SEM II CC Study Materials

CELL SIGNALING

Cell–signaling/ cellular conversation can be briefly explained in three stages:-

Reception: In this stage the signal molecule is detected by the receptor protein of target cell. The signal molecule generally comes from outside and is new to the target cell, where as the receptor molecules/proteins are located outside/ inside to the target cell. In other terms reception can be defined as the target cell detection of signal molecule that is coming from outside of the cell.

Transduction: This is second stage of cell signaling where the binding of signal molecule triggers the receptor protein of the target cell initiating the process of transduction.

Response: It is the third stage of cell signaling where the transduced signal finally triggers a specific cellular response. This response may be in the form of cellular activity–such as catalysis by an enzyme (Eg: Glycogen phosphorylase), rearrangement of the cytoskeleton, or activation of specific genes in the nucleus.

Signaling molecule binds to a receptor protein that is complementary in shape to a specific site of receptor, causing it to change shape and attaches there, like a key in a lock or a substrate in the catalytic site of an enzyme. Here the signaling molecule behaves as a ligand that generally causes a receptor protein to undergo a change in shape and causes the aggregation of two or more receptor molecules, which leads to further molecular events inside the cell.

All cells receive and respond to signals from their surroundings. This is accomplished by a variety of signal molecules that are secreted or expressed on the surface of one cell and bind to a receptor expressed by the other cells, thereby integrating and coordinating the function of the many individual cells that make up organisms. Each cell is programmed to respond to specific extracellular signal molecules. Extracellular signaling usually entails the following steps:

1. Synthesis and release of the signaling molecule by the signaling cell;
2. Transport of the signal to the target cell;
3. Binding of the signal by a specific receptor leading to its activation;
4. Initiation of signal-transduction pathways.

Classification::

Cell signaling can be classified as either mechanical or biochemical based on the type of the signal. Mechanical signals are the forces exerted on the cell and the forces produced by the cell. These forces can both be sensed and responded to by the cells. Biochemical signals are biochemical molecules such as proteins, lipids, ions, and gases. These signals can be categorized based on the distance between signaling and responder cells. Signaling within, between, and amongst cells is subdivided into the following classifications:

- *Intracrine* signals are produced by the target cell that stay within the target cell.
- *Autocrine* signals are produced by the target cell, are secreted, and affect the target cell itself via receptors. Sometimes autocrine cells can target cells close by if they are the same type of cell as the emitting cell. An example of this are immune cells.

- *Juxtacrine* signals target adjacent (touching) cells. These signals are transmitted along cell membranes via protein or lipid components integral to the membrane and are capable of affecting either the emitting cell or cells immediately adjacent.
- *Paracrine* signals target cells in the vicinity of the emitting cell. Neurotransmitters represent an example.
- *Endocrine* signals target distant cells. Endocrine cells produce hormones that travel through the blood to reach all parts of the body.

Cells communicate with each other via direct contact (juxtacrine signaling), over short distances (paracrine signaling), or over large distances and/or scales (endocrine signaling).

Some cell–cell communication requires direct cell–cell contact. Some cells can form gap junctions that connect their cytoplasm to the cytoplasm of adjacent cells. In cardiac muscle, gap junctions between adjacent cells allow for action potential propagation from the cardiac pacemaker region of the heart to spread and coordinate the contraction of the heart.

Many cell signals are carried by molecules that are released by one cell and move to make contact with another cell. *Endocrine* signals are called hormones. Hormones are produced by endocrine cells and they travel through the blood to reach all parts of the body. Specificity of signaling can be controlled if only some cells can respond to a particular hormone. *Paracrine* signals such as retinoic acid target only cells in the vicinity of the emitting cell. Neurotransmitters represent another example of a paracrine signal. Some signaling molecules can function as both a hormone and a neurotransmitter. For example, epinephrine and norepinephrine can function as hormones when released from the adrenal gland and are transported to the heart by way of the blood stream. Norepinephrine can also be produced by neurons to function as a neurotransmitter within the brain. Estrogen can be released by the ovary and function as a hormone or act locally via paracrine or autocrine signaling. Active species of oxygen and nitric oxide can also act as cellular messengers. This process is dubbed redox signaling.

Signaling receptors::

Cells receive information from their neighbors through a class of proteins known as receptors. Notch is a cell surface protein that functions as a receptor. Animals have a small set of genes that code for signaling proteins that interact specifically with Notch receptors and stimulate a response in cells that express Notch on their surface. Molecules that activate (or, in some cases, inhibit) receptors can be classified as hormones, neurotransmitters, cytokines, and growth factors, in general called receptor ligands. Ligand receptor interactions such as that of the Notch receptor interaction, are known to be the main interactions responsible for cell signaling mechanisms and communication notch acts as a receptor for ligands that are expressed on adjacent cells. While some receptors are cell-surface proteins, others are found inside cells. For example, estrogen is a hydrophobic molecule that can pass through the lipid bilayer of the membranes. As part of the endocrine system, intracellular estrogen receptors from a variety of cell types can be activated by estrogen produced in the ovaries.

A number of transmembrane receptors for small molecules and peptide hormones, as well as intracellular receptors for steroid hormones exist, giving cells the ability to respond to a great number of hormonal and pharmacological stimuli. In diseases, often, proteins that interact with receptors are aberrantly activated, resulting in constitutively activated downstream signals.

For several types of intercellular signaling molecules that are unable to permeate the hydrophobic cell membrane due to their hydrophilic nature, the target receptor is expressed on the membrane. When such a signaling molecule activates its receptor, the signal is carried into the cell usually by means of a second messenger such as cAMP.

CAMP dependent pathways::

cAMP was discovered by Earl Sutherland and Ted Rall. cAMP is considered a secondary messenger along with Ca^{2+}. Sutherland won the Nobel Prize in 1971 for his discovery of the mechanism of action of epinephrine in glycogenolysis, that requires cAMP as secondary messenger.

Mechanism:

G protein-coupled receptors (GPCRs) are a large family of integral membrane proteins that respond to a variety of extracellular stimuli. Each GPCR binds to and is activated by a specific ligand stimulus that ranges in size from small molecule catecholamines, lipids, or neurotransmitters to large protein hormones. When a GPCR is activated by its extracellular ligand, a conformational change is induced in the receptor that is transmitted to an attached intracellular heterotrimeric G protein complex. The G_s alpha subunit of the stimulated G protein complex exchanges GDP for GTP and is released from the complex.

In a cAMP-dependent pathway, the activated G_s alpha subunit binds to and activates an enzyme called adenylyl cyclase, which, in turn, catalyzes the conversion of ATP into cyclic adenosine monophosphate (cAMP). Increases in concentration of the second messenger cAMP may lead to the activation of

- cyclic nucleotide-gated ion channels
- exchange proteins activated by cAMP (EPAC) such as RAPGEF3
- popeye domain containing proteins (Popdc)
- an enzyme called protein kinase A (PKA).

The PKA enzyme is also known as cAMP-dependent enzyme because it gets activated only if cAMP is present. Once PKA is activated, it phosphorylates a number of other proteins including:

- enzymes that convert glycogen into glucose
- enzymes that promote muscle contraction in the heart leading to an increase in heart rate
- transcription factors, which regulate gene expression
- also phosphorylate AMPA receptors

Specificity of signaling between a GPCR and its ultimate molecular target through a cAMP-dependent pathway may be achieved through formation of a multiprotein complex that includes the GPCR, adenylyl cyclase, and the effector protein.

G protein coupled receptors:

G protein-coupled receptors (**GPCRs**), also known as **seven-(pass)-transmembrane domain receptors**, **7TM receptors**, **heptahelical receptors**, **serpentine receptor**, and **G protein-linked receptors** (**GPLR**), constitute a large protein family of receptors that detect molecules outside the cell and activate internal signal transduction pathways and, ultimately, cellular responses. Coupling with G proteins, they are called seven-transmembrane receptors because they pass through the cell membrane seven times.

G protein-coupled receptors are found only in eukaryotes, including yeast, choanoflagellates, and animals. The ligands that bind and activate these receptors include light-sensitive compounds, odors, pheromones, hormones, and neurotransmitters, and vary in size from small molecules to peptides to large proteins. G protein-coupled receptors are involved in many diseases.

There are two principal signal transduction pathways involving the G protein-coupled receptors:

- the cAMP signal pathway and
- the phosphatidylinositol signal pathway.

When a ligand binds to the GPCR it causes a conformational change in the GPCR, which allows it to act as a guanine nucleotide exchange factor (GEF). The GPCR can then activate an associated G protein by exchanging the GDP bound to the G protein for a GTP. The G protein's α subunit, together with the bound GTP, can then dissociate from the β and γ subunits to further affect intracellular signaling proteins or target functional proteins directly depending on the α subunit type ($G_{\alpha s}$, $G_{\alpha i/o}$, $G_{\alpha q/11}$, $G_{\alpha 12/13}$).

GPCRs are an important drug target and approximately 34% of all Food and Drug Administration (FDA) approved drugs target 108 members of this family. The global sales volume for these drugs is estimated to be 180 billion US dollars as of 2018.

GPCRs are involved in a wide variety of physiological processes. Some examples of their physiological roles include:

1. The visual sense: The opsins, gradually evolved from early GPCRs over 650 million years ago, use a photoisomerization reaction to translate electromagnetic radiation into cellular signals. Rhodopsin, for example, uses the conversion of *11-cis*-retinal to *all-trans*-retinal for this purpose.
2. The gustatory sense (taste): GPCRs in taste cells mediate release of gustducin in response to bitter-, umami- and sweet-tasting substances.
3. The sense of smell: Receptors of the olfactory epithelium bind odorants (olfactory receptors) and pheromones (vomeronasal receptors)
4. Behavioral and mood regulation: Receptors in the mammalian brain bind several different neurotransmitters, including serotonin, dopamine, histamine, GABA, and glutamate
5. Regulation of immune system activity and inflammation: chemokine receptors bind ligands that mediate intercellular communication between cells of the immune system; receptors such as histamine receptors bind inflammatory mediators and engage target cell types in the inflammatory response. GPCRs are also involved in immune-modulation, e. g. regulating interleukin induction or suppressing TLR-induced immune responses from T cells.
6. Autonomic nervous system transmission: Both the sympathetic and parasympathetic nervous systems are regulated by GPCR pathways, responsible for control of many automatic functions of the body such as blood pressure, heart rate, and digestive processes
7. Cell density sensing: A novel GPCR role in regulating cell density sensing.
8. Homeostasis modulation (e.g., water balance).
9. Involved in growth and metastasis of some types of tumors.
10. Used in the endocrine system for peptide and amino-acid derivative hormones that bind to GCPRs on the cell membrane of a target cell. This activates cAMP, which in turn activates several kinases, allowing for a cellular response, such as transcription.

GPCR Structure:

GPCRs are integral membrane proteins that possess seven membrane-spanning domains or transmembrane helices. The extracellular parts of the receptor can be glycosylated. These extracellular loops also contain two highly conserved cysteine residues that form disulfide bonds to stabilize the receptor structure. Some seven-transmembrane helix proteins (channelrhodopsin) that resemble GPCRs may contain ion channels, within their protein.

In 2000, the first crystal structure of a mammalian GPCR, that of bovine rhodopsin (1F88), was solved. In 2007, the first structure of a human GPCR was solved This human β_2-adrenergic receptor GPCR structure proved highly similar to the bovine rhodopsin. The structures of activated or agonist-bound GPCRs have also been determined. These structures indicate how ligand binding at the extracellular side of a receptor leads to conformational changes in the cytoplasmic side of the receptor. The biggest change is an outward movement of the cytoplasmic part of the 5th and 6th

transmembrane helix (TM5 and TM6). The structure of activated beta-2 adrenergic receptor in complex with G_s confirmed that the Gα binds to a cavity created by this movement.

GPCRs are evolutionarily related to some other proteins with seven transmembrane domains, such as microbial rhodopsins and adiponectin receptors 1 and 2 (ADIPOR1 and ADIPOR2). However, these 7TMH (7-transmembrane helices) receptors and channels do not associate with G proteins. In addition, ADIPOR1 and ADIPOR2 are oriented oppositely to GPCRs in the membrane (i.e. GPCRs usually have an extracellular N-terminus, cytoplasmic C-terminus, whereas ADIPORs are inverted)

MAP/ERK Pathways::

The **MAPK/ERK pathway** (also known as the **Ras-Raf-MEK-ERK pathway**) is a chain of proteins in the cell that communicates a signal from a receptor on the surface of the cell to the DNA in the nucleus of the cell.

The signal starts when a signaling molecule binds to the receptor on the cell surface and ends when the DNA in the nucleus expresses a protein and produces some change in the cell, such as cell division. The pathway includes many proteins, including MAPK (mitogen-activated protein kinases, originally called ERK, extracellular signal-regulated kinases), which communicate by adding phosphate groups to a neighboring protein (phosphorylating it), which acts as an "on" or "off" switch.

When one of the proteins in the pathway is mutated, it can become stuck in the "on" or "off" position, which is a necessary step in the development of many cancers. Components of the MAPK/ERK pathway were discovered when they were found in cancer cells. Drugs that reverse the "on" or "off" switch are being investigated as cancer treatments.

Overall, the extracellular mitogen binds to the membrane receptor. This allows Ras (a Small GTPase) to swap its GDP for a GTP. It can now activate MAP3K (e.g., Raf), which activates MAP2K, which activates MAPK. MAPK can now activate a transcription factor, such as Myc. In more detail:

Ras activation

Receptor-linked tyrosine kinases such as the epidermal growth factor receptor (EGFR) are activated by extracellular ligands, such as epidermal growth factor (EGF). Binding of EGF to the EGFR activates the tyrosine kinase activity of the cytoplasmic domain of the receptor. The EGFR becomes phosphorylated on tyrosine residues. Docking proteins such as GRB2 contain an SH2 domain that binds to the phosphotyrosine residues of the activated receptor.GRB2 binds to the guanine nucleotide exchange factor SOS by way of the two SH3 domains of GRB2. When the GRB2-SOS complex docks to phosphorylated EGFR, SOS becomes activated. Activated SOS then promotes the removal of GDP from a member of the Ras subfamily (most notably H-Ras or K-Ras). Ras can then bind GTP and become active.

Apart from EGFR, other cell surface receptors that can activate this pathway via GRB2 include Trk A/B, Fibroblast growth factor receptor (FGFR) and PDGFR.

Kinase cascade

Activated Ras activates the protein kinase activity of RAF kinase.[4] RAF kinase phosphorylates and activates MEK (MEK1 and MEK2). MEK phosphorylates and activates a mitogen-activated protein kinase (MAPK).

RAF, and ERK (also known as MAPK) are both serine/threonine-selective protein kinases. MEK is a serine/tyrosine/threonine kinase.

In the technical sense, RAF, MEK, and MAPK are all mitogen-activated kinases, as is MNK (see below). MAPK was originally called "extracellular signal-regulated kinases" (ERKs) and "microtubule associated protein kinase" (MAPK). One of the first proteins known to be phosphorylated by ERK was a microtubule-associated protein (MAP). As discussed below, many additional targets for phosphorylation by MAPK were later found, and the protein was renamed

"mitogen-activated protein kinase" (MAPK). The series of kinases from RAF to MEK to MAPK is an example of a protein kinase cascade. Such series of kinases provide opportunities for feedback regulation and signal amplification.

Regulation of translation and transcription

Three of the many proteins that are phosphorylated by MAPK are shown in the Figure. One effect of MAPK activation is to alter the translation of mRNA to proteins. MAPK phosphorylates 40S ribosomal protein S6 kinase (RSK). This activates RSK, which, in turn, phosphorylates ribosomal protein S6. Mitogen-activated protein kinases that phosphorylate ribosomal protein S6 were the first to be isolated.

MAPK regulates the activities of several transcription factors. MAPK can phosphorylate C-myc. MAPK phosphorylates and activates MNK, which, in turn, phosphorylates CREB. MAPK also regulates the transcription of the C-Fos gene. By altering the levels and activities of transcription factors, MAPK leads to altered transcription of genes that are important for the cell cycle.

The 22q11, 1q42, and 19p13 genes are associated with schizophrenia, schizoaffective, bipolar, and migraines by affecting the ERK pathway.