

# Lecture 11 Cell Communication

## Part II

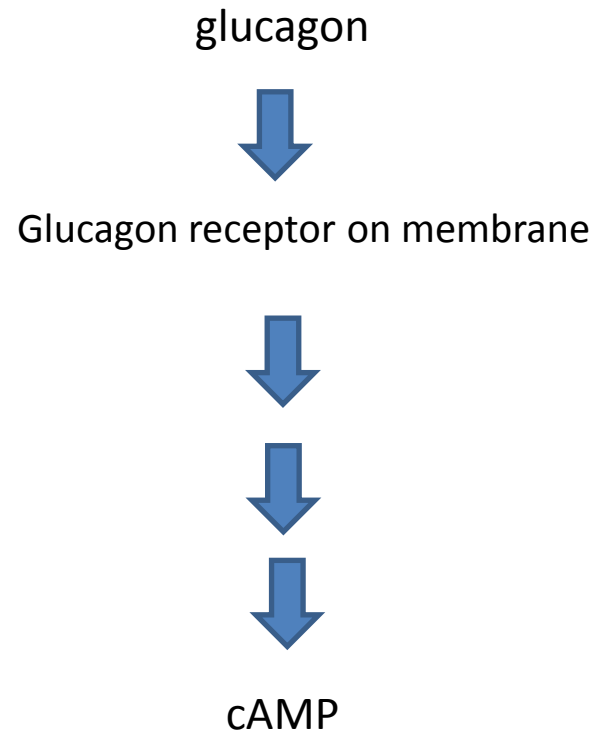
### Outline

- I. G-protein coupled receptor signaling
- II. Enzyme –linked receptor signaling

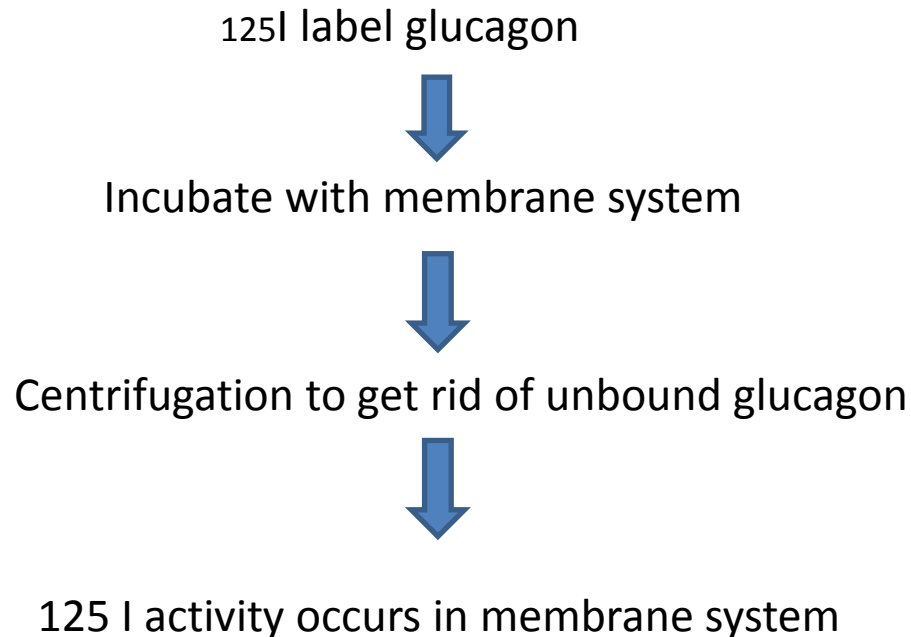
## A brief history

how is GTP found to be required for cAMP  
synthesis ---1971

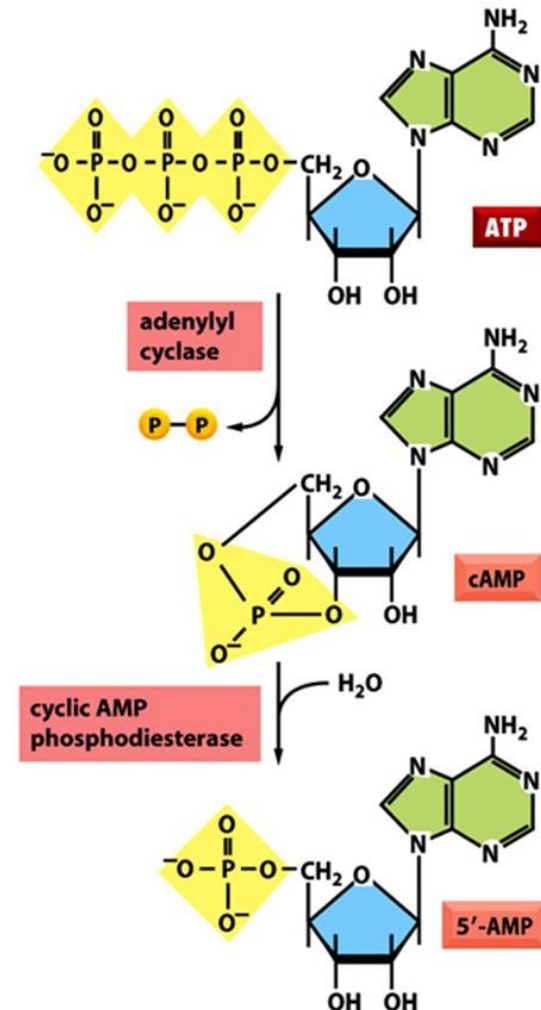
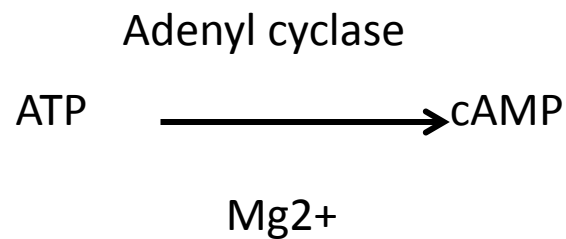
# What did they know at that time?



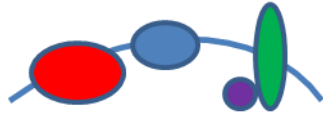
# How did they find glucagon binds to membrane proteins (receptors)?



# They also know how cAMP was produced biochemically



Now they performed this experiment



Purified rat liver membrane system



Add ATP and  $Mg^{2+}$  and glucagon

cAMP can be produced

However, they found that  $^{125}I$  activity on membrane was decreased by 50%



Later they found that glucagon dissociation naturally happens when glucagon trigger cAMP production

## The unique feature of GTP in triggering glucagon dissociation

ATP and **millimolar** of UTP, CTP all decrease glucagon binding to similar levels,

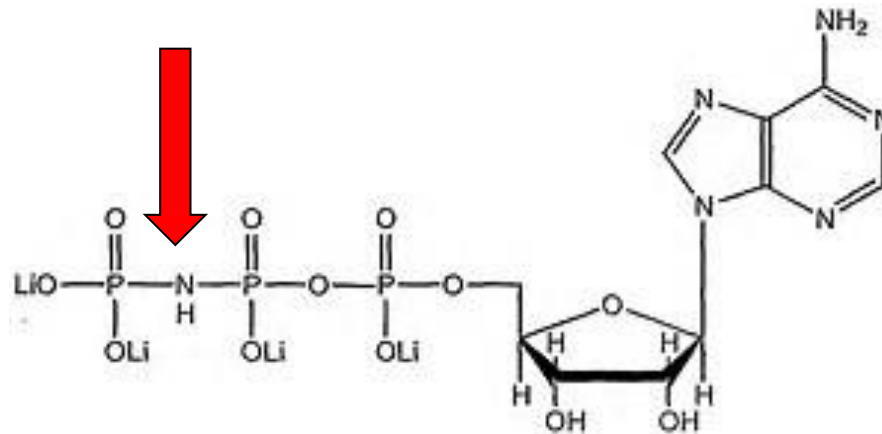


In contrast, **micromolar** GTP decreases glucagon binding.



Suggesting GTP encourages glucagon dissociation

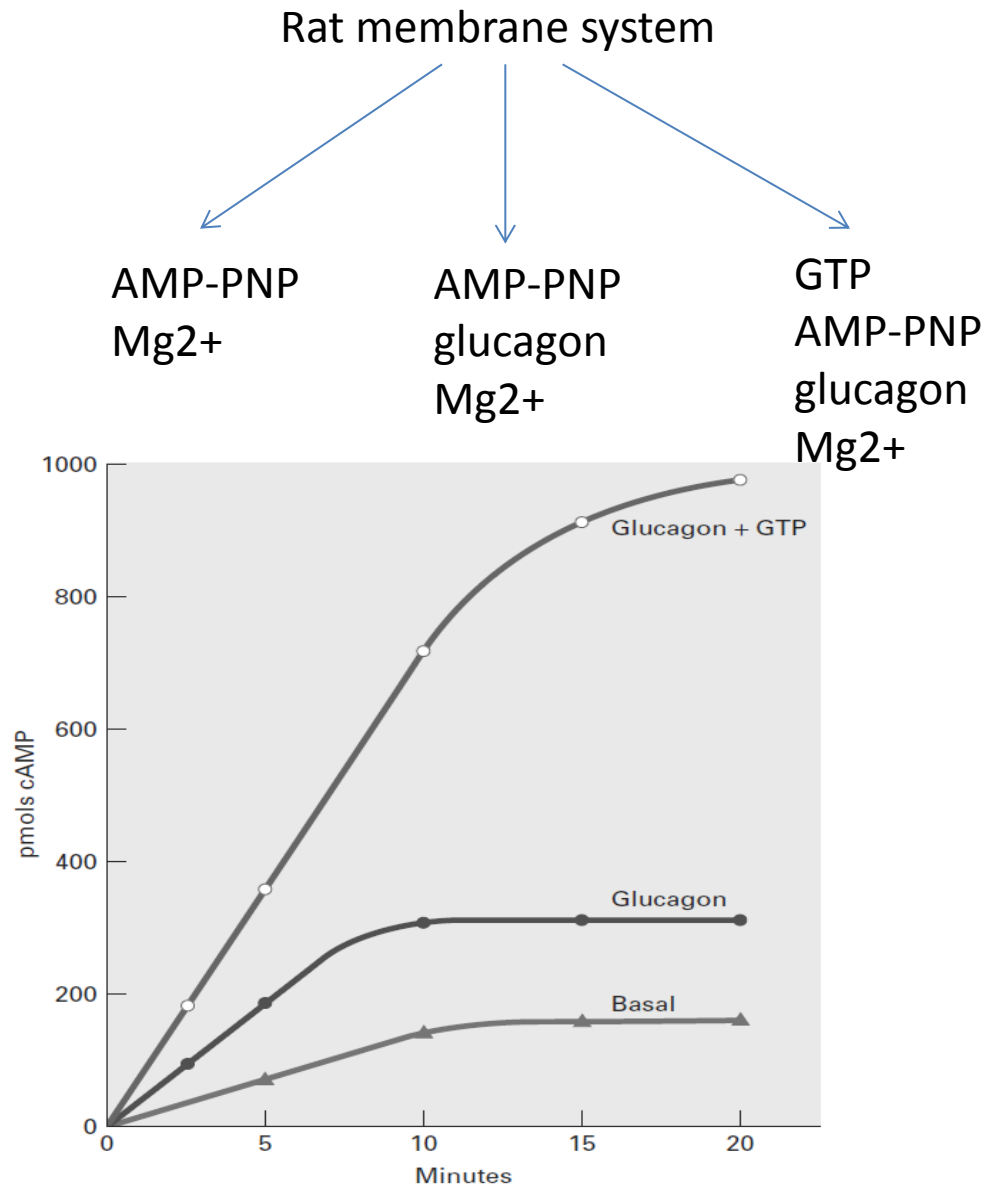
ATP is easily hydrolyzed by ATPase in the membrane system so they used non-hydrolyzable ATP analog:



This will allow the cyclic AMP to be produced in similar way.



## Here is what they eventually found



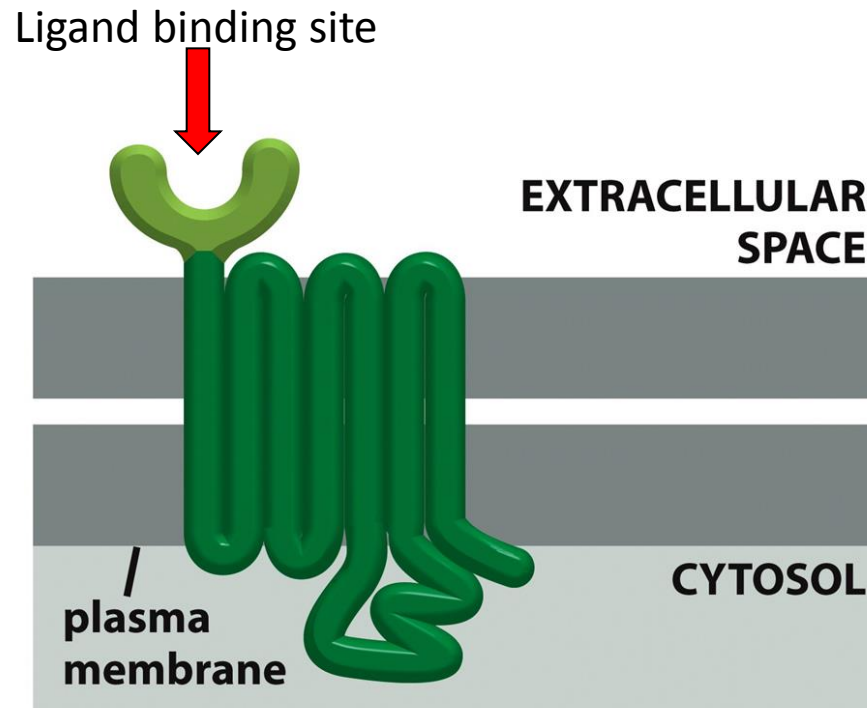
# I. GPCR signaling outline

1. Structure of GPCR and G-proteins
2. Activation of G protein by activated GPCR
3. Major families of G proteins and their function
4. Major effectors mediating G protein signaling
  - 4.1 cAMP
  - 4.2 Phospholipase C- $\beta$
  - 4.3  $\text{Ca}^{2+}$
  - 4.4 cAMP/cGMP gated ion channel in olfactory and vision
5. GPCR desensitization

# I. G-protein coupled receptor (GPCR) signaling overview

- >700 GPCRs in human , the largest cell surface receptor family
- Respond to sight, smell, taste, neurotransmitters, etc.
- ~50% of drugs target GPCR signaling
- ~150 GPCR ligands unknown.
- All GPCRs are 7-pass transmembrane protein and need trimeric GTP-binding protein to relay signals.

# 1. Structure of GPCR



GPCR

One single polypeptide, 7-pass transmembrane  
in a serpentine manner

## G-protein is coupled to GPCR

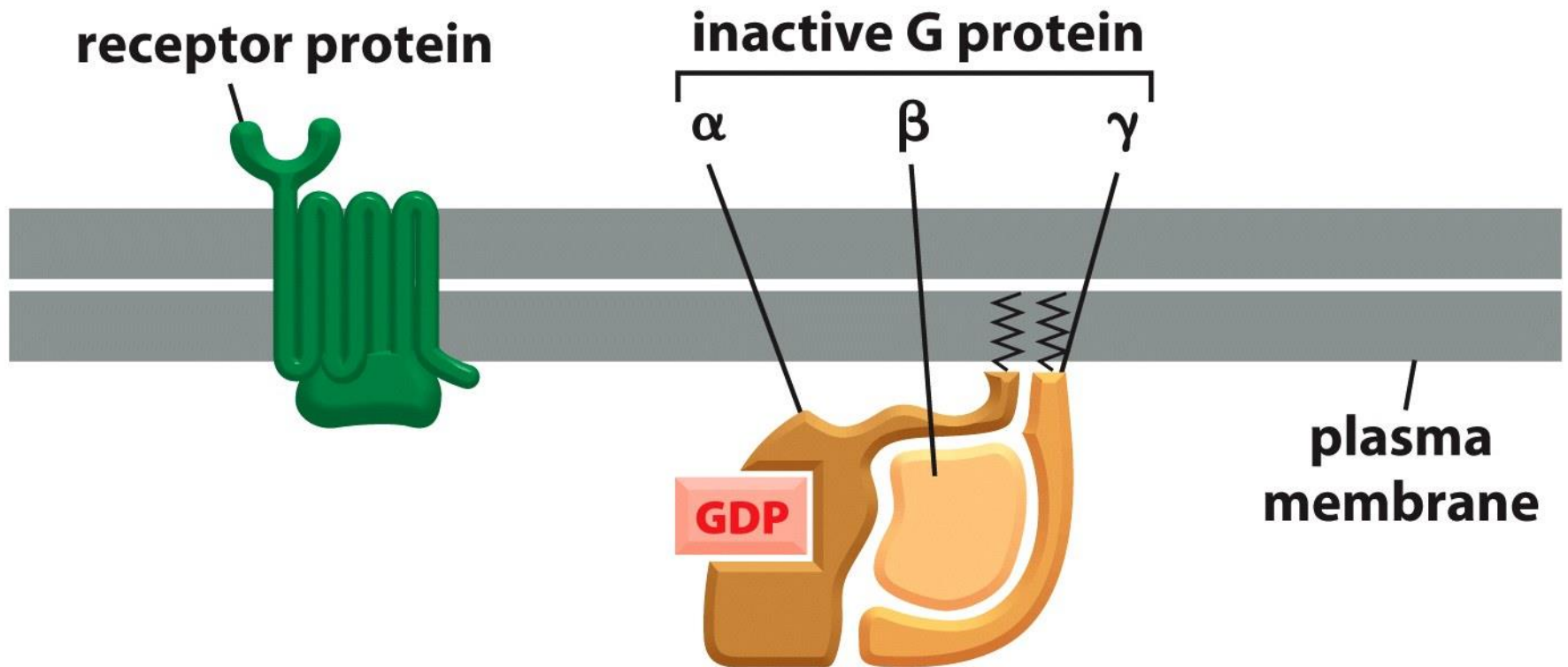
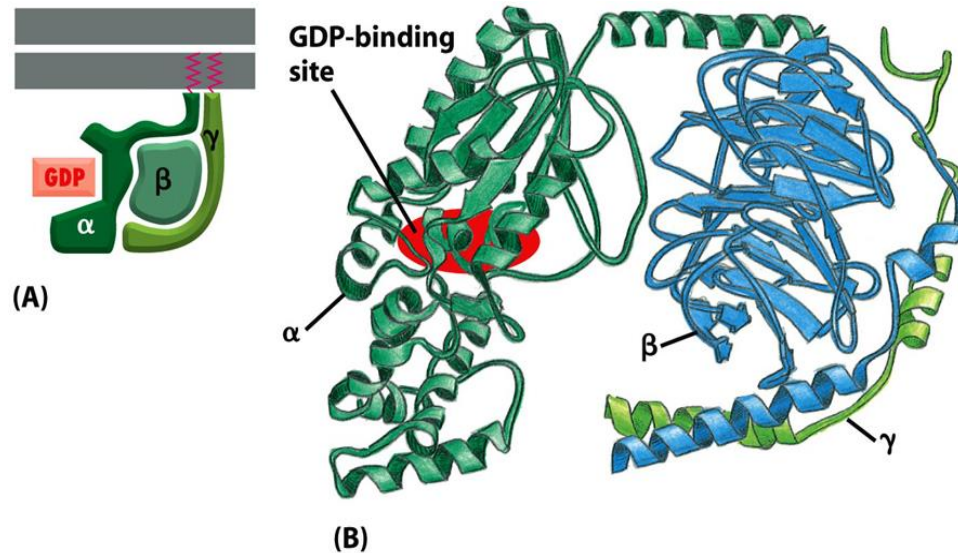


Figure 16-17a Essential Cell Biology 3/e (© Garland Science 2010)

# Trimeric G-protein



Subunits  $\alpha$  and  $\gamma$  are tethered to membrane  
Subunit  $\alpha$  binds to GTP/GDP and has GTPase Activity.

## 2. Activation of a G protein by an activated GPCR

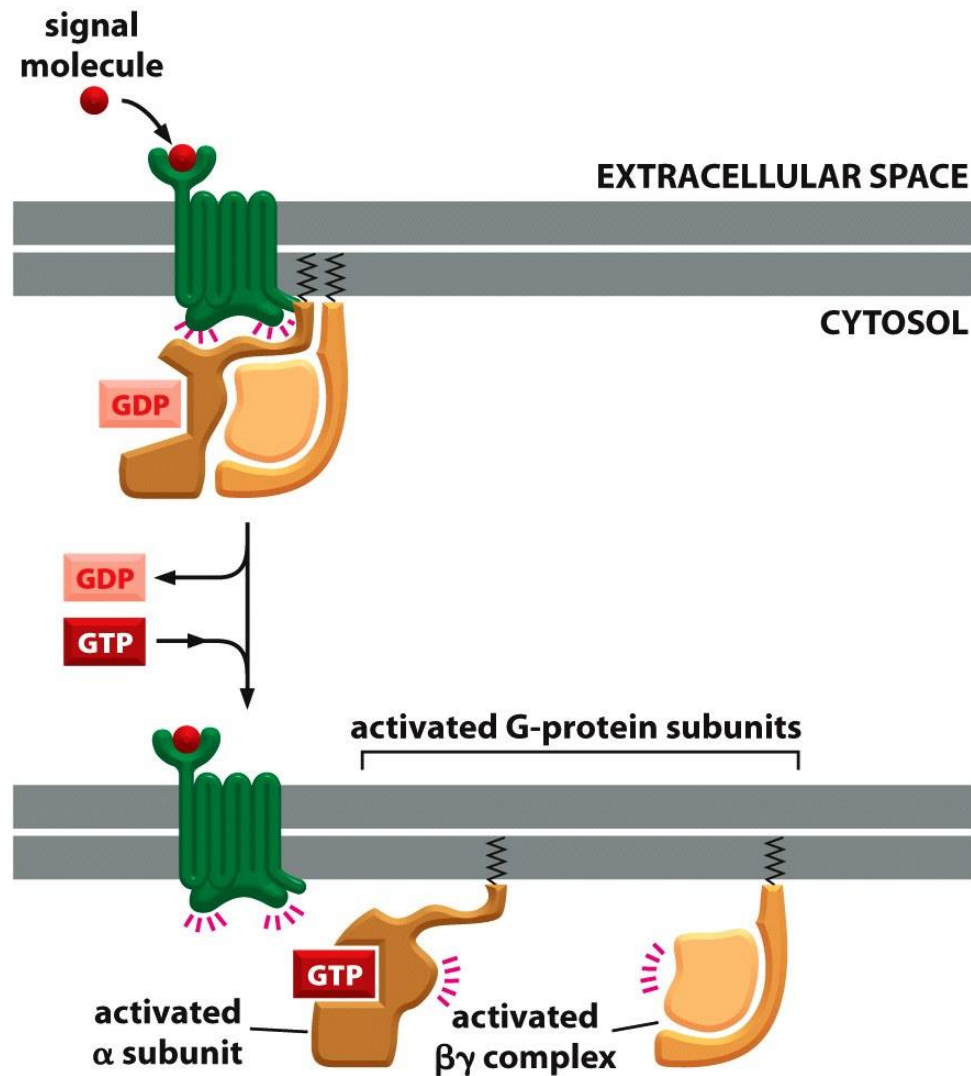


Figure 16-17b Essential Cell Biology 3/e (© Garland Science 2010)

# G protein can be deactivated by GTP hydrolysis

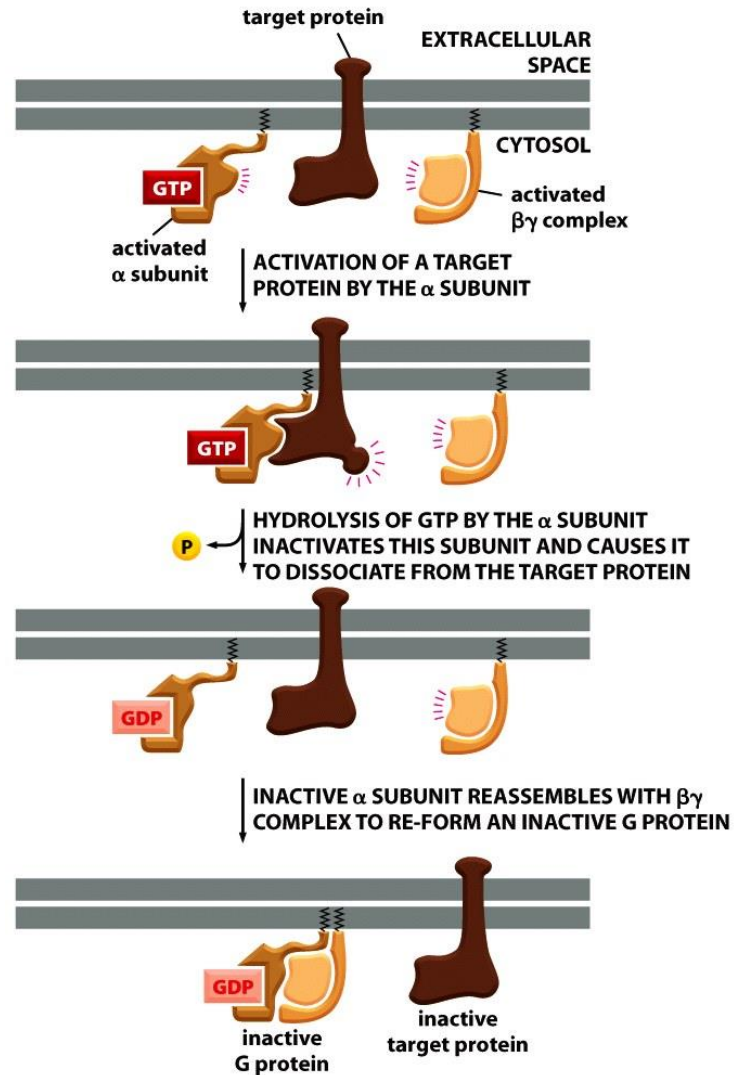


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## GPCR signaling

Ligand binding activates GPCR, which acts as an GEF to displace GDP and activate G protein;

Exposing functional groups in activated  $\alpha$  subunit and  $\beta\gamma$  complex.

Subunit  $\alpha$  then acts as GTPase to hydrolyze GTP into GDP, inactivating G-protein;

Or RGS( regulator of G protein signaling) acts as  $\alpha$ -Subunit specific GAPs to cause GTP hydrolysis.



15.3-G-protein\_signaling.mov

### 3. Four major families of trimeric G proteins

**Table 15–3 Four Major Families of Trimeric G Proteins\***

FAMILY	SOME FAMILY MEMBERS	SUBUNITS THAT MEDIATE ACTION	SOME FUNCTIONS
I	G <sub>s</sub>	α	activates adenylyl cyclase; activates Ca <sup>2+</sup> channels
	G <sub>olf</sub>	α	activates adenylyl cyclase in olfactory sensory neurons
II	G <sub>i</sub>	α	inhibits adenylyl cyclase
		βγ	activates K <sup>+</sup> channels
	G <sub>o</sub>	βγ	activates K <sup>+</sup> channels; inactivates Ca <sup>2+</sup> channels
		α and βγ	activates phospholipase C-β
	G <sub>t</sub> (transducin)	α	activates cyclic GMP phosphodiesterase in vertebrate rod photoreceptors
III	G <sub>q</sub>	α	activates phospholipase C-β
IV	G <sub>12/13</sub>	α	activates Rho family monomeric GTPases (via Rho-GEF) to regulate the actin cytoskeleton

\*Families are determined by amino acid sequence relatedness of the α subunits. Only selected examples are included. About 20 α subunits and at least 6 β subunits and 11 γ subunits have been described in humans.

# How does GPCR signaling activate K<sup>+</sup> channel?

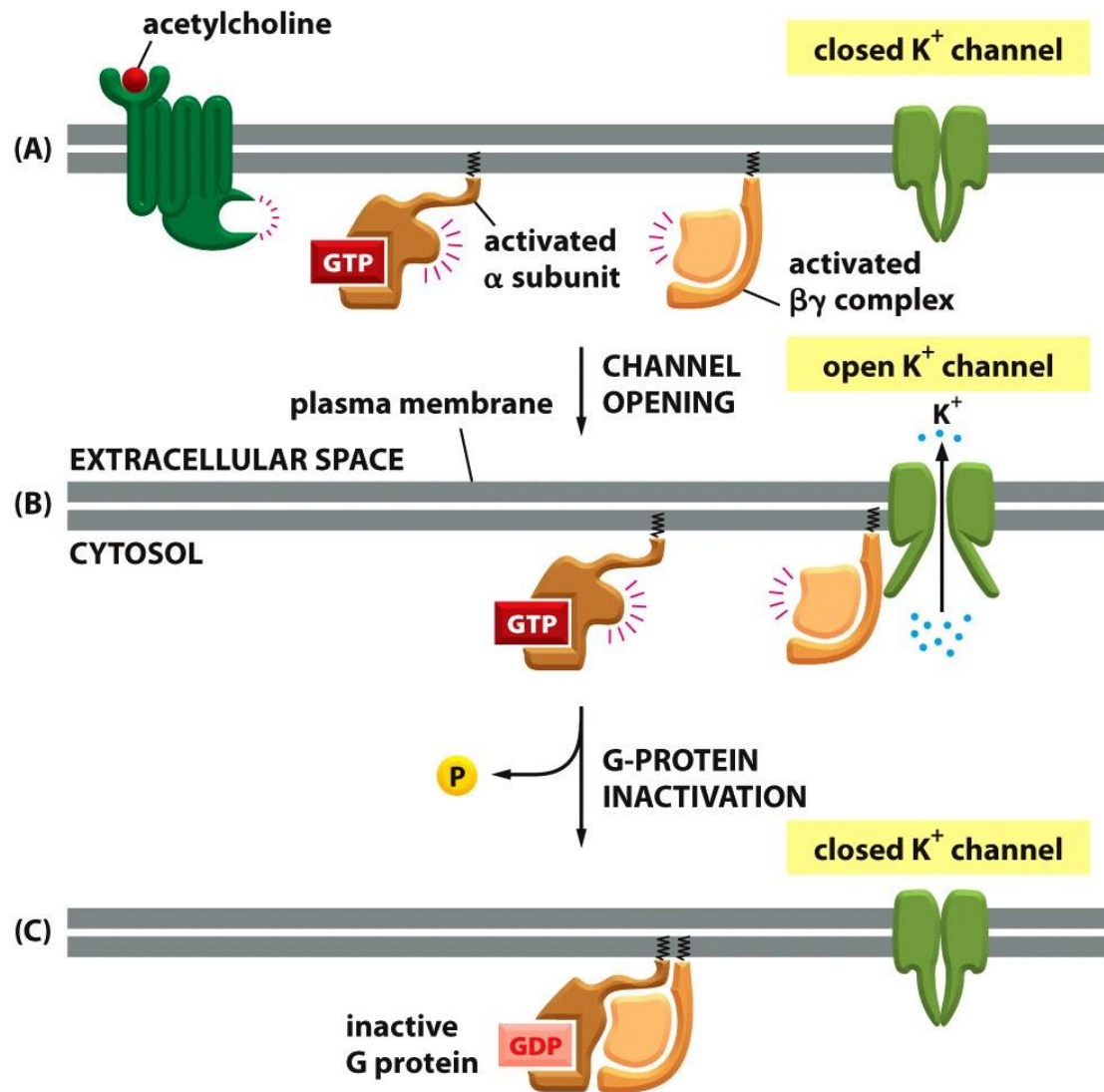


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## 4. How does GPCR signaling activates small messenger molecules?

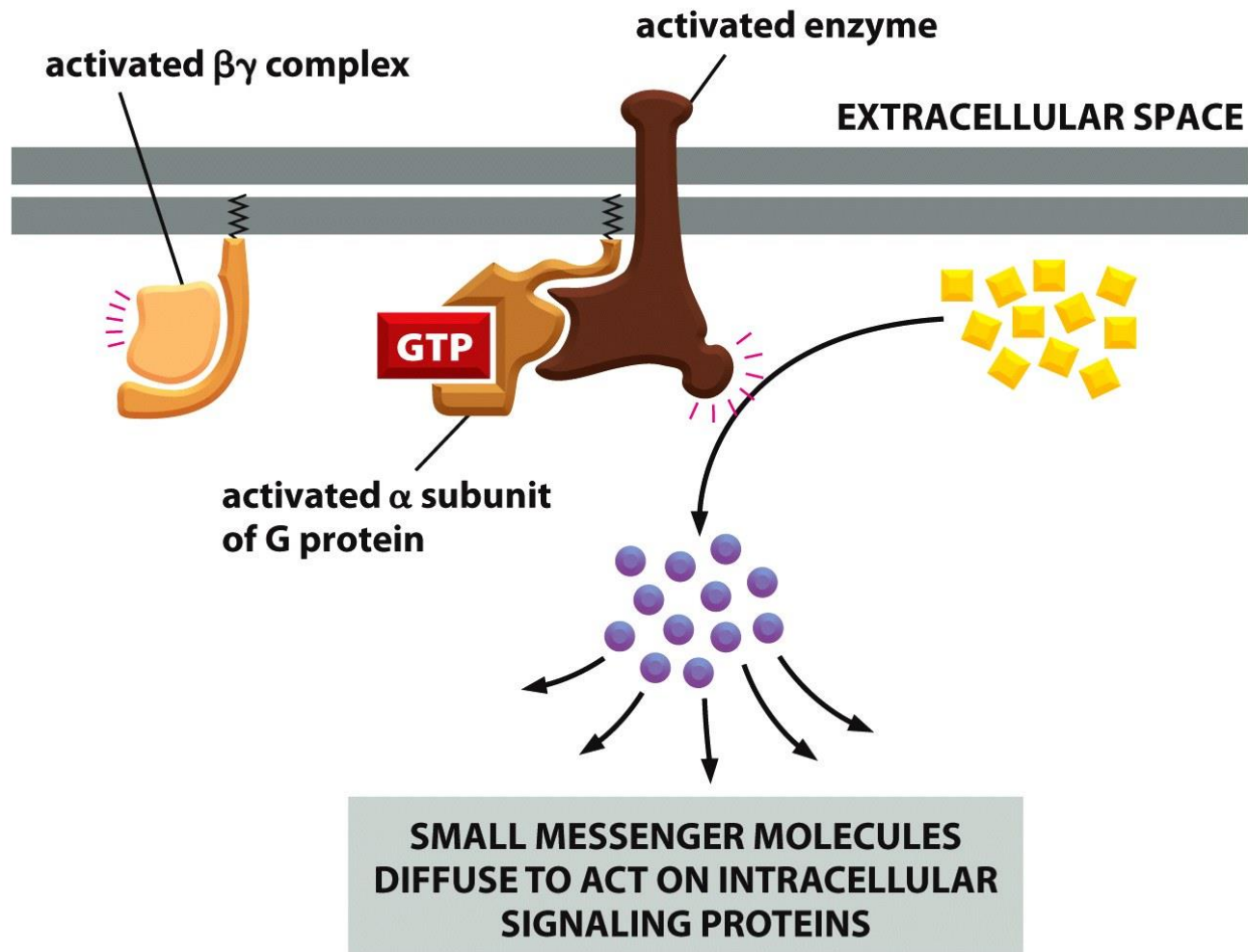


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## Small messenger molecules downstream of GPCR signaling

- ♥ cAMP

- ♥ DAG and IP3 through phospholipase C- $\beta$

- ♥ Ca<sup>2+</sup>

- ♥ cGMP/cAMP-gated ion channels in smell and vision

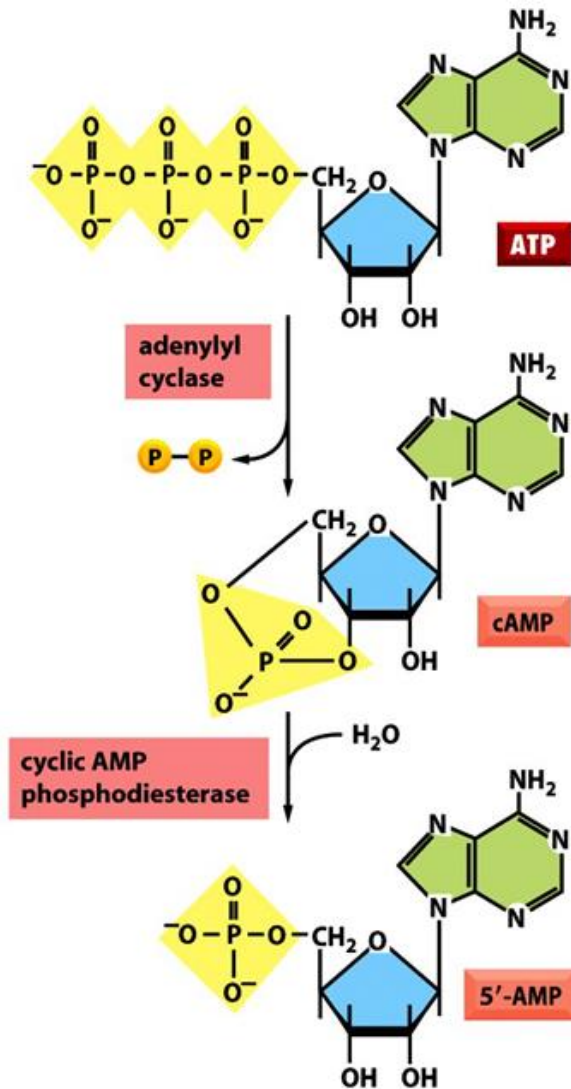
## 4.1 How does GPCR activates cAMP?

**Table 15–3 Four Major Families of Trimeric G Proteins\***

FAMILY	SOME FAMILY MEMBERS	SUBUNITS THAT MEDIATE ACTION	SOME FUNCTIONS
I	$G_s$	$\alpha$	activates adenylyl cyclase; activates $Ca^{2+}$ channels
	$G_{olf}$	$\alpha$	activates adenylyl cyclase in olfactory sensory neurons
II	$G_i$	$\alpha$	inhibits adenylyl cyclase
		$\beta\gamma$	activates $K^+$ channels
	$G_o$	$\beta\gamma$	activates $K^+$ channels; inactivates $Ca^{2+}$ channels
		$\alpha$ and $\beta\gamma$	activates phospholipase C- $\beta$
	$G_t$ (transducin)	$\alpha$	activates cyclic GMP phosphodiesterase in vertebrate rod photoreceptors
III	$G_q$	$\alpha$	activates phospholipase C- $\beta$
IV	$G_{12/13}$	$\alpha$	activates Rho family monomeric GTPases (via Rho-GEF) to regulate the actin cytoskeleton

\*Families are determined by amino acid sequence relatedness of the  $\alpha$  subunits. Only selected examples are included. About 20  $\alpha$  subunits and at least 6  $\beta$  subunits and 11  $\gamma$  subunits have been described in humans.

# cAMP as an important second messenger



1. cAMP levels are balanced by **adenylyl cyclase** and **Cyclic AMP phosphodiesterase**.
2. Adenylyl cyclase is a plasma-membrane bound Enzyme.
3. Two types of G proteins: **G<sub>s</sub>** ( stimulatory G protein) activates adenylyl cyclase; while **G<sub>i</sub>** ( inhibitory G protein) Inhibits adenylyl cyclase.



# Neuron cells senses serotonin to produce cAMP instantly

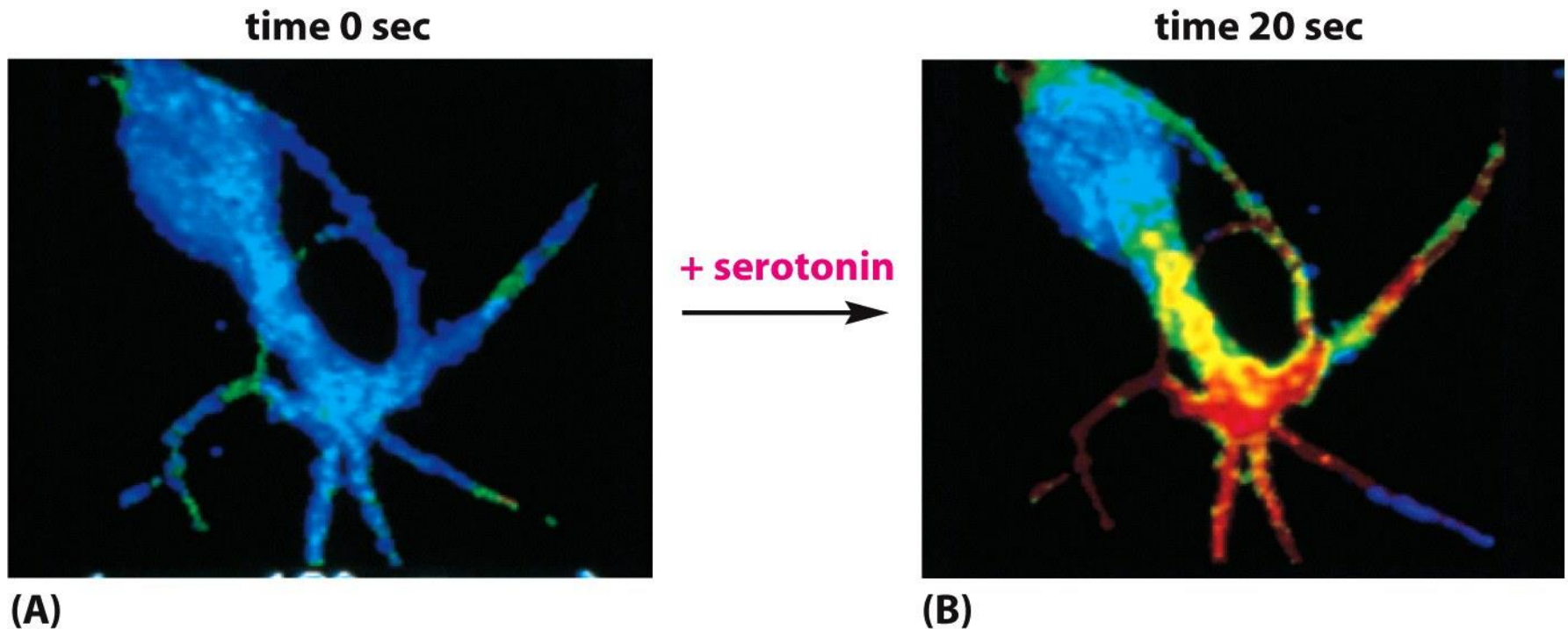
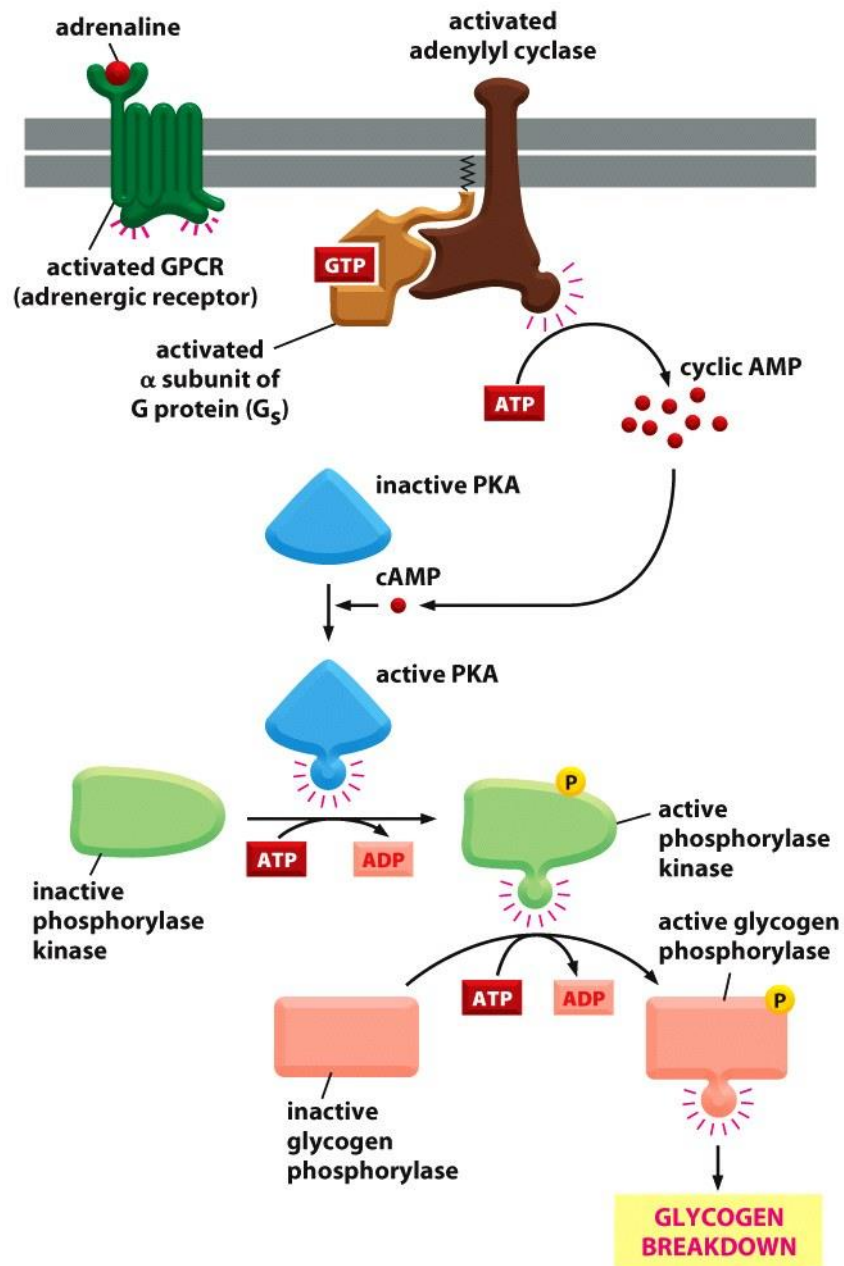


Figure 16-22 Essential Cell Biology 3/e (© Garland Science 2010)



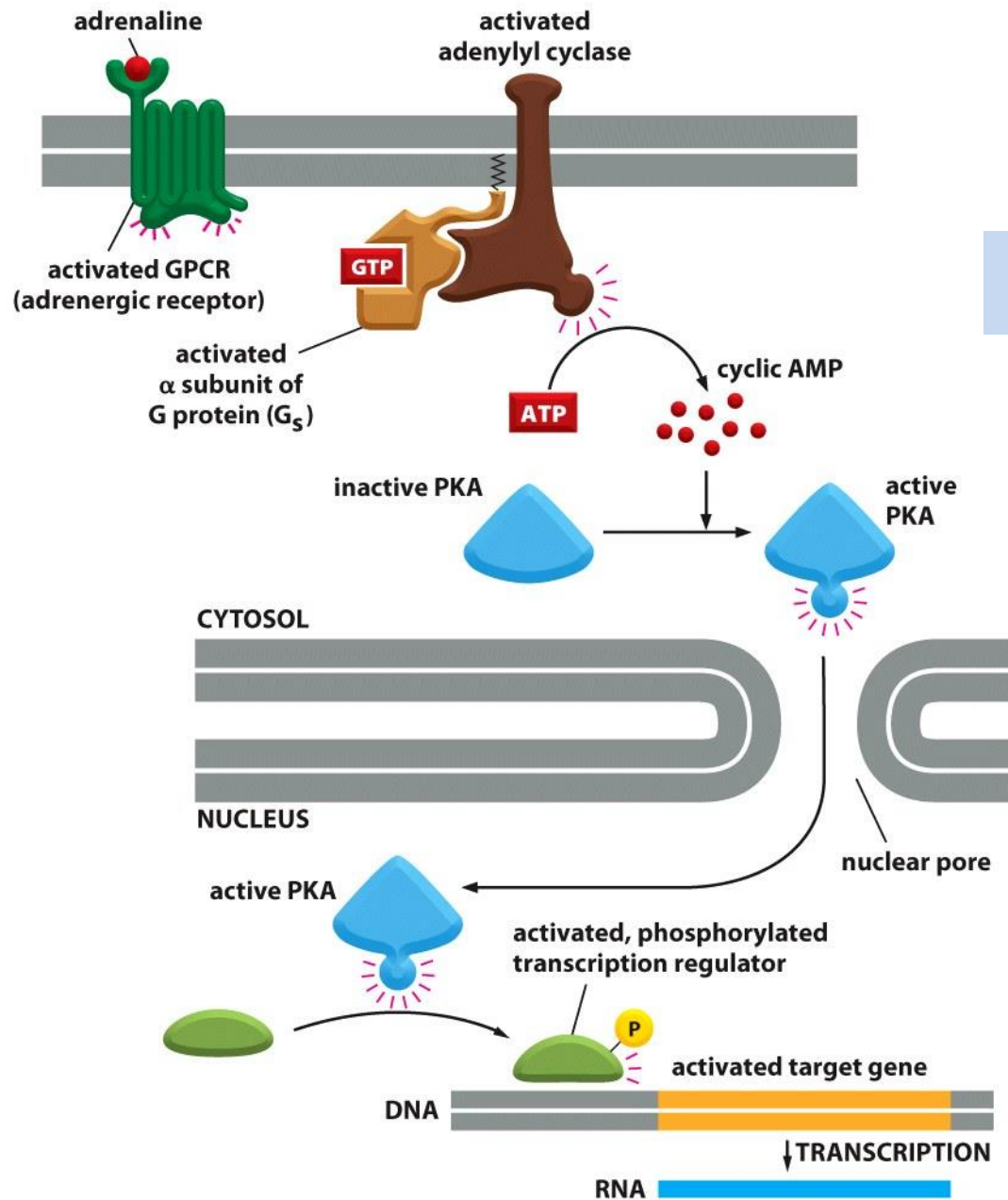
## PKA (cAMP-dependent protein kinase) mediates cAMP signaling

- PKA is a serine/Threonine protein kinase, through phosphorylation on substrates, PKA mediates GPCR signaling **in a fast manner**
- PKA also phosphorylates CREB (CRE-binding protein), which then recruits CBP (CREB-binding protein) and activates gene transcription **in a slow manner**



PKA functions in fast manner

Figure 16-23 Essential Cell Biology 3/e (© Garland Science 2010)



PKA works in slow manner

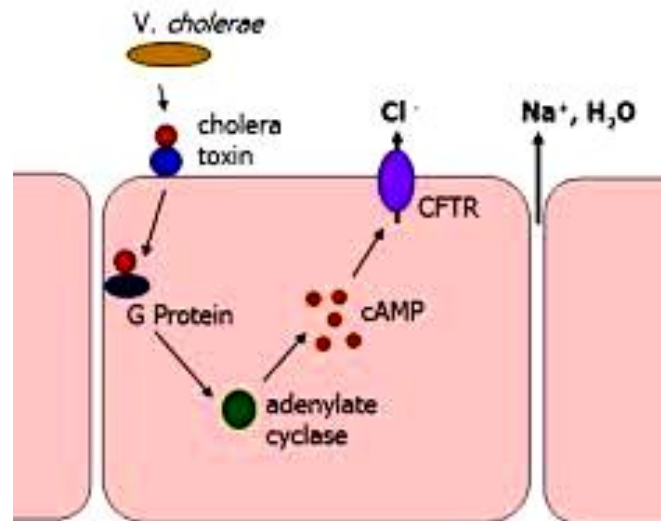


15.4-cAMP\_signaling.mov

Figure 16-24 Essential Cell Biology 3/e (© Garland Science 2010)

# Mechanism of cholera toxin

- Catalyze the transfer to ADP ribose from intracellular NAD<sup>+</sup> to the  $\alpha$  subunit of Gs.
- ADP ribosylation alters the  $\alpha$  subunit so that it can no longer hydrolyze its bound GTP, causing it to remain in an active state
- This stimulates adenyl cyclase indefinitely.
- The prolonged elevation in cAMP concentration causes influx of Cl<sup>-</sup> and water from intestinal epithelial cells into the gut , thereby causing the severe diarrhea that characterizes cholera.



## Mechanism of pertussis toxin

- Catalyze the ADP ribosylation of  $\alpha$  subunit of  $G_i$ .
- Lock G protein in GDP (inactive) state.
- Leads to increase in mucus secretion in the lung.
- Syndrome: whooping cough

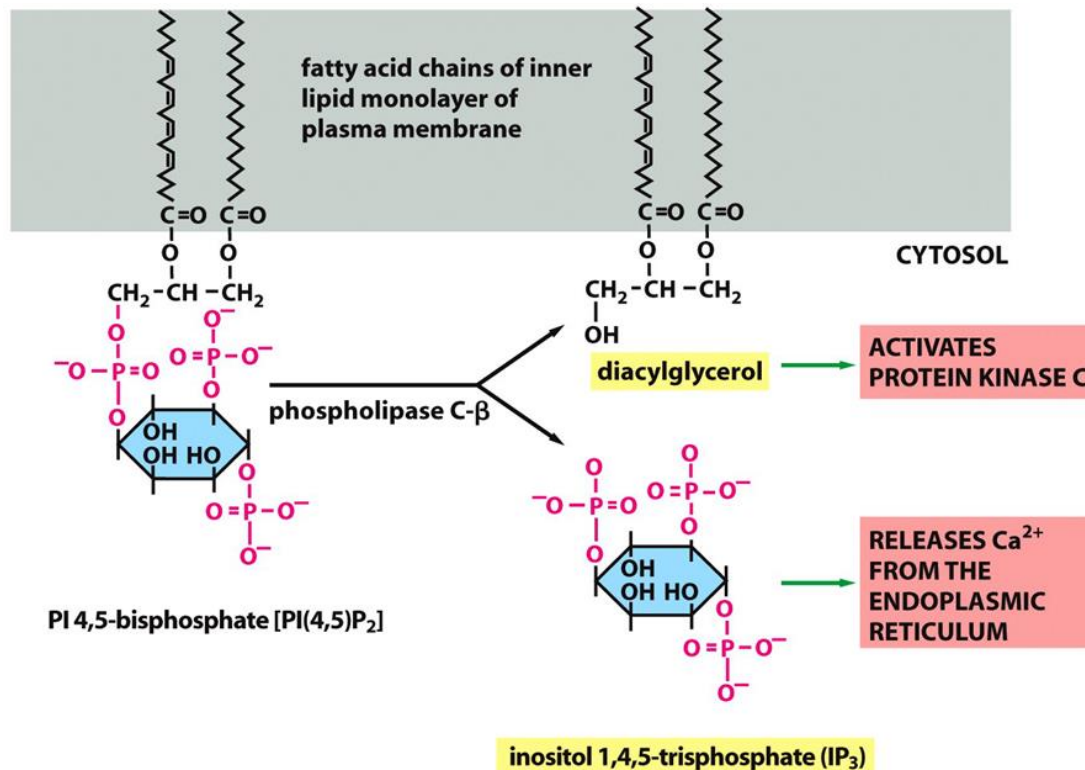
## 4.2. Signaling through phospholipase C- $\beta$

- **Gq** proteins activate phospholipase C- $\beta$ .
- Substrates for phospholipase C- $\beta$  is PI(4,5)P<sub>2</sub>
- Cleavage of PIP<sub>2</sub> results in production of IP<sub>3</sub> and diacylglycerol (DAG)

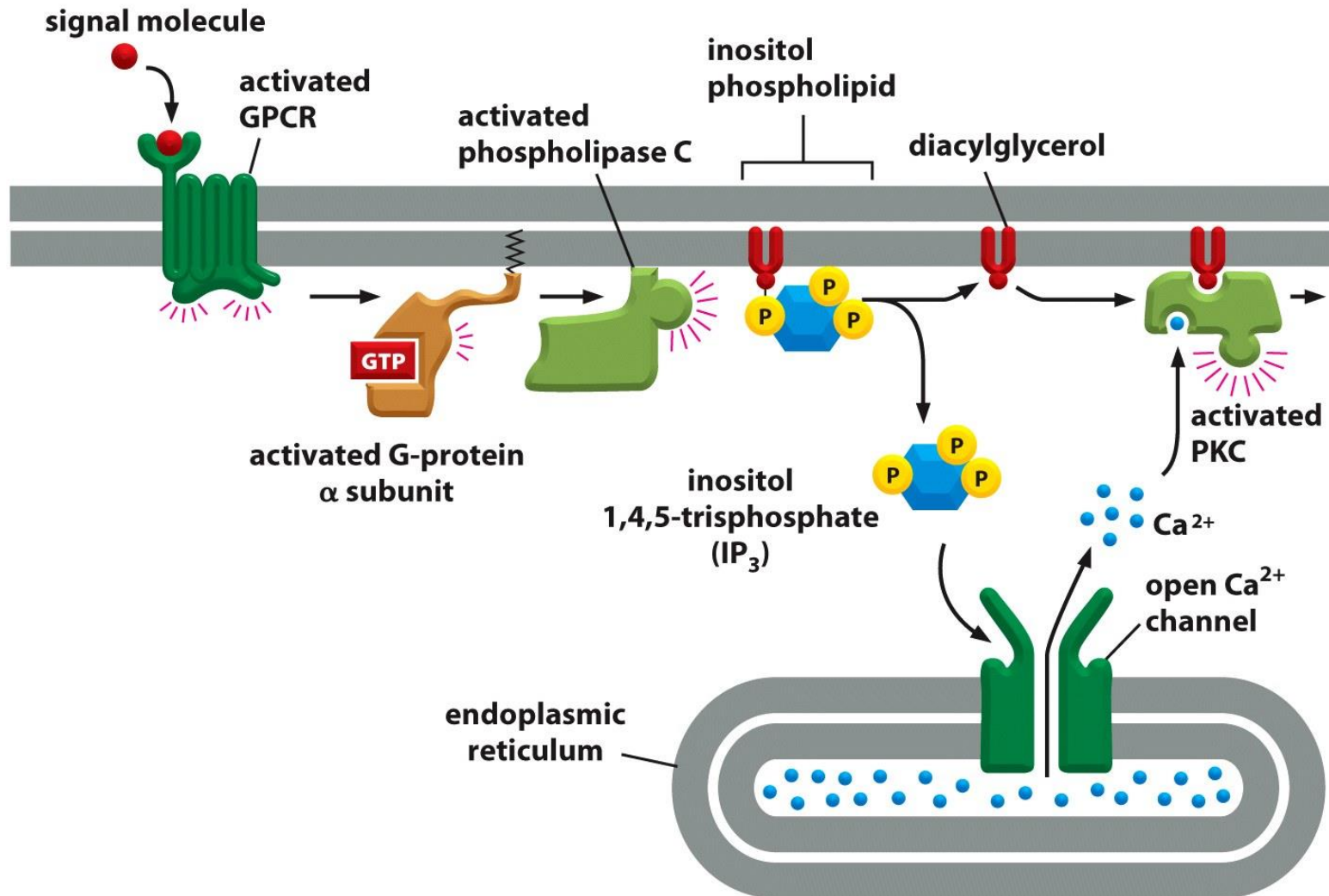
**Table 15–2 Some Cell Responses in Which GPCRs Activate PLC $\beta$**

TARGET TISSUE	SIGNAL MOLECULE	MAJOR RESPONSE
Liver	vasopressin	glycogen breakdown
Pancreas	acetylcholine	amylase secretion
Smooth muscle	acetylcholine	muscle contraction
Blood platelets	thrombin	platelet aggregation

# Production and action of second messengers by phospholipase C- $\beta$



## 4.3. How does GPCR trigger $\text{Ca}^{2+}$ release and PKC activation?





## Ca<sup>2+</sup> function

- Rise in Ca<sup>2+</sup> in fertilized egg cytosol initiates embryonic development
- Triggers muscle contraction
- Triggers secretory vesicle to secrete

Fertilized egg show waves of Ca<sup>2+</sup> from the site of sperm entry

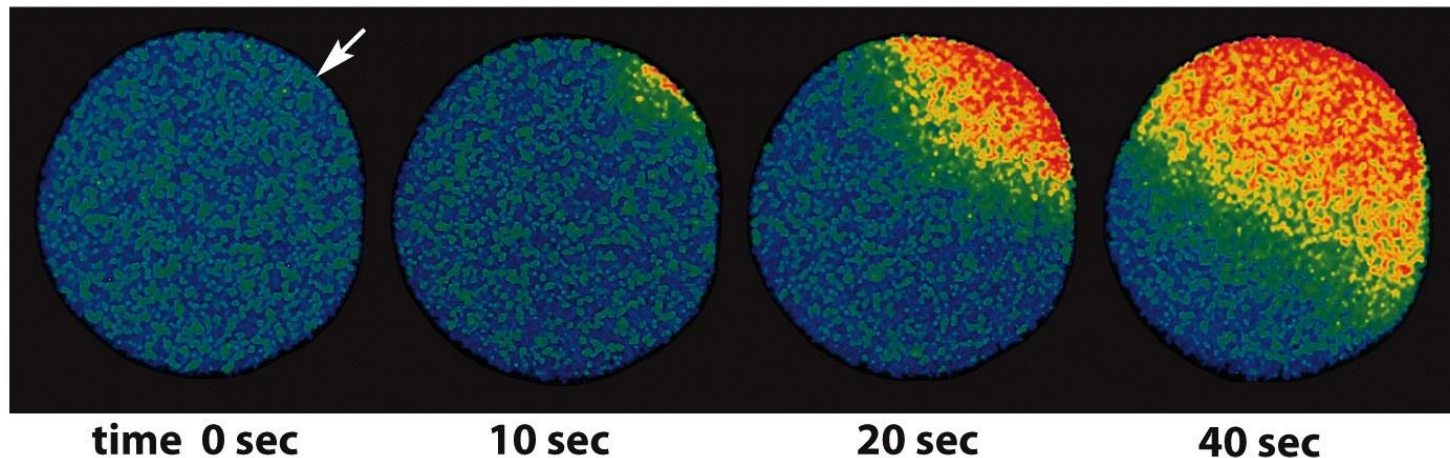
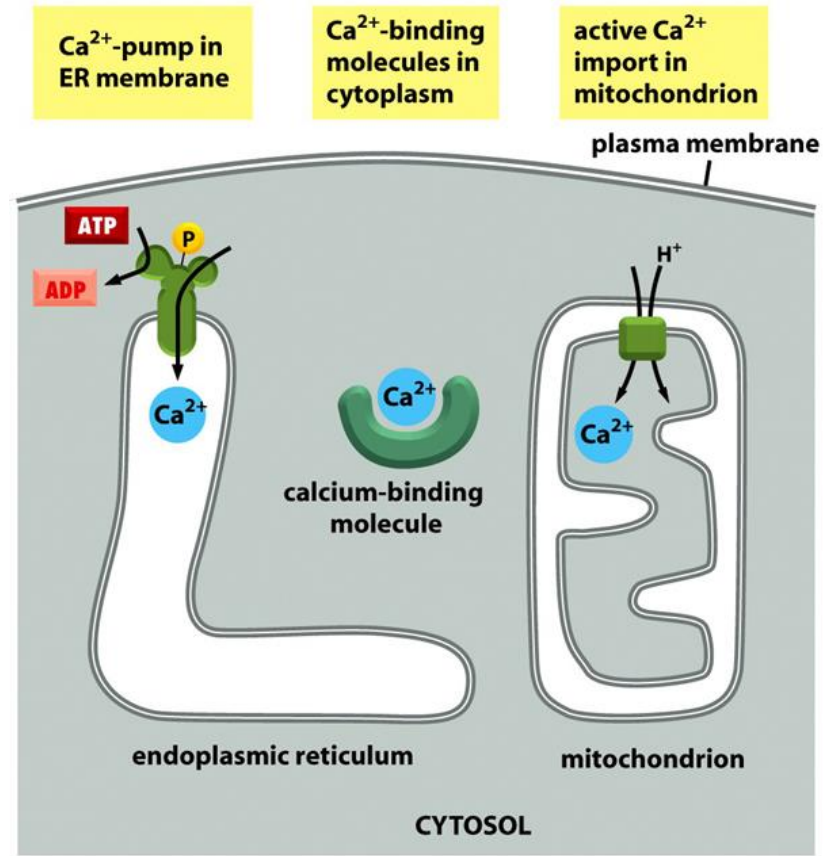
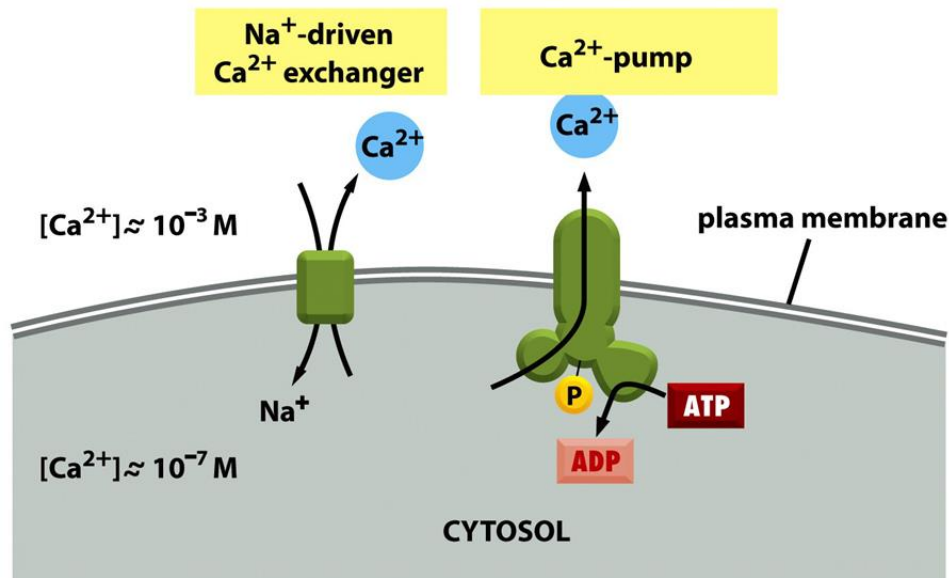


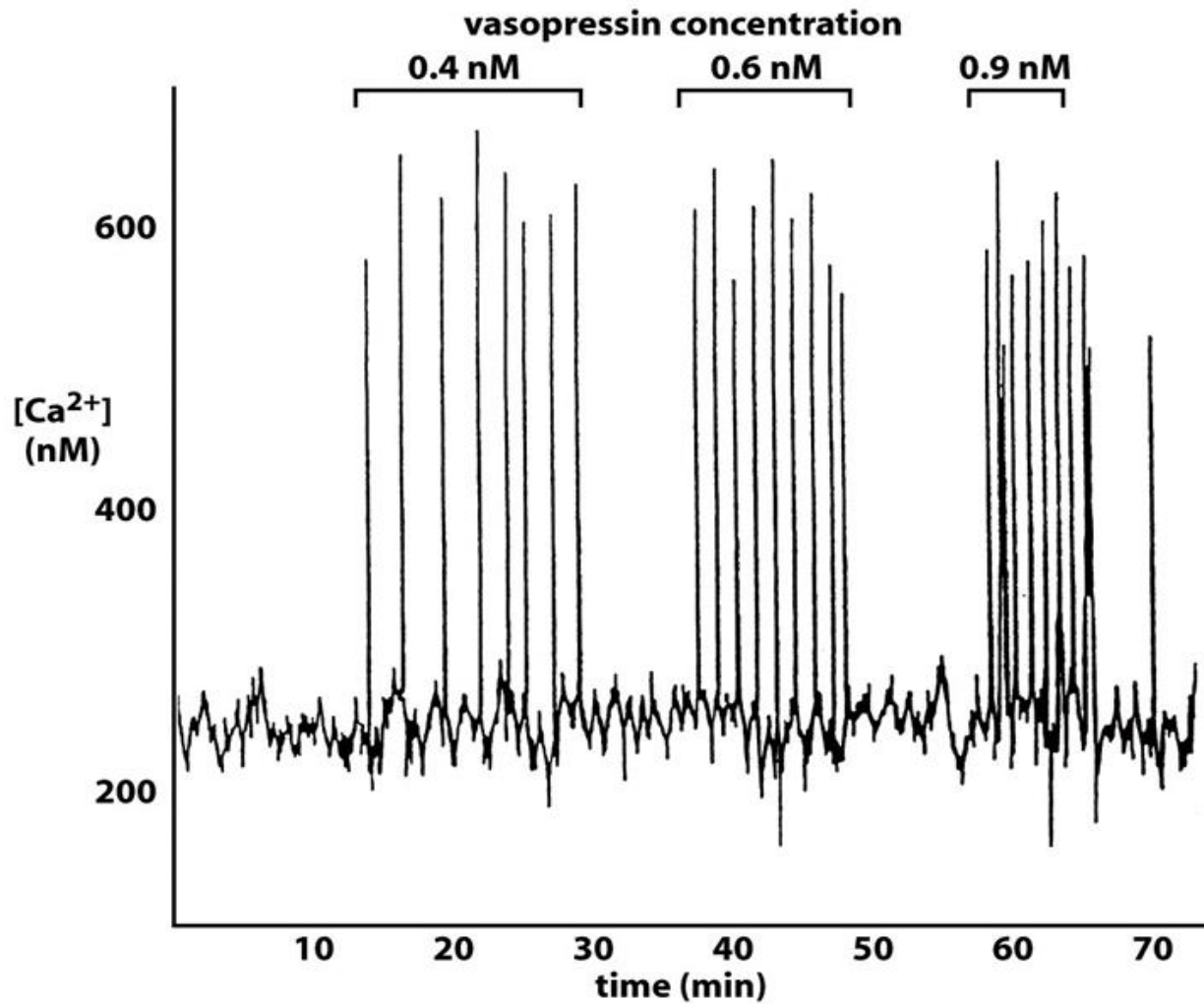
Figure 16-26 Essential Cell Biology 3/e (© Garland Science 2010)

# How do cells keep low $\text{Ca}^{2+}$ in cytosol?

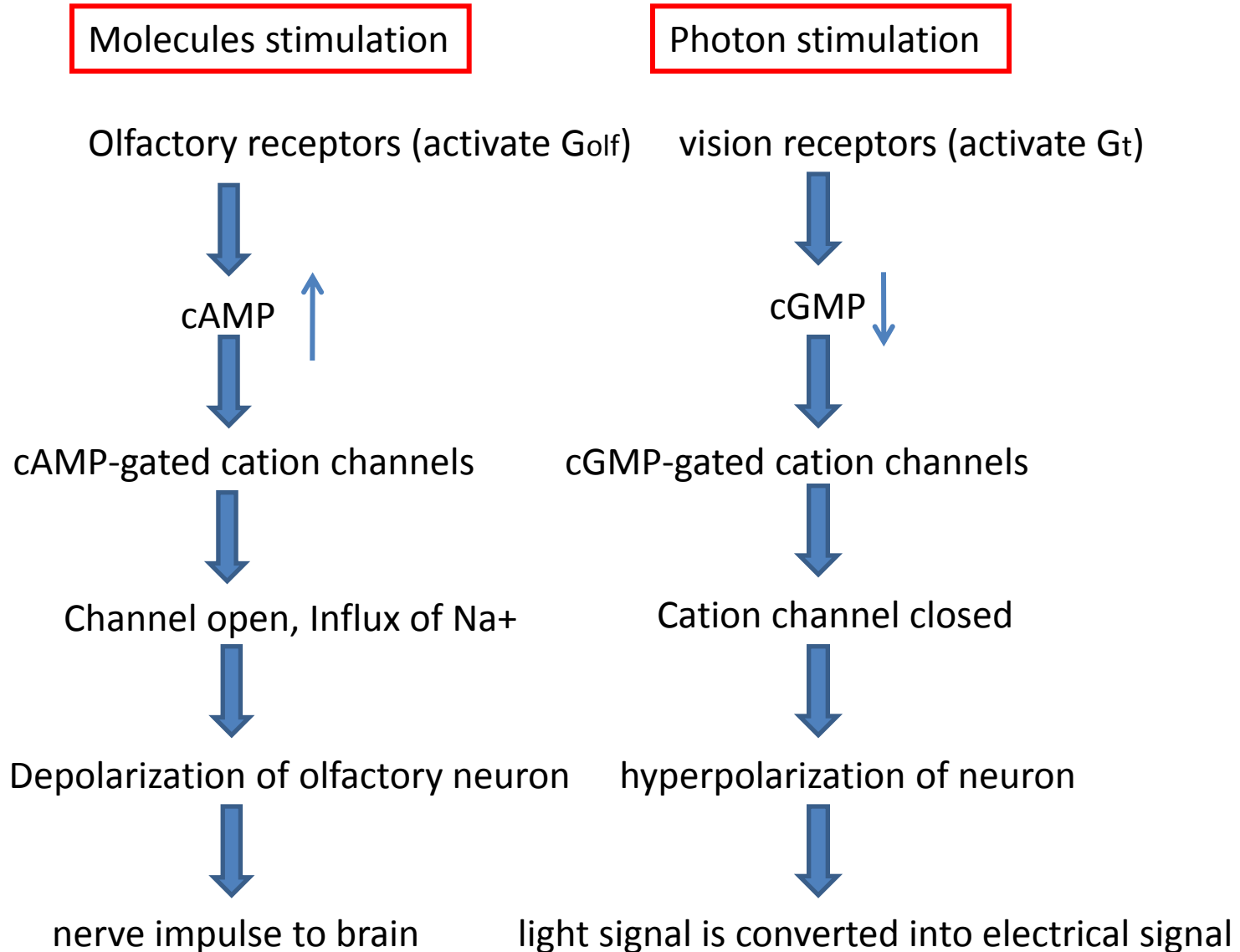
5 different ways to keep  $\text{Ca}^{2+}$  low:



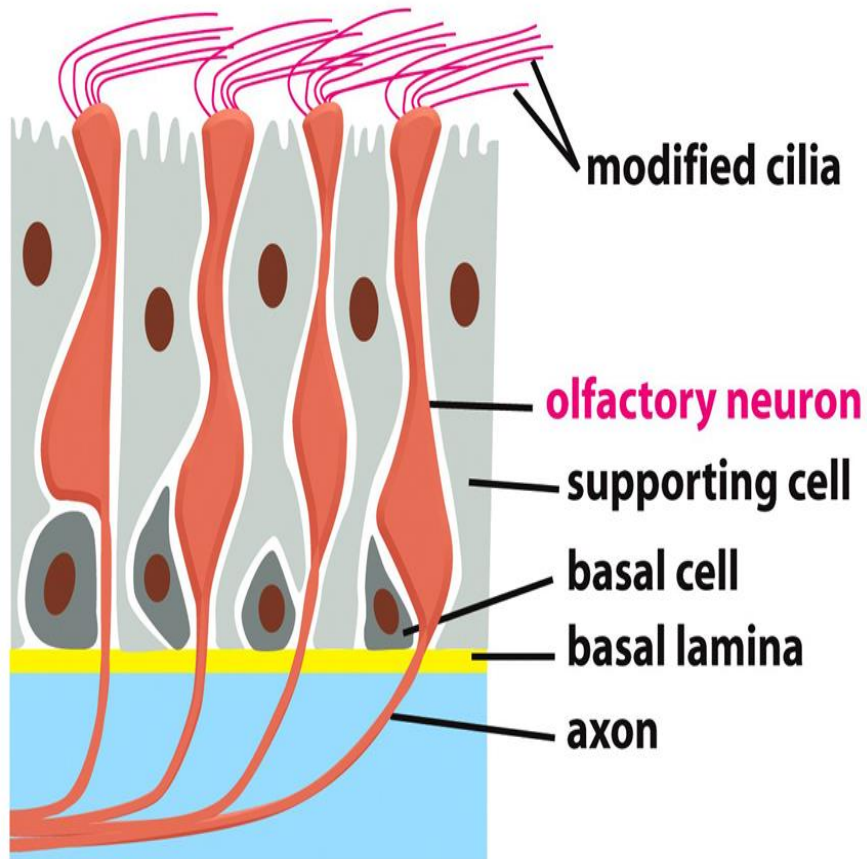
# Ca<sup>2+</sup> oscillation in cells in response to stimuli



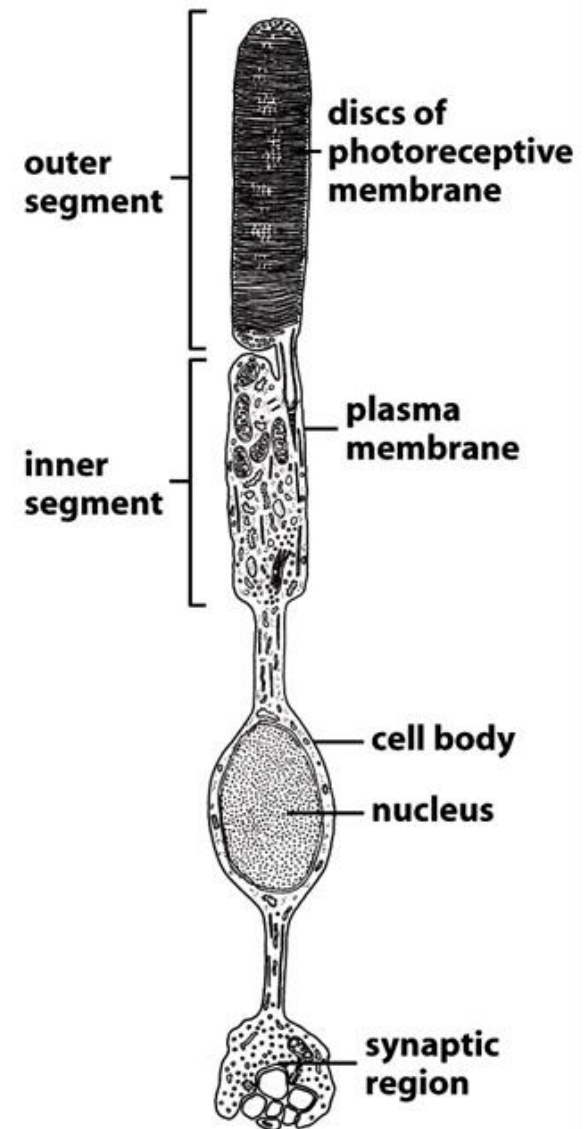
## 4.4. cyclic-nucleotide-gated ion channel downstream of GPCR in smell and vision



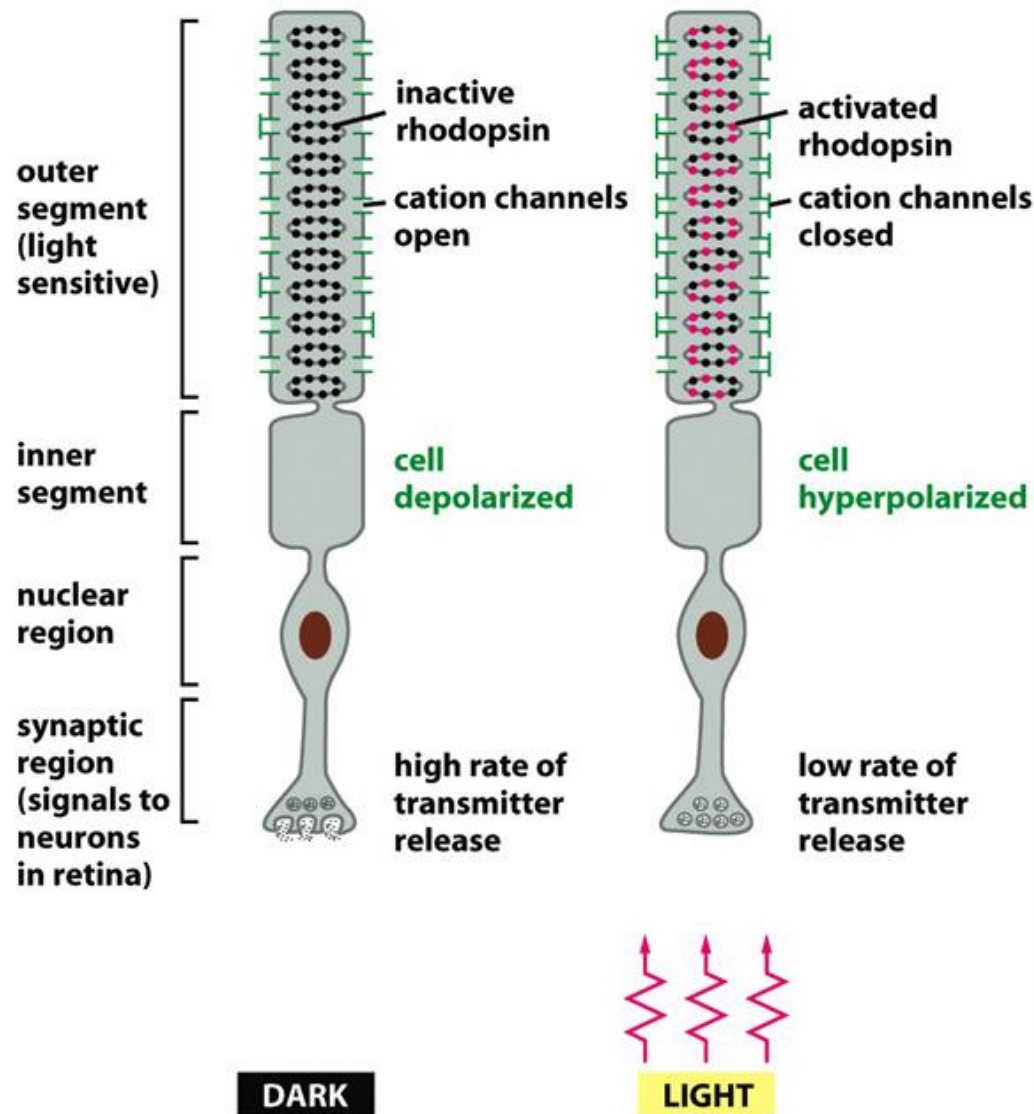
Over 10,000 smells can be differentiated by ~ 350 distinct receptors for human.



Rod photoreceptor cell senses Black and white; while cone Photoreceptor cell sense color



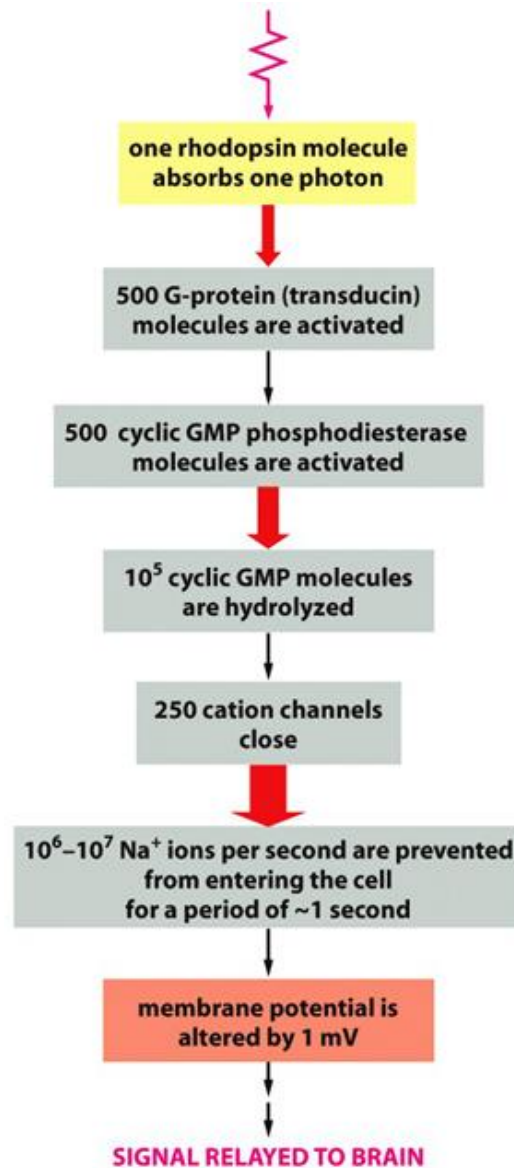
# Response of a rod photoreceptor cell to light



Photon  
↓  
11-cis-retinal to isomerize,  
↓  
Rhodopsin conformational change,  
↓  
activates Gt(transducin),  
↓  
activate cGMP phosphodiesterase.



# Signal amplification in transduction



A catalytic cascade due to enzymatic activity

## 5. GPCR desensitization

- (1). G-protein  $\alpha$ -subunit is stimulated by its target protein or RGS to cause GTP hydrolyzed into GDP
- (2). IP3 is dephosphorylated by lipid phosphatase or phosphorylated by lipase kinase.
- (3). cAMP/cGMP is hydrolyzed by phosphodiesterases.
- (4).  $\text{Ca}^{2+}$  is pumped out of cytosol.
- (5). Phosphorylated protein is dephosphorylated by phosphatases.
- (6). GPCRs are phosphorylated by GPCR kinases, triggering arrestin binding, uncoupling receptors from G proteins and promotes their endocytosis.



## II. Signaling through enzyme coupled cell surface receptors

- All these are single transmembrane receptor, which either is itself an enzyme or directly associates with an enzyme
- Can be divided into 6 classes:
  1. receptor tyrosine kinase (RTK)
  2. Tyrosine-kinase-associated receptors
  3. receptor Ser/Thr kinase
  4. Histidine-kinase-associated receptors
  5. Receptor guanylyl cyclases---produce cGMP
  6. Receptorlike tyrosine phosphatases

# 1. RTK outline

- 1.1. Types of RTK signaling
- 1.2. General configuration of RTK
- 1.3. How RTKs are activated and relay signal
- 1.4. Major pathways downstream of RTK
  - 1.4.1 Ras signaling
  - 1.4.2 Rho signaling
  - 1.4.3 PI3K/Akt signaling

# 1.1. Configurations of some RTKs

Three domains :

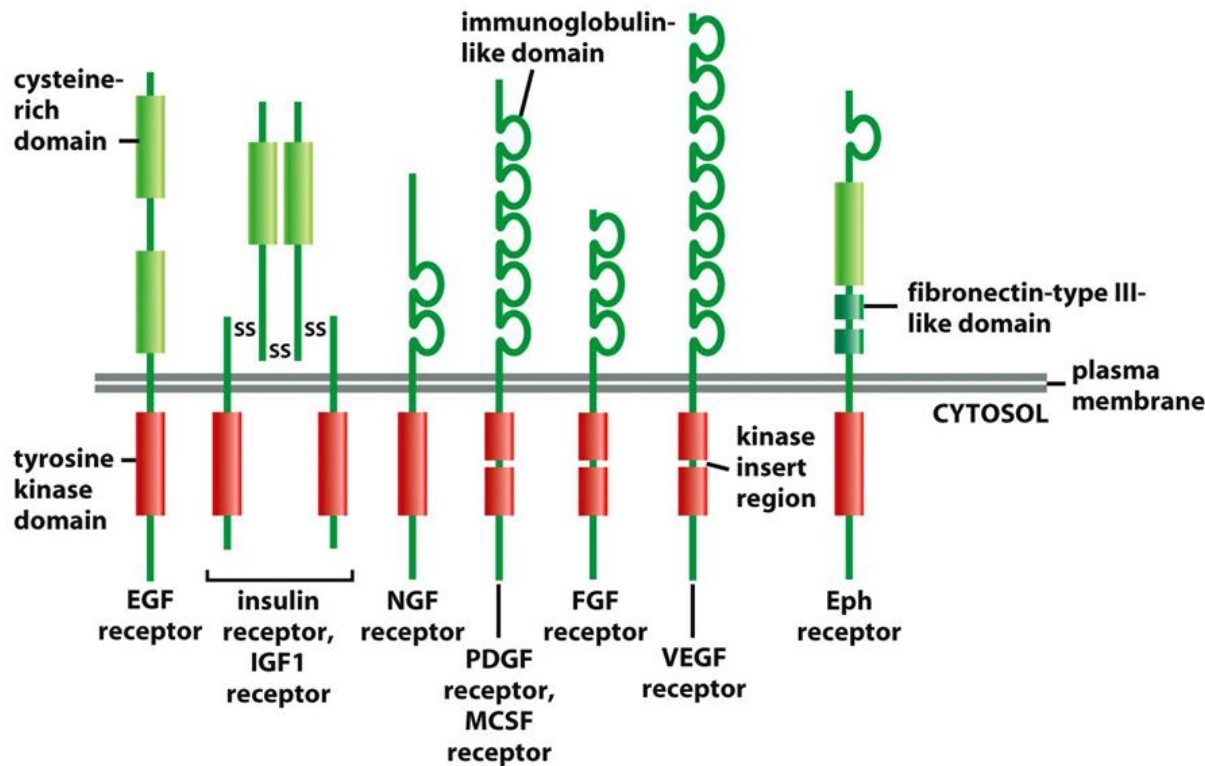
Extracellular region: interact with ligand

One single transmembrane domain

Intracellular region: tyrosine kinase activity

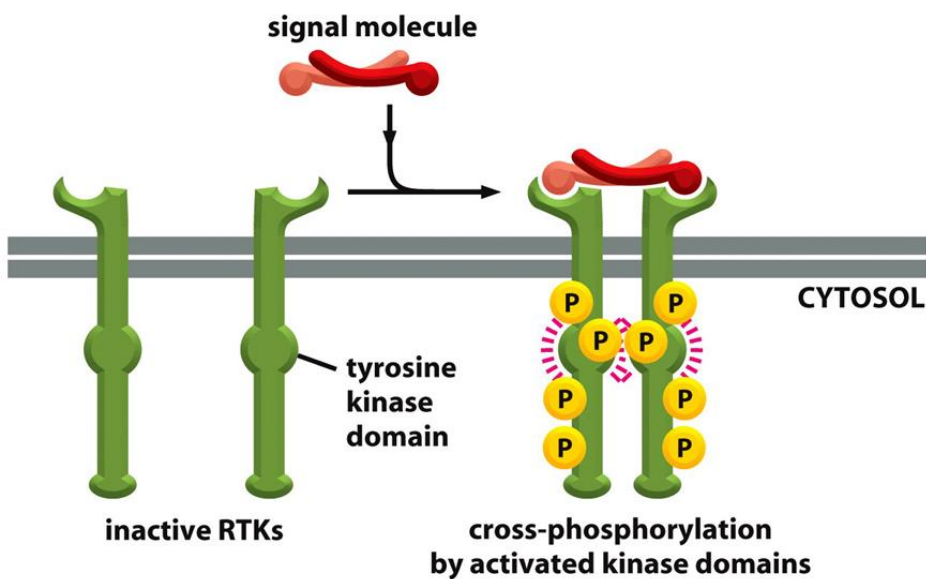
Human genome encodes ~ 60 RTK genes

Upon ligand binding, RTK usually **dimerize** and **transautophosphorylate** themselves

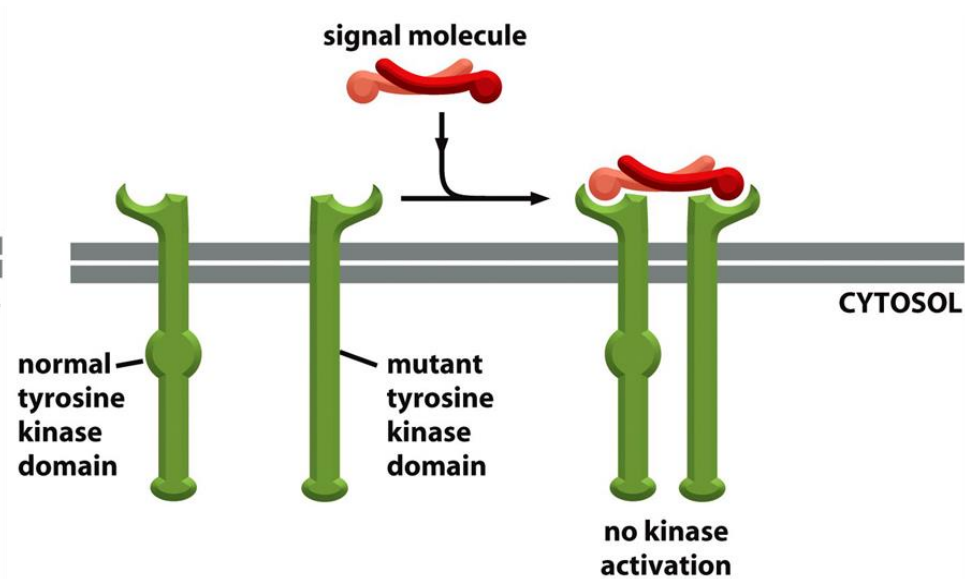


# How a mutant RTK acts as dominant negative manner

Dominant negative: a mutant protein exhibits an inhibitory manner for wild type protein.



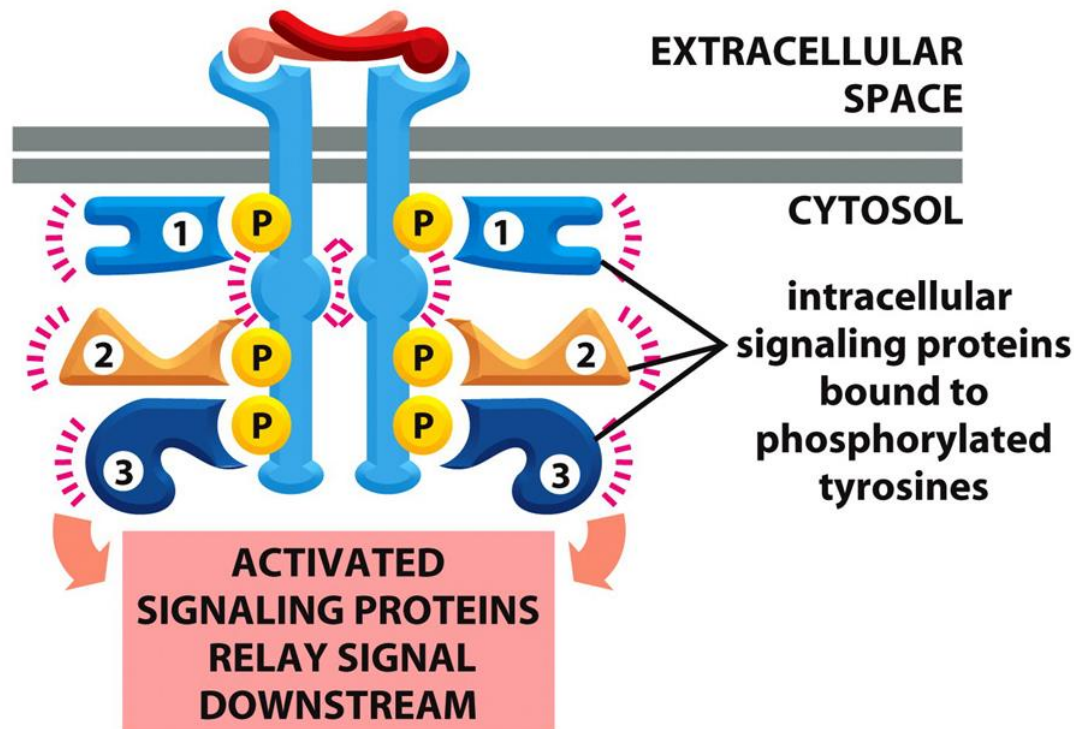
**NORMAL RTK ACTIVATION**



**DOMINANT-NEGATIVE INHIBITION BY MUTANT RTK**

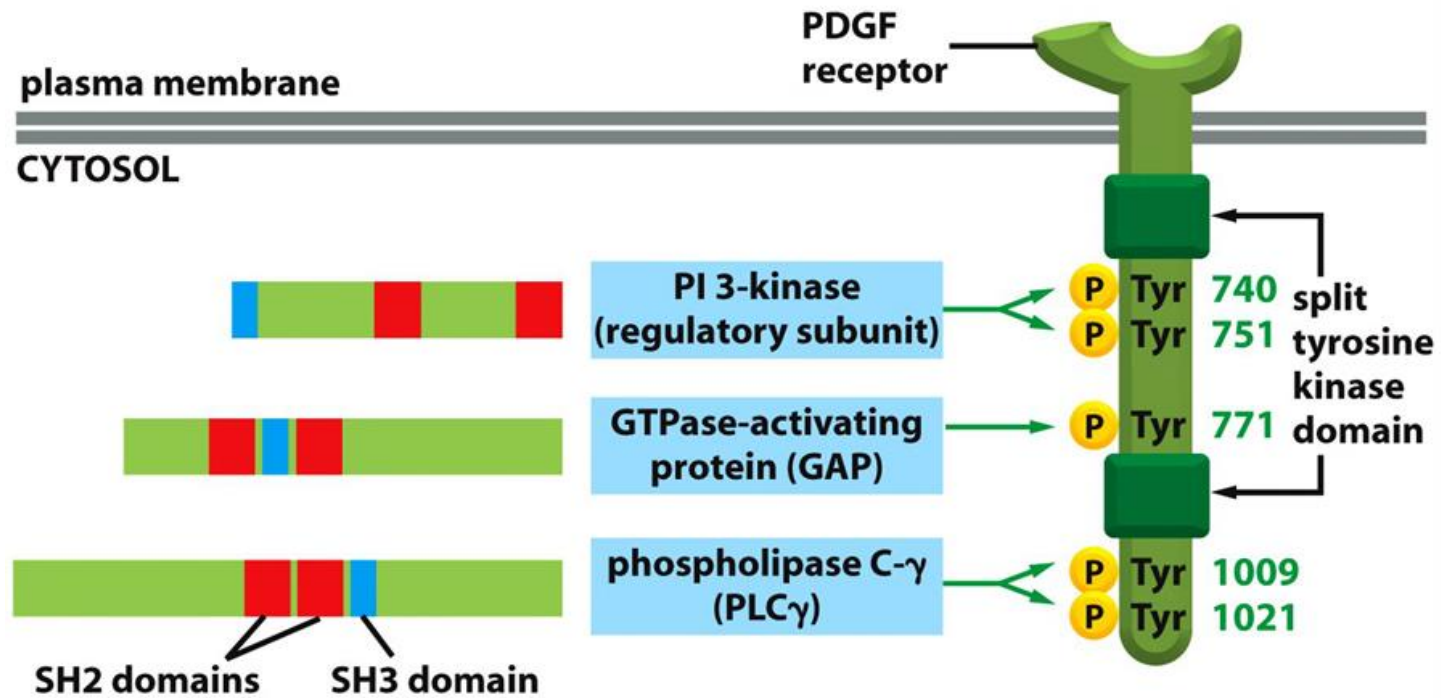
## 1.2 Phosphorylation on RTK has dual roles

- Activates RTK kinase activity
- Introduce phospho-Tyr that can recruit other protein factors to relay signals



Through SH2, PTB (phospho-Tyr binding) domain, or SH3 (Pro-rich Binding) domain, etc.

# For example: PDGF-receptor



## 1.3. Several important signal pathways downstream of RTK

- 1.3.1. Ras pathway
- 1.3.2. Rho pathway
- 1.3.3. PI3K pathway

## 1.3.1. Ras pathway

- Ras --- name derived from *Rat sarcoma virus* , found by RSV infection.
- Small monomeric GTPase, ~20KD, weak GTP hydrolysis activity.
- Highly mutated in human cancers, ~ 30%, in pancreatic cancer, mutation rate 95%.
- Pivotal roles in cell proliferation, survival, motility, etc.
- tethered on lipid membrane.

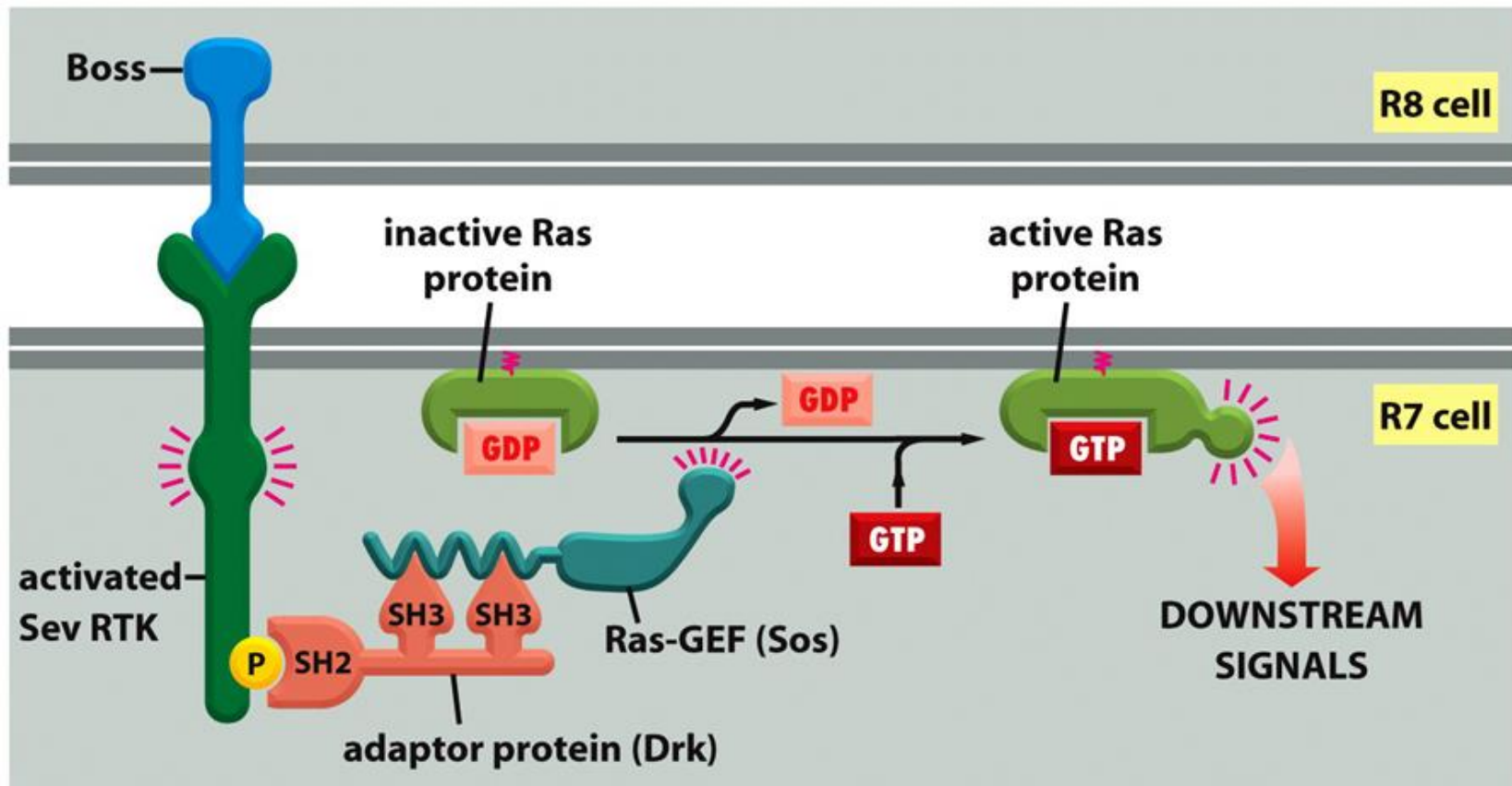
**Table 15–5 The Ras Superfamily of Monomeric GTPases**

FAMILY	SOME FAMILY MEMBERS	SOME FUNCTIONS
Ras	H-Ras, K-Ras, N-Ras Rheb Rep1	relay signals from RTKs activates mTOR to stimulate cell growth 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 3–72.



# Activation of Ras downstream of RTK mediated by Grb2 and Sos

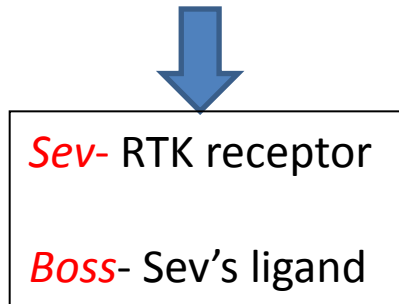


In human, Grb2 is homolog for Drk: adaptor protein, stands for Growth factor receptor binding protein 2

# How is Ras pathway found in Drosophilae eye development

Deficiency of *sevenless (Sev)* causes failure to detect UV light by R7 photoreceptor

Deficiency of *bride of sevenless (Boss)* causes this failure, too

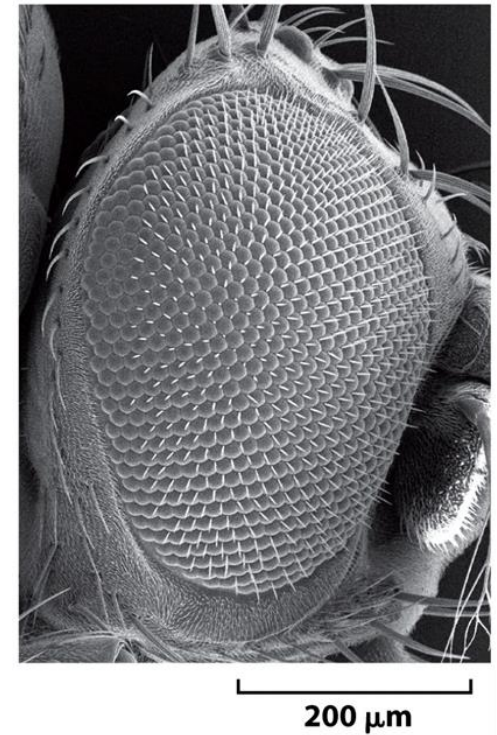


Using *Sev/Boss* partial mutant strains do genetic screen, found mutation of Ras leads to loss of R7; while hyperactive Ras rescues deficiency of both *Sev/Boss*

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graph TD; A[Using Sev/Boss partial mutant strains do genetic screen, found mutation of Ras leads to loss of R7; while hyperactive Ras rescues deficiency of both Sev/Boss] --> B[Ras locates downstream of Sev];
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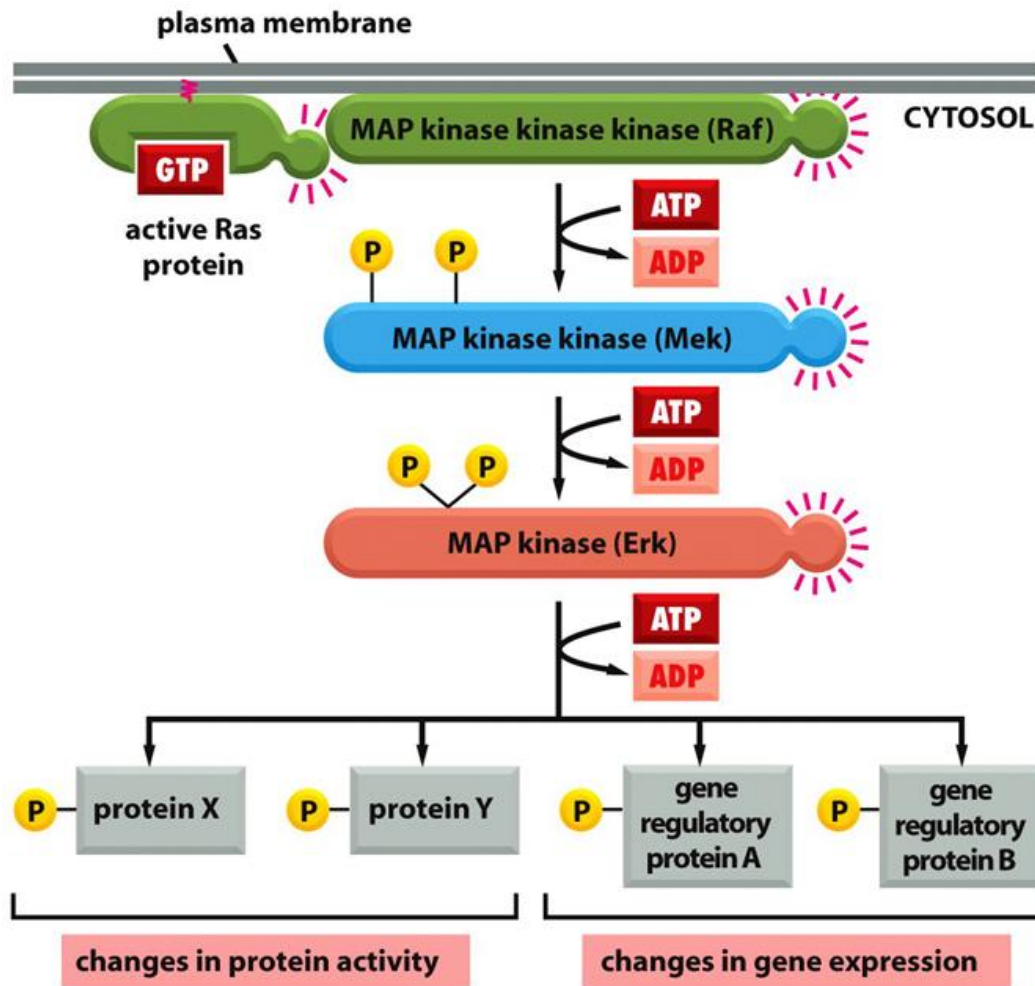
A diagram showing a box containing two lines of text. The first line is "Using *Sev/Boss* partial mutant strains do genetic screen, found mutation of Ras leads to loss of R7; while hyperactive Ras rescues deficiency of both *Sev/Boss*". A large blue arrow points down from the box to the text below it.

Ras locates downstream of *Sev*



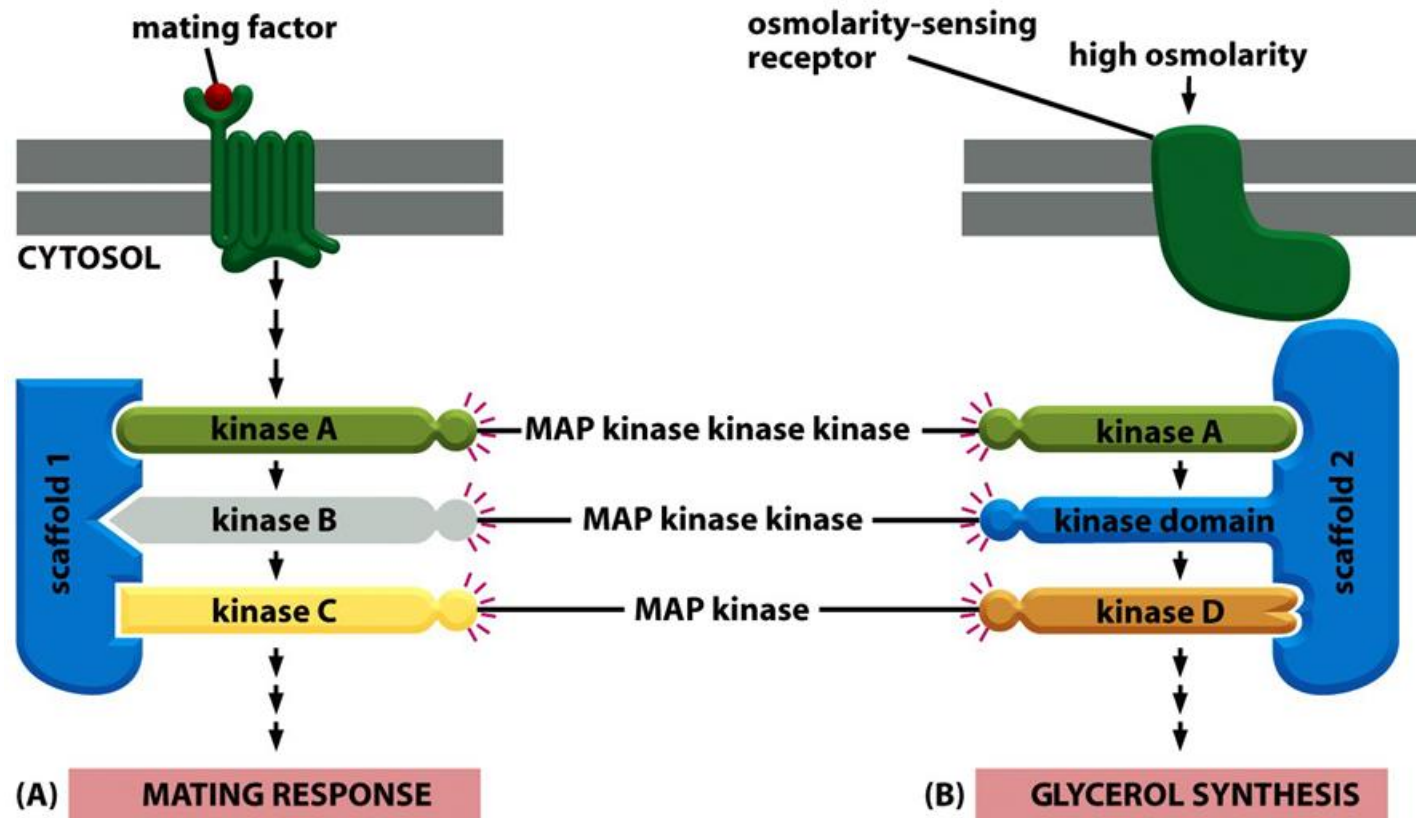
Further genetic screen identified *Son of sevenless (Sos)* AND *Drk*

# MAP (mitogen-activated protein) kinase signaling downstream of Ras



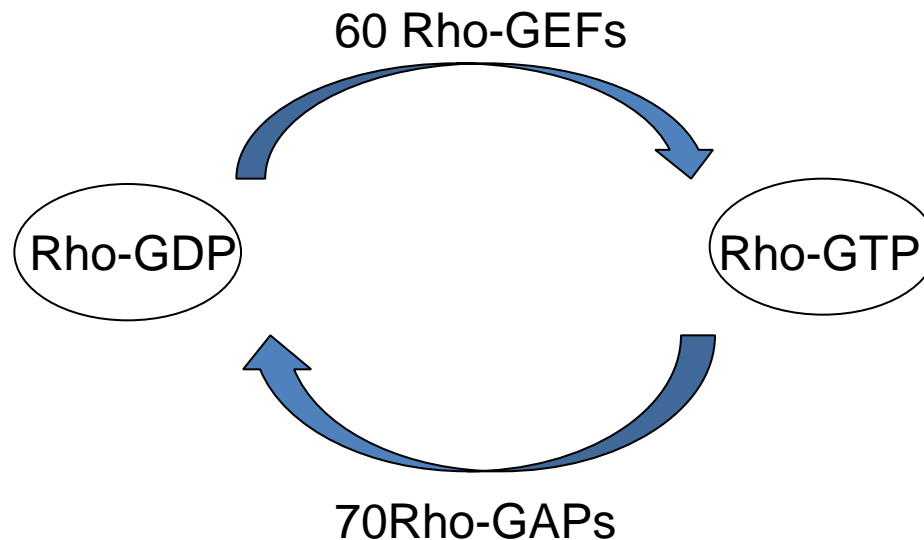
MAP kinase pathway controls both protein activity and gene transcription

# Scaffold proteins provide precision and avoid cross-talk between parallel MAP kinase modules

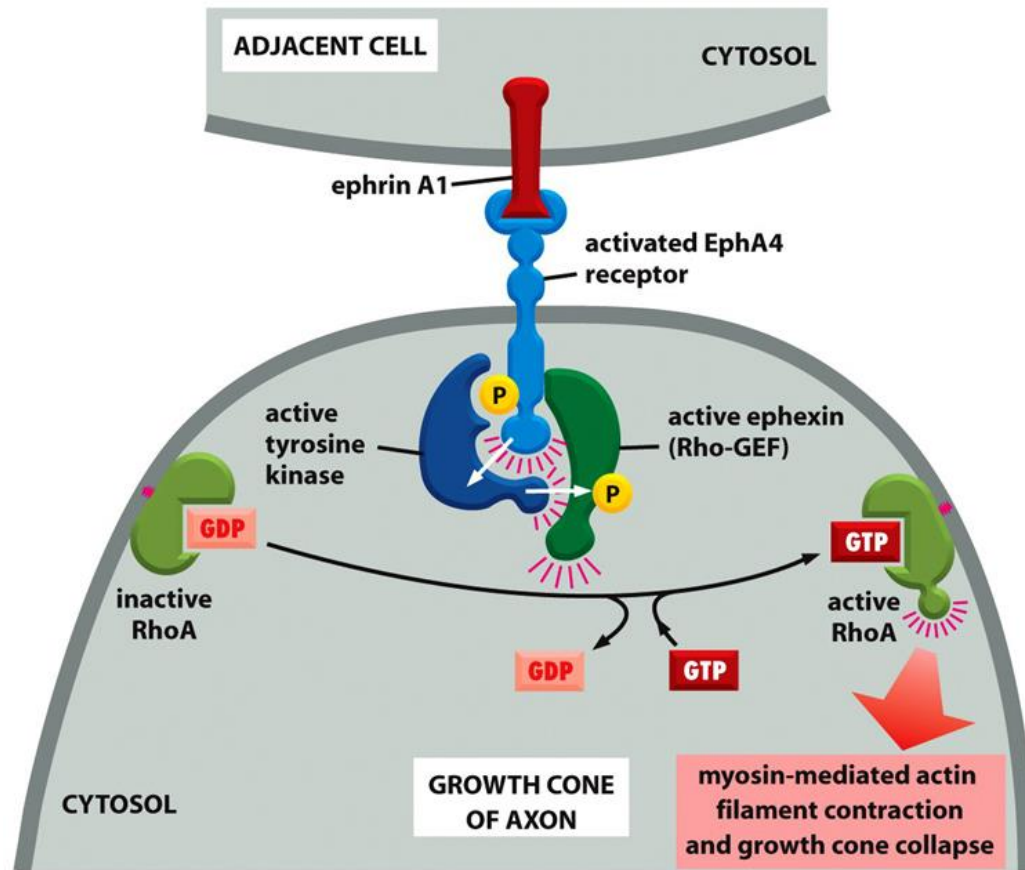


## 1.3.2. Rho-GTPase

- ◆ Couple cell surface receptors to the cytoskeleton
- ◆ Control cell shape, polarity, migration, and adhesion.
- ◆ When inactive, usually associates with *Guanine nucleotide dissociation inhibitor* (GDI)
- ◆ Three major Rho family members: Rho, Rac, Cdc42



For example: ephrin induces growth cone collapse



### 1.3.3. PI3K pathway promotes cell growth and survival

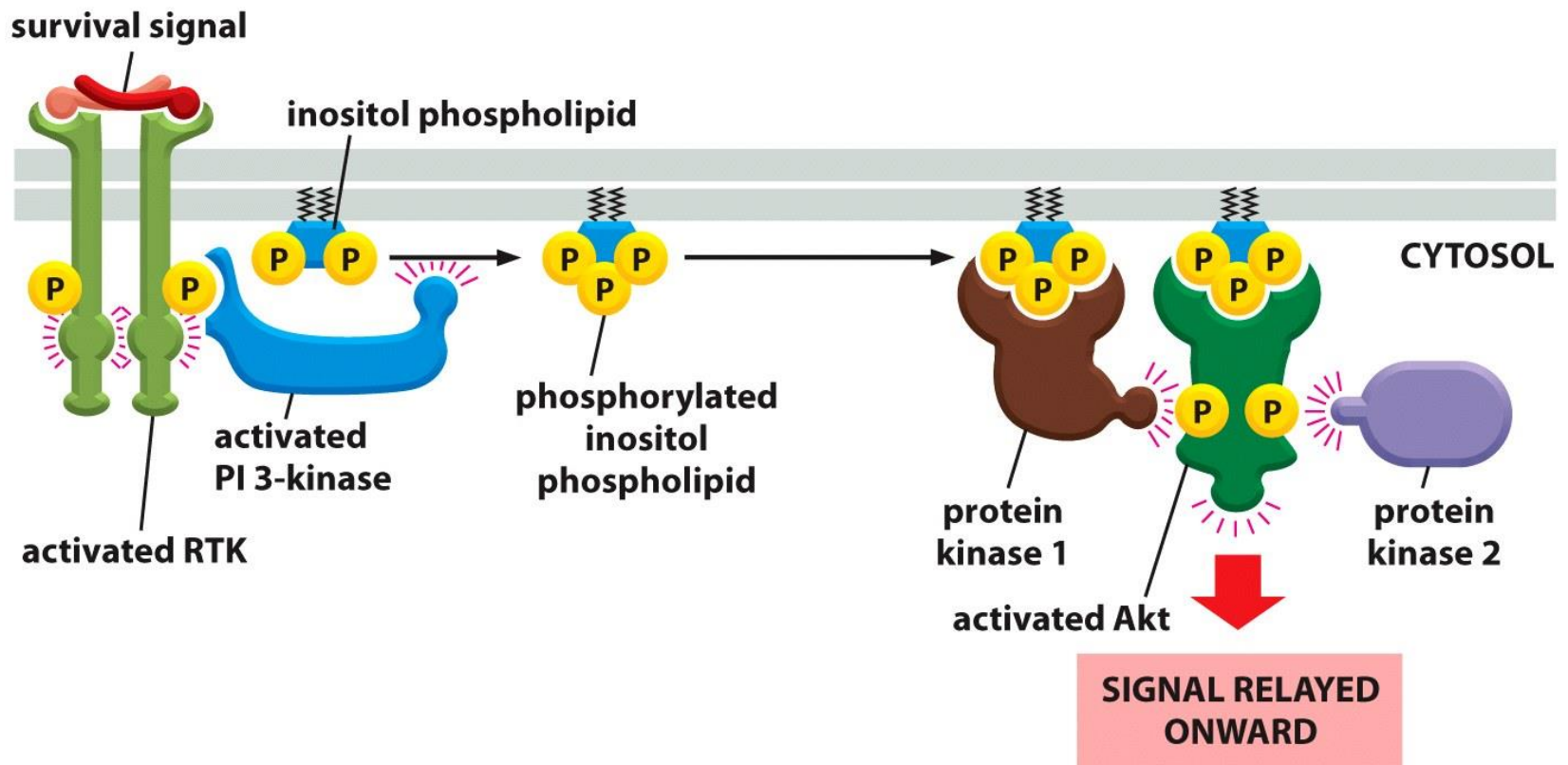
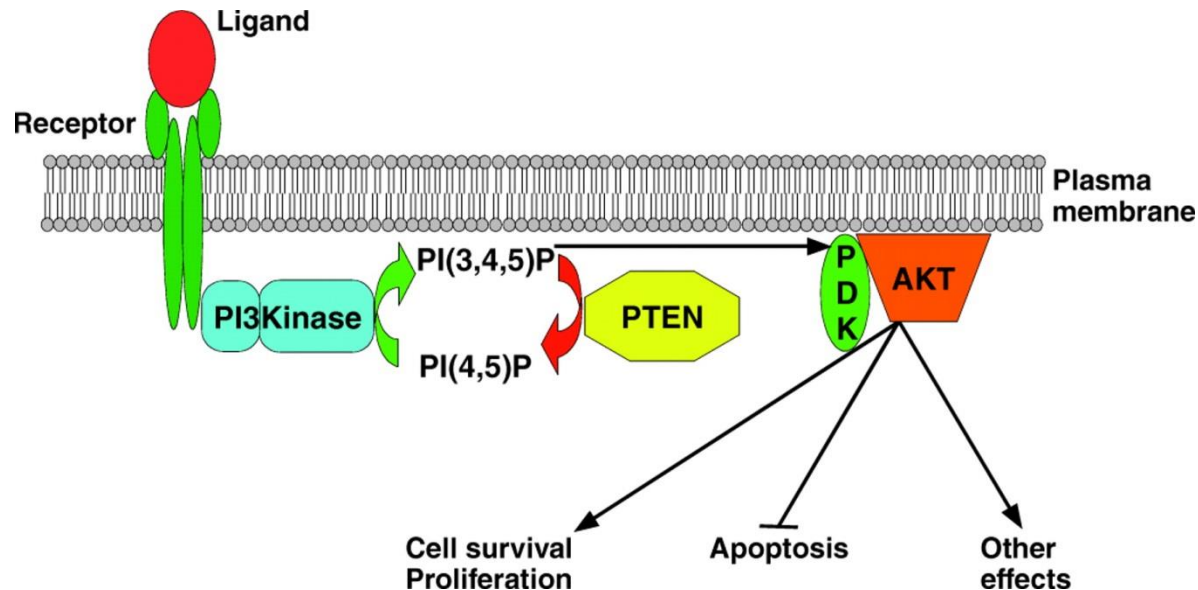


Figure 16-33 Essential Cell Biology 3/e (© Garland Science 2010)



# PI3K and PTEN in controlling PIP3

- PTEN: Phosphatase and tensin homolog



PI3K hyperactivation and PTEN loss of function frequently occur in human cancers



## 2. Tyrosine-kinase-associated receptors outline

### 2.1 overview

### 2.2 major types for non-receptor tyrosine kinases

#### 2.2.1 JAK-STAT

#### 2.2.2 Src family

#### 2.2.3 Focal adhesion kinase

## 2.1 Tyrosine-kinase-associated receptors

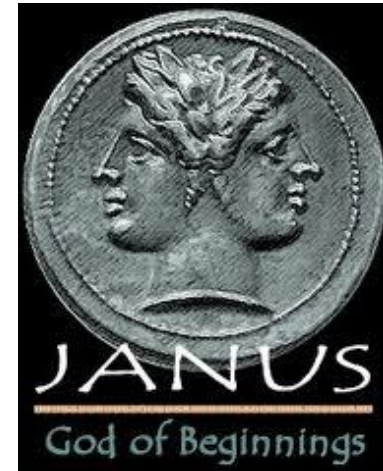
- Recruit **cytosolic tyrosine kinase** to relay signal
- Also form dimers upon ligand binding
- This family include:
  - Antigen receptor such as BCR, TCR
  - integrin;
  - interleukin;
  - receptors for many cytokines and growth hormones

## 2.2. Major types of non-receptor tyrosine kinase in cytosol

- **JAK**: the largest family, mediates cytokine signaling
- **Scr family**: cytosolic tyrosine kinase (Src--- sarcoma virus), proto-oncogene, controls cytoskeleton assembly, growth and proliferation.
- **Focal adhesion kinase**: mediate integrin signaling to cytoskeleton during cell adhesion.

## 2.2.1. JAK-STAT

- JAK-Janus kinase--- cytosolic tyrosine kinase;
- STAT-Signal transducers and activators of transcription  
--- transcription factors



**Table 15–6** Some Extracellular Signal Proteins That Act Through Cytokine Receptors and the JAK–STAT Signaling Pathway

SIGNAL PROTEIN	RECEPTOR-ASSOCIATED JAKs	STATS ACTIVATED	SOME RESPONSES
$\gamma$ -interferon	JAK1 and JAK2	STAT1	activates macrophages
$\alpha$ -interferon	Tyk2 and JAK2	STAT1 and STAT2	increases cell resistance to viral infection
Erythropoietin	JAK2	STAT5	stimulates production of erythrocytes
Prolactin	JAK1 and JAK2	STAT5	stimulates milk production
Growth hormone	JAK2	STAT1 and STAT5	stimulates growth by inducing IGF1 production
GMCSF	JAK2	STAT5	stimulates production of granulocytes and macrophages

# How does prolactin promote milk production?

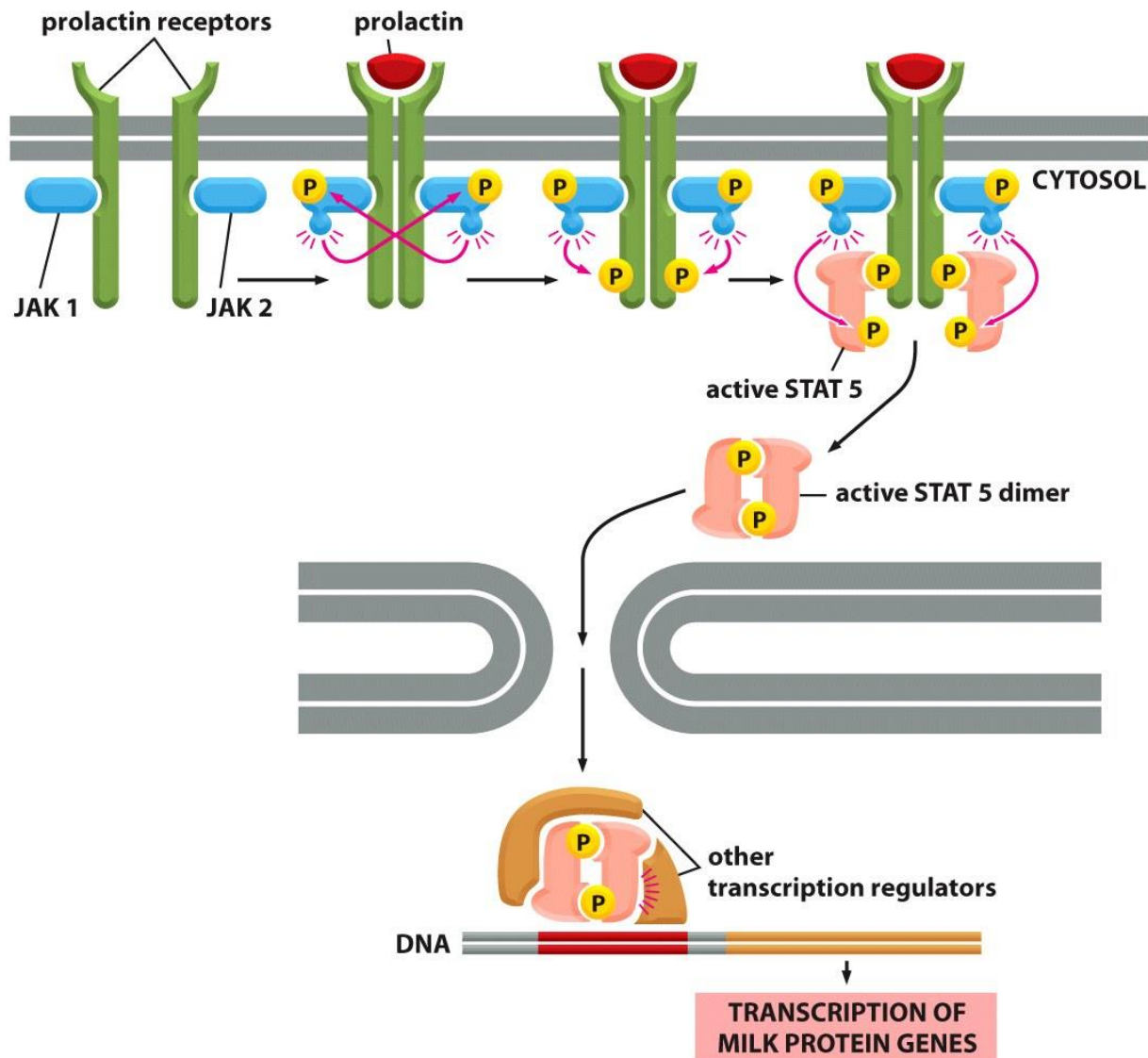
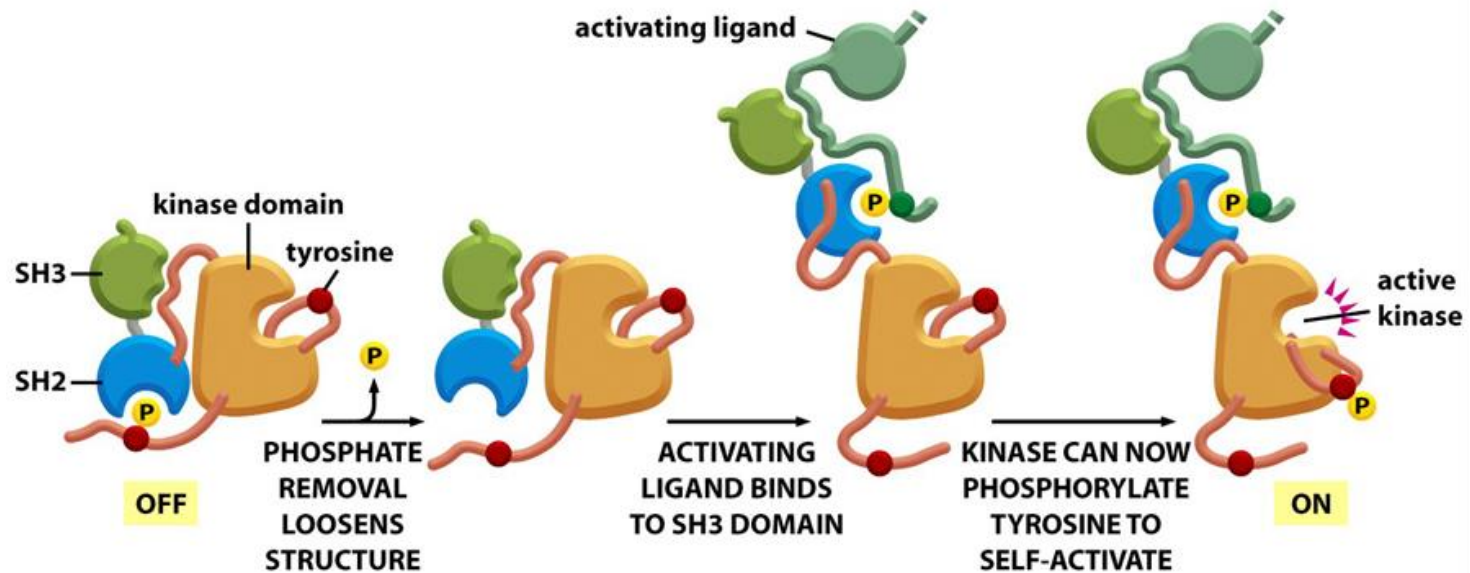


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## 2.2.2. Src tyrosine kinase family

- ◆The largest family of cytoplasmic tyrosine kinases
- ◆Family members: Src, Yes, Fgr, Fyn, Lck, Lyn, Hck, Blk, etc
- ◆All contain SH2, SH3 and kinase domains.
- ◆All within cytoplasmic region

# Src kinase has “on” and “off” states, as are many kinases



Three distinct domains: SH2, SH3, kinase domain

## How to deactivate phosphorylated Tyrosine?

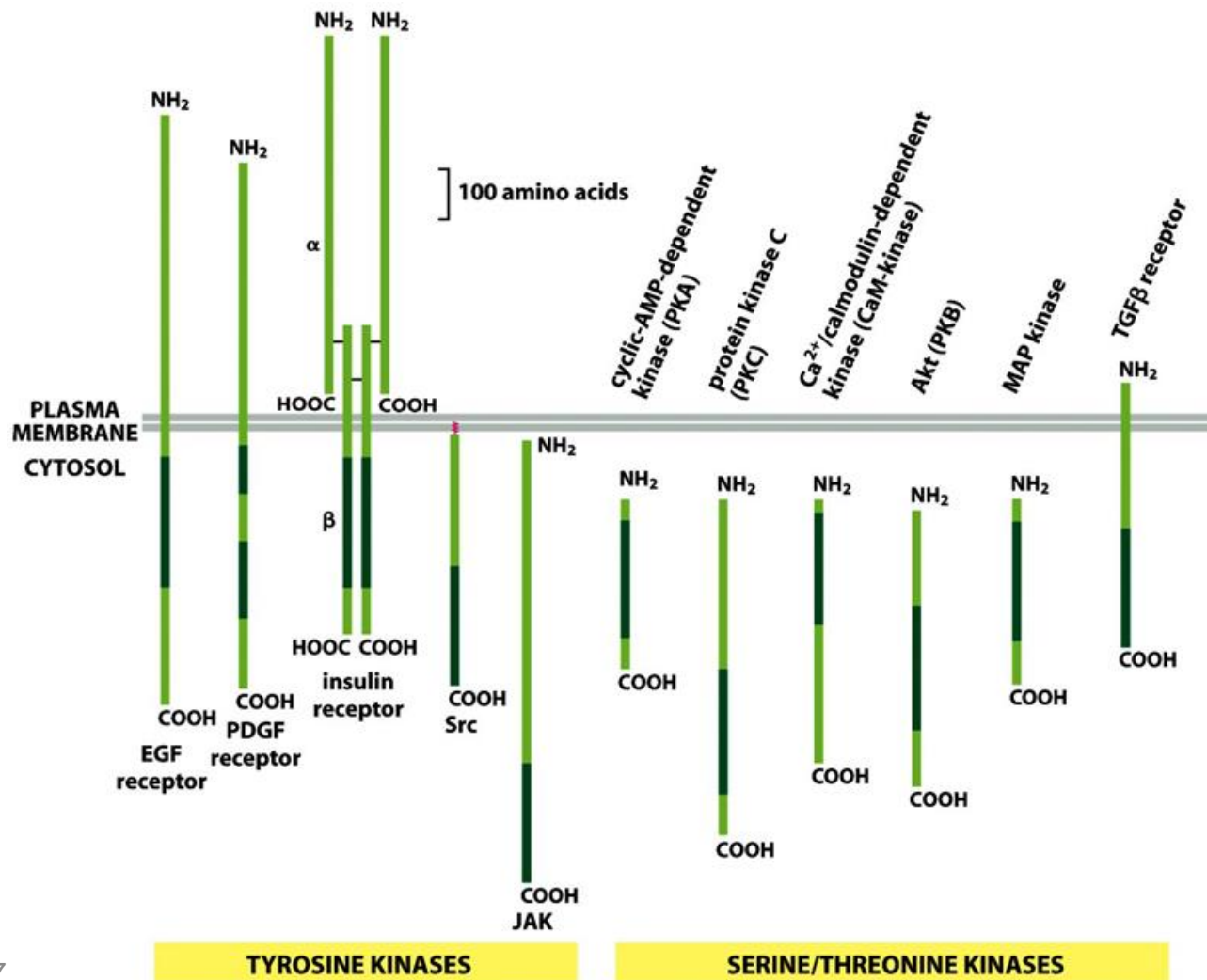
- Done by protein tyrosine phosphatases
- ~100 protein tyrosine phosphatases in human genome, each has exquisite specificity for a subset of proteins



### 3. Serine/Threonine kinase receptor

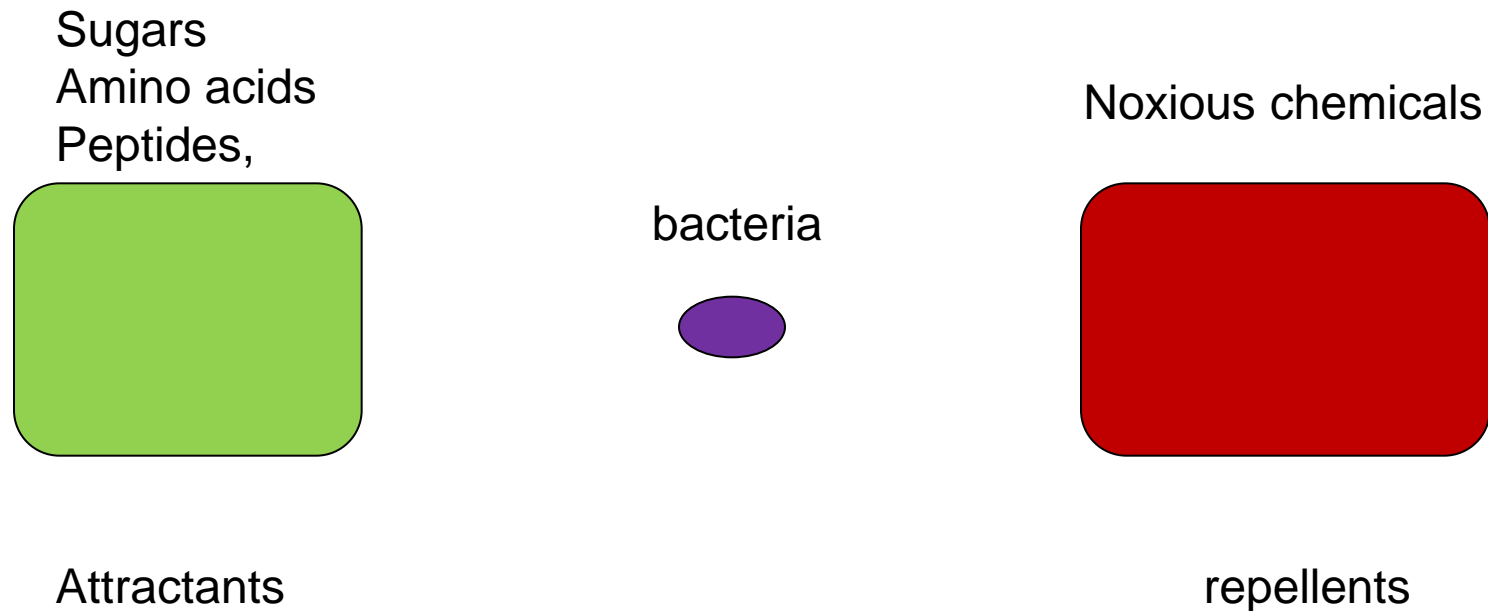
- Single transmembrane receptor and Ser/Thr kinase.
- Two classes: Type I and Type II which form homodimers, upon activation by ligand, Type II dimer phosphorylates Type I dimer to form active tetramer.
- They are receptors for : TGF  $\beta$  superfamily (derived from Tumor growth factor), Secreted and dimeric proteins, ~30-40 members for human, two categories:
  - TGF $\beta$ /activin family
  - bone morphogenetic protein (BMP) family
- Control diverse activity in differentiation, proliferation, cell death, development, etc.

# A summary and comparison for Tyrosine kinase and Ser/Thr kinase



## 4. Histidine-kinase-associated receptors

Mediates Bacterial chemotaxis response

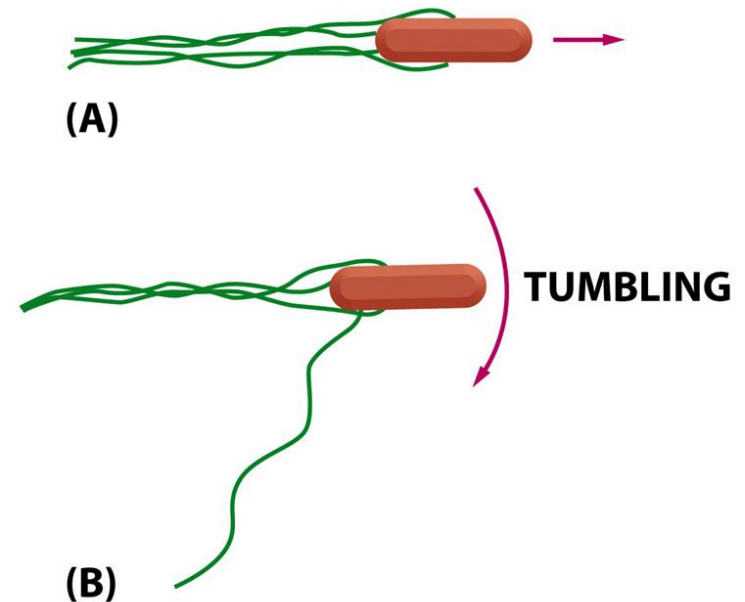
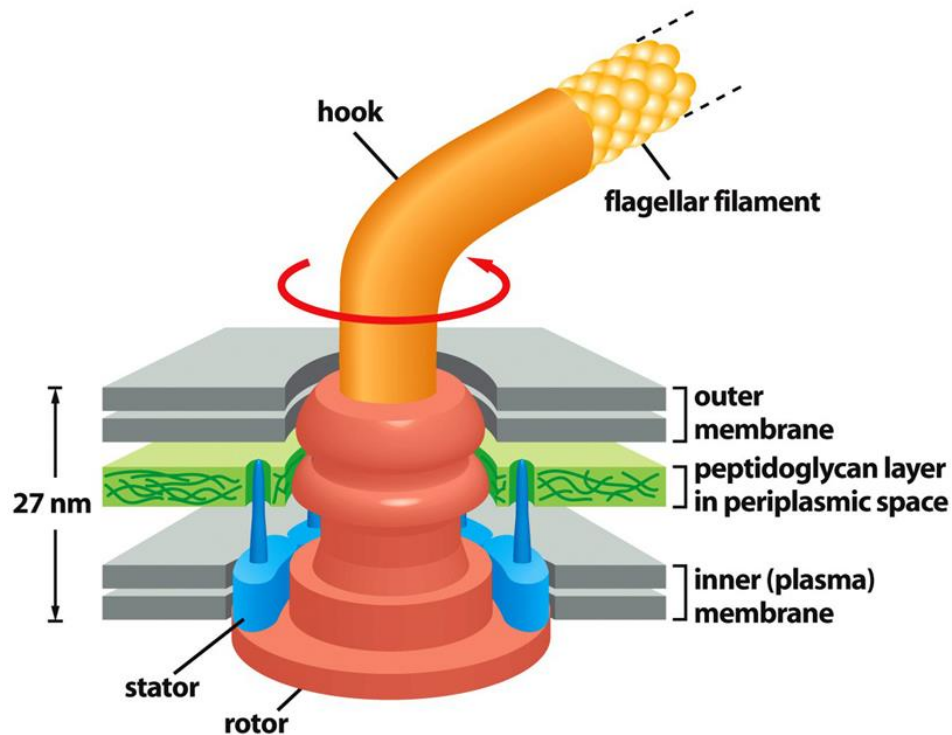


# Bacteria movement through flagella

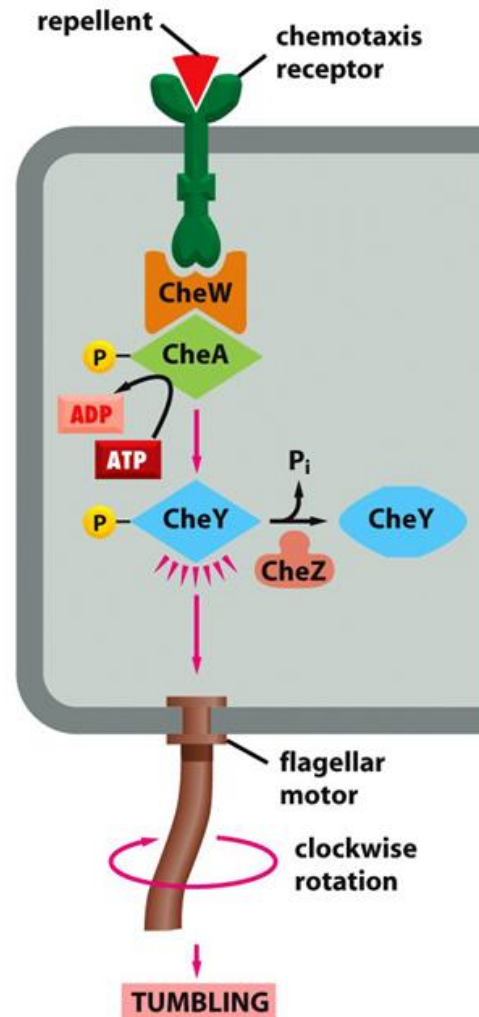
proton pumps couple proton gradient to motor rotation

Normally all motor rotate counterclockwise.

Every sec or two, some motor rotate clockwise---tumbling



# Mechanism for chemotaxis through Chemotaxis receptors---histidine-kinase-associated receptors



CheA--- Histidine kinase  
Which can phosphorylate itself  
On histidine, CheA then transfers  
Phosphate group to Asp on CheY

# Similar signaling pathways exist in plants to regulate plant growth

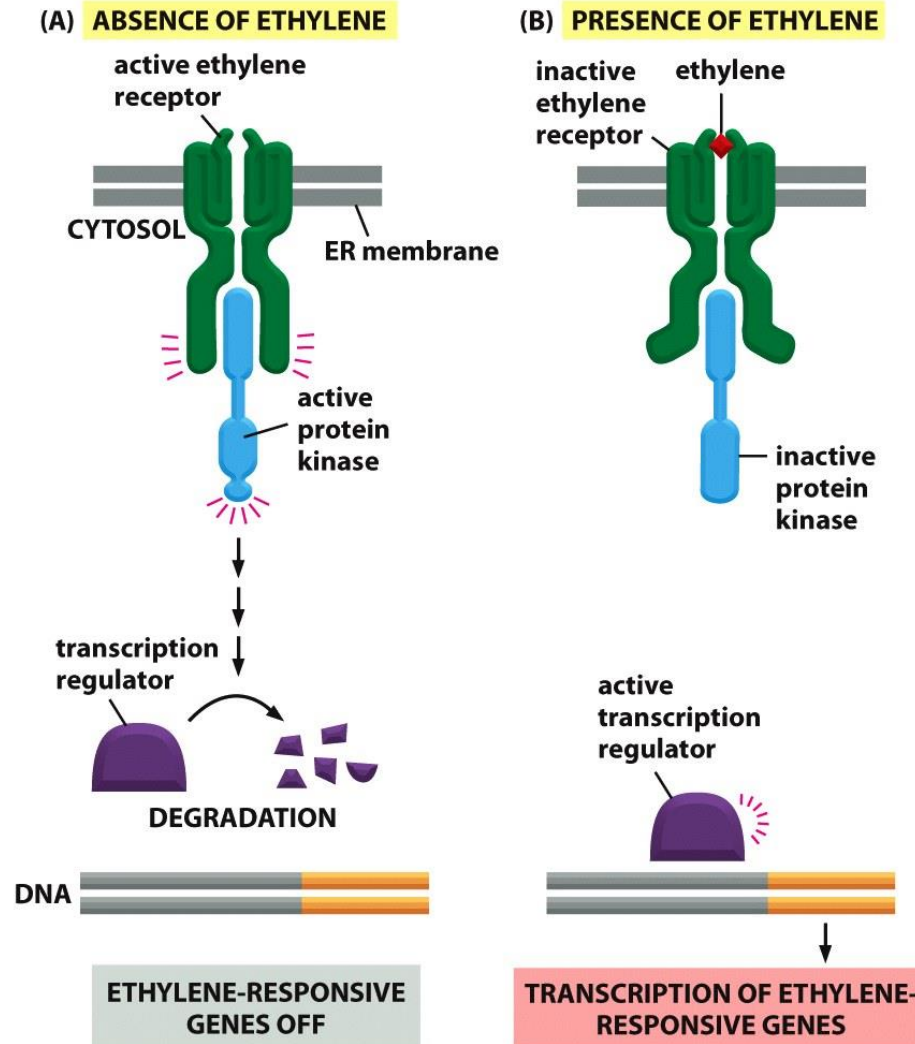


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