HN

SEASON 2

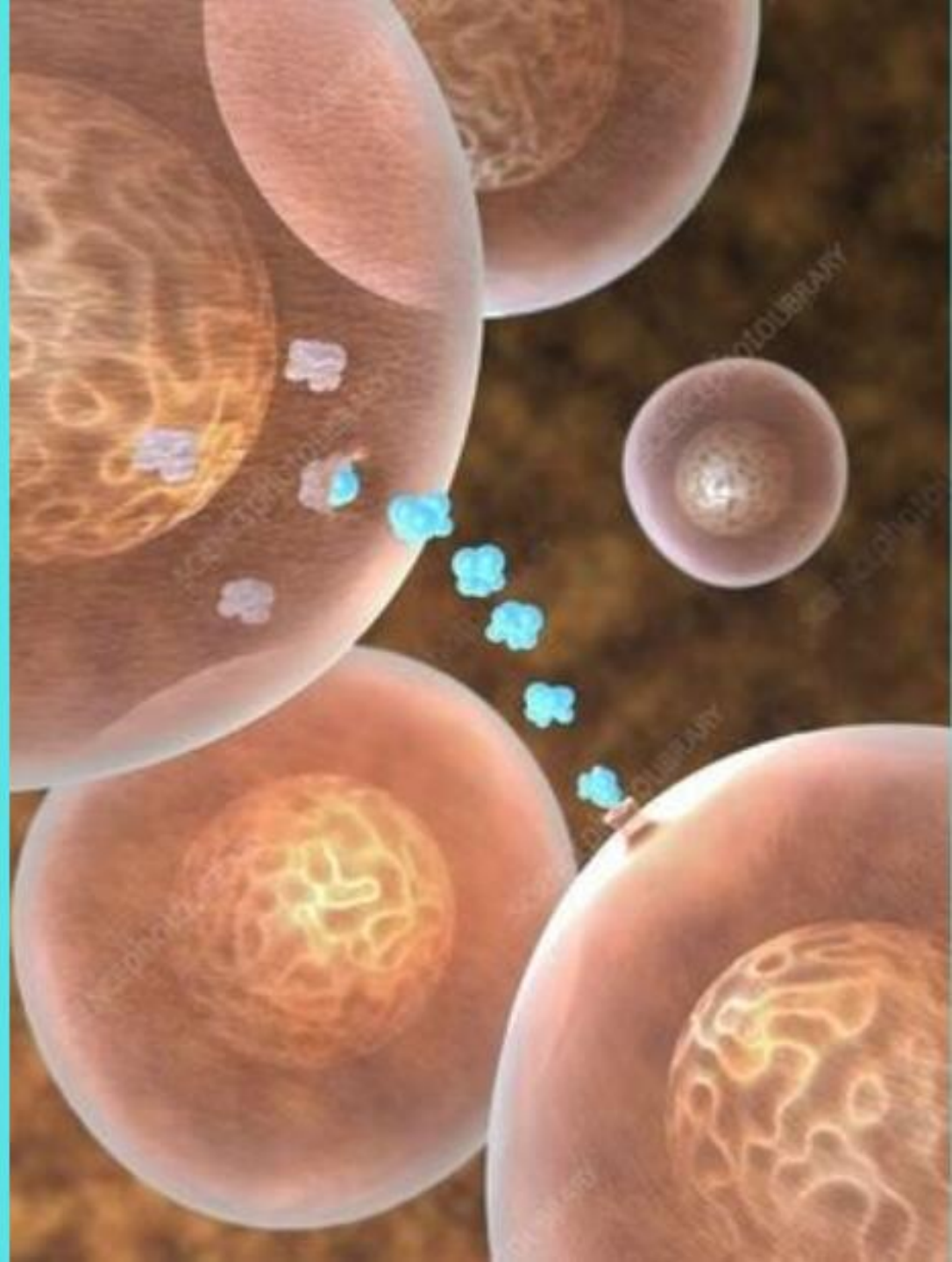
Farah Saeed FS Farah Saeed

# Understanding Cancer

## Lecture 5
## Signal transduction and cellular response

DR HAFSA WASEELA ABBAS

www.hafsaabbas.com



# RECAP:

## *What you hopefully should understand so far from Lecture 4*

- **Receptor activation** is the **first step of cell-to-cell communication (cell signalling).**
- **The ligand** is the **first messenger** that could be a **protein or a steroid** and can **complementary bind with the receptor like a lock and key.**
- Some receptors are found on the **cell surface** and other receptors are found **inside the cells i.e. in the nucleus and cytoplasm.**
- Some ligands are **hydrophilic i.e. proteins and cannot diffuse through the plasma membrane due to their size** and require cell surface receptors. Other ligands are **hydrophobic i.e. steroid hormones can diffuse through plasma membrane** and interact with **intracellular receptors.**
- **The rate of the binding between a ligand and receptor equals the rate of releasing the ligand from the receptor.**

# What will we learn today?

- ***What is signal transduction?***
- ***The importance of protein kinases.***
- ***What are secondary messengers?***
- ***Examples of secondary messengers.***
- ***How do cells respond?***
- ***What are transcription factors?***
- ***Types of Domains in transcription factors***

# GENTLE REMINDER
# An ideal way of learning:

| Monday | Tuesday | Wednesday | Thursday | Friday | Saturday | Sunday |
|---|---|---|---|---|---|---|

Mini-lectures.

Approximate total time: 1 hour

**Divide over 7 days** at your **own pace**.

**Challenge yourself** with a quiz!

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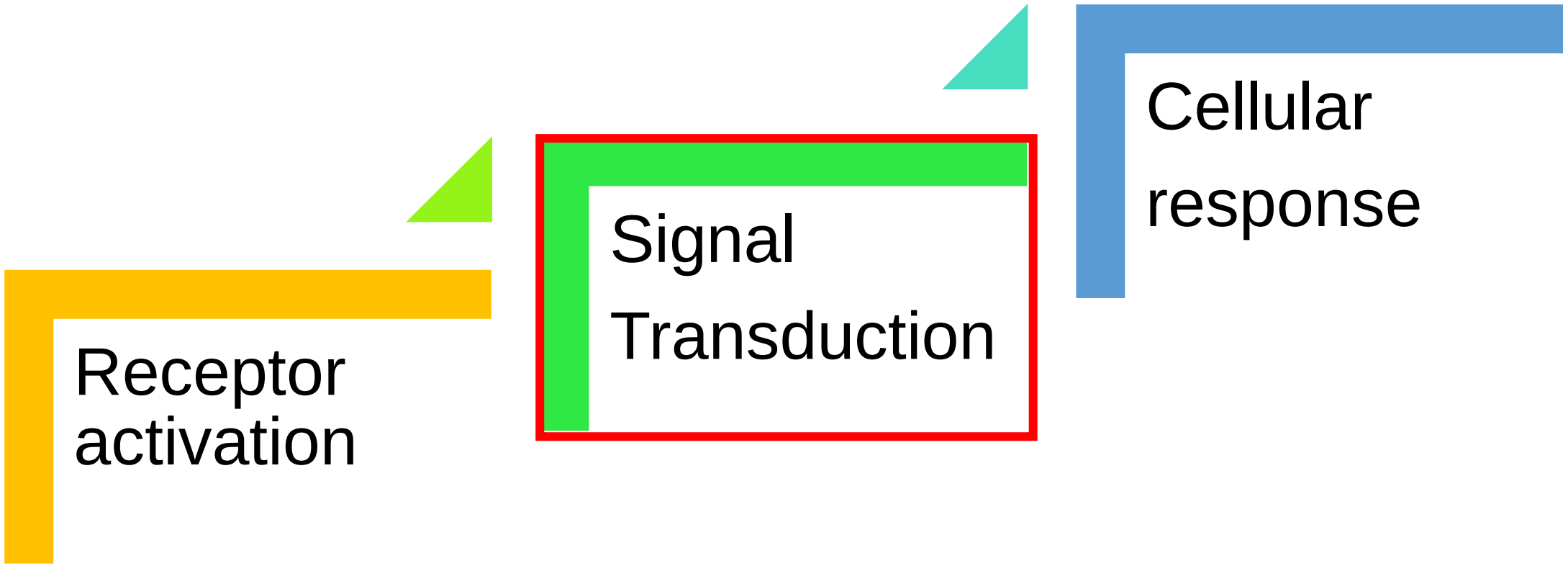
# RECAP: How to support your learning?

- **Key facts with diagrams by HN designs presented in a simplified way.**
- **Glossary to help understand what key words mean.**
- **Summary doodle revision posters by HN designs.**
- **Quizzes to test your knowledge and reflect.**
- **Reference list for further reading.**

**Acknowledgements: Special thanks to my parents, family, friends and colleagues for their support and the respected teachers and health professions who taught me and installed the passion of cancer/oncology.**

# What is signal transduction?

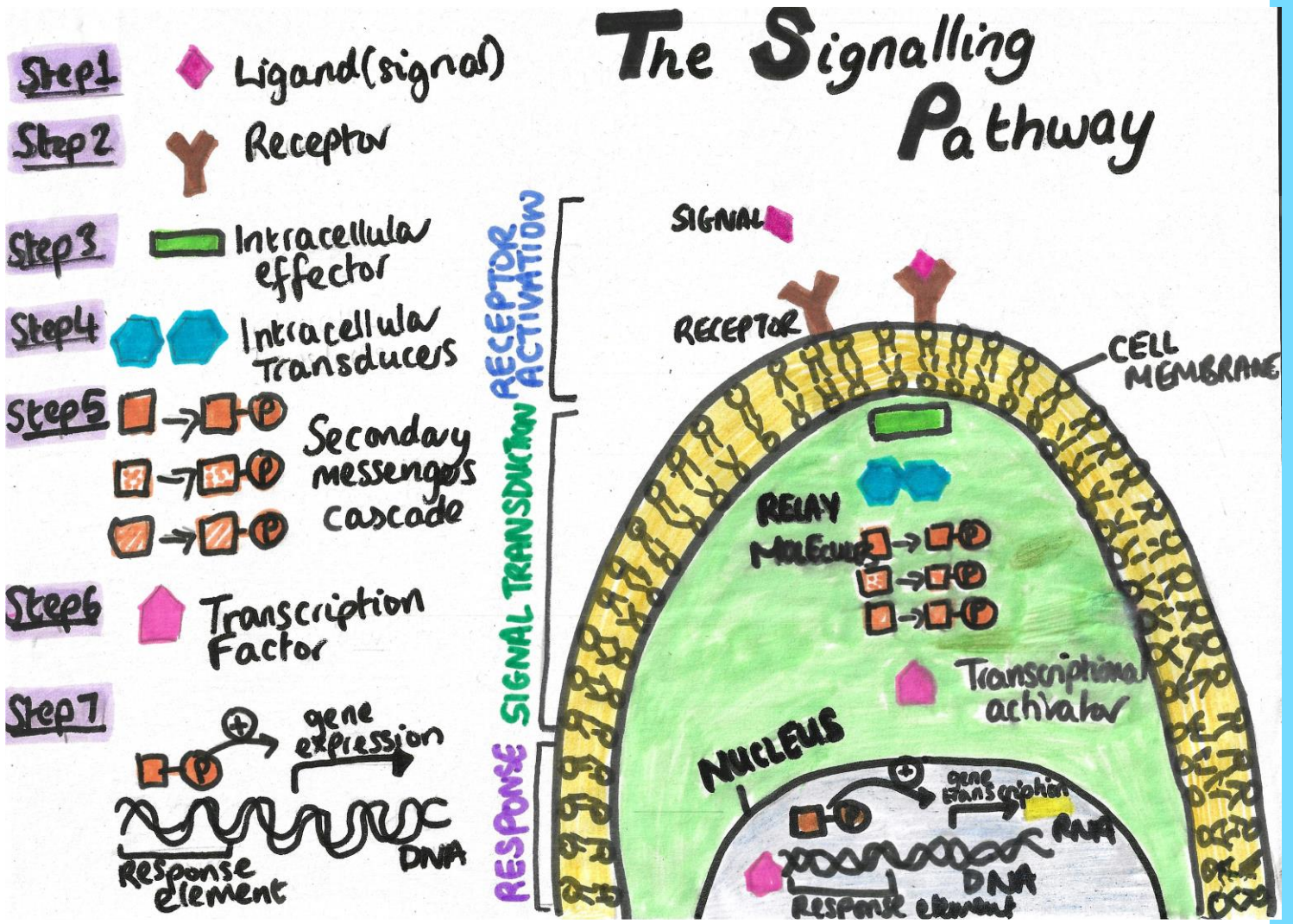



# The three steps in cell communication

# What is signal transduction?

The **signal transmitted through the plasma membrane after the receptor is activated** and **into the cytoplasm**.

This is known as **signal transduction pathway or cascade.**



# The importance of protein kinases.

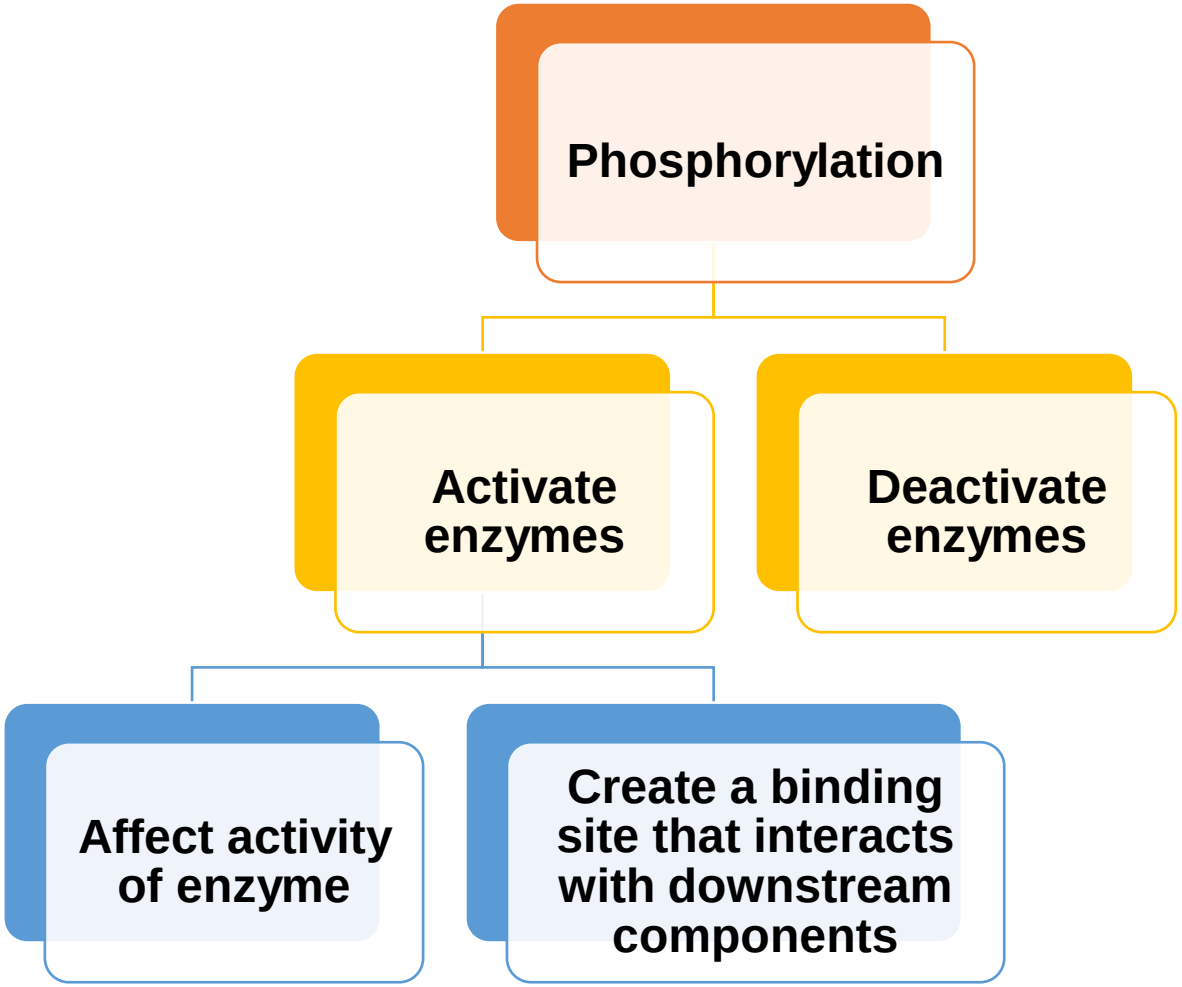
# What are protein kinases?

They are enzymes that help signal transduction via a process called **phosphorylation.**

Phosphorylation is **the addition of a phosphate group.**

**Dephosphorylation of protein kinases occurs** by:

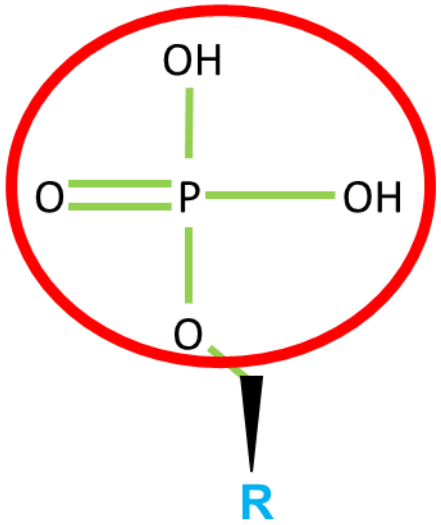
- ❑ **Protein kinase inhibitors**
- ❑ **Phosphatase**



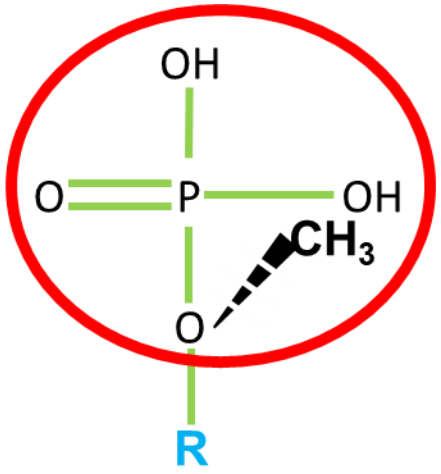
# Example: Phosphorylation of proteins

In each molecule, **a phosphate is attached to an oxygen on the amino acid.**

A **hydroxyl group (OH) is removed to place the phosphate group.**



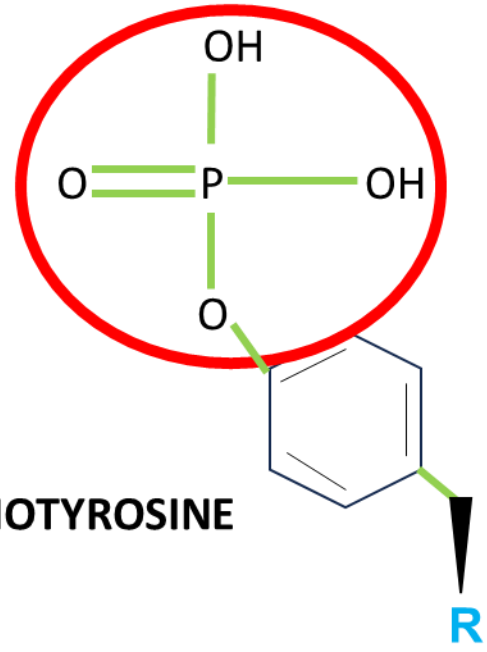

PHOSPHOSERINE




PHOSPHOTHREONINE

Examples of the amino acids are:

- ❑ **Serine**
- ❑ **Threonine**
- ❑ **Tyrosine**




PHOSPHOTYROSINE

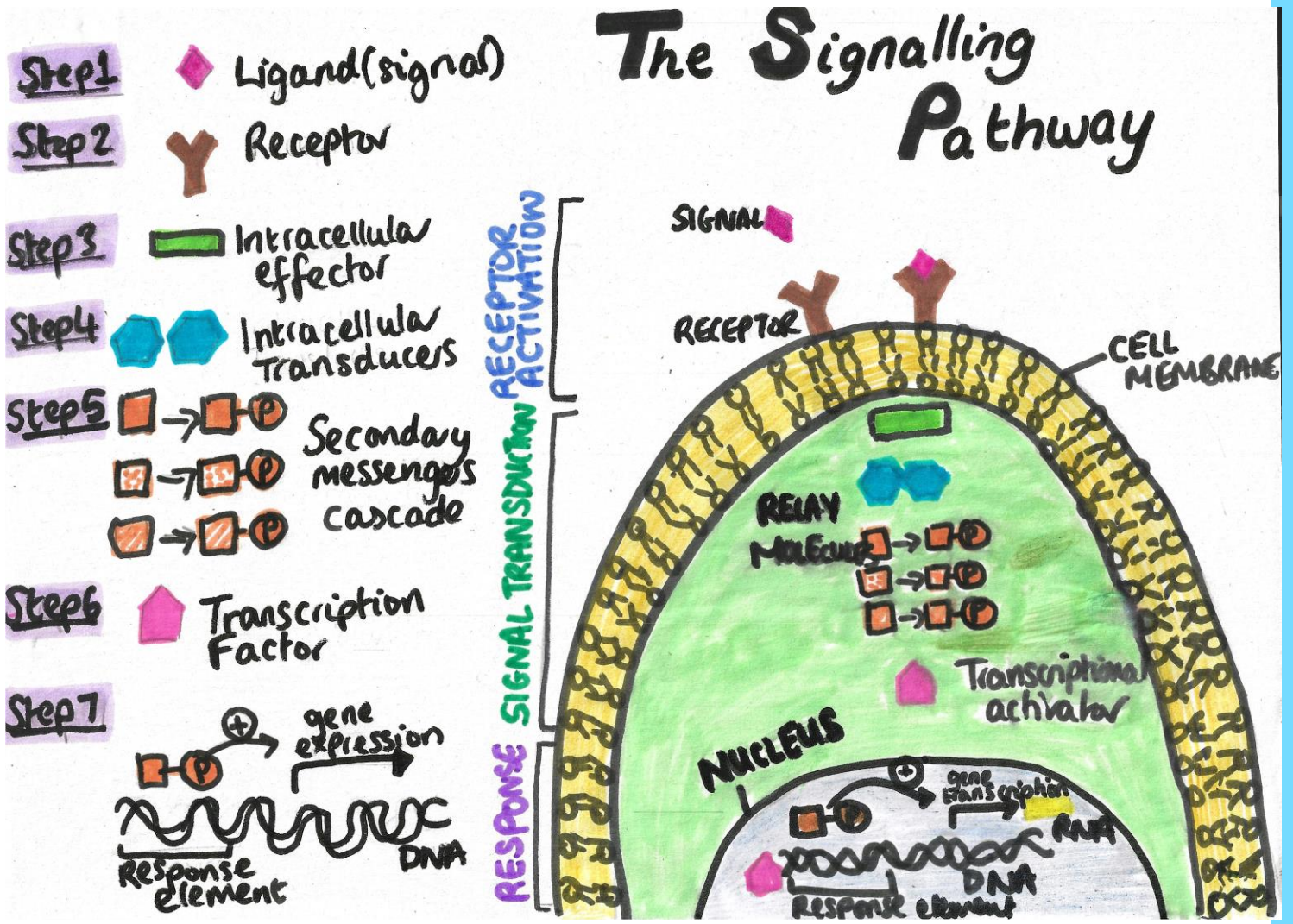
**Please revisit Lecture 4 on how ATP and GTP are formed.**

# What are second messengers?

# RECAP: What are first messengers?

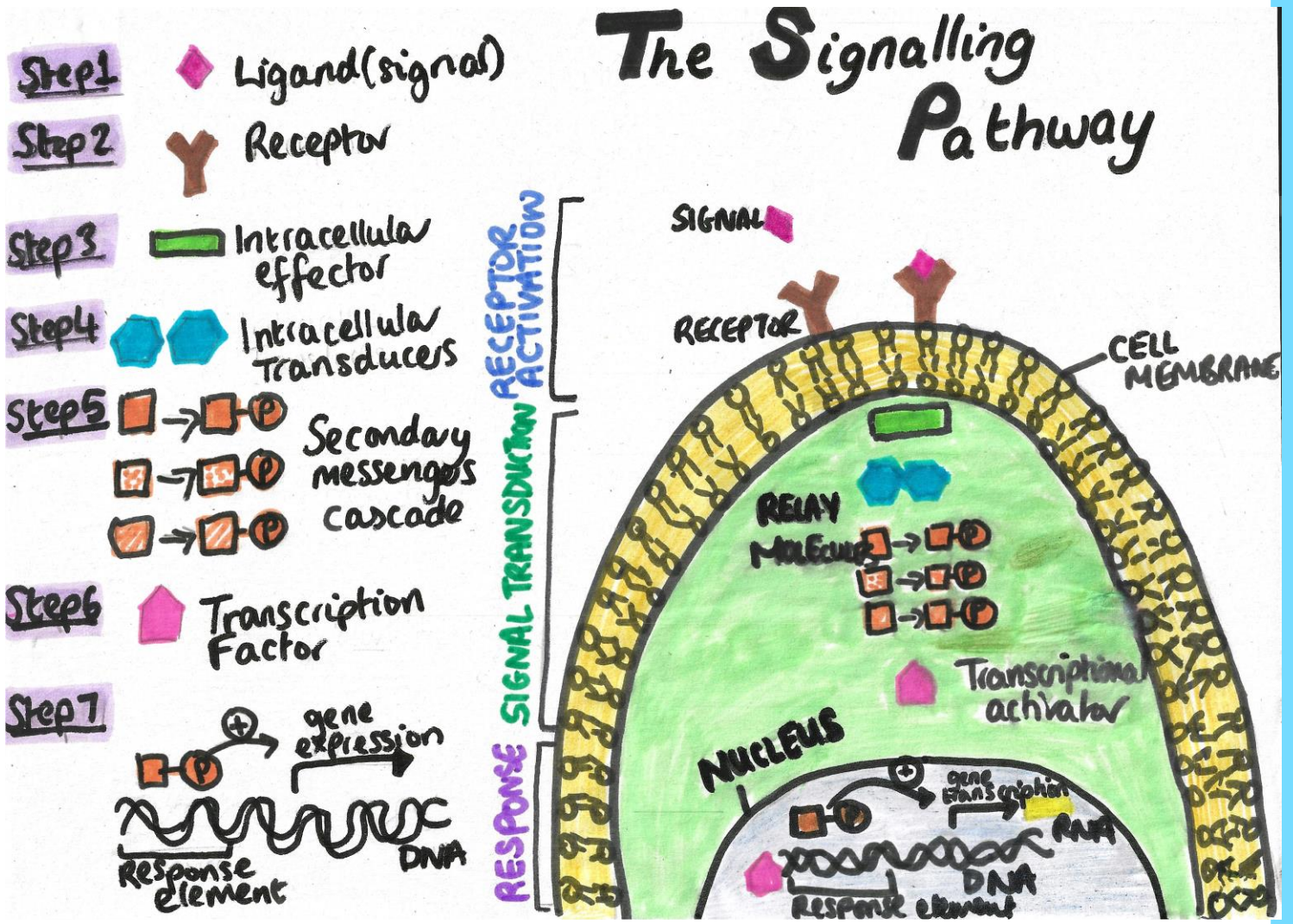
The **ligand or signalling molecule** is considered as the **first messenger:**

- **Peptide hormones bind to a receptor on the cell surface membrane.**
- **Steroid hormones are able to diffuse through the membrane.**



# What are second messengers?

The **second messengers** are **small, non-protein** that is used to **transmit the signal within the cytoplasm** of a cell by **altering or changing the behaviour of particular proteins.**



# Examples of secondary messengers

# Examples of secondary messengers

Calcium ions
($Ca^{2+}$)

Cyclic AMP
(cAMP)

Diacylglerol
(DAG)

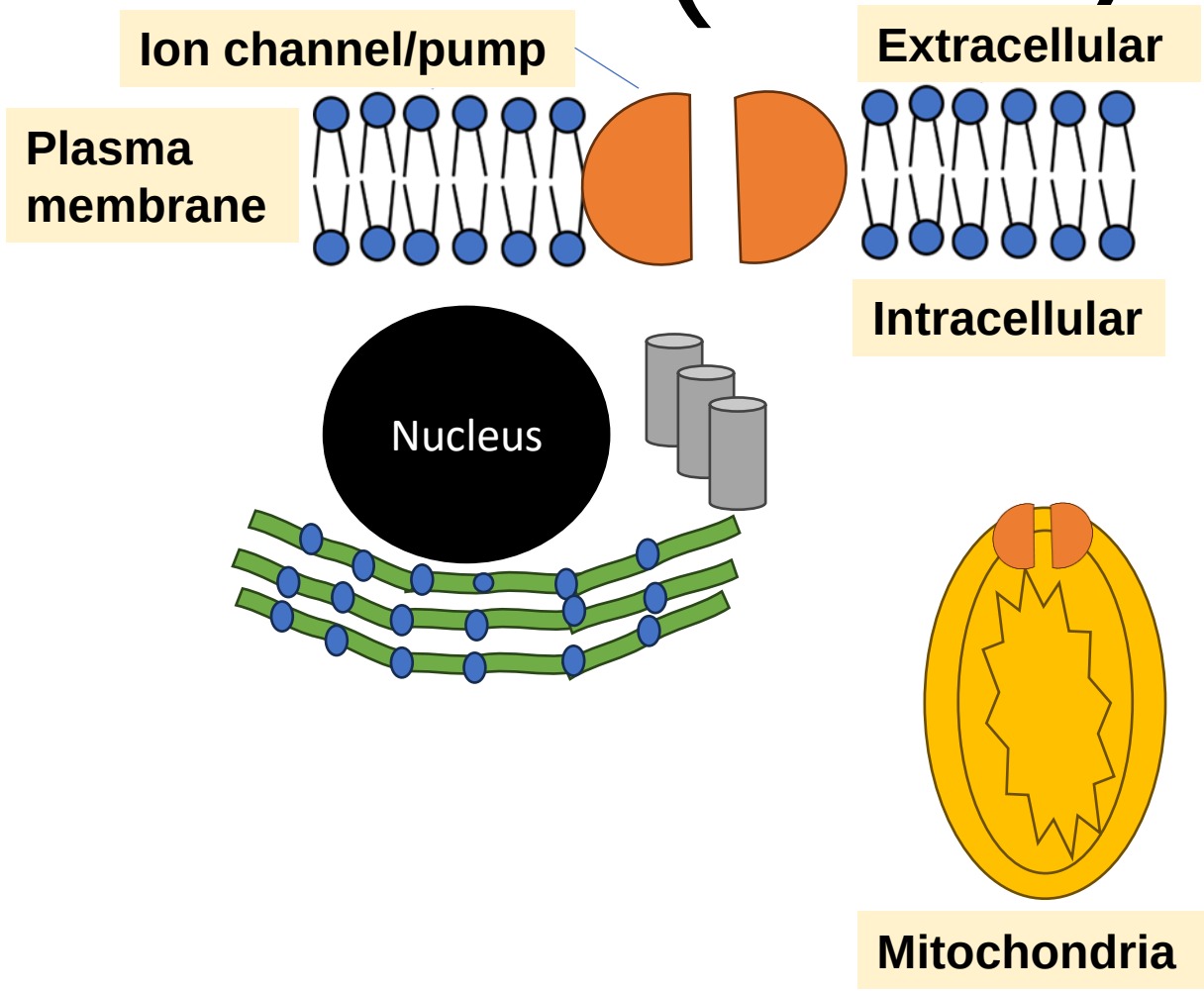
Inositol triphosphate
($IP_3$)

# Example 1: Calcium ions ($Ca^{2+}$)

This is **a common secondary messenger.**

Calcium ions are stored in structures called **vesicles.**

They are useful in signalling pathway of **water-soluble molecules.**




**Low levels of calcium ions in the cytoplasm compared to outside the cell, mitochondria and endoplasmic reticulum. More calcium ions enter organelles when channels open.**

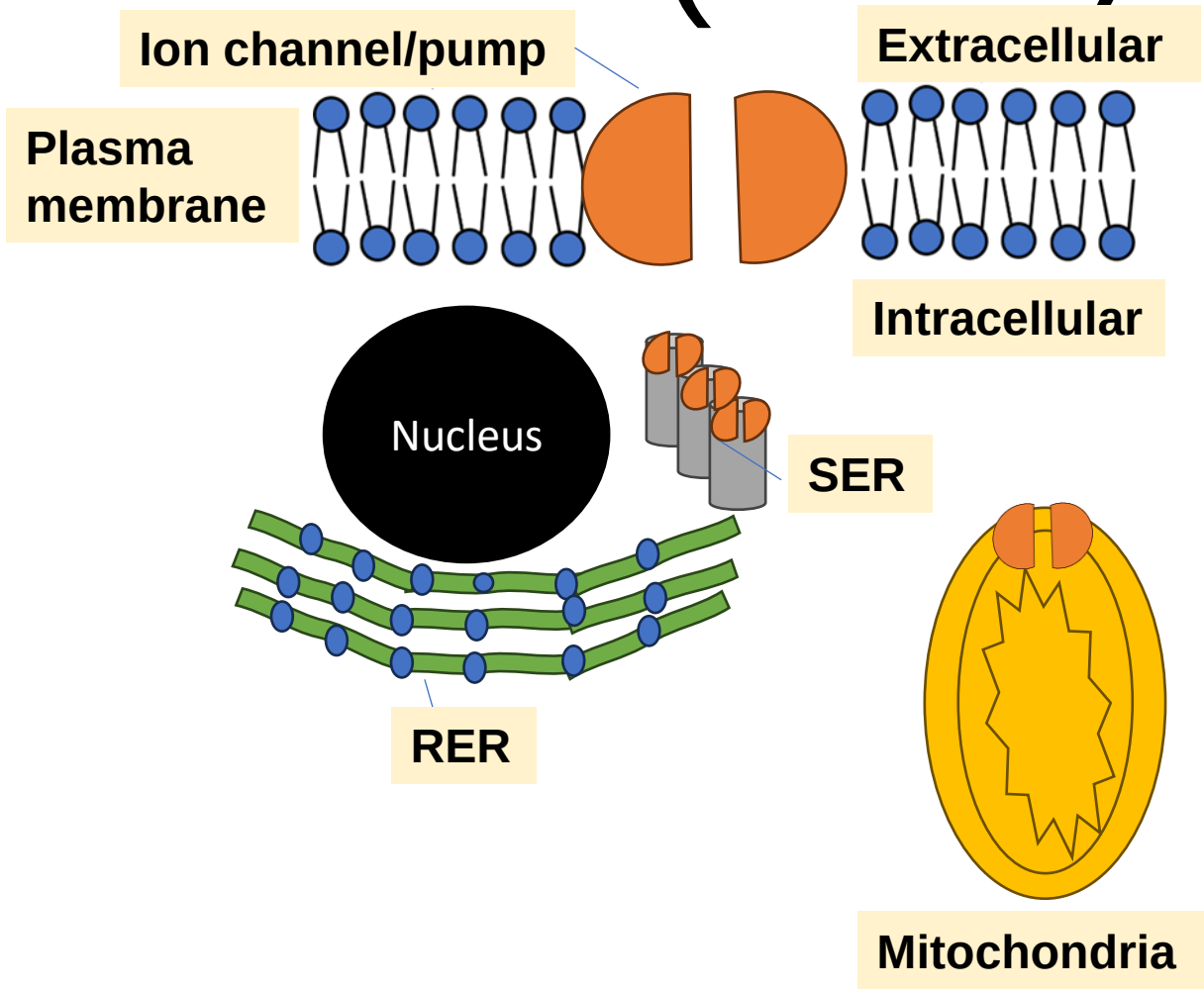
# Example 1: Calcium ions ($Ca^{2+}$)

**There are calcium pumps in the membrane of the following organelles:**

- ❑ **Cell membrane**
- ❑ **Mitochondria**
- ❑ **Endoplasmic reticulum**

***Smooth endoplasmic reticulum (SER):*** *It* accumulates calcium ions, produces and modifies fats/lipids.

***Rough endoplasmic reticulum (RER):*** *it has lots of ribosomes and is involved in production of proteins and sorting of proteins.*



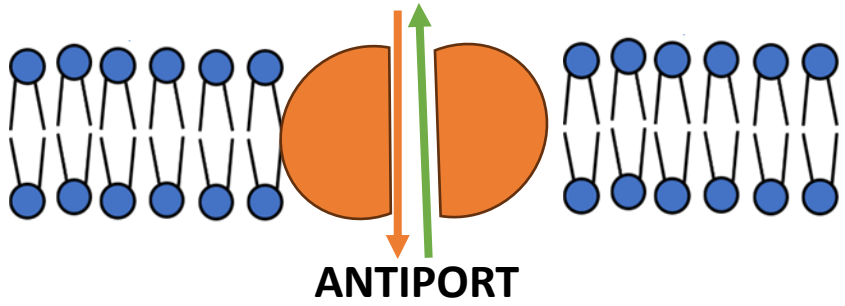

**Low levels of calcium ions in the cytoplasm compared to outside the cell, mitochondria and endoplasmic reticulum. More calcium ions enter organelles when channels open.**

*Why is the concentration of calcium ions low in cells despite they have pumps?*

# Why calcium ions ($Ca^{2+}$) are low?

There are **2 ion channels in the plasma membrane** of cells have a **large calcium ion concentration gradient**.

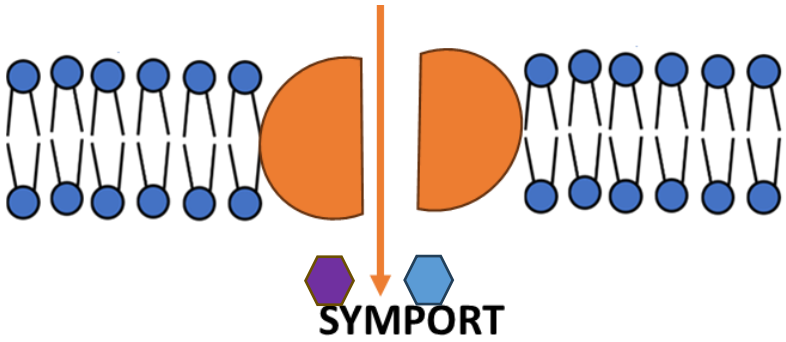
- **$Na^+/Ca^{2+}$ antiporters/exchangers (Sodium/Calcium ion antiporters)**
- **$H^+/Ca^{2+}$ antiporters/exchangers (Hydrogen/Calcium ion antiporters)**

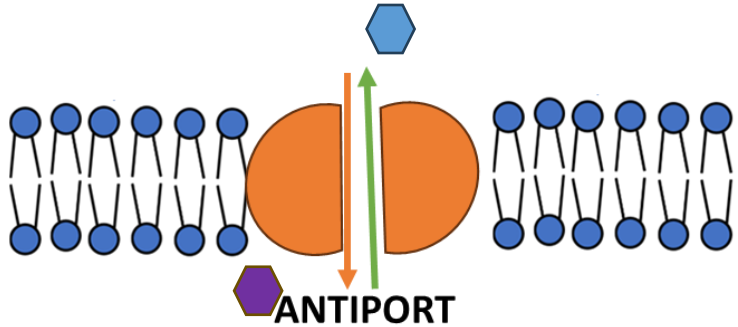


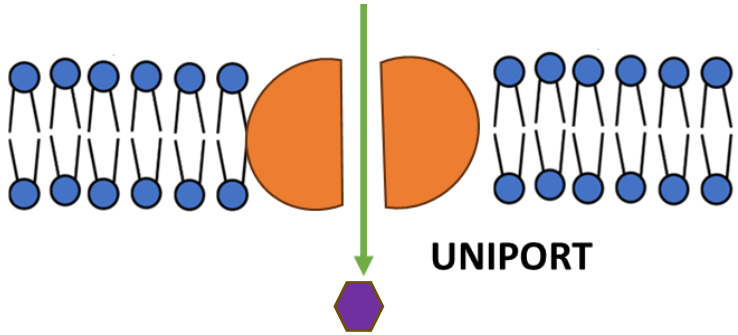

**10–20,000 fold concentration gradient of calcium across the plasma membrane.**

# What are antiporters?

There are **three types of transporters.**




**SYMPORTERS** binds **TWO OR MORE IONS OR MOLECULES** and transports them in **SAME DIRECTION.**

**SYM =SAME**

**ANTIPORTERS** binds **TWO OR MORE IONS OR MOLECULES** and transports them in **OPPOSITE DIRECTIONS.**

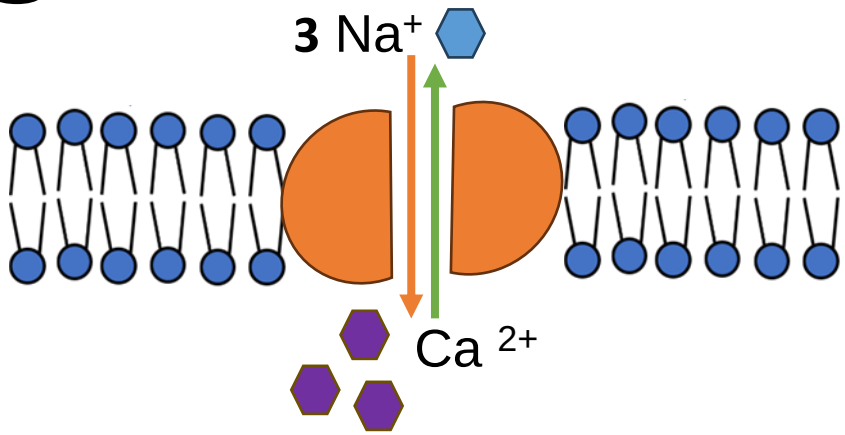
**ANTI=AGAINST**

**UNIPORTER binds a SINGLE MOLECULE OR ION and transports it ACROSS THE MEMBRANE.**

**UNI =ONE**

# $Na^+$/ $Ca^{2+}$ antiporters

**$Na^+/Ca^{2+}$ antiporters/exchangers** use **energy within sodium ion gradients** to **pump calcium ions across membrane.**



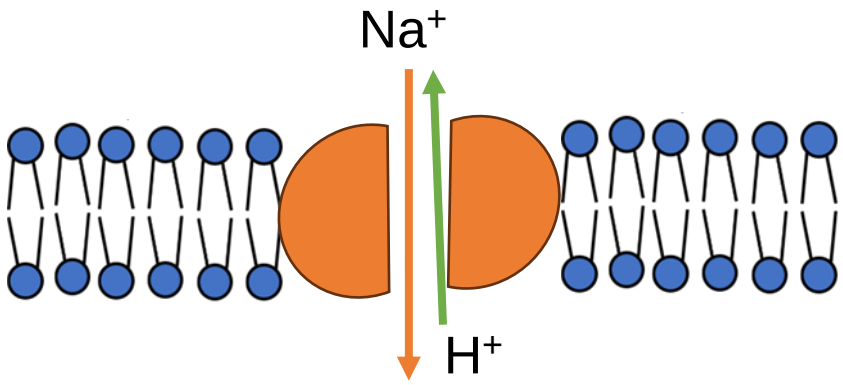

**3 Sodium ions IN**
**1 Calcium ion OUT**

**Calcium ions ATPases** use energy from **hydrolyzing/splitting ATP to pump calcium ions against the gradient.**

**ATP = Adenosine Triphosphate (energy source)**

# $H^+/Ca^{2+}$ antiporters

**$H^+/Ca^{2+}$ antiporters/exchangers** use **energy as ATP** within **sodium/hydrogen gradient pump** against the **ion gradient to remove calcium ions by pumping calcium ions across membrane.**




**1 Sodium ion IN**
**1 Hydrogen ion OUT**

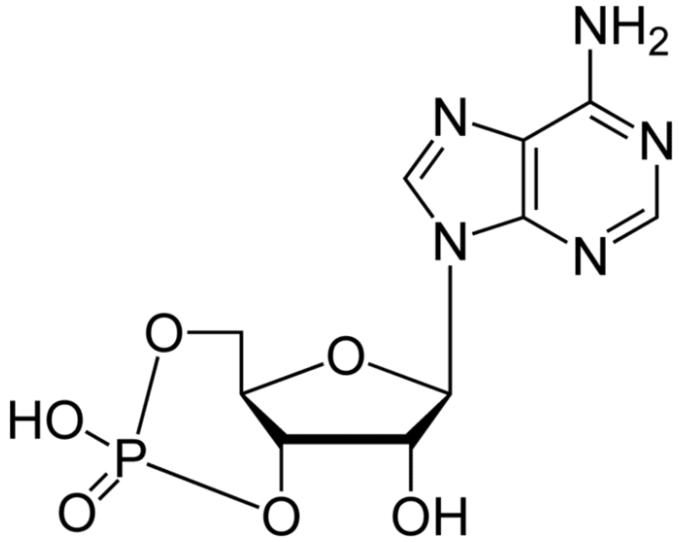
**Electrochemical gradients** allow cells to **control the direction ions** move **across membranes.**

# Example 2: cAMP

**cAMP** stands for **cyclic adenosine monophosphate.**

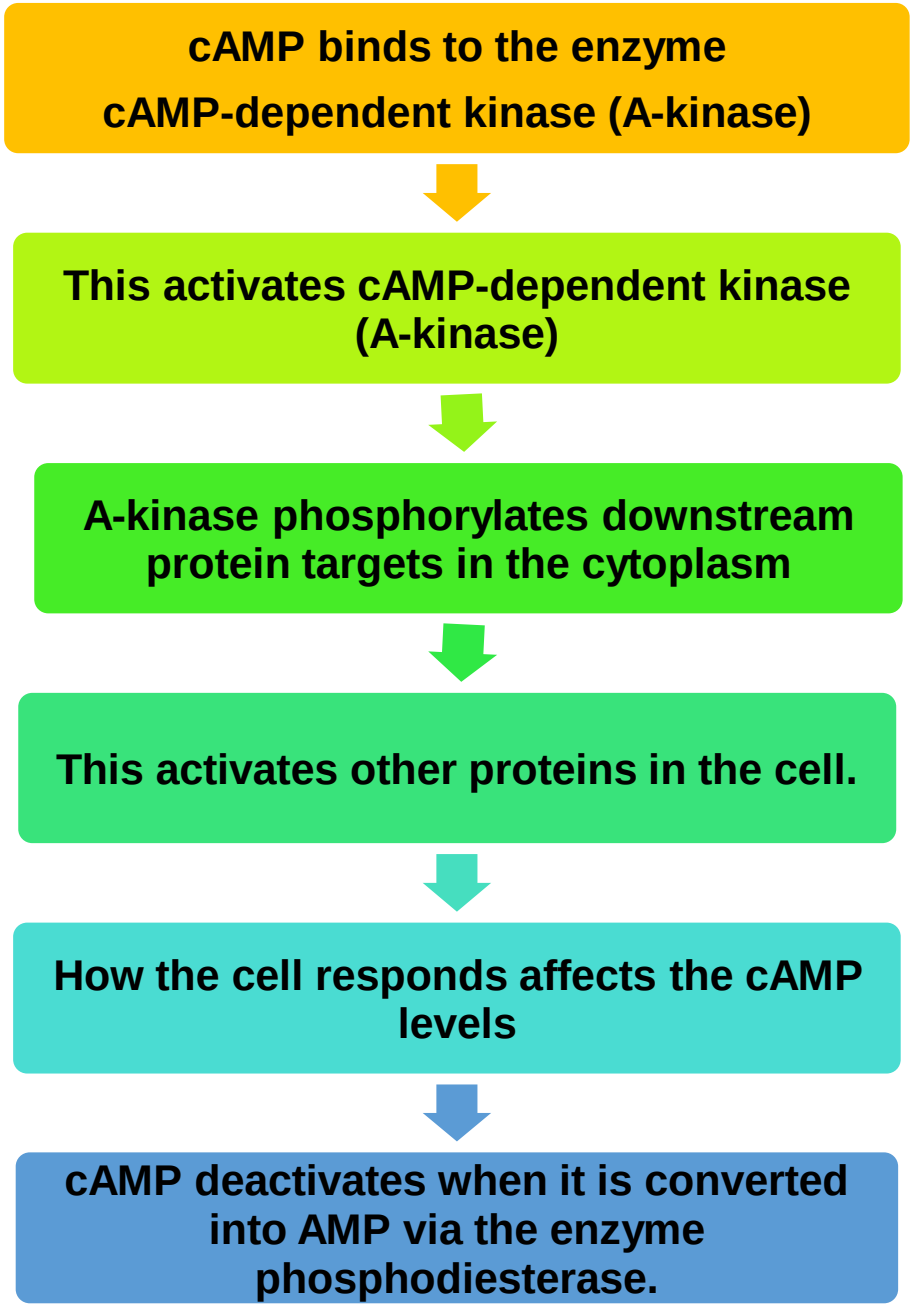
This cyclic **secondary messenger** is made by the enzyme **adenylyl cyclase from ATP.**

**The phosphate group** is attached to **two positions** on the **ribose sugar molecule** forming a circular ring.

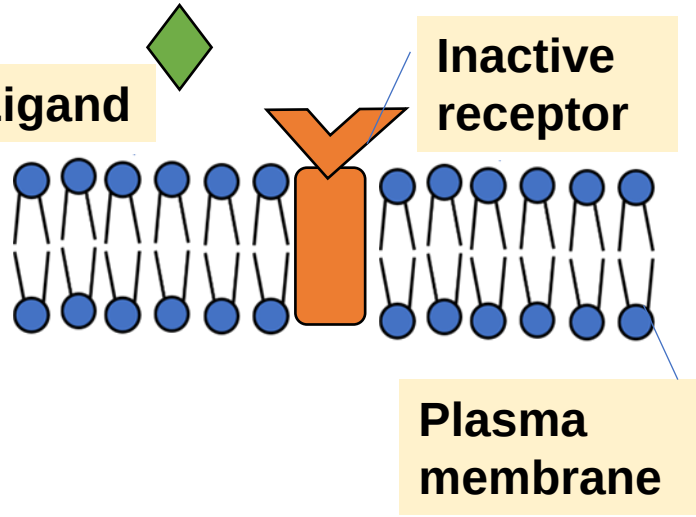



Source: (Alchetron, 2022)

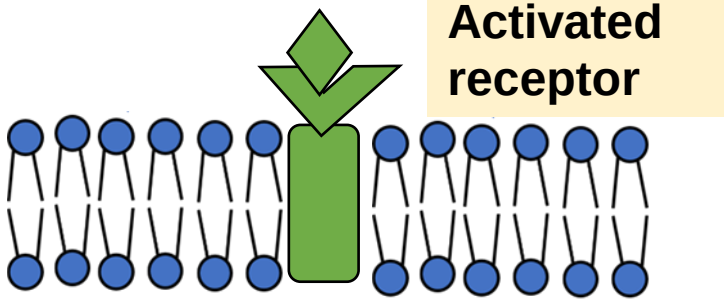
# What does cAMP do in signal transduction?



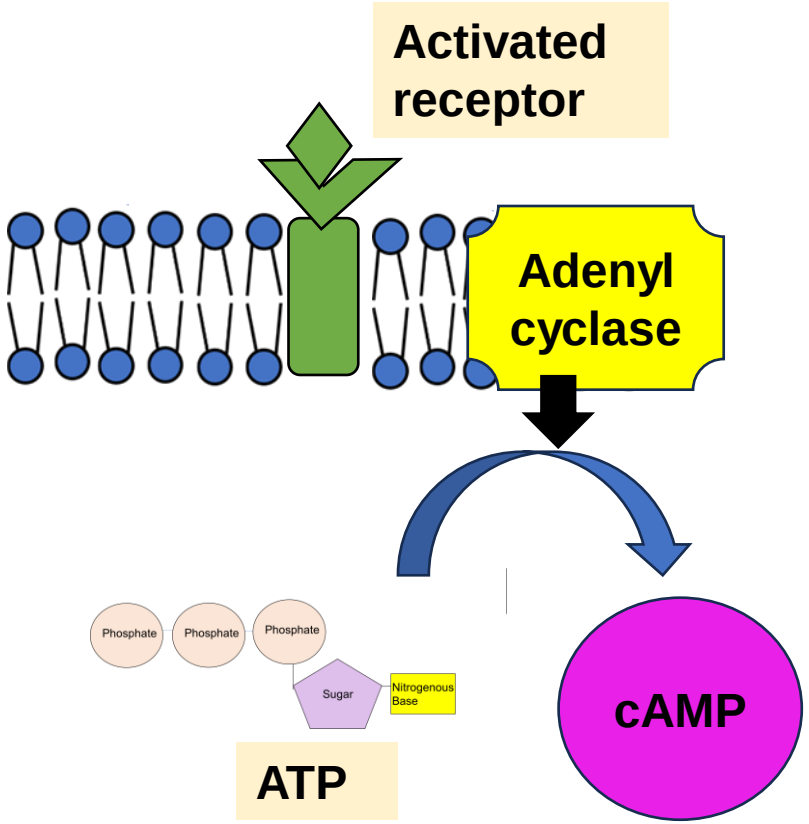
# Cyclic AMP (cAMP) – The process




A ligand binds to a receptor on the plasma membrane.

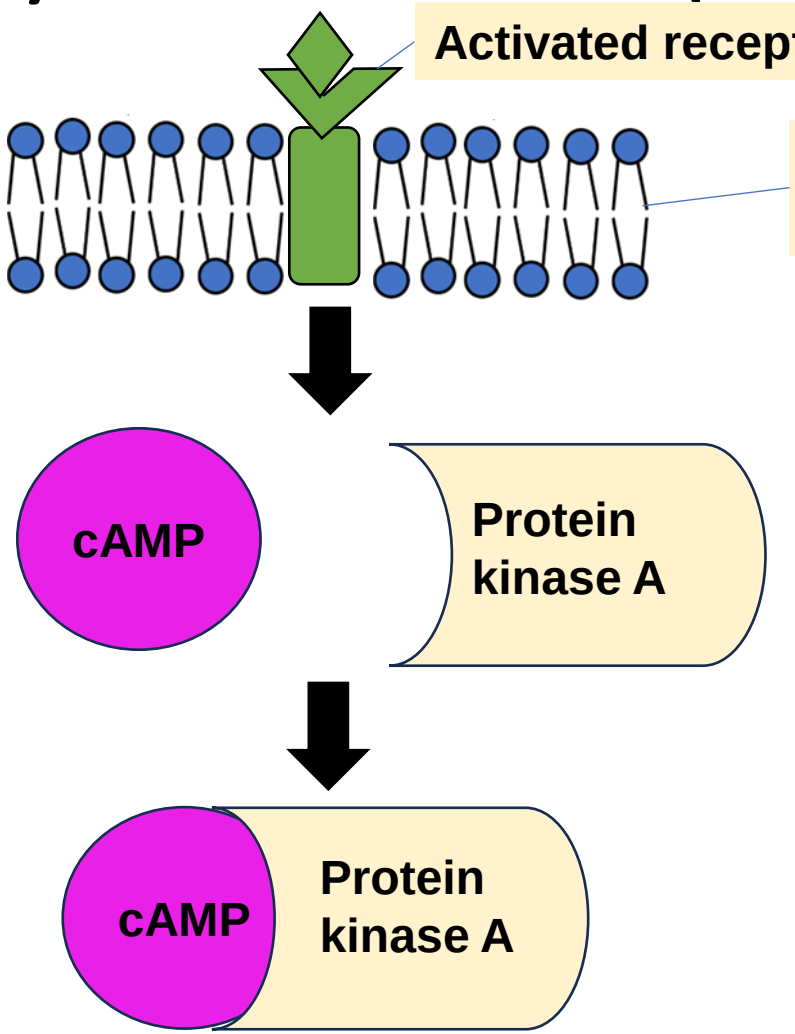



The receptor is activated.

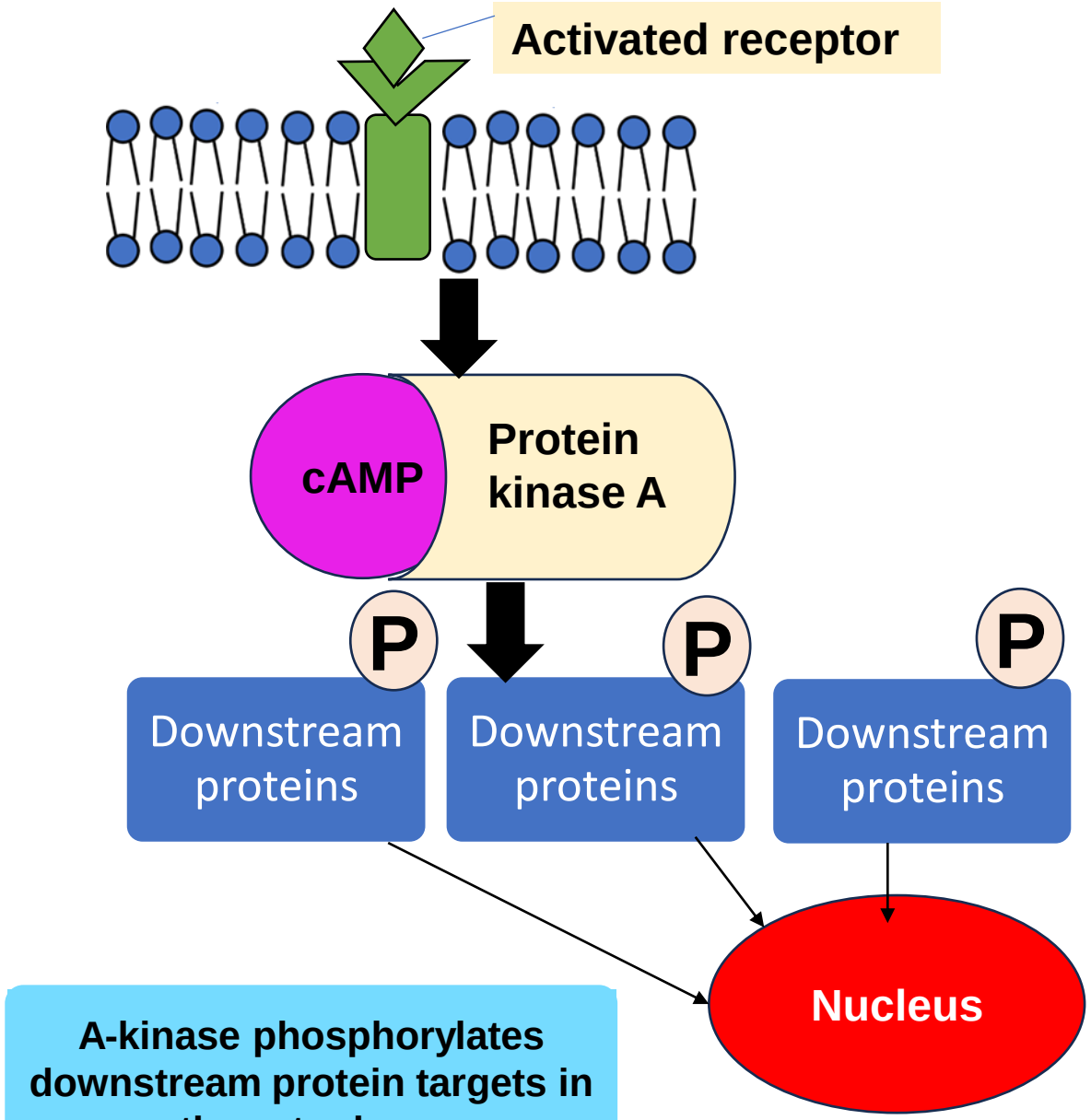



Adenylyl cyclase produces cAMP from ATP.

# Cyclic AMP (cAMP)



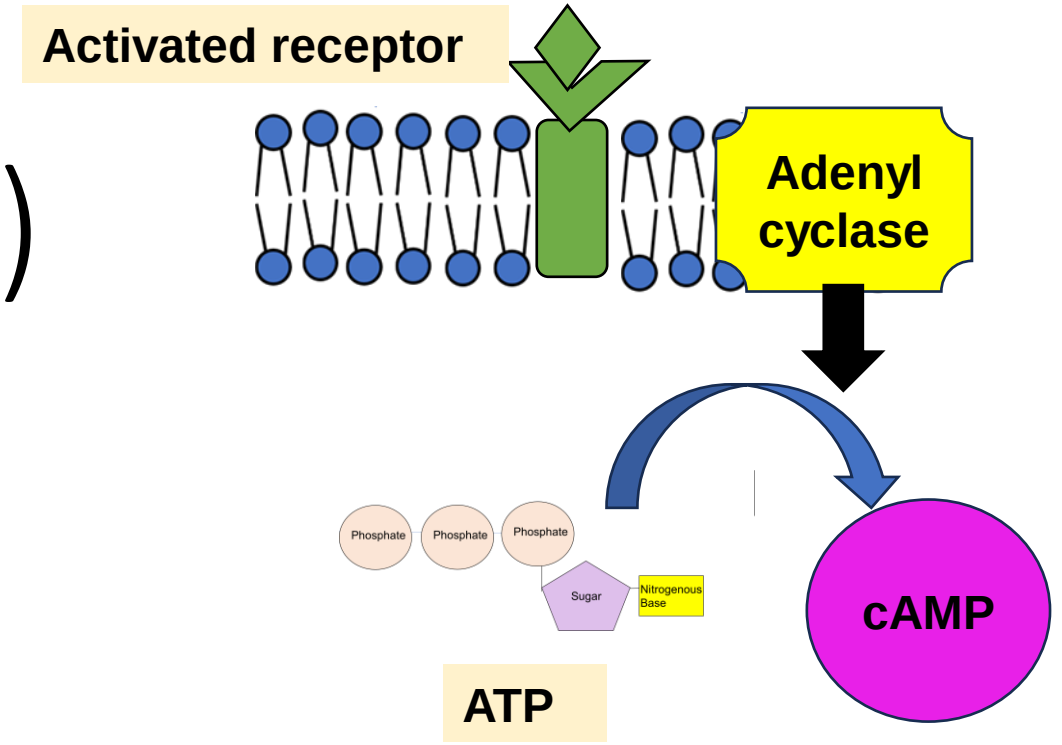

cAMP binds to the enzyme cAMP-dependent kinase (A-kinase)




A-kinase phosphorylates downstream protein targets in the cytoplasm

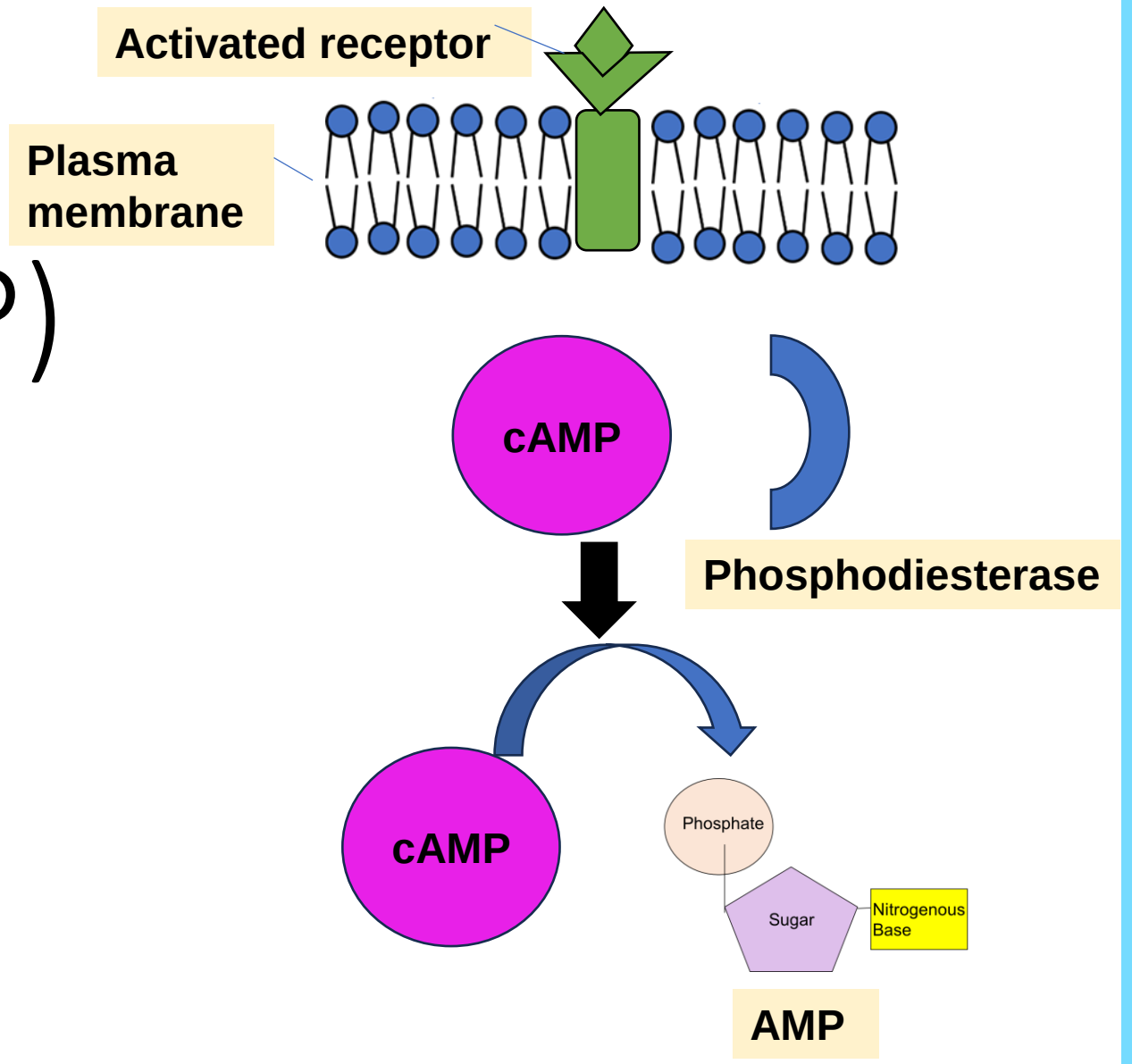
# Cyclic AMP (cAMP)

ON



# Cyclic AMP (cAMP)

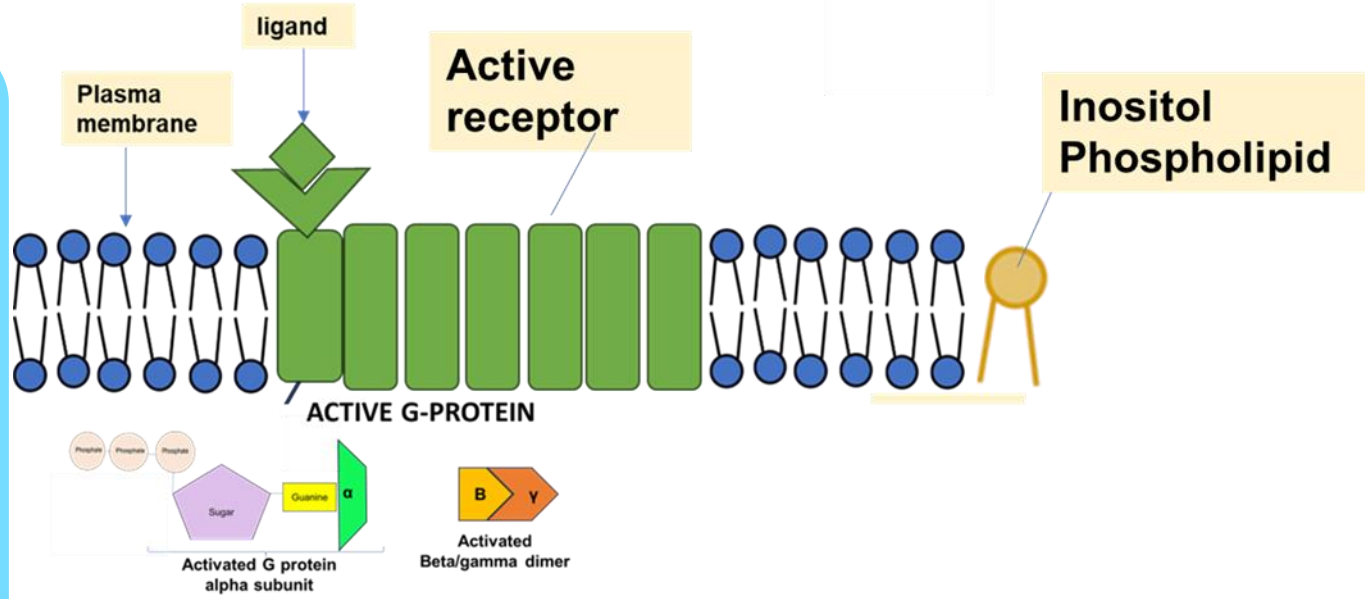
OFF



# Example 3 Diacylglerol (DAG) and Inositol triphosphate ($IP_3$)

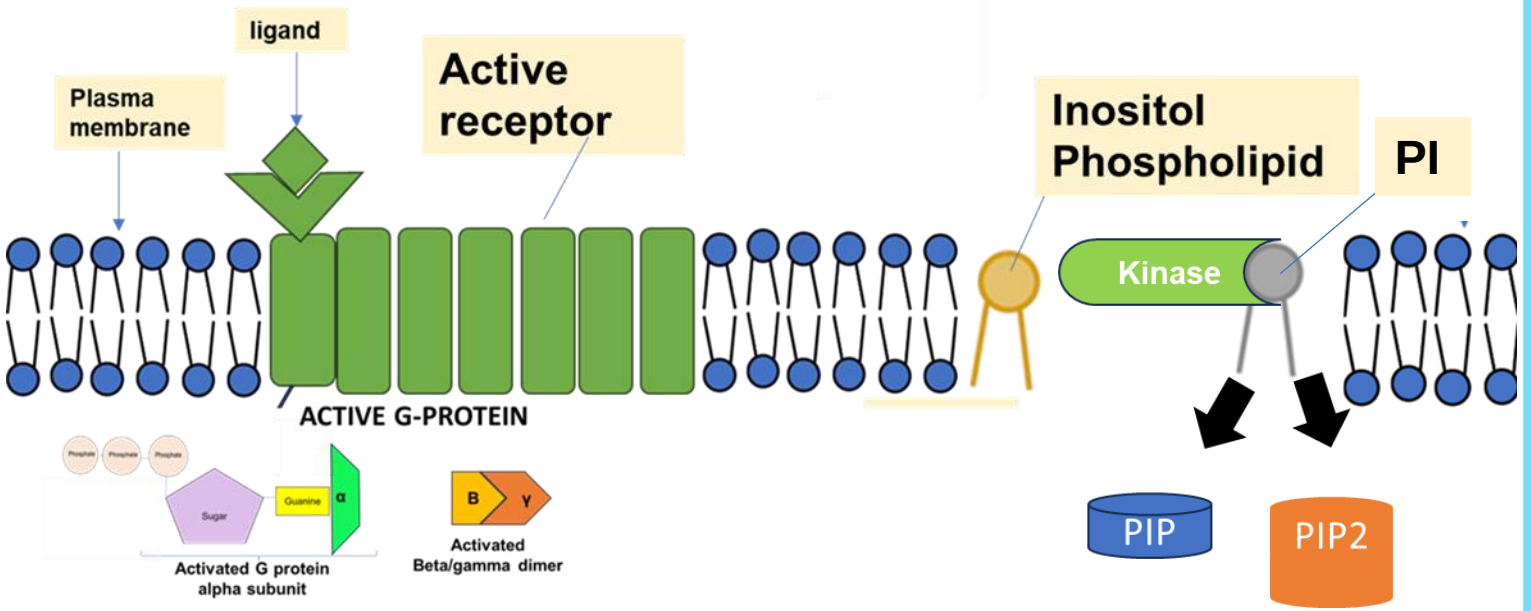
Inositol triphosphate ($IP_3$) is produced from membrane phospholipids. ***How?***

**1. Inositol phospholipid** is a **lipid/fat molecule** it has **inositol (a carbohydrate)** as its **hydrophilic head that acts as a secondary messenger because they are membrane-bound and found near cell surface receptors.**



# Example 3 Diacylglerol (DAG) and Inositol triphosphate ($IP_3$)

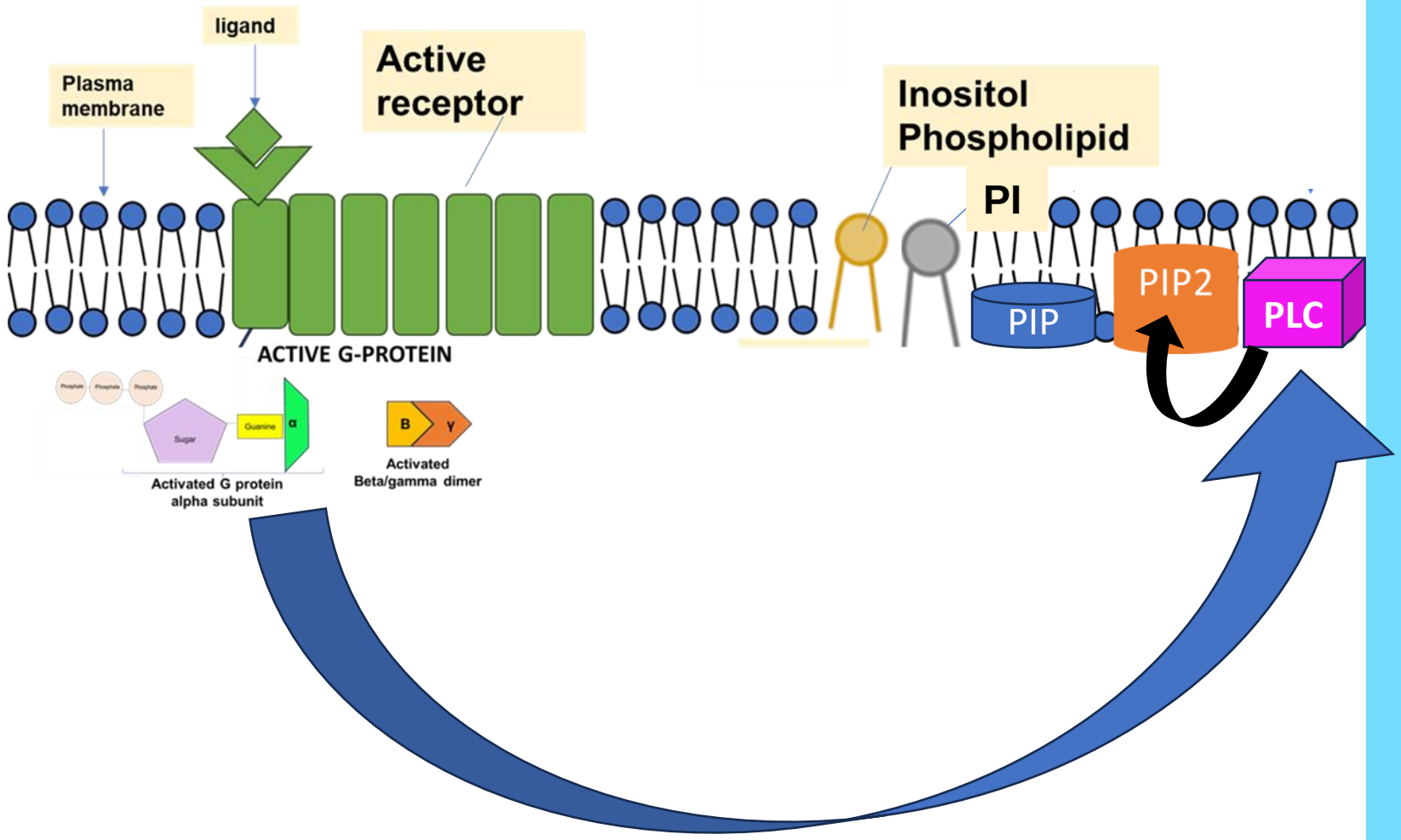
2. **Phosphatidylinositol (PI)** is a **phospholipid** and is **phosphorylated by kinase enzymes** to form **PI-phosphate (PIP) and PI-bisphosphate ($PIP_2$).**




Inositol triphosphate ($IP_3$) is produced from membrane phospholipids. ***How?***

# Example 3 Diacylglerol (DAG) and Inositol triphosphate ($IP_3$)

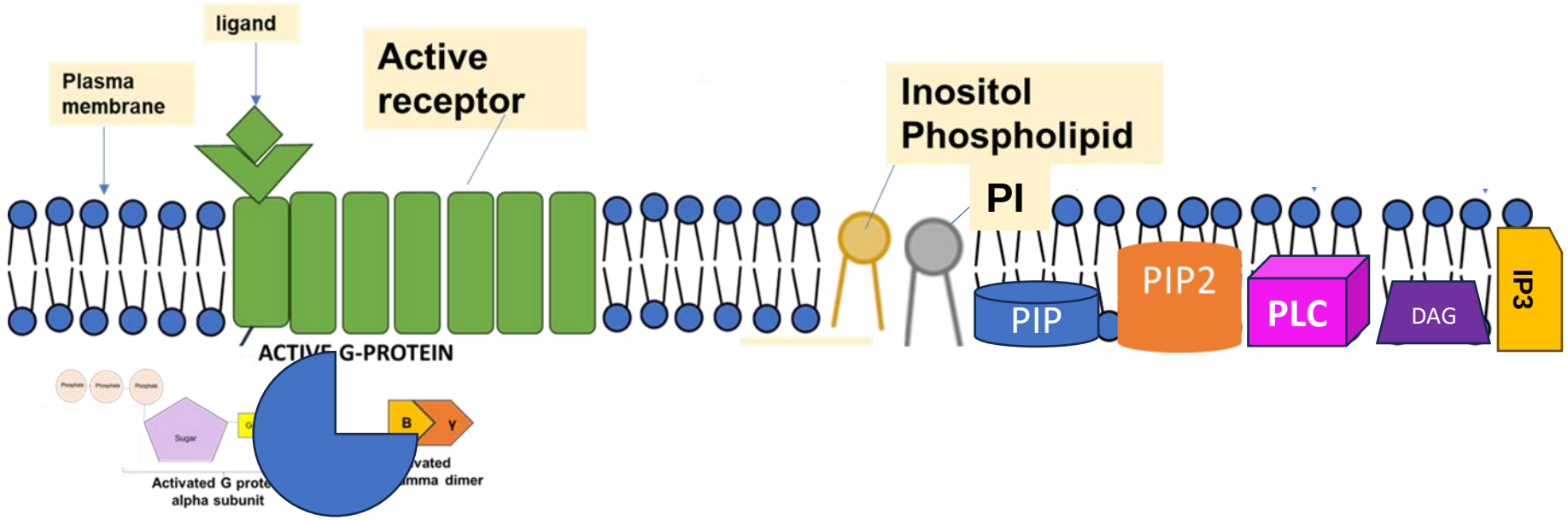
**3.The alpha subunit of G protein activates another enzyme called phospholipase c (PLC)who then cleaves $PIP_2$.**




Inositol triphosphate ($IP_3$) is produced from membrane phospholipids. ***How?***

# Example 3 Diacylglerol (DAG) and Inositol triphosphate ($IP_3$)

**4. PLC cleaves $PIP_2$ to form two second messengers: Diacylglerol (DAG) and Inositol triphosphate ($IP_3$)**

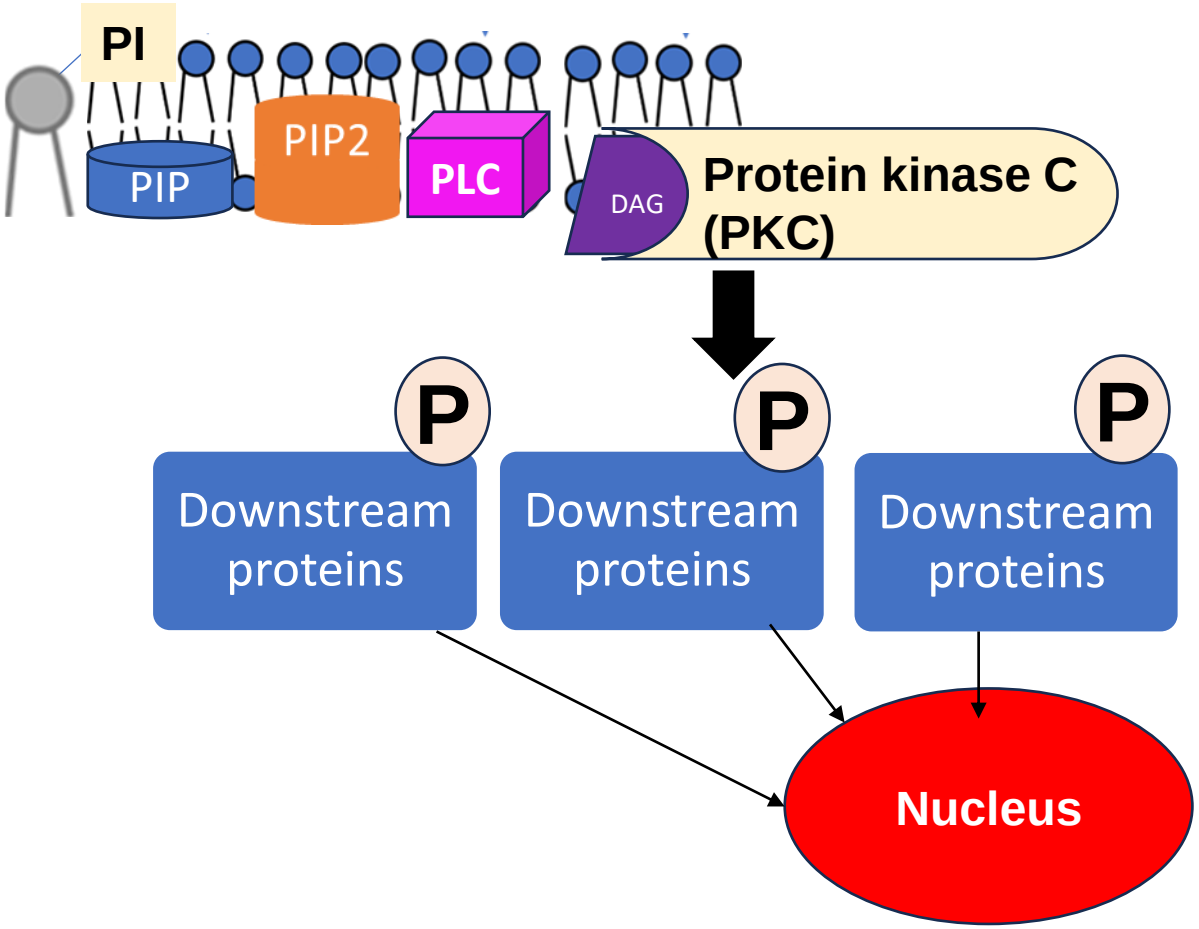



Inositol triphosphate ($IP_3$) is produced from membrane phospholipids. ***How?***

# Example 3 Diacylglerol (DAG) in action

**5. Diacylglycerol (DAG) stays in the plasma membrane, binds and activates protein kinase C (PKC).**

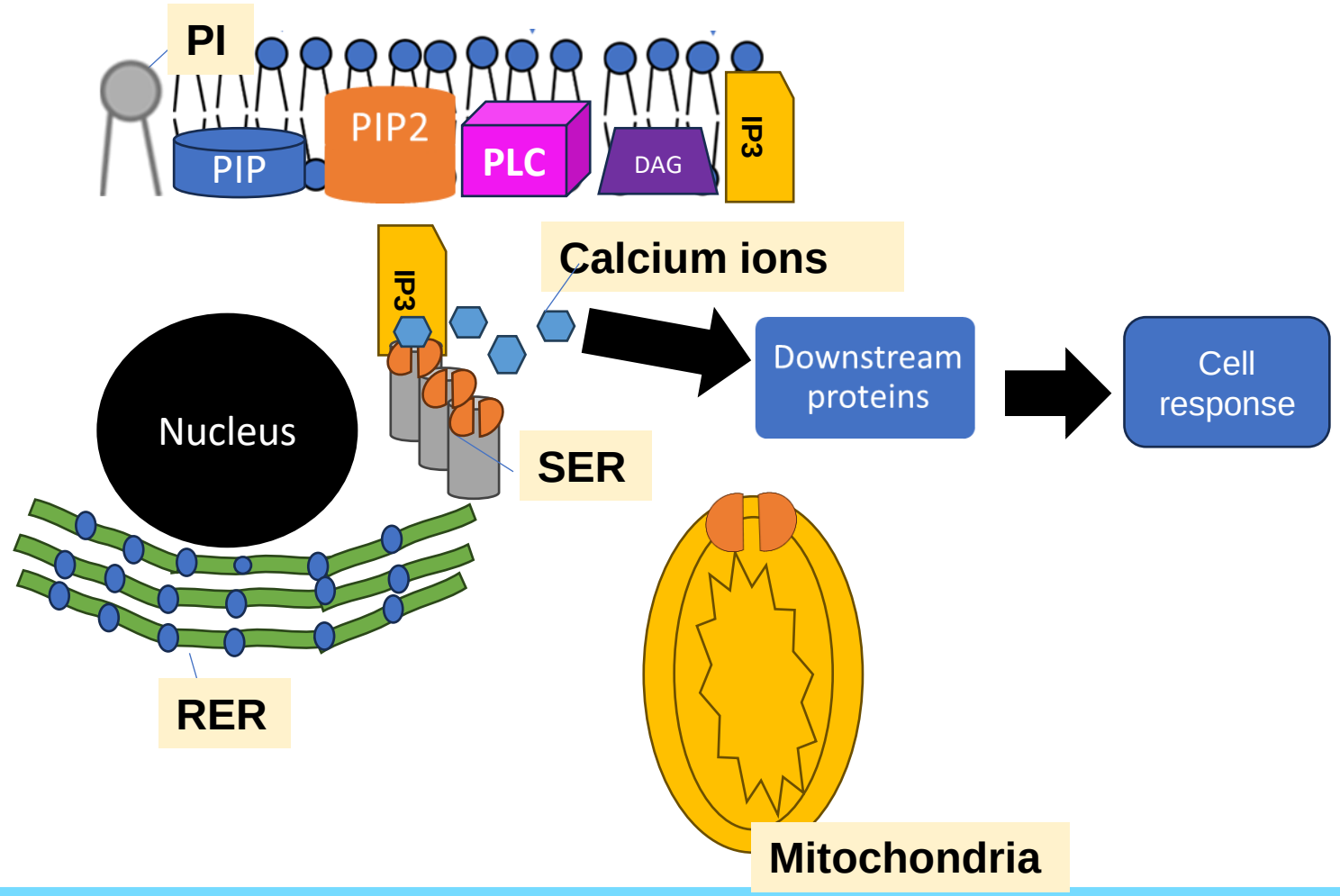
**Protein kinase C phosphorylates serine and threonine amino acid residues in its target cellular proteins.**



# Example 3 Diacylglerol (DAG) and Inositol triphosphate ($IP_3$)

**6. $IP_3$ diffuses into the cytoplasm** and **binds to calcium channels in the endoplasmic reticulum membrane.**

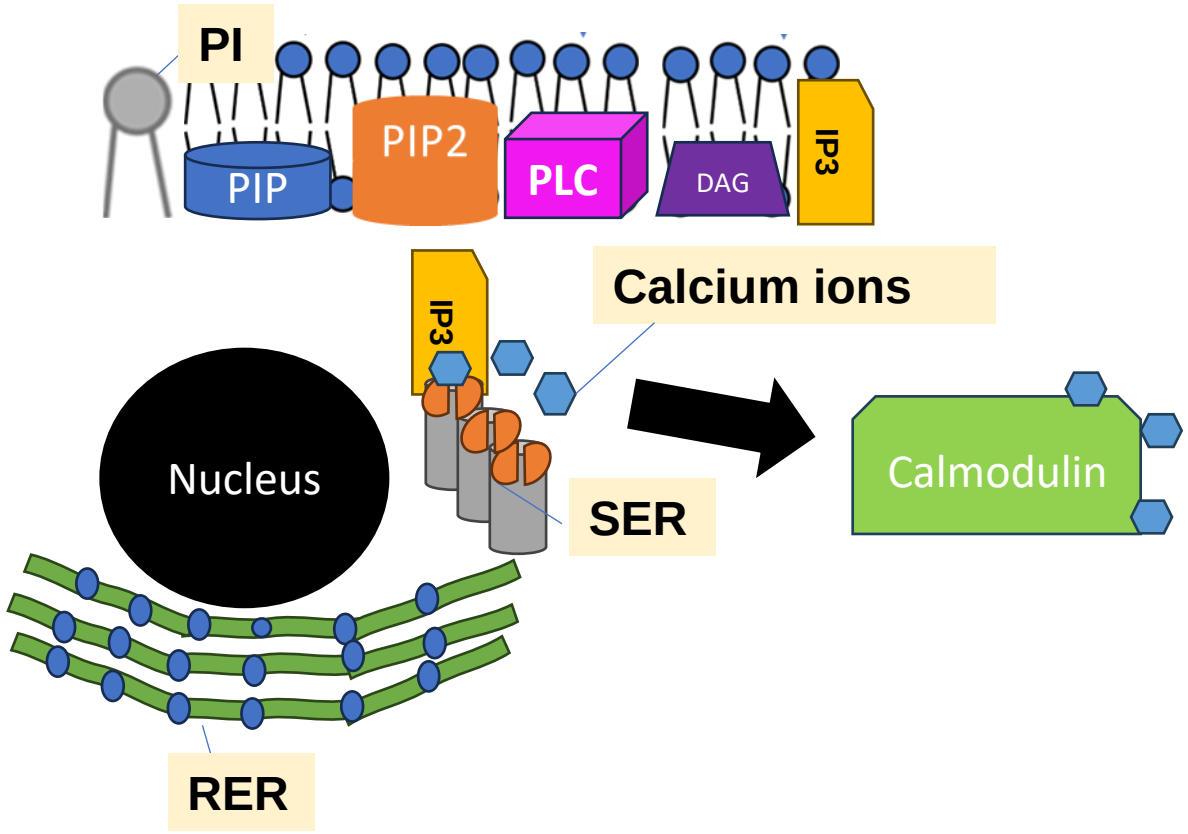
**Calcium channels open to release Ca2+** into the $IP_3$ that continues the **signal cascade.**



# Example 3 Diacylglerol (DAG) and Inositol triphosphate ($IP_3$)

**7. Calmodulin (CaM)** is a protein whose role is to **bind to calcium and regulate calcium-dependent pathways** e.g.

Carbohydrate breakdown in liver cells.

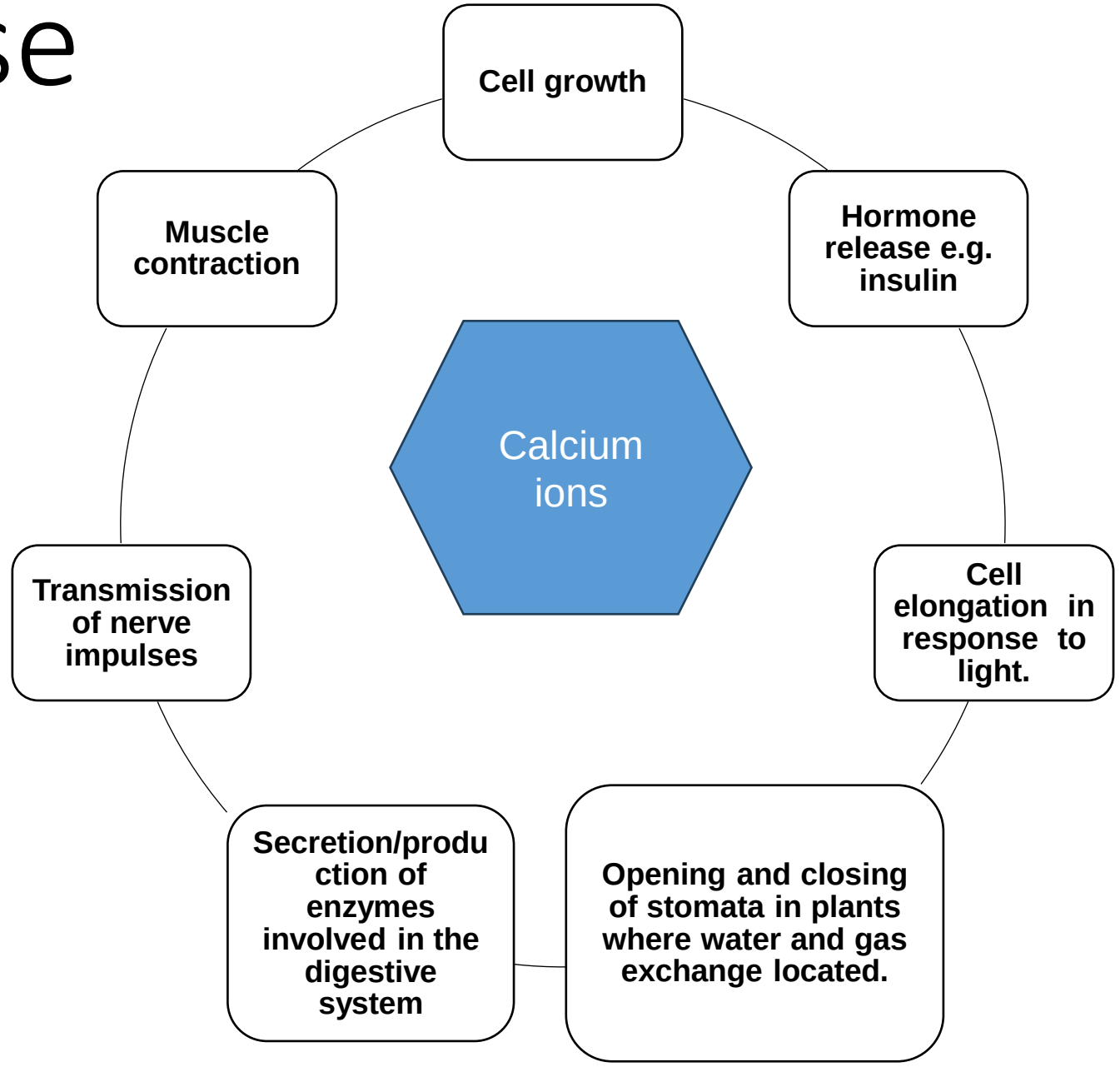


# Cellular response

# Cellular response

It depends on the **receptor, signal transduction and target cellular proteins.**

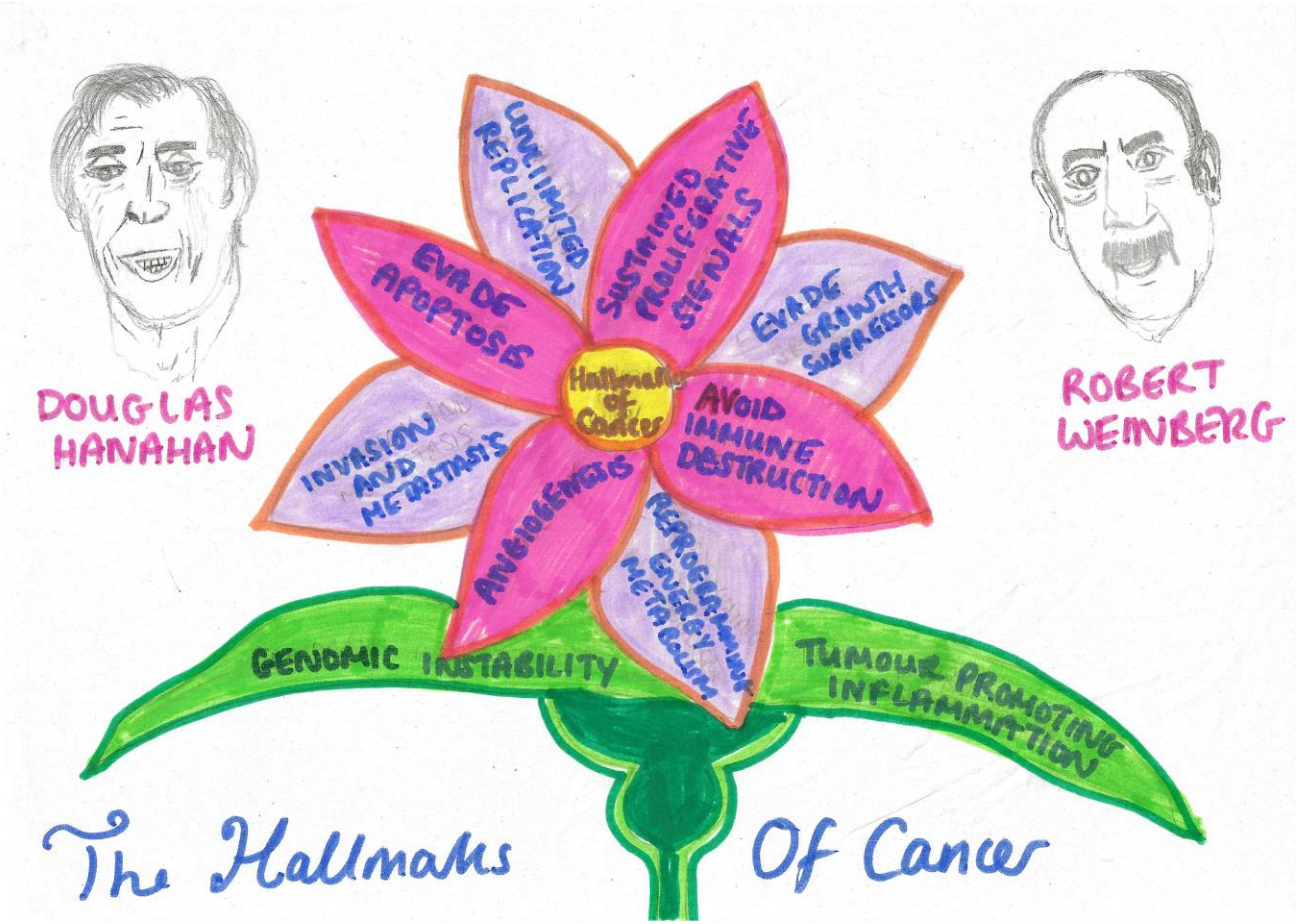
**For example, calcium ions requires proteins to perform the following functions:**



# Calcium ions in cancer cells.

**In cancer cells, calcium regulates many hallmarks of cancer:**

- ❑ **Self-sufficiency in growth signals**
- ❑ **Evading apoptosis.**
- ❑ **Insensitivity to anti-growth signals,**
- ❑ **Angiogenesis**
- ❑ **Invasion and metastasis**



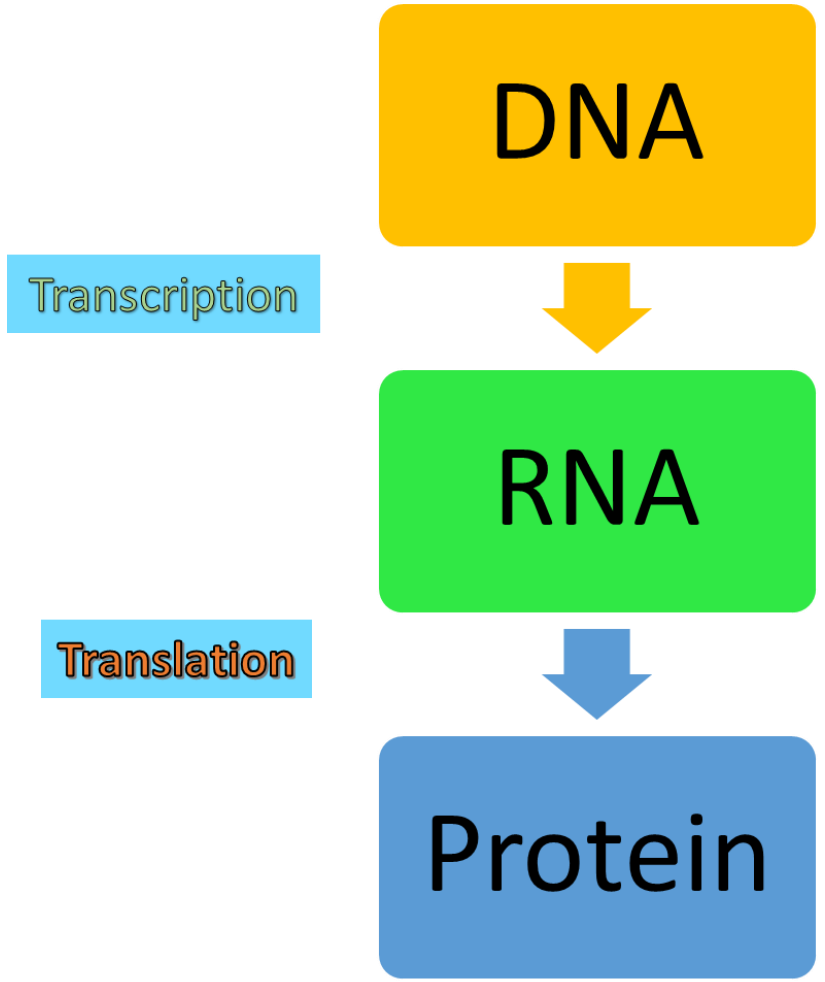
# Transcription factors

# What are Transcription factors?

**Transcription factors** are proteins involved in the **transcription of DNA to RNA.**

They **regulate the expression of genes which affects cellular response** e.g. **differentiation, development and immune system.**

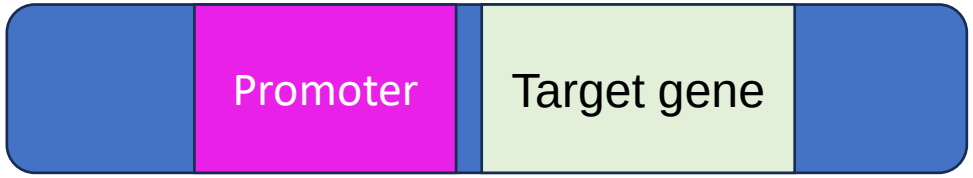
There are approximately. **3000 transcription factors regulate the 23,000 genes or so encoded in the human genome.**



# What are Transcription factors?

**Some transcription factors** can **bind to specific sequences of DNA called promoter sequences** near **the transcription start site**.

**Other transcription factors bind to regulatory sequences** e.g. enhancer sequences. They consist of many **base pairs that are near the target gene.**



**The activity of a transcription factor can be regulated by:**

- ❑ **Synthesis in particular cell types only.**
- ❑ **Phosphorylation**

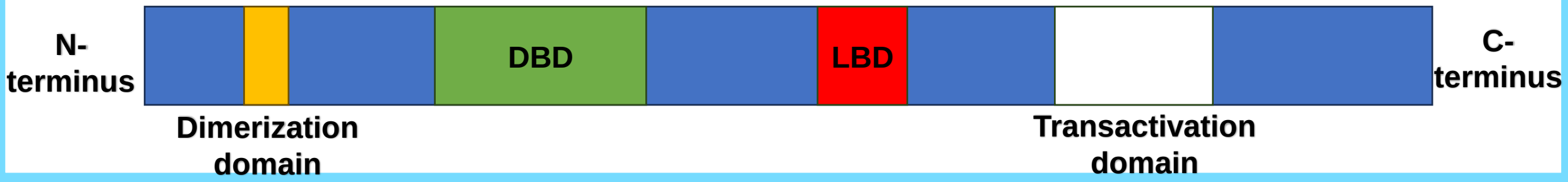
# *Types of Domains in transcription factors*

# Types of Domains

| Name of Domain | Sub-types | Description |
|---|---|---|
| **DNA-binding domain (DBD)** | **1) Helix-turn-helix motif**<br>**2) Leucine zipper motif.**<br>**3) Helix-loop-helix motif.**<br>**4) Zinc finger motif (approximately 30 amino acids long)**<br><br>A **collective conformational change helps with the binding to DNA.** | ❑ **DBDs direct transcription factors** to their **target regulatory regions by recognizing specific DNA sequences/response element** in the **major groove of DNA.**<br><br>❑ It is **conserved well which helps classifying transcription factors.** |

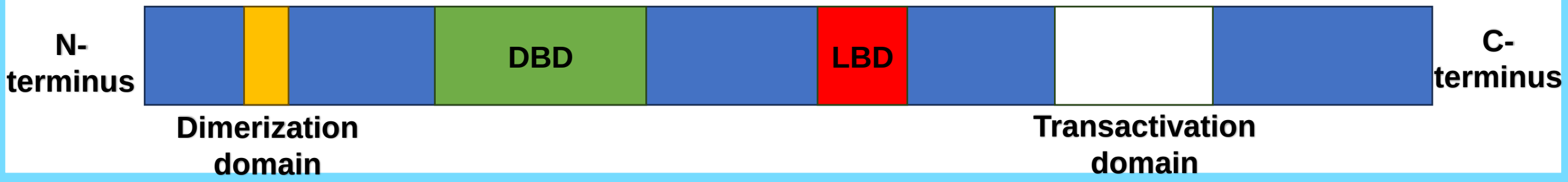
# Types of Domains

| Name of Domain | Sub-types | Description |
|---|---|---|
| Effector domains | 1) Transactivation domain<br>2) Repressor domain<br>3) Bifunctional domain (they can activate or repress gene expression) | ❑ It modulates transcriptional activity.<br>❑ It interacts and binds with cofactors and which leads to changes to histone proteins, DNA methylation and activating RNA polymerase II to facilitate transcription. |



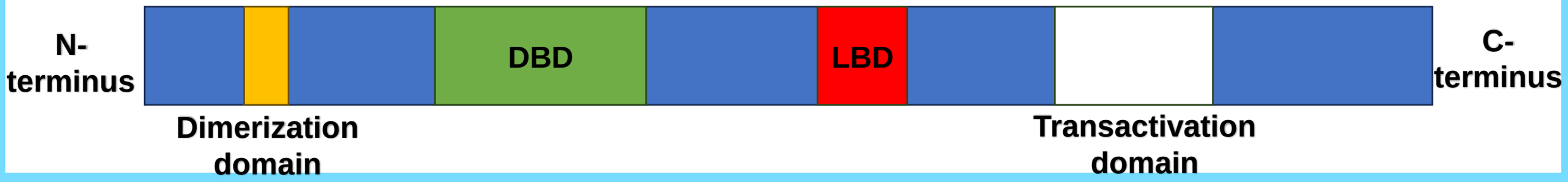
# Types of Domains

| Name of Domain | Sub-types | Description |
|---|---|---|
| **Dimerization domain** | **None** | ❑ It **helps transcription factors** that **work in pairs/dimers to facilitate transcription.**<br>❑ Transcription factors can bind to DNA as **homodimers or heterodimers** |



# Types of Domains

| Name of Domain | Sub-types | Description |
|---|---|---|
| Ligand-binding domains (LBD). | | ❑ **Some transcription factors only function upon binding of a ligand and require LBD.** |



# By the end of this lecture, you should understand

- **The first messenger in the cell-signalling pathway is the ligand. The secondary messenger helps transduce the signal to elicit a response.**
- **Calcium ion channels are found in the mitochondria, endoplasmic reticulum and plasma membrane. It is regulated to maintain its low concentration in cells. It binds specifically to the calcium-binding protein Calmodulin.**
- **cAMP plays a key role in signal transduction pathway. It is activated by adenyl cyclase where it then binds to protein kinase A to activate more proteins in the signal cascade. cAMP is deactivated by phosphodiesterase.**
- **Diacylglerol (DAG) and Inositol triphosphate ($IP_3$) are secondary messengers that are involved in the phosphorylation of downstream targets and stimulate other secondary messengers e.g. calcium ions respectively.**
- **Transcription factors regulate the expression of genes which affects cellular response.**

# Reference list for further reading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SEASON 2

# Understanding Cancer

## Lecture 6

## Types of signalling pathway: normal and dysregulated GPCR

DR HAFSA WASEELA ABBAS

www.hafsaabbas.com