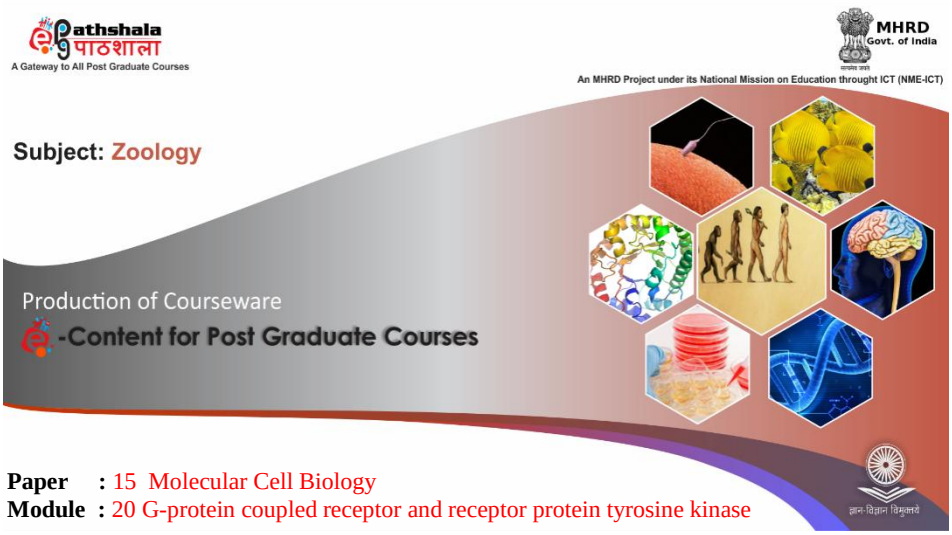

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ZOOLOGY

Molecular Cell Biology

G-protein coupled receptor and receptor protein tyrosine kinase

Description of Module	
Subject Name	ZOOLOGY
Paper Name	Zool 015: Molecular Cell Biology
Module Name/Title	Cellular energetic and regulatory mechanisms
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6. Summary

1. Learning objectives

- Purpose of cell signaling.
- How cells communicate with each other.
- Cellular components involved in cell signaling.
- Significance of G-protein coupled receptor and receptor tyrosine kinase.

2. Introduction

The process by which cells communicate with each other through extracellular messenger molecules is called cell signaling. Cell signaling can be of three types (Fig. 1.):

- Autocrine signaling
- Paracrine signaling
- Endocrine signaling

2.1. Autocrinesignaling

The cell producing the messenger, expresses receptors on its surface as well which can respond to that messenger. Therefore, the cell that releases the message either stimulates or inhibitsthem.

2.2. Paracrine signaling

The secreted messenger molecules travel only short distances through the extracellular space to the nearby cells. In Paracrine signaling the messenger molecules secreted travels only for a short distance.

2.3. Endocrine signaling

The secreted messenger molecules reach the target cells through the bloodstream. Endocrine messengers are also known as hormones, and they exclusively act on the target cells situated at distant place in the body.

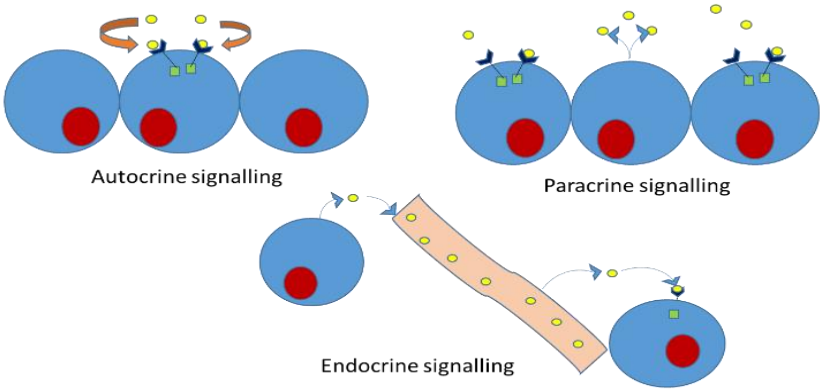

Fig.1: Different modes of signaling
Source:Author

3. Ligand, Target cell and Receptors

Cell signaling is started by the release of a **messenger** molecule also known as **ligand** by a cell that is involved in sending messages to the **target cell** by binding to the specific **receptors**.**Ligand,**messenger molecules that bind to the receptor include steroids and neurotransmitters, small, soluble protein hormones and huge glycoproteins.Cells tend to respond to a specific extracellular message only if they express receptors that exclusively recognize and bind to that messenger molecule. The cells that harbour those specific receptors are called as target cells. The most studied receptors are

- G-protein coupled receptor (GPCR)
- Receptor tyrosine kinase (RTK)

4. G-Protein coupled receptor

Heterotrimeric G proteins coupled receptors were discovered, purified, and characterized by Martin Rodbell and Alfred Gilman. G protein-coupled receptors (GPCRs) are named so as they are involved with G proteins. GPCR superfamily members are also known as seven transmembrane (7TM) receptors as they are composed of seven transmembrane helices. Fig.2.

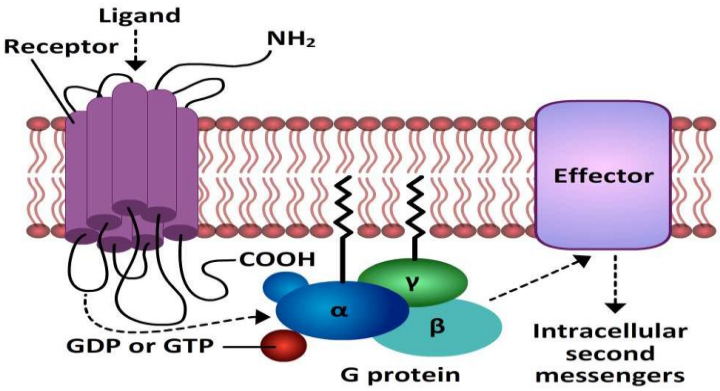

Fig.2: Structure of GPCR
Source: Departmental Artist

4.1. Ligands for GPCR

Encoded by animal genomes, GPCRs are the single largest family of proteins. A diverse group of the natural ligands that bind to GPCRs include:

- Hormones
- Neurotransmitters
- Opium derivatives
- Chemoattractants
- Odorants
- Tastants
- Photons

4.2. Receptors for GPCR

G protein-coupled receptor's amino-terminus end present outside the cell. Connected by loops are the seven helices traversing the plasma membrane, carboxyl-terminus end located inside the cell. Three loops present outside the cell, together, form the ligand-binding pocket, whose structurevaries among different GPCRs. Three loops are present on the cytoplasmic side; act as binding sites for signaling proteins present intracellularly. Heterotrimeric proteins consist of three subunits, α, β, and γ. The guanine nucleotide-binding site is present on the G_α subunit.G protein is "on" when it's α subunit is attached to GTP. G_α subunits can be turns off by hydrolysis of GTP to GDP and Pi. As a result, a conformational change leads to a decrease in affinity for the effector and rise in the affinity for βγ subunit.After hydrolysis of GTP, the

G_α subunit will part from the effector and rejoin βγ subunit to reform the inactive heterotrimeric G protein. These are turned on by the involvement with the activated receptor and turned off by the hydrolysis of bound GTP after a certain period of time. When active, G_α subunits can activate the downstream effectors. Heterotrimeric G proteins are of four types depending on the G_α subunits and the effector molecule to which they combine. The specific response caused by an active GPCR relies on the type of G protein with which it interacts.

- **Gs** family members combine receptors to adenylyl cyclase. Adenylyl cyclase in turn is activated by GTP-bound Gs subunits.
- **G_α** subunits of Gq family members activate PLC_β. PLC_β hydrolyzes phosphatidylinositol bisphosphate, generating inositol trisphosphate and diacylglycerol.
- **Gi** family act by inhibiting adenylyl cyclase after activation.
- Excessive cell proliferation and malignant transformation activate **G12/13**members.

4.3. Steps involved in cell signaling by GPCR

1. Ligand binds to the specific GPCR and activates it.
2. Following activation, GDP is replaced by GTP, resulting in a conformational change in the G_α subunit leading to dissociation of the trimeric complex.
3. Each dissociated G subunit (with GTP attached) is free to activate an effector protein, such as adenylyl cyclase.
4. Activation of the effector leads to the production of the second messenger cAMP, phospholipase C-β and cyclic GMP phosphodiesterase.
5. Second messengers, further activate one or more cellular signaling proteins.
6. After dissociation from the G_α subunit, the βγ complex can couple to a number of effectors e.g. PLC_β, K^+, Ca^{2+} ion channels, and adenylyl cyclase (Fig. 3).

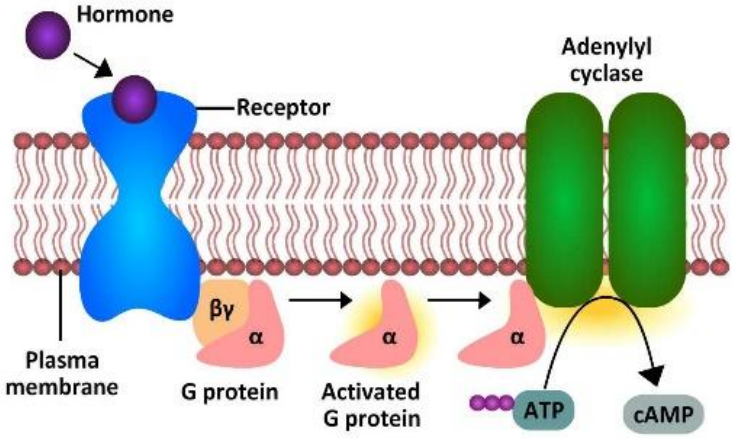

Fig.3: Steps involved in the signaling by GPCR
Source: Departmental artist

Regulation of GPCR

To discourage overstimulation, receptors need to be blocked from continuing to activate G proteins. To retainthe sensitivity in response to future stimuli, the G protein, receptor, and the effector should be returned to their original inactive state. Desensitization, the process which prevent active receptors from turning on additional G proteins takes place in two steps.

- Cytoplasmic domain of activated GPCR gets phosphorylated by a special kinase, called G protein-coupled receptor kinase (GRK). GRKs are a small family of serine-threonine protein kinases which specifically recognize activated GPCR and phosphorylate it.
- Arrestins bind to GPCR. Arrestins form a small family of proteins which upon binding to GPCRs compete for binding with heterotrimeric G proteins. As a result, arrestin binding does not allow further activation of more G proteins. Fig.4.

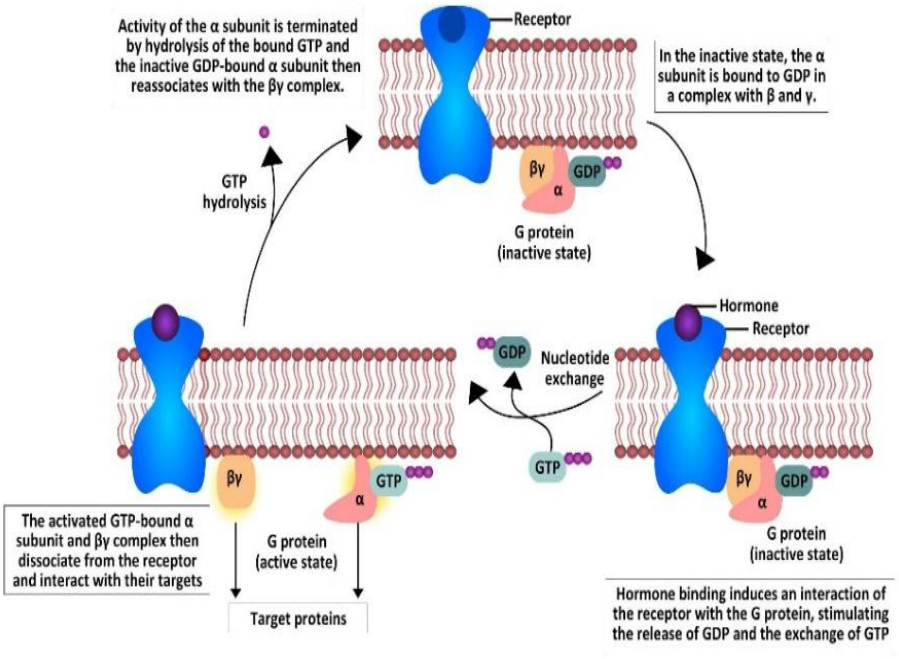

Fig.4: Regulation of cell signaling by GPCR
Source: Departmental artist

4.4. Types of G-Protein coupled receptor

There are four types of G-Protein coupled receptors:

- G Protein–Coupled receptors that activate or inhibit adenylyl cyclase:
- G Protein–Coupled Receptors that Regulate Ion Channels
- G Protein–Coupled Receptors that Activate Phospholipase C
- G Protein–Coupled Receptors that activate Gene Transcription

4.4.1. G Protein–Coupled receptors that activate or inhibit adenylyl cyclase

All epinephrine receptors (G protein– coupled receptors) combine with different types of G proteins. Two subtypes of α adrenergic receptors (α 1 and α 2) couple to a stimulatory G protein (Gs) which activate the enzyme adenylyl cyclase(membrane-bound). Upon activation, adenylyl cyclasecatalyzes the synthesis of cAMP (the second messenger). Two subtypes of adrenergic receptors (α-1 and α-2) couples to different G proteins. The α1-adrenergic receptor combines with a Gi protein that inhibits adenylyl cyclase. This effector enzyme combines with β-adrenergic receptors. The Gq protein-α-2-adrenergic receptor complex activates

another effector enzyme which generates different second messengers (adrenergic receptors. Activation of adenylyl cyclase, and in turn the cAMP level, is proportional to total concentration of Gs·GTP complex arising from binding of both hormones and their respective receptors.

4.4.2. G Protein–Coupled receptors that regulate ion channels

4.4.2.1. Neurotransmitter receptors

Neurotransmitter receptors are the example for this category of G protein–coupled receptors. The effector protein for these is Na^+ or K^+ channel. Upon binding of neurotransmitter to these receptors enables the associated ion channel to open or close, leading to changes in the membrane potential.

4.4.2.2. Cardiac muscarinic acetylcholine receptor

The cardiac muscarinic acetylcholine receptor is also a GPCR whose effector is a K^+ channel. Upon receptor activation it causes the release of G subunit, which in turn opens K^+ channels. The ultimate effect is the hyperpolarization of cell membrane and the rate of heart muscle contraction slows down.

4.4.2.3. Photoreceptors in the eye

Transducin (Gt) is the trimeric G protein which is coupled to rhodopsin, found only in the rod cell and are activated by light.Light-absorbing pigment 11-cis-retinal is covalently bound to the rod cell. After absorption of photon, the retinal terminal of rhodopsin is rapidly converted to all-trans isomer, leading to a conformational transformation in the opsin portion that induce its activation. Rhodopsin, the photosensitive GPCR in rod cells, consist of the opsin protein associated to 11-cis-retinal. Upon light induction isomerization of the 11-cis-retinal terminal generates activated opsin.Opsins then activates the coupled trimeric G protein transducin (Gt) by catalyzing exchange of free GTP for bound GDP on the Gt subunit.The effector protein catalysed by Gt·GTP is cGMPphosphodiesterase. Reduction in the cGMP level by this enzyme leads to closing of cGMP-gated Na^+/Ca^{2+} channels, hyperpolarization of the membrane, and decreased release of neurotransmitter. Like other G proteins, binding of

GTP to Gt causes conformational changes in the protein that disrupt its molecular interactions with G and enable Gt-GTP complex to bind to its downstream effector. Phosphorylation of light-activated opsin by rhodopsin kinase and further binding of arrestin to phosphorylated opsin disable them to activate transducin.

4.4.3. G Protein–coupled receptors that activate phospholipase C

Activation of some GPCRs and other cell-surface receptors leads to activation of phospholipase C, which generates two second messengers:

- diffusible IP3 (inositol triphosphate) and
- Membrane-bound DAG (diacylglycerol).

IP3 induces opening of IP3-gated Ca^{2+} channels present in the endoplasmic reticulum and increase the level of cytosolic free $Ca^{2+.}$ Due to elevated cytosolic Ca^{2+}, protein kinase C is introduced to the plasma membrane, where it can be activated by DAG. The Ca^{2+}/calmodulin complex regulates the activity of many different proteins, including

- cAMPphosphodiesterase,
- nitric oxide synthase, and
- Proteinkinases or phosphatases that control the activity of various transcription factors.

4.4.4. G protein–coupled receptors that activate gene transcription

Most pathways stimulated by G protein–coupled receptors are included in this category.The **tubby gene** is expressed mainly in some areas of the brain and is involved in the control of eating behaviour. Stimulation of phospholipase C by receptors linked to Go or Gq proteins releases Tubby transcription factor which binds to PIP2 (Phosphatidyl-bis-phosphate) attached to the plasma membrane in the resting cells.Activation of protein kinase A (PKA) through signal leads to phosphorylation of CREB protein.CREB protein along with the CBP/300 co-activator induces transcription of several target genes. The GPCR-arrestin complex activates many cytosolic kinases, starting cascades which lead to transcriptional activation of several genes that control the cell growth.

5. Receptor tyrosine kinase

Other signaling pathway control gene activity and involve receptor tyrosine kinases (Fig. 5). They exhibit their intrinsic protein tyrosine kinase activity in the cytosolic domains. The ligands/messengers for RTKs can be soluble or membrane-bound peptide or protein hormones that includes:

- Nerve growth factor (NGF)
- Insulin
- Fibroblast growth factor (FGF)
- Platelet-derived growth factor (PDGF)
- Epidermal growth factor (EGF)

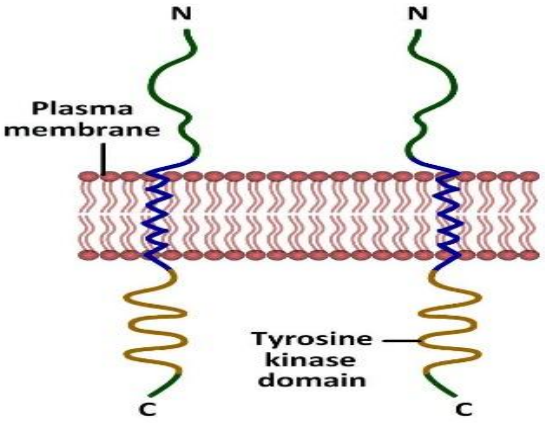

Fig.5: Receptortyrosine kinase
Source: Departmental artist

Activation of an RTK by its ligand commences its tyrosine kinase activity, which further induce the Ras–MAP kinase pathway with many other signal-transduction pathways. RTK signaling pathways has an array of functions that include

- Immune related function
- Cell survival promotion
- Cellular metabolism modulation
- Regulation of the process of cell proliferation and differentiation

5.1. Steps involved in the process of signal transduction by receptor tyrosine kinase

- Receptor dimerization

- Autophosphorylation
- Protein kinase activation
- Phosphotyrosine-dependent protein–protein interactions
- Activation of downstream signaling pathways
- Termination of response: internalization of the receptor

5.1.1. Receptor dimerization

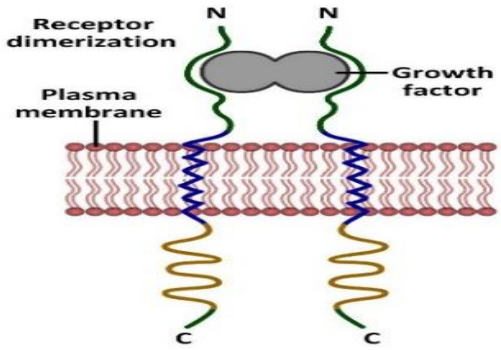

Fig.6: Dimerization of Receptor tyrosine kinase
Source: Departmental artist

Two mechanisms for receptor dimerizationhave been reported so far:

- ligand-mediated dimerization and
- receptor-mediated dimerization

5.1.1.1. Ligand-mediated dimerization

All RTKs has an extracellular domain that contains

- a ligand-binding site
- a single hydrophobic transmembrane helix, and
- a cytosolic domain that includes a region with protein tyrosine kinase activity.

These RTKs are monomeric. Ligand binding with the extracellular domain leads to formation of receptor dimers. Fig.7.

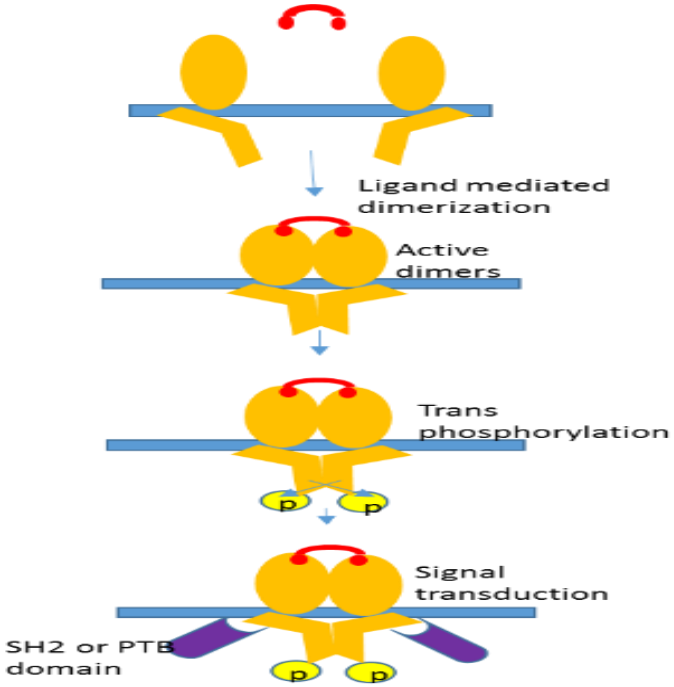

Fig.7: Ligand mediated receptor dimerization
Source:Author

5.1.1.2. Receptor-mediated dimerization

Many ligands that are monomers e.g. FGF, bind closely to heparin sulphate (a negatively charged polysaccharide molecule of the extracellular matrix).This type of association encourages ligand binding with the monomeric receptor and ultimately leads to the formation of a receptor-ligand complex (dimer). The ligands for some RTKs are dimer thus their binding brings two monomeric receptors together directly(Fig.8).

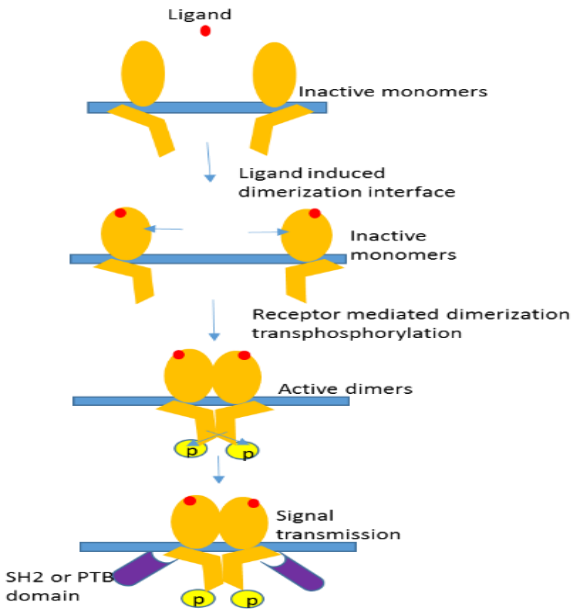

Fig.8: Ligand mediated receptor dimerization
Source: Author

5.1.2. Autophosphorylation

For most RTKs juxtapositioning of two protein-tyrosine kinase domains occurs on the cytoplasmic side of the plasma membrane due to receptor dimerization.Two kinase domains in close proximity lead to *trans-autophosphorylation*, wherein the protein kinase property of one receptor in the dimer phosphorylates tyrosine residues present in the cytoplasmic domain of another receptor of the dimer, and vice versa.

5.1.3. Outcome of Autophosphorylation

Autophosphorylation sites present on RTKs can implement two different functions:

- They regulate receptor's kinase activity and
- Act as binding sites for signaling molecules in the cytoplasm (Fig. 9).

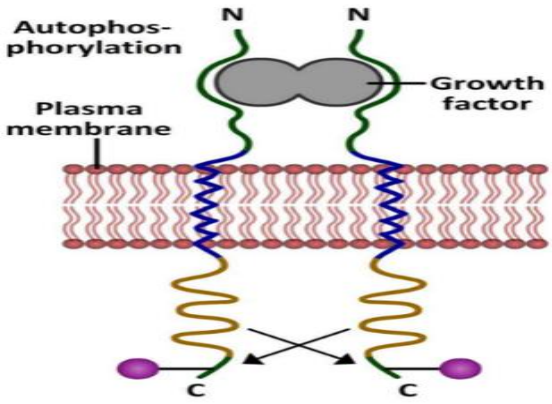

Fig.9: Receptor tyrosine kinase showing autophosphorylation.
Source: Departmental artist

5.1.4. Protein Kinase Activation

In the non-activated state, there is very low intrinsic kinase activity of an RTK.However, in the dimeric receptor (activated state), the kinase activity in one subunit may phosphorylate one or more tyrosine residues at the activation end in the proximity of the catalytic site in the other subunit. This leads to a conformational change that allows:

- Binding of ATP in certain receptors (e.g., insulin receptor) or
- Binding of protein substrates in other receptors (e.g., FGF receptor).

The resulting enhanced kinase activity then phosphorylates other sites in the cytosolic domain of the receptor. Autophosphorylation sites on RTKs can carry out two different functions:

- They can regulate the receptor's kinase activity or
- Theycan serve as binding sites for signaling molecules present in the cytoplasm.

5.1.5. Phosphotyrosine-Dependent Protein–Protein Interactions

In activated RTKs, phosphotyrosine residues serve as docking sites for proteins that leads to downstream signal transduction. Several phosphotyrosine residuespresent in activated RTKs involve with adapter proteins, (a small proteins that consist of SH2, PTB, or SH3 domains) (Fig.10).

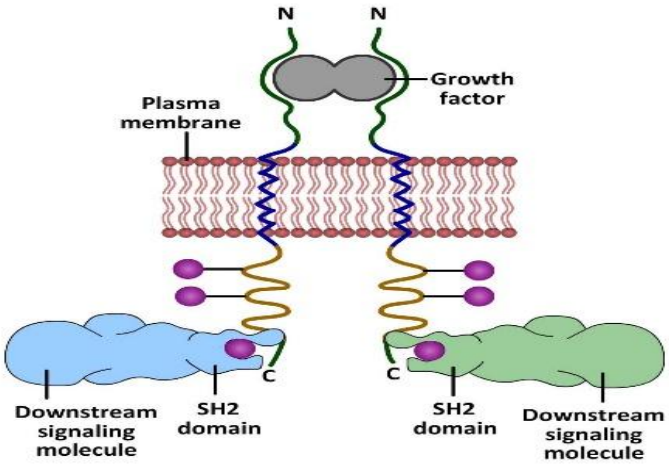

Fig.10: Phosphotyrosine-dependent protein–protein interactions in receptor tyrosine kinase
Source: Departmental artist

Adaptor proteins act as linkers, enabling two or more signaling proteins to join together as a component of signaling complex. Adaptor proteins consist of a SH2 domain and one or more protein–protein interaction domains e.g., the adaptor protein Grb2 consist of one SH2 and two SH3 (Src-homology 3) domains,

- SH3 domains bind to motifs that are proline-rich sequence.
- The SH3 domains of Grb2 bind to other proteins, e.g. Sos and Gab constitutively.
- Within TyrX-Asn motif the SH2 domain binds to tyrosine residues that are phosphorylated.
- Thus, tyrosine phosphorylation of the Tyr-X-Asn motif on an RTK leads to transfer of Grb2-Sos or Grb2-Gab from the cytosol to a receptor, that is present in the plasma membrane.

Docking proteins, such as IRS, provide certain receptors with surplus tyrosine phosphorylation sites. Docking proteins contain

- Either a PTB domain/ an SH2 domain and
- Anumber of tyrosine phosphorylation sites

Binding of an extracellular ligand to the receptor leads to autophosphorylation of the receptor, which supply a binding site for the PTB or SH2 domain of the docking protein. Once bound together, the receptor phosphorylates tyrosine residues present on the docking protein. These phosphorylation sites then act as binding sites for additional signaling molecules. Docking proteins provide diversity to the signaling process, because the ability of the receptor to turn on signaling molecules can change with the docking proteins that are expressed in a particular cell. Transcription factors that belong to the STAT family play an important role in the function of the immune system. STATs consist of SH2 domain along with a tyrosine phosphorylation site that can act as a binding site for the SH2 domain of another STAT molecule.Tyrosine phosphorylation of STAT SH2 binding sites located within a dimerized receptor leads to the involvement of STAT proteins. Being associated with the receptor complex, tyrosine residues in these STAT proteins get phosphorylated. Thus, due to the interaction between the phosphorylated tyrosine residue on one STAT protein and the SH2 domain on the second STAT protein, and vice versa, these transcription factors form dimers.Dimers then move to the nucleus wherein they stimulate the transcription of certain specific genes involved in an immune response.

5.1.6. Activation of Downstream Signaling Pathways

Several groups of signaling proteins that can interact with activated RTKs, include

- Adaptor proteins
- Docking proteins
- Transcription factors
- Enzymes

5.1.6.1. Adaptor proteins

Adaptor proteins act as linkers which enable two or more signaling proteins to join together as a part of the signaling complex. Adaptor proteins consist of

- SH2 domain and
- Additional one or more protein–protein interaction domains.

e.g., the adaptor protein Grb2 contains one SH2 and two SH3 (Src-homology 3) domains. SH3 domains associate with proline-rich sequence motifs. The SH3 domains of Grb2 bind constitutively to other proteins e.g. Sos and Gab. Within a Tyr-X-Asn motif the SH2 domain binds to phosphorylated tyrosine residues. As a result, tyrosine phosphorylation of the Tyr-X-Asn motif on an RTK leads to transfer of Grb2-Sos or Grb2-Gab from the cytosol to a receptor, which is present at the plasma membrane (Fig. 11).

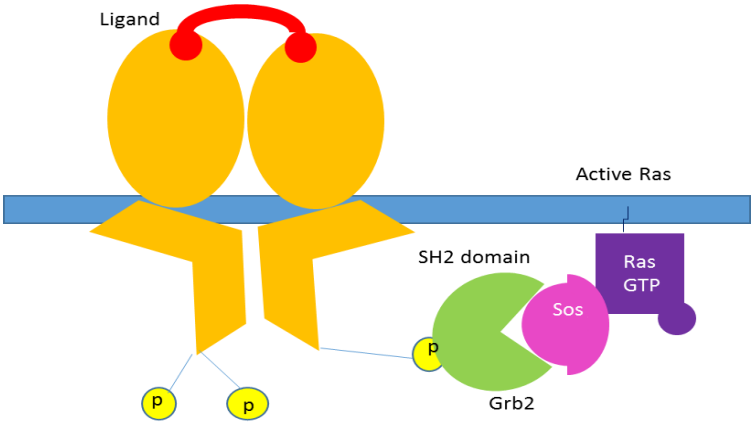

Fig.11: Adaptor proteins
Source: Author

5.1.6.2. Docking proteins

Docking proteins e.g. IRS, provide some receptors with additional tyrosine phosphorylation sites. Docking proteins consist of

- a PTB domain/an SH2 domain and
- Several tyrosine phosphorylation sites.

As an extracellular ligand bind to a receptor it leads to autophosphorylation of the receptor, which serve as a binding site for the PTB or SH2 domain of the docking protein. Upon binding, the receptor phosphorylates tyrosine residues located on the docking protein.These phosphorylation sites in turn act as binding sites for more signaling molecules. Docking proteins provide diversity to the signaling process, as the ability of the receptor to turn on signaling molecules can change with the docking proteins that are being expressed in a particular type of cell(Fig.12).

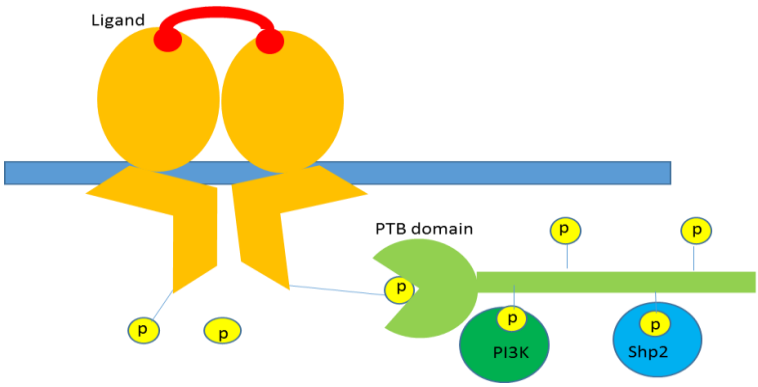

Fig.12: Docking proteins
Source: Author

5.1.6.3. Transcription factors

Transcription factors which belong to the STAT family play an important role in the functioning of the immune system. STATs consist of an SH2 domain along with a tyrosine phosphorylation site that acts as a binding site for the SH2 domain of another STAT molecule. Tyrosine phosphorylation of STAT SH2 binding sites located within a dimerized receptor leads to the involvement of STAT proteins. After association with the receptor complex, tyrosine residues on STAT proteins get phosphorylated. Interaction between the phosphorylated tyrosine residue on one STAT protein and the SH2 domain on another STAT protein, these transcription factors form dimers. These dimers translocate to the nucleus tostimulate the transcription of particular genes (Fig.13).

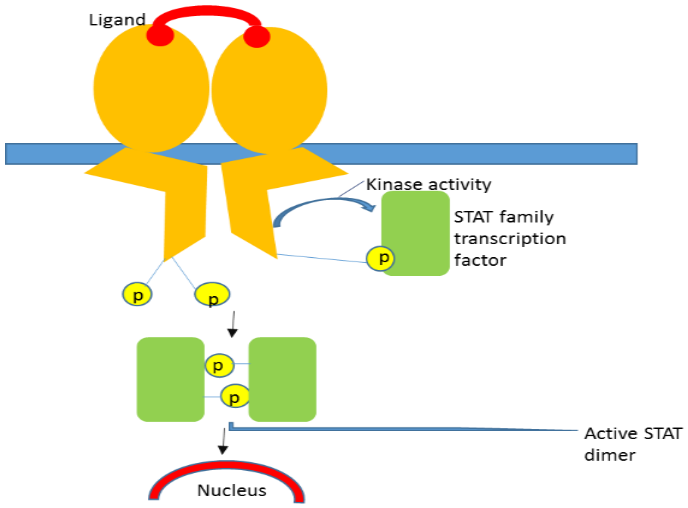

Fig.13: Transcription factors
Source: Author

5.1.6.4. Enzymes

Signaling enzymes include

- protein kinases,
- protein phosphatases,
- lipid kinases,
- phospholipases, and
- GTPase activating proteins.

When linked with SH2 domains, these enzymes joins together with activated RTKs and are turned on directly or indirectly as a result of this association (Fig.14). Three general pathways have been identified through which these enzymes can be activated after their association with a receptor.

- Enzymes can be activated as a result of translocation to the membrane, which locate them in close proximity to their substrates.

- Enzymes can also be activated by an allosteric mechanism. The binding to phosphotyrosine leads to a conformational change in the SH2 domain which causes a conformational change in the catalytic domain, leading to a change in the catalytic activity.

- Enzymes canalso be regulated directly through phosphorylation. Signaling proteins that assemble with activated RTKs initiate cascades of events which lead to the biochemical changes that is required in order to respond to the presence of extracellular messenger molecules

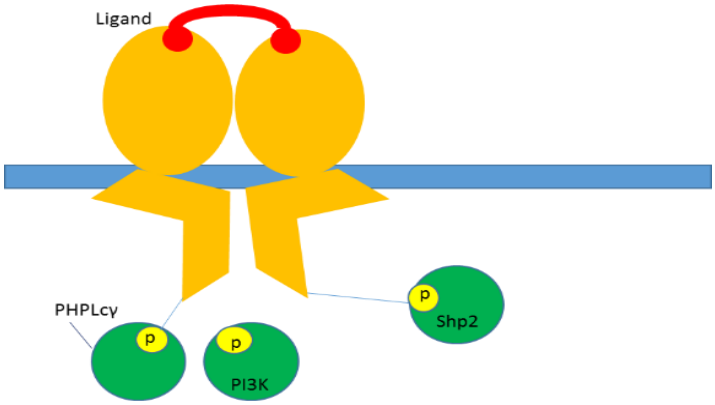

Fig.14: Enzymes
Source:Author

5.1.7. Ending the response: internalization of the receptor

Signal transduction by RTKs is generally terminated by the internalization of the receptor. Exactly what causes the internalization of the receptor is still not known. One such process incorporate the protein Cbl. Association of the Cbl complex to activated receptors leads to receptor ubiquitination and finally internalization.

6. Summary

Many different signaling molecules are secreted by one cell and bind to the receptors expressed by the target cell. Cell-cell signaling is divided into three general categories (endocrine, paracrine, and autocrinesignaling) depending upon the distance over which signals are transmitted. G Protein-Coupled Receptors, the largest family of cell surface receptors, includes the receptors for many hormones and neurotransmitters. They can transmit signals to the intracellular targets through the intermediary action of G proteins. There are four kinds of G-Protein Coupled receptors: G Protein-coupled receptors that activate or inhibit adenylyl cyclase, G Protein-coupled receptors that regulate ion channels, G Protein-coupled receptors that activate phospholipase C and G Protein-coupled receptors that activate gene transcription. Cyclic AMP is a crucial second messenger present in the animal cells in response to a variety of hormones and odorants. Cyclic GMP is another important second messenger in animal cells.

Another class of Receptorsis Receptor Tyrosine Kinases which regulate cell proliferation and differentiation, promotion of cell survival, modulation of cellular metabolism and Immune function. Every Receptor Tyrosine Kinases consist of an extracellular domain having a ligand-binding site, a single hydrophobic transmembrane helix, and a cytosolic domain which includes a region with protein tyrosine kinase activity. Most of the RTKs are monomeric, and binding of the ligand to the extracellular domain leads to the formation of receptor dimers. Through ligand-induced activation RTKstyrosine kinase activity is stimulated which subsequently leads to Phosphotyrosine-dependent protein–protein interactions that finally lead to Activation of downstream signaling Pathways. The response is ended by the internalization of the receptors.