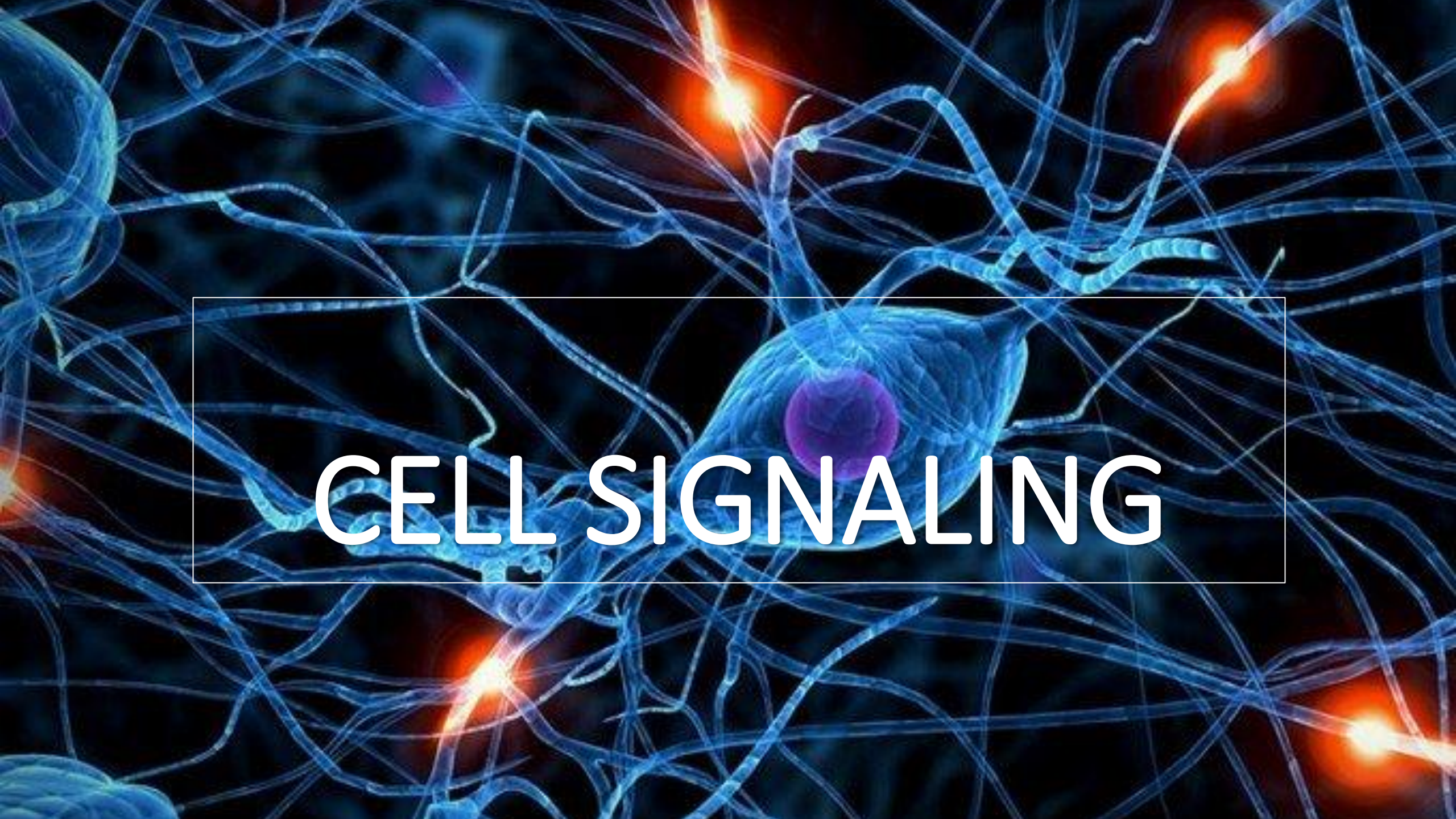

CELL SIGNALING

What is cell signaling?

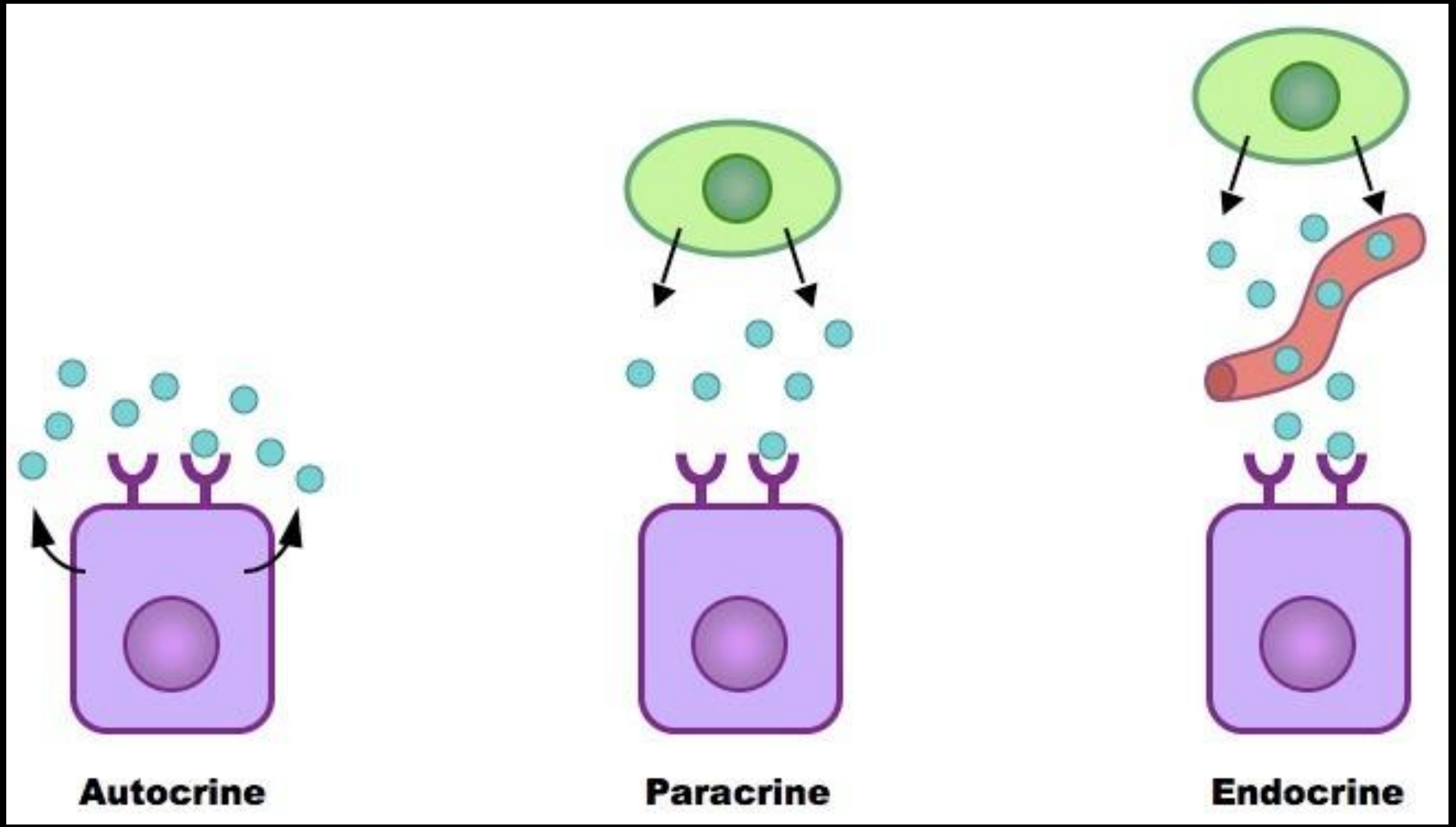
- It is the communication that occur between cells.
- Cell signaling makes it possible for cells to respond in an appropriate manner to a specific environmental stimulus.

Basic elements of cell signaling

- Cells usually communicate with each other through **extracellular messenger molecules**.

- Extracellular messengers can travel a short distance and stimulate cells that are in close proximity to the origin of the message, or they can travel throughout the body, potentially stimulating cells that are far away from the source.

- Three different types of signaling is seen;
  - Autocrine
  - Paracrine
  - Endocrine (Endocrine messengers are called hormones)


Autocrine
Paracrine
Endocrine

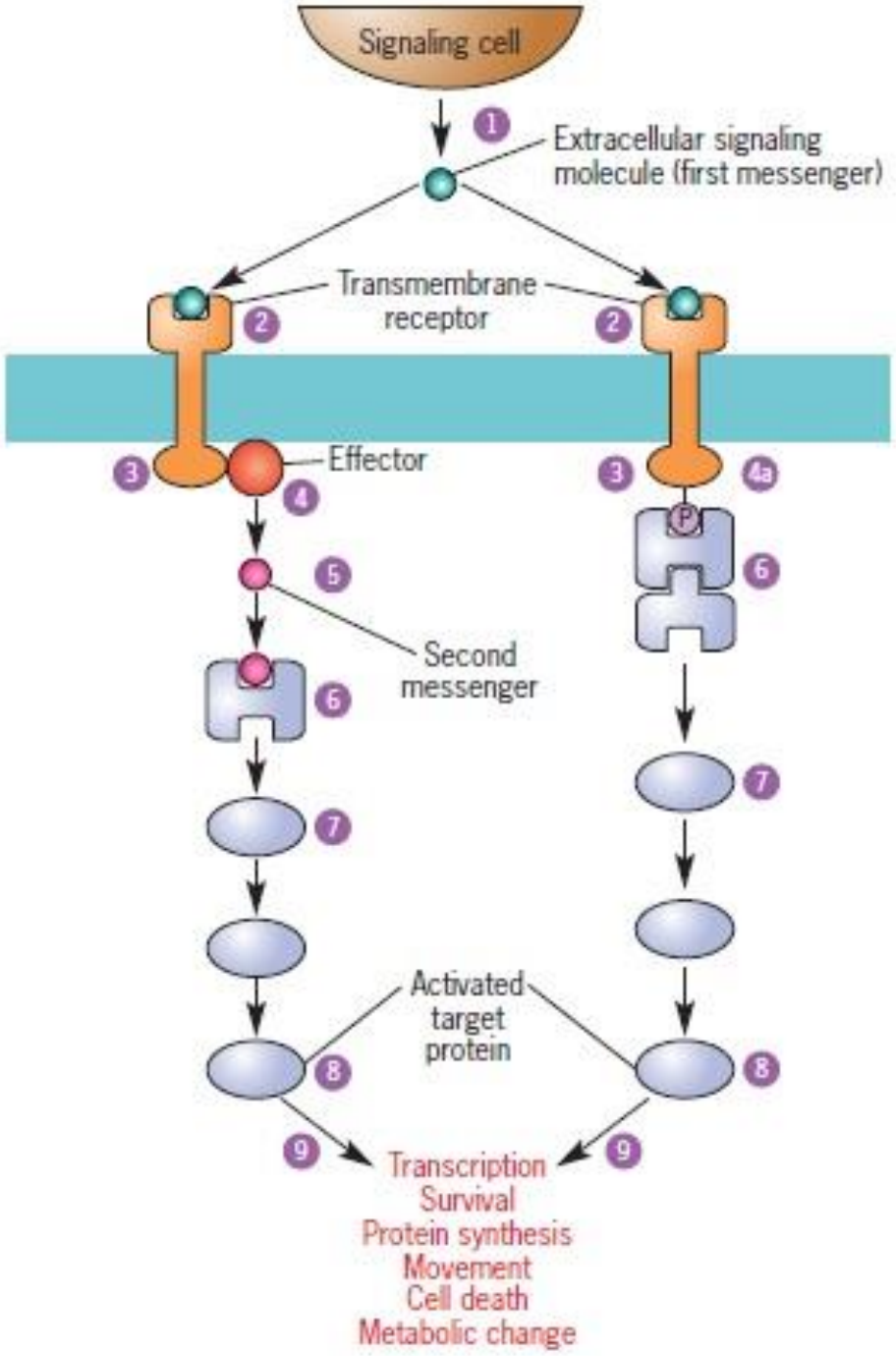
An overview of cellular signaling pathway

1. Cell signaling is initiated with the release of a messenger molecule by a cell that is engaged in sending messages to other cells in the body.

2. Cells can only respond to an extracellular message if they express **receptors** that specifically recognize and bind that particular messenger molecule.

3. In most cases, the messenger molecule (or ligand) binds to a receptor at the extracellular surface of the responding cell. This interaction causes a signal to be relayed across the membrane to the receptor's cytoplasmic domain.

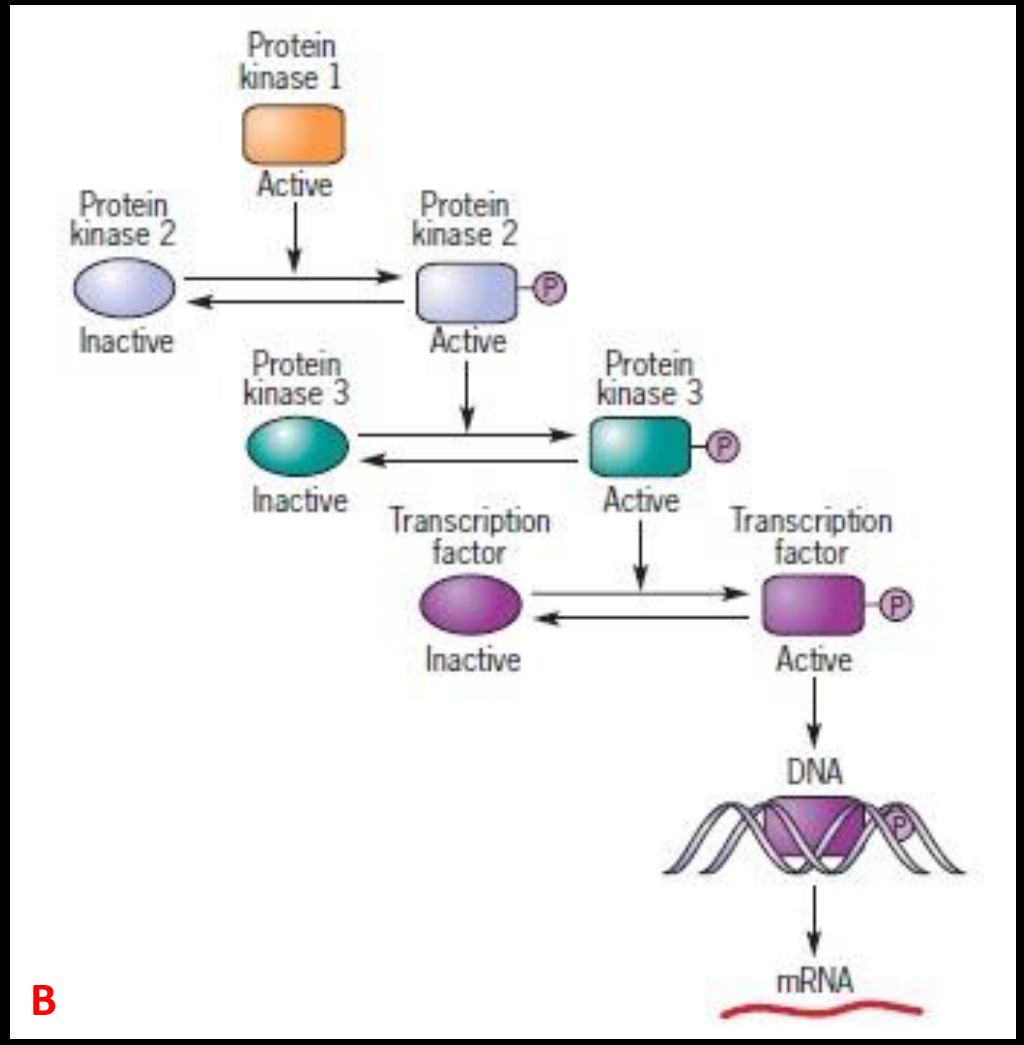
4. Once it has reached the inner surface of the plasma membrane, there are two major routes by which the signal is transmitted into the cell interior, where it elicits the appropriate response. The particular route taken depends on the type of receptor that is activated.


Signaling cell
1
Extracellular signaling molecule (first messenger)
Transmembrane receptor
2
2
Effector
3
4
3
4a
P
5
6
Second messenger
6
7
7
Activated target protein
8
8
9
9
Transcription
Survival
Protein synthesis
Movement
Cell death
Metabolic change

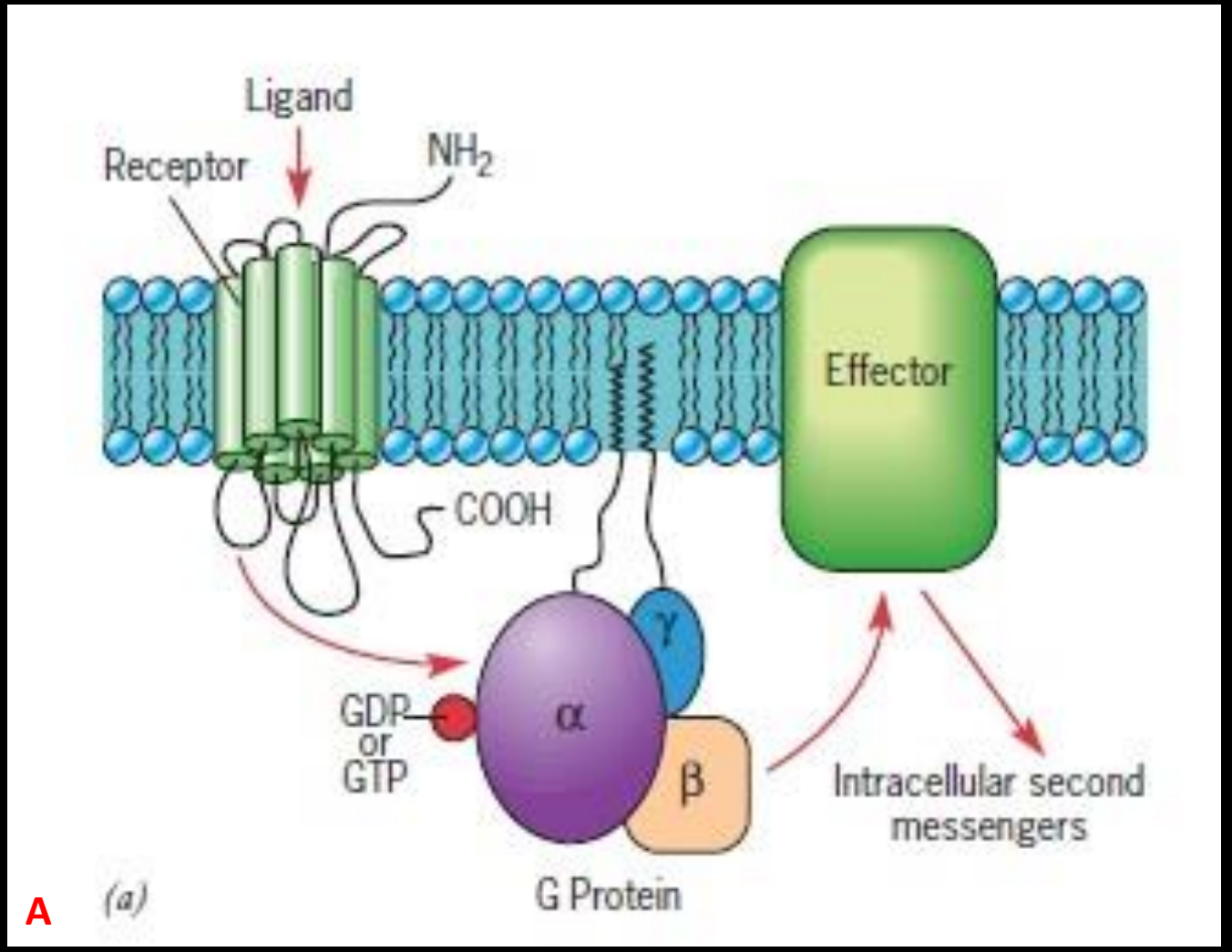
Two major routes of signal transduction

A. One type of receptor transmits a signal from its cytoplasmic domain to a nearby enzyme, which generates a **second messenger**. Because it brings about (effects) the cellular response by generating a second messenger, the enzyme responsible is referred to as an **effector**. Second messengers are small substances that typically activate (or inactivate) specific proteins. Depending on its chemical structure, a second messenger may diffuse through the cytosol or remain embedded in the lipid bilayer of a membrane.

B. Another type of receptor transmits a signal by transforming its cytoplasmic domain into a recruiting station for cellular signaling proteins. Proteins interact with one another, or with components of a cellular membrane, by means of specific types of interaction domains.


Protein kinase 1
Active
Protein kinase 2
Inactive
Protein kinase 2
Active
Protein kinase 3
Inactive
Protein kinase 3
Active
Transcription factor
Inactive
Transcription factor
Active
DNA
mRNA

B


Ligand
Receptor
NH_2
Effector
COOH
GDP or GTP
α
γ
β
Intracellular second messengers
(a)
G Protein

A

Outcomes of Signaling pathways

I. Change in gene expression
II. Alteration of the activity of metabolic enzymes
III. Reconfiguration of cytoskeleton
IV. Increase or decrease in cell mobility
V. Change in ion permeability of the cell
VI. Activation of DNA synthesis
VII. The death of the cell

How a signaling is terminated?

- Termination of signaling is important because cells have to be responsive to additional messages that they may receive.

- The first order of business is to;
  - Eliminate the extracellular messenger molecule. To do this, certain cells produce extracellular enzymes that destroy specific extracellular messengers.
  - Activated receptors are internalized. Once inside the cell, the receptor may be degraded together with its ligand, which can leave the cell with decreased sensitivity to subsequent stimuli.
  - Receptor and ligand separated within an endosome, after which the ligand is degraded and the receptor is returned to the cell surface.

Extracellular messenger and their receptors

1. Extracellular messengers

- Amino acids and amino acid derivatives. Examples include glutamate, glycine, acetylcholine, epinephrine, dopamine, and thyroid hormone. These molecules act as neurotransmitters and hormones.

- Gases, such as NO and CO.

- Steroids, which are derived from cholesterol. Steroid hormones regulate sexual differentiation, pregnancy, carbohydrate metabolism, and excretion of sodium and potassium ions.

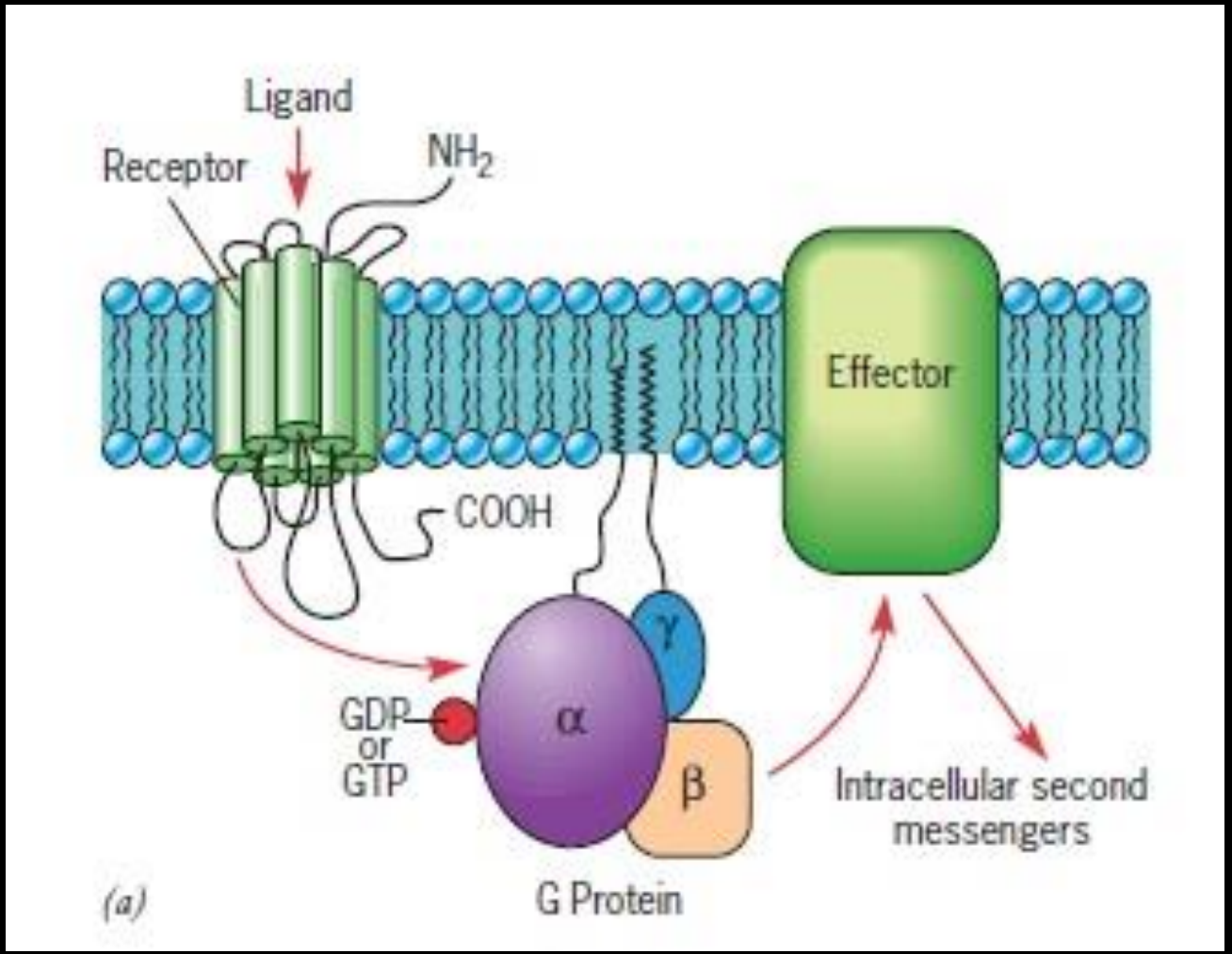
- Eicosanoids, that are derived from a fatty acid named arachidonic acid. Eicosanoids regulate a variety of processes including pain, inflammation, blood pressure and blood clotting.

- Wide variety of polypeptides and proteins. They are involved in regulating processes such as cell division, differentiation, the immune response, or cell death and cell survival.

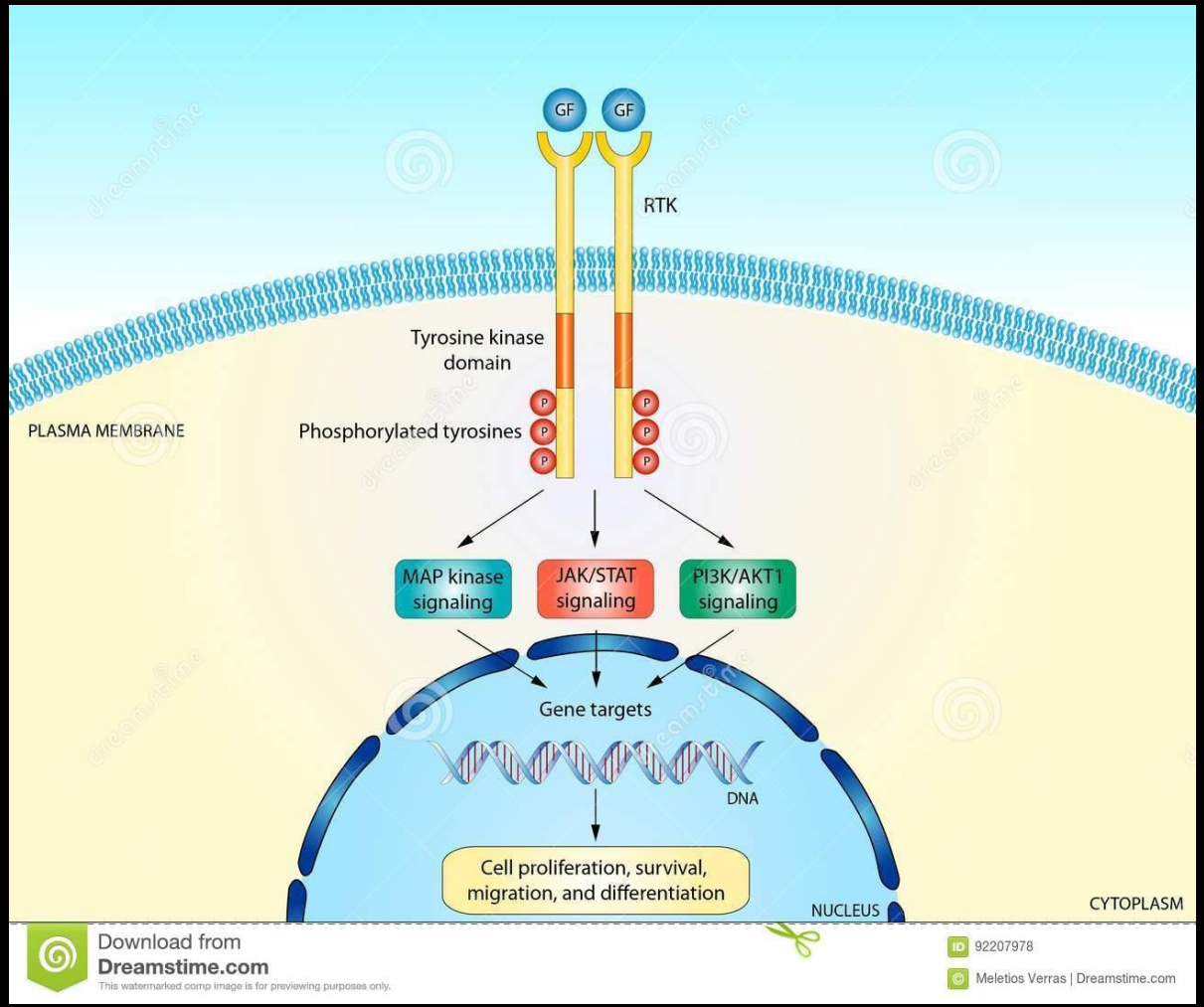
2. Receptors in signaling

- G protein-coupled receptors (GPCRs)
  - Are a huge family of receptors that contain seven transmembrane helices.
  - These receptors translate the binding of extracellular signaling molecules into the activation of GTP-binding proteins.
  - **GTP-binding proteins** (or **G proteins**) helps in transmitting messages along "cellular information circuits."

- Receptor protein-tyrosine kinases (RTKs)
  - Represent a second class of receptors that have evolved to translate the presence of extracellular messenger molecules into changes inside the cell.
  - Binding of a specific extracellular ligand to an RTK usually results in receptor dimerization followed by activation of the receptor's protein-kinase domain, which is present within its cytoplasmic region.
  - Upon activation, these protein kinases phosphorylate specific tyrosine residues of cytoplasmic substrate proteins, thereby altering their activity, their localization, or their ability to interact with other proteins within the cell.



GPCR Pathway

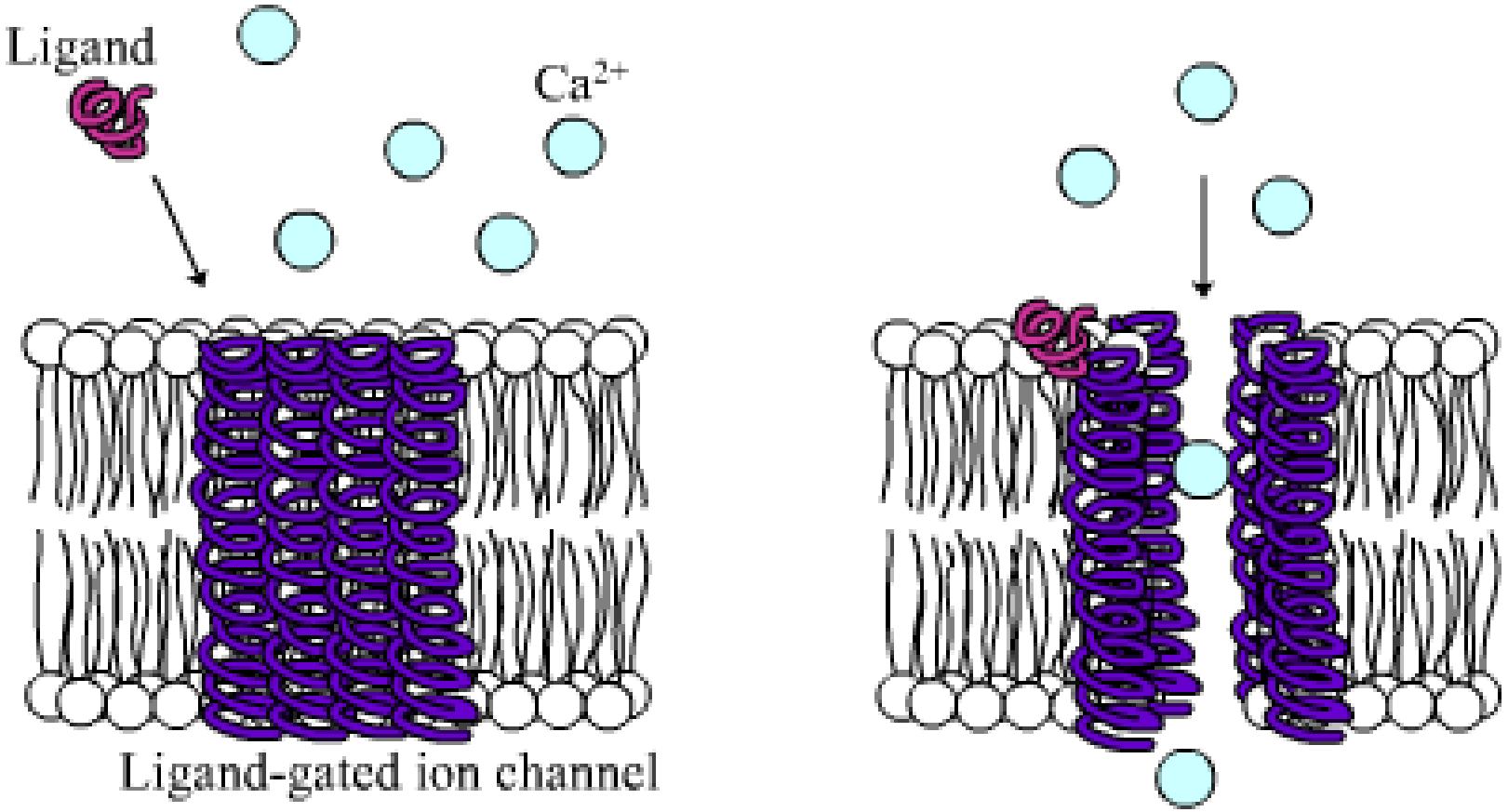


RTK Pathway

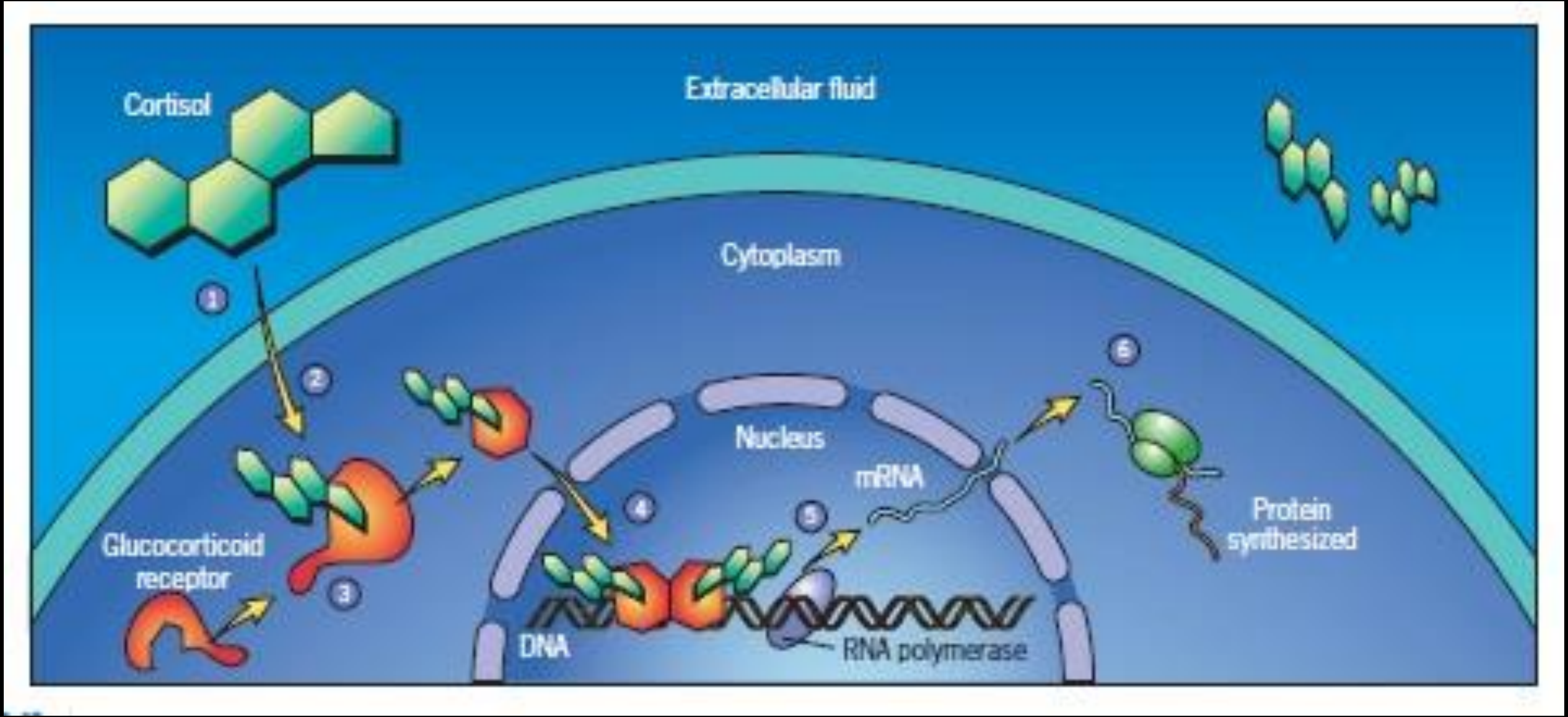
- Ligand-gated channels
  - Represent a third class of cell surface receptors that bind to extracellular ligands.
  - The ability of these proteins to conduct a flow of ions across the plasma membrane is regulated directly by ligand binding.
  - A flow of ions across the membrane can result in a temporary change in membrane potential, which will affect the activity of other membrane proteins, for instance, voltage-gated channels.
  - In addition, the influx of certain ions, such as Ca_2, can change the activity of particular cytoplasmic enzymes.

- Steroid hormone receptors
  - They function as ligand-regulated transcription factors.
  - Steroid hormones diffuse across the plasma membrane and bind to their receptors, which are present in the cytoplasm.
  - Hormone binding results in a conformational change that causes the hormone–receptor complex to move into the nucleus and bind to elements present in the promoters or enhancers of hormone-responsive genes. This interaction gives rise to an increase or decrease in the rate of gene transcription.

Ligand-gated channel



Steroid hormone receptor



G PROTEIN-COUPLED RECEPTORS AND THEIR SECOND MESSENGERS

(GPCR)

- **G protein-coupled receptors** (**GPCRs**) are so named because they interact with G proteins.

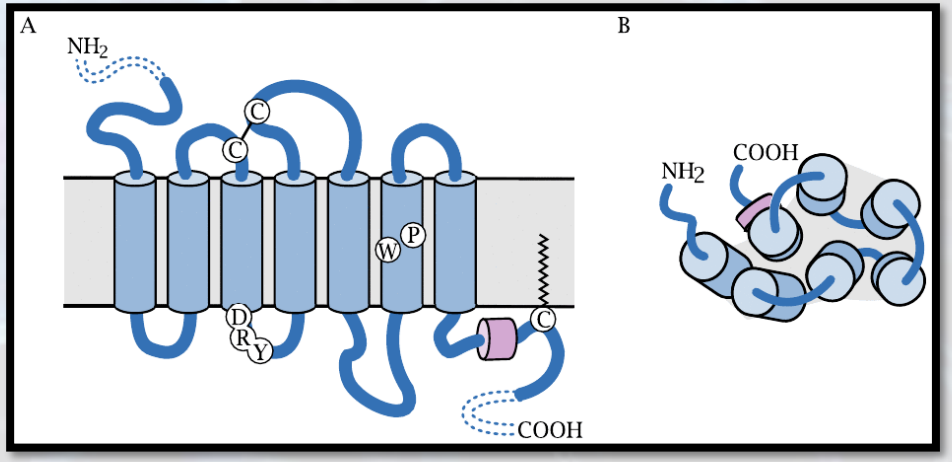
- Members of the GPCR superfamily are also referred to as seven-transmembrane (7TM) receptors because they contain seven transmembrane helices.

Signal transduction by G protein-Coupled receptors

1) Receptors

- GPCR have their amino-terminus is present on the outside of the cell
- Seven **α** helices traverse the plasma membrane and are connected by loops of varying lengths
- Carboxyl terminal is present on the inner side of the cell
- Three loops on the outside of the cell forms the ligand binding site.
- Three loops on the inside of the cell provides the binding sites for intracellular signaling proteins
- G protein binds to the third intracellular loop.

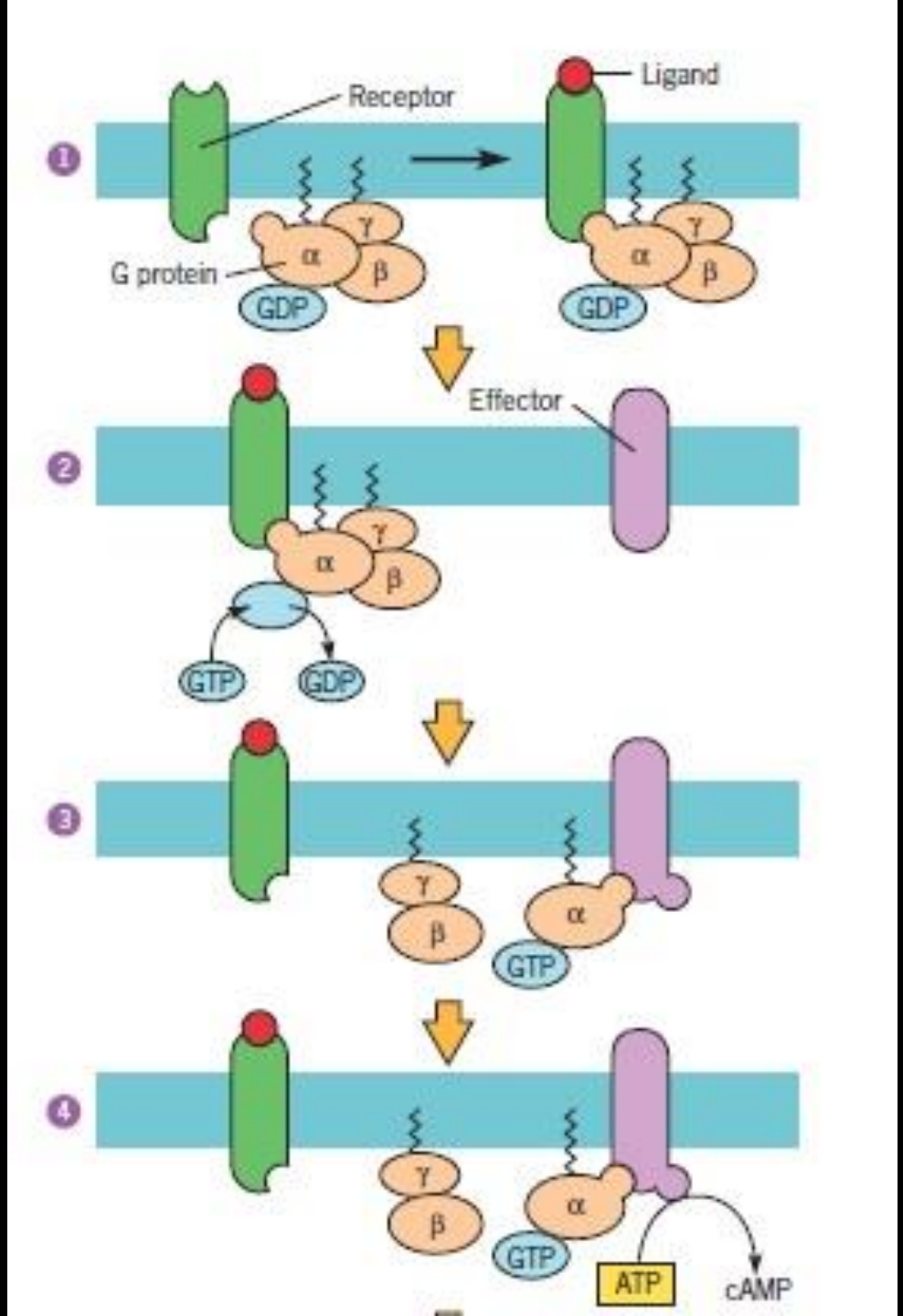
(Mechanism of action)

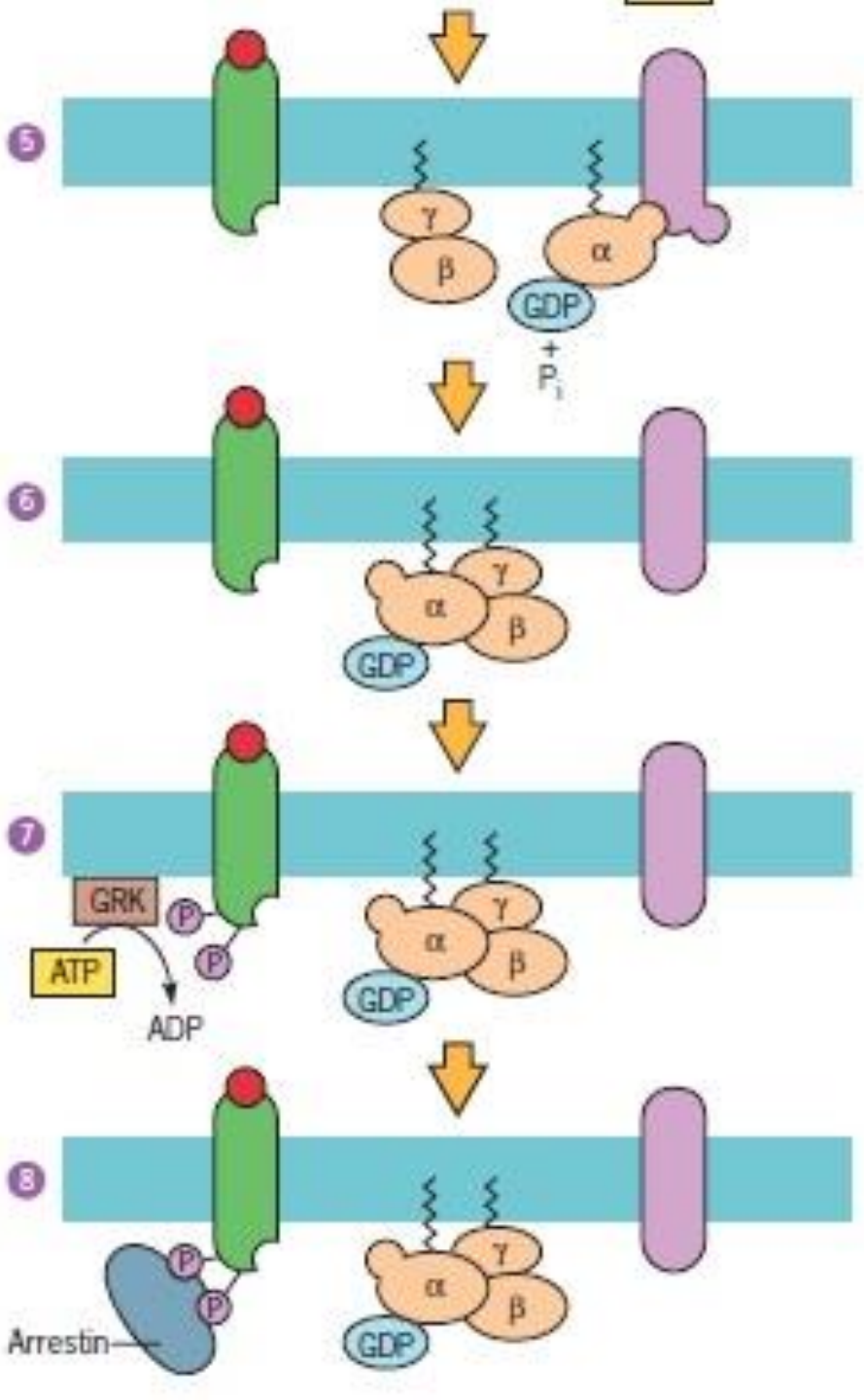


2. G proteins

- Called as G proteins because they bind guanine nucleotides (GDP or GTP)
- They are called heterotrimeric because all of them consist of three different polypeptide subunits, called **α** , **β** and **γ**.
- Heterotrimeric G proteins are held at the plasma membrane by lipid chains that are covalently attached to the α and γ subunits.
- The guanine nucleotide-binding site is present on the G_{α} subunit.
- Replacement of GDP by GTP on the G_{α} results in a conformational change in the G_{α} subunit.
- The G_{α} subunit now has a low affinity for $G_{\beta\gamma}$, leading to its dissociation from the complex.
- Each dissociated G_{α} subunit (with GTP attached) is free to activate an effector protein, such as adenylyl cyclase.
- Activation of the effector leads to the production of the second messenger like cAMP which activate one or more cellular signaling pathways.

GPCR SIGNALING PATHWAY





Termination of GPCR signaling

- To prevent overstimulation, receptors have to be blocked from continuing to activate G proteins.
- *Desensitization*, the process that blocks active receptors from turning on additional G proteins.
  - The cytoplasmic domain of the activated GPCR is phosphorylated by a specific type of kinase, called *G protein-coupled receptor kinase* (*GRK*). GRKs form a small family of serine-threonine protein kinases that specifically recognize activated GPCRs.
  - Phosphorylation of the GPCR sets the stage for the second step, which is the binding of proteins, called *arrestins*. Arrestins form a small group of proteins that bind to GPCRs and compete for binding with heterotrimeric G proteins. As a consequence, arrestin binding prevents the further activation of additional G proteins. This action is termed desensitization because the cell stops responding to the stimulus, while that stimulus is still acting on the outer surface of the cell.

- Termination of the response is accelerated by another class of proteins called *regulators of G protein signaling* (*RGSs*).
  - The interaction with an RGS protein increases the rate of GTP hydrolysis by the G_{α} subunit. Once the GTP is hydrolyzed, the G_{α} -GDP reassociates with the $G_{\beta\gamma}$ subunits to reform the inactive trimeric complex leading the system to resting state.

G-Protein coupled receptors regulate ion channels

- Acetylcholine decreases heart rate.

- Mechanism

  - Acetylcholine + Receptor → Opens K+ Channel → Movement of K+ into the cytosol → Produces a negative potential across the Plasma membrane (Hyperpolarized) → Reduces the frequency of heart muscle contraction → reduces the heart rate.

Regulation of blood glucose levels

- Controlled by hormones: Glucagon, Insulin and Epinephrine.
  - Glucagon stimulates breakdown of glycogen and release of glucose into the bloodstream, thereby causing glucose levels to rise. Glucagon is a small protein that is composed of 29 amino acids. (GPCR)
  - Insulin is produced by the beta cells of the pancreas in response to high glucose levels and stimulates glucose uptake and storage as glycogen. (RTK)
  - Epinephrine—which is sometimes called the "fight-or-flight" hormone—is produced by the adrenal gland in stressful situations. Epinephrine causes an increase in blood glucose levels to provide the body with the extra energy resources needed to deal with the stressful situation at hand. Epinephrine is a small molecule that is derived from the amino acid tyrosine. (GPCR)

- Epinephrine and Glucagon bind to GPCRs and stimulate the breakdown of glycogen into glucose 1-phosphate.

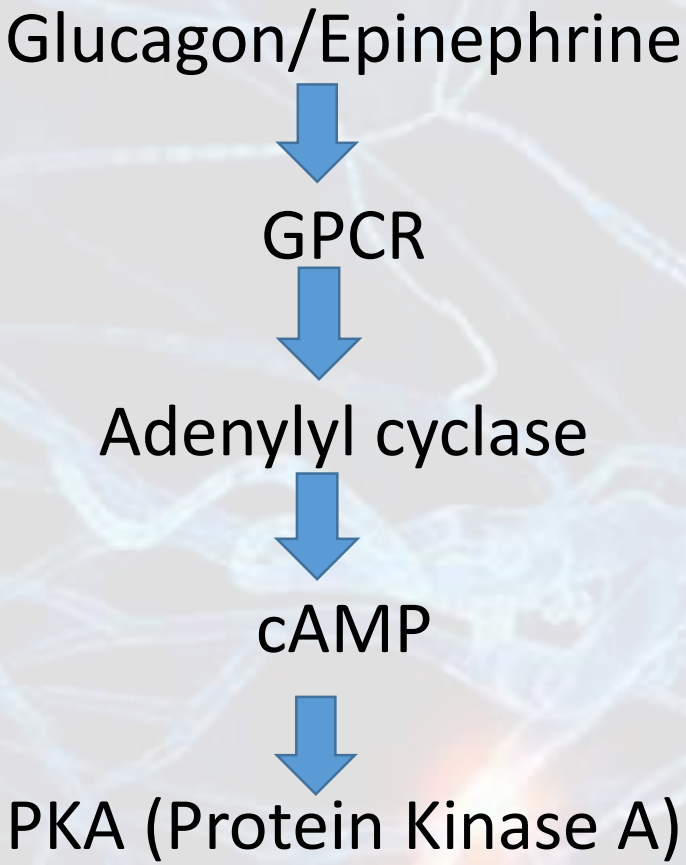
- In addition, the binding of either of these hormones leads to the inhibition of the enzyme glycogen synthase.

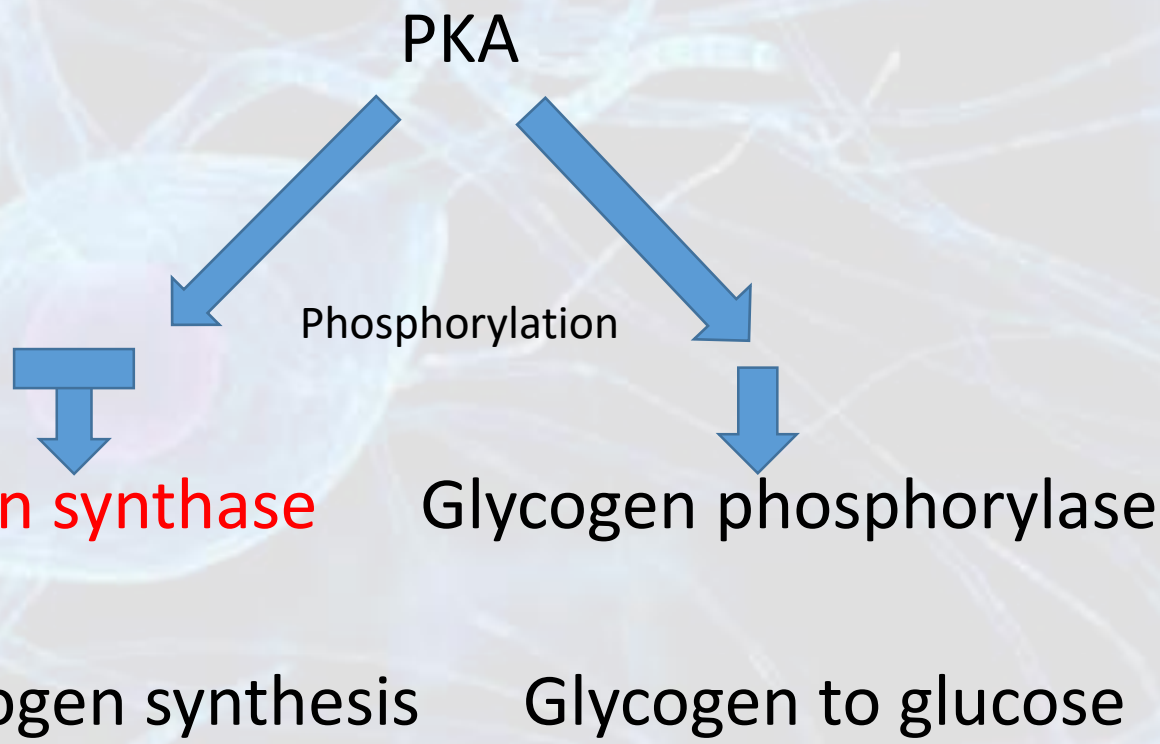
- The two different stimuli (glucagon and epinephrine) are recognized by different receptors but both induce the same response in a single target cell.

- The two receptors differ from one another primarily in the structure of the ligand-binding pocket on the extracellular surface of the cell.

- Once they get activated by their respective ligands, both receptors activate the same type of heterotrimeric G proteins that cause an increase in the levels of cAMP.

Mechanism of action





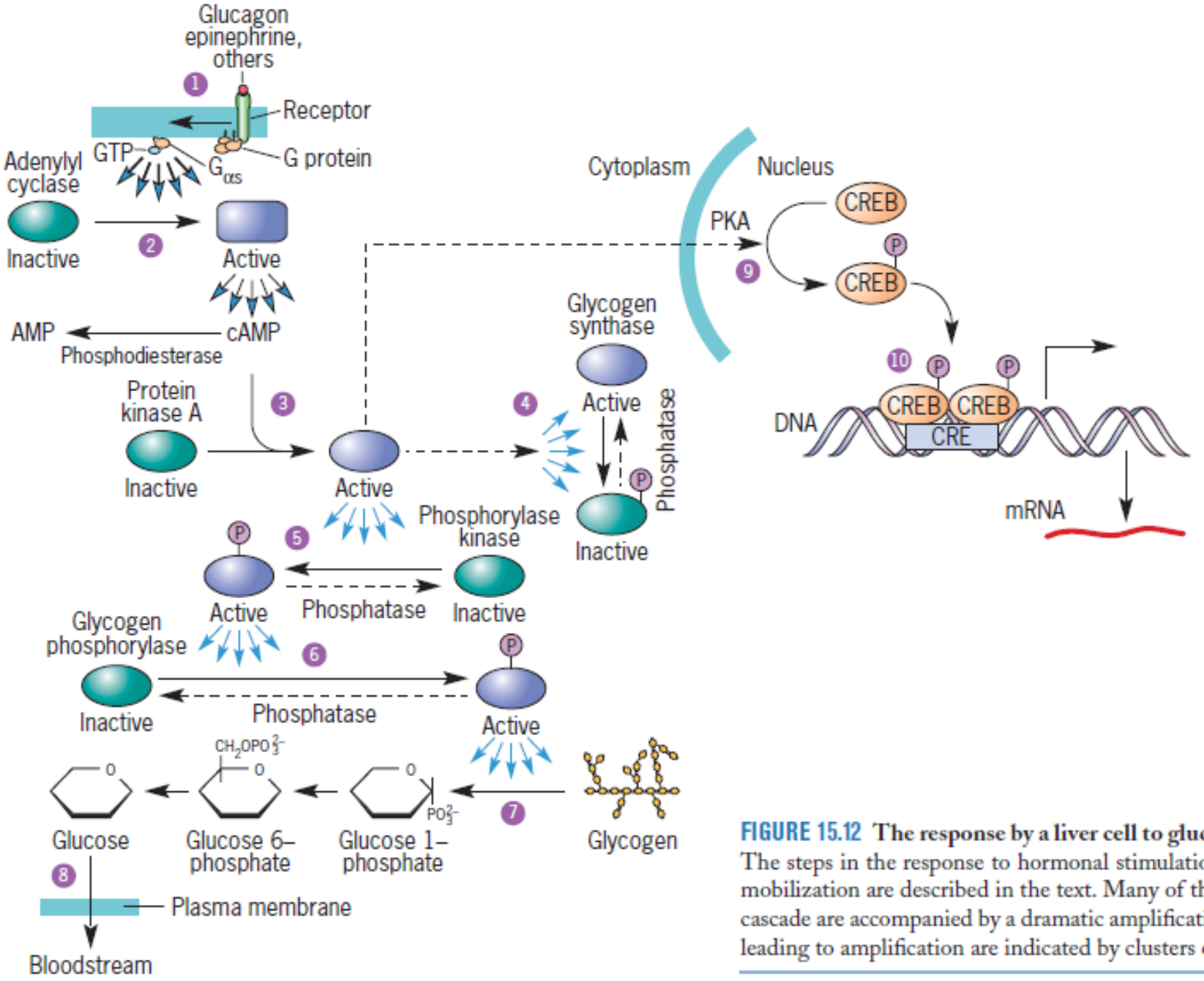
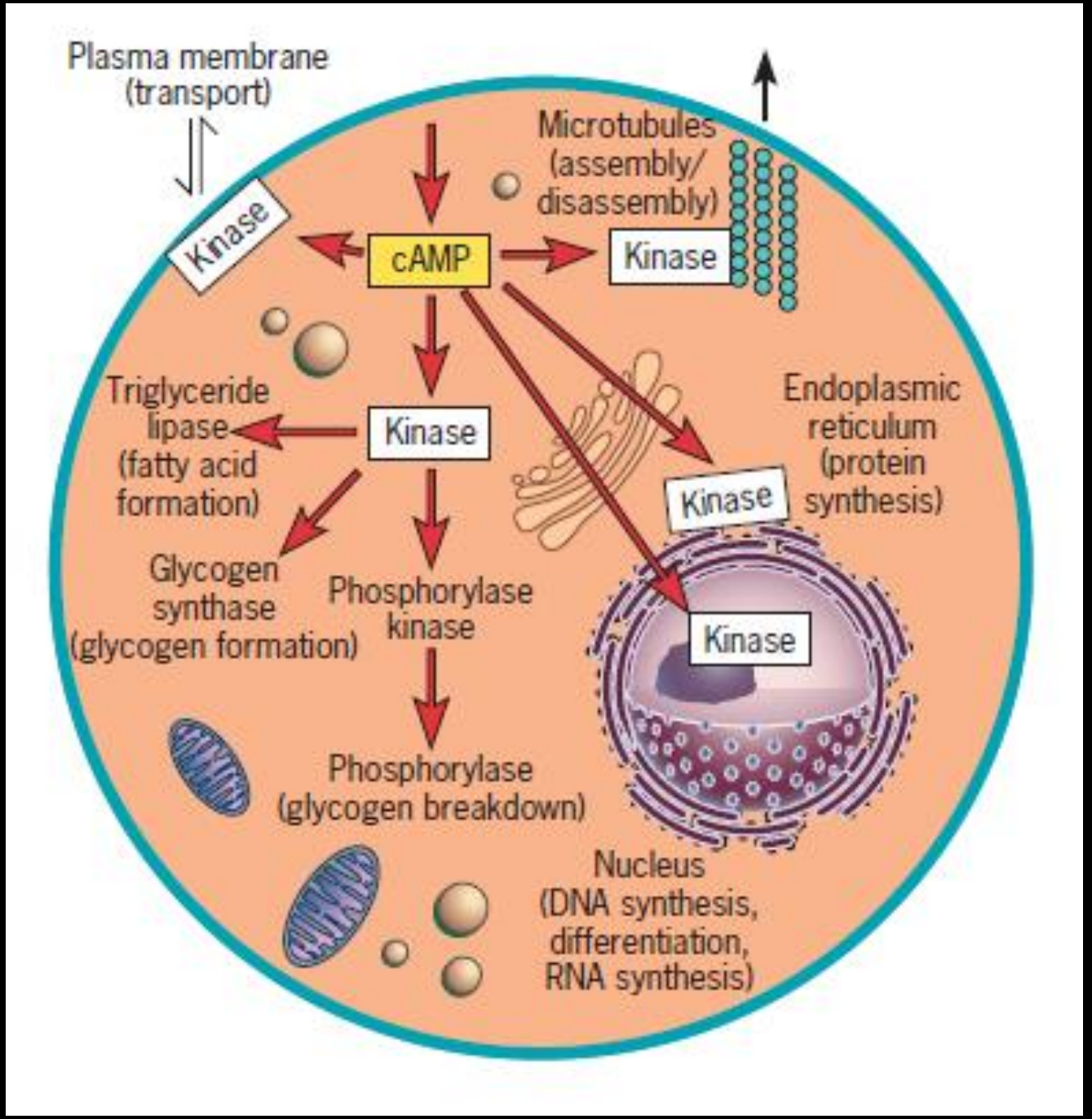


FIGURE 15.12 The response by a liver cell to glucagon or epinephrine. The steps in the response to hormonal stimulation that lead to glucose mobilization are described in the text. Many of the steps in the reaction cascade are accompanied by a dramatic amplification of the signal. Steps leading to amplification are indicated by clusters of blue arrows.

Schematic illustration of the variety of processes that
can be affected by changes in cAMP concentration.



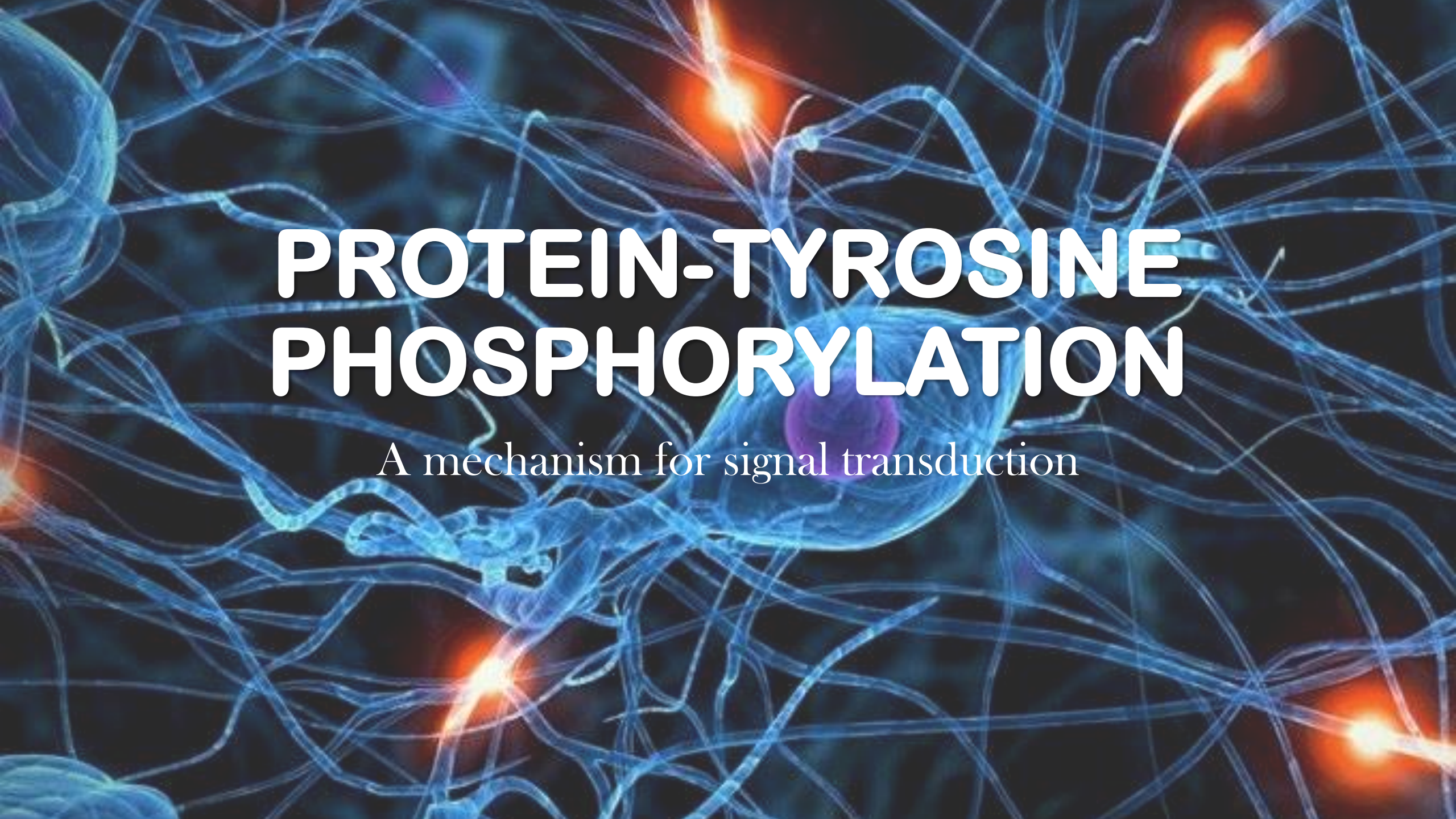
Second Messengers

1. Cyclic AMP

2. Phosphatidylinositol derived messengers

3. Phospholipase C

(Prepare notes and submit on next Thursday)



PROTEIN-TYROSINE PHOSPHORYLATION

A mechanism for signal transduction

Protein Tyrosine kinases

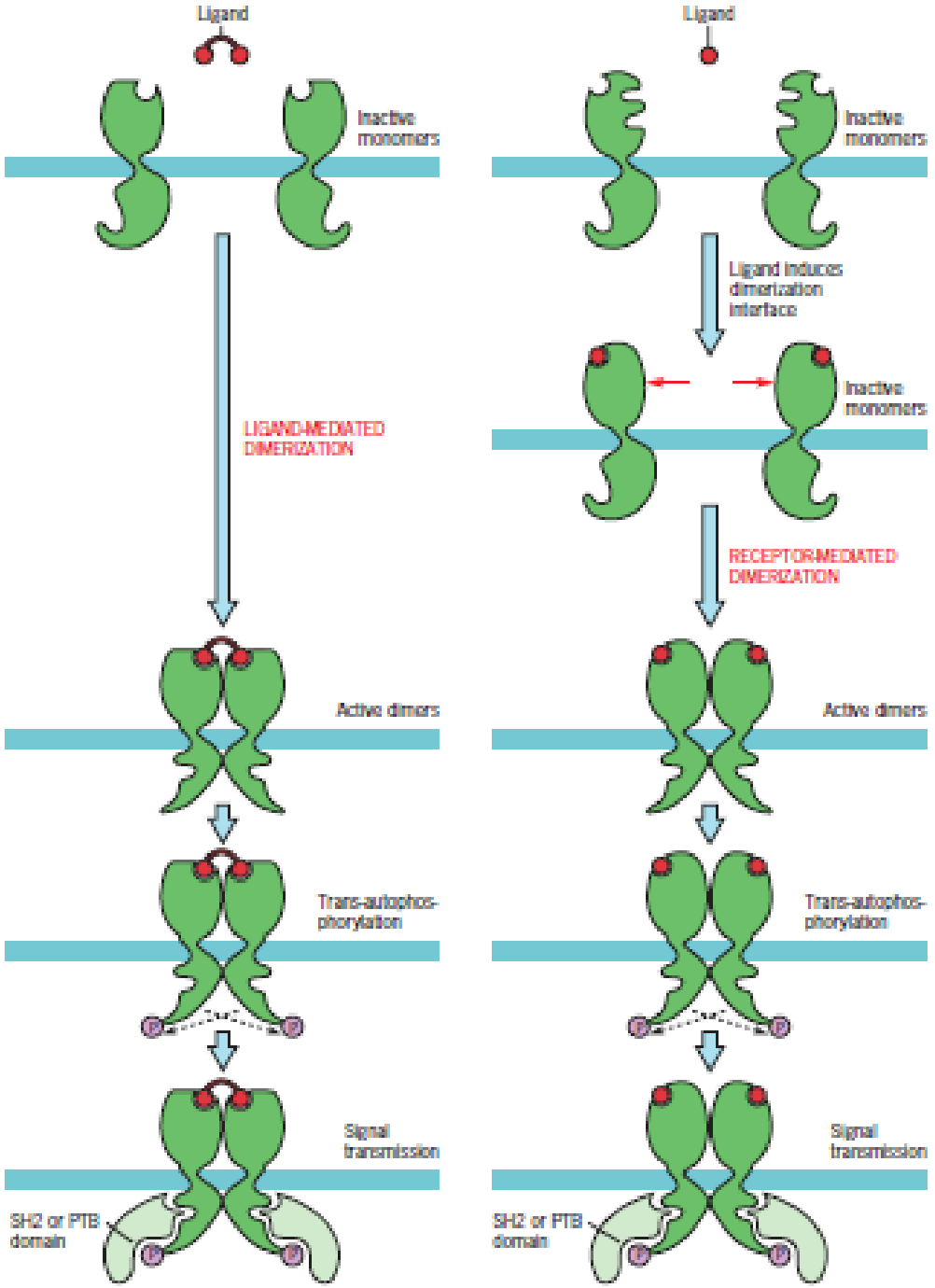
- **Protein-tyrosine kinases** are enzymes that phosphorylate specific tyrosine residues on protein substrates.

- These kinases are involved in the regulation of growth, division, differentiation, survival, attachment to the extracellular matrix, and migration of cells.

- Protein-tyrosine kinases can be divided in two groups:
  - **Receptor protein-tyrosine kinases (RTKs)**, which are integral membrane proteins that contain a single transmembrane helix and an extracellular ligand binding domain.
    - RTKs are activated directly by extracellular growth and differentiation factors such as epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) or by metabolic regulators such as insulin.
  - **Non-receptor or cytoplasmic protein-tyrosine kinases** are regulated indirectly by extracellular signals and they control processes as diverse as the immune response, cell adhesion, and neuronal cell migration.

Receptor dimerization

- Two mechanisms for receptor dimerization have been recognized:
  1. Ligand-mediated dimerization
     - Ligands of RTKs contains two receptor-binding sites.
     - This makes it possible for a single growth or differentiation factor molecule to bind to two receptors at the same time, thereby causing ligand-mediated receptor dimerization.
  2. Receptor mediated dimerization
     - Here ligand binding induces a conformational change in the extracellular domain of a receptor, leading to the formation or exposure of a receptor dimerization interface.
- Receptor dimerization results in the juxtapositioning of two protein tyrosine kinase domains on the cytoplasmic side of the plasma membrane.
- Bringing two kinase domains in close contact allows for *trans-autophosphorylation*, in which the protein kinase activity of one receptor of the dimer phosphorylates tyrosine residues in the cytoplasmic domain of the other receptor of the dimer, and vice versa.


Ligand
Inactive monomers
LIGAND-MEDIATED DIMERIZATION
Active dimers
Trans-autophos-phorylation
Signal transmission
SH2 or PTB domain
Ligand
Inactive monomers
Ligand induces dimerization interface
Inactive monomers
RECEPTOR-MEDIATED DIMERIZATION
Active dimers
Trans-autophos-phorylation
Signal transmission
SH2 or PTB domain

Protein Kinase Activation

- Autophosphorylation sites on RTKs can carry out two different functions:
  1. Regulate the receptor's kinase activity
  2. Serve as binding sites for cytoplasmic signaling molecules.

- Kinase activity is usually controlled by autophosphorylation on tyrosine residues that are present in the *activation loop* of the kinase domain.

- Once their kinase domain has been activated, the receptor subunits proceed to phosphorylate each other on tyrosine residues that are present in regions adjacent to the kinase domain.

- These autophosphorylation sites that act as binding sites for cellular signaling proteins.

Phosphotyrosine-Dependent Protein–Protein Interactions

- Signaling pathways consist of a chain of signaling proteins that interact with one another in a sequential manner.

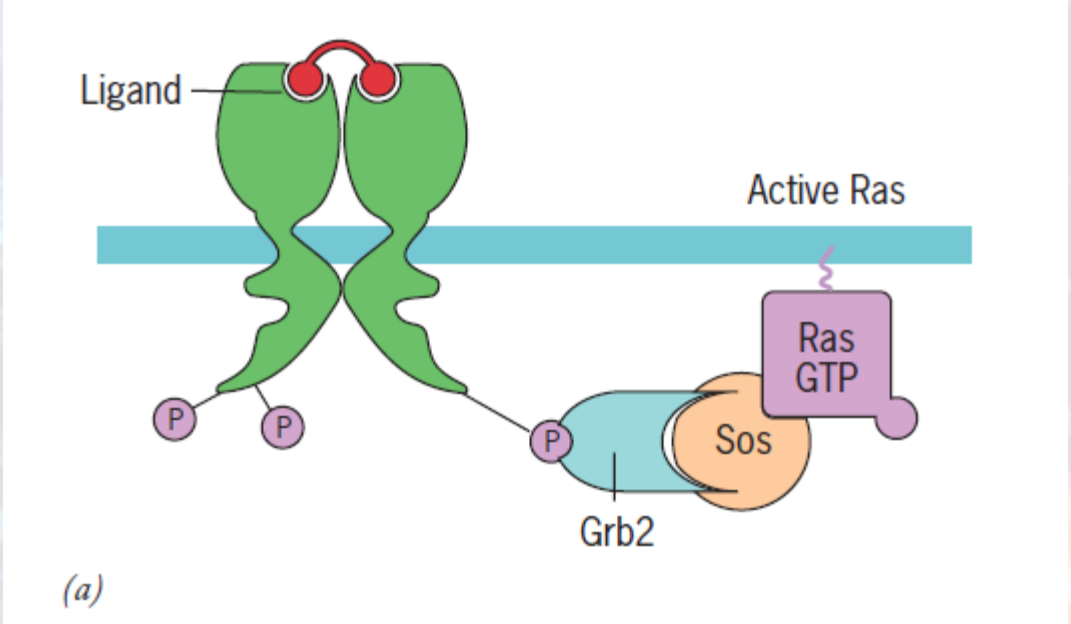
- Signaling proteins are able to associate with activated protein-tyrosine kinase receptors with domains that bind specifically to phosphorylated tyrosine residues.

- Two of these domains have been identified;
  1. *Src-homology 2 (SH2) domain*
     - *They are composed of approximately 100 amino acids and contain a conserved binding-pocket that accommodates a phosphorylated tyrosine residue.*
     - *Interactions occur following phosphorylation of specific tyrosine residues.*
     - *Specificity of the interactions is determined by the amino acid sequence immediately adjacent to the phosphorylated tyrosine residues.*

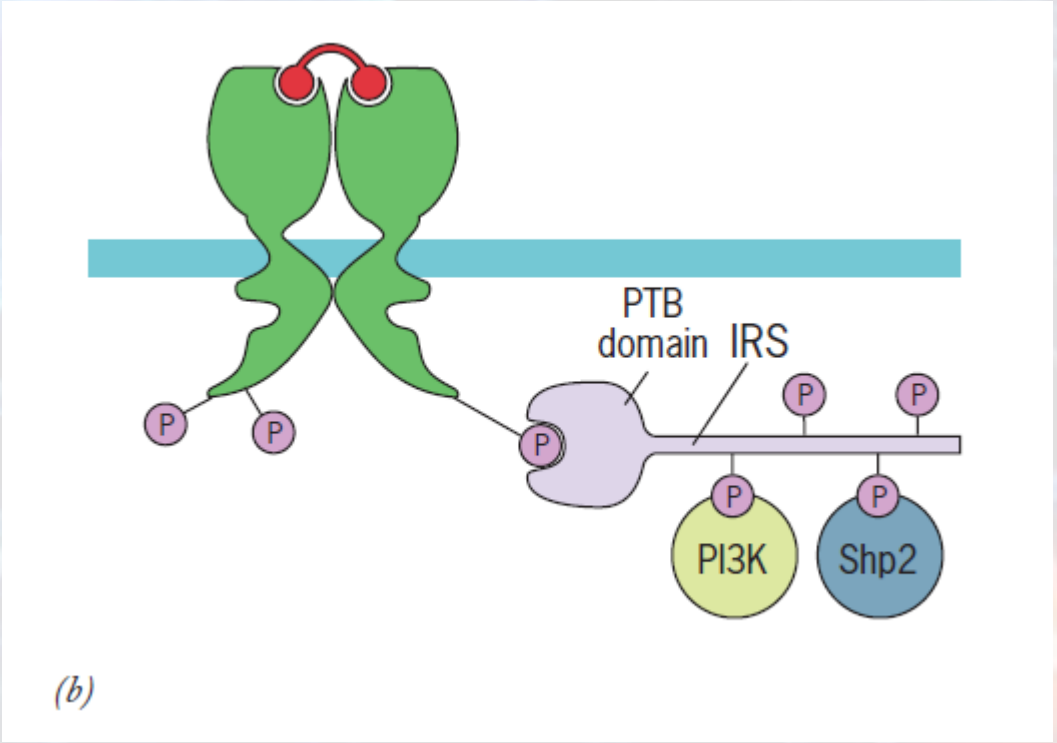
  2. *Phosphotyrosine-binding (PTB) domain*
     - *They can bind to phosphorylated tyrosine residues that are usually present as part of an asparagine-proline-X-tyrosine (Asn-Pro-X-Tyr) motif.*

Activation of downstream signaling pathways

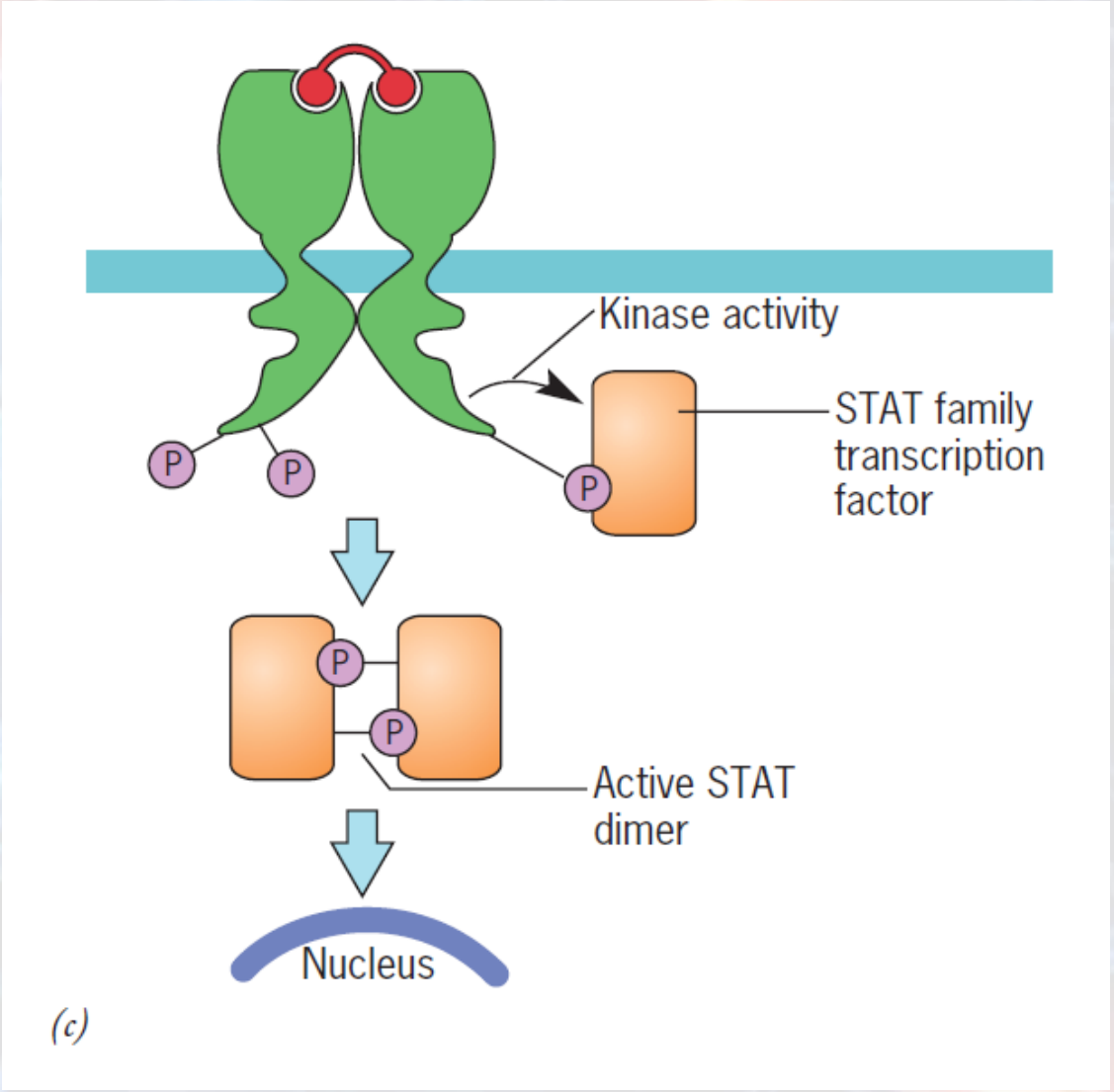
- Adaptor proteins function as linkers that enable two or more signaling proteins to become joined together as part of a signaling complex. Adaptor proteins contain an SH2 domain and one or more additional protein–protein interaction domains. For instance, the adaptor protein Grb2 contains one SH2 and two SH3 domains.



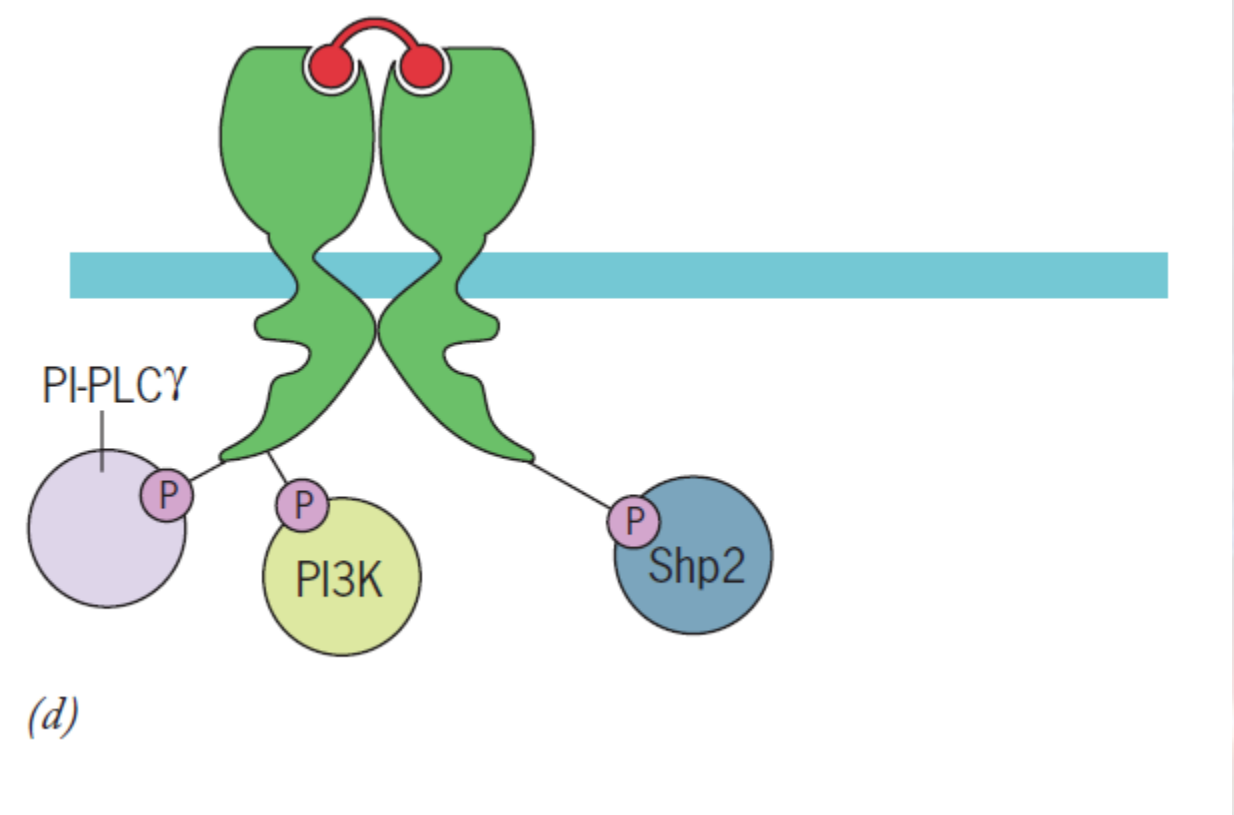
- Docking proteins, such as IRS, supply certain receptors with additional tyrosine phosphorylation sites. Docking proteins contain either a PTB domain or an SH2 domain and a number of tyrosine phosphorylation sites. Binding of an extracellular ligand to a receptor leads to autophosphorylation of the receptor, which provides a binding site for the PTB or SH2 domain of the docking protein. Once bound together, the receptor phosphorylates tyrosine residues present on the docking protein. These phosphorylation sites then act as binding sites for additional signalling molecules.



- Transcription factors that belong to the STAT family play an important role in the function of the immune system. STATs contain an SH2 domain together with a tyrosine phosphorylation site that can act as a binding site for the SH2 domain of another STAT molecule. Tyrosine phosphorylation of STAT SH2 binding sites situated within a dimerized receptor leads to the recruitment of STAT proteins. Upon association with the receptor complex, tyrosine residues in these STAT proteins are phosphorylated. As a result of the interaction between the phosphorylated tyrosine residue on one STAT protein and the SH2 domain on a second STAT protein, and vice versa, these transcription factors will form dimers. Dimers, but not monomers, move to the nucleus where they stimulate the transcription of specific genes involved in an immune response.



- Signaling enzymes include protein kinases, protein phosphatases, lipid kinases, phospholipases, and GTPase activating proteins. When equipped with SH2 domains, these enzymes associate with activated RTKs and are turned on directly or indirectly as a consequence of this association. Three general mechanisms have been identified by which these enzymes are activated following their association with a receptor.



Ending Response

- Signal transduction by RTKs is usually terminated by internalization of the receptor.

- One mechanism involves a receptor-binding protein named Cbl. When RTKs are activated by ligands, they autophosphorylate tyrosine residues, which can act as a binding site for Cbl, which possesses an SH2 domain. Cbl then associates with the receptor and catalyzes the attachment of a ubiquitin molecule to the receptor. Ubiquitin cause internalization of the receptor.

The Ras-MAP Kinase Pathway

- Retroviruses are small viruses that carry their genetic information in the form of RNA. Some of these viruses contain genes, called oncogenes, that enable them to transform normal cells into tumor cells.

- Ras was originally described as the product of a retroviral oncogene and, only later, determined to be derived from its mammalian host. It was subsequently discovered that approximately 30 percent of all human cancers contain mutant versions of RAS genes.

- It is important to note that Ras proteins are part of a superfamily of more than 150 small G proteins including the Rabs, Sar1 and RAN.

- These proteins are involved in the regulation of numerous processes, including cell division, differentiation, gene expression, cytoskeletal organization, vesicle trafficking, and nucleocytoplasmic transport.

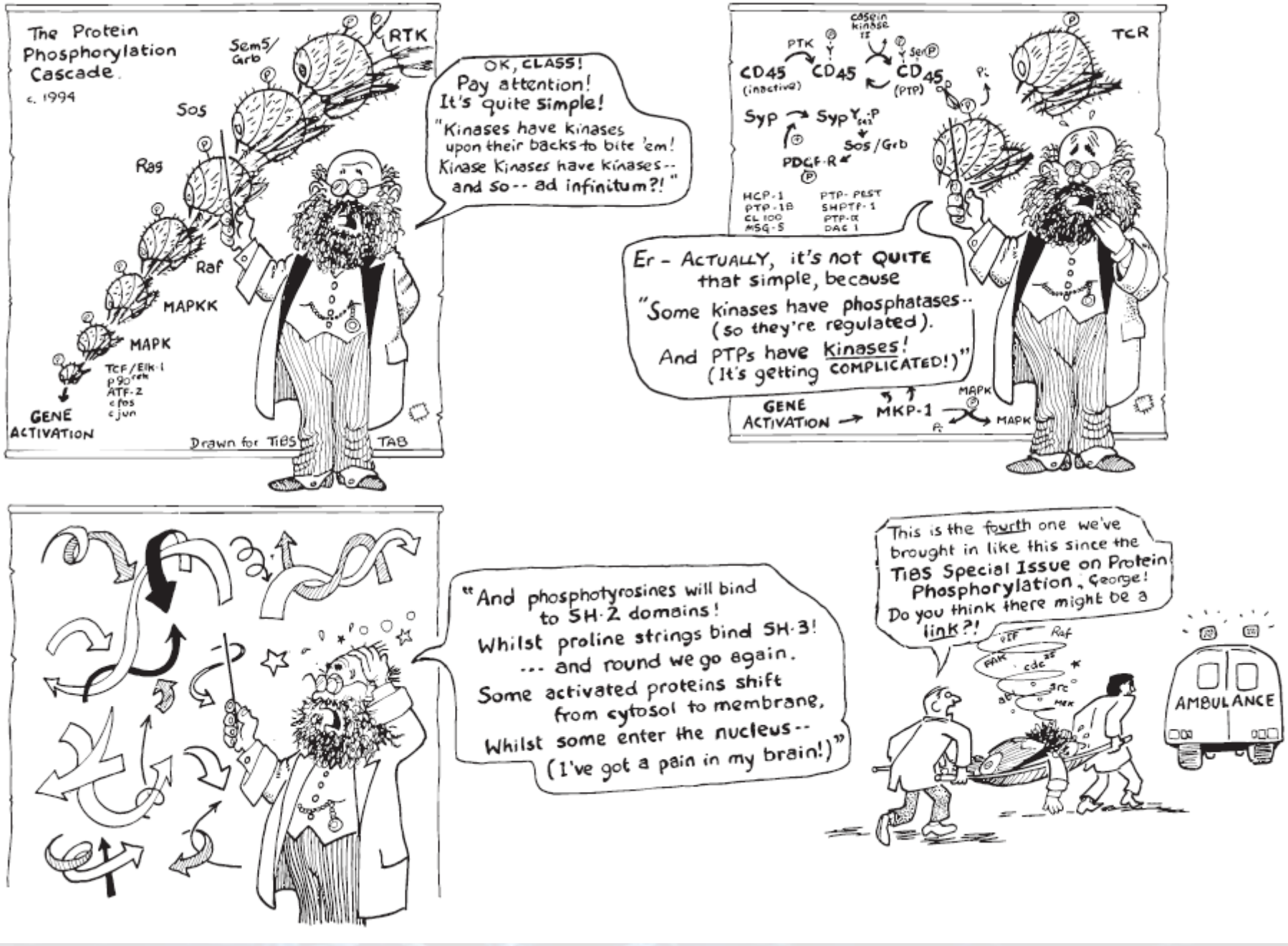
- Ras is a small GTPase that is anchored at the inner surface of the plasma membrane by a lipid group that is embedded in the inner leaflet of the bilayer.

- Ras is functionally similar to the heterotrimeric G proteins and acts as both a switch and a molecular timer.

- Ras consists of only a single small subunit.

- Ras proteins are present in two different forms: an active GTP-bound form and an inactive GDP-bound form.

- Ras-GTP binds and activates downstream signaling proteins. Ras is turned off by hydrolysis of its bound GTP to GDP. Mutations in the human RAS gene that lead to tumor formation prevent the protein from hydrolyzing the bound GTP back to the GDP form. As a result, the mutant version of Ras remains in the “on” position, sending a continuous message downstream along the signaling pathway, keeping the cell in the proliferative mode.


The Protein Phosphorylation Cascade.
c. 1994
RTK
Sem5/ Grb
Sos
Ras
Raf
MAPKK
MAPK
TCF/Elk-1
p90rsk
ATF-2
c fos
c jun
GENE ACTIVATION
Drawn for TIBS
TAB
OK, CLASS! Pay attention! It's quite simple!
"Kinases have kinases upon their backs to bite 'em! Kinase Kinases have kinases-- and so-- ad infinitum?!"
TCR
PTK
CD45 (inactive)
CD45
CD45 (PTP)
Syp
Sos/Grb
PDGF-R
HCP-1
PTP-1B
CL 100
MSG-5
PTP-PEST
SHPTP-1
PTP-R
DAC 1
Er - ACTUALLY, it's not QUITE that simple, because
"Some kinases have phosphatases-- (so they're regulated).
And PTPs have kinases! (It's getting COMPLICATED!)"
GENE ACTIVATION
MKP-1
MAPK
"And phosphotyrosines will bind to SH-2 domains!
Whilst proline strings bind SH-3!
--- and round we go again.
Some activated proteins shift from cytosol to membrane,
Whilst some enter the nucleus--
(I've got a pain in my brain!)"
This is the fourth one we've brought in like this since the TIBS Special Issue on Protein Phosphorylation, George! Do you think there might be a link?!
Raf
PAK
cdc
src
AMBULANCE

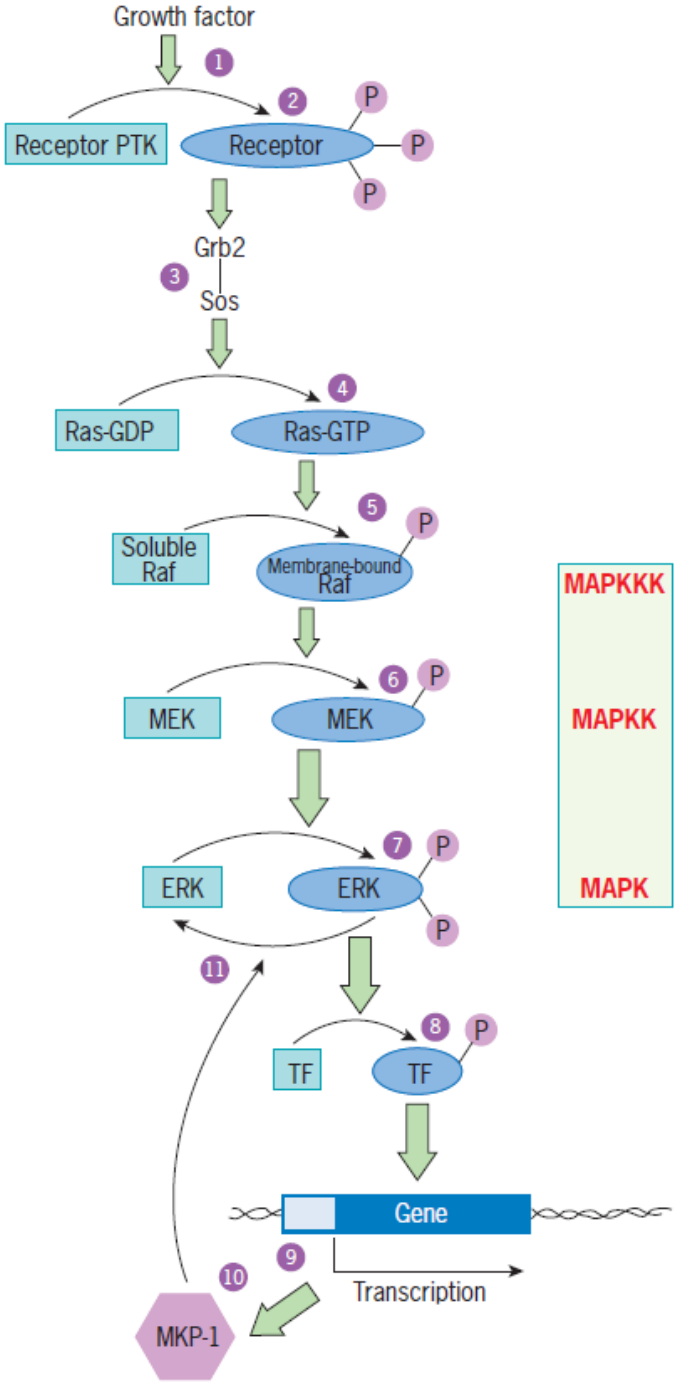


FIGURE 15.20 The steps of a generalized MAP kinase cascade. Binding of growth factor to its receptor (step 1) leads to the autophosphorylation of tyrosine residues of the receptor (step 2) and the subsequent recruitment of the Grb2-Sos proteins (step 3). This complex causes the GTP-GDP exchange of Ras (step 4), which recruits the protein Raf to the membrane, where it is phosphorylated and thus activated (step 5). In the pathway depicted here, Raf phosphorylates and activates another kinase named MEK (step 6), which in turn phosphorylates and activates still another kinase termed ERK (step 7). This three-step phosphorylation scheme shown in steps 5–7 is characteristic of all MAP kinase cascades. Because of their sequential kinase activity, Raf is known as a MAPKKK (MAP kinase kinase kinase), MEK as a MAPKK (MAP kinase kinase), and ERK as a MAPK (MAP kinase). MAPKKs are dual-specificity kinases, a term denoting that they can phosphorylate tyrosine as well as serine and threonine residues. All MAPKs have a tripeptide near their catalytic site with the sequence Thr-X-Tyr. MAPKK phosphorylates MAPK on both the threonine and tyrosine residue of this sequence, thereby activating the enzyme (step 7). Once activated, MAPK translocates into the nucleus where it phosphorylates transcription factors (TF, step 8), such as Elk-1. Phosphorylation of the transcription factors increases their affinity for regulatory sites on the DNA (step 9), leading to an increase in the transcription of specific genes (e.g., *Fos* and *Jun*) involved in the growth response. One of the genes whose expression is stimulated encodes a MAPK phosphatase (MKP-1; step 10). Members of the MKP family can remove phosphate groups from both tyrosine and threonine residues of MAPK (step 11), which inactivates MAPK and stops further signaling activity along the pathway. (AFTER H. SUN AND N. K. TONKS, TRENDS BIOCHEM. SCI. 19:484, 1994.)

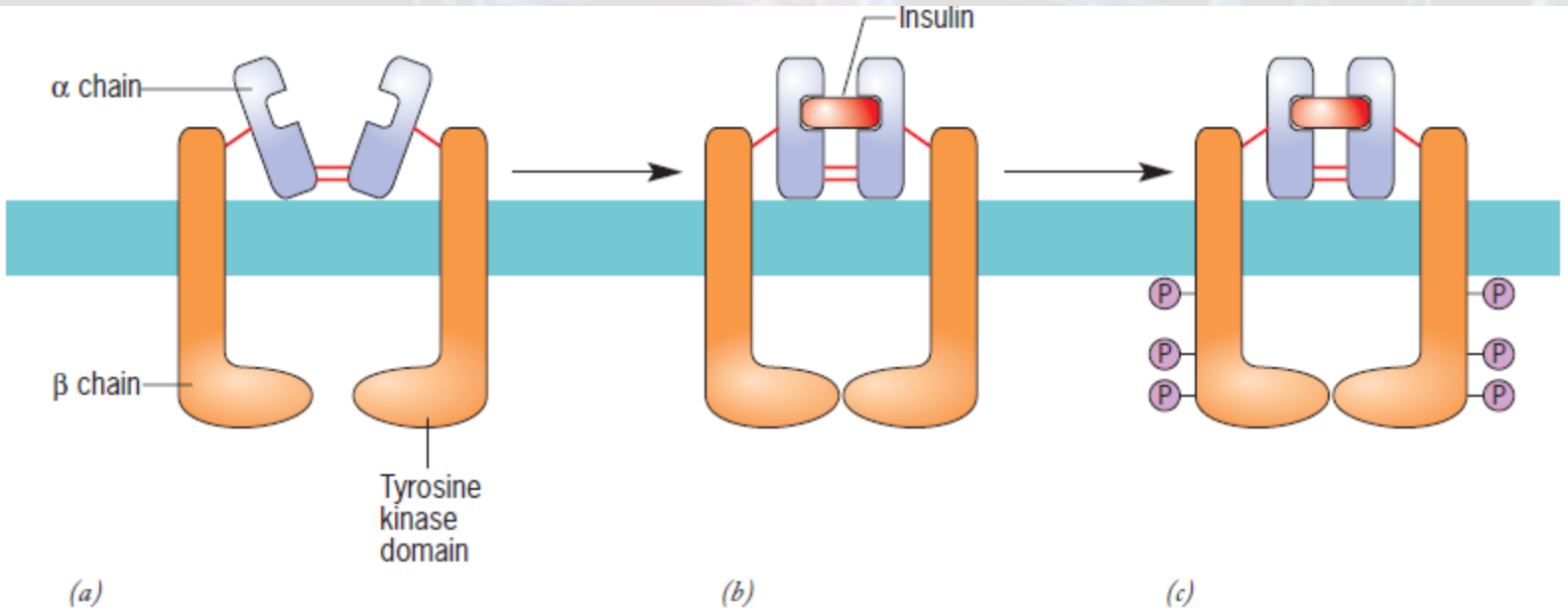


FIGURE 15.22 The response of the insulin receptor to ligand binding. (*a*) The insulin receptor, shown here in schematic form in the inactive state, is a tetramer consisting of two α and two β subunits. (*b*) Binding of a single insulin molecule to the α subunits causes a conformational change in the β subunits, which activates the tyrosine kinase activity of the β subunits. (*c*) The activated β subunits phosphorylate tyrosine residues located on the cytoplasmic domain of the receptor as well as tyrosine residues on several insulin receptor substrates (IRSs) that are discussed below.

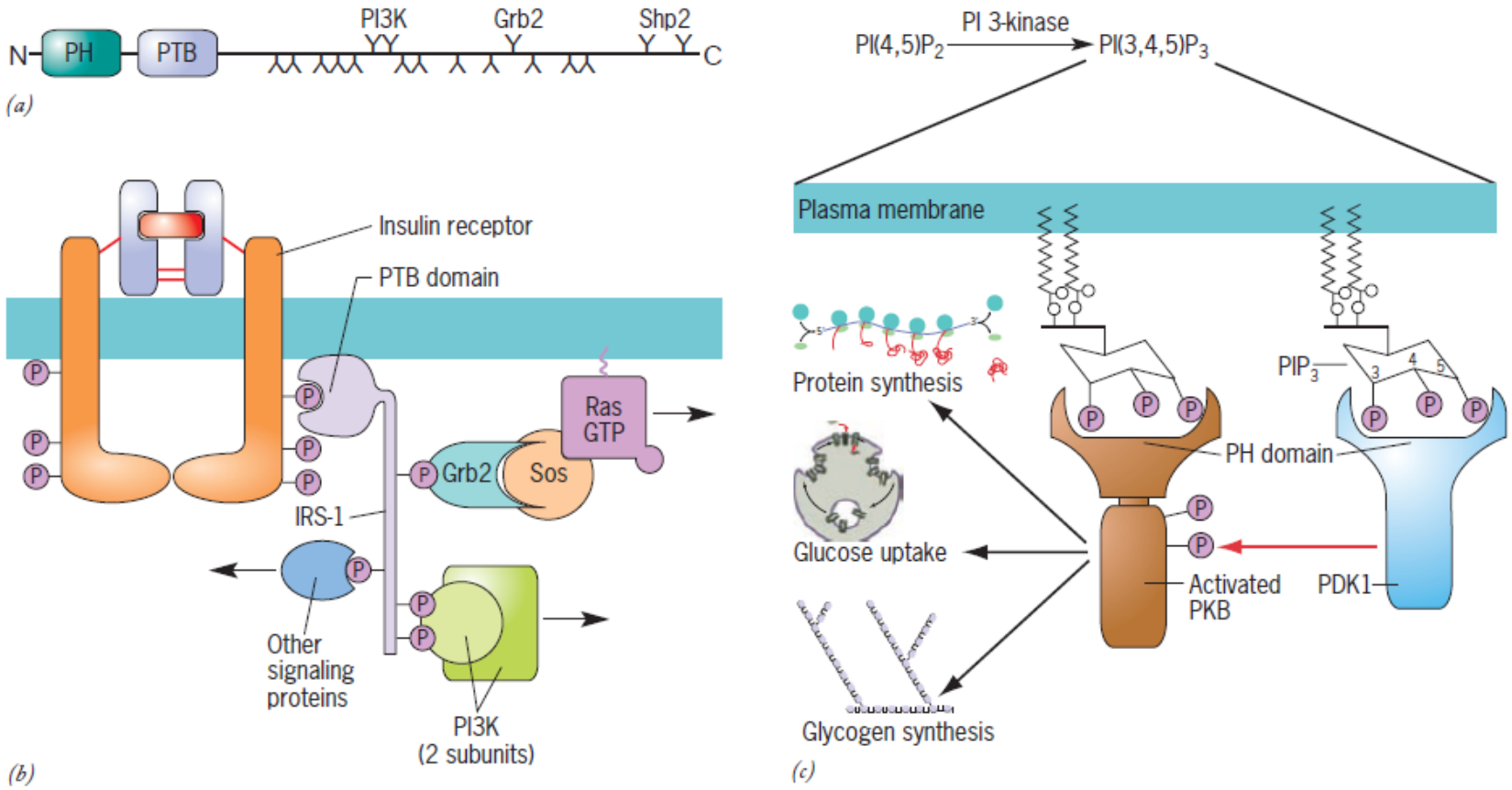


FIGURE 15.23 The role of tyrosine-phosphorylated IRS in activating a variety of signaling pathways. (*a*) Schematic representation of an IRS polypeptide. The N-terminal portion of the molecule contains a PH domain that allows it to bind to phosphoinositides of the membrane and a PTB domain that allows it to bind to a specific phosphorylated tyrosine residue (#960) on the cytoplasmic domain of an activated insulin receptor. Once bound to the insulin receptor, a number of tyrosine residues in the IRS may be phosphorylated (indicated as Y). These phosphorylated tyrosines can serve as binding sites for other proteins, including a lipid kinase (PI3K), an adaptor protein (Grb2), and a protein-tyrosine phosphatase (Shp2). (*b*) Phosphorylation of IRSs by the activated insulin receptor is known to activate PI3K and Ras pathways, both of which are discussed in the chapter. Other pathways that are less well defined are also activated by IRSs. (The IRS is drawn as an extended, two-dimensional molecule for purposes of illustration.) (*c*) Activation of PI3K leads to the formation of membrane-bound phosphoinositides, including PIP_3. One of the key kinases in numerous signaling pathways is PKB (AKT), which interacts at the plasma membrane with PIP_3 by means of a PH domain on the protein. This interaction changes the conformation of the PKB molecule, making it a substrate for another PIP_3-bound kinase (PDK1), which phosphorylates PKB. The second phosphate shown linked to PKB is added by a second kinase, mostly likely mTOR. Once activated, PKB dissociates from the plasma membrane and moves into the cytosol and nucleus. PKB is a major component of a number of separate signaling pathways that mediate the insulin response. These pathways lead to translocation of glucose transporters to the plasma membrane, synthesis of glycogen, and the synthesis of new proteins in the cell.

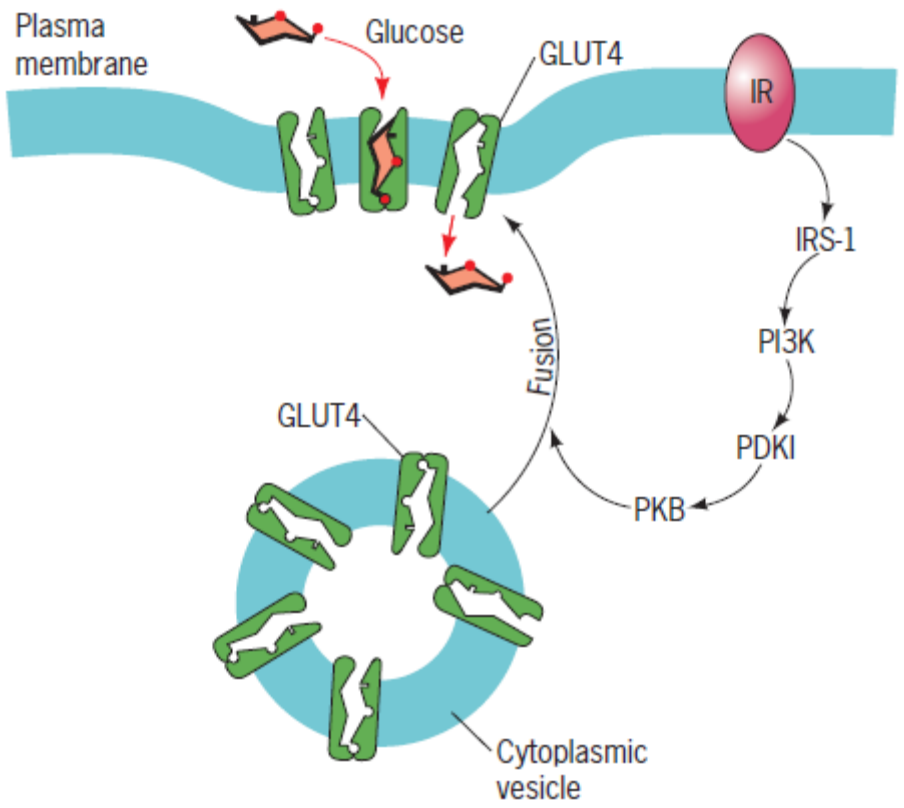


FIGURE 15.24 Regulation of glucose uptake in muscle and fat cells by insulin. Glucose transporters are stored in the walls of cytoplasmic vesicles that form by budding from the plasma membrane (endocytosis). When the insulin level increases, a signal is transmitted through the IRS-PI3K-PKB pathway, which triggers the translocation of cytoplasmic vesicles to the cell periphery. The vesicles fuse with the plasma membrane (exocytosis), delivering the transporters to the cell surface where they can mediate glucose uptake. A second pathway leading from the insulin receptor to GLUT4 translocation is not shown (see *Trends Biochem. Sci.* 31:215, 2006).