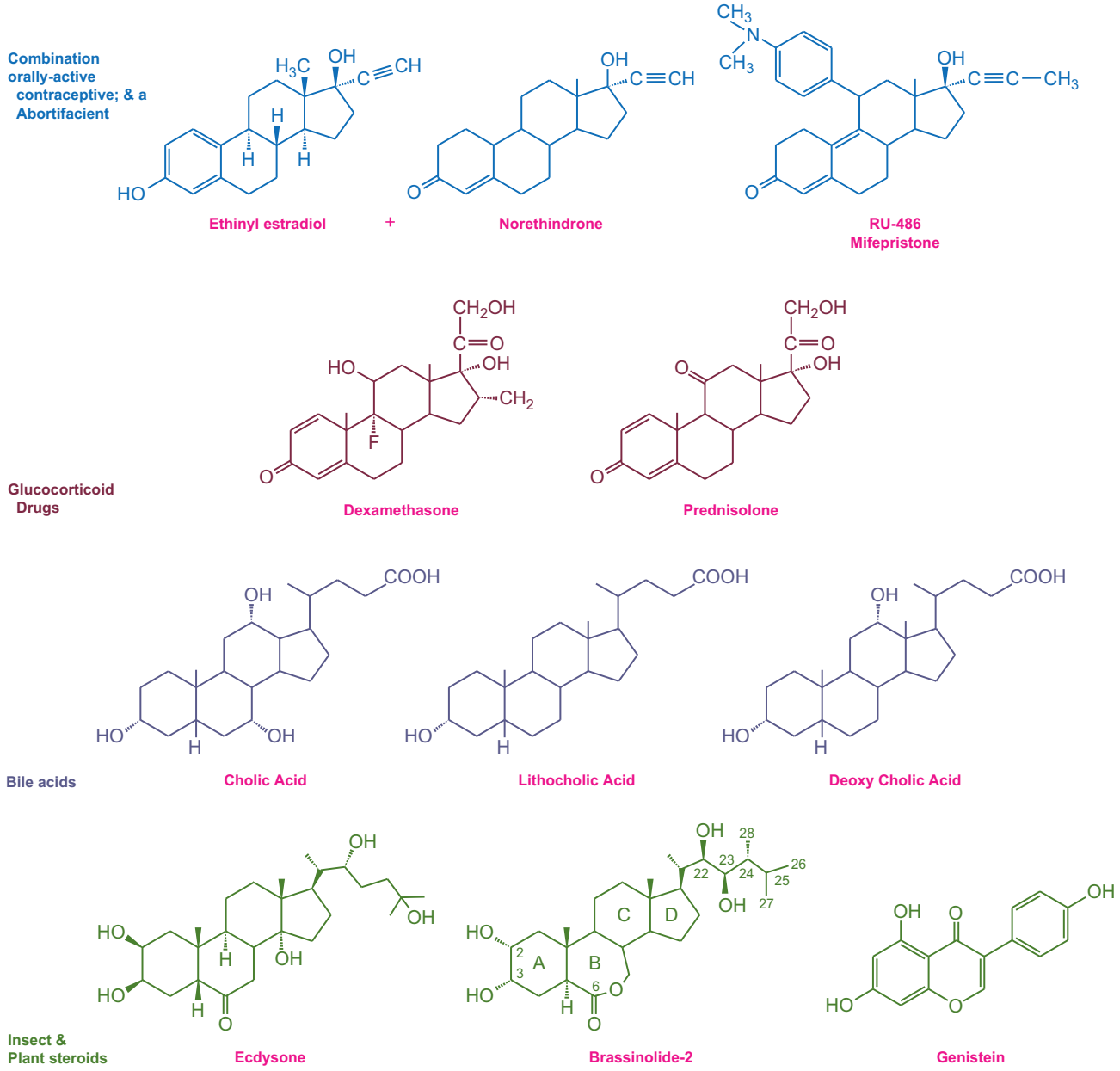


FIGURE 2.9 Structures of biologically significant steroids. Collectively the illustrated steroids emphasize the wide involvement of the backbone cyclopentanoperhydrophenanthrene template (see Fig. 2.1) as a central foundation for the wide diversity of structural additions and modifications that generate unique structures with unique biological functions. The top row (row 1) illustrates two steroids used together as a contraceptive and a third steroid that is an abortifacient or "morning after pill." Row 2 illustrates two widely prescribed drug forms of a glucocorticoid, dexamethasone and prednisolone. Row 3 illustrates three naturally occurring bile acids in man; they are cholic acid, lithocholic acid, and deoxy-cholic acid. Row 4 illustrates an insect steroid hormone, ecdysone, a plant growth regulator-steroid hormone, brassinolide, and a plant member of the family of isoflavonoids, genistein, which have in common phenolic ring structures. Genistein is able to bind to the estrogen receptor. Exposure to genistein occurs principally through foods made with soybeans and soy protein.