Mechanism of hormone actions II Cell Surface Receptors

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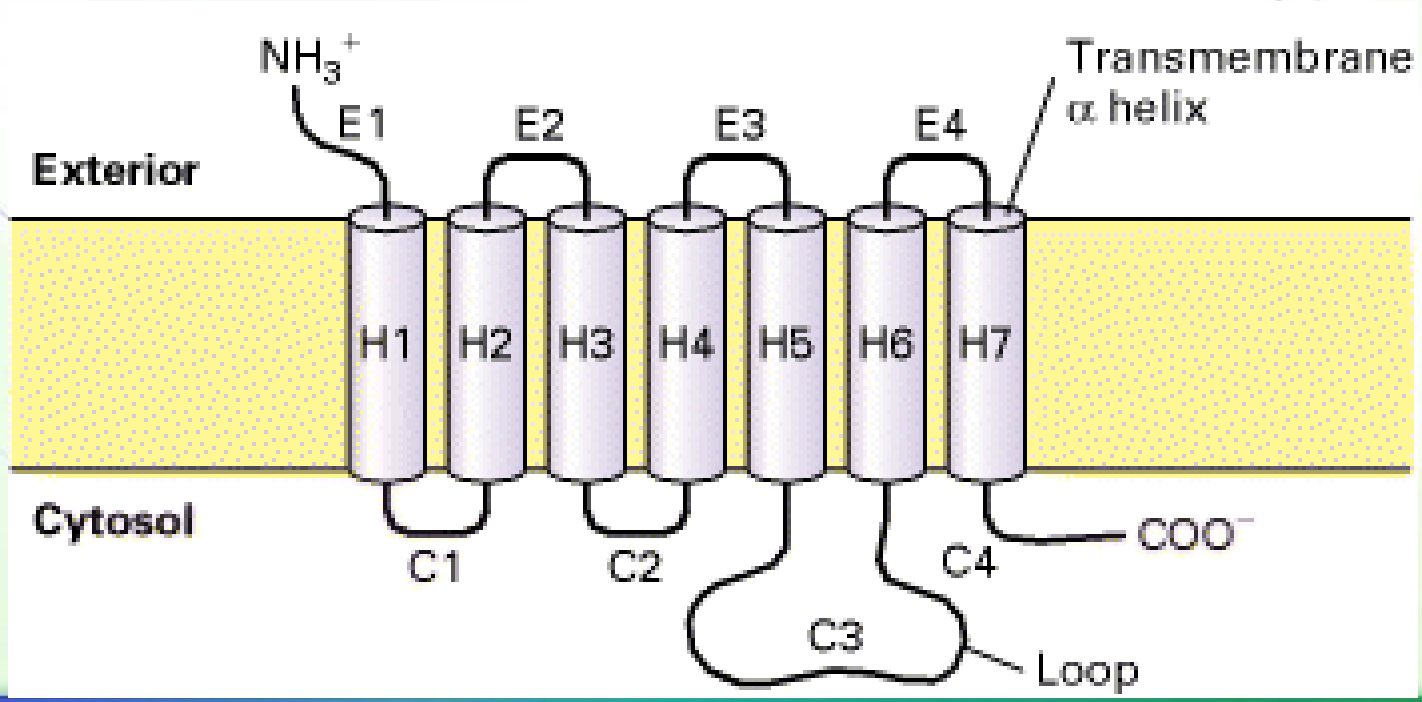
Second year, 2019

G protein-coupled receptors

G protein-coupled receptors

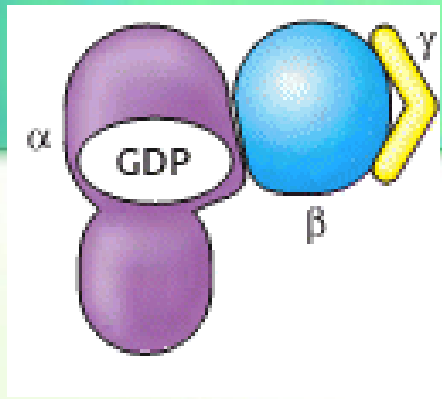
- All G protein-coupled receptors (GPCRs) contain seven membrane-spanning regions.
- They all mediate a similar signaling pathway.



Homology

- Although all GPCRs are structurally similar, their amino acid sequences generally are quite dissimilar.
  - β 1- and β 2-adrenergic receptors are 50 percent identical.
  - a - and β -adrenergic receptors exhibit even less homology.
- The specific amino acid sequence of each receptor determines:
  - Ligand binding
  - G proteins interaction

G proteins



- G proteins are intermediary in signal transduction from the seven transmembrane (7TM) receptors.
- G proteins are made of three subunits α, β, and γ.

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

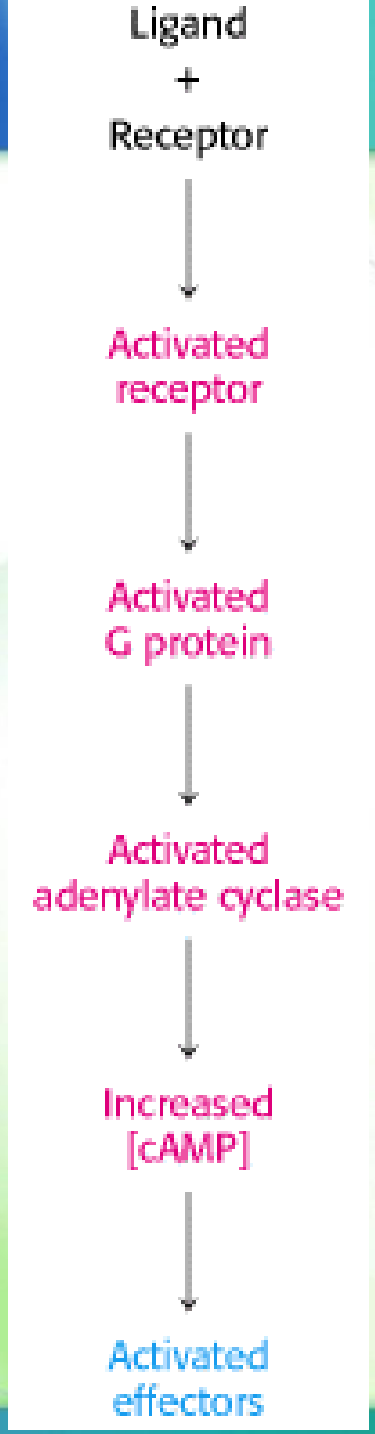
Second messengers

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

- Why are second messengers good?
  - Second messengers are often free to diffuse to other compartments of the cell.
  - The signal may be amplified significantly in the generation of second messengers
  - The use of common second messengers in multiple signaling pathways often results in cross-talk between different signaling pathways.

Classification according to second messengers

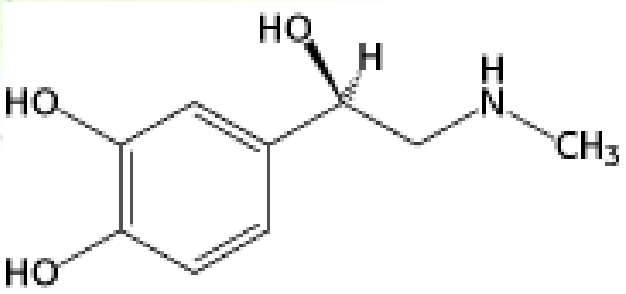
Group II. HORMONES THAT BIND TO CELL SURFACE RECEPTORS	
A. The second messenger is cAMP	
Adrenocortropic hormone (ACTH)	Parathyroid hormone (PTH)
Angiotensin II	Opioids
Antidiuretic hormone (ADH)	Acetylcholine
Follicle-stimulating hormone (FSH)	Glucagon
Human chorionic gonadotropin (hCG)	α_2-Adrenegic 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)


Ligand
+
Receptor
Activated receptor
Activated G protein
Activated adenylate cyclase
Increased [cAMP]
Activated effectors

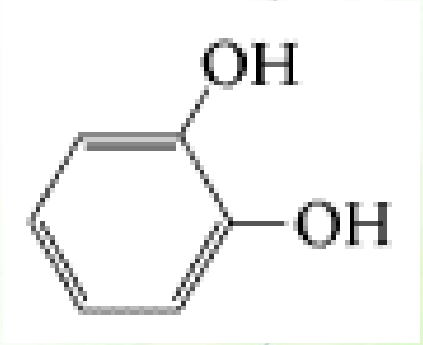
An example

Adrenergic receptors

- This protein binds epinephrine (also called adrenaline), a hormone responsible for the "fight or flight" response.



Epinephrine



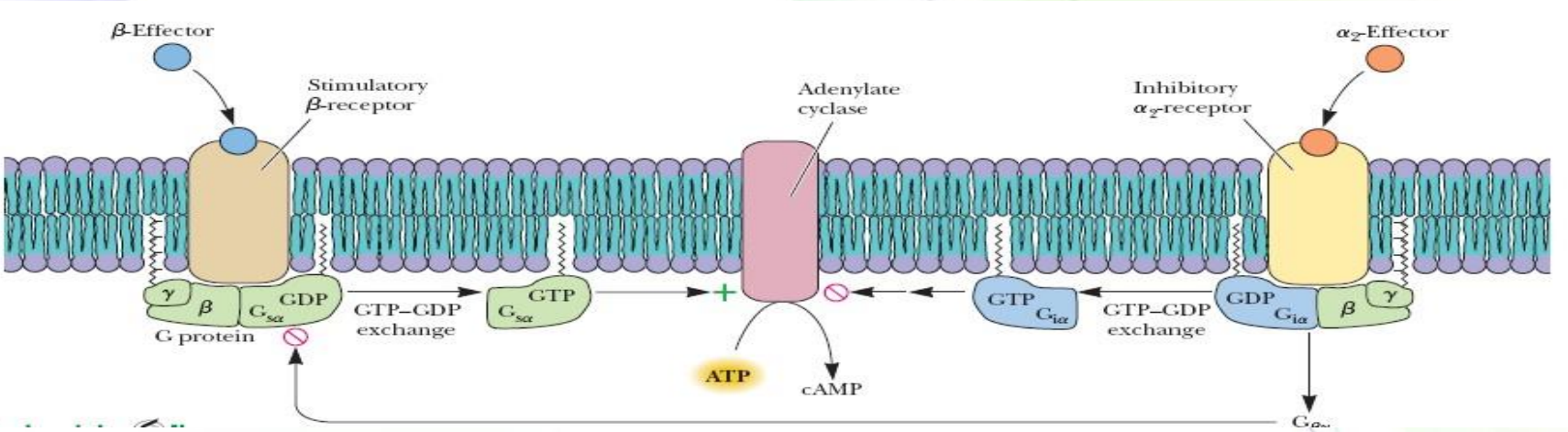
Actions of epinepherine (β-adrenergic receptor)

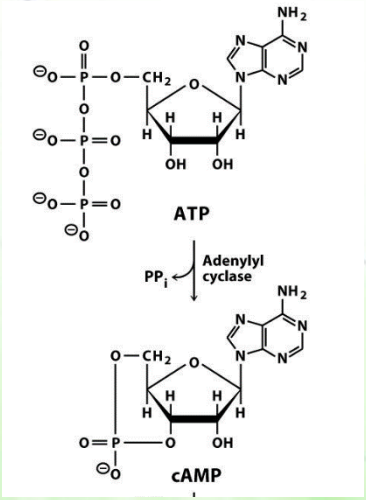
Beta Receptors	
β1 (postsynaptic)	**β2 (postsynaptic)**
Gs protein coupled Activates **Adenyl Cyclase** **ATP → cAMP**	
1. The heart a. ↑heart rate (+ chronotropic) b. ↑impulse conduction (+dromotropic) c. ↑contraction (+ inotropic) d. ↑ejection fraction 2. ↑renin release by Juxtaglomerular cells 3. ↑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

Actions of epinepherine (α1-adrenergic receptor)

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

The signal transduction of epinepherine





Signal Transduction

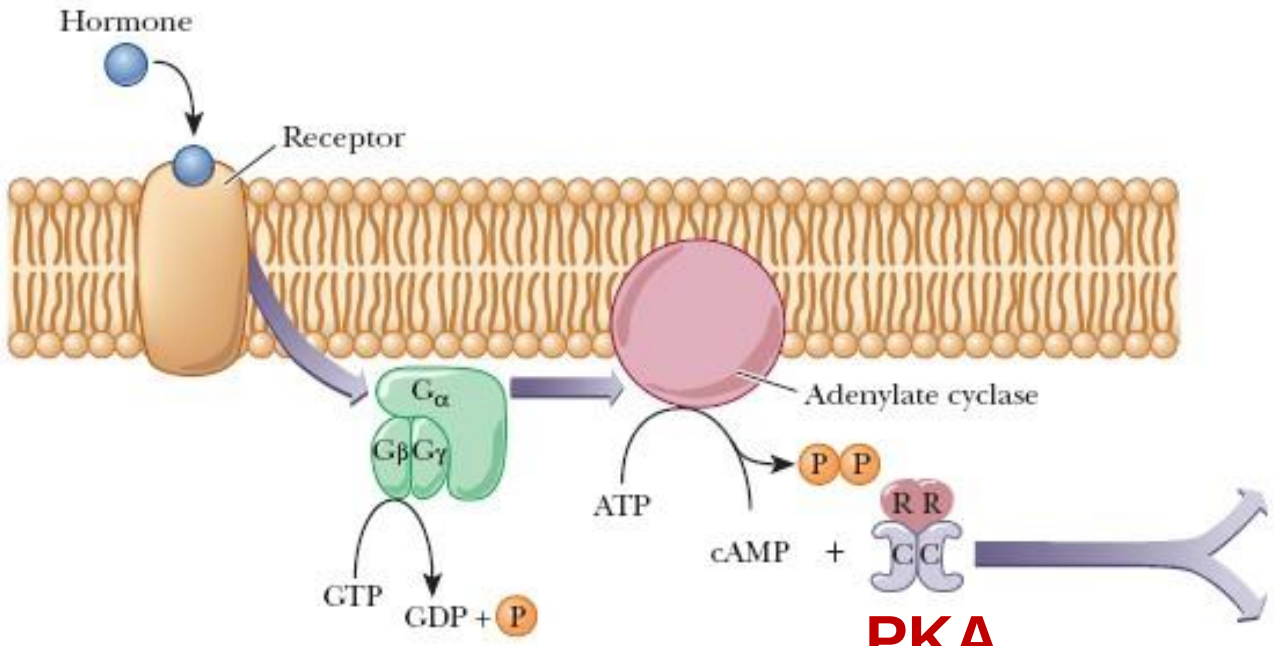
Cellular effects of cAMP

- ↑ degradation of storage fuels
- ↑ **secretion of acid by gastric mucosa**
- Dispersion of melanin pigment granules
- ↓ aggregation of blood platelets
- Opening of chloride channels

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

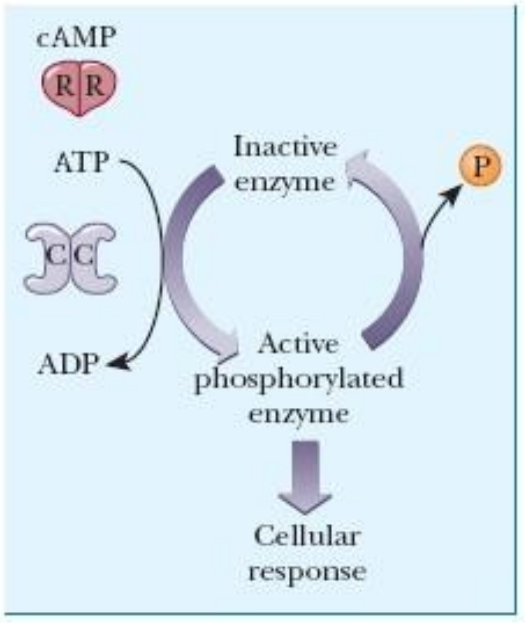
Caffeine induces gastric acid secretion via bitter taste signaling in gastric parietal cells

Then…



PKA

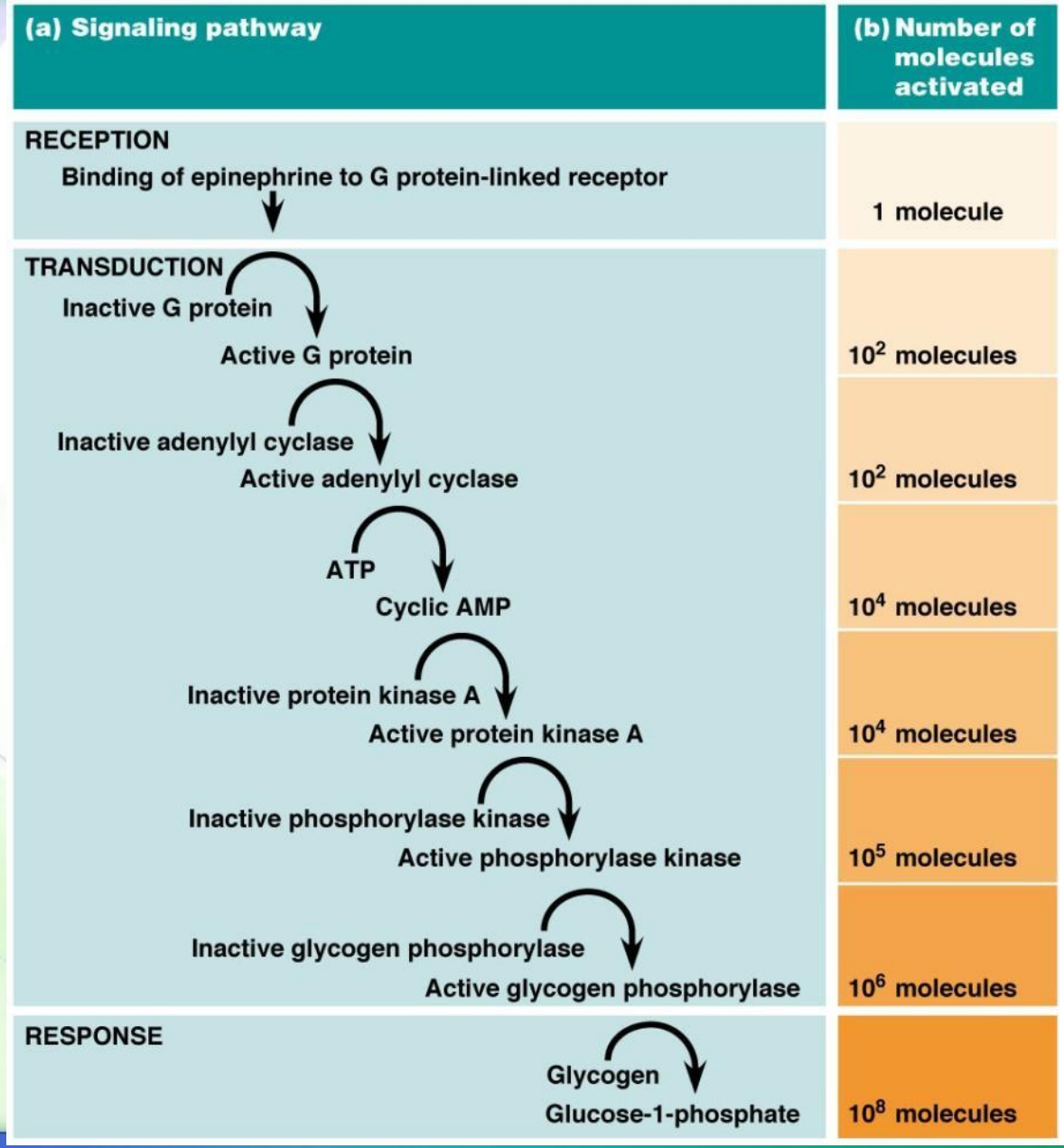
Usually:
Ser or Thr



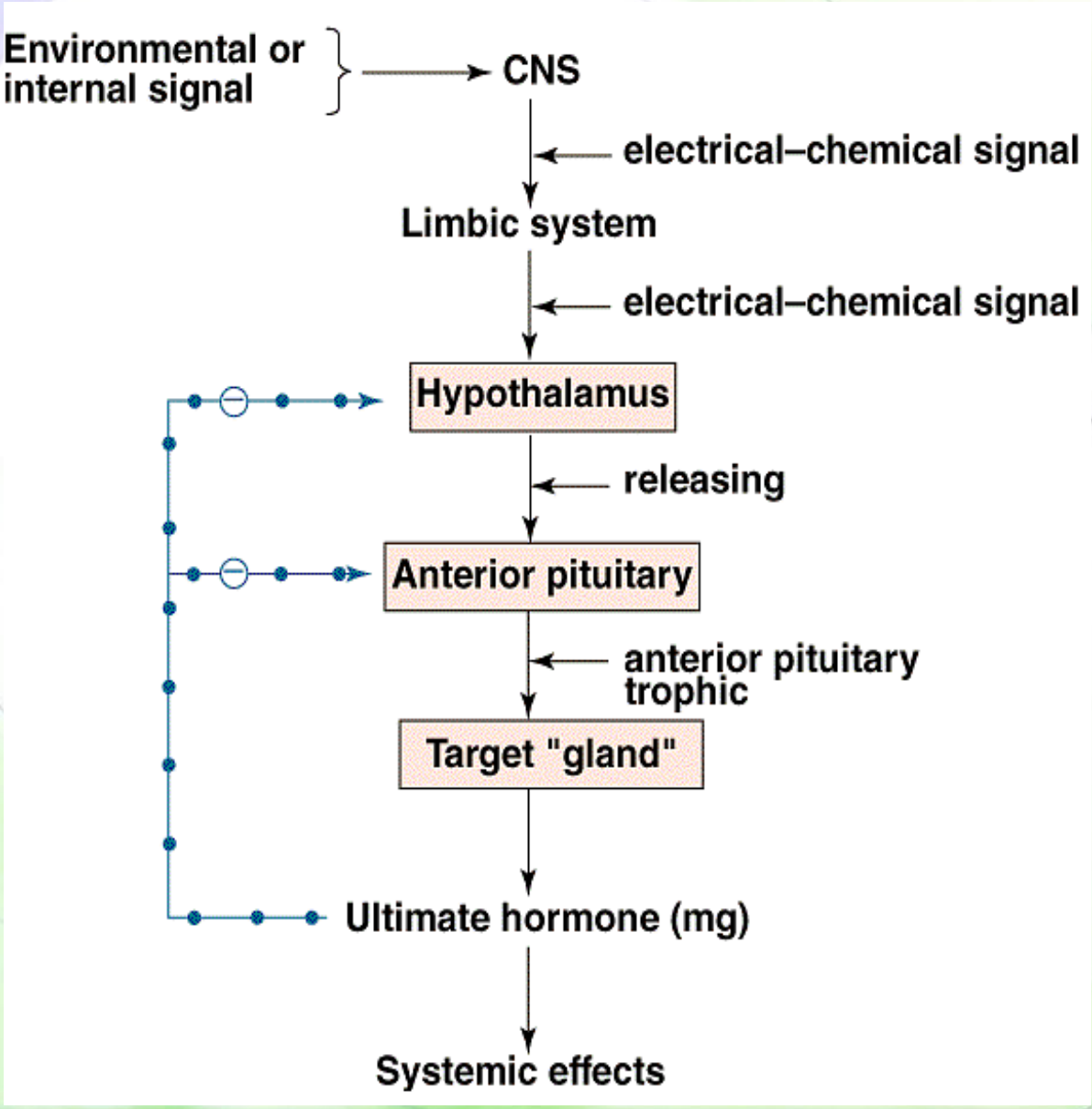
Glycogen Synthase!!

Signal Amplification

Signal Amplification

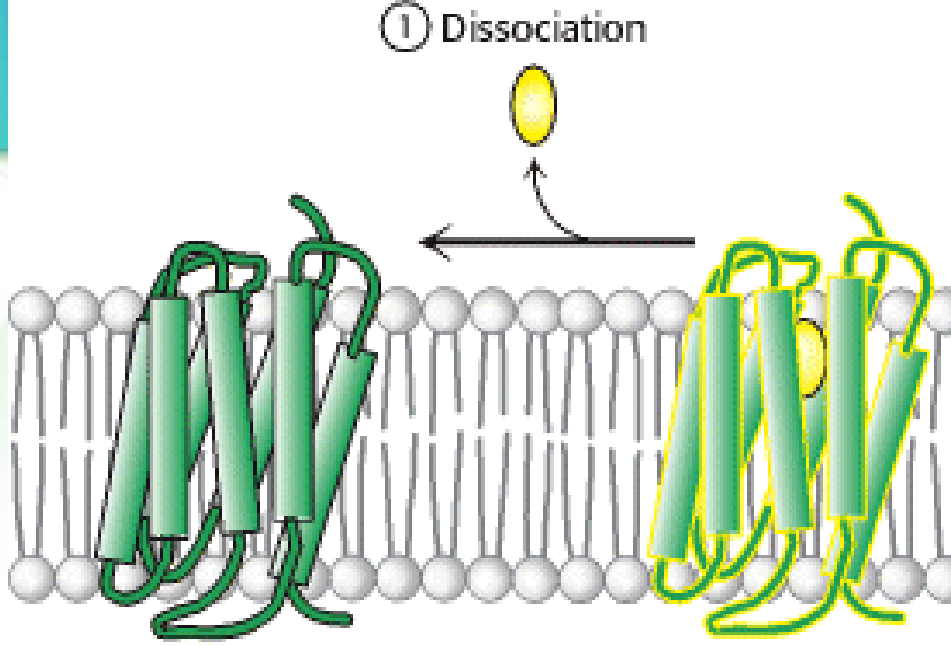


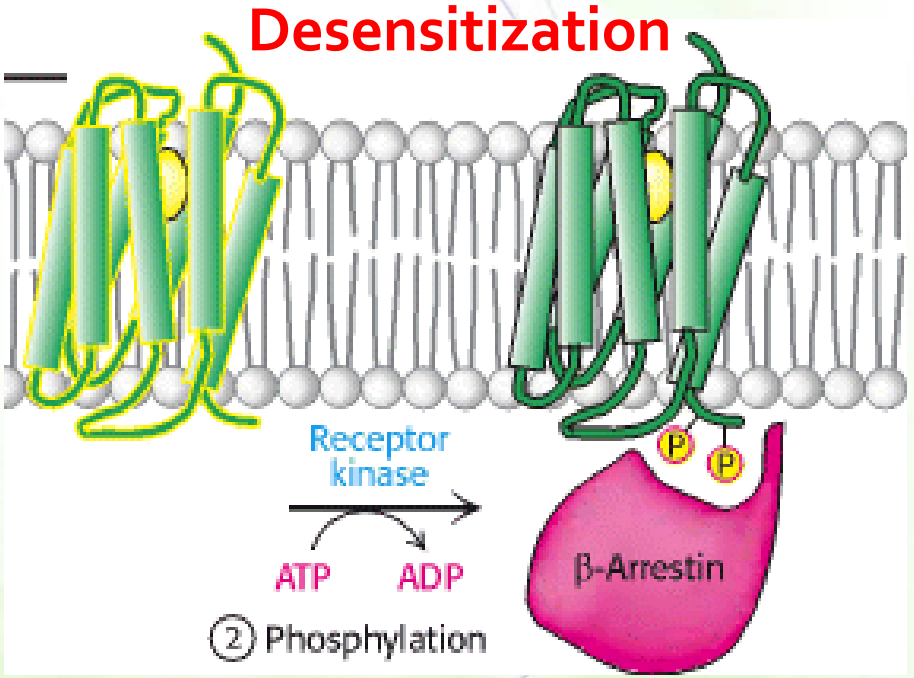
Amplification at the hormonal level as well

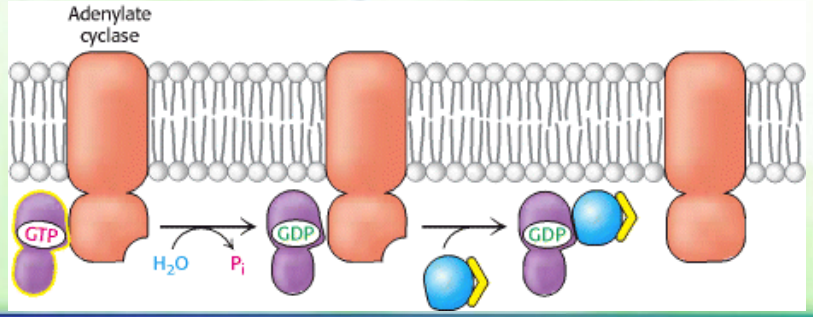


Termination

- **Dissociation** of the hormone
- **GTPase** activity of Gα subunit
- **Hydrolysis** of cAMP (phosphodiesterase)
- Phosphorylation of the hormone bound-receptor followed by binding to **β-Arrestin**

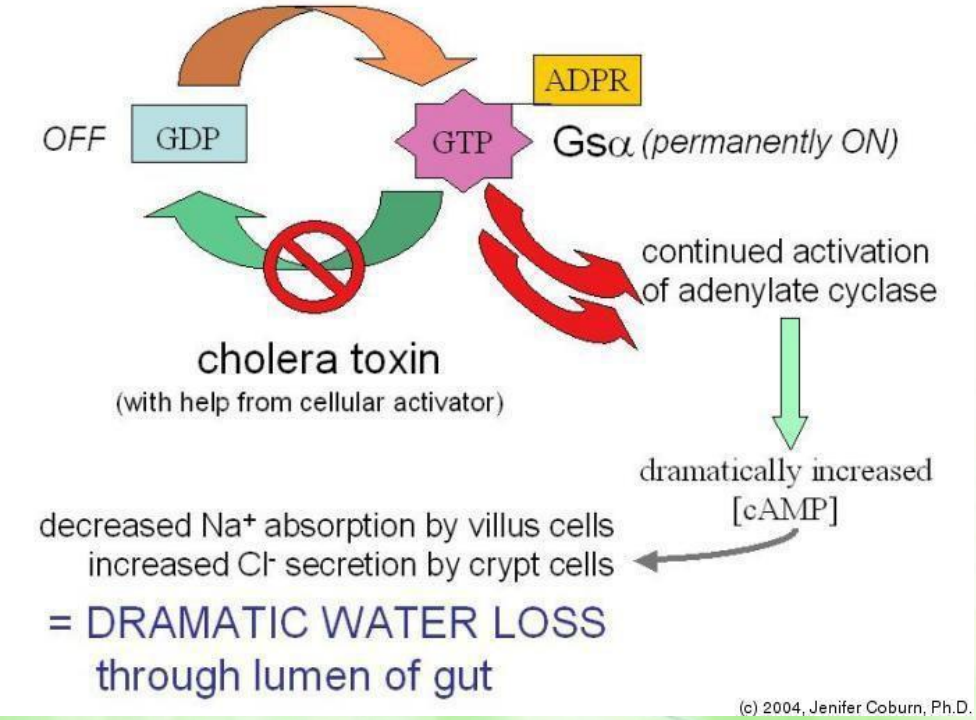


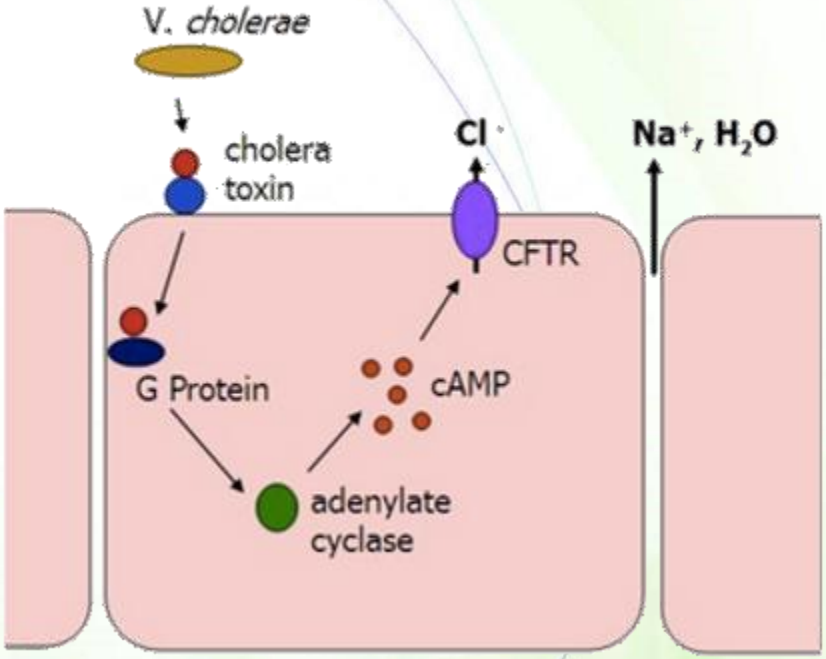




Cholera

- **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**





The phosphoinositide pathway

- Used by many hormones (e.g. ADH)
- Binding of a hormone to 7TM receptor

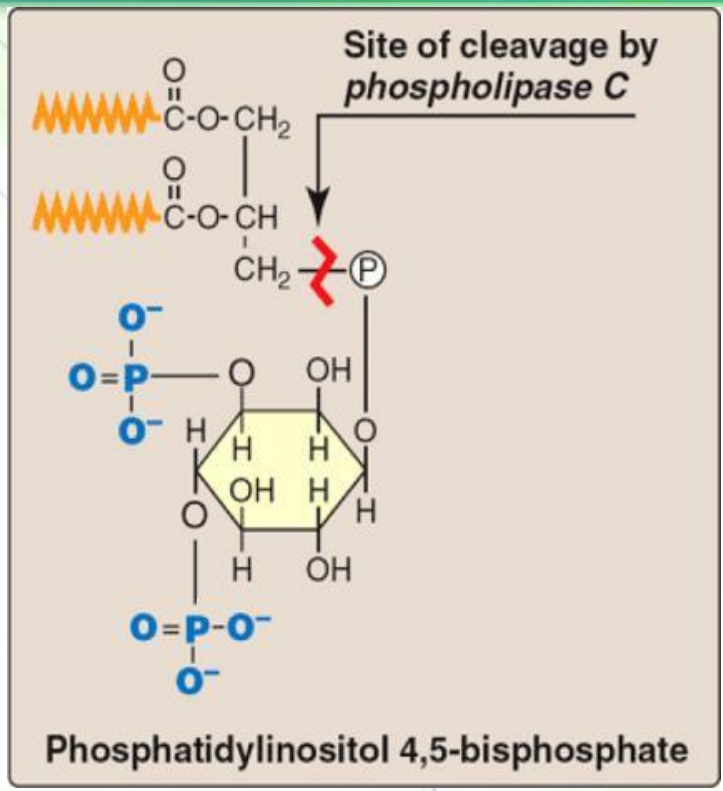
↓

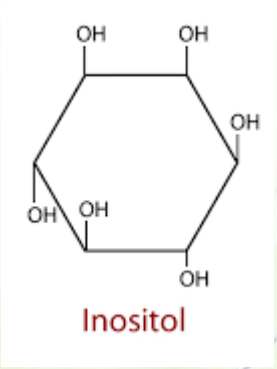
Activation of G Protein

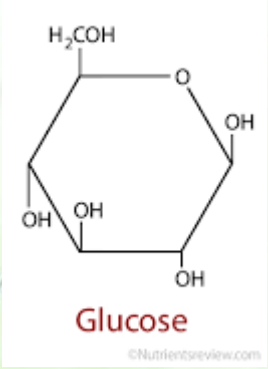
↓

Activation of Phospholipase C (many isoforms) – PIP2

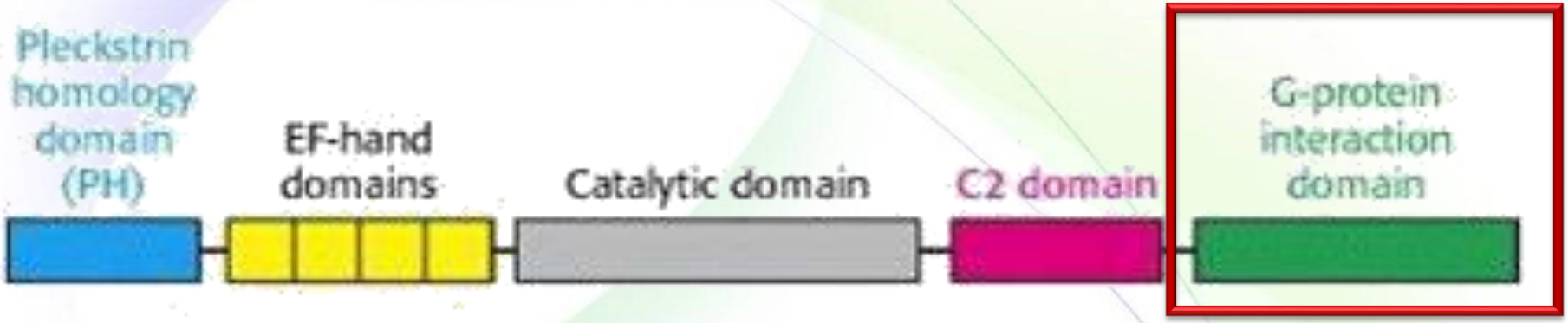
- Two messengers are produced
  - Inositol 1,4,5-trisphosphate, hydrophilic, (Soluble)
    - IP3 is the actual second messenger
  - Diacyclglycerol, amphipathic (membrane)

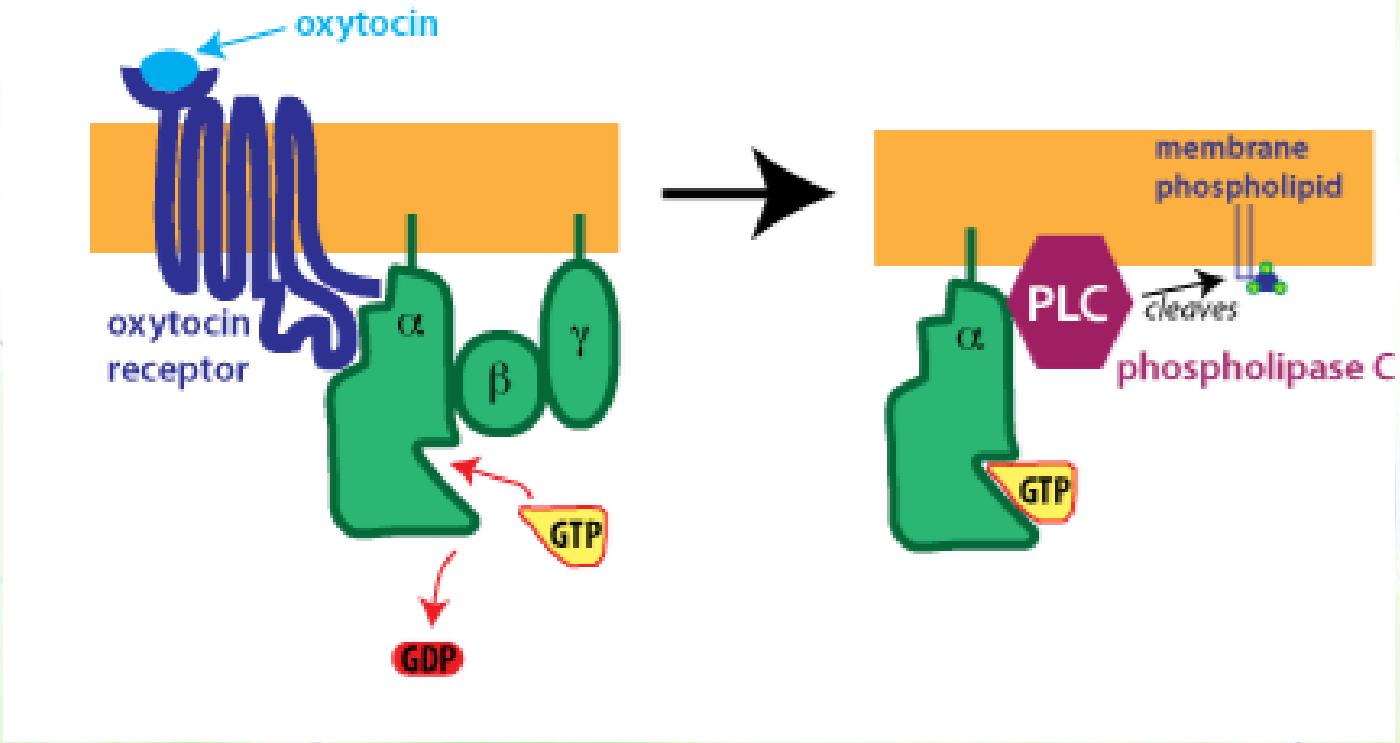






G protein interaction domain



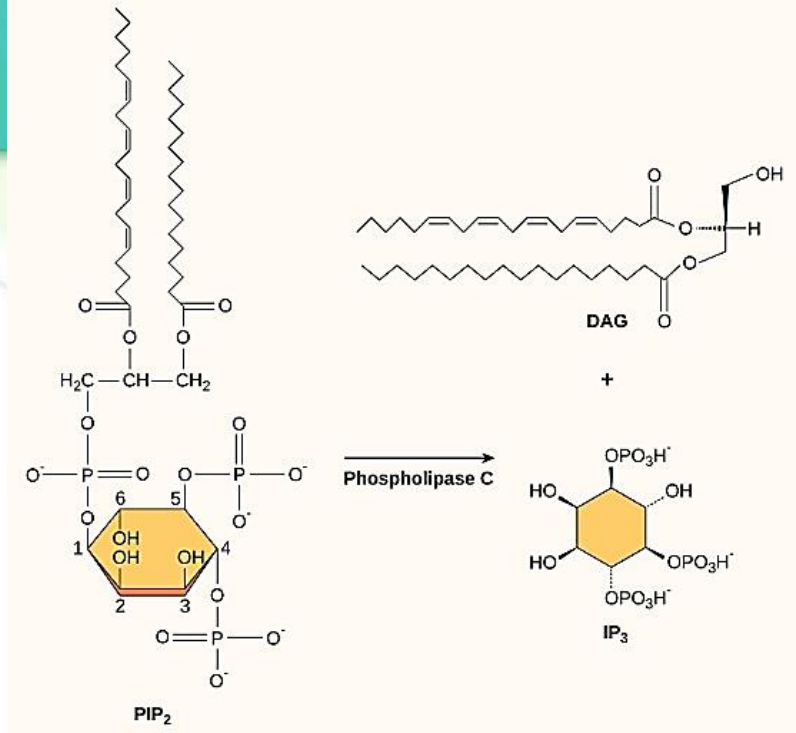


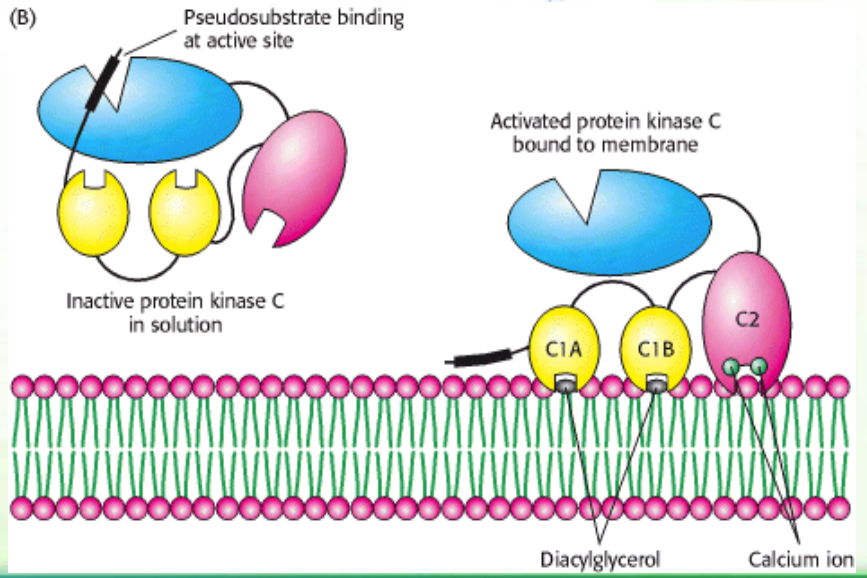
The biochemical effects of IP3

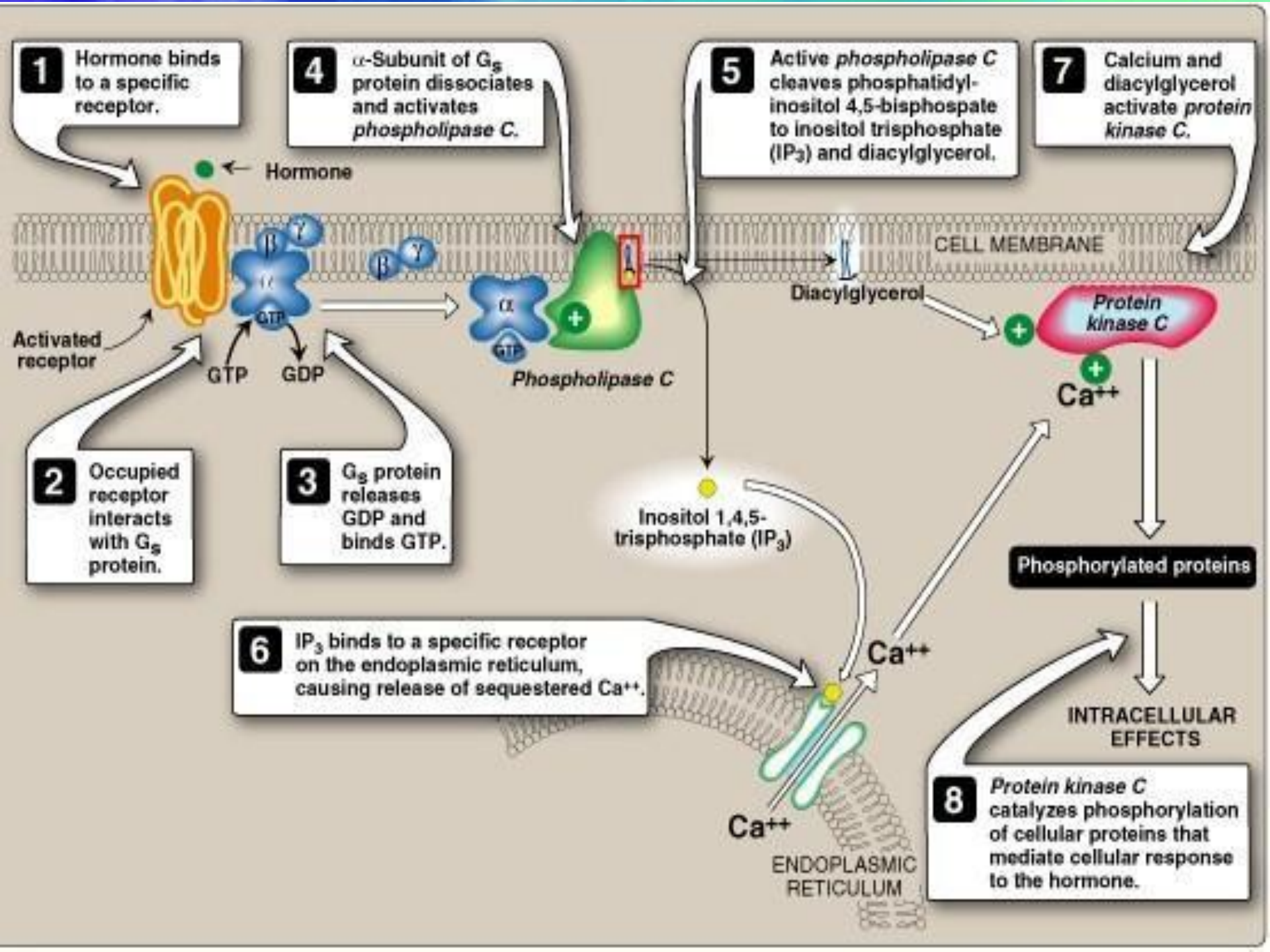
- IP3 binds to a membrane protein called the IP3 receptor, which forms an ion channel.
- The channel opens releasing Ca2+ from the endoplasmic reticulum and, in smooth muscle cells, the sarcoplasmic reticulum.
- Increased Ca2 triggers processes such as smooth muscle contraction, glycogen breakdown, and vesicle release (exocytosis).

Diacylglycerol

- DAG is formed by the hydrolysis of PIP2 by PLC.
- DAG activates many targets
  - Protein kinase C
- How?
  - PKC is inactivated by a self pseudosubstrate.
  - Increased Ca2+
  - allows enzyme binding to the membrane facilitating DAG binding to PKC, which pulls out the pseudosubstrate out of the active site.






1 Hormone binds to a specific receptor.
4 α-Subunit of G_s protein dissociates and activates *phospholipase C.*
5 Active *phospholipase C* cleaves phosphatidyl-inositol 4,5-bisphospate to inositol trisphosphate (IP_3) and diacylglycerol.
7 Calcium and diacylglycerol activate *protein kinase C.*
Hormone
CELL MEMBRANE
Activated receptor
GTP
GDP
Phospholipase C
Diacylglycerol
Protein kinase C
Ca^{++}
2 Occupied receptor interacts with G_s protein.
3 G_s protein releases GDP and binds GTP.
Inositol 1,4,5-trisphosphate (IP_3)
Phosphorylated proteins
6 IP_3 binds to a specific receptor on the endoplasmic reticulum, causing release of sequestered Ca^{++}.
Ca^{++}
INTRACELLULAR EFFECTS
Ca^{++}
ENDOPLASMIC RETICULUM
8 *Protein kinase C* catalyzes phosphorylation of cellular proteins that mediate cellular response to the hormone.

Ca-activated calmodulin

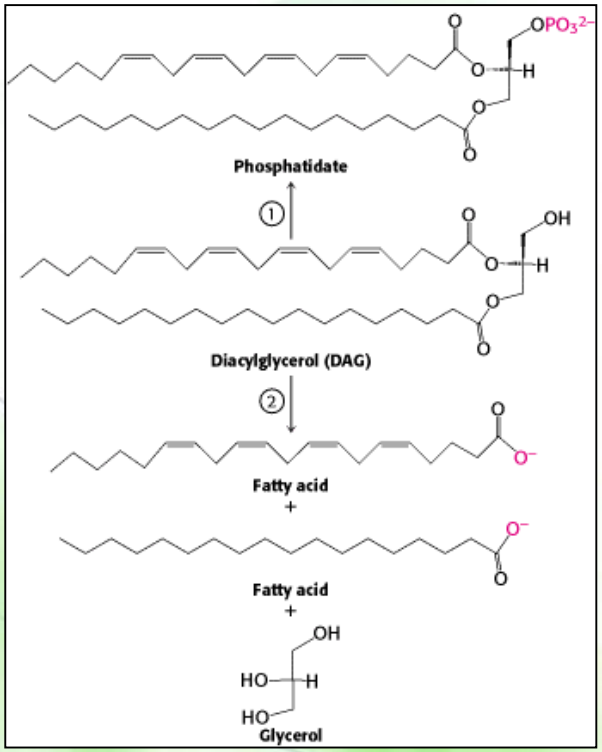
- Ca2+ also interacts with and activates calmodulin, which modulates the functions of many enzymes:
  - adenylate cyclase
  - phosphorylase kinase
  - pyruvate carboxylase
  - pyruvate dehydeogenase
  - glycerol-3-phosphate dehydrogenase
  - glycogen synthase
  - guanylate cyclase
  - myosin kinase
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

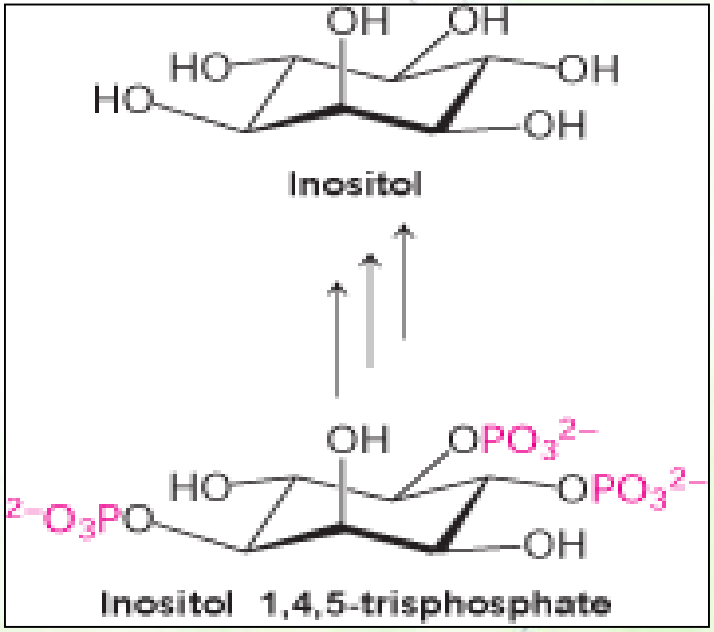
Ca-activated calmodulin

- These enzymes catalyze many important cellular responses
  - 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.

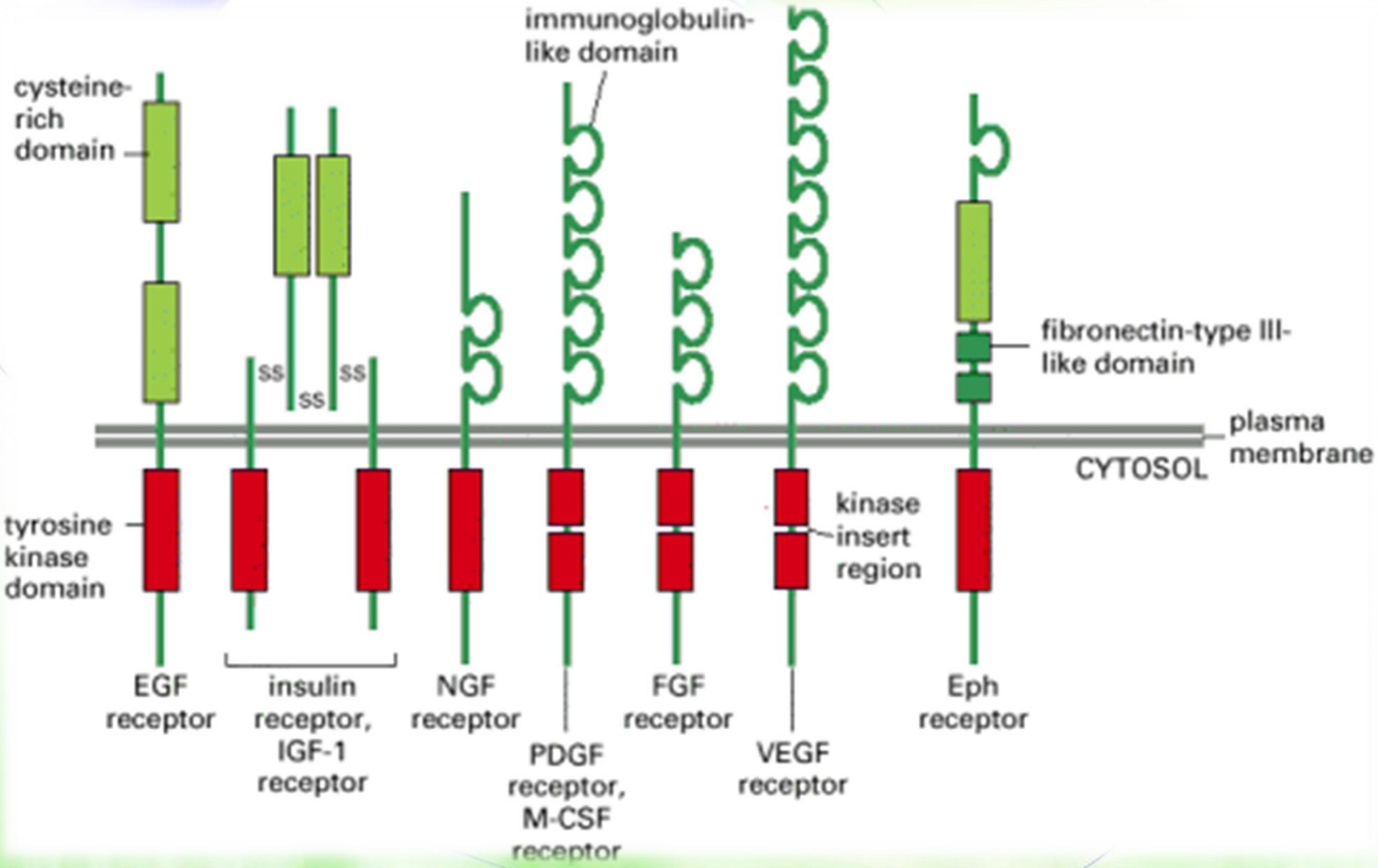




Signaling through Enzyme-Linked Cell-Surface Receptors

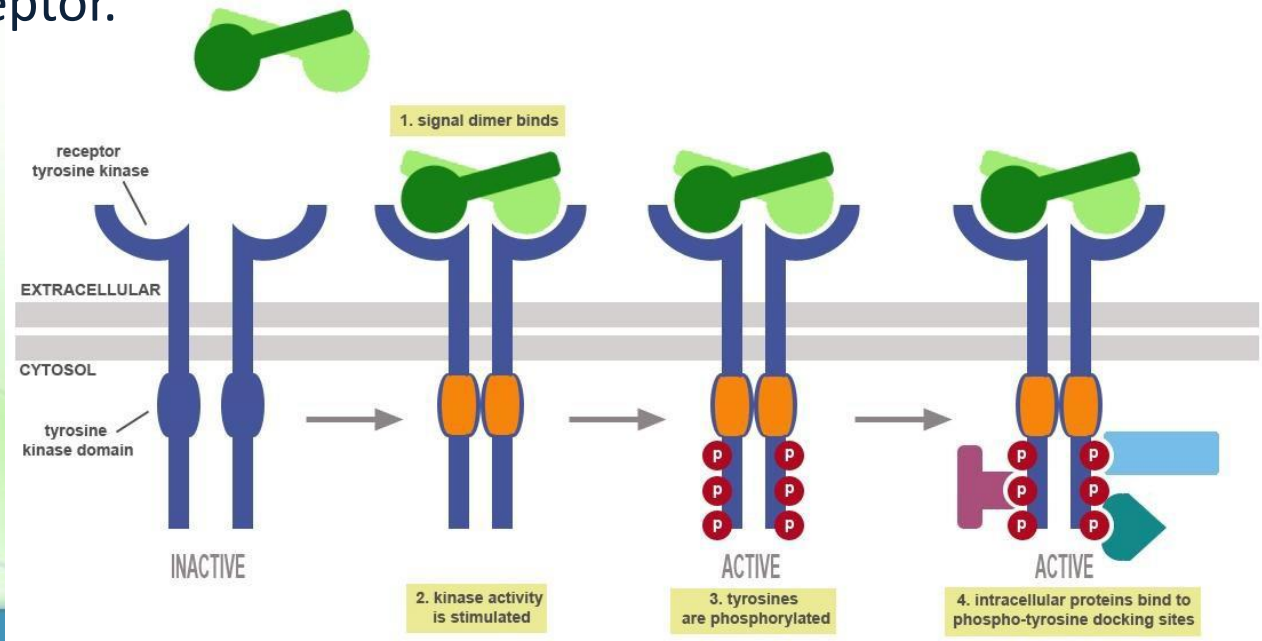
Enzyme-Linked Cell-Surface Receptors

- Enzyme-linked receptors are a major type of cell-surface receptors that promote cell growth, proliferation, differentiation, or survival.
- Their ligands are often called growth factors, which act at very low concentrations (about 10^{-9}-10^{-11} M).
- Receptors either mediate slower response (hours) that lead to changes in gene expression or faster responses (seconds) with effectson the cytoskeleton (cell movement and shape).


immunoglobulin-like domain
cysteine-rich domain
fibronectin-type III-like domain
plasma membrane
CYTOSOL
tyrosine kinase domain
kinase insert region
SS
SS
SS
EGF receptor
insulin receptor, IGF-1 receptor
NGF receptor
PDGF receptor, M-CSF receptor
FGF receptor
VEGF receptor
Eph receptor

Receptor tyrosine kinases

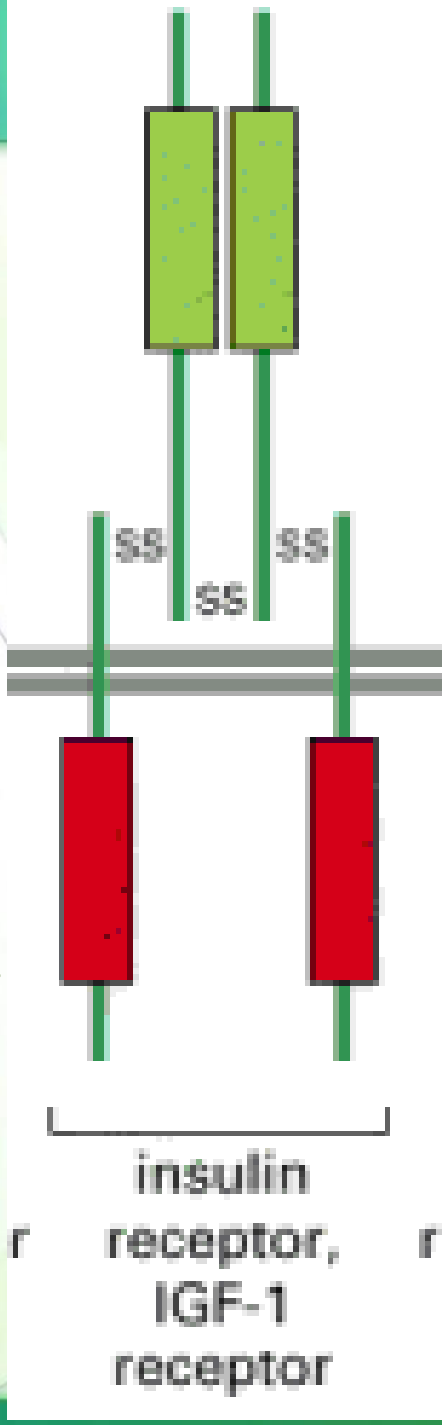
- An example of enzyme-linked receptors is receptor tyrosine kinases.
- These receptors also contain an intracellular kinase domain that phosphorylates specific tyrosines on a small set of intracellular signaling proteins.
- The binding of a signal protein to the ligand-binding domain induces dimerization of the receptor and activates the intracellular tyrosine kinase domain that phosphorylates itself (authophosphorylation) and other intracellular signaling proteins that subsequently bind to the phosphorylated receptor.



An example: insulin and IGF-1 receptor

The mechanism of receptor activation

- The receptors are tetramers where, when bound to the ligand, the two kinase domains come close together, phosphorylate each other (autophosphorylation).
- 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)
    - Grb2



Insulin signaling pathways

- Insulin can initiate multiple signaling pathways resulting in:
  - Immediate effects (minutes):
    - 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.

  These effects do not require new protein synthesis.

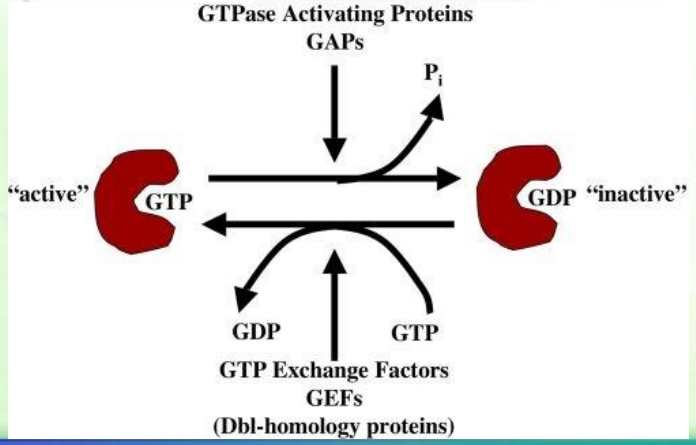
  - Longer-lasting effects (hours):
    - increased expression of enzymes that synthesize glycogen (liver) and triacylglycerols (adipocyte).

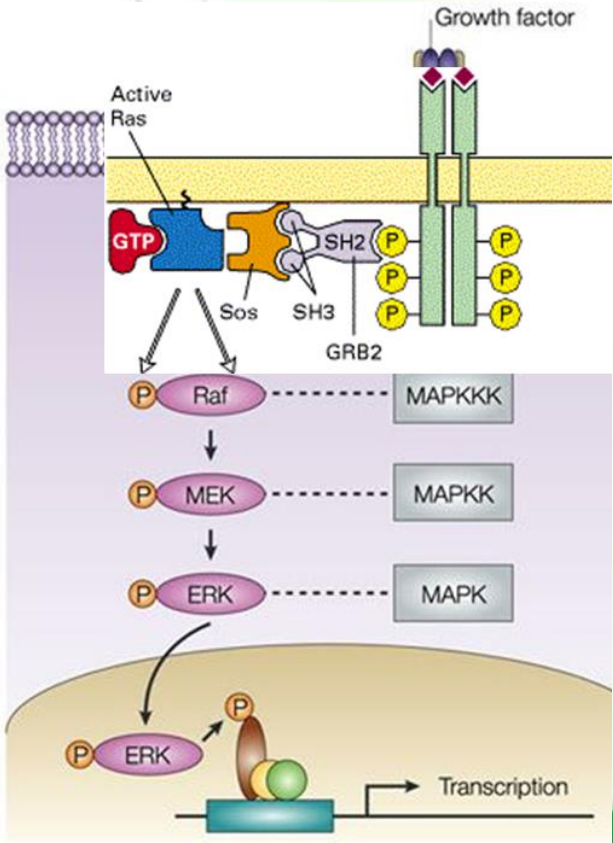
Insulin-activated signaling pathways

- Binding of insulin can initiate three distinct signaling pathways:
  - Ras-dependent pathway and
  - Ras-independent pathways
- Both depend on insulin receptor substrate 1 (IRS1).

Ras-Dependent Pathway

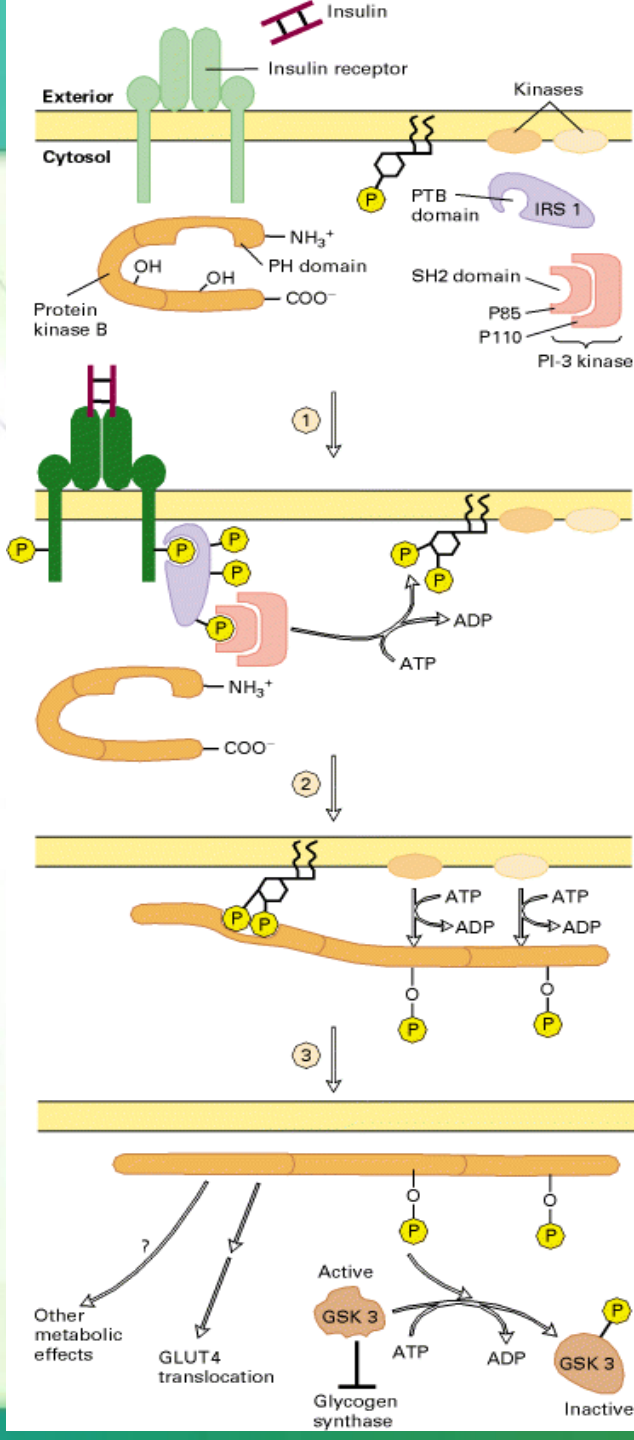
- IRS1 binds to the activated insulin receptor and is phosphorylated by the receptor's kinase
- Phosphorylated IRS1, not the activated insulin receptor, binds to Grb2, which binds to Sos protein.
- SOS is a GTP-exchange factor promoting exchange of GDP to GTP in Ras.
- GTP-Ras activates Raf (a kinase), which activates MAP kinase, which activates ERK.
- ERK can then be re-located into nucleus activating transcription factors.





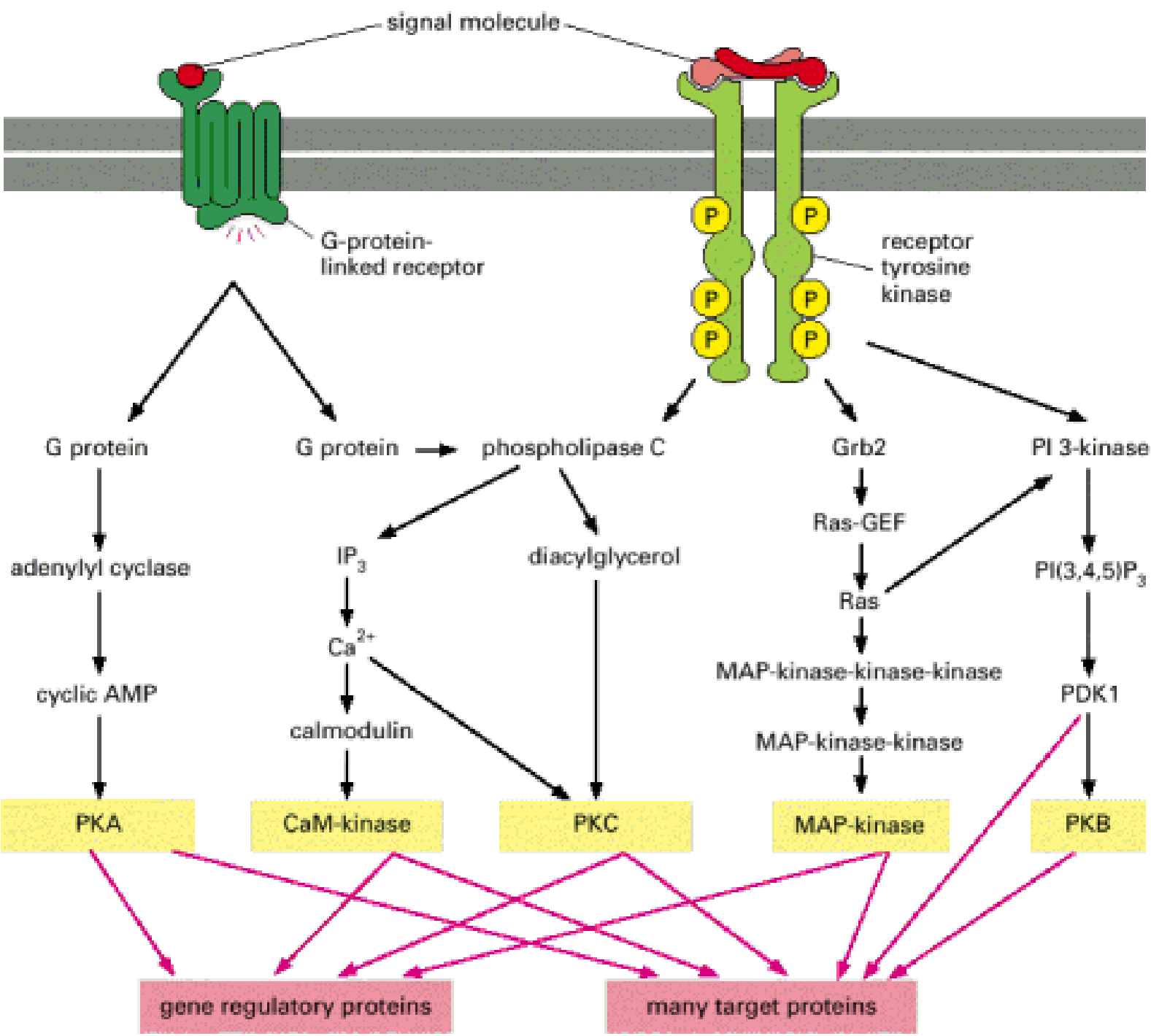
Ras-independent Pathways

- Phosphorylated IRS1 also binds PI-3 kinase activating it resulting in production of phosphoinositides. This lead to recruitment of protein kinase B (PKB) to the membrane.
- PKB is phosphorylated by membrane-associated kinases.
- Phosphorylated (active) PKB is released into the cytosol mediating many effects of insulin such as stimulation of glucose uptake and glycogen synthesis.



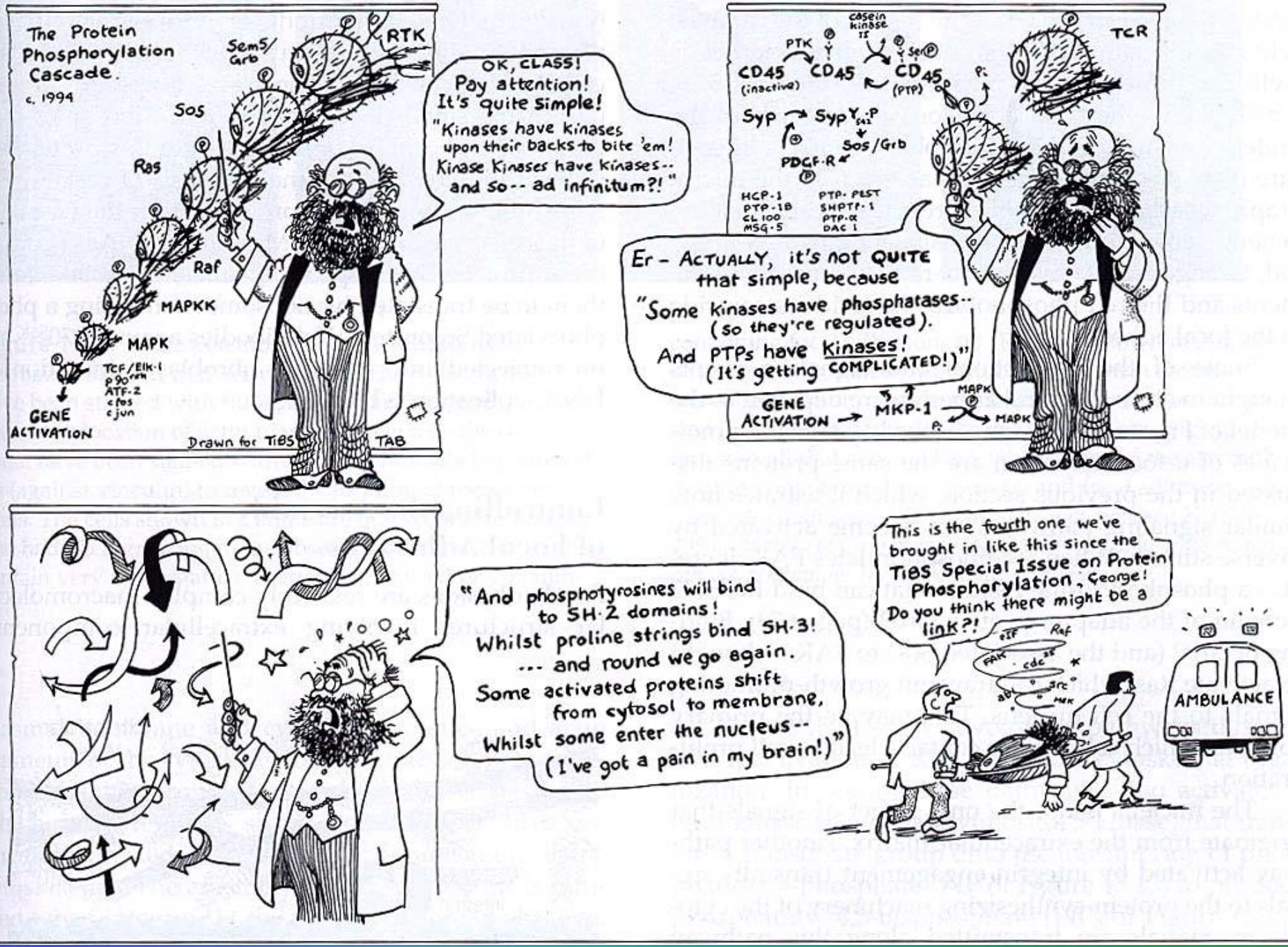
Phospholipase C

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


signal molecule
G-protein-linked receptor
receptor tyrosine kinase
P
G protein
G protein
phospholipase C
Grb2
PI 3-kinase
adenylyl cyclase
IP3
diacylglycerol
Ras-GEF
PI(3,4,5)P3
Ras
Ca2+
cyclic AMP
MAP-kinase-kinase-kinase
PDK1
calmodulin
MAP-kinase-kinase
PKA
CaM-kinase
PKC
MAP-kinase
PKB
gene regulatory proteins
many target proteins

Termination of signal

- Signals are terminated by phosphatases.


The Protein Phosphorylation Cascade.
c. 1994
RTK
Sem5/ Grb
Sos
Ras
Raf
MAPKK
MAPK
TCF/Elk-1
p90rsk
ATF-2
c fos
c jun
GENE ACTIVATION
Drawn for TiBS
TAB
OK, CLASS! Pay attention! It's quite simple!
"Kinases have kinases upon their backs to bite 'em! Kinase Kinases have kinases-- and so-- ad infinitum?!"
PTK
CD45 (inactive)
CD45
casein kinase II
CD45 (PTP)
Syp
Syp Y542 P
Sos/Grb
PDGF-R
HCP-1
PTP-1B
CL 100
MSG-5
PTP-PEST
SHPTP-1
PTP-α
DAC 1
TCR
Er - ACTUALLY, it's not QUITE that simple, because
"Some kinases have phosphatases-- (so they're regulated).
And PTPs have Kinases! (It's getting COMPLICATED!)"
GENE ACTIVATION
MKP-1
MAPK
MAPK
"And phosphotyrosines will bind to SH-2 domains!
Whilst proline strings bind SH-3!
... and round we go again.
Some activated proteins shift from cytosol to membrane,
Whilst some enter the nucleus--
(I've got a pain in my brain!)"
This is the fourth one we've brought in like this since the TiBS Special Issue on Protein Phosphorylation, George! Do you think there might be a link?!
eIF
Raf
FAK
cdc
src
MEK
AMBULANCE