TOPIC K: CELL SIGNALLING

Learning Outcome

Candidates should be able to

Core Topic 6

(o) Describe the three main stages of cell signalling – ligand-receptor interaction, signal transduction (inclusive of phosphorylation and signal amplification) and cellular responses. (Limited to an overview of insulin & RTK signalling and glucagon & G-protein signalling. Candidates will be expected to generalise their understanding of these systems in solving problems involving other cell signalling systems.) Advantages and significance of having a cell signalling system may be required.

Content Outline

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2. Types of Cell Signalling
   - (a) Local Signalling
   - (b) Long Distance Signalling
3. Main Stages of Cell Signalling
   - (a) Ligand-receptor interaction
     - (i) Cell surface receptors
       - Ion channel receptor
       - G protein-coupled receptor
       - Receptor tyrosine kinase
     - (ii) Intracellular receptor
   - (b) Signal transduction
     - (i) Phosphorylation cascade
     - (ii) Second messengers
     - (iii) Signal amplification
   - (c) Cellular response
4. Advantages and Significance of a Cell Signalling System
5. Specific Examples of Cell Signalling Pathways
   - (a) Glucagon-GPCR Signalling
   - (b) Insulin-RTK Signalling
   - (c) Generation of nerve impulses
   - (d) Activation of cellular defence mechanism

References

1. Campbell N. A. and Reece J. B. (2008). Biology. Chapter 11: Cell Communication. Eighth Edition. Benjamin Cummings Publishing, Inc.
2. Brooker R. J. *et. al.* (2008). Biology. Chapter 9: Cell Communication and the Regulation of the Cell Cycle. First Edition. McGraw-Hill Companies.
3. Alberts B. *et. al.* (1998). Essential Cell Biology – An Introduction to the Molecular Biology of the Cell. Chapter 15: Cell Communication. First Edition. Garland Publishing, Inc.
4. Karp G. (1996). Cell and Molecular Biology – Concepts and Experiments. Chapter 15: Cell Signalling: Communication between Cells and Their Environment. Second Edition. John Wiley and Sons, Inc.

1. Introduction

The trillions of cells in a multi-cellular organism must communicate with each other to coordinate their activities. They do so by means of signal molecules, which include proteins, small peptides, amino acids, nucleotides, steroids, fatty acid derivatives and even dissolved gases such as nitric oxide. The cell signalling process helps ensure that crucial activities occur in the correct cells, at the right time and in coordination with other cells in the organism.

2. Types of Cell Signalling

Cells in multi-cellular organisms usually communicate via **ligands** / **signal molecules** ("first messengers") targeted for cells that may be adjacent (local signalling) or may not be immediately adjacent (long distance signalling).

(a) Local Signalling

Direct contact between cells

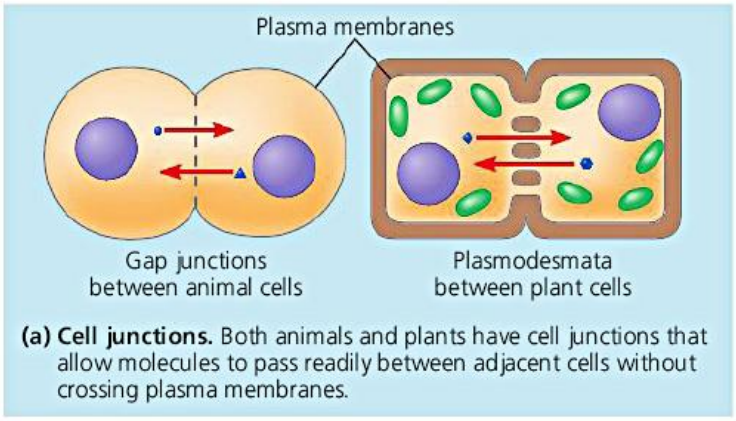
- Cell junctions

  In both plants and animals, cells may communicate via cell junctions, where cytoplasm of adjacent cells connect directly

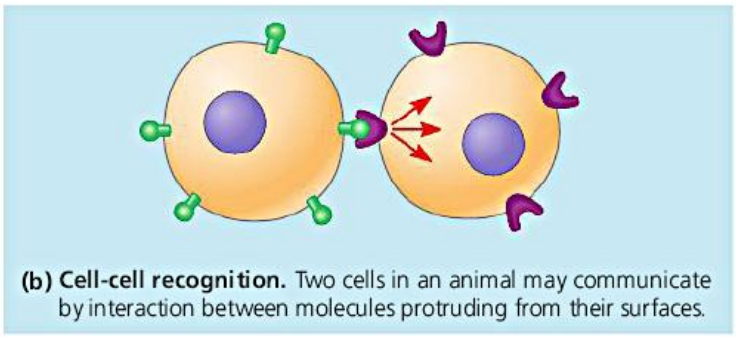
  e.g. gap junction in animals and plasmodesmata in plants.

- Cell-cell recognition

  In animals, cell may communicate between membrane bound molecules on the cell surface membrane.



(a) Cell junctions. Both animals and plants have cell junctions that allow molecules to pass readily between adjacent cells without crossing plasma membranes.



(b) Cell-cell recognition. Two cells in an animal may communicate by interaction between molecules protruding from their surfaces.

Communication by direct contact between cells

In other cases, signal molecules are secreted by the signalling cell, which travel short distances and influence cells in the vicinity

- Paracrine signalling

  e.g. growth factor promote cell division and growth. Numerous cells can simultaneously receive and respond to the molecules of growth factor produced by a single cell in their vicinity.

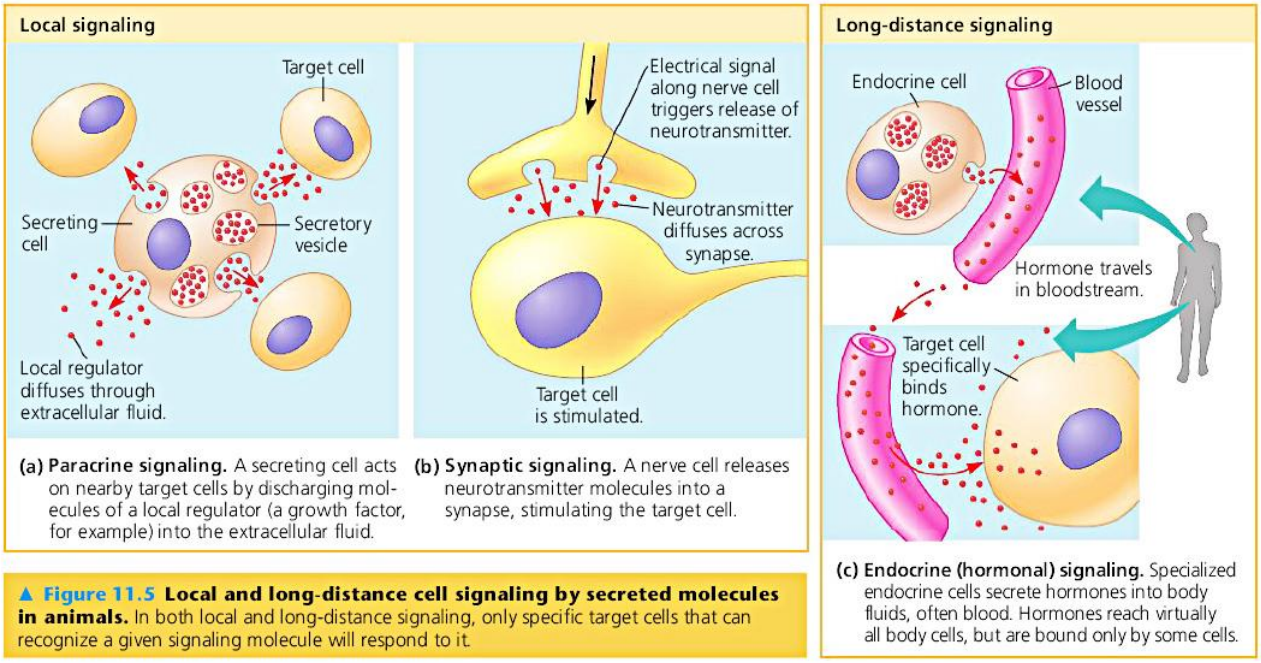
- Synaptic signalling

  e.g. neurotransmitters (such as acetylcholine) diffusing across synapses between neurones.

(b) Long Distance Signalling

For long distance signalling, signal molecules known as **hormones** are secreted into the circulatory system (e.g. bloodstream of an animal or the sap of a plant) to act on distant target cells.

- Specialised animal cells release hormone molecules (e.g. **insulin** and **glucagon**) into blood vessels of the circulatory system to other parts of the body, where they reach target cells that can recognise and respond to the hormones. This is known as **endocrine signalling**.
- Plant hormones (e.g. auxin, a growth hormone) sometimes travel in vessels but more often reach their targets by moving through cells or by diffusion through the air as a gas.



Local and long distance cell signalling

Experimental Evidence of Cell Signalling

Earl W. Sutherland (from Vanderbilt University, won Nobel Prize 1971) investigated how animal hormone adrenaline stimulates the breakdown of the storage polysaccharide, glycogen, within liver and skeletal muscle cells.

Glycogen breakdown releases the sugar glucose-1-phosphate, which the cell converts to glucose-6-phosphate. Glucose-6-phosphate can then be used by the cell (e.g. liver cell) for energy production. Alternatively, the compound can be stripped of phosphate and released from the liver cell into the blood as glucose, which can fuel cells throughout the body. Thus, one effect of adrenaline, which is secreted from the adrenal gland during times of physical or mental stress, is the mobilization of fuel reserves.

Sutherland's research team discovered that adrenaline stimulates glycogen breakdown by activating glycogen phosphorylase (cytosolic enzyme) in intact cells. When adrenaline was added to a test tube containing the enzyme glycogen phosphorylase and glycogen, no breakdown occurred.

This implies that adrenaline does not interact directly with the enzyme responsible for glycogen breakdown and that the cell surface membrane is somehow involved in the transmission of the signal.

3. Main Stages of Cell Signalling

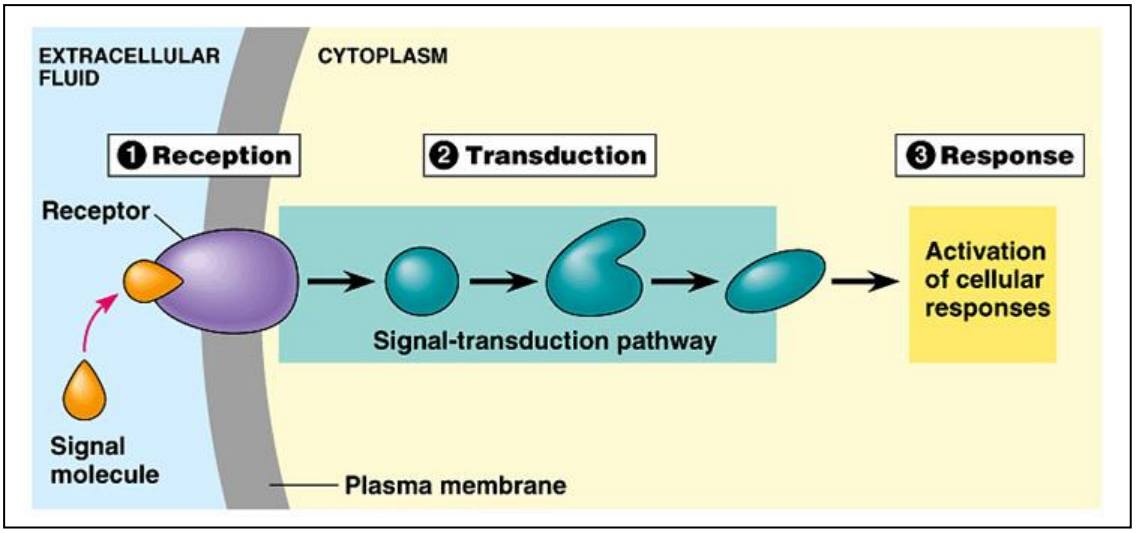
For a cell to respond when it encounters a signal, the signal must first be recognised by a specific receptor molecule on the cell surface and then transmitted to the cell's interior before an appropriate cellular response can occur.

Cell signalling can be divided into three stages:

(a) Ligand-receptor interaction

(b) Signal transduction

(c) Cellular Response



Three main stages in cell signalling

(a) Ligand-receptor interaction

The **ligand** / **signal molecule** is **complementary in shape** to a **binding site** on the **receptor** and attaches itself there. There is specificity in the ligand-receptor binding.

Binding of the ligand to the receptor **activates** the receptor.

Generally, there are two types of ligands:

(i) Molecules that are **large** and/or **hydrophilic**.

- A large class of ligand molecules cannot pass through the cell surface membrane of the target cell.
- Examples include insulin molecule and glucagon molecule.
- The receptor proteins for these signal molecules have to lie in the **cell surface membrane** of the target cell and relay the message across the membrane.

(ii) Molecules that are **small** and **hydrophobic**.

- A smaller, class of ligands consisting molecules that are able to diffuse across the cell surface membrane.
- Examples include steroid hormones (like cortisol, estrogen and testosterone) and thyroid hormones.
- The receptors for these signal molecules have to lie in the **interior of the target cell** and when bound to their ligands, they generally act as gene regulatory proteins.

Corresponding to the two classes of signal molecules, there are also two classes of receptors; cell surface receptors and intracellular receptors.

(i) Cell surface receptors

A cell surface receptor is usually a **transmembrane protein** embedded in the cell surface membrane of the target cell. It allows the cell to receive the signal coming from outside the cell and respond to it.

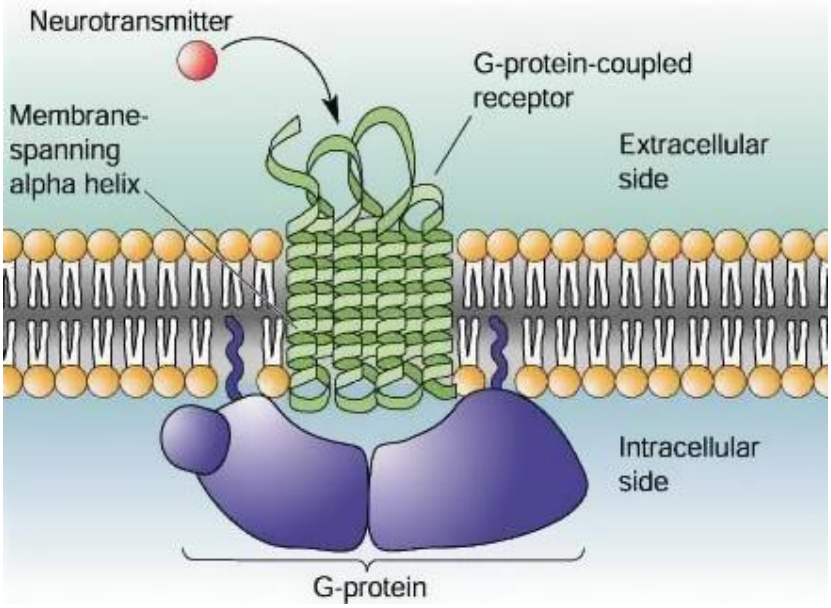
- **Ion-channel receptor**
  - A ligand-gated ion channel is a type of membrane-bound receptor.
  - When a ligand binds to the extracellular side of the receptor, the gate opens or closes, allowing or blocking the flow of specific ions, such as Na^+ or Ca^{2+}, through a channel in the receptor.
  - An example is the ligand-gated sodium ion channels found on the post-synaptic membrane.

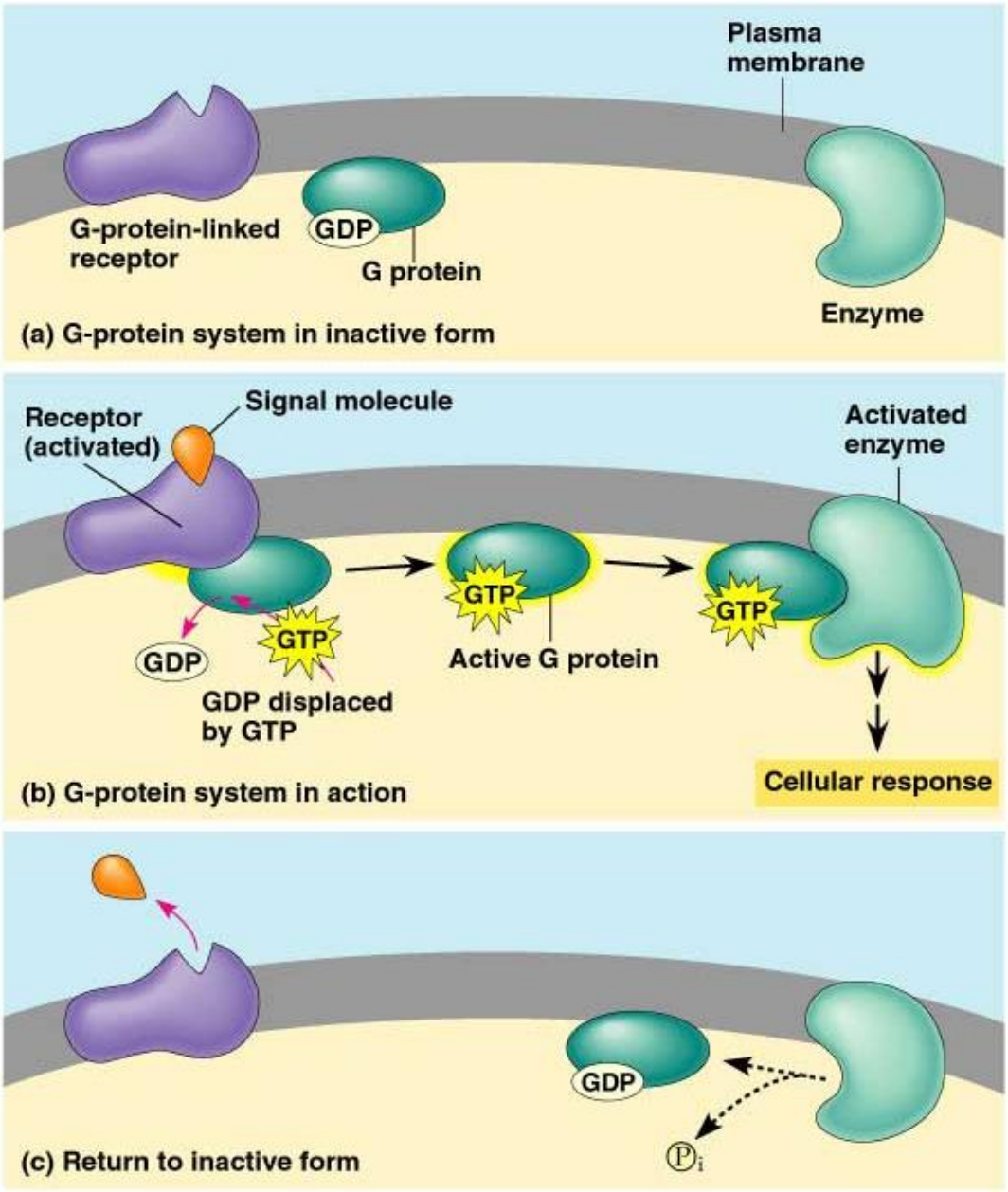
- **G protein-coupled receptor (GPCR)**

  The **G protein-coupled receptor (GPCR)** / G protein-linked receptor (GPLR) is closely associated with a **G protein**, a protein that binds to guanosine triphosphate (GTP) or guanosine diphosphate (GDP). GTP is an energy molecule similar to ATP.

  GPCR makes up a large family of eukaryotic receptor proteins. There are nearly 1000 GPCRs and many different signal molecules are specific for different types of GPCRs. These receptors vary in their binding sites for recognising signal molecules and for recognising different G proteins inside the cell. Yet, GPCRs are all remarkably similar in structure.

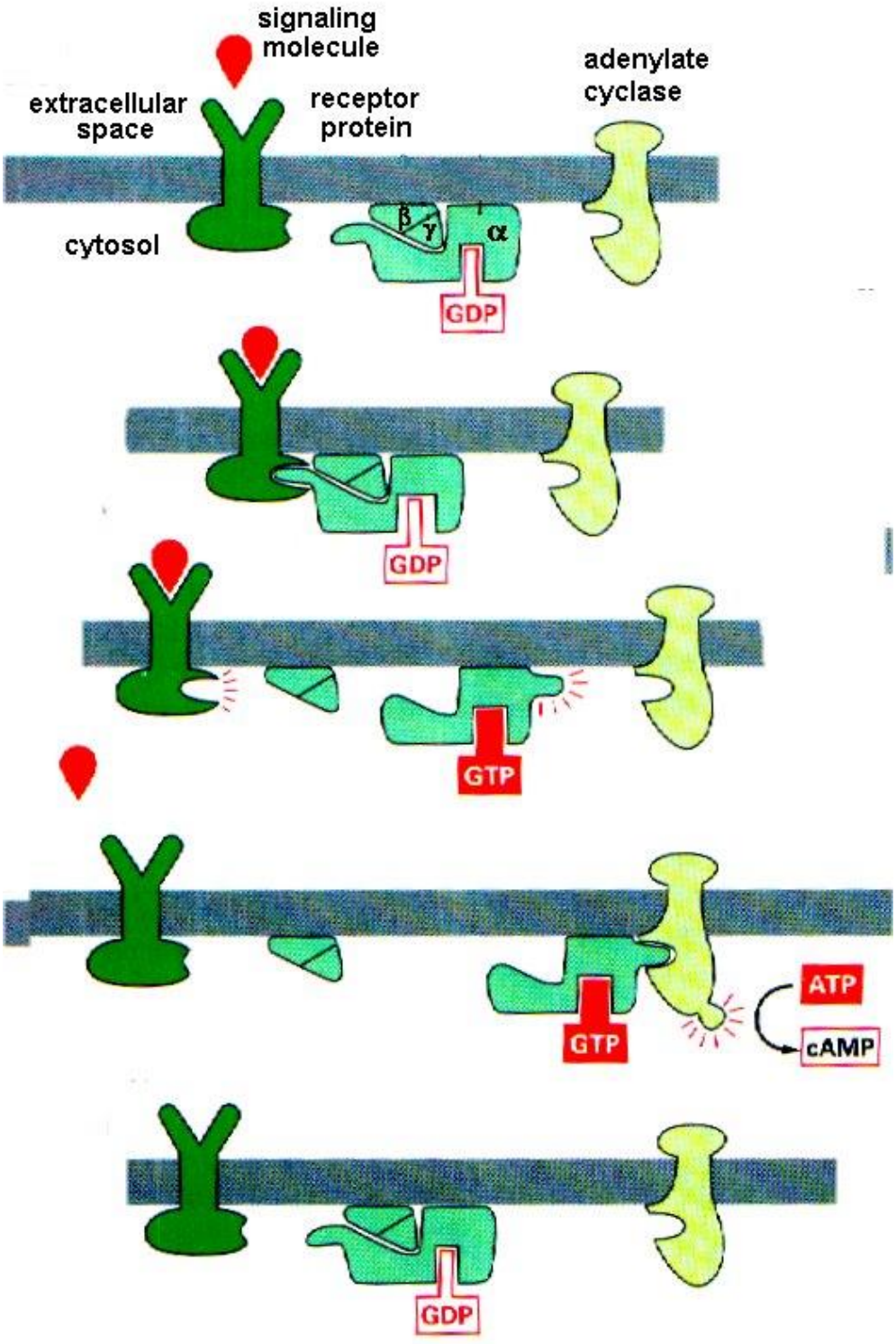
  The structure of the GPCR:
  - is a single polypeptide chain with **seven transmembrane α-helices**
  - has an extracellular **ligand binding site**
  - has an intracellular **G protein binding site**





How the G protein-coupled receptor work

- The GPCR is inactive when not bound to ligand. The G protein is inactive when bound to **GDP**.
- When the ligand binds to extracellular side of GPCR, the receptor is activated causing it to change its conformation.
- The cytoplasmic side of the receptor then binds an inactive G protein, causing **G protein to exchange its bound GDP for GTP**.
- The G protein is **activated** and dissociates from the receptor. Activated G protein binds to an enzyme, activating it. Once activated, the enzyme triggers signal transduction leading to cellular response.
- Once the signal molecule is absent, **GTP is hydrolysed back into GDP** by the **GTPase** enzyme found in the G protein subunit. The G protein thus dissociates from the enzyme and returns to its inactive form. The signal is switched off.



How the G protein-coupled receptor work

- **Receptor Tyrosine Kinases (RTKs)**

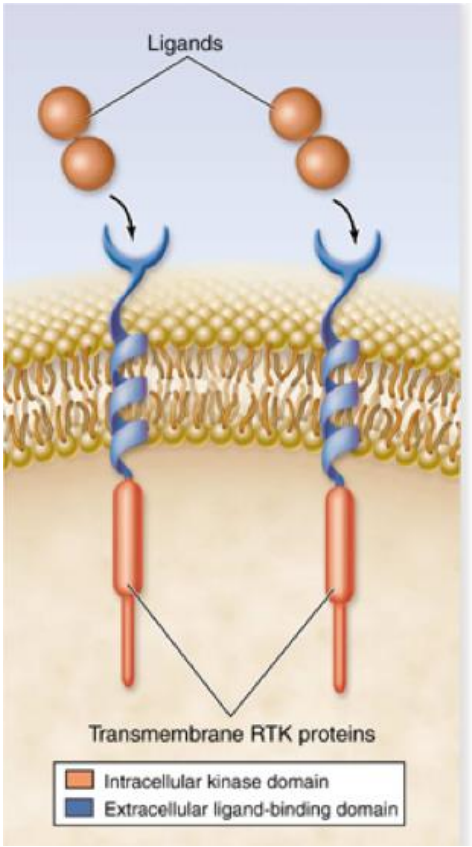
  Receptor tyrosine kinases (RTKs) belong to a major class of receptors characterised by having enzymatic activity.

  One RTK complex may activate ten or more different signal transduction pathways and cellular responses helping the cell to regulate and coordinate several cell activities simultaneously.

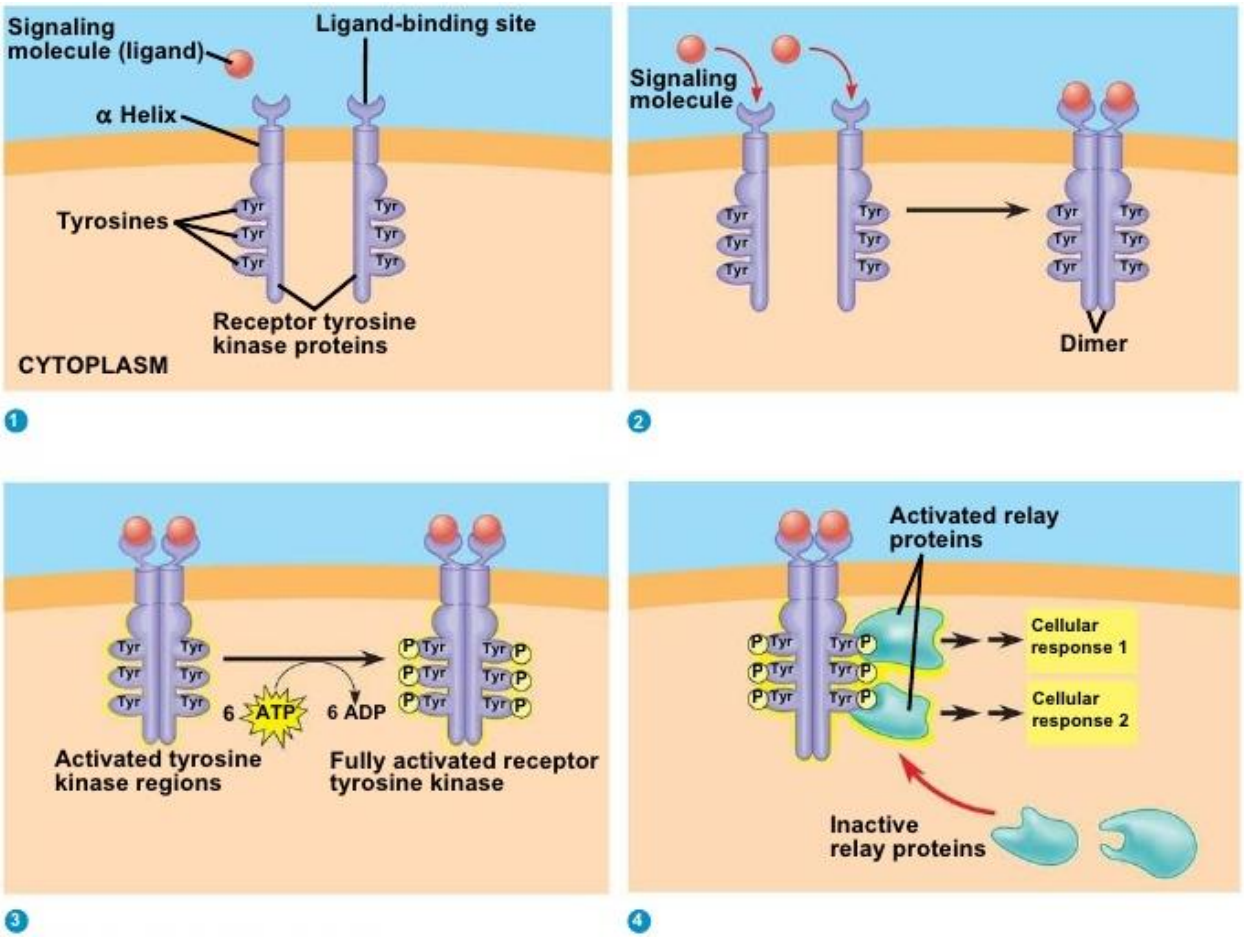
  The structure of receptor tyrosine kinase:

  - is a single polypeptide chain with a **single transmembrane α-helix**
  - has an extracellular **ligand binding site**
  - has an intracellular tail that functions as tyrosine kinase and also contains a number of tyrosine amino acid residues.

    A tyrosine kinase is an enzyme that catalyses the transfer of phosphate groups from ATP to the tyrosine (amino acid) residues on a substrate protein, activating it.



Structure of receptor tyrosine kinase

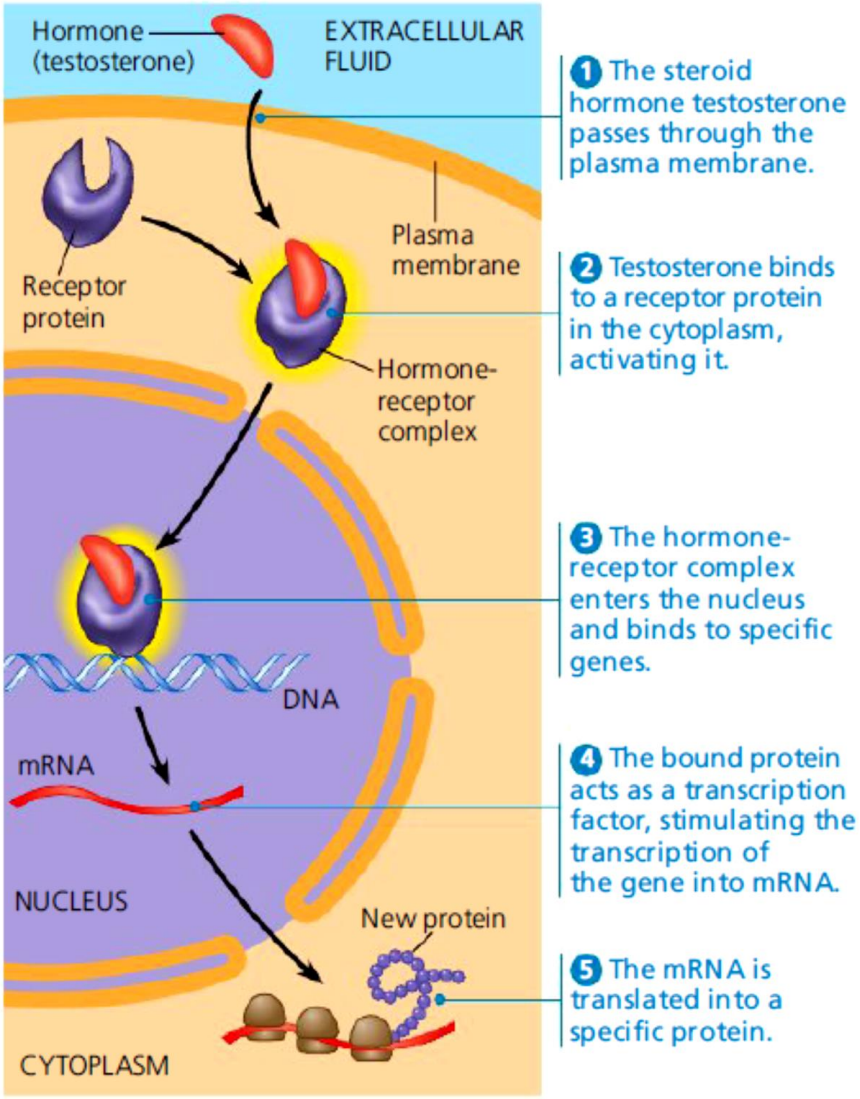


How receptor tyrosine kinase work

(image source: Campbell, 9th ed)

- Before a ligand binds, the receptors exist as individual RTK monomers.
- The binding of a ligand to the extracellular binding sites of RTKs causes two RTK proteins to come together in the membrane, forming a **dimer**.
- Dimerisation **activates the tyrosine kinase function** found in the intracellular tails of RTK.
- Tyrosine kinase adds **phosphate group** from ATP molecule to the tyrosine residues on the tail of the other RTK protein by **autophosphorylation**.
- The **activated RTK** will trigger the assembly of specific **relay proteins** on the receptor tails, activating them.
- Each activated protein triggers a signal transduction pathway, leading to a cellular response.

- Intracellular receptors
  - Intracellular receptors are located either in the **cytosol** or in the **nucleus** of target cells.
  - To reach an intracellular receptor, the ligand has to pass through the target cell's surface membrane. Thus, the signal molecule must be **small** and **hydrophobic** to pass through the hydrophobic core of the membrane.



How intracellular receptor works

  - Testosterone, a steroid hormone secreted by the cells of the testes travels through the blood and enters cells all over the body. However, only cells that contain receptor molecules for testosterone will respond.
  - In the cytoplasm of target cells, the hormone binds to the receptor, activating it. With the hormone attached, the activated ligand-receptor complex then enters the nucleus.
  - The activated ligand receptor complex acts as a transcription factor and bind to **regulatory sequence** in the DNA promoting **transcription** of specific genes, giving rise to male characteristics.

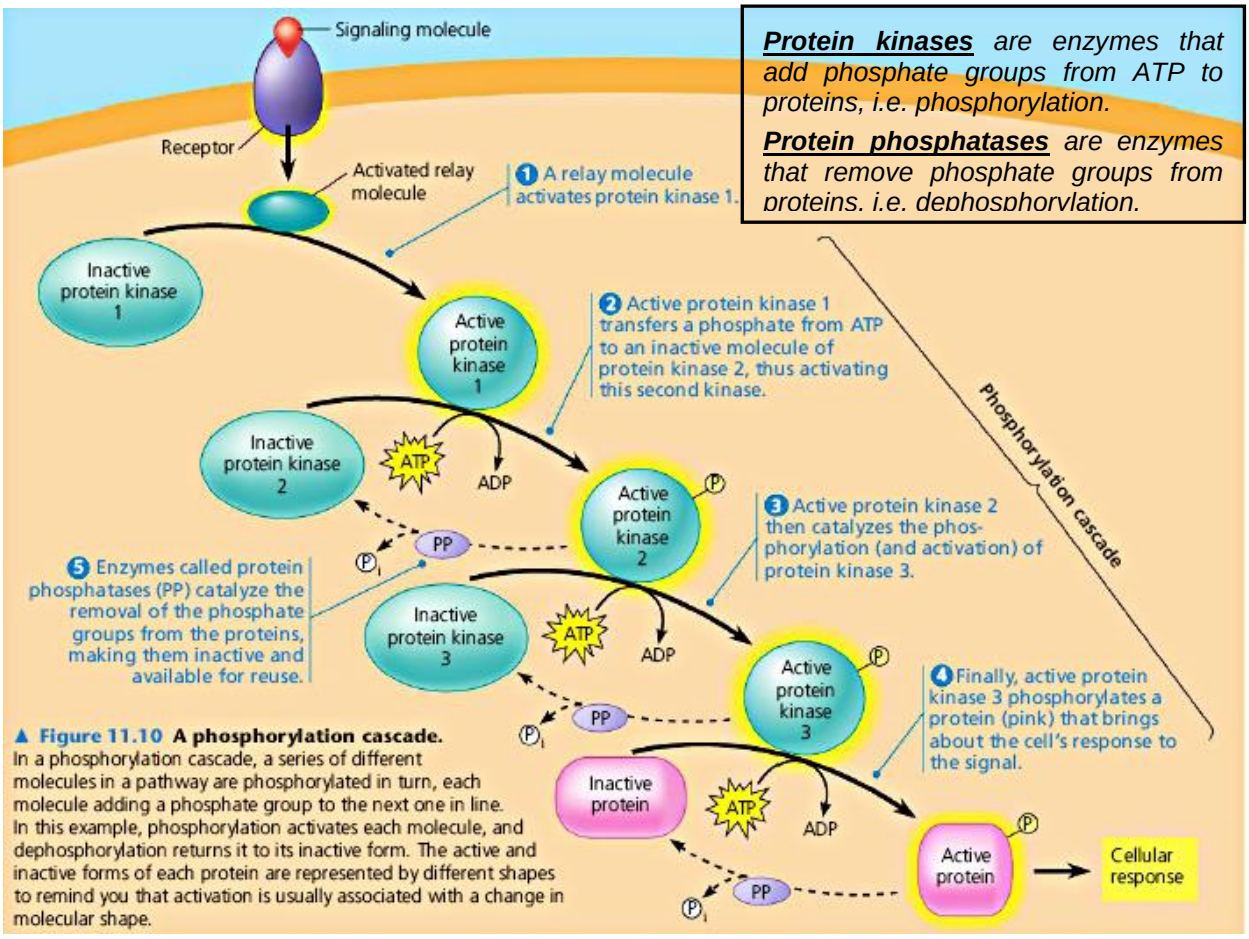
(b) Signal transduction

Transduction converts the signal to a form that can bring about a specific cellular response. The signal transduction pathway often requires a sequence of changes in a series of different molecules in a multistep pathway. These molecules in the pathway are often called **relay molecules**.

Signal transduction occurs via two main ways, protein phosphorylation in a **phosphorylation cascade** and the release of **second messengers**. Such pathways also allow for **signal amplification**.

(i) Phosphorylation cascade

The activated receptor activates other relay molecules, which activates another relay molecule, and so on, until the molecule (usually a protein) that produces the final cellular response is activated.



▲ **Figure 11.10 A phosphorylation cascade.** In a phosphorylation cascade, a series of different molecules in a pathway are phosphorylated in turn, each molecule adding a phosphate group to the next one in line. In this example, phosphorylation activates each molecule, and dephosphorylation returns it to its inactive form. The active and inactive forms of each protein are represented by different shapes to remind you that activation is usually associated with a change in molecular shape.

Phosphorylation cascade

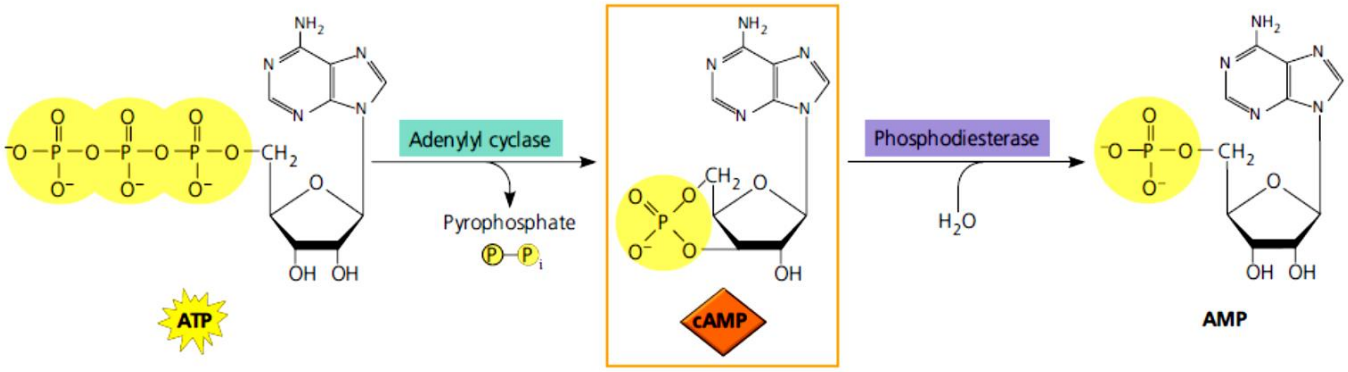
- Many of the relay molecules in signal transduction pathways are **protein kinases** and they often act on other protein kinases in the pathway.
- Each activated protein kinase will initiate **a sequential phosphorylation and activation** of other kinases, resulting in a **phosphorylation cascade**.
- Relay molecules are usually **activated** when they are **phosphorylated** and **deactivated** when they are **dephosphorylated**.
- As such, the phosphorylation and dephosphorylation acts as a molecular switch in the cell, turning activities on and off as required.

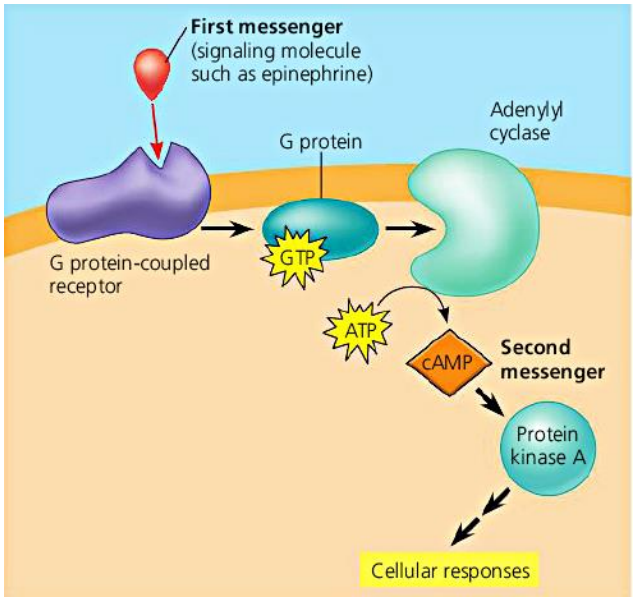
(ii) **Second messengers**

Not all relay molecules in the signal transduction pathway are proteins. Second messengers are **small**, **non-protein**, **water-soluble** molecules or ions that relay signals received at receptors on the cell surface to target molecules in the cytosol and / or nucleus. As second messengers are small and water-soluble, they can readily diffuse throughout the cell.

In addition to their job as relay molecules, second messengers serve to greatly **amplify** the strength of the signal. Most common second messengers are **cyclic AMP** and **calcium ion, Ca^{2+}**. Second messengers participate in pathways initiated by G protein-coupled receptors and receptor tyrosine kinases.

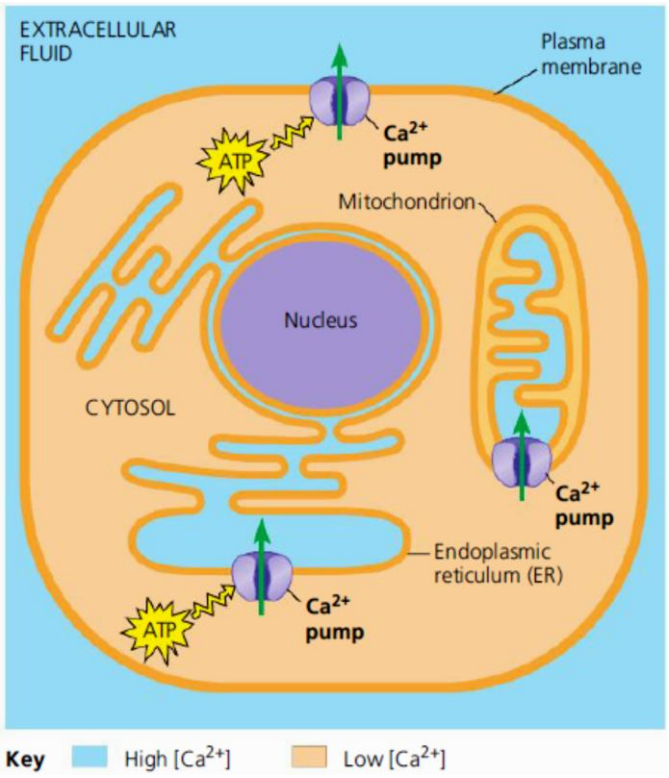
- **Cyclic AMP**
  - An enzyme embedded in the cell surface membrane, **adenylyl cyclase** / adenylate cyclase, when activated by the G protein, can **convert many ATP to cAMP** molecules.
  - The cytosolic concentration **cyclic adenosine monophosphate (cAMP)** is elevated twenty-fold in a matter of seconds, **amplifying** the signal in the cytoplasm.
  - It does not persist for long in the absence of the hormone, because another enzyme, called **phosphodiesterase**, **converts the cAMP to AMP**, resulting in signal termination.



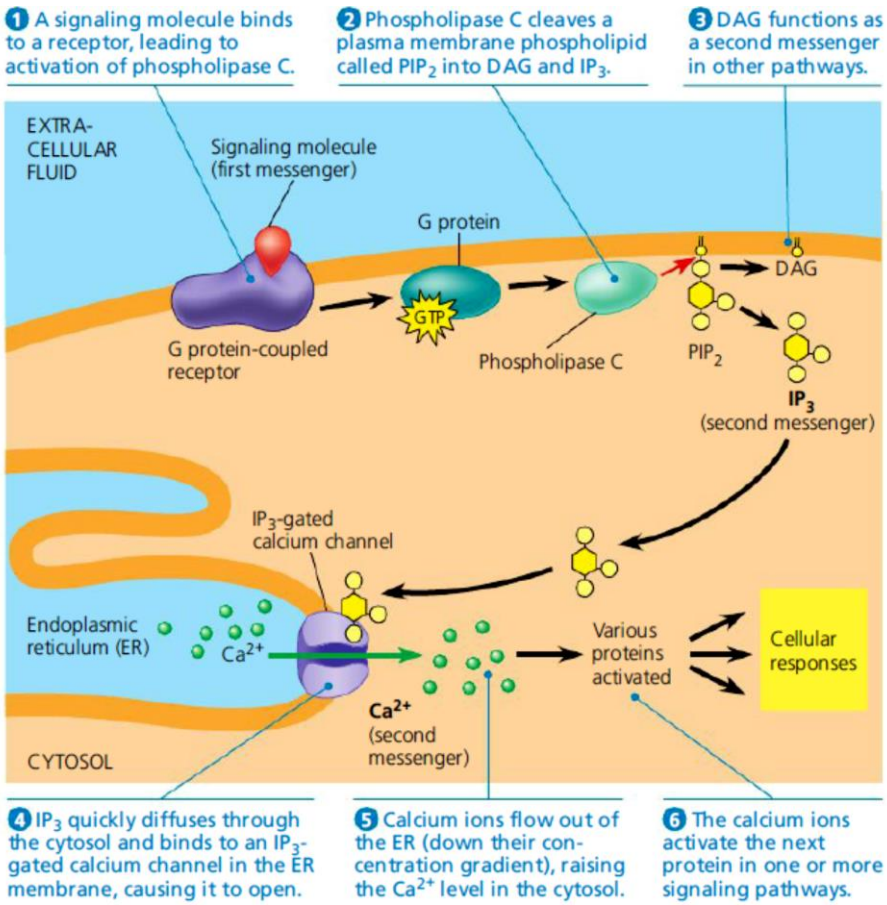


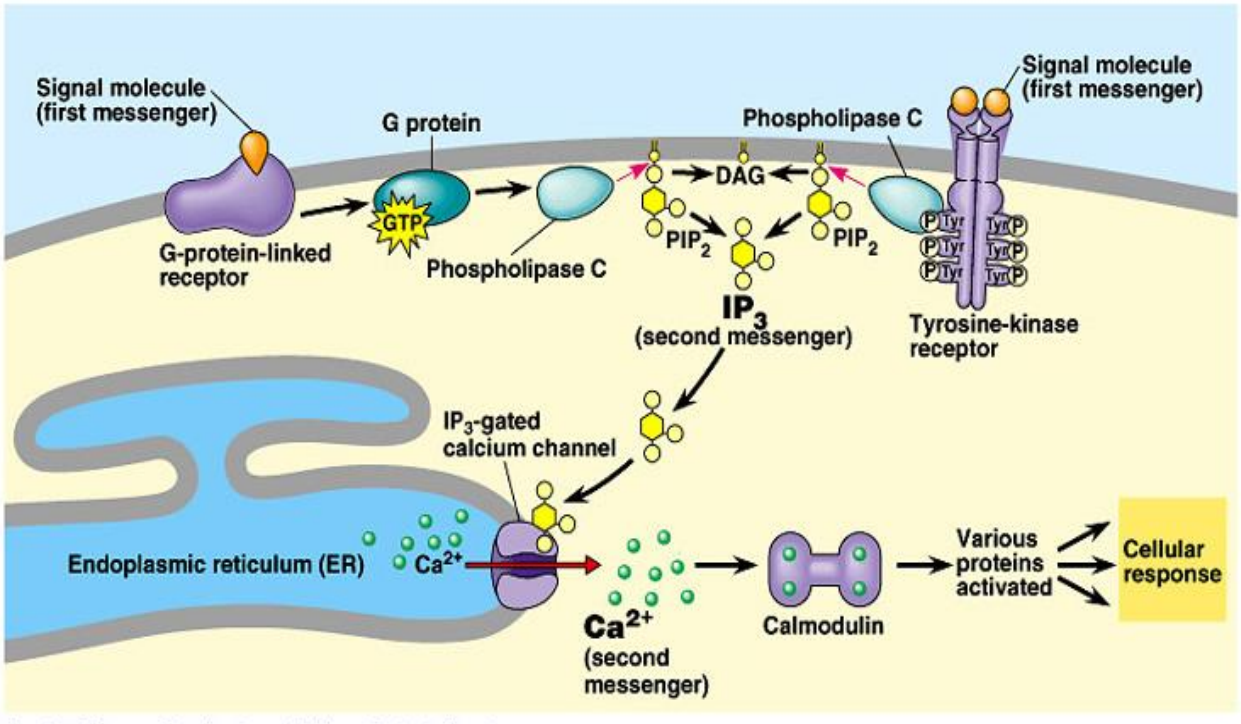
cAMP as a second messenger in a signalling pathway

- **Calcium ions (Ca^{2+})**
  - Many signal molecules in animals, including neurotransmitters, growth factors, and some hormones, induce responses in their target cells via signal transduction pathways that **increase the cytosolic concentration of Ca^{2+}**.
  - Increasing the cytosolic concentration of Ca^{2+} causes many responses in animal cells, including muscle cell contraction, secretion of substances (e.g. acetylcholine), and cell division. Cells use Ca^{2+} as a second messenger in both G protein-coupled receptor and receptor tyrosine kinase pathways.
  - Although cells always contain some Ca^{2+}, this ion can function as a second messenger because its **concentration in the cytosol is normally much lower than the concentration outside the cell**. Calcium ions are actively transported out of the cell and are actively transported from the cytosol into the endoplasmic reticulum ER by various protein pumps.



Calcium ion concentrations in the cell

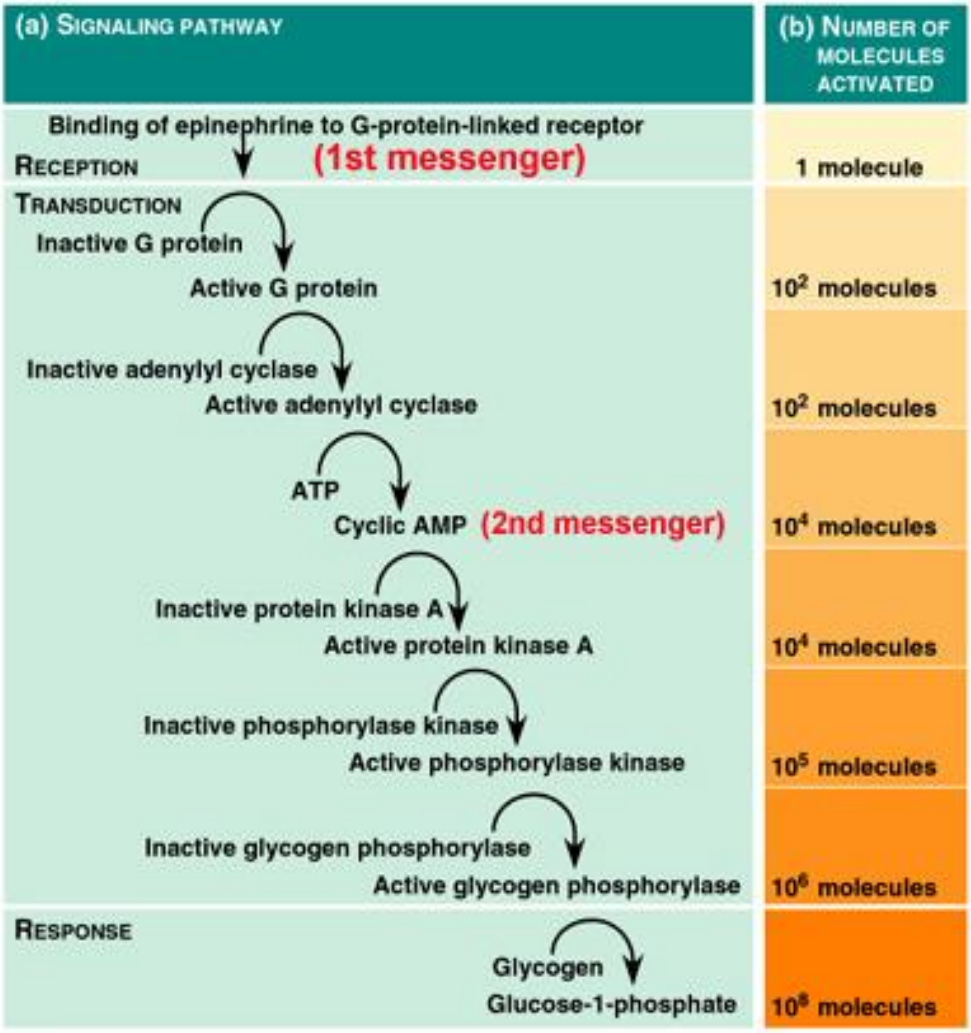




Calcium ion as second messenger

(iii) **Signal amplification**

- If some of the relay molecules in a pathway transmit the signal to multiple molecules of the next component in the series, the result can be a large number of activated molecules at the end of the pathway.
- In other words, a small number of extracellular **signal molecules** can produce a **large cellular response**.
- Enzyme cascades amplify the cell's response to a signal. **At each catalytic step in the cascade, the number of activated products is much greater than those in the preceding step**.



Signal amplification

- For example, each adenylate cyclase molecule catalyses the formation of many cAMP molecules, and each molecule of protein kinase A phosphorylates many molecules of the next kinase in the pathway, and so on.
- The amplification effect stems from the fact that these proteins persist in the active form long enough to process numerous molecules of substrate before they become inactive again.
- As a result of the signal's amplification, a small number of signal molecules binding to receptors on the surface of a liver or muscle cell can lead to the release of hundreds of millions of glucose molecules from glycogen.

(c) Cellular response

The signal transduction pathway leads to a specific **cellular response**, which is the regulation of one or more cellular activities. The response may occur in the nucleus or cytoplasm of a cell.

Depending on the type of cell and signal molecules, some cellular responses may include:

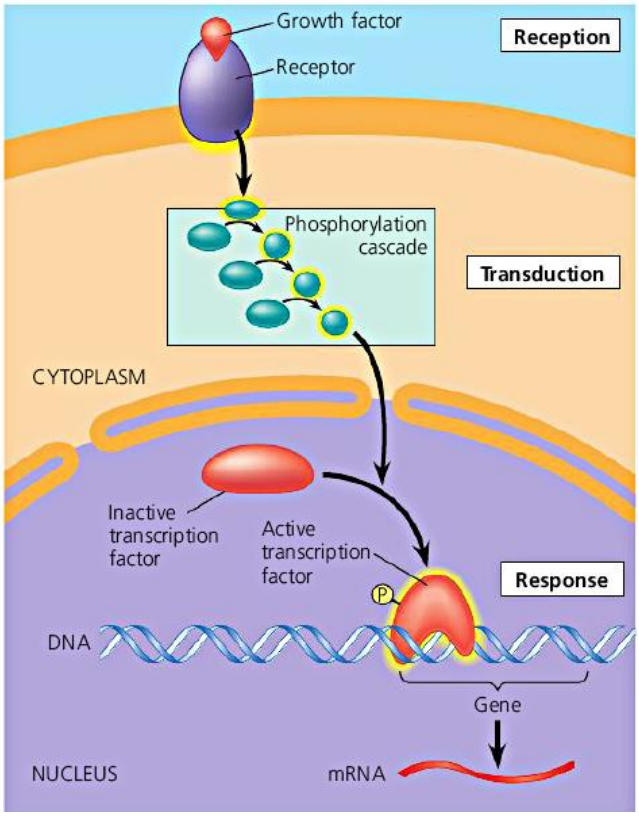
(i) Regulation of activity of protein (e.g. opening or closing of an ion channel in the cell surface membrane changes membrane permeability);

(ii) Regulation of synthesis of protein by turning specific gene expression on or off in the nucleus;

(iii) Regulation of activity of enzyme (e.g. glycogen phosphorylase);

(iv) Rearrangement of the cytoskeleton of the cell;

(v) Death of the cell (e.g. in apoptosis)



Nuclear response to a signal.

(image source: Campbell, 9th ed)

After the specific cellular response has been carried out, the signal is terminated. The ability of a cell to receive new signals depends on the reversibility of the changes produced by prior signals. Signal termination can occur in the following ways:

(i) Dissociation of ligand from receptor when concentration of signal molecules drops. The binding of ligand to receptors is reversible, thus when the ligand leaves the receptor, the receptor reverts to its inactive form.

(i) The GTPase activity intrinsic to a G protein hydrolyses its bound GTP to GDP.

(ii) Protein phosphatases inactivate protein kinases by dephosphorylation.

(iii) Phosphodiesterase converts cAMP to AMP.

4. Advantages and Significance of a Cell Signalling System

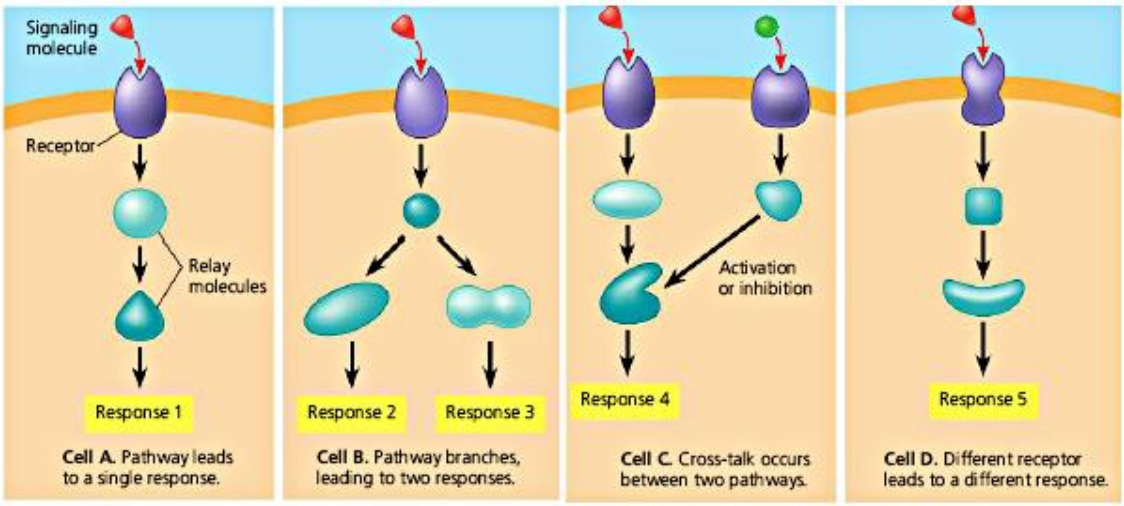
(a) **Specificity** in the ligand-receptor interaction allows signal molecule to elicit responses in specific target cells.

(b) The ability of a signal molecule to **activate many different target cells** simultaneously allow for regulation and control of response.

(c) Signal amplification allows for one signal molecule to trigger a **large cellular response**.

(d) One signal molecule can activate many signal transduction pathways to trigger numerous cellular reactions simultaneously.

(e) The binding of signal molecule to receptor at cell surface membrane can result in activation of gene transcription in the nucleus.



The specificity of cell signalling.

(image source: Campbell, 9th ed)

The same signal molecule can trigger different cellular responses in two different cells in the body. Because different kinds of cells turn on different sets of genes, different kinds of cells have different collections of proteins

Differential gene expression in different cells can affect the cellular response in the following manner:

- A cell may or may not express a receptor for a particular signal molecule.
- Different cell types may have different cell surface receptors that recognise the same signal molecule.
- Two (or more) receptors may work the same way in different cell types but have different affinities for the same signal molecule.
- The expression of proteins (e.g. relay proteins) involved in intracellular signal transduction pathways may vary in different cell types.
- The expression of proteins that are controlled by signal transduction pathways may vary in different cell types.

5. Specific Examples of Cell Signalling Pathways

(a) Glucagon and GPCR Signalling

(i) Ligand-receptor interaction

- During ligand-receptor interaction, binding of **glucagon** to extracellular side of **G protein-coupled receptor (GPCR)** activates the receptor and causing it to change its conformation.
- The cytoplasmic side of the receptor then binds an inactive G protein, causing **G protein to exchange its bound GDP for GTP**.
- The G protein is **activated** and dissociates from the receptor. Activated G protein binds and activates adenylate cylase, which catalyse the conversion of large number of ATP to cAMP.

(ii) Signal transduction

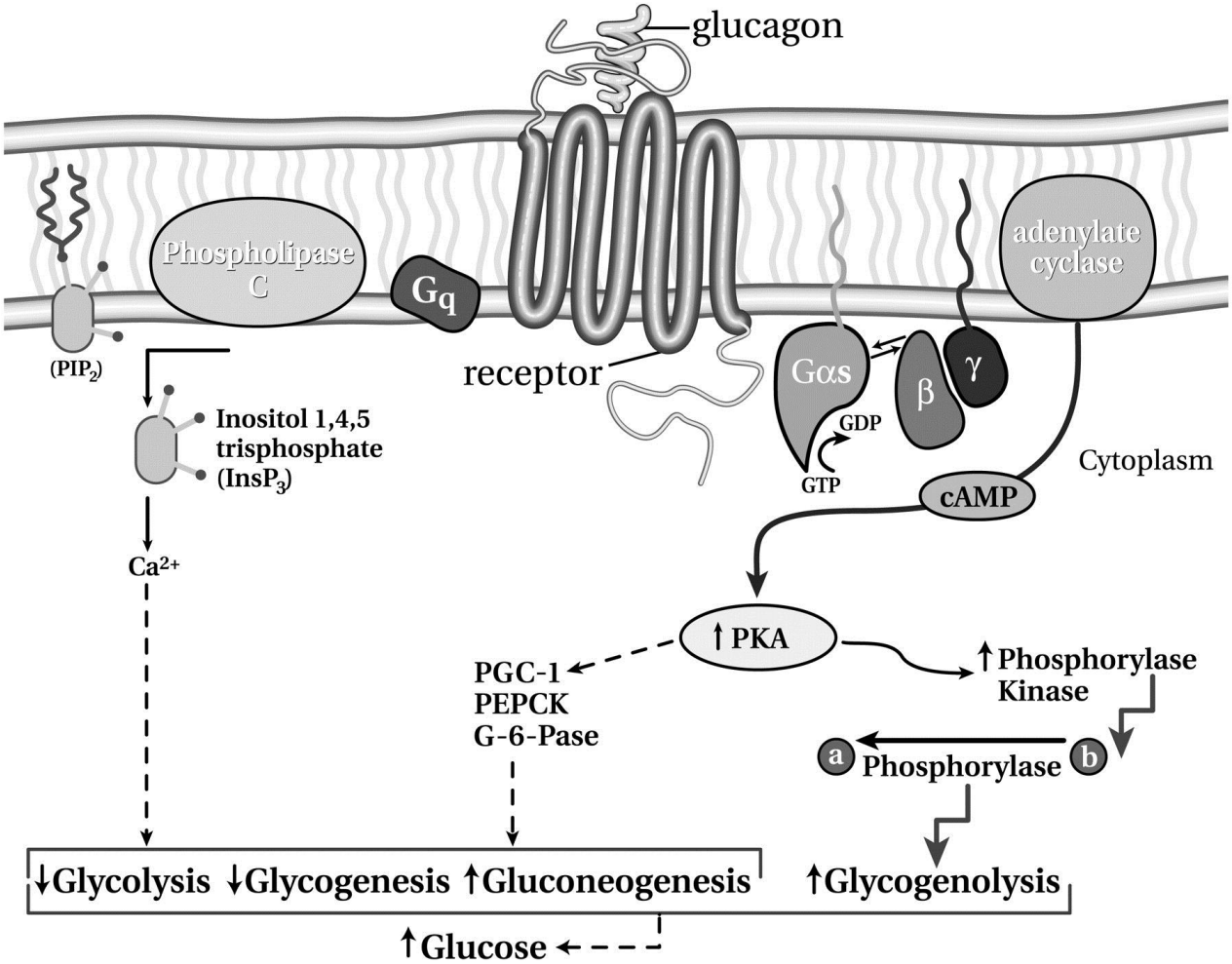
- During signal transduction, cAMP, a **second messenger**, binds and activates a large number of protein kinase A (PKA).
- Each activated protein kinase will initiate **a sequential phosphorylation and activation** of other kinases, resulting in a **phosphorylation cascade**.
- At each phosphorylation step, each activated kinase is able to activate a large number of the next kinase.
- At each catalytic step in the cascade, the number of activated product is always greater than those in the preceding step, resulting in **signal amplification**.
- Activated PKA phosphorylates and activates phosphorylase kinase, which in turn phosphorylates and activates glycogen phosphorylase.
- The final protein to be activated is glycogen phosphorylase.

(iii) Cellular Response

- During cellular response, a large number of glycogen phosphorylase is activated, which will catalyse the breakdown of glycogen into glucose (glycogenolysis).
- Cellular response also includes increase synthesis or activity of enzymes involved in gluconeogensis.

(iv) Signal Termination

- Glucagon is released from receptor.
- The GTPase activity intrinsic to a G protein hydrolyses its bound GTP to GDP.
- Phosphodiesterase converts cAMP to AMP.



Glucagon signalling pathway

(image source: http://ajpendo.physiology.org/content/ajpendo/284/4/E671/F1.large.jpg)

(b) Insulin and RTK Signalling

(i) Ligand-receptor interaction

- During ligand-receptor interaction, binding of **insulin** to extracellular binding sites of **receptor tyrosine kinase** causes two RTK proteins to form a **dimer**.
- Dimerisation **activates the tyrosine kinase function** found in the intracellular tails of RTK
- Tyrosine kinase adds **phosphate group** from ATP molecule to the tyrosine residues on the tail of the other RTK protein by **autophosphorylation**.

(ii) Signal Transduction

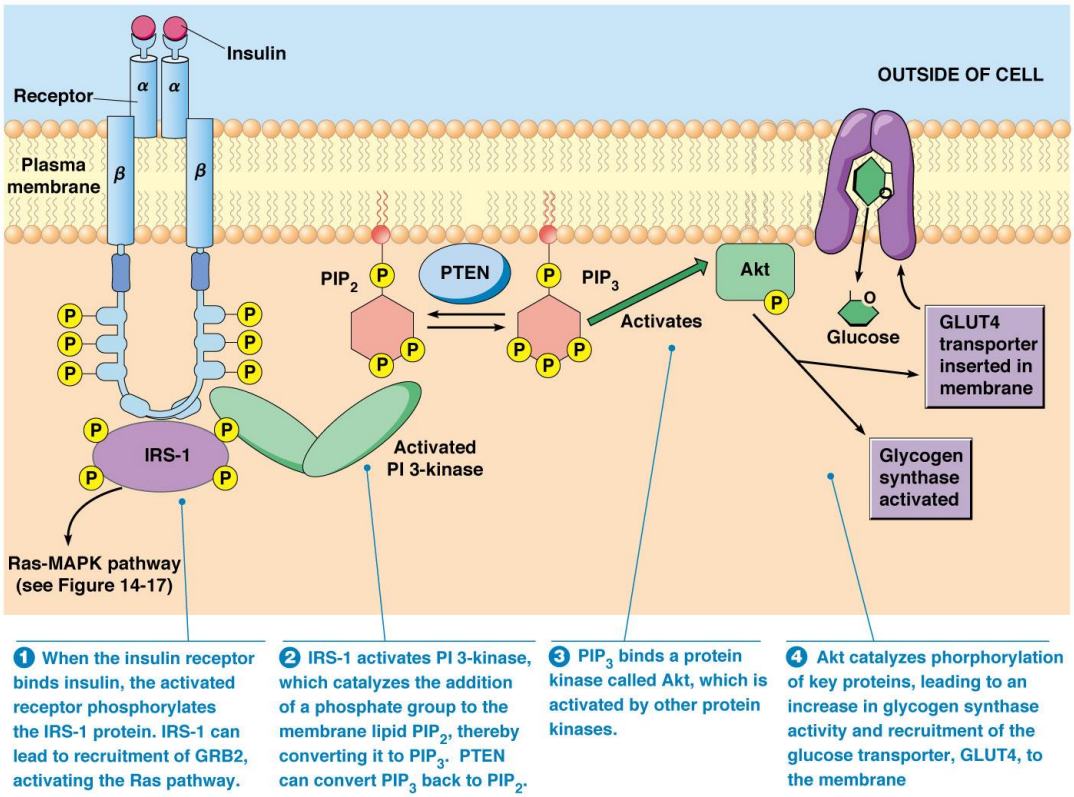
- During signal transduction, the **activated RTK** will trigger the assembly of **relay proteins** on the receptor tails, activating them.
- Activated relay proteins will further recruit and activate other downstream relay molecules and protein kinases.
- Each activated protein kinase will initiate **a sequential phosphorylation and activation** of other kinases, resulting in a **phosphorylation cascade**.
- At each phosphorylation step, each activated kinase is able to activate a large number of the next kinase.
- At each catalytic step in the cascade, the number of activated product is always greater than those in the preceding step, resulting in **signal amplification**.

(iii) Cellular Response

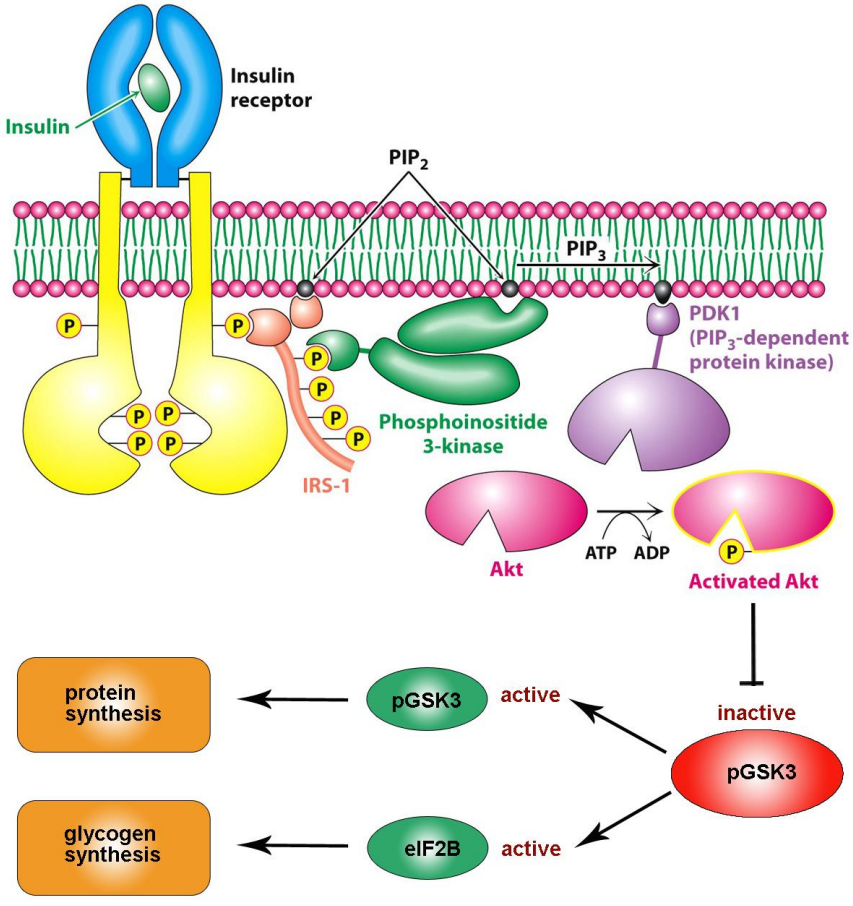
- Activated relay proteins cause vesicles embedded with glucose transporters to move to the cell surface membrane and fuse with it, thus inserting the transporters into the cell surface membrane.
  - This will result in the increase in uptake of glucose into muscle cells.
- Large number of of glycogen synthase is activated, which will catalyse the synthesis of glycogen from glucose (glycogenesis).
  - Thus, binding of 1 insulin molecule to receptors will lead to the synthesis of large amounts of glycogen.
- Decrease activity or synthesis of enzymes involved in glycogenolysis and gluconeogenesis.

(iv) Signal termination:

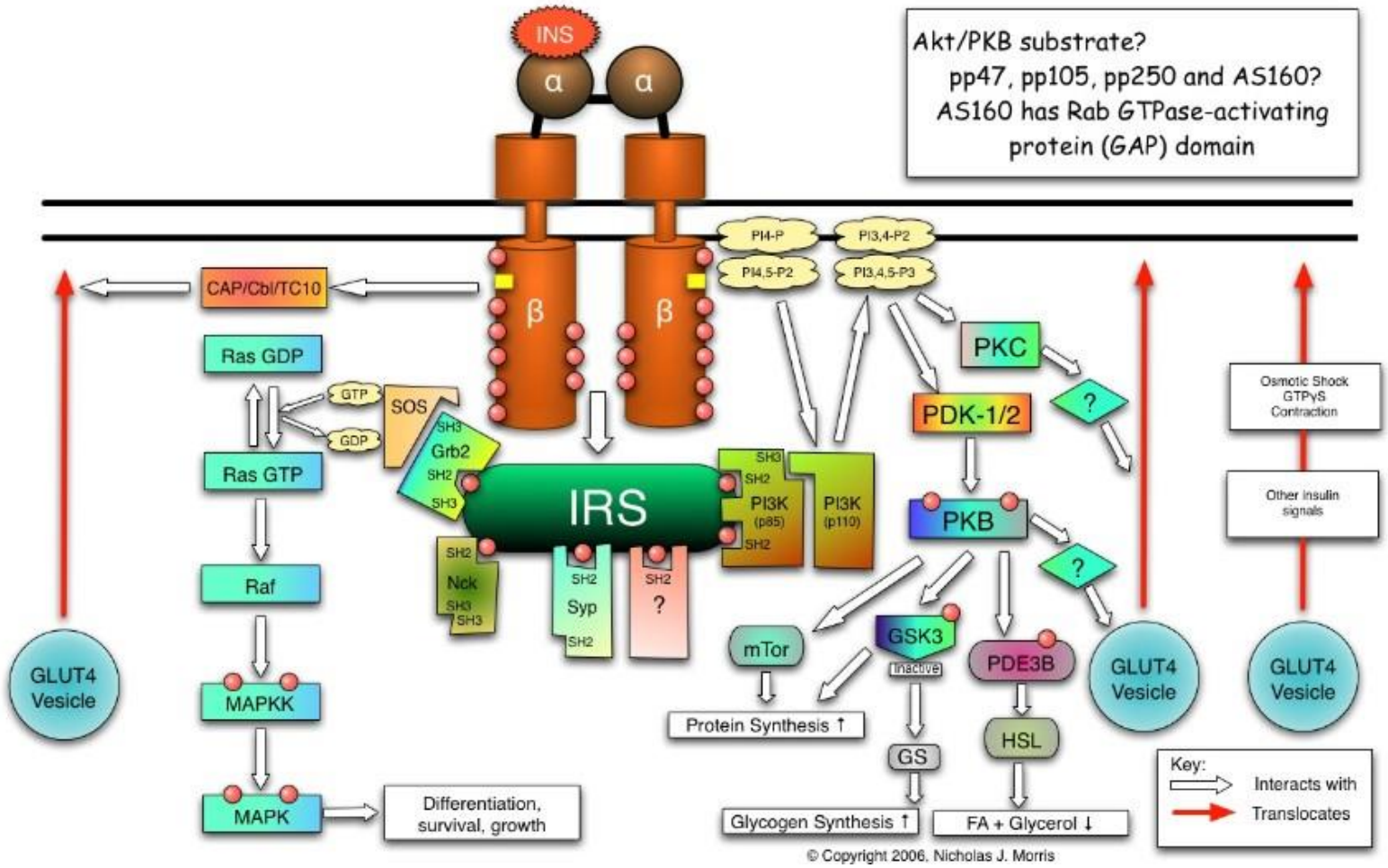
- Insulin is released from receptors, the tyrosine residues are dephosphorylated by phosphatases and the dimer dissociates back into individual RTK proteins.
- Protein phosphatases inactivate protein kinases by dephosphorylation.



Insulin-RTK signalling pathway leading to glycogen synthesis and glucose uptake



Insulin-RTK signalling pathway leading to protein and glycogen synthesis

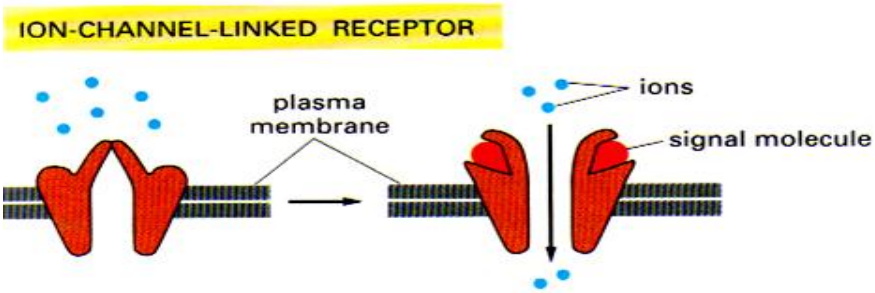


Insulin signalling pathway

(Adapted from http://www.staff.ncl.ac.uk/n.j.morris/gifs/insulin_signalling.jpg, 4 Feb 2009)

(c) Generation of nerve impulses via ligand-gated ion channels

Ligand-gated ion channels are very important in the nervous system. For example, the neurotransmitter molecules released at a synapse between two nerve cells bind as ligands to ion channels on the receiving cell, causing the channels to open. Ions flow in and trigger an electrical signal that propagates down the length of the receiving cell. Some gated ion channels are controlled by electrical signals instead of ligands; these voltage-gated ion channels are also crucial to the functioning of the nervous system.



Ligand gated ion channels

(d) Activation of cellular defence mechanism

Interferons are natural proteins produced by the cells of the immune system of most vertebrates in response to challenges by foreign agents such as viruses, parasites and tumor cells. Interferons are produced by a wide variety of cells in response to the presence of double-stranded RNA, a key indicator of viral infection. Interferons assist the immune response by inhibiting viral replication within host cells, activating natural killer cells and macrophages, increasing antigen presentation to lymphocytes, and inducing the resistance of host cells to viral infection.

The interferon receptor is similar to a tyrosine kinase receptor.

While there is evidence to suggest other signalling mechanisms exist, the JAK-STAT signalling pathway is the commonly accepted interferon signalling pathway.