Cell Biology
Lecture 10

Cell Signaling - principles

Sesilja Aranko

22.11.2023

Alberts • Johnson • Lewis • Morgan • Raff • Roberts • Walter

Molecular Biology of the Cell

Sixth Edition

Chapter 15

Cell Signaling, Part II

Pages: 832-843, 850-866

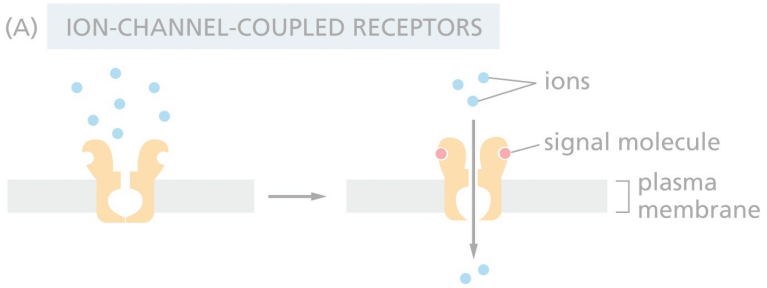
Course overview – Tentative schedule

Date	Lecture		Chapters & Topics	Assignments
25.10.	1	Part 1	*Course overview,* DNA, Chromosomes, Genome, Ch. 4	
27.10.	2 -G		Replication, Repair, Recombination, Ch. 5	
1.11.	3		From DNA to protein, Ch. 6	
3.11.	4		Control of gene expression, Ch. 7	
8.11.	5	Part 2	Membrane structures, Ch. 10 Membrane transport, Ch. 11	**Assignment I (Essay) Draft I** (8.11.)
10.11.	6 -G		Intracellular compartments and protein sorting, Ch. 12	
15.11.	7		Intracellular compartments and protein sorting, Ch. 12 **Susanna Mäkinen, Solar Foods**	**Assignment II – Draft I (15.11.)**
17.11.	8		Membrane Traffic, Ch. 13 **iGEM team 2023**	*+iGEM intro*
22.11.	9	Part 3	Cell signalling, Ch. 15	**Assignment II – Peer review** (22.11.)
24.11.	10 -G		Cell signalling, Ch. 15	**Assignment I (Essay) Draft II** (24.11.)
29.11.	11		Cell cycle, Ch. 17 **Jere Weltner, Folkhälsan**	
1.12.	12		Apoptosis, Ch. 18	**Assignment II – final version** (1.12.)
7.12.	EXAM		December 7th	
8.12.	Final version essay		December 8th	**Assignment I (Essay) Final version** (8.12.) Aim at finishing before exam date. Use last days for polishing.

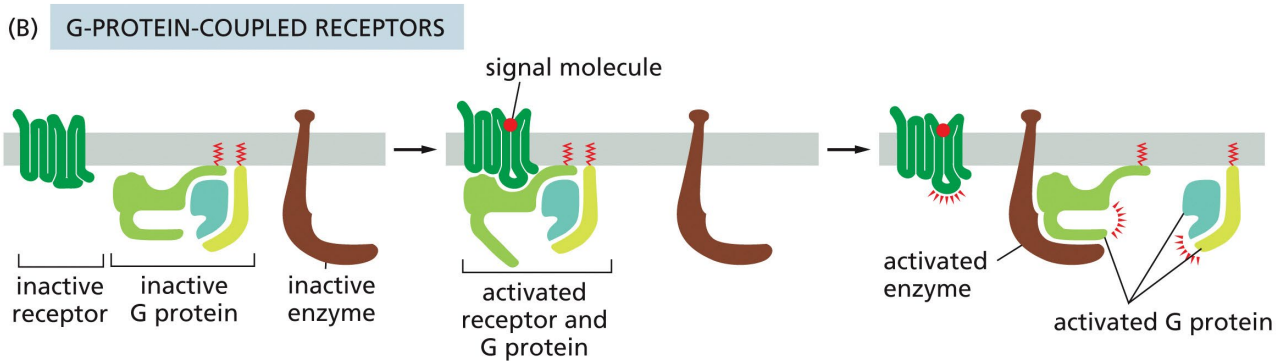
LEARNING OUTCOMES

- Can describe the mechanisms of G-protein coupled receptors and enzyme-coupled receptors and apply these to real cases in which they are used

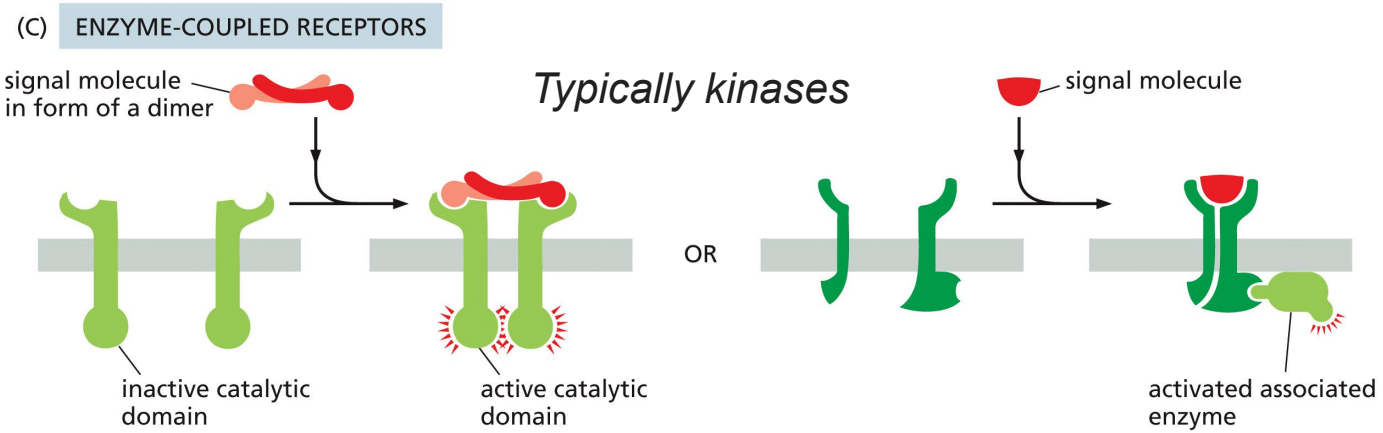
THREE CLASSES OF CELL-SURFACE RECEPTOR PROTEINS



- Synaptic signaling between nerve cells
- Signal molecules (neurotransmitters) transiently open ion channel



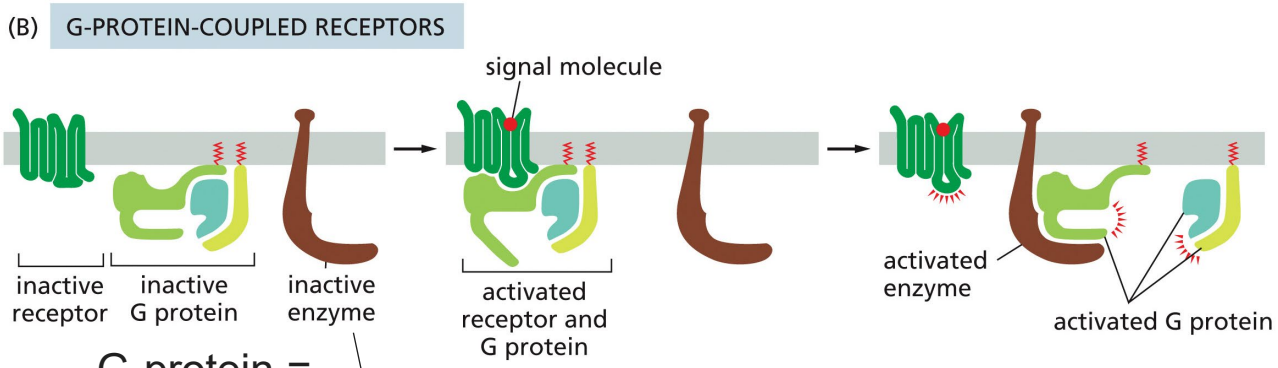
- G-protein mediates signal from receptor to target protein



- Receptor is an enzyme that act directly or by activating an associated enzyme

G-PROTEIN-COUPLED RECEPTOR (GPCR)

- Largest family of cell-surface receptors
- Ligands can we very diverse, proteins, peptides, small molecules or even photons of light
- All share similar structure



G-protein = trimeric GTP-binding protein

Target protein can be enzyme or ion channel

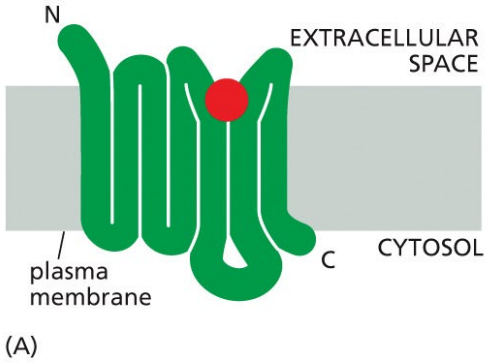
- G-protein mediates signal from receptor to target protein

Three modules:

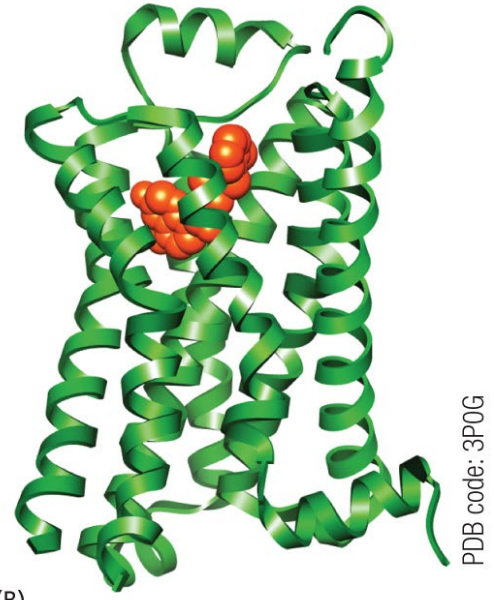
1. **GPCR**
2. **G-protein (trimeric GTP binding protein)**
3. **Enzyme or ion channel**

G-protein-coupled receptor (GPCR)

- Typical cylindrical arrangement of the *seven transmembrane helices* in a **GPCR**
- The **ligand** binds in a pocket between the helices → **conformational changes on the cytoplasmic surface** of the receptor → **G-protein activation**



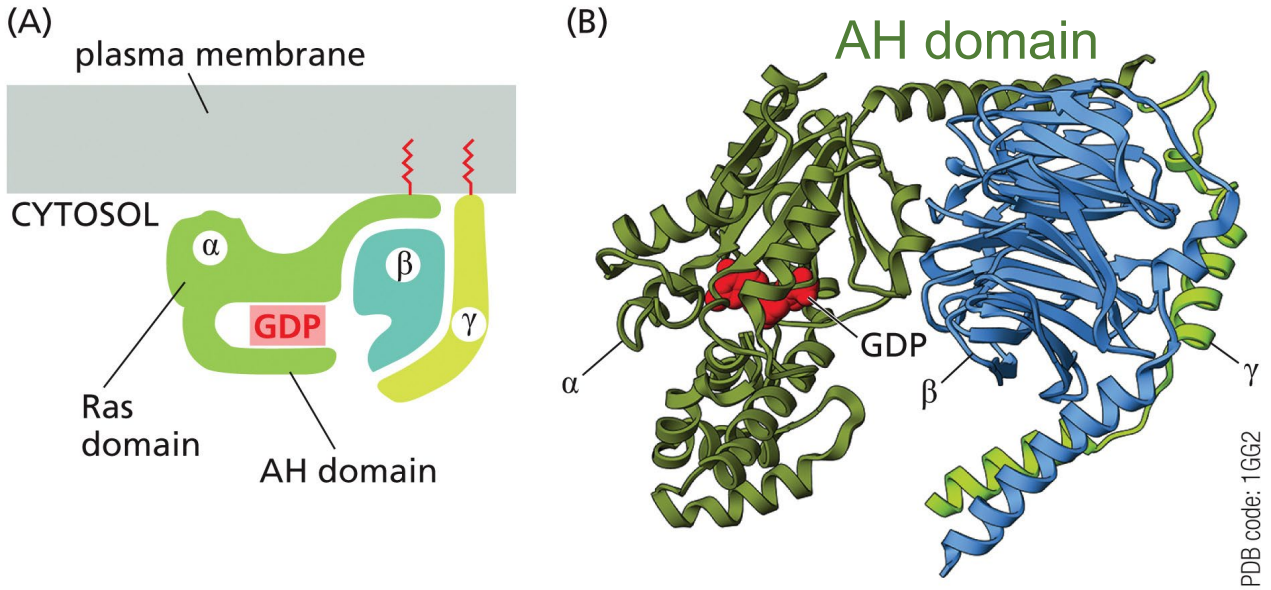
(A)



(B)

G-PROTEINS

- Heterotrimeric G proteins relay signals from GPCRS

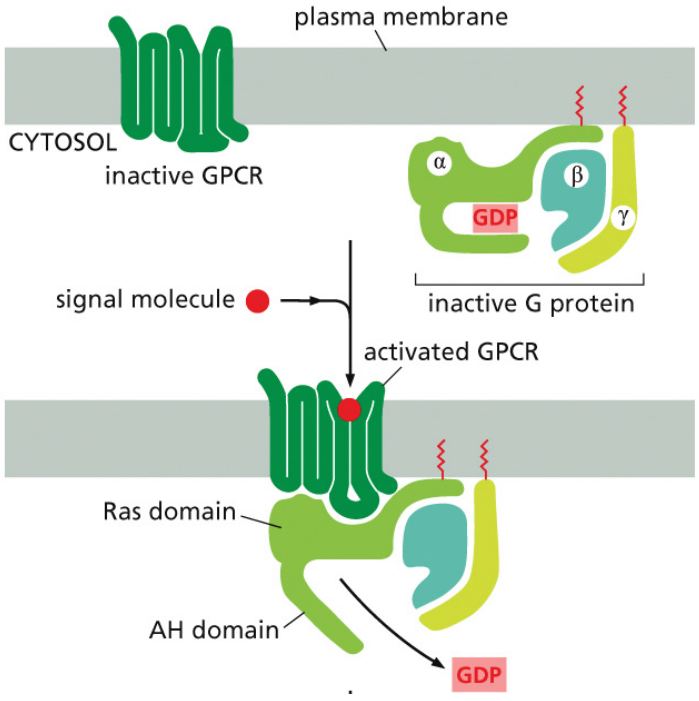


- Inactive G-protein shown
- α and γ subunits have covalently attached lipid molecules that help them bind to the plasma membrane,
- α subunit has GDP bound

ACTIVATION OF A G PROTEIN BY AN ACTIVATED GPCR

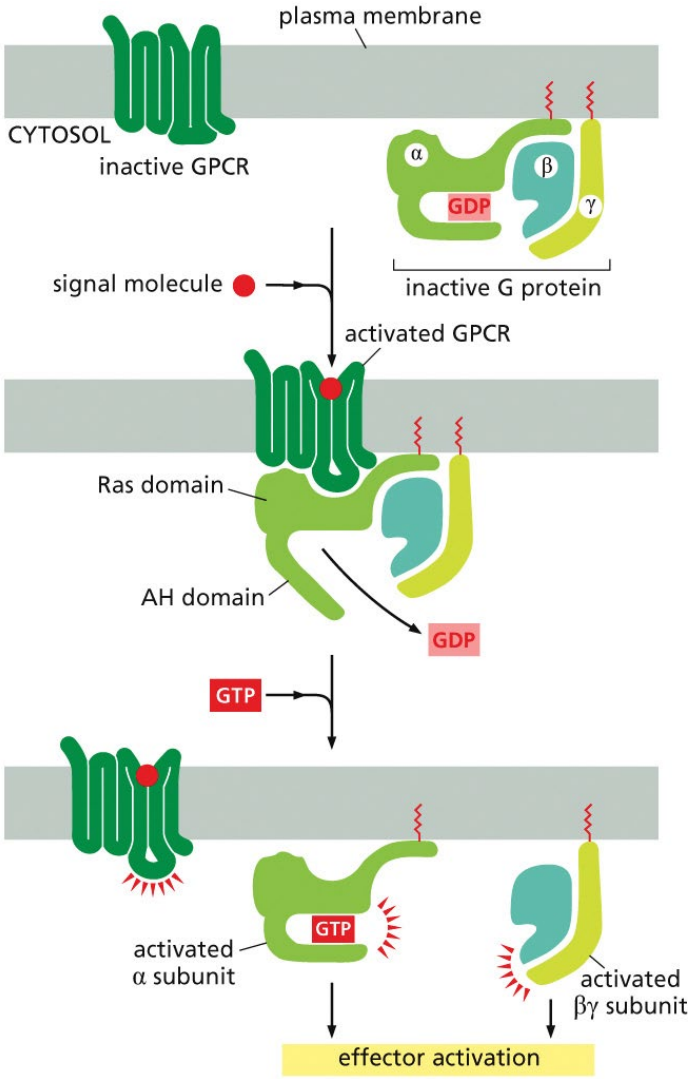
Heterotrimeric G proteins relay signals from GPCRs:

- Binding of an extracellular signal molecule to a GPCR → change in the conformation of the receptor
- Receptor binds G protein and alters its conformation
- The AH domain of the G protein α subunit moves outward to open the GTP-binding site, thereby promoting dissociation of GDP



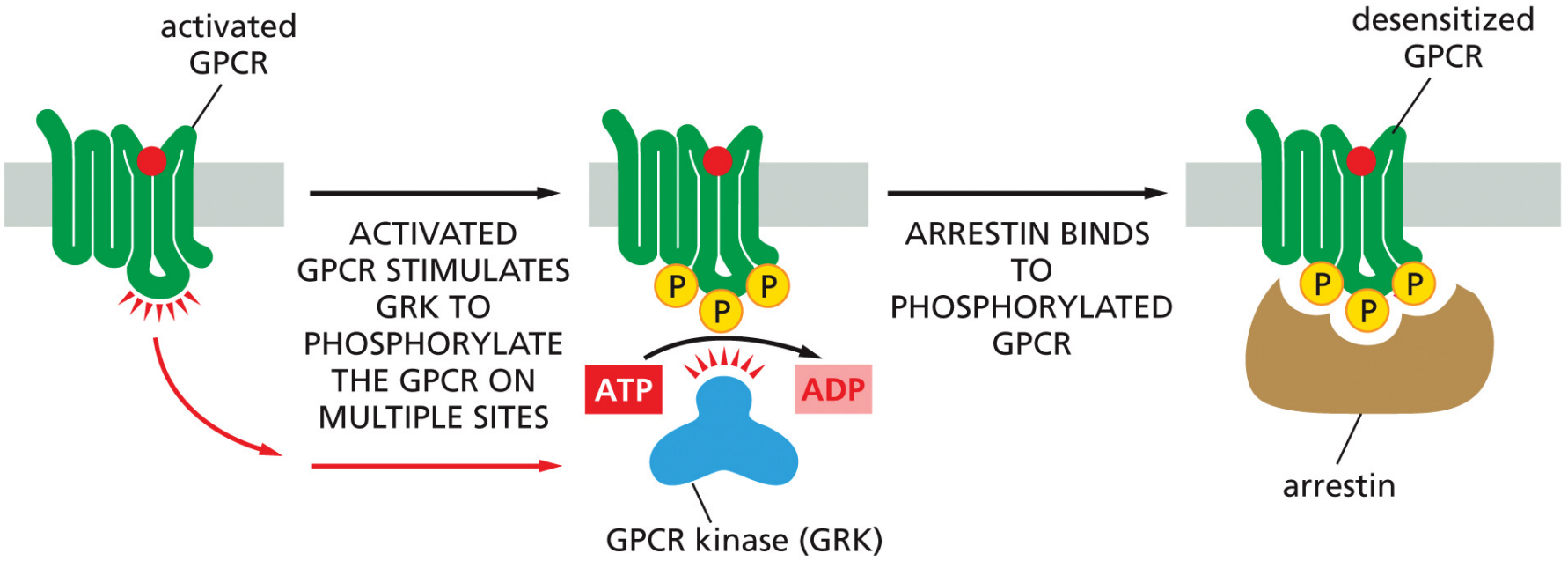
ACTIVATION OF A G PROTEIN BY AN ACTIVATED GPCR

- GTP binding promotes closure of the binding site → conformational changes → dissociation of the α subunit from the receptor and from the βγ complex
- The GTP-bound α subunit and the βγ complex each regulate the activities of downstream signaling molecules
- The receptor stays active while the extracellular signal molecule is bound to it, and it can therefore catalyze the activation of many G-protein molecules



SIGNALING THROUGH G-PROTEIN-COUPLED RECEPTORS

- GPCR Desensitization depends on receptor phosphorylation



A GRK phosphorylates only activated receptors because it is the activated GPCR that turns on the GRK. The binding of an arrestin to the phosphorylated receptor prevents the receptor from binding to its G protein and also directs its endocytosis

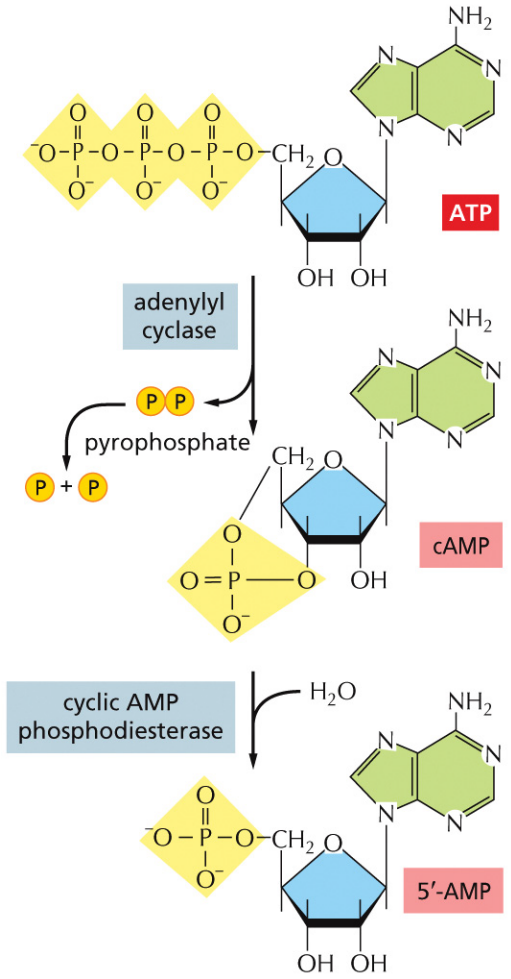
GPCR PATHWAYS

Examples of GPCR pathways:

- cAMP + PKA pathway
- Inositol phospholipid pathway
- Ca2+ mediated
- Directly to ion gates

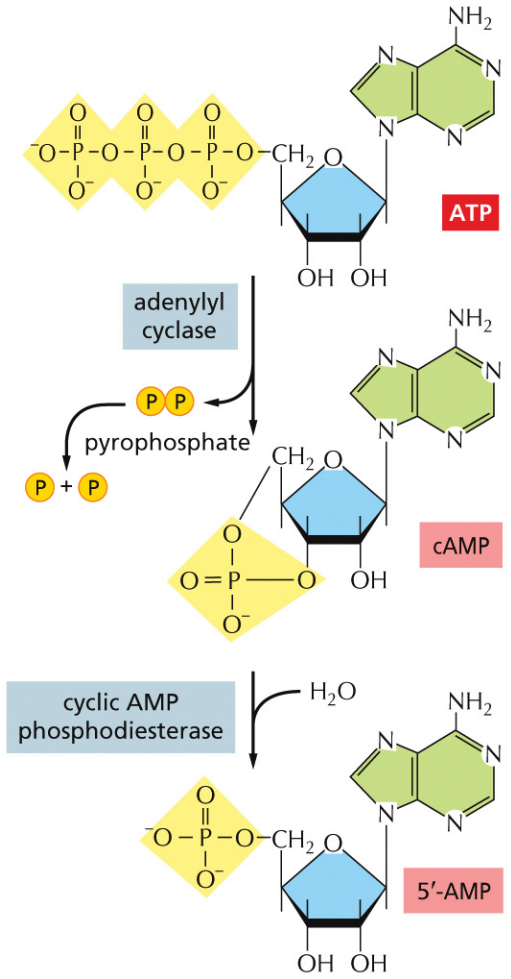
CYCLIC AMP (CAMP) AND GPCRS

- Some G proteins regulate the production of cyclic AMP
  - **Catalysis of cyclic AMP (cAMP) synthesis** by enzyme *adenylyl cyclase*
    - Large transmembrane protein, active site in cytosol
  - cAMP is synthesized from ATP through a cyclization reaction that removes two phosphate groups as pyrophosphate (PP)
    - pyrophosphatase drives this synthesis by hydrolyzing the released pyrophosphate to phosphate
  - Cyclic AMP is short-lived (unstable) in the cell because it is hydrolyzed by specific phosphodiesterases to form 5′-AMP



CYCLIC AMP (CAMP) AND GPCRS

- Some G proteins regulate the production of cyclic AMP
  - Stimulatory G protein (G_s) activates adenylyl cyclase
  - Inhibitory G-protein (G_i) inactivates adenylyl cyclase

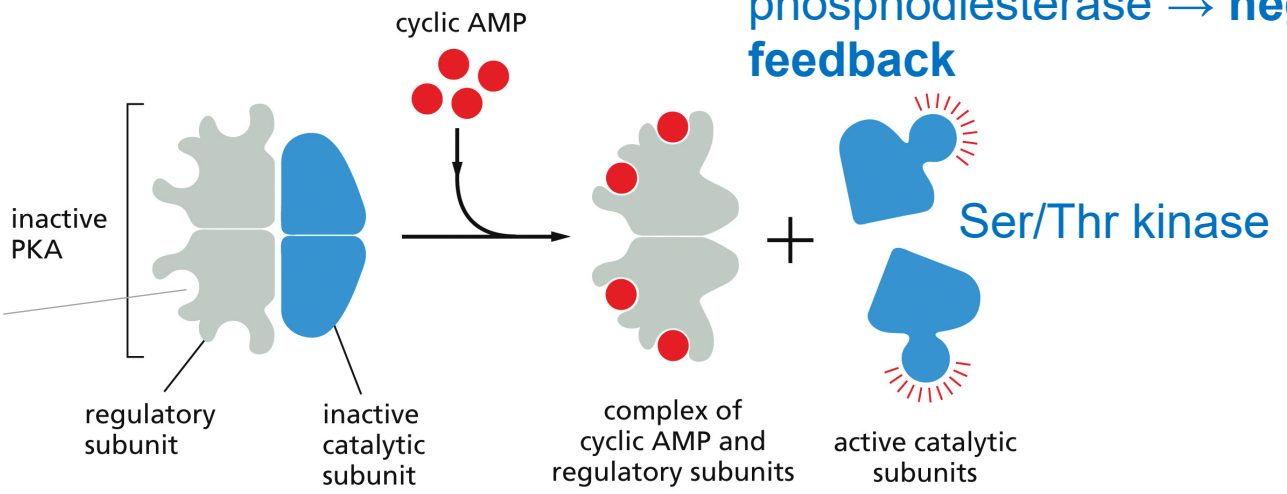


CYCLIC-AMP-DEPENDENT PROTEIN KINASE (PKA)

- **Cyclic-AMP-dependent protein kinase (PKA)** mediates most of the effects of cyclic AMP
- PKA is a Ser/Thr kinase

One of the targets cAMP phosphodiesterase → **negative feedback**

Localize to particular compartments



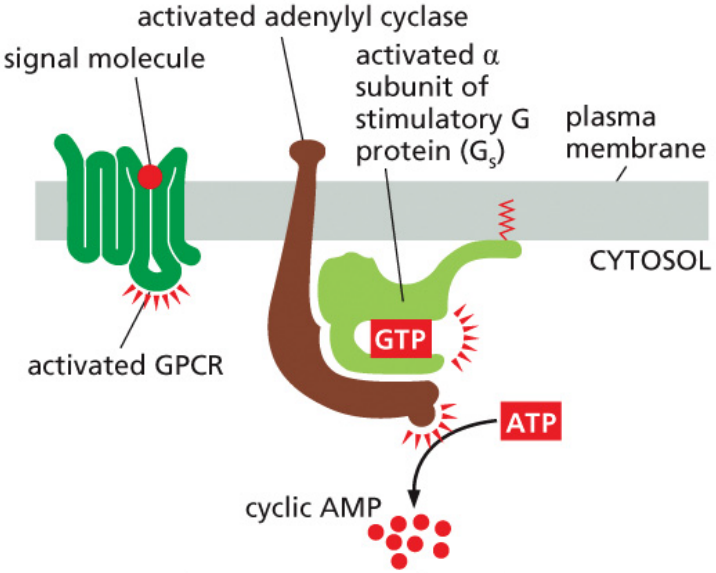
- **Binding of cAMP** to the *regulatory subunits* of the PKA tetramer → conformational change → subunits dissociate from the catalytic subunits → **kinase activity of the *catalytic* subunits activated**
- The release of the catalytic subunits *requires the binding of more than two cAMP molecules* to the regulatory subunits in the tetramer → *sharpens the response* of the kinase to changes in cAMP concentration (lecture 9)

Cyclic-AMP-dependent protein kinase (PKA)

Effect of PKA can be in seconds or in hours

Slow effect e.g. by *altering gene transcription*

Cyclic-AMP-dependent protein kinase (PKA)



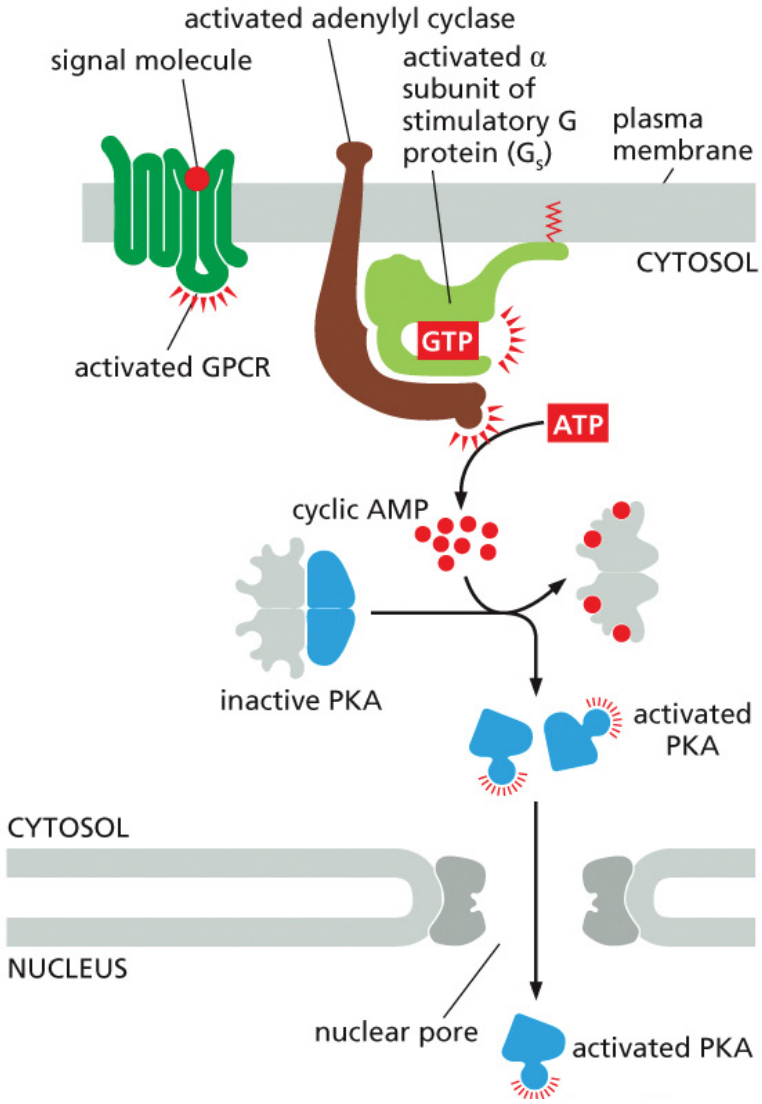
A rise in intracellular cyclic AMP concentration can alter gene transcription:

- The *binding* of an extracellular *signal molecule to its GPCR activates adenylyl cyclase* via G_s → **increases cAMP concentration in the cytosol**
- Activates PKA → released catalytic subunits of PKA enter the nucleus → phosphorylate the transcription regulatory protein CREB
- Phosphorylated CREB recruits the coactivator CBP → stimulates gene transcription

Cyclic-AMP-dependent protein kinase (PKA)

A rise in intracellular cyclic AMP concentration can alter gene transcription:

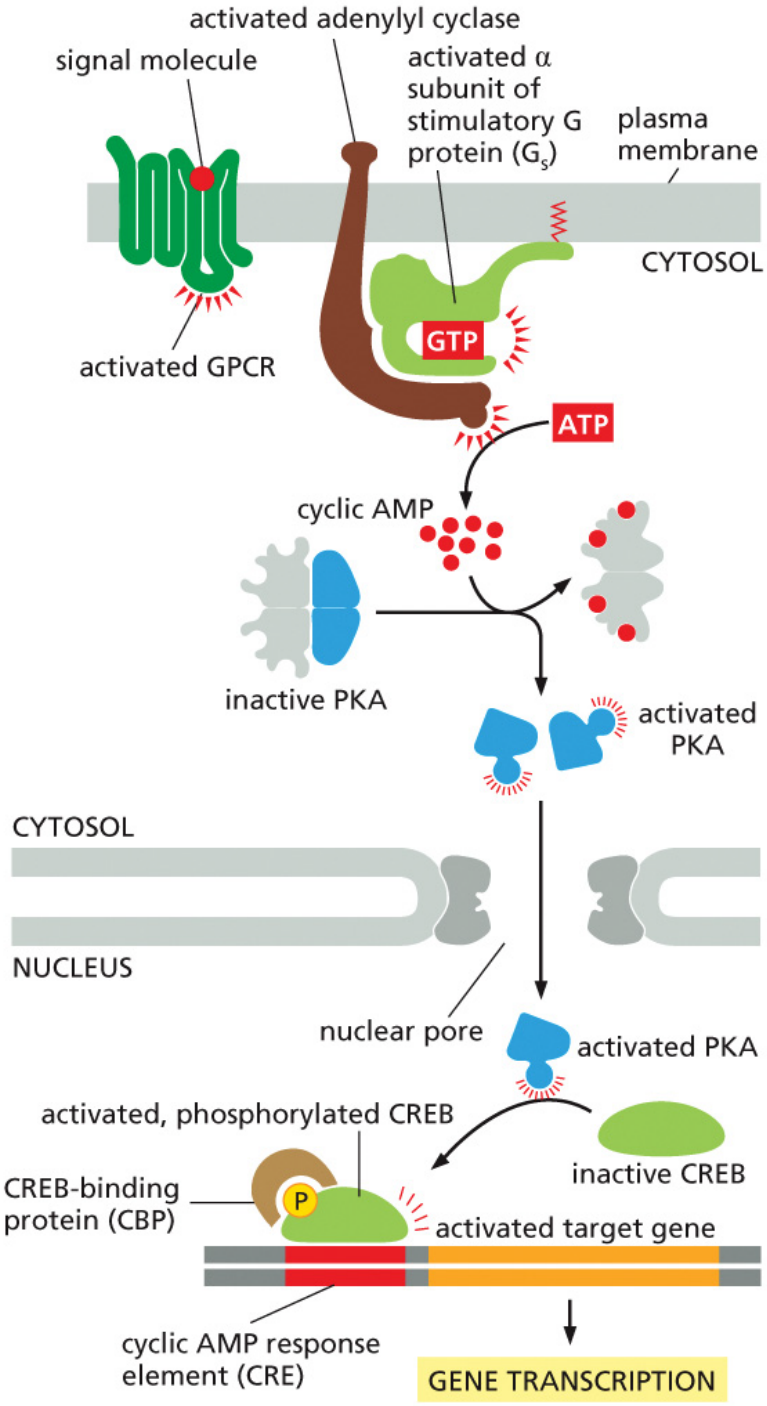
- The *binding* of an extracellular *signal molecule to its GPCR activates adenylyl cyclase* via G_s → **increases cAMP concentration in the cytosol**
- Activates PKA → released catalytic subunits of PKA enter the nucleus



Cyclic-AMP-dependent protein kinase (PKA)

A rise in intracellular cyclic AMP concentration can alter gene transcription:

- The *binding* of an extracellular *signal molecule to its GPCR activates adenylyl cyclase* via G_s → **increases cAMP concentration in the cytosol**
- Activates PKA → released catalytic subunits of PKA enter the nucleus
- Catalytic subunits of PKA phosphorylate the transcription regulatory protein CREB
- Phosphorylated CREB recruits the coactivator CBP → stimulates gene transcription

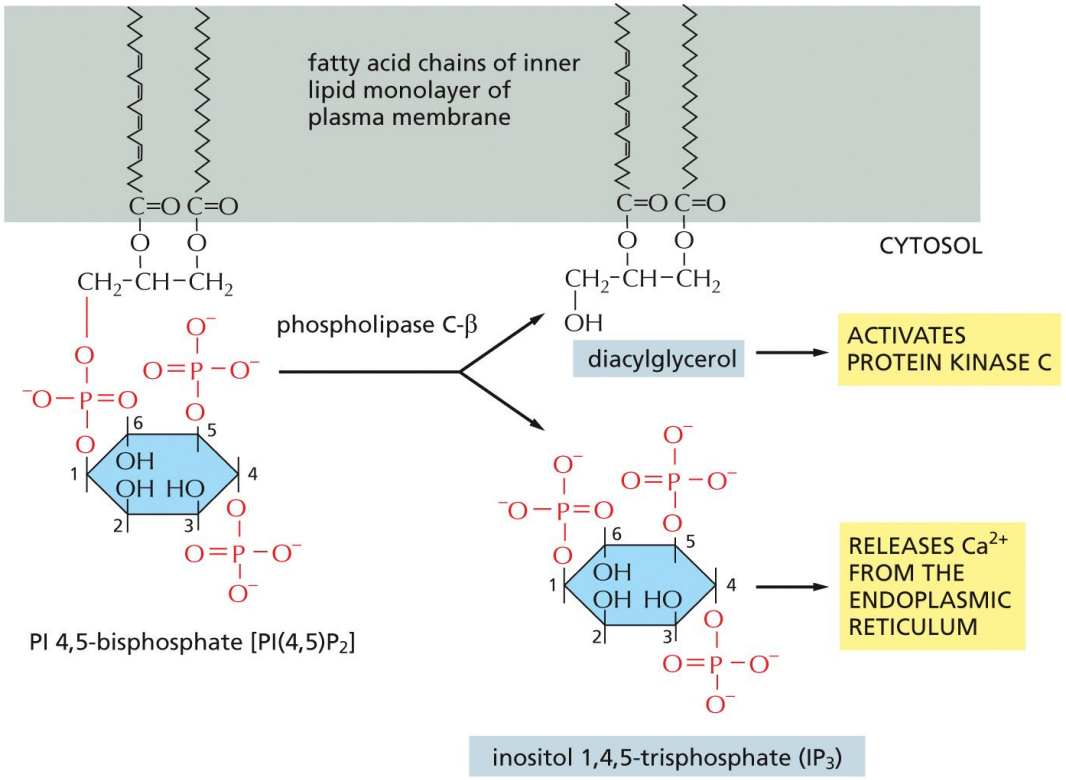


G PROTEINS AND PHOSPHOLIPIDS

- Some G proteins signal via phospholipids

Phospolipase C-β

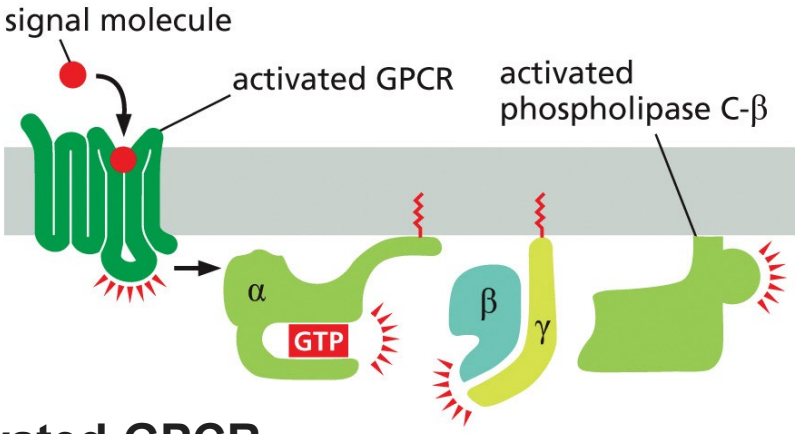
- A plasma membrane bound enzyme
- Cleaves PI(4,5)P to IP3 and diaglycerol



There are several classes of phospholipase C:

- β class is activated by GPCRs
- γ class is activated by a class of enzyme-coupled receptors called receptor tyrosine kinases (RTKs)

GPCRS INCREASE CYTOSOLIC CA^{2+} AND ACTIVATE PROTEIN KINASE C

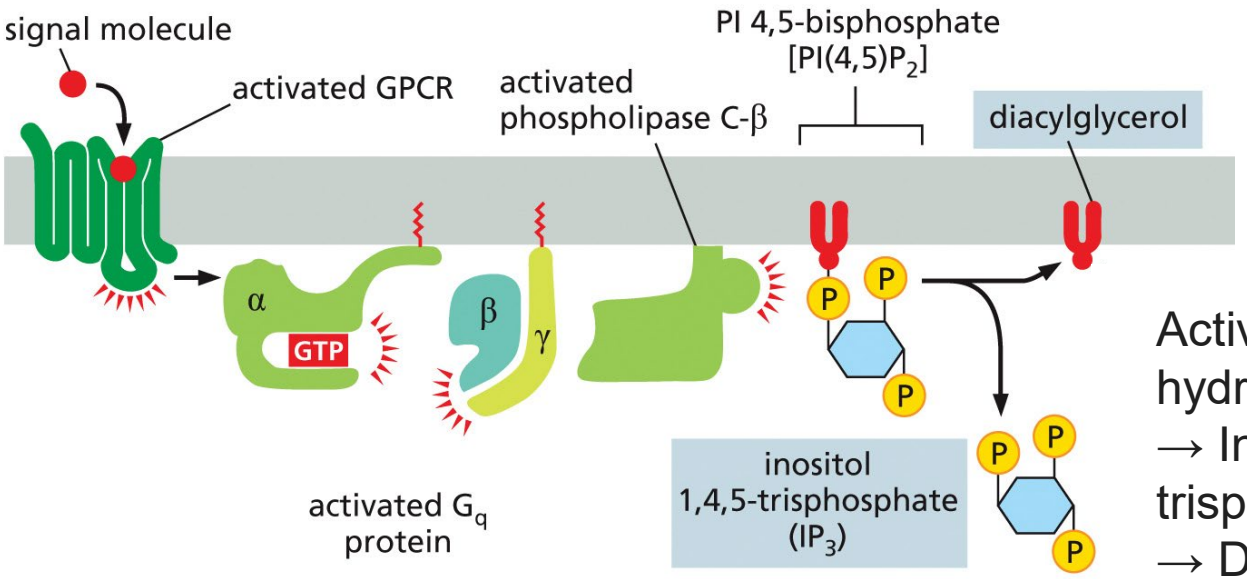


The **activated GPCR** stimulates the plasma-membrane-bound **phospholipase C-β** (PLCβ) via a **G protein called G_q**

activated G_q protein

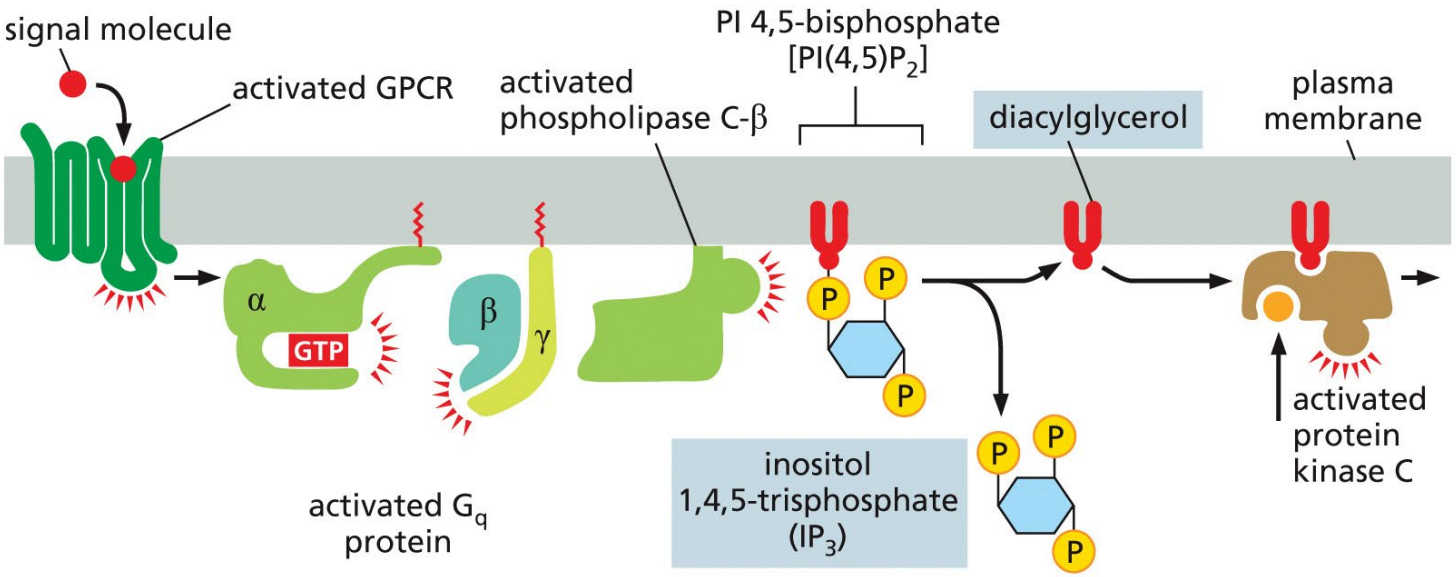
The α subunit and βγ complex of G_q are both involved in this activation.

GPCRS INCREASE CYTOSOLIC CA^{2+} AND ACTIVATE PROTEIN KINASE C



Activated PLC hydrolyzes $PI(4,5)P_2$ → Inositol 1,4,5-trisphosphate (IP_3) → Diacylglycerol

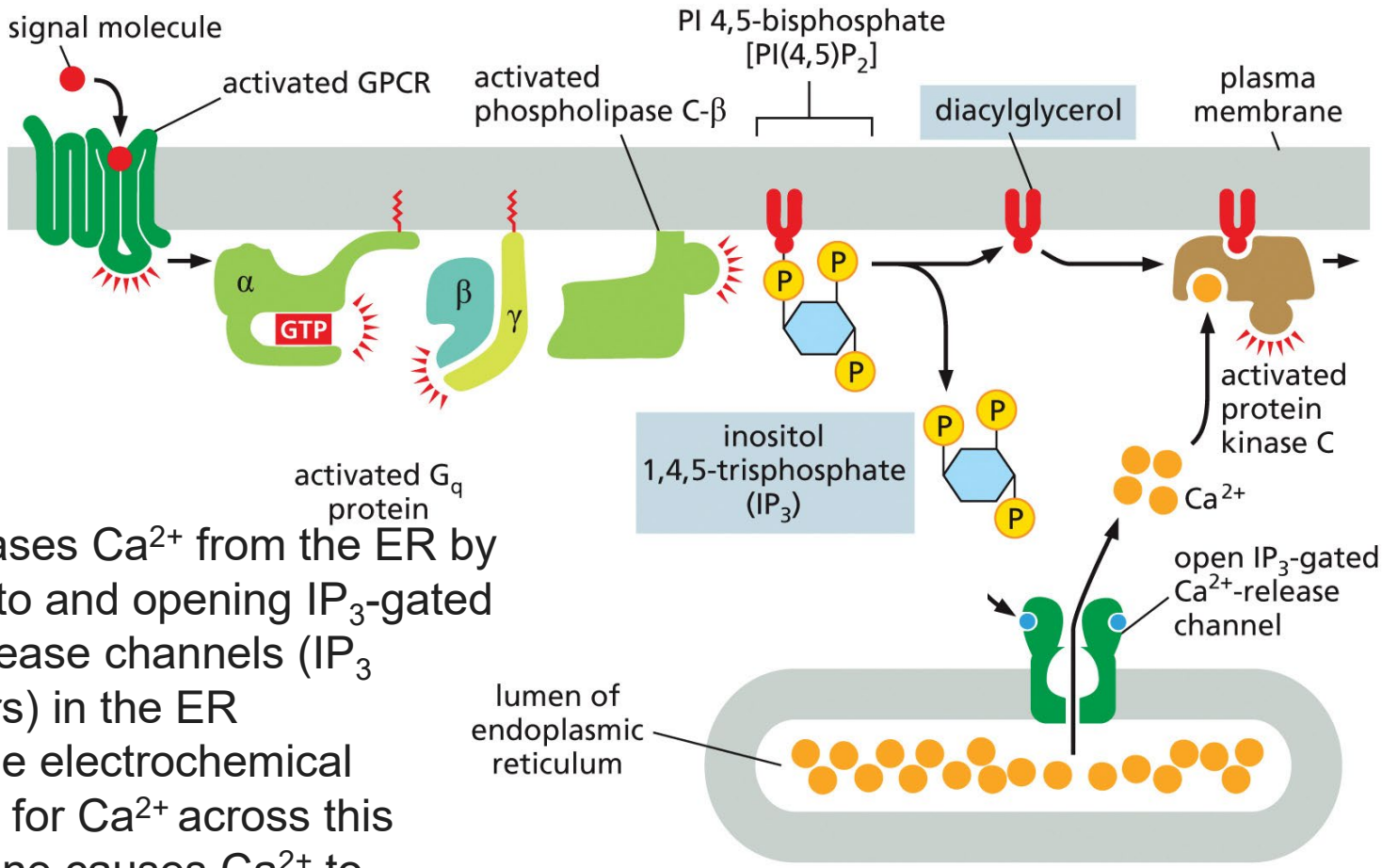
GPCRS INCREASE CYTOSOLIC CA^{2+} AND ACTIVATE PROTEIN KINASE C



Activated PLC hydrolyzes $PI(4,5)P_2$ → Inositol 1,4,5-trisphosphate (IP_3) → Diacylglycerol

Diacylglycerol remains in the plasma membrane and helps to activate protein kinase C (PKC), which is recruited from the cytosol to the cytosolic face of the plasma membrane

GPCRS INCREASE CYTOSOLIC CA^{2+} AND ACTIVATE PROTEIN KINASE C



- IP_3 releases Ca^{2+} from the ER by binding to and opening IP_3-gated Ca^{2+}-release channels (IP_3 receptors) in the ER
- The large electrochemical gradient for Ca^{2+} across this membrane causes Ca^{2+} to escape into the cytosol when the release channels are opened

Ca^{2+}, helps to activate protein kinase C (PKC) (makes PKC to translocate to membrane)

SIGNALING THROUGH G-PROTEIN-COUPLED RECEPTORS

- Ca^{2+} functions as a ubiquitous intracellular mediator, e.g. muscle contraction and secretion in nerve cells

TABLE 11–1 A Comparison of Inorganic Ion Concentrations Inside and Outside a Typical Mammalian Cell*

Component	Cytoplasmic concentration (mM)	Extracellular concentration (mM)
Cations		
Na^+	5–15	145
K^+	140	5
Mg^{2+}	0.5	1–2
Ca^{2+}	10^{-4}	1–2
H^+	7×10^{-5} ($10^{-7.2}$ M or pH 7.2)	4×10^{-5} ($10^{-7.4}$ M or pH 7.4)
Anions		
Cl^-	5–15	110

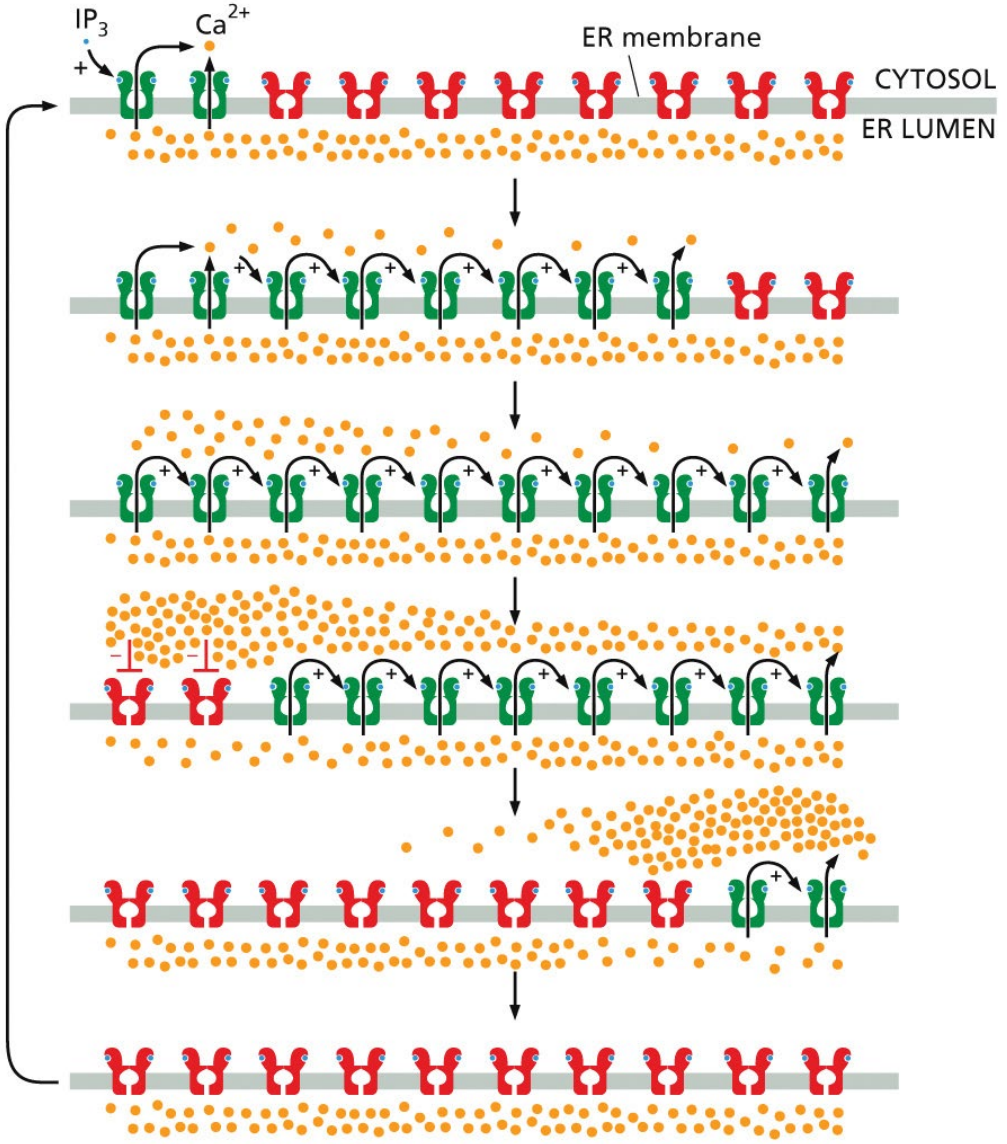
*The cell must contain equal quantities of positive and negative charges (that is, it must be electrically neutral). Thus, in addition to Cl^-, the cell contains many other anions not listed in this table; in fact, most cell constituents are negatively charged (HCO_3^-, PO_4^{3-}, nucleic acids, metabolites carrying phosphate and carboxyl groups, etc.). The concentrations of Ca^{2+} and Mg^{2+} given are for the free ions: although there is a total of about 20 mM Mg^{2+} and 1–2 mM Ca^{2+} in cells, both ions are mostly bound to other substances (such as proteins, free nucleotides, RNA, etc.) and, for Ca^{2+}, stored within various organelles (such as the endoplasmic reticulum and mitochondria).

- High concentrations also in ER

FEEDBACK GENERATES CA^{2+} WAVES AND OSCILLATIONS

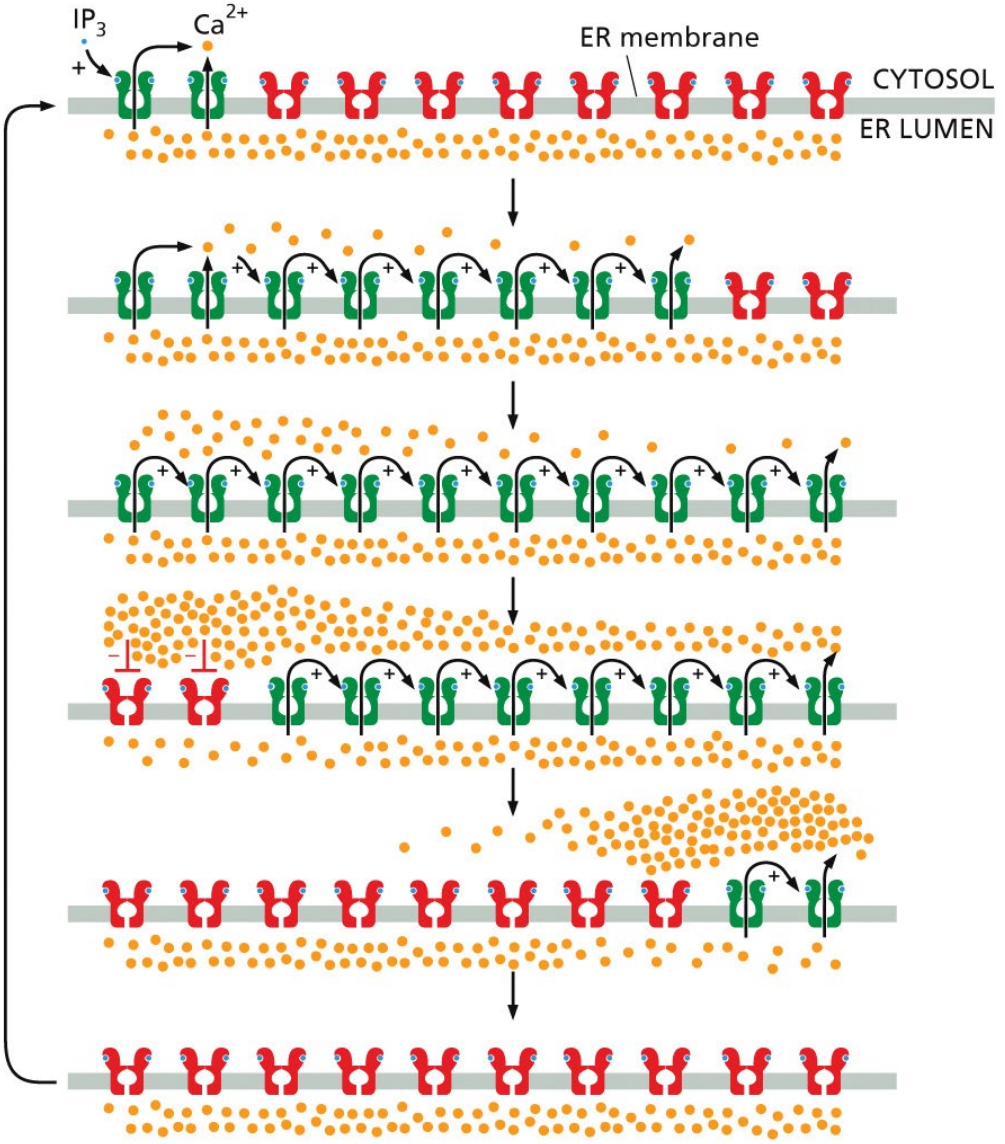
Positive and negative feedback produce cytosolic Ca^{2+} waves and oscillations

- Cytosolic IP_3 rises to high levels in response to a strong extracellular signal → occupies most IP_3 receptors on the ER membrane.
- A few IP_3-bound receptors are activated by the low amount of cytosolic Ca^{2+} present in the unstimulated cell
- *The local release of Ca^{2+} by an activated receptor cluster promotes the opening of nearby IP_3 receptors, resulting in more Ca^{2+} release*
- This **positive feedback** produces a *regenerative wave of Ca^{2+} release* that spreads across the cell
- The regenerative wave produces *a high Ca^{2+} concentration across the entire cell*



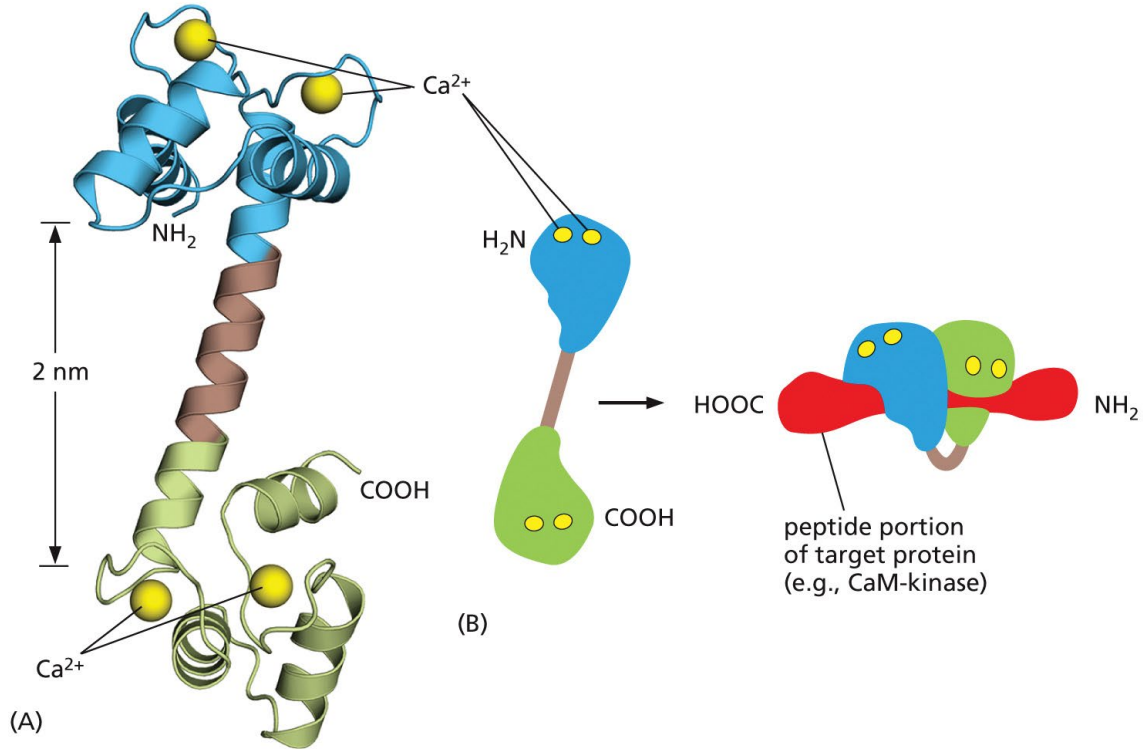
FEEDBACK GENERATES Ca^{2+} WAVES AND OSCILLATIONS

- When it *reaches high concentrations, Ca^{2+} inactivates IP_3 receptors* and ryanodine, shutting down the Ca^{2+} release
- *Ca^{2+}-pumps reduce the local cytosolic Ca^{2+} concentration to its low resting levels*
- The result is a cytosolic **Ca^{2+} pulse**: positive feedback drives a rapid rise in cytosolic Ca^{2+}, and negative feedback sends it back down again.
- The Ca^{2+} channels remain refractory to further stimulation for some period of time, delaying the generation of another Ca^{2+} spike
- Eventually the negative feedback wears off, allowing IP_3 to trigger another Ca^{2+} wave.
- The end result is **repeated Ca^{2+} oscillations**



CALMODULIN

- Two globular ends, which can bind to many different target proteins.
- The globular ends are connected by a long, exposed α helix, which allows the protein to adopt a number of different conformations, depending on the target protein it interacts with.
- Each globular head has two Ca^{2+}-binding sites



A, PDB code: 1CLL; B, PDB codes: 1CDL and 2BBM.

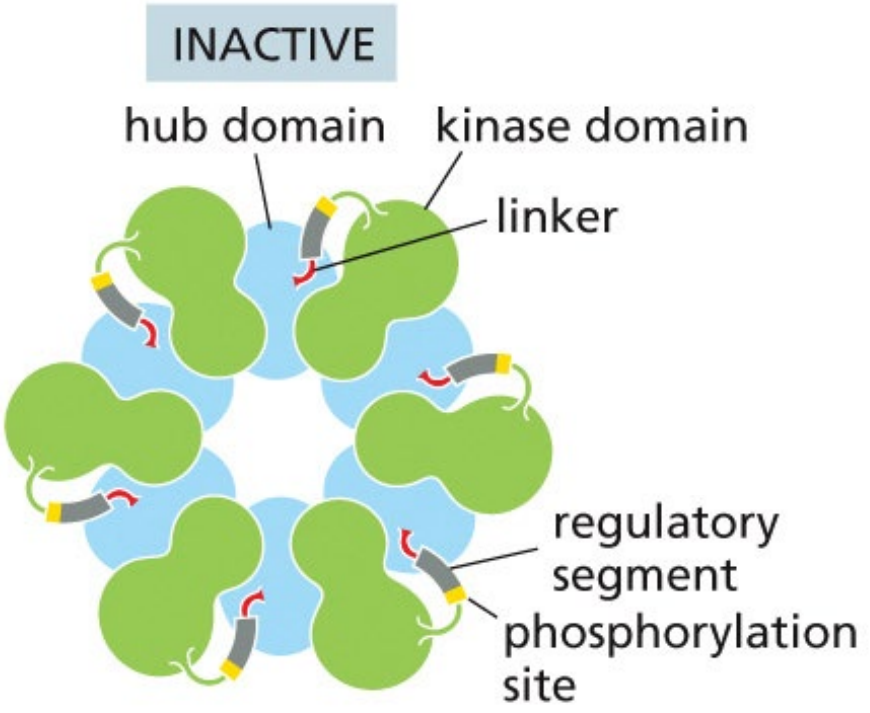
Binding of Ca^{2+} causes conformational change

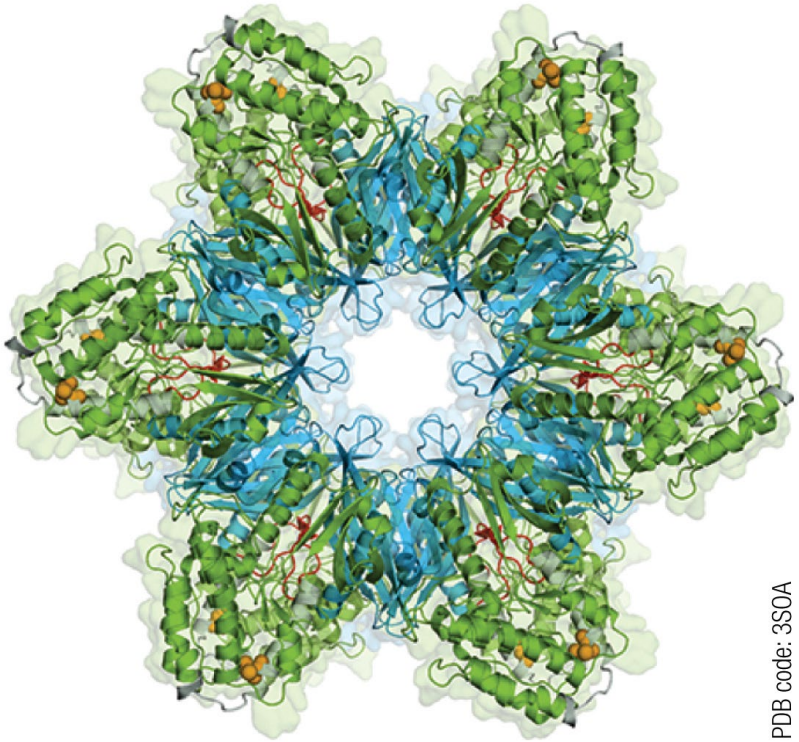
2^{nd} major structural change occurs in Ca^{2+}/calmodulin when it binds to a target protein

E.g. activates Ca2+ pump that reduces Ca2+ concentration – *negative feedback*

CAM KINASES

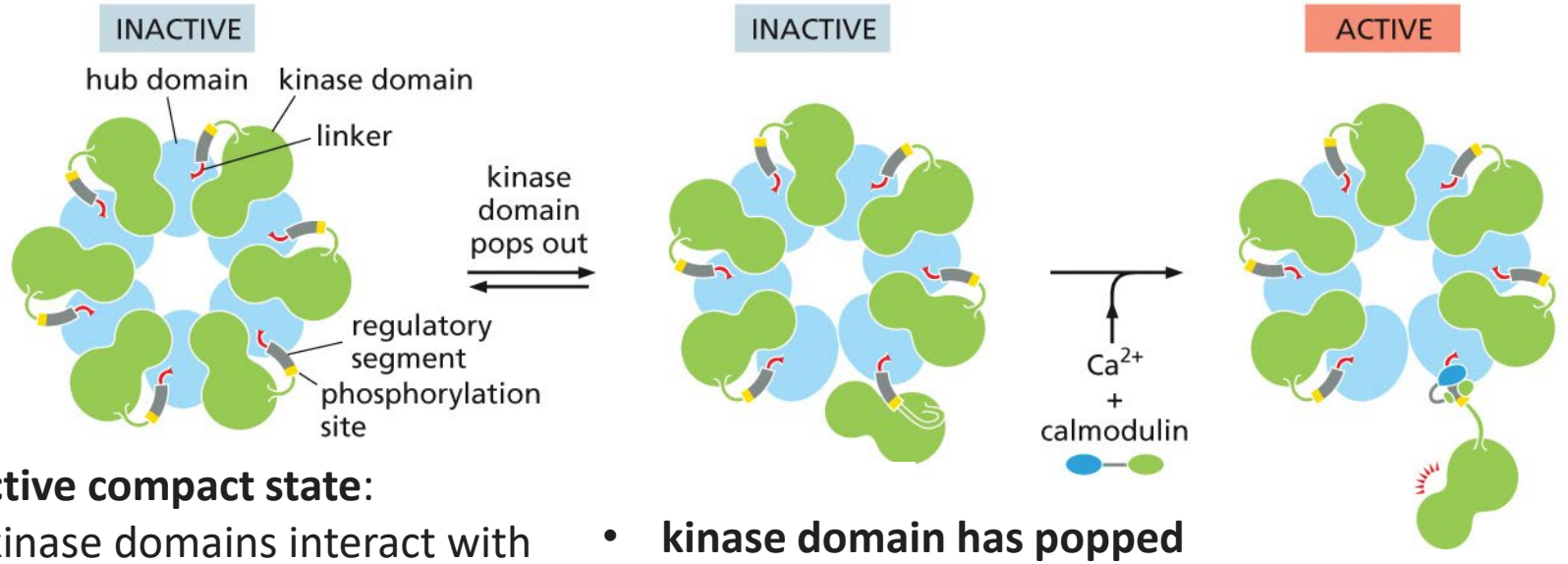
- Ca^{2+}/calmodulin-dependent **protein kinases** mediate many responses to Ca^{2+} signals





PDB code: 3S0A

- Six CaM-kinase II proteins are assembled into a giant ring
- The complete enzyme contains two stacked rings, for a total of 12 kinase proteins

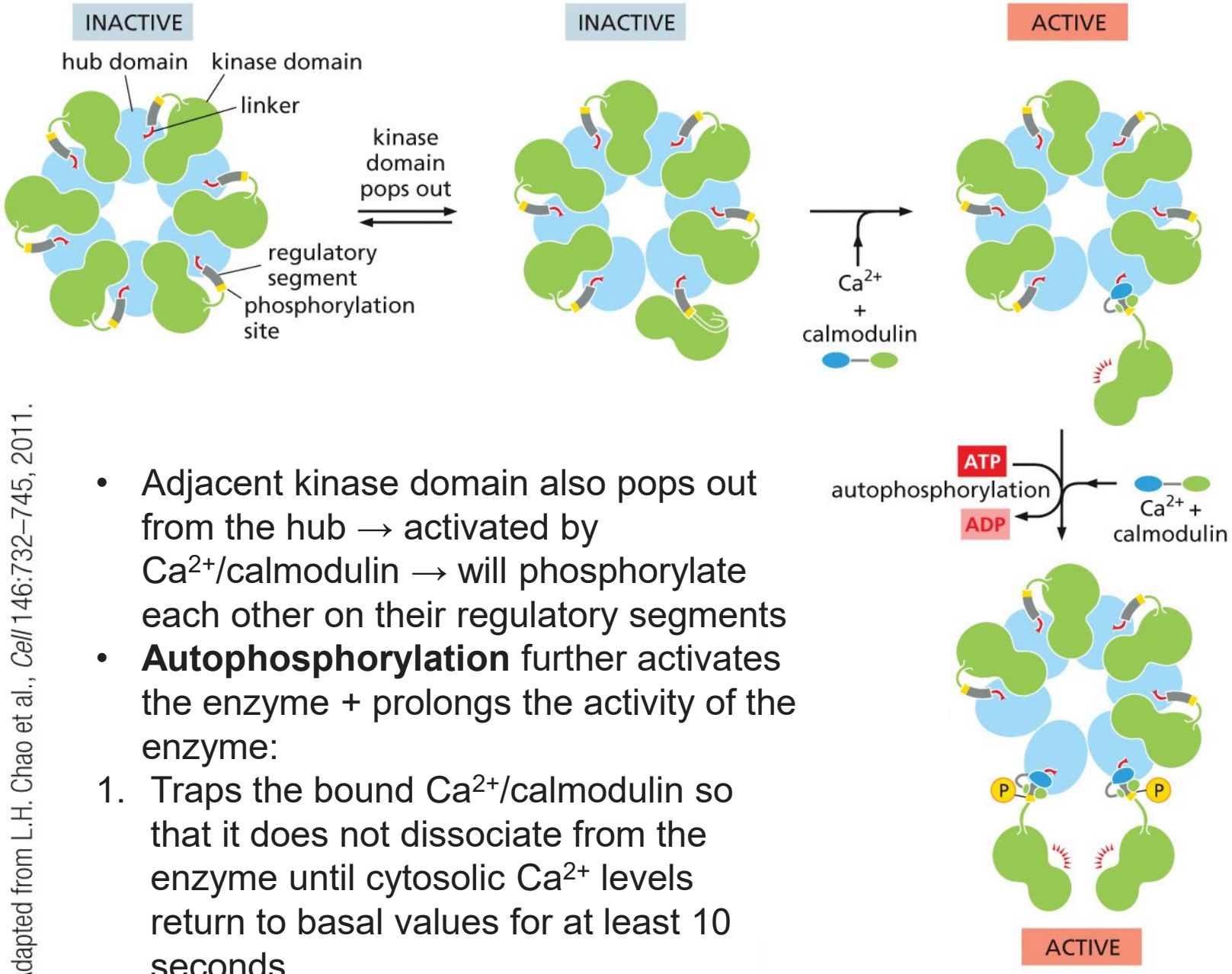


Inactive compact state:

- kinase domains interact with the hub domains
- *regulatory segments are buried in the kinase active sites and block catalytic activity*

- **kinase domain has popped out**, linked to its hub domain by its regulatory segment,
- continues to inhibit the kinase domain but is now **accessible to Ca^{2+}/calmodulin**

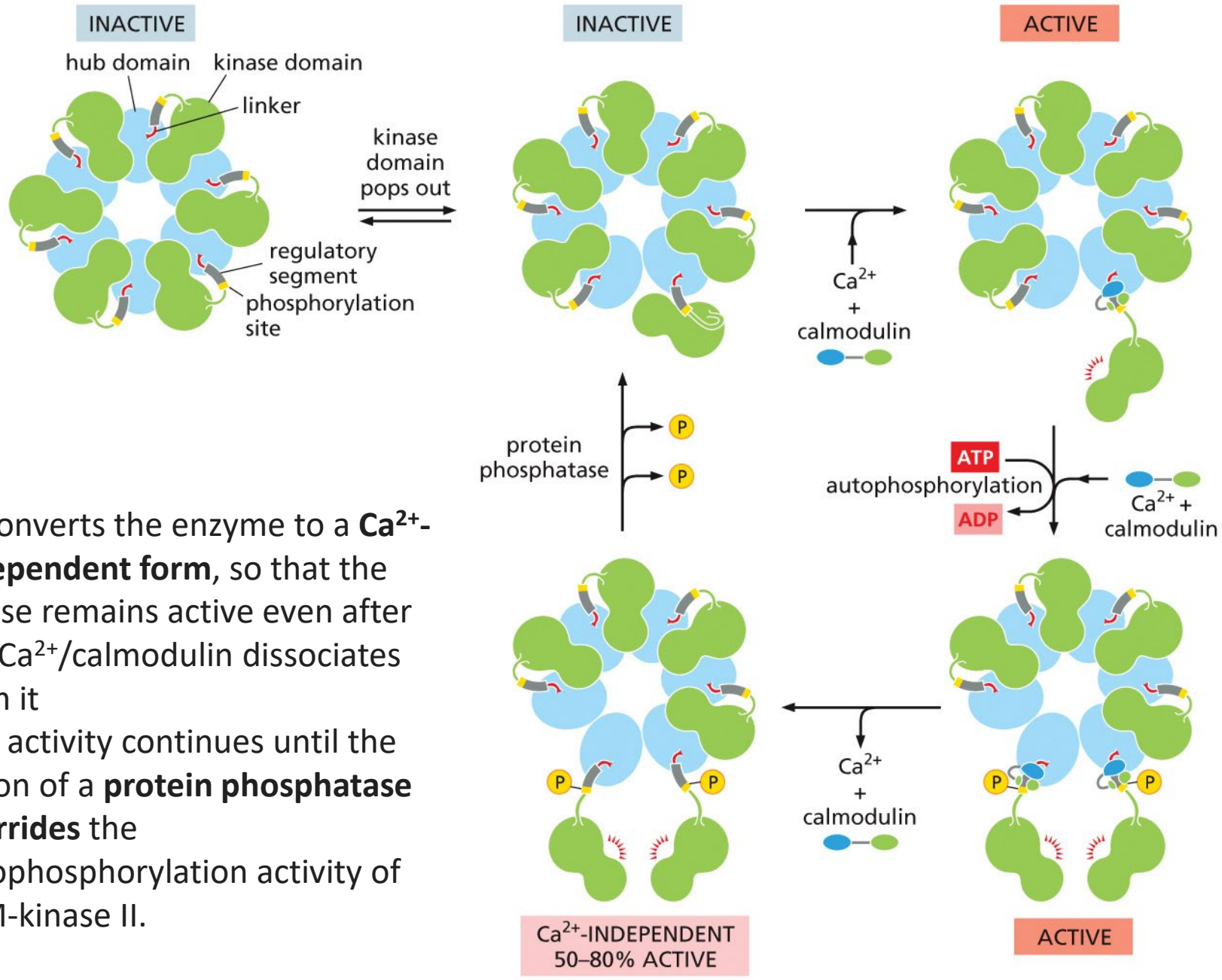
- If present, **Ca^{2+}/calmodulin will bind the regulatory segment** and prevent it from inhibiting the kinase, thereby *locking the kinase in an active state*



- Adjacent kinase domain also pops out from the hub → activated by Ca^{2+}/calmodulin → will phosphorylate each other on their regulatory segments
- **Autophosphorylation** further activates the enzyme + prolongs the activity of the enzyme:

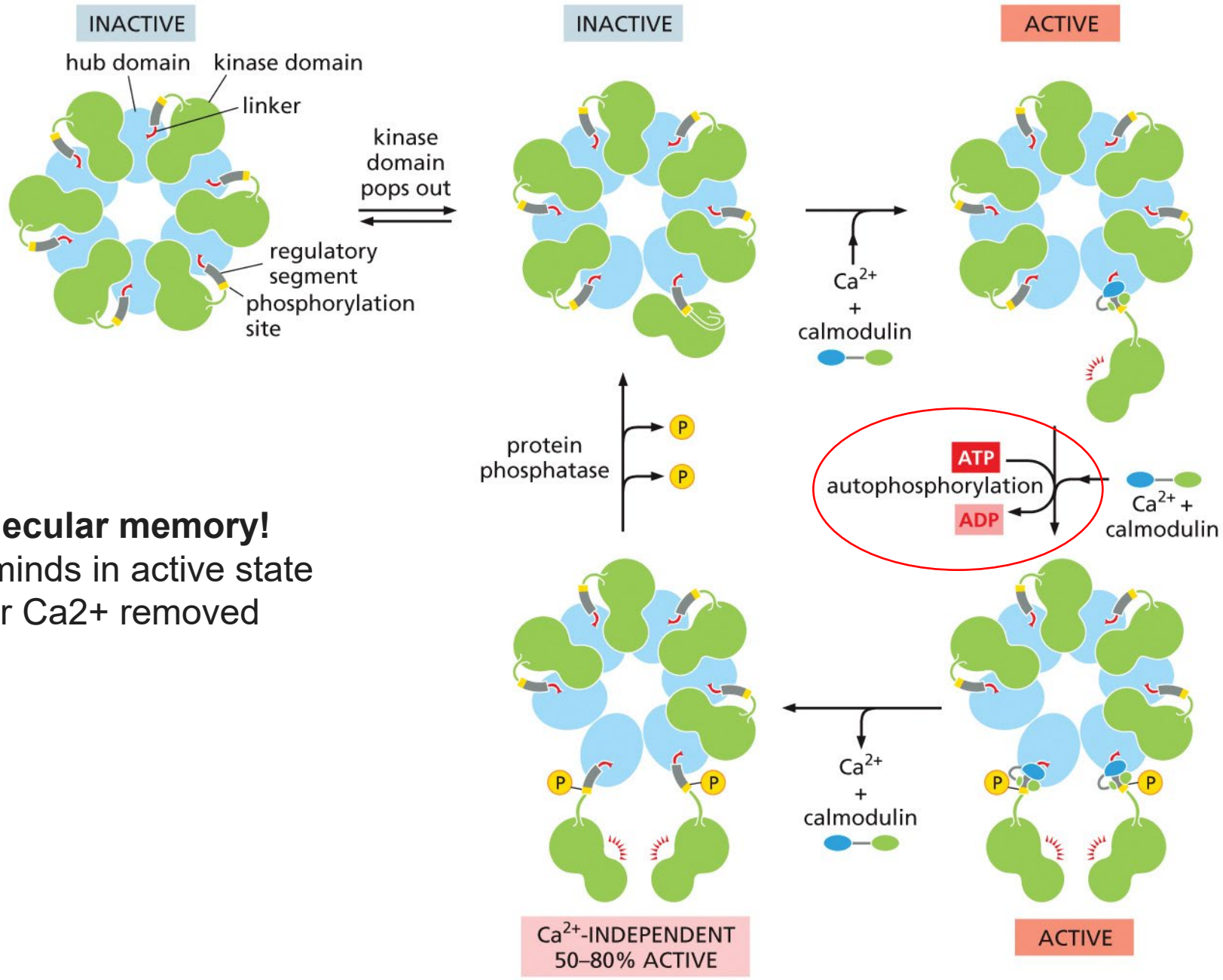
1. Traps the bound Ca^{2+}/calmodulin so that it does not dissociate from the enzyme until cytosolic Ca^{2+} levels return to basal values for at least 10 seconds

Adapted from L.H. Chao et al., *Cell* 146:732–745, 2011.



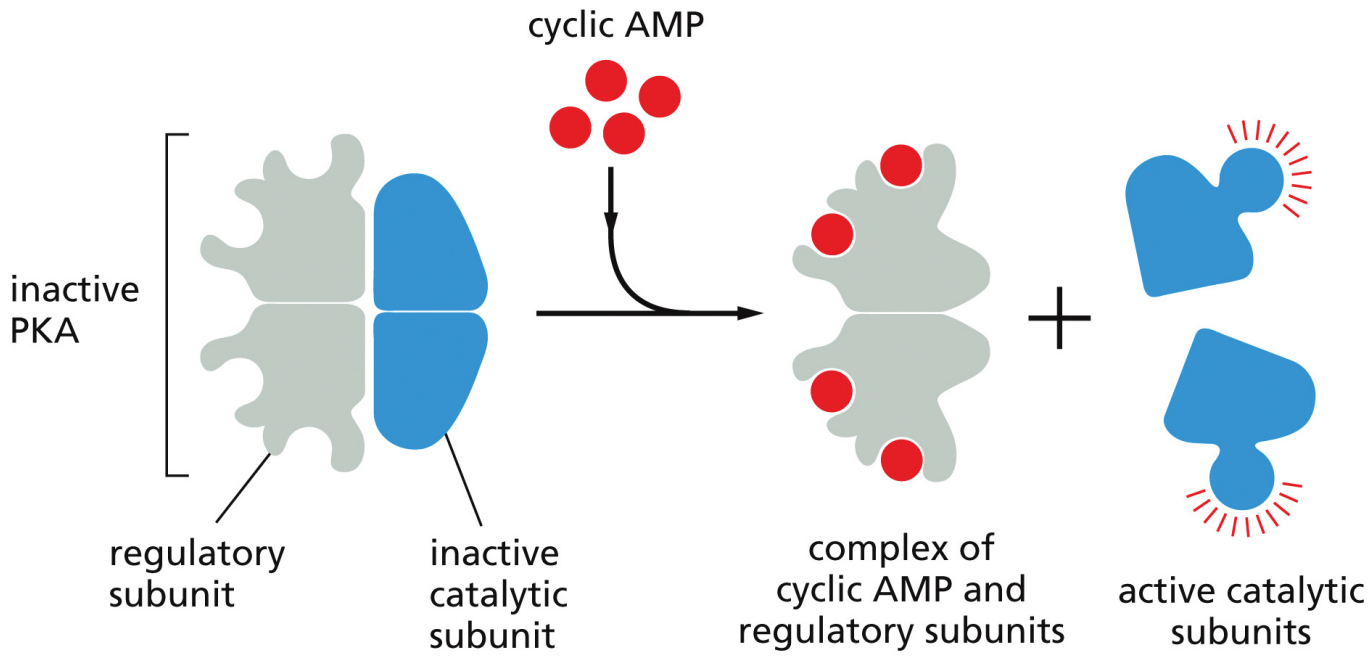
2. Converts the enzyme to a **Ca^{2+}-independent form**, so that the kinase remains active even after the Ca^{2+}/calmodulin dissociates from it

This activity continues until the action of a **protein phosphatase overrides** the autophosphorylation activity of CaM-kinase II.

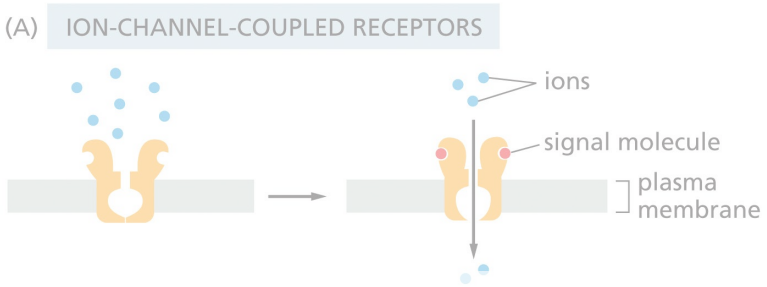


Molecular memory!
Reminds in active state after Ca2+ removed

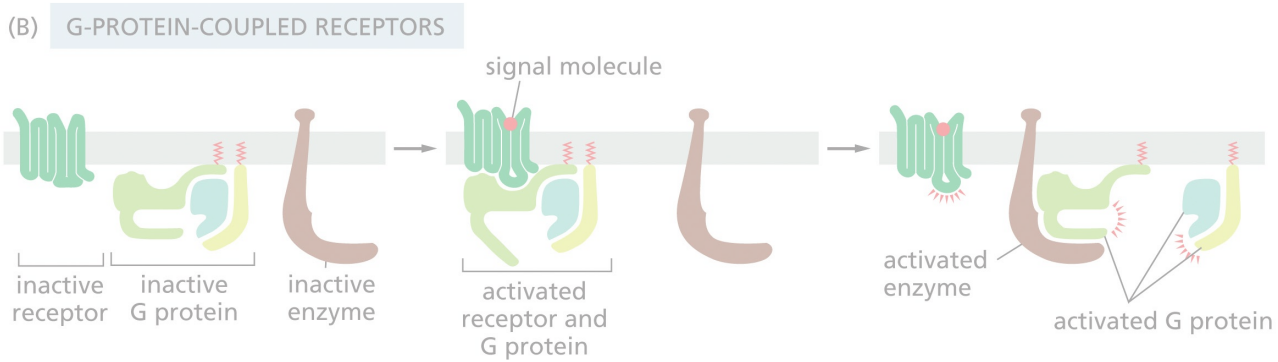
Propose specific types of mutations in the gene for the regulatory subunit of cyclic-AMP-dependent protein kinase (PKA) that could lead to either a permanently active PKA or a permanently inactive PKA?



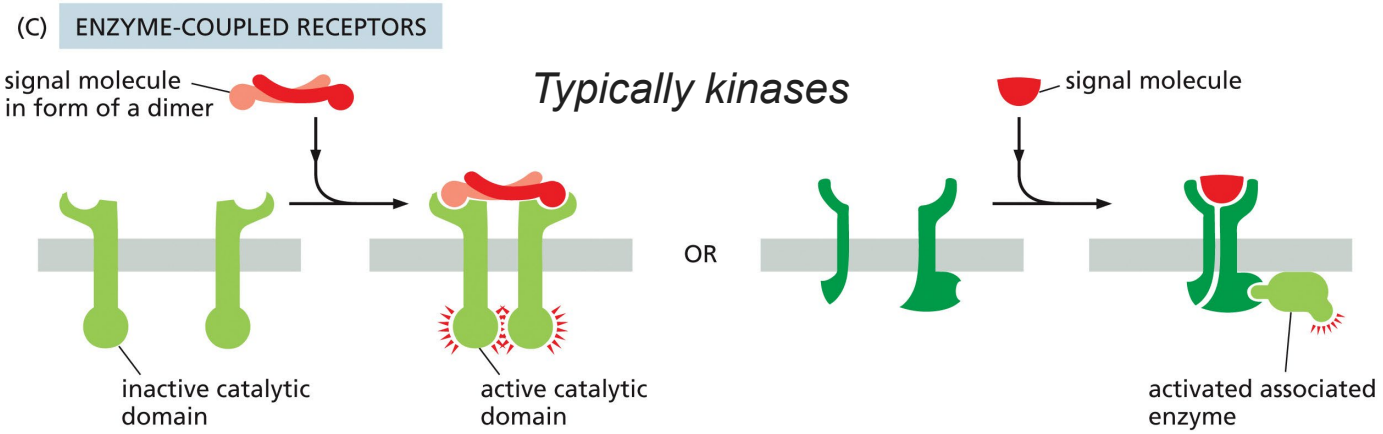
ENZYME-COUPLED RECEPTORS



- Synaptic signaling between nerve cells
- Signal molecules (neurotransmitters) transiently open ion channel



- G-protein mediates signal from receptor to target protein



- Receptor is an enzyme that act directly or by activating an associated enzyme

RECEPTOR TYROSINE KINASES (RTKS)

- ~60 in human
- Signals typically small proteins

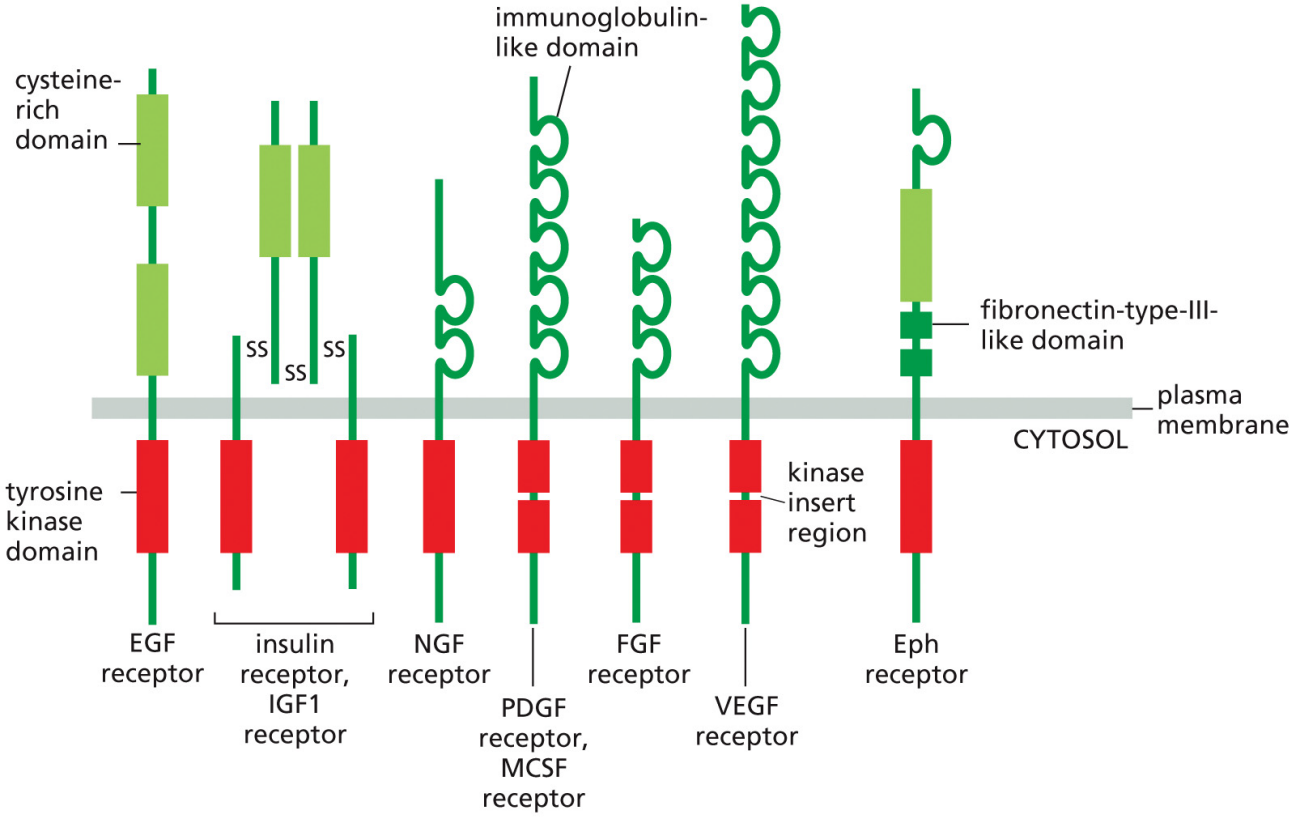
TABLE 15–4 Some Extracellular Signal Proteins That Act Via RTKs

Signal protein family	Receptor family	Some representative responses
Epidermal growth factor (EGF)	EGF receptors	Stimulates cell survival, growth, proliferation, or differentiation of various cell types; acts as inductive signal in development
Insulin	Insulin receptor	Stimulates carbohydrate utilization and protein synthesis
Insulin-like growth factor (IGF1)	IGF receptor-1	Stimulates cell growth and survival in many cell types
Nerve growth factor (NGF)	Trk receptors	Stimulates survival and growth of some neurons
Platelet-derived growth factor (PDGF)	PDGF receptors	Stimulates survival, growth, proliferation, and migration of various cell types
Macrophage-colony-stimulating factor (MCSF)	MCSF receptor	Stimulates monocyte/macrophage proliferation and differentiation
Fibroblast growth factor (FGF)	FGF receptors	Stimulates proliferation of various cell types; inhibits differentiation of some precursor cells; acts as inductive signal in development
Vascular endothelial growth factor (VEGF)	VEGF receptors	Stimulates angiogenesis
Ephrin	Eph receptors	Stimulates angiogenesis; guides cell and axon migration

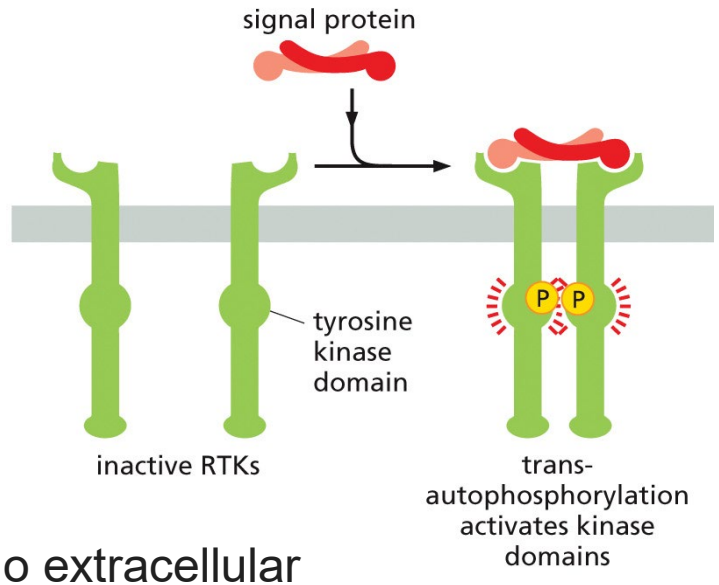
RECEPTOR TYROSINE KINASES (RTKS)

- Activated receptor tyrosine kinases (RTKs) phosphorylate themselves

- Typically have 1 transmembrane segment



ACTIVATION OF RTKS BY DIMERIZATION

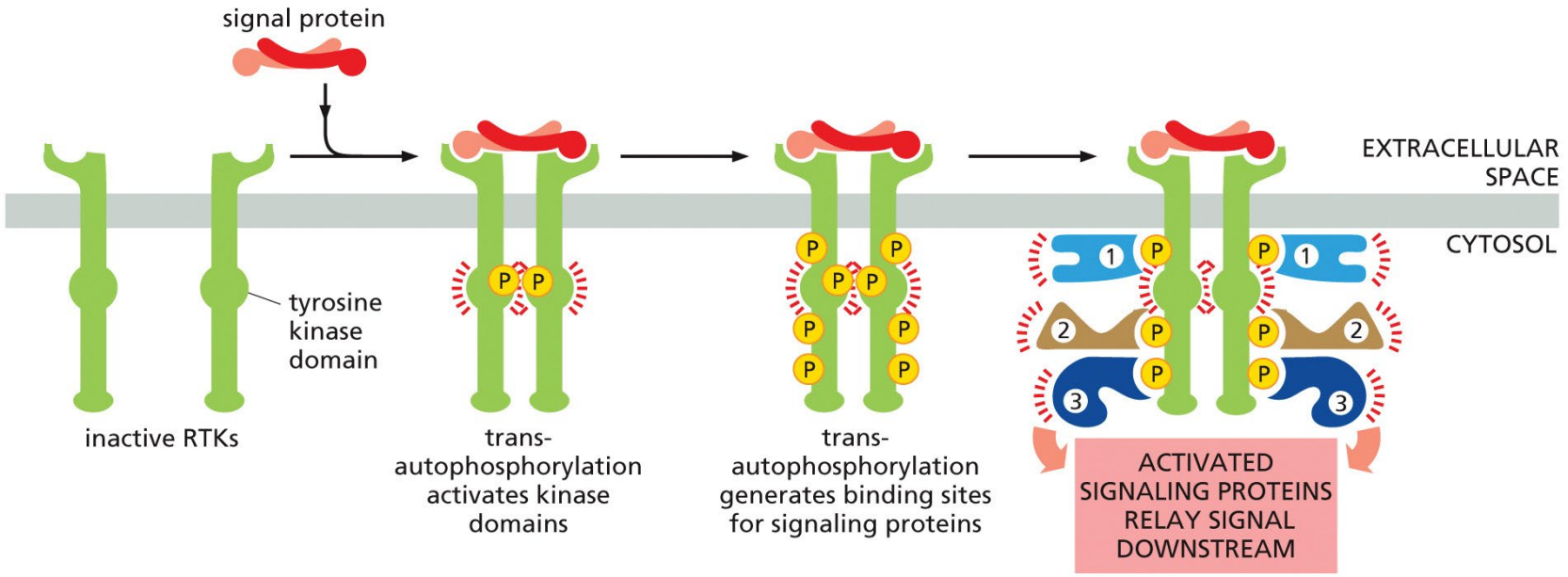


The ligand itself can be a dimer or two ligands can bind independently on two receptors to promote receptor dimerization

No extracellular signals → monomers → the internal kinase domain is inactive

Binding of ligand → dimerization → two domains phosphorylate each other

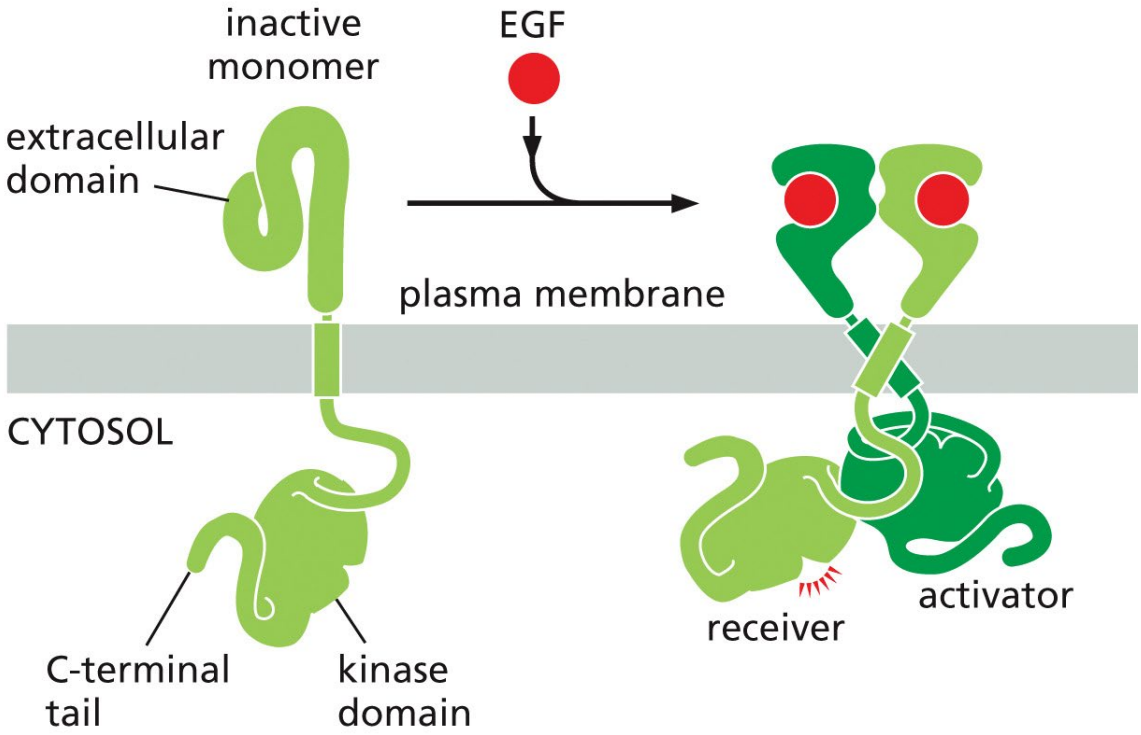
ACTIVATION OF RTKS BY DIMERIZATION



1. Phosphorylation at some tyrosines in the kinase domains → complete activation of the domains

2. Phosphorylation at tyrosines in other parts of the receptors → docking sites for intracellular signaling proteins → large signaling complexes

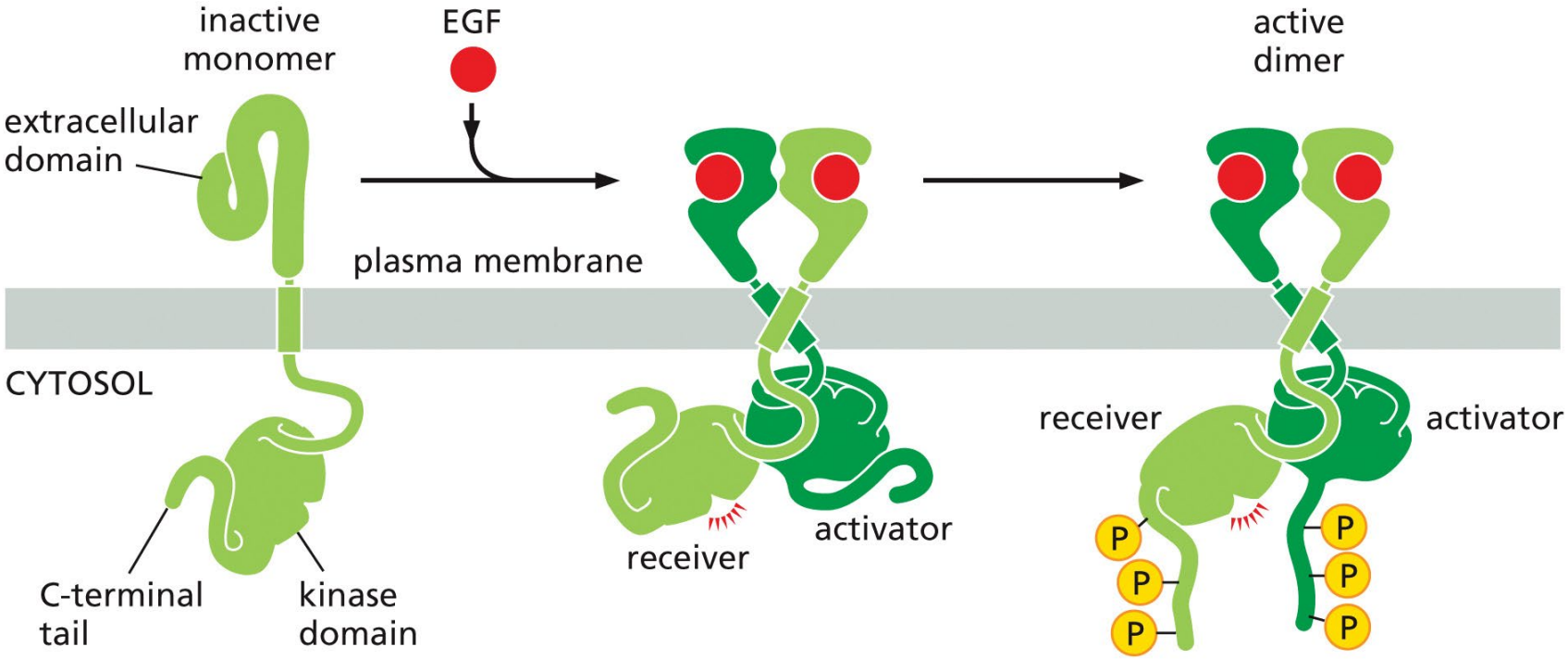
ACTIVATION OF RTKS BY CONFORMATIONAL CHANGE



In the absence of ligand, the EGF receptor exists primarily as an inactive monomer.

EGF binding results in a conformational change that promotes dimerization of the external domains.

ACTIVATION OF RTKS BY CONFORMATIONAL CHANGE



In the dimer one kinase domain (the *activator*) y causing an activating conformational change in the other domain (*receiver*)

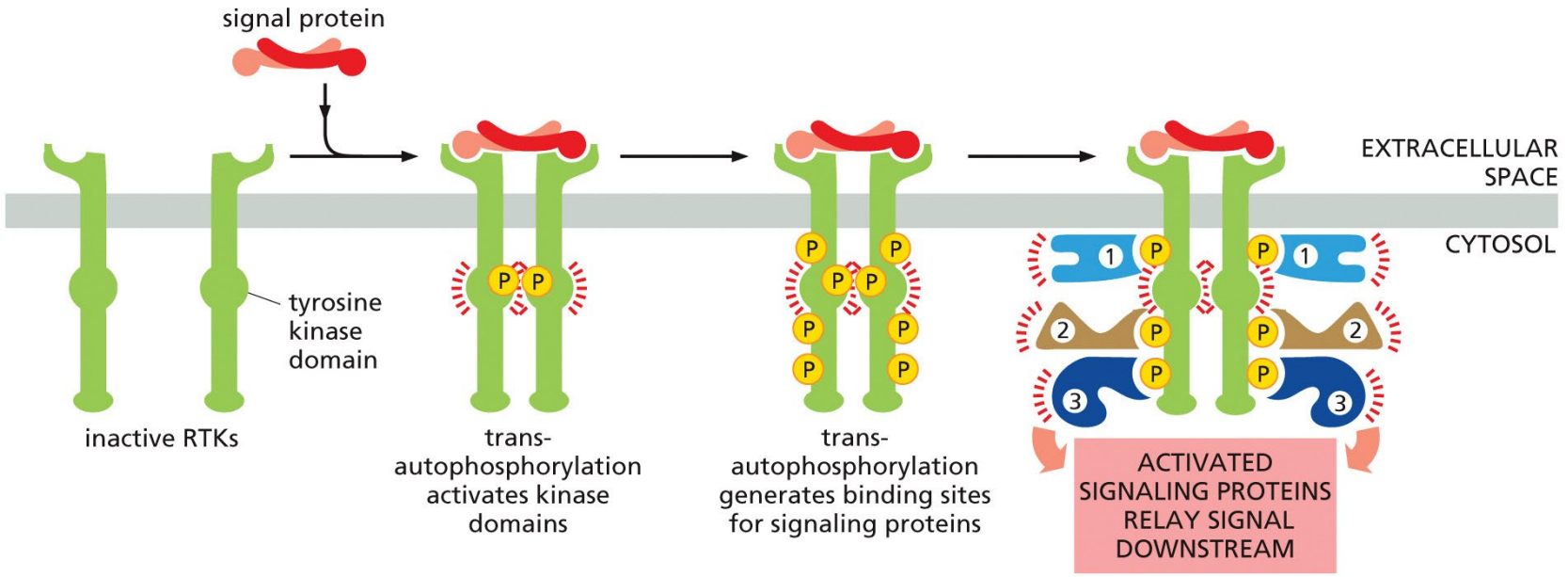
The active receiver domain phosphorylates multiple tyrosines in the C-terminal tails of both receptors, generating docking sites for intracellular signaling proteins

Not transautophosphorylation!

SIGNALING THROUGH ENZYME-COUPLED RECEPTORS

- Proteins with SH2 Domains Bind to Phosphorylated Tyrosines

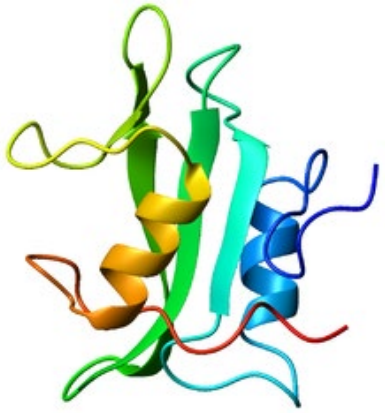
Phosphorylated RTKs as docking sites for intracellular signaling proteins



- **Phosphorylated tyrosines on RTKs serve as docking sites for intracellular signaling proteins** (typically in intrinsically disordered regions)
- Binding molecules recognize phospotyrosine+flanking residues
- Activation by 1) phosphorylation, 2) conformational change, or 3) proximity
- Scaffold proteins grow the complexes further
- Can also mediate inhibition by e.g. directing to lysosomes

PRINCIPLES OF CELL SIGNALING

- Modular interaction domains mediate interactions between intracellular signaling proteins

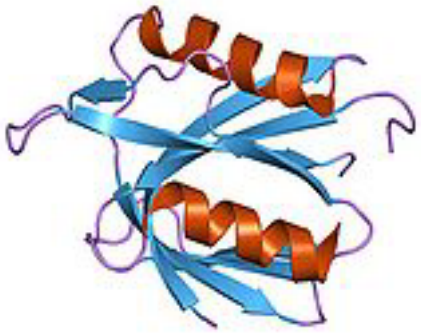


SH2
Src homology 2

Phosphorylated tyrosine

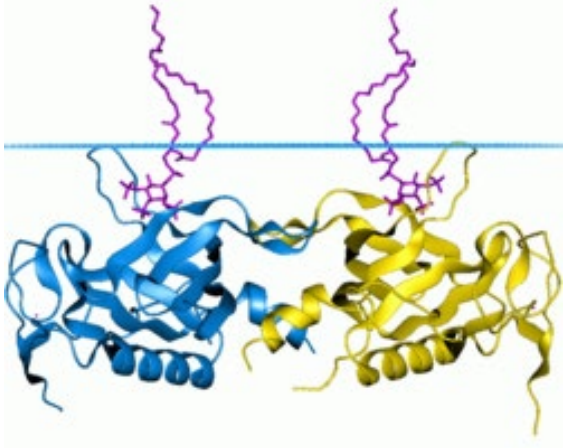
SH3
Src homology 3

Pro-rich regions



PTB
Phosphotyrosine binding

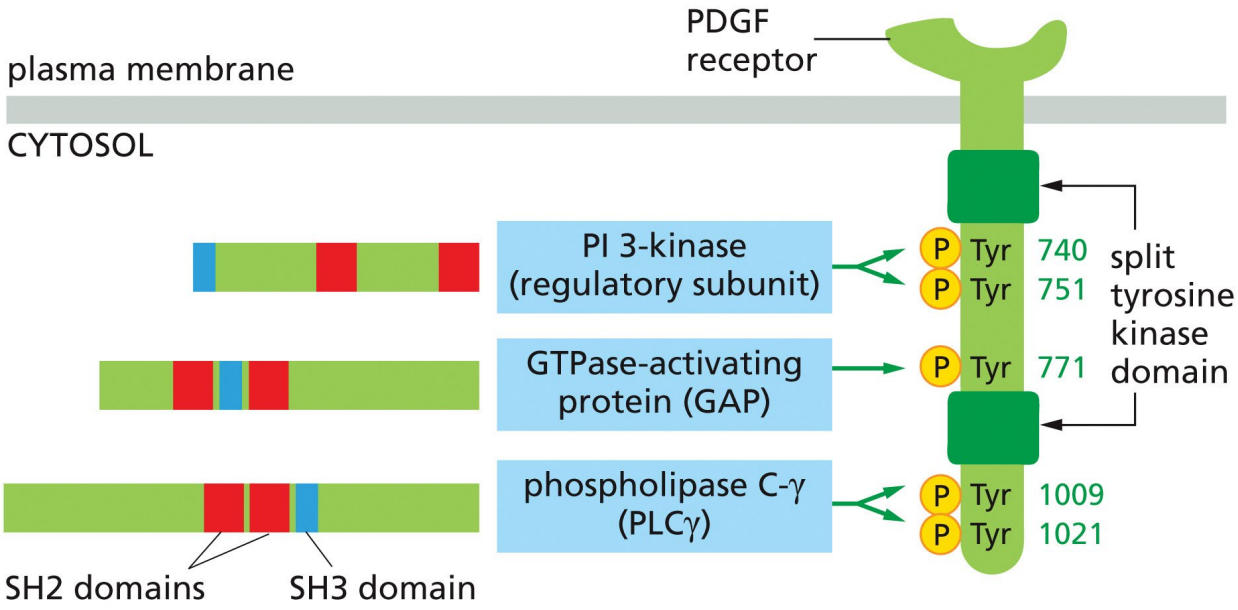
Phosphorylated tyrosine



PH
Pleckstrin homology

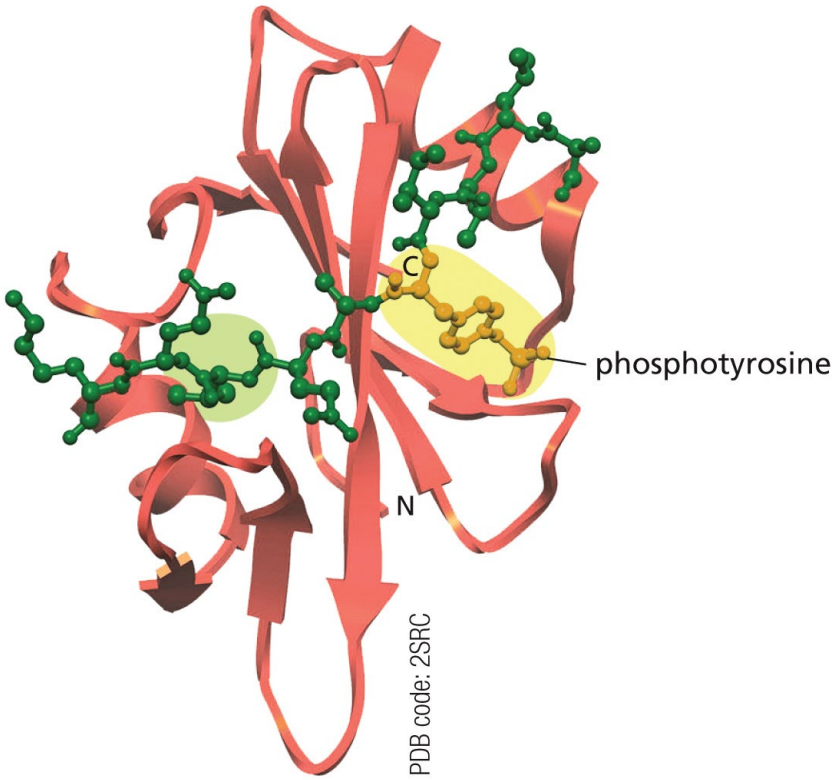
Phosphoinositides

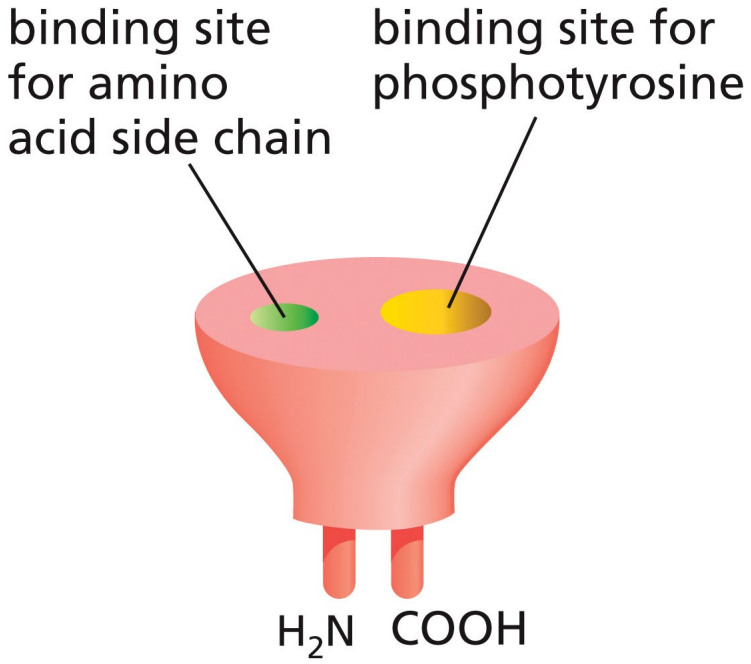
MULTIPLE DOCKING SITES IN AN ACTIVATED RTK



Five phosphotyrosines are shown, three in the kinase insert region and two on the C-terminal tail; these form three docking sites, each of which binds a different signaling protein as indicated

THE BINDING OF SH2-CONTAINING INTRACELLULAR SIGNALING PROTEINS TO AN ACTIVATED RTK





The binding pocket for phosphotyrosine, and a pocket for binding a specific amino acid side chain (valine, in this case). The RTK polypeptide segment binds the SH2 domain

The SH2 domain is a compact, “plug-in” module, which can be inserted in disordered regions of a protein without disturbing the protein’s folding or function

RAS SUPERFAMILY

- Consists of families of monomeric GTPases
- Ras and Rho families mediate signals from cell-surface receptors

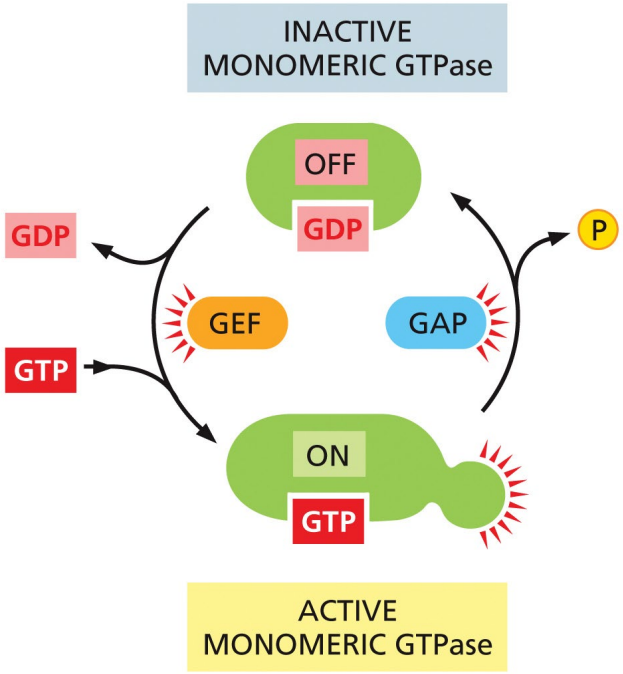


TABLE 15–5 The Ras Superfamily of Monomeric GTPases

Family	Some family members	Some functions
Ras	H-Ras, K-Ras, N-Ras	Relay signals from RTKs
	Rheb	Activates mTOR to stimulate cell growth
	Rap1	Activated by a cyclic-AMP-dependent GEF; influences cell adhesion by activating integrins
Rho*	Rho, Rac, Cdc42	Relay signals from surface receptors to the cytoskeleton and elsewhere
ARF*	ARF1–ARF6	Regulate assembly of protein coats on intracellular vesicles
Rab*	Rab1–60	Regulate intracellular vesicle traffic
Ran*	Ran	Regulates mitotic spindle assembly and nuclear transport of RNAs and proteins

*The Rho family is discussed in Chapter 16, the ARF and Rab proteins in Chapter 13, and Ran in Chapters 12 and 17. The three-dimensional structure of Ras is shown in Figure 3–64.

Recap: **Ran** GTPase imposes directionality on nuclear transport

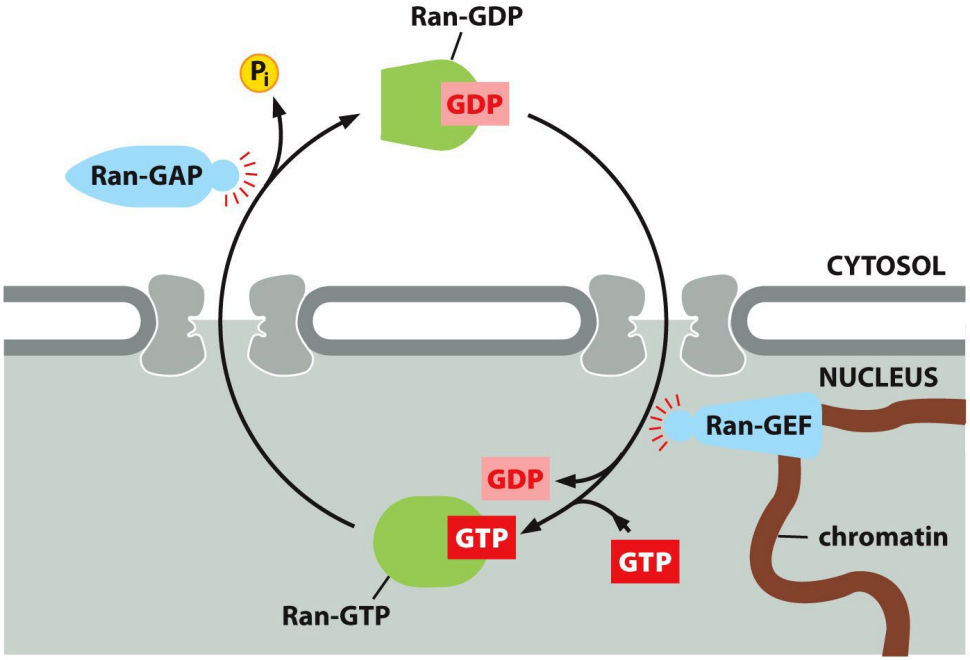


Figure 12-12 Molecular Biology of the Cell 6e (© Garland Science 2015)

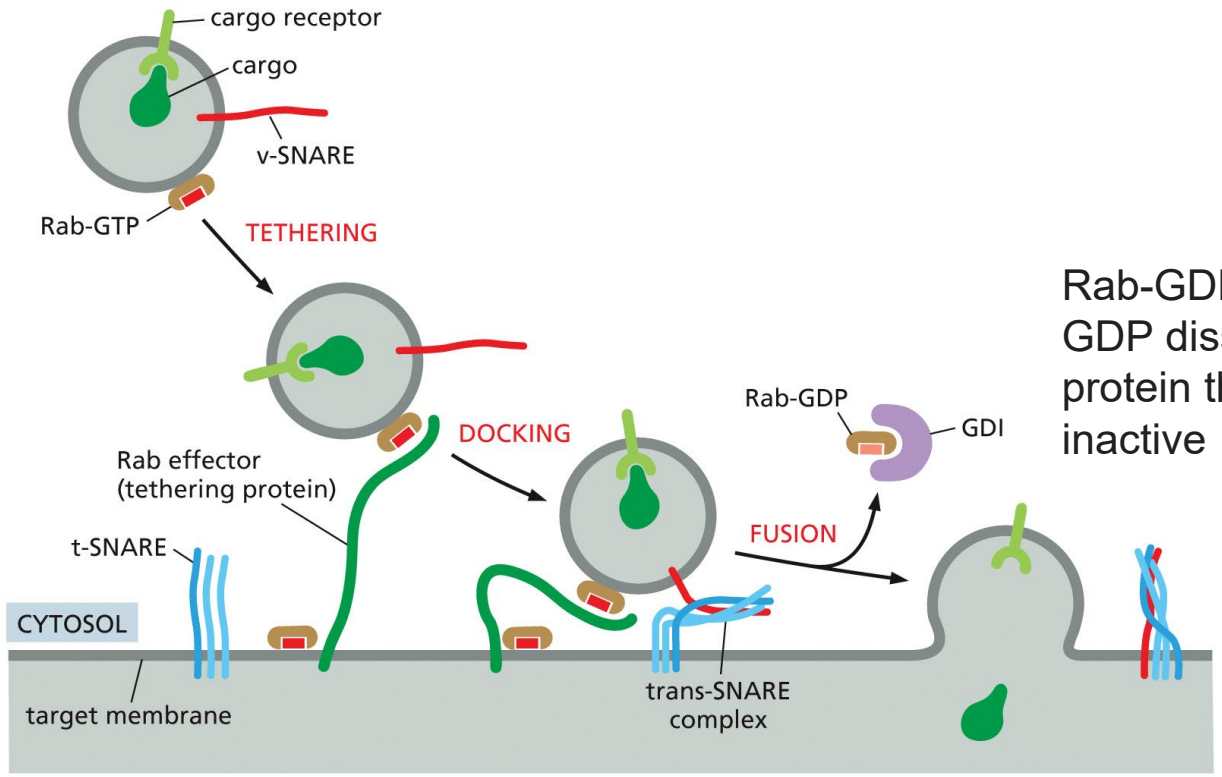
Players:

- Ran GTPase
- Ran **GTPase activating protein** (Ran-GAP) Cytosol
- Ran **guanine exchange factor** (Ran-GEF) anchored to chromatin

- Ran is a molecular switch that exists in two conformational states (GTP and GDP bound)
- Cytosol contains mainly Ran-GAP, nucleus Ran-GEF
- Gradient of the two forms (GTP/GDP form) drives nuclear transport

RECAP: **RAB PROTEINS** GUIDE TRANSPORT VESICLES TO THEIR TARGET MEMBRANE

- *Membrane bound* state, bind *GTP* (**active**)
  - Interact with *Rab effector proteins* on the target and/or vesicle membrane
- *Soluble state*, bind *GDP* (+GDI) (**inactive**)
  - *Hydrolysis* of GTP to GDP during docking causes Rab to *dissociate*



Rab-GDP in cytosol is bound by a GDP dissociation inhibitor (GDI) protein that keeps it soluble and inactive

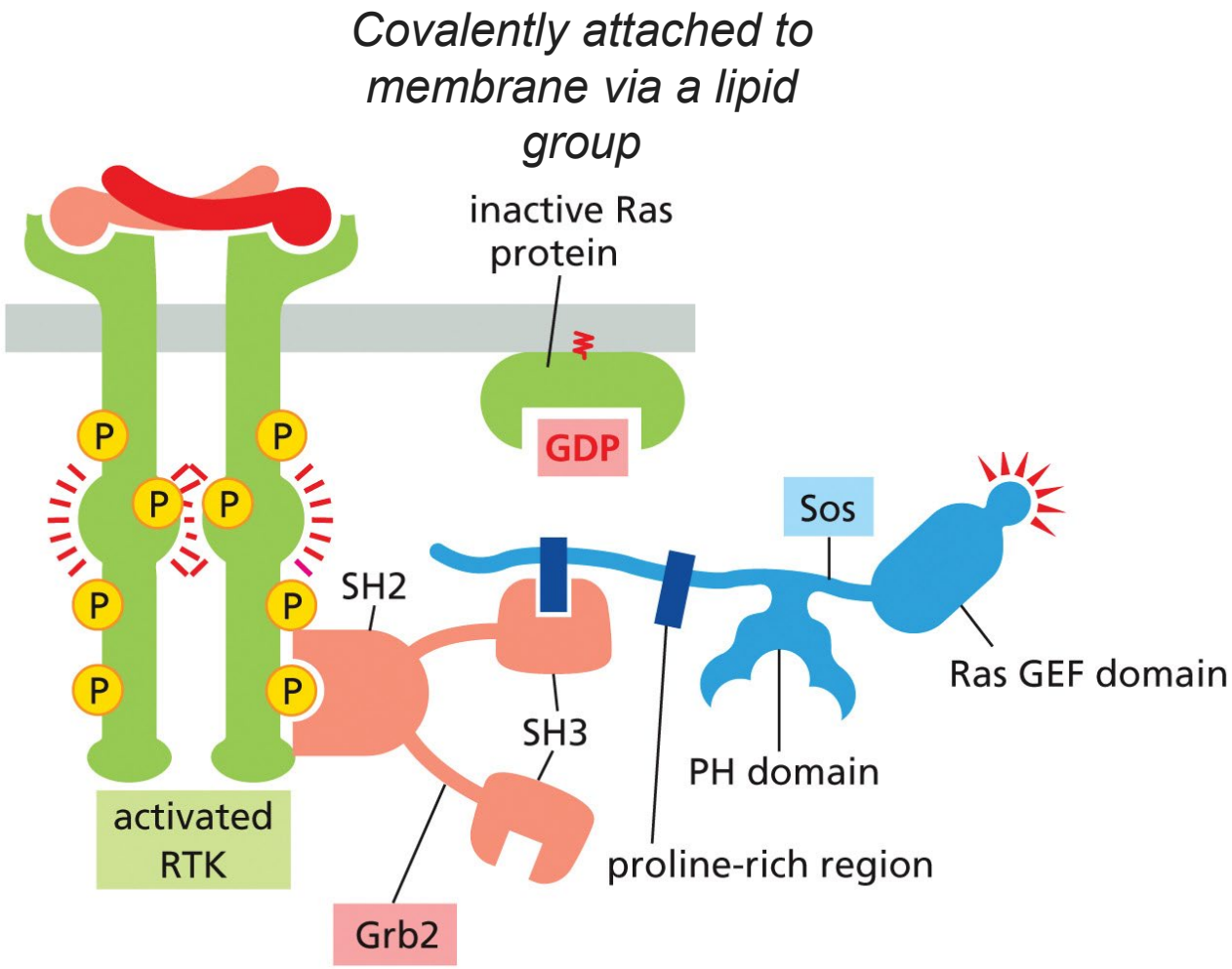
RAS SUPERFAMILY

- Consists of families of monomeric GTPases
- **Ras and Rho families mediate signals from cell-surface receptors**

TABLE 15–5 The Ras Superfamily of Monomeric GTPases

Family	Some family members	Some functions
Ras	H-Ras, K-Ras, N-Ras	Relay signals from RTKs
	Rheb	Activates mTOR to stimulate cell growth
	Rap1	Activated by a cyclic-AMP-dependent GEF; influences cell adhesion by activating integrins
Rho*	Rho, Rac, Cdc42	Relay signals from surface receptors to the cytoskeleton and elsewhere
ARF*	ARF1–ARF6	Regulate assembly of protein coats on intracellular vesicles
Rab*	Rab1–60	Regulate intracellular vesicle traffic
Ran*	Ran	Regulates mitotic spindle assembly and nuclear transport of RNAs and proteins

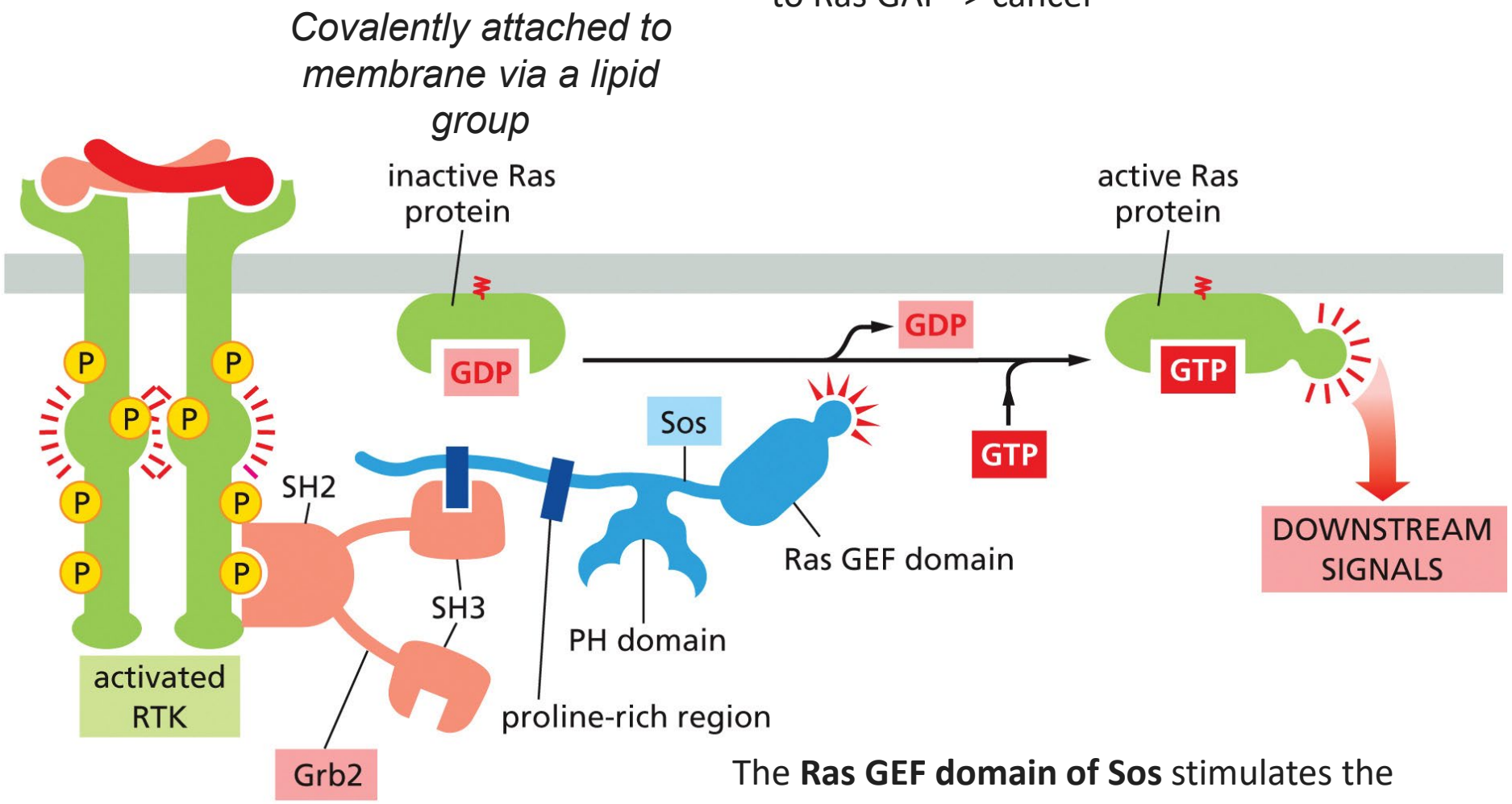
*The Rho family is discussed in Chapter 16, the ARF and Rab proteins in Chapter 13, and Ran in Chapters 12 and 17. The three-dimensional structure of Ras is shown in Figure 3–64.



The **Grb2 adaptor** protein recognizes a specific **phosphorylated tyrosine** on the activated receptor by means of an **SH2** domain

Recruits the Ras GEF Sos by means of an interaction between its **SH3** domains and **proline-rich** regions in Sos

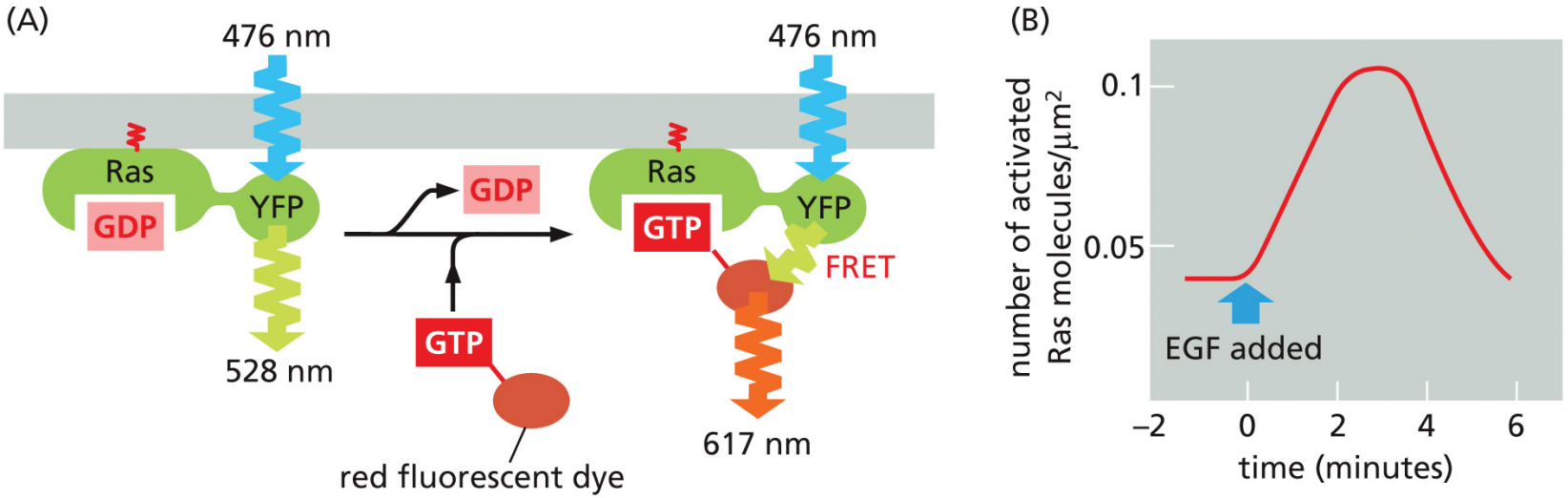
The Ras GAP increases hydrolysis of bound GTP in Ras -> inactivates. Hyperactive Ras=resistant to Ras GAP -> cancer



The **Ras GEF domain of Sos** stimulates the inactive Ras protein to replace its bound GDP by GTP, which **activates Ras** to relay the signal downstream.

SIGNALING THROUGH ENZYME-COUPLED RECEPTORS

- Both Tyr phosphorylation and activation of Ras typically transient (tyrosine phosphatases and Ras GAPs)

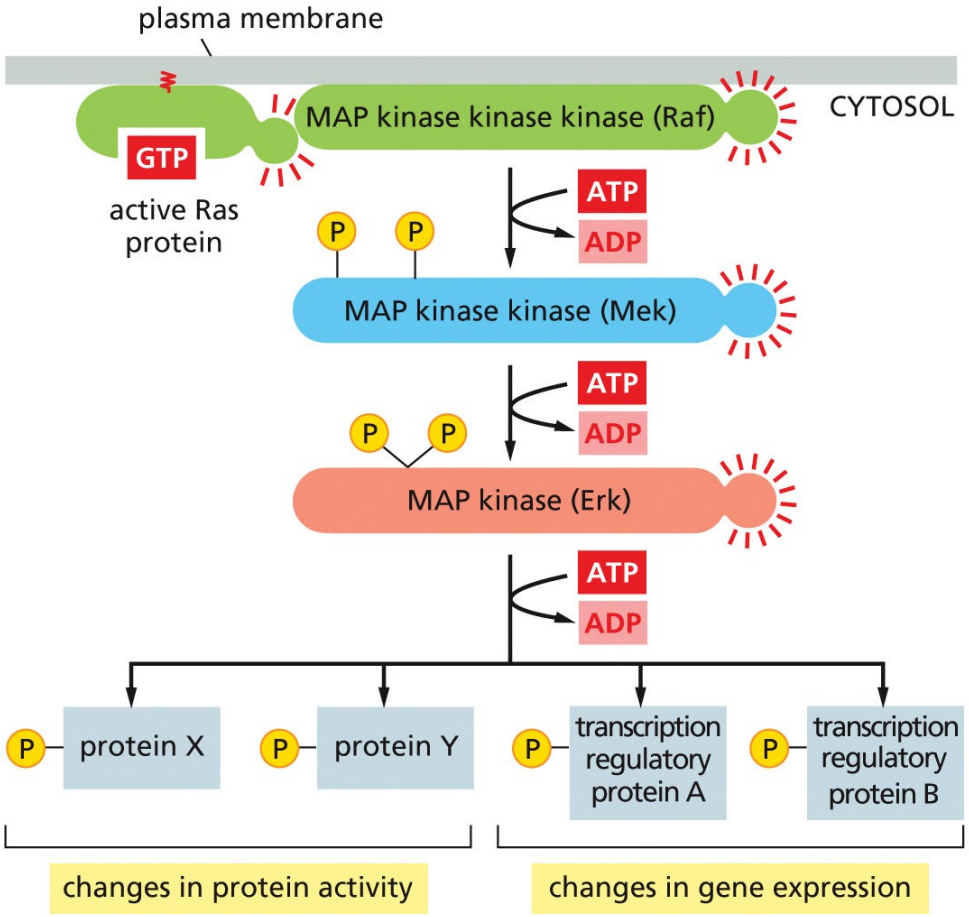


Modified from H. Murakoshi et al., *Proc. Natl. Acad. Sci. USA* 101:7317–7322, 2004. Copyright 2004 National Academy of Sciences, USA.
With permission from National Academy of Sciences.

SIGNALING THROUGH ENZYME-COUPLED RECEPTORS

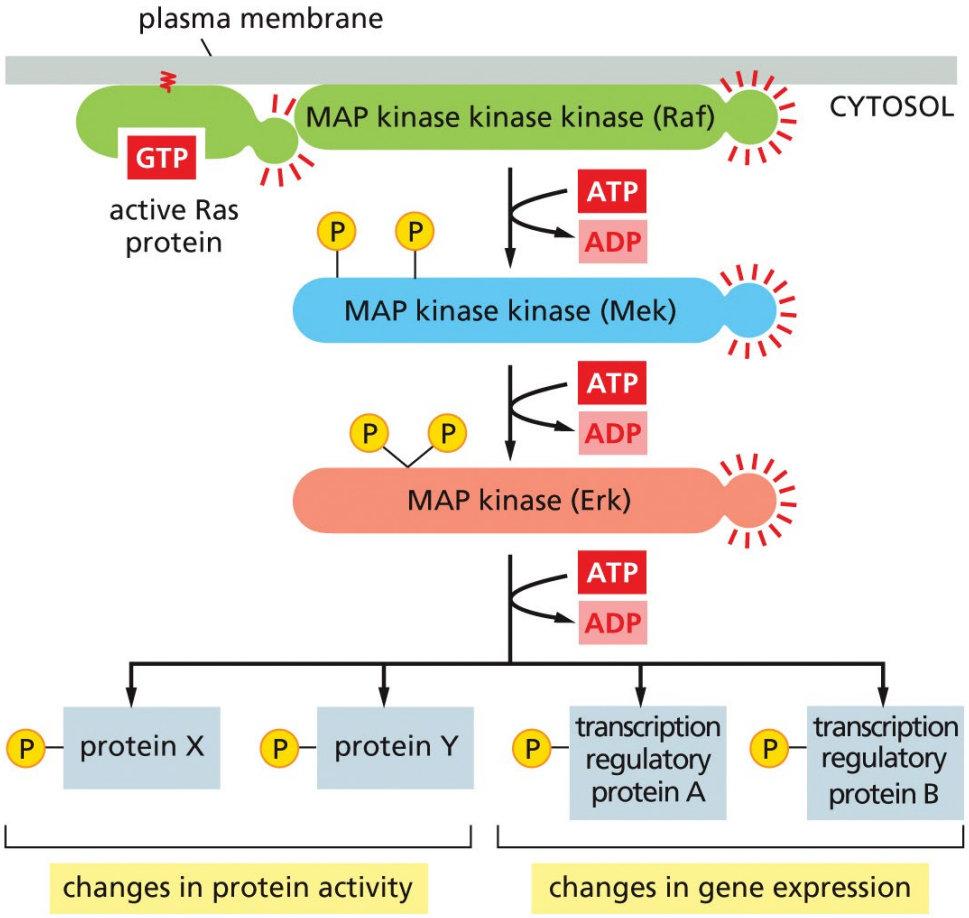
- Ras activates a MAP (mitogen-activated protein) kinase signaling module

- Signal downstreams + sustained after primary signal runed off
- MAP kinase (MAPK) → phosphorylates diverse set of proteins → changes in protein activities, gene expression



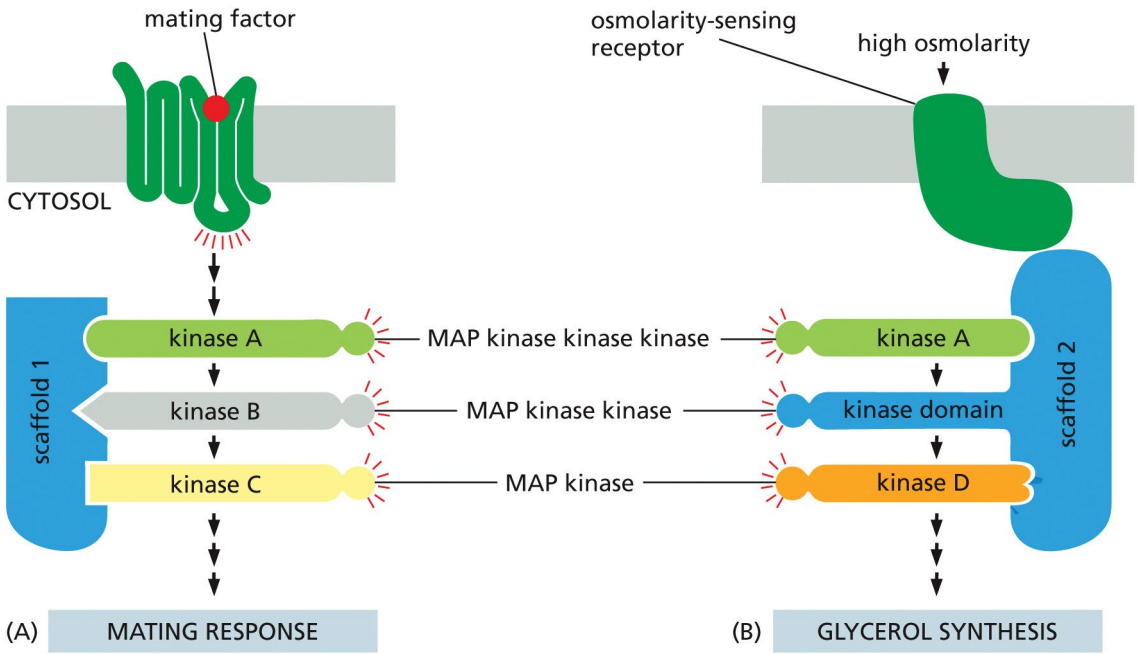
MAP KINASE SIGNALING MODULE

- Ras activates a MAP (mitogen-activated protein) kinase signaling module
  - MAPK activates effector protein
  - MAPKK activates MAPK
  - MAPKKK activates MAPK



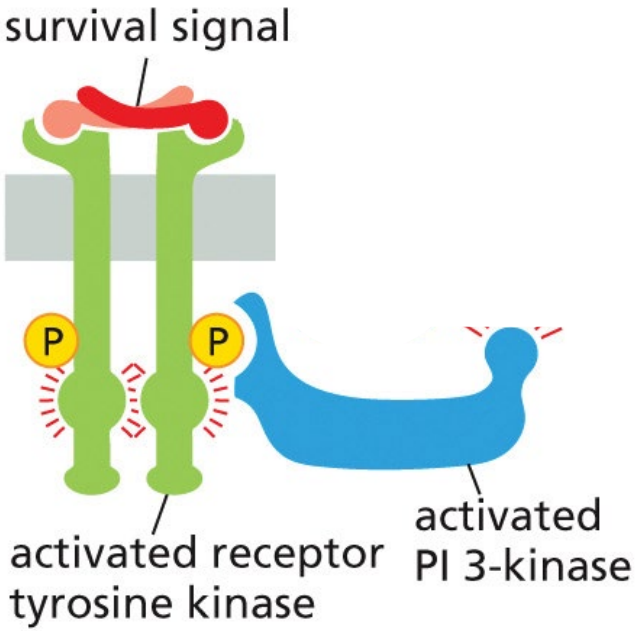
SIGNALING THROUGH ENZYME-COUPLED RECEPTORS

- Scaffold proteins reduce cross-talk between different MAP kinase modules
- Same kinase “unit” can be used in different pathways without crosstalk

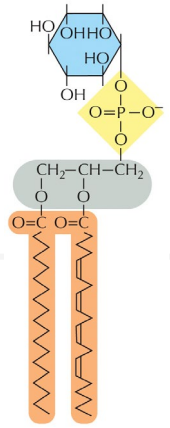


PI 3-KINASE

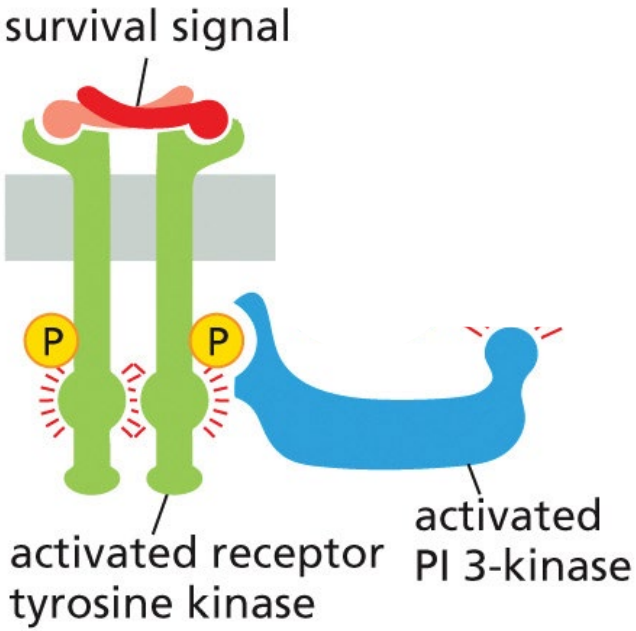
- PI 3-kinase binds the phosphorylated tail of RTK receptors

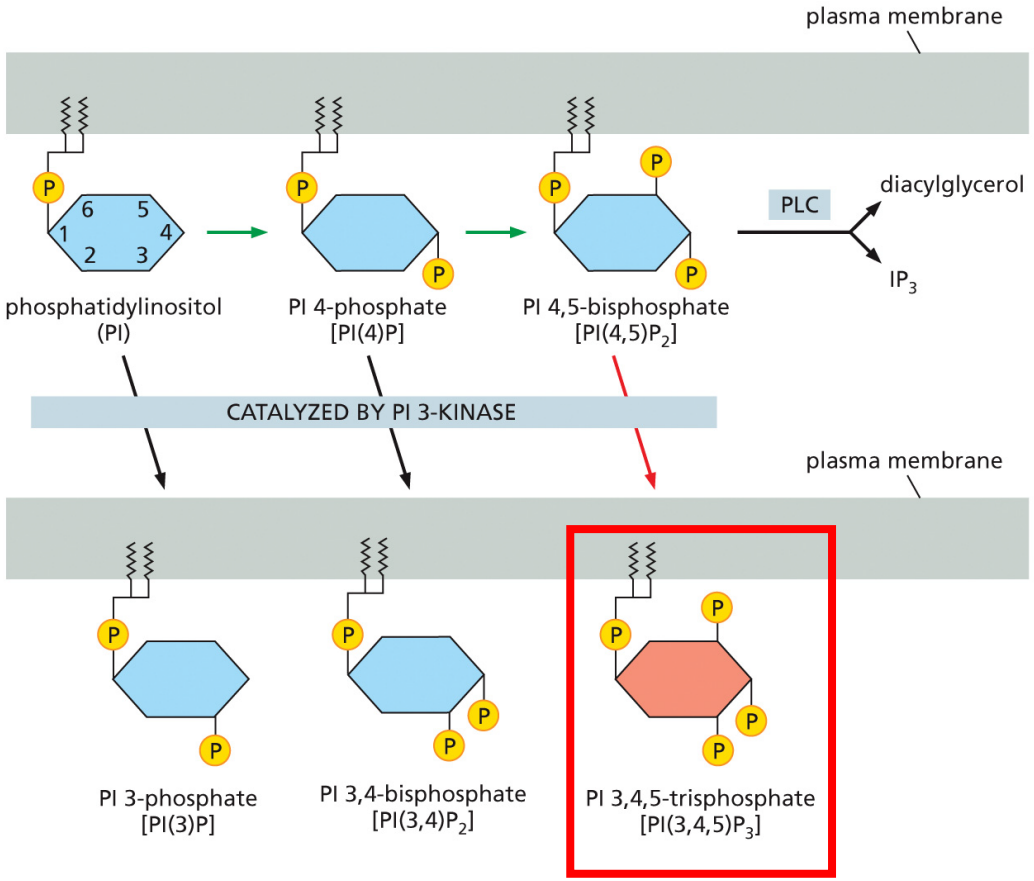


PI 3-KINASE



- PI 3-kinase produces lipid docking sites in the plasma membrane

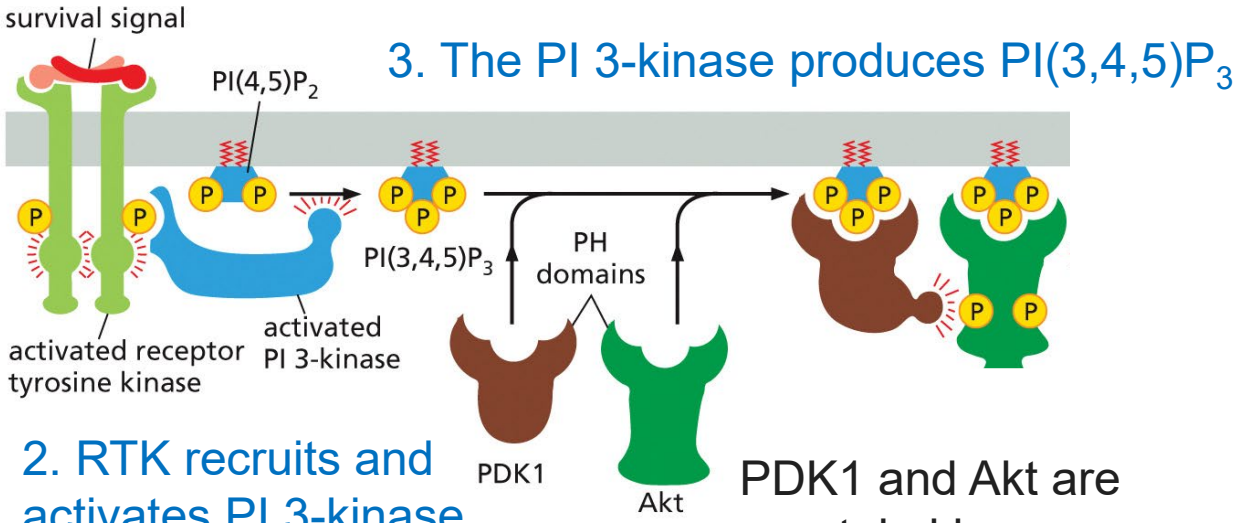




PI-3-KINASE–AKT SIGNALING PATHWAY

- The PI-3-kinase–Akt signaling pathway stimulates animal cells to survive and grow

1. An extracellular survival signal activates an RTK



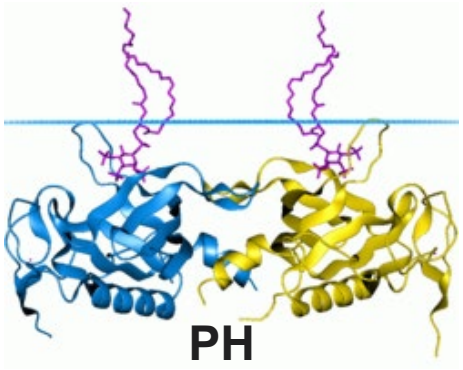
3. The PI 3-kinase produces $PI(3,4,5)P_3$

2. RTK recruits and activates PI 3-kinase

PDK1 and Akt are protein kinases

4. $PI(3,4,5)P_3$ serves as a docking site for two serine/threonine kinases with PH domains

Phosphoinositides

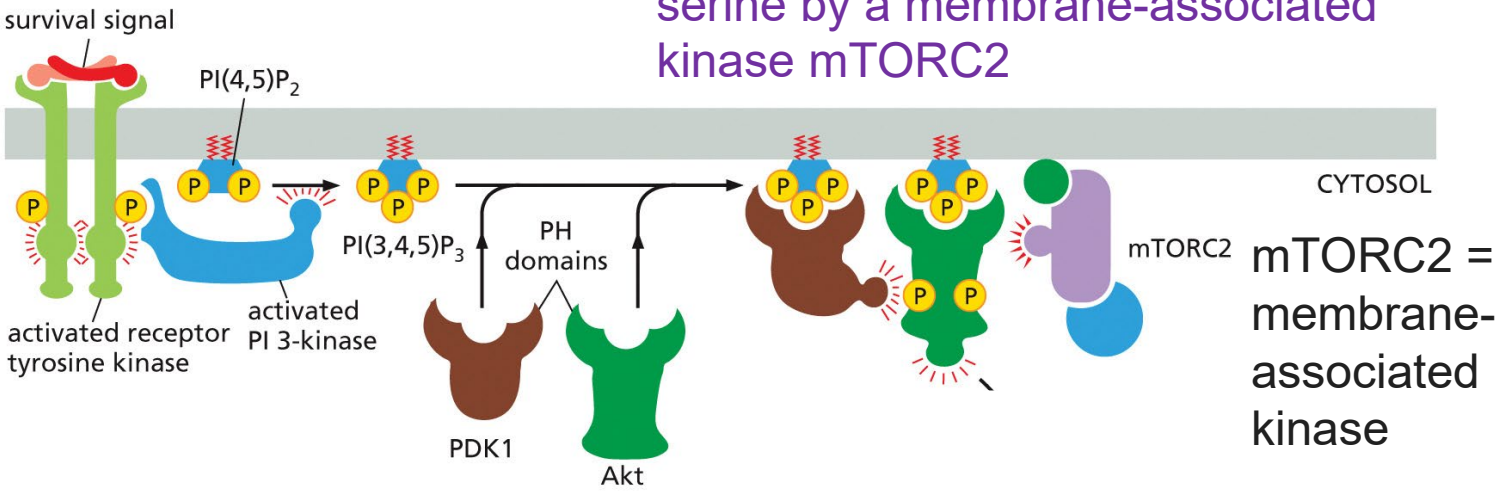


Pleckstrin homology

PI-3-KINASE–AKT SIGNALING PATHWAY

- The PI-3-kinase–Akt signaling pathway stimulates animal cells to survive and grow

5. Akt is phosphorylated on a serine by a membrane-associated kinase mTORC2

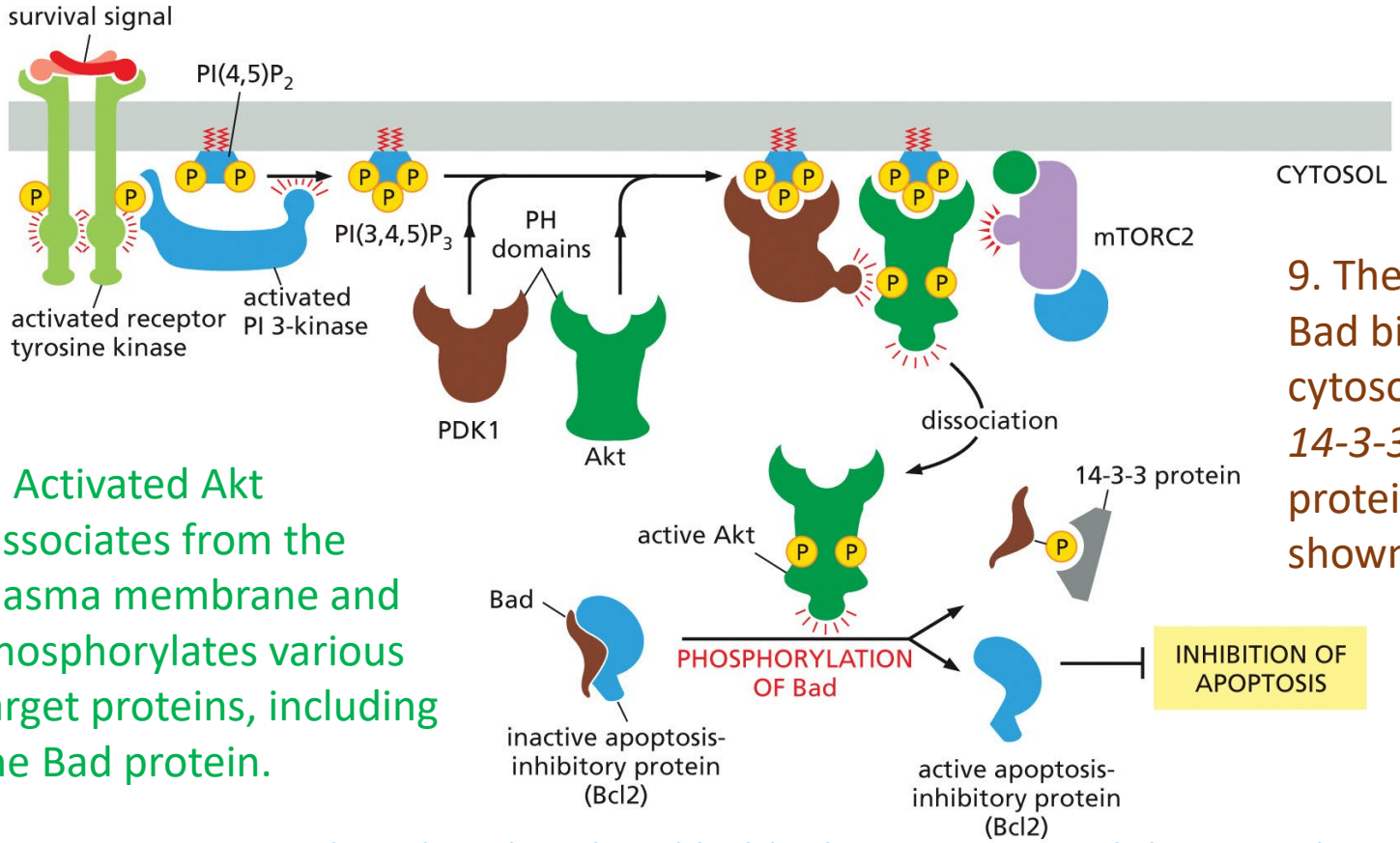


mTORC2 = membrane-associated kinase

6. Phosphorylation alters the conformation of the Akt → can be phosphorylated on a threonine by PDK1 → activates Akt

PI-3-KINASE–AKT SIGNALING PATHWAY

- The PI-3-kinase–Akt signaling pathway stimulates animal cells to survive and grow

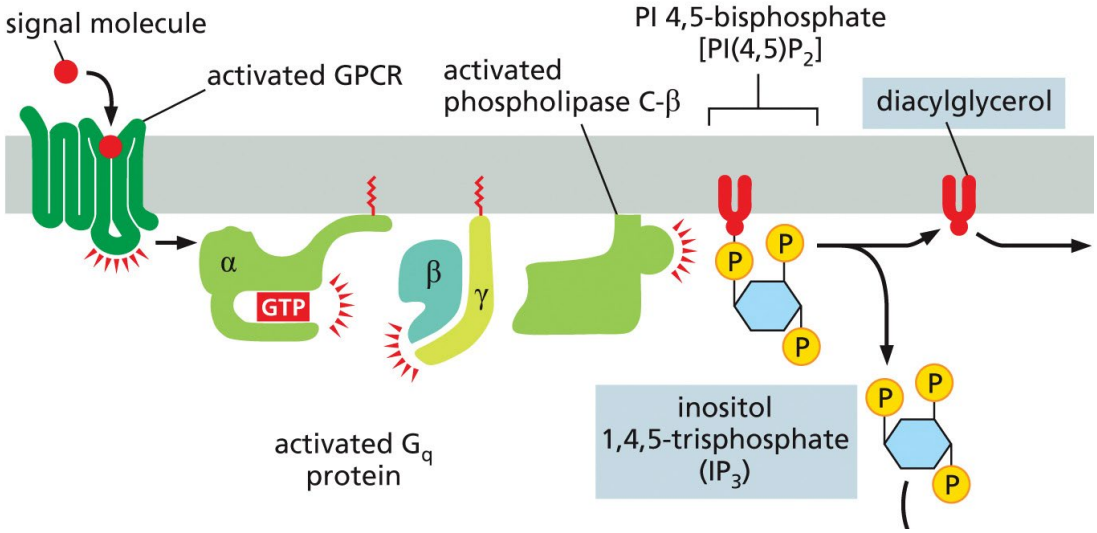


7. Activated Akt dissociates from the plasma membrane and phosphorylates various target proteins, including the Bad protein.

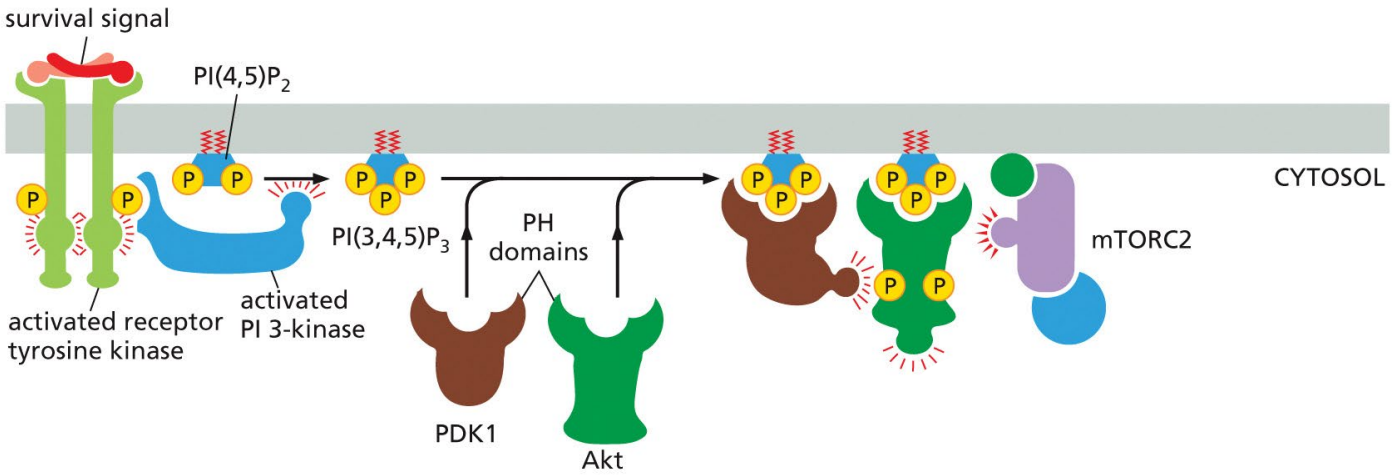
9. The phosphorylated Bad binds to a ubiquitous cytosolic protein called *14-3-3*, which keeps the protein out of action, as shown.

8. Unphosphorylated Bad holds the apoptosis-inhibitory Bcl2 in an inactive state (lecture 12). Phosphorylated, Bad releases Bcl2, which now can block apoptosis and thereby promote cell survival.

Two mechanisms for PIPs to take part in signaling

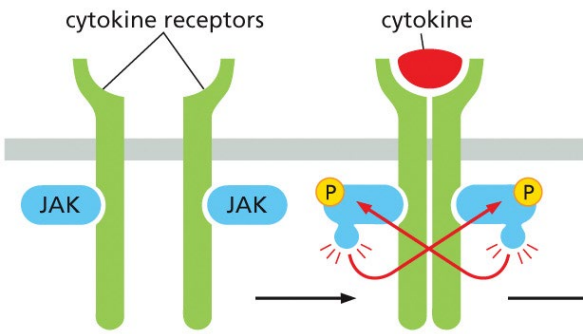


Activated PLC hydrolyzes $PI(4,5)P_2$ → Inositol 1,4,5-trisphosphate (IP_3) → Diacylglycerol



SIGNALING THROUGH ENZYME-COUPLED RECEPTORS

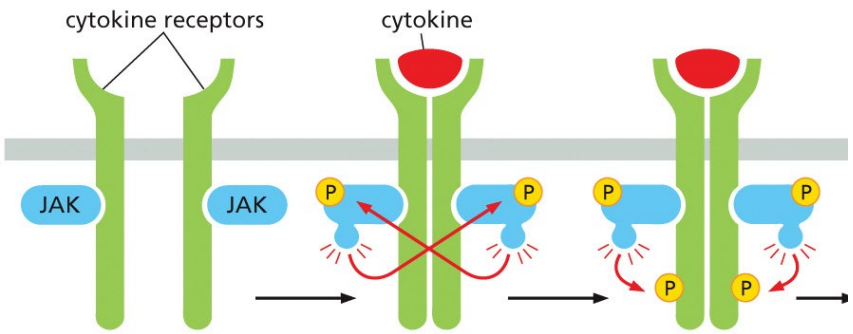
- Cytokine receptors activate the JAK–STAT signaling pathway



1. The binding of the cytokine either causes two separate receptor polypeptide chains to dimerize or re-orients the receptor chains in a preformed dimer

SIGNALING THROUGH ENZYME-COUPLED RECEPTORS

- Cytokine receptors activate the JAK–STAT signaling pathway



2. The associated JAKs are brought together so that they can phosphorylate each other on tyrosines to become fully activated, after which they phosphorylate the receptors to generate binding sites for the SH2 domains of STAT proteins

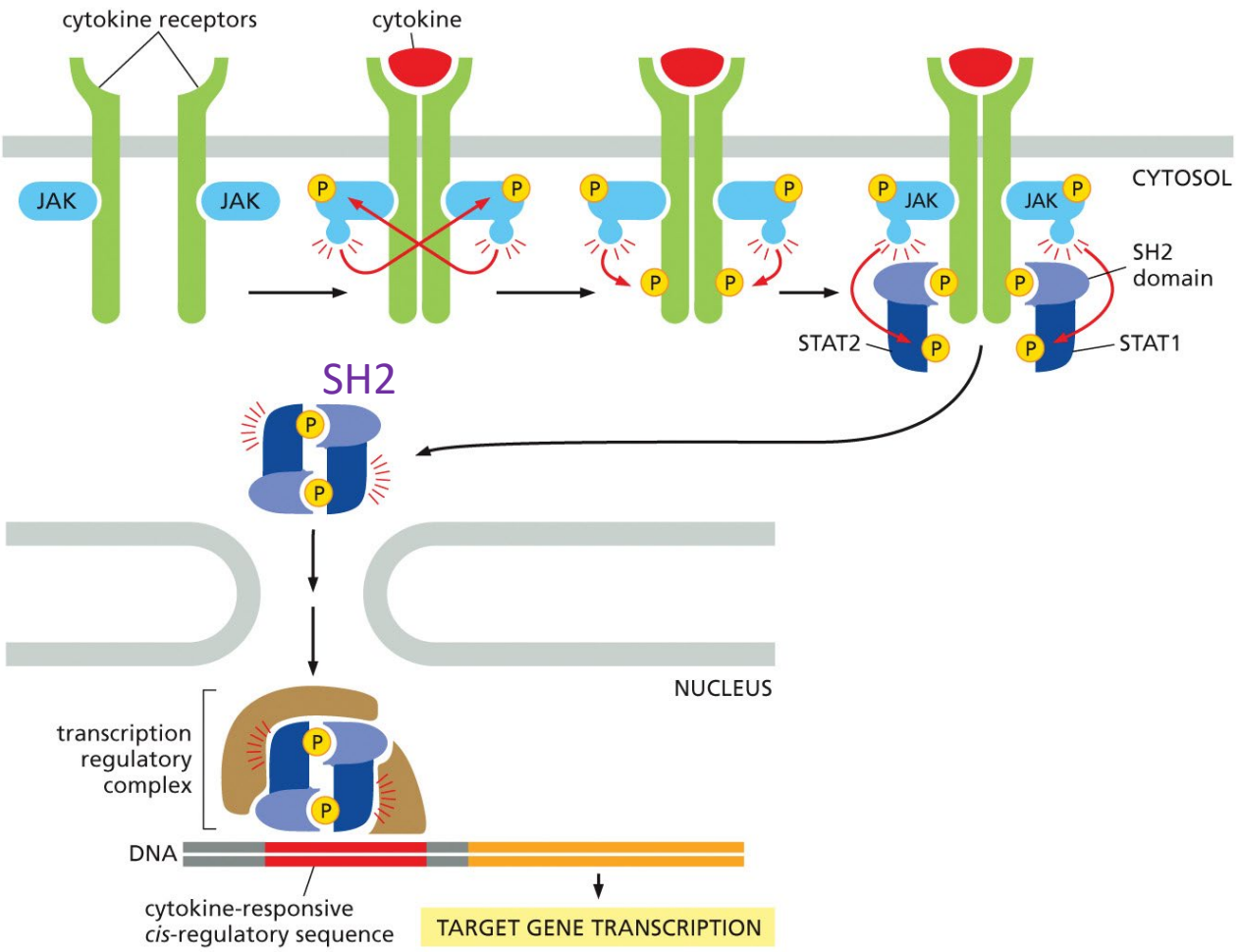
SIGNALING THROUGH ENZYME-COUPLED RECEPTORS

- Cytokine receptors activate the JAK–STAT signaling pathway

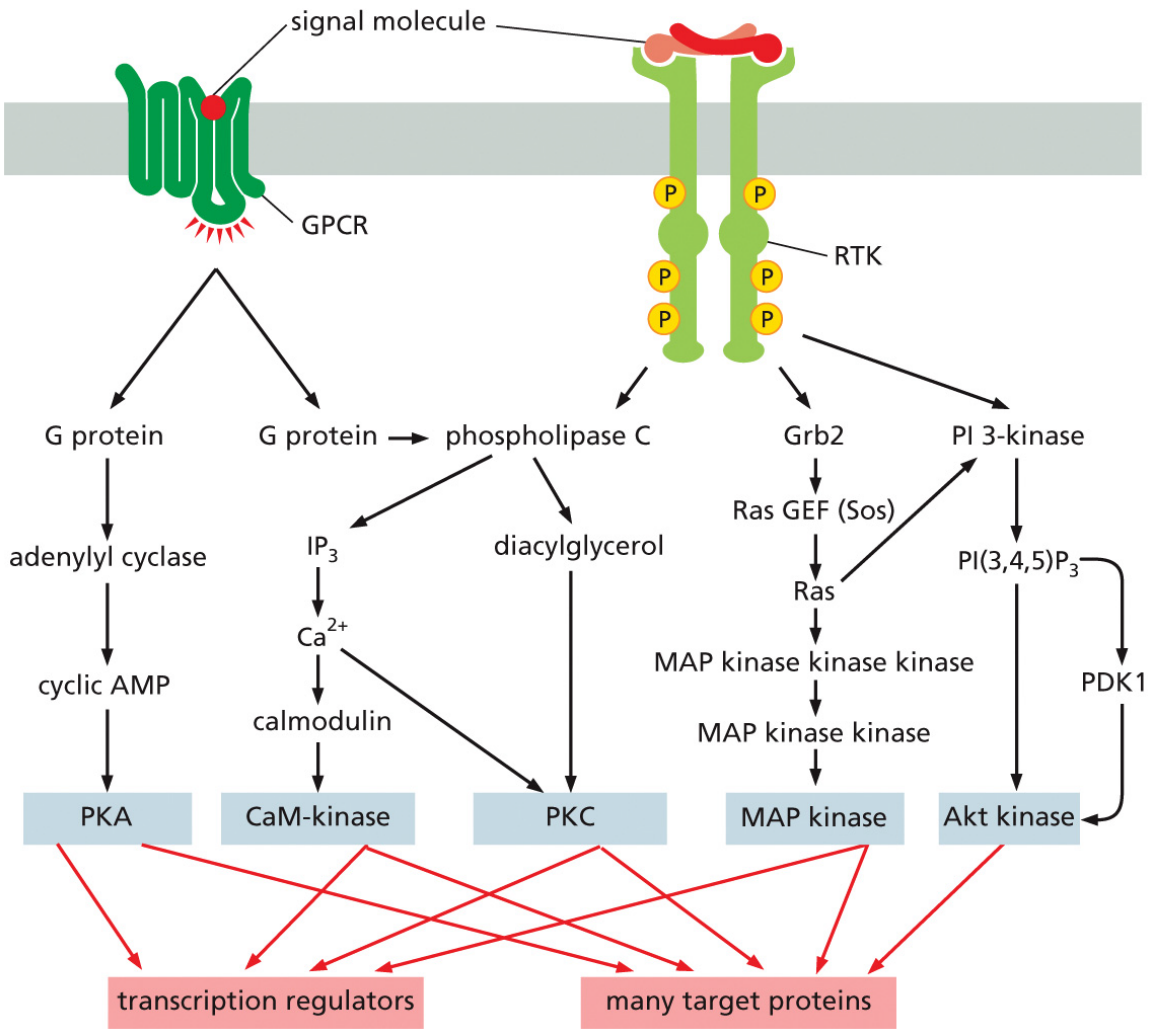
3. JAKs phosphorylate the STAT proteins

4. Phosphorylated STAT proteins dissociate from the receptor to form dimers

5. STAT dimers enter the nucleus to control gene expression.



SUMMARY


signal molecule
GPCR
RTK
P
G protein
G protein
phospholipase C
Grb2
PI 3-kinase
adenylyl cyclase
IP3
diacylglycerol
Ras GEF (Sos)
PI(3,4,5)P3
Ras
Ca2+
cyclic AMP
MAP kinase kinase kinase
PDK1
calmodulin
MAP kinase kinase
PKA
CaM-kinase
PKC
MAP kinase
Akt kinase
transcription regulators
many target proteins