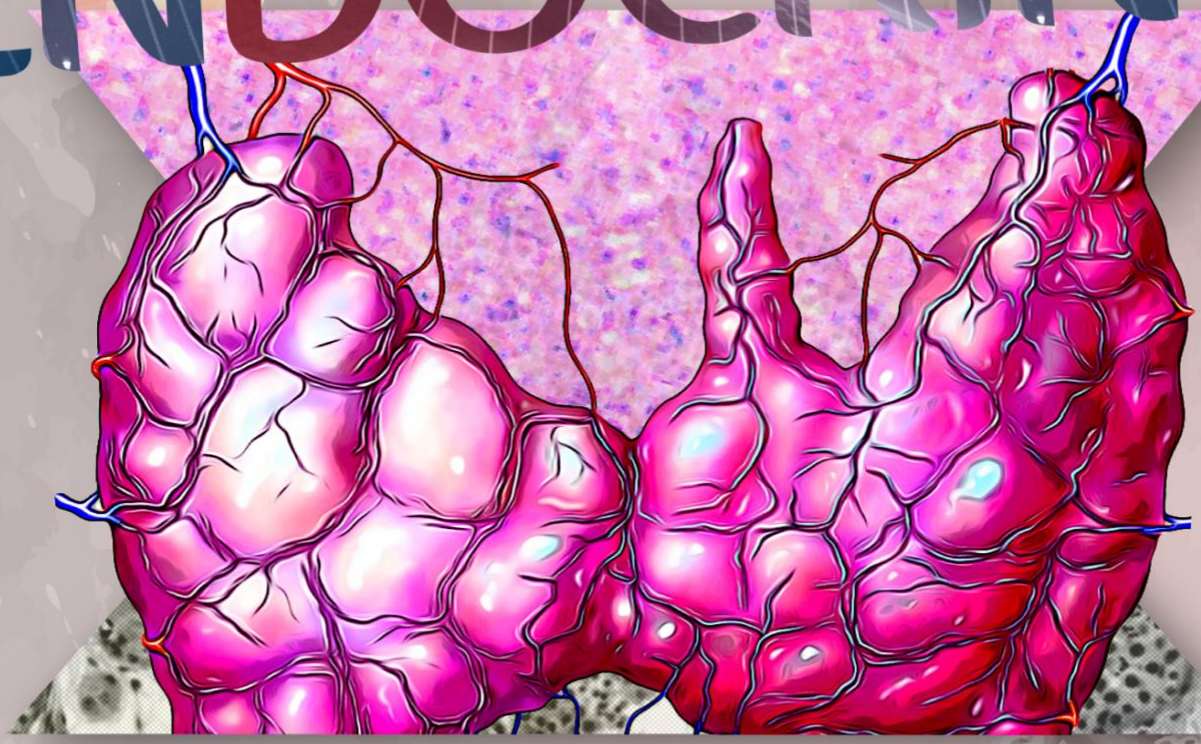
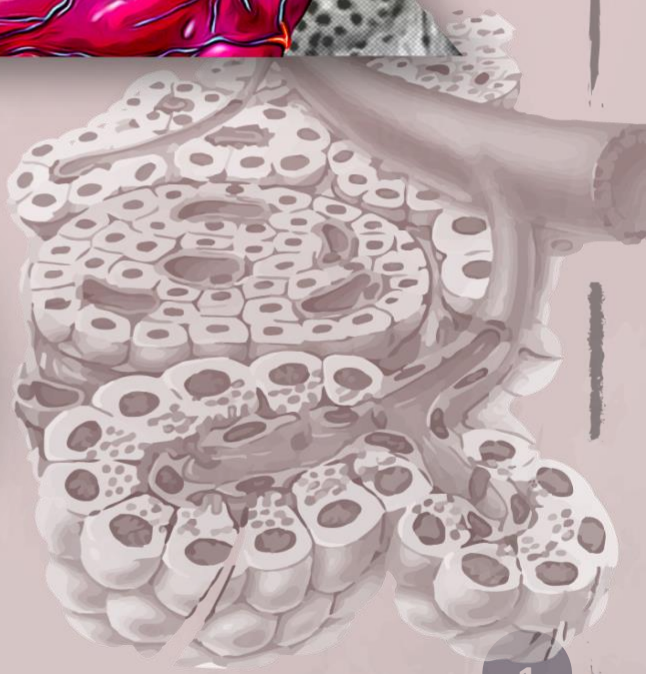
No

ENDOCRINE





SUBJECT: Biochemistry

DONE BY: Lujain Hamdan

CORRECTED BY: Yara AlAdwan

DOCTOR: M'amoun Ahram

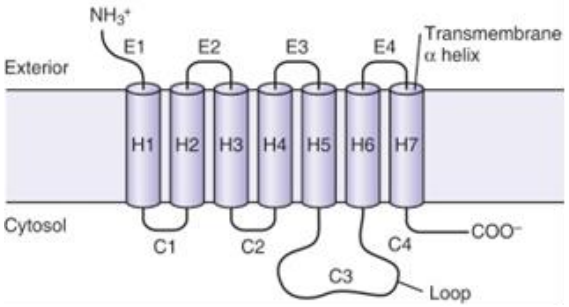
المعذرة، ولكن عدد الصفحات ما هو إلا بسبب كثرة الصور، وما كثرة الصور إلا لتسهيل الفهم وتوضيح الفكرة !

Cell Surface Receptors

Receptors are extremely important, around 40% of drugs target them.

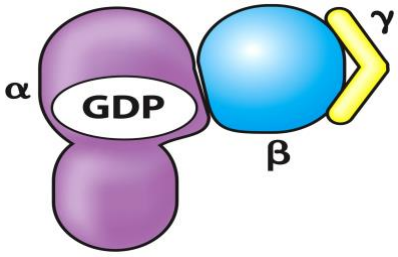
A) G protein-coupled receptors

- G protein-coupled receptors (GPCRs) are cell surface receptors contain seven membrane-spanning regions (7 transmembrane domains).
- They have portions extend outside the cell or to the inside of the cell.
- GPCRs are important in pharmacology, about 25% of drugs target them.
- Although they function similarly , they're different in homology.



- Although all GPCRs are structurally similar, their amino acid sequences generally are quite dissimilar, for example:
  - β 1- and β 2-adrenergic receptors are 50 percent identical.
  - α - and β -adrenergic receptors exhibit even less homology.
- The specific amino acid sequence of each receptor determines: Ligand binding region and G protein binding region, which are the most important regions.
- Overall there are two important domains(segments) of these receptors that are important:the ligand binding domain which extends to the outside and the G protein binding domain which extends to the inside ((((They have homology in terms of that, but there are differences as well to specify the type of G protein the binds to the receptor))))

G proteins



✓ G proteins are heterotrimers proteins, made of three subunits α, β, and γ. They are intermediary in signal transduction from the seven transmembrane (7TM) receptors, to the effector molecules.

- ✓ Alpha subuit is the functional subunit, Beta and Gamma are regulatory subunits.It's not an enzyme but it has an(intrinsic) enzymatic activity.
- ✓ When α subunit binds to GTP, G protein is activated, alpha subunit dissociates from beta-gamma complex, moves in the membrane to activate adenylate cyclase.
- ✓ The movment in the membrane makes the process effecicent, easier and faster because it's a two-dimentional system.
- ✓ Alpha subunit has an intrensic enzymatic activity (GTPase), it hydrolizes GTP to GDP, so alpha subunit becomes inactive then it binds to beta-gamma complex.

Types of G proteins:

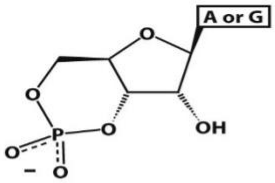
1. Stimulatory (Gαs) : activates adenylate cyclase, works on b-adrenergicreceptors, with different types of ligands.
2. Inhibitory (Gαi): inhibits adenylate cyclase.
3. Gαq: activates phospholipase C, functions via second messengers which are mainly lipids and calcium.

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

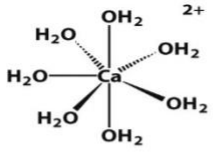
Second messengers

G proteins function through enzymes and what they do is that they utilize second messengers .

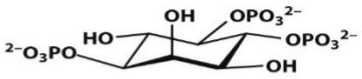
Information is transduced via changes in the concentration of second messengers: *cyclic AMP and cyclic GMP, calcium ion, inositol 1,4,5trisphosphate (IP3), diacylglycerol (DAG).*



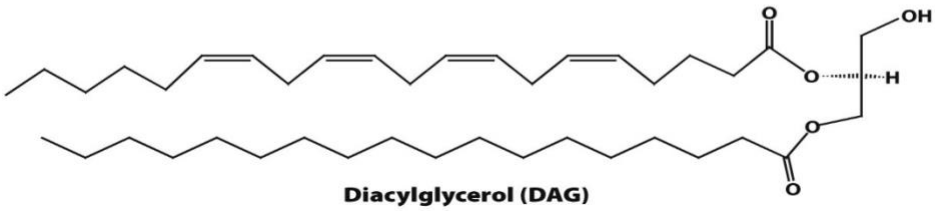
cAMP, cGMP



Calcium ion



Inositol 1,4,5-trisphosphate (IP_3)



Diacylglycerol (DAG)

- Why are second messengers good?

1. They are often small molecules free to diffuse to other compartments of the cell.
2. The signal can be amplified significantly in the generation of second messengers.
3. The use of common second messengers in multiple signaling pathways often results in cross-talk between different signaling pathways.

- How can one second massenger regulate many pathways?

Many different pathways can be regulated by the same secondary messenger and the same receptor as well using these two mechanisms:

a- Fine-tuning: the extent of the activation of the receptor, G protein, adenylyl cyclase, etc.

b- Utilization of other secondary messengers and regulatory molecules in each pathway, for example: phosphofructkinase is regulated by ATP, AMP, citrate, fructose 2,6-biphosphate, etc.

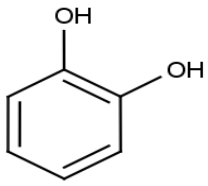
- Receptors can be calssified according to the secondary massenger that they use.

(only memorize the enzymes that we will explain in details)

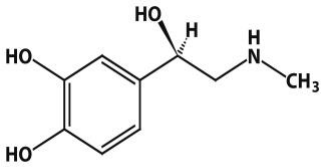
Group II. HORMONES THAT BIND TO CELL SURFACE RECEPTORS	
A. The second messenger is cAMP	
Adrenocortropic hormone (ACTH)	Parathyroid hormone (PTH)
Angiotensin II	Opioids
Antidiuretic hormone (ADH)	Acetylcholine
Follicle-stimulating hormone (FSH)	Glucagon
Human chorionic gonadotropin (hCG)	α_2-Adrenegic catecholamines
Lipotropin (LPH)	Corticotropin-releasing hormone (CRH)
Luteinizing hormone (LH)	Calcitonin
Melanocyte-stimulating hormone (MSH)	Somatostatin
Thyroid-stimulating hormone (TSH)	β-Adrenergic catecholamines
B. The second messenger is calcuim or phosphatidylinositides (or both)	
α_1-Adrenergic catecholamines	Acetylcholine (muscarinic)
Cholecystokinin	Substance P
Gastrin	Angiotensin II
Thyrotropin-releasing hormone (TRH)	Gonadotropin-releasing hormone(GnRH)
Vasopressin	
C. The intracell messenger is a protein kinase cascade (started by tyr phosphorylation)	
Growth hormone (GH)	Oxytocin
Insulin	Nerve growth factor (NGF)
Insulin-like growth factors (IGF-1, IGF-II)	Epidermal growth factor(EGF)
Prolactin (PRL)	Platelet-derived growth factor
	Fibroblast growth factor (FGF)

Adrenergic receptors: an example for GPCRs.

Catechol ring



- This protein binds epinephrine (also called adrenaline), a hormone responsible for the "fight or flight" response, it belongs to catecholamines (hormones that have a catechol ring).



Epinephrine

- When epinephrine binds to beta adrenergic receptors, it causes relaxation of smooth muscles and stimulation of: glycolysis, gluconeogenesis, lipolysis, the purpose is to produce energy.
- Epinephrine can also bind to Alpha 1 adrenergic receptors and it has an opposite effect, it causes vasoconstriction,but it also induces glucolysis and gluconeogenesis.

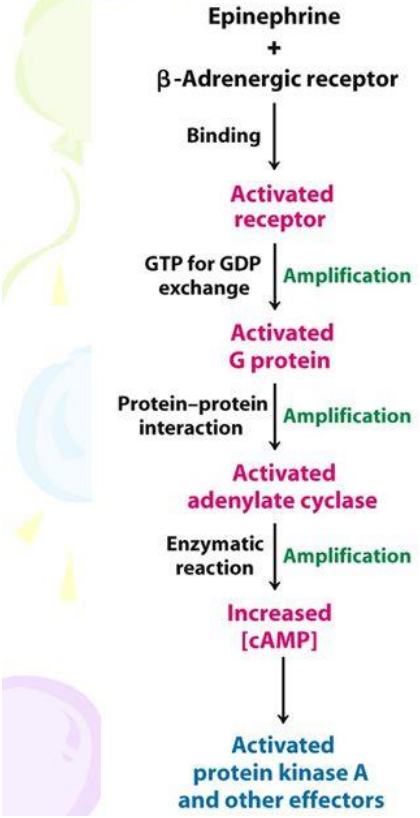
Alpha Receptors		Beta Receptors	
1. Vasoconstriction of a. Coronary arteries b. Veins 2. ↓motility of GIT smooth muscle cells			
α1 (postsynaptic)	**α2 (presynaptic)**	**β1 (postsynaptic)**	**β2 (postsynaptic)**
Gq protein coupled Activates **Phospholipase C** **PIP2 →IP3 + DAG**	**Gi protein** coupled 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

Cellular effects of cAMP

1. *Increased degradation of storage fuels*
2. *Dispersion of melanin pigment granules*
3. *Decreased aggregation of blood platelets*
4. *Opening of chloride channels*
5. ***Increased secretion of acid by gastric mucosa.***

Now can you think why coffee increases gastric secretions and causes ulcers?

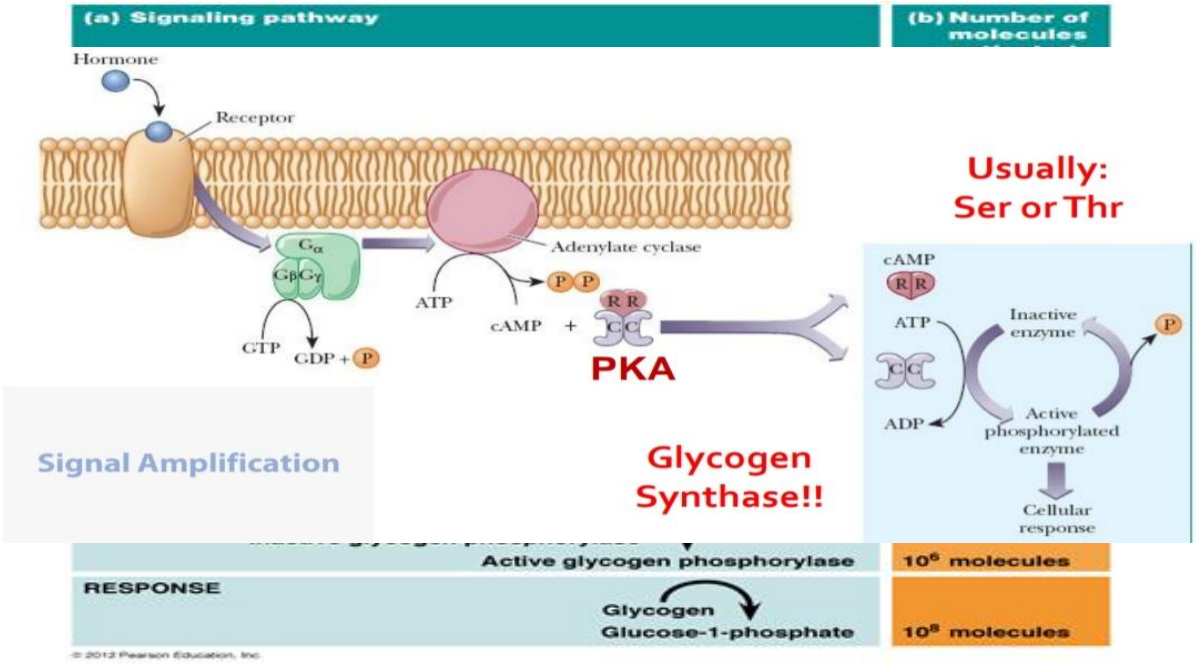
- *Caffeine induces acid secretion from parietal cells via adenylate cyclase activation.*
- *Then cAMP binds to protein kinase A, it's now active, so it can phosphorylates many proteins including glycogen synthase and inhibiting it and activating glycogen phosphorylase.*
- *Remember: Phosphorylation can activate or inhibit proteins.*



Signal amplification:

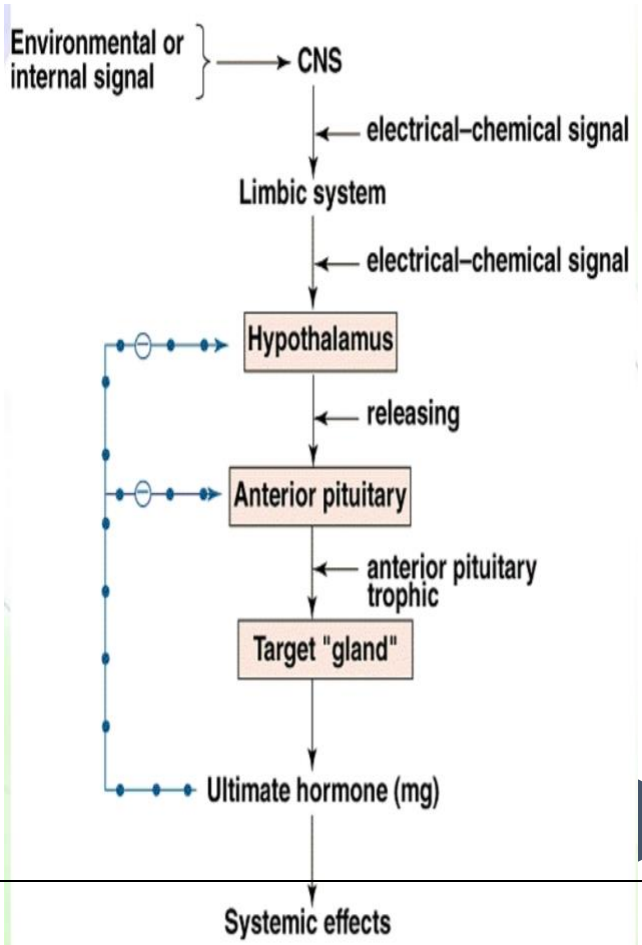
a. At the level of signal transduction

- 1 molecule of epinephrine activates many many G proteins, each G proteins activates a lot of adynelate cyclase molecules and so on.
- So the amount of hormones is little but the effect is huge.



b. At the level of hormones secretion

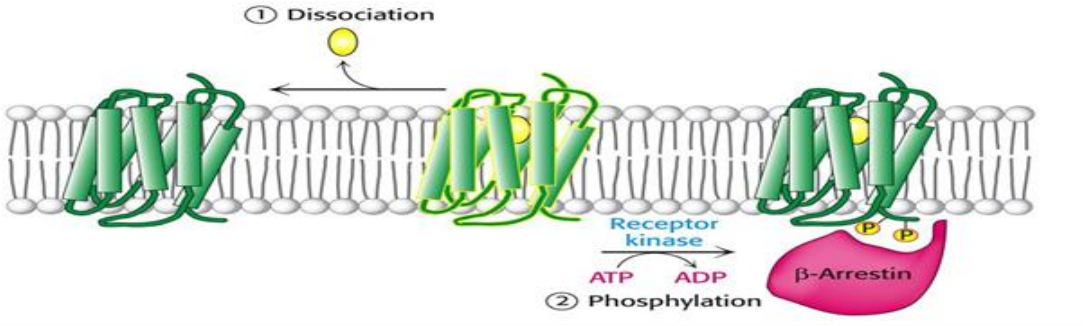
For example one hormone molecule from hypothalamus causes secertion of thousandas of certain hormone molecules from the anterior piutarity gland, each one of them can target certain tissue again producing hunderds and thousands of hormones .



Termination of the signal:

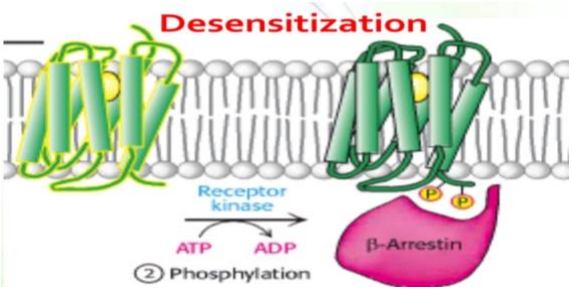
1) Dissociation of the hormone from the receptor.

2) *Inactivation of G protein by GTPase activity in Alpha subunit.*
3) *Hydrolysis of cAMP to AMP by phosphodiesterase.*
4) *Phosphorylation of the receptor by receptor kinases, the receptor will bind to a protein known as β-Arrestin that makes the receptor inactive even if the ligand is still bound to it.*



Desensizatin

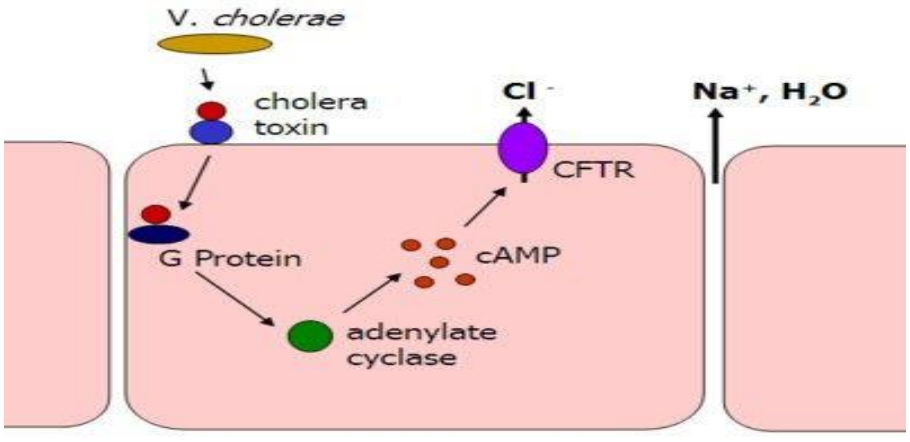
occurs when the signal hasn't been terminated.



Cholera toxin:

It induces continuos diarrehea which cause dehydration and can be fatal.

1. Cholera toxin makes G protein locked in active form and inhibits GTPase activity of alpha subunit
2. Alpha subunit becomes constitutively active and overactivates adenylate cyclase Excessive cAMP causes active transport of Na+
3. large flow of Na+ and water from the mucosa causes diarrhea then dehydration.

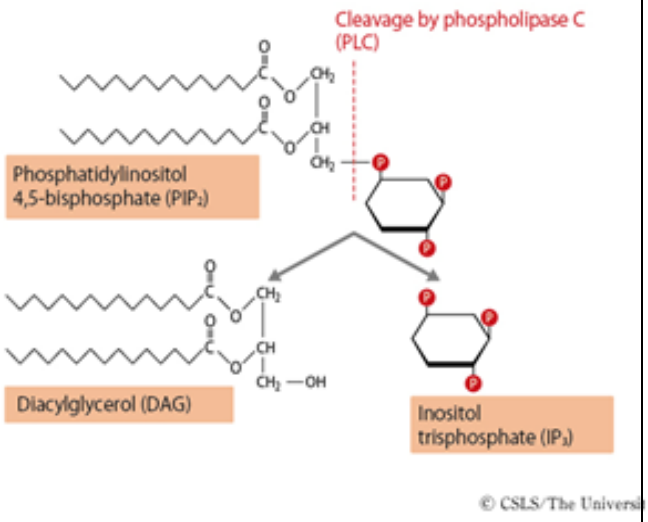


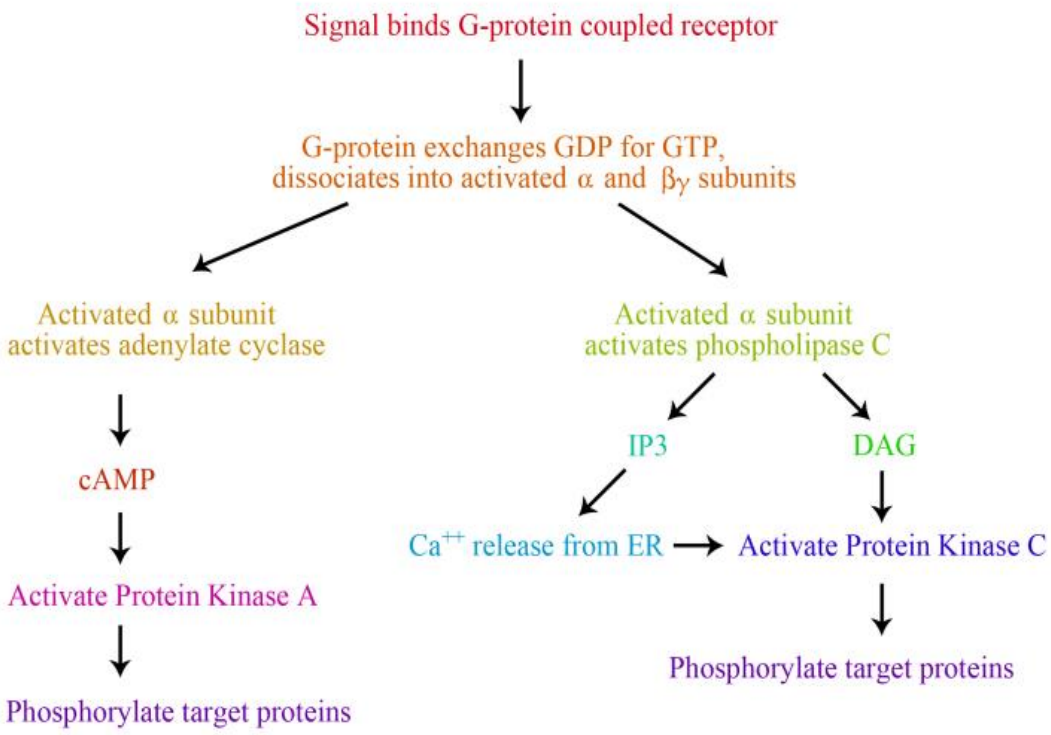
The phosphoinositide pathway

1) Binding of a hormone (e.g. ADH) to 7TM receptor
2) Activation of G Protein (it uses Gaq).
3) Activation of Phospholipase C, that is an enzyme located in the membrane to facilitate the interaction with G protein.
4) Phospholipase C cleaves PIP2 to IP3 and DAG.
5) Two messengers are produced:

a- *Inositol 1,4,5-trisphosphate (IP3), hydrophilic (Soluble), is the actual second messenger.*

b- *Diacyclglycerol, amphipathic (located in the membranes) it's also a second massenger works with IP3.*





The biochemical effects of IP3

I. IP3 is released from plasma membrane and binds to a membrane protein located in the endoplasmic reticulum membrane called the IP3 receptor. Together, they form an ion channel.

II. The channel opens releasing Ca2+ from the endoplasmic reticulum and, in smooth muscle cells, the sarcoplasmic reticulum.

III. Increased cytosolic concentration of Ca2+ triggers processes such as smooth muscle contraction, glycogen breakdown, and vesicle release (exocytosis).

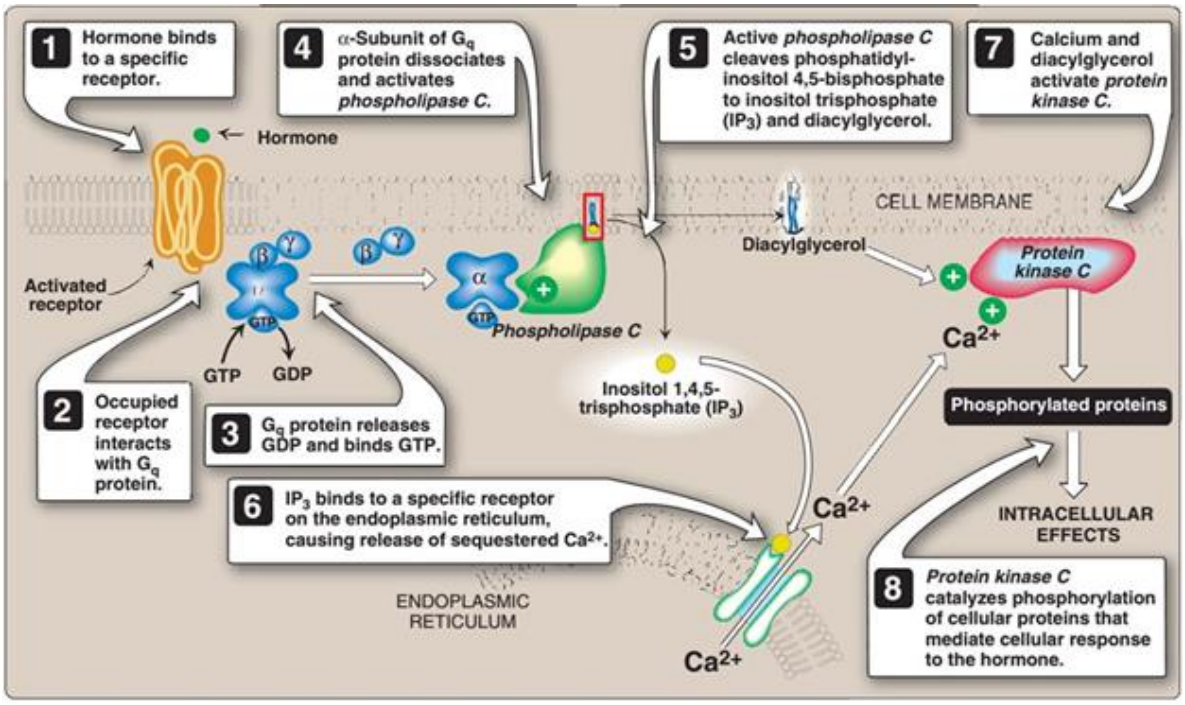
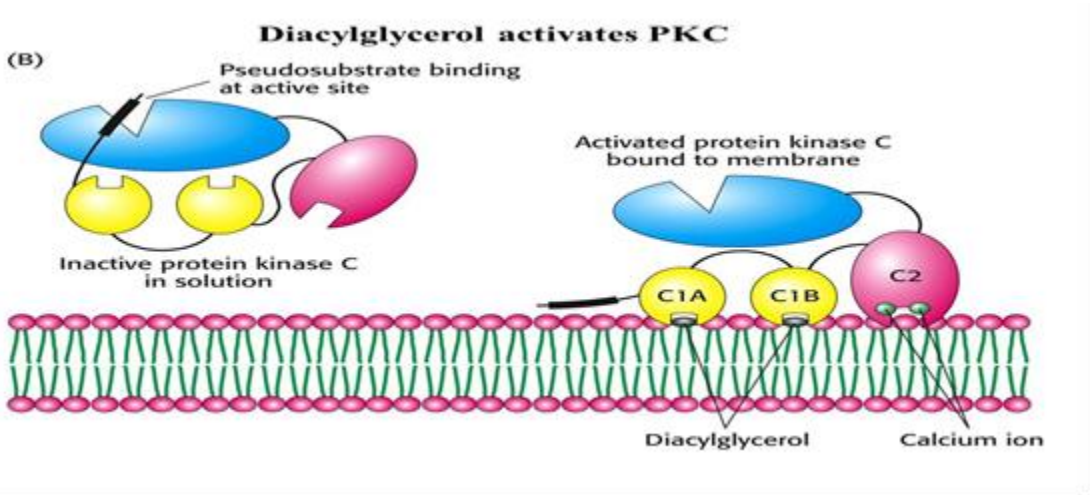


Figure 17.8: Role of inositol trisphosphate and diacylglycerol in intracellular signaling.

Diacylglycerol

DAG activates many targets of Protein kinase C, How?

PKC is inactivated by amino acids sequence called self-pseudo-substrate. Increased Ca2+ allows enzyme binding to the membrane facilitating DAG binding to PKC, which pulls out the pseudo-substrate out of the active site, so PKC is now ready to phosphorylate many substrates.



Ca-activated calmodulin

Ca2+ also interacts with and activates calmodulin, which modulates the functions of many enzymes such as: adenylate cyclase, phosphorylase kinase, pyruvate carboxylase, pyruvate dehydrogenase, glycerol-3-phosphate dehydrogenase, glycogen synthase, guanylate cyclase, myosin kinase, phospholipase A2, calmodulin-dependent kinase

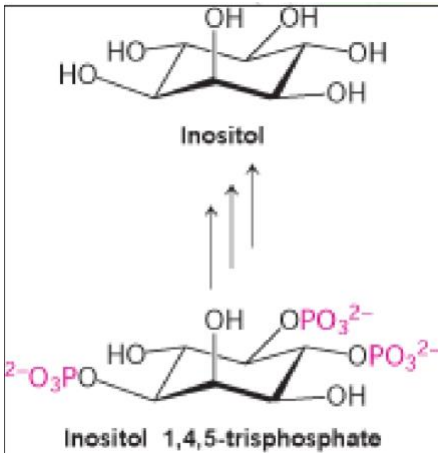
These enzymes catalyze many important cellular responses like:

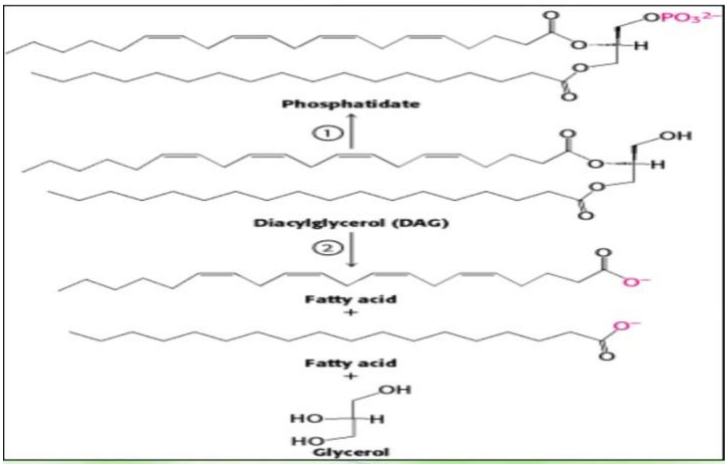
- ✓ Glycogenolysis in liver cells
- ✓ Histamine secretion by mast cells
- ✓ Insulin secretion by pancreatic islet cells
- ✓ Aggregation of blood platelets
- ✓ Epinephrine secretion by adrenal chromaffin cells
- ✓ Smooth muscle contraction
- ✓ Visual transduction
- ✓ Gene transcription

Termination

- *IP3 is a short-lived messenger (less than a few seconds) because it is rapidly degraded to inositol.*

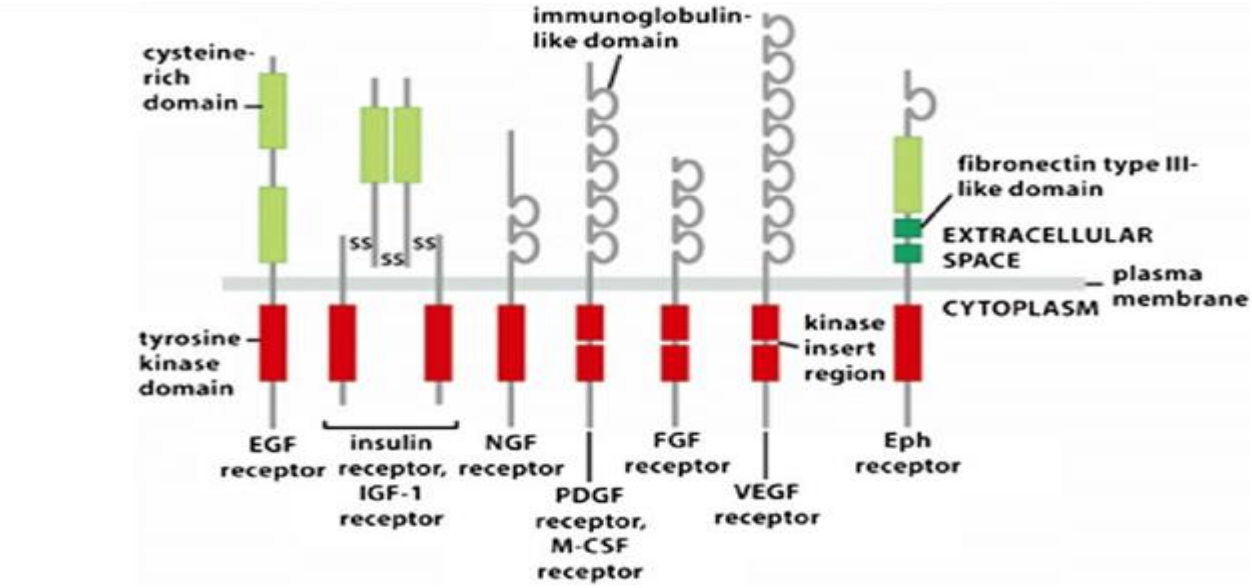
- *DAG is phosphorylated to phosphatidate or hydrolyzed to glycerol and fatty acids.*
- *The termination happens enzymatically, so it really a fast process.*





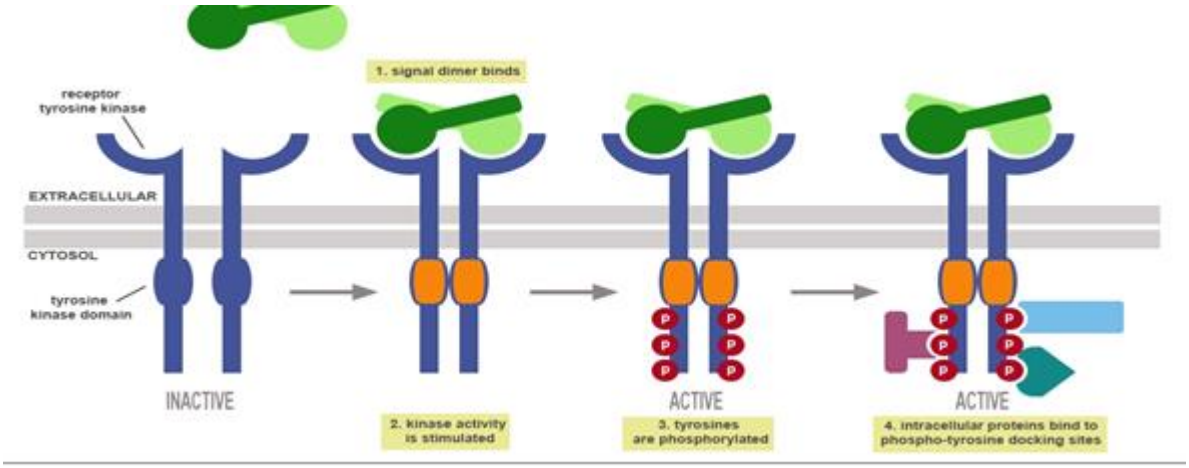
B) Enzyme-Linked Cell-Surface Receptors

I. *Enzyme-linked receptors are a major type of cell surface receptors that promote cell growth, proliferation, differentiation, or survival.*
II. *Their ligands are often called growth factors, which act at very low concentrations (about 10-9-10-11 M).*
III. *Receptors either mediate slower response (hours) that lead to changes in gene expression or faster responses (seconds) with effects on the cytoskeleton (cell movement and shape).*
IV. *They can be monomer or polymers, with different type and number of domains.*
V. *They all have an intracellular kinase domain.*



Receptor tyrosine kinases

- Enzyme-linked receptors contain an intracellular kinase domain that phosphorylates specific tyrosine residues on a small set of intracellular signaling proteins.
- The binding of a signal protein to the ligand-binding domain induces dimerization of the receptor and activates the intracellular tyrosine kinase domain that phosphorylates itself (auto-phosphorylation) and other intracellular signaling proteins that subsequently bind to the phosphorylated receptor.



- **The mechanism of receptor activation:**

I. The receptors are tetramers where, when bound to the ligand, the two kinase domains come close together, phosphorylate each other (auto-phosphorylation).

II. Auto-phosphorylation activates signaling by:

✓ Phosphorylation of tyrosine residues within the kinase domain increases the kinase activity.

✓ Phosphorylation of tyrosine residues outside the kinase domain creates high-affinity binding sites for the binding of other signaling proteins such as: Insulin receptor substrate-1 (IRS-1), Grb2.

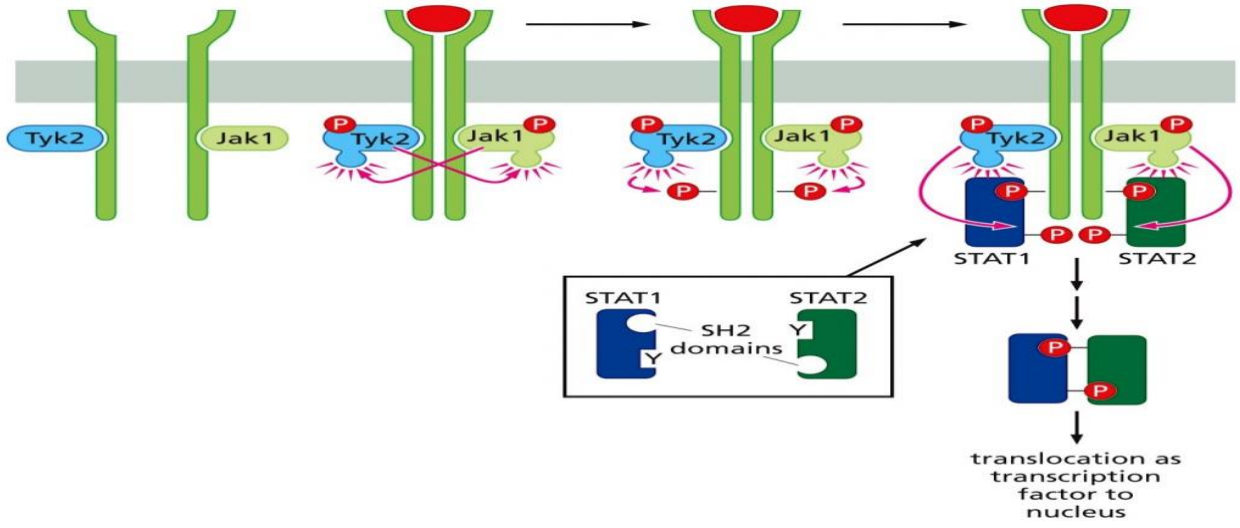


Figure 6.22 The Biology of Cancer (© Garland Science 2014)

Insulin signaling pathways

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

Both Ras-dependent pathway and Ras-independent pathway depend on **Insulin Receptor Substrate 1 (IRS 1).**

Ras is a small molecule has GTPase activity, activated by binding to GTP. *(It is a monomeric G protein but different from the trimeric G protein)*

These signaling pathways result in:

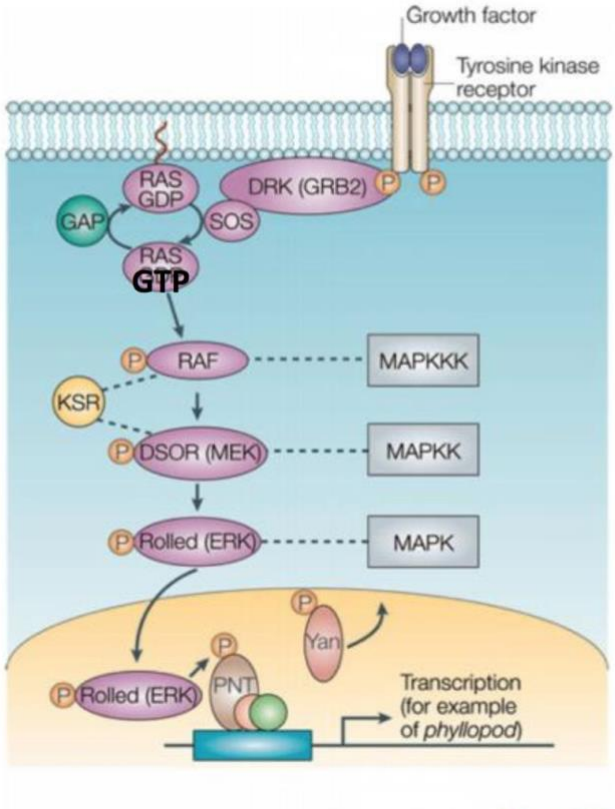
a. Immediate effects (minutes):

- These effects do not require new protein synthesis, 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.

b. Longer-lasting effects (hours):

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

Ras-Dependent Pathway

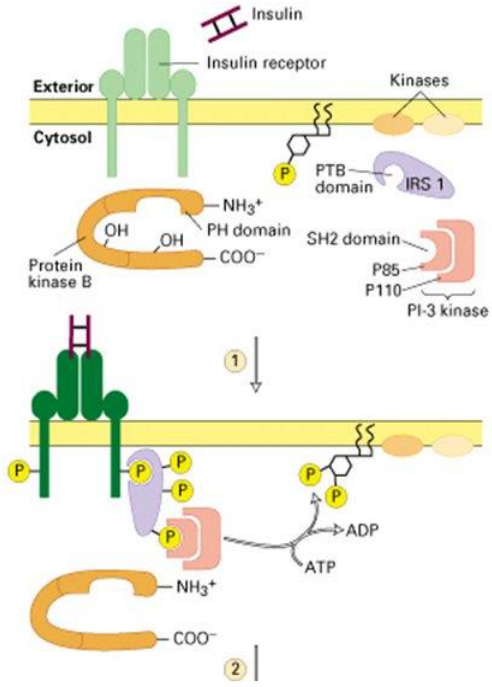


1) *Insulin binds to its receptor and activates it.*
2) *IRS1 binds to the activated insulin receptor, then it is phosphorylated by the receptor's kinase*
3) *Phosphorylated IRS1, not the activated insulin receptor, binds to Grb2, which binds to Sos protein.*
4) *SOS is a GTP-exchange factor promoting 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 nucleus activating transcription factors (it causes longer lasting effects).*

Ras-independent Pathway

1) *Insulin binds to its receptor and activates it.*
2) *IRS1 binds to the activated insulin receptor, then it is phosphorylated by the receptor's kinase*

3) *Phosphorylated IRS1 also binds PI-3 kinase activating it resulting in production of phosphoinositides.*
4) *This lead 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).*



The phosphoinositide pathway

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

Termination of signal

Signals are terminated by phosphatases (remove a phosphate group from their substrates).

Finally, here you can notice cross-talk between different pathways.