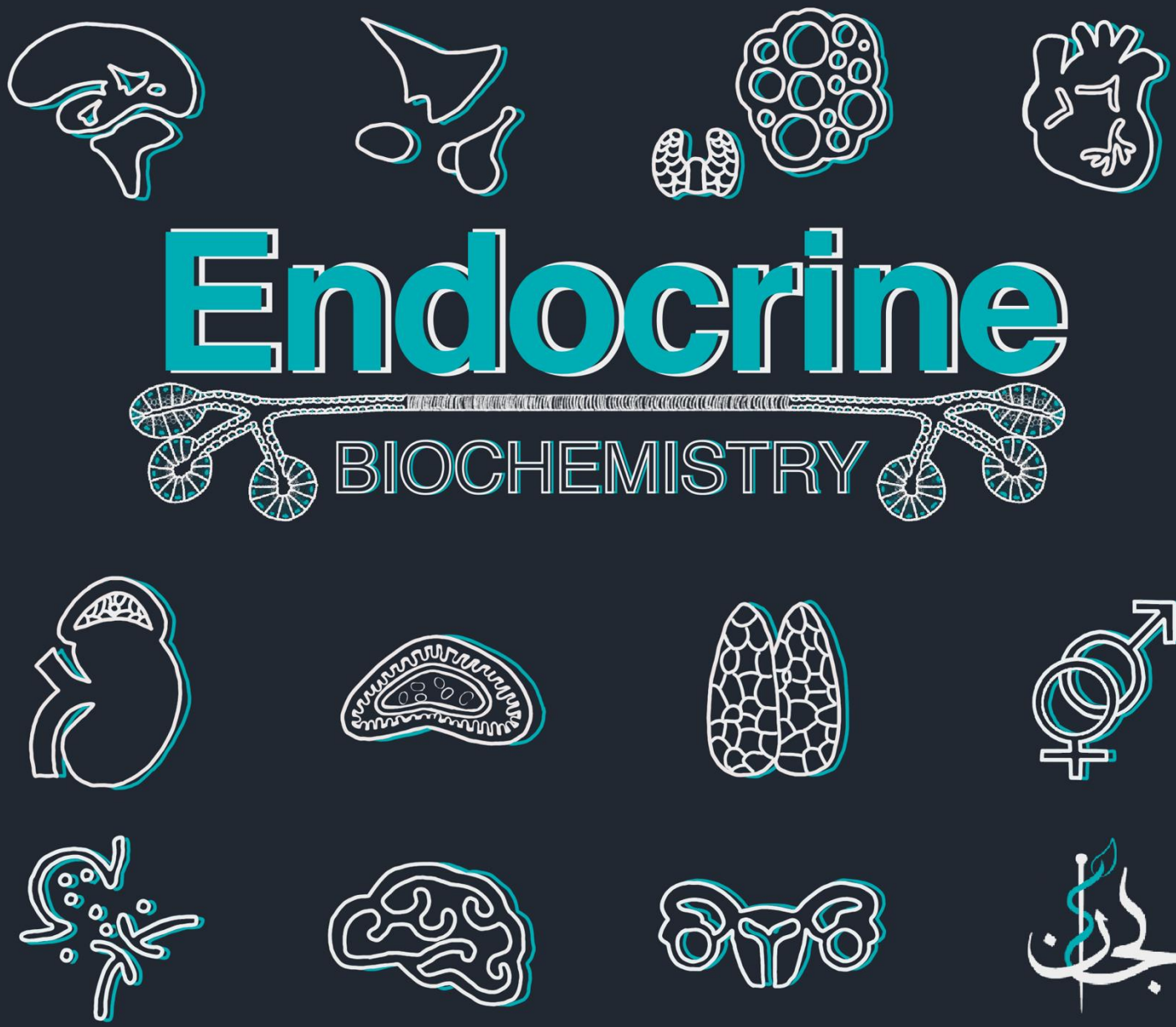


Title: Sheet 3 – Cell Surface Receptors

Writer: Mohammad Aboshaban
Science: Mohammed Bushnaq
Grammar: Dena Kofahi
Doctor: Mamoun Ahram

What's in red and between [] is written in the slides but wasn't mentioned by the doctor.

QUICK RECAP: In the previous lectures we talked about hormones, their mechanism of action and how they're regulated (introduction). Then we said that hormones can be classified into two types according to their solubility and type of receptor to which they bind to:

1. Lipid-soluble hormones: Which bind to a receptor **INSIDE** the cell. (discussed in the previous sheet)
2. Water-soluble hormones: Which bind to a **cell surface receptor**. (our topic)

CELL SURFACE RECEPTORS 00:00

Although we talked about some of these receptors before, their mechanism of action and how they TRANSDUCE signals (a signal is transmitted from one molecule to another), this lecture wraps up all of the information we have discussed before.

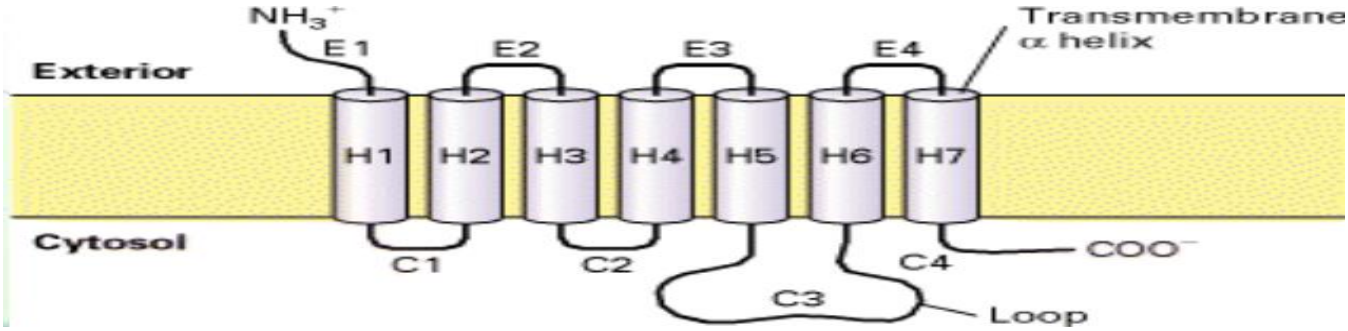
Cell surface receptors can be divided into 2 categories:

1- GPCR (G protein-coupled receptors).
2- Enzyme-linked cell-surface receptors.

G PROTEIN-COUPLED RECEPTOR 00:55

-All G protein-coupled receptors (GPCRs) contain seven membrane-spanning regions or seven domains. Portions of these domains can extend to the outside or inside of the cell.

-[They all mediate a similar signaling pathway].



HOMOLOGY التجانس: The state of having the same relation or structure.

- There are different types of receptors and they are really important because about 40% of drugs target (يهاجم) receptors, and 25% of drugs overall target (يهاجم) GPCRs. Although all GPCRs are structurally similar, their amino acid sequences generally are quite dissimilar. They function similarly but in terms of homology they are very different. To clarify what I said:

- β1- and β2-adrenergic receptors are 50 percent identical.
- α- and β-adrenergic receptors exhibit even less homology.

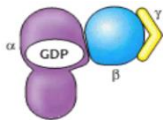
*But, overall, there are 2 important domains or segments that are important in a GPCR and it's determined by the specific amino acid of sequence of each receptor, these 2 domains are:

1- Ligand Binding Domain, which extends to the outside.
2- G-protein Binding Domain.

-They have homology in terms of having these two domains, but they have differences as well to specify the size of the G-protein that binds to the receptor.

/I know it's a silly note but please recall that a GPCR is not the same as a G-protein/

G-PROTEIN 02:37



-[G proteins are intermediary in signal transduction from the seven transmembrane (7TM) receptors.]

-There are different types of G-proteins. They are heterotrimers composed of three subunits: α, β, and γ. The α subunit is the important functional subunit while β and γ are regulatory.

G_{α} class	Initiating signal	Downstream signal
$G_{\alpha s}$	β-Adrenergic: amines, glucagon, parathyroid hormone, many others	Stimulates adenylate cyclase
$G_{\alpha i}$	Acetylcholine, α-adrenergic: amines, many neurotransmitters	Inhibits adenylate cyclase
$G_{\alpha q}$	Acetylcholine, α-adrenergic: amines, many neurotransmitters	Increases IP_3 and intracellular calcium
$G_{\alpha t}$	Photons	Stimulates cGMP phosphodiesterase
$G_{\alpha 13}$	Thrombin, other agonists	Stimulates Na^+ and H^+ exchange

- Information is transduced via changes in the concentration of second messengers.

Examples of second messengers:

cyclic AMP and cyclic GMP, calcium ion, inositol 1,4,5- trisphosphate (IP3), diacylglycerol (DAG).

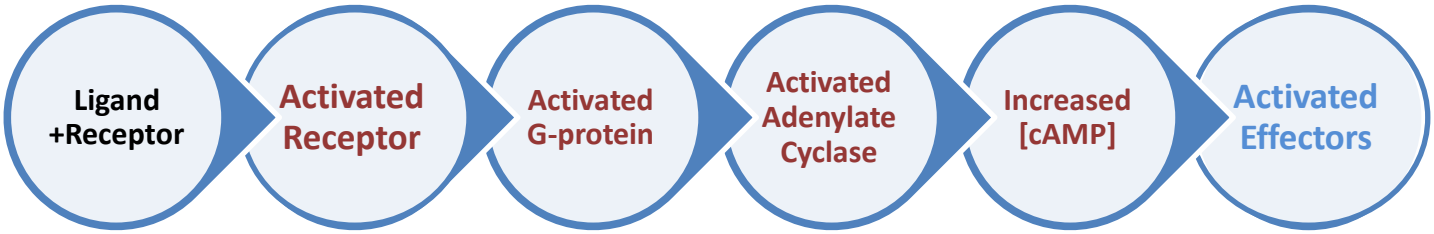
-Why do cells use secondary messengers? Or, in other words, why are they good?

- Second messengers are often free to diffuse to other compartments of the cell since they are small in size.
- The signal may be amplified significantly in the generation of second messengers. This is a very important aspect in the endocrine system. Why? Because we have a very small amount of hormones in the system. In order for the system to be efficient, the signal must be amplified.
- The use of common second messengers in multiple signaling pathways often results in cross-talk between different signaling pathways.

/The doctor said something that is not in the scope of our lecture, please refer to what he said: 05:34 -07:11/

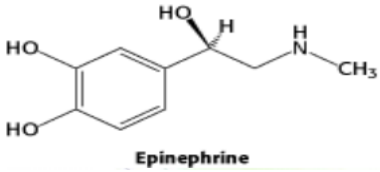
-Hormones are classified according to many factors. One of these factors is according to second messengers. Refer to the table in slide 6 (it's not for memorizing).

The Overall Signal Transduction Pathway: 07:58

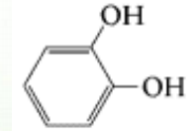


ADRENERGIC RECEPTORS: An example 08:25

-This protein binds epinephrine (also called adrenaline), a hormone responsible for the "fight or flight" response. Epinephrine is a catecholamine hormone. Epinephrine is classified as a catecholamine because it has a "catechol" ring in its structure.



Epinephrine



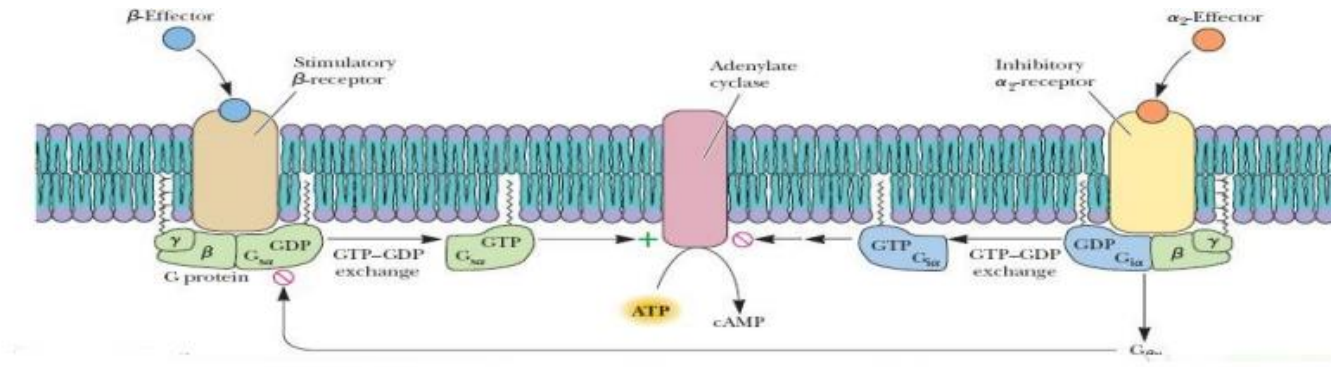
-As we learned in pharmacology, **Epinephrine** activates both α and β receptors.

Alpha Receptors		Beta Receptors	
1. Vasoconstriction of a. Coronary arteries b. Veins 2. Motility of GIT smooth muscle cells			
α1 (postsynaptic)	**α2 (presynaptic)**	**β1 (postsynaptic)**	**β2 (postsynaptic)**
Gq protein coupled Activates **Phospholipase C** **PIP2 →IP3 + DAG**	**Gi protein** coupled Inhibits **Adenyl Cyclase** **ATP →X→cAMP**	**Gs protein** coupled Activates **Adenyl Cyclase** **ATP → cAMP**	
1. Vasoconstriction of blood vessels of a. Skin b. GIT c. Kidney d. Brain 2. Contraction of smooth muscles of a. Ureter b. Vas deferens c. Urethral spinchter d. Uterus e. Cilliary body (mydiarisis) 3. Glucose metabolism a. Gluconeogenesis b. Glucolysis	1. Glucose metabolism a. Inhibits insulin release b. Stimulates glucagon release 2. Contraction of anal spinchter 3. Inhibits release of Norepinephrine	1. The heart a. ↑heart rate (+ chronotropic) b. ↑impulse conduction (+dromotropic) c. ↑contraction (+ inotropic) d. ↑ejection fraction 2. ↑renin release by Juxtaglomerular cells 3. ↑hunger a. ↑ghrelin release by stomach	1. Smooth muscle relaxation of a. Bronchus b. Bronchioles c. Detrusor muscle d. Uterine muscle 2. Contraction of urethral spinchter 3. ↑renin release by Juxtaglomerular cells 4. Glucose metabolism a. Inhibits insulin release b. Stimulate i. Gluconeogenesis ii. Glucolysis 5. Lipolysis 6. Thickened salivary secretion

/I'm not really sure if these tables are for memorizing or not although we studied them in detail before. I think you should know that α1 is associated with Gq, α2 with Gi, and β1 with Gs/

Optimism is the faith that leads to achievement ☺

THE SIGNAL TRANSDUCITON OF EPINEPHRINE 09:29

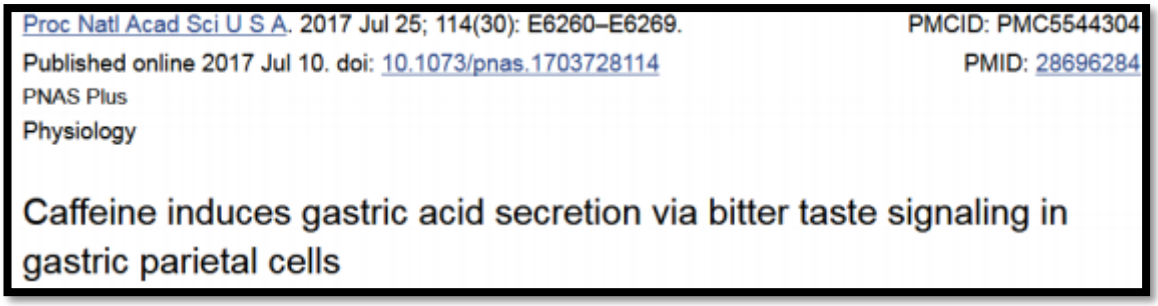


-The idea here is that the effector (ligand) binds to the receptor, then the α subunit (of the G-protein) is released and travels ALONG the membrane which makes it easier to find its target "Adenylate cyclase" instead of dissociating and moving in a three-dimensional area (cytoplasm), depending on the random collisions of molecules.

-What's important about the α subunit is that it is not an enzyme, but it does have intrinsic enzymatic activity (acts on the molecule itself). The activity is GTPase activity which hydrolyzes GTP to GDP, which allows the α subunit to rebind to the β and γ subunits in order to terminate the current signal and get ready to be activated again by another signal.

**So, when the α subunit reaches its target adenylate cyclase, cAMP is produced. What are the cellular effects of cAMP?*

- ↑ degradation of storage fuels.
- ↑ **secretion of acid by gastric mucosa**:
  Why does coffee increase the secretion of acid which causes ulcers?


Proc Natl Acad Sci U S A. 2017 Jul 25; 114(30): E6260–E6269. PMCID: PMC5544304
Published online 2017 Jul 10. doi: 10.1073/pnas.1703728114 PMID: 28696284
PNAS Plus
Physiology

Caffeine induces gastric acid secretion via bitter taste signaling in gastric parietal cells

- Dispersion of melanin pigment granules.
- ↓ aggregation of blood platelets.
- Opening of chloride channels.

NOW YOU SHOULD OPEN SLIDE NO.15 VERY QUICKLY AND STUDY THE FOLLOWING:

-So, the story started when the hormone (ligand) has bound to the receptor, and then the cAMP concentration increased in the cytosol. What's next?

cAMP will bind to the regulatory units of protein kinase A which results in the dissociation of the catalytic subunits. Oh no, Protein kinase A (PKA) is active now! PKA then phosphorylates many proteins like glycogen synthase, which will inhibit its activity. Also, it will phosphorylate glycogen phosphorylase, activating it and inducing the breakdown of glycogen to generate energy.

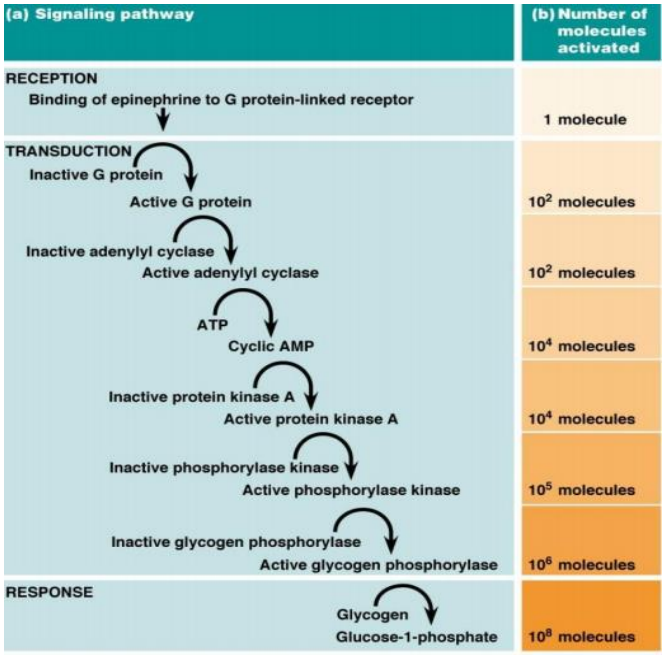
-[The protein kinase usually phosphorylates either Serine or Threonine.]

*Q: Which type of G-protein is/are the cause of increasing cAMP, thus activating protein kinase A?
Answer: Gs.

*Ok another Q:
Which type of G-protein is/are associated with gluconeogenesis and glycolysis?
Answer: Gs & Gq

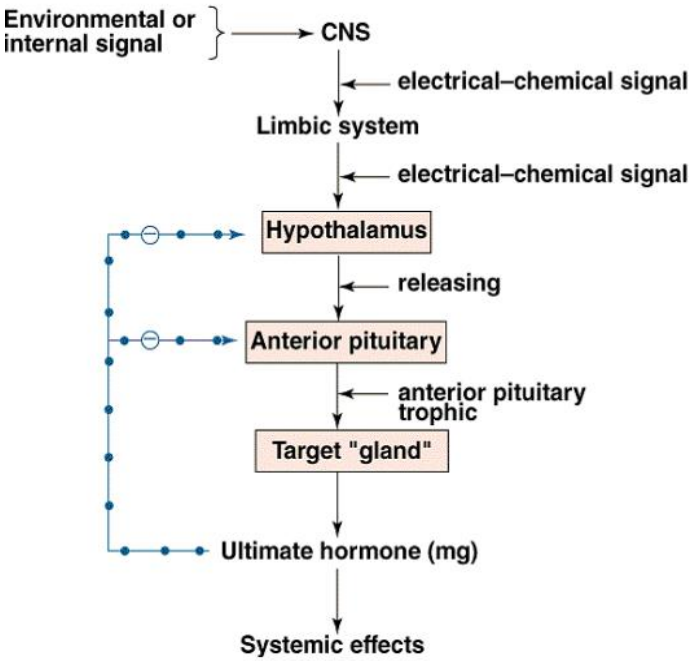
SIGNAL AMPLIFICATION 12:56

-Although we have a very small amount of hormone (Atto- to nano-molar range (10^{-18} to 10^{-9} mol/L)) present in the body, its effects are humongous because of signal amplification. The idea is that one molecule of epinephrine can active one receptor and this receptor can activate multiple G-proteins and each one of them can activate a **SINGLE** adenylate cyclase. Then each adenylate cyclase can produce thousands of cAMP molecules that can then bind to protein kinase A, and so on.



-SIDE NOTE: One epinephrine molecule can bind to more than one receptor because it can dissociate and bind to another receptor as well.

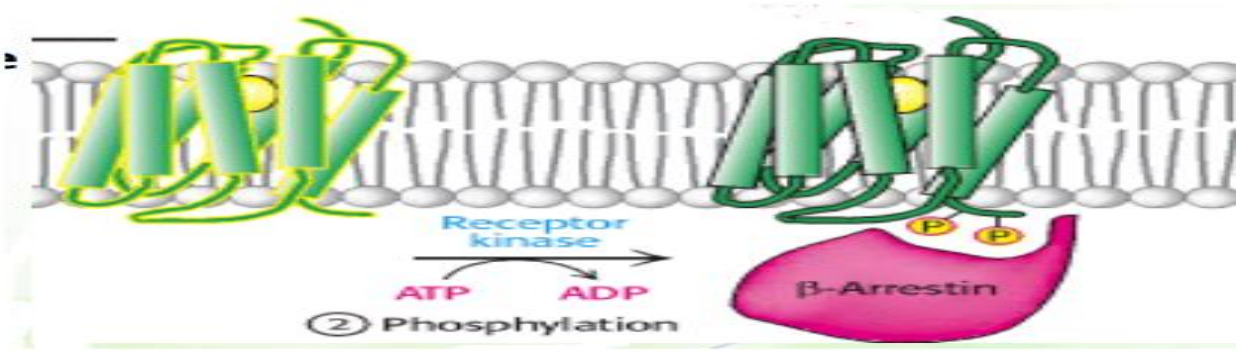
-The amplification can occur at the level of hormones themselves as well. We said earlier that hormones secreted from the hypothalamus can affect the anterior pituitary gland, inducing it to release its hormones that can then affect other target glands. Well, you can have one hormone secreted from the hypothalamus resulting in the secretion of hundreds or thousands of hormones from the anterior pituitary gland and each hormone can then target a certain gland or tissue. These tissues can then produce hundreds or thousands of hormone molecules.



TERMINATION 15:11

-Termination of the signal is important to prevent **Desensitization** (it was defined in sheet 1 at page 9). So how is the signal terminated?

- **Dissociation of the hormone**: Once it's dissociated, the receptor is inactivated.
- **GTPase activity of Gα subunit:** Hydrolyzes GTP to GDP.
- **Hydrolysis of cAMP (by phosphodiesterase)** to AMP.
- **Phosphorylation of the hormone bound-receptor by receptor kinase.** So when it's phosphorylated, it binds to a protein known as **β-Arrestin,** which makes the receptor inactive even if the ligand is bound to it.

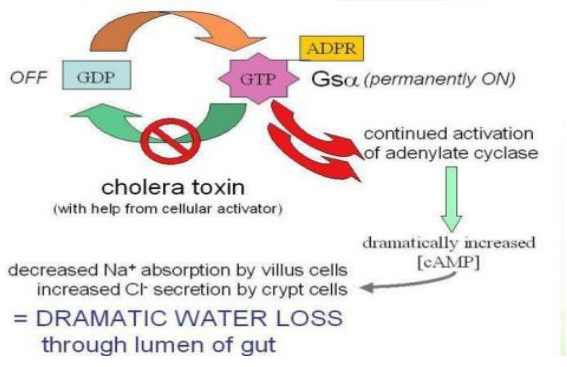


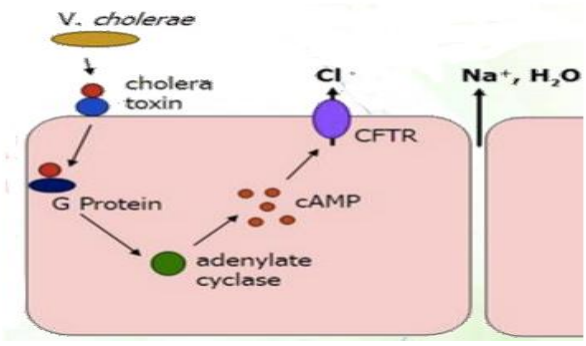
CHOLERA 16:36

-As we know, Cholera induces continuous diarrhea that causes dehydration which can be fatal. So how does the cholera toxin function?

*The toxin keeps the α subunit of the G-protein constitutively active by inhibiting GTPase activity. This leads to the production of a lot of cAMP due to the continuous activation of adenylate cyclase.
-Increasing [cAMP] will cause a flow of Na^+ as well as Cl^- out from the mucosa.

<u>Summary: Cholera toxin → G protein is locked in active form → Overactive adenylate cyclase → Excessive cAMP → Active transport of Na^+ → Large flow of Na^+ and water from the mucosa → Diarrhea</u>





THE PHOSPHOINOSITIDE PATHWAY 17:46

-We discussed together the Gαs pathway that relies on adenylate cyclase and cAMP, but we have another pathway that utilizes Gαq. This pathway results in the production of different secondary messengers: IP3 and Diacylglycerol.

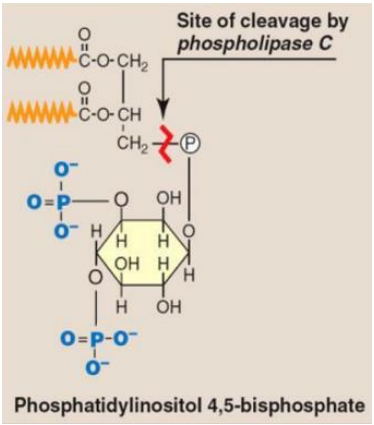
-[used by many hormones (e.g. ADH)

The idea here is: Binding of a hormone to the 7TM receptor → Activation of G Protein (Gαq) → Activation of Phospholipase C (many isoforms) which cleaves PIP2 into two messengers:

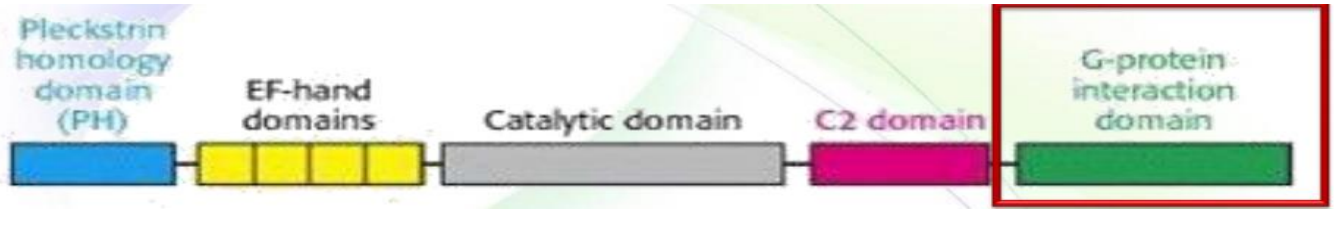
1- Inositol 1,4,5-trisphosphate, hydrophilic, (Soluble). [IP3 is the actual second messenger].
2- Diacylglycerol, amphipathic (membrane).

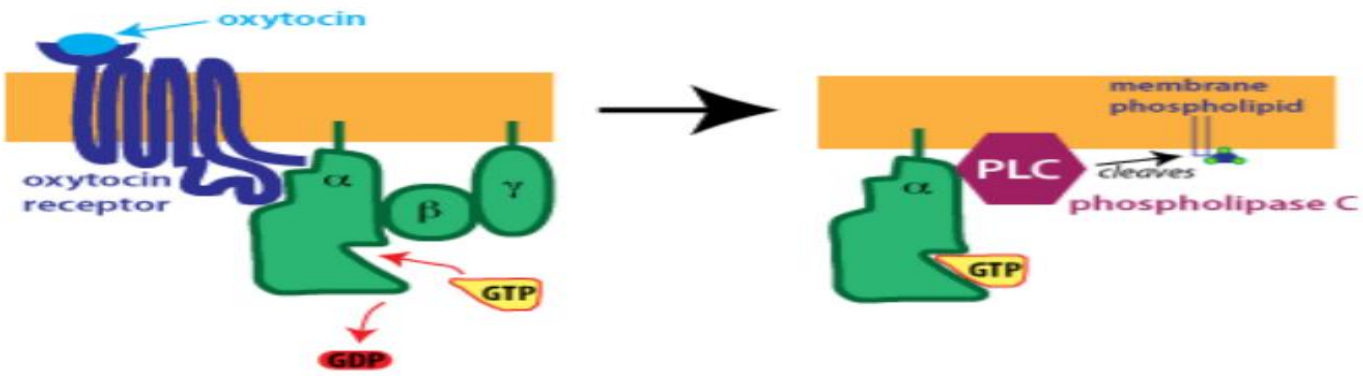
-They both act as secondary messengers and they also work in tandem (=working together, not by themselves).

**Notice that PLC is bound to the plasma membrane to speed up the interaction between molecules.*



-Phospholipase C has different domains. One of them is the G-protein interaction domain, which is responsible for interacting with the α subunit (Gαq). Once it is bound to the α subunit it gets activated and cleaves PIP2 -*which exists in the plasma membrane*- into IP3 & diacylglycerol.

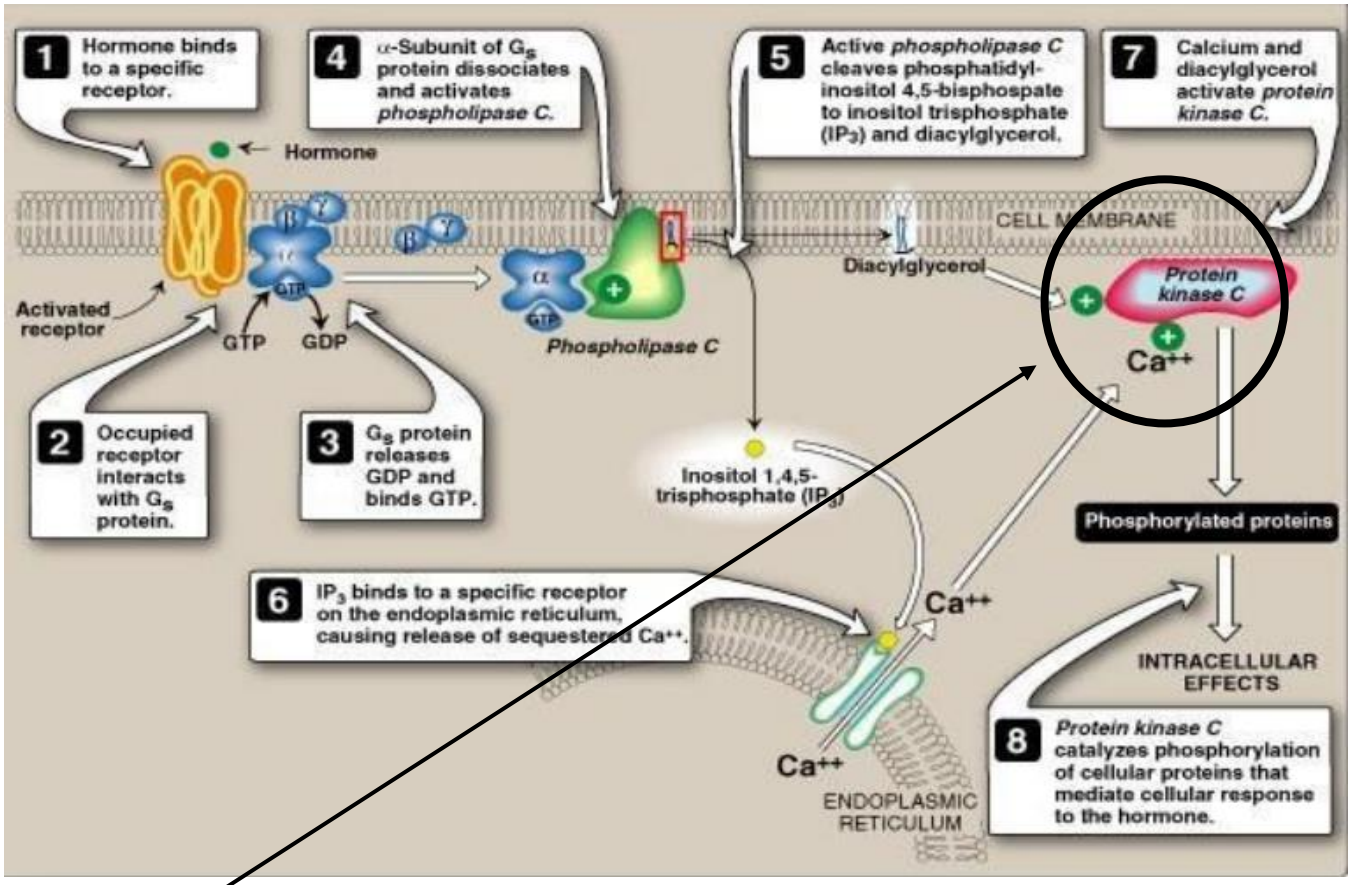




THE BIOCHEMICAL EFFECTS OF IP3 19:58

- [IP3 binds to an endoplasmic membrane protein called the IP3 receptor, which forms an ion channel.]
- [The channel opens releasing Ca^{2+} from the endoplasmic reticulum and, in smooth muscle cells, the sarcoplasmic reticulum.]
- [Increased Ca^{2+} triggers processes such as smooth muscle contraction, glycogen breakdown, and vesicle release (exocytosis).]

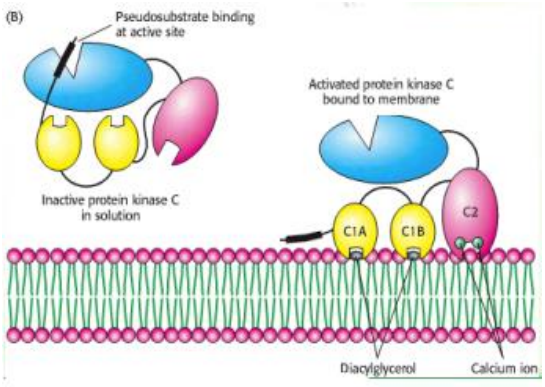
*Look at the picture and study it carefully. (it's clear and doesn't need explanation)



-IMPORTANT: What happens here is that you have the production of diacylglycerol from phospholipase C, which then interacts with other proteins or regulatory molecules, specifically protein kinase C, activating it. But the activation of protein kinase C does not only depend on interaction with diacylglycerol, it also depends on interaction with calcium ions which come from the ER. That's how diacylglycerol and IP3 work in TANDEM (as we said before), because IP3 releases calcium from the ER.

DIACYLGLYCEROL: As we know it's formed by the hydrolysis of PIP2 by PLC, it also activates many targets including Protein Kinase C. How?

- If you look at the structure of Protein Kinase C, it has multiple domains in different shapes and colors. These domains are:
  1- **C1A,C1B**: One of these two domains uses DAG to interact with plasma membrane.
  2- **C2:** Binds to calcium.
  3- **Catalytic domain.**



In the N-terminus of PKC, there is a sequence knows as the **PSEUDOSUBSTRATE**, which looks like a substrate but it's not effective (fake substrate). What it does is that it covers the active site of the enzyme (Protein Kinase C), making it inactive. Once the **C1A,C1B** domains bind to DAG and **C2** binds to Ca^{2+}, protein kinase C can bind to the membrane and the **DIACYLGLYCEROL** pulls out the pseudosubstrate from the active site so Protein kinase C can now phosphorylate many targets. [Increased Ca^{2+} allows enzyme binding to the membrane facilitating DAG binding to PKC, which pulls out the pseudosubstrate out of the active site.]

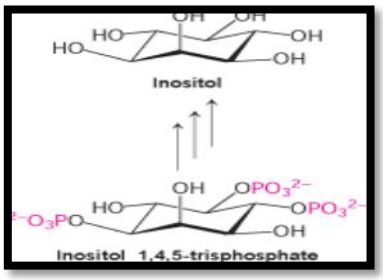
Ca^{2+}-Activated Calmodulin 23:16

-Ca^{2+} also interacts with and ***activates calmodulin***, which modulates the functions of many enzymes: (look at them briefly, don't memorize)
*Adenylate cyclase/ *phosphorylase kinase/ *pyruvate carboxylase/ *pyruvate dehydrogenase/ *glycerol-3-phosphate dehydrogenase/ *glycogen synthase/ *guanylate cyclase/ *myosin kinase/ *phospholipase A2/ *calmodulin-dependent kinase.

-And these enzymes catalyze many important cellular responses: (also don't memorize)
*glycogenolysis in liver cells/ *histamine secretion by mast cells/ *insulin secretion by pancreatic islet cells/ *aggregation of blood platelets/ *epinephrine secretion by adrenal chromaffin cells/ *smooth muscle contraction/ *visual transduction/ *gene transcription.

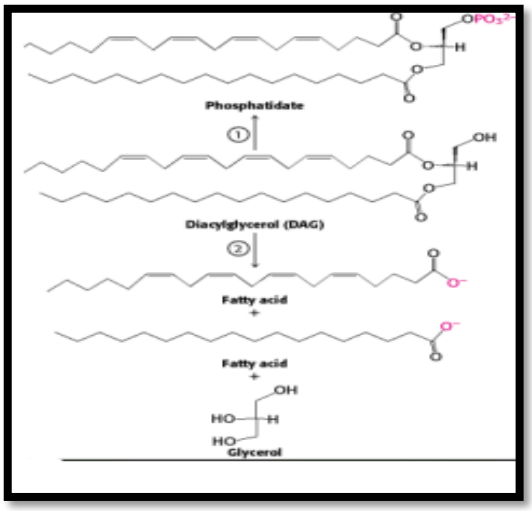
-IMPORTANT: These different pathways (cellular responses) are regulated on DIFFERENT cells by the same exact hormone, so the response is cell-specific.

-Termination:



- IP3 is a short-lived messenger (less than a few seconds) because it is rapidly degraded to inositol by two different mechanisms, but we won't mention them.

- DAG is phosphorylated to phosphatidate or hydrolyzed to glycerol and fatty acids.



-Notice that all the termination signals are enzymatic. That's why the termination process is quick.

ENZYME-LINKED CELL-SURFACE RECEPTORS 24:57

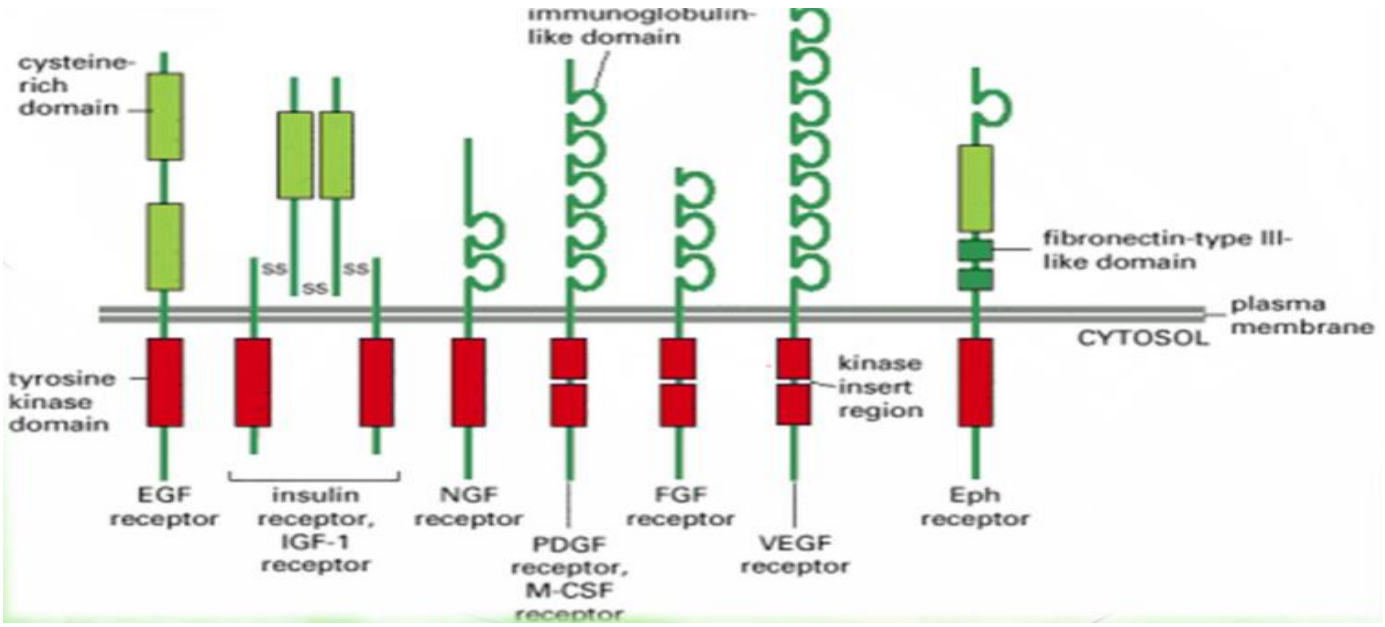
- Enzyme-linked receptors are a major type of cell-surface receptors that promote cell growth, proliferation, differentiation, death, or survival. Their ligands are often called growth factors, which act at very low concentrations (about 10^{-9} – 10^{-11} M).

-These receptors either mediate:

- **A Slower response**: Occurs through a genomic effect: it regulates gene expression, and it takes time to transcribe and translate a gene.
- **A Faster response**: It's enzymatic, for example it effects the cytoskeleton (cell movement and shape) which takes place within a few seconds or hours.

-There are different types and shapes of these receptors, some of them are heterodimers, like the insulin receptor, others are monomers; just a single chain. Also, there are receptors that have repetitive domains, some of them have the same common domain but the domain is repeated in different amounts. What they all have in common is that they have an intracellular kinase domain. So, when a ligand binds to the receptor, this kinase domain is activated, and it can activate or regulate many signals. That's why they're called enzyme-linked cell-surface receptors.

look at the picture in the next page

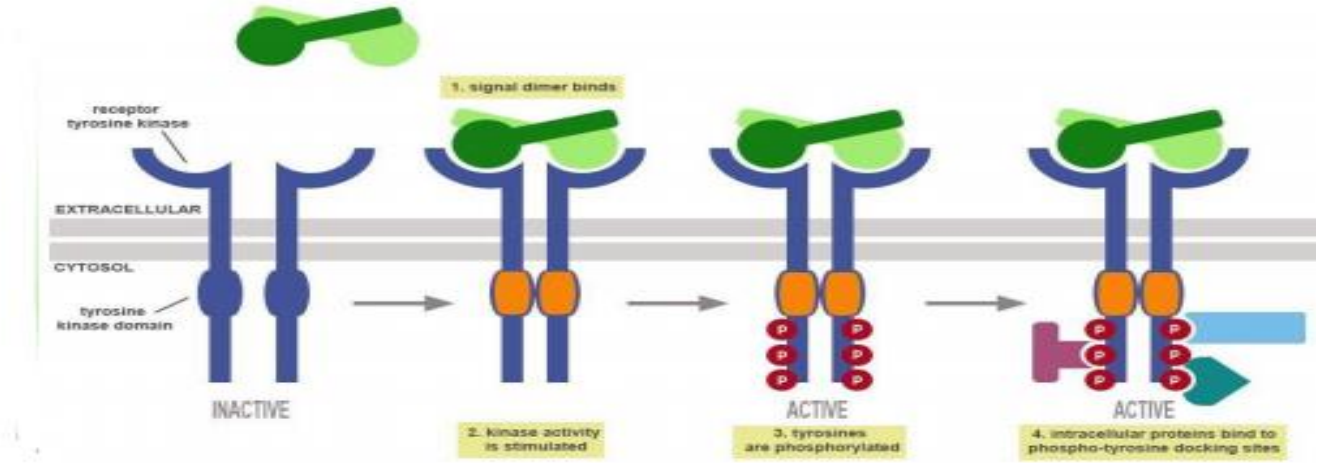


RECEPTOR TYROSINE KINASES 27:07

- An example of enzyme-linked receptors is receptor tyrosine kinases. These receptors also contain an intracellular kinase domain that phosphorylates specific tyrosines on a small set of intracellular signaling proteins.

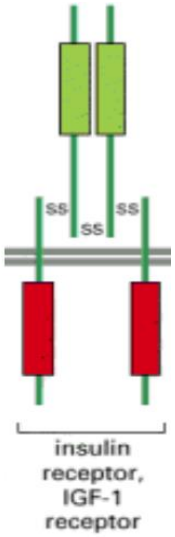
-So the story is (شوف الصورة الي تحت بالعقل وانت بتقرأ القصة) the ligand binds to the receptor, the receptor dimerizes. Notice how before ligand binding the two chains are located a bit far from each other but once the ligand binds it induces the dimerization of the receptor forming a homodimer. Why this dimerization occur? 🤔

-When the two receptors get close to each other, the kinase domains also get close, so they phosphorylate each other. This is called "**Autophosphorylation.**" The presence of the phosphate groups facilitate the interaction of other molecules with the receptor.



Summary: The ligand binds → Dimerization → Autophosphorylation → Association with different regulatory molecules.

INSULIN AND IGF-1 RECEPTOR 28:23



-Insulin and IGF-1 receptors are examples of receptor tyrosine kinases. They are hetero-tetramers and *when bound to the ligand*, the two kinase domains come close together, phosphorylate each other (autophosphorylation), and then associate with different molecules.

-Mechanism of Action:

Autophosphorylation activates signaling by:

- First, phosphorylation of tyrosines within the kinase domain increases the kinase activity.
- Second, phosphorylation of tyrosines outside the kinase domain creates high-affinity binding sites for the binding of other signaling proteins such as: **Insulin receptor substrate-1 (IRS-1)** and **Grb2**.

-Insulin Signaling Pathways:

Binding of insulin can initiate three distinct signaling pathways: Ras-dependent pathway, Ras-independent pathway and the phosphoinositide pathway.

Both the Ras-dependent pathway and Ras-independent pathway depend on **Insulin Receptor Substrate 1 (IRS 1).** Ras is a small molecule that has GTPase activity and is activated by binding to GTP. *(It is a monomeric G protein and different from the trimeric G protein discussed earlier.*)

These signaling pathways result in:

- **Immediate effects (minutes):**
  - These effects do not require synthesis of new proteins, such as:

  *An increase in the rate of glucose uptake from the blood into muscle cells and adipocytes.

  *Modulation of the activity of various enzymes involved in glucose metabolism.

- **Longer-lasting effects (hours):**

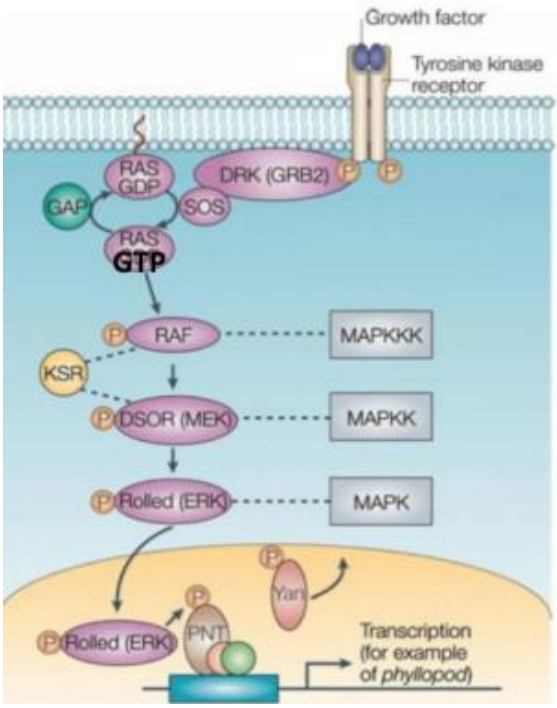
  - They are genomic effects and require protein synthesis.
  - Increased expression of enzymes that synthesize glycogen (liver) and triacylglycerols (adipocyte).

-Insulin Activated Signaling Pathways: 29:51

*Binding of insulin can initiate two distinct signaling pathways:

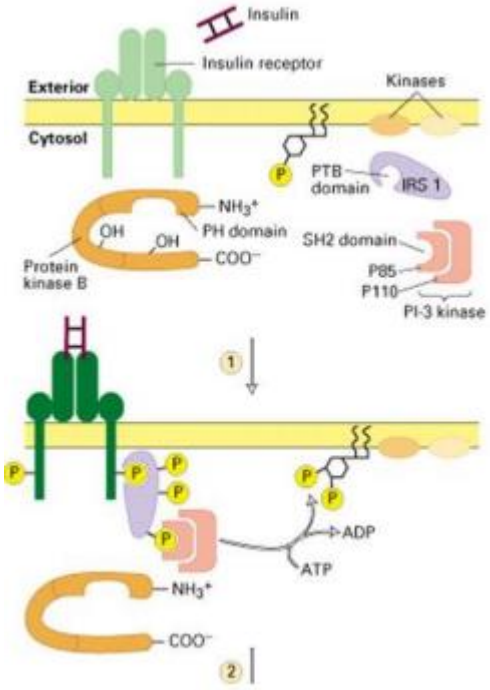
- **Ras-Dependent Pathway**: 30:40

1) Insulin binds to its receptor and activates it.
2) IRS1 binds to the activated insulin receptor and is then phosphorylated by the receptor's kinase.
3) Phosphorylated IRS1 (not the activated insulin receptor) binds to Grb2, which binds to the SOS protein.
4) SOS is a GTP-exchange factor promoting the exchange of GDP to GTP in Ras.
5) GTP-Ras activates Raf (a kinase), which activates MAP kinase, which activates ERK.
6) ERK can then be re-located into the nucleus, activating transcription factors (it causes longer lasting effects).

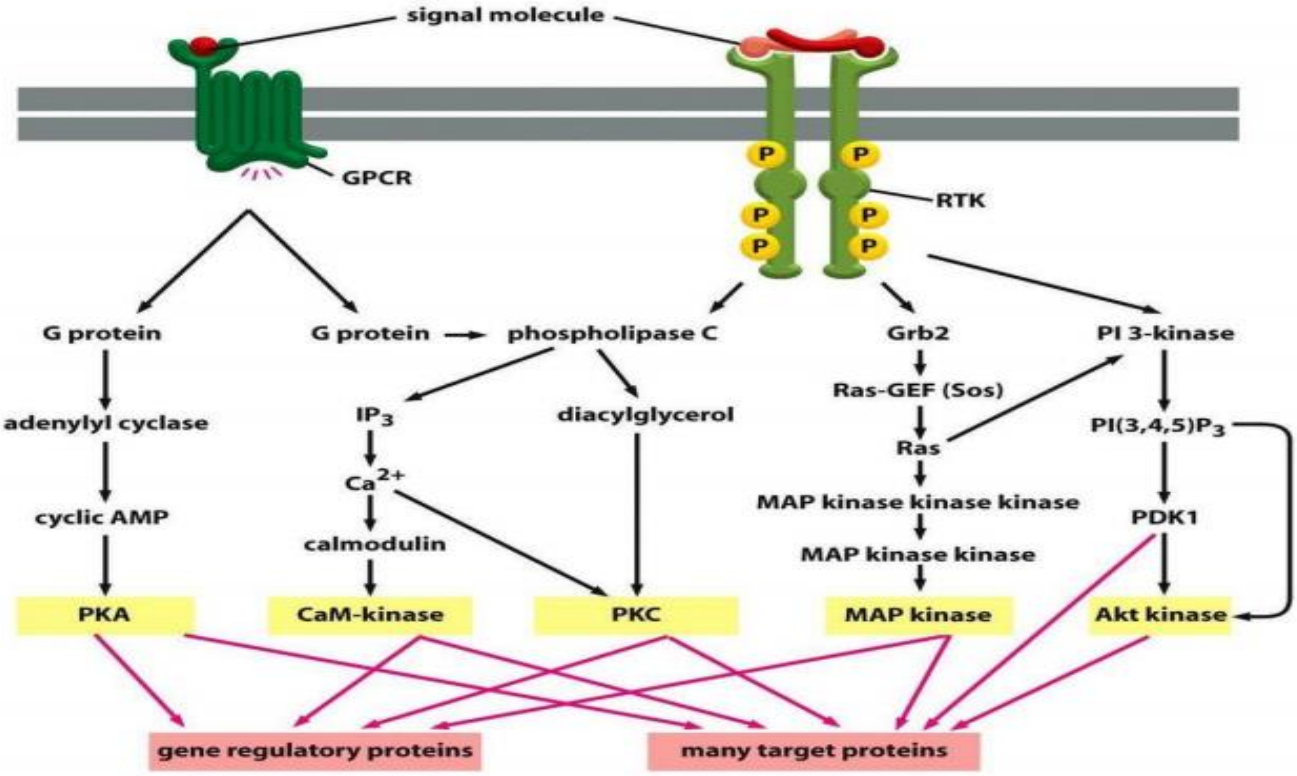


- **Ras-independent Pathway:** 32:02

1) Insulin binds to its receptor and activates it.
2) IRS1 binds to the activated insulin receptor and is then phosphorylated by the receptor's kinase.
3) Phosphorylated IRS1 also binds PI-3 kinase and activates it. This results in production of phosphoinositides.
4) This leads to recruitment of protein kinase B (PKB) to the membrane.
5) PKB is phosphorylated by membrane associated kinases.
6) Phosphorylated (active) PKB is released into the cytosol mediating many effects of insulin such as stimulation of glucose uptake and glycogen synthesis by activating glycogen synthase (it causes immediate effects).



- Similar to G-protein mediated signaling, the insulin receptor can lead to the activation of phospholipase C.



- Notice that you can have a single ligand bound to its receptor and this receptor can activate multiple pathways like the Phospholipase C pathway or Grb2 pathway, resulting in different effects. Also note the cross-talk between different pathways.

- This is all balanced and coordinated within the cell, so you don't have activation of one pathway only.

Termination of the Signal 34:08

Signals are terminated by phosphatases, which either remove phosphate groups on the receptor or on the other molecules that were phosphorylated by different kinases.