Cell surface receptors

Most ligands responsible for cell-cell signaling (including neurotransmitters, peptide hormones, and growth factors) bind to receptors on the surface of their target cells. Consequently, a major challenge in understanding cell-cell signaling is unraveling the mechanisms by which cell surface receptors transmit the signals initiated by ligand binding. Some neurotransmitter receptors are ligand-gated ion channels that directly control ion flux across the plasma membrane. Other cell surface receptors, including the receptors for peptide hormones and growth factors, act instead by regulating the activity of intracellular proteins. These proteins then transmit signals from the receptor to a series of additional intracellular targets, frequently including transcription factors. Ligand binding to a receptor on the surface of the cell thus initiates a chain of intracellular reactions, ultimately reaching the target cell nucleus and resulting in programmed changes in gene expression. The major classes of cell surface receptors are:

1. G Protein-Coupled Receptors:

The largest family of cell surface receptors transmit signals to intracellular targets via the intermediary action of guanine nucleotide-binding proteins called G proteins. More than a thousand such G protein-coupled receptors have been identified, including the receptors for many neurotransmitters, neuropeptides, and peptide hormones. In addition, the G protein-coupled receptor family includes a large number of receptors that are responsible for smell, sight, and taste.

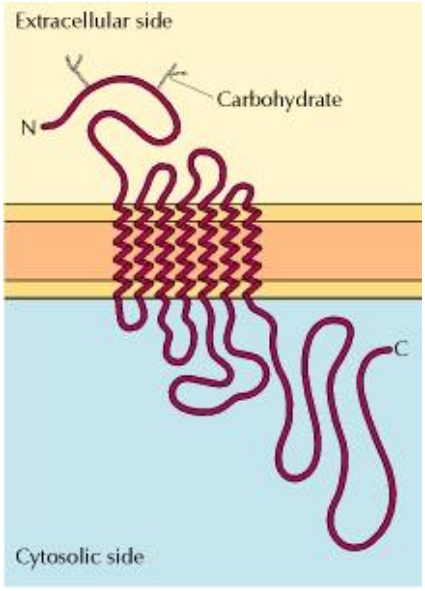

Fig. Structure of a G protein-coupled receptor [The G protein-coupled receptors are characterized by seven transmembrane α helices.]

The G protein-coupled receptors are structurally and functionally related proteins characterized by seven membrane-spanning α helices. The binding of ligands to the extracellular domain of these receptors induces a conformational change that allows the cytosolic domain of the receptor to bind to a G protein associated with

the inner face of the plasma membrane. This interaction activates the G protein, which then dissociates from the receptor and carries the signal to an intracellular target, which may be either an enzyme or an ion channel.

The discovery of G proteins came from studies of hormones (such as epinephrine) that regulate the synthesis of cyclic AMP (cAMP) in their target cells. cAMP is an important second messenger that mediates cellular responses to a variety of hormones. In the 1970s, Martin Rodbell and his colleagues made the key observation that GTP is required for hormonal stimulation of adenylyl cyclase (the enzyme responsible for cAMP formation). This finding led to the discovery that a guanine nucleotide-binding protein (called a G protein) is an intermediary in adenylyl cyclase activation. Since then, an array of G proteins have been found to act as physiological switches that regulate the activities of a variety of intracellular targets in response to extracellular signals.

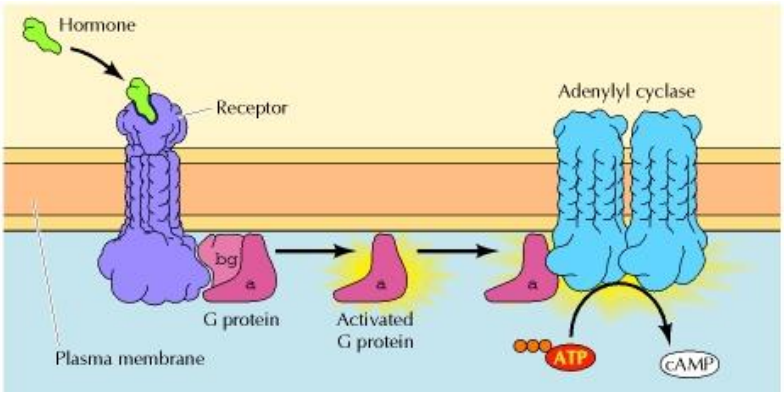

Fig. Hormonal activation of adenylyl cyclase [Binding of hormone promotes the interaction of the receptor with a G protein. The activated G protein α subunit then dissociates from the receptor and stimulates adenylyl cyclase, which catalyzes the conversion of ATP to cAMP.]

G proteins consist of three subunits, designated α, β, and γ. They are frequently called heterotrimeric G proteins to distinguish them from other guanine nucleotide-binding proteins, such as the Ras proteins. The α subunit binds guanine nucleotides, which regulate G protein activity. In the resting state, α is bound to GDP in a complex with β and γ. Hormone binding induces a conformational change in the receptor, such that the cytosolic domain of the receptor interacts with the G protein and stimulates the release of bound GDP and its exchange for GTP. The activated GTP-bound α subunit then dissociates from β and γ, which remain together and function as a βγ complex. Both the active GTP-bound α subunit and the βγ complex then interact with their targets to elicit an intracellular response. The activity of the α subunit is terminated by hydrolysis of the bound GTP, and the inactive α subunit (now with GDP bound) then reassociates with the βγ complex, ready for the cycle to start anew.

Mammalian genomes encode at least 20 different α subunits, 6 β subunits, and 12 γ subunits. Different G proteins associate with different receptors, so this array of G proteins couples receptors to distinct intracellular targets. For example, the G protein associated with the epinephrine receptor is called G_s because its α subunit stimulates adenylyl cyclase. Other G protein α and βγ subunits act instead to inhibit adenylyl cyclase or to regulate the activities of other target enzymes.

In addition to regulating target enzymes, both the α and βγ subunits of some G proteins directly regulate ion channels. A good example is provided by the action of the neurotransmitter acetylcholine on heart muscle, which is distinct from its effects on nerve and skeletal muscle. The acetylcholine receptor on nerve and skeletal muscle cells is a ligand-gated ion channel. Heart muscle cells have a different acetylcholine receptor, which is G protein-coupled. This G protein is designated G_i because its α subunit inhibits adenylyl cyclase. In addition, the G_i βγ subunits act directly to open K^+ channels in the plasma membrane, which has the effect of slowing heart muscle contraction.

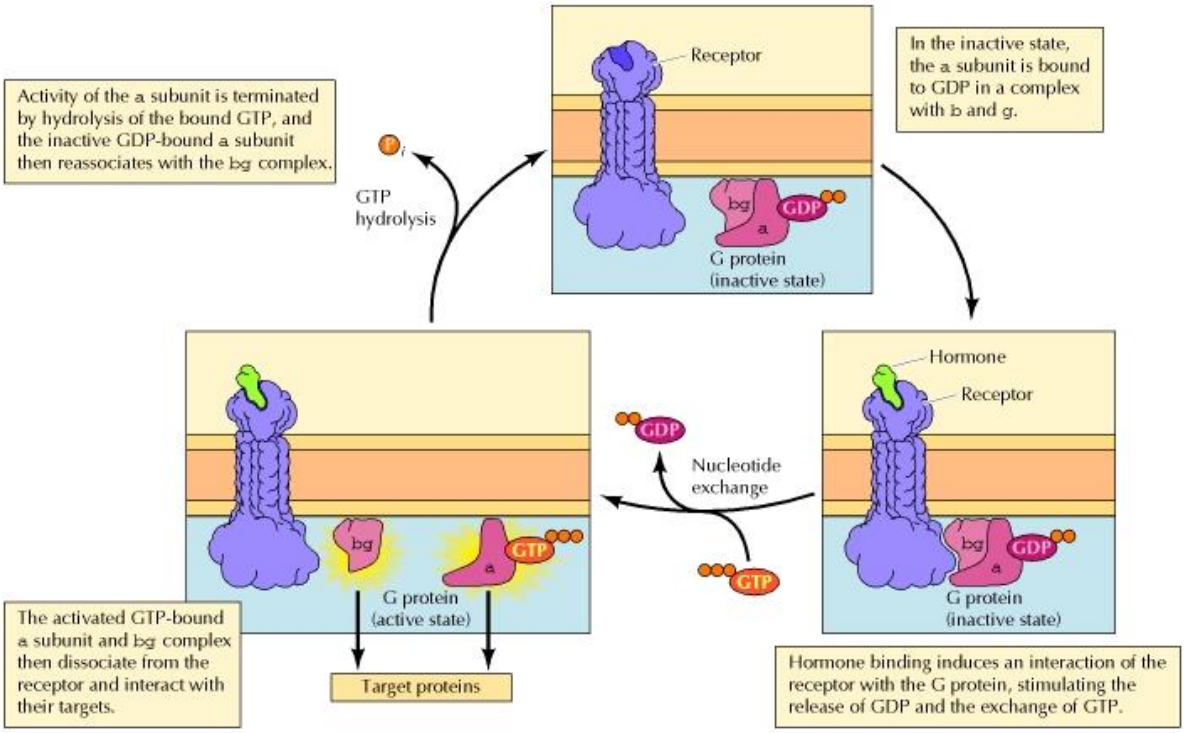

Fig. Regulation of G proteins

2. Receptor Protein-Tyrosine Kinases

In contrast to the G protein-coupled receptors, other cell surface receptors are directly linked to intracellular enzymes. The largest family of such enzyme-linked receptors are the receptor protein-tyrosine kinases, which phosphorylate their substrate proteins on tyrosine residues. This family includes the receptors for most polypeptide growth factors, so protein-tyrosine phosphorylation has been particularly well studied as a signaling mechanism involved in the control of animal cell growth and differentiation. Indeed, the first protein-tyrosine kinase was discovered in 1980 during studies of the oncogenic proteins of animal tumor viruses, in particular Rous sarcoma virus, by Tony Hunter and Bartholomew Sefton. The EGF receptor was then found to function as a protein-tyrosine kinase by Stanley Cohen and his colleagues, clearly establishing protein-tyrosine phosphorylation as a key signaling mechanism in the response of cells to growth factor stimulation.

By now more than 50 receptor protein-tyrosine kinases have been identified, including the receptors for EGF, NGF, PDGF, insulin, and many other growth factors. All these receptors share a common structural organization: an N-terminal extracellular ligand-binding domain, a single transmembrane α helix, and a

cytosolic C-terminal domain with protein-tyrosine kinase activity. Most of the receptor protein-tyrosine kinases consist of single polypeptides, although the insulin receptor and some related receptors are dimers consisting of two pairs of polypeptide chains. The binding of ligands (e.g., growth factors) to the extracellular domains of these receptors activates their cytosolic kinase domains, resulting in phosphorylation of both the receptors themselves and intracellular target proteins that propagate the signal initiated by growth factor binding.

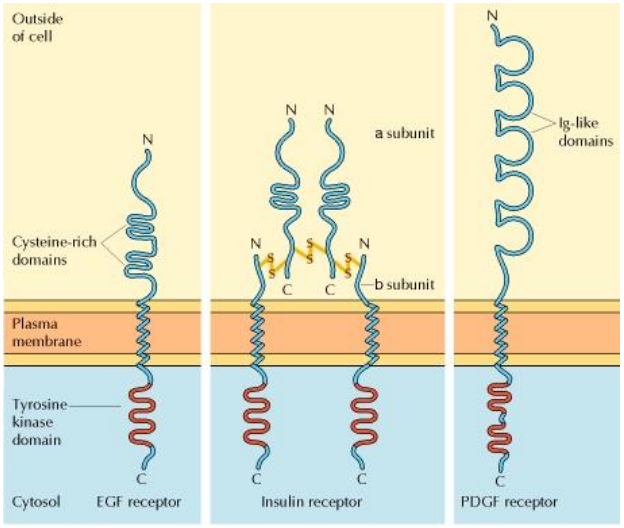

Fig. Organization of receptor protein-tyrosine kinases [Each receptor consists of an N-terminal extracellular ligand-binding domain, a single transmembrane α helix, and a cytosolic C-terminal domain with protein-tyrosine kinase activity. The structures of three distinct subfamilies of receptor protein-tyrosine kinases are shown. The EGF receptor and insulin receptor both have cysteine-rich extracellular domains, whereas the PDGF receptor has immunoglobulin (Ig)-like domains. The PDGF receptor is also noteworthy in that its kinase domain is interrupted by an insert of approximately a hundred amino acids unrelated to those found in most other protein-tyrosine kinase catalytic domains. The insulin receptor is unusual in being a dimer of two pairs of polypeptide chains (designated α and β).]

The first step in signaling from most receptor protein-tyrosine kinases is ligand-induced receptor dimerization. Some growth factors, such as PDGF and NGF, are themselves dimers consisting of two identical polypeptide chains; these growth factors directly induce dimerization by simultaneously binding to two different receptor molecules. Other growth factors (such as EGF) are monomers but have two distinct receptor binding sites that serve to crosslink receptors.

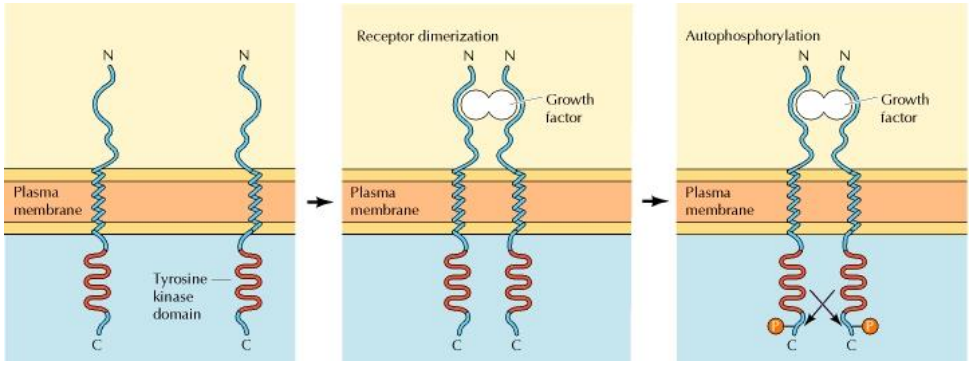

Fig. Dimerization and autophosphorylation of receptor protein-tyrosine kinases [Growth factor binding induces receptor dimerization, which results in receptor autophosphorylation as the two polypeptide chains phosphorylate one another.]

Ligand-induced dimerization then leads to **autophosphorylation** of the receptor as the dimerized polypeptide chains cross-phosphorylate one another. Such autophosphorylation plays two key roles in signaling from these receptors. First, phosphorylation of tyrosine residues within the catalytic domain may play a regulatory role by increasing receptor protein kinase activity. Second, phosphorylation of tyrosine residues outside of the catalytic domain creates specific binding sites for additional proteins that transmit intracellular signals downstream of the activated receptors.

The association of these downstream signaling molecules with receptor protein-tyrosine kinases is mediated by protein domains that bind to specific phosphotyrosine-containing peptides. The best-characterized of these domains are called SH2 domains (for *S*rc *h*omology *2*) because they were first recognized in protein-tyrosine kinases related to Src, the oncogenic protein of Rous sarcoma virus. SH2 domains consist of approximately a hundred amino acids and bind to specific short peptide sequences containing phosphotyrosine residues. The resulting association of SH2-containing proteins with activated receptor protein-tyrosine kinases can have several effects: It localizes the SH2-containing proteins to the plasma membrane, leads to their association with other proteins, promotes their phosphorylation, and stimulates their enzymatic activities. The association of these proteins with autophosphorylated receptors thus represents the first step in the intracellular transmission of signals initiated by the binding of growth factors to the cell surface.

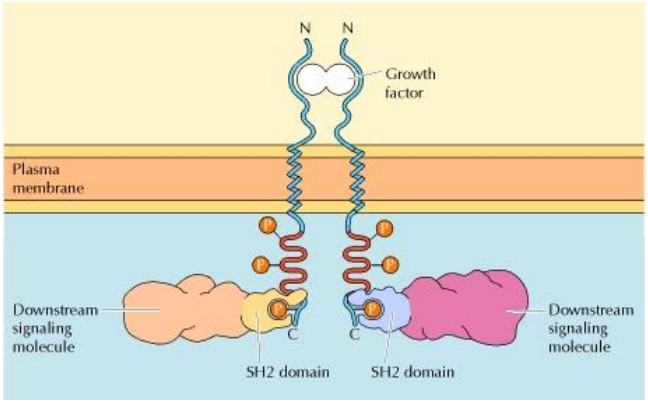

Fig. Association of downstream signaling molecules with receptor protein-tyrosine kinases [SH2 domains bind to specific phosphotyrosine-containing peptides of the activated receptors.]

3. Cytokine Receptors and Nonreceptor Protein-Tyrosine Kinases:

Rather than possessing intrinsic enzymatic activity, many receptors act by stimulating intracellular protein-tyrosine kinases with which they are noncovalently associated. This family of receptors (called the **cytokine receptor superfamily**) includes the receptors for most cytokines (e.g., interleukin-2 and erythropoietin) and for some polypeptide hormones (e.g., growth hormone). Like receptor protein-tyrosine kinases, the cytokine receptors contain N-terminal extracellular ligand-binding domains, single transmembrane α helices, and C-terminal cytosolic domains. However, the cytosolic domains of the cytokine receptors are devoid of any known catalytic activity. Instead, the cytokine receptors function in association with nonreceptor protein-tyrosine kinases, which are activated as a result of ligand binding.

The first step in signaling from cytokine receptors is thought to be ligand-induced receptor dimerization and cross-phosphorylation of the associated nonreceptor protein-tyrosine kinases. These activated kinases then phosphorylate the receptor, providing phosphotyrosine-binding sites for the recruitment of downstream signaling molecules that contain SH2 domains. Combinations of cytokine receptors plus associated nonreceptor protein-tyrosine kinases thus function analogously to the receptor protein-tyrosine kinases discussed in the previous section.

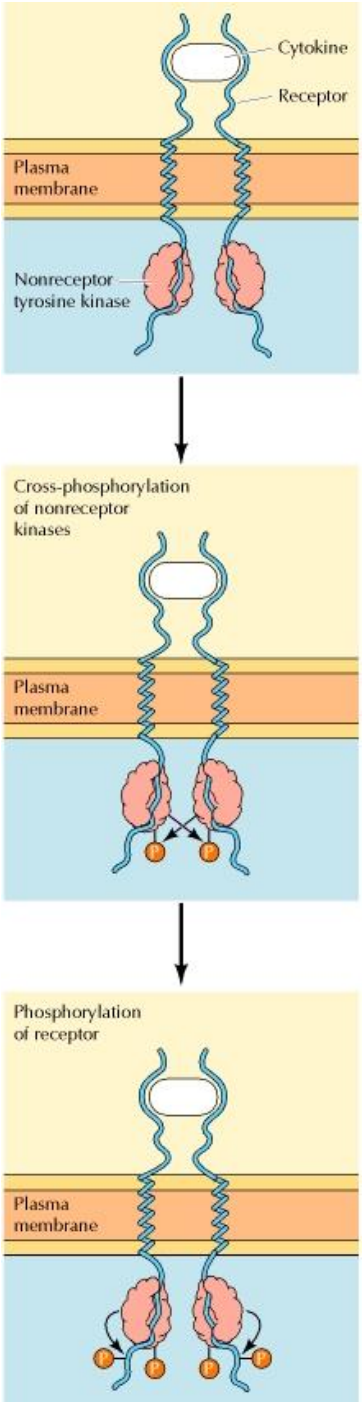

Fig. Signaling from cytokine receptors [Ligand binding induces receptor dimerization and leads to the activation of associated nonreceptor protein-tyrosine kinases as a result of cross-phosphorylation. The activated kinases then phosphorylate tyrosine residues of the receptor, creating phosphotyrosine-binding sites for downstream signaling molecules.]

The nonreceptor protein-tyrosine kinases associated with the cytokine receptors fall into two major families. Many of these kinases are members of the Src family, which consists of Src and eight closely related proteins. As already noted, Src was initially identified as the oncogenic protein of Rous sarcoma virus and was the first protein shown to possess protein-tyrosine kinase activity, so it has played a pivotal role in experiments leading to our current understanding of cell signaling. In addition to Src family members, the cytokine receptors are associated with nonreceptor protein-tyrosine kinases belonging to the Janus kinase, or JAK, family. Members of the JAK family appear to be universally required for signaling from cytokine receptors, indicating that JAK family kinases play a critical role in coupling these receptors to the tyrosine phosphorylation of intracellular targets. In contrast, members of the Src family play key roles in signaling from antigen receptors on B and T lymphocytes but do not appear to be required for signaling from most cytokine receptors.

4. Receptors Linked to Other Enzymatic Activities:

Although the vast majority of enzyme-linked receptors stimulate protein-tyrosine phosphorylation, some receptors are associated with other enzymatic activities. These receptors include protein-tyrosine phosphatases, protein-serine/threonine kinases, and guanylyl cyclases. The functions of most of these receptors are less well understood than those of either the G protein-coupled receptors or the receptors associated with protein-tyrosine kinase activity.

Protein-tyrosine phosphatases remove phosphate groups from phosphotyrosine residues, thus acting to counterbalance the effects of protein-tyrosine kinases. In many cases, protein-tyrosine phosphatases play negative regulatory roles in cell signaling pathways by terminating the signals initiated by protein-tyrosine phosphorylation. However, some protein-tyrosine phosphatases are cell surface receptors whose enzymatic activities play a positive role in cell signaling. A good example is provided by a receptor called CD45, which is expressed on the surface of T and B lymphocytes. Following antigen stimulation, CD45 is thought to dephosphorylate a specific phosphotyrosine that inhibits the enzymatic activity of Src family members. Thus, the CD45 protein-tyrosine phosphatase acts (somewhat paradoxically) to stimulate nonreceptor protein-tyrosine kinases.

The receptors for transforming growth factor β (TGF-β) and related polypeptides are protein kinases that phosphorylate serine or threonine, rather than tyrosine, residues on their substrate proteins. TGF-β is the prototype of a family of polypeptide growth factors that control proliferation and differentiation of a variety of cell types, generally inhibiting proliferation of their target cells. The cloning of the first receptor for a member of the TGF-β family in 1991 revealed that it is the prototype of a unique receptor family with a cytosolic protein-serine/threonine kinase domain. Since then, receptors for additional TGF-β family members have similarly been found to be protein-serine/threonine kinases. The binding of ligand to these receptors results in the association of two distinct polypeptide chains, which are encoded by different members of the TGF-β receptor family, to form heterodimers in which the receptor kinases cross-phosphorylate one another. The

activated TGF-β receptors then phosphorylate members of a family of transcription factors called SMADs, which translocate to the nucleus and stimulate expression of target genes.

Some peptide ligands bind to receptors whose cytosolic domains are guanylyl cyclases, which catalyze formation of cyclic GMP. As discussed earlier, nitric oxide also acts by stimulating guanylyl cyclase, but the target of nitric oxide is an intracellular enzyme rather than a transmembrane receptor. The receptor guanylyl cyclases have an extracellular ligand-binding domain, a single transmembrane α helix, and a cytosolic domain with catalytic activity. Ligand binding stimulates cyclase activity, leading to the formation of cyclic GMP—a second messenger whose intracellular effects are discussed in the next section of this chapter.

Other receptors bind to cytoplasmic proteins with additional biochemical activities. For example, the cytokine tumor necrosis factor (TNF) induces cell death, perhaps (as discussed later in this chapter) as a way of eliminating damaged or unwanted cells from tissues. The receptors for TNF and related death-signaling molecules are associated with specific proteases, which are activated in response to ligand binding. Activation of these receptor-associated proteases triggers the activation of additional downstream proteases, ultimately leading to degradation of a variety of intracellular proteins and death of the cell.

Courtesy: The Cell: A Molecular Approach (Geoffrey M Cooper.)