Hormones

THIRD EDITION

ELSEVIER science & technology books

Companion Web Site:

http://store.elsevier.com/product.jsp?&isbn=9780123694447

Hormones, Third Edition

Anthony W. Norman and Helen L. Henry, Authors

Resources for Professors:

- All figures from the book available as both Power Point slides and .jpeg files

ELSEVIER

ACADEMIC PRESS

Hormones

THIRD EDITION

Anthony W. Norman, Ph.D.

Distinguished Professor of Biochemistry & Biomedical Sciences
University of California, Riverside

Helen L. Henry, Ph.D.

Professor of Biochemistry
University of California, Riverside

AMSTERDAM • BOSTON • HEIDELBERG • LONDON
NEW YORK • OXFORD • PARIS • SAN DIEGO
SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO
Academic Press is an imprint of Elsevier

Academic Press is an imprint of Elsevier
32 Jamestown Road, London NW1 7BY, UK
225 Wyman Street, Waltham, MA 02451, USA
525 B Street, Suite 1800, San Diego, CA 92101-4495, USA
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, UK

Notices
Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

With respect to any drug or pharmaceutical products identified, readers are advised to check the most current information provided (i) on procedures featured or (ii) by the manufacturer of each product to be administered, to verify the recommended dose or formula, the method and duration of administration, and contraindications. It is the responsibility of practitioners, relying on their own experience and knowledge of their patients, to make diagnoses, to determine dosages and the best treatment for each individual patient, and to take all appropriate safety precautions.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

ISBN: 978-0-12-369444-7

For information on all Academic Press publications visit our website at http://store.elsevier.com

Typeset by MPS Limited, Chennai, India
www.adi-mps.com

Printed in China

15 16 17 18 19 10 9 8 7 6 5 4 3 2 1

Cover Illustrator: Marie C. Dean
Interior Illustrators: Marie C. Dean and Matrix Art Service
Please see page XV for further information on each cover image

Preface

The objective of this third edition of *Hormones* is to provide a comprehensive update of 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. This new edition of *Hormones* is intended to be used principally by advanced undergraduates in the biological sciences and graduate students. The authors anticipate that *Hormones* will continue to be used by researchers at all levels. We also anticipate that it will provide useful background information for first-year medical students as they engage in studies that are increasingly problem-based rather than discipline-focused. As the field of endocrinology itself has continued to expand in the past two decades, the up-to-date presentation of the basics in this book will be a solid foundation on which more specialized considerations can be based.

The first and second editions of *Hormones* were published in 1987 and 1997; the authors were Anthony W. Norman and Gerald Litwack. Both editions were very successful, based on sales and the favorable comments provided by Elsevier. The present authors, Anthony W. Norman and Helen L. Henry are on the faculty at the University of California–Riverside. We have been using *Hormones* for over 30 years in a popular class of ~150 biochemistry majors dedicated largely to students intending to pursue a career in the health sciences. We have, however, become acutely aware over the last 10 years that it was time to revise *Hormones* once again.

Hormones presumes that the individual reader and classes of students will have been exposed in detail to the areas of knowledge fundamental to biochemistry, including the structure and function of macromolecules and the other bioorganic substances of intermediary metabolism, as well as to a wide array of topics in molecular biology. In addition, an understanding of cell biology, cellular and subcellular organization, and mammalian physiology will be useful. It is the pentad of biochemistry, structural biology, molecular biology, and cell and organ physiology that forms the foundation for the principles of the biological "facts of life" that are critical to the development of our modern understanding of the molecular endocrinology of hormones and their actions.

The book is organized to provide two introductory chapters, followed by 15 chapters on selected topics of the molecular biology of the major endocrine systems operative in humans. The opening chapter is concerned with a delineation of the first principles of hormone action. These include a discussion of the structural and functional classification of hormones and a detailed presentation of current general theories of mechanisms of hormone action at both the cellular and the subcellular level. In this third edition, this includes the addition of information on the families of hormone receptors (steroid and growth factors) and a detailed introduction to the topic of signal transduction, which describes how the chemical message of the hormone is transformed to generate specific biological responses. Chapter 2 provides a detailed presentation of the seven classes of steroid hormones and their chemistry, biosynthesis, and metabolism. These two introductory chapters are followed by 15 chapters, each of which, with the exception of Chapter 8 on eicosanoids, then focuses on a classical human endocrinology system and its associated hormones. For example, Chapter 6 focuses on the classical pancreatic hormones: insulin and glucagon along with leptin, pancreatic polypeptide, somatostatin, and amylin, whose hormonal and major clinical properties are described in Chapter 6. Five newly discovered and characterized hormones—kisspeptin, ghrelin, oxyntomodulin, FGF-23, and adrenomedullin—are each included in their appropriate chapter. Also there are six appendices: A, a compilation of ~100 known hormones in higher mammals and humans; B, a table of the blood concentrations of major human hormones; C, a list of prominent endocrine disorders; D, a table of the genetic code; E, a table of three-letter and single-letter amino abbreviations for amino acids; and F, a table of scientific measurements in biological systems.

A dramatic change in this third edition of *Hormones* is that there are 344 color panels associated with the 277 figures in the 17 chapters. PowerPoint slides of all the figures in *Hormones* are available to the reader on the Companion website.

On the whole, this text is related to the biochemical endocrinology courses we have taught to our first-year medical students and graduate students at the University of California–Riverside. The authors hope that the uniform organizational framework of the chapters as well as the division of chapter topics into separate hormone systems will allow instructors to selectively identify varying levels of coverage. Thus it should be possible to prepare a teaching syllabus that is comprehensive or one that focuses only on the subcellular mode of action of selected hormones divorced

from a detailed understanding of their human anatomy and physiology.

We hope that this revised textbook will fulfil a need for comprehensive resource materials that currently exist for teaching cellular and molecular endocrinology. We visualize that this book could be employed as an equivalent source of information with most of the standard biochemistry textbooks to provide a comprehensive and balanced coverage of the realm of biology.

Anthony W. Norman
Helen L. Henry

Anthony W. Norman was born in 1938 in Ames, Iowa. He received his B.S. in 1955 in Chemistry from Oberlin College and earned his Ph.D. in Biochemistry at the University of Wisconsin, Madison in 1963. He was a Postdoctoral fellow in the laboratory of the Nobel Laureate, Paul Boyer at UCLA. Norman joined the Department of Biochemistry at the University of California, Riverside in 1964 where he is currently Distinguished Professor of Biochemistry and Biomedical Sciences.

Helen L. Henry was born in 1944 in Fairborn, Ohio. She received her B.A. in Biology in 1965 and her PhD, also in Biology, in 1970 from Washington University in St. Louis. After two years as a Postdoctoral fellow in animal reproduction at Ohio State she moved to the Department of Biochemistry at the University of California, Riverside as an Assistant Research Biochemist. She joined the faculty of the Department of Biochemistry in 1978 and is currently Professor of Biochemistry.

We dedicate **HORMONES** to our grandchildren:

Guinivere, Ophelia

Zavdi, Chagall, ZsaZsa

Charlotte, Lucie, Elliott, Raphael

About the Cover

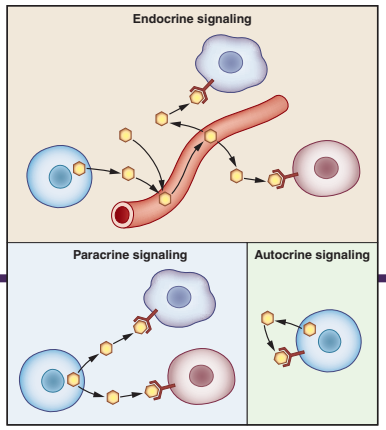
FRONT COVER IMAGE

The crystal structure of a G-protein-coupled receptor (GPCR) (**on the outside front cover**) with a hormone ligand in the outer cell membrane in association with the G-protein on the intracellular side of the membrane is presented on the outside front cover. This is a representation of the proteins' structural elements. The receptor (*green)* consists of seven membrane-spanning helices with an extracellular N-terminal region forming the ligand binding pocket. The intracellular loops and carboxy terminus connect the receptor to the α subunit (*blue*) of the G-protein, which is responsible for transmitting the signal of the hormone to intracellular effector proteins. Here the α subunit is shown in contact with the β (*gold*) and γ (*red*) subunits of the G protein. The Nobel Prize in Chemistry for 2012 was awarded to Brian Kobilka (senior author on the Nature paper; see citation below) and to Robert Lefkowitz for their work that was "crucial for understanding how G-protein–coupled receptors function." The human genome has over 800 genes for G-protein-coupled receptors also known as seven-transmembrane domain receptors. A GPR receptor protein has >500 amino acids.

The citation of the article in NATURE is as follows: S.G.F. Rasmussen & B.K. Kobilka + 19 co-authors from 9 institutions. "Crystal structure of the β_2 adrenergic receptor-Gs protein complex". in Nature **477**:549–555 (2011).

BACK COVER IMAGE

This paragraph is to honor the splendid work of Professor Dorothy Crowfoot Hodgkin in determining the crystal structure of insulin. (**See the outside back cover**) Dorothy's early x-ray foundation involved being the first person to use the newly emerging procedure of x-ray crystallography to learn the three-dimensional orientation of every atom in the target being studied. These included the following small molecules; the steroid cholesterol (MW 386; 1937), vitamin D_3 (MW 384; her Ph.D. dissertation, 1945), penicillin (MW 334; 1945) and vitamin B12 (MW 1,356; 1954). These significant accomplishments all used tedious hand computations since computers were not available until the 1970s. In 1964, Professor Crowfoot Hodgkin was awarded the Nobel Prize in Chemistry for her pioneering work that made x-ray crystallography an essential tool to understand the shapes and interactions of large molecules like insulin (MW 5,808; 51 amino acids in two peptide chains with two key disulfide bonds) and large proteins like hemoglobin (MW 68,880; 574 amino acids), steroid receptors (500-700 amino acids) and now large G-protein-coupled membrane receptors. She completed the insulin structure in 1969. The X-ray structure revealed that it crystalized first as a dimer, and then 3 dimers co-crystalize as a hexamer; see the figure on the back cover. Each of the three dimers uses a histidine to stabilize its interactions with a single central zinc atom (Zn^{2+}) that emphasizes the threefold symmetry. Insulin is stored in the body as a hexamer in the pancreas beta cells.



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 whose importance 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—i.e., 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: (a) the anatomical description of cells A and B and their immediate environment, as well as the distance between A and B; (b) the chemical structure of the hormone(H); (c) the details of the biosynthesis of the hormone by cell A; (d) the mode of transfer of H from cell A to cell B; (e) the detailed mechanism by which cell B uses receptors to detect the presence of H; (f) how cell B transduces the presence of H to initiate and sustain a biological response; and (g) 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 1900–1960, 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 1955–1960 and extends to the present time. The availability of radioactive isotopes of carbon

Hormones. DOI: http://dx.doi.org/10.1016/B978-0-12-369444-7.00001-2

(^{14}C), hydrogen (^{3}H), phosphorus (^{32}P), among others, coupled with advances in chemical methodology (chromatography, mass spectrometry, nuclear magnetic resonance spectroscopy (NMR), 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 the more specific considerations of individual hormonal systems in the chapters to follow.

B. Review of Animal Cell Structure

In order 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 Figure 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 transport of macromolecules, proteins, and RNA, between the two

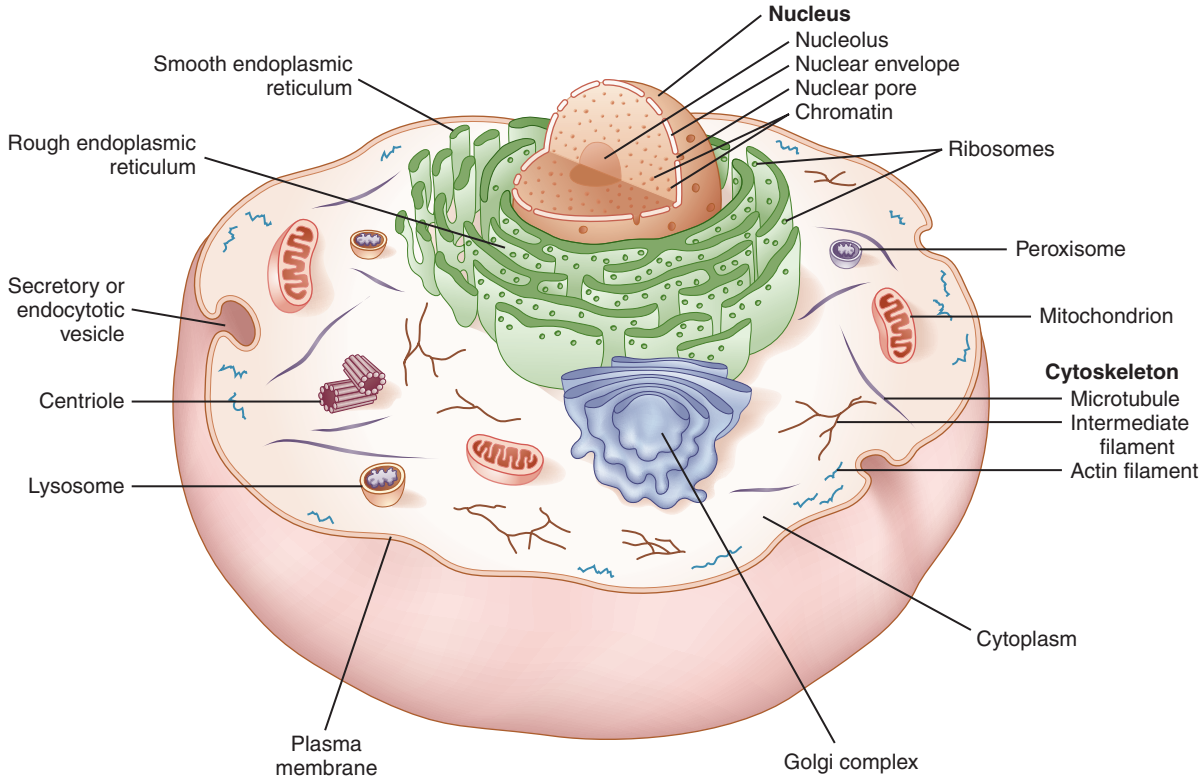


Figure 1-1.
Structural elements of animal cells. The major features shared by animal cells are shown. In eukaryotes the nuclear membrane separates the genetic material, the chromatin, from the cytoplasm. Molecules move between the two compartments through nuclear pores and a portion of the chromatin (see Figure 1-2), 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 Figure 1-3.

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 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.

Figure 1-2A 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,

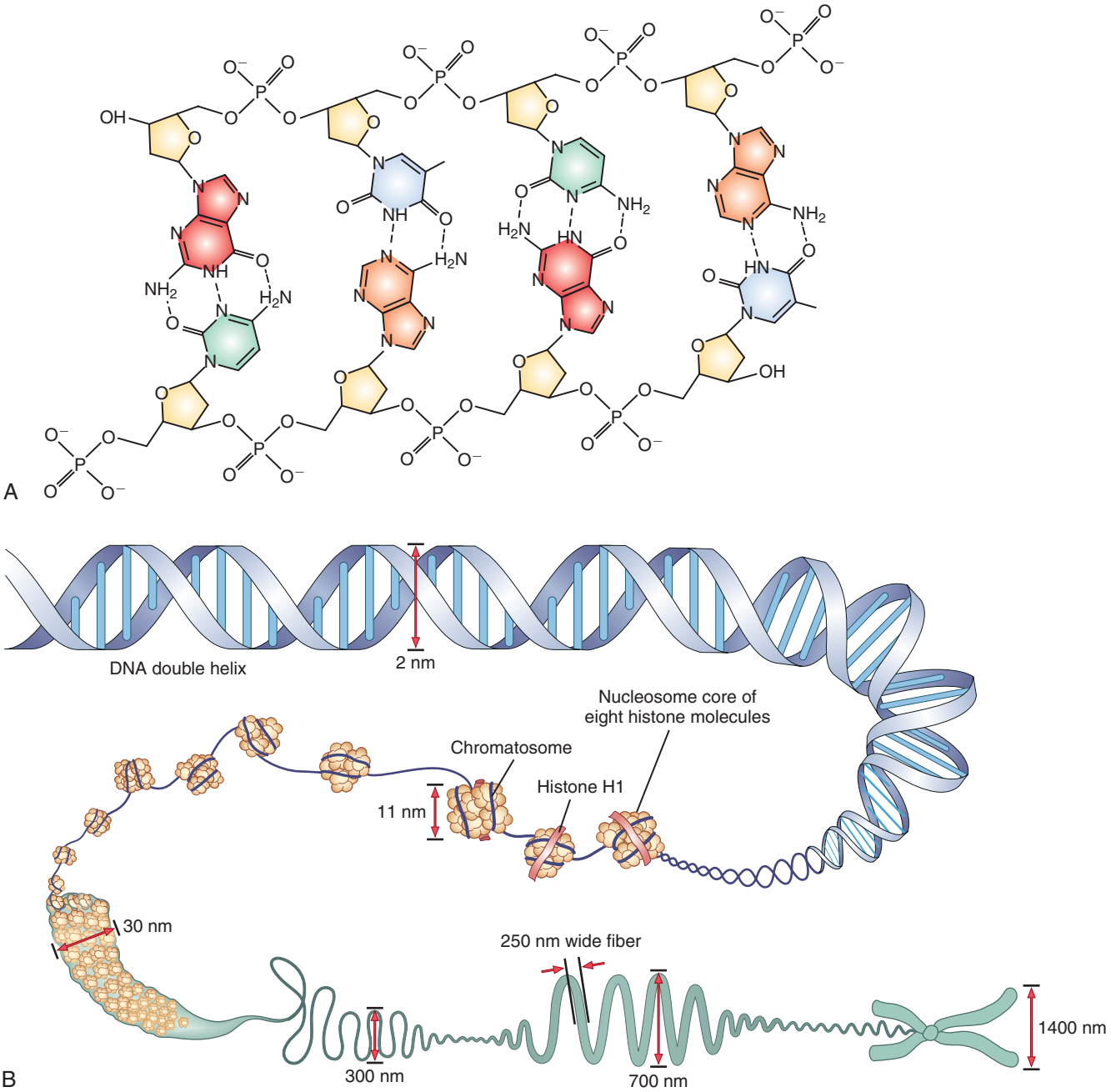


Figure 1-2.

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 anti-parallel 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.

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 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 Figure 1-2B. The result of this process is the packing of a linear molecule of DNA that is about 10^5 μM long into a nucleus with a diameter of about 10 μM.

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 Figure 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 Figure 1-3. 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 Figure 1-3, are found in or associated with the plasma membrane.

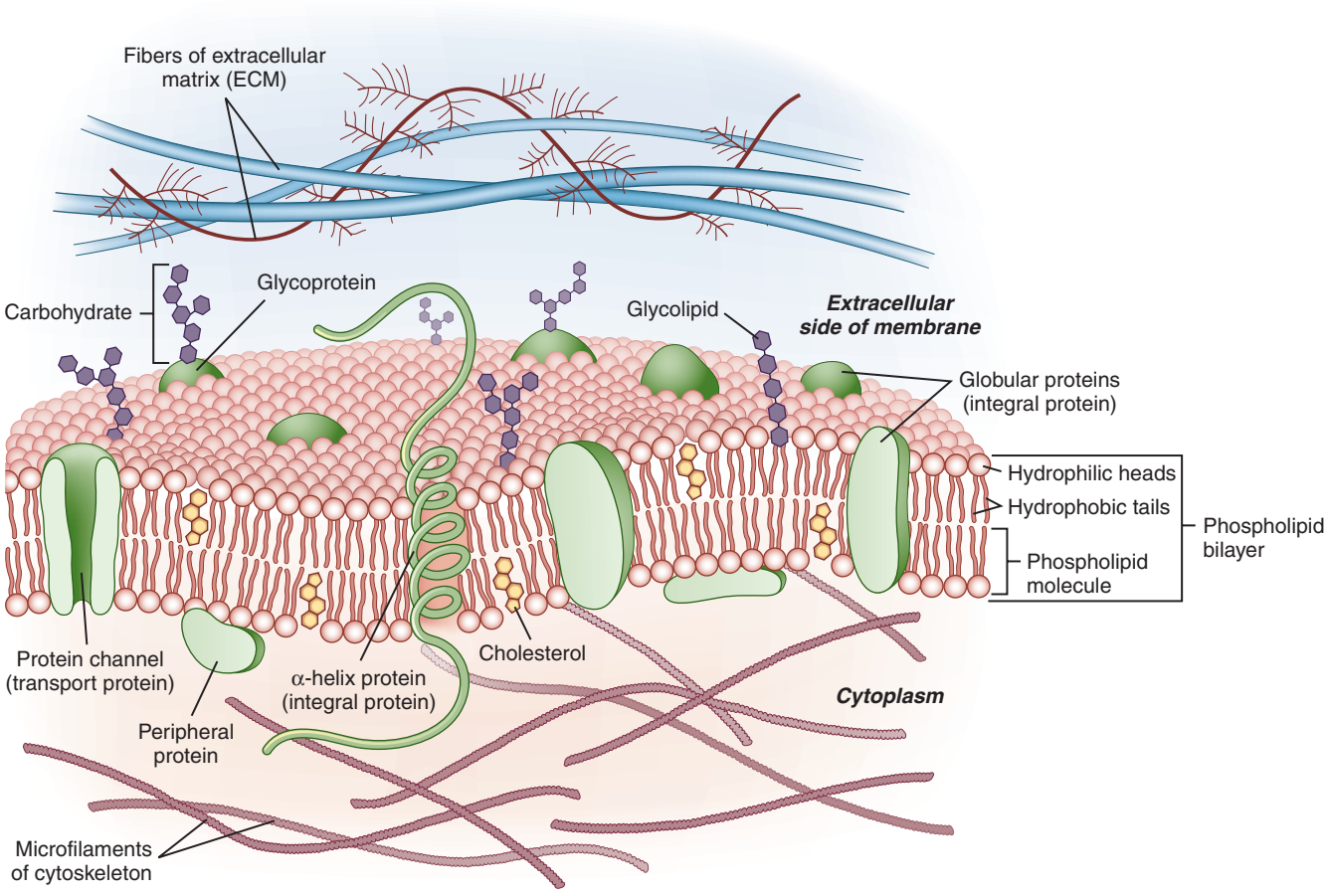


Figure 1-3.
The plasma membrane. The phospholipids that make up all cellular membranes are oriented with their polar head groups (usually choline, ethanolamine, or serine) facing the aqueous environments inside and outside the cell and their hydrophobic fatty acid side chains towards the interior of 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).

In many chapters in this book we will be considering the actions of membrane hormone receptors which 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. These carbohydrate moieties may play important recognition functions or in the case of 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 Figure 1-1. 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 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.

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) is at one extreme of the size range; the pituitary gonadotropins (Chapter 3) 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 three amino acids (TRH, Chapter 3) to over 100 per subunit. Thyroid hormone (Chapter 5) and epinephrine (Chapter 11) 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). Arachidonic acid, cleaved from membrane phospholipids, is the main precursor of the prostaglandins and other eicosanoids (Chapter 8).

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: thyroid hormone 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 Figure 1-4.

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 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.

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; Figure 3-5).

The lower left panel of Figure 1-4 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 which

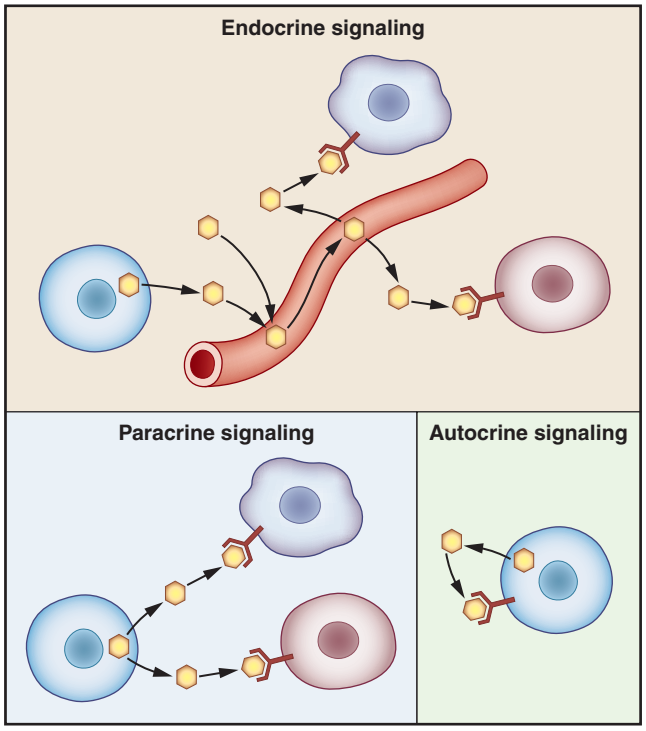


Figure 1-4.
Types of hormonal signaling. Three of the ways that hormones secreted by one cell can carry out its signaling function are illustrated. The top panel shows the classical endocrine system with a specialized hormone-synthesizing cell secreting its product into the bloodstream. It is carried 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.

are reached by diffusion: i.e., 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) 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). IGF-1 (Chapters 3 and 17) 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 describes the production of the steroid hormones, while Chapters 5, 8, and 11 describe the biosynthesis of the thyroid hormones, 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, 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. Figure 1-5 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 calcitonin-gene-related peptide (CGRP) was one of the first examples of alternative splicing to be elucidated (see Figure 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 post-translational 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

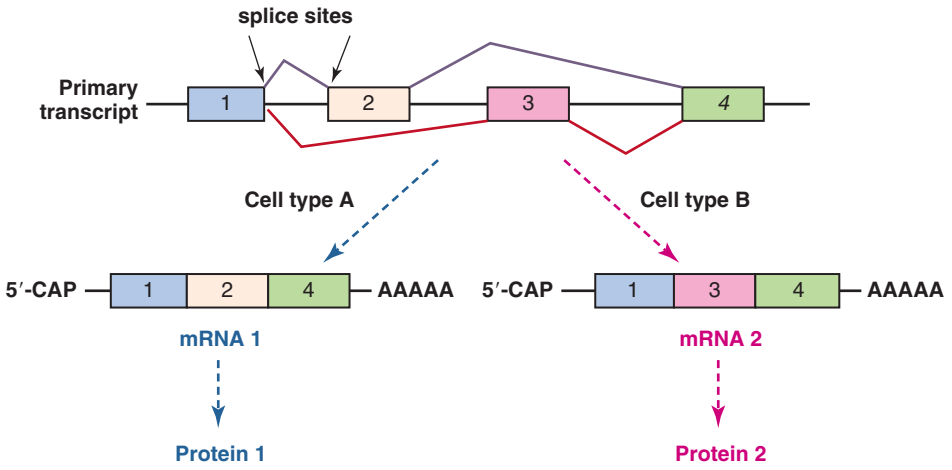


Figure 1-5.
Alternative splicing of mRNA for hormones. In eukaryotes almost all genes for proteins consist of regions of DNA that carry the code for the protein (exons; colored boxes) interrupted by noncoding sequences (gray line) in the primary mRNA transcript. Maturation of the primary transcript involves 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 Figure 9-10 for the example of calcitonin and calcitonin-gene-related peptide.

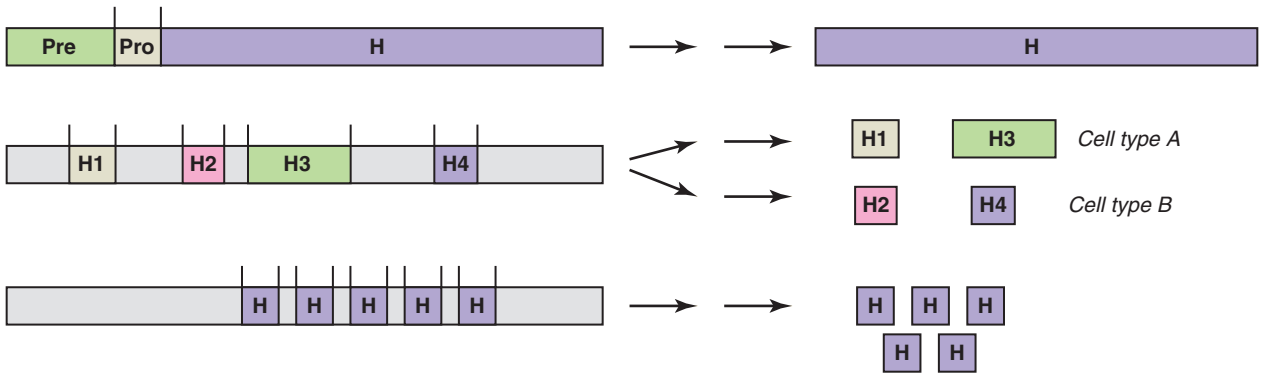


Figure 1-6.
Processing of pre- and pro-hormones. Many protein and peptide hormones (right) are synthesized within a larger precursor protein (left), of which three examples are shown here. The process is catalyzed by specific proteases that cleave the protein at specific sites (vertical lines), usually 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 which are sequentially removed to form the active hormone; see parathyroid hormone, Figure 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 proopiomelanocortin (POMC), Figure 3-15. Thirdly, 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 (Figure 3-9).

as the hormone is being prepared for movement into secretory vesicles.

Figure 1-6 illustrates some examples of the post-translational 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, Figure 9-9) contains 84 amino acids from which pre- and pro-sequences of 25 and 6 amino acids, respectively, have been removed.

The second example in Figure 1-6 shows a precursor protein that contains within its sequence several biologically active peptides, and which can be differentially processed in different cell types. Such a situation is exemplified by proopiomelanocortin (POMC; Figure 3-15). ACTH (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 (thyrotropin releasing hormone, Figure 3-9). This example, as well as that of insulin (not shown in Figure 1-6 but see Figure 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 Figure 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 pro-protein.

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 Figure 1-7: 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 parathyroid hormone (PTH) is considerably more complex than shown in this figure (see Chapter 9), 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 Figure 1-7 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 central nervous system, specific neurons of the hypothalamus secrete a given hormone (e.g., thyrotrophin releasing hormone, 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, TSH). 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., thyroid hormone). Thyroid hormone 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), the gonads (Chapters 12 and 13), and the adrenal cortex (Chapter 10).

The cellular and molecular details of how the synthesis and secretion of hormones is regulated by the players described above 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) and are released as synthesized, not stored in the gland. Thyroid hormone, 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 thyroid hormone and, in some cases, testosterone. Secondly, 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.

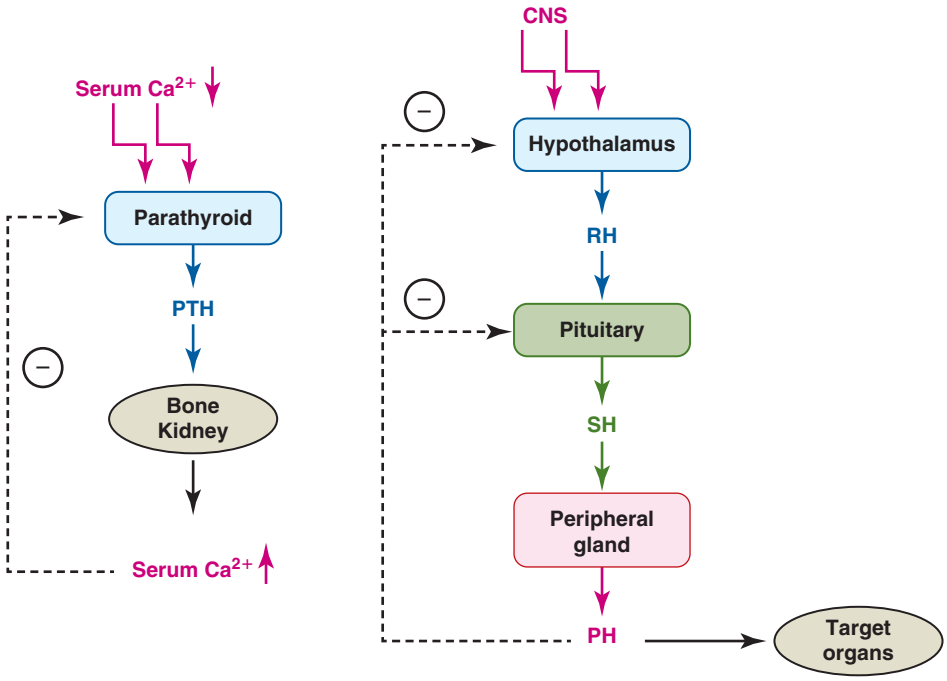


Figure 1-7.

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 parathyroid hormone (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 and especially Figure 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 central nervous system (CNS) and responds by secreting a hormone (releasing 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.

2. Binding Proteins

As discussed in Chapter 2, most steroid hormones have limited solubility in plasma due to their intrinsic hydrophobic character; accordingly, steroid hormones (see Table 2-5) as well as thyroid hormone (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 for its cognate hormone. These ligand domains display little amino acid sequence homology with the ligand binding of the cognate receptors. Nevertheless, the PTP ligand-binding domain also displays a high affinity (see section II.D following) 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 that 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 thyroid hormones 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: (a) a ligand-binding domain that noncovalently but stereospecifically binds the correct hormone for that receptor and (b) 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 following). In general, steroid hormones and thyroid hormone interact with receptors that are within the cell, either the nucleus or the cytoplasm, 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. G-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. 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. Figure 1-8 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 Figure 1-8. 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 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 Figure 1-8. G-proteins are composed of three subunits, α, β, and γ—i.e., 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, GDP occupies this site, and the complex is inactive. When ligand is bound, 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. Figure 1-9 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 for more details). In some cases, closely related receptors (e.g., EGFR 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 Figure 1-9, 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 IgG (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

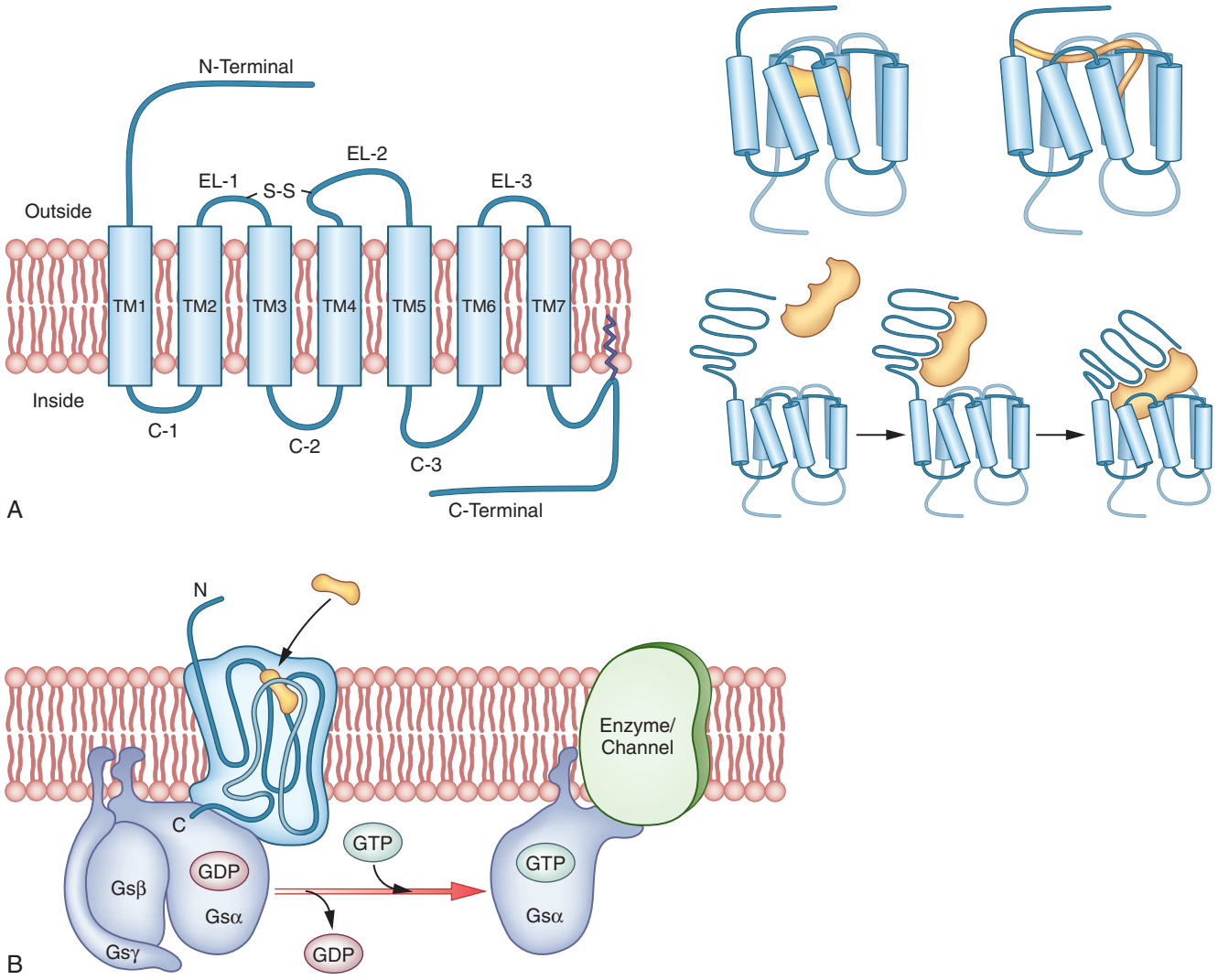


Figure 1-8.

G-protein coupled receptors (GPCRs). A. General structure of GPCRs. The G protein-coupled receptors 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 Figure 1-16).

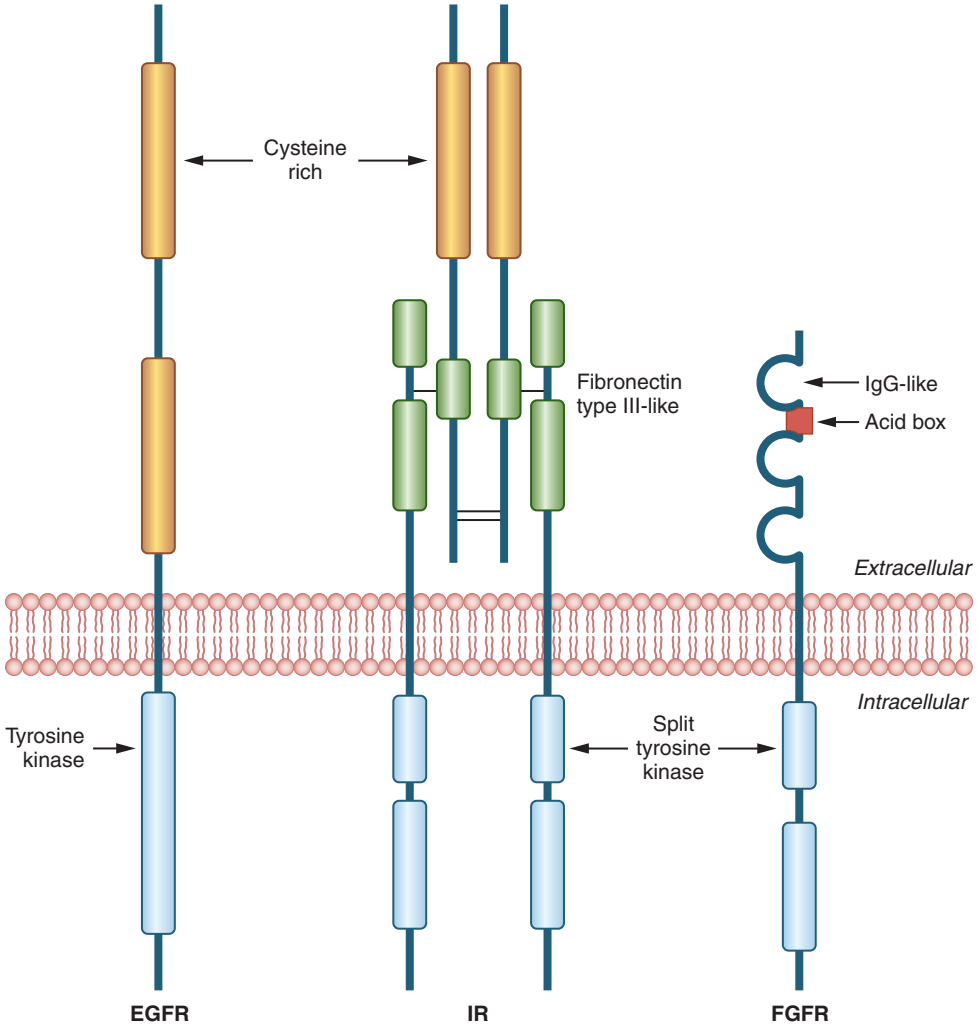


Figure 1-9.

General structure of receptor tyrosine kinases. The structural features of the receptor tyrosine kinases (RTK) are illustrated in three examples: the epidermal growth factor receptor (EGFR); the Ins/IGF-1 receptor; and the fibroblast growth factor receptor (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 non-tyrosine kinase sequence. Examples of N-terminal region motifs include cysteine rich sequences (gold), fibronectin type III-like regions (green), a series of IgG (immunogammaglobulin; blue) regions, and the acid box (red) as seen between the first and second IgG sequence 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 for more details on the structures of these and related receptors and their interactions with their ligands.

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 Figure 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, i.e., no activity-modulating ligand has yet been identified for them. We will focus our attention on the ten separately encoded proteins whose activity 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, thyroid hormone, and retinoic acid is shown in Figure 1-10A. 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 thyroid hormone (TR) and $1\alpha,25(OH)_2$-vitamin D_3 (VDR) are typically found in the nucleus of target cells where they (especially TR)

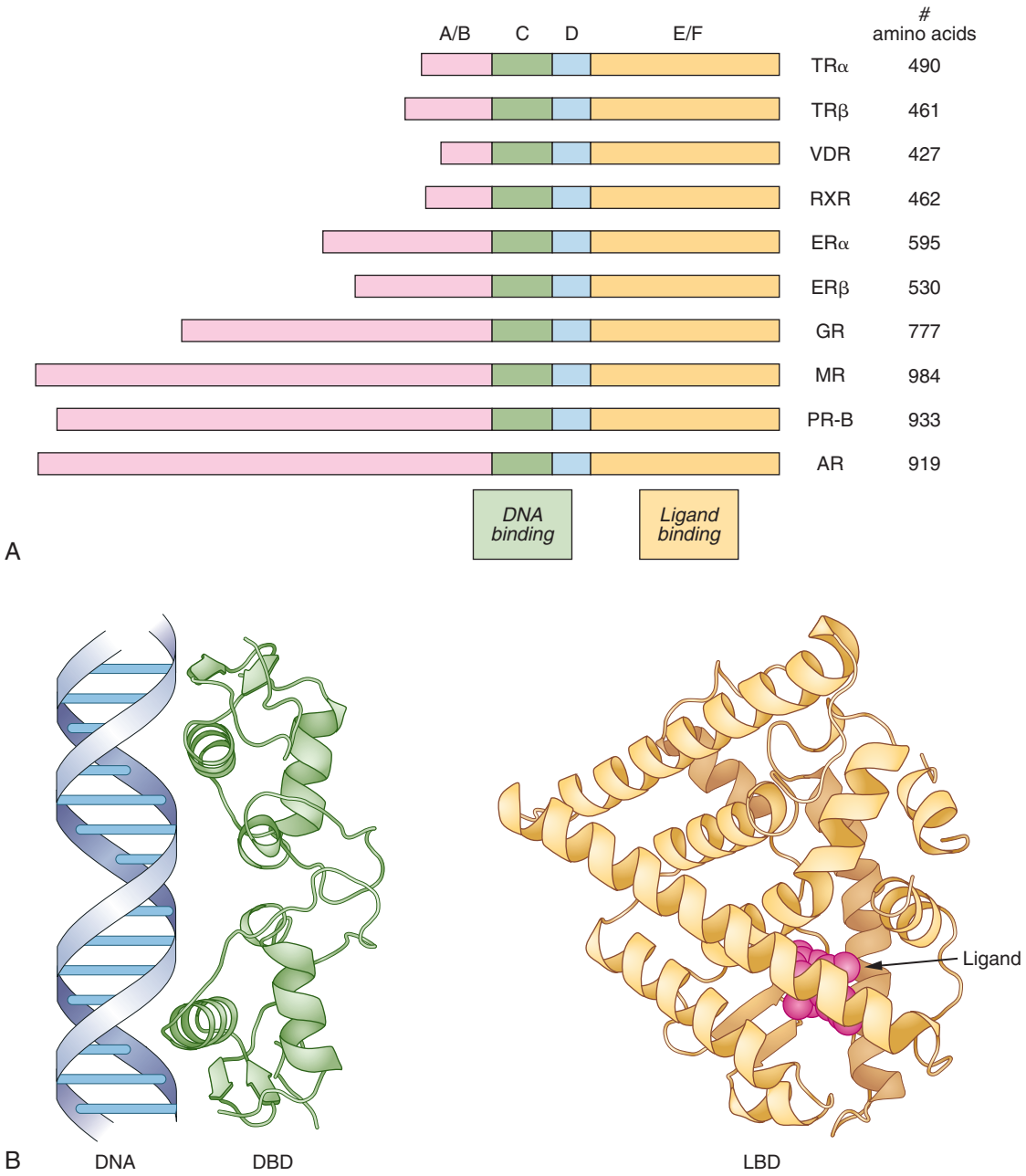


Figure 1-10.

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 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 Figure 1-11. 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 twelve 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.

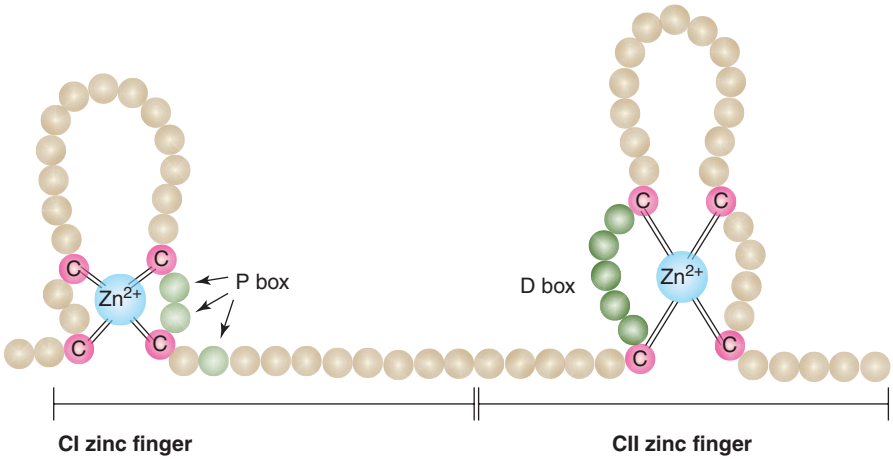


Figure 1-11.
Steroid hormone receptor zinc fingers. The amino acids in the DNA binding domain (see Figure 1-10) of a steroid hormone nuclear receptor are represented by circles. The coordination of a Zn^{2+} atom (blue) by four cysteines (pink) causes the formation of a loop. One of these, C1, which contains the P-box (light green), is involved in binding to the specific DNA binding site (hormone response element, see Figure 1-21) 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.

may be bound to corepressor molecules which suppress DNA transcription (see Chapter 5). These receptors form heterodimers with RXR (also in the nucleus) to bind to specific DNA sequences. The receptors for cortisol (GR) and aldosterone (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 Figure 1-10A, the nuclear receptors consist of a single polypeptide chain divided into six domains. The N-terminal sequence is highly variable in both 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 can regulate gene transcription independently of ligand binding, but can also be controlled by ligand binding. This section of the protein can undergo post-translational 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 Figure 1-10B. 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 Figure 1-11 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 twelve α-helices (Figure 1-10B) 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 among receptors in order 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 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.

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

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

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 = 5,730 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 Eq. 1.1.

$$K_a = \frac{[RH]}{[H][R]} = \frac{k_{+1}}{k_{-1}} = 1/K_d \qquad (1.1)$$

As shown in Figure 1-12, 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 Figure 1-12) 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 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 Figure 1-13. 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

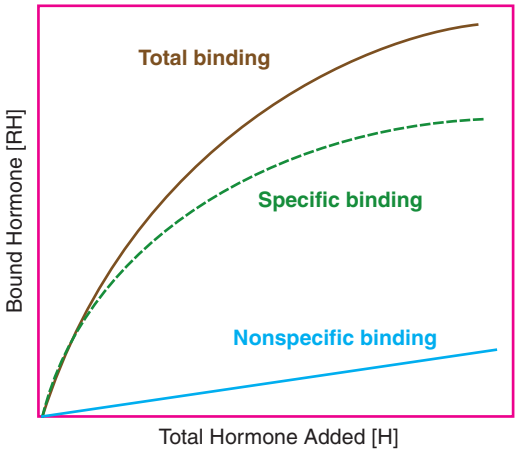


Figure 1-12.
A typical plot showing the concentration dependency for the hormone [H] under experimental evaluation. The horizontal axis shows increasing concentrations of [H] that generate the data shown on the vertical axis of the increasing amounts of Bound Hormone [RH]. There are two categories of 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.

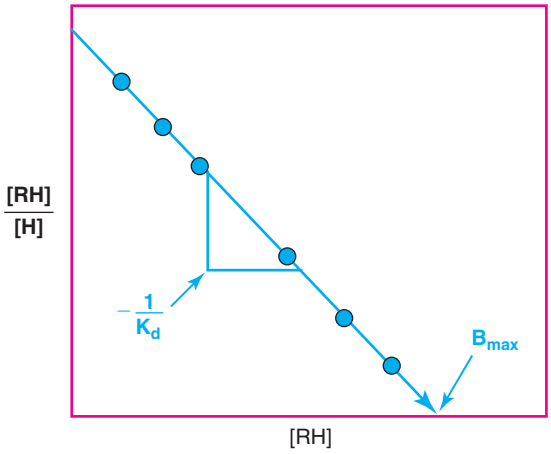


Figure 1-13.
Typical plot of a Scatchard analysis of specific binding of hormone to its receptor. The vertical axis shows the ratio of bound ligand [RH] to free hormone [H].

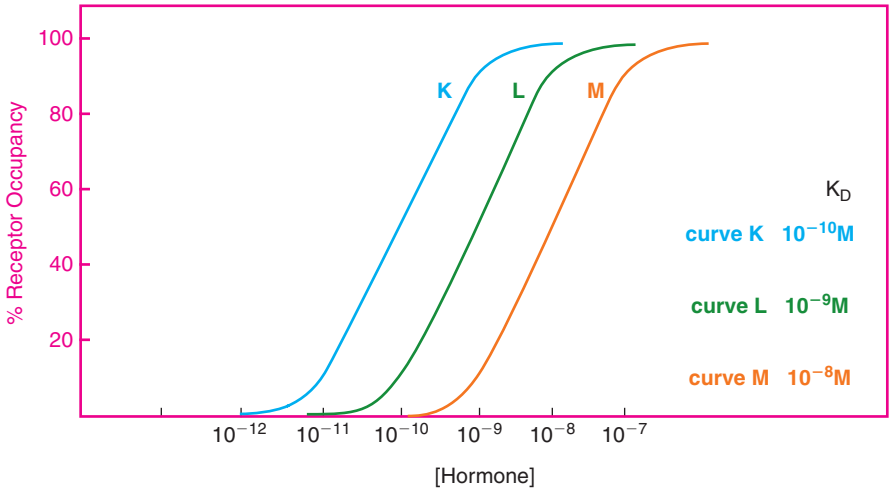


Figure 1-14.
Regulation of receptor performance, by determination of the K_d. The horizontal line shows the concentration dependency of three different hormones (K, L & M), which have different affinities for binding to the receptor from 10^{-11} to 10^{-8} molar. The specific K_d values for K, L & M 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.

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,

$$\frac{\text{bound}}{\text{free}} = \frac{[RH]}{[H]} = K_a(B_{max} - [RH]) = \frac{1}{K_d}(B_{max} - [RH]) \tag{1.3}$$

When the line in Figure 1-13 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. Figure 1-14 illustrates the regulation of steroid receptor performance for binding the same ligand to three related receptors which have similar but not identical amino acid sequences. The three receptors' K_ds for binding curves K, L and M are 10^{-10} M, 10^{-9} M 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.

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 in order 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 Figure 1-8, 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 5 β- and 14 γ-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

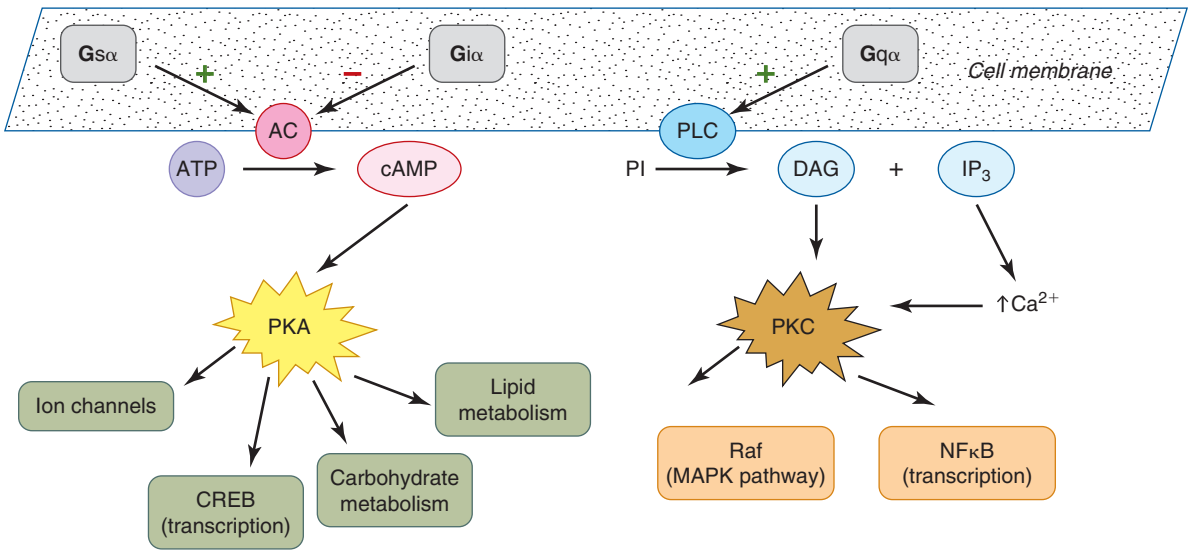


Figure 1-15.

Hormonal signaling by G-protein coupled receptors. 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 Figure 1-8 the G-protein molecules are shown after being released from their βγ partners through interaction with a G-protein coupled receptor (see Figure 1-8). 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 (cyclic AMP response element binding 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 diacylglycerol (DAG). See Figure 1-16 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 Figure 1-18) or the transcription factor NFκB to affect gene transcription.

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.

Figure 1-15 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 (adenosine-3′-5′-cyclic monophosphate) the first intracellular second message to be discovered in the 1970s. Cyclic AMP binds to the regulatory subunits of 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 Figure 1-15, 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 Figure 1-15 $G_q\alpha$ is shown interacting with phospholipase C. This enzyme catalyzes the reaction shown in Figure 1-16, the hydrolysis of the membrane lipid phosphatidyl inositol 4,5-bis-phosphate into IP_3 (inositol 1,4,5-triphosphate) and diacylglyderol (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 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 MAP kinase pathway (see Figure 1-19 in section III.B) and the phosphorylation of the nuclear transcription factor NFκB. The increased intracellular Ca^{2+} may activate exocytosis, 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 17kDa calcium-sensing protein. Calmodulin is present in the cytoplasmic compartment of virtually all cells of higher organisms.

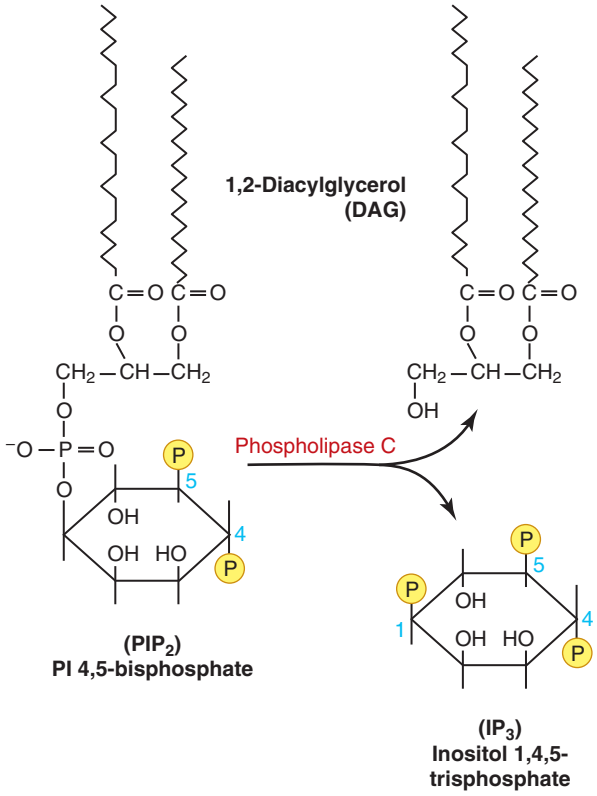


Figure 1-16.
Phospholipase C reaction. The cleavage by phospholipase C of phosphatidyl inositol 4,5-bisphosphonate into diacylglycerol (DAG) and inositol 1,4,5,-triphosphate (IP_3) is shown.

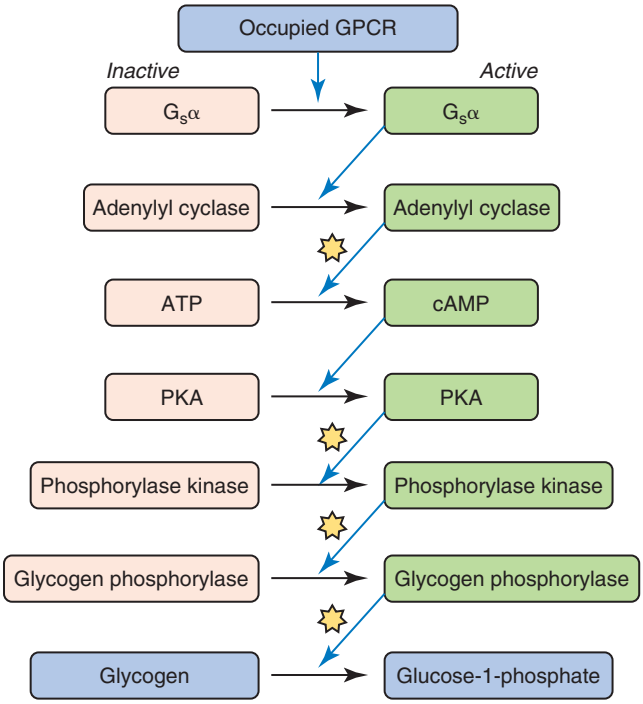


Figure 1-17.
Amplification of hormonal signaling. The cascade of events from the binding of epinephrine to its G-protein coupled receptor in skeletal muscle to the breakdown of glycogen is shown as one example of the amplification of hormonal signaling. On the left are molecules that are inactive in the cascade prior to the initial binding event and on the right are the active forms of the molecules. As shown in Figures 1-8 and 1-15, $G_s\alpha$ is activated by hormone binding and in turn activates adenylyl cyclase in the membrane, generating the second messenger cAMP. Protein kinase A (cyclic AMP dependent protein kinase, PKA) 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.

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 phosphatidyl inositol-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 GTP-ase 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 Figure 1-17 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 central nervous system, hypothalamus, pituitary, and peripheral target organ, as described in Figure 3-1.

2. Receptor Tyrosine Kinases

Figure 1-18 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 beginning with that of the kinase Raf (MAPKKK) by a small G-protein, Ras. Ras in turn is activated by the guanine nucleotide exchange factor, SOS, recruited to the phosphorylated RTK by the adaptor protein GRB2.