SIGNAL TRANSDUCTION

JAMES KAPIYA

6TH YEAR MEDICAL STUDENT

UNZA-SOM

INTRODUCTION

- In human body, numerous processes are required for coordinating individual cells to support the entire body.
- At the cellular level, Sensing of environments and **cell communication** for coordination relies on signal transduction; modeling signal transduction 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 these processes in human biology and medicine

- **Cell communication** occurs through chemical signals and cellular receptors by either the
- 1)direct contact of molecules on two cells surfaces or the
- 2)release of a "chemical signal" recognized by another cell (near or far).
- *Hormones* are carried by the circulatory systems to many sites.
- *Growth factors* are released to act on nearby tissues
- *Ligands* are signals that bind cell surface receptors or that can pass into the cell and bind an internal receptor.

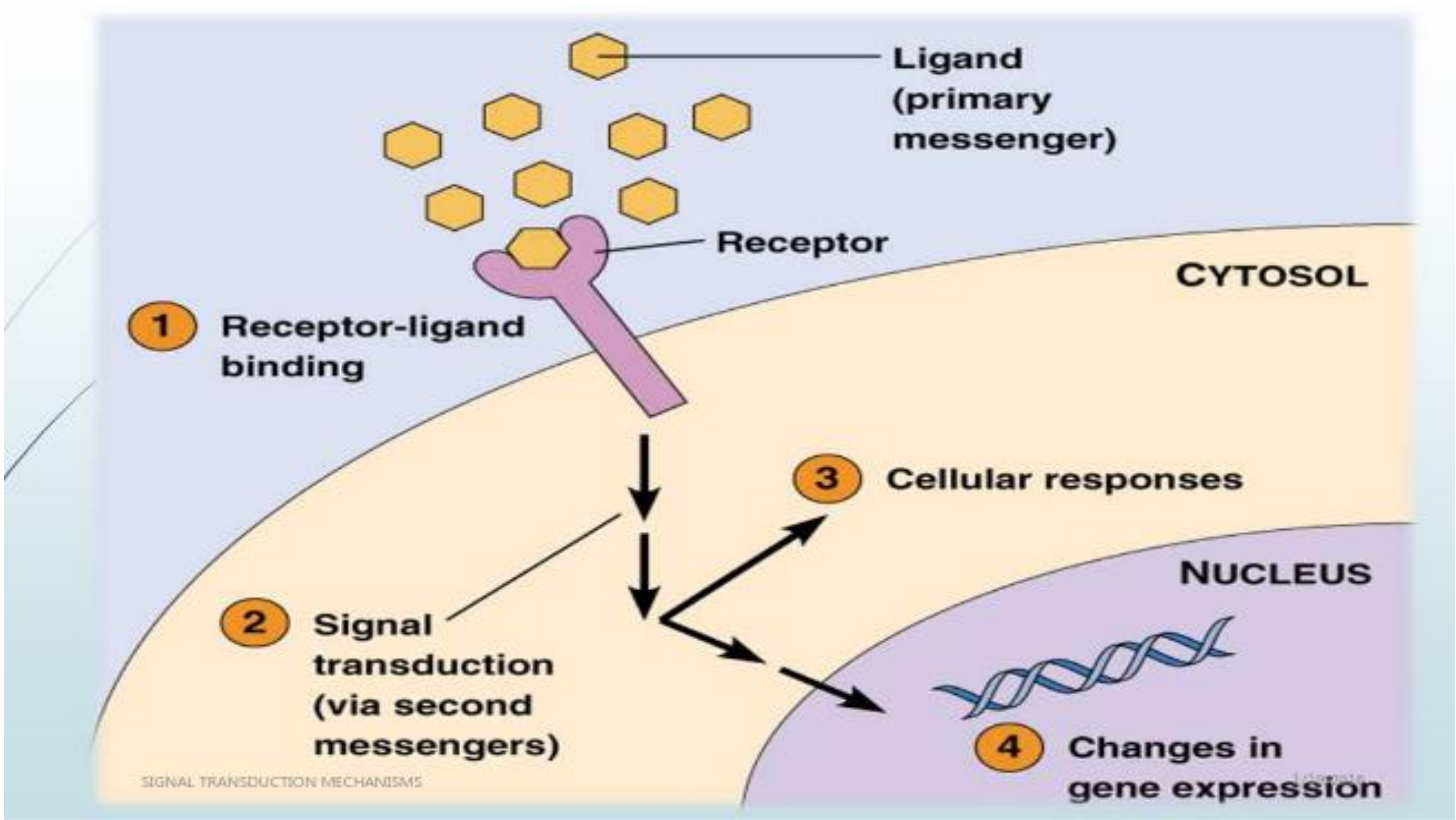
SIGNAL TRANSDUCTION

- Any process occurring within cells that convert one kind of signal/stimulus into another type.
- It also known as **cell signaling** in which the transmission of molecular signal from a cell's exterior to its interior.
- Signal received by cells must be transmitted effectively into the cell to ensure an appropriate response. This step is initiated by cell-surface receptors which triggers a biochemical chain of events inside the cell, creating a response.
- It is also defined as the ability of a cell to change behavior in response to a receptor-ligand interaction.(signal)

- The ligand is the *primary messenger* *and as the* result of binding the receptor, other molecules or *second messengers* are produced within the target cell.

- *Second messengers* relay the signal from one location to another (such as from plasma membrane to nucleus) leading to cascade of events/changes within a cell

Pictorial



Ligand
(primary
messenger)
Receptor
CYTOSOL
1 Receptor-ligand binding
3 Cellular responses
NUCLEUS
2 Signal transduction (via second messengers)
4 Changes in gene expression
SIGNAL TRANSDUCTION MECHANISMS

- Messenger molecules may be amino acids, peptides, proteins, fatty acids, lipids, nucleosides or nucleotides.
- Hydrophilic messengers bind to cell membrane receptors
- Hydrophobic messengers bind to intracellular receptors which regulate expression of specific genes.
- A ligand binds its receptor through a number of specific weak non-covalent bonds by fitting into a specific binding site or *"pocket"*
- In situations where even low concentrations of a ligand will result in binding of most of the corresponding receptors, the receptor affinity is considered to be high.

- Low receptor affinity occurs when a high concentration of the ligand is required for most receptors to be occupied.
- The *dissociation constant (Kd)* is the concentration of ligand required to occupy one half of the total available receptors.
- With prolonged exposure to a **ligand** cells often become desensitized.
- *Desensitization* of the cell to a ligand depends upon receptor down-regulation.
- *Desensitization* may lead to tolerance, a phenomenon that results in the loss of medicinal effectiveness of some medicines that are over prescribed.

RECEPTORS

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

1. EXTRACELLULAR RECEPTORS

•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).

Various Extracellular Receptors

- G protein-coupled receptors
- Receptors with Kinase activity.
- Integrin receptors.
- Toll gate receptors.
- Ligand-gated ion channel receptors

G PROTEIN-COUPLED RECEPTORS (GPCRs)

Also known as seven-transmembrane domain receptors, 7TM receptors, heptahelical receptors, and G protein-linked receptors (GPLR).

- These constitute a large protein family of receptors that sense molecules outside the cell and activate inside 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.
- 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, and are also the target of approximately 40% of all modern medicinal drugs.
- G proteins, also known as guanine nucleotide-binding proteins.
- They are family of proteins that act as molecular switches inside cells, and are involved in transmitting signals from a variety of stimuli outside a cell to its interior.
- When they are bound to GTP, they are 'on', and, when they are bound to GDP, they are 'off'.
- G proteins belong to the larger group of enzymes called *GTPases*

There are two classes of G proteins

- The first function as monomeric small *GTPases*,
- The second form and function as heterotrimeric G protein complexes.

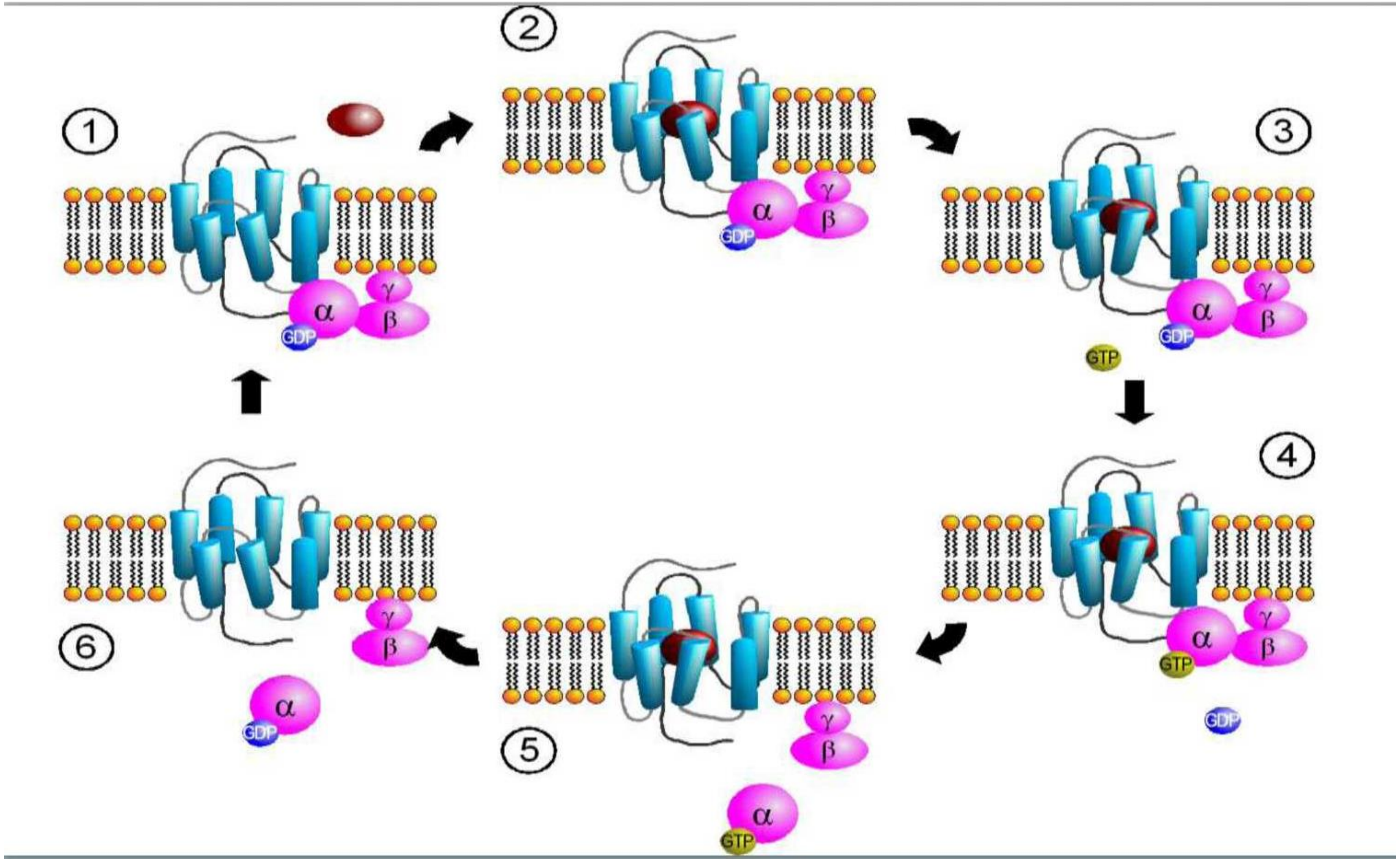
•Heterotrimeric class of complexes is made up of *alpha* (a), *beta* (|3) and *gamma* (y) subunits. The *beta* and *gamma* subunits can form a stable dimeric complex referred to as

•the *beta-gamma* complex while *alpha* subunit dissociates on activation.

MECHANISM

- It is known that in the inactive state, the GPCR is bound to a heterotrimeric G protein complex.
- Binding of an agonist to the GPCR results in a conformation change in the receptor that is transmitted to bound *$G_a$subunit* of the heterotrimeric G protein.
- The activated G_a subunit exchanges GTP in place of GDP which in turn triggers the dissociation of G_a subunit from the G» dimer and from the receptor.
- The dissociated G_a and G_{By} subunits interact with other intracellular proteins to continue the signal transduction cascade
- While the freed GPCR is able to rebind to another heterotrimeric G protein to form a new complex that is ready to initiate another round of signal transduction.

Pictorial Presentation



- There are two principal signal transduction pathways involving the G protein-coupled receptors:
- A. the *cAMPsignal pathway* and
- B. the *phosphatidylinositol signal pathway.*

- A. cAMP-DEPENDENT PATHWAY.
- It is also known as *adenylyl cyclase pathway.*
- 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

-an enzyme called Protein Kinase(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

Molecules that activate cAMP pathway include:

- cholera toxin - increase cAMP levels
- caffeine and theophylline inhibit cAMP phosphodiesterase, which degrades cAMP - thus enabling higher levels of cAMP than would otherwise be had.
- pertussis toxin, which increase cAMP levels by inhibiting Gi to its GDP (inactive) form.
- This leads to an increase in adenylyl cyclase activity, thereby increasing cAMP levels, which can lead to an increase in insulin and therefore hypoglycemia

RECEPTORS WITH KINASE ACTIVITY

- A *kinase* is a type of enzyme that transfers *phosphate* groups from high-energy donor molecules, such as *ATP* to specific target molecules (substrates); the process is termed *phosphorylation.*
- For every phosphorylation event, there is a *phosphatase,* an enzyme that can remove phosphate residue and thus modulate signaling
- Kinase enzymes that specifically phosphorylate tyrosine amino acids are termed *tyrosine kinases.*

▪ RECEPTOR TYROSINE KINASE(RTKs)

It is a cell surface receptor that also has a tyrosine kinase activity.

- The *signal binding domain* of the receptor tyrosine kinase is on the cell surface, while the *tyrosine kinase enzymatic activity* resides in the cytoplasmic part of the protein.
- A transmembrane alpha helix connects these two regions of the receptor.
- In this case, the G proteins are members of the *Ras, Rho,* and Raf families, referred to collectively as small G proteins

- The most important groups of signals that bind to receptor tyrosine kinases are:
- *peptide growth factors* like *nerve growth factor* (NGF) and *epidermal growth factor* (EGF)
- *peptide hormones*, like insulin.

- An important protein that is subsequently activated by the signaling complexes on the receptor tyrosine kinases is called *RAS*.

- Activated *RAS* triggers a *phosphorylation cascade* of protein kinases, which relay and distribute the signal.

- These protein kinases are members of a group called the *MAP kinases* (Mitogen Activated Protein Kinases
- The final kinase in this cascade phosphorylates various target proteins, including

- *transcriptional activators* (e.g.myc) that regulate gene expression.

- *RAS* is the most common mutated proto-oncogene in human tumors. Indeed, approximately 30% of all human tumors contain mutated versions of the *RAS* gene, and the frequency is even higher in some specific cancers (e.g colon and pancreatic adenocarcinomas)

NON RECEPTOR TYROSINE KINASE (nRTKs)

- *Non-receptor tyrosine kinases* are a subgroup of protein family *tyrosine kinases,* enzymes that can transfer the phosphate group from ATP to a tyrosine residue of a protein (phosphorylation).
- They are cytoplasmic enzymes
- **nRTKs** regulate cell's growth, proliferation, differentiation, adhesion, migration and apoptosis and they are critical components in the regulation of the immune system
- The main function of nRTKs is their involvement in signal transduction in activated T- and B-cells in the immune system.
- CD4 and CD8 receptors on T lymphocytes require for their signaling the Src family member *Lck.*

- Src contains unique functional regions such as Src-homology 2 (SH2) and Src- homology 3 (SH3).
- SH2 domains typically bind to receptors phosphorylated by another kinase, allowing the aggregation of multiple enzymes
- SH3 domains mediate other protein-protein interactions.

JAK-STAT Signaling pathway

- The **JAK-STAT signaling pathway** transmits information from chemical signals outside the cell, through the cell membrane, and into gene promoters on the DNA in the cell nucleus, which causes DNA transcription and activity in the cell.
- The JAK-STAT system is a major signaling alternative to the second messenger system and is an example of nRTK.
- The JAK-STAT system consists of three main components:

•1. a receptor

•2. Janus kinase (JAK) and

•3. Signal Transducer and Activator of Transcription (STAT).

- Many JAK-STAT pathways are expressed in white blood cells, and are therefore involved in regulation of the immune system.

MECHANISM.

•The binding of the *ligand* to the receptor triggers activation of JAKs.

•With increased kinase activity, they phosphorylate tyrosine residues on the receptor and create sites for interaction with proteins that contain phosphotyrosine-binding SH2 domains.

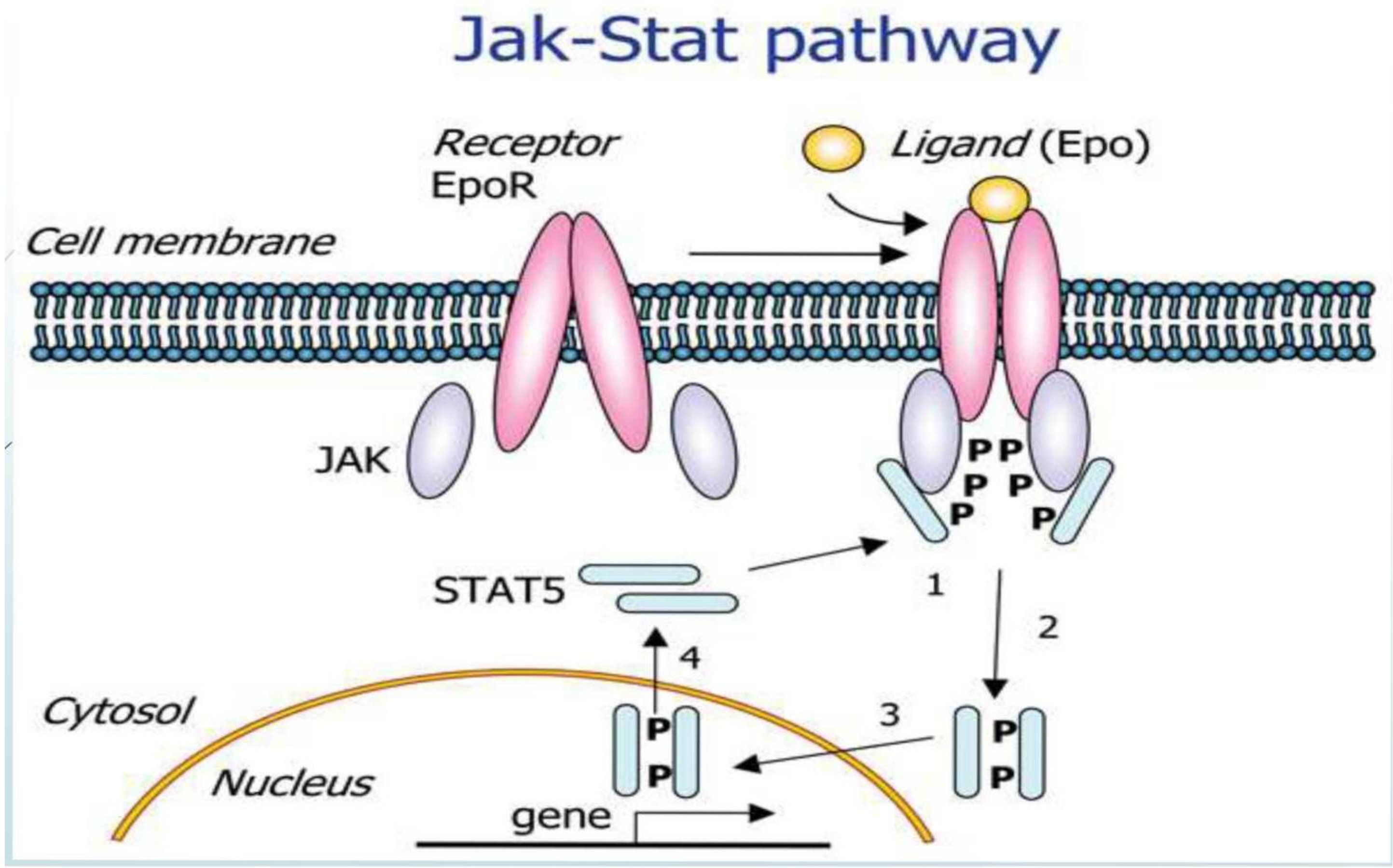
•STATs possessing SH2 domains capable of binding these phosphotyrosine residues are recruited to the receptors, and are themselves tyrosine-phosphorylated by JAKs

- These phosphotyrosines then act as binding sites for SH2 domains of other STATs, mediating their dimerization. Different STATs form hetero- or homodimers.
- Activated STAT dimers accumulate in the cell nucleus and activate transcription of their target genes

•JAK-STAT pathway mutations are associated with many hematological malignancies caused by gaining constitutive functions e.g.

- MYELOPROLIFERATIVE NEOPLASMS; Ph negative MPNs
- MDS
- MULTIPLE MYELOMA.

JAK-STAT PATHWAY



INTEGRINS

Integrins are produced by a wide variety of cells; they play a role in:

1. Cell attachment to other cells and the extracellular matrix and
2. 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.*

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- Integrin signaling exist in two places mainly;
- Integrin-signaling in circulating blood cells and non-circulating cells such as epithelial cells.
- Important differences exist between two is that 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.

TOLL GATE RECEPTORS

- **Toll-like receptors (TLRs)** are a class of proteins that play a key role in the innate immune system.
- TLRs are a type of *pattern recognition receptor* (PRR)
- They recognize molecules that are broadly shared by pathogens but distinguishable from host molecules, collectively referred to as *pathogen-associated molecular patterns* (PAMPs).

LIGAND-GATED ION CHANNEL

- Ligand-gated ion channels (LGICs) are a group of transmembrane ion channel proteins which open to allow ions such as;
- Na^+, K^+, Ca^{2+}, or Cl^- to pass through the membrane in response to the binding of a chemical messenger (i.e.a ligand), such as a neurotransmitter
- 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.
- *Ligand-gated ion channels* are likely to be the major site at which anaesthetic agents and ethanol have their effects, in particular, the GABA and NMDA receptors are affected by anaesthetic agents.

2.INTRACELLULAR RECEPTORS

- Intracellular receptors are receptors located inside the cell rather than on its cell membrane.

  •Classic hormones that use intracellular receptors include thyroid and steroid hormones.

  •Examples are:

  •Class of nuclear receptors located in the cell nucleus and cytoplasm

  •IP_3 receptor located on the endoplasmic reticulum.

- 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.

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- Due to their enabling gene transcription, they are alternatively called inductors of gene expression.

CONCLUSION

- The entire signal transduction system normally works astonishingly well, but serious problems can occur.
- Cancer is unregulated cell growth and occurs when the machinery tightly regulating cell growth breaks down.
- Mutations in growth factor receptors, *Gproteins, MAP kinases*, and other molecules frequently contribute to cancer,
- And this result in these molecules losing their normal switching function, staying in the activated form
- and therefore inappropriately stimulating these important enzyme cascades.

How does signal transduction Relate to Cancer?

- ST play key roles in the initiation, progression and dissemination of cancer. This signaling molecules are attractive targets for cancer therapeutics.
- Abnormal changes in signaling pathways frequently underlie the initiation and progression of cancer. Activation/overexpression of oncogenes or loss of inhibition tumor suppressor genes connected to signal transduction can lead to multiple manifestations of cancer.
- This includes both changes to the tumor cells themselves and alteration of the tumor microenvironment .

Which signal transduction pathway lead to cancer?

- The two RAS signaling pathway mostly prominently associated with cancer are ;

(i) MAP kinase pathway regulating cell proliferation

(ii) The phosphoinositide 3-kinase (PI3K) pathway that regulates cell metabolism and survival.

Role of signal transduction in cancer treatment and drug resistance

- ST involves cell differentiation, proliferation and cell death with alterations in these mechanisms being involved in the pathogenesis of cancer.
- It has been postulated that such pathways could be linked to anti-cancer drug resistance.
- Recently, novel approaches to overcome anti-cancer drug resistance through manipulation of signal transduction pathway, have been introduced in clinical trials.

THANK YOU

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