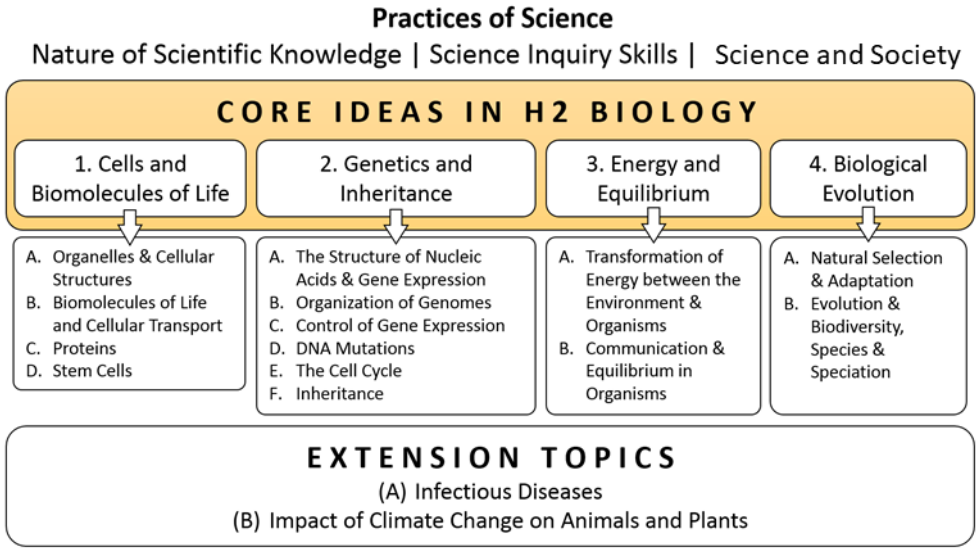
Core Idea 3B

12. Energy and Equilibrium – Communication in Multicellular Organisms



SYLLABUS OVERVIEW		
No.	**Overarching Idea**	**Topics**
1	**Core Idea 1** The Cell and Biomolecules of Life	Cell – The Basic Unit of Life
2		Biomolecules of Life and Cellular Transport
3	**Core Idea 3** Energy and Equilibrium	Transformation of Energy – Photosynthesis and Cellular Respiration
4	**Core Idea 2** Genetics and Inheritance	Genetics and Inheritance (I) – The Cell Cycle
5		Genetics and Inheritance (II) – DNA Replication and Gene Expression
6		Genetics and Inheritance (III) – DNA Mutations and their Consequences
7		Genetics and Inheritance (IV) – Molecular Techniques in DNA Analysis
8		Genetics and Inheritance (V) – Organization of Genome & Control of Gene Expression in Eukaryotes *[Includes Core Idea 1D: Stem Cells]*
9		Genetics and Inheritance (VI) – Organization and Inheritance of Viral Genomes
10		Genetics and Inheritance (VII) – Organization of Genome & Control of Gene Expression in Prokaryotes
11		Genetics and Inheritance (VIII) - Inheritance
12	**Core Idea 3** Energy and Equilibrium	Communication and Equilibrium in Multicellular Organisms
13	**Core Idea 4** Biological Evolution	Biological Evolution
14	**Extension Topic A** Infectious Diseases	Immunity and Infectious Diseases
15	**Extension Topic B** Impact of Climate Change on Animals & Plants	Climate Change – Causes and Impacts on Animals and Plants

What's the Big Picture?
The following question should help students frame their learning: • How do organisms **respond** to **internal** and **external changes**? **Communication is needed for organisms to respond to the environment and maintain equilibrium** Organisms should be able to detect changes both from the surrounding environment and within themselves so that they are able to respond to these changes to maintain a **constant internal environment**. This ability to respond to changes is made possible due to coordination across the various biological systems as well as communication between cells. Communication between cells can take the form of **electrical** or **chemical transmission** via the **nervous** or **endocrine system** respectively. The endocrine system facilitates communication between different cells through the release of hormones into the bloodstream. **Binding** of **hormones** to **receptors** on or within target cells initiates **signal transduction** and eventually results in a **change** in **gene expression** to bring about certain **physiological changes**. **Defects** in any part of the **signalling pathway** often lead to detrimental conditions such as **metabolic diseases** and **cancer**.
Core Idea 3: Energy and Equilibrium **Topic B: Communication and Equilibrium in Organisms** The emphasis of this section is on how **cell signalling processes** can cause a **physiological response** in an organism. The **circulatory system** transports **hormones** from where they are secreted to the **target cells**. Hormones bind to **specific binding sites – receptors** found on the **cell surface membrane** or **within the cell** – to **initiate** the **process of cell signalling**. **Cell signalling** comprises the following stages: **ligand-receptor interaction, signal transduction** and **amplification,** and **cellular response.** Various molecules such as **second messengers, kinases** and **transcription factors** mediate the processes of converting information from the **signal molecule (hormone)** into a **cellular response**. **Insulin** and **glucagon** are examples of hormones that trigger **cell signalling pathways** to bring about **responses** to **regulate blood glucose level**. It is important to appreciate the complexity and inter-connectedness of how the communication systems within and between cells interact to achieve the required response. The **maintenance of blood glucose levels** will be used to illustrate how **physiological responses** are **regulated by controlling gene expression**. Sufficient glucose in the blood is necessary to provide cells with respiratory substrates. The **pancreas** detects the **level of blood glucose** and secretes either **insulin or glucagon** to maintain a stable level of glucose in blood. These hormones trigger **cellular responses in liver, muscle and adipose cells** when the **hormones bind to receptors**. **Signal transduction** occurs through **various proteins and molecules** to **amplify** and **transduce** the **signal** and eventually, **elicit** a **cellular response**. Thus, **cell signalling and communication** result in a **relatively stable internal environment** for **cells** in an organism to **function optimally**.

LEARNING OUTCOMES

Core Idea 3B: Communication and Equilibrium in Organisms

Candidates should be able to:

a) Outline the main stages of cell signalling:
   i. ligand-receptor interaction,
   ii. signal transduction (phosphorylation cascade and signal amplification)
   iii. cellular response (change in gene expression).
   (Knowledge of intracellular receptors is not required.)

b) Explain the roles and nature of second messengers (including cyclic AMP).

c) Explain the role of kinases and phosphatases in signal amplification.

d) Outline how insulin and glucagon regulate the concentration of blood glucose level through the respective tyrosine kinase receptor and G-protein linked receptor. (The outline should be limited to describing how the ligand induces a conformational change in membrane-bound receptor to trigger downstream signalling pathways that elicit physiological changes in blood glucose level. Details of different second messengers and specific kinases activated in the pathway are not required).

Use the knowledge gained in this section in new situations or to solve related problems.

LECTURE OUTLINE

1. Overview
- 1.1 Why is Communication within the Multicellular Organism Important?
- 1.2 Types of Signal Molecules / Chemical Messengers
- 1.3 Types of Cell Signalling
- 1.4 Cell Signaling - How do cells receive and communicate signals?

2. Signal Reception
- 2.1 G Protein-Linked Receptor Signaling
- 2.2 Tyrosine Kinase Receptor Signaling
- 2.3 Ion Channel Receptor Signaling
- 2.4 Intracellular Receptor Signaling

3. Signal Transduction
- 3.1 Important Features
- 3.2 Transduction by Protein Phosphorylation and Dephosphorylation
- 3.3 Transduction by Second Messengers

4. Cellular Responses to Signals
- 4.1 Nature of Cellular Responses to Signals
- 4.2 Termination of Signal

5. Advantages and Significance of Cell Signaling Systems in Multicellular Organisms

6. Achieving Equilibrium in the Internal Environment of Multicellular Organisms
- 6.1 Overview
- 6.2 Regulation of Blood Glucose Concentration
  - (A) Regulation of Blood Glucose Concentration by Insulin
  - (B) Regulation of Blood Glucose Concentration by Glucagon

REFERENCES

1. Reece, J., *et al*. (2014) *Campbell Biology*. Pearson Education, Inc., San Francisco, Tenth Edition. Pages 232 – 248.

2. Animations:

Introduction to cell signalling	https://www.youtube.com/watch?v=-dbRterutHY
Common cell signalling pathways (good overview!)	https://www.youtube.com/watch?v=9sF_h-bAnIE
Signal transduction	https://www.dnatube.com/video/1162/Signal-transduction
G protein coupled receptor	https://www.youtube.com/watch?v=xT0mAQ4726s https://www.youtube.com/watch?v=kLOwGKEvZF0
Second messengers: cAMP	https://www.youtube.com/watch?v=re2dNETlvw0
Signal amplification	https://www.youtube.com/watch?v=wo_fXINA9U0 https://www.youtube.com/watch?v=PQxQ1joKd1o

CELL SIGNALING – OVERVIEW

This table serves as an overview. You must refer to the notes for details.
You need to know the cell signalling pathways triggered in response to insulin and glucagon.
For other signalling pathways, you will need to draw upon your understanding of the molecules involved.

Step 1: Signal Reception

Binding of signal molecules (ligands / first messengers) with a **shape complementary** to the binding site of receptors forms the ligand-receptor complex → receptor protein undergoes **conformation change** and is activated

Possible types of cell surface membrane receptors:

- G protein-linked receptors
- Tyrosine kinase receptors
- Ligand-gated ion channels

Signal molecules that are **hydrophilic** cannot traverse the hydrophobic phospholipid bilayer, hence, need to bind to cell surface membrane receptor. e.g. of cell surface membrane receptors - peptide hormones such as insulin, glucagon and ions such Na^+, K^+, Cl^-, etc.

Step 2: Signal Transduction

Multi-step, cascaded pathways that **amplify** and relay the signal received.

Possible types of signal transduction:

(1) Phosphorylation Cascade

- Involves **activated protein kinases phosphorylating** (adding phosphate group) the next protein in cascade to **activate** the inactive proteins.
- Involves protein **phosphatases** that **remove** phosphate groups to **inactivate** proteins.

(2) Transduction by 2nd messengers (e.g. cAMP)

Second messengers are small, non-protein molecules that **relay signals** received by the receptors **to activate** intracellular pathways.

Step 3: Cellular Response

Various types of cellular responses (e.g. gene expression, enzymatic processes) triggered in response to the specific signal molecule

Possible types of cellular responses:

NOTE: The type/s of cellular responses triggered **depends on the signal** that binds to the specific receptor (signal reception) and the subsequent enzyme/molecules activated (signal transduction)

(1) Expression of specific gene(s) to produce specific proteins

(2) Enzymatic pathways

Examples:

(a) Glycogenesis –
Glucose→glucose-6-phosphate → → → glycogen

(b) Glycogenolysis –
Glycogen → → →glucose

(3) Other Interactions among molecules *(not in syllabus)* e.g.

- interact with myosin leading to muscle contractions

Step 4: Signal Termination

Cellular responses are terminated to ensure cell's continued responsiveness to incoming signals

Possible ways of signal termination:

(1) At receptors

- Chemical modification to inactivate receptors
- Inactivation of signalling proteins
- Down regulation via endocytosis / degradation by lysosomes

(2) At relay proteins

- Hydrolysis of bound GTP to GDP by GTPase
- Converting cAMP to AMP by cAMP phosphodiesterase
- Increase phosphatase activity to inactivate relay proteins

1. Overview

1.1 Why is Communication within a Multicellular Organism Important?

Key Concept 1:
Cells in a **multicellular organism** must **communicate** through various **chemical messengers** to **coordinate** their activities.

- In multicellular organisms, **cell communication** is important to allow for:
  - multiple cell types to **coordinate their activities** to initiate a synchronized response.
  - specialization of groups of cells.

- This communication is achieved by releasing **chemical messengers / ligands / signal molecules** which may travel,
  - **short distances** to influence cells in the vicinity (Fig. 1.1a), or **long distances** via the circulatory system to influence cells distantly located (Fig. 1.1b),
  - to **target specific cells** that will **recognize** and **respond** to the given messenger (Fig. 1.1a/b)

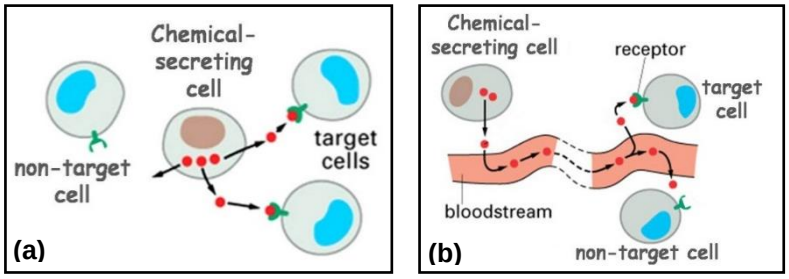


Fig. 1.1: (a) Short-distant cell signaling. **(b)** Long-distant cell signaling via the bloodstream.

1.2 Types of Signal Molecules / Chemical Messengers / Ligands

- **Signal molecules / ligands** may be proteins, peptides or amino acids, nucleotides, steroids or fatty acid derivatives and dissolved gases.

- A signal molecule that is **hydrophilic/polar** in nature (Fig. 1.2a) **cannot traverse** the hydrophobic phospholipid bilayer of the cell surface membrane. Hence, it binds to **receptor** molecules on the **cell surface membrane**.

- A signal molecule that is **hydrophobic/non-polar** in nature (Fig. 1.2b) **can traverse** the hydrophobic phospholipid bilayer of the cell surface membrane and binds to **intracellular receptor**s within the cell. *(not in syllabus)*

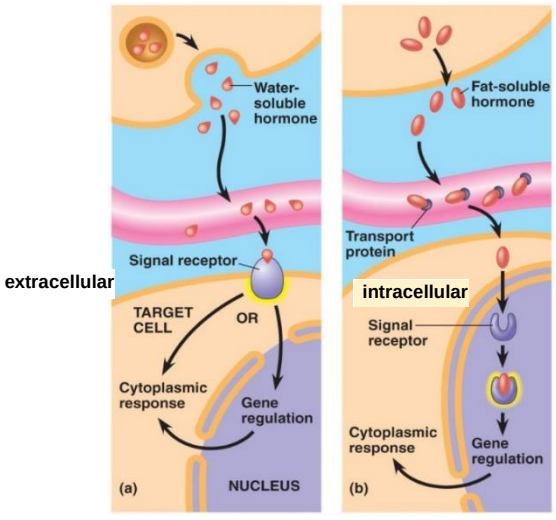


Fig. 1.2 (a): Hydrophilic/polar hormone secreted by a cell is carried in the bloodstream (hydrophilic environment) unaided, eventually exits the bloodstream and binds to the cell surface receptor of another cell.

Fig. 1.2 (b): Hydrophobic/non-polar hormone secreted by a cell is carried in the bloodstream with the help of a transport protein, eventually exits the bloodstream, enters another cell and binds to a intracellular receptor in the nucleus of the cell.

1.3 Types of Cell Signalling

- Cell signaling can be classified into 4 main categories.
  a) Paracrine signaling
  b) Autocrine signaling
  c) Hormonal/endocrine signaling
  d) Synaptic signaling between neurons of the nervous system *(FYI only; not in syllabus)*

a) Paracrine Signaling

- A type of **<u>local</u> signaling** where a **secreting cell** discharges substances (**chemical signals**) into the **interstitial/tissue fluid** to act on **<u>nearby</u> target cells** (Fig. 1.3).

- These signal molecules are rapidly removed from the interstitial/tissue fluid to prevent them from diffusing and exerting their effects over long distances.

- This removal occurs via
  1. uptake by target cells
  2. destruction by extracellular enzymes

- E.g. growth factors secreted by neutrophils during inflammation to stimulate cell growth and division.

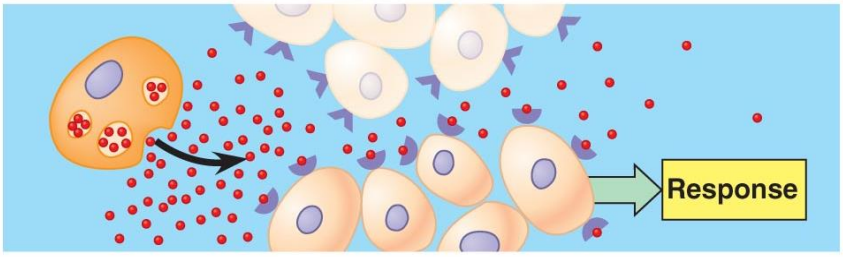


Fig. 1.3: Paracrine signaling – a secreting cell discharges chemical messengers into its vicinity, which affects target cells but not non-target cells.

b) Autocrine Signaling

- **Local signaling** where a cell secretes signal molecules that bind to its **<u>own</u> cell surface receptors** (Fig. 1.4).

- E.g. In response to bacterial antigens, monocytes (a type of leukocyte) secretes interleukin-1 (IL-1) which binds to its own cell surface receptor called IL-1 receptor.

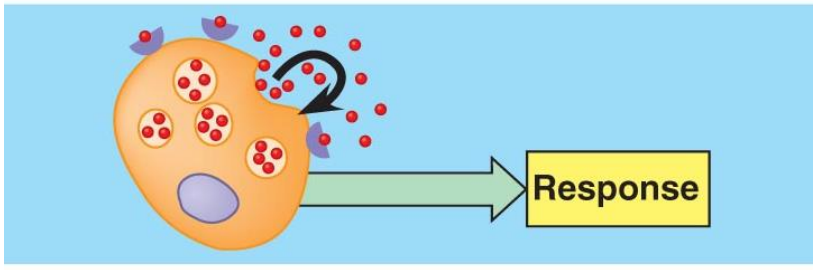


Fig. 1.4: Autocrine signaling

c) Hormonal / Endocrine Signaling

- **Long-distance signaling** where specialized **endocrine cells** secrete hormones into the **bloodstream** and is transported in the blood to **other cells in the body** (Fig. 1.5).

- E.g. insulin secreted by the β cells of the Islets of Langerhans of the pancreas is transported by the bloodstream to target liver cells and muscle cells.

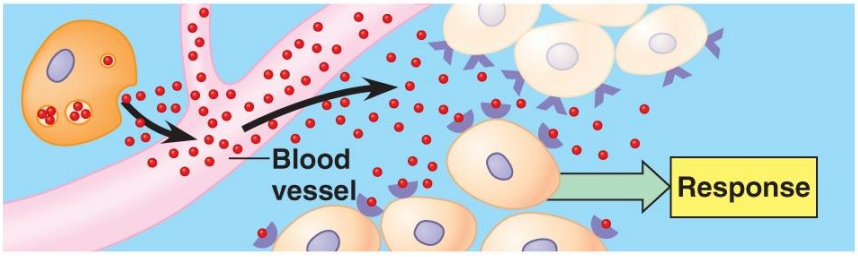


Fig. 1.5: Hormonal/endocrine signaling – hormones are secreted directly into the bloodstream by endocrine cells

1.4 Cell Signaling – How do cells receive and communicate the signals?

Key Concept 2:
Cell signaling ensures that crucial **activities** occur in the **right cells**, at the **right time** and in **proper coordination with** the **other cells** of the organism.
It comprises 3 main stages: (1) **signal reception**, (2) **transduction**, (3) **response**.

- There are hundreds of different specific types of cell signaling pathways, but all of them share the same features [(1) **signal reception**, (2) **transduction**, (3) **response**].

- Examples:
  - Ras signaling in the regulation of cell division
  - Glucagon and insulin signaling in the regulation of blood glucose concentration
  - Cytokine signaling in the activation of gene transcription during immune response

1.4.1 Process

- **Signal molecules/ligands** are molecules that carries information from the environment. These signal molecules bind **specifically and reversibly** to a **complementary receptor** to form the receptor-ligand complex **(reception)**. The information carried will then be **converted into another chemical form** (**transduced**) inside the cell before the cell can **respond** (Fig. 1.6).

- Cell signaling involves 3 main stages: (1) **signal reception**, (2) **transduction**, (3) **response**.

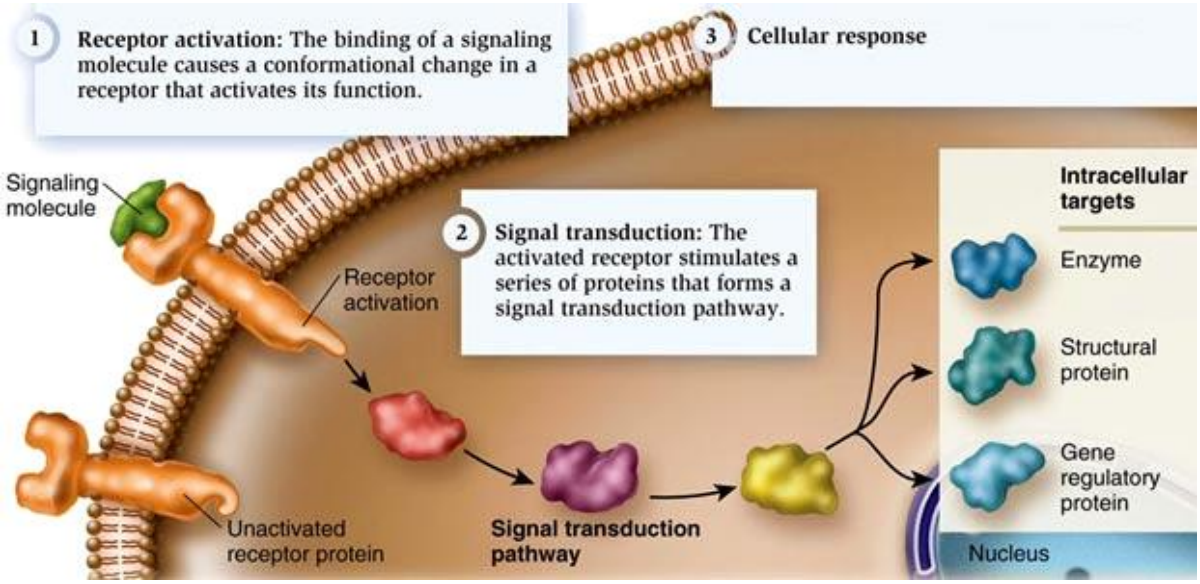


Fig.1.6: The three main stages of cell signaling

1 ***Signal Reception***

- Membrane receptors transfer information from the environment to the cell's interior.
- A cell targeted by a particular chemical signal has **receptor molecules at the cell surface membrane** which are **complementary in shape** to the signal molecule. Most of these signal receptors are **transmembrane proteins.**
- The **signal molecule** is detected when it **binds** to the binding site of **specific complementary receptor protein**.
- This interaction causes a **conformational change** in the **receptor protein** and **activates** it.
- The activated receptor then **interacts** with **another molecule** or **aggregate with another receptor molecule**.

2 ***Transduction***

- Transduction is induced by the activated receptor which converts the signal to specific cellular responses **via a cascade of reactions** (*i.e.* a series of) **that amplifies the signal.**
  - E.g. the phosphorylation cascade
- In some cases, transduction may occur in a **single step**.

3 ***Cellular Response***

- The transduced signal triggers a **specific cellular response**.
- Examples:
  - **Enzyme catalysis** (glycogen phosphorylase catalyses the breakdown of glycogen)
  - **Rearrangement of cytoskeleton** (movement of synaptic vesicles containing neurotransmitter)
  - **Activation of specific genes** in the nucleus (testosterone or progesterone needed for the development of secondary sexual characteristics).

2. Signal Reception

Receptors associated with membranes are known as membrane receptors, some examples include:

3. G protein-linked receptor (a.k.a. G protein-coupled receptor)
4. Tyrosine kinase receptor

Note: *You are expected to use the knowledge gained in this section to apply to novel situations involving other types of cell signaling.*

2.1 G Protein-linked Receptor Signalling

a) Features of the G protein-linked receptor

- The G protein-linked receptor is a group of similar proteins that activate G protein.

- It is a cell surface membrane protein with **7 α-helices spanning the membrane**. **Specific loops** between the helices form **binding sites** for (Fig. 2.1):
  1. extracellular **signal molecule**, and
  2. intracellular **G protein** (a trimeric protein)

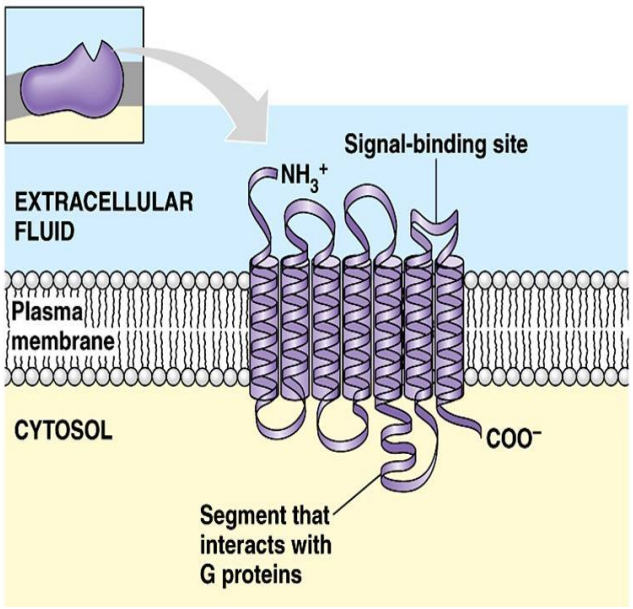


Fig. 2.1: The G Protein-linked receptor

- In turn, the **G protein** acts as an **on-off switch**
  1. If **GDP** (guanosine diphosphate) is **bound**, the G protein is **inactive**
  2. If **GTP** (guanosine triphosphate) is **bound**, the G protein is **active**

- Examples of G protein-linked receptors in different cell types include:
  3. Olfactory neuron receptors of the nose – responsible for sense of smell
  4. Rod photoreceptors of the eyes– responsible for sight
  5. Glucagon receptors of the liver cells – responsible for glucagon-induced responses

b) Signal Reception & Transduction (Fig. 2.2)

- 1 When the ligand/signal molecule (e.g. hormone glucagon) **binds** to the G protein-linked receptor, the G Protein-linked receptor changes conformation, becomes **activated,** and **binds to an inactive G protein** in membrane.

- 2 This binding causes the G protein to **<u>replace</u> GDP with GTP**, and is **activated.**
- 3 The active G protein then **dissociates** from the G protein-linked receptor, **diffuses** along the membrane and **binds** to, alters the conformation of another **membrane protein**, often an **enzyme.**

  These enzymes include:
  - Adenylyl cyclase (most common)
    - that converts ATP to cAMP, which in turn triggers and lead to cellular responses.
  - Phospholipase C
    - which splits PIP2 into IP3 and DAG, which in turn triggers and lead to cellular responses.

- 4 After the extracellular signal is removed, transduction is shut down by:

  ➢ **<u>deactivating</u> the <u>G protein</u>.**
  The active G protein acts as a **GTPase enzyme** and **<u>hydrolyzes</u>** the **GTP to GDP**. This deactivates the G protein, which then dissociates from the adenylyl cyclase to prevent its action.

  Other ways of termination of signal will be covered in Section 4.2.

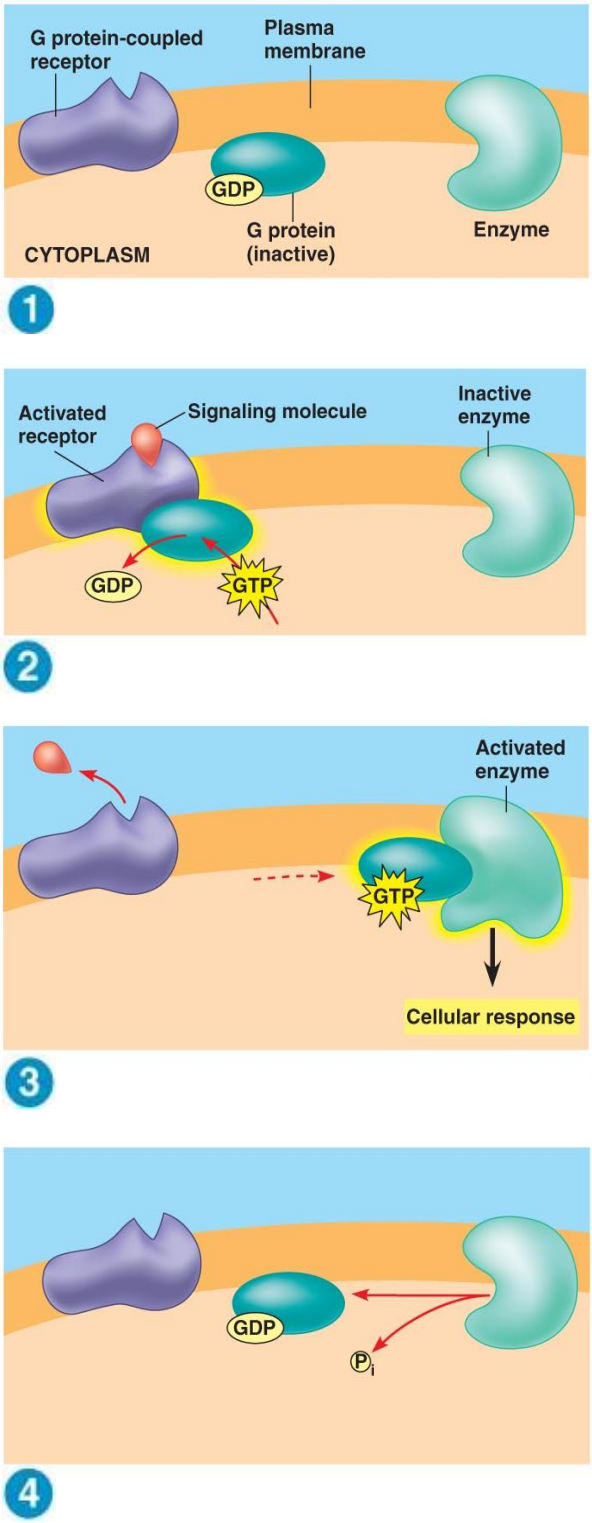


Fig. 2.2: How the G Protein-linked receptor signaling works

2.2 Tyrosine Kinase Receptor Signaling

a) Features of the Tyrosine Kinase Receptor

- The tyrosine kinase receptor is a group of similar proteins **with kinase activity** and is capable of **phosphorylating tyrosine residues** in the protein.

- A transmembrane protein with a **single alpha helix spanning the membrane** (Fig. 2.3) with:
  1. an **extracellular** ligand **binding site**, and
  2. an **intracellular catalytic tail** containing **intrinsic tyrosine kinase (enzyme)** and **several tyrosine residues**. *(Recall: tyrosine is an amino acid)*

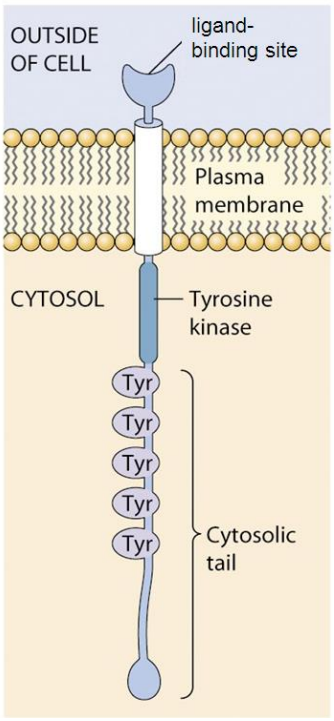


Fig. 2.3: Tyrosine kinase receptor

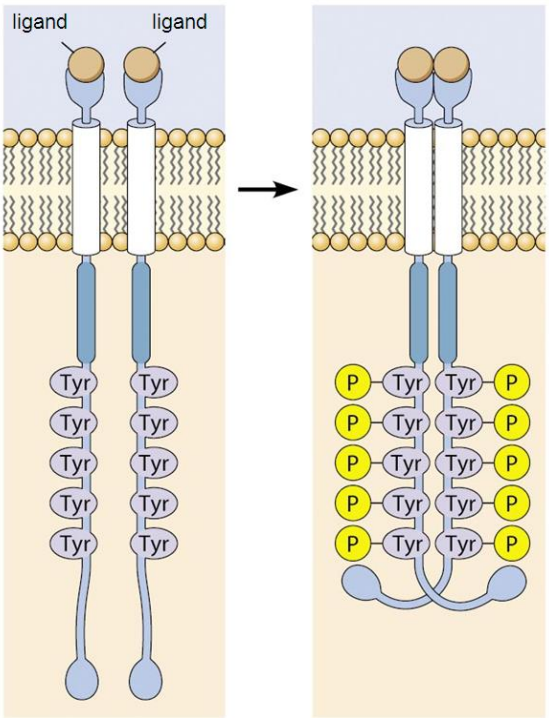


Fig. 2.4: Tyrosine kinase tail phosphorylates the tyrosine residues of intracellular tail of another tyrosine kinase receptor.

- The **intracellular tyrosine kinase tail** functions as a **kinase enzyme** that **catalyzes** the **transfer of phosphate groups from ATP** molecules to tyrosine residues of intracellular tail of another tyrosine kinase receptor (Fig. 2.4).

- The tyrosine kinase receptor system is effective in regulating and coordinating a variety of activities, and triggering several signaling pathways simultaneously (e.g. during cell growth and reproduction).

- Examples of tyrosine kinase receptors include:
  - Insulin receptor
  - Epidermal growth factor (EGF) receptor
  - Fibroblast growth factor (FGF) receptor

b) Tyrosine Kinase Receptor Signal Reception & Transduction Process (Fig. 2.5)

- 0 In the absence of extracellular signals, most tyrosine kinase receptors exist as monomers where its kinase domain is inactive.
- 1 When ligand /signal molecules (e.g. insulin) bind to the two receptor proteins, the receptors **dimerize** to form a **dimer**.
- 2 This **dimerization activates** the **catalytic tyrosine kinase** tail of each receptor protein to **cross-phosphorylates** each other on **multiple tyrosine residues**.
- 3 The **fully-activated receptor dimer activates** a variety of **specific intracellular relay proteins** that recognize and bind to its specific phosphorylated tyrosine residues of the cytoplasmic tails of the receptors.
- 4 Relay proteins undergo **structural changes** triggering **multiple responses** simultaneously.

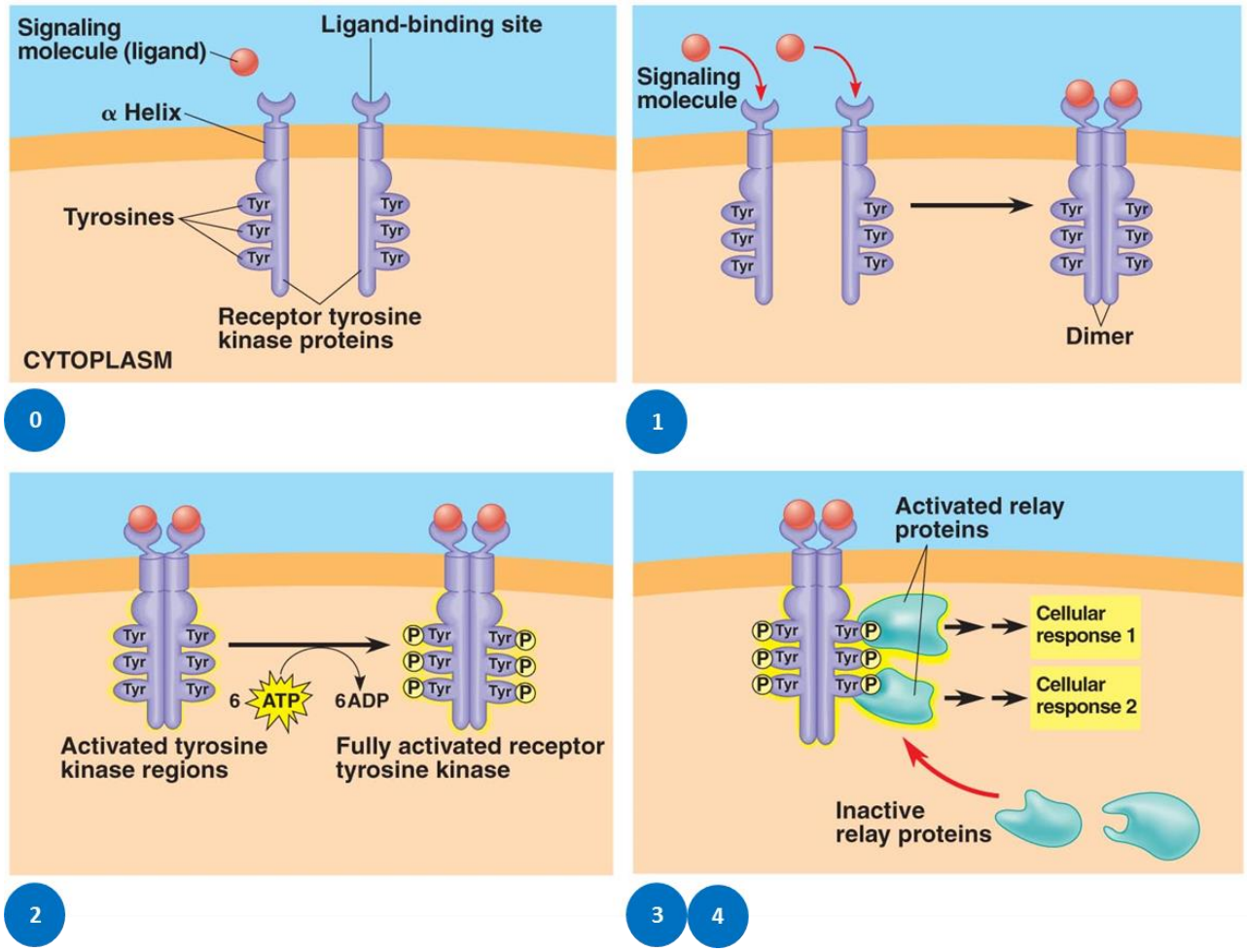


Fig. 2.5: Tyrosine Kinase Receptor signal reception and transduction

2.3 Ion Channel Receptor Signaling

a) Features of Ion Channel Receptors

- The ligand-gated ion channel consists of **transmembrane protein subunits** (Fig. 2.6), with:
  - an **extracellular ligand/signal molecule binding site,** and
  - a **gated hydrophilic channel** which allows or blocks the flow of specific ions (e.g. Na^+, K^+ and Ca^{2+}) through the receptor channel.

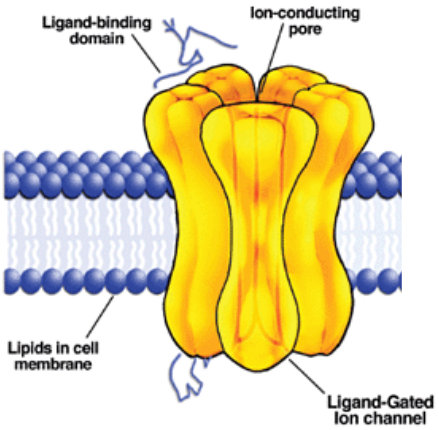


Fig. 2.6 The structure of a typical ion channel receptor

b) Ligand-Gated Ion Channel Receptor Signal Transduction Process (Fig. 2.7)

1. When a ligand (e.g. acetylcholine) binds to the extracellular side of the receptor, it induces a **conformational change** in the receptor, resulting in the **opening of the channel**.
2. Ion flow **changes** its **concentration inside** the **cell** rapidly which in turn **affects cellular activity**.
3. When the **ligand dissociates**, the **channel closes**.

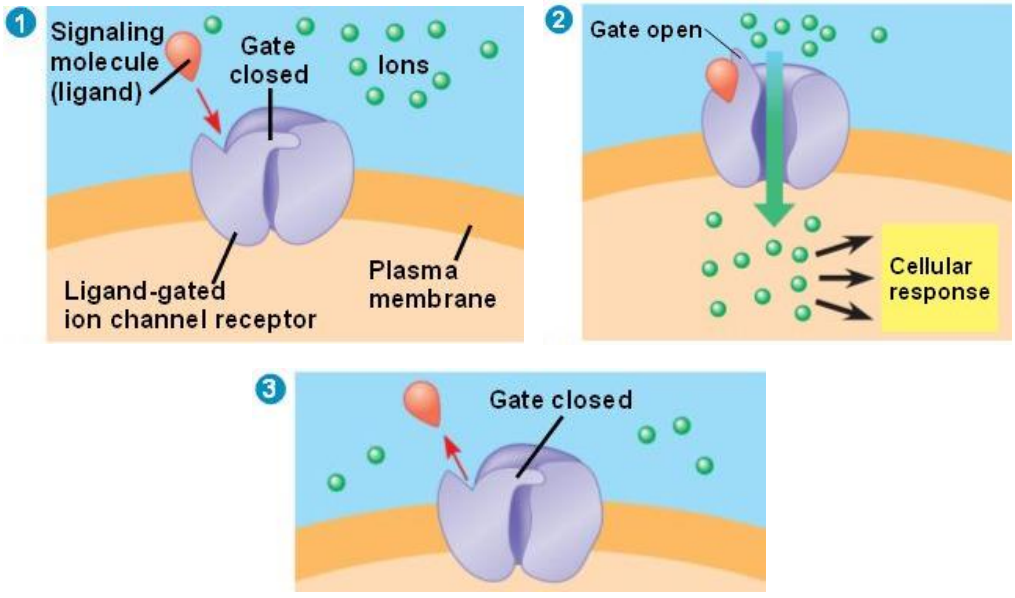


Fig. 2.7: How the ion channel receptor works

2.4 Intracellular Receptor Signaling *(details are not required in the syllabus)*

a) Features of Intracellular Receptors

- These are **signal receptors** that are found in the **cytosol** (e.g. testosterone receptor) or in the **nucleus** (e.g. thyroid hormone receptor) of target cells (Fig. 2.8).
- These **receptors bind to ligands/signal molecules that are small** (e.g. nitric oxide) and **hydrophobic** in nature (e.g. steroid hormones such as testosterone and oestrogen).
- Serve as **both intracellular receptors and effectors** for the signal molecule

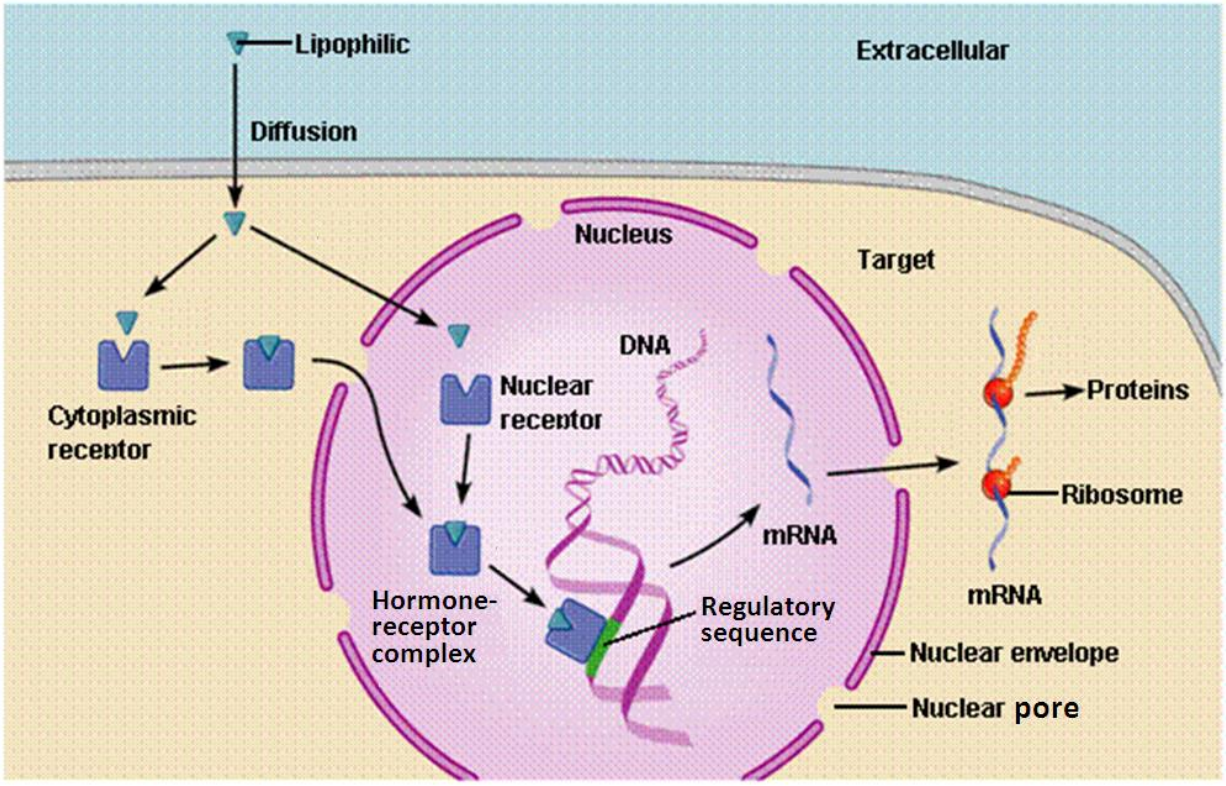


Fig. 2.8: Intracellular receptor signaling. The hormone-receptor complex acts as an effector (transcription factor) which activates the transcription of genes.

b) Intracellular Receptor Signal Transduction Process (Fig. 2.8)

1) Signals which are usually **steroidal** in nature (e.g. steroid hormones) are able to directly **diffuse** through the plasma membrane, **bind** to and **activate intracellular receptor proteins** (either cytosolic or in nucleus, depending on the ligand).

2) These activated proteins (in the form of hormone-receptor complexes) enter the nucleus and act as **transcription factors** that **switches on specific genes**, which are then **transcribed** into **messenger RNA** (mRNA).

3) The mRNA molecules leave the nucleus and carry information that **directs the synthesis** of **specific proteins** (via **translation**) at the ribosome.

3. Signal Transduction

Key Concept 3:
Signal Transduction is a **multistep**, **cascaded** pathway that **amplifies the signal** by (i) **phosphorylation–dephosphorylation reactions** and/or (ii) **second messengers**.

3.1 Important Features

- In a signal transduction pathway, the signal molecule is not passed along the pathway. Instead, **information** is passed on by molecules that **relay** the signal in multistep processes such as
    - Phosphorylation-dephosphorylation reactions of subsequent proteins in the signal transduction pathway

    and/or

    - Second messengers **(**e.g. cyclic AMP)

★ • This relaying of signals is a **multistep** process that allows for **greater fine-tuning of cellular responses** and for **amplification** of the signal. Such a series of signal amplification is known as **cascade amplification** (Fig. 3.1)
    - Each catalytic step in a cascade produces a **larger number of activated products** than in the **preceding step**.
    - Thus, a **very small amount** of **signal** will give a **large response** as each activated enzyme molecule can convert many substrate molecules into products per unit time before being inactivated.

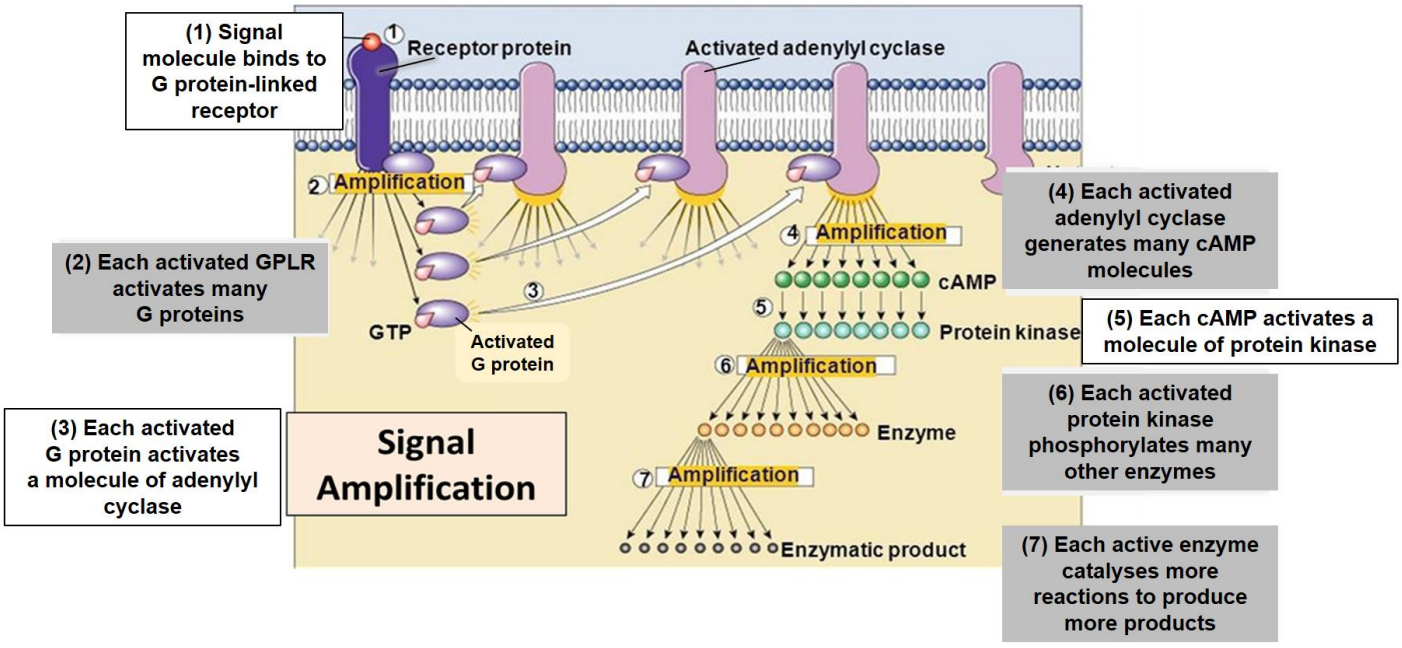


Fig. 3.1: Signal amplification

3.2 Transduction by Protein Phosphorylation and Dephosphorylation

- Protein phosphorylation is the **transfer of phosphate groups from ATP to a target protein** by **protein kinase.** The phosphorylation causes the target protein to undergo a **conformational change** and **convert** from the **inactive to active** form.

- It is a widespread cellular mechanism for **regulating protein activity**. It can also be seen as a **post-translational control** of gene expression.

- The protein kinases act on amino acid **serine** or **threonine** of its substrate (usually another protein kinase) in a signal transduction pathway leading to a **phosphorylation cascade** (Fig. 3.2).

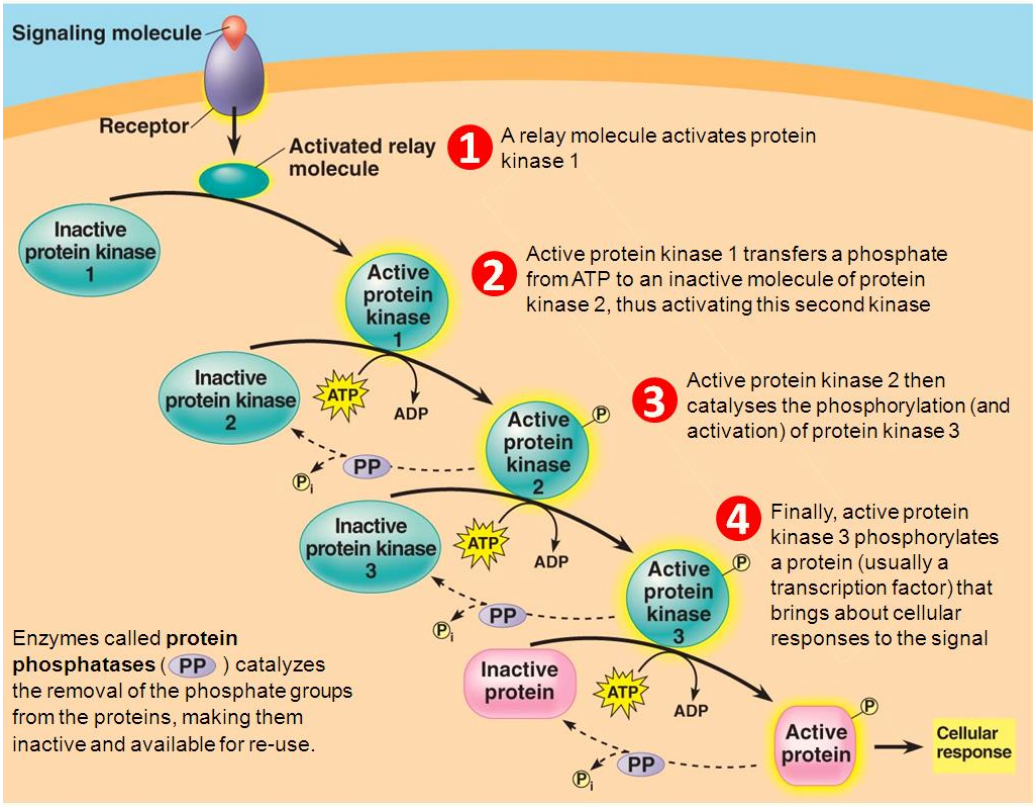


Fig. 3.2: A phosphorylation cascade

★• A **phosphorylation cascade** is **sequence of phosphorylation reactions** where the activated relay protein phosphorylates and activates a protein kinase which in turn **phosphorylates** and **activates the subsequent protein kinase** leading to an amplified and divergent cellular response.

★• Conversely, **protein phosphatases remove phosphate groups from proteins** thus **inactivating protein kinases,** in the absence of the extracellular signal. This consequently turns off the signal transduction pathway.

3.3 Transduction by Second Messengers

a) Nature of second messengers

- Are **small**, **non-protein molecules or ions** which may be:
    - **water-soluble** and rapidly diffuse throughout the cell (e.g. cAMP, Ca^{2+} ions and inositol triphosphate, IP_3),

    OR

    - **lipid-soluble** and diffuse within the plasma membrane (e.g. diacylglycerol, DAG).

b) Roles of second messengers

- Second messengers **relay** information from the receptor-ligand complex **to other proteins** in the transduction pathway.
    - They **bind** to and **alter** the **conformation** of **other proteins**, hence, elicit a response to the signal.
- Second messengers participate in pathways initiated by both **G protein-linked receptors** and **tyrosine kinase receptors**.
- They are **short-lived** because they are eventually **removed or sequestrated** from the cytosol.

  Examples:
    - Phosphodiesterase converts cAMP to AMP (Fig. 3.3a), thus reducing their concentration in the cytosol.
    - Cytosolic Ca^{2+} is pumped back into the ER or out of the cell (Fig. 3.3b), thus sequestrating them from the cytosol.

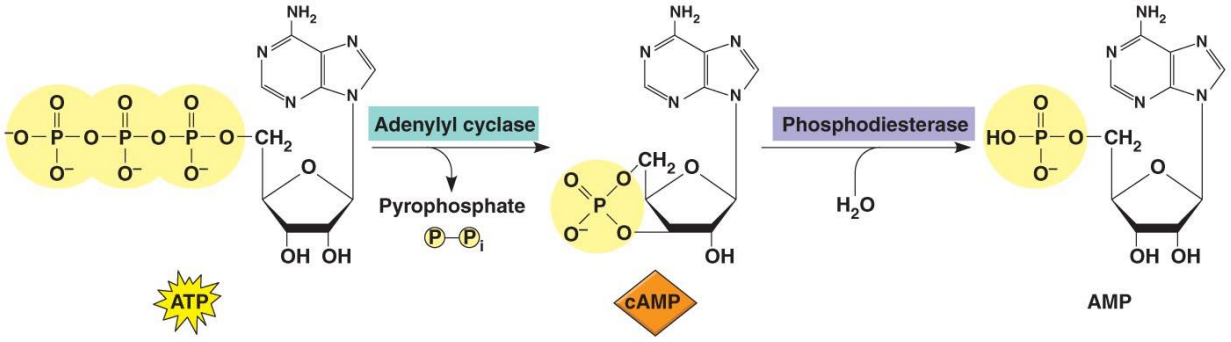


Fig. 3.3a: Upon signal reception, membrane-anchored adenylyl cyclase converts ATP to cAMP, which acts as a second messenger for downstream signaling. cAMP is eventually converted to AMP by cAMP phosphodiesterase.

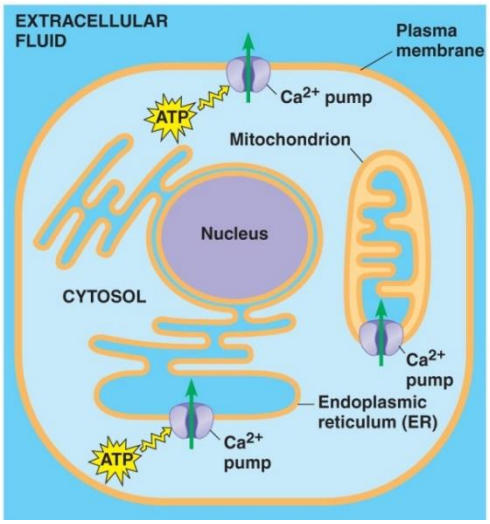


Fig. 3.3b: Calcium ion sequestration from the cytosol.

c) Examples of second messengers

1. Cyclic AMP (cAMP)

- When a signal molecule such as the hormone adrenaline or glucagon **binds to** the **G protein-linked receptors**, G-protein is activated, which in turn, binds to and **activates adenylyl cyclase** in the plasma membrane (Fig. 3.4).
- Activated adenylyl cyclase catalyses the **conversion of ATP to cAMP**.
- The cAMP acts as a **second messenger** and diffuses throughout the cell and **activates protein kinase A** (a cAMP-dependent protein kinase).
- This in turn **phosphorylates other proteins** which **activates transcription of target genes**.
- The cAMP is then rapidly hydrolysed by cAMP phosphodiesterases to form AMP.

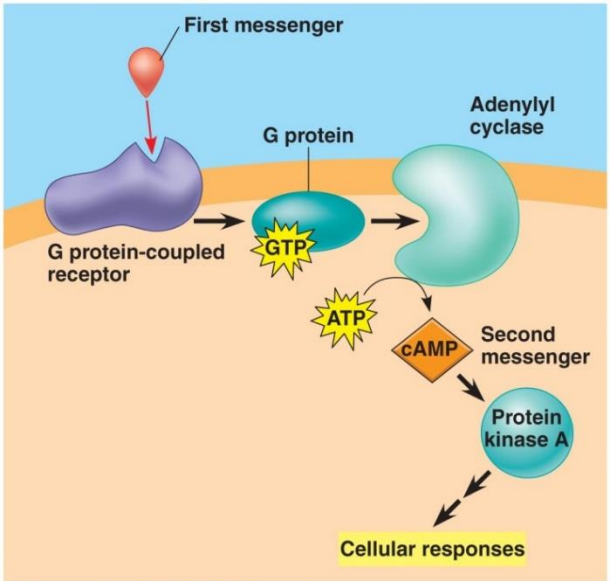


Fig. 3.4: cAMP as the second messenger.

2. Calcium ions *(details not required in syllabus)*

- Normally, Ca^{2+} is **maintained** at a **much lower concentration in the cytosol** than in the extracellular/tissue/interstitial fluid, endoplasmic reticulum, and under certain conditions, the mitochondria and chloroplast.

- This is due to the **active transport of Ca^{2+}** from the cytosol into the extracellular/tissue/interstitial fluid and these organelles by various protein pumps.

- Cytosolic Ca^{2+} can be increased in 2 ways:

    - **cAMP** activates the **opening of plasma membrane Ca^{2+} channels** allowing facilitated diffusion of extracellular Ca^{2+} into the cytosol (e.g. in olfactory sensory neurons).

    - **IP_3** (inositol trisphosphate) activates the **release of intracellular store of Ca^{2+}** in the **endoplasmic reticulum** (Fig. 3.5):

        1. A signal molecule binds to G-protein linked membrane receptors or tyrosine kinase receptors and **activates phospholipase C**.

        2. Phospholipase C in turn **cleaves a membrane phospholipid (PIP_2)** to give two <u>other</u> second messengers, **diacylglycerol (DAG)** and **inositol trisphosphate (IP_3)**.

        3. IP_3 diffuses through the cytosol, binds to, activates and opens **IP_3-gated Ca^{2+} channels** on the **endoplasmic reticulum (ER) membrane**, releasing Ca^{2+} from the ER thereby increasing cytosolic Ca^{2+} concentration.

        4. Ca^{2+} ions also acts in a **positive feedback loop** where increased cytosolic Ca^{2+} activate more IP_3-gated Ca^{2+} channels triggering the release of more Ca^{2+} stores, thus amplifying the response.

        5. DAG and Ca^{2+} ions also participate in the activation of protein kinase C which phosphorylates specific target proteins.

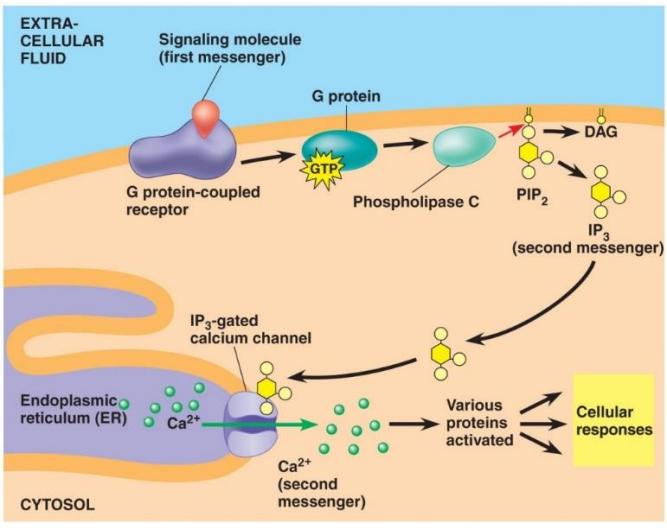


Fig. 3.5: How the calcium ion store in the ER can be released into the cytosol.

- An **increase in cytosolic Ca^{2+}** concentration usually **activates a wide variety of enzymatic processes** e.g. smooth muscle contraction, exocytosis and glycogen metabolism.

- Ca^{2+} ions are eventually pumped out of the cell into the extracellular fluid or back into the ER to **terminate the initial Ca^{2+}** response and restore the low cytosolic Ca^{2+} concentration.

4. Cellular Responses to Signals

4.1 Nature of Cellular Responses to Signals

Key Concept 4:
The **type(s) of cellular responses** (e.g. cell metabolism and protein synthesis) triggered depends on (a) **the signal** and the **type of receptor** that it binds to (signal reception) and (b) the **subsequent enzyme/molecules activated** (signal transduction).

a) Cells respond to signals by
- by **regulating protein and enzyme activities** in the **cytoplasm**, hence **changing** its **metabolism**;
- **gene expression** through **transcription factors in** the **nucleus** (Fig. 4.1)

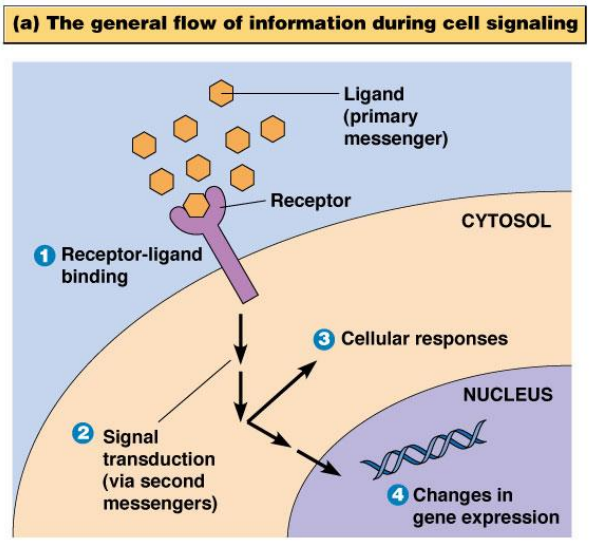


Fig. 4.1: Cytoplasmic or transcriptional responses to signals

b) Various **types of cells** may **receive the same signal** but **respond very differently** (Fig. 4.2).

- The response of a target cell to a signal depends on the particular **collection** of **receptor proteins**, **relay proteins**, and **other proteins** that are present to carry out the response.

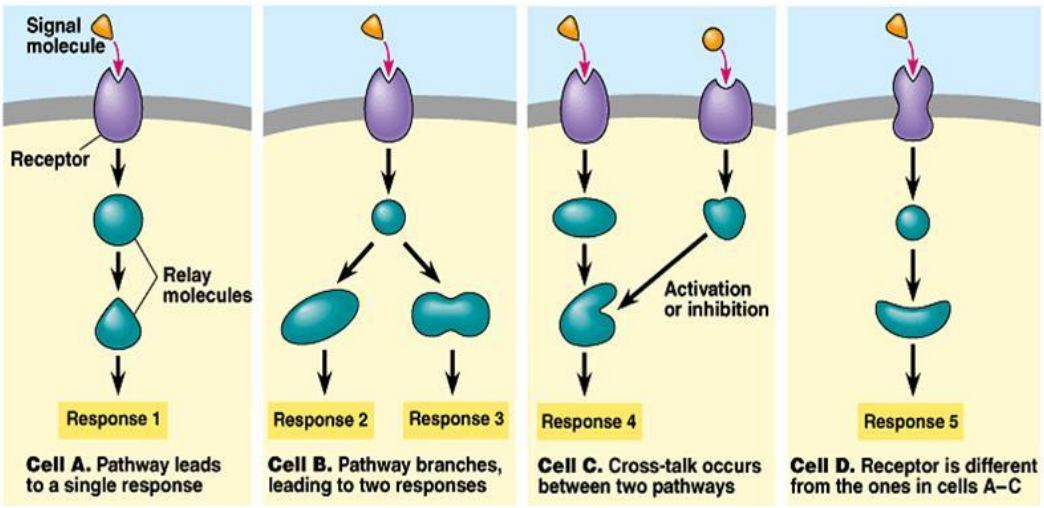


Fig. 4.2: How a signal molecule can give rise to different cellular response in different cell types.

- Examples:
    - **Adrenaline** triggers **liver** or **skeletal muscle cells** to **break down glycogen**, but stimulate **cardiac muscle cells** to **contract**, leading to a rapid heartbeat.
    - **Acetylcholine** decreases rate and force of contraction in cardiac muscles but stimulates contraction of skeletal muscle & secretion of enzymes by salivary gland.

c) **Same cell** with **different receptors** can receive **different signals** to trigger a **coordinated cellular response**. Branching of pathways and interactions ("cross-talk") between pathways (Fig. 4.2, Cell C) **allow fine-tuned regulation and coordination of a cell's response to incoming information from different sources**.

d) The **efficiency** of signal transduction may be **increased** by the presence of **scaffold proteins** (Fig. 4.3).

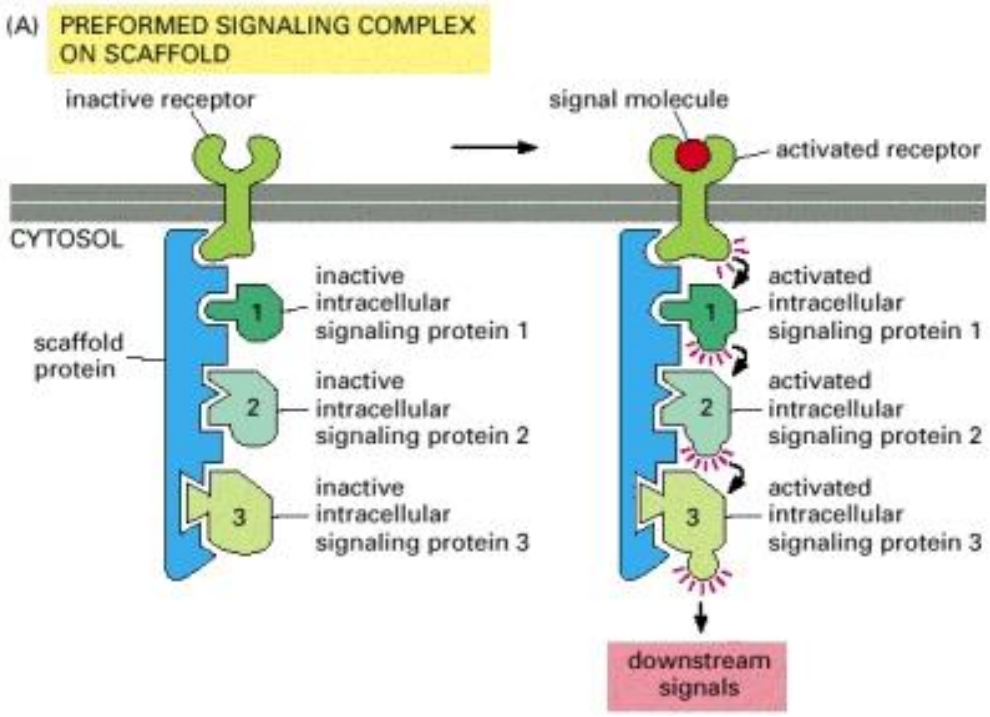


Fig. 4.3: The presence of a scaffold protein facilitates the signaling cascade.

- Scaffold proteins are large relay proteins to which several other relay proteins (usually protein kinases) are simultaneously attached.
- Scaffolding proteins facilitate the activation of specific phosphorylation cascade(s) thereby **enhancing** the **speed** and **accuracy** of signal transfer between intracellular proteins.
- Some scaffolding proteins also participate in more than one pathway in different cell types or at different times in the same cell thus forming a complex signaling network.

4.2 Termination of Signal

- The purpose of signal termination is to ensure **cell's continued responsiveness to incoming signals**.

- Cells can terminate signals by acting on the **receptors** or the **relay proteins** along the pathways. Examples include (Fig. 4.4):

    - **Receptor sequestration** – the receptor can be internalized into the cell via **endocytosis**. This reduces the number of available receptors on the cell surface for the ligands to bind to. The sequestrated receptor could be **recycled** to the cell surface for use.

    - **Receptor down-regulation** – Internalized receptor is sent to the **lysosomes** for **degradation**.

    - **Receptor inactivation** – The receptor can be inactivated via **chemical modification** to its intracellular tail. The receptor is hence unable to activate other intracellular signaling proteins.

    - **Inactivation of signaling protein** – The intracellular signaling protein can be inactivated via **chemical modification**. It is hence unable to activate other intracellular signaling proteins.

    - When the **signal molecule detaches** from the **receptor**, the receptor and relay molecules revert back to their inactive form:

        1. **GTPase** hydrolyses its bound **GTP to GDP**.
        2. **cAMP phosphodiesterase** converts **cAMP to AMP**.
        3. **Protein phosphatases** removes phosphate groups from the phosphorylated kinases and other proteins, thus, inactivating these protein molecules.

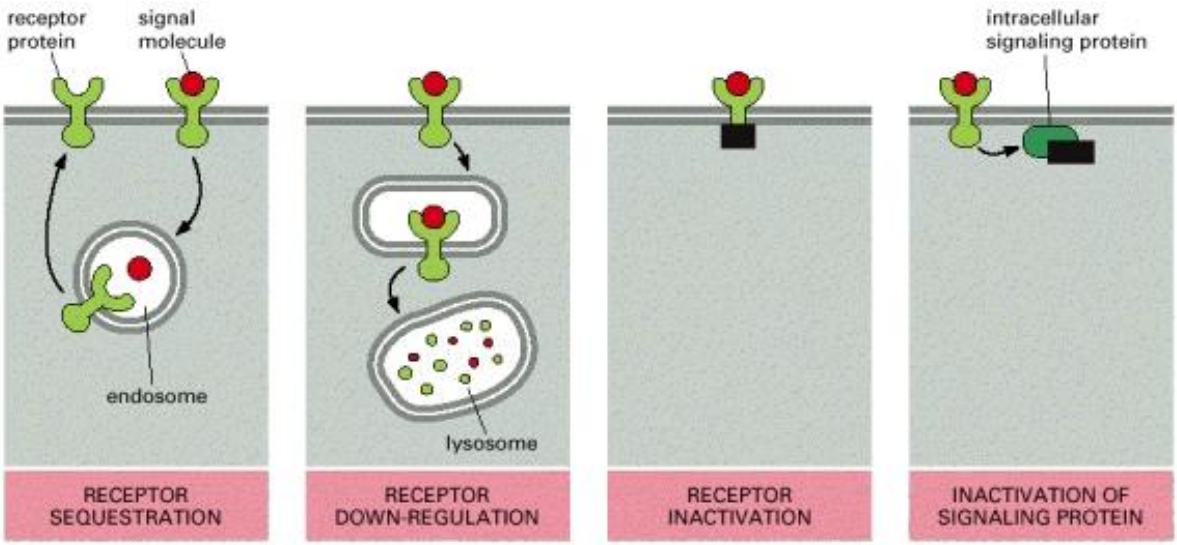


Fig. 4.4: Some ways in which target cells can become desensitized to an extracellular signal molecule.

5. Advantages and Significance of Cell Signaling Systems in Multicellular Organisms

1. **Signal amplification** (Fig. 3.1) – A very small amount of signal is sufficient to give a large response because each catalytic step in a cascade produces a larger number of activated products than in the preceding step.

2. **Regulated and controlled cellular response** – The multi-step signaling cascade enables a greater fine-tuning and control as each enzyme-catalyzed step can be controlled independently of the preceding steps, ensuring an appropriate cellular response**.** In addition, signal termination (Section 4.2) ensures that ligand reception will not lead to unintended prolonged cellular responses.

3. **Specificity** – Each ligand is specific only to certain receptor(s) found on certain cell type(s). This ensures that only the appropriate cells are being targeted. For example, the hormone erythropoietin (Epo) is only specific to erythropoietin receptor (EpoR) found on bone marrow stem cells, which then differentiate to give erythrocytes.

4. **Reception on the cell surface membrane can lead to activation of genes in the nucleus** – Many ligands are hydrophilic in nature. Cell signaling system ensures that these ligands are able to activate gene transcription even though they are unable to traverse through the hydrophobic core of the cell membrane into the cell.

5. **A single signal molecule can trigger numerous cellular reactions at once** – At certain point, the signaling pathway may branch off (Fig. 4.2, Cell B) such that different kinds of intracellular signaling proteins are activated. These activated proteins then activate the respective sets of genes, leading to different cellular response.

6. **Coordinated cellular response** – Coordinated responses can be initiated within the same cell responding to different ligands at the same time due to interactions ("cross-talk") between the different pathways activated by the different receptors (Fig. 4.2, Cell C).

7. **A single type of signal molecule can activate many *different cell types* simultaneously** to **trigger numerous different cellular reactions** at once

    - e.g. Adrenaline triggers liver or striated muscle cells to break down glycogen, but stimulate cardiac muscle cells to contract, leading to a rapid heartbeat

6. Achieving Equilibrium in the Internal Environment

6.1 Overview

- For **optimal** function, the internal environment of an organism needs to be **maintained constant** (within narrow limits) despite changes in the external environment.

- The internal environment refers to the **intracellular (inside the cell) and extracellular (in the tissue fluid surrounding the cell) environment** of the body.

- Some important parameters in the internal environment include

    - **Temperature**
        - Low temperatures inactivate enzymes, hence, slowing metabolic reactions.
        - High temperatures denature proteins and enzymes.

    - **pH**
        - The pH of blood and tissue fluid, as well as the pH of cytosol, has to be kept within narrow limits to prevent denaturation of enzymes and proteins.

    - **Concentration of glucose**
        - Glucose is the primary respiratory substrate. A lack of it causes respiration to slow down or stop.
        - Excess blood glucose (a solute) may cause water to leave cells into the blood by osmosis. Excessive retention of water in the blood can cause high blood pressure.

    - **Volume of water in the blood**
        - Lack of water in the tissue fluid causes water to leave the cells by osmosis, causing metabolic reactions to slow down or to stop.
        - Too much water entering the cell may cause dilution of metabolites.

- Thus, maintaining an equilibrium in the internal environment is important to :

    - ensure that enzymes and metabolic processes can function **optimally** for organisms to live and reproduce.
    - ensure that **limits are not exceeded**, hence, preventing dangerous fluctuations.
    - provide the organism with a degree of **independence of the external environment**.

- The roles of signal molecules in maintaining this equilibrium is illustrated in Sections 6.2 and 6.3. The signal molecules are a pair of protein hormones, insulin and glucagon, which act antagonistically to maintain blood glucose concentration within the tight limit of 70-110 mg glucose /100ml blood. The normal level or set point of blood glucose is 90 mg /100ml blood.

6.2 Case Study: Regulation of Blood Glucose Concentration by Insulin Signaling

(A) <u>Regulation of blood glucose level by Insulin Signaling in Liver and Muscle Cells</u> (Fig. 6.1)

- When blood glucose level **rises above the set point** of 90mg glucose /100m of blood (e.g. after a meal), insulin is released by β **cells of the Islets of Langerhans of pancreas** to bring about responses in liver cells, muscle cells and other respiring cells that would **<u>lower blood glucose</u> level back to the set point**:

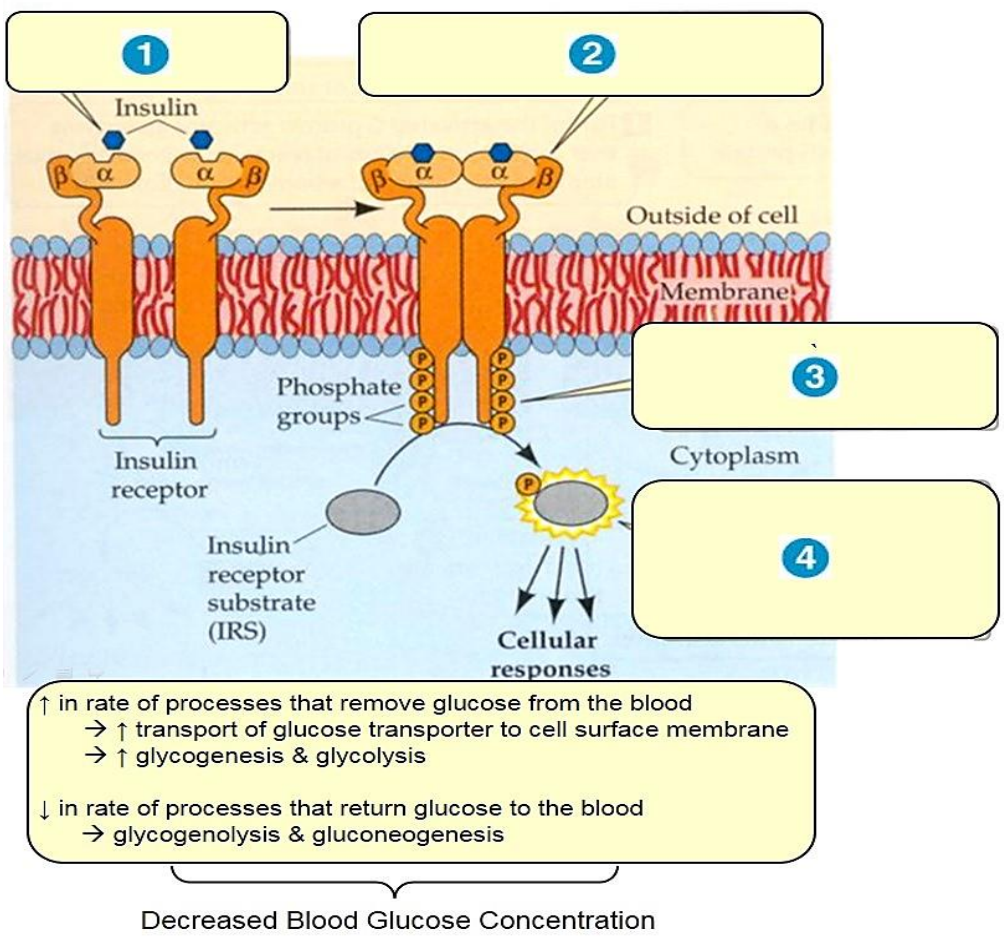


Fig. 6.1a: Insulin receptor signaling

1. **Two insulin molecules bind** to the **complementary** α-subunits of **2 insulin receptors** on the cell surface membrane of liver and muscle cells.

   This binding triggers a **conformation change** in the receptors, and **the 2 receptors dimerized**.

2. The **activated dimer** causes the β-subunit of the insulin receptor to **transmit a signal to the catalytic tyrosine kinase tail** of each receptor protein.

3. The tyrosine kinase **cross-phosphorylates each other** on multiple **tyrosine residue**s, fully activating the insulin receptor.

   The **fully activated insulin receptor** then **activates the insulin receptor substrate (IRS).**

4. Activated IRS in turn, trigger a variety of relay proteins (Fig. 6.1b), resulting in various **cellular responses**:

    - ↑ in rate of processes that remove glucose from the blood →
        - ↑ transport of glucose transporterS to cell surface membrane to increase glucose uptake into the cell
        - ↑ glycogenesis (synthesis of glycogen from glucose for storage)
        - ↑ glycolysis (oxidation of glucose), hence increased ATP synthesis
        - ↑ fatty acid synthesis

    - ↓ in rate of processes that return glucose to the blood →
        - ↓ glycogenolysis (hydrolysis of glycogen to glucose)
        - ↓ gluconeogenesis (conversion of non-carbohydrate sources to glucose)

- These responses **decrease blood glucose concentration back to the set-point.**

Fig. 6.1b shows a detailed example of a insulin receptor signalling pathway.

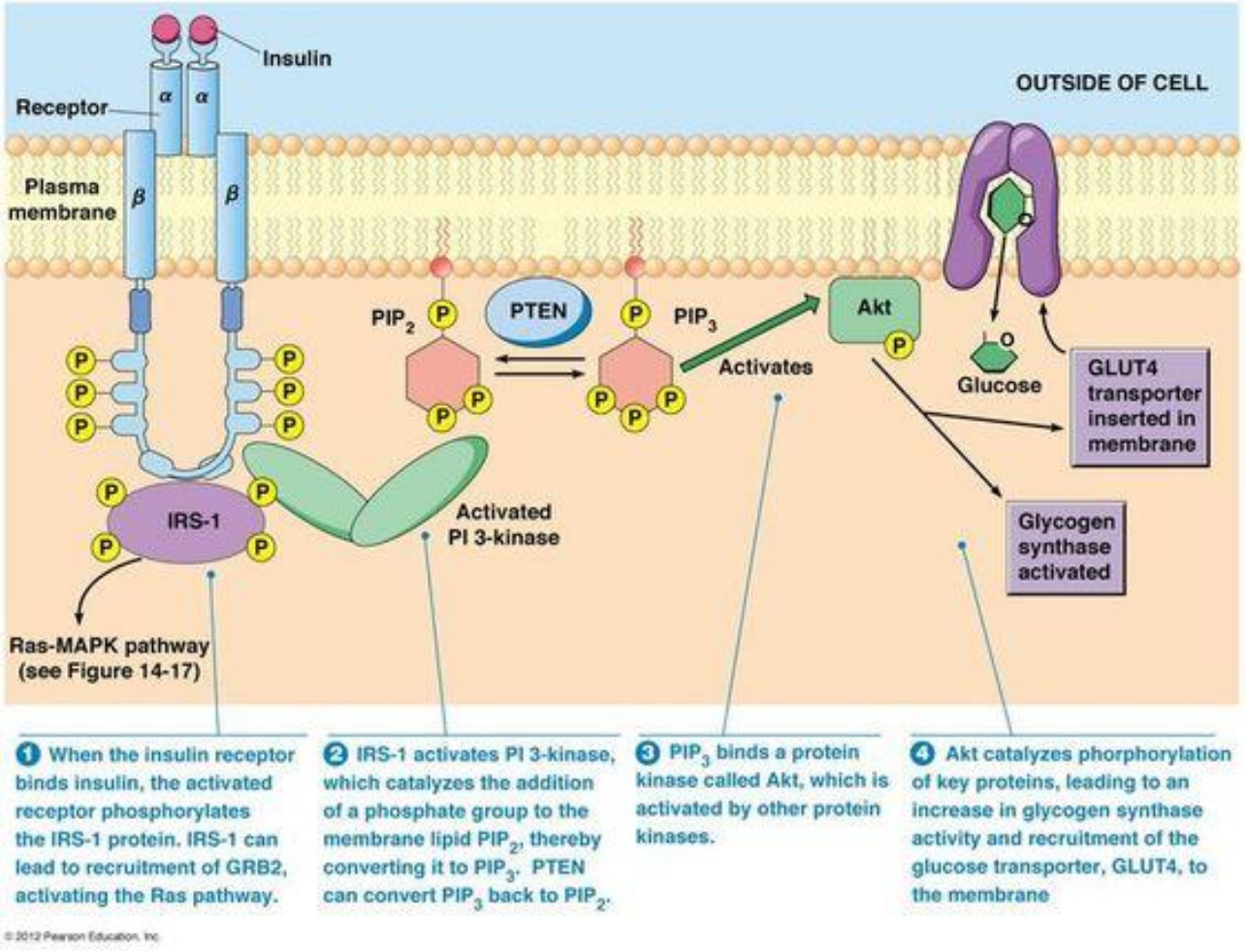


Fig. 6.1b: Detailed example of an insulin receptor signaling pathway

(B) <u>Regulation of blood glucose level by Glucagon Signaling In Liver Cells</u> (Fig. 6.2 and 6.3)

- When blood glucose level **falls below the set point** (e.g. during fasting), glucagon is released by α **cells of the Islets of Langerhans of pancreas** to bring about responses in **liver** cells (but NOT muscle cells).

- In the liver cell, **glucagon activates different transduction pathways** to bring about the same set of cellular responses that release glucose back to the blood to **restore the set point**.

Transduction pathway via cAMP as second messenger (Fig. 6.2)

1. **Glucagon binds** to **complementary G protein-linked receptor**, **activating the receptor**. Activated receptor binds to an **inactive G protein** in membrane.

2. The binding causes the G protein to **replace GDP** with **GTP** and **becomes activated.** The (α-subunit of) **activated** G protein then **diffuses** along the membrane and **bind** to **adenylyl cyclase**.

3. **Adenylyl cyclase becomes activated** and catalyses the **synthesis of cAMP from ATP**.

4. **Increased cytosolic concentration of cAMP activates protein kinase A (PKA)**,

5. PKA in turn, **phosphorylates** and **activate other proteins**, which results in
   a. ↑ gluconeogenesis and glycogenolysis,
   b. ↓ of glycolysis and glycogenesis.
   c. Eventually, resulting in increased blood glucose concentration back to the set-point.

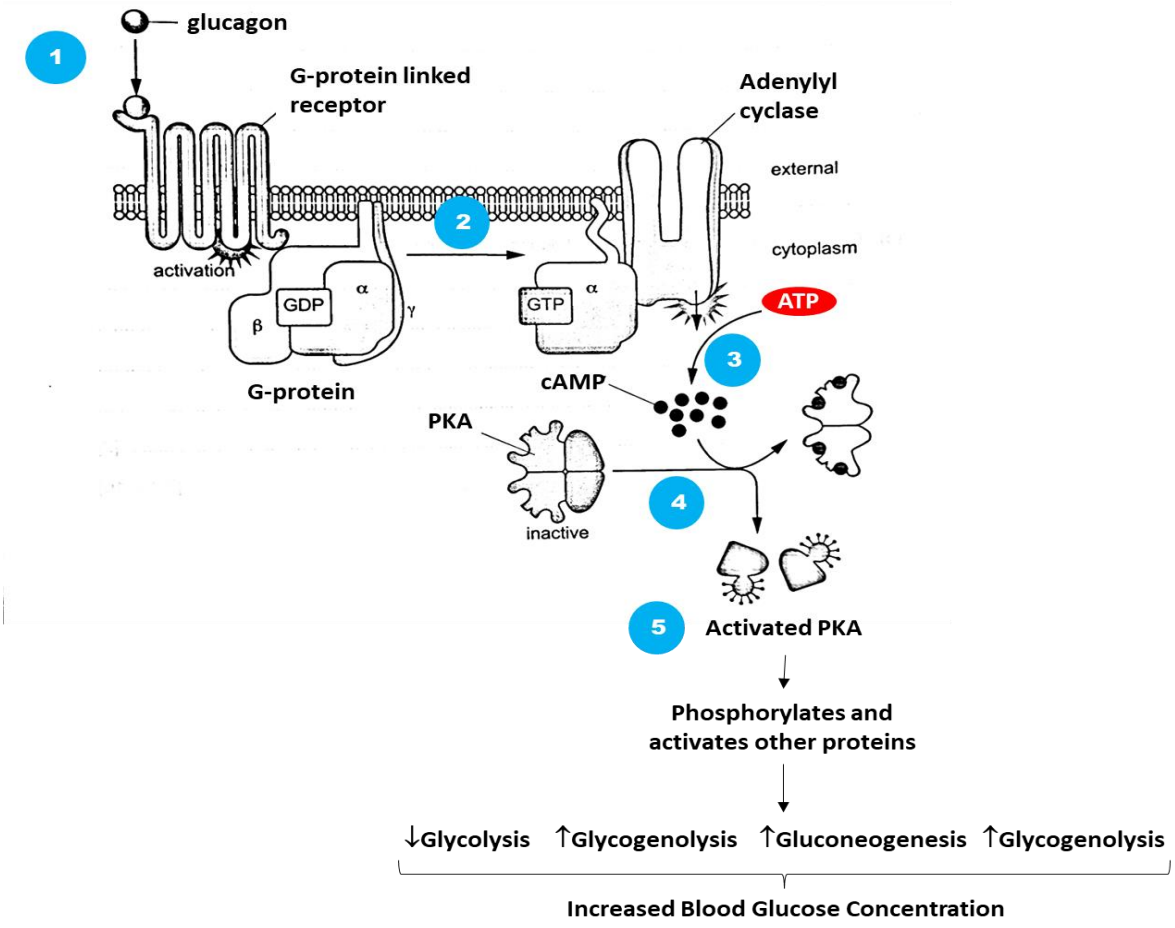


Fig. 6.2: Glucagon signaling pathway involving cAMP as second messenger.

Transduction pathway via Calcium Ions as second messenger (Fig. 6.3) *(details not required)*

1. Upon **binding of glucagon** to the G protein-linked receptor (G protein-linked glucagon receptor), the receptor becomes activated and binds to an inactive G protein in membrane.

2. This activates **another G-protein** which in turn **activates phospholipase C**.

3. Phospholipase C in turn **cleaves** a membrane phospholipid (**PIP_2**) into two <u>other</u> second messengers:

   - **Diacylglycerol (DAG)** – activates protein kinase C (PKC)
   - **Inositol trisphosphate (IP_3)** – binds to and opens the calcium channel on the ER, thus increasing cytosolic Ca^{2+} concentration

4. Increased cytosolic Ca^{2+} concentration and activated PKC increases gluconeogenesis and glycogenolysis and inhibits glycolysis and glycogenesis.

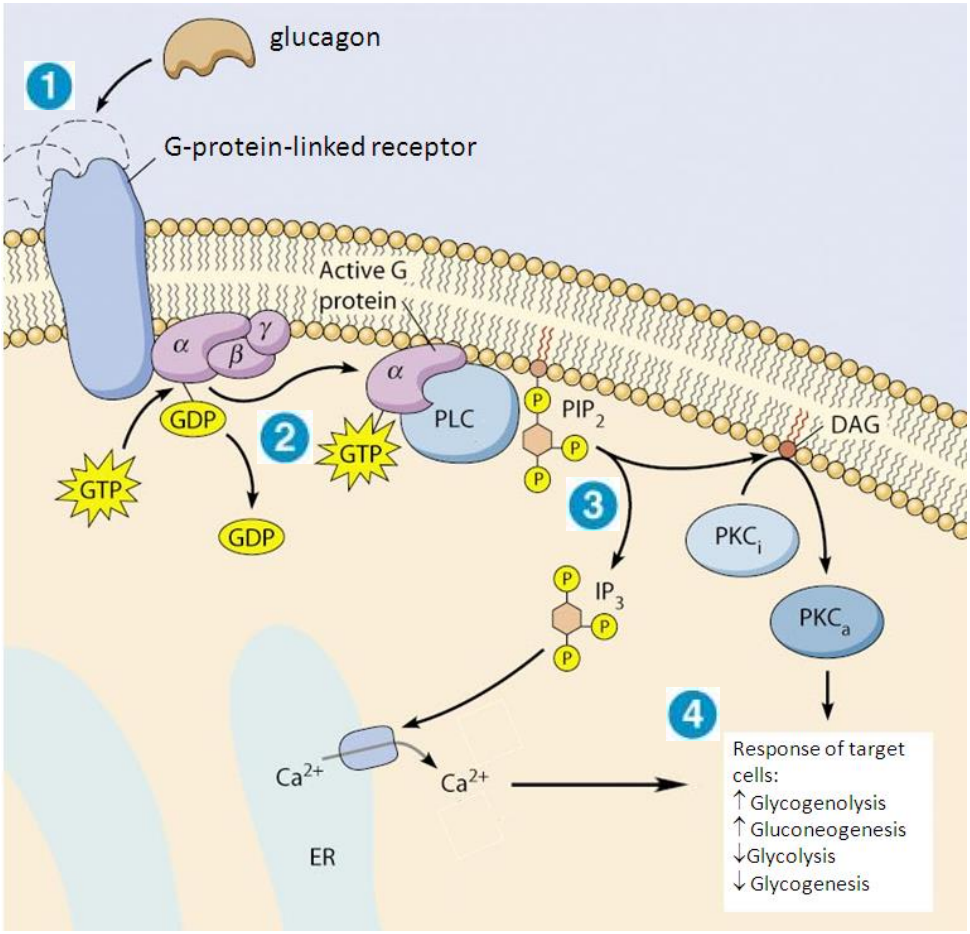


Fig. 6.3: Glucagon signaling involving IP_3, DAG and Ca^{2+} as the second messengers. *(details not required by syllabus)*

Summary Map on Control of Blood Glucose Level by Insulin & Glucagon

3 Main Stages of Cell Signalling:	**By Insulin**	**By Glucagon**
	An **increase** in **blood sugar level above set point** (90mg/100ml of blood)	A **decrease** in **blood sugar level below set point** (90mg/100ml of blood)
	↓	↓
	Is detected by the **beta cells** of **islets of Langerhans** of pancreas	Is detected by the **alpha cells** of **islets of Langerhans** of pancreas
	↓	↓
	Which secretes **insulin (1st messenger)**	Which secretes **glucagon (1st messenger)**
	↓	↓
1. Signal Reception	2 insulin molecules bind to the complementary **receptor tyrosine kinase (RTK)** on the **liver (or muscle)** cell	Glucagon binds to complementary **G-protein linked receptor (GPLR)** on **liver cell**
	↓	↓
	This binding causes RTK to undergo **changes in conformation** and form **a dimer**	This binding causes GPLR to undergo **changes in conformation.**
	↓	↓
	The dimer activates the **catalytic tyrosine kinase tail** to **cross-phosphorylate** each other on **tyrosine residues**	This conformational change causes the GPLR to be **activated** and binds to an **inactive G protein**
	↓	↓
2. Signal Transduction	The activated receptors in turn activates the downstream signal proteins such as protein kinases	The binding causes the inactive G protein to **replace GDP with GTP** and G protein becomes **activated**.
		↓
		The activated G-protein will then **diffuse along the membrane and bind to adenyl cyclase to activate it**
		↓
		Activated adenyl cyclase will convert **ATP to cyclic AMP (cAMP), the 2nd messenger**, which **activates protein kinase A (PKA)**. Activated PKA in turn **activates target enzymes** by phosphorylating them.
	↓	↓
	Thus a **phosphorylation cascade** is initiated.	Thus a **phosphorylation cascade** is initiated.
3. Cellular Response	Phosphorylation activates protein kinase in a cascade which eventually activates **glycogen synthase** which catalyses glycogen synthesis from glucose (i.e. increase **glycogenesis** in liver and muscle)	**Protein kinase A activates phosphorylase kinase** which activates **glycogen phosphorylase** which catalyses glucose synthesis from glycogen (i.e**. glycogenolysis**)
	Other cellular responses also occur Examples: 1. **Translocation of glucose transporters** from cytoplasmic vesicles to the cell membrane. Hence increased glucose uptake into cells. 2. **Increased rate of glycolysis (i.e. oxidation of glucose)** 3. **Increased lipid & protein synthesis** 4. **Inhibit gluconeogenesis and glycogenolysis**	Other cellular responses also occur. Example: 1. Increased **gluconeogenesis** (synthesis of glucose from non-carbohydrate sources). 2. **Inhibit glycolysis and glycogenesis**
	↓	↓
	Thus **blood glucose levels decreases** to **set point (90mg/100ml of blood)**	Thus **blood glucose level increases**.
	↓	↓
	This is detected by the **beta cells of islets of Langerhans** which then **decreases insulin production**.	This is detected by the **receptor (detector, i.e. alpha cells)** which then **decreases glucagon production**.
4. Signal Termination	Cellular responses can be terminated by acting on the: **(1)** Receptors • Chemical modification to inactivate receptors • Inactivation of signalling proteins • Down regulation via endocytosis / degradation by lysosomes **(2)** Relay proteins • Hydrolysis of bound GTP to GDP by GTPase • Converting cAMP to AMP by cAMP phosphodiesterase • Increase phosphatase activity to inactive relay proteins	

Advantages of multi-step signal cascade:

1. Greatly amplifies the signal
   - Each step in a cascade produces a **larger number of activated products than in the preceding step**
   - Thus, a very **small amount of signal** will give a **large cellular response**
2. Allows for greater fine-tuning of cellular responses

End of Notes