CELL SIGNALING

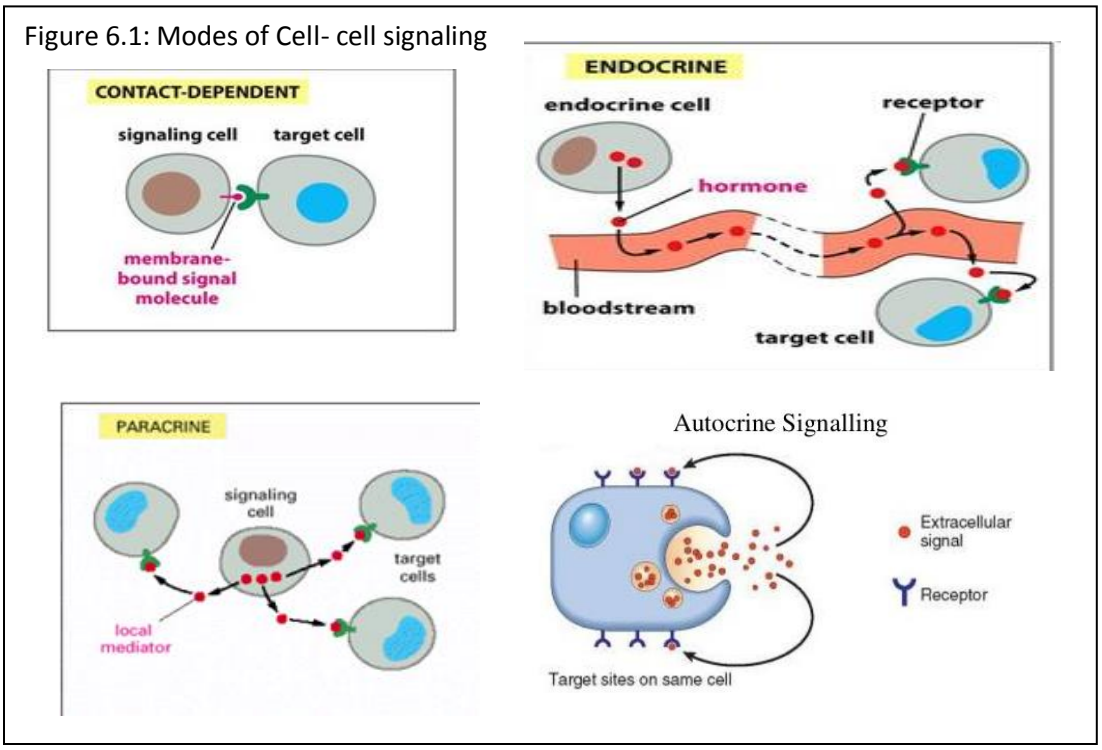
Cell signaling includes, binding of these to appropriate receptors, initiating series of intracellular reactions that regulate various aspects of cell behavior, including metabolism, movement, proliferation, survival and differentiation.

Modes of Cell – Cell signaling: Cell signaling can result either the direct interaction of a cell with the neighboring cell or from the action of secreted signaling molecules (Figure 6.1). The signaling system involving secretion of several molecules are categorized into three- Endocrine signaling, Paracrine signaling and Autocrine signaling.

In direct cell-cell signaling or contact dependent cell signaling. In this the cells exchange signals with neighboring cell by way of integrins and cadherins or via cell adhesion molecules. This is required for cell involved in development.

Endocrine signaling involves release of hormones that are carried by circulation to target cells. Animals produce more than 50 different types of signals by various endocrine glands. In Paracrine signaling, a molecule released by one cell, acts on neighboring target cells, Eg:nerve impulse transmission across neurons via acetylcholine. Autocrine signaling is the release of signal molecules that bind to the same cell. Eg:T_H cells secrete IL-2 to stimulate themselves.

Figure 6.1: Modes of Cell- cell signaling



I. CELL SIGNALING MOLECULES

Various cell signaling molecules are categorized as follows:

1. Steroid hormones
2. Nitric oxide and carbon monoxide
3. Neurotransmitters
4. Peptide hormones and growth hormones
5. Eicosnoids
6. Plant hormones

1) Steroid hormones: These include hormones that are synthesized from cholesterol – testosterone, estrogen, progesterone, the corticosteroids and ecdysone, thyroid hormones, Vitamin D3 and retinoic acid. Testosterone, estrogen and progesterone are the sex steroids which are produced by the gonads and Corticosteroids are produced by the adrenal gland. Corticosteroids include Glucocorticoids that stimulate production of glucose and the mineralocorticoids which act on kidney to regulate salt and water balance. Ecdysone is a insect hormone that triggers metamorphosis of larvae to adults. Brassinosteroids are plant specific steroid hormones that control developmental processes, including cell growth and differentiation. Thyroid hormones are synthesized from tyrosine in the thyroid gland which plays important role in regulation of metabolism. Vitamin D_3 regulates Ca^{2+} metabolism and bone growth while retinoic acid synthesized from vitamin A plays important role in vertebrate development.

All the bind to a family of receptors known as Nuclear receptor superfamily, which can enter the cells by diffusion through the plasma membrane, bind to intracellular receptors, which inturn bind to specific sites on DNA leading to gene regulation (Activation/ suppression) (figure 6.2).

2) Nitric oxide and carbon monoxide: Nitric oxide is a major Paracrine signaling molecule in the nervous, immune and circulatory systems. It can directly diffuse across plasma membrane of its target cells. NO is synthesized from amino acid arginine by the enzyme nitric oxide synthase and can affect cells nearby too. But its half life is few seconds and is eliminated. The major intracellular target for NO is guanylyl cyclase. NO is produced by endothelial cells of blood vessels when stimulated by aetylcholine. This can diffuse down to the smooth muscles present

below the blood vessel, binds to this enzyme guanylyl cyclase resulting in the synthesis of cyclic GMP that that induces smooth muscle relaxation and increases blood flow to the heart (figure 6.3).

Carbon monoxide (CO) functions as a signal molecule in the nervous system. Synthesis of CO in brain cells can stimulate guanylyl cyclase which finally leads relaxation of smooth muscles.

Figure 6.2: Steroid hormone and a nuclear binding receptor mediated gene regualtion

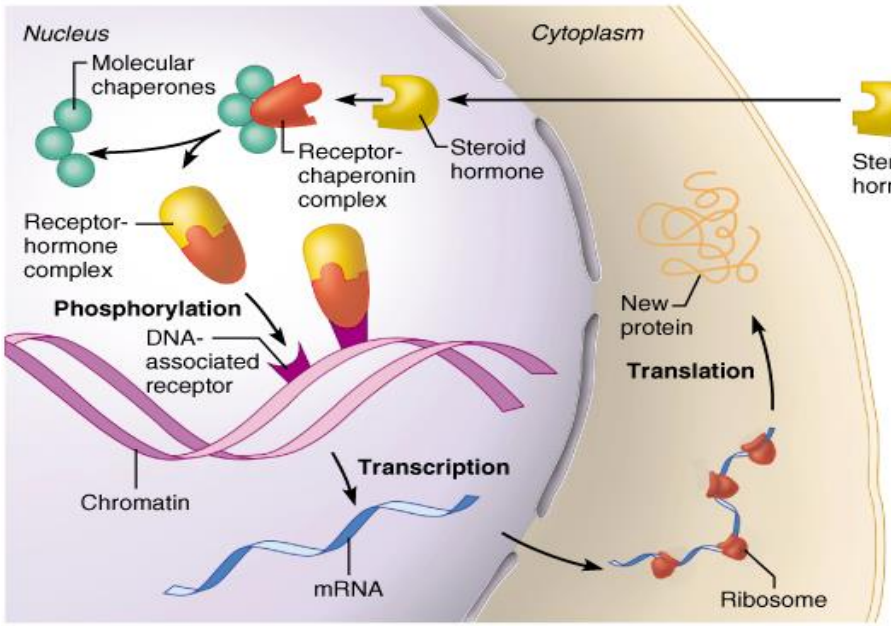
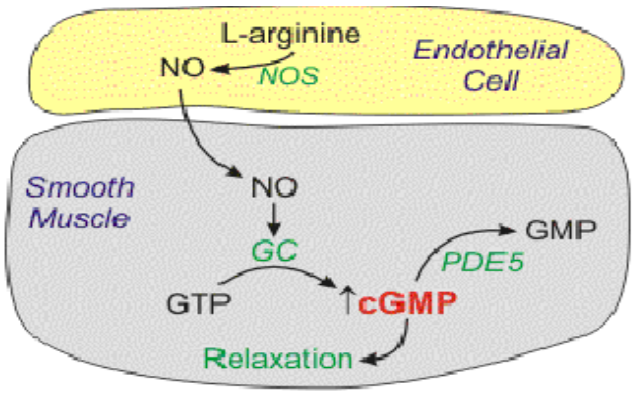


Figure 6.3: NO signaling smooth muscle contraction



3) Neurotransmitters: They carry signals between neurons or between neurons and other target cells. These hydrophilic molecules include- acetylcholine, dopamine, epinephrine, serotonin, histamine, glutamate, glycine and gama- aminobutyric acid (GABA). These bind to cell curface receptors there by inducing conformational changes, that open ion channels directly resulting in changes in ion flux in the target cell. While few of these bind to G-protein receptors and regulate cell activities.

4) Peptide Hormones and Growth factors:

This group includes peptide hormones, neuropeptides, and a diverse array of polypeptide growth factors and are listed table below. Peptide hormones are produced by pituitarygland, neuropeptides are secreted by neurons. Among these enkephalins and endorphins decrease pain responses in central nervous system. Polypeptide growth hormones include variety of signals that control animal cell growth and differentiation. Platelet derived growth factor (PDGF) is produced in wound healing and is stored in Blood platelets.

Signaling Molecules	Activities
Peptide Hormones	
Insulin	Regulation of glucose uptake; Stimulation of cell proliferation
Glucagon	Stimulation of glucose synthesis
Growth hormone	General stimulation of growth
Follicle- stimulating hormone (FSH)	Stimulation of the growth of oocytes and ovarian follicles
Prolactin	Stimulation of milk production
Neuropeptides and neurohormones	
Substance P	Sensory synaptic transmission
Oxytocin	Stimulation of smooth muscle contraction
Vasopressin	Stimulation of water reabsorption in the kidney
Enkephalin	Analgesic
β– Endorphin	Analgesic
Growth factors	
Nerve growth factor (NGF)	Differentiation and survival of neurons
Epidermal growth factor (EGF)	Proliferation of many types of cells
Platelet-derived growth factor (PDGF)	Proliferation of fibroblasts and other types of cells
Interleukin – 2	Proliferation of T lymphocytes
Erythropoietin	Development of RBC

5. Eicasanoids: These are lipids which serve as signaling molecules and act by binding to cell surface receptors. These include- prostaglandins, prostacyclin, thromoxanes and leukotrienes. These are a part of autocrine and paracrine signaling pathways. They are involved in variety of responses like blood platelet aggregation, inflammation and smooth muscle contraction.

These are synthesized from arachidonic acid which is formed from phospholipids. The first step in the pathway pathway leading to synthesis of either prostaglandins or thromboxanes is the conversion of arachidonic acid to prostaglandin H_2. The sme enzyme cyclooxygenase is the target of aspirin and other nonsteroidal anit-inflammatory drugs (NSAIDs). Reduction in the synthesis of prostaglandins by aspirin reduces inflammation and pain. By inhibiting synthesis of thromboxanes, aspirin also reduced platelet aggregation and blood clotting. Because of this activity, small daily doses of aspirin are frequently prescribed for prevention of strokes. In addition, aspirin and NSAIDs have been found to reduce the frequency of colon cancer in human by inhibiting synthesis of prostaglandins that actually stimulate cell proliferation and promote cancer development.

6. Plant hormones: Plant growth and development is regulated by these, and their levels are modified by environmental factors like sun light, infection etc. These are divided into five major groups- auxins, gibberellins, cytokinins, abscisic acid and ethylene. Auxins induce plant cell proliferation by weakening the cell wall. They regulate cell division and differentiation. Gibberlins are responsible for stem elongation; ethylene is involved in fruit ripening, cytokinins in cell division and abscisic acid onset of dormancy.

II. CELL SURFACE RECEPTORS

These are the sites to which cell signaling molecules bind. The receptors under study include:

1. G-protein coupled receptors
2. Receptor Protein –Tyrosine kinase
3. Cytokine receptors and Nonreceptor Protein- Tyrosine Kinases
4. Receptors linked to other enzymatic activities.

1. G-protein coupled receptors:

The largest family of cell surface receptors are the G-protein coupled receptors (GPCRs). There are hundreds of different GPCR proteins, and they are the targets of many drugs. A diverse set of ligands bind to this type of receptors including peptide hormones, neurotransmitters, neuropeptides and eicosonoids. These receptors all have a similar structure with seven transmembrane domains. On the basis of their seven transmembrane domain structure, many GPCRs have been identified in the human genome. These are known as orphan receptors, because their ligands have not been identified.

GPCRs are associated with heterotrimeric G-proteins i.e., G-proteins composed of three different subunits: alpha, beta, and gamma. The subunits are tethered at the membrane surface by covalently attached lipid molecules.

When a ligand binds, the receptor activates the attached G-protein by causing the exchange of GTP for GDP. The activated G-protein then dissociates into an alpha (G-alpha) and a beta-gamma complex. G-alpha bound to GTP is active, and can diffuse along the membrane surface to activate target proteins, often enzymes that generate second messengers. Likewise, the beta-gamma complex is also able to diffuse along the inner membrane surface and activate proteins, typically affecting ion channels.

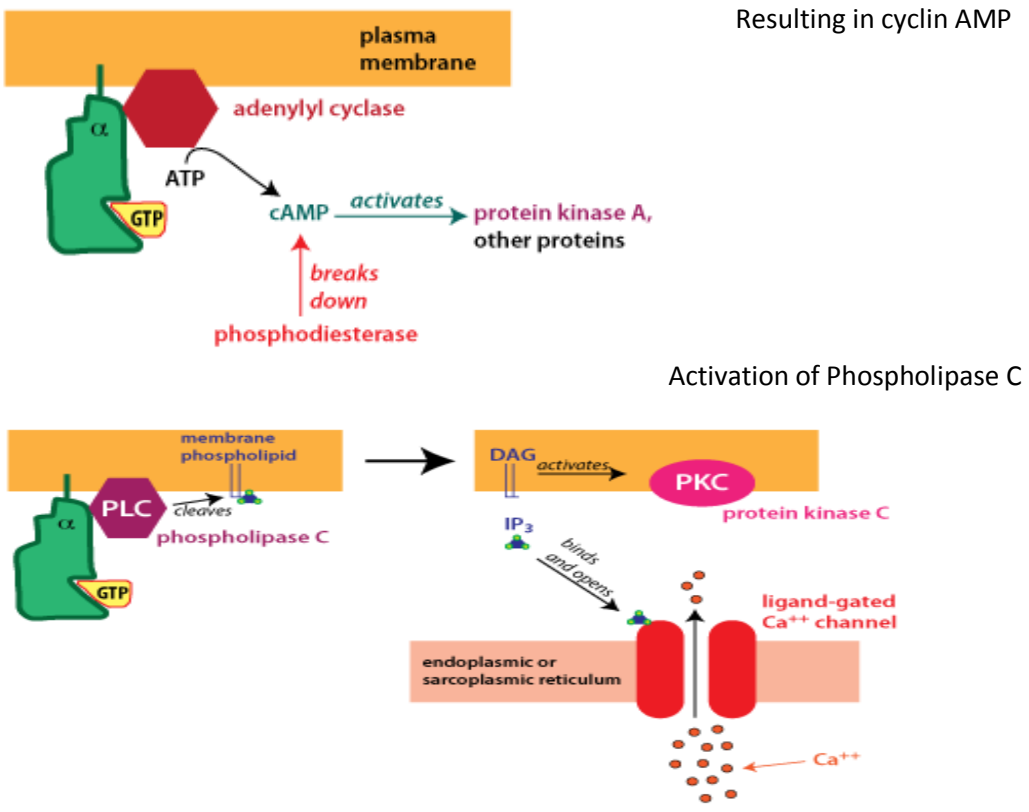
Inactivation occurs because G-alpha has intrinsic GTPase activity. After GTP hydrolysis, G-alpha bound to GDP will reassociate with a beta-gamma complex to form an inactive G-protein that can again associate with a receptor. The GTPase activity of the G-alpha can be made faster by other proteins which include the target protein or by a separate regulatory protein.

Different G-alpha proteins activate different second messenger pathways (Figure 6.4)

There are several different classes of heterotrimeric G-proteins that are defined by their different G-alpha subunits. One type of G-alpha activates the enzyme adenylyl cyclase, which catalyzes the formation of the second messenger cyclic AMP (cAMP). Because an activated adenylyl cyclase can generate many molecules of cAMP it will result in signal amplification. cAMP can have several effects, but a major effect is to bind to and activate protein kinase A (PKA; also known as cAMP-dependent kinase). PKA then phosphorylates target proteins in the cell. cAMP is rapidly broken down by phosphodiesterases, limiting the length of the signal.

Another type of G-alpha activates the enzyme phospholipase C. Phospholipase C cleavesPI P2, a membrane phospholipid, to generate two second messengers, IP_3 and diacylglycerol (DAG). IP_3 is water soluble, diffusing through the cytosol to bind to and open a ligand-gated Ca^{++} channel in the endoplasmic reticulum (or sarcoplasmic reticulum in muscle cells). Thus, stimulation of a receptor linked to this G-alpha is a way to increase Ca^{++} inside the cytosol. Ca^{++} in the cytosol exerts its effects by binding to Ca^{++}-binding proteins such as calmodulin. DAG is lipid soluble and stays in the membrane. It activates protein kinase C (PKC), which, like PKA phosphorylates particular target proteins.

Figure 6.4 G-Protein activities and the two secondary messenger pathways



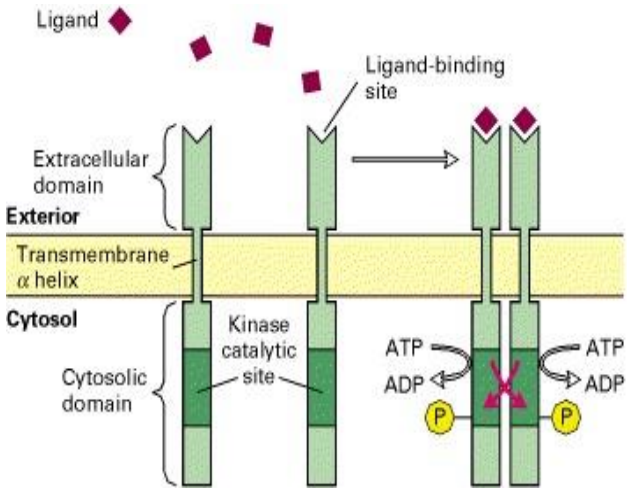
Desensitization

In the continuing presence of ligand, many GPCRs show desensitization. The mechanism is shown in the figure. A protein known as a G-proteinReceptor Kinase (GRK) phosphorylates the receptor on particular residues. This increases its affinity for a protein called beta-arrestin, that

binds to the receptor and reduces signaling by preventing the association with the G-protein. Beta arrestin can target the receptor for endocytosis, leading to receptor down regulation.

2) Receptor Protein- Tyrosine Kinases: These phosphorylate their substrate proteins on tyrosine residues. Human genome encodes 59 receptor protein –tyrosine kinases, including receptors for EGF, NGF, PDGF, Insulin and many other growth factors. All these receptors have a common structural organization: an N-terminal extracellular Ligand-binding domain, a single transmembrane alpha helix, and a cytosolic C-terminal domain with protein- tyrosine kinase activity. First step in signaling is, dimerization of receptor which is Ligand induced. Some factors lead to dimerization by binding to a different site on the dimer promoting binding of different receptor molecules, while few induce conformational changes. These dimerized polypeptides cross phosphorylate each other, which is autophosphorylation of the receptor(Figure 6.5). Fist phosphorylation increases catalytic activity while second one at catalytic domain creates specific binding sites for additional proteins that transmit intracellular signals downstream of the activated receptor. The association of these downstream signaling molecules with receptor protein – tyrosine kinases is mediated by protein domains that bind to specific phosphotyrosine- containing peptides. 1) SH2 domains (Src homology 2) and 2) PTB domains.

Figure 6.5: General structure and activation of Receptor Tyrosine kinases

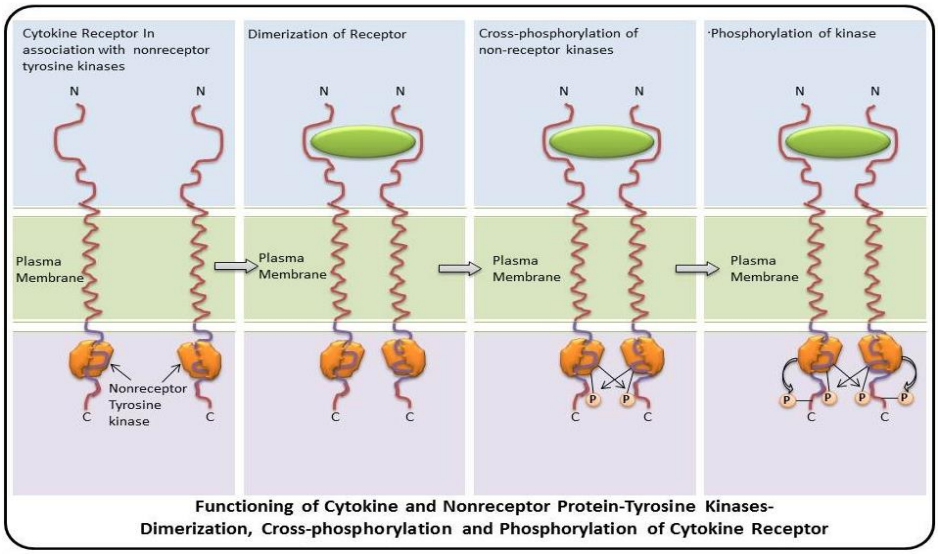


3) Cytokine receptors and Nonreceptor Protein- Tyrosine Kinases:

These include receptors for cytokines like Interleukin- 2, erythropoietin etc and even few polypeptide hormones like growth hormone. Cytokine receptors contain N-terminal extracellular Ligand-binding domains, single transmembrane alpha helix, and C-terminal cytoplasmic domains. These function in association with nonreceptor protein-tyrosine kinases, which are activated as a result of ligand binding. First step in their activation is Ligand induced dimerization and cross phosphorylation of the associated non-receptor protein tyrosine kinases(figure 6.6). These activated kinases then phosphorylate the receptor, providing phosphorylation binding sites for the recruitment of downstream signaling molecules that contain SH-2 domains.

Kinases associated with cytokine receptors belong to the family of Janus kinases (JAK) family. These are required universally for signaling from cytokine receptors.

Figure 6.6: Signaling from cytokine receptors



4) Receptors linked to other Enzymatic activities:

Though most of the enzyme-linked receptors stimulate protein-tyrosine phosphorylation, some receptors are associated with other enzymatic activities. These receptors include Protein-tyrosine Phosphatases, Protein-serine/Threonine kinases and guanylyl cyclases. Protein Tyrosine Phosphatases remove phosphate groups from phosphotyrosine residues, and this activity plays a

role of counterbalancing the effect of protein tyrosine kinases. There are certain Phosphatases which are cell surface receptors. There are 21 such receptor protein-tyrosine Phosphatases encoded in the human genome. Eg: CD45 dephosphorylase, transforming growth factor-β etc.

III. Pathways of Intracellular Signal Transduction

These are a chain of reactions transmitted from cell surface to a variety of intracellular targets and this phenomenon is called Intracellular signal transduction. The targets of such signaling pathways frequently include transcription factors that function to regulate gene expression. Intracellular signaling pathways thus connect the cell surface to the nucleus, leading to changes in gene expression in response to extracellular stimuli. These pathways include:

1. The cAMP pathway: secondary messengers and protein phosphorylation
2. Cyclic GMP
3. Phospholipids and Ca^{2+}
4. The PI 3-kinase/Akt and mTOR pathways
5. MAP kinase pathways
6. The JK/STAT and TGFβ / Smad Pathways
7. NF- κB signaling
8. The Hedgehog, Wnt and Notch pathways

1. The cAMP pathway: secondary messengers and protein phosphorylation:

Intracellular signaling was first elucidated by studies of the action of hormones such as epinephrine, which signals the breakdown of glycogen to glucose in anticipation of muscular activity. Epinephrine mediates this by an increase in the intracellular concentration of cyclic AMP (cAMP), leading to the concept that cAMP is a second messenger in hormonal signaling (the first messenger being the hormone itself). Cyclic AMP is formed from ATP by the action of adenylyl cyclase and degraded to AMP by cAMP phosphodiesterase (Figure 6.7). The epinephrine receptor is coupled to adenylyl cyclase via a G protein that stimulates enzymatic activity, thereby increasing the intracellular concentration of cAMP (Figure 6.8).

Most other effects of cAMP in animal cells are mediated by the action of cAMP-dependent protein kinase, or protein kinase A. The inactive form of protein kinase A is a tetramer consisting of two catalytic and two regulatory subunits. Cyclic AMP binds to the regulatory subunits,

leading to their dissociation from the catalytic subunits. The free catalytic subunits are then enzymatically active and able to phosphorylate serine residues on their target proteins. In the regulation of glycogen metabolism, protein kinase A phosphorylates two key target enzymes (Figure 6.9). The first is another protein kinase, phosphorylase kinase, which is phosphorylated and activated by protein kinase A. Phosphorylase kinase in turn phosphorylates and activates glycogen phosphorylase, which catalyzes the breakdown of glycogen to glucose-1-phosphate. In addition, protein kinase A phosphorylates the enzyme glycogen synthase, which catalyzes glycogen synthesis. In this case, however, phosphorylation inhibits enzymatic activity. Elevation of cAMP and activation of protein kinase A thus blocks further glycogen synthesis at the same time as it stimulates glycogen breakdown (figure 6.10).

In many animal cells, increases in cAMP activate the transcription of specific target genes that contain a regulatory sequence called the cAMP response element, or CRE (Figure 6.11). In this case, the signal is carried from the cytoplasm to the nucleus by the catalytic subunit of protein kinase A, which is able to enter the nucleus following its release from the regulatory subunit. Within the nucleus, protein kinase A phosphorylates a transcription factor called CREB (for CRE-binding protein), leading to the activation of cAMP-inducible genes. Such regulation of gene expression by cAMP plays important roles in controlling the proliferation, survival, and differentiation of a wide variety of animal cells.

Figure 6.7: Synthesis and degradation of cAMP

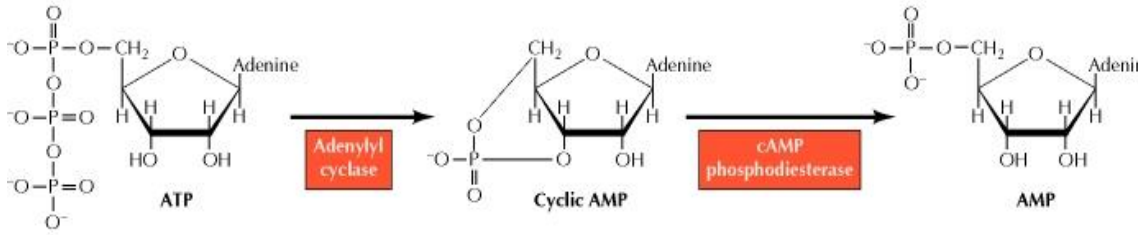


Figure 6.8: G Protein mediated induction of Adenylyl cyclase increase cAMP

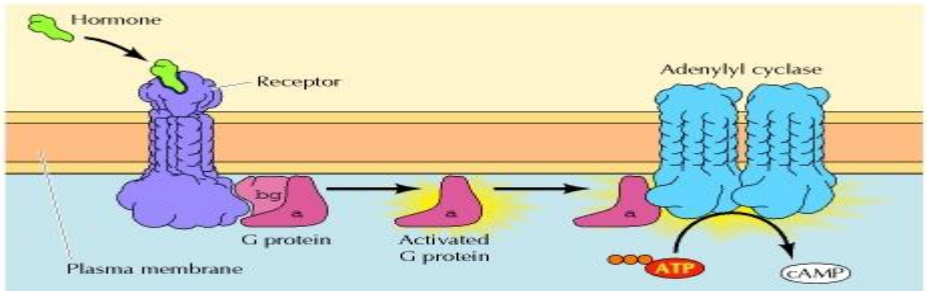


Figure 6.10: Regulation of Glycogen metabolism by Protein Kinase A

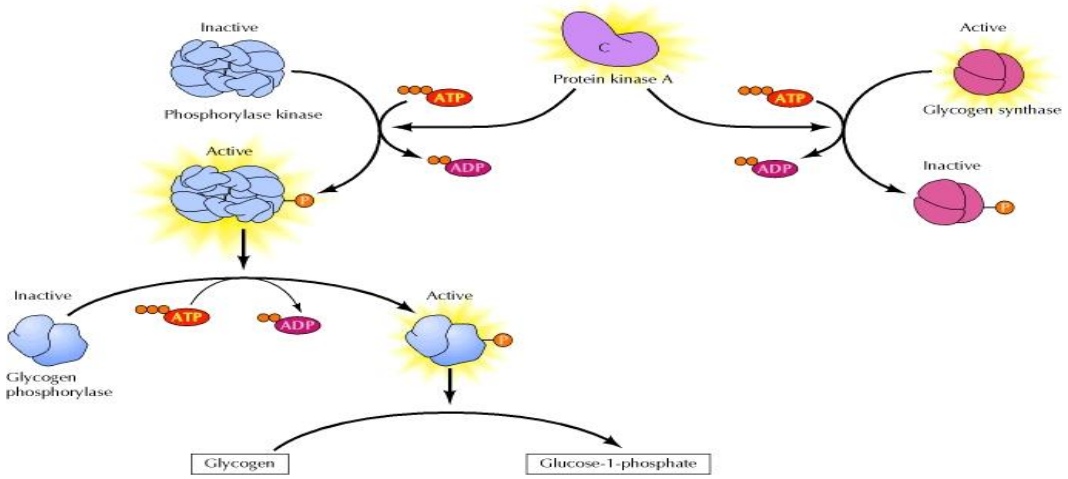
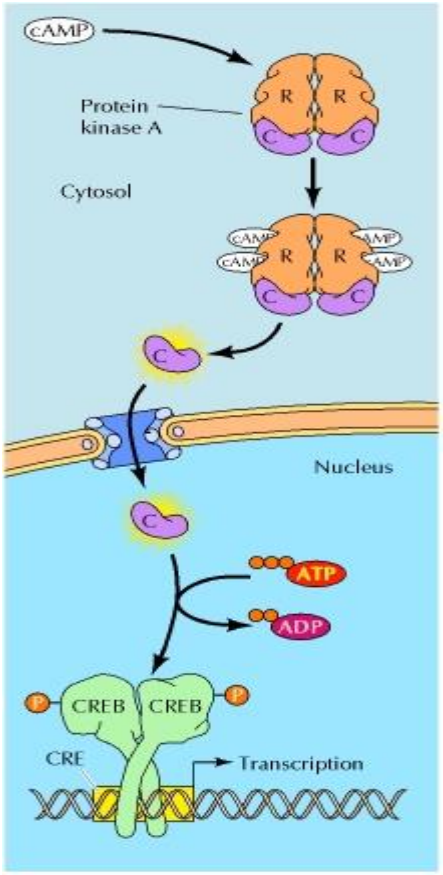


Figure 6.11: CyclicAMP induced gene expression

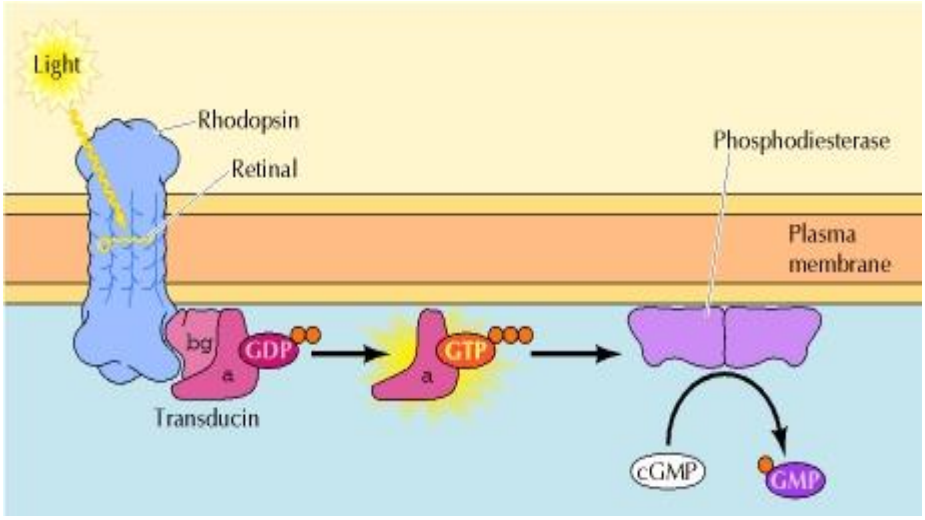


2. Cyclic GMP

Cyclic GMP is formed from GTP by guanylyl cyclases and degraded to GMP by a phosphodiesterase. Different types of guanylyl cyclases are activated by both nitric oxide and peptide ligands. Stimulation of these guanylyl cyclases leads to elevated levels of cGMP, which then mediate biological responses, such as blood vessel dilation. The action of cGMP is frequently mediated by activation of a cGMP-dependent protein kinase, although cGMP can also act to regulate other targets, including ion channels.

The best-characterized role of cGMP is in the vertebrate eye, where it serves as the second messenger responsible for converting the visual signals received as light to nerve impulses. The photoreceptor in rod cells of the retina is a G protein-coupled receptor called rhodopsin (Figure 6.12). Rhodopsin is activated as a result of the absorption of light by the associated small molecule 11-cis-retinal, which then isomerizes to all-trans-retinal, inducing a conformational change in the rhodopsin protein. Rhodopsin then activates the G protein transducin, and the α subunit of transducin stimulates the activity of cGMP phosphodiesterase, leading to a decrease in the intracellular level of cGMP. This change in cGMP level in retinal rod cells is translated to a nerve impulse by a direct effect of cGMP on ion channels in the plasma membrane, similar to the action of cAMP in sensing smells.

Figure 6.12: Role of cGMP in Photoreception



3. Phospholipids and Ca^{2+}:

Many pathways of intracellular signaling is based on the use of second messengers derived from the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP_2). PIP_2 is a minor component of the plasma membrane, localized to the inner leaflet of the phospholipid bilayer. A variety of hormones and growth factors stimulate the hydrolysis of PIP_2 by phospholipase C—a reaction that produces two distinct second messengers, diacylglycerol and inositol 1,4,5-trisphosphate (IP_3). Diacylglycerol and IP_3 stimulate distinct downstream signaling pathways (protein kinase C and Ca^{2+} mobilization, respectively), so PIP_2 hydrolysis triggers a two-armed cascade of intracellular signaling.

Hydrolysis of PIP_2 is activated downstream of both G protein-coupled receptors and protein-tyrosine kinases. This occurs because one form of phospholipase C (PLC-β) is stimulated by G proteins, whereas a second (PLC-γ) contains SH2 domains that mediate its association with activated receptor protein-tyrosine kinases. This interaction localizes PLC-γ to the plasma membrane as well as leading to its tyrosine phosphorylation, which increases its catalytic activity (figure 6.13).

The diacylglycerol produced by hydrolysis of PIP_2 activates protein-serine/threonine kinases belonging to the protein kinase C family, many of which play important roles in the control of cell growth and differentiation. Whereas diacylglycerol remains associated with the plasma membrane, the other second messenger produced by PIP_2cleavage, IP_3, is a small polar molecule that is released into the cytosol, where it acts to signal the release of Ca^{2+} from intracellular stores. IP_3 acts to release Ca^{2+} from the endoplasmic reticulum (where it is present bound to calmodulin) by binding to receptors that are ligand-gated Ca^{2+}channels. As a result, cytosolic Ca^{2+} levels increases, which affects the activities of a variety of targetproteins, including protein kinases and phosphatases. For example, some members of the protein kinase C family require Ca^{2+} as well as diacylglycerol for their activation, so these protein kinases are regulated jointly by both arms of the PIP_2signaling pathway (figure 6.14).

Many effects of Ca^{2+} are mediated by the Ca^{2+}-binding protein calmodulin, which is activated by Ca^{2+} binding when the concentration of cytosolic Ca^{2+} increases to about 0.5 μM. Ca^{2+}/calmodulin then binds to a variety of target proteins, including protein kinases. One example of such a Ca^{2+}/calmodulin-dependent protein kinaseis myosin light-chain kinase, which

signals actin-myosin contraction by phosphorylating one of the myosin light chains (Figure 6.15). Other protein kinases that are activated by Ca^{2+}/calmodulin include members of the CaM kinase family, which phosphorylate a number of different proteins, including metabolic enzymes, ion channels, and transcription factors. One form of CaM kinase is particularly abundant in the nervous system, where it regulates the synthesis and release of neurotransmitters. In addition, CaM kinases can regulate gene expression by phosphorylating transcription factors. Interestingly, one of the transcription factors phosphorylated by CaM kinase is CREB, which is phosphorylated at the same site by protein kinase A. This phosphorylation of CREB illustrates one of many intersections between the Ca^{2+} and cAMP signaling pathways. Other examples include the regulation of adenylyl cyclases and phosphodiesterases by Ca^{2+}/calmodulin, the regulation of Ca^{2+} channels by cAMP, and the phosphorylation of a number of target proteins by both protein kinase A and Ca^{2+}/calmodulin-dependent protein kinases. The cAMP and Ca^{2+} signaling pathways thus function coordinately to regulate many cellular responses.

4) The PI 3-Kinase/Akt and mTOR Pathway

PIP_2 not only serves as the source of diacylglycerol and IP_3, but is also the starting point of a distinct second messenger pathway that plays a key role in regulating cell survival. In this pathway, PIP_2 is phosphorylated on the 3 position of inositol by the enzyme phosphatidylinositide (PI) 3-kinase (Figure 6.16). Like phospholipase C, one form of PI 3-kinase is activated by G proteins, while a second has SH2 domains and is activated by association with receptor protein-tyrosine kinases. Phosphorylation of PIP_2 yields phosphatidylinositol 3,4,5-trisphosphate (PIP_3), which functions as a distinct second messenger. A key target of PIP_3, which is critical for signaling cell survival, is a protein-serine/threonine kinase called Akt. PIP_3 binds to a domain of Akt known as the pleckstrin homology domain (figure 6.17). This interaction recruits Akt to the inner face of the plasma membrane, where it is phosphorylated and activated by other protein kinases (called PDKs) that also contain pleckstrin homology domains and bind PIP_3. The formation of PIP_3 thus results in the association of both Akt and PDKs with the plasma membrane, leading to phosphorylation and activation of Akt. Once activated, Akt phosphorylates a number of target proteins, including proteins that are direct regulators of cell survival, transcription factors, and other protein kinases that regulate cell metabolism and protein synthesis.

Figure 6.13: Activation of Phospholipase C by Protein-Tyrosine Kianses

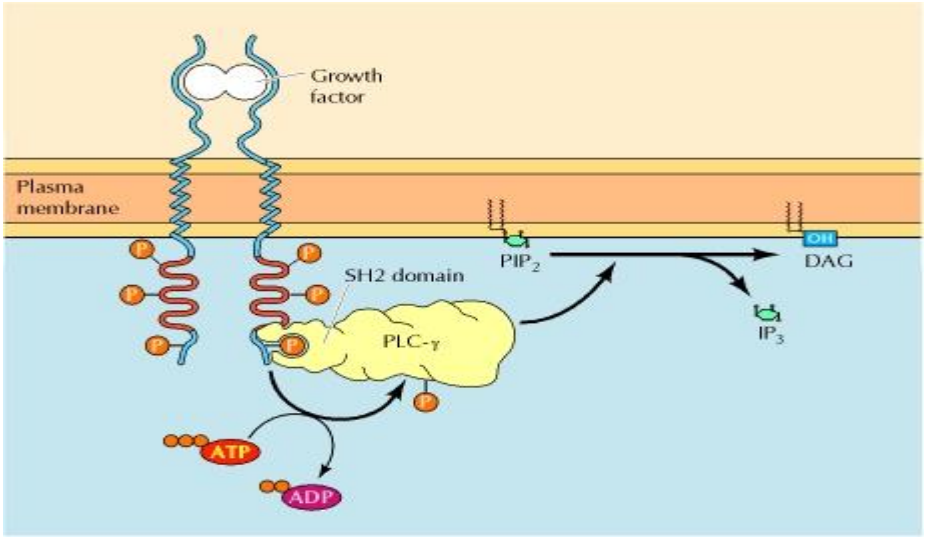


Figure 6.14: Ca^{2+} mobilization by IP3

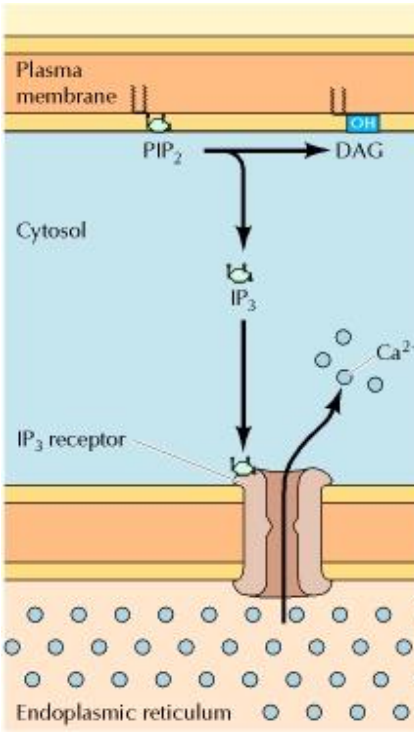


Figure 6.15: Function of calmodulin

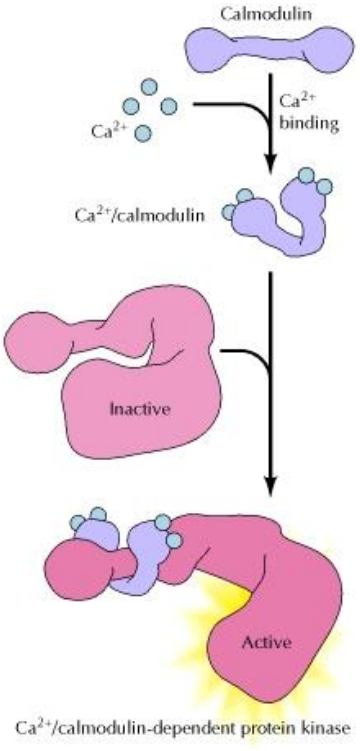


Figure 6.16:ctivity of PI 3 Kinase

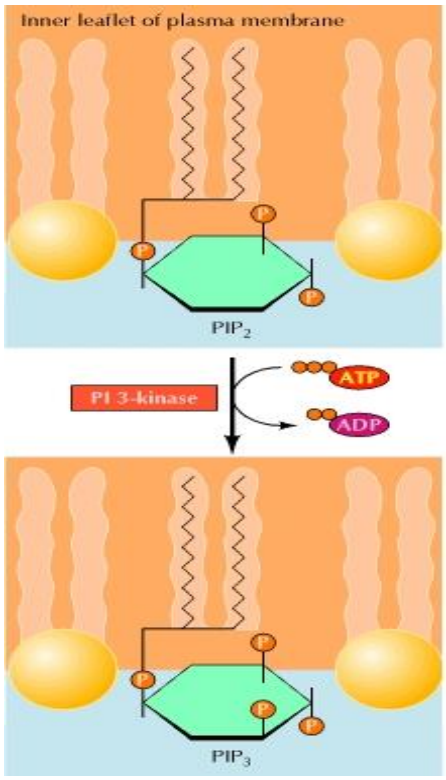
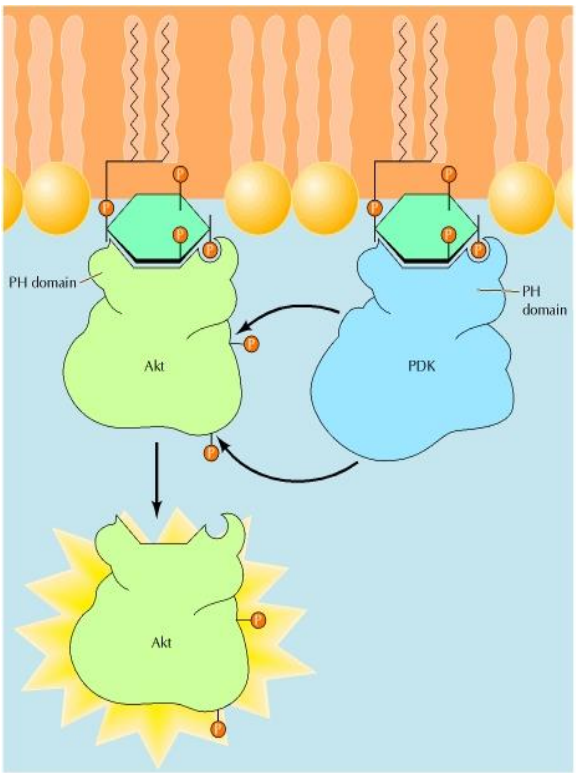


Figure 6.17: Activation of Akt Protein Kinase



5. MAP kinase Pathway:

The MAP kinase pathway refers to a cascade of protein kinases that are highly conserved in evolution and play central roles in signal transduction in all eukaryotic cells, ranging from yeasts to humans. The central elements in the pathway are a family of protein-serine/threonine kinases called the MAP kinases (for mitogen-activated protein kinases) that are activated in response to a variety of growth factors and other signaling molecules. In yeasts, MAP kinase pathways control a variety of cellular responses, including mating, cell shape, and sporulation. In higher eukaryotes (including C.elegans, Drosophila, frogs, and mammals), MAP kinases are ubiquitous regulators of cell growth and differentiation.

The best-characterized forms of MAP kinase in mammalian cells belong to the ERK (extracellular signal-regulatedkinase) family that plays a central role in signaling cell

proliferation induced by growth factors that act through either protein-tyrosine kinase or G protein-coupled receptors.

Activation of ERK is mediated by two upstream protein kinases, which are coupled to growth factor receptors by a GTP-binding protein called Ras (figure 6.18). Activation of Ras leads to activation of the Raf protein-serine/threonine kinase, which phosphorylates and activates a second protein kinase called MEK (for MAP kinase/ERK kinase). MEK is a dual-specificity protein kinase that activates members of the ERK family by phosphorylation of both threonine and tyrosine residues separated by one amino acid. Once activated, ERK phosphorylates a variety of targets, including other protein kinases and transcription factors.

The Ras proteins are guanine nucleotide-binding proteins that function analogously to the α subunits of G proteins, alternating between inactive GDP-bound and active GTP-bound forms (Figure 6.19). In contrast to the G protein α subunits, however, Ras functions as a monomer rather than in association with βγ subunits. Ras activation is mediated by guanine nucleotide exchange factors that stimulate the release of bound GDP and its exchange for GTP. Activity of the Ras-GTP complex is then terminated by GTP hydrolysis, which is stimulated by the interaction of Ras-GTP with GTPase-activating proteins. It is interesting to note that the mutations of ras genes in human cancers have the effect of inhibiting GTP hydrolysis by the Ras proteins. These mutated Ras proteins therefore remain continuously in the active GTP-bound form, driving the unregulated proliferation of cancer cells even in the absence of growth factor stimulation.

The Ras proteins are prototypes of a large family of approximately 50 related proteins, frequently called small GTP-binding proteins because Ras and its relatives are about half the size of G protein α subunits. While the Ras proteins regulate cell growth and differentiation, other subfamilies of small GTP-binding proteins control distinct cellular activities. The best understood mode of Ras activation is that mediated by receptor protein-tyrosine kinases. Autophosphorylation of these receptors results in their association with Ras guanine nucleotide exchange factors as a result of SH2-mediated protein interactions. One well-characterized example is provided by the guanine nucleotide exchange factor Sos, which is bound to the SH2-containing protein Grb2 in the cytosol of unstimulated cells. Tyrosine phosphorylation of receptors (or of other receptor-associated proteins) creates a binding site for

the Grb2 SH2 domains. Association of Grb2 with activated receptors localizes Sos to the plasma membrane, where it is able to interact with Ras proteins, which are anchored to the inner leaflet of the plasma membrane by lipids attached to the Ras C terminus. Sos then stimulates guanine nucleotide exchange, resulting in formation of the active Ras-GTP complex. In its active GTP-bound form, Ras interacts with a number of effector proteins, including the Raf protein-serine/threonine kinase. This interaction with Ras recruits Raf from the cytosol to the plasma membrane, where it is activated as a result of phosphorylation by both protein-tyrosine and protein-serine/threonine kinases.

Activation of Raf initiates a protein kinase cascade leading to ERK activation. ERK then phosphorylates a variety of target proteins, including other protein kinases. Importantly, a fraction of activated ERK translocates to the nucleus, where it regulates transcription factors by phosphorylation (Figure 6.20). In this regard, it is notable that a primary response to growth factor stimulation is the rapid transcriptional induction of a family of approximately 100 genes called immediate-early genes. The induction of a number of immediate-early genes is mediated by a regulatory sequence called the serum response element (SRE), which is recognized by a complex of transcription factors including the serum response factor (SRF) and Elk-1. ERK phosphorylates and activates Elk-1, providing a direct link between the ERK family of MAP kinases and immediate-early gene induction. Many immediate-early genes themselves encode transcription factors, so their induction in response to growth factor stimulation leads to altered expression of a battery of other downstream genes, thereby establishing new programs of gene expression.

Both yeasts and mammalian cells have multiple MAP kinase pathways that control distinct cellular responses. Each cascade consists of three protein kinases: a terminal MAP kinase and two upstream kinases (analogous to Raf and MEK) that regulate its activity. In the yeast S. cerevisiae, five different MAP kinase cascades regulate mating, sporulation, filamentation, cell wall remodeling, and response to high osmolarity. In mammalian cells, at least five MAP kinases have been identified. In addition to members of the ERK family, these include the JNK and p38 MAP kinases, which are preferentially activated in response to inflammatory cytokines and cellular stress (e.g., ultraviolet irradiation) (Figure 6.21). Whereas ERK signaling principally leads to cell proliferation, survival, and differentiation, the JNK and p38 MAP kinase pathways often lead to inflammation and cell death. Like ERK, the JNK and

p38 MAP kinases can translocate to the nucleus and phosphorylate transcription factors that regulate gene expression. Multiple MAP kinase pathways thus function in all types of eukaryotic cells to control cellular responses to diverse environmental signals.

Figure 6.18: Activation of ERK MAP Kinase

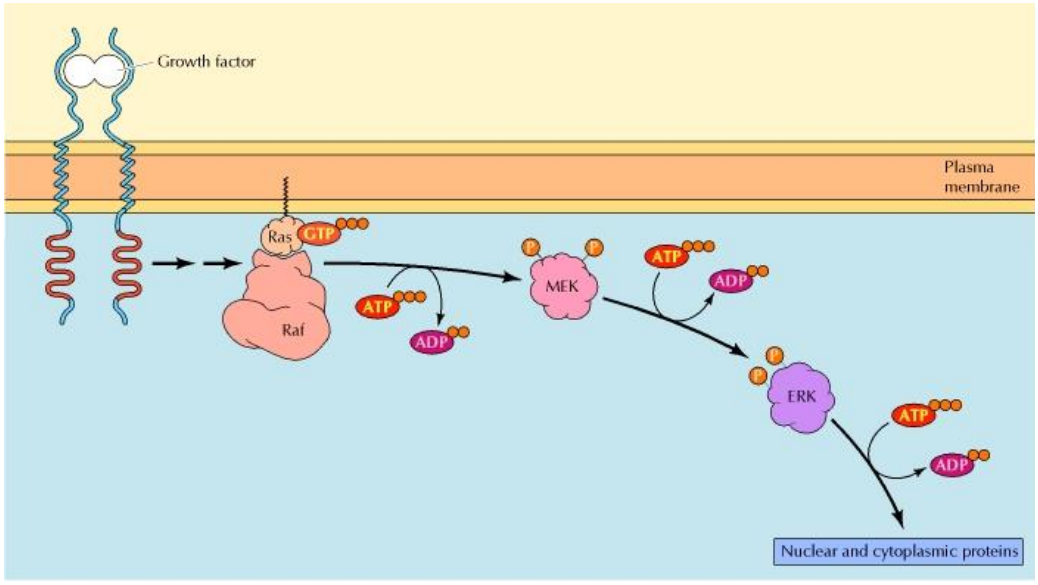


Figure 6.19: Regulation of Ras protein

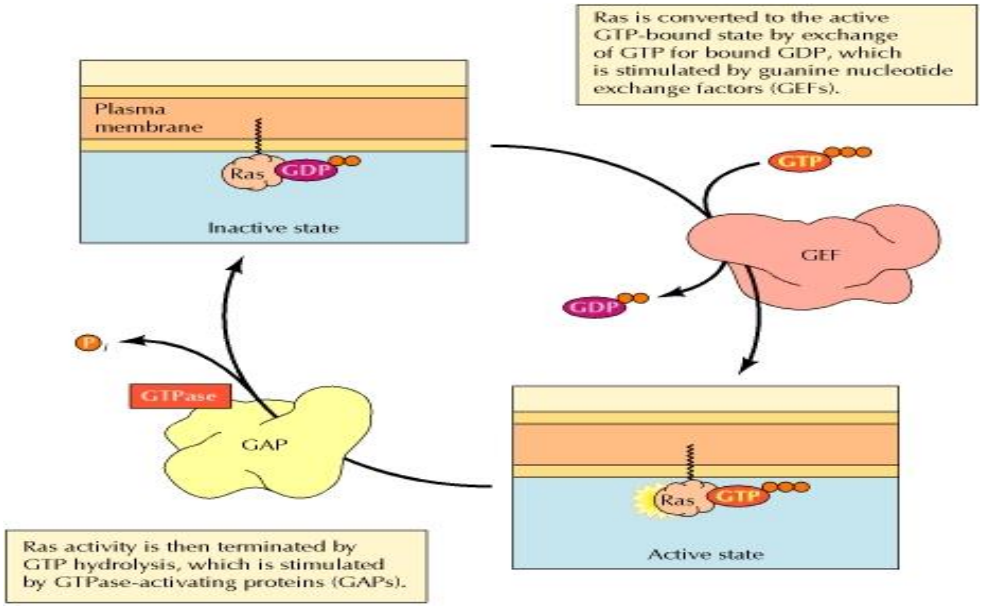


Figure 6.20 Induction of Immediate early genes by ERK

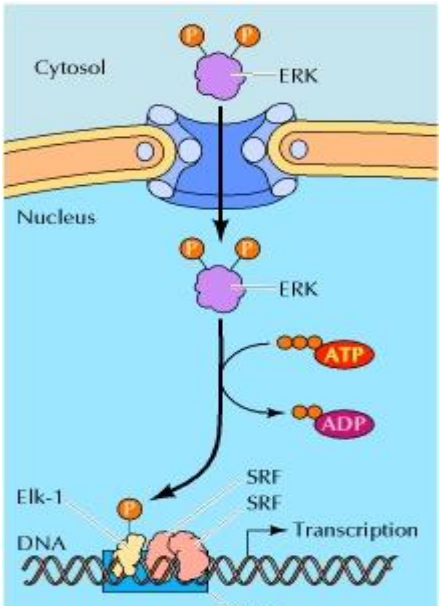
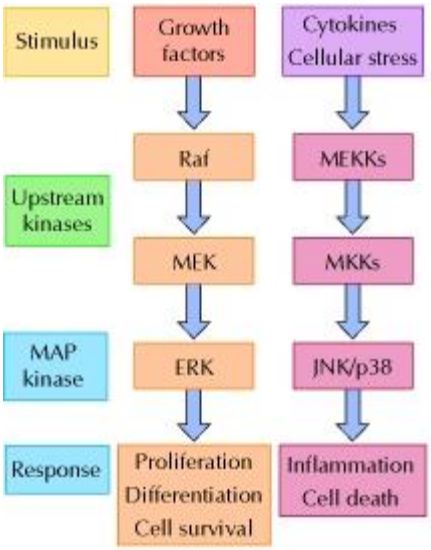


Figure 6.21: Pathways of MAP kinase activation in Mammals



6. The JK/STAT and TGFβ / Smad Pathways

. An alternative pathway, known as the JAK/STAT pathway, provides a much more immediate connection between protein-tyrosine kinases and transcription factors apart from MAP kinases. In this pathway protein-tyrosine phosphorylation directly affects transcription factor localization and function (figure 6.22). The key elements in this pathway are the STAT proteins (signal transducers and activators of transcription), are a family of transcription factors that contain SH2 domains. They are inactive in unstimulated cells, where they are localized to the cytoplasm. Stimulation of cytokine receptors leads to recruitment of STAT proteins, which bind via their SH2 domains to phosphotyrosine-containing sequences in the cytoplasmic domains of receptor polypeptides. Following their association with activated receptors, the STAT proteins are phosphorylated by members of the JAK family of nonreceptor protein-tyrosine kinases, which are associated with cytokine receptors. Tyrosine phosphorylation promotes the dimerization of STAT proteins, which then translocate to the nucleus, where they stimulate transcription of their target genes.

Further studies have shown that STAT proteins are also activated downstream of receptor protein-tyrosine kinases, where their phosphorylation may be catalyzed either by the receptors themselves or by associated nonreceptor kinases. The STAT transcription factors thus serve as direct links between both cytokine and growth factor receptors on the cell surface and regulation of gene expression in the nucleus.

Figure 6.22: JAK/STAT patway

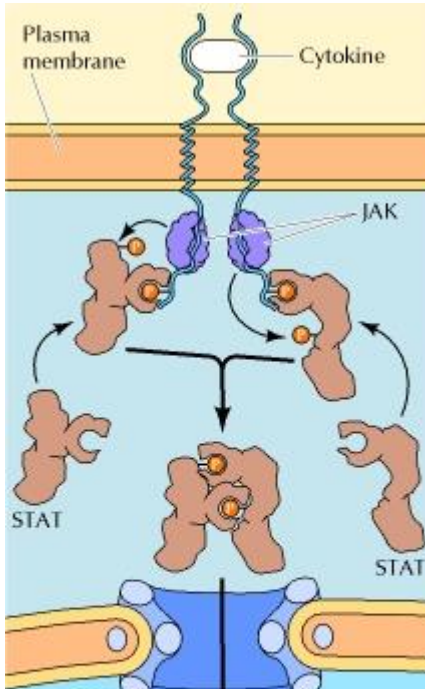
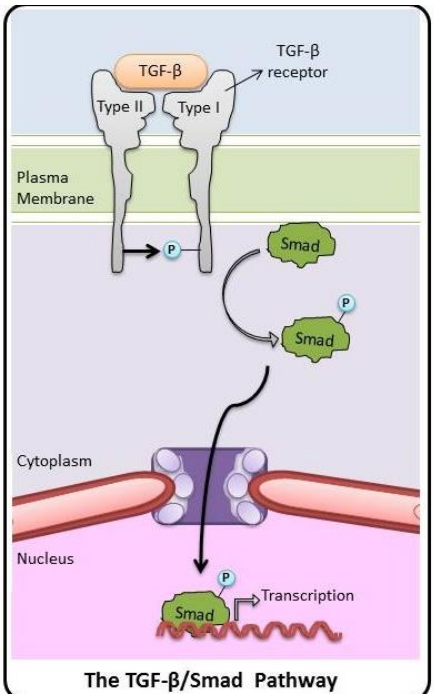


Figure 6.23: Signaling from TGF-β receptors



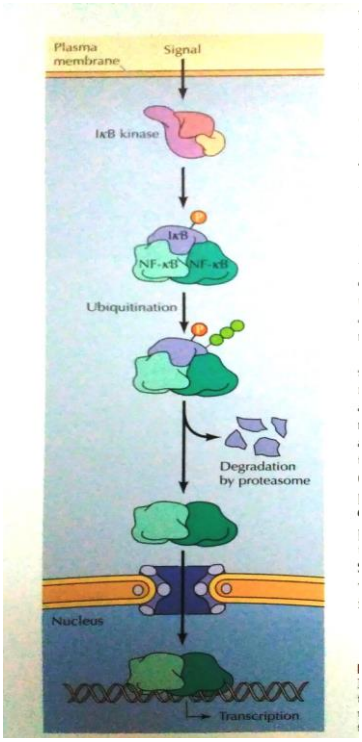
The receptors for members of the TGF-β family of growth factors are protein-serine/Threonine kinases, which directly phosphorylate transcription factors of the smad family (figure 6.23). The receptors are composed of Type I and Type II polypeptides, which become associated following Ligand binding. The Type II receptors then phosphorylates the type I receptor, which in turn phosphorylates a Smad protein. The phosphorylated Smads then translocate to the nucleus and regulate gene expression. Human genome codes for 42 different members of TGF-β , which elicit different responses in their target cells. This is accomplished by comabnitorial interactionof seven different type I receptors and five type II receptors, which lead to activation of different members of Smad family. Smad family members can also be phosphorylated by ERK, and this

intersection between the TGFβ / Smad pathway and ERK plays a critical role in embryonic development.

7. NF- κB signaling

NF- κB signaling is another example of a signaling pathway that directly targets a specific family of transcription factors. The NF- κB family consists of five transcription factors that play key roles in the immune system and in inflammation as well as in regulation of proliferation and survival of many types of animal cells. Members of this transcription factor family are activated in response to a variety of stimuli, including cytokines, growth factors, viral infections and DNA damage. In unstimulated cells NF- κB proteins are bound to inhibitory 1κB proteins that maintain NF- κB in an inactive state in the cytosol (figure 6.24). Activation of NF- κB results from signals that activate the 1κB kinase, which phosphorylates 1κB. this phosphorylation targets 1κB fir ubiquitination and degradation by the proteosome, freeing NF- κB to translocate to the nucleus and induce expression of its target genes.

Figure 6.24 NF- κB signaling



8.The Hedgehog, Wnt and Notch pathways

The Hedgehog and Wnt pathways are closely related and play important role during embryonic development. The Hedgehog genes encode secreted proteins that are modified by the addition of lipids. The functional receptor for Hedgehog consists of two transmembrane proteins, patched and smoothed. Hedgehog binds to Patched, which acts as a negative regulator for smoothed (figure 6.25). This binding to Patched allows Smoothened to propagate an intracellular signal, leading to the activation of a transcriptional factor called Gli in mammals and Ci in Drosophila, which otherwise is found bound to a protein that anchors the complex of microtubules. Hedgehog signaling promotes interaction of Smoothened with this protein, to release Ci, which enables to translocate to the nucleus and activate transcription of target genes.

The Wnt proteins are a family of secreted growth factors that bind to receptors of the LRP and Frizzled family, which are related to activation of cytoskeletal protein called Dishevelled, and inhibition of a complex of the proteins axin, APC and the protein kinase GSK-3β. Within the complex GSK-3β phosphorylates β-catenin, leading to ubiquitination and degradation, there by Wnt signaling results in increase of β-catenin levels (figure 6.26).

The Notch pathway is another highly conserved signaling pathway that control cell fate during animal development. Notch is a large protein with a single transmembrane domain that serves as a receptor of signaling by transmembrane proteins. Ligand binding leads to proteolytic cleavage of Notch, and an intracellular domain of Notch is then translocated to nucleus that interacts with transcriptional factor that converts it from repressor state to an activator state of its target gene (figure 6.27).

Figure6.25: Hedgehog signaling

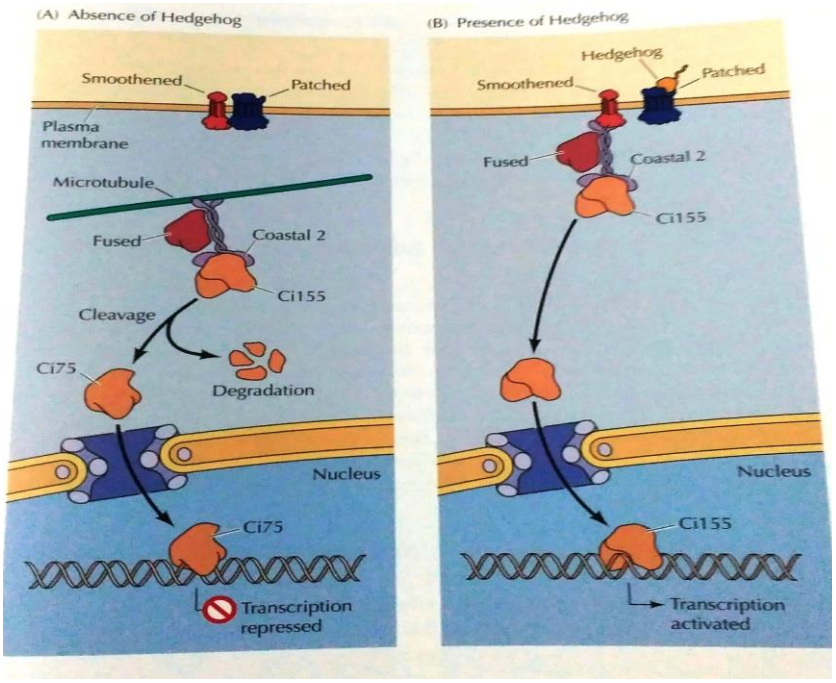


Figure 6.26: Wnt Pathway

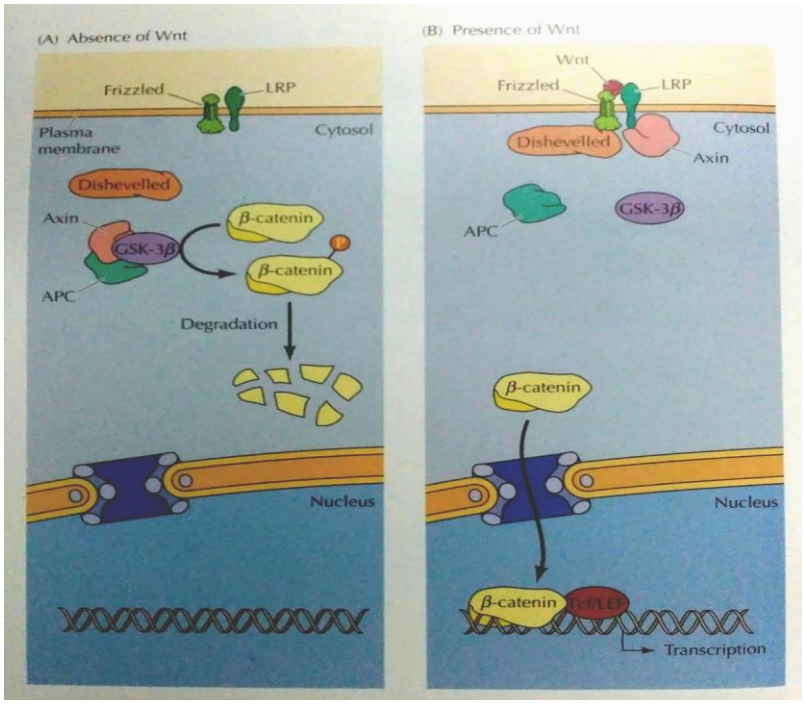
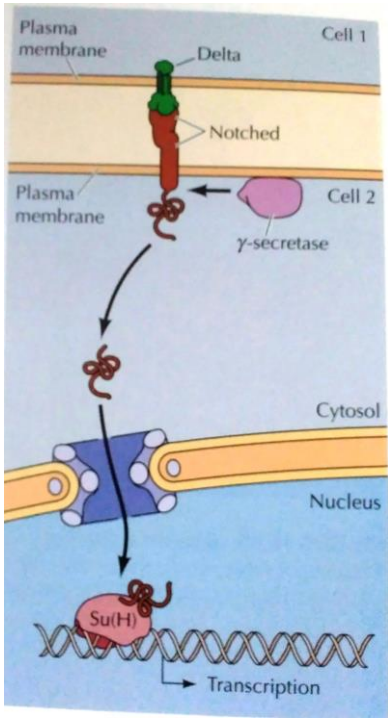


Figure 6.27: Notch Signaling



IV:Signal Transduction and the Cytoskeleton

The functions of most cells are also directly affected by cell adhesion and the organization of the cytoskeleton. The receptors responsible for cell adhesion thus act to initiate intracellular signaling pathways that regulate other aspects of cell behavior, including gene expression. Conversely, growth factors frequently act to induce cytoskeletal alterations resulting in cell movement or changes in cell shape. Components of the cytoskeleton thus act as both receptors and targets in cell signaling pathways, integrating cell shape and movement with other cellular responses.

1. Integrins and Signal Transduction:

The integrins are the major receptors responsible for the attachment of cells to the extracellular matrix. At two types of cell-matrix junctions, the integrins also interact with components of the cytoskeleton to provide a stable linkage between the extracellular matrix and adherent cells. In addition to this structural role, the integrins serve as receptors that activate intracellular signaling pathways, thereby controlling gene expression and other aspects of cell behavior in response to adhesive interactions.

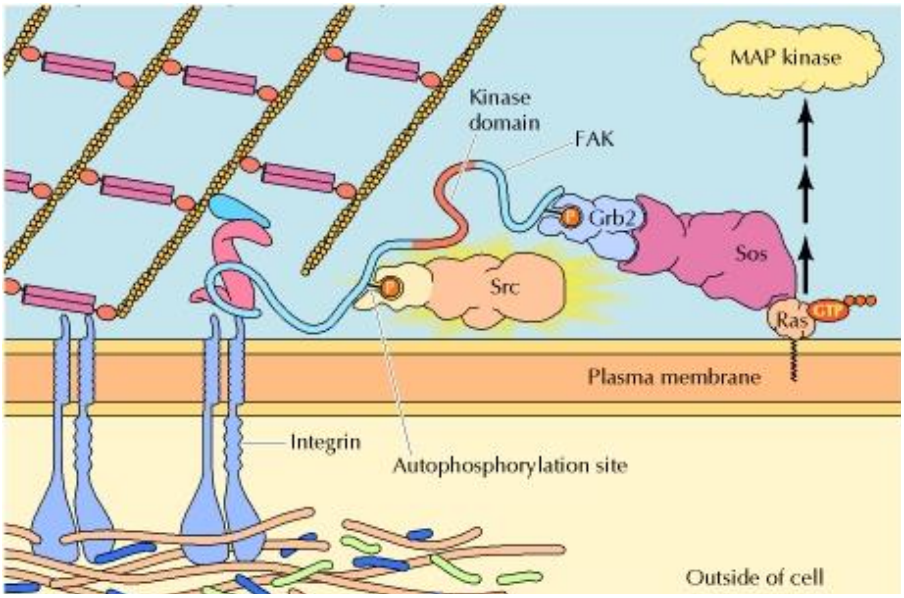
Like members of the cytokine receptor superfamily, the integrins have short cytoplasmic tails that lack any intrinsic enzymatic activity. However, protein-tyrosine phosphorylation is an early response to the interaction of integrins with extracellular matrix components, suggesting that the integrins are linked to nonreceptor protein-tyrosine kinases. In particular, a nonreceptor protein-tyrosine kinase called FAK (focal adhesion kinase) plays a key role in integrin signaling (Figure 6.27). FAK is localized to focal adhesions and rapidly becomes tyrosine-phosphorylated following the binding of integrin to extracellular matrix components, such as fibronectin. Like other protein-tyrosine kinases, the activation of FAK is thought to involve autophosphorylation induced by the clustering of integrins bound to the extracellular matrix.

In addition to FAK, members of the Src family of nonreceptor protein-tyrosine kinases also associate with focal adhesions and are involved in integrin signaling. Interestingly, Src and FAK appear to function in association with each other as a result of the binding of the Src SH2

domain to an autophosphorylation site of FAK. Src then phosphorylates additional sites on FAK, so these two nonreceptor protein-tyrosine kinases act jointly in signaling from integrin receptors.

Tyrosine phosphorylation of FAK creates binding sites for the SH2domains of other downstream signaling molecules, including PI 3-kinase and the Grb2-Sos complex. Recruitment of the Sos guanine nucleotide exchange factor leads to activation of Ras, which in turn couples integrins to activation of the ERK MAP kinase signaling pathway. Integrin activation of the FAK and Src nonreceptor protein-tyrosine kinases thus links cell adhesion to changes in gene expression and cell behavior that are analogous to those induced by the binding ofgrowth factors to their cell surface receptors.

Figure 6.27: Model for signaling from the FAK Protein -Tyrosine kinase



2. Regulation of Actin cytoskeleton:

Cellular responses to extracellular signals, including growth factors, frequently include changes in cell movement and cell shape and these aspects of cell behavior are governed principally by the actin cytoskeleton. In particular, many types of cell movement are based on the dynamic assembly and disassembly of actin filaments underlying the plasma membrane. Remodeling of the actin cytoskeleton therefore represents a key element of the response of many cells to growth factors and other extracellular stimuli.

Members of the Rho subfamily of small GTP-binding proteins (including Rho, Rac, and Cdc42) play central roles in regulating the organization of the actin cytoskeleton and thus control a variety of cell processes, including cell motility, cell adhesion, and cytokinesis. The role of Rho family members in regulating different aspects of actin remodeling was first elucidated by studies of the response of fibroblasts to growth factor stimulation (Figure 6.28). The cytoskeletal alterations resulting from growth factor stimulation include the production of cell surface protrusions (filopodia, lamellipodia, and membrane ruffles) as well as the formation of focal adhesions and stress fibers.

Further Rho family members play similar roles in regulating the actin cytoskeleton in all types of eukaryotic cells. For example, Rho is required for cytokinesis (cell division following mitosis), which is mediated by an actin-myosin contractile ring (figure 6.29). In neurons, Rac, Cdc42 and Rho regulate the extension and retraction of axons during development of the nervous system. In smooth muscle cells, Rho contributes to regulation of contraction. In epithelial cells, Rho family members regulate the formation of adherens junctions, which involve the linkage of cadherins to the actin cytoskeleton (figure 6.30). Members of the Rho family thus serve as universal regulators of the actin cytoskeleton, linking extracellular signals to changes in cell shape and movement. In addition, Rho family members can activate MAP kinase signaling pathways, so these small GTP-binding proteins function as dual regulators of cytoskeletal remodeling and gene expression.

A large number of proteins have been identified as potential targets of Rho, Rac, and Cdc42, and the cellular roles of many of these candidate target proteins remain to be elucidated. However, recent experiments have clarified some of the pathways by which Rho family members regulate cytoskeletal alterations. A key target of Rho appears to be a protein-serine/threonine kinase called Rho kinase. Activation of Rho kinase increases the phosphorylation of the light chain of myosin II by two mechanisms: Rho not only directly phosphorylates the myosin light chain but also phosphorylates and inhibits myosin light chain phosphatase. The resulting increase in myosin light chain phosphorylation activates myosin and leads to the assembly of actin-myosin filaments, resulting in cytoskeletal alterations such as the formation of stress fibers and focal adhesions, adherens junctions, and cytokinesis. In addition to affecting phosphorylation of myosin light chain, Rho kinase phosphorylates another protein kinase (LIM-kinase), which in turn phosphorylates the actin-binding protein cofilin. Cofilin is a key regulator of actin filament

disassembly, so its phosphorylation by LIM-kinase directly links Rho to regulation of actin filament dynamics.

A key target of both Rac and Cdc42 is the protein-serine/threonine kinase called PAK. Like Rho kinase, PAK also affects phosphorylation of the myosin light chain, but in the opposite direction. PAK phosphorylates and inhibits myosin light chain kinase, leading to a decrease in myosin light chain phosphorylation. This results in inactivation of myosin II and a decrease in actin-myosin interaction, consistent with the ability of Rac and Cdc42 to stimulate the formation of cell surface protrusions (filopodia and lamellipodia) rather than stress fibers and focal adhesions. Activation of Rac also leads to stimulation of LIM-kinase and phosphorylation of cofilin, linking Rac to remodeling of the actin cytoskeleton. Other targets of Rac and Cdc42 also participate in the formation of filopodia and lamellopodia, as well as in the regulation of cell-cell interactions at adherens junctions.

Figure 6.28: Regualtion of actin remodeling by Rho family proteins

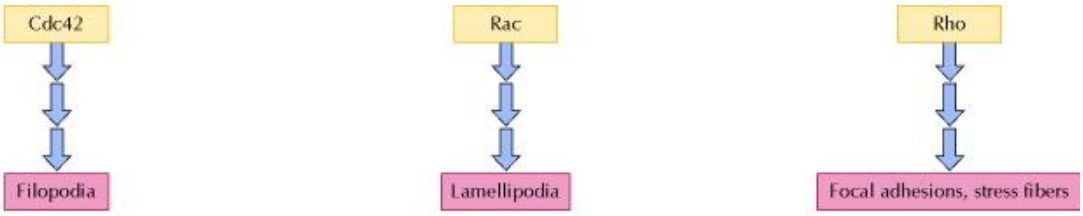


Figure 6.29 Contractile ring consisting of actin filaments

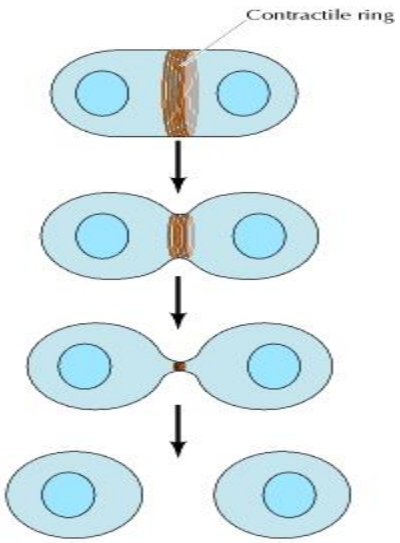
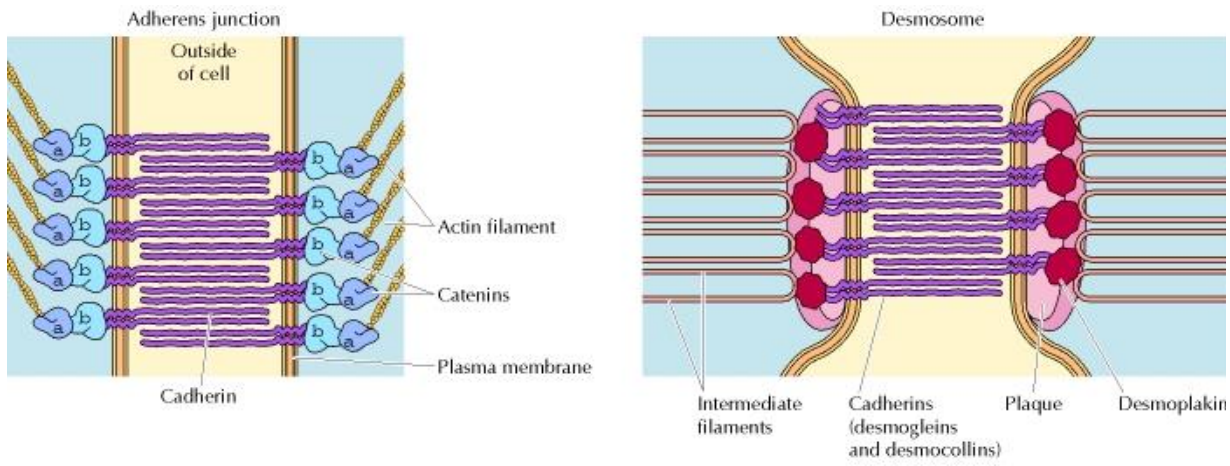


Figure 6.30: Cell – cell junctions mediated by Cadherins



V. Signaling networks:

Signaling pathways are rarely linear. The activities of individual pathways are regulated by feedback loops that control the extent and duration of signaling activity. Signaling pathways do not work in isolation , rather there is frequent cross talk between the pathways, and hence intracellular signal transduction is to be understood as a network of connected pathways.

1. Feedback and Crosstalk: The activity of signaling pathways is controlled by feedback loops, which are similar in principal to feedback regulation of metabolic pathways.

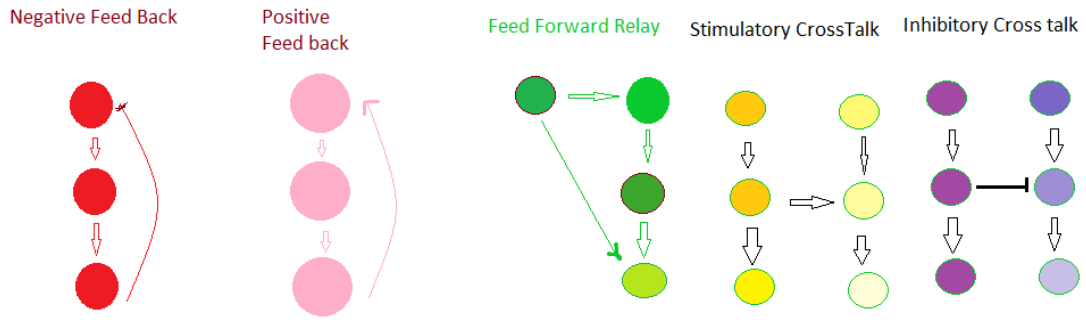
Eg: NF- κB is activated by signals that lead to proteolysis of the inhibitor 1κB, allowing NF- κB to translocate to the nucleus and induce expression of its target genes. One of the target genes induced by NF- κB encodes 1κB, so NF- κB signaling leads to the synthesis of new 1κB which inhibits continued NF- κB activity.

Cross talk refers to the interaction of one signaling pathway with another. Examples for cross talk between pathways are- Ca^{2+}- cAMP signaling, between cAMP and ERK pathways, between ERK and TGFβ/Smad pathways, and between integrins signaling and receptor protein-tyrosine kinase.

2. Networks of cellular Signal Transduction:

The extensive crosstalk between individual signal transduction pathways mean that multiple pathways interact with one another to form signaling networks within the cell. Some of the pathways can connect within a networks as shown in figure 6.31.

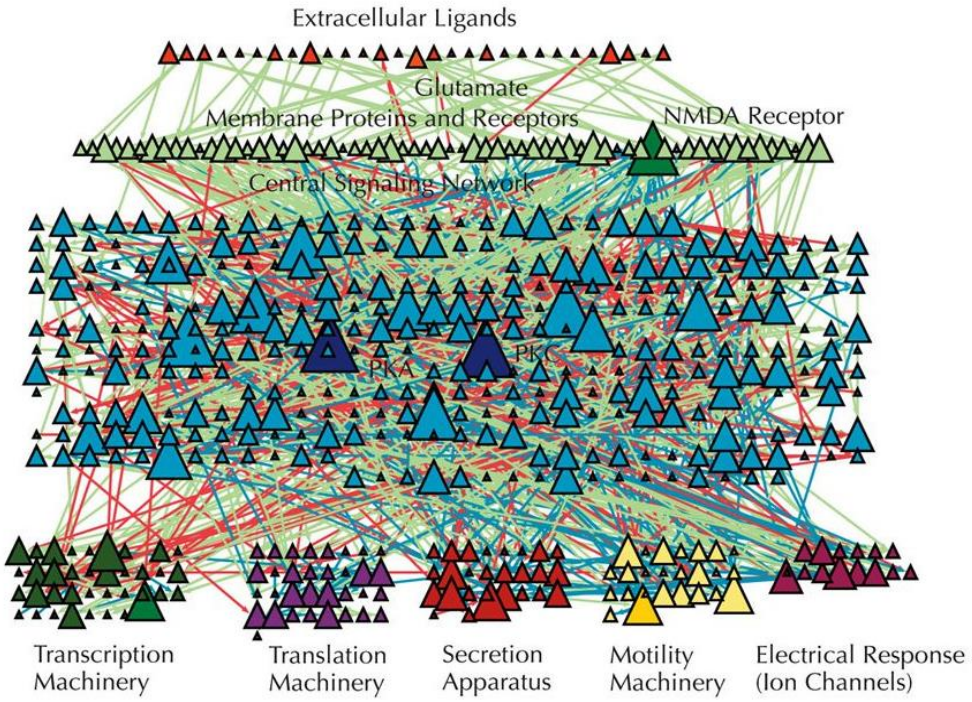
6.31: Elements of signaling networks



In feedback loops a downstream element of a pathway either inhibits (negative feed back) or stimulates (Positive feedback) an upstream element. In feed forward relays, an upstream element of a pathway stimulates both its immediate targets and another element further downstream. Cross talk occurs when an element of one pathway either stimulates or inhibits an element of a second pathway.

A full understanding of cell signaling will require the development of network models that predict the dynamic behavior of the interconnected signaling pathways that ultimately result in a biological response. It is an integrated system and includes several signaling pathways involving receptors that stimulate cascade of protein kinases that ultimately effect one gene expression by regulating transcription factors. The human genome encodes approximately 1500 different receptors, 700 protein kinases and Phosphatases and nearly 2000 transcription factors, indicating the potential for cross regulation among various pathways. The figure 6.32 represents an imaginative model for signaling in a mammalian neuron. Understanding mammalian signaling network is a tough task yet it is important to understand it in quantitative terms for the better understanding of cell biology.

Figure 6.32: A mammalian Signaling network



The network depicts connection between 545 signaling elements in a mammalian neuron. Green arrows are stimulatory, red arrows are inhibitory and blue arrows are neutral links between elements