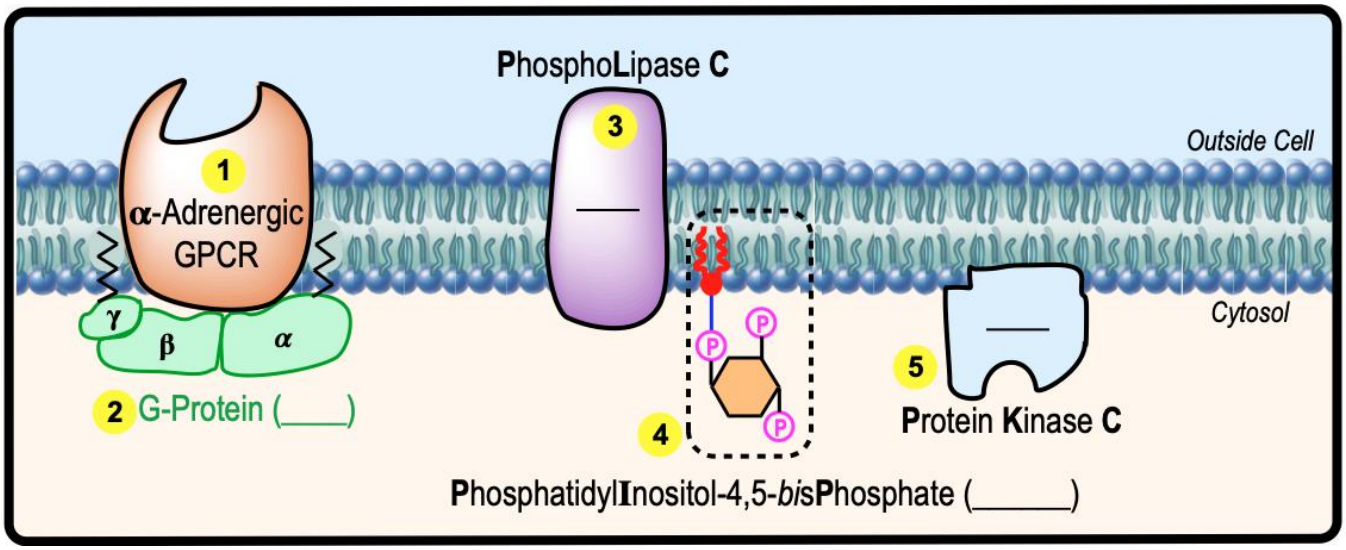
CONCEPT: PHOSPHOINOSITIDE GPCR SIGNALING

●***P**hosphoinositide* (or *Inositol-Phospholipid*) ***S**ignaling **P**athway* (_____): another classic example of a *GPCR signaling*.

□ Mediates a _____ of hormonal effects (ex. epinephrine, angiotensin, vasopressin).

□ Many functions including blood platelet & injured-cell signaling to stimulate growth & injury-repair process.

●*Phosphoinositide signaling* requires these _____ components:

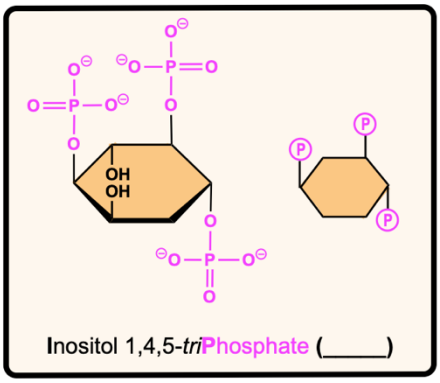


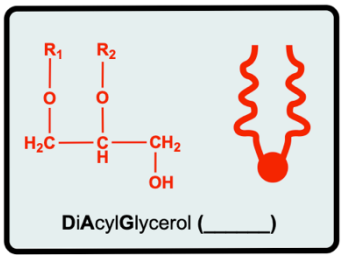
3 Phosphoinositide Secondary Messengers

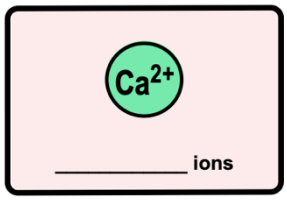
●PSP generally involves production of _____ *secondary messengers*:

1) **I**nositol-1,4,5-*tri***p**hosphate (IP__). 2) 1-2-**dia**cyl**g**lycerol (_____). 3) *Calcium* Ions (Ca^{2+}).

EXAMPLE: Phosphoinositide Secondary Messengers:







PRACTICE: Which of the following are secondary messengers produced in the PSP pathway?

a) cGMP & K^+. b) IP_2 & IP_3. c) IP_3 & DAG. d) DAG & cAMP.

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●Many variations of **P**hosphoinositide **S**ignaling **P**athways (*PSP*), but can be condensed into _____ general steps:

α-Adrenergic Pathway

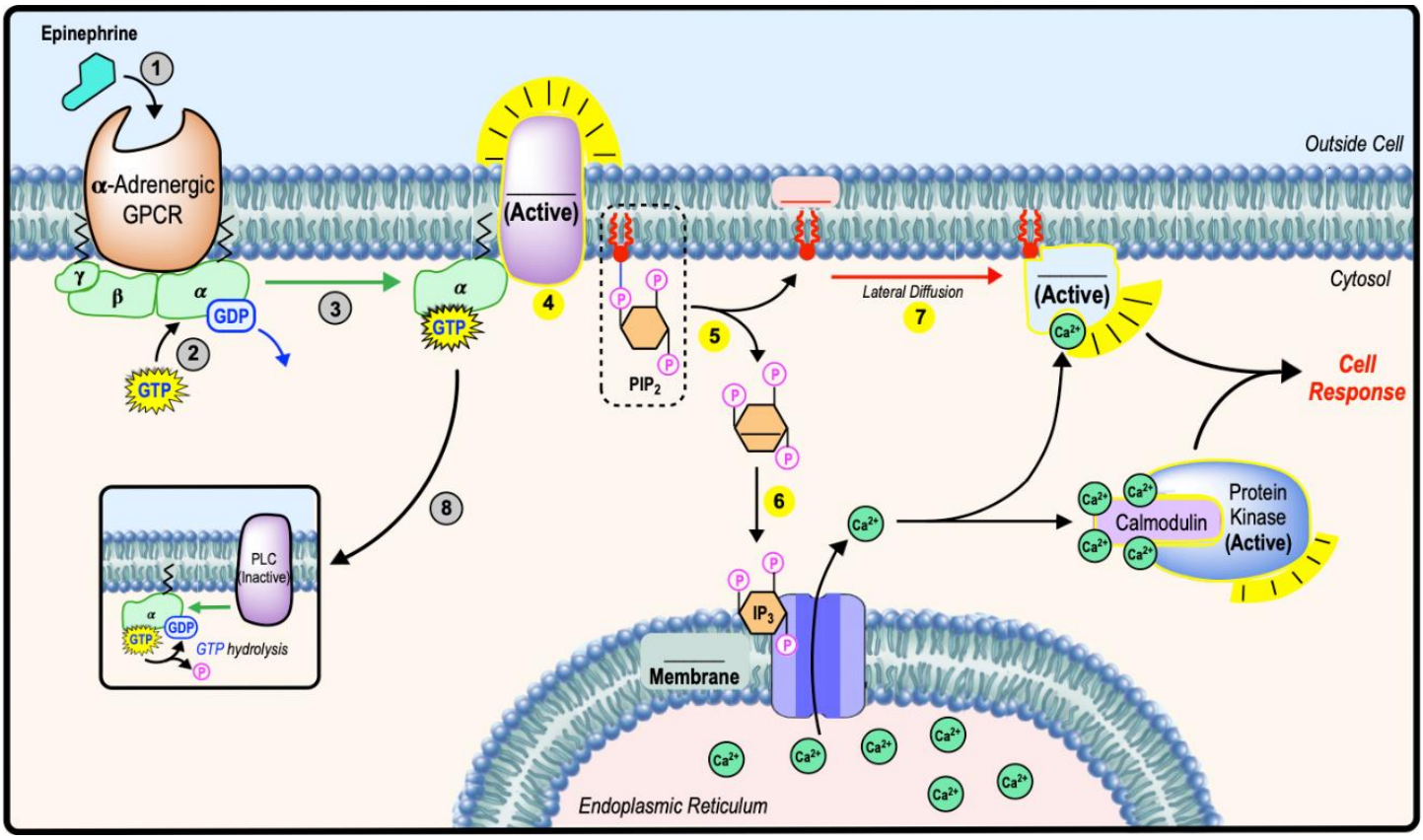
Same as Before

1. Hormone signaling ligand (*epinephrine*) binds GPCR (*α-adrenergic receptor*) causing a _____ *shift*.
2. GPCR conformational shift _____ G protein (G_q) by promoting α subunit to *release* GDP & bind GTP.
3. With bound GTP, ___ subunit of G_q _____ from β-γ subunits & *diffuses* to nearby *effector enzyme*.

PSP Pathway

New

4. The activated/GTP-bound α-subunit binds & *activates* the effector enzyme (***p**hospho**l**ipase **C***, _____).
5. Activated *PLC* hydrolyzes its substrate, ***p**hosphat**i**dylinositol-4,5-bis**p**hosphate* (_____).
   - □ Active *PLC* generates two _____ *messengers* (_____ & _____).
6. IP_3 diffuses in cytoplasm to *endoplasmic reticulum* to bind/open _____ *transport channels*, ___ *cytosolic* [Ca^{2+}].
   - □ Ca^{2+} Activates→ *Calmodulin* (_____) Activates→ *cytosolic protein kinases* ⟶ *Cell Response*.
7. *DAG* diffuses in membrane &, along with released Ca^{2+}, *activates* a membrane-bound ***p**rotein **k**inase **C*** (_____).



Same as Before

8. G protein α subunit slowly _____ itself (& *PLC*) by *hydrolyzing* its GTP → GDP.
   - □ α subunit assembles its original, *inactive* form & ligand *dissociates* from GPCR to _____ pathway.

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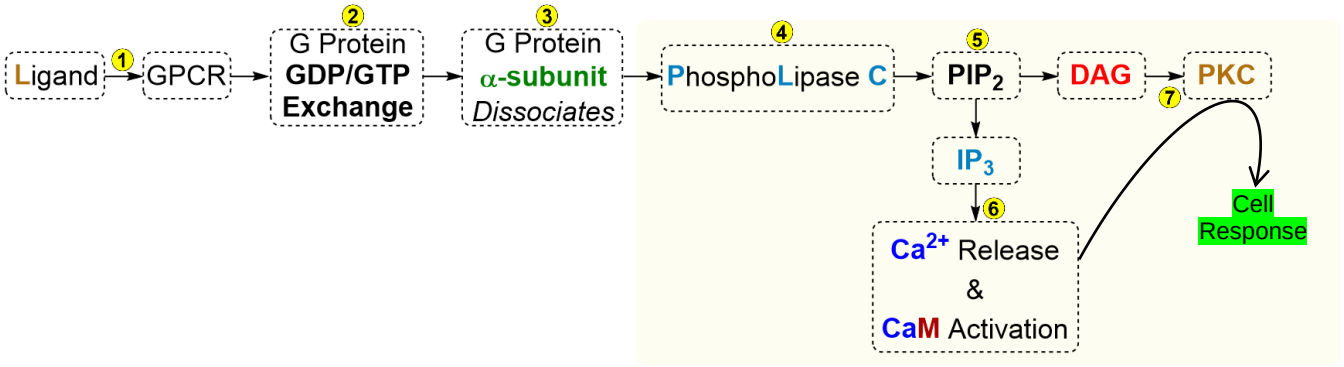
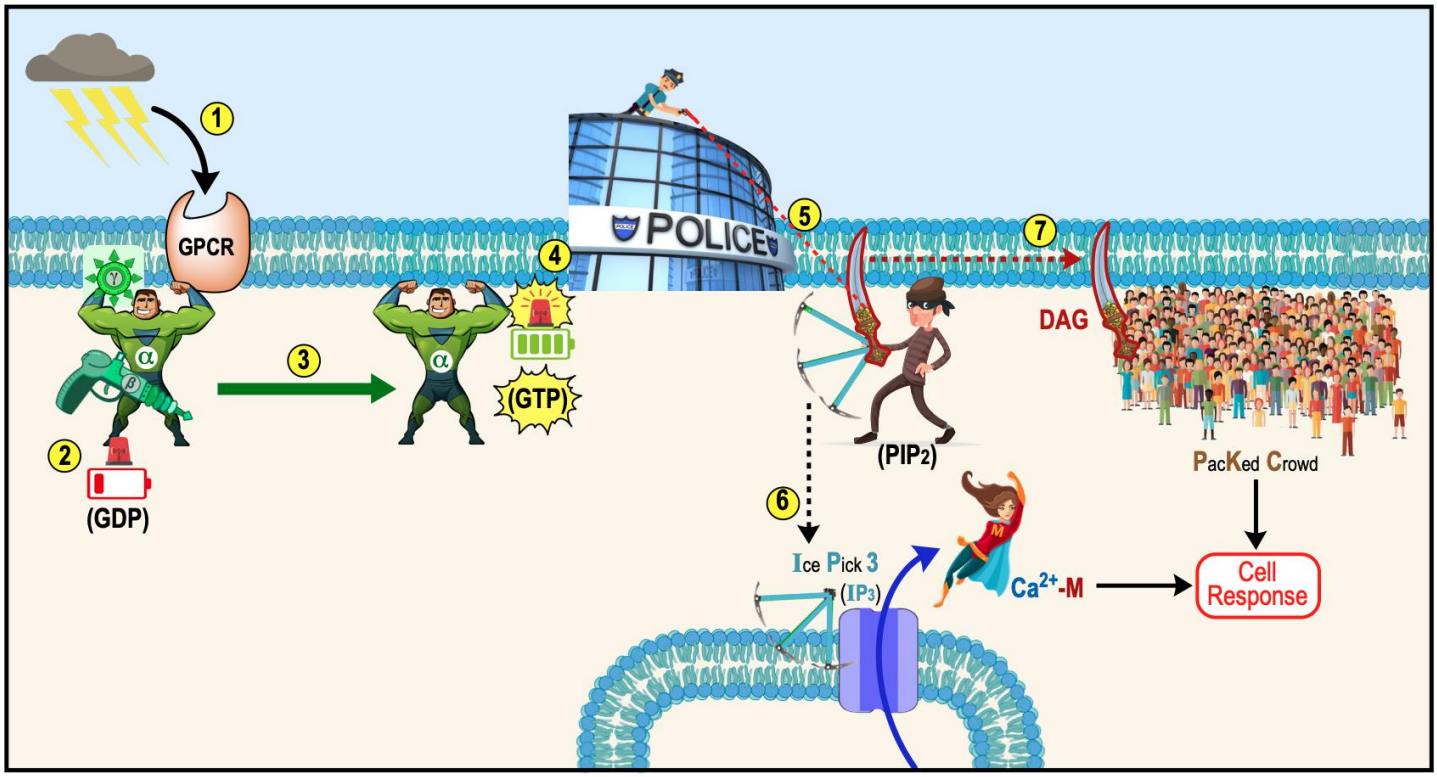
How to Remember Phosphoinositide GPCR Signaling?



Story: Cell wants to *save the day* (***Cell Response***) from the villain *"**Pip**"* and his ***2*** weapons (***PIP_2***).

1. ***L***ightining strike provides a warning of *danger* (***L***igand binding).
2. ***Alpha****-hero* (G protein α-subunit) *exchanges* **empty siren battery** with **full siren battery** (GTP exchange).
3. *Alpha*-hero alarms ***P***o***L***i***C***e but *forgets* β-blaster & γ-gadget (G protein α-subunit dissociation to ***P***hospho***L***ipase ***C***).
4. *Alpha*-hero *tells* ***P***o***L***i***C***e to target ***PIP***s ***2*** weapons (***PIP_2***) (***P***hospho***L***ipase ***C*** *activation*).
5. ***P***o***L***i***C***e *tasers* ***PIP***s ***2*** weapons, separating them into ***DAG***ger & ***3*** ***I***ce ***P***icks (***DAG*** & ***IP_3*** production).
6. ***3*** ***I***ce ***P***icks drop to *release* ***Ca***ptain ***M***arvelous to help *save the day* (***Ca^{2+}*** *release*, ***CaM*** *activation* & Cell Response).
7. ***DAG***ger flips toward a ***P***ac***K***ed ***C***rowd (***PKC***), who catch it & help *save the day* (***PKC*** *activation* & *Cell Response*).



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EXAMPLE: Place the following steps of the PSP signaling pathway in order from 1-7:

a) Conformational shift of the G-protein and G_{α} exchanges GDP for GTP _____.

b) Activation of Phospholipase C _____.

c) DAG and Ca^{2+} ions activate Protein Kinase C _____.

d) Ligand binds to the GPCR _____.

e) G_{α} dissociates from $G_{\beta\gamma}$ _____.

f) IP_3 diffuses to the ER membrane to release Ca^{2+} into the cytoplasm _____.

g) Phospholipase C hydrolyzes PIP_2 into IP_3 and DAG _____.

PRACTICE: When epinephrine binds to a(n) _____ receptor, _____ is activated, causing an increase in the cellular concentration of _____.

a) Beta ; $G_{\alpha i}$; Inositol triphosphate.

b) Alpha ; $G_{\alpha q}$; Inositol triphosphate.

c) Beta ; $G_{\alpha i}$; Diacylglycerol.

d) Alpha ; $G_{\alpha s}$; cAMP.

e) Beta ; $G_{\alpha q}$; Diacylglycerol & Inositol triphosphate.

PRACTICE: Which of the following secondary messengers, when released by phospholipase C will directly activate PKC?

a) Inositol triphosphate.

b) Phosphatidylinositol bisphosphate.

c) Diacylglycerol.

d) cGMP.

PRACTICE: _____, when formed by phospholipase C, triggers the release of calcium from the endoplasmic reticulum?

a) Inositol triphosphate.

b) Phosphatidylinositol bisphosphate.

c) Diacylglycerol.

d) cGMP.

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PRACTICE: In cells, epinephrine binding to the α-adrenergic receptor activates $G_{\alpha q}$. All of the following happen EXCEPT:

a) Protein Kinase C activity will be decreased.
b) Inositol triphosphate will cause the release of Ca^{2+} from the endoplasmic reticulum.
c) Increased binding of Ca^{2+} to the calmodulin will occur.
d) Increased Ca^{2+}-Calmodulin complex will bind to and activate calmodulin-binding proteins.
e) Protein Kinase C will be activated by increased levels of diacylglycerol.

PRACTICE: All G proteins are activated by GDP/GTP exchange (binding to GTP), but lose their activity when they hydrolyze GTP to GDP. However, different G proteins can have different roles. Which of the following is INCORRECT?

a) When active, $G_{\alpha s}$ activates adenylate cyclase.
b) When active, $G_{\alpha s}$ results in increased production of cAMP.
c) When active, $G_{\alpha i}$ results in the inhibition of adenylate cyclase.
d) When active, $G_{\alpha q}$ results in the activation of phospholipase C.
e) When active, $G_{\alpha s}$ results in increased production of inositol triphosphate.

PRACTICE: The reaction catalyzed by Phospholipase C can use all of the following EXCEPT:

a) Phosphatidylinositol bisphosphate as a substrate.
b) Diacylglycerol as a product.
c) Diacylglycerol phosphate as a product.
d) Inositol triphosphate as a product.
e) Water as a substrate.