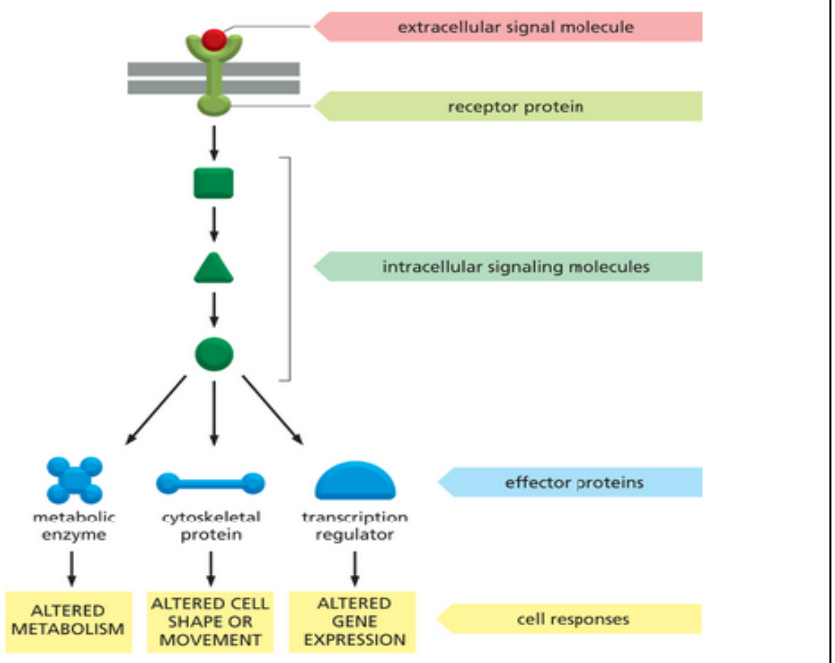
CELL- CELL COMMUNICATION

Cell communication is required for governing the activities of a multicellular organism. These communication mechanisms depend on the extracellular signal molecules produced by the neighboring cells. Organisms have developed specific signals to communicate to the cells to act in a specified way. These are proteins which include intracellular or extracellular signal molecules and cell surface receptors. Among the intracellular signaling proteins, which bind the signal molecules that distribute the signal to appropriate parts of the cell. The intracellular signaling proteins include kinases, Phosphatases, GTP binding proteins and many other proteins with which they interact and alter pathways. Depending on the signals effect, the target proteins can be gene regulatory proteins, Ion channels, components of a metabolic pathway, part of a cytoskeleton etc (figure 5.1).

Figure 5.1: Overview of Intracellular signaling pathways



General principles of cell communication:

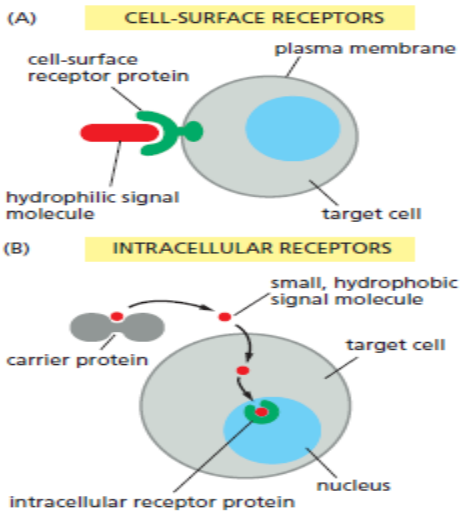
1) Extra cellular signal molecules bind to specific receptors:

Higher animal communicate by means of hundreds of signal molecules. These include proteins, small peptides, amino acids, nucleotides, steroids, retinoids, fatty acid derivatives, dissolved gases like nitric oxide and carbon monoxide. These are secreted by signaling cells into

extracellular space by exocytosis. Most signal molecules are hydrophilic and hence they cannot cross membrane by diffusion, while few small ones do cross by diffusion.

Regardless of the nature of signal molecules, target cells respond by expressing specific binding proteins called receptors. These signals are produced at very low concentrations of ~10^{-6}M, with a high affinity constant $K_\alpha \geq 10^8$ liters per mole. Binding of signal to receptor generates a cascade of intracellular signals that alter the behavior of cells. Few signals are to penetrate the cells and bind to intracellular receptors; such signals are usually small, hydrophobic and can cross plasma membrane (figure 5.2).

Figure 5.2: Extra cellular molecules and types of receptors



2. Forms of intracellular signals: There are four different types of intracellular signaling (Figure 5.3):

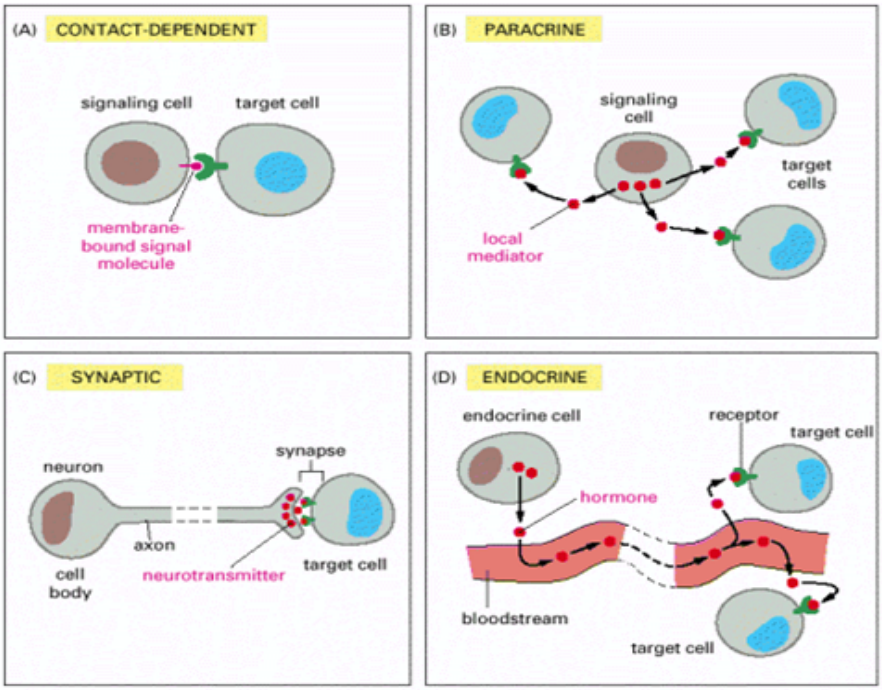
a) Contact dependent signaling: Important during development and in immune responses. The secreted signals molecules are carried far afield to act on distant targets.

b) Paracrine signaling: These affect the cells in the immediate environment and act as local mediators. These act on cells around, i.e., onto their proper target cells and must not diffuse too far. These are taken up immediately by cells, and are also destroyed by extracellular enzymes or enzymes present in the extracellular matrix.

c) Synaptic signaling: These are short range signals and play specific role in cell communication. These are seen in specialized cells and have evolved to communicate with parts of body. Eg: nerve impulse transmission by the neurotransmitter acetylcholine across synaptic space.

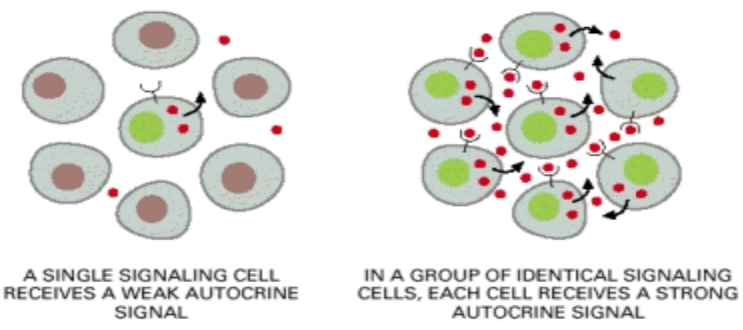
d) Endocrine signals: These are specialized signals that control behavior of the organism. These signals are called hormones or endocrine secretions which travel through blood stream to reach target cells.

Figure 5.3: Forms of Intracellular signaling



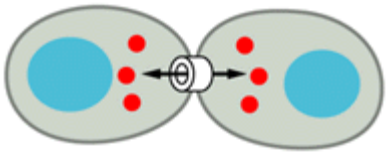
3. Autocrine signaling: Signals sent by cells onto cells of same type and also for themselves are called autocrine signals. Eg: The cells during development if they have to take up a pathway for differentiation the cells secrete autocrine signals to reinforce themselves for developmental activities. Autocrine signals also stimulate cells of similar type (identical cells) which can respond to differentiation inducing signals (figure 5.4).

Figure 5.4: Autocrine signaling



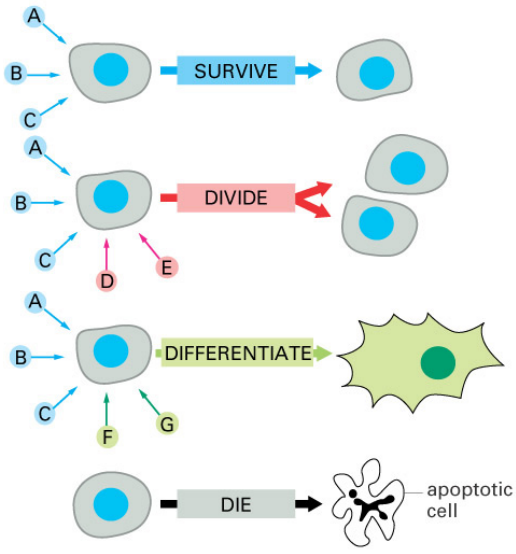
4. Role of gap junctions: These are specialized cell-cell junctions that are formed between closely apposed plasma membranes. These are water filled channels that connect adjacent cells and allow exchange of small intracellular signals Ca^{2+} and cyclic AMP to pass through (Figure 5.5).

Figure 5.5: Signaling via gap junctions



5. Cells respond to several signals: Cells in a multicellular organism are exposed to different signals. They have the ability to respond to them differently and every response is programmed, leading to execution of specialized functions (figure 5.6). They have set of receptors that bind to signal molecules. Invitro, if cells are deprived of these signals, they undergo apoptosis.

Figure 5.6: Animal cell dependence on multiple signals for survival



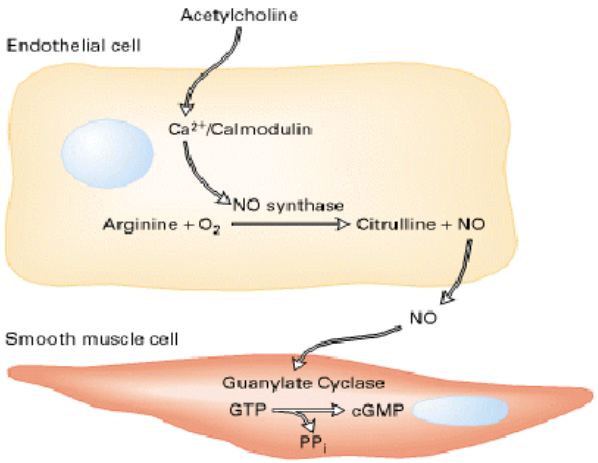
6. Concentration and life span of signals: These two depend upon the necessity of their persistence within the cells. Few signal molecules presence is necessary for long duration as in the case of cells in the stages of development. In an adult tissue, responses to fade and signals

have to cease after certain span. There is rapid turnover of signal molecules, as in case of nerve impulse transmission, acetylcholine is destroyed by acetylcholine esterase and fresh signal at axon causes release of new molecules of acetylcholine to transmit the signal to muscle cells, if not the muscle would be left in a continuous state of contraction. They are released in micro to nanomolar concentrations and destroyed every time. Few proteins (intracellular) serve for long duration (≤ 10 minutes) as they have key regulatory roles.

7. Some signals directly bind to target proteins:

Nitric oxide gas signal binds to guanylyl cyclase. Acetylcholine released by nerve ending activates NO synthase in the endothelial cells of blood vessels to produce NO. Nitric oxide diffuse out of the endothelial cells into the underlying smooth muscles, binds to guanylyl cyclase to produce cyclic GMP. This triggers relaxation of blood vessels and enhances flow of blood through them (figure 5.7). The life span of NO is very short and it is converted into nitrates and nitrites by oxygen and water. Even Carbon Monoxide is another gas signal that is used as intercellular signal to stimulate guanylyl cyclase.

Figure 5.7: Responses induced by acetyl choline via NO resulting in smooth muscle contraction

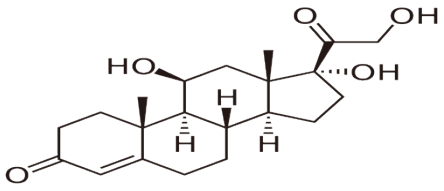


8. Signals that bind to nuclear receptors:

Some signal molecules like steroid hormones, thyroid hormones, retinoids and vitamin D, bind to receptors which are part of Nuclear Receptor Super Family. They bind to receptor proteins, activate them to bind to DNA to regulate transcription of specific genes.

Eg 1. Steroid hormones – cortisol(figure5.8): It is produced by cortex of adrenal gland and influences metabolism in various ways. It is located in cytosol and enters nucleus after binding to nuclear hormone receptor protein.

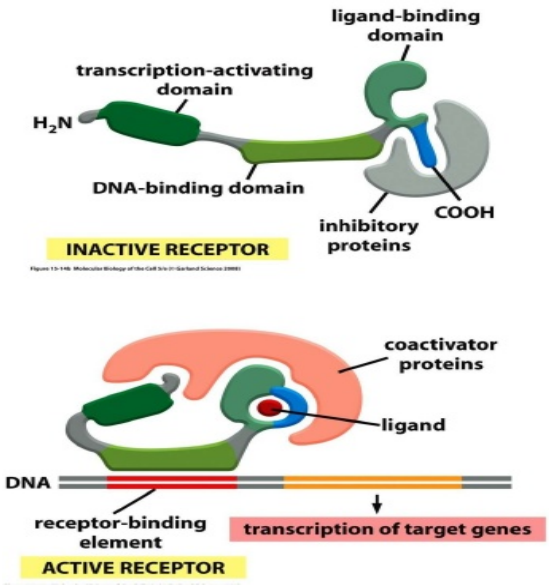
Figure 5.8: Structure of cortisol



These DNA binding proteins are either homodimers or heterodimers. Binding of ligand to the inactive receptor changes its conformation, this involves dissociation of inhibitory complexes from them. Ligand binding promotes binding of receptor to coactivator protein, then binding to Receptor's transcription activating domain, which increases gene transcription (figure 5.8).

This response induced by activation of Nuclear hormone receptors occurs in two phases- Early primary response and delayed secondary response. Primary response lasts for around 30 minutes and results in protein products synthesis, which inturn activates other genes to produce their products (secondary response) (figure 5.9).

Figure 5.9: Nuclear Receptor Binding protein stages of activity



9) Classes of cell surface receptors:

There are three major classes of cell surface receptors (figure 5.10):

i) Ion – channel – linked receptors

ii) G- Protein linked receptors

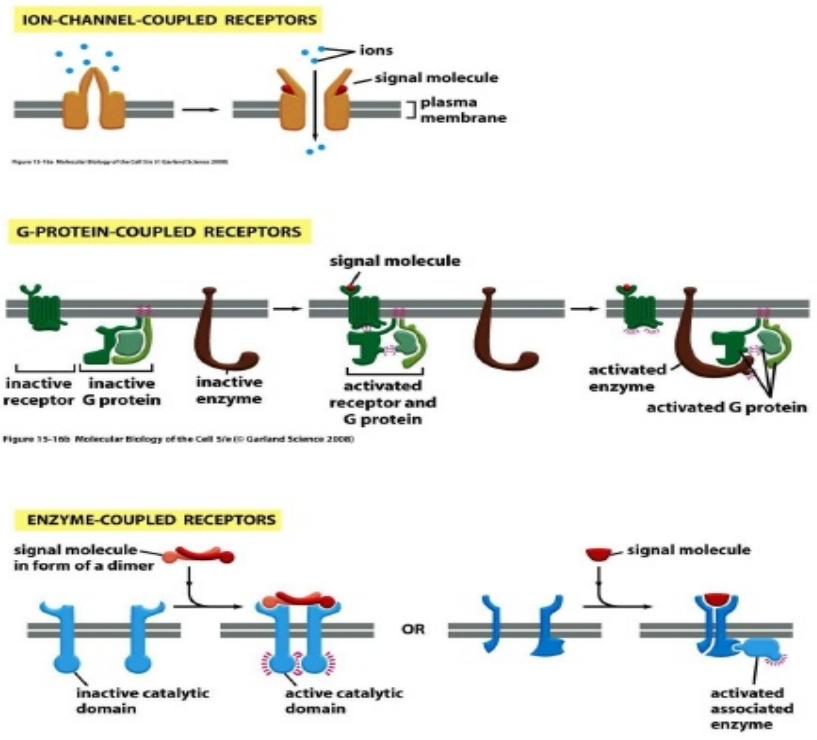
iii) Enzyme linked receptors

i) Ion-channel linked receptor: These are known as Transmitter-gated ion channels/ Ionotrophic receptors. They are involved in rapid synaptic signaling between electrically excitable cells. This is mediated by few neurotransmitters that transiently open/close ion channels formed by proteins to which they bind.

ii) G-Protein linked receptors: These regulate the activity of separate plasma membrane bound target proteins, which can be enzymes or Ion channels. The interaction between receptor and target proteins is mediated by a trimeric GTP-binding protein(G-protein).

iii)Enzyme –linked receptors: These when activated , function either directly as enzymes or are directly associated with enzymes that they activate.

Figure 5.10: Three major classes of cell surface receptors



10) Intra cellular Signaling Proteins:

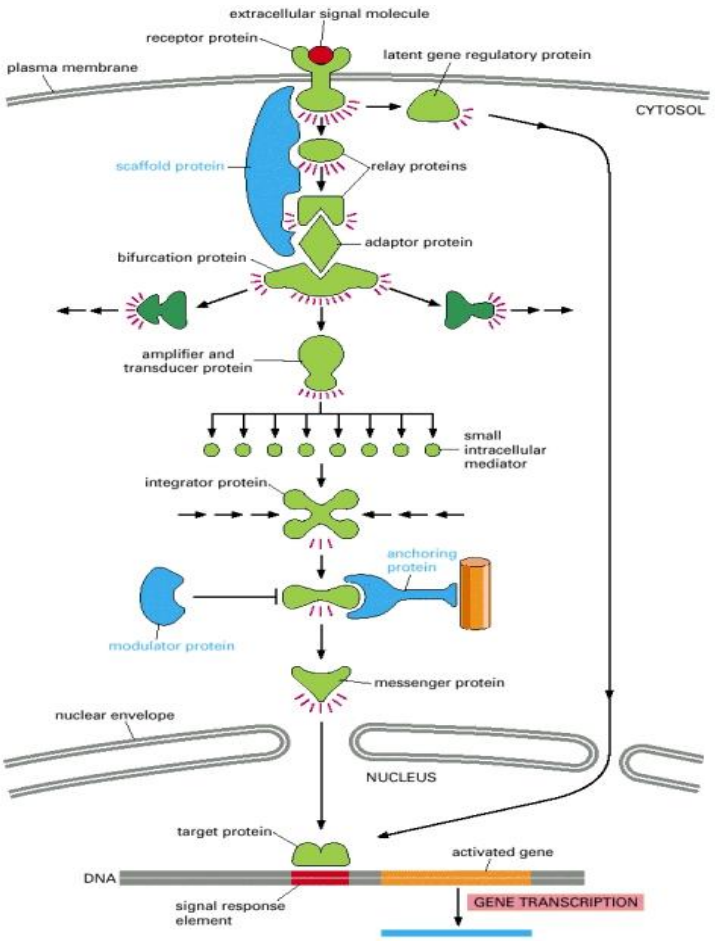
The intracellular mediators or secondary messengers are produced by the binding of G-protein linked or Enzyme- linked receptor binding to their receptors. The secondary messengers are generated in large numbers in response to receptor activation and they rapidly diffuse from source. Eg: Cyclic AMP and Ca^{2+}, which are water soluble and diacyl glycerol which is lipid soluble and diffuses even along plasma membrane.

Intra cellular signaling proteins act in a cascade manner. A series of signaling proteins and small intracellular mediators relay the extracellular signal into the cell causing a change in gene expression (figure 5.11).

Proteins involved in intracellular signaling are categorized as follows:

1. Relay proteins: They simply pass the message to the next signaling component in the chain.
2. Messenger Proteins: Carry signal from one part of the cell to another, such as from cytosol to the nucleus.
3. Adaptor proteins: Link one signaling protein to another, without themselves conveying a signal.
4. Amplifier proteins: These are usually enzymes or ion channels, that amplify the signal they receive either by producing large amounts of small intracellular mediators or by activating large number of downstream intracellular signaling proteins. When there are multiple amplification steps in a relay chain it is referred to as Signaling cascade.
5. Transducer proteins: they convert the signal into different form. The enzyme that makes cyclic-AMP- it converts the signal and amplifies it (Transducer + amplifier).
6. Bifurcation proteins: Spread the signal from one signaling pathway to another.
7. Integrator proteins: Receive signals from two or more signaling pathways and integrate them before relaying a signal onward.
8. Latent gene regulatory proteins: they are activated at the cell surface by activated receptors and then migrate to the nucleus to stimulate gene transcription.

Figure 5.11: Different kinds of Intracellular signaling proteins

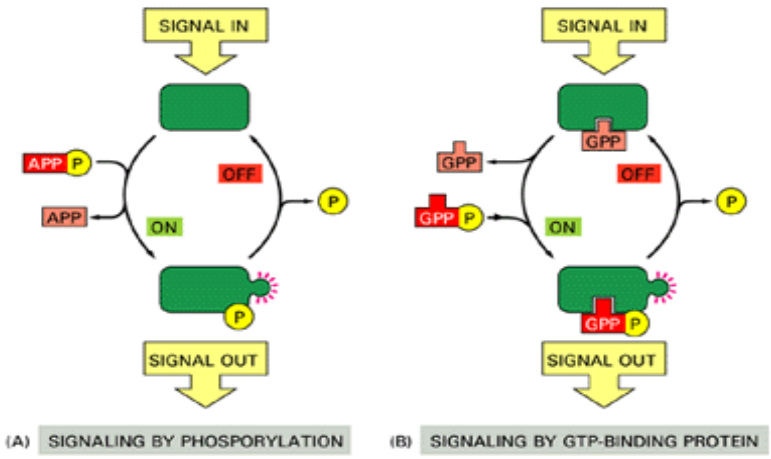


11. Molecular switches: Upon receipt of a signal they switch from inactive state to active state, until another process switches them off. Molecular switches fall into two main classes, that operate in different ways. Both classes of proteins involve activation and inactivation of phosphate groups. For one class phosphate is added by protein kinase using ATP as phosphate source. In the other class the signaling protein is induced to exchange its bound GDP with GTP (figure5.12).

- Many of the signaling proteins controlled by phosphorylation are protein kinases and are often organized into phosphorylation cascades. The two main protein kinases are Serine/Threonine kinase, which phosphorylates Serine and Threonine, and the other is Tyrosine kinase which phosphorylates tyrosine residues in the proteins.

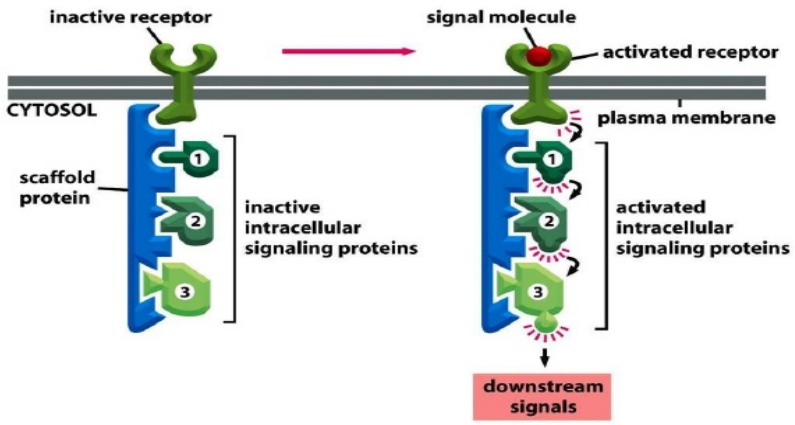
- GTP- binding protein alters between active state with GTP bound to them and the other inactive state when GDP is bound. The two major types of GTP binding proteins are – A large trimeric GTP- binding protein and a small monomer GTPase.

Figure 5.12: Types of intracellular proteins that act as Molecular switches



12) Intracellular signaling complexes: These are extracellular signals acting through a single type of G-protein linked or Enzyme-linked receptor usually activates multiple parallel signaling pathway and can thereby influence multiple aspects of cell behavior such as shape, movement , metabolism and gene expression. Cells use Scaffold proteins, which organize groups of interacting signaling proteins into signaling complexes. Scaffold guides the interactions between the successive components in such a complex, the signal can be relayed with precision, speed and efficiency; moreover, unwanted cross talk between signaling pathways is avoided. These complexes form transiently, as and when signaling proteins assemble around a receptor after an extracellular signal molecule attaches to it. The cytoplasmic tail of activated protein gets activated by Phosphorylation and this acts as docking site for intracellular signaling proteins (figure 5.13).

Figure 5.13: Preformed signaling proteins complex on scaffold



13. Modular binding domains: These are compact protein modules that bind to a particular motif in the protein or lipid, with which the signaling protein interacts. They bind to signaling proteins forming the route of the signaling pathways.

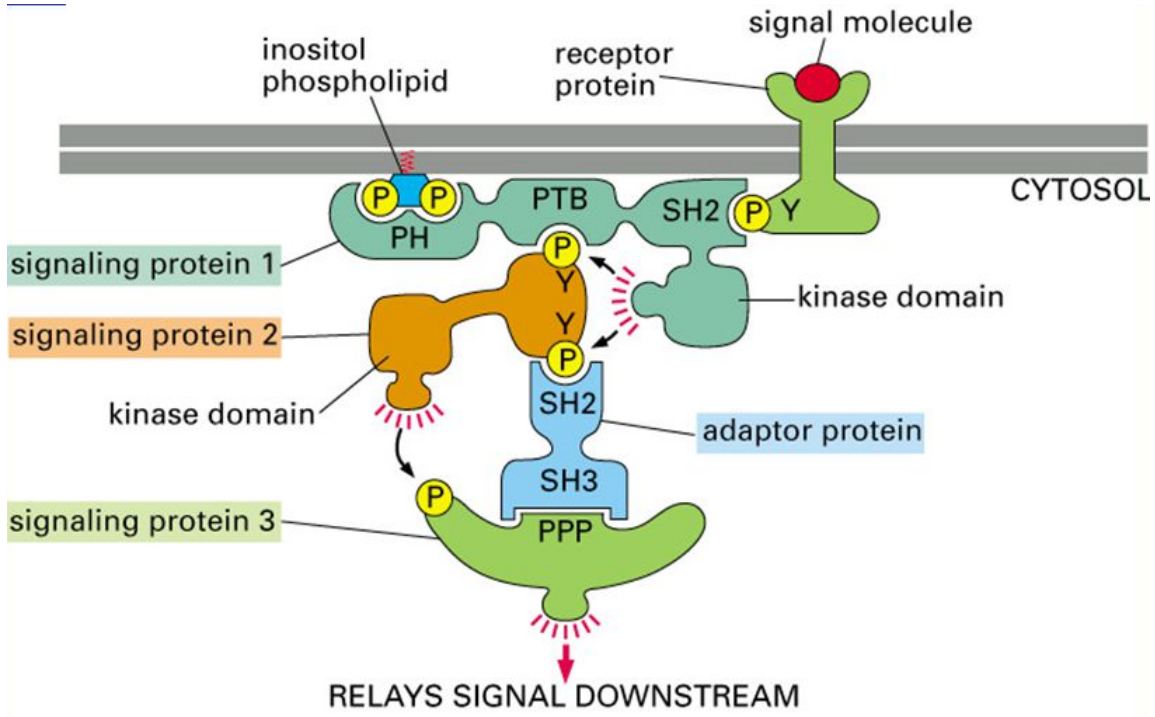
Eg: Src homology 2 (SH2) domains and phosphotyrosine –binding (PTB) domains (figure 5.14).

It is signaling protein I that contains three different binding domains and a catalytic protein kinase domain. It moves to the plasma membrane when extra cellular signals lead to the creation of various phosphorylated docking sites on the cytosolic face of the membrane. Its SH2 domain binds to phosphorylated tyrosines on the receptor protein and its PH domain binds to phosphorylated inositol phospholipids in the inner leaflet of the lipid bilayer.

Protein I phosphorylates signaling protein 2 on tyrosines, which allows protein2 to bind to the PTB domain on protein1 and to the SH2 domain on an adopter protein that links protein2 and protein 3, causes phosphorylation of protein 3 by protein 2.

The adapter protein shown consists of 2 binding domains, one SH2 domain, which binds to a phosphotyrosine on protein 2 and an SH3 domain, which binds to a protein rich motif on protein 3.

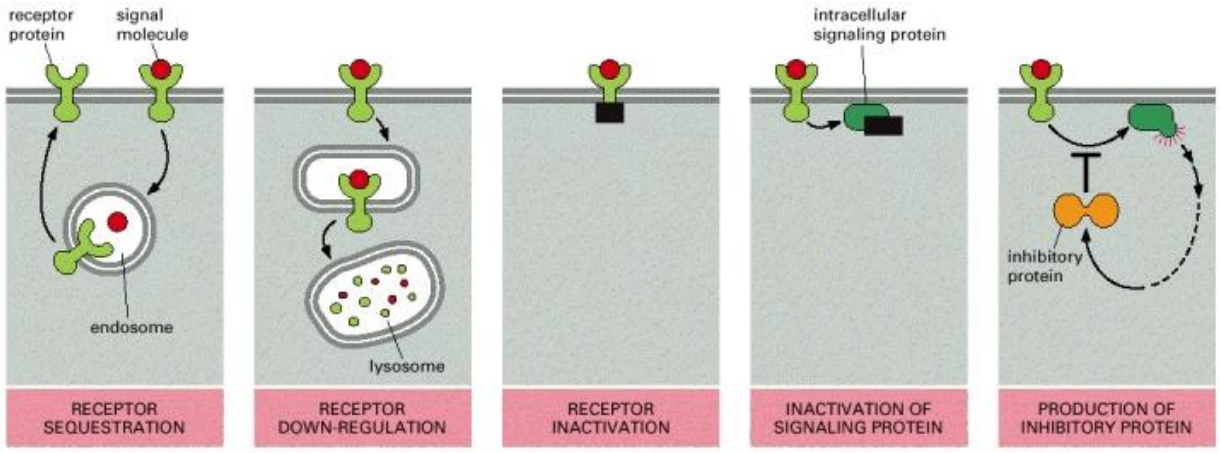
Figure 5.14 Src homology 2 (SH2) domains and phosphotyrosine –binding (PTB) domains



14. Desensitization of cell signaling:

The target cells of cell signaling undergo a reversible process of adaptation or sensitization where by a prolonged exposure to a stimulus decreases the cells response to that level of exposure. There are five ways of desensitization of signal molecules (figure 5.15). Ligand binding to cell surface receptors, may induce their endocytosis and temporary sequestration in endosomes. Such Ligand- induced receptor endocytosis can lead to the destruction of the receptors in lysosomes, a process referred to as receptor down-regulation. In other cases, desensitization occurs due to rapid inactivation of receptor due to its phosphorylation. Production of an inhibitor that blocks transduction process can also be a reason for desensitization.

Figure 5.15: Five ways of desensitization to signal molecules



CLASSES OF CELL SURFACE RECEPTORS

1) Ion channel linked receptors: Most of the membrane transport proteins on the plasma membrane are concerned with transport of inorganic ions like Na^+, K^+, Ca^{2+} or Cl^- and are referred to as Ion channels. These ions diffuse rapidly sown their concentration gradient through the channel across the membrane and control many cell functions. Few important channel families are listed in Table below:

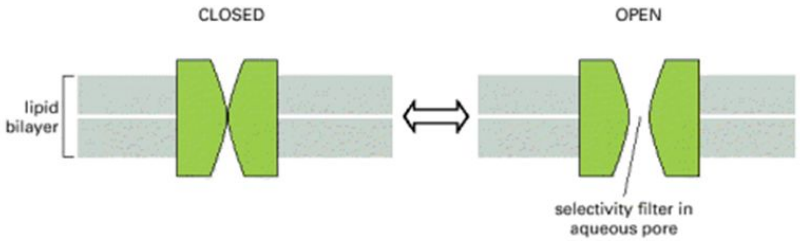
Family	Representative Subfamilies	
Voltage gated cation channels	Voltage gated Na^+ channels	
	Voltage gated K^+ channels	
	Voltage gated Ca^{2+} channels	
Transmitter gated ion channels	Acetylcholine gated cation channels	Excitatory
	Glutamate gated Ca^{2+} channels	
	Serotonin gated cation channels	
	GABA gated Cl^- channels	Inhibitory
	Glycine gated Cl^- channels	

Few characteristics of these channels:

a) **Fluctuation between open and closed state:** as the ions channels are selective they allow movement of ions, based on the concentration gradient. Their opening and closing is associated with conformation changes. When pores open, it is associated with lining up of polar groups along the pore, while hydrophobic amino acids interact with lipid bilayer. This

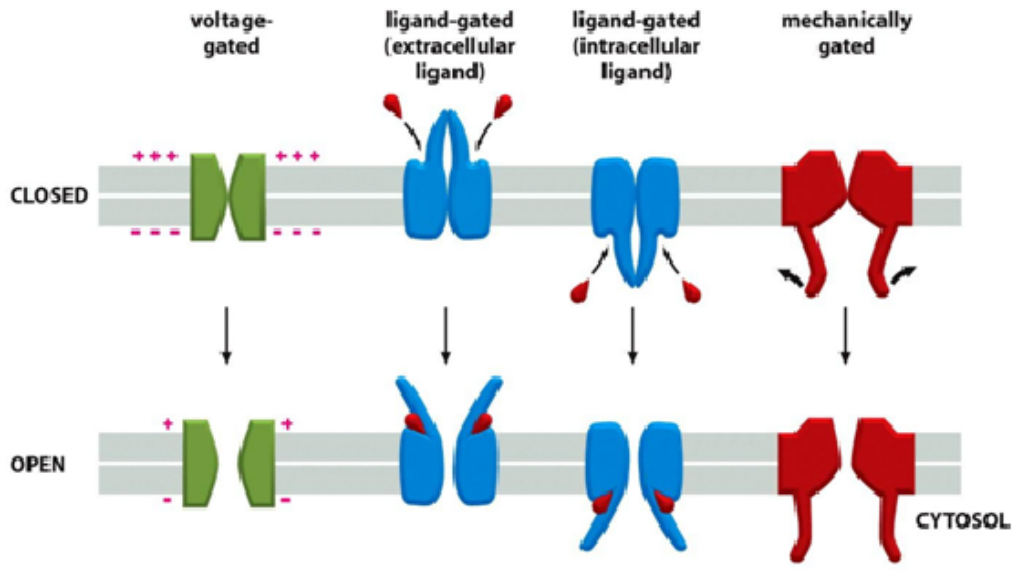
opening allows penetration of ions that have shed most of their water molecules to pass though the nearest channel called as selective filter (figure 5.16).

Figure5.16: Opening and closing of Ion channels



b) Gating of Ion channels: few channels open due to some stimulus and these are called gated channels. The types of stimuli that can open these gated channels are negative voltage across the membrane (Voltage gated channels), a mechanical stress (mechanically gated channels), or binding of a ligand (Ligand- gated channels). The Ligand can be either extracellular mediator (as in transmitter gated channels) or a intracellular mediator (Ion-gated channels)or a nucleotide (nucleotide gated channel) (figure 5.17).

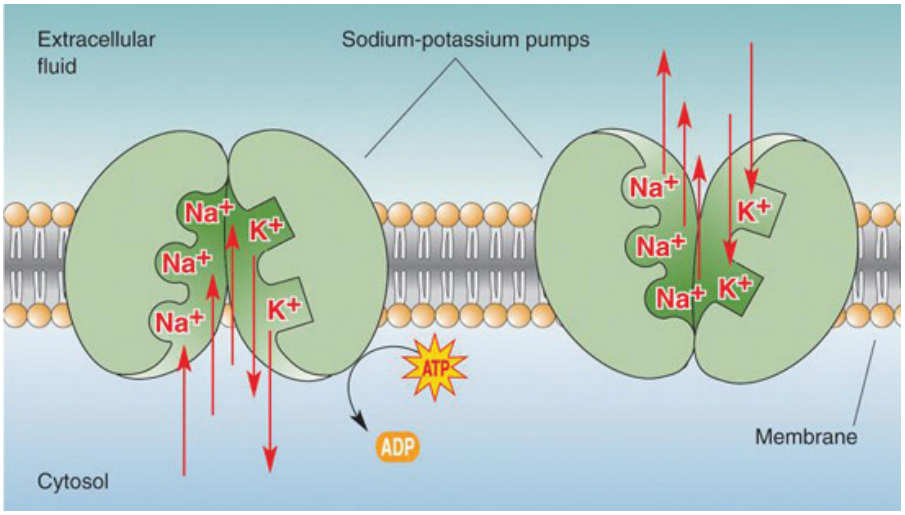
Figure 5.17: Types of Gated Ion channels



c) Membrane potential role in Na^+- K^+ pumps: Membrane potential arises due to differences in the electrical charge across the membrane. Usually animal cells have low Na^+ inside them

while K^+ ions are more in the cytosol. The balancing if Na^+ charge gradient is done by K^+ ions. K^+ ions can freely move through K^+ leaky channels across the membrane thereby balancing the concentration of these ions. A state when there is no net flow of ions is called resting potential and the cell is called a polarized cell. A stimulus can disturb this cell and cause penetration of Na^+ ions into the cells and this is called Avalanche effect, which continues until equilibrium is established. At one point Na^+ inflow stops and this cell is called a depolarized cell, the potential of cell at this state is called action potential. The cell again has to restore back to polarized state and this is by pumping out of Na^+ ions and in flow of K^+ through Na^+ - K^+ pumps. Cells start pumping our 3 Na^+ ions for every 2 K^+ ions until equilibrium is reached (figure 5.18). In animal cells, the potential difference varies from – 20 to 200mV depending on the type of cell.

Figure 5.18: Functioning of Na^+ - K^+ pumps



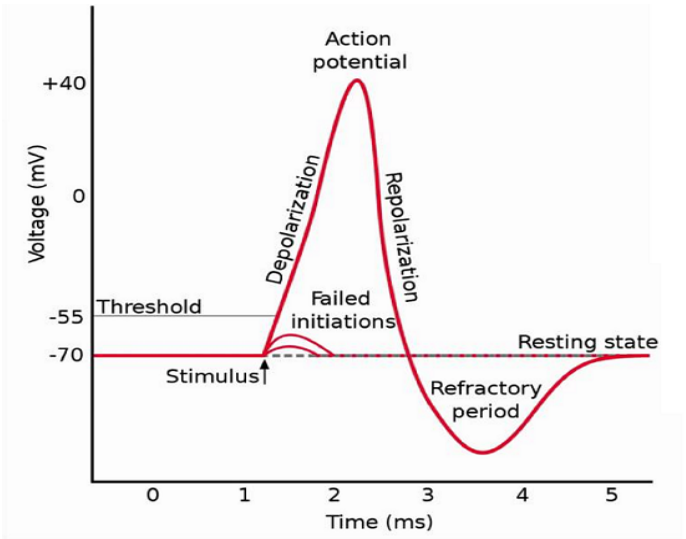
d) Functioning of neuron by Voltage gated channels:

A neuron can receive input from other neurons via a chemical called a neurotransmitter. If this input is strong enough, the neuron will pass the signal to downstream neurons. Transmission of a signal within a neuron (in one direction only, from dendrite to axon terminal) is carried out by the opening and closing of voltage-gated ion channels, which cause a brief reversal of the resting membrane potential to create an action potential . As an action potential travels down the axon, the polarity changes across the membrane. Once the signal reaches the axon terminal, it stimulates other neurons.

Depolarization and the Action Potential

When neurotransmitter molecules bind to receptors located on a neuron's dendrites, voltage-gated ion channels open. At excitatory synapses, positive ions flood the interior of the neuron and depolarize the membrane, decreasing the difference in voltage between the inside and outside of the neuron. A stimulus from a sensory cell or another neuron depolarizes the target neuron to its threshold potential (-55 mV), and Na^+ channels in the axon hillock open, starting an action potential. Once the sodium channels open, the neuron completely depolarizes to a membrane potential of about +40 mV (figure 5.19). The action potential travels down the neuron as Na+ channels open.

Figure 5.19: Action potential curve of neuron



Hyperpolarization and Return to Resting Potential

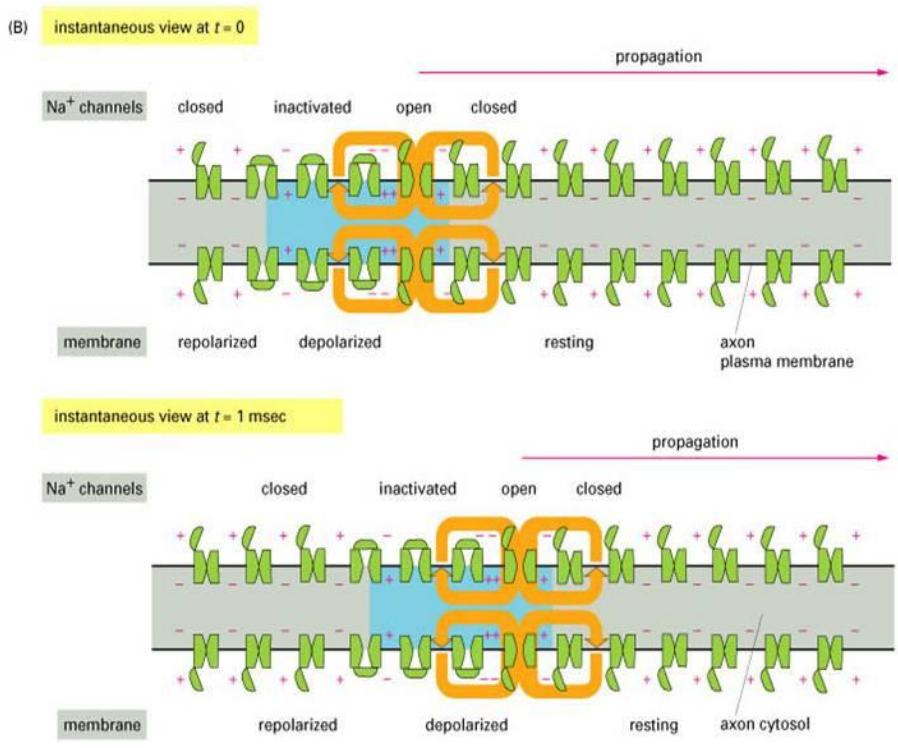
Action potentials are considered an "all-or nothing" event. Once the threshold potential is reached, the neuron completely depolarizes. As soon as depolarization is complete, the cell "resets" its membrane voltage back to the resting potential. The Na^+ channels close, beginning the neuron's refractory period. At the same time, voltage-gated K^+ channels open, allowing K^+ to leave the cell. As K^+ ions leave the cell, the membrane potential once again becomes negative. The diffusion of K^+ out of the cell hyperpolarizes the cell, making the membrane potential more negative than the cell's normal resting potential(figure 5.20). At this point, the sodium channels return to their resting state, ready to open again if the membrane potential again exceeds the

threshold potential. Eventually, the extra K^+ ions diffuse out of the cell through the potassium leakage channels, bringing the cell from its hyperpolarized state back to its resting membrane potential.

Myelin and Propagation of the Action Potential

For an action potential to communicate information to another neuron, it must travel along the axon and reach the axon terminals where it can initiate neurotransmitter release. The speed of conduction of an action potential along an axon is influenced by both the diameter of the axon and the axon's resistance to current leak. Myelin acts as an insulator that prevents current from leaving the axon, increasing the speed of action potential conduction. Diseases like multiple sclerosis cause degeneration of the myelin, which slows action potential conduction because axon areas are no longer insulated so the current leaks.

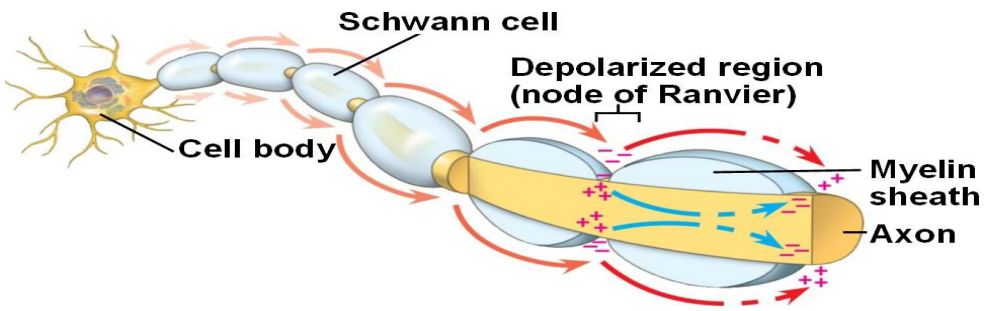
Figure 5.20: Propagation of action potential along axon ofneuron



Node of Ranvier: A node of Ranvier is a natural gap in the myelin sheath along the axon. These unmyelinated spaces are about one micrometer long and contain voltage gated Na^+ and K^+ channels. The flow of ions through these channels, particularly the Na^+ channels, regenerates

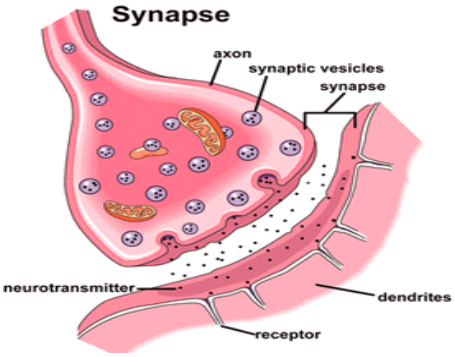
the action potential over and over again along the axon. Action potential "jumps" from one node to the next in saltatory conduction (figure 5.21). If nodes of Ranvier were not present along an axon, the action potential would propagate very slowly; Na^+ and K^+ channels would have to continuously regenerate action potentials at every point along the axon. Nodes of Ranvier also save energy for the neuron since the channels only need to be present at the nodes and not along the entire axon.

Figure 5.21 Nodes of Ranvier and salutatory conduction of nerve impulse



Synapse usually occurs between the axon of a pre-synaptic neuron and a dendrite or cell body of a post-synaptic neuron (Figure 5.22). At a synapse, the end of the axon is swollen and referred to as the end bulb or synaptic knob. Within the end bulb are found many synaptic vesicles that have the neurotransmitter and mitochondria that provide ATP. Between the end bulb and the dendrite of the post – synaptic neuron or onto the surface of skeletal muscle membrane, the gap is referred to as synaptic cleft. The impulse has to be transmitted through this space.

Figure 5.22: Synapse

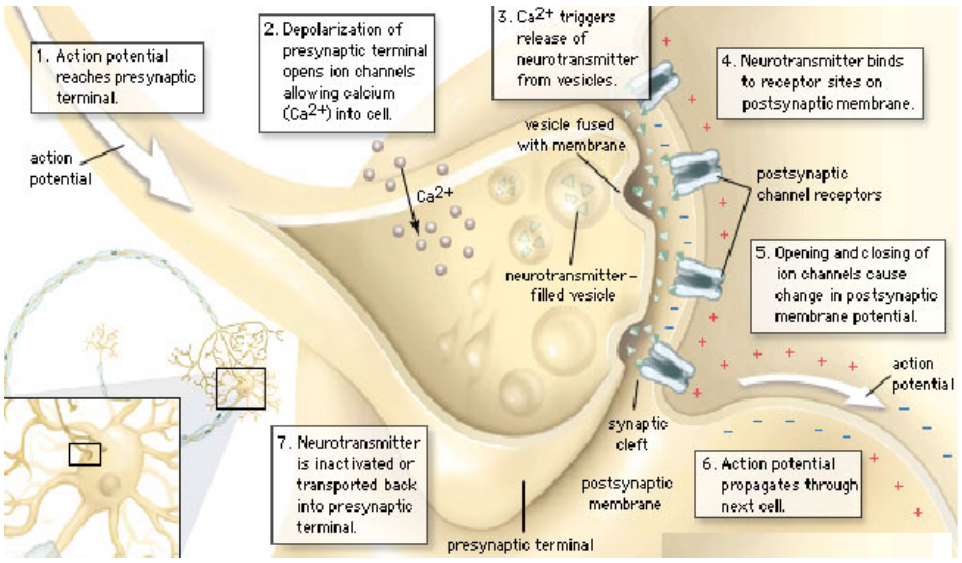


Mechanism of action

Synaptic transmission at the neuromuscular junction begins when an action potential reaches the presynaptic terminal of a motor neuron, which activates voltage-dependent calcium channels to

allow calcium ions to enter the neuron. Calcium ions bind to sensor proteins (synaptotagmin) on synaptic vesicles, triggering vesicle fusion with the cell membrane and subsequent neurotransmitter release from the motor neuron into the synaptic cleft (figure 5.23). In vertebrates, motor neurons release acetylcholine (ACh), a small molecule neurotransmitter, which diffuses across the synaptic cleft and binds to nicotinic acetylcholine receptors (nAChRs) on the cell membrane of the muscle fiber, also known as the sarcolemma. nAChRs are ionotropic receptors, meaning they serve as ligand-gated ion channels. The binding of ACh to the receptor can depolarize the muscle fiber, causing a cascade that eventually results in muscle contraction.

Figure 5.23: Events at Motor end plate



Acetylcholine receptors at the neuromuscular junction

1. Ion channel linked receptor
2. Ions
3. Ligand (such as acetylcholine)

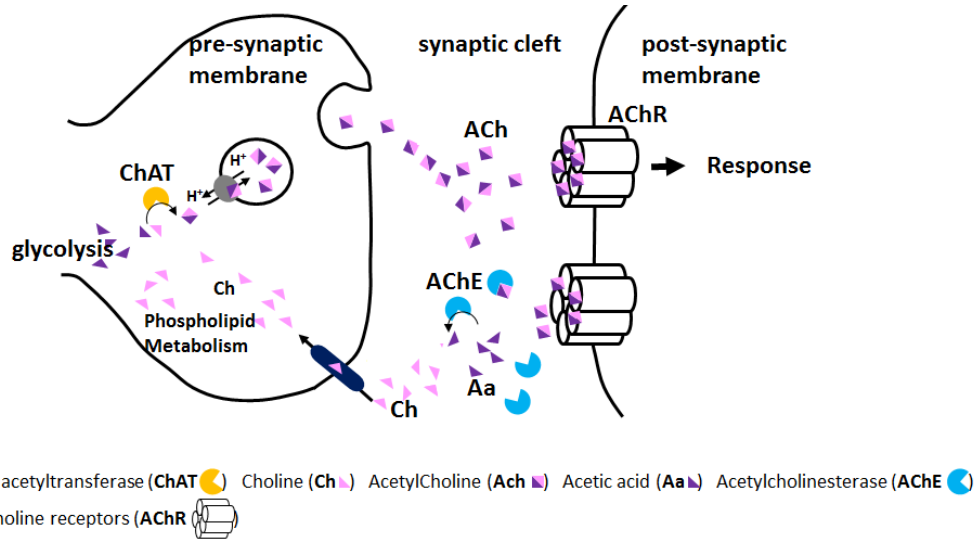
When ligands bind to the receptor, the ion channel portion of the receptor opens, allowing ions to pass across the cell membrane.

Acetylcholine is a neurotransmitter synthesized from dietary choline and acetyl-CoA (ACoA), and is involved in the stimulation of muscle tissue in vertebrates. The acetylcholine receptor subtype that is found at the neuromuscular junction of skeletal muscles is the nicotinic

acetylcholine receptor (nAChR), which is a ligand-gated ion channel. Each subunit of this receptor has a characteristic "cys-loop," which is composed of a cysteine residue followed by 13 amino acid residues and another cysteine residue. The two cysteine residues form a disulfide linkage which results in the "cys-loop" receptor that is capable of binding acetylcholine and other ligands.

AChRs at the skeletal neuromuscular junction form heteropentamers composed of two α, one β, one ε, and one δ subunits. When a single ACh ligand binds to one of the α subunits of the ACh receptor it induces a conformational change at the interface with the second AChR α subunit. This conformational change results in the increased affinity of the second α subunit for a second ACh ligand. AChRs therefore exhibit a sigmoidal dissociation curve due to this cooperative binding . The presence of the inactive, intermediate receptor structure with a single-bound ligand keeps ACh in the synapse that might otherwise be lost by cholinesterase hydrolysis or diffusion. The persistence of these ACh ligands in the synapse can cause a prolonged post-synaptic response.

Figure 5.24: Events at synaptic cleft involving Acetylcholine and its receptor, followed by acetylcholine hydrolysis by acetylcholine esterase



2) G-protein linked cell surface receptors:

G-protein-linked receptors form the largest family of cell-surface receptors and are found in all eucaryotes. These receptors mediate the responses to an enormous diversity of signal molecules, including hormones, neurotransmitters, and local mediators. These signal molecules that activate them are as varied in structure as they are in function: the list includes proteins and small peptides, as well as derivatives of amino acids and fatty acids. The same ligand can activate many differentreceptor family members; at least 9 distinct G-protein-linked receptors are activated by adrenaline, for example, another 5 or more by acetylcholine, and at least 15 by the neurotransmitter serotonin.

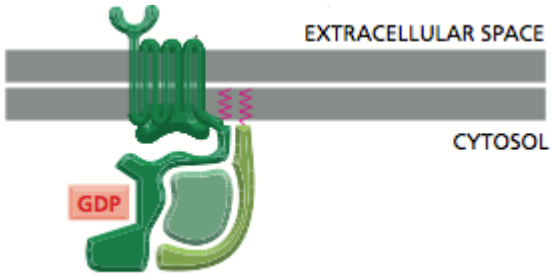
Despite the chemical and functional diversity of the signal molecules that bind to them, all G-protein-linked receptors have a similar structure. They consist of a single polypeptide chain that threads back and forth across the lipid bilayer seven times and are therefore sometimes called serpentine receptors. In addition to their characteristic orientation in the plasma membrane, they have the same functional relationship to the G proteins they use to signal the cell interior that an extracellular ligand is present.

G-protein:

Trimeric GTP-binding proteins (G proteins) are attached to the cytoplasmic face of the plasma membrane, where they serve as relay molecules, functionally coupling the receptors to enzymes or ion channels in this membrane. There are various types of G proteins, each specific for a particular set of serpentine receptors and for a particular set of downstream target proteins in the plasma membrane. All have a similar structure, however, and they operate in a similar way.

G proteins are composed of three protein subunits—α, β, and γ. In the unstimulated state, the α subunit has GDP bound and the G protein is inactive (Figure 15-27). When stimulated by an activated receptor, the α subunit releases its bound GDP, allowing GTP to bind in its place. This exchange causes the trimer to dissociate into two activated components—an α subunit and a βγ complex (figure 5.25).

Figure 5.25: GTP binding protein (G protein)

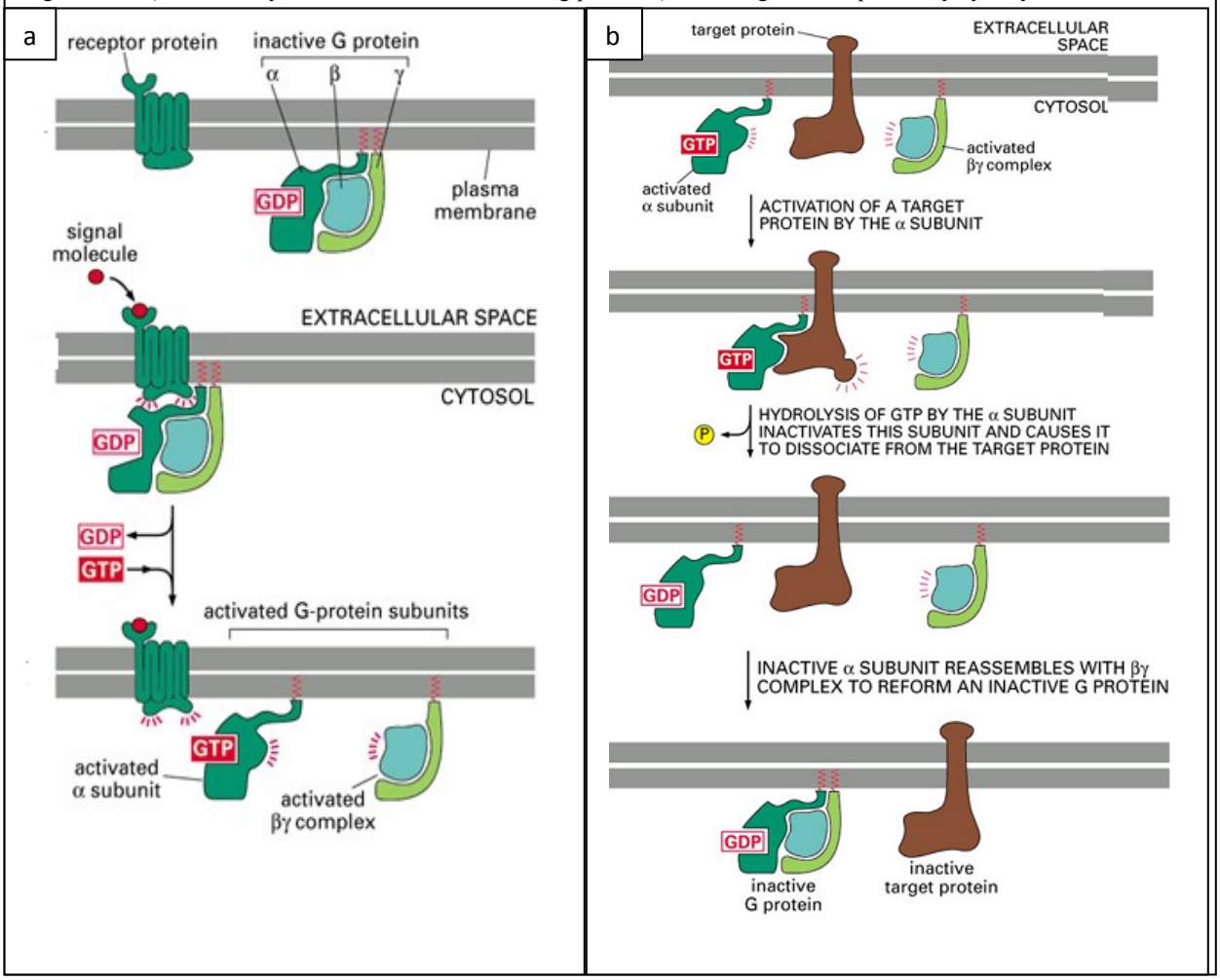


Activation of G Protein

The dissociation of the trimeric G protein activates its two components in different ways. GTP binding causes a conformational change that affects the surface of the α subunit that associates with the βγ complex in the trimer. This change causes the release of the βγ complex, but it also causes and the α subunit to adopt a new shape that allows it to interact with its target proteins. The βγ complex does not change its conformation, but the surface previously masked by the α subunit is now available to interact with a second set of target proteins. The targets of the dissociated components of the G protein are either enzymes or ion channels in the plasma membrane, and they relay the signal onward.

The α subunit is a GTPase, and once it hydrolyzes its bound GTP to GDP, it reassociates with a βγ complex to re-form an inactive G protein, reversing the activation process (Figure 15-29). The time during which the α subunit and βγ complex remain apart and active is usually short, and it depends on how quickly the α subunit hydrolyzes its bound GTP. An isolated α subunit is an inefficient GTPase, and, left to its own devices, the subunit would inactivate only after several minutes. Its activation is usually reversed much faster than this, however, because the GTPase activity of the α subunit is greatly enhanced by the binding of a second protein, which can be either its target protein or a specific modulator known as a regulator of G protein signaling (RGS). **RGS proteins** act as α-subunit-specific GTPase activating proteins (GAPs), and they are thought to have a crucial role in shutting off G-protein-mediated responses in all eukaryotes. There are about 25 RGS proteins encoded in the human genome, each of which is thought to interact with a particular set of G proteins.

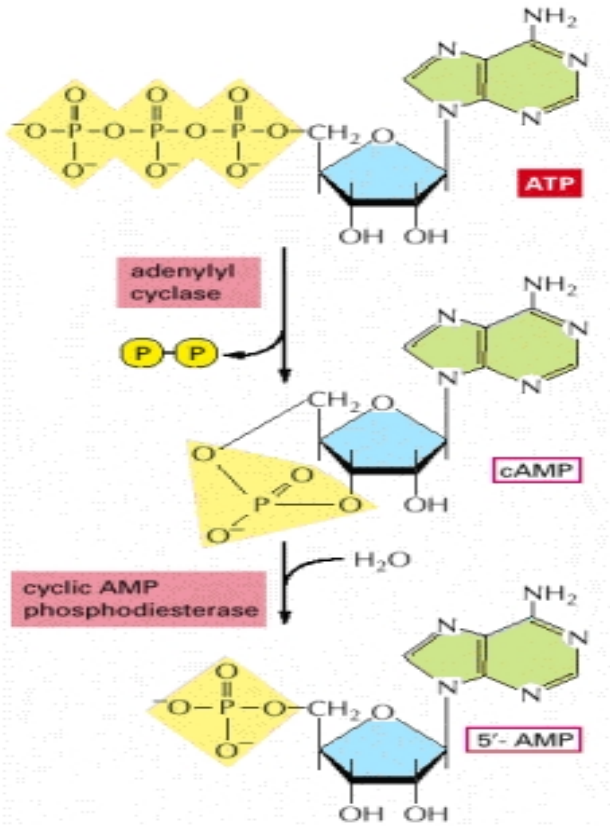
Figure 5.26: a)Disassembly and activation of GTP binding protein b) Switching off of G-protein by hydrolysis of ATP



Some G proteins signal by regulating the production of cyclic AMP

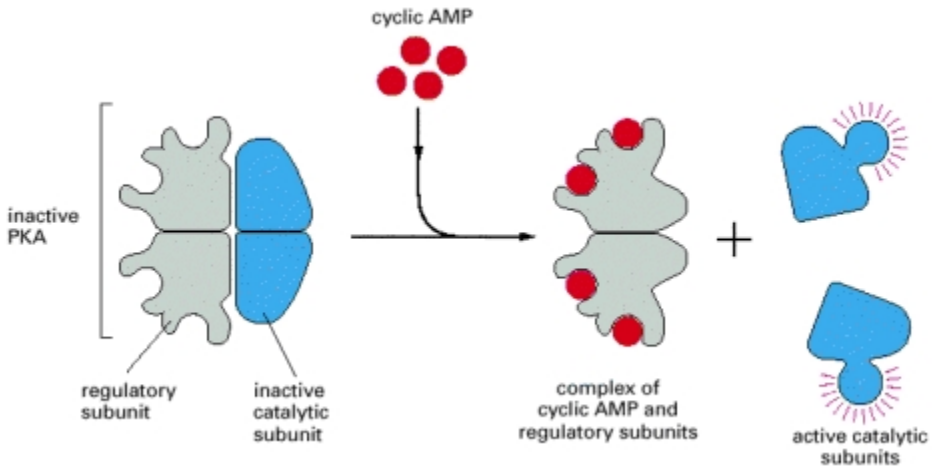
Cyclic AMP (cAMP) was first identified as a small intracellular mediator in the 1950s. It has since been found to act in this role in all prokaryotic and animal cells that have been studied. The normal concentration of cyclic AMP inside the cell is about 10^{-7} M, but an extracellular signal can cause cyclic AMP levels to change by more than twentyfold in seconds (Figure 15-30). As explained earlier (see Figure 15-10), such a rapid response requires that a rapid synthesis of the molecule be balanced by its rapid breakdown or removal. In fact, cyclic AMP is synthesized from ATP by a plasma-membrane-bound enzyme adenylyl cyclase, and it is rapidly and continuously destroyed by one or more cyclic AMP phosphodiesterases that hydrolyze cyclic AMP to adenosine 5′-monophosphate (5′-AMP) (Figure 5.27).

Figure5.27: Synthesis and degradation of cyclic AMP



Cyclic-AMP-dependent protein kinase(PKA): This enzyme catalyzes the transfer of the terminal phosphate group from ATP to specific serines or threonines of selected target proteins, thereby regulating their activity. In the inactive state, PKA consists of a complex of two catalytic subunits and two regulatory subunits. The binding of cyclic AMP to the regulatory subunits alters their conformation, causing them to dissociate from the complex. The released catalytic subunits are thereby activated to phosphorylate specific substrate protein molecules (Figure 5.28). The regulatory subunits of PKA also are important for localizing the kinase inside the cell: special PKA anchoring proteins bind both to the regulatory subunits and to a membrane or a component of the cytoskeleton, thereby tying up the enzyme complex to a particular subcellular compartment. Some of these anchoring proteins also bind other kinases and some phosphatases, creating a signaling complex.

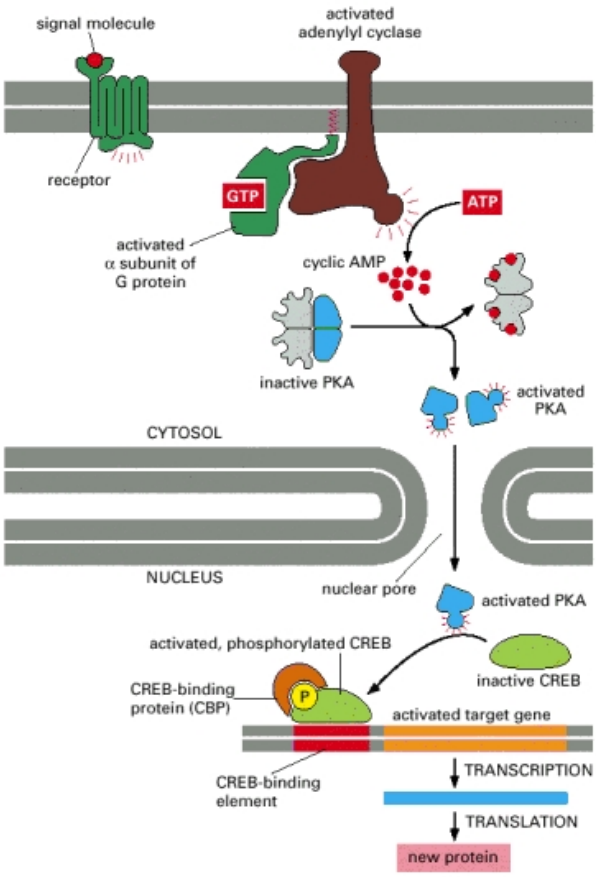
Figure 5.28 Activation of PKA



Cyclic AMP role in activating genes:

In cells that secrete the peptide hormone somatostatin, for example, cyclic AMP activates the gene that encodes this hormone. The regulatory region of the somatostatin gene contains a short DNA sequence, called the cyclic AMP response element (CRE) that is also found in the regulatory region of many other genes activated by cyclic AMP. A specific gene regulatory protein called CRE-binding (CREB) protein recognizes this sequence. When CREB is phosphorylated by PKA on a single serine, it recruits a transcriptional coactivator called CREB-binding protein (CBP), which stimulates the transcription of these genes (Figure 5-29).

Figure 5.29: Gene transcription activation by cyclic AMP



Dephosphorylation of PKA activated proteins by Phosphatases:

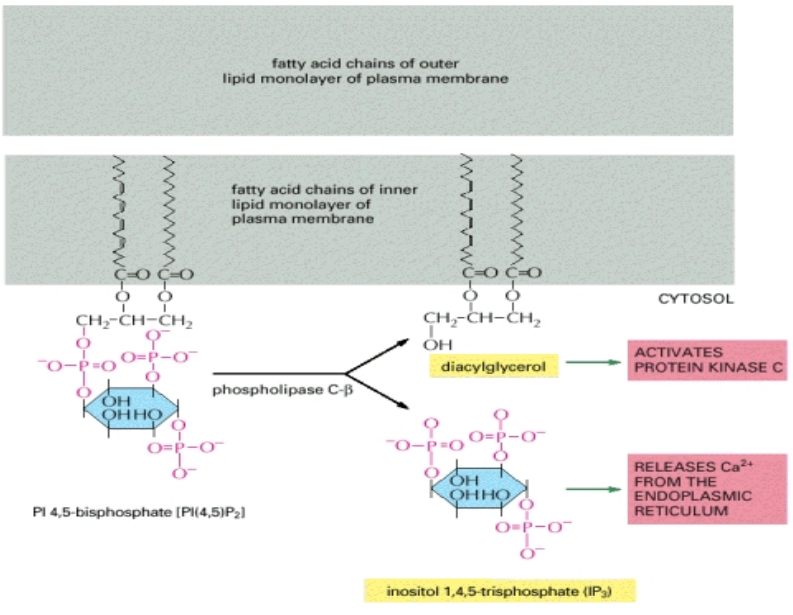
Since the effects of cyclic AMP are usually transient, cells must be able to dephosphorylate the proteins that have been phosphorylated by PKA. In general, the dephosphorylation of phosphorylated serines and threonines is catalyzed by four types of serine/threonine phosphoprotein phosphatases—protein phosphatases I, IIA, IIB, and IIC. Except for protein phosphatase-IIC (which is a minor phosphatase, unrelated to the others), all of these phosphatases are composed of a homologous catalytic subunit complexed with one or more of a large set of regulatory subunits; the regulatory subunits help to control the phosphatase activity and enable the enzyme to select specific targets. Protein phosphatase I is responsible for dephosphorylating many of the proteins phosphorylated by PKA. It inactivates CREB, for example, by removing its activating phosphate, thereby turning off the transcriptional response

caused by a rise in cyclic AMP concentration. Protein phosphatase IIA has a broad specificity and seems to be the main phosphatase responsible for reversing many of the phosphorylations catalyzed by serine/threonine kinases. Protein phosphatase IIB, also called calcineurin, is activated by Ca^{2+} and is especially abundant in the brain.

Inositol Phospholipid Signaling Pathway activation by G Protein

Many G-protein-linked receptors exert their effects mainly via G proteins that activate the plasma-membrane-bound enzyme phospholipaseC-β. The phospholipase acts on an inositol phospholipid called phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$], which is present in small amounts in the inner half of the plasma membrane lipid bilayer. Receptors that operate through this inositol phospholipid signaling pathway mainly activate a G protein called G_q, which in turn activates phospholipase C-β, in much the same way that G_s activates adenylyl cyclase. The activated phospholipase cleaves $PI(4,5)P_2$ to generate two products: inositol 1,4,5-trisphosphate and diacylglycerol (Figure 5-30), which diffuses through the cytosol and releases Ca^{2+} from the ER, and diacylglycerol, which remains in the membrane and helps to activate the enzyme protein kinase C.

Figure 5.30: Hydrolysis of $PI(4,5)P_2$ by phospholipase C-β



Calcium as a Ubiquitous messenger:

Calcium ions play several roles in animal cells. In muscle cells, Ca^{2+} triggers contraction, and in many secretory cells, including nerve cells, it triggers secretion. Ca^{2+} can be used as a signal in this way because its concentration in the cytosol is normally kept very low ($\sim 10^{-7}$ M), whereas its concentration in the extracellular fluid ($\sim 10^{-3}$ M) and in the ER lumen is high. Thus, there is a large gradient tending to drive Ca^{2+} into the cytosol across both the plasma membrane and the ER membrane. When a signal transiently opens Ca^{2+} channels in either of these membranes, Ca^{2+} rushes into the cytosol, increasing the local Ca^{2+} concentration by 10–20-fold and triggering Ca^{2+}-responsive proteins in the cell.

Three main types of Ca^{2+} channels can mediate this Ca^{2+} signaling:

1.Voltage-dependent Ca^{2+} channels in the plasma membrane open in response to membrane depolarization and allow, for example, Ca^{2+} to enter activated nerve terminals and trigger neurotransmitter secretion.

2. IP_3-gated Ca^{2+}-release channels allow Ca^{2+} to escape from the ER when the inositol phospholipid signaling pathway is activated, as just discussed.

3.Ryanodine receptors (so called because they are sensitive to the plant alkaloid ryanodine) react to a change in plasma membrane potential to release Ca^{2+} from the sarcoplasmic reticulum and thereby stimulate the contraction of muscle cells; they are also present in the ER of many nonmuscle cells, including neurons, where they can contribute to Ca^{2+} signaling.

Families of Trimeric G-proteins and their function: They are summarized in the table below:

FAMILY	SOME FAMILY MEMBERS	ACTION MEDIATED BY	FUNCTIONS
I	G_s	α	activates adenylyl cyclase; activates Ca^{2+} channels
	G_{olf}	α	activates adenylyl cyclase in olfactory sensory neurons
II	G_i	α	inhibits adenylyl cyclase
		βγ	activates K^+ channels
	G_o	βγ	activates K^+ channels; inactivates Ca^{2+} channels
		α and βγ	activates phospholipase C-β
	G_t (transducin)	α	activates cyclic GMP phosphodiesterase in vertebrate rod photoreceptors
III	G_q	α	activates phospholipase C-β

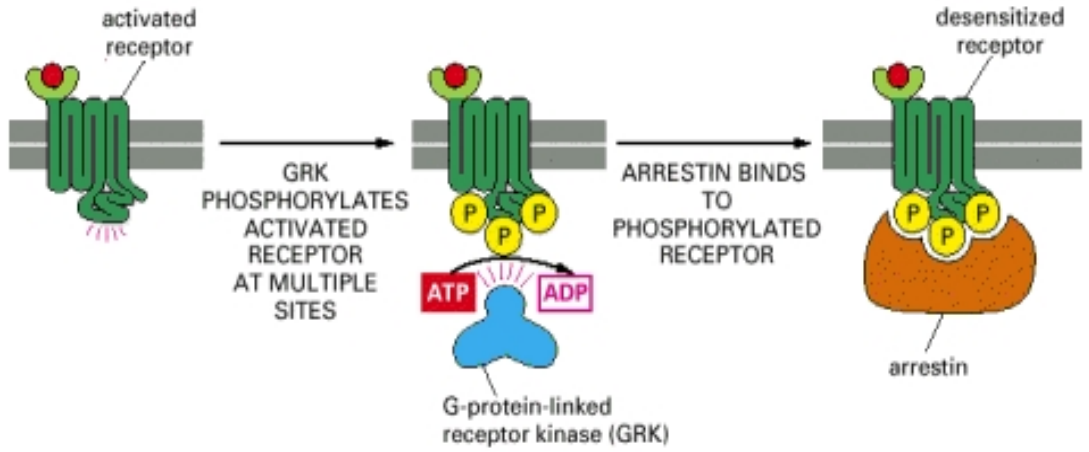
Desensitization of G-protein linked receptor: Target cells use a variety of mechanisms to desensitize, or adapt, when they are exposed to a high concentration of stimulating ligand for a prolonged period. Few mechanisms that involve an alteration in G-protein-linked receptors themselves.

These receptors can desensitize in three general ways:

1. They can become altered so that they can no longer interact with G proteins (receptor inactivation).
2. They can be temporarily moved to the interior of the cell (internalized) so that they no longer have access to their ligand (receptor sequestration).
3. They can be destroyed in lysosomes after internalization (receptor down-regulation).

In each case, the desensitization process depends on phosphorylation of the receptor, by PKA, PKC, or a member of the family of G-protein-linked receptor kinases (GRKs). The GRKs phosphorylate multiple serine and threonines on a receptor, but they do so only after the receptor has been activated by ligand binding. The binding of an arrestin to the phosphorylated receptor prevents the receptor from binding to its G protein and can direct its endocytosis (Figure 5.31).

Figure 5.31: The roles of G-protein-linked receptor kinases (GRKs) and arrestins in receptor desensitization



3) Signaling through Enzyme linked Cell Surface Receptors:

Enzyme-linked receptors are a second major type of cell-surface receptor. They were recognized initially through their role in responses to extracellular signal proteins that promote the growth, proliferation, differentiation, or survival of cells in animal tissues. These signal proteins are often collectively called growth factors, and they usually act as local mediators at very low concentrations (about 10^{-9}-10^{-11} M). The responses to them are typically slow (on the order of hours) and usually require many intracellular signaling steps that eventually lead to changes in gene expression. Enzyme-linked receptors have since been found also to mediate direct, rapid effects on the cytoskeleton, controlling the way a cell moves and changes its shape. Disorders of cell proliferation, differentiation, survival, and migration are fundamental events that can give rise to cancer, and abnormalities of signaling through enzyme-linked receptors have major roles in this class of disease.

These are transmembrane proteins with their ligand-binding domainon the outer surface of the plasma membrane and cytosolic domain either has an intrinsic enzyme activity or associates directly with an enzyme.

Six classes of enzyme-linked receptors include:

1.Receptor tyrosine kinases: They phosphorylate specific tyrosines on a small set of intracellular signaling proteins.

2.Tyrosine-kinase-associated receptors – They associate with intracellular proteins that have tyrosine kinase activity.

3.Receptor like tyrosine Phosphatases: They remove phosphate groups from tyrosines of specific intracellular signaling proteins.

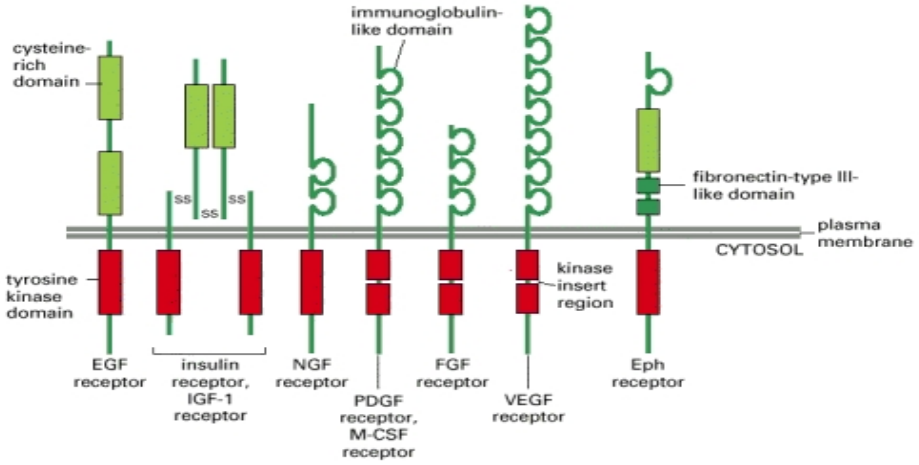
4.Receptor serine/threonine kinases – They phosphorylate specific serines or threonines on associated latent gene regulatory proteins.

5.Receptor guanylyl cyclases- they directly catalyze the production of cyclic GMP in the cytosol.

6.Histidine-kinase-associated receptors activate a "two-component" signaling pathway in which the kinase phosphorylates itself on histidine and then immediately transfers the phosphate to a second intracellular signaling protein.

1. Tyrosine receptor Kinases: Receptor tyrosine kinases consist of a large variety of secreted growth factors and hormones. Many cell-surface-bound signal proteins also act through these receptors. Few of these include: epidermal growth factor (EGF),platelet-derived growth factor (PDGF), fibroblast growth factors (FGFs), hepatocyte growth factor (HGF), insulin, insulinlike growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF), macrophage-colony-stimulating factor(M-CSF), and all the neurotrophins, including nerve growth factor (NGF).

Figure 5.32: Seven subfamilies of Receptor Tyrosine kinases



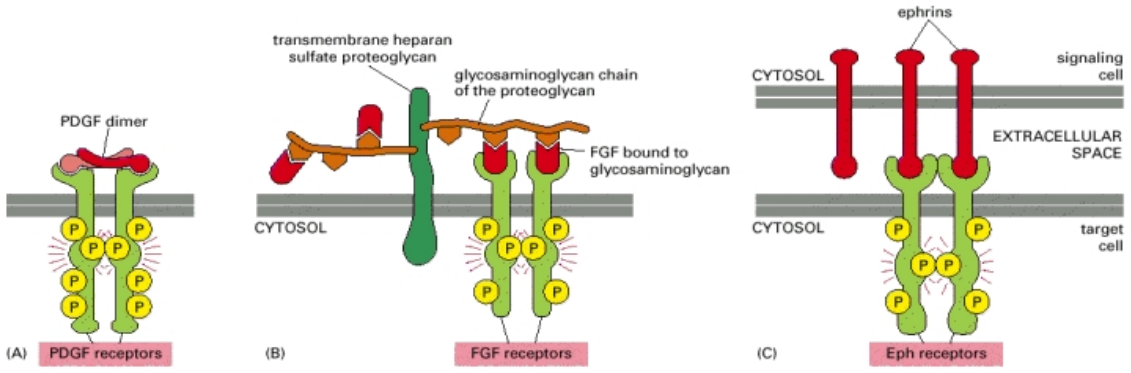
The largest class of these membrane-bound ligands is the ephrins, which regulate the cell adhesion and repulsion responses that guide the migration of cells and axons along specific pathways during animal development. The receptors for ephrins, called Eph receptors, are also the most numerous receptor tyrosine kinases. The ephrins and Eph receptors can simultaneously act as both ligand and receptor: on binding to an Eph receptor, some ephrins not only activate the Eph receptor but also become activated themselves to transmit signals into the interior of the ephrin-expressing cell. In this way, an interaction between an ephrin protein on one cell and an Eph protein on another cell can lead to bidirectional reciprocal signaling that changes the behavior of both cells.

Receptor tyrosine kinases can be classified into more than 16 structural subfamilies, each dedicated to its complementary family of protein ligands. Once activated, the kinase domain transfers a phosphate group from ATP to selected tyrosine side chains, both on the receptor proteins themselves and on intracellular signaling proteins that subsequently bind to the phosphorylated receptors.

When the receptor chains are cross-linked, the kinase domains of adjacent receptors cross-phosphorylate each other, stimulating the kinase activity of the receptor and creating docking sites for intracellular proteins. (A) Platelet-derived growth factor (PDGF) is a covalently linked dimer with two receptor-binding sites, so it can directly cross-link adjacent receptors to initiate the intracellular signaling process. The PDGF dimers are composed of A and B chains in different combinations, and they can activate different combinations of two types of PDGF receptor chains (α and β), which have somewhat different signaling properties. (B) Some monomeric ligands, such as fibroblast growth factors (FGFs), bind in clusters to proteoglycans, enabling the ligands to cross-link their receptors. The proteoglycans can be in the extracellular matrix or, as shown here, on the cell surface. There are more than 20 types of FGFs and more than 4 types of FGF receptors. (C) Membrane-bound signaling proteins, such as ephrins, can cross-link their receptors even though they are monomeric, because they cluster in the plasma membrane of the signaling cell. Some ephrins (B-type) are transmembrane proteins (as shown), whereas others (A-type) are linked to the membrane by a glycosylphosphatidylinositol (GPI)

anchor (Figure 5.33). For the most part, A-type ephrins activate A-type Eph receptors, and B-type ephrins activate B-type Eph receptors.

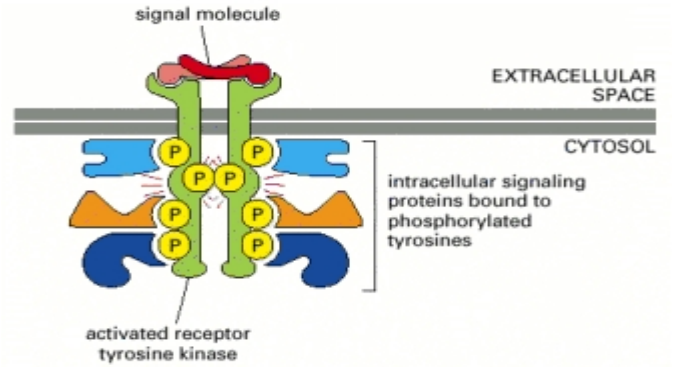
Figure 5.33: Three ways in which signaling proteins can cross-link receptor chains



Phosphorylated Tyrosine serves as docking site for proteins:

Autophosphorylation of the cytosolic tail of receptor tyrosine kinases contributes to the activation process in two ways. First, phosphorylation of tyrosines within the kinase domain increases the kinase activity of the enzyme. Second, phosphorylation of tyrosines outside the kinase domain creates high-affinity docking sites for the binding of a number of intracellular signaling proteins in the target cell. Each type of signaling protein binds to a different phosphorylated site on the activated receptor because it contains a specific phosphotyrosine-binding domain that recognizes surrounding features of the polypeptide chain in addition to the phosphotyrosine. Once bound to the activated kinase, the signaling protein may itself become phosphorylated on tyrosines and thereby activated; alternatively, the binding alone may be sufficient to activate the docked signaling protein. In summary, autophosphorylation serves as a switch to trigger the transient assembly of a large intracellular signaling complex, which then broadcasts signals along multiple routes to many destinations in the cell.

Figure 5.34: Docking of intracellular signaling proteins on an activated receptor tyrosine kinase



Phosphorylated Tyrosines acting as Docking sites:

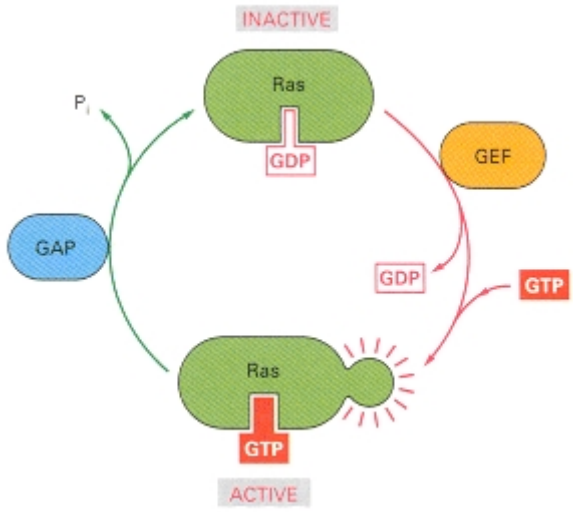
Although the intracellular signaling proteins that bind to phosphotyrosines on activated receptor tyrosine kinases and docking proteins have varied structures and functions. These can be either SH2 domains or, less commonly, PTB domains (phosphotyrosine-binding). By recognizing specific phosphorylated tyrosines, these small domains serve as modules that enable the proteins that contain them to bind to activated receptor tyrosine kinases, as well as to many other intracellular signaling proteins that have been transiently phosphorylated on tyrosines. Many signaling proteins also contain other protein modules that allow them to interact specifically with other proteins as part of the signaling process. These include the SH3 domain, which binds to proline-rich motifs in intracellular proteins. Some signaling proteins are composed almost entirely of SH2 and SH3 domains and function as adaptors to couple tyrosine-phosphorylated proteins to other proteins that do not have their own SH2 domains. Such adaptor proteins help to couple activated receptors to the important downstream signaling protein Ras.

Ras is activated by Guanine Nucleotide Exchange factor:

Ras proteins belong to the large Ras superfamily of monomeric GTPases, involved in relaying signals from cell-surface receptors to the actin cytoskeleton and the Rab family, involved in regulating the traffic of intracellular transport vesicles. The Ras proteins contain a covalently attached lipid group that helps to anchor the protein to the plasma membrane where the protein functions. There are multiple Ras proteins, and different ones act in different cell types.

Ras helps to broadcast signals from the cell surface to other parts of the cell. It is often required, for example, when receptor tyrosine kinases signal to the nucleus to stimulate cell proliferation or differentiation by altering gene expression. Ras functions as a switch, cycling between two distinct conformational states—active when GTP is bound and inactive when GDP is bound. Two classes of signaling proteins regulate Ras activity by influencing its transition between active and inactive states. Guanine nucleotide exchange factors (GEFs) promote the exchange of bound nucleotide by stimulating the dissociation of GDP and the subsequent uptake of GTP from the cytosol, thereby activating Ras. GTPase-activating proteins (GAPs) increase the rate of hydrolysis of bound GTP by Ras, thereby inactivating Ras (Figure 5-35). Mutation of Ras leads to cancer.

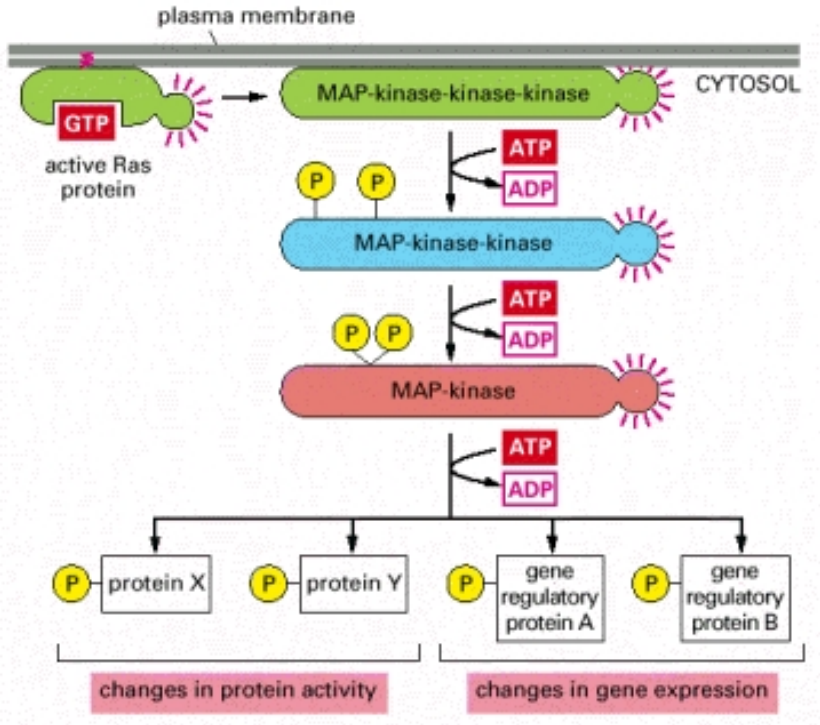
Figure 5.35 Regulation of Ras activity



Once activated, Ras in turn activates various other signaling proteins to relay the signal downstream along several pathways. One of the signaling pathways Ras activates is a serine/threonine phosphorylation cascade that is highly conserved in eukaryotic cells from yeasts to humans. A crucial component in this cascade is a novel type of protein kinase called MAP-kinase. Once activated, Ras in turn activates various other signaling proteins to relay the signal downstream along severalpathways. One of the pathways is Serine/Threonine phosphorylation

cascade. Crucial components of this cascade is Protein kinase MAP Kinase (Mitotic Activated Protein Kinase) (Figure 5.36).

Figure 5.36: MAP-kinase serine-threonine phosphorylation pathway activated by Ras



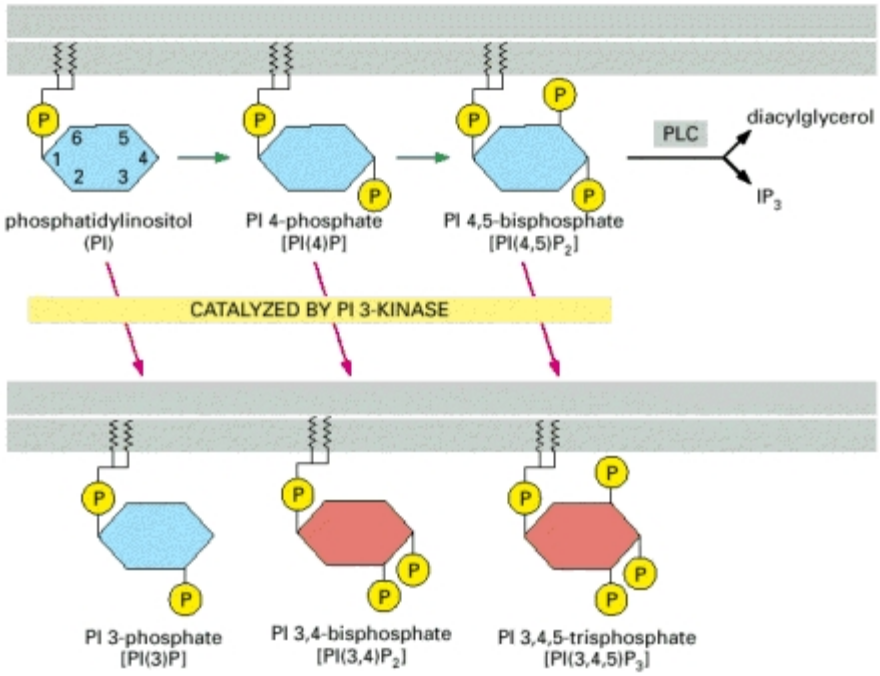
P13-Kinase produces Inositol Phospholipid Socking Sites

One of the major intracellular signaling pathways leading to cell growth involves phosphatidylinositol 3-kinase (PI 3-kinase). This kinase principally phosphorylates inositol phospholipids rather than proteins; it can be activated by receptor tyrosine kinases, as well as by many other types of cell-surface receptors, including some that are G-protein-linked.

Phosphatidylinositol (PI) is unique among membrane lipids because it can undergo reversible phosphorylation at multiple sites to generate a variety of distinct inositol phospholipids. When activated, PI 3-kinase catalyzes the phosphorylation of inositol phospholipids at the 3 position of the inositol ring to generate lipids called $PI(3,4)P_2$ or$PI(3,4,5)P_3$ (Figure 5.37). The $PI(3,4)P_2$ and $PI(3,4,5)P_3$ then serve as docking

sites for intracellular signaling proteins, bringing these proteins together into signaling complexes, which relay the signal into the cell from the cytosolic face of the plasma membrane.
The generation of inositol phospholipid docking sites by PI 3-kinase.
They remain in the plasma membrane until they are dephosphorylated by specific inositol phospholipid phosphatases that remove phosphate from the 3 position of the inositol ring. There are various types of PI 3-kinases. The one that is activated by receptor tyrosine kinases consists of a catalytic and regulatory subunit. The regulatory subunit is an adaptor protein that binds to phosphotyrosines on activated receptor tyrosine kinases through its SH2 domains. Another PI 3-kinase has a different regulatory subunit and is activated by the βγ complex of a trimeric G protein when G-protein-linked receptors are activated by their extracellular ligand. The catalytic subunit, which is similar in both cases, also has a binding site for activated Ras, which allows Ras to directly stimulate PI 3-kinases.

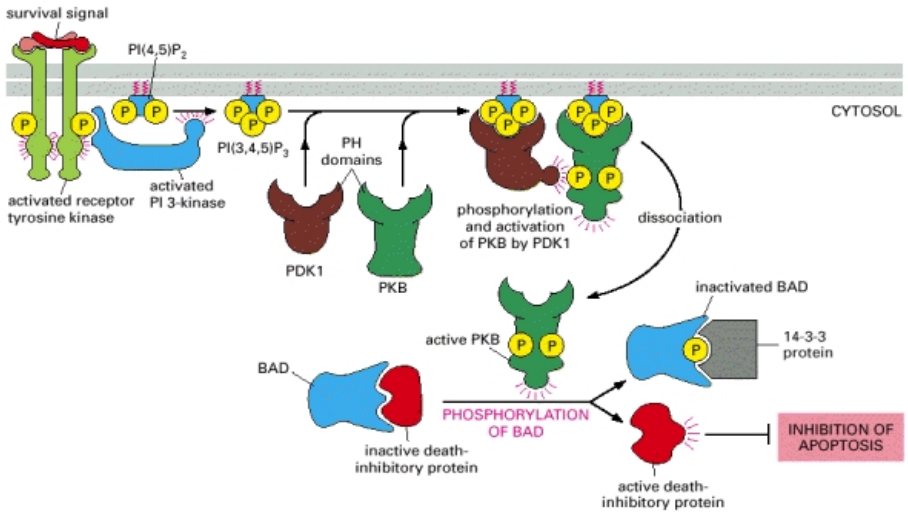
Figure 5.37: Generation of Inositol Phospholipid docking sites by PI 3- Kinases



PI 3-kinase promotes cell survival

An extracellular survival signal activates a receptor tyrosine kinase, which recruits and activates PI 3-kinase (figure 5.38). The PI 3-kinase produces $PI(3,4,5)P_3$ and $PI(3,4)P_2$ (not shown), both of which serve as docking sites for two serine/threonine kinases with PH domains—protein kinase B (PKB) and the phosphoinositol-dependent kinase PDK1. The binding of PKB to the inositol lipids alters its conformation so that the protein can be phosphorylated and activated by PDK1. The activated PKB now dissociates from the plasma membrane and phosphorylates the BAD protein, which, when unphosphorylated, holds one or more death-inhibitory proteins in an inactive state. Once phosphorylated, BAD releases the inhibitory proteins, which now can block programmed cell death (apoptosis) and thereby promote cell survival.

Figure 5.38: One way in which signaling thorugh PI 3 kinase promotes cell survival



Cytokine Receptor and Jak-STAT pathway:

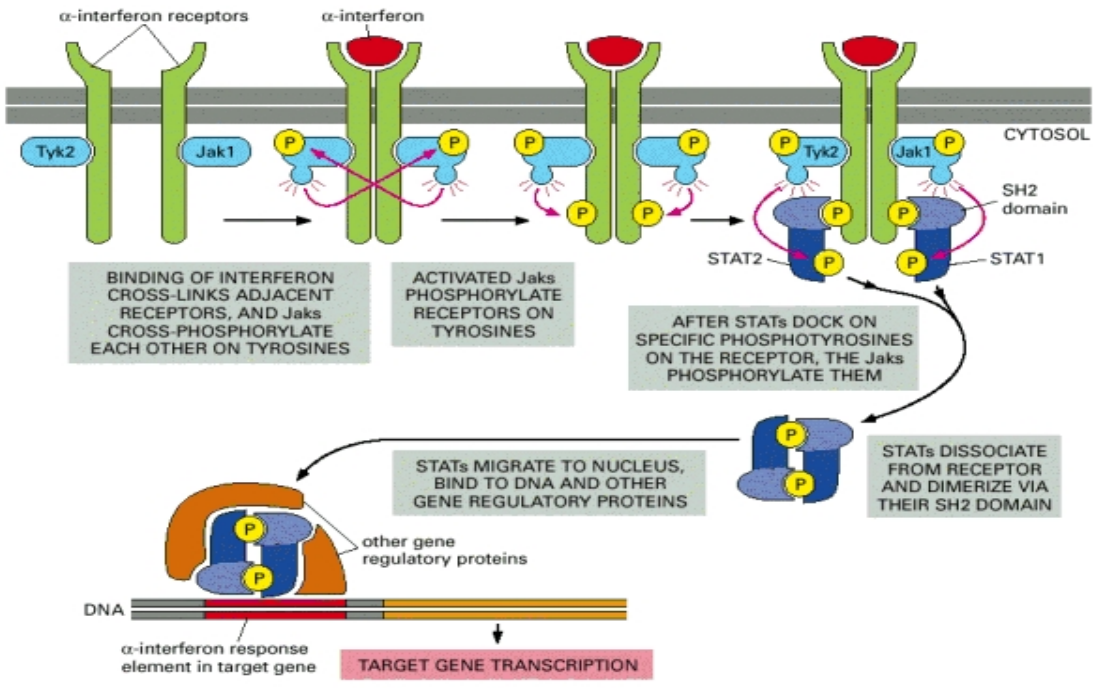
When activated, interferon receptors activate a novel class of cytoplasmic tyrosine kinases called Janus kinases (Jaks) (after the two-faced Roman god). The Jaks then phosphorylate and activate a set of latent gene regulatory proteins called STATs (signal transducers and activators oftranscription), which move into the nucleus and stimulate the transcription of specific genes. More than 30 cytokines and hormones activate the Jak-STAT pathway by binding to cytokine receptors. All STATs also have an SH2 domain that enables them to dock onto

specific phosphotyrosines on some activatedreceptor tyrosine kinase receptors. These receptors can directly activate the bound STAT, independently of Jaks.

Cytokine receptors are composed of two or more polypeptide chains. All cytokine receptors, however, are associated with one or more Jaks. There are four known Jaks—Jak1, Jak2, Jak3, and Tyk2—and each is associated with particular cytokine receptors. The receptors for α-interferon, for example, are associated with Jak1 and Tyk2, whereas the receptors for γ-interferon are associated with Jak1 and Jak2. The receptor for the hormone erythropoietin, which stimulates erythrocyte precursor cells to survive, proliferate, and differentiate, is associated with only Jak2.

There are seven known STATs, each with an SH2 domain that performs two functions. First, it mediates the binding of the STAT protein to a phosphotyrosine docking site on an activated cytokine receptor ; once bound, the Jaks phosphorylate the STAT on tyrosines, causing it to dissociate from the receptor. Second, the SH2 domain on the released STAT now mediates its binding to a phosphotyrosine on another STAT molecule, forming either a STAT homodimer or heterodimer which moves into the nucleus, where, in combination with other gene regulatory proteins, it binds to a specific DNA response element in various genes and stimulates their transcription (Figure 5.39).

Figure 5.39: The Jak-STAT signaling pathway activated by Alpha Interferon



Protein Tyrosine Phosphatases as cell surface receptors:

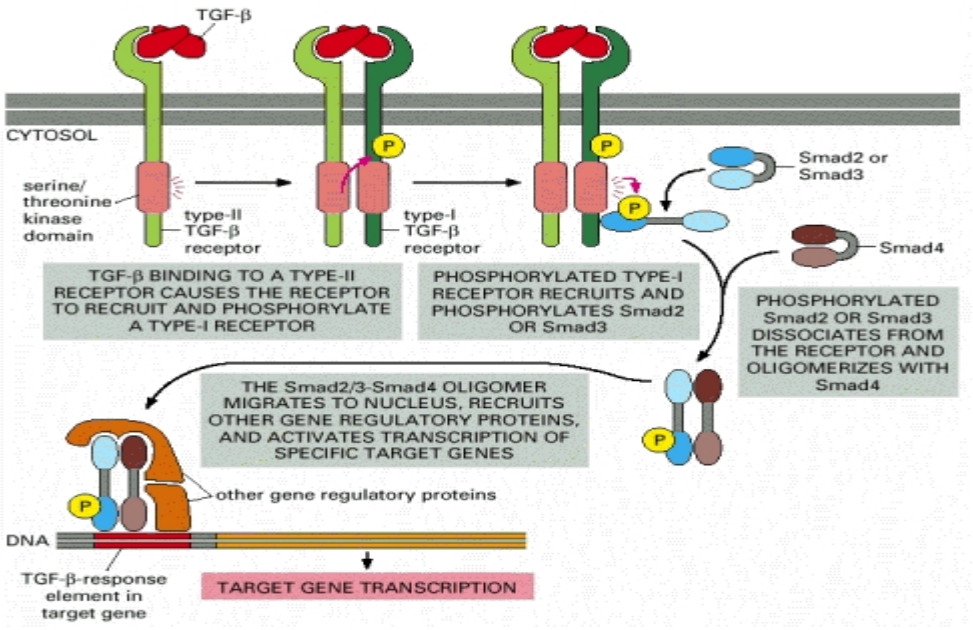
ProteinTyrosine Phosphatases (PTPs) are involved in removal of phosphate groups from selected phosphotyrosines on a subset of tyrosine-phosphorylated proteins. There are two tyrosine Phosphatases in vertebrates, having SH2 domains and these are SHP-1 and SHP-2. SHP-1helps to terminate some cytokine responses in blood cells by dephosphorylating activated Jacks, while both SHP-1 and SHP-2 help to terminate responses mediated by some receptor tyrosine kinases. Some receptor like tyrosine phosphatases display features of cell-adhesion proteins and can even mediate homophilic cell-cell binding in cell adhesion assays. Transmembrane tyrosine phosphatases can also serve as signaling ligands that activate receptors on a neighboring cell.

Signal proteins of TGF-β superfamily

The transforming growth factor -β superfamily consists of large number of structurally related, secreted, dimeric proteins, which either act as hormones or local mediators. During development they regulate pattern formation and influence various cell behaviors, including proliferation, differentiation, extracellular matrix production and cell death.

There are two classes of these receptorserine/threonine kinases—type I and type II—which are structurally similar. Each member of the TGF-β superfamilybinds to a characteristic combination of type-I and type-II receptors, both of which are required for signaling. Typically, the ligand first binds to and activates a type-II receptor homodimer, which recruits, phosphorylates, and activates a type-I receptor homodimer, forming an active tetrameric receptor complex. The type-I receptor directly binds and phosphorylates a latent gene regulatory protein of the Smad family. Activated TGF-β receptors and activin receptors phosphorylate Smad2 or Smad3, while activated BMP receptors phosphorylate Smad1, Smad5, or Smad8. Once one of these Smads has been phosphorylated, it dissociates from the receptor and binds to Smad4, which can form a complex with any of the above five receptor-activated Smads. The Smad complex then moves into the nucleus, where it associates with other gene regulatory proteins, binds to specific sites in DNA, and activates a particular set of target genes.

Figure 5.40: A smad dependent signaling pathway activated by TGF-β



Receptor guanylyl cyclases: These are single-pass transmembrane proteins with an extracellular binding site for a signal molecule and an intracellular guanylyl cyclase catalytic domain. The binding of the signal molecule activates the cyclase domain to produce cyclic GMP, which in turn binds to and activates a cyclic GMP-dependent protein kinase (PKG),which phosphorylates specific proteins on serine or threonine. Thus, receptor guanylyl cyclases use cyclic GMP as an intracellular mediator in the same way that some G-protein-linked receptors use cyclic AMP, except that the linkage between ligand binding and cyclase activity is a direct one.

Among the signal molecules that use receptor guanylyl cyclase receptors are the natriuretic peptides (NPs), a family of structurally related secreted signal peptides that regulate salt and water balance and dilate blood vessels. There are several types of NPs, including atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP). Muscle cells in the atrium of the heart secrete ANP when blood pressure rises. The ANP stimulates the kidneys to secrete Na^+ and water and induces the smooth muscle cells in blood vessels walls to relax. Both of these effects tend to lower the blood pressure.

Histidine- kinase associated Receptors:

Bacterial movements are mediated by histidine-kinase-associated chemotaxis receptors, which typically are dimeric transmembrane proteins that bind specific attractants and repellents on the outside of the plasma membrane. The cytoplasmic tails of the receptors are stably associated with an adaptor protein CheW and a histidine kinase CheA, which help to couple the receptors to the flagellar motor. Repellent binding activates the receptors, whereas attractant binding inactivates them; a single receptor can bind either type of molecule, with opposite consequences. The binding of a repellent to the receptor activates CheA, which phosphorylates itself on a histidine and almost immediately transfers the phosphate to an aspartic acid on a messenger protein CheY. The phosphorylated CheY dissociates from the receptor, diffuses through the cytosol, binds to the flagellar motor, and causes the motor to rotate clockwise, so that the bacterium tumbles. CheY has intrinsic phosphatase activity and dephosphorylates itself in a process that is greatly accelerated by the CheZ protein (Figure 5.41).

Figure 5.41: Two component signal pathway that enables chemotactic receptors to control bacterial chemotaxis