# CHAPTER 7

# Cell Signalling and Signal Transduction Pathways

☛ **Q.1. What is signal transduction? What are the pathways involved in signal transduction**

**Ans.** It occurs when an extracellular signaling molecule activates a specific receptor located on the cell surface or inside the cell. In turn, this receptor triggers a biochemical chain of events inside the cell, creating a response. Depending on the cell, the response alters the cell's metabolism, shape, gene expression, or ability to divide. The signal can be amplified at any step. Thus, one signaling molecule can cause many responses.

Signal transduction involves the binding of extracellular signaling molecules also called ligands to receptors that trigger events inside the cell. The combination of signaling molecule with a receptor causes a change in the conformation of the receptor, known as receptor activation. This activation is always the initial step (the cause) leading to the cell's ultimate responses (effect) to the messenger. Despite the myriad of these ultimate responses, they are all directly due to changes in particular cell proteins. Intracellular signaling cascades can be started through cell-substratum interactions; examples are the integrin that binds ligands in the extracellular matrix and steroids. Most steroid hormones have receptors within the cytoplasm and act by stimulating the binding of their receptors to the promoter region of steroid-responsive genes. Examples of signaling molecules include the hormone melatonin, the neurotransmitter acetylcholine and the cytokine interferon ?. The classifications of signaling molecules do not take into account the molecular nature of each class member; neurotransmitters range in size from small molecules such as dopamine to neuropeptides such as endorphins. Some molecules may fit into more than one class; for example, epinephrine is a neurotransmitter when secreted by the central nervous system and a hormone when secreted by the adrenal medulla.

## Environmental Stimuli

In single-celled organisms, the variety of signal transduction processes influence its reaction to its environment. In multicellular organisms, numerous processes are required for coordinating individual cells to support the organism as a whole; the complexity of these processes tends to increase with the complexity of the organism. At the cellular level, Sensing of environments relies on signal transduction; modeling signal transduction

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systems as self-organizing allows one to explain how equilibria are maintained, many disease processes, such as diabetes and heart disease arise from defects or dysregulations in these pathways, highlighting the importance of this process in biology and medicine. Various environmental stimuli exist that initiate signal transmission processes in multicellular organisms; examples include photons hitting cells in the retina of the eye, and odorants binding to odorant receptors in the nasal epithelium. Certain microbial molecules, such as viral nucleotides and protein antigens, can elicit an immune system response against invading pathogens mediated by signal transduction processes. This may occur independent of signal transduction stimulation by other molecules, as is the case for the toll-like receptor. It may occur with help from stimulatory molecules located at the cell surface of other cells, as with T-cell receptor signaling. Unicellular organisms may respond to environmental stimuli through the activation of signal transduction pathways. For example, slime molds secrete cyclic adenosine monophosphate upon starvation, stimulating individual cells in the immediate environment to aggregate, and yeast cells use mating factors to determine the mating types of other cells and to participate in sexual reproduction.

## Receptors

Receptors can be roughly divided into two major classes: intracellular receptors and extracellular receptors.

## Extracellular

Extracellular receptors are integral transmembrane proteins and make up most receptors. They span the plasma membrane of the cell, with one part of the receptor on the outside of the cell and the other on the inside. Signal transduction occurs as a result of a ligand binding to the outside region of the receptor (the ligand does not pass through the membrane). Ligand-receptor binding induces a change in the conformation of the inside part of the receptor. This results in either the activation of an enzyme domain of the receptor or the exposure of a binding site for other intracellular signaling proteins within the cell, eventually propagating the signal through the cytoplasm.

In eukaryotic cells, most intracellular proteins activated by a ligand/receptor interaction possess an enzymatic activity; examples include tyrosine kinase and phosphatases. Some of them create second messengers such as cyclic AMP and $IP_3$, the latter controlling the release of intracellular calcium stores into the cytoplasm. Other activated proteins interact with adaptor proteins that facilitate signalling protein interactions and coordination of signalling complexes necessary to respond to a particular stimulus. Enzymes and adaptor proteins are both responsive to various second messenger molecules.

Many adaptor proteins and enzymes activated as part of signal transduction possess specialized protein domains that bind to specific secondary messenger molecules. For example, calcium ions bind to the EF hand domains of calmodulin, allowing it to bind and activate calmodulin-dependent kinase. $PIP_3$ and other phosphoinositides do the same thing to the Pleckstrin homology domains of proteins such as the kinase protein AKT.

## G Protein-coupled

G protein-coupled receptors (GPCRs) are a family of integral transmembrane proteins that possess seven transmembrane domains and are linked to a heterotrimeric G protein. Many receptors are in this family, including adrenergic receptors and chemokine receptors.

Signal transduction by a GPCR begins with an inactive G protein coupled to the receptor; it exists as a heterotrimer consisting of $G\alpha$, $G\beta$, and $G\gamma$. Once the GPCR recognizes a ligand, the conformation of the receptor changes to activate the G protein, causing $G\alpha$ to bind a molecule of GTP and dissociate from the other two G-protein subunits. The dissociation exposes sites on the subunits that can interact with other molecules. The activated G protein subunits detach from the receptor and initiate signaling from many downstream effector proteins such as phospholipases and ion channels, the latter permitting the release of second messenger molecules. The total strength of signal amplification by a GPCR is determined by the lifetimes of the ligand-receptor complex and receptor-effector protein complex and the deactivation time of the activated receptor and effectors through intrinsic enzymatic activity.

A study was conducted where a point mutation was inserted into the gene encoding the chemokine receptor CXCR2; mutated cells underwent a malignant transformation due to the expression of CXCR2 in an active conformation despite the absence of chemokine-binding. This meant that chemokine receptors can contribute to cancer development.

## Tyrosine and Histidine Kinase

Receptor tyrosine kinases (RTKs) are transmembrane proteins with an intracellular kinase domain and an extracellular domain that binds ligands; examples include growth factor receptors such as the insulin receptor. To perform signal transduction, RTKs need to form dimers in the plasma membrane; the dimer is stabilized by ligands binding to the receptor. The interaction between the cytoplasmic domains stimulates the autophosphorylation of tyrosines within the domains of the RTKs, causing conformational changes. Subsequent to this, the receptors' kinase domains are activated, initiating phosphorylation signaling cascades of downstream cytoplasmic molecules that facilitate various cellular processes such as cell differentiation and metabolism.

As is the case with GPCRs, proteins that bind GTP play a major role in signal transduction from the activated RTK into the cell. In this case, the G proteins are members of the Ras, Rho, and Raf families, referred to collectively as small G proteins. They act as molecular switches usually tethered to membranes by isoprenyl groups linked to their carboxyl ends. Upon activation, they assign proteins to specific membrane subdomains where they participate in signaling. Activated RTKs in turn activate small G proteins that activate guanine nucleotide exchange factors such as SOS1. Once activated, these exchange factors can activate more small G proteins, thus amplifying the receptor's initial signal. The mutation of certain RTK genes, as with that of GPCRs, can result in the expression of receptors that exist in a constitutively activate state; such mutated genes may act as oncogenes.

Histidine-specific protein kinases are structurally distinct from other protein kinases and are found in prokaryotes, fungi, and plants as part of a two-component signal transduction mechanism: a phosphate group from ATP is first added to a histidine residue within the kinase, then transferred to an aspartate residue on a receiver domain on a different protein or the kinase itself, thus activating the aspartate residue.

### Integrin
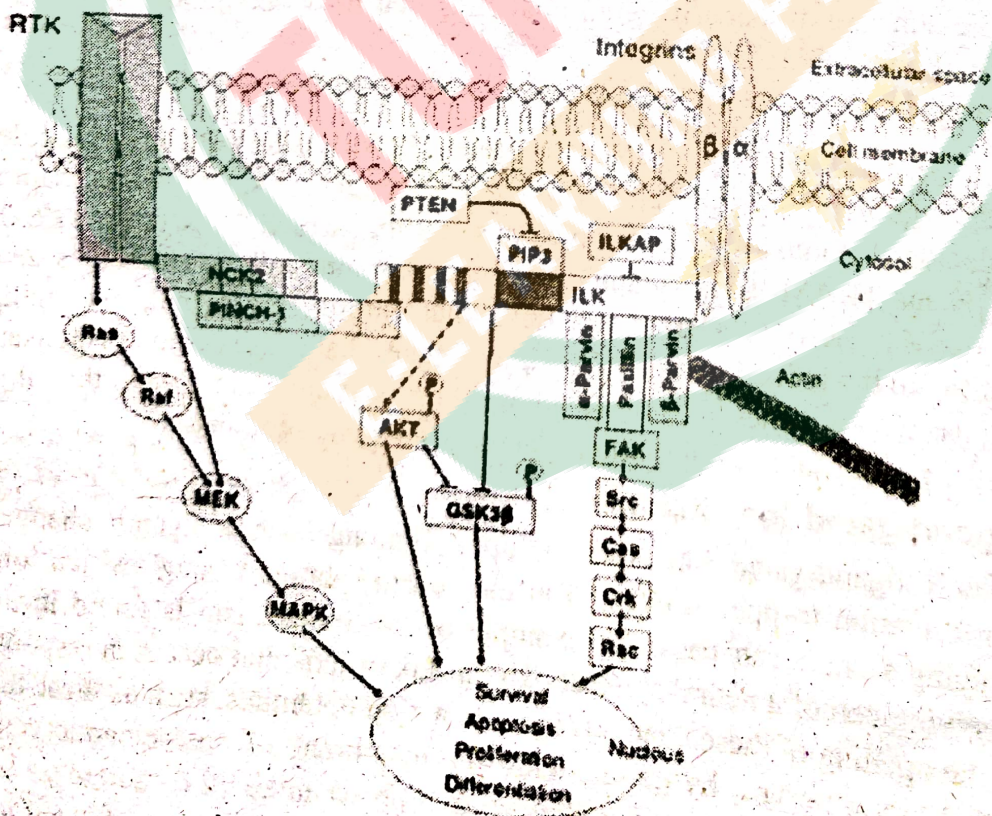


Integrin-mediated signal transduction

Integrins are produced by a wide variety of cells; they play a role in cell attachment to other cells and the extracellular matrix and in the transduction of signals from extracellular matrix components such as fibronectin and collagen. Ligand binding to the extracellular domain of integrins changes the protein's conformation, clustering it at the cell membrane to initiate signal transduction. Integrins lack kinase activity; hence, integrin-mediated signal transduction is achieved through a variety of intracellular protein kinases and adaptor molecules, the main coordinator being integrin-linked kinase. As shown in the picture to the right, cooperative integrin-RTK signalling determines the timing of cellular survival, apoptosis, proliferation, and differentiation.

Important differences exist between integrin-signalling in circulating blood cells and non-circulating cells such as epithelial cells; integrins of circulating cells are normally inactive. For example, cell membrane integrins on circulating leukocytes are maintained in an inactive state to avoid epithelial cell attachment; they are activated only in response to stimuli such as those received at the site of an inflammatory response. In a similar manner, integrins at the cell membrane of circulating platelets are normally kept inactive to avoid thrombosis. Epithelial cells (which are non-circulating) normally have active integrins at their cell membrane, helping maintain their stable adhesion to underlying stromal cells that provide signals to maintain normal functioning.

## Toll Gate

When activated, toll-like receptors (TLRs) take adapter molecules within the cytoplasm of cells in order to propagate a signal. Four adaptor molecules are known to be involved in signaling, which are Myd88, TIRAP, TRIF, and TRAM. These adapters activate other intracellular molecules such as IRAK1, IRAK4, TBK1, and IKKi that amplify the signal, eventually leading to the induction or suppression of genes that cause certain responses. Thousands of genes are activated by TLR signaling, implying that this method constitutes an important gateway for gene modulation.

## Ligand-gated Ion Channel

A ligand-gated ion channel, upon binding with a ligand, changes conformation to open a channel in the cell membrane through which ions relaying signals can pass. An example of this mechanism is found in the receiving cell of a neural synapse. The influx of ions that occurs in response to the opening of these channels induces action potentials, such as those that travel along nerves, by depolarizing the membrane of post-synaptic cells, resulting in the opening of voltage-gated ion channels.

An example of an ion allowed into the cell during a ligand-gated ion channel opening is $Ca^{2+}$; it acts as a second messenger initiating signal

transduction cascades and altering the physiology of the responding cell. This result in amplification of the synapse response between synaptic cells by remodelling the dendritic spines involved in the synapse.

## Intracellular

Intracellular receptors, such as nuclear receptors and cytoplasmic receptors, are soluble proteins localized within their respective areas. The typical ligands for nuclear receptors are non-polar hormones like the steroid hormones testosterone and progesterone and derivatives of vitamins A and D. To initiate signal transduction, the ligand must pass through the plasma membrane by passive diffusion. On binding with the receptor, the ligands pass through the nuclear membrane into the nucleus, altering gene expression.

Activated nuclear receptors attach to the DNA at receptor-specific hormone-responsive element (HRE) sequences, located in the promoter region of the genes activated by the hormone-receptor complex. Due to their enabling gene transcription, they are alternatively called inductors of gene expression. All hormones that act by regulation of gene expression have two consequences in their mechanism of action; their effects are produced after a characteristically long period of time and their effects persist for another long period of time, even after their concentration has been reduced to zero, due to a relatively slow turnover of most enzymes and proteins that would either deactivate or terminate ligand binding onto the receptor.

Nucleic receptors have DNA-binding domains containing zinc fingers and a ligand-binding domain; the zinc fingers stabilize DNA binding by holding its phosphate backbone. DNA sequences that match the receptor are usually hexameric repeats of any kind; the sequences are similar but their orientation and distance differentiate them. The ligand-binding domain is additionally responsible for dimerization of nucleic receptors prior to binding and providing structures for transactivation used for communication with the translational apparatus.

Steroid receptors are a subclass of nuclear receptors located primarily within the cytosol. In the absence of steroids, they associate in an aporeceptor complex containing chaperone or heatshock proteins (HSPs). The HSPs are necessary to activate the receptor by assisting the protein to fold in a way such that the signal sequence enabling its passage into the nucleus is accessible. Steroid receptors, on the other hand, may be repressive on gene expression when their transactivation domain is hidden. Receptor activity can be enhanced by phosphorylation of serine residues at their N-terminal as a result of another signal transduction pathway, a process called crosstalk.

Retinoic acid receptors are another subset of nuclear receptors. They can be activated by an endocrine-synthesized ligand that entered the cell by

diffusion, a ligand synthesised from a precursor like retinol brought to the cell through the bloodstream or a completely intracellularly synthesised ligand like prostaglandin. These receptors are located in the nucleus and are not accompanied by HSPs. They repress their gene by binding to their specific DNA sequence when no ligand binds to them, and vice versa.

Certain intracellular receptors of the immune system are cytoplasmic receptors; recently identified NOD-like receptors (NLRs) reside in the cytoplasm of some eukaryotic cells and interact with ligands using a leucine-rich repeat (LRR) motif similar to TLRs. Some of these molecules like NOD2 interact with RIP2 kinase that activates NF-κB signaling, whereas others like NALP3 interact with inflammatory caspases and initiate processing of particular cytokines like interleukin-1ß.

## Second Messengers

First messengers are the signaling molecules (hormones, neurotransmitters, and paracrine/autocrine agents) that reach the cell from the extracellular fluid and bind to their specific receptors. Second messengers are the substances that enter the cytoplasm and act within the cell to trigger a response. In essence, second messengers serve as chemical relays from the plasma membrane to the cytoplasm, thus carrying out intracellular signal transduction.

## Calcium

The release of calcium ions from the endoplasmic reticulum into the cytosol results in its binding to signaling proteins that are then activated; it is then sequestered in the smooth endoplasmic reticulum and the mitochondria. Two combined receptor/ion channel proteins control the transport of calcium: the $InsP_3$-receptor that transports calcium upon interaction with inositol triphosphate on its cytosolic side; and the ryanodine receptor named after the alkaloid ryanodine, similar to the $InsP_3$ receptor but having a feedback mechanism that releases more calcium upon binding with it. The nature of calcium in the cytosol means that it is active for only a very short time, meaning its free state concentration is very low and is mostly bound to organelle molecules like calreticulin when inactive.

Calcium is used in many processes including muscle contraction, neurotransmitter release from nerve endings, and cell migration. The three main pathways that lead to its activation are GPCR pathways, RTK pathways, and gated ion channels; it regulates proteins either directly or by binding to an enzyme.

## Lipophilics

Lipophilic second messenger molecules are derived from lipids residing in cellular membranes; enzymes stimulated by activated receptors activate the

lipids by modifying them. Examples include diacylglycerol and ceramide, the former required for the activation of protein kinase C.

## Nitric Oxide

Nitric oxide (NO) acts as a second messenger because it is a free radical that can diffuse through the plasma membrane and affect nearby cells. It is synthesised from arginine and oxygen by the NO synthase and works through activation of soluble guanylyl cyclase, which when activated produces another second messenger, cGMP. NO can also act through covalent modification of proteins or their metal co-factors; some have a redox mechanism and are reversible. It is toxic in high concentrations and causes damage during stroke, but is the cause of many other functions like relaxation of blood vessels, apoptosis, and penile erections.

## Redox Signaling

In addition to nitric oxide, other electronically activated species are also signal-transducing agents in a process called redox signaling. Examples include superoxide, hydrogen peroxide, carbon monoxide, and hydrogen sulfide. Redox signaling also includes active modulation of electronic flows in semiconductive biological macromolecules.

## Cellular Responses

Gene activations and metabolism alterations are examples of cellular responses to extracellular stimulation that require signal transduction. Gene activation leads to further cellular effects, since the products of responding genes include instigators of activation; transcription factors produced as a result of a signal transduction cascade can activate even more genes. Hence, an initial stimulus can trigger the expression of a large number of genes, leading to physiological events like the increased uptake of glucose from the blood stream and the migration of neutrophils to sites of infection. The set of genes and their activation order to certain stimuli is referred to as a genetic program. Mammalian cells require stimulation for cell division and survival; in the absence of growth factor, apoptosis ensues. Such requirements for extracellular stimulation are necessary for controlling cell behavior in unicellular and multicellular organisms; signal transduction pathways are perceived to be so central to biological processes that a large number of diseases are attributed to their disregulation. Three basic signals determine cellular growth:

1. Stimulatory (growth factors)

(a) Transcription dependent response

For example steroids act directly as transcription factor (gives slow response, as transcription factor must bind DNA, which needs to be

transcribed. Produced mRNA needs to be translated, and the produced protein/peptide can undergo Posttranslational_modificátion (PMT))

(b) Transcription independent response for example epidermal growth factor (EGF) binds the epidermal growth factor receptor (EGFR), which causes dimerization and autophosphorylation of the EGFR, which in turn activates the intracellular signaling pathway.

2. Inhibitory (cell-cell contact)

3. Permissive (cell-matrix interactions)

The combination of these signals is integrated in altered cytoplasmic machinery which leads to altered cell behavior.

Following are some major signaling pathways, demonstrating how ligands binding to their receptors can affect second messengers and eventually result in altered cellular responses.

(a) MAPK/ERK pathway: A pathway that couples intracellular responses to the binding of growth factors to cell surface receptors. This pathway is very complex and includes many protein components. In many cell types, activation of this pathway promotes cell division, and many forms of cancer are associated with aberrations in it.

(b) cAMP-dependent pathway: In humans, cAMP works by activating protein kinase A (PKA, cAMP-dependent protein kinase) (see picture), and, thus, further effects depend mainly on cAMP-dependent protein kinase, which vary based on the type of cell.

(c) $IP_3$/DAG pathway: PLC cleaves the phospholipid phosphatidylinositol 4,5-bisphosphate (PIP2) yielding diacyl glycerol (DAG) and inositol 1,4,5-triphosphate (IP3). DAG remains bound to the membrane, and $IP_3$ is released as a soluble structure into the cytosol. $IP_3$ then diffuses through the cytosol to bind to $IP_3$ receptors, particular calcium channels in the endoplasmic reticulum (ER). These channels are specific to calcium and allow the passage of only calcium to move through. This causes the cytosolic concentration of Calcium to increase, causing a cascade of intracellular changes and activity. In addition, calcium and DAG together works to activate PKC, which goes on to phosphorylate other molecules, leading to altered cellular activity. End-effects include taste, manic depression, tumor promotion, etc.