بسم الله الرحمن الرحيم

Physiology | Lecture 7

Signal Transduction 3

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Signal Transduction Lec 3

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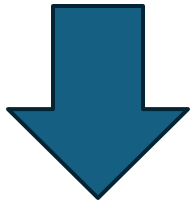
SECOND MESSENGER MECHANISMS FOR MEDIATING INTRACELLULAR HORMONAL FUNCTIONS

- **Most water-soluble extracellular chemical messengers activate second-messenger pathways.**
- Hormones can exert intracellular actions is to stimulate formation of the second messenger inside the cell membrane.
- The second messenger then causes subsequent intracellular effects of the hormone.



The only direct effect that the hormone has on the cell is to activate a single type of membrane receptor.

The second messenger does the rest.

Water-soluble hormones, messenger, chemicals, ligands, and anything like that will bind to a receptor on the plasma membrane. This receptor will then trigger a cascade of chemical interactions or signaling pathways that continue the downstream pathway inside the cell.

We already discussed G-protein coupled receptors and tyrosine kinase receptors, which either activate enzymes directly or, in the case of tyrosine kinases, may activate pathways like the the “juxta” pathway.

Second messenger formation and function:
The main role of second messengers is to help **transmit information from the extracellular environment to the intracellular environment**. When a ligand (such as a hormone or signaling molecule) binds to a receptor on the plasma membrane, this binding **triggers the formation of a second messenger** inside the cell.

The ligand’s job is simply to bind to the receptor—**after that, the second messenger takes over** and continues the signaling process inside the cell to produce the desired cellular response.

SECOND MESSENGER MECHANISMS FOR MEDIATING INTRACELLULAR HORMONAL FUNCTIONS

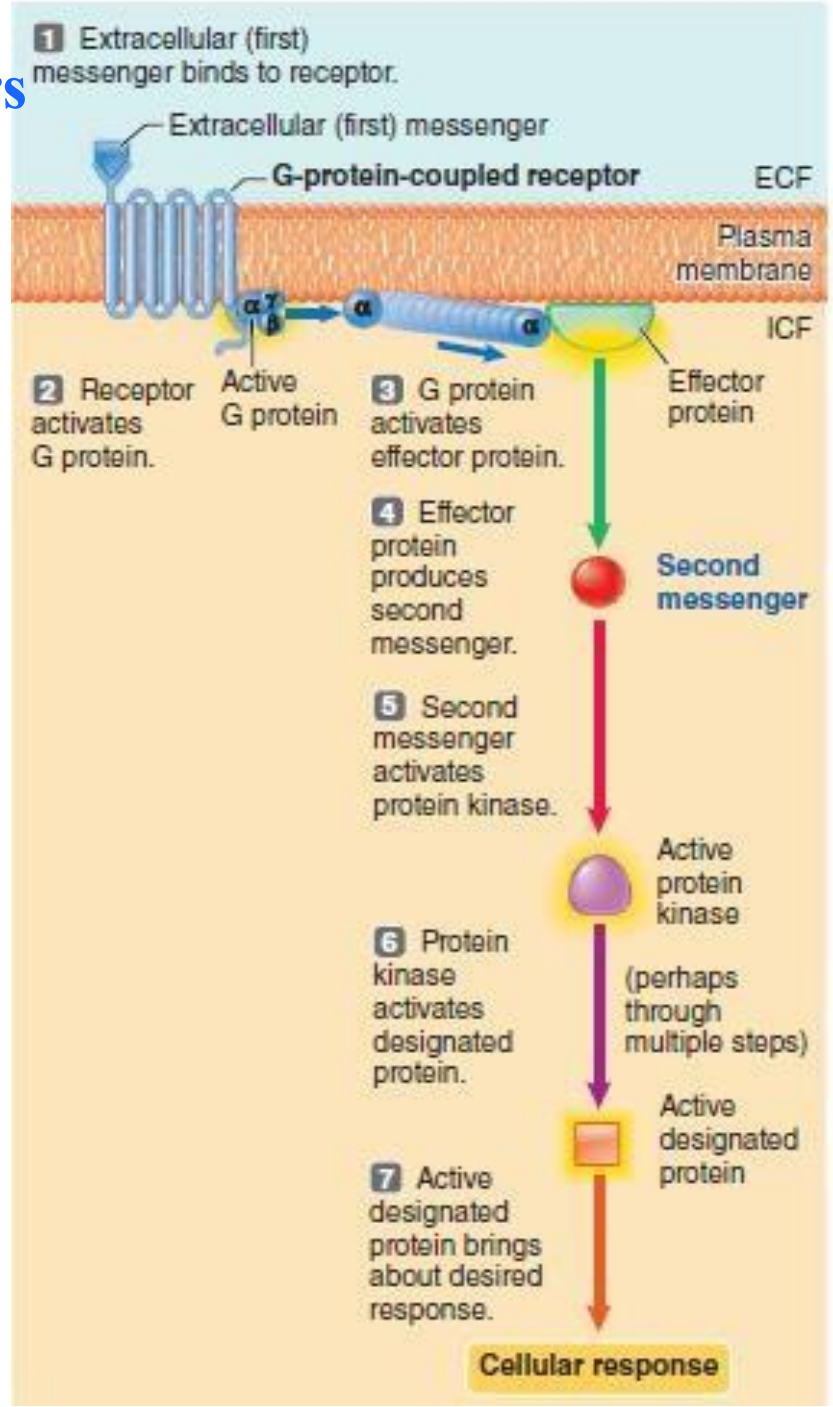
- Types of Second Messenger: We have different types for different functions
- 1. Calcium ions and associated *calmodulin*
- 2. Products of membrane phospholipid breakdown.
- 3. cAMP
- 4.cGMP

- In some cases, a hormone may stimulate more than one second messenger system in the same target tissue

A single ligand may use **more than one receptor** to carry out its function. It's not always the case that a hormone uses only one specific pathway to do something. In fact, **many hormones can activate multiple signaling pathways**. So, the **same ligand can act through different receptors** and trigger **more than one pathway** inside the cell, depending on the cell type and context.

Most water-soluble extracellular chemical messengers activate second-messenger pathways via G-protein-coupled receptors

- Binding of the first messenger to the receptor activates the **G protein**,
- On activation, a portion of the G protein shuttles along the membrane to alter the activity of a nearby membrane protein called the **effector protein**.
- Once altered, the effector protein leads to an increased concentration of an intracellular messenger, known as the **second messenger**.
- The second messenger relays the orders through a cascade of chemical reactions inside the cell that cause a change in the shape and function of designated proteins.



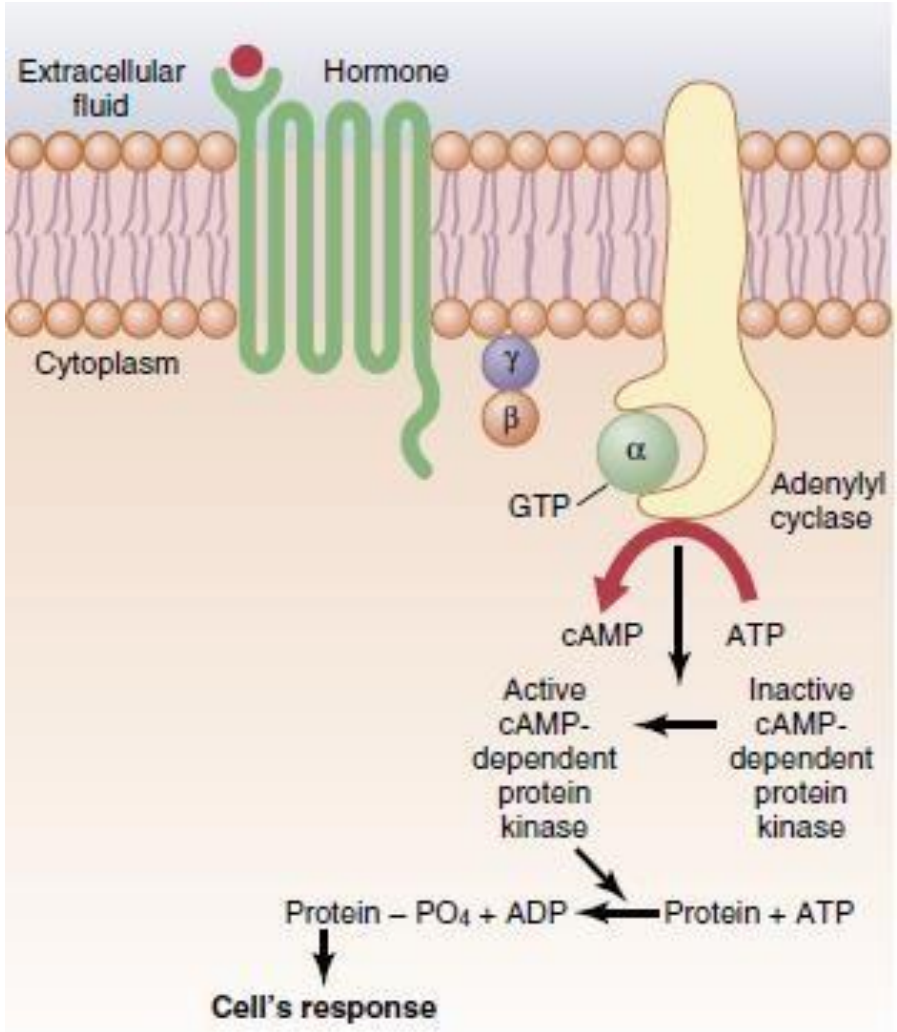
This figure shows how **second messengers** work in the signaling of **water-soluble hormones**. These hormones **cannot enter the cell**, so they bind to **G-protein coupled receptors** (GPCRs) on the **plasma membrane**.

Once the ligand binds to the receptor, the **alpha subunit** of the G-protein **dissociates** from the complex and binds to **GTP**, which then **activates an enzyme** like **adenylate cyclase**. This enzyme produces **second messengers** such as **cAMP**, which will continue the signal inside the cell.

The main role of second messengers (or the receptor itself) is to:
- **Activate protein kinases**,
- Which leads to **phosphorylation of target proteins** in the cell,
- These activated proteins may enter the nucleus and **affect gene transcription**,
- Leading to the creation of **new proteins** or the **activation of existing proteins**,
- And finally resulting in a **specific cellular response**.

Adenylyl Cyclase–cAMP Second Messenger System

- Stimulation of adenylyl cyclase, by the Gs protein
- Catalyzes the conversion of a small amount of cytoplasmic *Adenosine triphosphate ATP* into cAMP inside the cell.
- Then activates *cAMP-dependent protein kinase.*
- Phosphorylates specific cell proteins, triggering biochemical reactions that ultimately lead to the cell's response to the hormone.



NOTES

If the G protein stimulates the adenylyl cyclase–cAMP system, it is called a *Gs protein,* denoting a stimulatory G protein

If binding of the hormone to its receptors is coupled to an inhibitory G protein (denoted *Gi* protein), adenylyl cyclase will be inhibited, reducing formation of Camp and ultimately leading to an inhibitory action in the cell.

C amp as a second messenger

How is it formed?

cAMP (cyclic AMP) is formed through the action of the enzyme **adenylate cyclase**. Here's how the process works:

· A **water-soluble hormone** binds to a **receptor on the cell membrane** (a G-protein coupled receptor).

· This activates the **G-protein** inside the cell.

· The **alpha subunit** of the G-protein **binds GTP** and **dissociates** from the beta and gamma subunits.

· The activated alpha subunit then **binds to adenylate cyclase**, which is an enzyme attached to the inner side of the plasma membrane.

· Adenylate cyclase becomes activated and **converts ATP into cAMP**, which acts as the **second messenger**.

· cAMP then **activates protein kinase** , which goes on to **phosphorylate target proteins** and cause various **cellular responses**, including the activation of new proteins.

However, if the hormone activates an **inhibitory G-protein (Gi)** instead:

· The **inhibitory alpha subunit** binds to adenylate cyclase and **inhibits** its activity.

· This results in **decreased cAMP formation**, leading to **inhibition of protein activation and new protein production**.

A. cAMP:

❖ Regulation of adenylate cyclase:

Receptors that cause increase in cAMP do so by activating G_s, a stimulatory protein that activates adenylyl cylcase

Adenylyl cyclase is turned off by G_i, an inhibitory protein.

PKA enters the nucleus and phosphorylates CREB (CRE binding protein), which binds to the cAMP response element (CRE), a regulatory DNA sequence associated with specific genes. This results in activation of transcription of those genes.

B. cGMP:

1. produced from GTP by guanylyl cyclase;
2. activates cGMP-dependent kinases or other targets
3. example: G-prot. Coupled rhodopsin photoreceptor in rod cells of retina The rods that present in the eye

cGMP and cAMP as Second Messengers:
· **cGMP** is formed from **GTP** (guanosine triphosphate),
· While **cAMP** is formed from **ATP** (adenosine triphosphate).

Almost all body cells use **either cAMP or cGMP pathways** to carry out different cellular functions. These second messengers help **transmit signals inside the cell** after a hormone or ligand binds to a receptor on the cell surface.

Each cell type may rely more on **one pathway than the other**, depending on its function and the type of receptors it expresses.

Summary of how cAMP activates transcription:

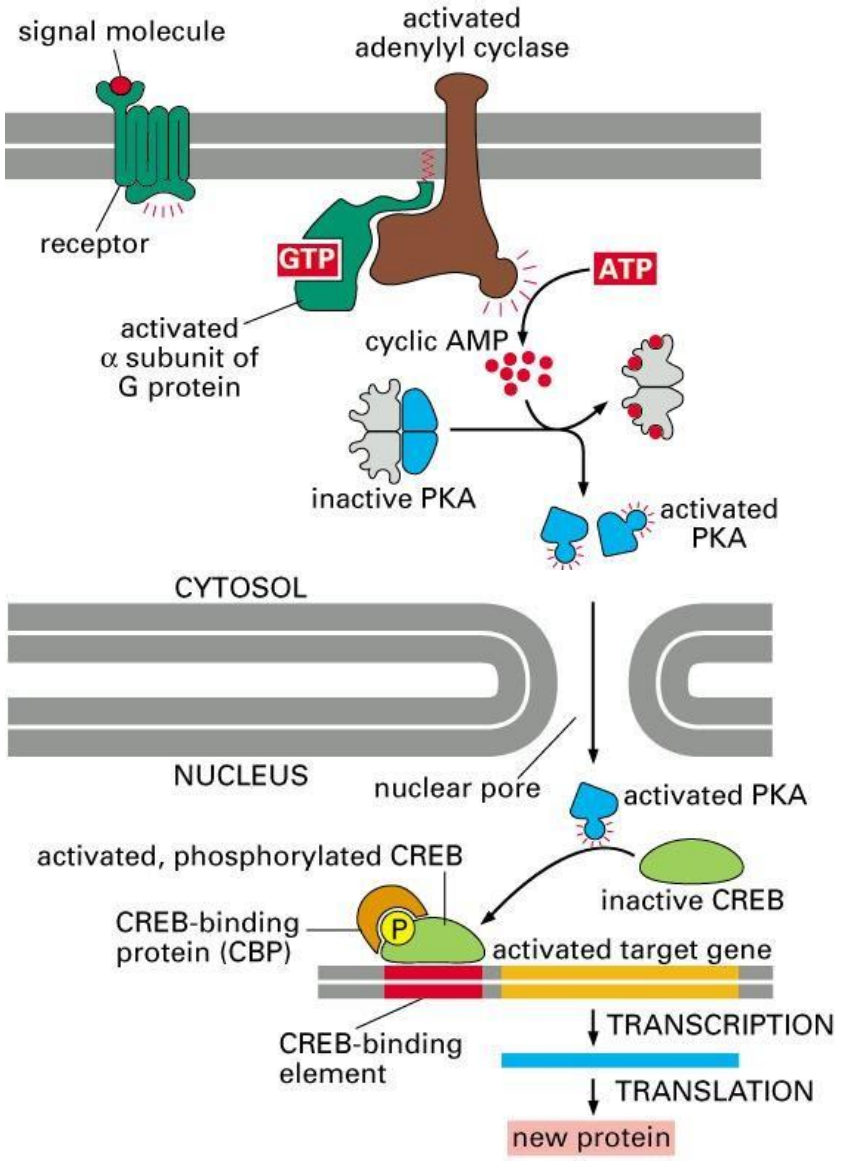


Figure 15–33 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

How cAMP Activates Protein Kinase A (PKA):

· **Protein Kinase A (PKA)** is made up of **four subunits**: two **regulatory** and two **catalytic (active)** subunits.
· In the inactive state, the two **catalytic subunits are bound to the regulatory ones**, preventing them from functioning.
· When **cAMP** is produced inside the cell (via activation of adenylate cyclase), it **binds to the regulatory subunits** of PKA.
· This binding causes the **regulatory and catalytic subunits to dissociate**.
· The **free catalytic subunits** are now **active**, and they can **enter the nucleus**.

What Happens in the Nucleus:

· Inside the nucleus, active PKA **phosphorylates** a transcription factor called **CREB** (cAMP Response Element Binding protein).
· The **CREB** protein then binds to a specific DNA sequence called the **CRE** (cAMP Response Element).
· This binding **initiates transcription** of specific genes into **mRNA**.
· The **mRNA exits the nucleus**, is translated in the cytoplasm, and leads to the **formation of new proteins**.

Cholera Toxin Example:

· **Cholera toxin** causes continuous activation of the **stimulatory alpha subunit (Gs alpha)**.
· This leads to **constant activation of adenylate cyclase**, which results in **persistent cAMP production**.
· As a result, **PKA stays active**, and **new proteins keep being produced**

NOTES

PKA enters the nucleus and phosphorylates CREB (CRE binding protein), which binds to the cAMP response element (CRE), a regulatory DNA sequence associated with specific genes. This results in activation of transcription of those genes.

❖ Pathogens alter cAMP production:

Cholera toxin active subunit catalyzes transfer of ADP ribose from intracellular NAD to the α subunit of G_s, causing it to be continuously active, stimulating adenylyl cyclase indefinitely. This causes ion channels that export chloride to produce a net efflux of Cl- and water, leading to severe diarrhea characteristic of cholera.

Table 75-3 Hormones That Use the Adenylyl Cyclase–cAMP Second Messenger System

Adrenocorticotropic hormone (ACTH)
Angiotensin II (epithelial cells)
Calcitonin
Catecholamines (beta receptors)
Corticotropin-releasing hormone (CRH)
Follicle-stimulating hormone (FSH)
Glucagon
Growth hormone–releasing hormone (GHRH)
Human chorionic gonadotropin (hCG)
Luteinizing hormone (LH)
Parathyroid hormone (PTH)
Secretin
Somatostatin
Thyroid-stimulating hormone (TSH)
Vasopressin (V_2 receptor, epithelial cells)

cAMP, Cyclic adenosine monophosphate.

Cell Membrane Phospholipid Second Messenger System

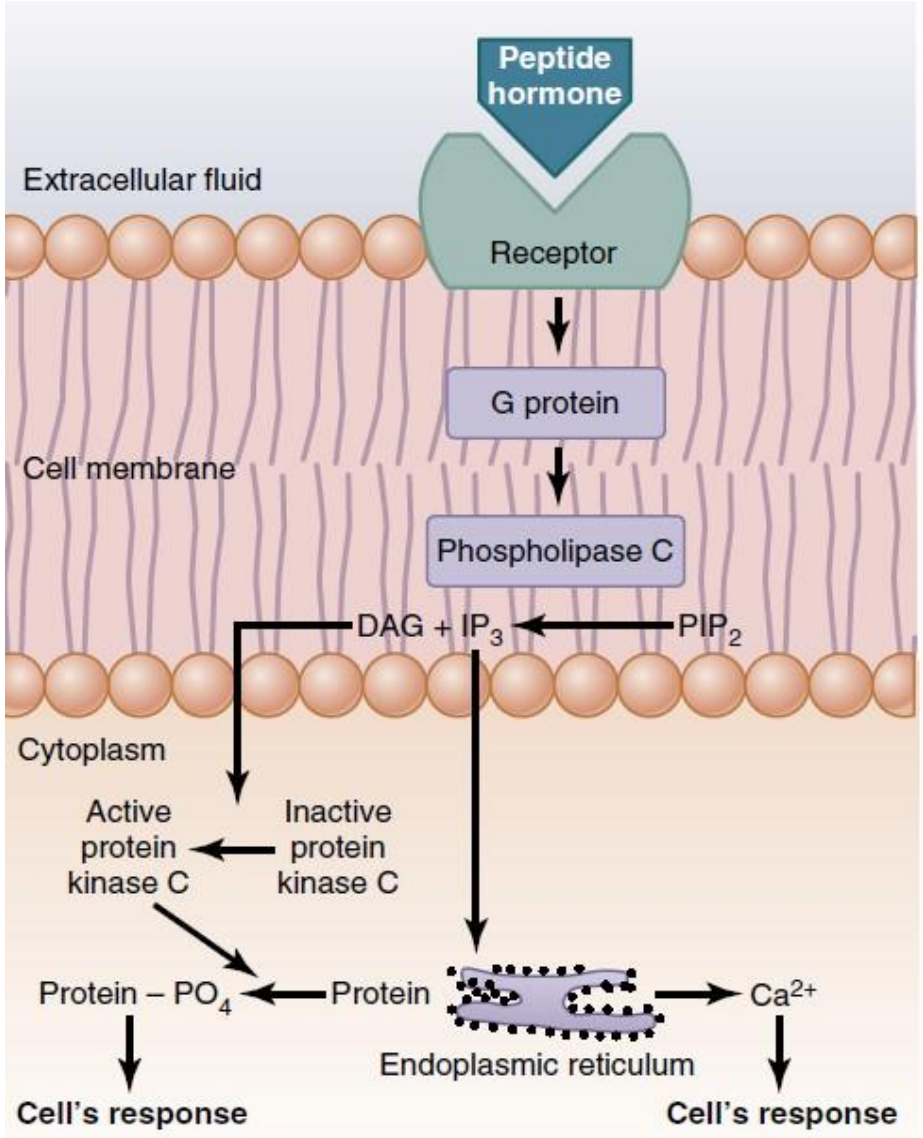
- Some hormones activate transmembrane receptors that activate the enzyme *phospholipase C*
- PLC catalyzes the breakdown of some phospholipids

in the cell membrane, especially *phosphatidylinositol bi-phosphate* (PIP2), into two different second messenger

- products:
- *Inositol triphosphate* (IP3)
- *Diacylglycerol* (DAG).

IP_3 mobilizes calcium ions from mitochondria and the endoplasmic reticulum, Ca ions can lead to different cellular responses

DAG, the other lipid second messenger, activates the enzyme *protein kinase C*, which then phosphorylates a large number of proteins, leading to the cell's response



Phospholipid Hydrolysis as a Second Messenger System:

- When a **peptide hormone** binds to its **receptor** on the **plasma membrane**, this receptor (which has extracellular and intracellular domains) becomes activated.

- Within the **inner leaflet** of the membrane, there are phospholipids such as **PIP2 (phosphatidylinositol 4,5-bisphosphate)**. PIP2 is an important membrane-bound molecule.
- Upon hormone binding, the receptor activates a specific G-protein — **Gq** — which in turn activates the enzyme **phospholipase C (PLC)**.
- **Phospholipase C** hydrolyzes PIP2 into **two second messengers**:
- · **IP3 (inositol 1,4,5-trisphosphate):**
- This is **water-soluble** and diffuses through the cytoplasm to the **endoplasmic reticulum (ER)**, where it triggers the **release of calcium ions (Ca^{2+})** into the cytosol.
- · **DAG (diacylglycerol):**
- This is **lipid-soluble** and stays embedded in the **plasma membrane**. It activates **Protein Kinase C (PKC)**, which then phosphorylates various target proteins, leading to a **cellular response**.
- **Summary:**
- · **PIP2 hydrolysis** produces **two second messengers**: **IP3** and **DAG**.
- · Each messenger has its own pathway:
- · **IP3 → Ca^{2+} release** from ER,
- · **DAG → Activation of PKC**.
- · Together, they mediate the cellular effects of the hormone.

Overview of PIP2

1. Phosphotidylinositol 4,5 bisphosphate (PIP2) triggers a 2-armed signaling pathway
   a. PIP2 is a minor PL in inner leaflet of PM bilayer that is produced by phosphorylation of phosphatidyl-inositol and is involved in signaling
   b. Ligand binding to certain receptors stimulates PIP2 hydrolysis by phospholipase C (PLC)
   c. This produces diacylglycerol (DAG) and inositol 1,4,5-phosphate (IP3), both of which are 2nd messengers
   d. PIP2 hydrolysis is activated by both GPRs and TKRs via different forms of PLC
   e. PLC-ß is stimulated by G_q proteins (specifically **α subunit** in GPCR, $G\alpha_q$) while PLC-γ has SH2 domains that allow binding to activated tyrosine kinases

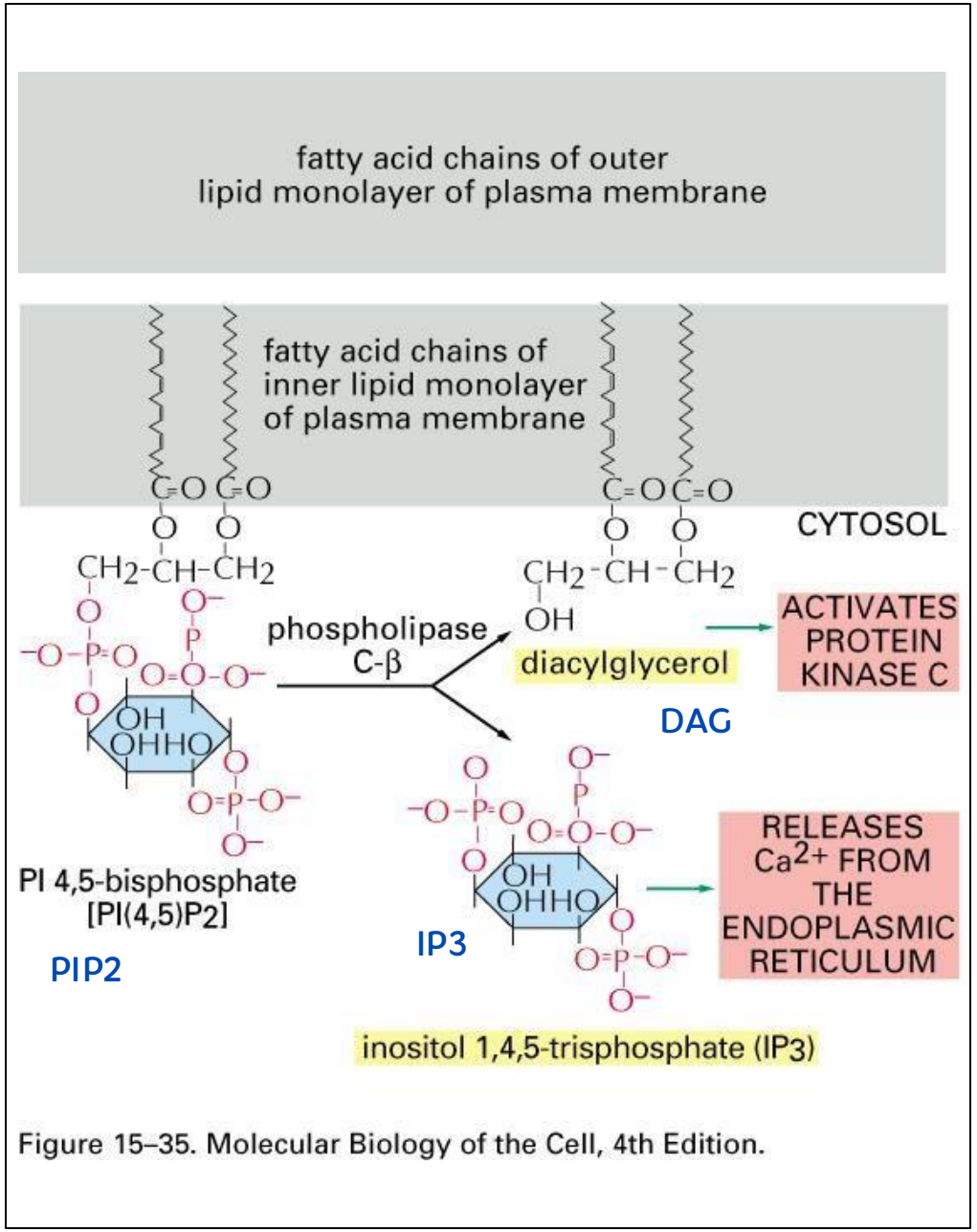


Figure 15–35. Molecular Biology of the Cell, 4th Edition.

PLC- signaling pathway

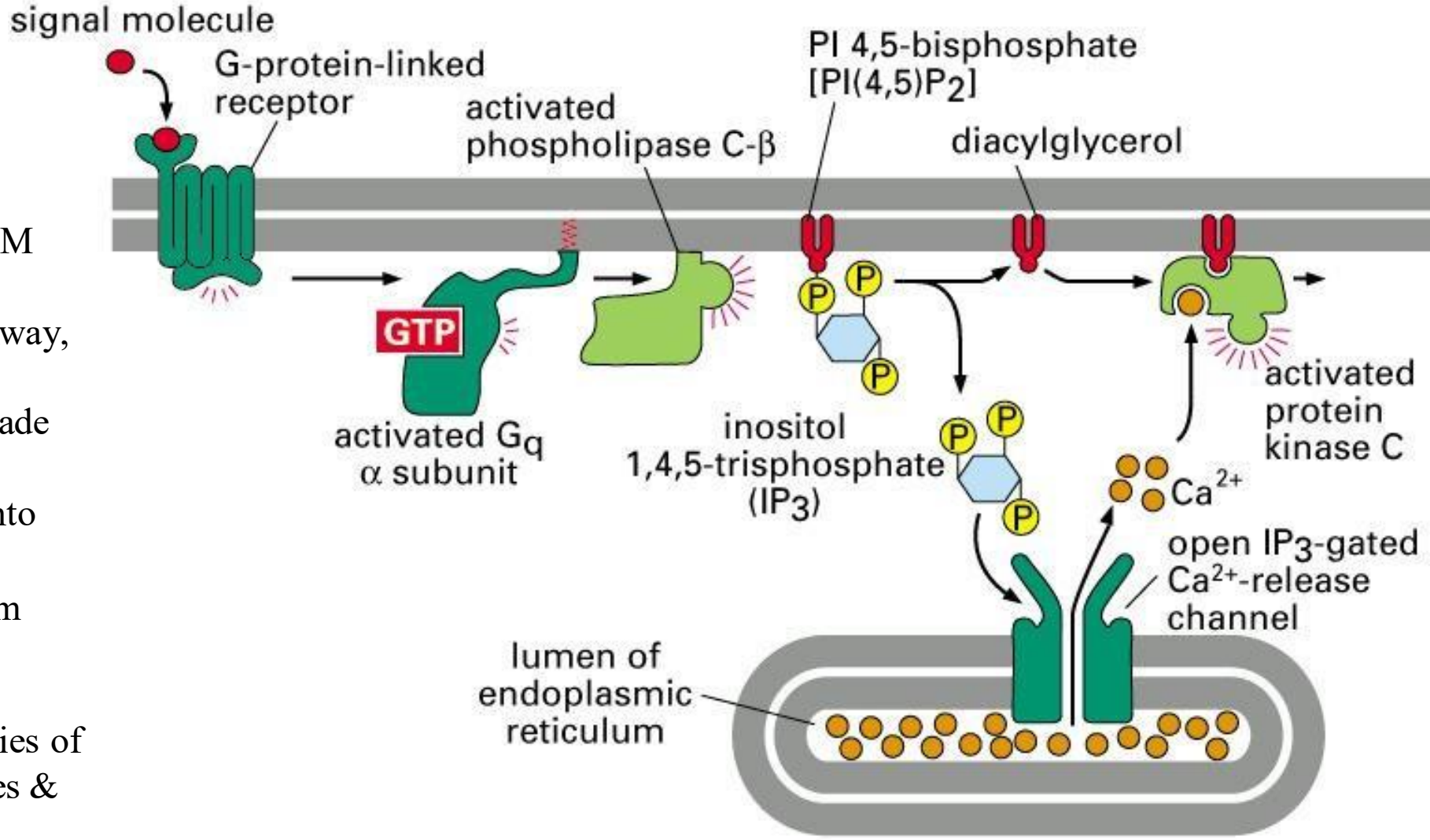
(as we said before) **cAMP** activates **Protein kinase A**, and now we know that **DAG** activates **Protein kinase C**

DAG: Remains associated with the PM
Stimulates the Ca^{+2}-dependent protein kinase C signaling pathway, which activates other targets including the MAP kinase cascade

IP3: Small polar molecule released into cytosol

a. Stimulates Ca^{+2} release from intracellular stores

b. Elevated Ca^{+2} alters activities of target proteins including kinases & phosphatases

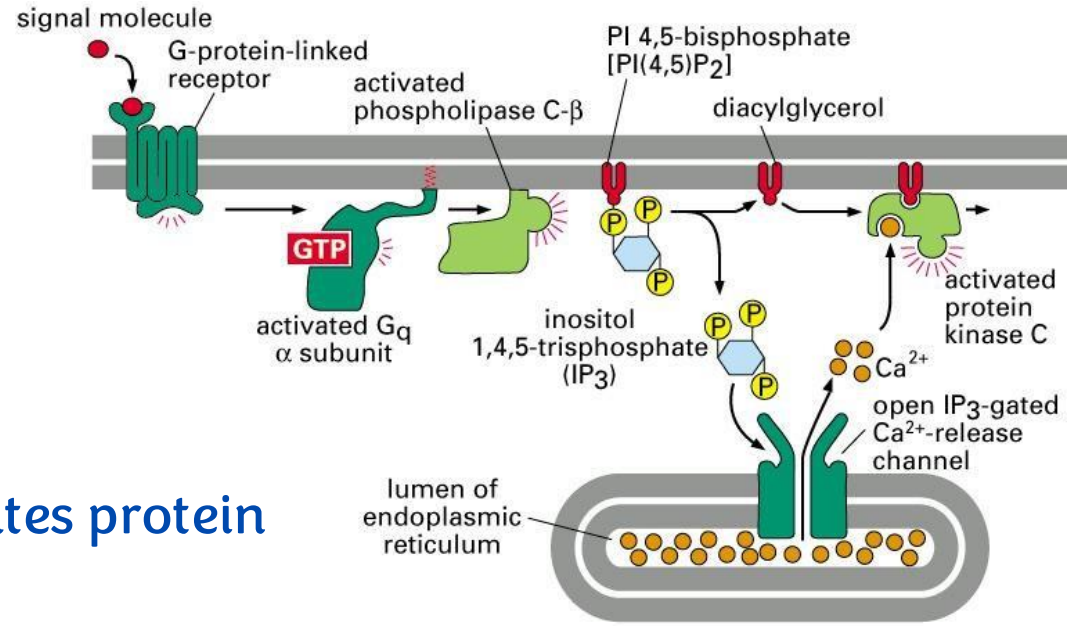


NOTE

PKC Phosphorylates many substrates, can activate kinase pathway,gene regulation

PLC- signaling pathway

- Signal molecule is bound to GPCR
- $G\alpha_q$ is then bound to GTP for it to be activated
- Activated $G\alpha_q$ then activates PLC-β
- PLC-β breaks down PIP2 into DAG and IP3
  - ➢ DAG remains associated with the PM and activates protein kinase C
  - ➢ IP3 enters the cytoplasm to the ER and releases Ca^{+2}



❑ Assuming this process occurs in skeletal muscle, smooth muscle, or any other cell type, both DAG and IP_3 will act complementary to fulfill their roles.

1. IP_3 binds to receptors on the ER, releasing Ca^{2+} essential for muscle contraction (actin-myosin interaction)
2. concurrently, DAG motivates PKC to increase the number of Ca^{2+} receptors , enhancing the muscles sensitivity to Ca^{2+}, thereby strengthening the contraction.

❖ This illustrates the synergistic roles of IP_3 and DAG in muscle contraction.

Table 75-4 Hormones That Use the Phospholipase C Second Messenger System

Angiotensin II (vascular smooth muscle)
Catecholamines (α receptors) (Epinephrine and norepinephrine)
Gonadotropin-releasing hormone (GnRH)
Growth hormone–releasing hormone (GHRH)
Parathyroid hormone (PTH)
Oxytocin
Thyrotropin-releasing hormone (TRH)
Vasopressin (V_1 receptor, vascular smooth muscle)

Some of these hormones can also activate cAMP pathways or affect PL breakdown. This means they may act through more than one signaling system, but all these pathways ultimately achieve the effect required by the ligand.

Calcium-Calmodulin Second Messenger System

- Calcium entry may be initiated by:

(1) Changes in membrane potential that open calcium channels

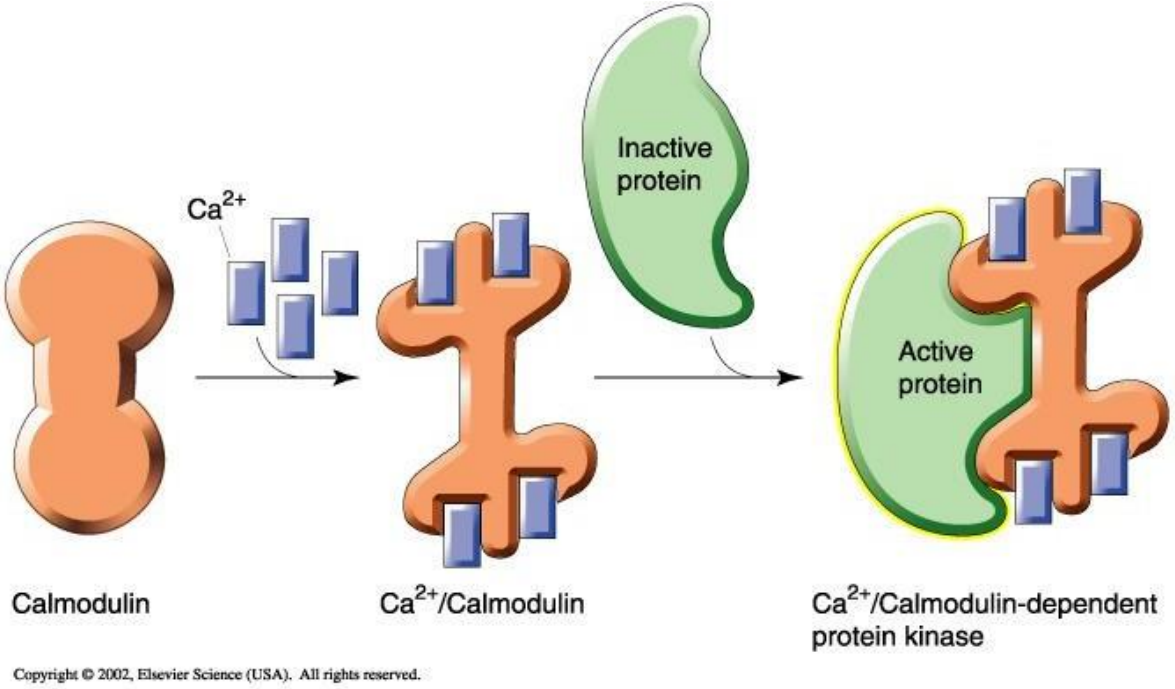
(2) Hormone interacting with membrane receptors that open calcium channels.

- Thus, another second messenger system operates in response to entry of calcium into the cells.

Calcium's concentration inside the cell is usually so small (about 10^{-8} or 10^{-7})
When intracellular Ca^{+2} levels rise, calcium acts as a second messenger.

Calcium-Calmodulin Second Messenger System

- Calcium ions bind with the protein *calmodulin.* (when Ca^{+2} concentration reaches 10^{-5} or 10^{-6})
- This protein has four calcium sites, and when three or four of these sites have become bound with calcium
- The calmodulin changes its shape
- Then initiates multiple effects inside the cell (cellular functions/ response), including activation or inhibition of protein kinases



Calcium-Calmodulin Second Messenger System

Many of the Ca^{2+} -dependent cellular events are triggered by activation of **calmodulin**, an intracellular Ca^{2+} -binding protein

- The Ca^{2+} – calmodulin complex activates **Ca^{2+} –calmodulin dependent protein kinase (CaM kinase)** (or activates another kinase)
- Activation of CaM kinase by the Ca^{2+} – calmodulin complex (through auto phosphorylation) is similar to activation of PKA by cAMP.

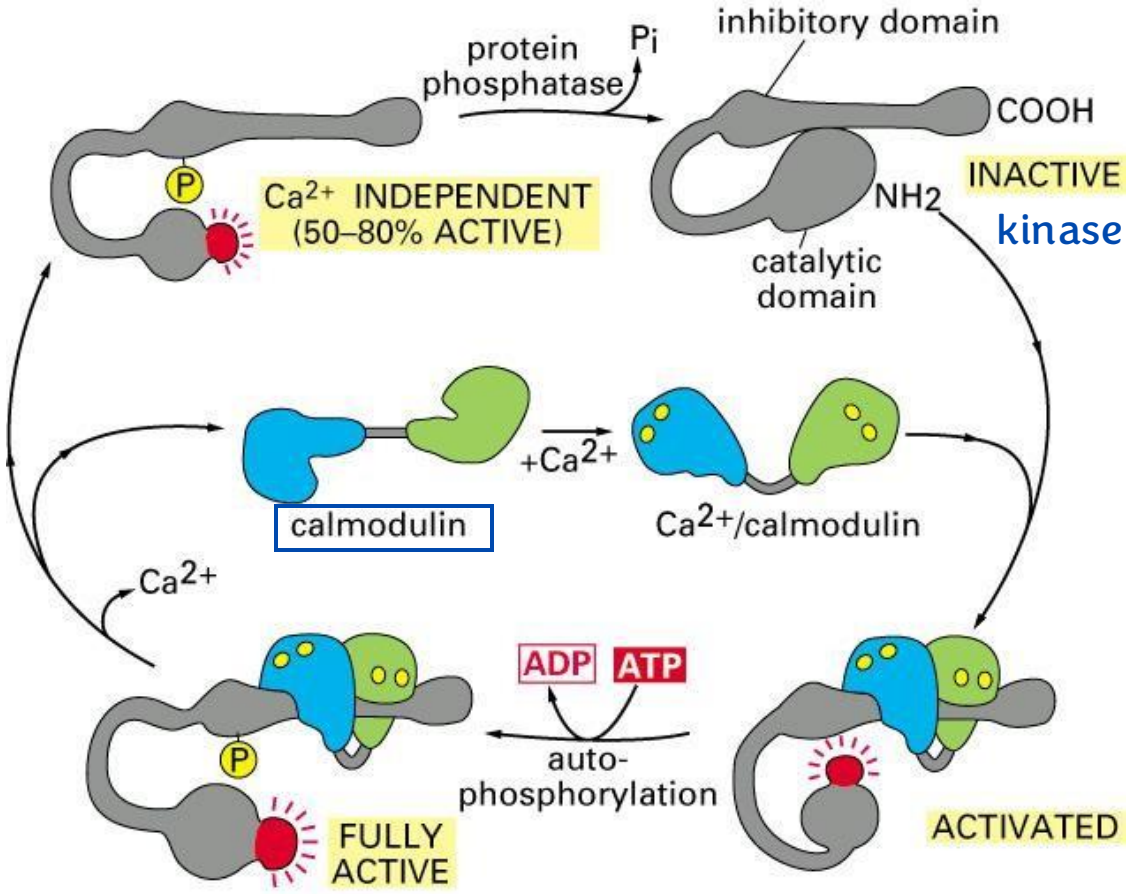


Figure 15–41. Molecular Biology of the Cell, 4th Edition.

- A hormone binds to a GPCR.
- This activates Gq protein.
- Gq activates PLC-β
- PLC-β breaks down PIP2 into DAG and IP3.

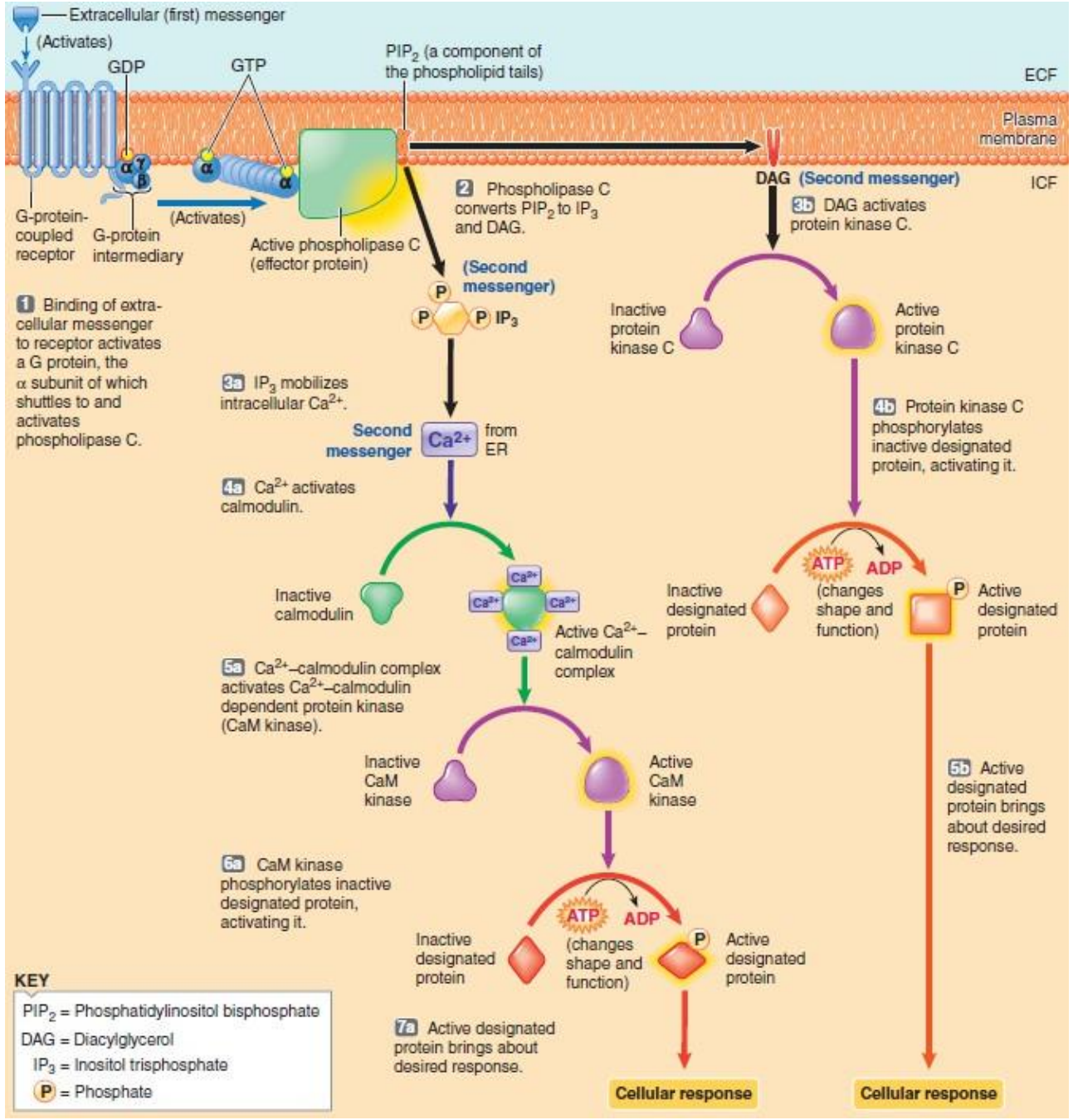
- DAG activates protein kinase C (PKC).
- PKC uses ATP to phosphorylate and activate other proteins

- IP3 releases Ca^{2+} from the ER into the cytosol

Ca^{2+} binds to calmodulin, forming a Ca^{2+}/calmodulin complex. This complex activates CaM kinases and other proteins by phosphorylation, leading to a cellular response.

- There are 3 second messengers in this pathway:

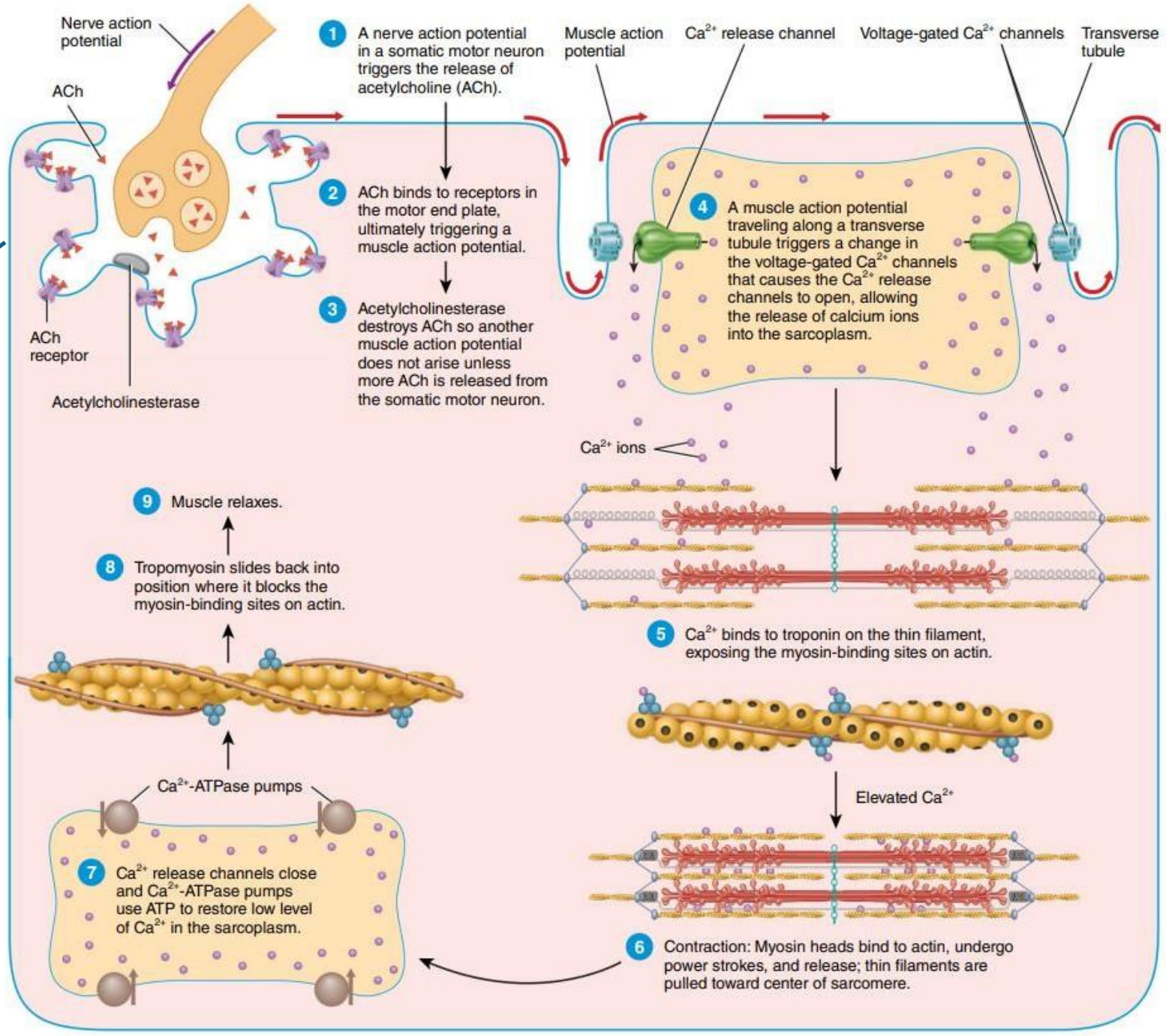
1-DAG 2-IP3 3-Ca^{2+}/calmodulin complex.



-This figure wraps up and summarizes what the Dr. explained before-

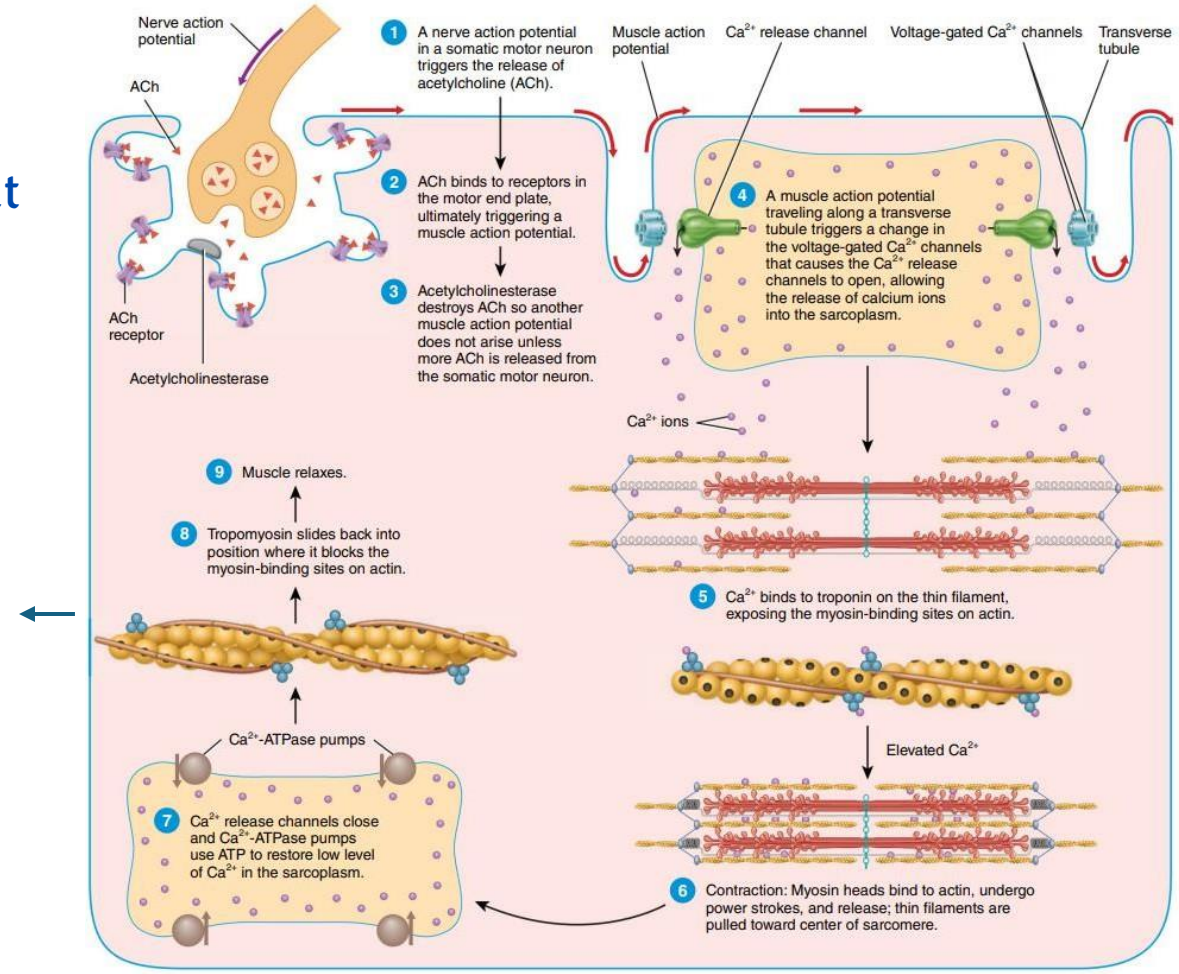
Other means by which Ca^{+2} can enter the cells

- When an action potential reaches the presynaptic terminal, voltage-gated calcium channels open, allowing Ca^{2+} to enter the neuron. The influx of calcium triggers exocytosis, causing the release of neurotransmitters. **This is one mechanism by which intracellular calcium levels increase** – through ion channels in the nerve terminal.



- Then if the neurotransmitter binds to receptors on the postsynaptic cell—for example, a skeletal muscle fiber—it triggers an action potential in that muscle fiber. Once the action potential reaches the endoplasmic reticulum (sarcoplasmic reticulum in the case of muscles), it causes the release of calcium ions. These calcium ions bind to actin filaments, which initiates the sliding of actin and myosin filaments, leading to muscle contraction.

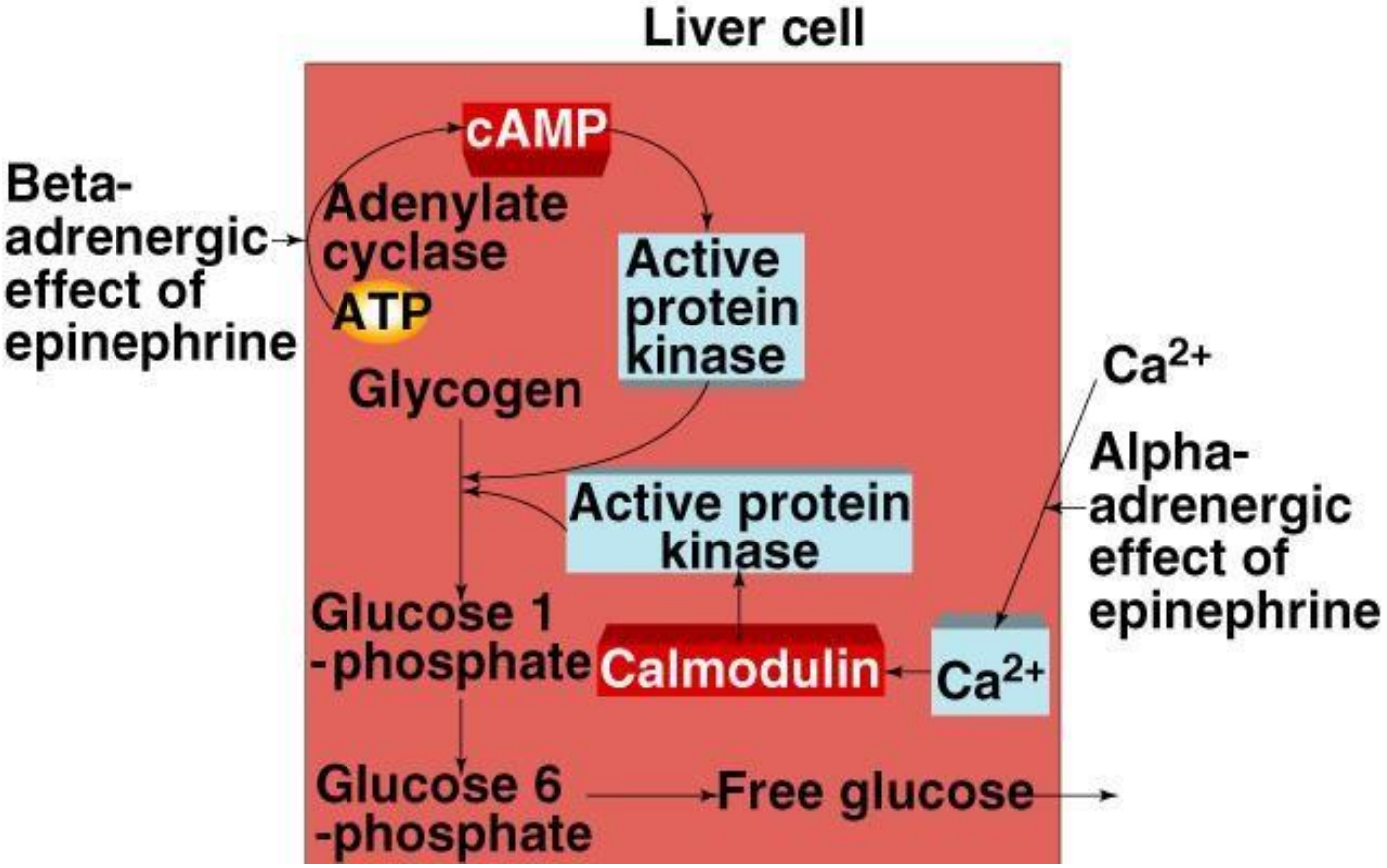
- Once calcium is released, it binds to calmodulin, forming a calcium-calmodulin complex. This complex activates specific protein kinases, which phosphorylate target proteins and trigger the appropriate cellular response.



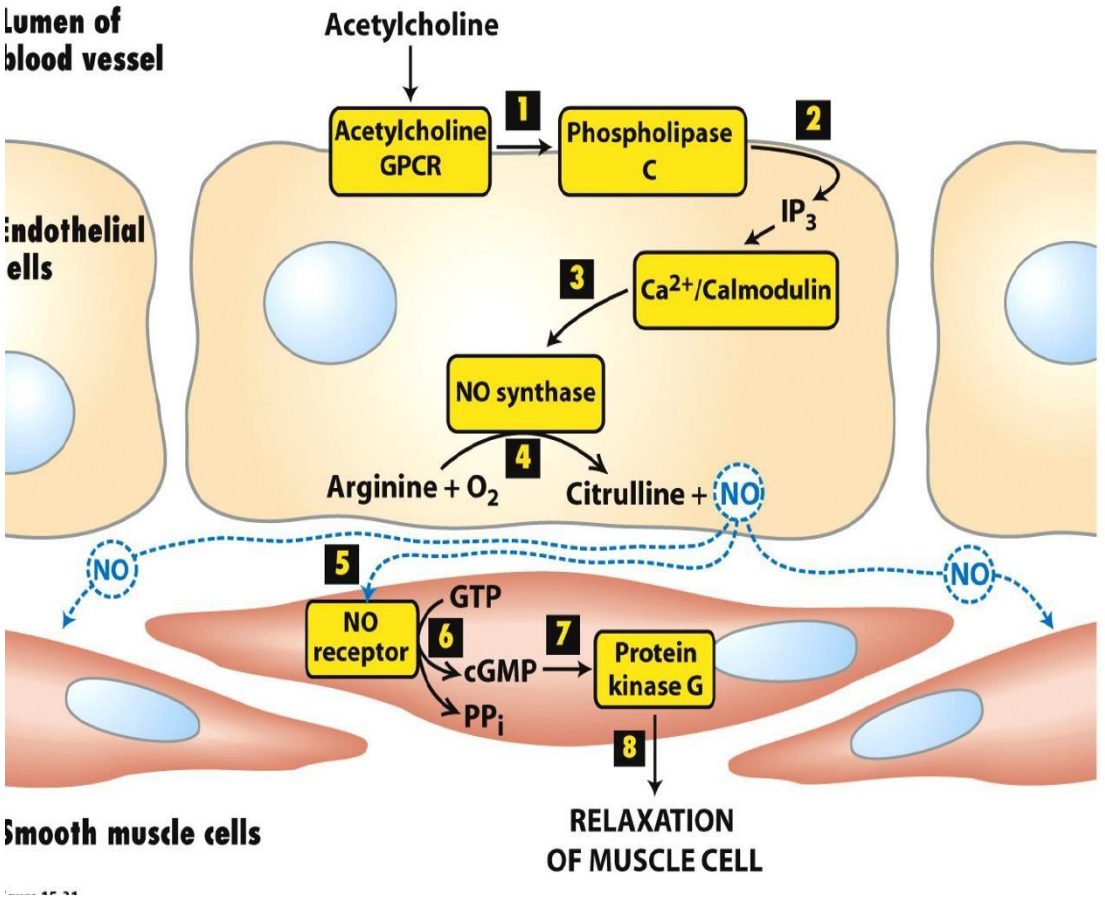
❖ To sum it up, Calcium levels in the cytosol can be increased not only through hormonal signaling but also through other mechanisms. These pathways allow calcium to function as a second messenger and carry out its role in various cellular responses.

Examples of Second messenger pathways

Epinephrine effect using 2 second messenger systems

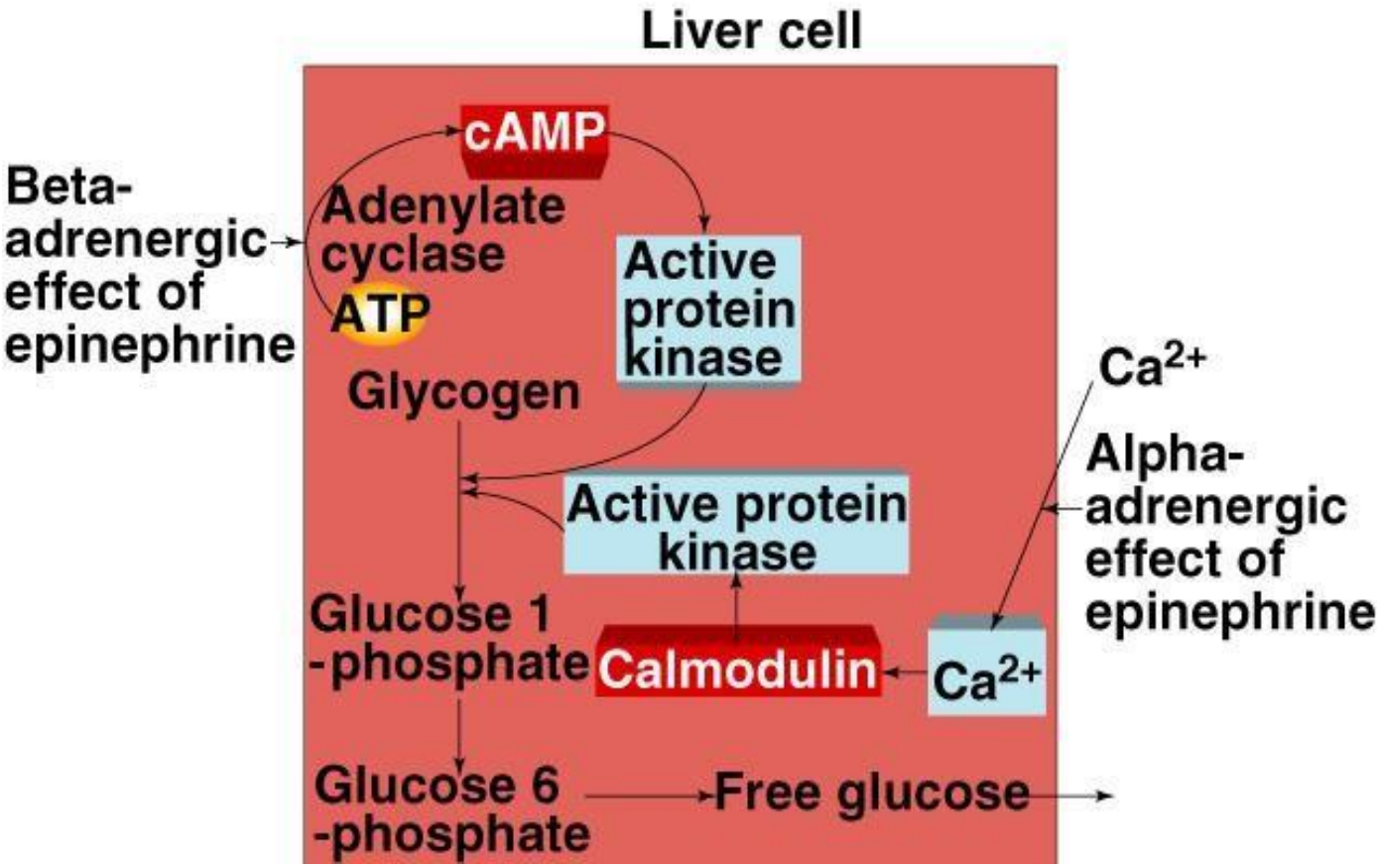


NO signaling through Ca-M second messenger system



Examples of Second messenger pathways

Epinephrine effect using 2 second messenger systems



We know that catecholamines (amino acids derivatives) like epinephrine can act through either the cAMP pathway or the PLC pathway, depending on the receptor.

- If epinephrine binds to an alpha-adrenergic receptor, it activates the PLC pathway, leading to the formation of the calcium-calmodulin complex, which then activates protein kinase and promotes the conversion of glycogen to glucose.
- On the other hand, if epinephrine binds to a beta-adrenergic receptor, it activates the cAMP pathway, which also leads to protein kinase activation and glycogen breakdown.

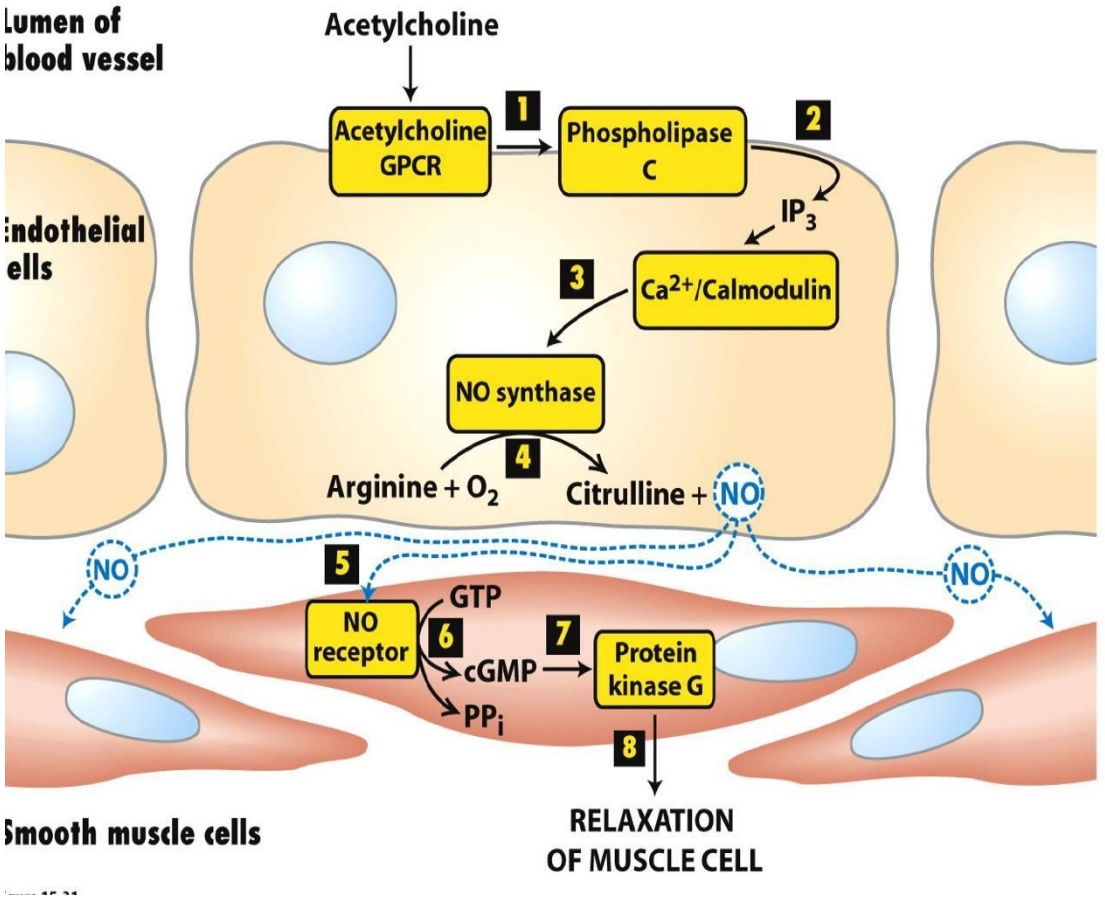
In both cases, the final effect is increased blood glucose, but the second messenger system used depends on the receptor type. That's why epinephrine can work through more than one second messenger pathway.

Examples of Second messenger pathways

1. When acetylcholine binds to GPCRs on post-synaptic cells, it activates the PLC pathway, leading to the formation of DAG and IP_3 from PIP_2.
2. IP_3 then triggers Ca^{2+} release from the ER in muscle cells.
3. The released Ca^{2+} binds to calmodulin, forming the calcium-calmodulin complex, which activates the enzyme nitric oxide synthase (NOS).
4. NOS converts arginine into citrulline and produces nitric oxide (NO). Since NO is lipid-soluble, it diffuses freely into neighboring smooth muscle or endothelial cells.
5. Inside these cells, NO activates guanylyl cyclase,
6. This leads to the production of cGMP, another second messenger.
7. cGMP then activates protein kinase G (PKG), which promotes smooth muscle relaxation.

❖ So, in this pathway, we have 4 second messengers:
IP_3 and calcium-calmodulin (inside endothelial cells) that led to NO production,
NO acting without a membrane receptor due to its lipid solubility, and cGMP inside target cells that drives the final cellular response.
(NO and cGMP inside smooth muscle cells)

NO signaling through Ca-M second messenger system



Amplification of signal

- Signal amplification means that a small amount of ligand doesn't need to bind to all available receptors to generate a maximal cellular response.
- From the first lecture (Signal Transduction 1) , we learned that the cellular signaling system can amplify the signal – meaning the output is much larger than the input (as you can see in the figure, 1 molecule of ligand/hormone activated 10 adenylyl cyclase which gave us 1000 cAMP, and so on).

❑In this way, even the slightest amount of hormone acting on the cell surface can initiate a powerful cascading activating force for the entire cell. (eventually leading to a full cellular/physiological response)

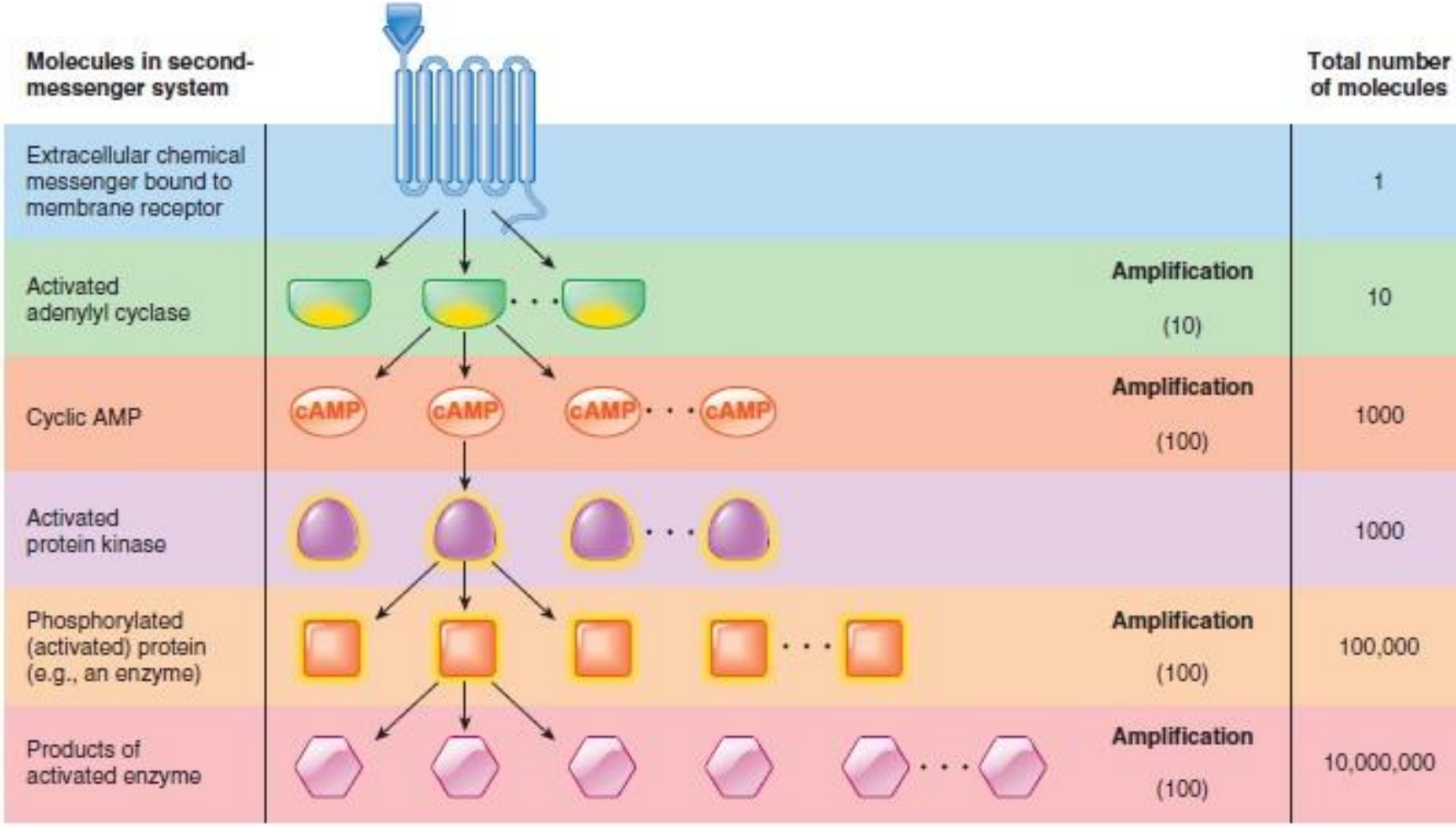


Figure 4-27 Amplification of the initial signal by a second-messenger pathway. Through amplification, very low concentrations of extracellular chemical messengers, such as hormones, can trigger pronounced cellular responses.

NOTE *Amplification* means that the output of a system is much greater than the input.

5 downstream kinases activated by different signaling cascades

Summary

There are different types of receptors, and when they bind to a signaling molecule, they trigger distinct intracellular signaling pathways/cascades. These cascades serve to transduce the extracellular signal into an intracellular response through a series of reactions, where each step activates the next. Ultimately, these pathways converge on the activation of protein kinases. These kinases either enter the nucleus to regulate gene transcription and protein synthesis, or they activate existing proteins to produce an immediate cellular response.

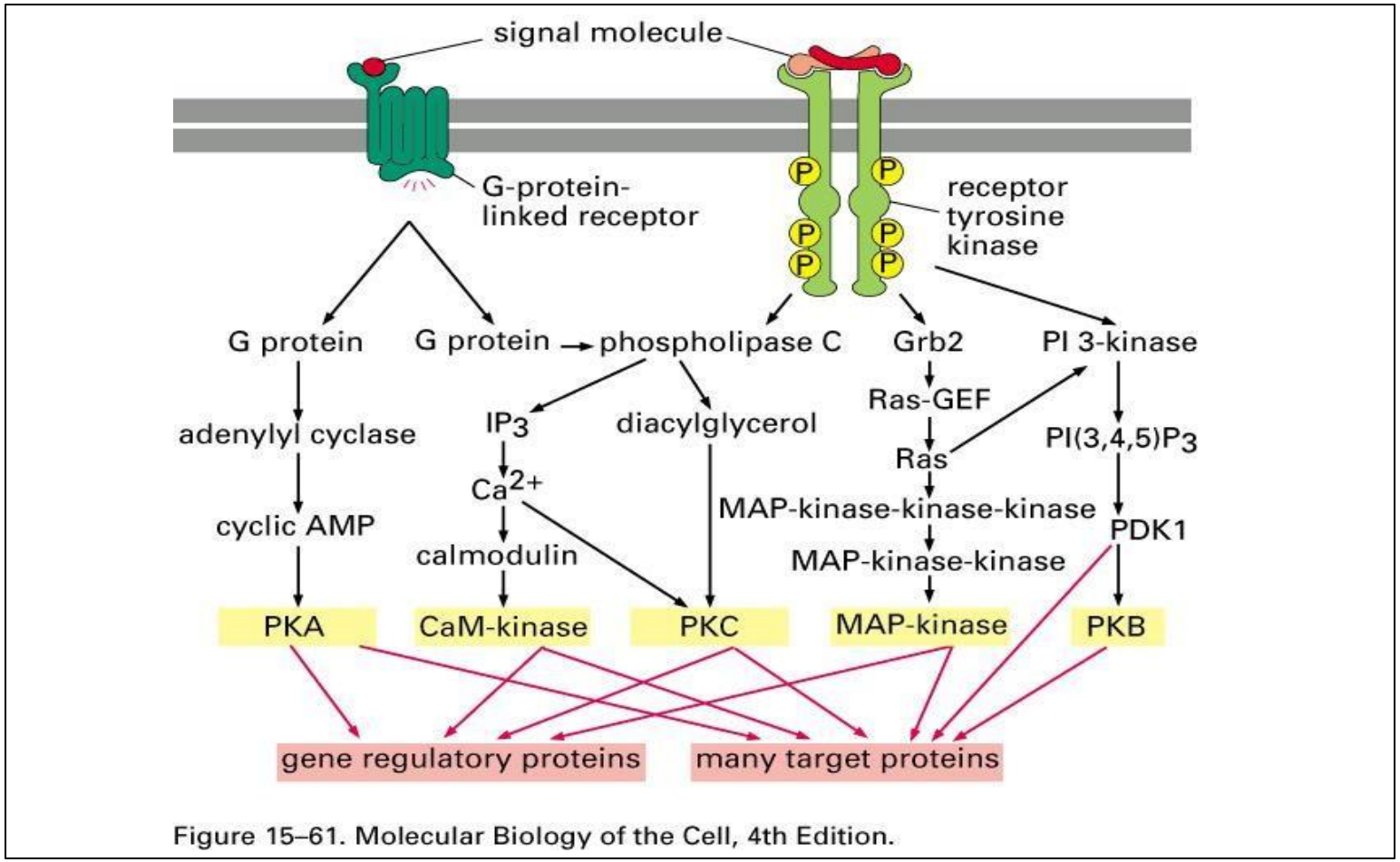


Figure 15–61. Molecular Biology of the Cell, 4th Edition.

NOTE mitogen-activated protein kinase (MAPK or MAP kinase)

Additional Resources:

رسالة من الفريق العلمي:

Extra References for the Reader to Use:

IP3 DAG Calcium Pathway YouTube short video
https://youtu.be/uuDm8nxPtQU?si=ZkeI_zaYOn0nARWj

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			PowerPoint notes from the doctor's file have been added wherever mentioned, under the title '**NOTES**'
V1 → V2			