Clinical Pediatric Endocrinology

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FIFTH EDITION

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Foreword

It is an honour to write this foreword. *Clinical Pediatric Endocrinology*, first edited solely by Charles Brook, has deservedly acquired a reputation as an essential textbook for everyone concerned with the care of children and adolescents with endocrine disease. The fifth edition, which Charles Brook co-edits with Peter Clayton and Rosalind Brown, maintains the very high standard of previous editions and also presents what I believe to be the most authoritative account of the scientific and clinical 'state of the art' related to pediatric endocrinology.

The book opens with three outstanding chapters on the scientific basis of current clinical practice. These are up-to-date, readable, and extremely informative. The description of disorders in the broad field of pediatric endocrinology are well organized and the international contributors are universally recognized for expertise in their respective areas. Again, there is attention to clear presentation and readability, the hallmarks of good writing and editorial rigor.

In particular, I was impressed by the accounts of topics of rapidly evolving scientific interest such as the development of the reproductive systems, fetal programing, and monogenic obesity, all now clearly embraced by the field of pediatric endocrinology.

This publication does not rely on its previous reputation, but enhances it as a modern textbook of exceptional quality. Despite the availability of a massive amount of online information, a detailed textbook, with the clear aim of contributing to the care of the child with endocrine disease, offers a far more useful source of authoritative information. The fifth edition of *Clinical Pediatric Endocrinology* emphatically achieves this aim.

Martin O. Savage

Preface to the fifth edition

Looking back over the quarter century since I embarked on the first edition of this book, I am impressed more by what has not changed in the clinical practice of pediatric endocrinology than by the advances. Clinical assessment remains the core function and, although the advances in molecular biology have illumined some of the mechanisms of disease, their clinical applicability is limited. Perhaps genomics and proteomics will serve us better. Some diagnostic and therapeutic advances, for example the hypothalamic peptides, have changed clinical practice, but we still debate the uses and abuses of steroids, thyroxine, and growth hormone.

The book has once again been almost entirely rewritten with the intention of reflecting the themes of 2005, and my co-editors and I have been served very well by the contributors. The choice of whom to invite was based on the wise advice of Rosalind Brown and Peter Clayton and it was due to their input that only one author actually failed to deliver – 'twas ever thus. These co-editors oversaw the science in the book but I take entire responsibility for the errors of commission and omission in the editing of the text.

It is a pleasure to acknowledge my continuing friendship with Blackwell Publishing, this time represented by Alison Brown, but the quantum leap in book production is due to Gillian Whytock of Prepress Projects Ltd. Gillian's charm, patience, and courtesy have been a continual comfort to all involved in completing the project.

It is fitting that the book will be launched at another joint meeting of the European Society for Paediatric Endocrinology and the Lawson Wilkins Pediatric Endocrine Society. It is to celebrate the contribution of Martin Savage to the former that we have invited him to write the foreword on this occasion. It is a matter of great pleasure that other pediatric endocrine societies now join our meetings to make them truly a world endeavor to bring endocrinology to its proper place in the care of children.

C.G.D. Brook

Preface to the first edition

Endocrine problems are not uncommon in paediatric practice and are mostly, *faute de mieux*, rather badly managed by non-specialists. This is especially true in England, where paediatric specialities are relatively newly defined. The same is certainly less true of Europe as a whole and this book has its origins in the friendship of the European Society for Paediatric Endocrinology, which has acted as a focus for the subject and which benefits greatly from its transatlantic corresponding members. If the book were to have a dedication, it would be to the health of the Society, coupled with a toast to its American counterpart, the Lawson Wilkins Society.

I hope that the book will be of service to general paediatric departments and of help and interest to departments of (adult) endocrinology in their dealings with patients who are still growing. In a book of this size, there may well be sins of omission and commission and for these I alone can take responsibility and I apologize for them in advance. If any readers were to take the trouble to let me know about such sins for future reference, I would be very grateful.

In the completion of my editorial task I have been greatly assisted by Miss Lynette Napper and Mrs Sue Shorvon, my secretaries at The Middlesex Hospital, and by Mr Jony Russell and Mr Peter Saugman at Blackwell Scientific Publications. My co-authors and I thank them for assisting at the birth of our work.

C.G.D. Brook

1 Principles of hormone action

Melissa Westwood

Introduction

Hormones elicit their effects on target cell function by interacting with specific receptors. These are located either at the cell surface or within intracellular compartments, such as the cytoplasm and nucleus. Receptor location, which forms the basis of their classification into subgroups (Fig. 1.1), reflects ligand characteristics. Receptors for hydrophilic hormones, such as the pituitary-derived proteins, insulin, and the catecholamines, are present at the plasma membrane; lipid-soluble steroid and thyroid hormones cross this barrier to access intracellular binding sites.

Receptors are specific and usually have a high affinity for their particular ligand, but the forces involved in ligand/receptor binding (ionic attractions, van der Waal's forces, hydrogen bonding, or hydrophobic interactions) are weak. The reaction is therefore reversible, and the receptor can be reused. K_d values, the concentration of ligand at which half the receptors are occupied, approximate the physiological concentration of the hormone (usually ranging between pico- and nanomolar concentrations), so that the receptor is sensitive to changes in hormone concentrations.

The concentration of each receptor can vary, and a cell may become more or less sensitive to a given extracellular concentration of ligand. Sensitization can occur by increasing the number of binding sites available. This is achieved through a combination of increased receptor synthesis and decreased degradation. Cells can become refractory (desensitized) to ligand by altering receptor localization (e.g. by internalizing cell surface receptors), reducing receptor levels, or recruiting molecules that deactivate intracellular signaling pathways. Internalization of cell-surface receptors involves endocytosis: the receptors relocate into clusters within the membrane, which then invaginates to form first a pit and then an endosomic vesicle. Once part of an endosome, the signal is terminated because the receptor/ligand complex dissociates as a result of the acidic pH within this compartment. Degradation usually involves the ubiquitin–proteosome pathway.

Cell-surface receptors

There are two major groups of cell-surface receptors linked to intracellular signals. The first relies upon tyrosine kinase for the initiation of signaling. The second group tends to activate serine or threonine kinases by coupling to G-proteins. However, there is an underlying structural unity in all cell-surface receptors because each is made up of three segments, an extracellular domain, a transmembrane region, and a cytoplasmic domain (Fig. 1.2).

The N-terminus of the protein forms the extracellular component of the receptor, and this domain is responsible for hormone recognition and binding. The extracellular domain is heavily glycosylated and comparatively rich in cysteine residues. These are necessary for disulfide bond formation and correct protein folding, but the functional significance of the oligosaccharide moieties is not known. The extracellular domain of some receptors [e.g. the growth hormone receptor and the receptor for thyroid-stimulating hormone (TSH)] can be cleaved from the plasma membrane so that it forms a separate entity that can be detected in the circulation, where it may function as hormone-binding proteins (see below).

The transmembrane region varies in structure from a simple linear stretch of approximately 25 hydrophobic amino acids to a more complex arrangement that threads the plasma membrane crossing it seven times. This segment of the receptor is often regarded as a passive lipid anchor, but there is evidence to suggest that it can influence receptor function as, for example, mutations in the transmembrane region of the fibroblast growth factor (FGF) receptor are associated with achondroplasia.

The cytoplasmic C-terminus of the receptor generally forms the effector region of the molecule because it initiates an intracellular signaling cascade, often involving protein phosphorylation, that eventually results in the cellular response.

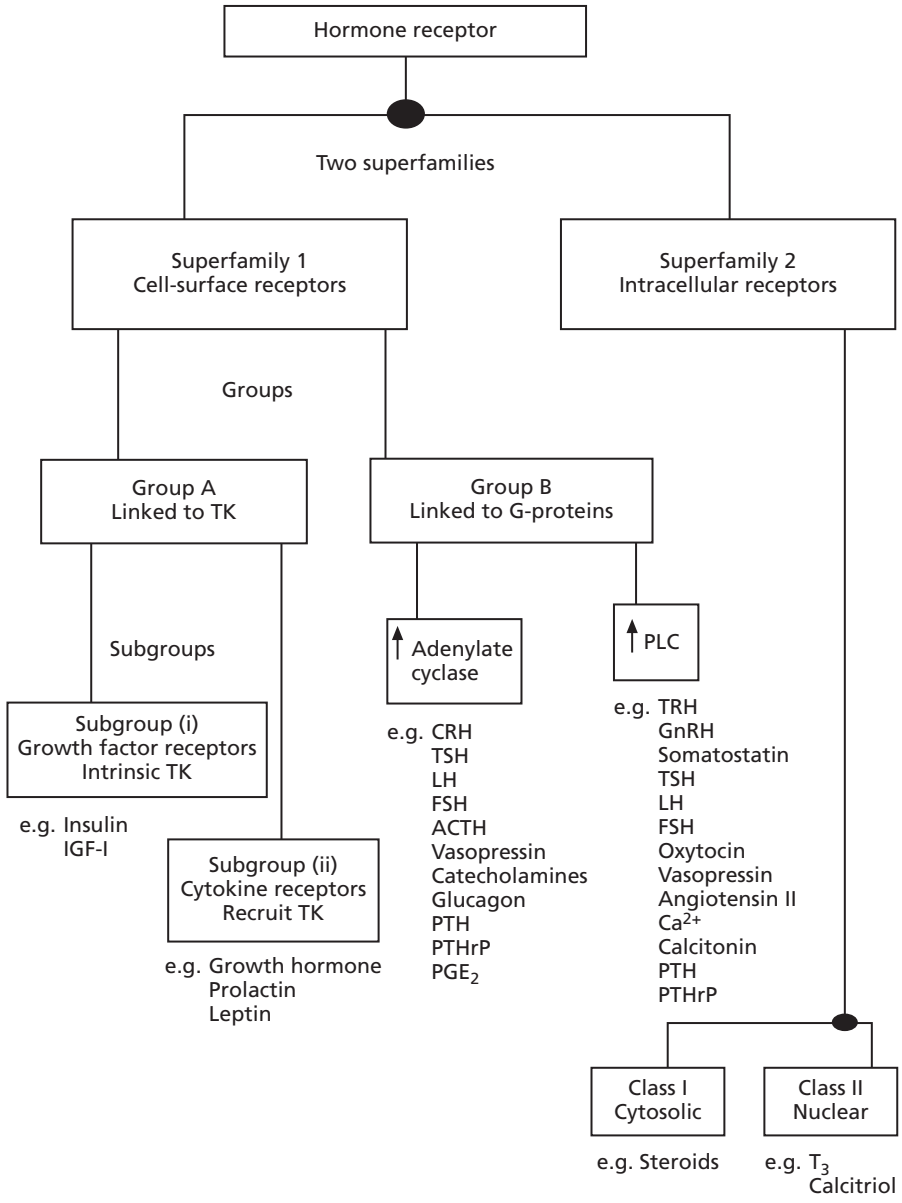


Fig. 1.1. A composite diagram showing the different classes of hormone receptors. Receptors for some hormones can occur in more than one grouping. For example, different types of PTH receptors link to different G-proteins and therefore couple to either adenylate cyclase or phospholipase C (PLC). TK, tyrosine kinase.

Protein phosphorylation

The amino acids serine, threonine, and tyrosine each carry a polar hydroxyl group that can be exchanged for a phosphate group from adenosine triphosphate (ATP) by enzymes collectively referred to as protein kinases. The energy generated during this reaction leads to a conformational change in the tertiary structure of the phosphorylated protein and, once activated in this way, many molecules within signaling pathways relay the signal by acting as protein kinases themselves. Each protein may be phosphorylated on more than one residue, and a protein may be the substrate for more than one kinase, in many cases allowing the convergence of several signaling molecules. The target sequence of most kinases has been identified, although the presence of a consensus motif within a protein's primary sequence does not mean that the protein will automatically be phosphorylated as the tertiary structure may prevent kinase access.

Kinases are grouped according to which amino acid they target. Serine/threonine kinases account for approximately 350 of the known phosphorylating enzymes and are responsible for the majority of the 10% of proteins that are phosphorylated at any given time in a mammalian cell.

Tyrosine kinases account for only 0.05% of the phosphoamino acid content of a cell, but they are key regulators of many cellular signaling pathways. In addition to protein activation, phosphorylation of tyrosine residues generates binding sites necessary for subsequent protein–protein interactions.

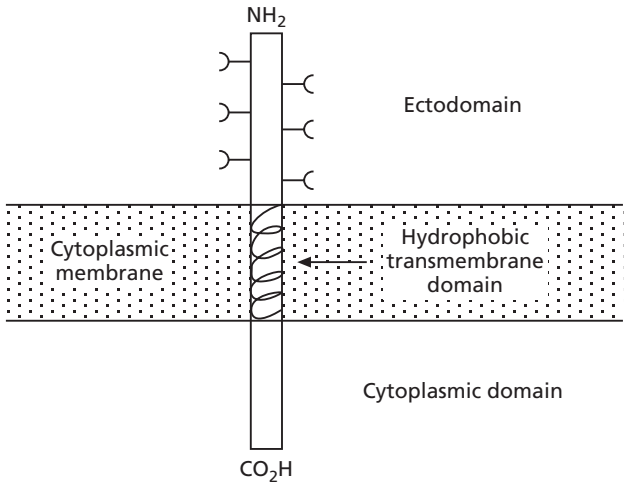


Fig. 1.2. Schematic representation of a membrane-spanning cell-surface receptor with three clearly identifiable domains: the extracellular domain is bridged by a membrane-spanning component to the intracellular cytoplasmic domain. Each domain has characteristic structural features that reflect its location and function.

The relatively long side-chain of phosphotyrosine enables it to dock with proteins containing "deep pockets" resulting from the presence of one or more consensus sequences (approximately 100 amino acids) known as the Src homology (SH2 or SH3) domain. Phosphorylation is a reversible process, and this molecular switch can be rapidly overturned through the action of enzymes termed phosphatases.

Tyrosine kinase-linked cell-surface receptors

These have a relatively simple transmembrane domain and either possess intrinsic tyrosine kinase activity or recruit such enzymes after activation by ligand binding.

Receptors with intrinsic tyrosine kinase activity

This family contains the insulin receptor, the structurally related type 1 insulin-like growth factor (IGF) receptor, and the receptors for epidermal growth factor (EGF), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF). They are often referred to as growth factor receptors.

The structure of these receptors is shown in Figure 1.2. Some ligands, for example FGF and EGF, stimulate dimerization of two adjacent monomeric receptors, whereas others, for example insulin and IGF-I, bind to receptors that exist as dimers in their unoccupied state. Either way, ligand/receptor coupling results in activation of the tyrosine kinase located in the cytosolic domain of the receptor.

Insulin/type 1 IGF receptors

Both receptors are heterotetrameric structures comprising two extracellular α-subunits linked to two membrane-spanning β-subunits by disulfide bonds [1,2] (Fig. 1.3). The α-subunits confer specificity for their cognate ligand, whereas the β-subunits possess the motifs required for recruiting the major signaling adaptor proteins and a tyrosine kinase domain, which is essential for the catalytic activity of the receptor. As a result of the considerable homology between the insulin and type 1 IGF receptors, cells expressing both can form a hybrid of an insulin αβ-hemireceptor coupled to an IGF-I αβ-hemireceptor [3]. The functional significance of this phenomenon has yet to be determined.

Insulin signaling pathways

Following autophosphorylation of tyrosine residues on the receptor β-subunit, a cascade involving more than 50 enzymes is activated; this includes primarily members of the insulin receptor substrate (IRS) family of proteins [4] (Fig. 1.3). Four mammalian IRS proteins have been identified, and evidence from transgenic mice suggests that they may display tissue and functional specificity [5], as IRS-1 seems to be important for somatic cell growth and insulin action in muscle and adipose tissue, whereas IRS-2 appears to be the main signaling molecule in the liver and is necessary for β-cell survival. Phosphorylation of IRS creates docking sites for proteins with Src homology 2 (SH2) domains. These include the regulatory (p85) subunit of phosphatidylinositol (PI) 3-kinase and growth factor receptor-binding protein 2 (Grb2). The effect of insulin depends on which of these effector molecules are expressed and recruited and which signaling pathways are activated as a result.

Stimulation of PI 3-kinase leads to the generation of PI 3-phosphate, by phosphorylation of phosphatidylinositol lipids at the D-3 position of the inositol ring and then activation of PI 3-dependent kinases (PDK) [6]. PDKs activate protein kinase B (PKB; also known as Akt) via phosphorylation of a critical serine and threonine residue, which results in the translocation of glucose transporters, predominantly GLUT-4, to the plasma membrane and the initiation of glycogen synthesis through activation of glycogen synthase (Fig. 1.3).

The mitogenic effects of insulin are mediated via Grb2. This adaptor protein links tyrosine-phosphorylated receptors or cytoplasmic tyrosine kinases to the guanine nucleotide exchange factor, SOS (son of sevenless protein) and, along with Ras, Raf, and MEK, is part of the pathway that leads to the activation of mitogen-activated protein kinase (MAPK; Fig. 1.3) [7]. This acts on multiple proteins to result in cytoplasmic and nuclear responses. The latter lead to stimulation of gene expression, protein synthesis, and cell growth. The MAPK pathway can be stimulated independently of IRS, because Shc, which is a substrate of the activated insulin receptor, can also associate with Grb2 (Fig. 1.3) [7].

IGF-I signaling pathways

Insulin and IGF-I have overlapping roles in metabolism, cell growth, differentiation, and cell survival, and their receptors

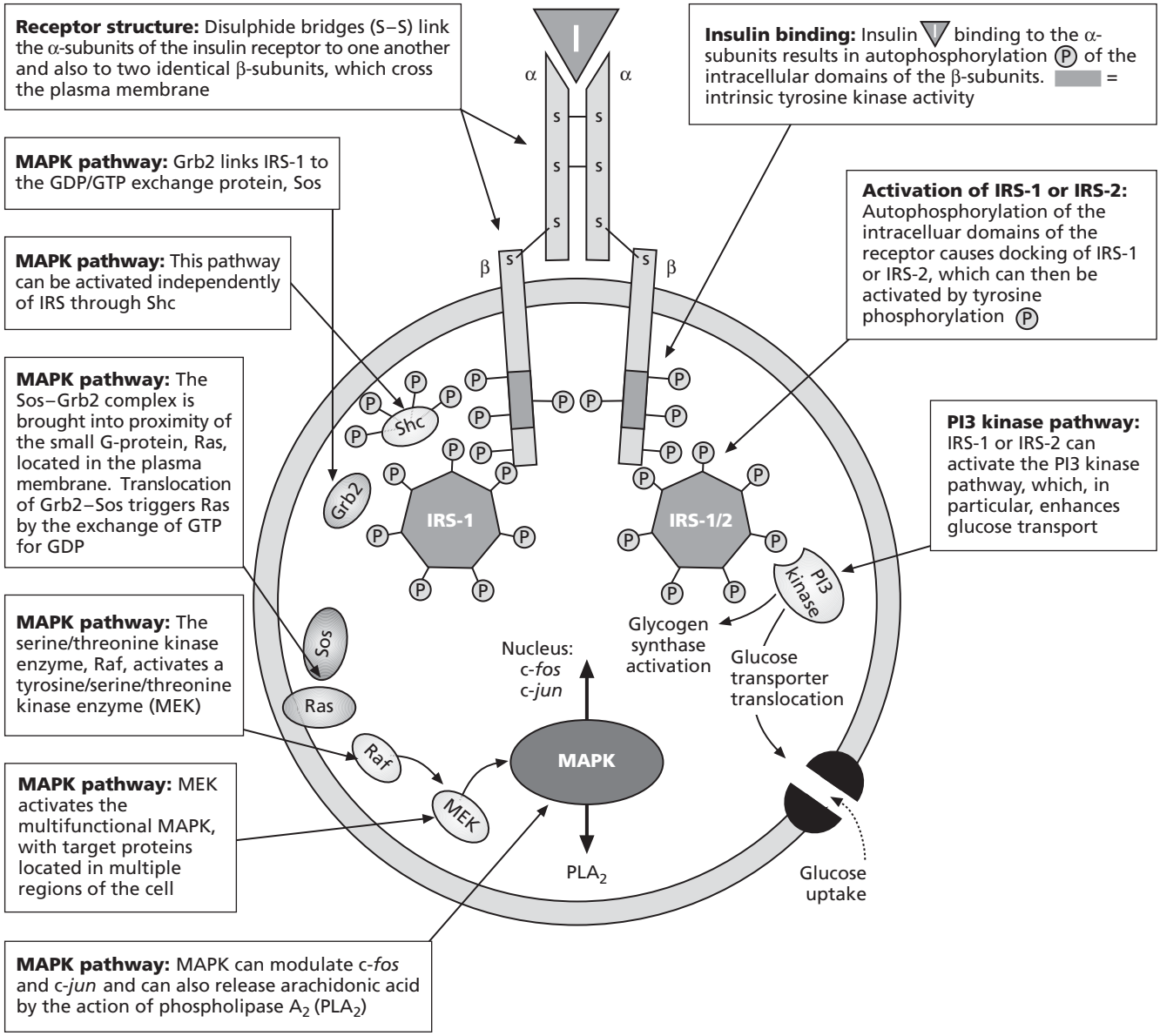


Fig. 1.3. Signaling pathways initiated in response to activation of the insulin receptor, an example of a receptor with intrinsic tyrosine kinase activity. The type 1 IGF receptor shares many of these pathways.

have structural similarity, so it is not surprising that activation of the type 1 IGF receptor activates many of the intracellular signaling events described above. IRS proteins, Grb2, and Shc are all involved in the cellular response to IGF-I (Fig. 1.3) [8]. However, it is thought that the two receptors can elicit distinct biological responses by using specific or preferential substrates, adaptor molecules, or signaling pathways. For example, through PI 3-kinase and generation of PI 3-phosphate, insulin activates protein kinase C (PKC) to stimulate proliferation of murine keratinocytes, whereas PKC is not involved in the proliferative response to IGF-I in these cells [9].

Desensitization

Protein tyrosine phosphatases (PTPs) play a key role in terminating the signal generated through the insulin or type 1 IGF receptor. This family of enzymes includes PTPα, SHP2, LAR, and PTP1B, but current evidence favors the last, particularly with regard to the negative regulation of insulin signaling pathways [10]. PTP1B *in vitro* dephosphorylates activated insulin receptors, IRS proteins, and possibly other downstream molecules as well. PTP1B-deficient mice display enhanced insulin sensitivity and increased insulin-stimulated phosphorylation of the insulin receptor in muscle and glucose. PTP1B gene variants in humans are associated with changes

in insulin sensitivity, which has prompted an interest in developing specific PTP1B inhibitors for the treatment of type 2 diabetes. Such compounds may also prove to be useful in cancer therapy as inappropriate activation of the type 1 IGF receptor has been linked to cellular transformation [11].

Defects

Mutations in the gene coding for the insulin receptor are associated with syndromes of severe insulin resistance, namely type A insulin resistance, Rabson–Mendenhall syndrome, and leprechaunism. All have impaired glucose metabolism in association with raised insulin levels, but only patients with leprechaunism completely lack functional insulin receptors, and they rarely survive beyond the first year of life [5]. Some patients with type A insulin resistance are reported to have normal insulin receptors, and these may harbor as yet unidentified mutations in any of other of the critical insulin signaling molecules described above. Mice deficient in the gene for IRS-1 display marked pre- and postnatal growth failure, insulin resistance, impaired glucose tolerance, and other features of the metabolic syndrome, but they do not develop diabetes, unlike the IRS-2 knockout animals [5]. This has led to the suggestion that IRS-2 is a diabetes-predisposing gene, although this has not been substantiated by clinical studies.

There have been no reports of humans completely lacking the gene for the type 1 IGF receptor, but knockout mice show severe growth failure, widespread developmental defects, and usually die at birth as a result of respiratory failure [12]. A recent study of children with intrauterine and/or postnatal growth restriction revealed a number of mutations associated with reduced receptor number and function [13].

Receptors that recruit tyrosine kinase activity

This group of receptors is referred to as the cytokine receptor superfamily. From an endocrine perspective, the most important members are the receptors for growth hormone (GH) and prolactin (PRL), which form the class 1 subgroup along with the receptors for erythropoietin, granulocyte–macrophage colony-stimulating factor (GM-CSF), leptin, and the interleukins (IL) 2–7, IL-9, IL-11, and IL-12 [14].

Like the tyrosine kinase receptors, cytokine receptors are expressed at the cell surface and are composed of a ligand-binding extracellular domain, a transmembrane region, and an intracellular carboxy tail (Fig. 1.2). Here the similarity ends, because members of the cytokine receptor superfamily do not possess enzymatic activity in their cytoplasmic domain. Instead, these receptors couple physically and functionally with non-receptor tyrosine kinases.

GH receptor activation

Early crystallographic studies revealed that ligand and receptor exist in a complex of one GH molecule and two molecules of receptor [15]. Subsequent work involving mutational analysis of residues within the ligand demonstrated that each molecule of GH has two distinct sites, a high-affinity "site 1" and a lower affinity "site 2," both of which are capable of binding to the extracellular domain of a GH receptor. The unoccupied receptor has not been crystallized, and it is not known whether dimerization occurs before or after GH binding, although there is evidence to suggest the latter. Hence, it is thought that GH initially binds to GHR via site 1 and that this then facilitates site 2 binding with a second receptor molecule (Fig. 1.4) [16]. Such homodimerization has also been reported for PRL [17] and erythropoietin [18], but members of the other subgroups of the cytokine receptor superfamily form heterodimers and oligomers.

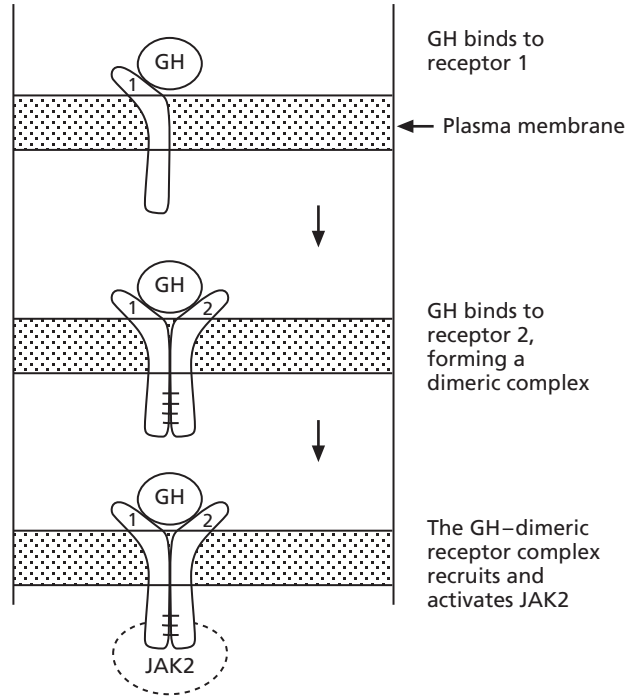


Fig. 1.4. Diagrammatic representation of GH binding to its cell-surface receptors and, via the formation of receptor dimers, subsequently recruiting Janus-associated kinase 2 (JAK2). The two receptors depicted (1 and 2) have identical structures.

Tyrosine kinase recruitment

Recent data suggest that the receptor undergoes a conformational change to the status required for tyrosine kinase recruitment and initiation of signaling following GH binding. Thirty-two mammalian non-receptor tyrosine kinases have been identified, which are classified into 11 groups based on sequence similarity in their Src homology (SH) 1, SH2, and SH3 domains. Each has the ability to bind to the intracellular motif of different receptor molecules. Early studies of GH signaling pathways used cross-linking and immunoprecipitation techniques to demonstrate that the activated GH receptor recruits predominantly members of the Janus family of tyrosine kinases [19,20].

Janus-associated kinases (JAK)

Four evolutionarily conserved members of the JAK family have been identified, JAK1, JAK2, JAK3, and Tyk2. JAK3 is expressed primarily in hematopoietic cells, although the others are found in most cell types. GH usually recruits JAK2 (Fig. 1.4), although GH-induced phosphorylation of JAK1 and JAK3 has also been reported. They all possess seven conserved JH regions (JH1–7), of which JH1 is the functional domain and JH2 a pseudokinase domain necessary to regulate the catalytic domain negatively so that the enzyme is inactive in the absence of a stimulus [21].

Although JAKs associate constitutively with the receptor through a proline-rich site known as Box 1 in the receptor's intracellular domain, they are spatially positioned and/or conformationally modified upon receptor activation so that transphosphorylation and activation of the kinase domain occur. This results in phosphorylation of the intracellular domain of the GH receptor and provision of docking sites for a variety of signaling molecules that contain SH2 or other phosphotyrosine-binding (PTB) motifs. These include SHC, IRS, and members of the signal transducer and activator of transcription (STAT) family of proteins (Fig. 1.5) [22].

STATs

In mammals, there are seven STAT family members, STAT1–4, STAT5A, STAT5B, and STAT6. Only STAT1, STAT3, and the STAT5 molecules are usually recruited in response to GH-induced JAK2 activation.

STAT proteins are localized in the cytoplasm in unstimulated cells, but they are rapidly recruited to the intracellular domain of the receptor after ligand/receptor coupling through binding between STAT SH2 domains and phosphorylated tyrosine residues on the receptor [23]. This interaction is highly specific and represents a critical step in determining the specificity of receptor-mediated STAT activation. Once bound, the STATs become phosphorylated. This leads to the formation of STAT homo- and heterodimers, which translocate rapidly to the nucleus for DNA binding (Fig. 1.5). Most STAT dimers recognize an 8- to 10-bp inverted repeat DNA element with a consensus sequence of 5′-TT($N_{4–6}$)AA-3′, usually referred to as a GAS element as a result of its initial characterization as a γ-interferon activation sequence recognized by STAT1 homodimers.

Following DNA binding, activated STAT dimers initiate the transcription of immediate early response genes that regulate

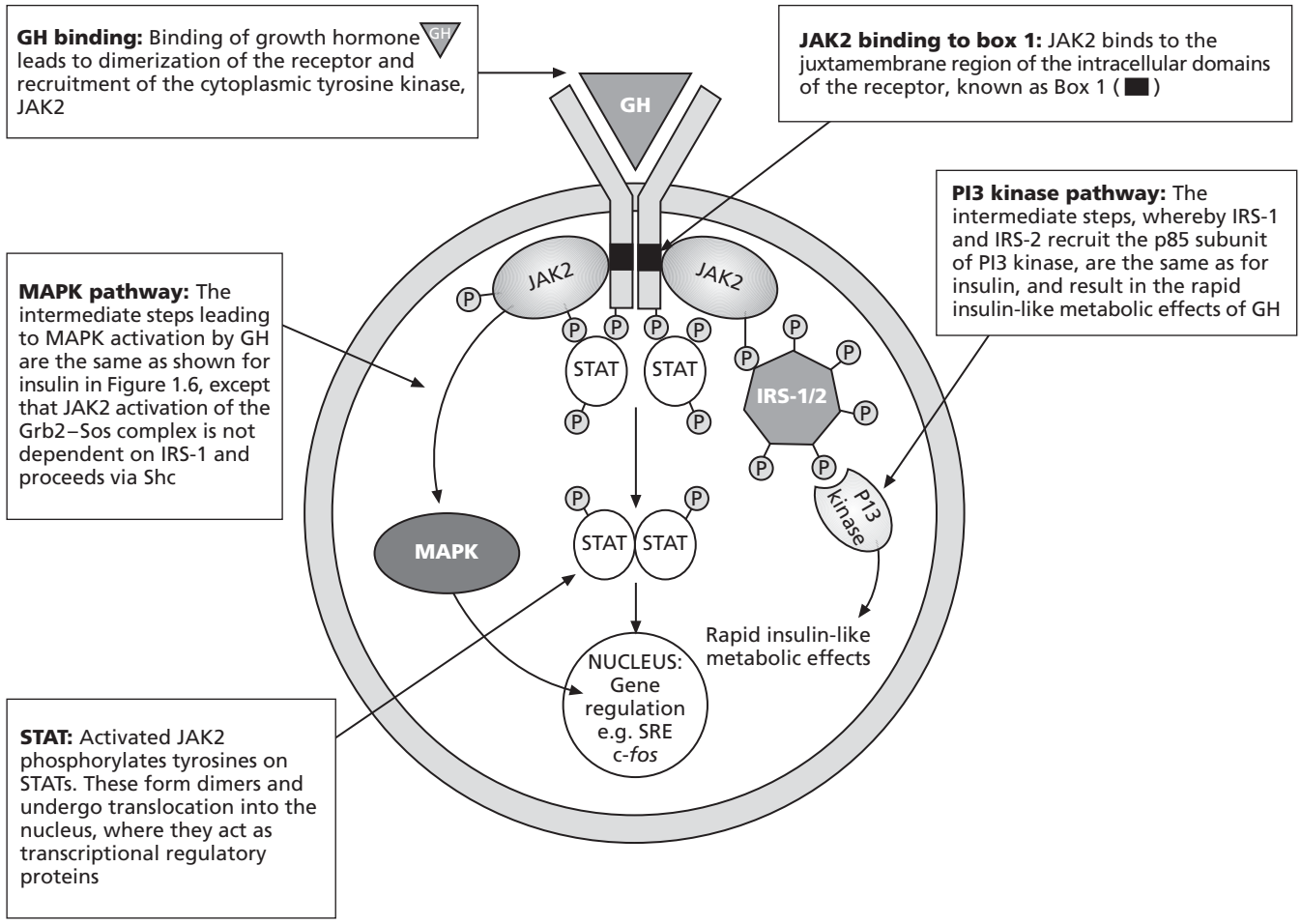


Fig. 1.5. Signaling pathways initiated in response to activation of the growth hormone receptor, an example of a receptor that recruits tyrosine kinase activity.

proliferation of more specific genes that determine the functional status of the cell [23].

Desensitization

Following activation, there is a rapid attenuation of receptor responsiveness to GH. This process is achieved by removal of the receptor from the cell surface (internalization) and degradation of the GH receptor/JAK complex [24]. In addition, JAK/STAT signaling pathways are inhibited by at least three families of proteins: phosphatases, suppressors of cytokine signaling (SOCS), and protein inhibitors of activated STATs (PIAS).

In vitro evidence for the involvement of protein tyrosine phosphatases (PTP) comes from the demonstration of prolonged GH-stimulated JAK2 and STAT5 phosphorylation in the presence of phosphatase inhibitors. Furthermore, in mice deficient in the enzyme SHP1, these signaling molecules are superactivated in response to GH [25].

Activation of the JAK/STAT pathway also induces expression of the SOCS proteins; these interact with JAK and also with the GH receptor to result in proteosomal degradation [26]. Finally, PIAS have been shown to regulate signal transduction negatively in response to prolactin, although less is known about the involvement of PIAS in GH regulation of STAT-mediated transcription [27].

Alternative pathways

JAK/STAT pathways are important in the cellular response to GH and prolactin, but there is evidence to suggest that other non-receptor tyrosine kinases may also mediate signal transduction of the cytokine receptor superfamily [28].

Members of the Src family of kinases (s-Src and c-Fyn) are activated by GH–receptor coupling, and this may lead to phosphorylation of focal adhesion kinase (FAK), recruitment of Grb2, and stimulation of the MAPK pathway. c-Fyn has also been implicated in the activation of PI 3-kinase by prolactin. Furthermore, Src kinases can associate with STAT1, STAT3, and STAT5, and so it is possible that this pathway is also involved in the transcriptional events regulated by GH or PRL. The use of signaling molecules from the transduction pathways associated with insulin may explain the acute insulin-like effects of GH. In addition, GH and prolactin have been reported to increase intracellular free calcium through activation of phospholipase C (see below).

Defects in GH and PRL signaling

Abnormalities in GH signal transduction result in GH resistance and severe growth impairment despite normal or elevated levels of circulating GH (Laron syndrome). Such patients have exceptionally low levels of IGF-I and its principal carrier protein, IGF binding protein-3 (IGFBP-3), and these cannot be elevated by the administration of exogenous GH. This observation gave the first clue that GH resistance resulted from non-responsive receptor or signaling pathways, mainly as a result of mutations in the gene for the GH receptor [29].

Deletions, nonsense, missense, splice, and frameshift mutations have all been detected in the exons of the GH receptor that code for the extracellular domain (exons 2–7). Some affect the ability of the receptor to bind GH, whereas others result in reduced GH-stimulated dimerization. Mutations in exons 8–10, which code for the transmembrane and intracellular domains, can lead to defective GH receptor–JAK coupling. Some patients, however, have no apparent defect in their GH receptor, suggesting that the problem must lie in genes further downstream. Indeed, STAT5b knockout mice fail to respond effectively to GH, and GH activation of STAT5 and MAP kinase is defective in fibroblasts isolated from patients with Laron syndrome [30]. There has been a report of one patient with severe intrauterine growth retardation followed by postnatal growth failure, sensorineural deafness, mild mental retardation, and GH resistance in whom there appears to be a partial deletion in the gene coding for IGF-I [31]. Clearly, this would render the GH–IGF-I axis ineffective.

There have been no reports of human disease resulting from gene defects in the prolactin receptor. This suggests that either mutations of the PRL receptor have no detectable effect *in vivo* or such mutations are lethal [17]. Evidence from PRL receptor knockout mice supports the former hypothesis, as these animals are viable but they do display a number of reproductive, behavioral, and bone abnormalities.

Circulating receptors

The extracellular domain of the GH receptor can be cleaved by an enzyme thought to be the metalloprotease TACE (tumor necrosis factor alfa converting enzyme) to form a circulating binding protein with high affinity for GH (GHBP) [32]. The physiological significance of GHBP is poorly understood, but "receptor decapitation" presents an alternative mechanism for receptor desensitization. The binding protein itself has the potential to modulate GH function either by prolonging its half-life and providing a circulating reservoir or by competing with GH for GH binding and inhibiting GH signaling through the formation of non-functional GHBP/GH receptor heterodimers.

Serum GHBP levels approximate GH receptor expression and are therefore used as a reflection of GH receptor status: for example, 75–80% of patients with Laron syndrome have low or undetectable levels of GHBP.

G-protein-coupled receptors

G-protein-coupled receptors (GPCRs) form a superfamily of more than 1000 membrane proteins that accounts for approximately 1% of the genes found within mammalian genomes. These receptors have a diverse range of ligands and, in addition to transducing hormonal signals, they also mediate the

cellular response to neurotransmitters, lipids, including prostaglandins and leukotrienes, nucleotides, ions, and sensory stimuli, such as light, smell, and taste [33].

As their name suggests, activation of GPCRs generally leads to the recruitment of intracellular G (guanine)-proteins and then the generation of second messengers, for example cyclic adenosine monophosphate (cAMP) and inositol 1,4,5-triphosphate (IP_3). Some of these receptors can signal through G-protein-independent pathways.

Structure

Although GPCRs have the same basic design as the tyrosine kinase-linked receptors, in that they possess extracellular, transmembrane, and intracellular domains, they can be defined structurally by their more elaborate "serpentine" transmembrane region [33]. This contains seven α-helices linked by alternating intracellular and extracellular loops, which can be arranged to form a hydrophobic pore (Fig. 1.6a and b). In general, each of the transmembrane segments consists of 20–27 amino acids. The extracellular N-terminal segment, loops, and intracellular C-terminal domain are much more variable in size; consequently, GPCRs range from the gonadotropin-releasing hormone receptor, at only 337 amino acid residues, to the calcium-sensing receptor, which has 1085 residues. The latter has a disproportionately long N-terminus (> 600 amino acids), because, in general, the length of the N-terminal segment is weakly correlated with ligand size. It has been suggested that this domain, along with the extracellular loops and transmembrane pore, has an important role in ligand recognition. Like the tyrosine kinase-linked receptors, the intracellular domains (both loops and C-terminus) are necessary for interaction with intracellular signaling partners.

GPCRs can be grouped into three families, A, B, and C (Table 1.1), on the basis of sequence similarity within the transmembrane region. There is little similarity between the groups, apart from the characteristic tertiary structure facilitated by the seven transmembrane helices [33]. Group A, which is the largest family and contains the receptors for light and adrenaline, has a putative fourth intracellular loop due to palmitoylation of cysteine residues in the C-terminal domain. Group B includes receptors for a variety of hormones and neuropeptides and is characterized by a long amino-terminus containing several cysteine residues, which presumably form a network of disulfide bridges. Group C includes the receptors for glutamate, γ-aminobutyric acid, and calcium.

G-proteins

Upon binding ligand, the conformation of the transmembrane domain, particularly the third and sixth helices, is

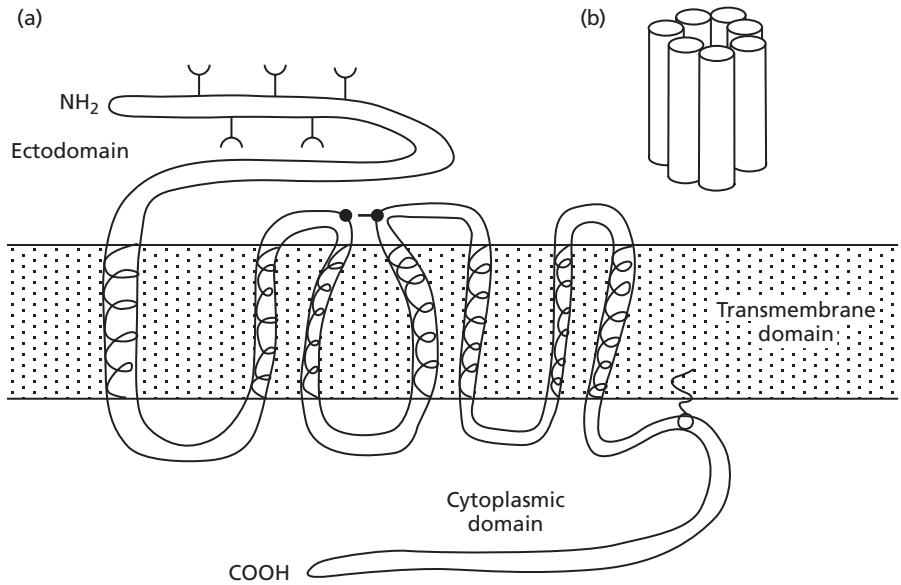


Fig. 1.6. A schematic representation of G-protein-coupled receptors (GPCRs) showing the seven transmembrane domains. (a) The structure is an elaborate variation of the three-segment design depicted in Figure 1.2. The size of the N-terminal extracellular domain is generally in proportion to the size of the cognate ligand. Homology of this region, which is obviously important for ligand binding, is less than that of the transmembrane and cytoplasmic domains. This region can be heavily glycosylated, and the carbohydrate moieties may contribute as much as 40% of its mass. The transmembrane domain has a characteristic heptahelical structure, most of which is embedded in the plasma membrane and provides a hydrophobic core. Conserved cysteine residues may form a disulfide bridge between the second and third extracellular loops. The cytoplasmic domain links the receptor to the signal-transducing G-proteins. Evidence from the β-adrenergic receptor suggests that specific regions in the third intracellular loop and sections of the C-terminal tail are critical for G-protein coupling. A fourth intracellular loop may be formed by a cysteine residue in the C-terminal tail, which could be palmitoylated in some GPCRs. (b) The hydrophobic pore formed by the seven transmembrane α-helices of the GPCR.

Table 1.1. Examples of GPCRs and their associated G-proteins/second messengers (AC, adenylate cyclase; PLC, phospholipase C). For somatostatin, vasopressin, calcitonin, and PTH/PTHrP, different receptor subtypes determine α-subunit specificity, and there may be differential tissue distributions of these receptor subtypes. This phenomenon provides opportunities to develop selective therapeutic antagonists.

Family	Characteristics	Examples	G-protein	Second messenger
A	Disulfide bridge connecting second and third extracellular loop Putative fourth intracellular loop	TRH receptor GnRH receptor Oxytocin	Gqα	PLC
		Biogenic amine receptors FSH receptor LH receptor TSH receptor Vasopressin	Gsα/Gqα	AC/PLC
		Somatostatin	Giα/Gqα	AC/PLC
		Melanocortin receptor	Gsα	AC
B	Disulfide bridge connecting second and third extracellular loop Long amino-terminus containing several cysteine residues	Calcitonin receptor	Gsα/Giα/Gqα	AC/PLC
		CRH receptor Glucagon receptor	Gsα	AC
		PTH receptor PTHrP receptor	Gsα/Gqα	AC/PLC
C	Very long (>600 amino acids) amino-terminus Very short and highly conserved third intracellular loop	Calcium receptors	Gqα/Giα	AC/PLC
		Glutamate receptors	Gsα/Gqα	AC/PLC

altered. This leads to a conformational change in the intracellular domains to uncover binding sites for heterotrimeric G-proteins previously masked. G-proteins consist of α-, β-, and γ-subunits. The β- and γ-subunits associate with such high affinity that G-proteins are usually described as having two functional units, Gα and Gβγ; to date, 23 α-subunits, six β-, and 12 γ-subunits have been described [34].

Activation by GPCRs induces a conformational change in the α-subunit, which results in the exchange of a molecule of GDP for a molecule of GTP and dissociation of the α-subunit from both the receptor and the βγ-dimer. Both the GTP-bound α-subunit and the βγ-dimer independently regulate a number of downstream signaling pathways.

Based on their primary effector molecules, the α-subunits can be grouped into four families. $G\alpha_s$ and $G\alpha_i$ activate or inhibit adenylate cyclase (AC) respectively. $G\alpha_q$ activates phospholipase C (PLC). Less is known about the $G\alpha_{12}$-subunits, although it appears that their effects are mediated through members of the Rho family of GTPases [35]. In addition to the effectors used by the α-subunits, Gβγ-dimers are known to target ion channels and protein kinases, and the list continues to increase [34].

The existence of numerous G-protein subunits in combination with a variety of downstream effectors enables the diversity and selectivity of intracellular signals in response to GPCR activation. Each receptor has the possibility of interacting with many G-proteins (Table 1.1). Recruitment of a particular Gα-subunit depends on many factors [36], including receptor subtype (Fig. 1.7a), structural features of the cytoplasmic domain, and the concentration of the ligand. For example, at low concentrations, TSH, calcitonin, and luteinizing hormone (LH)/human chorionic gonadotropin (hCG) receptors activate adenylate cyclase through $G\alpha_s$, whereas at higher concentration, $G\alpha_q$ is recruited to activate PLC. Further complexity is introduced by the potential for receptors simultaneously or successively to couple with distinct G-proteins (Fig. 1.7b) and the ability of a particular G-protein to activate multiple intracellular signaling cascades (Fig. 1.7c).

Intracellular second messengers

cAMP

Activation of membrane-bound adenylate cyclase catalyzes the conversion of ATP to the potent second messenger cAMP (Fig. 1.8) [37]. This cyclic nucleotide activates the heterotetrameric protein kinase A (PKA) by binding to repressive regulatory subunits (R), which then dissociate from the two catalytic subunits (C) so that phosphorylation of serine/threonine residues in proteins containing the consensus sequence Arg-Arg-X-Ser/Thr-X can occur. These include intermediaries of lipolysis, glycogenolysis, and steroidogenesis (for example, glycogen synthase, hormone-sensitive lipase, cholesterol ester hydrolase) as well as the transcription factor CREB (cAMP response element binding protein). Phosphorylated CREB translocates to the nucleus where it binds to a short palindromic sequence, the CRE or cAMP response

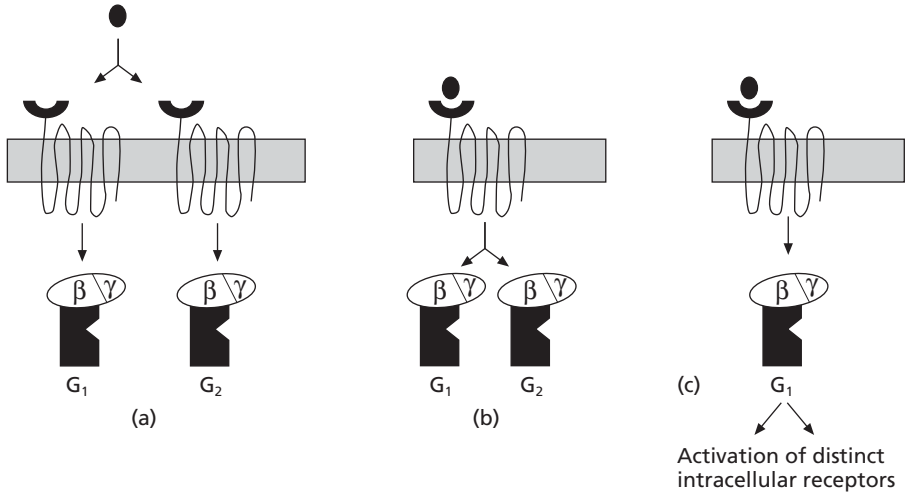


Fig. 1.7. Various mechanisms of G-protein selection and subsequent activation of intracellular second messengers. Adapted from Hermans E. Biochemical and pharmacological control of the multiplicity of coupling at G-protein coupled receptors. *Pharmacol Ther* 2003; 99: 25–44 [36], with permission from Elsevier.

element, of cAMP-regulated genes (e.g. somatostatin). In this way, the generation of cAMP can have a direct effect on gene transcription.

cAMP does not act exclusively through PKA, and there is a growing list of alternative cAMP targets [38]. The physiological effects of cAMP are also produced by direct regulation of monovalent and divalent cation channels and the ubiquitous guanine exchange factors Epac 1 and 2.

The cAMP-mediated signal is terminated by members of the phosphodiesterase (PDEs) family of proteins. These hydrolyze cAMP rapidly to the inactive 5′-AMP in response to phosphorylation by PKA and other mechanisms.

Diacylglycerol and Ca^{2+}

Occupancy of numerous GPCRs, including TRH, GnRH, and oxytocin, results in G-protein activation of the enzyme phospholipase C (PLC; Fig. 1.9) [39]. This leads to the hydrolysis of phospholipids, specifically phosphatidylinositol-4,5-bisphosphate (PIP_2), which resides in the inner leaflet of the plasma membrane, to yield diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP_3). DAG, together with a cofactor phosphatidylserine, recruits another protein kinase, the membrane-bound PKC, which, in the presence of calcium, phosphorylates a wide variety of proteins and peptides to bring about the cellular response. Ca^{2+} is provided by IP_3, which diffuses through the cytoplasm to bind receptors on the endoplasmic reticulum, causing Ca^{2+} mobilization and a rapid increase in cytosolic free Ca^{2+}. In addition to PKC, the rise in intracellular Ca^{2+} also activates the protein kinase calmodulin and phospholipase A_2. Phospholipase A_2 liberates arachidonate from phospholipids and thereby generates potent local tissue activators known collectively as eicosanoids. These include thromboxanes, leukotrienes, and prostaglandins. Prostaglandins are well-recognized paracrine and autocrine mediators that may amplify or prolong the response to the original hormone stimulus. Intracellular Ca^{2+} concentrations are restored to resting levels by several mechanisms including Ca^{2+} pumps and deactivation of G-proteins.

Receptor dimerization

Receptor dimerization is required for signal transduction through some types of receptor, but models that describe the activation of GPCRs are usually based on the assumption that GPCRs exist as monomers and that they couple to G-proteins in a 1:1 molar ratio. Increasing evidence from *in vitro* studies suggests that these models may need to be refined [33]. Homodimers of the β_2-adrenergic receptor, the δ-opioid receptor, and the dopamine D_1, D_2, and D_3 receptors have been described. Intriguingly, ligand appears to regulate dimer formation, which suggests that homodimerization could have a role either in the receptor activation mechanism or in the subsequent desensitization and internalization process.

The possibility of heterodimerization between closely related receptor subtypes has been proposed. This process may be important for targeting functional receptors to the cell surface, and it is possible that heterodimerization could generate novel ligand binding and signal transduction pathways to result in functional properties distinct from those of either of the receptors. The physiological significance of homo- and heterodimers remains to be determined.

Non-G-protein pathways

GPCRs do not always signal through G-proteins. There is a rapidly growing list of new GPCR effector molecules [40]. In some cases, these are known receptor-interacting proteins, such as the arrestins, which, in addition to their well-established role in receptor desensitization, appear to link GPCRs into MAP kinase pathways. In other cases, novel binding partners have been identified, and the challenge will be to understand how the classical and new effector pathways are integrated to achieve specificity of GPCR signal transduction.

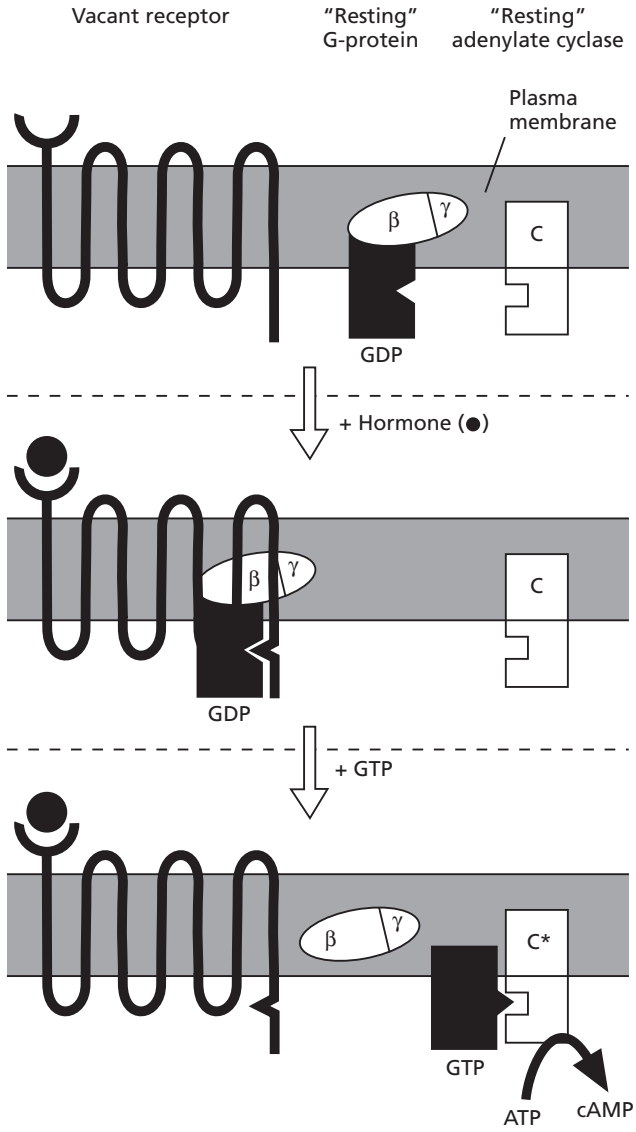


Fig. 1.8. A representation of G-protein-modulated activation of a membrane-bound enzyme such as adenylate cyclase. A hormone, e.g. adrenaline, binds to the extracellular region of the receptor. The third intracellular loop and the C-terminus of the receptor associate with a G-protein, for example $G\alpha_s\beta\gamma$. This leads to displacement of GDP by GTP and dissociation of $G\alpha_s$ from the βγ-dimer. The α-subunit diffuses in the lipid bilayer and activates the catalytic subunit (C*) to generate many molecules of cAMP.

GPCR desensitization

GPCR desensitization results from changes to either the receptor or the intracellular G-proteins [41]. The extent varies from complete termination of signaling, which occurs in the sensory systems, to a reduction in the potency of ligand, as is observed with the β-adrenergic receptors.

Internalization of receptors to intracellular compartments and reduced expression as a result of decreased mRNA and protein synthesis both lead to desensitization, but this can be achieved more rapidly (in seconds rather than minutes or hours) by uncoupling the receptor from G-protein-mediated signaling pathways. It is widely accepted that both second messenger-dependent protein kinases [e.g. protein kinase A (PKA) and PKC] and G-protein-coupled receptor kinases (GRK) are responsible for uncoupling GPCRs from G-proteins by phosphorylating serine and threonine residues within the intracellular loop and carboxy-terminal tail domains of the receptor. GRK phosphorylation of GPCRs also promotes the binding of cytosolic cofactor proteins known as arrestins, which target GPCR for endocytosis by clathrin-coated vesicles.

GPCR signals can also be terminated at the G-protein level. Gα-subunits possess intrinsic GTPase activity, which can cleave phosphate from GTP to result in GαGDP. This process can be enhanced by a family of proteins called regulators of G-protein signaling (RGS), which accelerate the rate of hydrolysis of GTP bound to both $G\alpha_i$ and $G\alpha_q$ to dampen $G\alpha_i$- and $G\alpha_q$-mediated signaling pathways. Hydrolysis of GTP allows the Gα-subunit to associate with a Gβγ-dimer again, and the heterotrimeric complex returns to the G-protein pool so that it can be activated by subsequent receptor occupation by ligand.

Defects

Given their numerous and varied ligands, it is not surprising that mutations in GPCRs or their interacting G-proteins are associated with endocrine disease [42]. Mutations that alter the extracellular (ligand-binding) domains of the receptor lead to hormone resistance (e.g. the TSH receptor), whereas aberrations in the transmembrane region of the receptor can result in altered receptor function.

Germline mutations in Xq28, which codes for the vasopressin V2 receptor, cause receptor misfolding and loss of receptor function so that circulating vasopressin, despite being present at very high levels, cannot increase urine concentration, and nephrogenic diabetes insipidus results. Some cases of early-onset severe obesity may be explained by functional defects in the melanocortin-4 receptor [43].

Activating mutations are also detrimental, presumably by altering crucial helix–helix interactions so that the receptor is active even in the absence of ligand. Familial male precocious puberty (testotoxicosis) is the result of such a mutation in the gene coding for the LH receptor, and activating mutations in the transmembrane domain of the TSH receptor have been reported in association with neonatal hyperthyroidism and toxic thyroid adenomas in adults.

Mutations resulting in the loss of $G\alpha_s$ function are linked to pseudohypoparathyroidism (Albright's hereditary osteodystrophy). If the mutation is maternally transmitted, resistance to the multiple hormones that activate $G\alpha_s$ in their target tissues occurs. Mutations resulting in the constitutive activation of $G\alpha_s$ cause McCune–Albright syndrome and some cases of acromegaly.

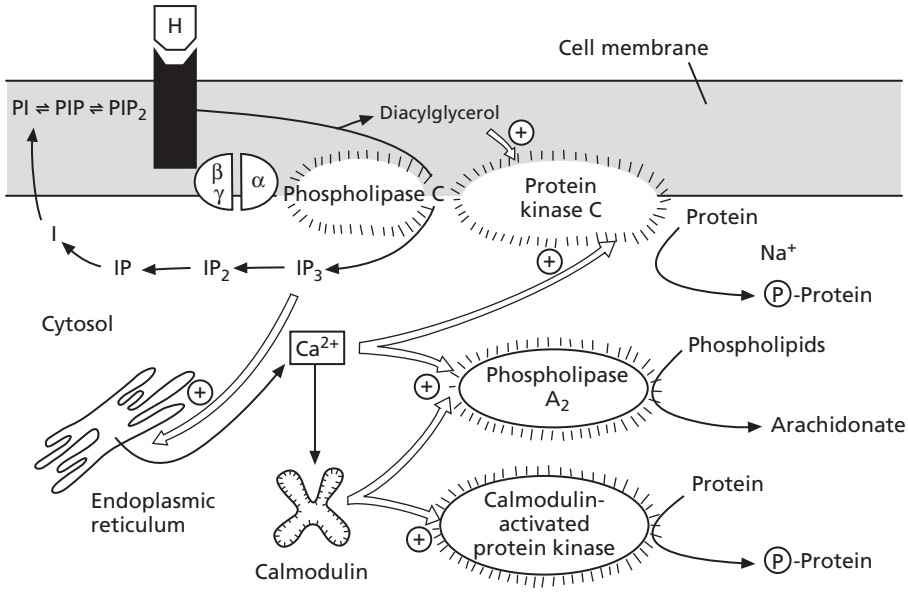


Fig. 1.9. A representation of hormone-stimulated phospholipid turnover and calcium metabolism as a result of G-protein-coupled receptors activating phospholipase C.

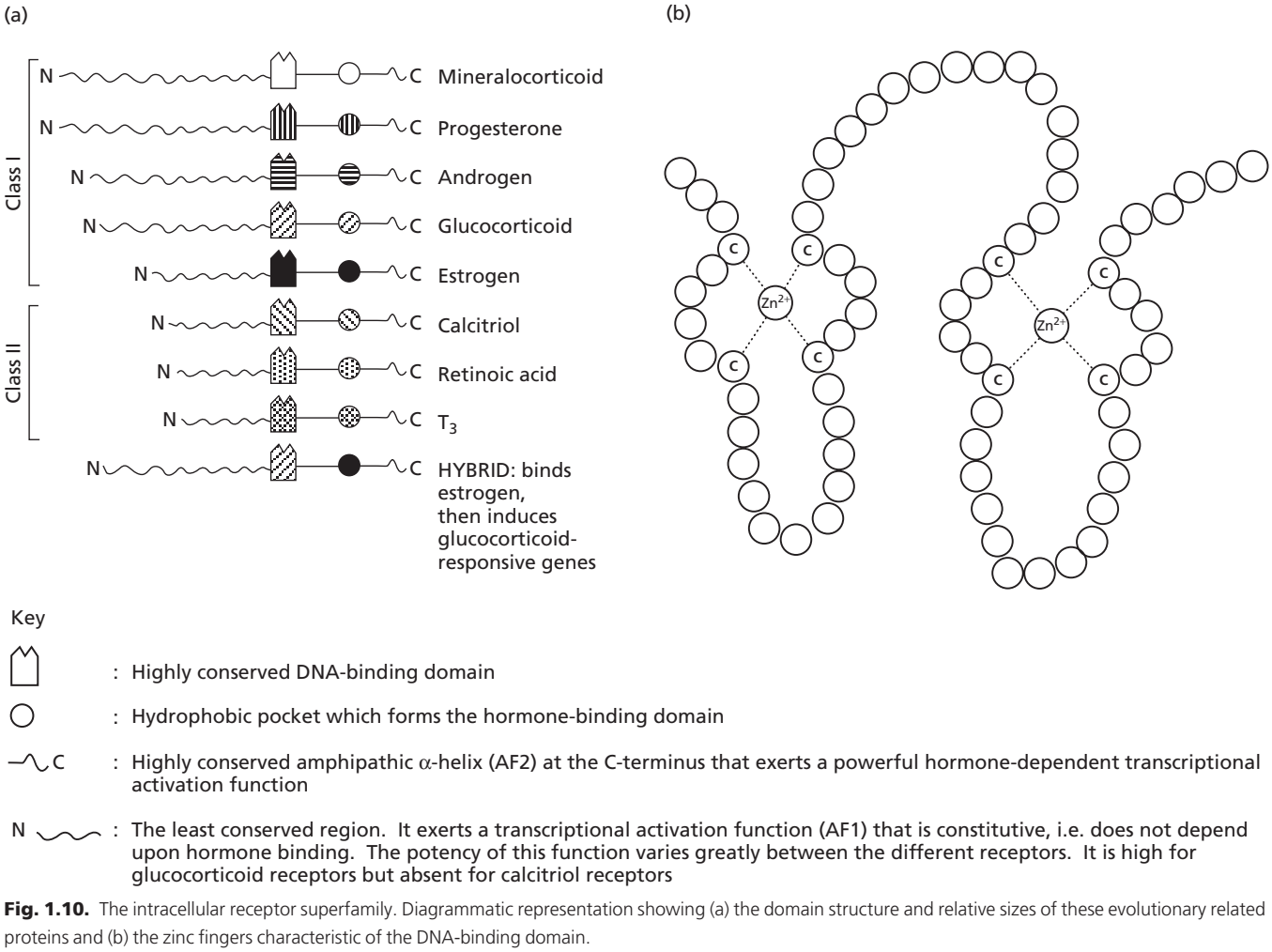


Fig. 1.10. The intracellular receptor superfamily. Diagrammatic representation showing (a) the domain structure and relative sizes of these evolutionary related proteins and (b) the zinc fingers characteristic of the DNA-binding domain.

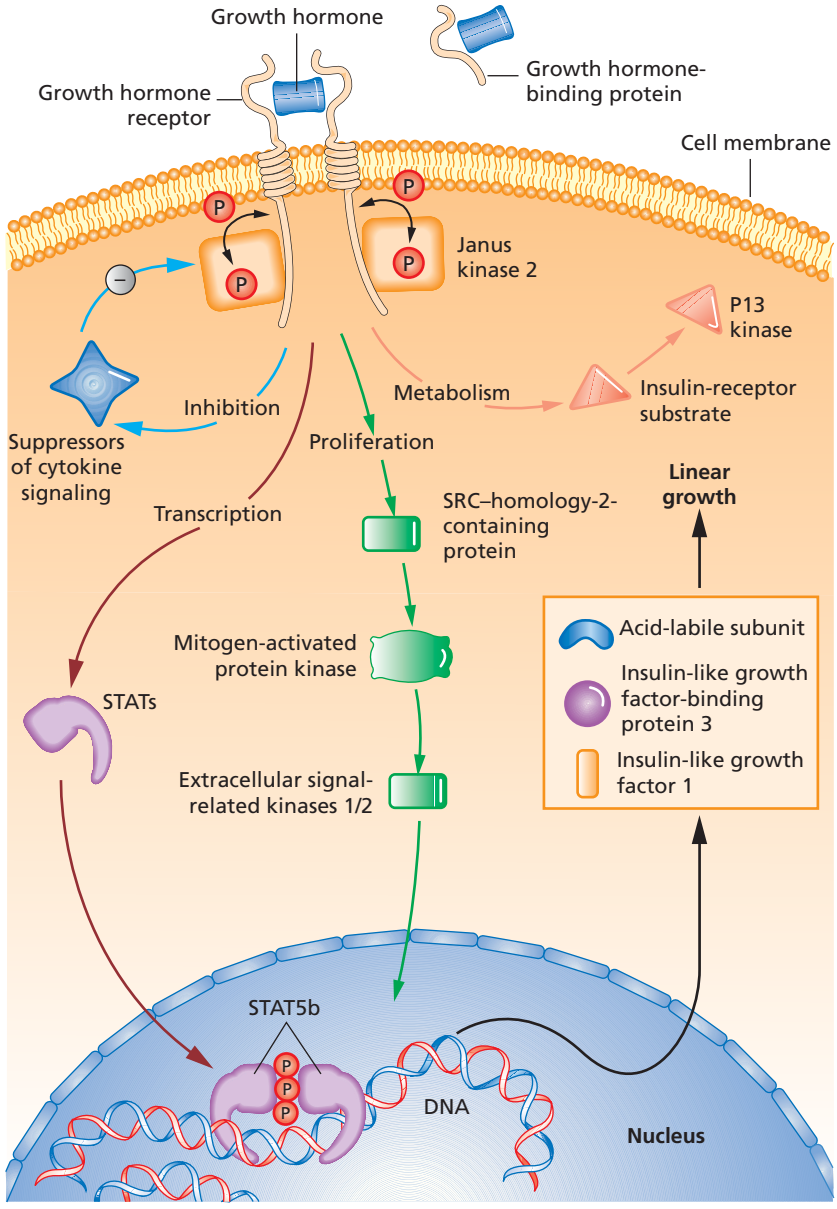
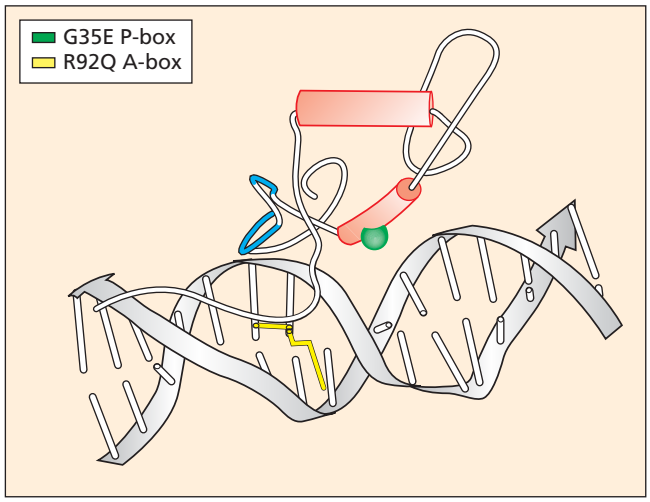
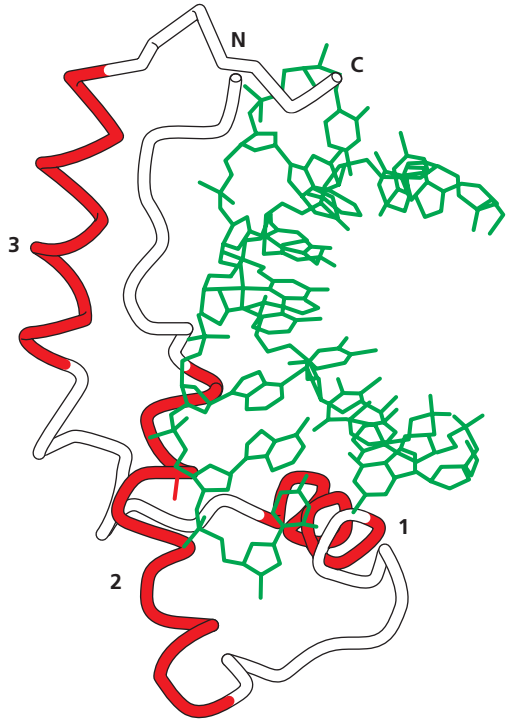


Plate 6 Growth hormone-activated intracellular signaling. Phosphorylation of the growth hormone receptor is followed by the activation of metabolic, proliferative, and transcriptional pathways. STAT5b stimulates the transcription of factors (shown in the box) that are critical for normal linear growth. P, phosphorylation; STAT, signal transducer and activator of transcription (from Eugster EA, Pescovitz OH. *N Engl J Med* 2003; 349: 1110–12.)



(a)



(b)

Plate 7 Models of key transcription factors bound to DNA. (a) The nuclear receptor steroidogenic factor 1 (SF1) regulates an array of genes involved in gonadal and reproductive development and binds as a monomer to extended DNA response elements (PyCA AGGTCA) in the promoters of target genes. A heterozygous mutation (G35E, shown in green) in the primary DNA-binding region ("P-box") of SF1 is associated with a severe clinical phenotype, whereas a homozygous change (R92Q, shown in yellow) in a secondary DNA-binding structure ("A-box") is necessary for expression of clinical features. (b) SRY binds to an AACAAT/A DNA response element and may influence transcription by inducing a structural "bend" in target DNA sequences. These conformational changes affect chromatin remodeling and influence target gene expression. Reproduced with permission from [17] and [10]. Copyright 2002, 2003, The Endocrine Society.

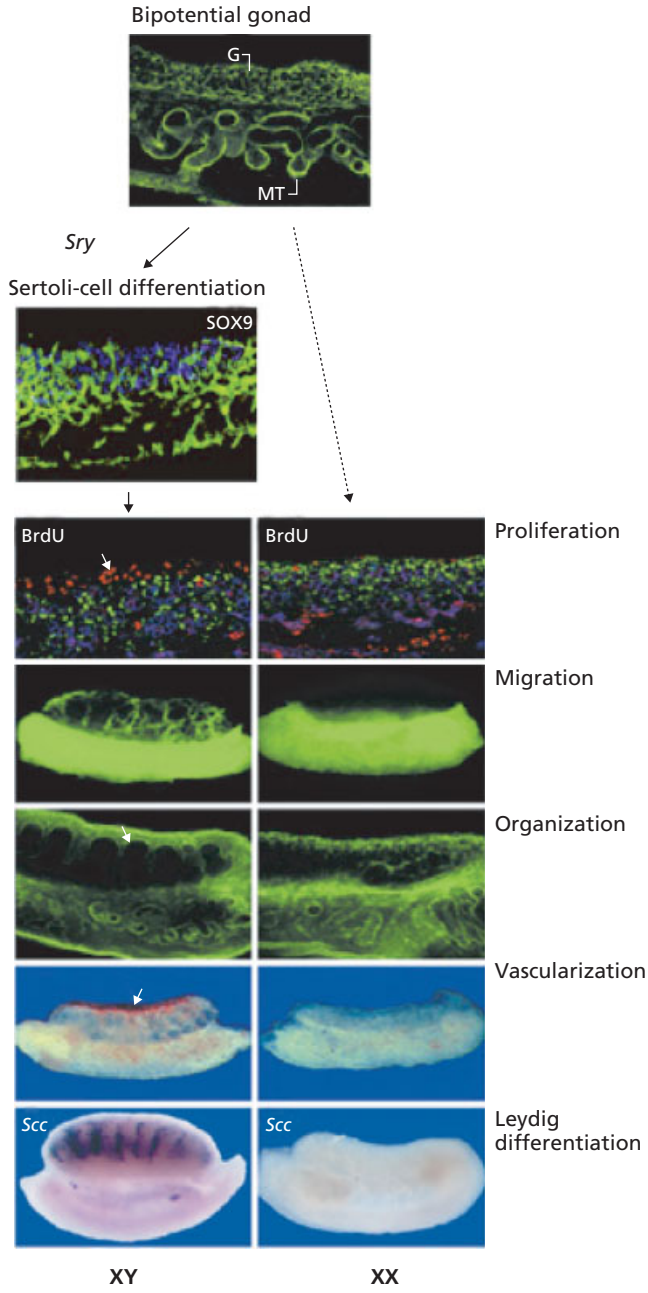


Plate 8 Morphological changes in gonadal development in male (left) and female (right) mice. No morphological differences between XY and XX gonads are seen during the bipotential gonad stage [10.5–11.5 days post coitum (dpc)]. In XY gonads, Sry upregulates nuclear Sox9 (blue) in pre-Sertoli cells and initiates Sertoli cell differentiation by 11.5 dpc (vasculature and germ cells are labeled with platelet endothelial cell adhesion molecule and appear green). Between 11.5 and 12.5 dpc, distinct changes occur in the XY gonad, which are not seen in the XX gonad. These changes include: proliferation of celomic epithelial cells; migration of cells from the mesonephros; structural organization of testis cords; male-specific vascularization; and Leydig cell differentiation. Modified with permission from [26]. Copyright 2004, Nature Publishing Group.

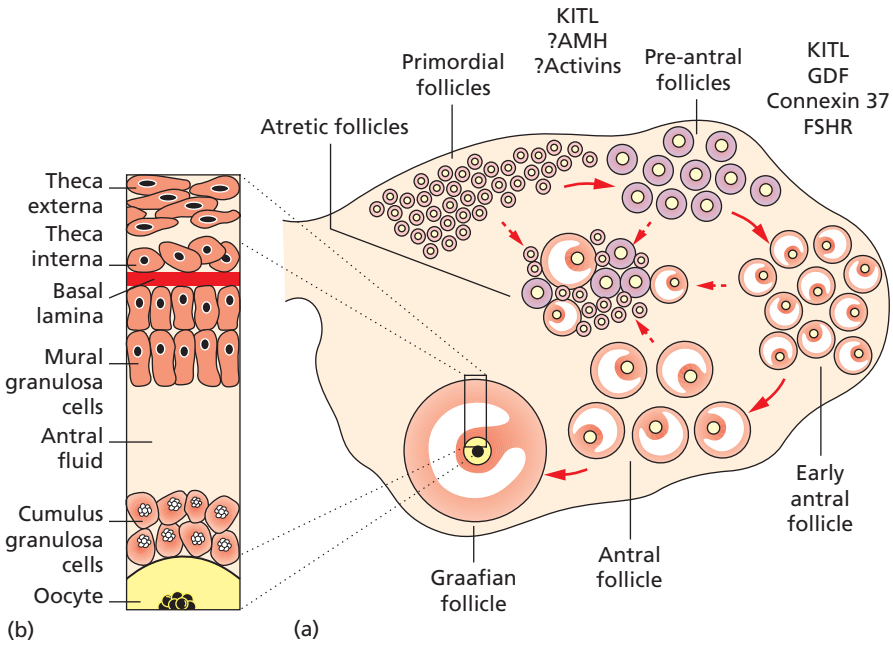
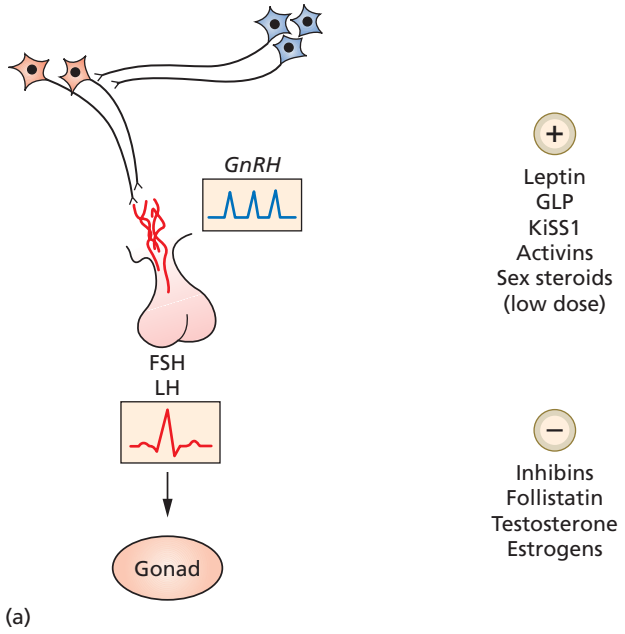


Plate 9 Folliculogenesis in the ovary. (a) Primordial follicles can develop following meiotic division of oogonia, but most primordial germ cells are destined for apoptosis. Different stages of follicular development require the presence of specific factors such as GDF9 and the FSH receptor. (b) Although Graafian follicles containing theca and granulosa cells (inset) can develop in the third trimester, co-ordinated ovulation does not become established until the time of puberty. Modified with permission from [39]. Copyright 2003, Chapterhouse Codex.



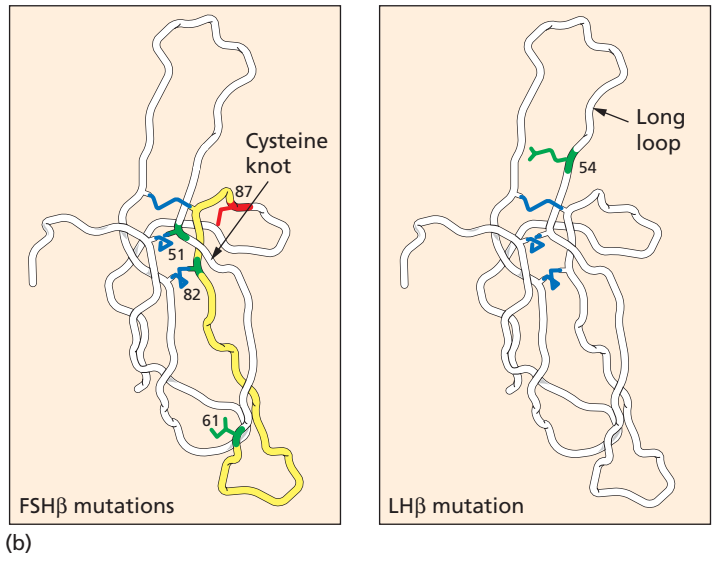


Plate 10 (a) Overview of the hypothalamic–pituitary (gonadotrope)–gonad axis. Several factors can stimulate or inhibit gonadotropin release at the level of the hypothalamus or pituitary (GLP, galanin-like peptide). (b) Locations of reported mutations in the FSH and LH β-subunits mapped on to the crystal structure of human chorionic gonadotropin (hCGβ). Point or deletion mutations in FSHβ (left) interfere with the "cysteine knot" motif and impair dimer stability. In contrast, the single point mutation in LHβ (right) affects the long loop, a region implicated in receptor binding (reproduced with permission from [55] and [56]). Copyright 2002, The Endocrine Society.

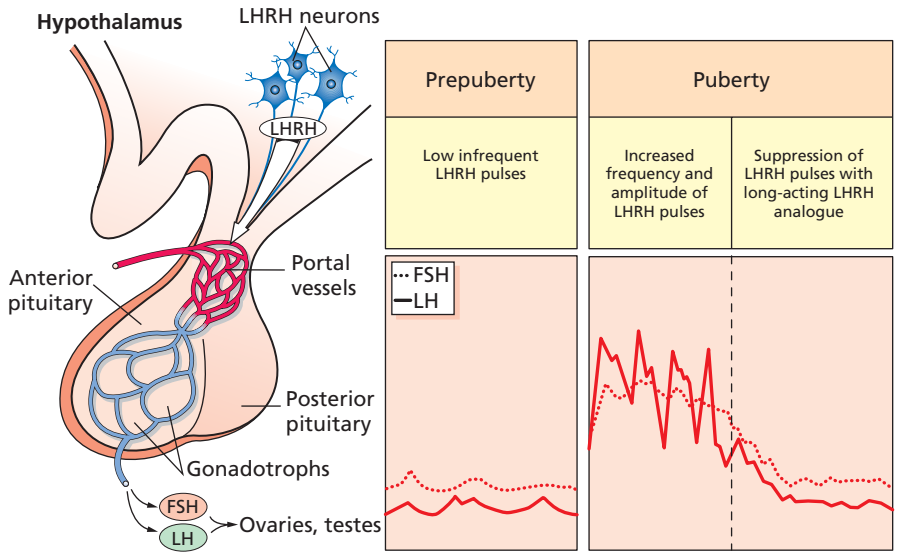


Plate 11 Anatomical arrangement of the gonadotropin system and the secretory pattern of luteinizing and follicle-stimulating hormones during puberty and prepuberty.

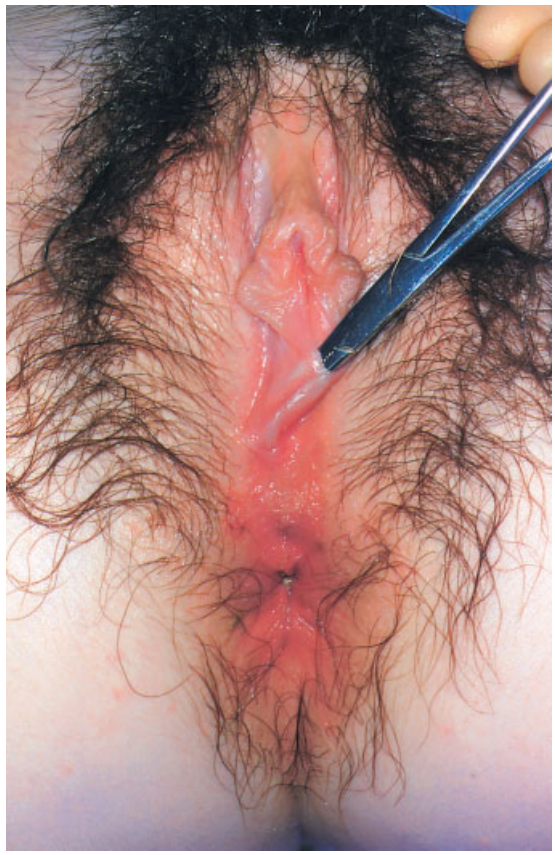


Plate 12 Labial adhesions in a pubertal girl with two previous surgical separations.

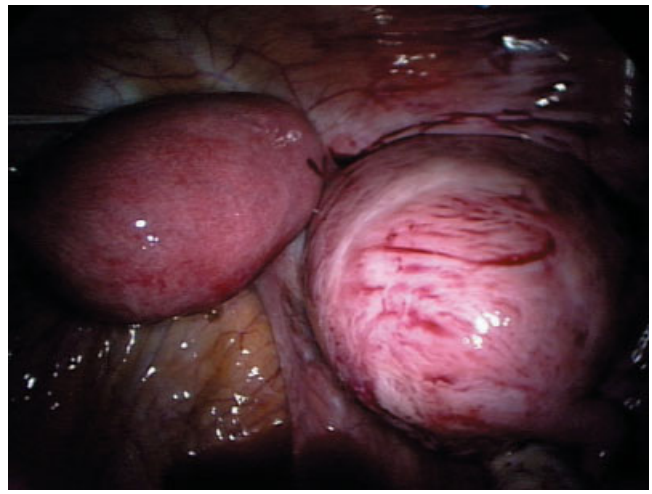


Plate 13 Laparoscopic picture of an obstructed uterine horn.