Hormone Receptors

8

Contents

Hormone receptors belong mainly to two large receptor groups: heptahelical G-protein-coupled membrane receptors and cytosolic nuclear receptors for steroids and thyroid hormones. The exceptions are particularly the insulin and the growth hormone receptors

The signals initiated by the main receptor groups have characteristic differences:

- *Fast-acting receptor signals.* G-protein-coupled receptors (GPCRs) trigger immediate reactions which are converted into long-lasting regulations by other intracellular interaction partners. Ion channel openings, membrane depolarization, release of calcium from intracellular calcium stores, activation of kinases, and lipid conversions by phospholipases are influenced by GPCRs. These reactions occur within seconds or faster. If these immediate reactions are transduced into the cellular nucleus and genes are activated, long-lasting modifications can occur.
- *Slow, genomic changes.* Cytosolic receptors for steroids or the thyroid hormones do not trigger immediate reactions. Hormones which have reached the cytosol by diffusion through the cell membrane initiate hormone receptor dimerization.

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By this dimerization, a nuclear import signal for the entire complex is generated. After transfer to the cellular nucleus, the dimer associates with characteristic recognition motifs on the DNA. Such an interaction modulates the activity of the associated gene, either activation of transcription or its inhibition. Whereas GPCRs trigger fast signal cascades at the membrane, nuclear receptors act in the cellular nucleus and modify gene activities. These reactions take longer than seconds: they need minutes or hours.

8.1 Nuclear Receptors

In 2004, the Lasker Award was given to three biochemists: Elwood Jensen, Ronald Evans, and Pierre Chambon. They had presented evidence for receptors which are today called nuclear receptors and studied their features (see Fig. 8.1). Forty-eight human nuclear receptors have been identified which recognize steroids, thyroxine, triiodothyronine, vitamin D_3, and vitamin A (Table 8.1). In the liver, many of these nuclear receptors target drugs and poisons.

There are three characteristic features of each nuclear receptor:

1. *Ligand binding.* All nuclear receptors have a domain where the ligand is accepted—for example, the hormone, the vitamin, or the toxin.
2. *Dimerization.* After ligand binding, two ligand-bound receptors form a dimer.

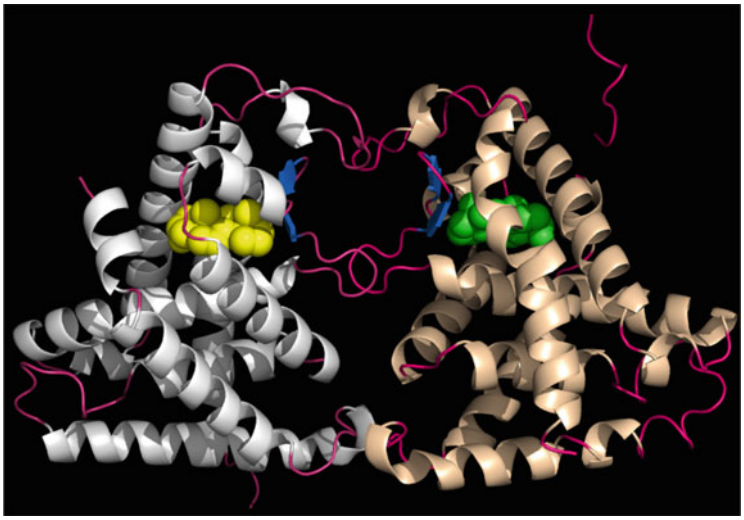

Fig. 8.1 Model of a nuclear receptor: The murine androstane receptor. Androstane (*yellow spheres* and *green spheres*) is embedded into the receptor structure, which consists mainly of helices and a single β sheet (*blue*). Two receptor molecules adhere at the side with the β sheet. This generates the nuclear import signal. The dimer is imported into the cellular nucleus; by binding to recognition motifs, it modulates gene activity (Produced with PyMOL using Protein Data Bank entry 1XNX)

Table 8.1 Nuclear receptors

Hormone/ligand	Receptor	Type	OMIM entries
Thyroid hormone	ErbA (TR)	α, β	190120, 190160
Estrogen	ER	α, β	133430, 601663
Progesterone	PR		607311
Testosterone, DHT	AR		133700
Mineralocorticoid	MR (NR3C2)	α, β	600983
Glucocorticoid	GR		138040
Vitamin D	VDR		601769
All-*trans*-retinoic acid	RAR	α, β, γ	180240, 180220, 180190
9-*cis*-Retinoic acid	RXR	α, β, γ	180245, 180246, 180247
Oxysterol	LXR	α, β	602423, 600380
Bile acids	FXR		603826
Fatty acids	PPAR	α, γ, δ	170998, 601487, 600409
Pregnanes, xenobiotics	PXR (NR1I2)		603065
Xenobiotics	CAR		603881

AR androgen receptor, *CAR* constitutive androstane receptor, *DHT* dihydrotestosterone, *ER* estrogen receptor, *FXR* farnesoid X receptor, *GR* glucocorticoid receptor, *LXR* liver X receptor, *MR* mineralocorticoid receptor, *NR1I2* nuclear receptor subfamily 1, group I, member 2, *NR3C2* nuclear receptor subfamily 3, group C, member 2, *OMIM* Online Mendelian Inheritance in Man (http://www.omim.org/), *PPAR* peroxisome-proliferator-activated receptor, *PR* progesterone receptor, *PXR* pregnane X receptor, *RAR* retinoic acid receptor, *RXR* retinoid X receptor, *TR* thyroid hormone receptor, *VDR* vitamin D receptor

3. *DNA binding.* The dimerization of two nuclear receptors creates a nuclear import signal. This signal triggers transport of the dimer into the cellular nucleus and allows the dimer to bind to its recognition sites. Any functional nuclear receptor has its own recognition sites. After binding of the dimer to the DNA, gene activity in that chromosomal region is modulated. The transcriptional activity might be enhanced or reduced. This will eventually stimulate or suppress cellular functions.

8.2 Heptahelical Transmembrane Receptors

In contrast to steroids, which dock to intracellular nuclear receptors, peptide/protein hormones bind to receptors on the surface of cells. Most of these receptors belong to a protein family where the membrane is spanned sevenfold ("seven" is *hepta* in Greek) by helices, so-called heptahelical receptors (Tables 8.2 and 8.3, Fig. 8.2).

By binding the receptor ligand on the cellular surface, these receptors initiate signal transduction by coupling to intracellular, guanosine triphosphate (GTP)-binding proteins, the G proteins.

Table 8.2 Class A heptahelical receptors: receptors related to rhodopsin and β-adrenergic receptor

Hormone/ligand	Receptor	Subtypes	OMIM entries
Adenosine	Adenosine receptor	A1, A2a, A2b, A3	102775, 102776, 600446, 600445
MSH, ACTH	MC1-R (MSH-R in melanocytes), MC2-R (ACTH-R in the adrenal cortex), MC3-R (in the CNS), MC4-R (AgRP in the hypothalamus), MC5-R (in exocrine glands)		155555, 202200, 155540, 155541, 600042
Noradrenaline, adrenaline	α-Adrenergic receptors	1A, 1B, 1D	104221, 104220, 104219
		2A, 2B, 2C	104210, 104260, 104250
	β-Adrenergic receptors	1, 2, 3	109630, 109690, 109691
Dopamine	DPR	1a, 1b, 2	126449, 126453, 126450
Serotonin (5-hydroxytryptamine)	HTR	1a, 1b, 1d, 1e, 1f	109760, 182131, 182133, 182132, 182134
		2a, 2b, 2c	182135, 601122, 312861
		3a, 3b, 5a, 6, 7	182139, 604654, 601305, 601109, 182137
Acetylcholine	Muscarinic AChR[a]	1, 2, 3, 4, 5	118510, 118493, 118494, 118495, 118496
Angiotensin II	AGTR	1, 2	106165, 300034
Bradykinin	BDKR	B1, B2	600337, 113503
Bombesin	BRS3		300107
Gastrin-releasing peptide	GRPR		305670
Cholecystokinin	CCK-R	A, B	119444, 119445
Neuromedin B	NMBR		162341
Neuromedin U	NMUR	1, 2	604153, 605108
Neuropeptide Y	NPYR	1, 2, (3)[b], 5	162641, 162642, (162643), 602001
Oxytocin	OXTR		167055
Arginine vasopressin	AVPR	1a, 1b	600821, 600264
Galanin	GALR	1, 2, 3	600377, 603691, 603692
Somatostatin	SSTR	1, 2, 3, 4, 5	182451, 182452, 182453, 182454, 182455
GnRH	GnRHR		138850

(continued)

Table 8.2 (continued)

Hormone/ligand	Receptor	Subtypes	OMIM entries
TRH	TRHR		188545
Melatonin	MTNR	1a, 1b	600665, 600804
FSH	FSHR		136435
LH/hCG	LHCGR		152790
TSH	TSHR		603372

AChR acetylcholine receptor, *ACTH* adrenocorticotropic hormone, *ACTH-R* adrenocorticotropic hormone receptor, *AgRP* agouti-related peptide receptor, *AGTR* angiotensin receptor, *AVPR* arginine vasopressin receptor, *BDKR* bradykinin receptor, *BSR3* bombesin receptor subtype 3, *CCK-R* cholecystokinin receptor, *CNS* central nervous system, *DPR* dopamine receptor, *FSH* follicle-stimulating hormone, *FSHR* follicle-stimulating hormone receptor, *GALR* galanin receptor, *GnRH* gonadotropin-releasing hormone, *GnRHR* gonadotropin-releasing hormone receptor, *GRPR* gastrin-releasing peptide receptor, *hCG* human choriogonadotropin, *HTR* 5-hydroxytryptamine receptor, *LH* luteinizing hormone, *LHCGR* luteinizing hormone/human choriogonadotropin receptor, *MC1-R* melanocortin 1 receptor, *MC2-R* melanocortin 2 receptor, *MC3-R* melanocortin 3 receptor, *MC4-R* melanocortin 4 receptor, *MC5-R* melanocortin 5 receptor, *MNTR* melatonin receptor, *MSH* melanocyte-stimulating hormone, *MSH-R* melanocyte-stimulating hormone receptor, *NMBR* neuromedin B receptor, *NMUR* neuromedin U receptor, *NPYR* neuropeptide Y receptor, *OXTR* oxytocin receptor, *SSTR* somatostatin receptor, *TRH* thyrotropin-releasing hormone, *TRHR* thyrotropin-releasing hormone receptor, *TSH* thyroid-stimulating hormone, *TSHR* thyroid-stimulating hormone receptor

[a]Nicotinic AChRs do not belong to the G-protein-coupled receptors

[b]NPYR3 does not bind neuropeptide Y, instead in binds stromal-cell-derived factor; NPYR3, aka CXCR4, is a co-receptor of HIV

Table 8.3 Class B heptahelical receptors: receptors related to the secretin receptor

Hormone/ligand	Receptor	Subtypes	OMIM entries
Glucagon	GcgR		138033
Glucagon-like peptide	GlP-R		138032
Secretin	SctR		182098
Vasoactive intestinal peptide	VIPR	1, 2	192321, 601970
GHRH	GHRH-R		139191
Calcitonin	CTR		114131
Calcitonin-gene-related peptide	CGRPR		114190[a]
Corticotropin-releasing hormone	CRHR	1, 2	122561, 602034[b]

CGRPR calcitonin-gene-related peptide receptor, *CRHR* corticotropin-releasing hormone receptor, *CTR* calcitonin receptor, *GcgR* glucagon receptor, *GHRH* growth-hormone-releasing hormone, *GHRH-R* growth-hormone-releasing hormone receptor, *GlP-R* glucagon-like peptide receptor, *SctR* secretin receptor, *VIPR* vasoactive intestinal peptide receptor

[a]Active only together with receptor-activity-modifying protein 1; OMIM entry 605153

[b]CRHR2 has another specific ligand, urocortin III

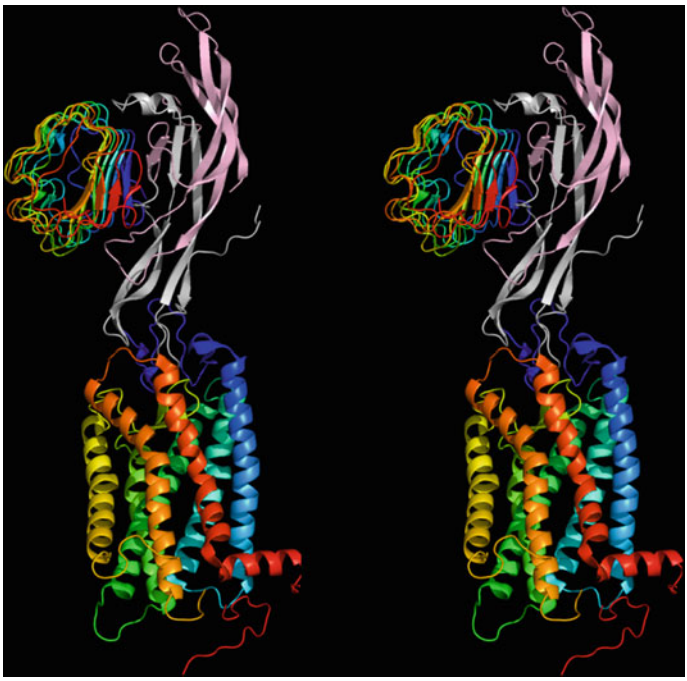

Fig. 8.2 Composite model of a heptahelical receptor. The follicle-stimulating hormone (FSH) receptor has a heptahelical membrane domain coupled to an N-terminal leucine-rich-repeat ectodomain like other glycoprotein hormone receptors and a few other so-called leucine-rich-repeat-containing G protein receptors (LGR). Since no complete LGR structure has been published, we modeled the ectodomain bound by FSH (Protein Data Bank entry 1XWD) to a rhodopsin heptahelical domain (Protein Data Bank entry 1F88) to show receptor–ligand interaction on the surface of cells. The heptahelical domain is inserted into the membrane, and the ectodomain lies on the outside of the cell. FSH binds with its single α-chain helix and the long β-chain loop enclosing the α chain (see Fig. 4.22) to the ten parallel ectodomain β sheets. At the bottom of the FSH dimer, both chains interact with the membrane domain in an as yet unknown stereochemistry. The interaction is testable with FSH mutants (Produced with PyMOL using Protein Data Bank entries 1XWD and 1F88)

8.2.1 G Proteins

G proteins are characterized by changes in conformation depending on whether GTP or guanosine diphosphate (GDP) is bound. The features of G proteins are as follows:

- *GDP/GTP binding.* All G proteins are able to bind either GDP or GTP. The nucleotide is placed into a characteristic ligand pocket.
- *GTP hydrolysis.* In this binding pocket, GTP can be hydrolyzed to GDP and phosphate. There is a faint intrinsic GTPase activity in any G protein;

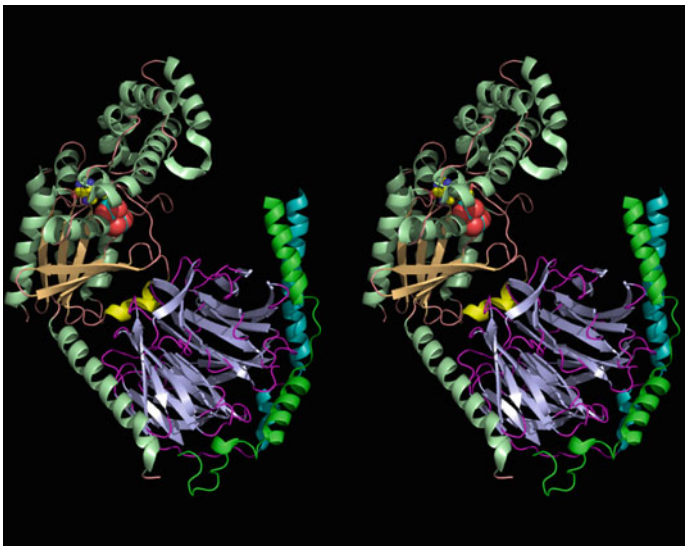

Fig. 8.3 Stereo-model of a heterotrimeric G protein. Three subunits—alpha subunit (*entire upper part* and *lowermost left helix*) with GDP bound, beta subunit (*lower part light blue* β *sheets* and *blue helix and pink loops*), and gamma subunit (*green*)—form the inactive molecule. GDP is shown with *yellow* carbon atoms, *blue* nitrogen atoms, *red* oxygen atoms, and *turquoise* phosphorus atoms. When the receptor binds to the G protein and induces GDP–GTP exchange, the next phosphate group presses on the β-sheet structure (*light orange*), and this in turn forces the contact helix (*yellow*) to press the beta–gamma complex away. An activated G protein consists of only the alpha subunit (From Wall et al. 1995; produced with PyMOL using Protein Data Bank entry 1GP2)

this activity, however, can be enhanced by interaction with GTPase-activating proteins.

- *Guanosine nucleotide exchange*. The hormone receptor with its ligand bound also changes its conformation. These conformational changes allow the interaction of the receptor with a G protein, which triggers GDP–GTP exchange.

Those G proteins binding to heptahelical membrane receptors are formed from three polypeptide chains called alpha, beta, and gamma subunits. The GDP/GTP-binding pocket is within the alpha subunit (Fig. 8.3).

8.2.2 Receptor–G Protein Interactions

The heptahelical membrane receptor changes in conformation after ligand binding. This change makes the intracellular loops between the different membrane domains of the receptor interact with G proteins. The first effect of this interaction is exchange of GDP for GTP. Activated heptahelical GPCRs are GDP–GTP exchange proteins.

With the exchange of GDP for GTP, the alpha subunit of the G protein undergoes structural changes which loosen the interaction of the alpha subunit with the beta–gamma complex and which generate a single alpha subunit with GTP bound and a beta–gamma dimer. The GTP–alpha monomer interacts with a variety of cellular targets. Eventually the intrinsic GTPase activity hydrolyzes GTP to GDP, rendering the GDP–alpha monomer inactive and allowing trimerization with the beta–gamma dimer. GTPase catalyzes hydrolysis, but this can be strongly enhanced by other proteins interacting with the GTP–alpha monomer. The beta–gamma dimer itself can stimulate enzymes within the cells—for example, adenylate cyclase—but its targets are less numerous than those of the GTP–alpha complex.

8.2.3 Target of G Proteins

A variety of intracellular enzymes can be activated by G proteins:

- *Adenylate cyclase.* This enzyme converts adenosine triphosphate to cyclic adenosine monophosphate (cAMP).
- *Guanylate cyclase.* This enzyme converts GTP to cyclic guanosine monophosphate (cGMP).
- *Phospholipases.* These cleave phospholipids. By this mechanism, messengers such as inositol trisphosphate, phosphocholine, and diacylglycerol or eicosanoids such as arachidonic acid and lysophosphatides are generated.
- *Sphingomyelinase.* If this enzyme is activated, then the membrane lipid sphingomyelin is cleaved to ceramide and phosphocholine.
- *Ion channels.* The G-protein alpha subunit subtypes o1 and o2 act on potassium channels.

Inositol trisphosphate, phosphocholine, diacylglycerol, arachidonic acid, ceramide, cAMP, and cGMP are messengers inducing other modifications in cells. Since hormones were called the primary messengers, first cAMP and later all these substances were called second messengers.

8.2.4 Variability by Differentially Expressed Receptor Subtypes: Somatostatin Receptors

Somatostatin is a hormone circulating in the blood. It is made in the hypothalamus as well as in the gastrointestinal tract and the endocrine pancreas, and its target cells are various other cells of the endocrine system. The specific action of somatostatin on the given target cells is achieved not by structural variation of somatostatin (14 versus 28 amino acids) but by variation of the somatostatin receptors.

There are five somatostatin receptors (Fig. 8.4), and they are active as monomers, but also as homodimers or heterodimers; this allows fine-tuned specific reactions (Rocheville et al. 2000b). Furthermore, somatostatin receptors dimerize not only

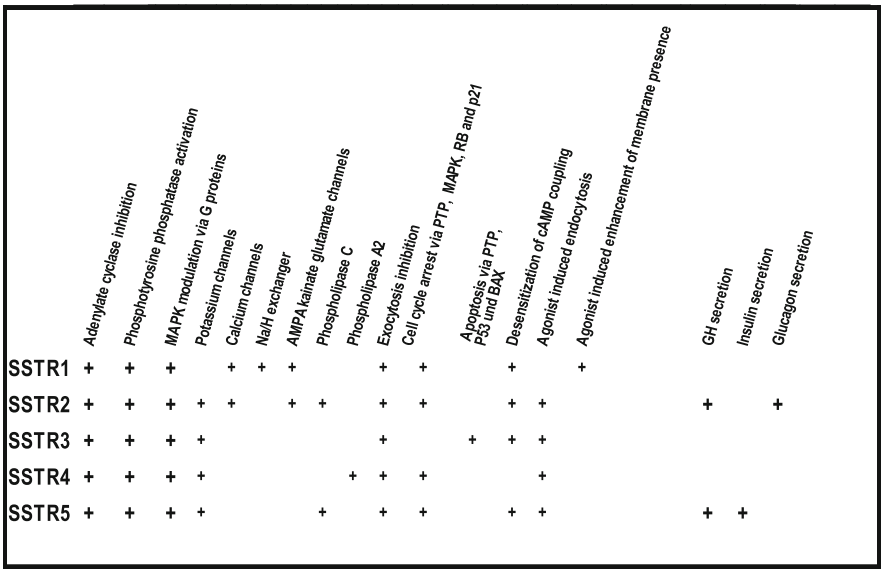

Fig. 8.4 Somatostatin receptor (*SSTR*)-mediated signal transduction and functions. *AMPA* α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, *cAMP* cyclic adenosine monophosphate, *GH* growth hormone, *MAPK* mitogen-activated protein kinase, *PTP* protein tyrosine phosphatase, *RB* retinoblastoma protein (Modified from Patel 1999)

with each other, but also with other receptors—for example, dopamine receptor (Rocheville et al. 2000a).

8.3 Receptors with Tyrosine Kinase Activity

The typical example of a tyrosine kinase membrane receptor for a hormone is the insulin receptor (Table 8.4). Insulin receptor consists of two pairs of two polypeptides. The extracellular part is formed by two identical polypeptide chains coupled by disulfide bridges. Each of these chains associates with another polypeptide crossing the membrane and enzymatically active within the cell (Fig. 8.5). This enzymatic activity adds phosphate to tyrosine residues.

Insulin receptors are considered as prototypic class I tyrosine kinase receptors. Other examples are platelet-derived growth factor receptor, vascular endothelial growth factor receptor, and colony stimulating factor 1 receptor. These receptors are class V tyrosine kinase receptors possessing several extracellular immunoglobulin-like domains and a tyrosine kinase domain with an additional 100 amino acids.

In most tyrosine kinase receptors, the receptor tyrosines are autophosphorylated. Other intracellular proteins called “Src” (Rous sarcoma virus protein) bind to these phosphorylated tyrosines. The binding domain of these proteins is called

Table 8.4 Tyrosine kinase receptors

Hormone/ligand	Receptor	Subtypes	OMIM entries
Insulin	Ins-R	α/β	147670
Insulin-like growth factor	IGF-R	I (II)	146370 (147280)
Neurotrophin	NTRK1 (TRKA)		191315
Platelet-derived growth factor	PDGF-R	α/β	173490/173410

IGF-R insulin-like growth factor receptor, *Ins-R* insulin receptor, *NTRK1* neurotrophic tyrosine kinase receptor type 1, *PDGF-R* platelet derived growth factor receptor
a IGF-R2 is not a tyrosine kinase receptor!

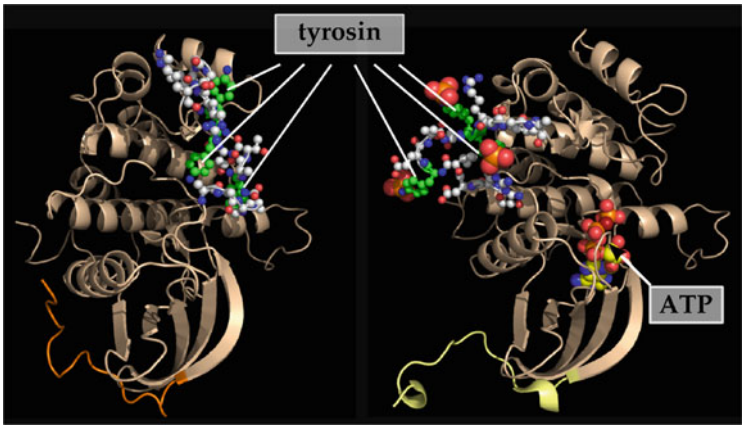

Fig. 8.5 The tyrosine kinase domain of insulin receptor. The same domain is shown on the *left*, in its inactive form, and on the *right*, activated and binding ATP. The loop with the three *green* tyrosines changes its position from the inactive to the active form. Phosphorylation (*enlarged orange/red spheres*) of the tyrosines and loading of ATP change not only the position of this loop, but also the entire structure, as can be seen. The lower β sheet has been kept constant between the two structures, which enables us to identify the subtler changes too. Opening access to the ATP molecules by flipping aside the tyrosine-containing loop activates the enzyme and allows protein phosphorylation to occur (Produced with PyMOL using Protein Data Bank entries 1IRK and 1IR3)

Src homology domain 2 (SH2). Such domains are common to various intracellular signaling proteins.

In insulin receptor, tyrosines are autophosphorylated, but they do not form an SH2 motif. For signal transduction, insulin receptor requires an additional protein, insulin receptor substrate. On phosphorylation, this protein has several SH2 motifs to communicate the insulin signal.

Figure 8.5 exemplifies how phosphorylation and associated structural adaptations modify the molecular topology, demonstrating signal transduction by conformational changes.

Table 8.5 Serine/threonine kinase receptors

Hormone/ligand	Receptor	Subtypes	OMIM entries
Activin	ACVR1	1a, 1b, 1c	102576, 601300, 608981
	ACVR2	2, 2a, 2b	102581, 602730
TGF-β	TGFβ-R	I, II	190181, 190182

ACVR1 activin receptor type 1, *ACVR2* activin receptor type 2, *TGF-β* transforming growth factor β, *TGFβ-R* transforming growth factor β receptor

8.4 Membrane Receptors with Serine/Threonine Kinase Activity

Activin/inhibin receptors (and other receptors for the transforming growth factor β supergene family proteins) are serine kinases (Table 8.5). Induced by ligand binding to the activin/inhibin receptor, the transcription factor SMAD5 is phosphorylated at serine. The phosphorylated SMAD5 is imported into the cellular nucleus, where it binds to SMAD5 motifs to regulate gene activity.

8.5 Membrane Receptors Without Kinase Activity

Leptin, prolactin, erythropoietin, and growth hormone are structurally similar. It is not surprising that their receptors are similar too, and they belong to the same class of membrane receptors (Table 8.6). These receptors, with colony stimulating factor receptor as a prototype, consist of five distinct extracellular domains, a transmembrane region, and a cytoplasmic domain. The genes for such receptors have up to 20 exons. Growth hormone, with its four helices, is bound by two extracellular domains (Fig. 8.6).

These receptors are not enzymatically active themselves. Their activity is mediated by signal transducers and activators of transcription (STAT proteins). These receptors become active when two hormonal ligands and two receptors interact. A STAT protein binds to the intracellular part of the receptors. This STAT protein with its SH2 domain initiates further signal transduction.

Whereas insulin receptor remains enzymatically active, these leptin, prolactin, or growth hormone receptors have no activity by themselves.

Leptin receptor exists in different splice variants. The OB-Rb variant has a 303 amino acid long cytoplasmic domain which transduces signals from the membrane receptor into the cell. Such a variant is expressed in cells where leptin blocks release of neuropeptide Y or agouti-related peptide—for example, on neurons of the arcuate nucleus. Shorter OB-R molecules lack this domain, and they are not capable of inducing signal transduction. Their expression in the choroid plexus or in blood capillaries in the brain suggests that they are active as leptin transporters through the blood–brain barrier. Whether such transport interferes with the in situ expression of leptin in the brain inhibited by hunger and fasting is an important research topic (Tartaglia 1997; Morash et al. 1999).

Table 8.6 Receptors without kinase activity

Hormone/ligand	Receptor	Subtypes	OMIM entries
Growth hormone	GH-R	(Alternative splicing)	600946
Leptin	LEP-R, OB-R	(Alternative splicing)	601007
Prolactin	PRL-R		176761
Erythropoietin	EPO-R		133171
Granulocyte/macrophage colony stimulating factor	GMCSF-R	α, β	306250, 138981

EPO-R erythropoietin receptor, *GH-R* growth hormone receptor, *LEP-R* leptin receptor, *PRL-R* prolactin receptor, *GMCSF-R* granulocyte/macrophage colony stimulating factor receptor

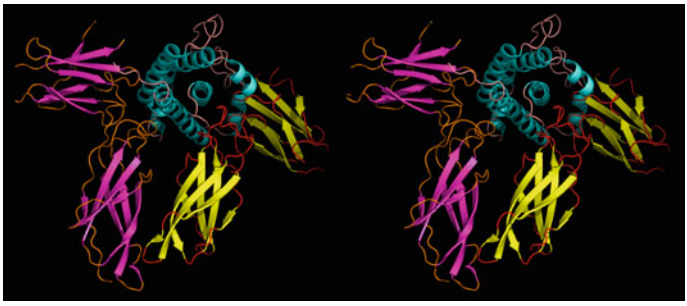

Fig. 8.6 Growth hormone and its receptor. To growth hormone with its *blue* helices bind two different growth hormone receptors with two extracellular domains each, characterized by *yellow* or *magenta* β-sheet structures. The interaction occurs by the *red loops* and *orange loops* and the helices of growth hormone (Produced with PyMOL using Protein Data Bank entry 3HHR)

8.6 Membrane Steroid Receptors: Still Unknown?

The fast action which aldosterone, for example, exerts on lymphocytes or smooth muscle cells cannot be explained by the slow action of nuclear receptors. The hunt for such fast-acting steroid receptors has taken more than 10 years, and is not yet finished. The fast-acting aldosterone receptor is still evasive—its action on the Na^+/H^+ exchanger can be blocked, signal transduction can be measured, but the darned receptor remains unknown.

In the meantime, researchers have extended this search for membrane steroid receptors for cortisol, testosterone, estradiol, vitamin D_3, and thyroid hormones. Since the human genome has been sequenced, candidates for such receptors have become limited. Orphan nuclear receptors, however, do not come into question.

In 2003, Zhu et al. (2003a) reported isolation of a human progesterone-binding GPCR after they had identified an analogous membrane progestin receptor in fish (Zhu et al. 2003b). Two years later, an estrogen-binding receptor was reported, which, on expression in previously negative cells, resulted in an estrogen-induced

cAMP level increase—typical for membrane receptors. This molecule was not related to progesterone receptor or any nuclear receptor (Thomas et al. 2005).

An alternative explanation for membrane-receptor-mediated actions of estradiol was presented by Levin (2005, 2008). Membrane actions of estradiol are possible only in a cell having an intact estrogen receptor α gene (*ESR1*). Levin (2005) demonstrated that estrogen receptor α, estrogen receptor β, androgen receptor, and progesterone receptor posses a common **FxxxxxxLL** motif by which, for example, GPCRs are sorted into the plasma membrane. The motif of the nuclear receptors additionally bears a cysteine residue which was shown to be palmitoylated (Pedram et al. 2007). Levin has presented convincing evidence that such palmitoylation inserts the nuclear receptors into membranes, and that mutation of the motif first blocks membrane insertion and inhibits membrane-associated fast reactions as well. This elegant solution avoids the search for additional receptors and provides a new feature for old molecules.

So far this mechanism has not been extended to the membrane aldosterone receptor.