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REVIEW ARTICLE

Activation of membrane receptors*

Tae H. Ji, William J. Murdoch & Inhae Ji

Department of Molecular Biology, University of Wyoming, Laramie, Wyoming 82071-3944, USA

Many extracellular messengers interact with discriminate receptors on the cell surface. Some of bound ligands activate receptors whereas others fail to do so. Only activated receptors are capable of generating and transferring signal through the membrane. Recent advances in our understanding of agonist-induced and constitutive receptor activation suggest several molecular mechanisms for receptor activation, signal generation and transmembrane signal transfer.

Keywords: hormone receptors; receptor activation; signal generation; transmembrane signal transfer

Introduction

The first experimental evidence that water soluble ligands do not have to enter the cytoplasm to elicit a cellular response was demonstrated when it was shown that lectin binding at the cell surface causes an organizational alteration along the inner surface of the plasma membrane (Ji & Nicolson, 1974). It is now apparent that ligand binding to complementary receptors on the cell surface triggers a cascade of intermediary transmembrane, cytoplasmic, and nuclear events leading toward a specific biological response. Investigations to date have focused mainly on structural analyses of hormone-receptor binding, stimulation of receptor kinases, G proteins, intracellular signal pathways, and receptor desensitization; numerous recent review articles have addressed these topics (Cambarnous *et al.*, 1992; Probst *et al.*, 1992; Spiegel *et al.*, 1992; Minegishi *et al.*, 1993; Segaloff & Ascoli, 1993; Spaulding, 1993; Lefkowits *et al.*, 1993; Neer, 1994; Strader *et al.*, 1994).

Receptor activation, signal generation and transmembrane signal transfer

The objective of this review is to consider the mechanistics of membrane receptor activation, signal generation and transmembrane signal transfer. Lately, receptor activation has popularly quoted in an abstract term and the structure of an activated receptor is unclear. The notion of 'activated' receptors necessitates an elaboration on the classical model of hormonal action; hormone + receptor → hormone-receptor complex → effector activation → cellular response. In most cases receptors must undergo multiple transient steps between hormone-receptor complexing and effector activation (Figure 1). This is manifested by the fact that some hormone-receptor complexes such as antagonist-receptor complexes (Strader *et al.*, 1994), defective hormone-normal receptor complexes (Yoo *et al.*, 1993) and normal hormone-defective receptor complexes (Ji & Ji, 1993) are incapable of activating effectors. On the other hand, mutant receptors exist that are constitutively activated in the absence of agonist to stimulate effectors (Lefkowitz *et al.*, 1993).

Surface membrane receptors are composed of hydrophilic extracellular, hydrophobic transmembrane and hydrophilic intracellular domains. A simple mechanism

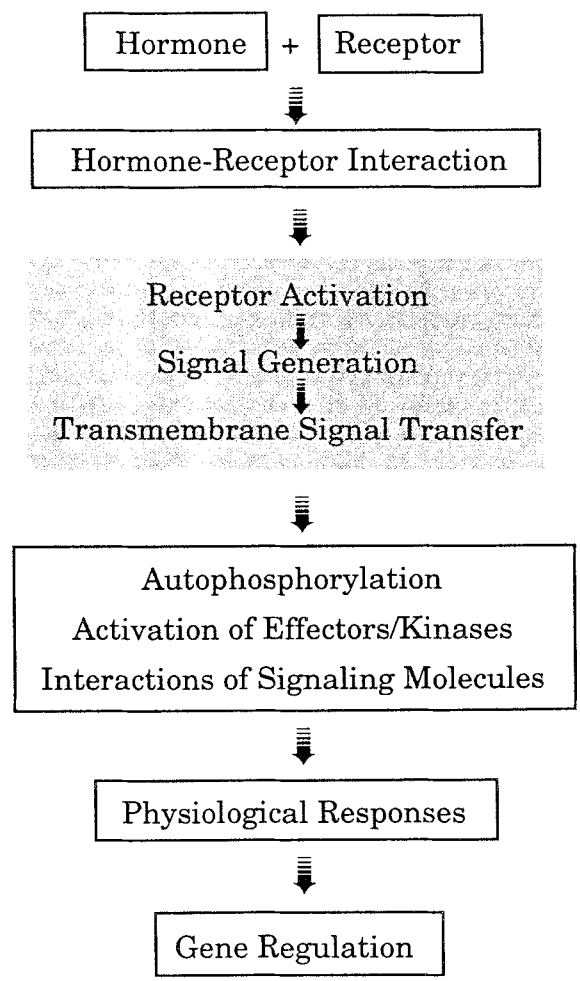

Figure 1 Hormone induced signaling steps. Receptors must undergo multiple transient steps between hormone-receptor complexing and effector activation

Correspondence: Tae H. Ji
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for a receptor to activate its effector in the cytoplasm is a conformational change of the receptor at the interface between the receptor and the effector. Because such a receptor alteration has to originate from the hormone-receptor contact point, the receptor is expected to undergo a conformational change at the hormone-receptor contact point upon hormone binding. Such a change leading to another (allosteric) change at another part of the receptor is considered as a signal (Figure 2). The conversion of an inactive receptor to an activated receptor which is capable of generating a signal is considered as receptor activation. The transfer of a signal from the hormone-receptor contact point to the hormone-effector interface in the cytoplasm is transmembrane signal transfer (transduction).

In spite of their vital role in hormone action, receptor activation, signal generation and transmembrane signal transfer still are ill defined and poorly understood steps. This is in part due to their relatively short half lives and a lack of informative assay system, causing a difficulty in studying them and allowing only before and after snap shots. Recently developed novel systems of mutant receptors and hormones and crystal structures of hormone receptor complexes, however, have provided an opportunity to examine these important steps of hormonal action.

Two major classes of receptors: Multi-transmembrane receptors and single transmembrane receptors

There are two major classes of hormone receptors which appear to utilize distinct mechanisms of transmembrane signal (Figure 3). Receptors with multi-transmembrane domains were G protein coupled recepters appear to accomplish transmembrane signal transfer through the transmembrane domains. Little is known, however, whether these G protein coupled receptors dimerize except for that the lutropin/choriogonadotropin receptors behave as a dimer on size exclusion chromatography (Dufau *et al.*, 1975).

On the other hand, hormone receptors with a single transmembrane domain and a cytoplasmic tail are often dimerized upon hormone binding for transmembrane signal transfer. Conceptually, in such a case, the transmembrane domain is not necessary for transmembrane signal transfer because dimerization at the extracellular domain may alter the structure of the cytoplasmic domain without involving the transmembrane domain. Growth hormone, prolactin, growth factors, cytokins, and arterial natriuretic peptide are among some examples of ligands that bind single transmembrane receptors.

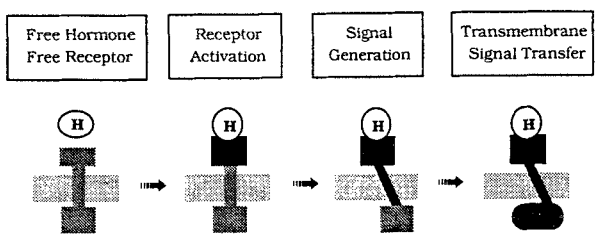

Figure 2 Receptor activation, signal generation and transmembrane signal transfer. A Hormone receptor is expected to undergo a conformational change at the hormone-receptor contact point upon hormone binding. Such a change leading to another (allosteric) change at another part of the receptor is considered as a signal. The conversion of an inactive receptor to an activated receptor which is capable of generating a signal is considered as receptor activation. The transfer of a signal from the hormone-receptor contact point to the hormone-effector interface in the cytoplasm is transmembrane signal transfer

Multi-transmembrane receptors

Seven transmembrane receptors

The major group in multi transmembrane receptors is the G protein coupled receptor superfamily (Lefkowitz, 1993). These receptors have an N-terminal extracellular extension, seven transmembrane domains, three cytoplasmic loops and three extracellular loops that link the seven transmembrane domains, and a C-terminal cytoplasmic tail. The identified members of this superfamily are comprised of several hundred distinct receptors for a wide variety of ligands such as proteins, peptides, neurotransmitters, biogenic amines, odorants and even, photons (Probst *et al.*, 1992; Lefkowitz *et al.*, 1993). The size of the corresponding ligands ranges from 35 kD to less than a few hundred dalton. Interestingly, a weak correlation exists such that the larger the ligand, the longer the N-terminal extension of the receptor. For example, the N-terminal extensions of the glycoprotein hormone receptors are the largest with ~350 amino acids, in comparison to the 11–30 amino acids long N-terminal extensions of adrenergic receptors (Probst *et al.*, 1992).

Four transmembrane receptors

Another group with multi-transmembrane domains is receptors with ligand gated ion channel. An ion channel is part of these receptors and binding of ligand neurotransmitters to the receptors opens the ion channel and allow specific ions to pass the channel. It includes the nicotinic acetylcholine receptor with a Na^+ and K^+ channel, the glycine and the γ-aminobutyrate with a Cl^- channel. These receptors are oligomeric and each subunit has four transmembrane domains (Hucho *et al.*, 1994).

Mechanisms of receptor activation and signal generation of multi-transmembrane receptors

Existing evidence suggests at least four distinct mechanisms for activation of these various sizes of

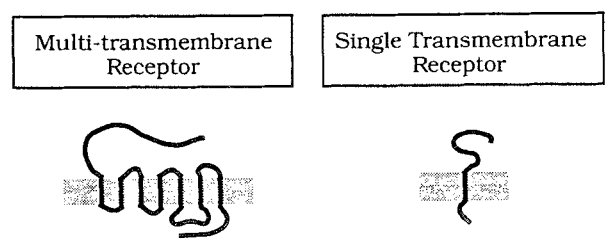

Figure 3 Two classes of hormone receptors. There are two major classes of hormone receptors. The first group includes receptors with multi-transmembrane domains like G protein coupled receptors and receptors with a ligand gated ion channel. The second group is hormone receptors with a single transmembrane domain

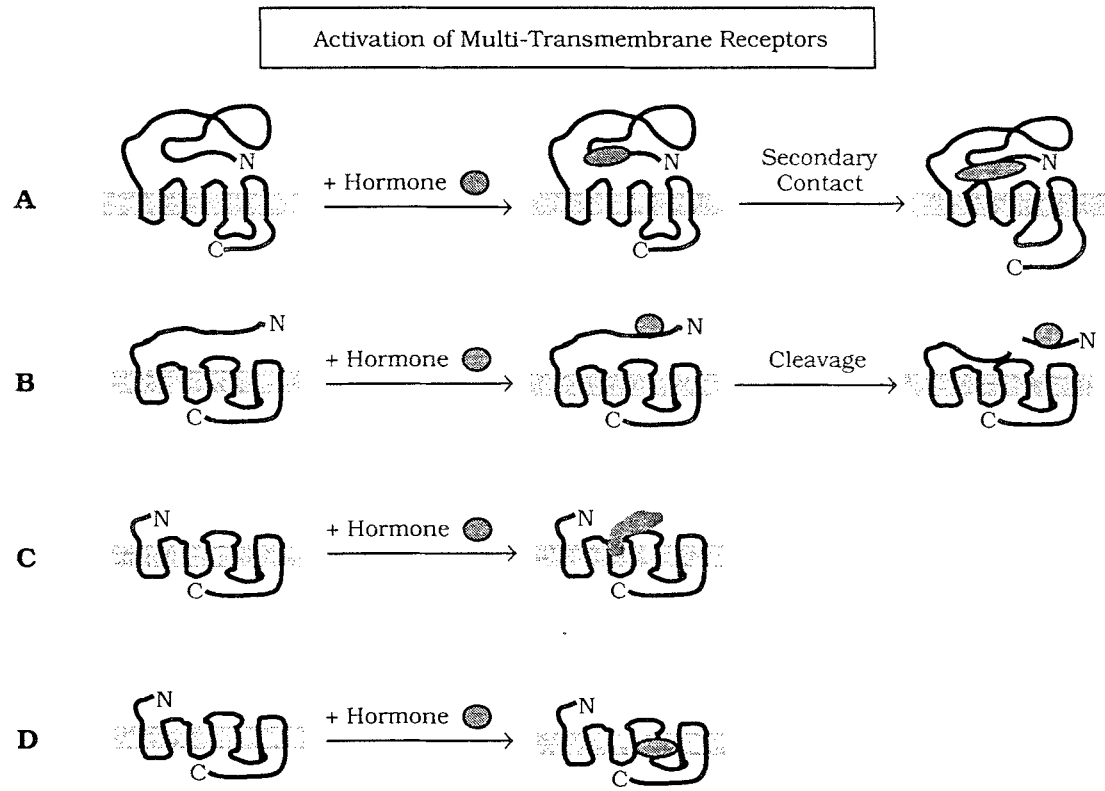

Figure 4 Activation of seven-transmembrane receptors. (**A**) Domain interaction: Certain seven transmembrane (G protein coupled) receptors with a long extracellular N-terminal extension bind their ligand at a high affinity site in the extracellular N-terminal extension and the resulting hormone-N-terminal complex interacts with juxtaposed domains in the C-terminal region of the receptor. This secondary interaction has low affinity and is responsible for activating receptor and generating signal. (**B**) Proteolytic activation of receptors: When thrombin binds to its receptor's N-terminal extension, the extracellular N-terminal 41 amino acids of the receptor is cleaved at a specific sequence. This cleavage generates a new N-terminal that functions as a tethered ligand and activates the receptor. (**C**) Modulation of the exoplasmic domain and transmembrane tunnel of receptors. Some peptide hormones bind the extracellular (exoplasmic) domains of their receptors and may, at least in part, penetrate the transmembrane tunnel. (**D**) Receptor activation within the transmembrane tunnel. Biogenic amines bind to their receptor in the polar transmembrane tunnel to activate receptors

receptors. The glycoprotein hormone receptors with the large extracellular N-terminal extension (N-terminal half) undergo stepwise ternary interactions (domain interaction) of the receptors' N-terminal half, the hormone and the C-terminal half as shown in Figure 4A. The thrombin receptor is activated by proteolytic cleavage which is catalyzed by thrombin (Figure 4B). Peptide ligands such as gonadotropin releasing hormone, tachykinins and formyl peptides interact the extracellular domain and penetrate the transmembrane tunnel to modulate the receptor (Figure 4C). The smallest ligands including bioactive amines and photons penetrate and modulate the transmembrane tunnel (pocket) as seen in Figure 4D.

Domain interactions

Certain seven transmembrane receptors with a long extracellular N-terminal extension interact with juxtaposed domains to activate receptor and generate signal. N-terminal halves lacking the membrane associated C-terminal halves of the luteinizing hormone/choriogonadotropin (LH/CG) receptor and the thyroid stimulating hormone receptor can bind their respective ligand with an affinity comparable to intact receptors (Tsai-Morrison *et al.*, 1990; Xie *et al.*, 1990; Ji & Ji, 1991a; Seetharamaiah *et al.*, 1994). However, high affinity hormone interaction at the N-terminal extension in itself is not sufficient for receptor activation and signal generation (Ji & Ji, 1991b; Remy *et al.*, 1993). On the other hand, C-terminal halves lacking an N-terminal half bind the ligand with low affinities (Ji & Ji, 1991b; Renny *et al.*, 1993), that activates the receptors and generates signal. When the two independent halves of the LH/CG receptor were co-expressed in a cell, the hormone was able to bind and to activate them with a high affinity, leading to successful signal generation and effector stimulation (Remy *et al.*, 1993).

These results of two distinct binding sites with different affinities suggest that receptor activation, signal generation and transmembrane signal transfer occur independent of the initial hormone-receptor interaction and require secondary contact (Figure 4A). These multistep hormone-receptor interactions are consistent with kinetic studies (Moyle, 1980; Roche & Ryan, 1985). The secondary contact suggests that the initial hormone-receptor complex may not be thermodynamically stable, perhaps because of changes in the environment and the structure of the hormone and receptor in the newly formed complex. Such an energetically unfavorable structure can drive a complex to refold. Recently it had been shown that an environmental change in the influenza virus receptor results in a

major refolding and the translocation of a group nearly 10 nm (Bullough *et al.*, 1994).

The secondary contact includes the hormone and the membrane associated C-terminal half of the receptor (Ji *et al.*, 1993) or the hormone and both of the receptor's N-terminal and C-terminal halves. Reciprocal mutagenesis is one of the approaches used to demonstrate interacting domains and pairing amino acids between interacting domains. The principle is as follows. When two different amino acids of a putative pair are reciprocally substituted with each other, the resulting mutant molecule or molecules may function if the pair of amino acids indeed interacts together. One set of pairing residues between the α subunit of human choriogonadotropin (hCG) and the boundary region of the second transmembrane domain and the first extracellular loop of its receptor has been identified (hCG αLys^{91}-receptor Asp^{397}) by reciprocal mutagenesis (Ji *et al.*, 1993). Such an ionic attractive force can generate several k calories of energy particularly in an apolar environment (Cantor & Schimmel, 1980) to impact the global structure of the receptor and to play a key step in receptor activation and signal generation.

Proteolytic activation of receptors

The thrombin receptor has been proven to be activated upon its proteolytic cleavage by the ligand (Vu *et al.*, 1991; Chen *et al.*, 1994), while the glucagon receptor is proposed to be cleaved and activated by glucagon (Unson & Merrifield, 1994). Proteolytic cleavage of a receptor will yield two new products, the released fragment and the truncated receptor with a new N-terminus. Both products can potentially regulate receptor functions. Interestingly, these receptors are members of the G-protein coupled receptor family. Therefore, structural alterations at the cell surface are likely to affect interactions of receptors with G-protein on the cytoplasmic surface of the membrane.

Thrombin is an established proteolytic enzyme which recognizes the consensus Leu-Asp-Pro-Arg-Ser sequence and cleaves the amide between Arg and Ser (Vu *et al.*, 1991). When thrombin binds to its receptor, the extracellular N-terminal 41 amino acids of the receptor is cleaved at the specific sequence. This cleavage generates a new N-terminal that functions as a tethered ligand and activates the receptor (Vu *et al.*, 1991; Chen *et al.*, 1994). Apparently, the released peptide acts as a suppressor of receptor activation before its cleavage from intact receptor. A question arises as to how the cleaved and activated receptor is deactivated, since a receptor should not be permanently left activated.

Unlike thrombin, glucagon does not have innate proteolytic activity, but does contain three essential residues (His^1, Asp^2 and Ser^{16}) analogous to the catalytic triad of serine proteases. These amino acids may behave as a coenzyme-like factor and catalyze the specific hydrolysis of a peptide bond within the receptor, resulting in receptor activation (Unson & Merrifield, 1994). Consistent with this hypothesis is the observation that proteolytic inhibitor blocks glucagon action.

The luteinizing hormone/choriogonadotropin receptor is apparently activated by proteolysis (Reichert & Ryan, 1977a). Proteolytic enzymes mimic and inhibitors attenuate luteinizing hormone action (Reichert & Ryan, 1977b). The source of such proteolytic enzyme is unknown.

Modulation of the exoplasmic domain and transmembrane tunnel

Some peptide hormones bind the extracellular (exoplasmic) domains of their receptors. In analogy with the ligand binding in the transmembrane tunnel of rhodopsin and adrenergic receptors, these peptide hormones are suggested to, at least in part, penetrate the transmembrane tunnel but the evidence is indirect. Hormone binding and receptor activation involve a number of amino acids residues of receptors but their precise role is unknown at this time. Therefore, it is yet to be determined whether peptide hormones undergo two or more steps to bind and activate their receptors and what the mechanism of receptor activation is.

Gonadotropin releasing hormone (GnRH) binds to the first and third exoloops of the GnRH receptor and Glu^{301} in the third exoloop is suggested to interact with Arg^8 of GnRH (Flanagan *et al.*, 1994). In addition, Asn^{87} of the second transmembrane domain and Asp^{318} of the seventh transmembrane domain are also involved in hormone binding and receptor activation (Cook *et al.*, 1993). Thyrotropin releasing hormone is suggested to bind the receptor by hydrogen bonding between the pyroglutamly carbonyl group of the hormone and the hyrdoxyl group of Tyr^{106} of the receptor's third transmembrane domain (Perlman *et al.*, 1994).

Substance P binding to the receptor requires Asn^{23} and Phe^{25} in the extracellular N-terminal extension, several amino acid residues in the transmembrane domains, two, four, five and six (Huang *et al.*, 1994; Werger, 1994). Glu^{78} of the second transmembrane domain and Tyr^{287} of the seventh transmembrane domain are important for receptor activation (Huang *et al.*, 1994). It is not clear, however, whether hormone binding and receptor activation occur simultaneously or subsequentially in a distinct steps. In the bradykinin receptor, Asp^{268} and Asp^{186} of the third exoloop and several amino acid residues of the sixth transmembrane domain are involved in hormone binding (Novotny *et al.*, 1994; Nardone & Hogan, 1994).

Bacterial formyl peptides are recognized by amino $acids^{2-11}$ of the formyl receptor of neutrophils (Perez *et al.*, 1994). Other residues of exoplasmic loops 1–3, including Arg^{84} and Lys^{85} of the exoloop one, are necessary for ligand binding (Perez *et al.*, 1994; Radel *et al.*, 1994), while the N-terminal four amino acid residues of bound formyl peptides are sequestered (Fay *et al.*, 1993). Whether these sequestered residues of the ligand are in the transmembrane tunnel has not been proven.

Receptor activation within the transmembrane tunnel

Each of the seven transmembrane domains of G protein coupled receptors is thought to behave as a transmembrane column. One side of these columns is comprised of primarily hydrophilic amino acids and the other side consists of hydrophobic residues according to the helical wheel analysis. The most stable organization of the seven transmembrane columns is to form a tunnel in which the hydrophobic side of the columns

faces the lipid bilayer. This arrangement sequesters the hydrophilic face of the columns, thus forming a stable polar tunnel. Biogenic amines bind to their receptor in the polar transmembrane tunnel. The positively charged amine group of agonists and antagonists ionically pairs with a negatively charge acidic residue present in the lining of the receptor tunnel (Strader *et al.*, 1994). However, this ion pairing in the ligand receptor interaction does not appear to be sufficient for receptor activation as is the case of that the high affinity glycoprotein hormine binding to the N-terminal half of the receptor (Ji & Ji, 1991b; Remy *et al.*, 1993). Rather, a highly conserved Asp in the second transmembrane domain is involved in receptor activation and signal generation (Strader *et al.*, 1994).

Regulation of ion channel

The nicotinic acetylcholine receptor consists of five subunits, each with four transmembrane domains. Some of the 20 transmembrane columns line the ion channel (Hucho *et al.*, 1994). Studies on agonist and antagonist binding and ion transport indicate that agonist binding is physically and biochemically distinct for channel opening (Reddy *et al.*, 1993). Agonist binding near or on the exoplasmic domain (O'Leary & White, 1992) is nearly 5 nm away (Johnson *et al.*, 1987) from a channel gate near the cytoplasmic surface (Reddy *et al.*, 1993). It is obvious that signal has to travel such a distance. It is interesting to see how the receptor is activated and the signal is transferred through the transmembrane domains and channel.

Transmembrane signal transfer

As described above, receptor activation and signal generation of multi-transmembrane receptors begin at the extracellular surface or within the transmembrane tunnel of receptors. A simple mechanism to relay signal to the cytoplasmic surface is to modulate transmembrane columns and to cause an allosteric structural change in the cytoplasmic domains of receptors. There are several ways to accomplish this: rotation (Figure 5A), piston (Figure 5B), pulse (Figure 5C), pivot (Figure 5D) and/or shuffling of transmembrane column(s) to alter their spatial relationship (Figure 5E). This can be accomplished without an alteration in their secondary structure because a transmembrane column in a hydrophobic environment like a lipid bilayer is extremely stable and therefore rigid. Transmembrane columns are primarily comprised of hydrophobic amino acids and assumed to be five or more turns of helices largely by analogy with the bacteriorhodopsin. However, these putative helices have not been experimentally demonstrated. In fact, some of transmembrane columns have been suggested to be at least in part a beta structure (Hucho *et al.*, 1994). In either case, a group of hydrophobic columns in the lipid bilayer is likely to form a stable and rigid structure.

When hormones interact with the extracellular portion of receptors, it can cause rotation, piston, pivot, pulse and/or alteration in the spatial relation of the transmembrane column(s). For example, when Asp^{397} of the LH/CG receptor interacts with Lys^{91} of hCGα this may rotate, pull up, push down, pivot, or attract the second transmembrane column. As a result the topology of the seven transmembrane columns may change. When ligands interact directly with the transmembrane columns of receptors as is the case for rhodopsin and adrenergic receptors (Lefkowitz & Caron, 1988; Khorana, 1992), they may link two or more transmembrane columns, resulting their rotation, piston, pivot, pulse and/or shuffle, leading to an allosteric change in the cytoplasmic domain of the receptors. Putative allosteric effects are expected to be greater when multiple transmembrane columns and other receptor regions are involved in contacting hormone.

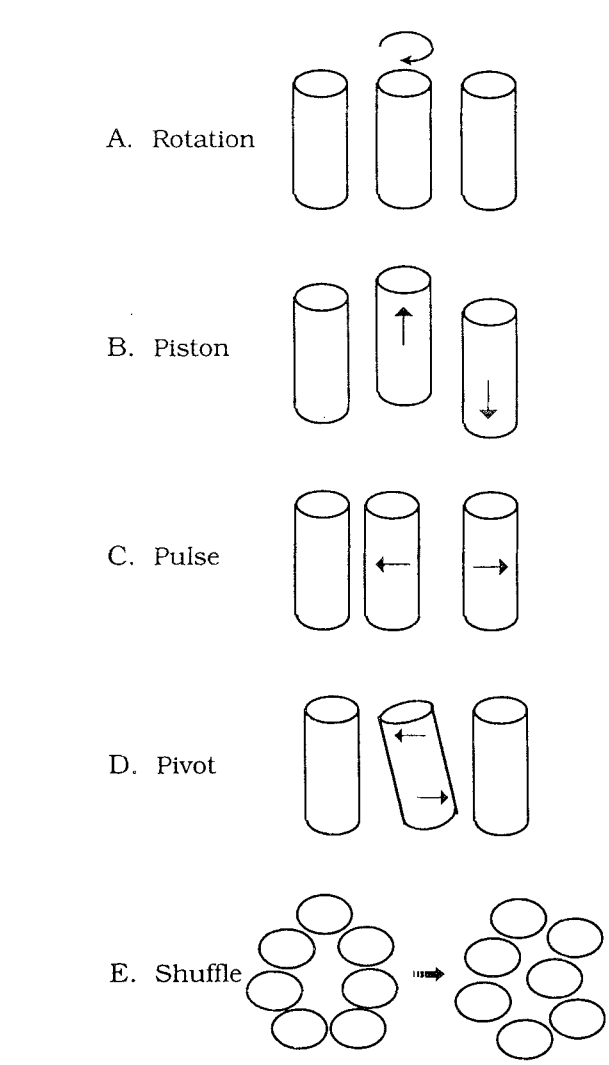

Figure 5 A simple mechanism to relay signal to the cytoplasmic surface is to modulate transmembrane columns and to cause an allosteric structural change in the cytoplasmic domains of receptors. There are several ways to accomplish this: (**A**) rotation, (**B**) piston, (**C**) pulse, (**D**) pivot and (**E**) shuffling of transmembrane column(s) to alter their spatial relationship. Any of these mechanisms, independently or in combination may be involved in transmembrane signal transfer

Constitutively active receptors

Considering the crucial role of transmembrane columns in linking the cytoplasmic domains and transmembrane signal transfer, it is not surprising to find that a number of hormone receptors is constitutively activated when an amino acid residue in a transmembrane column is mutated: $Asp^{578} \rightarrow Gly$ of the luteinizing hormone/choriogonadotropin receptor (Shenker *et al.*, 1993), $Pro^{556} \rightarrow Leu$ of the thyroid stimulating hormone receptor (Stein *et al.*, 1994) and $Val^{664} \rightarrow Glu$ the epidermal growth factor receptor (Cao *et al.*, 1992). The substitution of Arg for a Gly in the transmembrane domain of the fibroblast growth factor receptor 3 results in dwarfism in humans (Shiang *et al.*, 1994). However, whether the mutant receptor is constitutively active or dominant negative is unknown. Substitution of amino acids in transmembrane columns may rotate and pulse the columns or alter their spatial relationship, thus sufficiently impacting on the role of the cytoplasmic domain of receptors.

In addition, some substitution of an amino acid at the receptor-effector interface also results in constitutive activation of receptors: $Leu^{266} \rightarrow Ser$, $Lys^{267} \rightarrow Arg$, $His^{269} \rightarrow Lys$ and $Leu^{272} \rightarrow Ala$ of the β adrenergic receptor (Lefkowitz *et al.*, 1993).

Oligomerization of single transmembrane receptors

Transmembrane signal transfer by ligand-dependent dimerization involves those receptors containing a single transmembrane domain (Figure 6). Extracellular domains of receptors lacking the transmembrane and cytoplasmic domains are capable of dimerization, but do not induce intracellular responses because they are not attached to the membrane and lack a cytoplasmic tail (de Vos *et al.*, 1992; Herren *et al.*, 1993; Zhou *et al.*, 1993). Apparently, dimerization of extracellular domains of intact receptors imparts sufficient structural changes in the cytoplasmic domains to confer hormonal action. However, the exact nature of the changes in dimeric receptor's cytoplasmic domains is unknown as is whether the two cytoplasmic domains of the receptor dimers interact together or they simply undergo independent allosteric changes. Some receptors dimerize with the same receptor species, whereas others interact with different types of receptors (Figure 6).

Homo-dimerization of receptors

Growth hormone has two unique binding sites capable of recognizing two identical receptor molecules (de Vos *et al.*, 1992). When the high affinity site (site I) binds receptor, it activates site II to bind a second receptor; site II can only bind receptor when site I is occupied. The two receptors interact with each other near the C-terminus of the extracellular domains providing stability to the hormone-$(receptor)_2$ ternary complex. Mutant hormones altered within site II are not biologically active. Divalent, but not monovalent, receptor antibodies mimic natural hormone (Cunningham *et al.*, 1991; Fuh *et al.*, 1992). Nonetheless, receptor dimerization is not entirely obligatory for transmembrane signal transfer. For example, placental lactogen activates the growth hormone receptor in its monomeric form for transmembrane signal transfer (Staten *et al.*, 1993).

Receptors for platelet-derived growth factor and epidermal growth factor also are dimerized by their specific ligands. This dimeric interaction correlates with stimulation of receptor tyrosine kinase activity (Bishayee *et al.*, 1989; Heldin *et al.*, 1989; Ullich & Schlessinger, 1991), autophosphorylation of receptor tyrosines (Williams, 1989), and interchain transphosphorylation (Kelly *et al.*, 1991). The platelet-derived growth factor receptor, however, dimerizes and autophosphorylates at high concentrations in the absence of agonist (Herren *et al.*, 1993). Thus, unoccupied receptors appear to have a low affinity for each other; binding of the growth factor simply enhances that affinity. Clearly, that differs from the receptor dimerization by a divalent growth hormone. Dimeric epidermal growth factor receptors bind ligand 30–40-fold more tightly than monomeric receptor (Zhou *et*

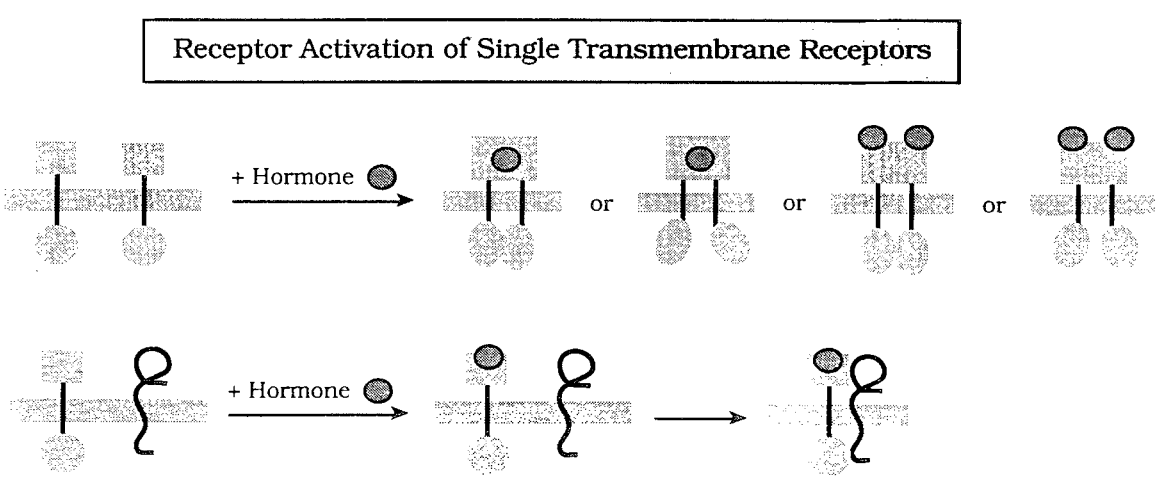

Figure 6 Oligomerization of receptors with a single transmembrane domain. Certain hormone receptors with a single transmembrane domain interact with their own species (homo-dimerization) upon hormone binding or complex with other species (hetero-oligomerization). In the case of growth hormone, it binds one growth hormone receptor, which activates a second receptor binding site in the hormone and facilitates binding of the second receptor

al., 1993) due to a slower dissociation rate of the factor from receptor dimer. Efficient biological responses are probably propagated by stable ternary complex. Whether one molecule of these growth factors have two different receptor binding sites is unknown.

Hetero-oligomerization

Crosslinking and reconsitution studies suggest formation of hetero-dimeric and multimeric receptors. The family of cytokines have more than one receptor subunit. A generic model was proposed that a molecular of cytokines binds initially an α subunit and then two β subunits (Stahl & Yancopoulos, 1993). The sequence of receptor binding is similar to the sequential interactions of growth hormone with its receptor (de Vos *et al.*, 1992).

Table 1 Mechanisms of receptor activation

Mode of receptor activation	*Ligands*
Multi-transmembrane domain	
Domain interaction	LH/CG
Proteolytic activation	Thrombin, glucagon
Modulation of extracellular domains and transmembrane tunnel	GmRH, parathyroid, hormone, tachykinins, Formyl peptide
Modulation of transmembrane tunnel	Bioactive amines, thyrotropin RH, photon
Single transmembrane domain	
Homo-dimerization	Growth hormone, EGF, FGF, PDGF, TNF, Neu
Hetero-oligomerization	Cytokines, NDF

Conclusions and future directions

A number of working mechanisms of the currently ill defined receptor activation have been surfaced in recent years (Table 1). The proposed definition of the initial steps of receptor activation, signal generation and transmembrane signal transfer (Figure 2) are likely to be better defined and modified. These complex processes are likely to involve numerous chemical groups of both ligands and receptors. When important amino acid residues are thoroughly examined as currently being identified, it will be necessary to determine whether they are direct contact sites or play an indirect and global role. Some of direct contact sites and interacting pairs are now beginning to be identified in a few hormone systems. Following the chemical identification, it will be necessary to determine the progress of rapidly changing interactions between chemical moieties of hormones and receptors. This structural study will necessarily involve physical methods including real time analyses.

The knowledge gained from the studies on the critical early steps of hormone action will open new challenges. Furthermore, it will not only lead to a better understanding of hormone-receptor action but also benefit clinical and industrial applications.

References

Bishayee, S., Majumdar, S., Khire, J. & Das, M. (1989). *J. Biol. Chem.*, **264,** 11699–11705.

Bullough, P.A., Hughson, F.M., Skehel, J.J. & Wiley, D.C. (1994). *Nature*, **371,** 37–43.

Cantor, C.R., Schimmel, P.R. (1980). *Biophysical Chemistry Part I: The Conformation of Biological Macromolecules.* Freedman and Co., New York, pp. 289–291.

Cao, H., Bangalore, L., Bormann, B.J. & Stern, D.F., (1992). *EMBO J.*, **11,** 923–932.

Chen, J., Ishii, M., Wang, L., Ishii, K. & Coughlin, S.R. (1994). *J. Biol. Chem.*, **269,** 16041–16045.

Combarnous, Y. (1992). *Endocrine Rev.*, **13,** 670–691.

Cook, J.V., Faccenda, E., Anderson, L., Couper, G.G., Eidne, K.A. & Taylor, P.L. (1993). *J. Endocrinol.*, **139,** R1–4.

Cunningham, B.C., Ultsch, M., De Vos, A.M., Mulkerrin, M.G., Clauser, K.R. & Wells, J.A. (1991). *Science*, **254,** 821–825.

Dufau, M., Ryan, D.W., Baukal, A.J. & Catt, K.J. (1975). *J. Biol. Chem.*, **250,** 4882–4824.

De Vos, A.M., Ultsch, M. & Kossiakoff, A.A. (1992). *Science*, **255,** 306–312.

Fay, S.P., Domalewski, M.D. & Sklar, L.A. (1993). *Biochemistry*, **32,** 1627–1631.

Flanagan, C.A., Becker, I.I., Davidson, J.S., Wakefield, I.K., Xhou, W., Sealfon, S.T. & Millar, R.P. (1994). *J. Biol. Chem.*, **269,** 22636–22641.

Fuh, G., Cunningham, B.C., Fukunaga, R., Nagata, S., Goeddel, D.V. & Wells, J.A. (1992). *Science*, **256,** 1677–1680.

Heldin, C-H., Ernlund, A., Rorsman, C. & Ronnstrand, L. (1989). *J. Biol. Chem.*, **264,** 8905–8912.

Herren, B., Rooney, B., Weyer, K.A., Iberg, N., Schmid, G. & Pech, M. (1993). *J. Biol. Chem.*, **268,** 15088–15095.

Huang, R-R.C., Yu, H., Strader, C.D. & Fong, T.M. (1994). *Biochemistry*, **33,** 3007–3013.

Hucho, F., Gorne-Tschelnowkow, U. & Strecker, A. (1994). *TIBS*, **19,** 383–387.

Ji, T.H. & Nicolson, G.L. (1974). *Proc. Natl. Acad. Sci. USA*, **71,** 2212–2216.

Ji, I. & Ji, T.H. (1991a). *Endocrinology*, **128,** 2648–2650.

Ji, I. & Ji, T.H. (1991b). *J. Biol. Chem.*, **266,** 13076–13079.

Ji, I. & Ji, T.H. (1993). *J. Biol. Chem.*, **268,** 20851–20854.

Ji, I., Zeng, H. & Ji, T.H. (1993). *J. Biol. Chem.*, **268,** 22971–22974.

Johnson, D.A., Brown, R.D., Herz, J.M., Berman, H.A., Andreasen, G.L. & Talyor, P. (1987). *J. Biol. Chem.*, **262,** 14022–14029.

Kelly, J.D., Haldeman, B.A., Grant, F.J., Murray, M.J., Seifert, R.A., Bowen-Pope, D.F., Cooper, J.A. & Kazlauskas, A. (1991). *J. Biol. Chem.*, **266,** 8987–8992.

Khorana, H.G. (1992). *J. Biol. Chem.*, **267,** 1–4.

Lefkowitz, R.J. & Caron, M.R. (1988). *J. Biol. Chem.*, **263,** 4993–4996.

Lefkowitz, R.J., Cotecchia, S., Samama, P. & Costa, T. (1993). *TIPS*, **14,** 303–307.

Moyle, W.R. (1980). *Oxford Reviews of Reproductive Biology*. Finn, C.A. (eds.). Oxford University Press: Oxford. pp. 128–204.

Nardone, J. & Hogan, P.G. (1994). *Natl. Acad. Sci. USA*, **91,** 4417–4421.

Neer, E.J. (1994). *Protein Sci.*, **3,** 3–14.

Novotny, E.A., Bednar, D.L., Connolly, M.A., Stormann, T.M. (1994). *Biochem. Biophys. Res. Commun.*, **201,** 523–530.

O'Leary, M.E. & White, M.M. (1992). *J. Biol. Chem.*, **267,** 8360–8365.

Perez, H.D., Vilander, L., Andrews, W.H. & Holmes, R. (1994). *J. Biol. Chem.*, **269,** 22485–22487.

Perlman, J.H., Thaw, C.N., Laakkonen, L., Bowers, C.Y., Osman, R. & Gershengorn, M.C. (1994). *J. Biol. Chem.*, **269,** 1610–1613.

Probst, W.C., Snyder, L.A., Schuster, D.I., Brosius, J. & Sealfon, S.C. (1992). *DNA Cell Biol.*, **11,** 1–20.

Radel, S.J., Genco, R.J. & De Nardin, E. (1994). *Infect Immun.*, **62,** 1726–1732.

Reddy, G.L., Iwamoto, T., Tomich, J.M. & Montal, M. (1993). *J. Biol. Chem.*, **268,** 14608–14615.

Reichert, N.D. & Ryan, R.J. (1977a). *Biochem. Biophys. Res. Commun.*, **78,** 799–805.

Reichert, N.D. & Ryan, R.J. (1977b). *Proc. Natl. Acad. Sci. USA*, **74,** 4857–4861.

Remy, J.J., Bozon, V., Couture, L., Goxe, B., Salesse, R. & Garnier, J. (1993). *Biochem. Biophys. Res. Commun.*, **193,** 1023–1030.

Roche, P.C. & Ryan, R.J. (1985). *Luteinizing Hormone Action and Receptors*. A. Ascoli, (eds.). CRC press Inc.: Boca Raton. pp. 17–56.

Segaloff, D.L. & Ascoli, M. (1993). *Endocrine Rev.*, **14,** 324–347.

Seetharamaiah, G.S., Kurosky, S., Desai, R.K., Dallas, J.S. & Prabhaker, B.S. (1994). *Endocrinology*, **134,** 549–554.

Shenker, A., Laue, L., Kosugi, S., Merendino, J.J., Minegishi, T. & Cutler, G.B. (1993). *Nature*, **365,** 652–654.

Spaulding, S.W. (1993). *Endocrine Rev.*, **14,** 632–650.

Spiegel, A.M., Weinstein, L.S. & Shenker, A. (1993). *J. Clin. Invest.*, **92,** 1119–1125.

Stein, S.A., Oates, E.L., Hall, C.R., Grumbles, R.M. Fernandez, L.M., Taylor, N.A., Puett, D. & Jin, S. (1994). *Molecular Endocrinology*, **8,** 129–138.

Stahl, N. & Yancopopoulos, G.D. (1993). *Cell*, **74,** 587–590.

Strader, C.D., Fong, T.M., Tota, M.R. & Underwood, D. (1994). *Annu. Rev. Biochem.*, **63,** 101–132.

Tsai-Morris, C.H., Buczko, E., Wang, W. & Dufau, M.L. (1990). *J. Biol. Chem.*, **265,** 19385–19388.

Ullrich, A. & Schlessinger, J. (1991). *Cell*, **61,** 203–212.

Unson, C.G. & Merrifield, R.B. (1994). *Proc. Natl. Acad. Sci. USA*, **91,** 454–458.

Vu, T-K.H., Hung, D.T., Wheaton, V.I. & Coughlin, S.R. (1991). *Cell*, **64,** 1057–1066.

Werger, T.M. (1994). *J. Biol. Chem.*, **269,** 22054–22058.

Williams, L.T. (1989). *Science*, **243,** 1564–1570.

Xie, Y-B., Wang, H. & Segaloff, D.L. (1990). *J. Biol. Chem.*, **265,** 21411–21414.

Yoo, J., Zeng, H., Ji, I., Murdoch, W.J. & Ji, T.H. (1993). *J. Biol. Chem.*, **268,** 13034–13042,

Zhou, M., Felder, S., Rubinstein, M., Hurwitz, D.R., Ullich, A., Lax, I. & Schlessinger, J. (1993). *Biochemistry*, **32,** 8193–8198.