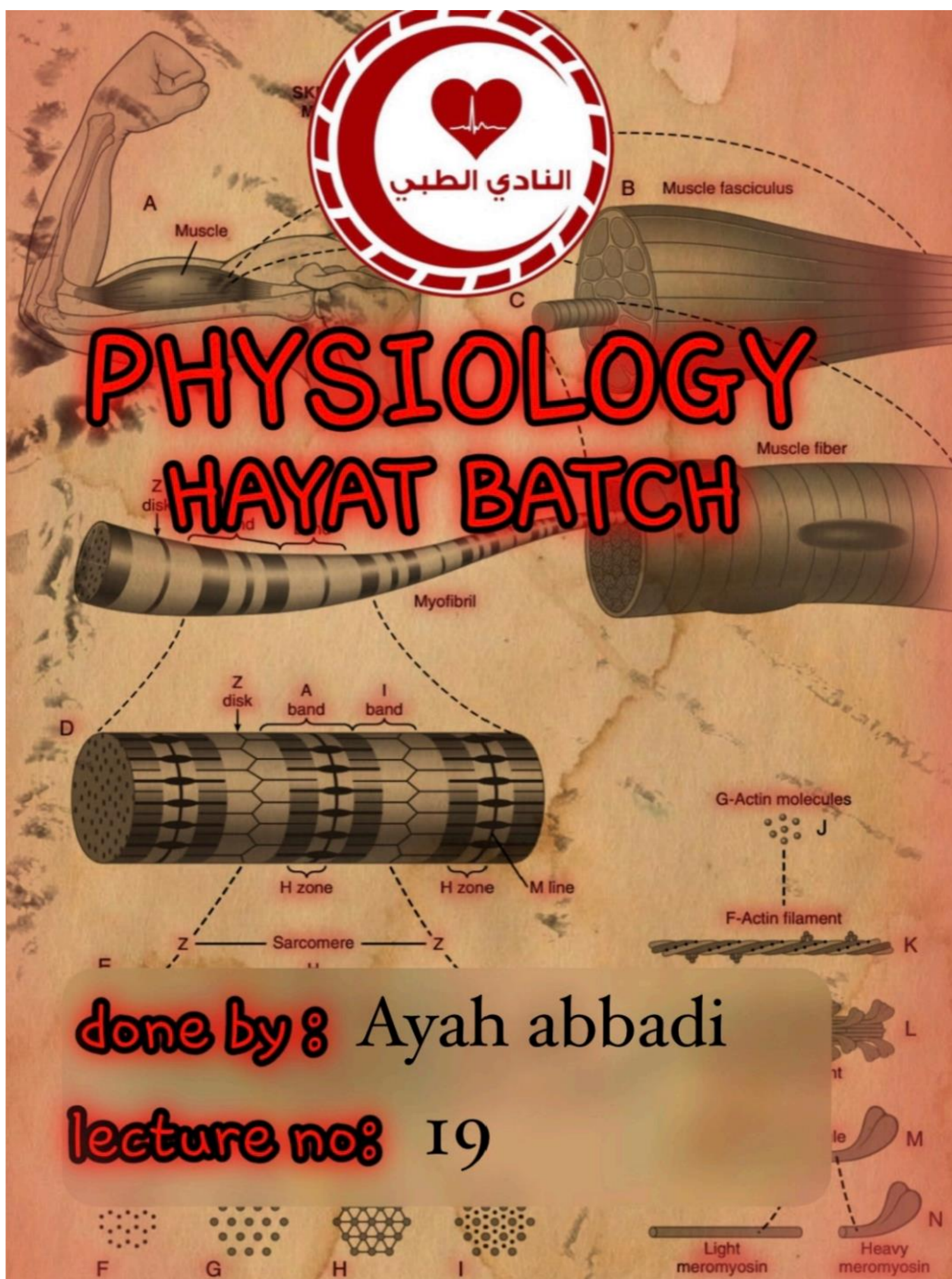




PHYSIOLOGY HAYAT BATCH



done by: Ayah abbadi

lecture no: 19

Figure 6-1. Organization of skeletal muscle, from the gross to the molecular level. F, G, H, and I are cross sections at the levels indicated.

Intracellular Signaling

Physiology lecture 19

Dr. Waleed R. Ezzat

Lecture objectives:

- ❖ Be familiar with ionic channels.
- ❖ Describe how second messengers (cAMP, cGMP, etc) regulate and amplify signal transduction.
- ❖ Describe how intracellular calcium concentration is regulated and used in intracellular signal transduction.

إذا ال signals ممكن تكون ionotropic or metabotropic
و محاضرة اليوم: كيف تستجيب الخلية للإشارة الي بتيجيها من الخارج

Ion Channel–Linked Receptors:

- ❖ All the neurotransmitter substances, such as Ach and NE, → *Acetylcholine* *Norepinephrine (noradrenaline)* combine with receptors in the postsynaptic membrane.
- ❖ This combination usually causes a change in the structure of the receptor, resulting in opening or closing a channel for one or more ions (such as channels for Na^+ , K^+ , and Ca^{2+}).
يدخل ال +Na لجوا الخلية ويقلل ال negativity وبالتالي يعمل activation
- ❖ Few neurohormones and hormones exert their actions through activation of ion channel receptors, most hormones that open or close ions channels do this indirectly by coupling with G protein-linked or enzyme-linked receptors.

اخذنا في اشي اسمه ionic channels ولما تفتح بتدخل يا اما ايون موجب وتعمل activation للخلية او بتطلع ايون سالب زي البوتاسيوم وتعمل inhibition للخلية

هلا عنا السؤال هو شو اللي بخلي هاي القنوات تفتح وتشتغل، حسب ما اخدنا بالبداية انه بيجي (i.g neurotransmitter (acetylcholine) بربط بال receptor protein او بالبروتين المجاور اله ومما يؤدي الى فتح هاي القنوات، ولكن هل هاي الطريقة الوحيدة اللي بتفتح هاي القنوات؟؟ وهل هاي الطريقة الوحيدة اللي بتعمل activation or inhibition للخلايا؟؟ الجواب طبعاً لا وهاد اللي حنعرفه بمحاضرة اليوم .

الهرمونات وال neurotransmitters الضخمة ما بتربط بمستقبل جنب القناة و ثم تفتح القناة لا ما بصير هيك هون بربط ويؤدي لتغيرات جوا الخلية وهاي التغيرات اثرت عالخلية يعني بطريقة indirectly هاي العملية بتصير indirectly by coupling with G-protein .

بدنا نعرف شو يعني G- protein coupling with ال G protein هو بروتين اسمه G اما coupling يعني هاد البروتين مربوط عليه receptor للهرمونات (لهرمون معين)

حكينا طبعاً انه ال receptor هو بروتين بال cell membrane عادةً ما يكون protein transmembrane يعني بروتين عملاق بارز من الجهتين للغشاء هذا البروتين اللي بارز من الجهتين هو ال receptor وهذا البروتين من الداخل رابط ب بروتين ثاني وهذا التركيب كامل بنسميه G protein coupled receptor يعني المستقبل المربوط ب G protein هذا معناه بالعربي

G Protein-Linked Hormone Receptors:

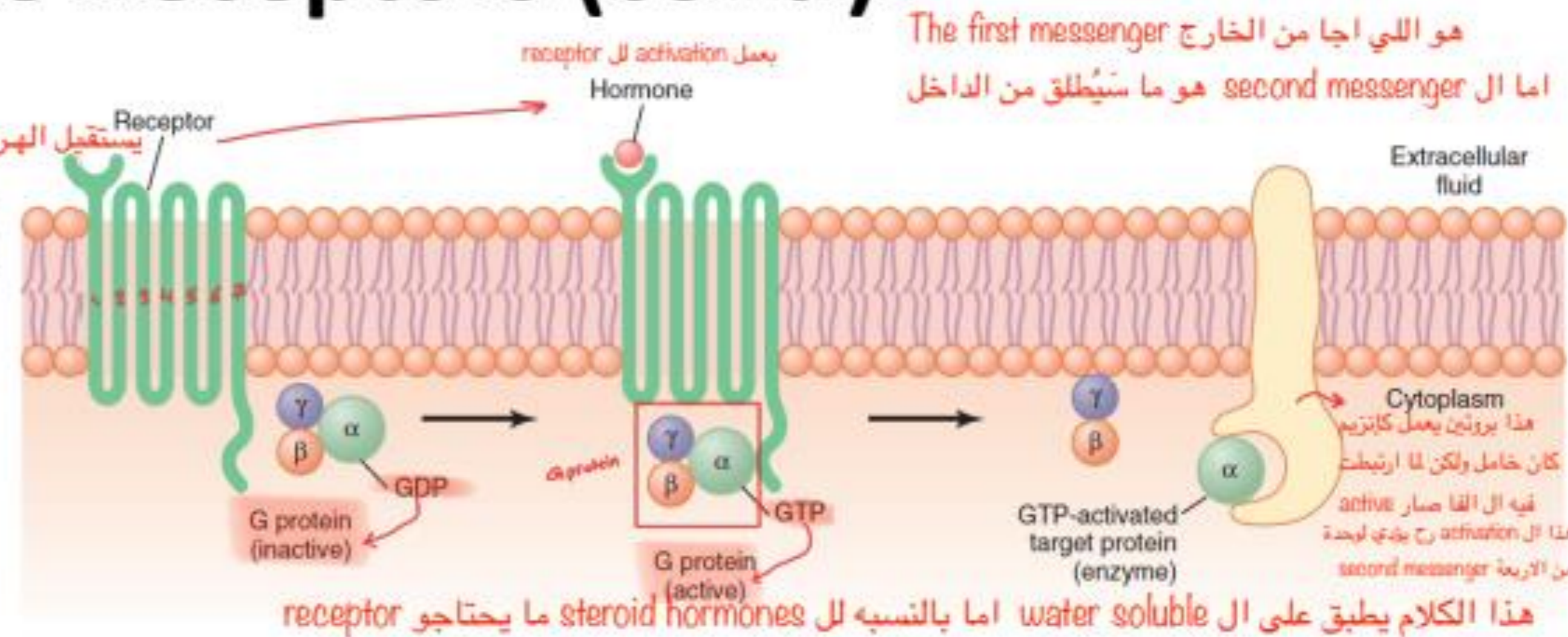
- ❖ Many hormones activate receptors that indirectly regulate the activity of target proteins (e.g., enzymes or ion channels) by coupling with groups of cell membrane proteins called **G proteins** (guanine nucleotide-binding proteins).
- ❖ There are more than 1000 G protein-coupled receptors; all have **seven transmembrane** segments that loop in and out of the cell membrane.

- Imp* ❖ Large G protein = macromolecule composed of **three subunits** (heterotrimeric) made up of alpha (α), beta (β) and gamma (γ) **subunits**. G proteins are **GTPases** enzymes. صح هو بروتين ولكن برضو يعمل كإنزيم.
- Guanine tri phosphate*
- ❖ G proteins have the ability to bind to and **hydrolyze** guanosine triphosphate (GTP) to guanosine diphosphate (GDP). *ال α بتشتغل as a GTPases enzyme that turns GTP \rightarrow GDP*

طيب هلا منيجي ليش سموه G protein ؟ اخدنا بال biochemistry انه nucleic acids نوعين DNA & RNA وتركيبيهم واساس صناعتهم انهم موجود فيهم nucleic acid واحد منهم هو ال guanine وهون هو اللي داخل بتركيب البروتين وهذا البروتين داخل بتركيب الخليه وبارز من الجهتين والجزء اللي بارز من الداخل من ال receptor وهو اللي لازق فيو بروتين ثاني بنسميه G protein ولحد الآن تم اكتشاف اكثر من 1000 شكل

G Protein-Linked Hormone Receptors (cont.):

- ❖ The alpha subunit is attached to either a GTP ("on" or active state) or GDP ("off" or inactive state). As such, the alpha subunit serves as an **on-off switch** for the activation of G-protein.



- ❖ Activation of the G-protein is achieved by exchanging the GDP on the alpha subunit to GTP. This exchange is mediated by the intracellular G protein-linked receptor domain when a **signal molecule** binds to the extracellular **domain**.

- ❖ The binding of GTP to the alpha subunit results in a structural change and its dissociation from the rest of the G-protein.

- ❖ The alpha subunit binds membrane-bound target proteins (enzymes) that initiate intracellular signals (i.e. downstream signaling cascade).

مين اللي حي يعمل اطلاق second messenger؟؟ ال activated (α) unit

G Protein-Linked Hormone Receptors (cont.):

- ❖ The activation of the G proteins and induction of intracellular signals either:
 1. Open or close cell membrane ion channels. هذا دور ثانوي يعني هاي الطريقة الثانيه لفتحهم، الاولى كانت تأثير من الخارج ويفتحو والثانيه هاي.
 2. Change the activity of an enzyme in the cytoplasm of the cell, such as adenylyl cyclase or phospholipase C.
 3. Activate gene transcription.
- ❖ The signaling event is terminated when the hormone is removed and the α subunit inactivates itself by converting (hydrolyzing) its bound GTP to GDP; then the α subunit once again combines with the β and γ subunits to form an inactive, membrane-bound trimeric G protein.
- ❖ Some hormones are coupled to **inhibitory G proteins** (G_i), whereas others are coupled to **stimulatory G proteins** (G_s). Thus, depending on the coupling of a hormone receptor to an inhibitory or stimulatory G protein, a hormone can either increase or decrease the activity of intracellular enzymes.

الخليه فيها معمل يصنع بروتين اللي هو عبارته عن سلسله من ال amino acids هذا المعمل يحتاج امر (بصمه او خريطة) عشان يصنع البروتين بترتيب معين من ال amino acids بتسلسل معين وهذا الامر بنسميه messenger RNA ، يعني بالبداية الامر الاصلي جاي من ال DNA بحكيه انه يرسل بصمه او امر للمصنع ال DNA ثابت مكانه دائماً بالنواة ف شو بعمل بيعت رساله اللي هي البصمه للبروتين المطلوب صناعته بتطلع من النواه للساييتوبلازم للرايبوزوم هناك في المصانع او المعمل بوصله الرساله ف يصنع البروتين ف صناعه البروتين بلشت اول شي من الجين وعملنا messengerRNA وطلع هذا كله بنسميه (gene transcription) هاي العمليه لازم تصير بالبداية عشان نصنع البروتين ومتى بتنتهي العمل لل G protein coupled receptor اذا راح الهرمون من الخارج ف بروح ال activation من الداخل وهاد ما حيبيب النا GTP جديد ف ال الفا حيكسر

عنا مثال الانسولين لما يجي للكبد ينشطها ويطلب منها تعمل glycogen يعني ال glucose اللي يدخل للكبد بتأثير الانسولين بيخليه يصنع glycogen ف بنسمي الانسولين الهرمون الذي يخزن الطاقة لانه ما بنحرق ال glucose كله بتفس الوقت لهيك بنخزنه عبي شكل glycogen يعني اوامر الهرمون اجت للخلية عن طريق G protein receptor اللي أدى بالآخر الى صناعة glycogen هذا امر صناعة عنا هرمون آخر عكسه بالضبط glucagon لما يربط بال receptor على سطح خلية الكبد بطلع امر بخليه يوقف انتاج ال glycogen ف صار الامر inhibitory ويمكن بنقدر نعمل العكس نشغل انزيم تاني يكسر ال glycogen. وتحولة ل glucose

Notes:

- ❖ Some receptors are enzyme-linked receptors that pass through the membrane **only once**, in contrast to the seven transmembrane G protein-coupled receptors. Example is the **cytokine receptors** such as the **leptin receptor**.
- ❖ Enzyme linked receptors have their hormone-binding site on the outside of the cell membrane and their catalytic or enzyme-binding site on the inside.
- ❖ When the hormone binds to the extracellular part of the receptor, an enzyme immediately inside the cell membrane is activated (or occasionally inactivated).

ال water soluble vitamins بعضها
مثل vitamin C , B(1,6,12) وبعضها
lipid soluble مثل vitamin A, D

Intracellular Hormone Receptors:

- ❖ ^{لا يحتاج G protein} Steroid hormones, ^{لا يحتاج G protein} thyroid hormones, ^{Vitamin A} retinoid hormones, and vitamin D, bind with protein receptors **inside the cell** rather than in the cell membrane.
- ❖ The activated hormone-receptor complex binds with a specific regulatory (promoter) sequence of the DNA called the hormone response element, and activates or represses **transcription** of specific genes and formation of **messenger RNA (mRNA)**.
- ❖ After certain period following the entrance of the hormone inside the cell, newly formed proteins appear in the cell and become the controllers of new or altered cellular functions.

◆ Second Messenger Mechanisms:

- ❖ Second messengers are intracellular signaling molecules released by the cell in response to exposure to extracellular signaling molecules (the first messengers such as the hormones).
- ❖ The **second messenger** include (1) cAMP, (2) cGMP, (3) Calcium ions and associated *Calmodulin* and (4) products of membrane phospholipid breakdown.

1- Adenylyl Cyclase–cAMP Second Messenger System:

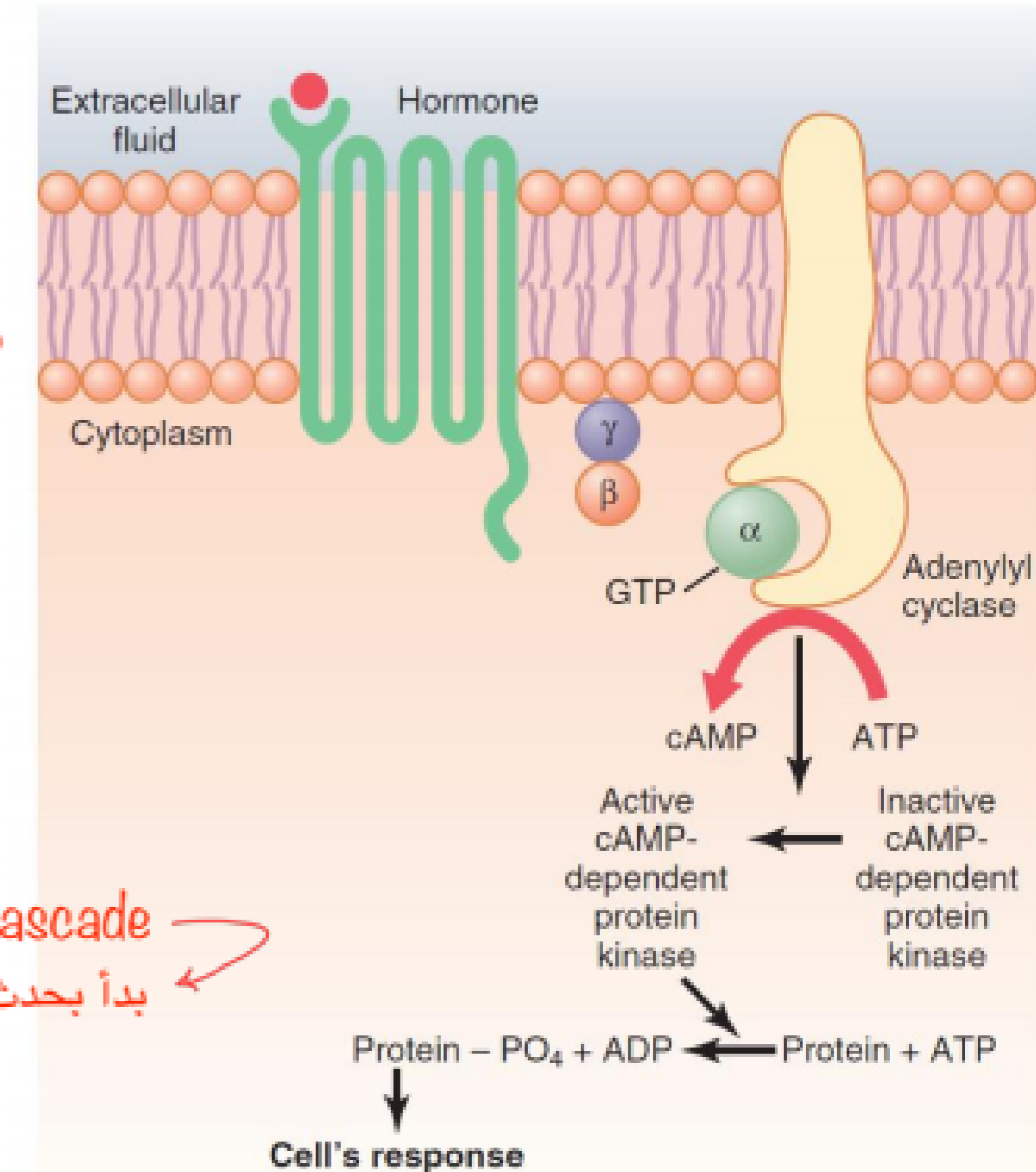
❖ If the Gs protein stimulates the adenylyl cyclase-cAMP system, adenylyl cyclase catalyzes the conversion of a **small** amount of cytoplasmic adenosine triphosphate (ATP) into cAMP inside the cell.

هون بعمله Phosphorylation يعني بيرتبط ب PO_4 وتبدأ سلسلة الاحداث بالتضخيم (زي ما حكينا بالمحاضرة الماضية)

❖ This then activates cAMP-dependent protein kinase (also called protein **kinase** A or PKA). PKA phosphorylates specific cell proteins, triggering biochemical reactions that ultimately lead to the cell's response to the hormone (i.e. downstream signaling **cascade** to greatly **amplify** the strength of the original first messenger signal). Example, the action of **ADH** hormone in the kidney.

بدأ بحدث صغير وكبر تدريجياً وصار حدث ثاني اكبر من الاصيلي الدكتور حكا انه اصل هاد المصطلح من النافورة المي بتنزل وبتعزل شلالات كثير

❖ If binding of the hormone to its receptors is coupled to an inhibitory G_i protein, adenylyl cyclase will be inhibited, reducing formation of cAMP and ultimately leading to an inhibitory action in the cell. Example, **somatostatin** hormone.



هذا ال cAMP عنا ال α unit اثر بالانزيم وعمله activation هذا الانزيم يشتغل عال ATP الموجود بال cytoplasm ويحوله الى ADP ثم الى AMP وهون بال AMP الراس الاول بربط مع الراس الاخير وبصير cyclic وهون عنا مرسوم بشكل نهائي ال $ATP \rightarrow cAMP$ وال cAMP هو اللي بكمل باقي الشغلات بحيث انه كثير من الانزيمات تتنشط عن طريقه

مثال عليه ADH بيطلع من ال pituitary gland لل kidney بربط ب G protein coupled receptor ويؤدي لتكوين ايا second messenger ؟؟ cAMP (هذا مثال على excitatory) مثال على inhibitory بعمل inhibition لل cAMP هرمون اسمه somatostatin بربط بال G protein coupled receptor يؤدي لمنع تكون cAMP ف هو inhibition

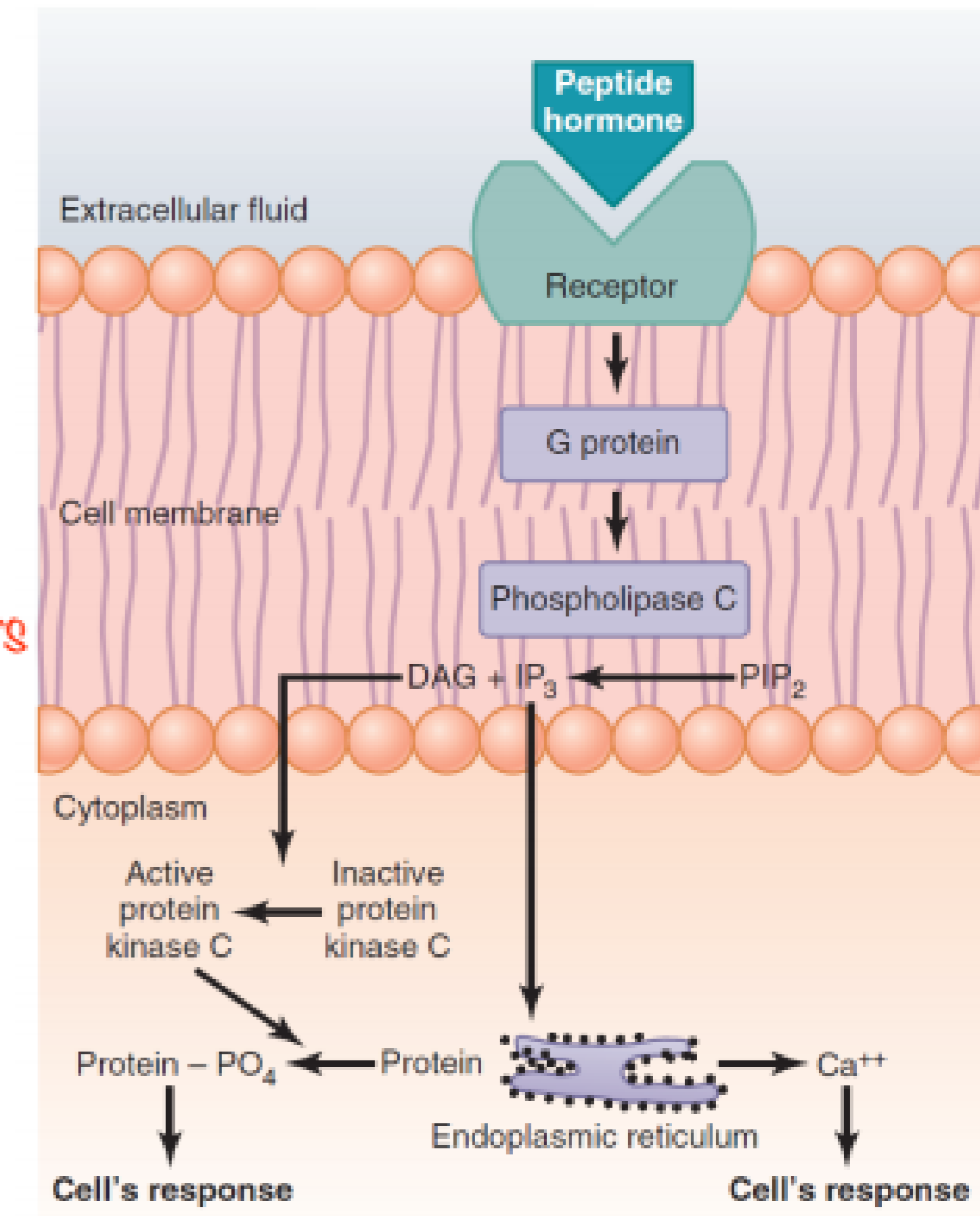
2- Guanylyl Cyclase–cGMP Second Messenger System:

- ❖ The second messenger cGMP is generated by the enzyme guanylyl cyclase (GC).
- ❖ There are **two forms of guanylyl cyclase**, a soluble, cytoplasmic form and a membrane-localized form. Soluble GC is a target for the paracrine-signaling molecule **nitric oxide (NO)**. *First messenger*
- ❖ It is a cyclic nucleotide derived from guanosine triphosphate (GTP). **cGMP acts as a second messenger** much like cAMP.
- ❖ **Nitric oxide** is a famous stimulator of cGMP synthesis. **cGMP relaxes smooth muscle tissues**. In blood vessels, relaxation of vascular smooth muscles lead to vasodilation and increased blood flow.
- ❖ cGMP is involved in the regulation of some protein-dependent kinases. Example, protein kinase G (PKG). Activation of PKG reduces cytoplasmic Ca^{2+} concentrations, resulting in smooth muscle relaxation.
- ❖ The transmembrane form of guanylyl cyclase is a receptor for **atrial natriuretic (ANP)** peptide. Binding of ANP to transmembrane guanylyl cyclase in the kidney increases cGMP, which stimulates Na^+ excretion to reduce blood volume.

بعض ال phospholipids اللي موجوده بال cell membrane ال receptor يؤدي لتكسيرها ب phospholipase c ويؤدي لتكوين مادتين ← (IP3)(DAG)

3- Cell Membrane Phospholipid Second Messenger System:

- ❖ Some hormones activate transmembrane receptors that activate the enzyme phospholipase C attached to the inside projections of the receptors.
- ❖ Phospholipase C catalyzes the breakdown of some phospholipids in the cell membrane, especially phosphatidylinositol biphosphate (PIP₂), into two different second messenger products: inositol triphosphate (IP₃) and diacylglycerol (DAG). *Second messengers*
- ❖ The IP₃ mobilizes calcium ions from mitochondria and the endoplasmic reticulum, and the calcium ions then have their own second messenger effects, such as smooth muscle contraction and changes in cell secretion.
- ❖ 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.



4- Calcium-Calmodulin Second Messenger System:

الكالسيوم قليل الذوبان في الماء لذلك يحتاج لبروتين لحتى يحمله هذا البروتين اللي بشيل الكالسيوم
اسمه calmodulin فالكالسيوم لما بطلع بكون محمول عليه ف هم التنين بيعملو ك second messenger

- ❖ Calcium influx may be initiated by (1) opening of voltage-gated calcium channels or (2) a hormone interacting with membrane receptors that open calcium channels (ligand-gated).
- ❖ Upon entering a cell, calcium ions bind with the protein **calmodulin**.
- ❖ Calmodulin has **four calcium sites**, and when three or four of these sites have become bound with calcium, the calmodulin changes its shape and initiates multiple effects inside the cell, including activation or inhibition of protein kinases.
- ❖ Example, calmodulin activates myosin light chain kinase, which acts directly on the myosin of smooth muscle to cause smooth muscle contraction.
- ❖ **Troponin C** in skeletal and cardiac muscle is similar to calmodulin in both function and protein structure.

Test Question:

- Q. In chemical synapses that involve a so-called second messenger, typically a G-protein linked to the postsynaptic receptor is activated when neurotransmitter binds to that receptor. Which of the following represents an activity performed by the activated second messenger?**
- A. Closure of a membrane channel for sodium or potassium
 - B. Activation of cyclic AMP or cyclic GMP
 - C. Inactivation of enzymes that initiate biochemical reactions in the postsynaptic neuron
 - D. Inactivation of gene transcription in the postsynaptic neuron
 - E. Opening of ligand-gated ion calcium channels

Done by: Ayah Abbadi

Good Luck 🍀