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?

glucagon

Glucagon receptor on membrane

cAMP

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

^{125}I label glucagon

Incubate with membrane system

Centrifugation to get rid of unbound glucagon

125 I activity occurs in membrane system

They also know how cAMP was produced biochemically

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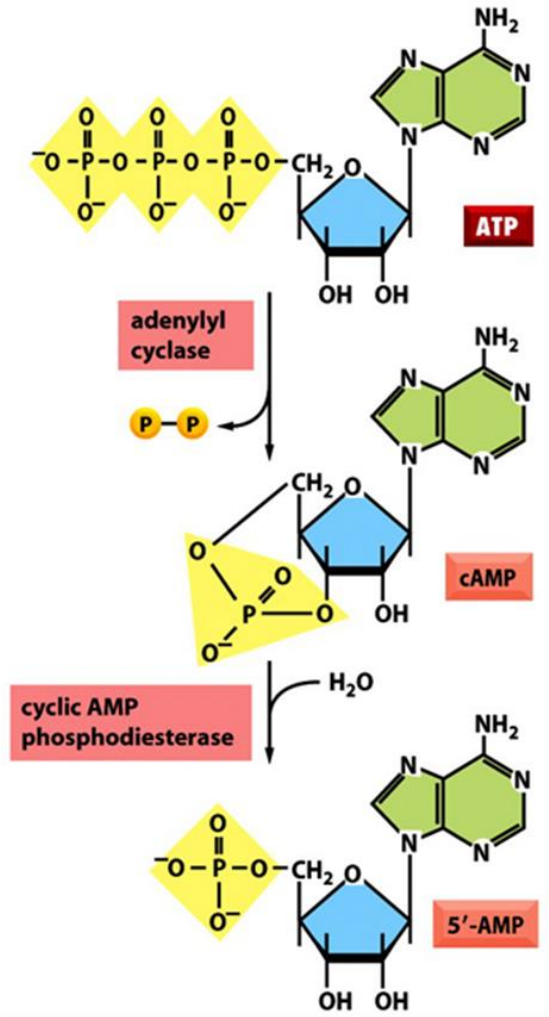
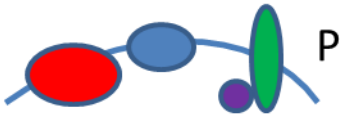
Adenyl cyclase

ATP ⟶ cAMP

Mg2+



Now they performed this experiment



Purified rat liver membrane system

Add ATP and Mg2+ and glucagon

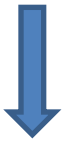
cAMP can be produced

However, they found that 125I activity on membrane was decreased by 50%

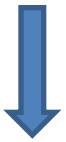
Later they found that glucagon dissociation naturally happens when glucagon trigger cAMP production

The unique feature of GTP in triggering glucagon dissociation

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

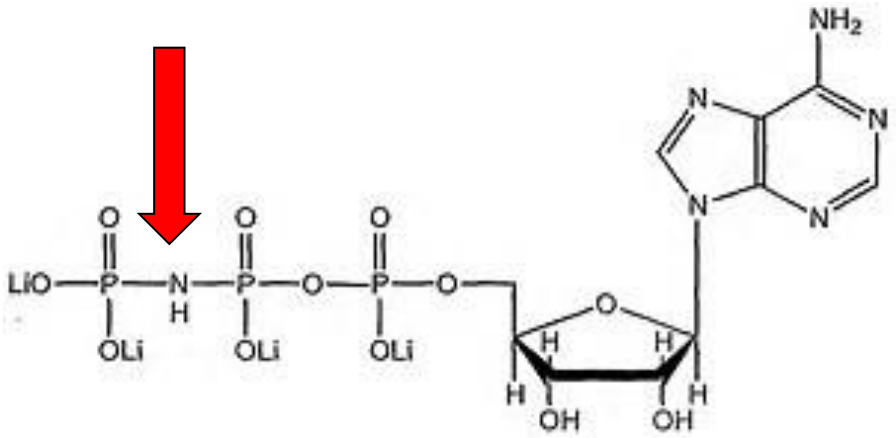


In contrast, micromolar GTP decreases glucagon binding.



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.

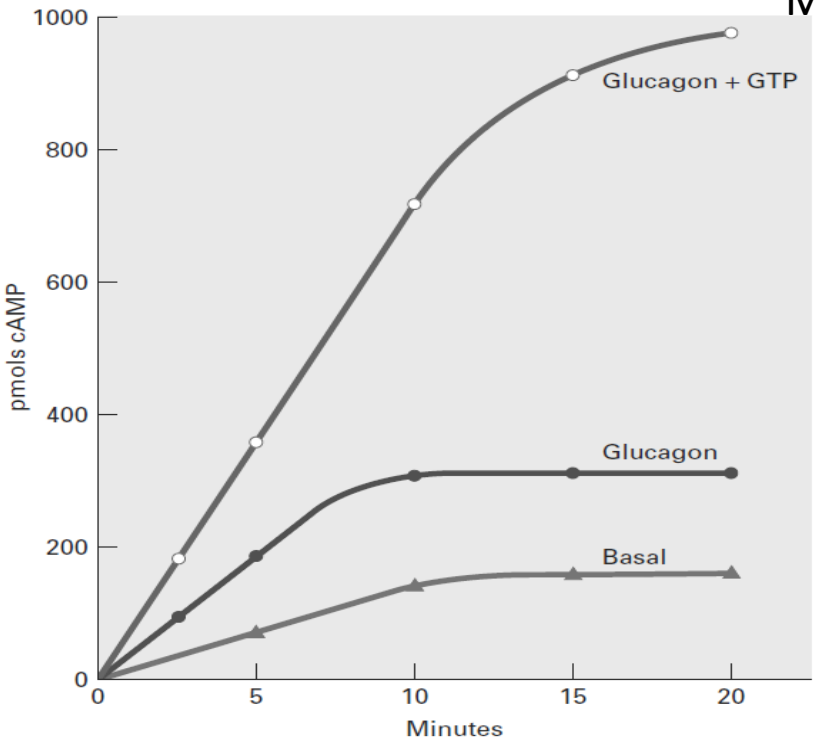
Here is what they eventually found

Rat membrane system

AMP-PNP
Mg2+

AMP-PNP
glucagon
Mg2+

GTP
AMP-PNP
glucagon
Mg2+



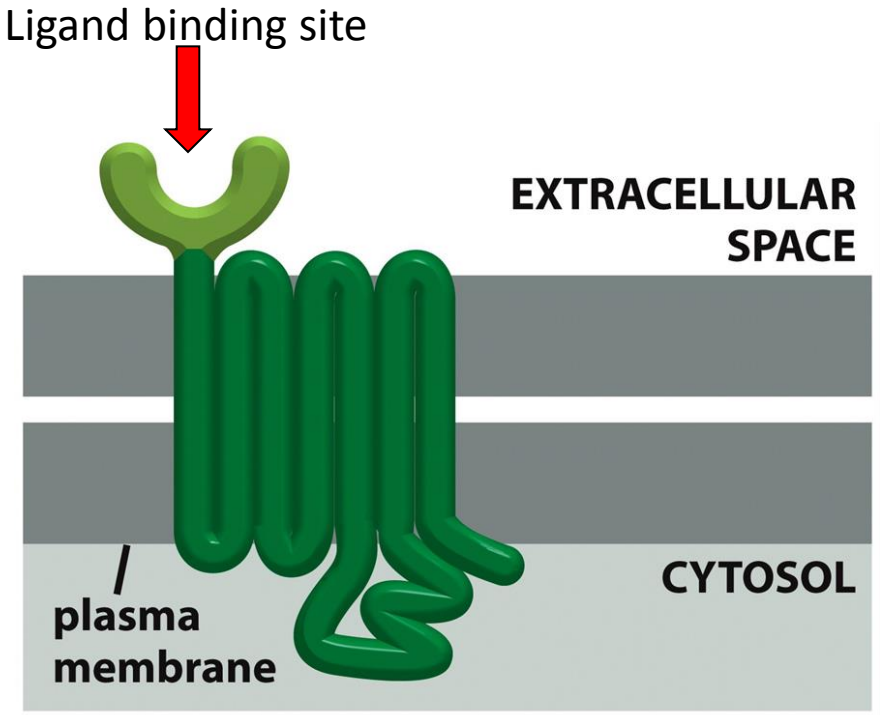
I. GPCR signaling outline

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-β
   - 4.3 Ca2+
   - 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

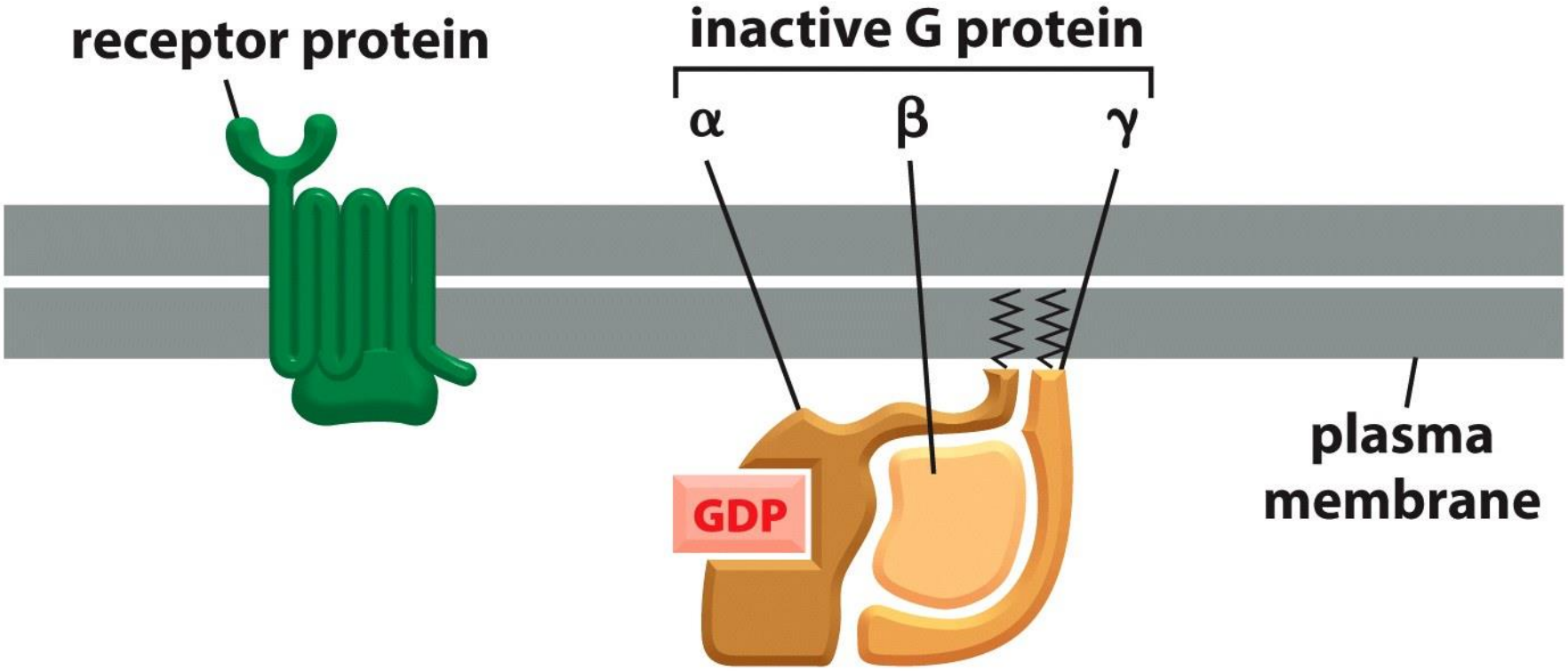
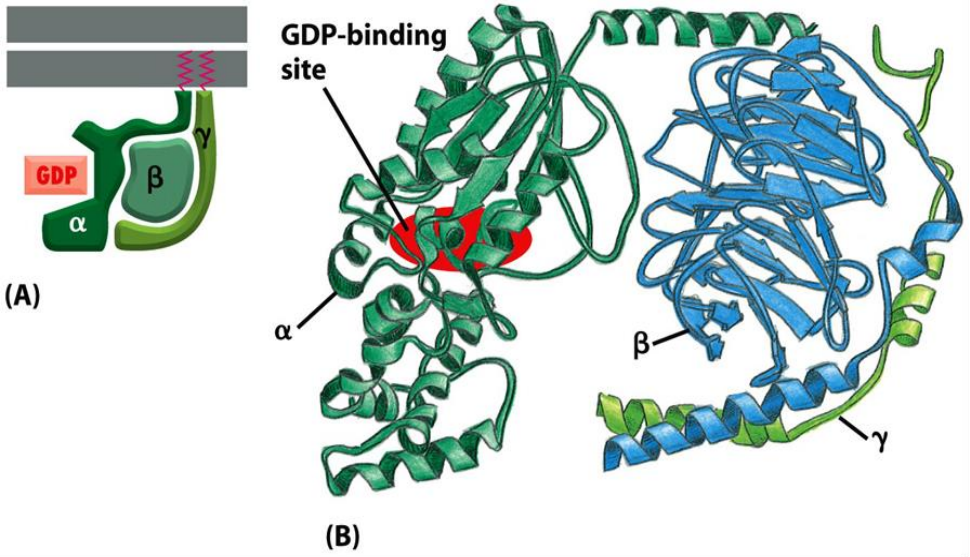


Figure 16-17a Essential Cell Biology 3/e (© Garland Science 2010)

Trimeric G-protein



Subunits α and γ are tethered to membrane
Subunit α binds to GTP/GDP and has GTPase Activity.

2. Activation of a G protein by an activated GPCR

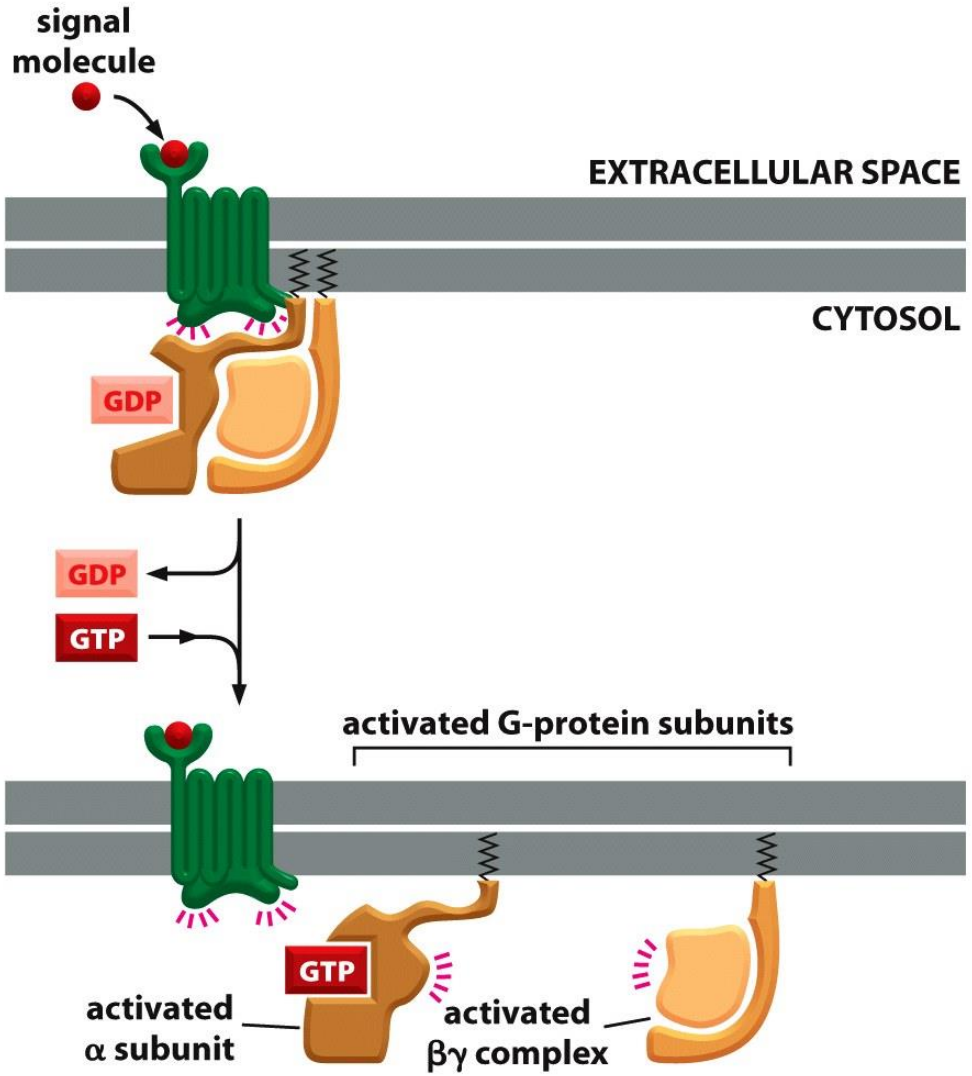
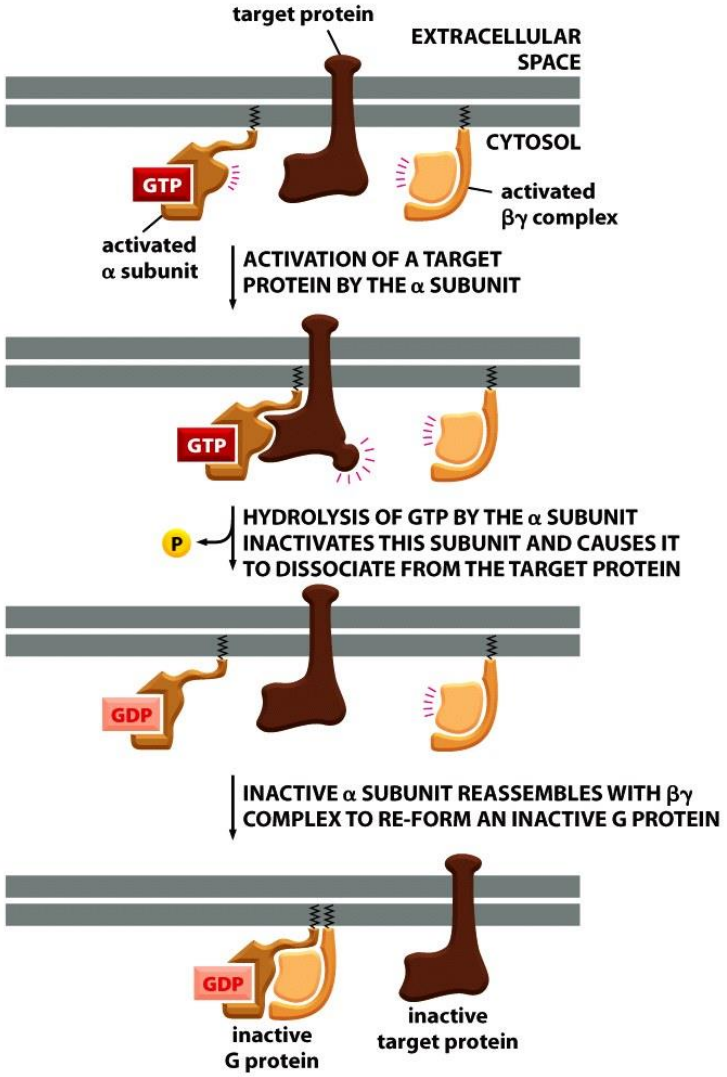


Figure 16-17b Essential Cell Biology 3/e (© Garland Science 2010)

G protein can be deactivation 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;

Epidermal growth factor (EGF) stimulates cell growth and differentiation by binding to its receptor, EGFR

Exposing functional groups in activated α subunit and βγ complex.

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

Or RGS(regulator of G protein signaling) acts as α-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_s	α	activates adenylyl cyclase; activates Ca^{2+} channels
	G_{olf}	α	activates adenylyl cyclase in olfactory sensory neurons
II	G_i	α	inhibits adenylyl cyclase
		βγ	activates K^+ channels
	G_o	βγ	activates K^+ channels; inactivates Ca^{2+} channels
		α and βγ	activates phospholipase C-β
	G_t (transducin)	α	activates cyclic GMP phosphodiesterase in vertebrate rod photoreceptors
III	G_q	α	activates phospholipase C-β
IV	$G_{12/13}$	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+ channel?

乙酰胆碱

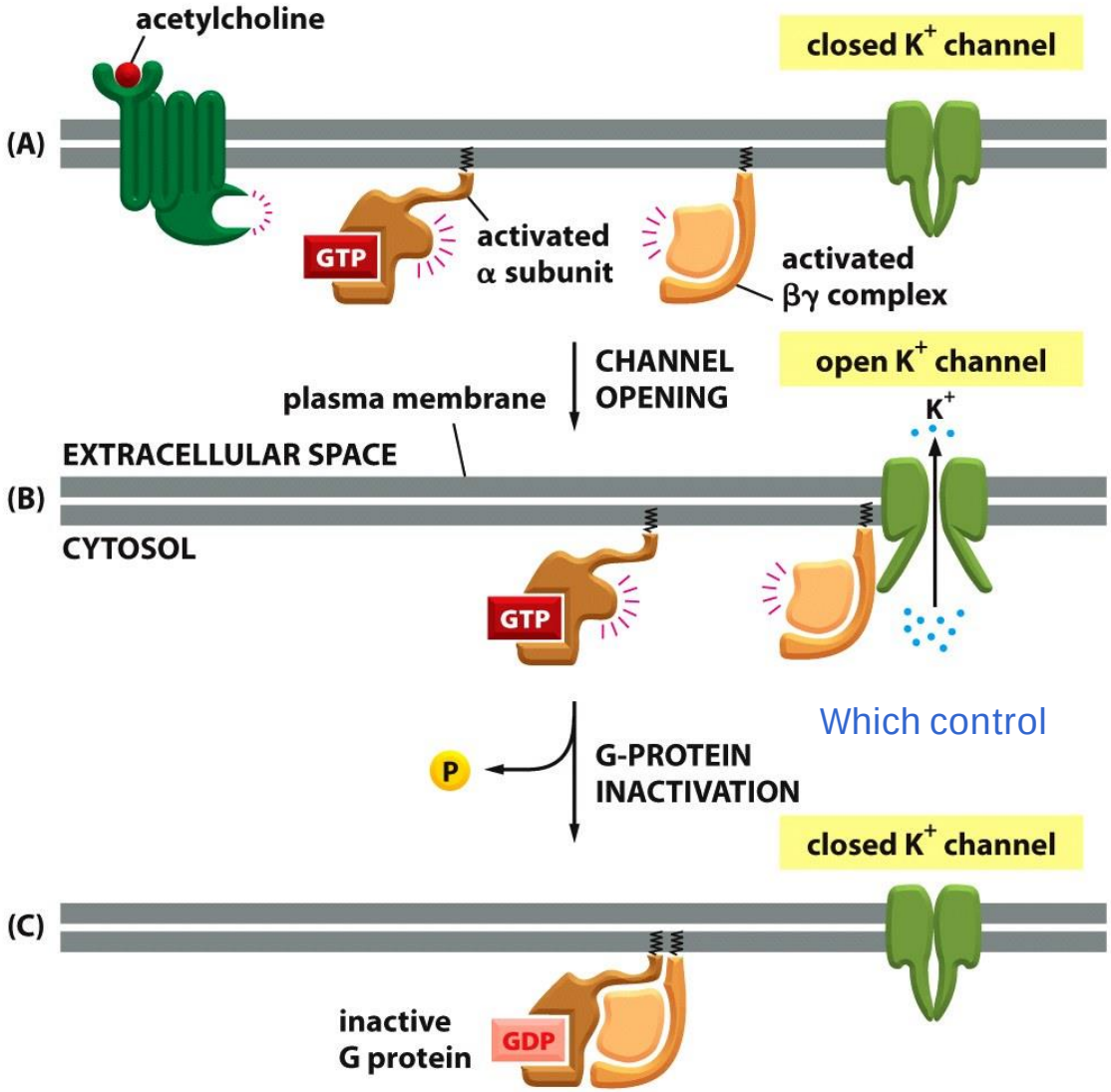

Figure 16-19 Essential Cell Biology 3/e (© Garland Science 2010)

4. How does GPCR signaling activates small messenger molecules?

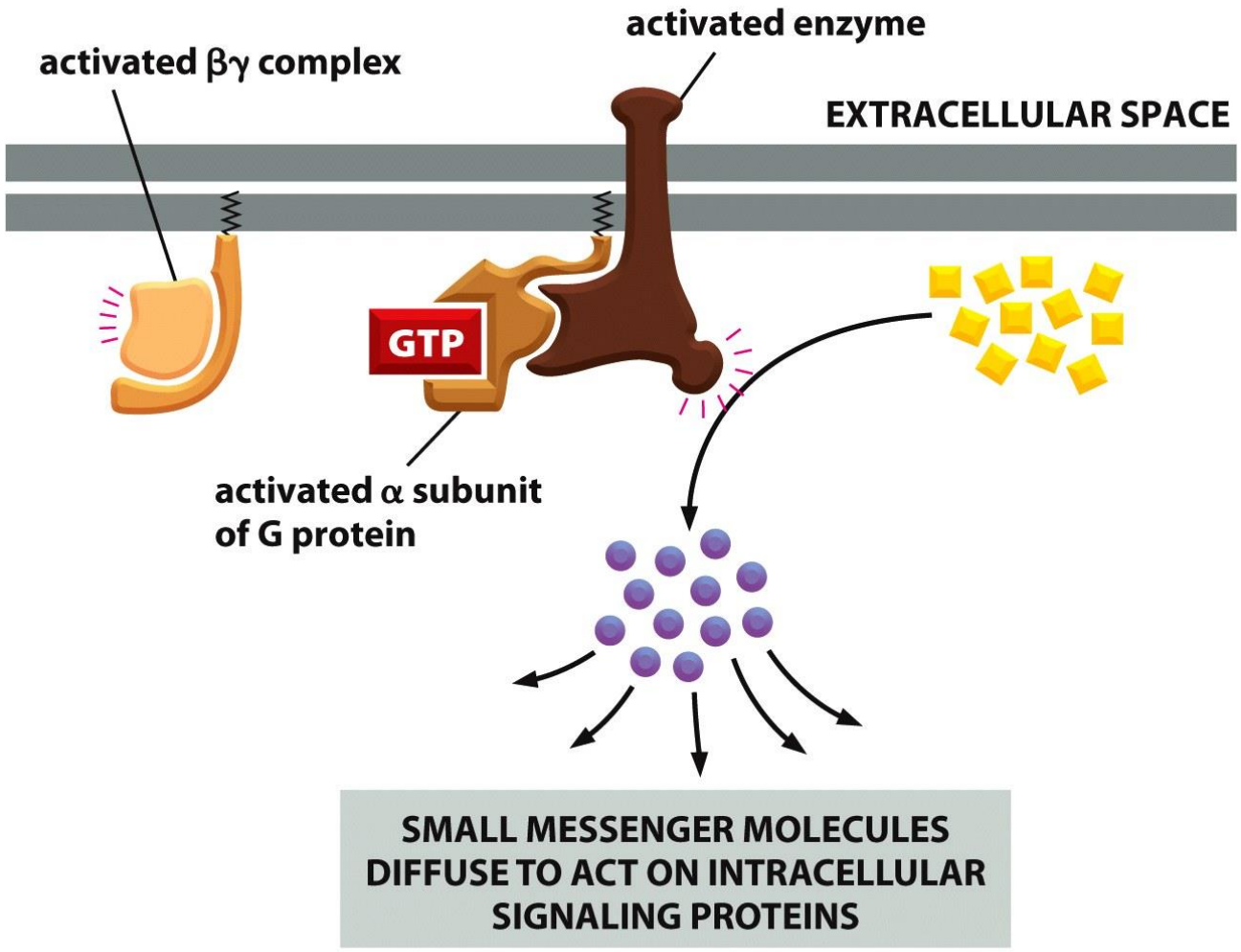

Figure 16-20 Essential Cell Biology 3/e (© Garland Science 2010)

Small messenger molecules downstream of GPCR signaling

- ♥cAMP
- ♥DAG and IP3 through phospholipase C-β
- ♥Ca2+
- ♥cGMP/cAMP-gated ion channels in smell and vision

4.1 How does GPCR activates cAMP?

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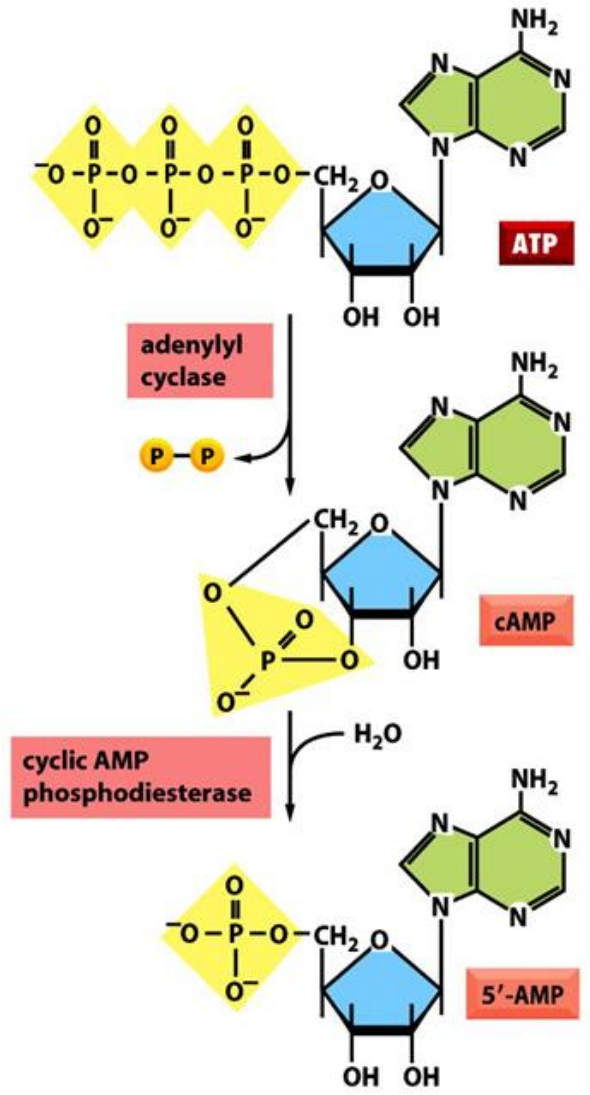
cAMP as an important second messenger



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: Gs (stimulatory G protein) activates adenylyl cyclase; while Gi (inhibitory G protein) Inhibits adenylyl cyclase.

血清素

Neuron cells senses serotonin to produce cAMP instantly

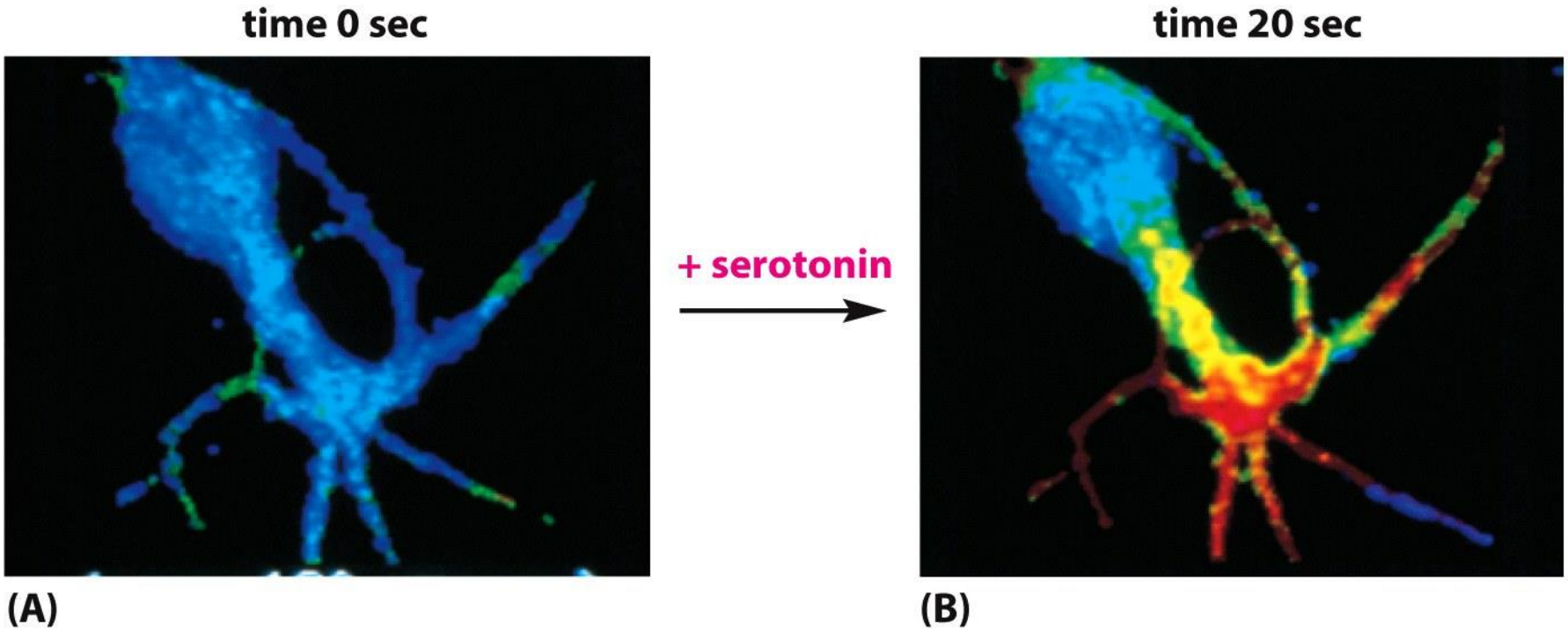
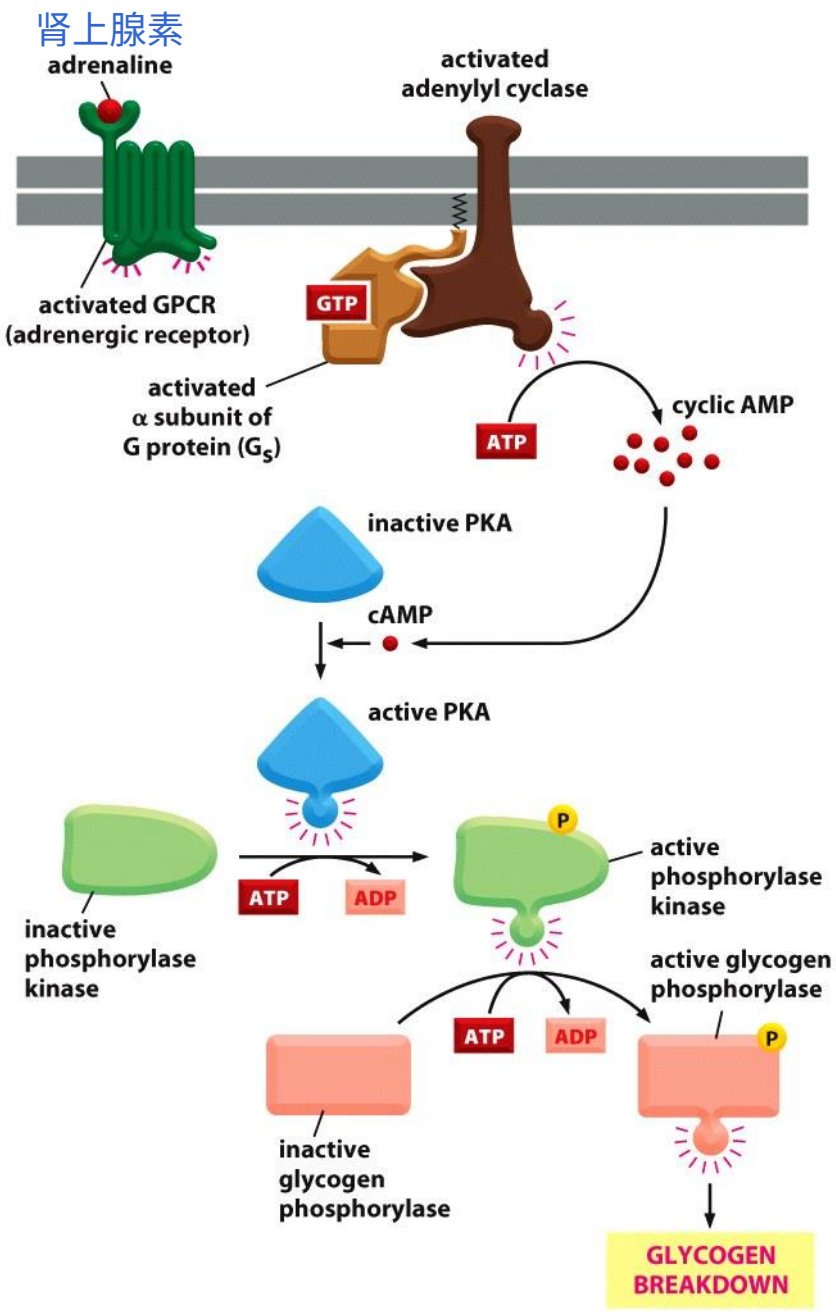


Figure 16-22 Essential Cell Biology 3/e (© Garland Science 2010)

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

肾上腺素



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PKA functions in fast manner

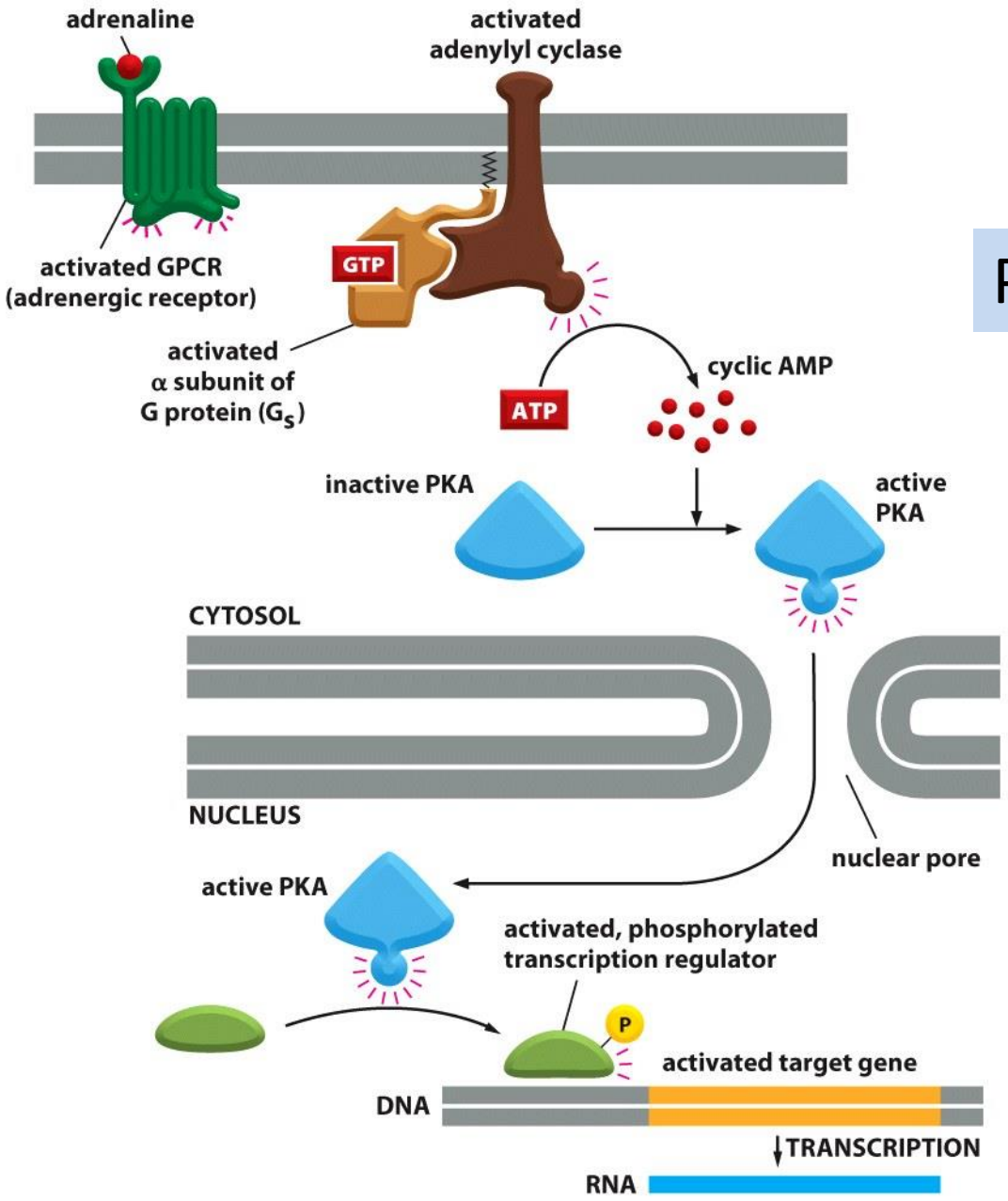


Figure 16-24 Essential Cell Biology 3/e (© Garland Science 2010)

PKA works in slow manner

The transport time

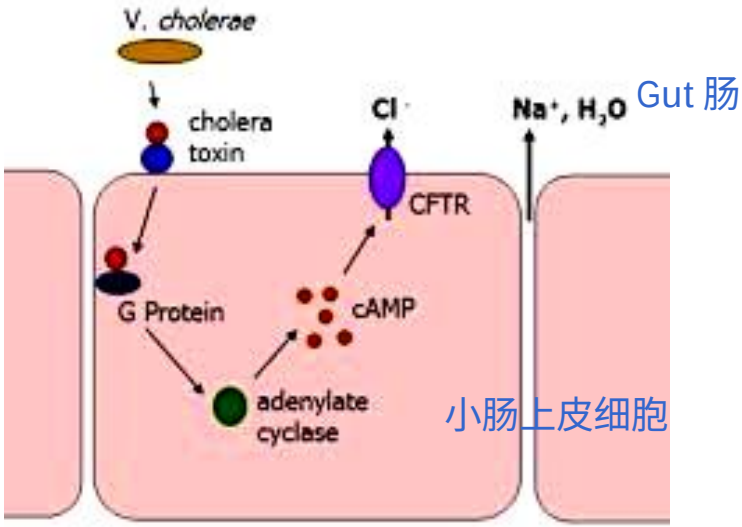
15.4-cAMP_signaling.mov

霍乱病毒

Mechanism of cholera toxin

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

痢疾，腹泻



Mechanism of pertussis toxin

百日咳

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

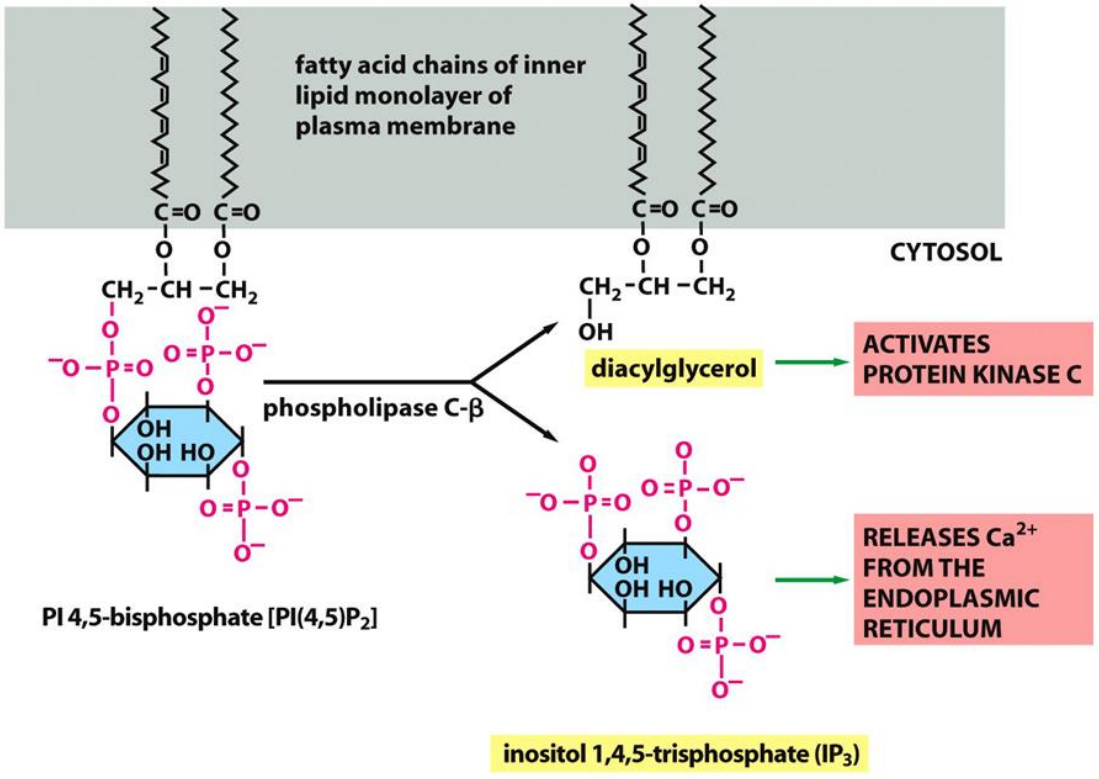
4.2. Signaling through phospholipase C-β

- Gq proteins activate phospholipase C-β.
- Substrates for phospholipase C-β is PI(4,5)P2
- Cleavage of PIP2 results in production of IP3 and diacylglycerol (DAG)

Table 15–2 Some Cell Responses in Which GPCRs Activate PLCβ

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-β



4.3.How does GPCR trigger Ca2+ release and PKC activation?

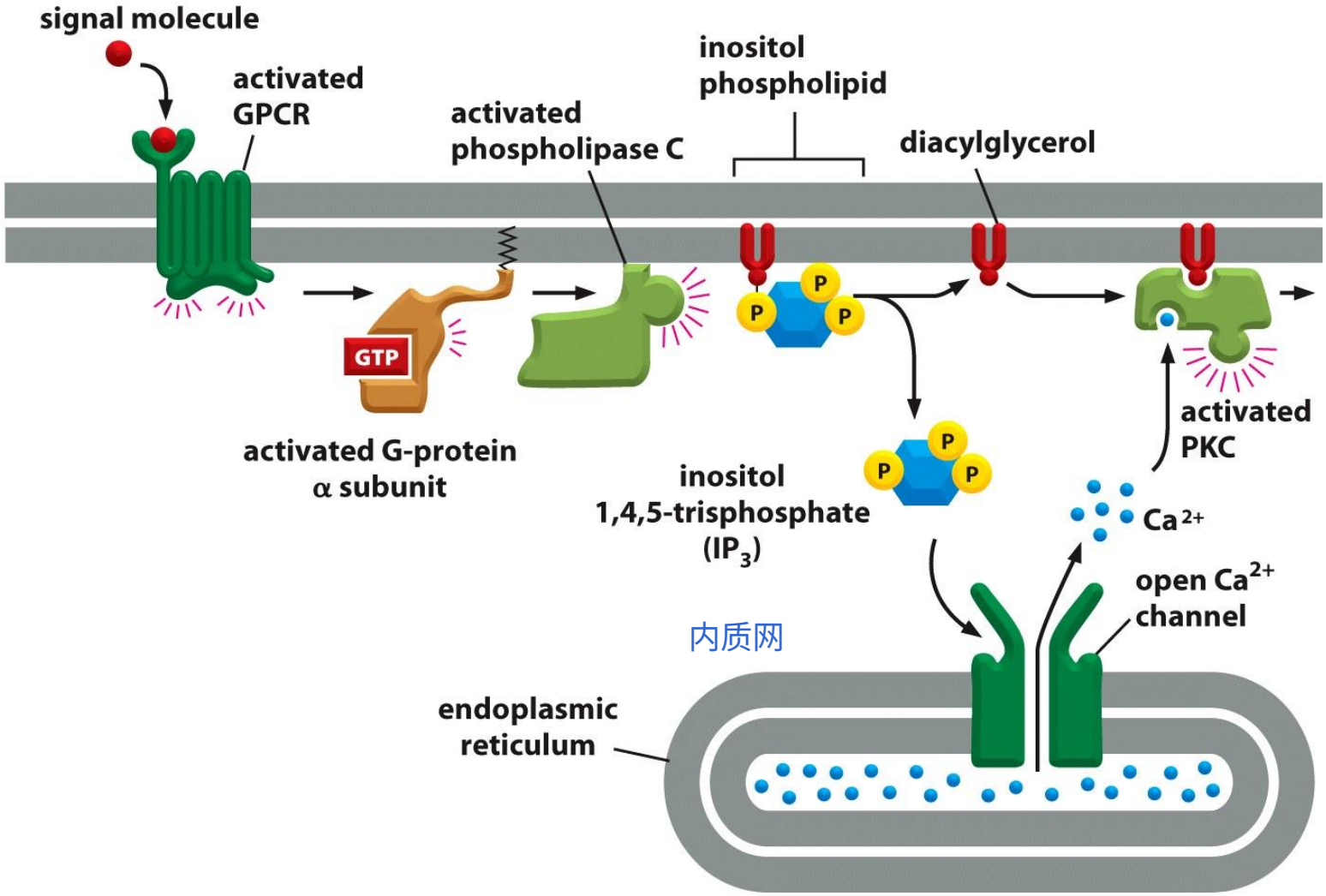


Figure 16-25 Essential Cell Biology 3/e (© Garland Science 2010)

Ca2+ function

- Rise in Ca2+ in fertilized egg cytosol initiates embryonic development
- Triggers muscle contraction
- Triggers secretory vesicle to secret
  分泌 囊泡

Fertilized egg show waves of Ca2+ from the site of sperm entry

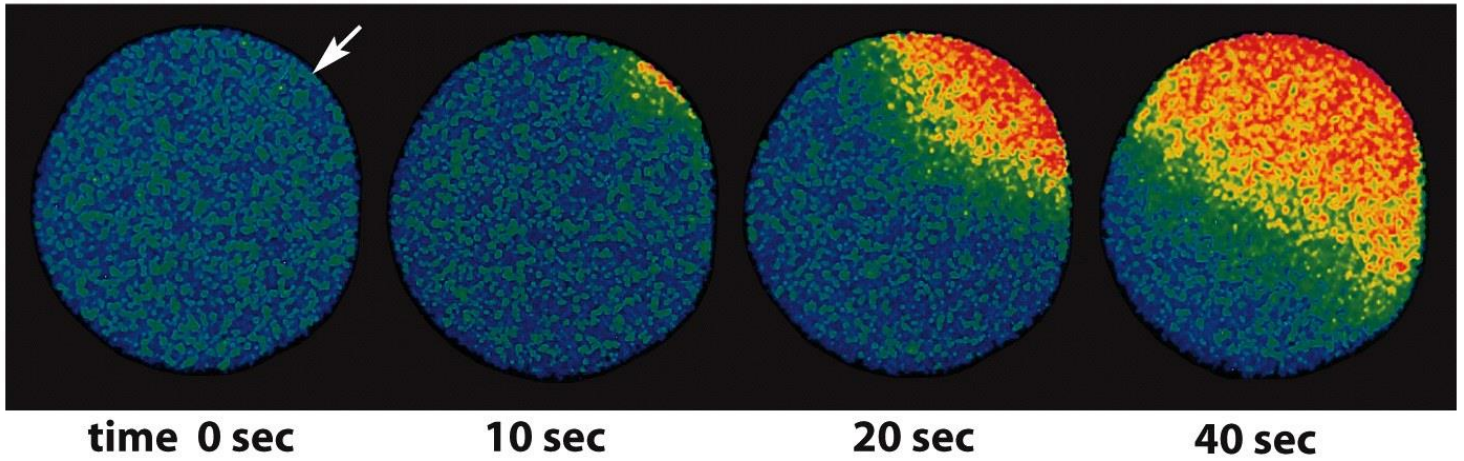
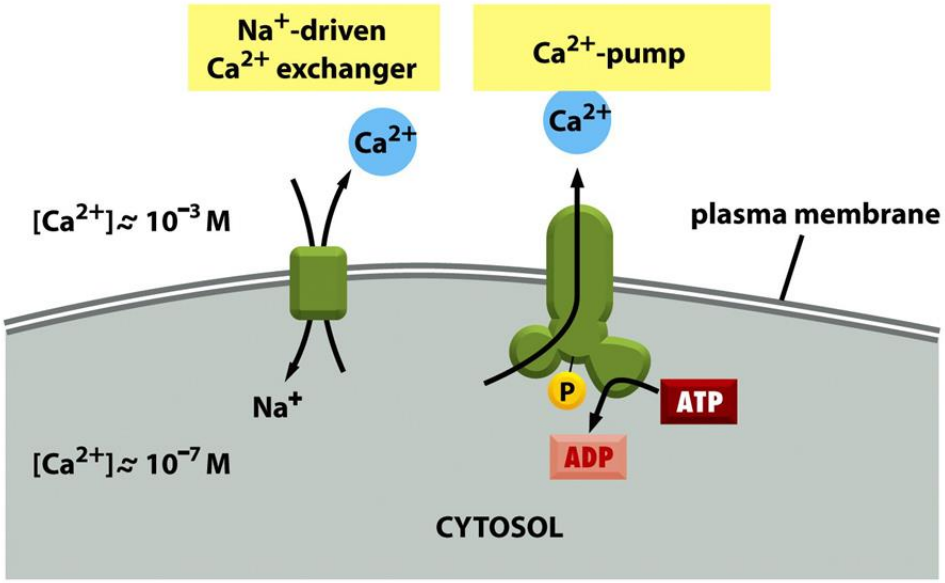
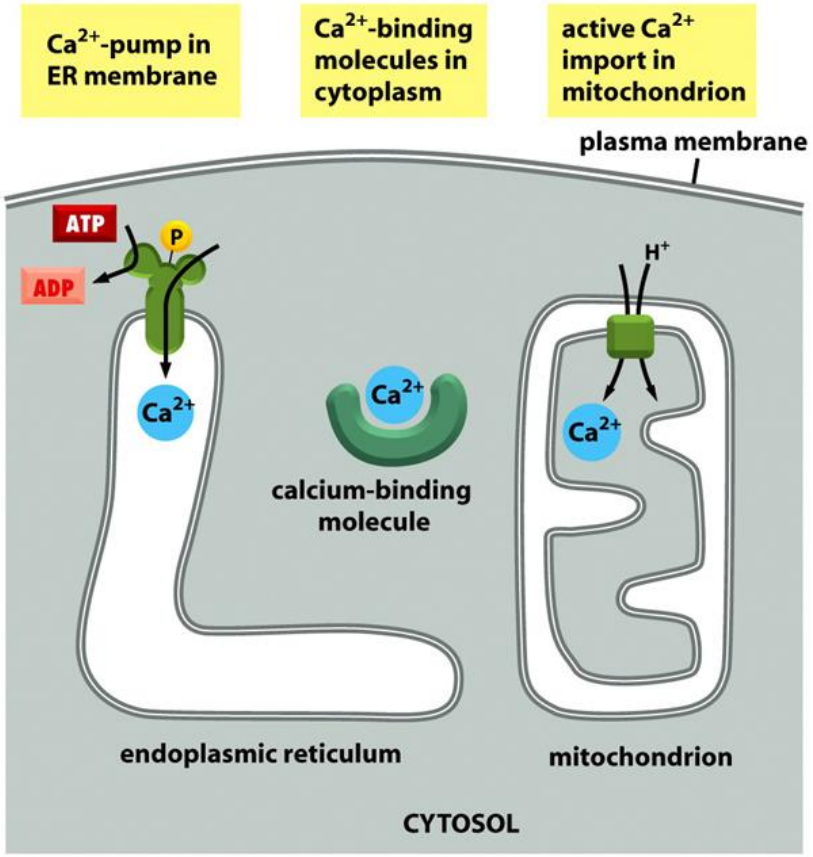


Figure 16-26 Essential Cell Biology 3/e (© Garland Science 2010)

How do cells keep low Ca2+ in cytosol?

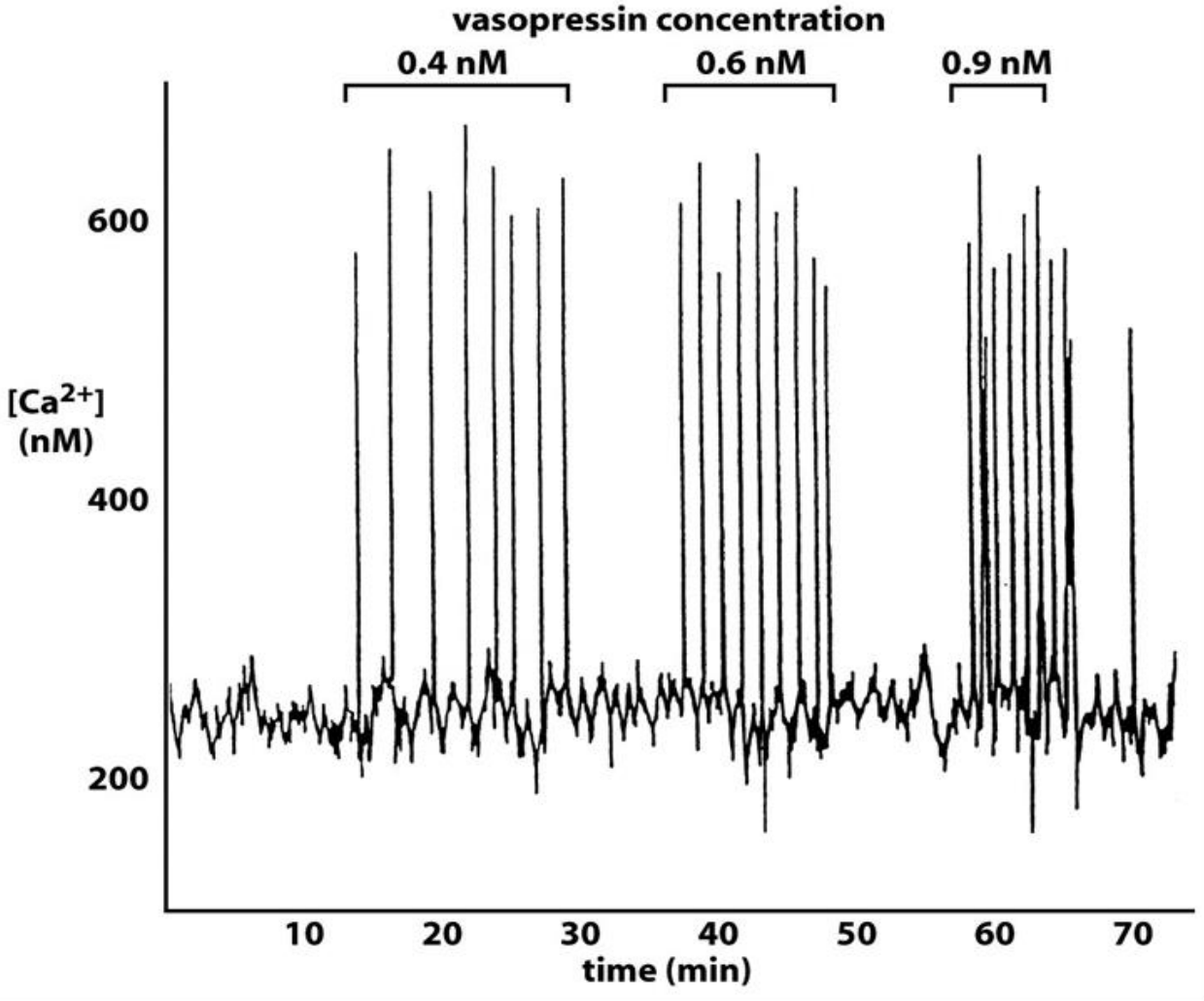
5 different ways to keep Ca2+ low:



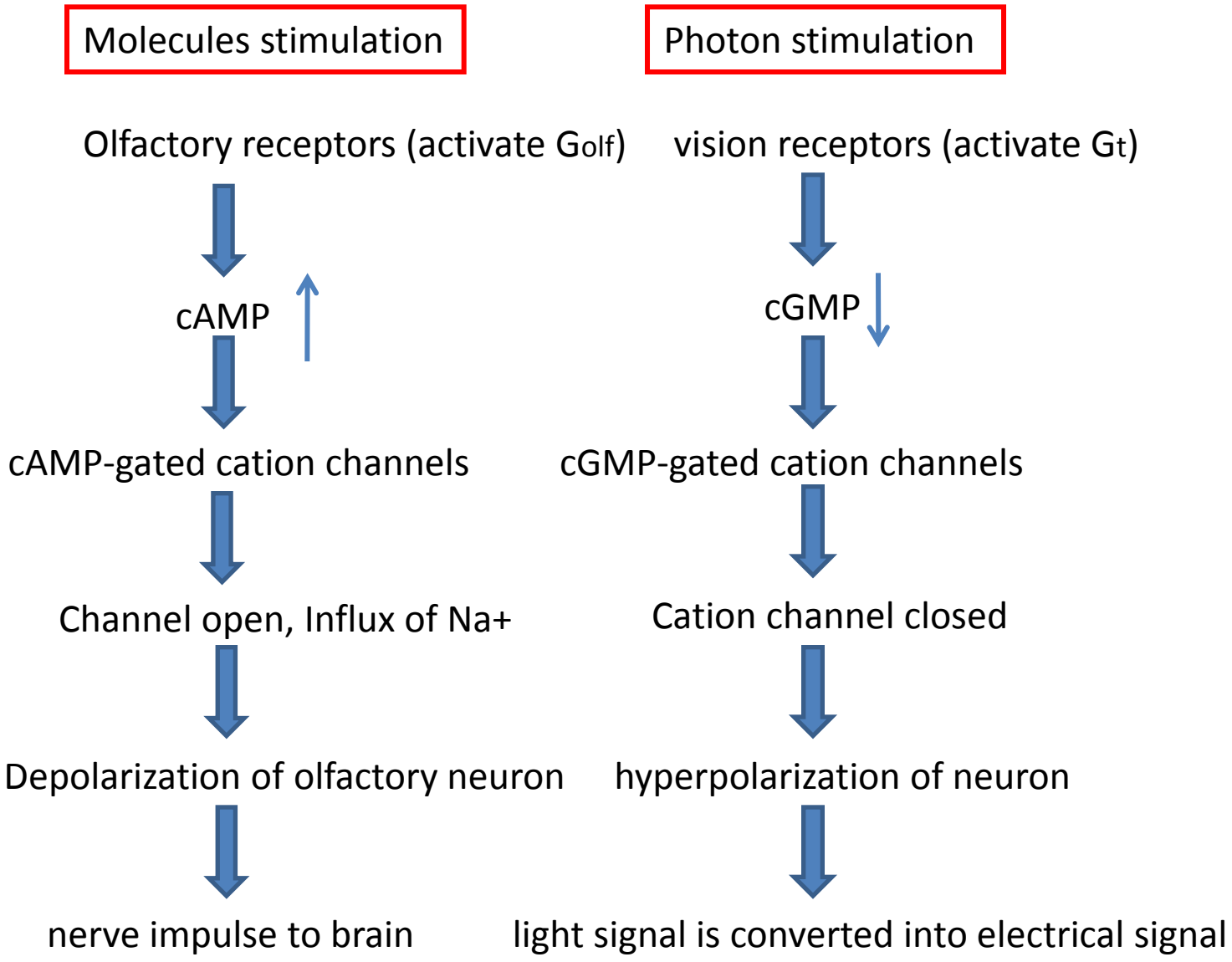


Ca2+ oscillation in cells in response to stimuli

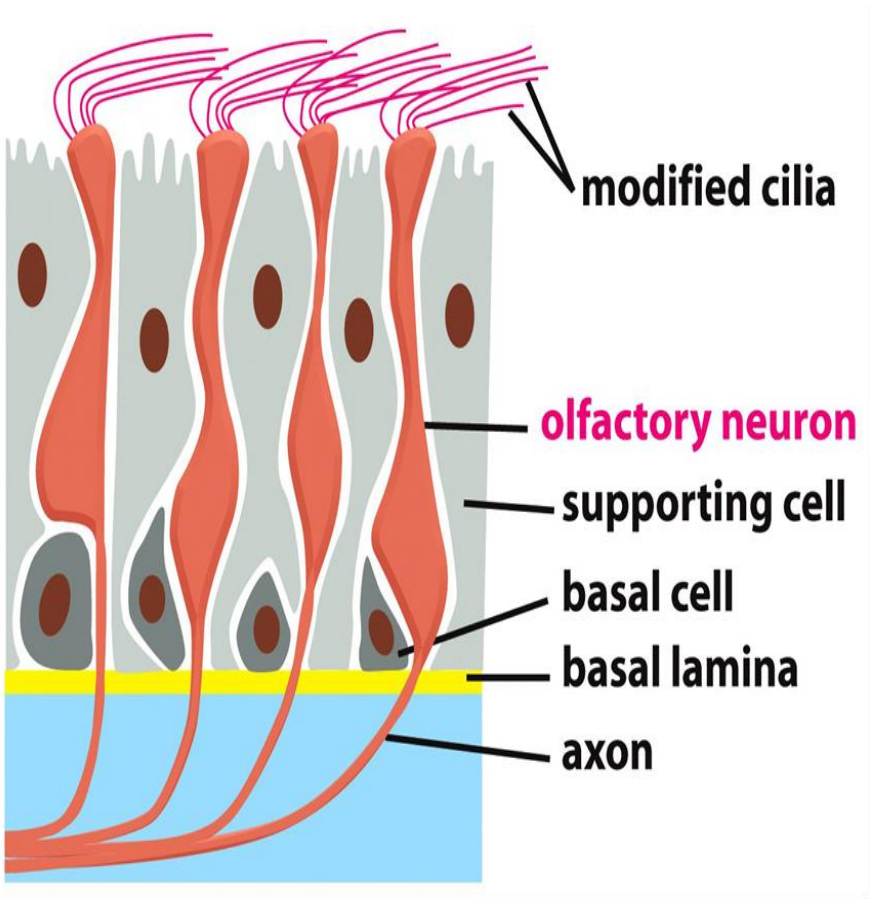
抗利尿激素



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

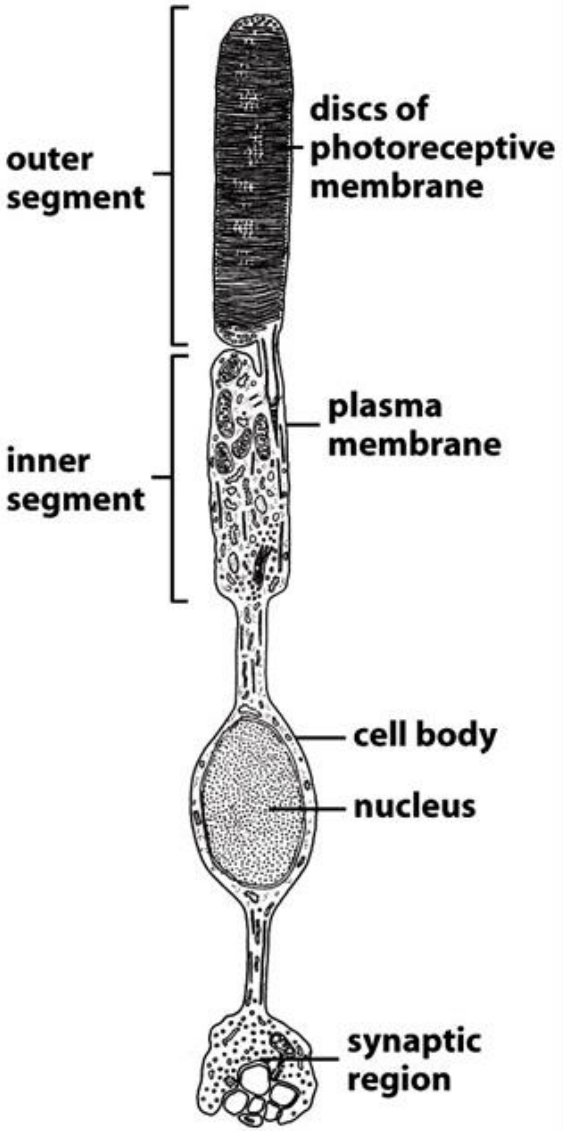


Over 10,000 smells can be differentiated by ~ 350 distinct receptors for human.

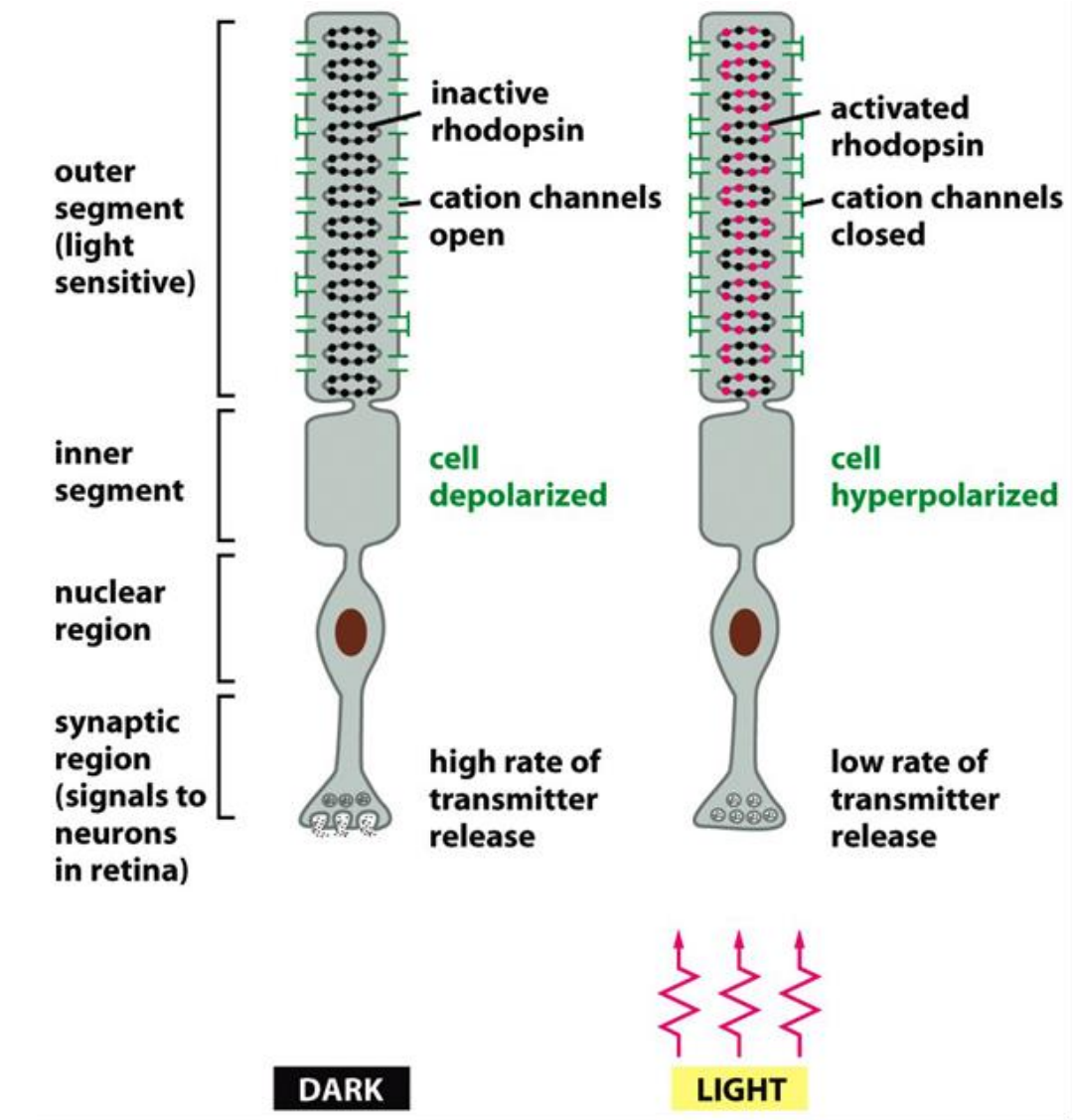


视杆细胞

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,

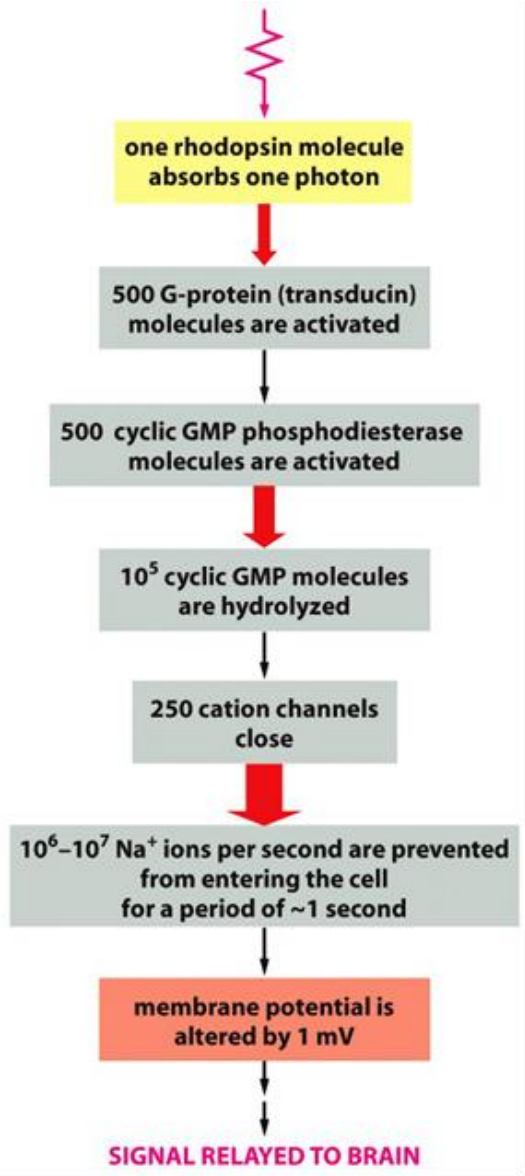
↓

activates Gt(transducin),

↓

activate cGMP phosphodiesterase.

Signal amplification in transduction



A catalytic cascade due to enzymatic activity

5. GPCR desensitization

- (1). G-protein α-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). Ca2+ 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. Tryosine-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

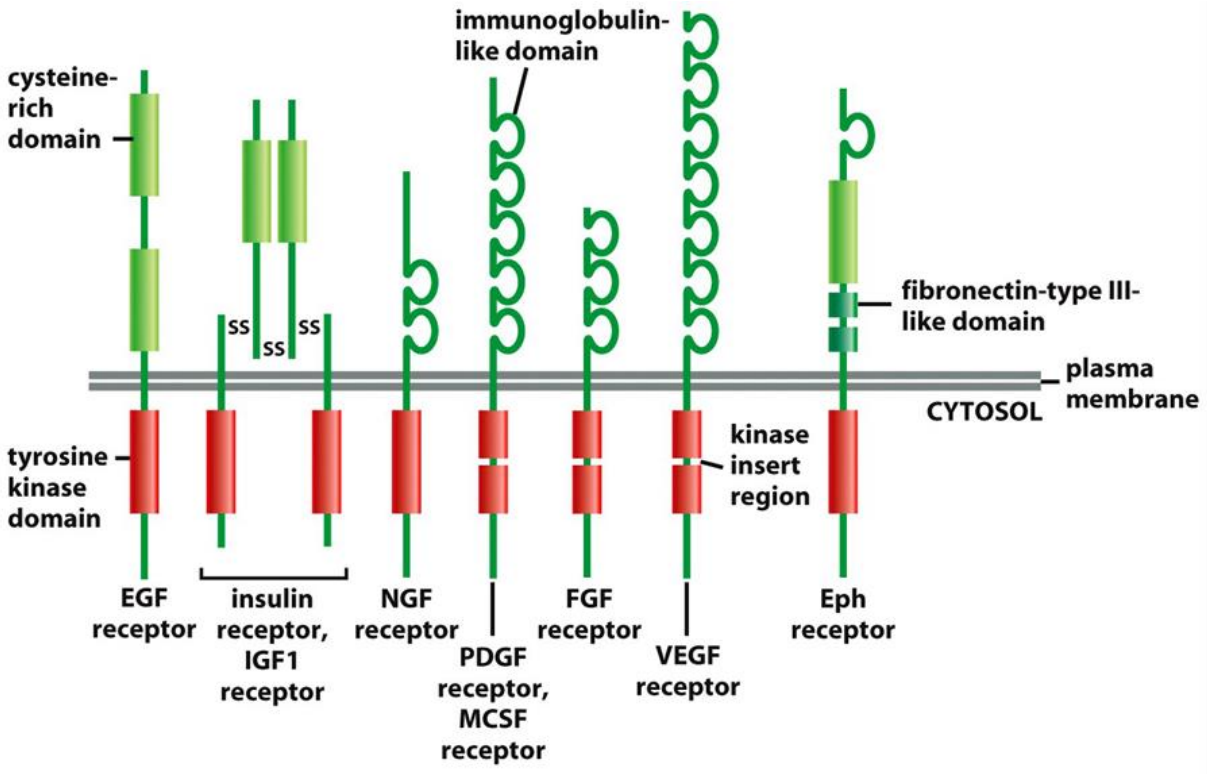
1.1. Configurations of some RTKs

Three domains :

- Extracellular region: interact with ligand
- One single transmembrane domain
- Intracellular region: tyrosine kinase activity

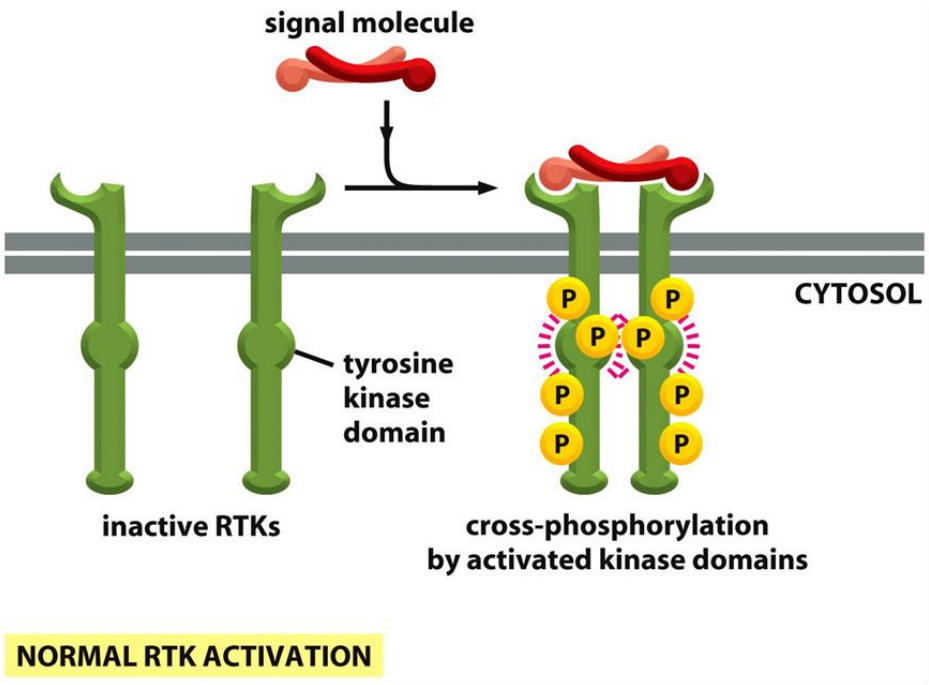
Human genome encodes ~ 60 RTK genes

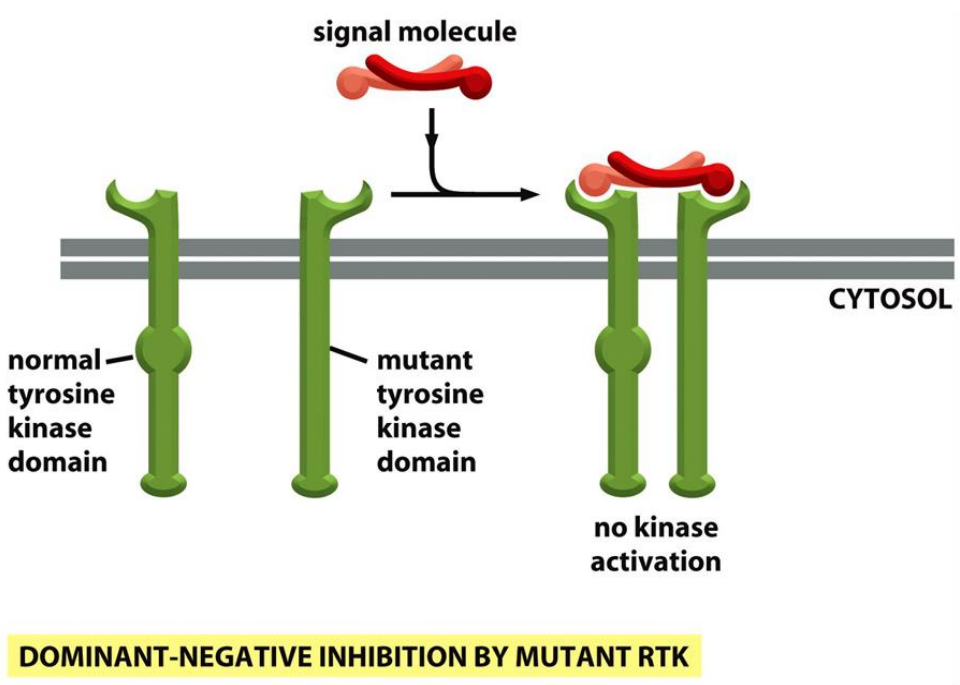
Upon ligand binding, RTK usually dimerize and transautophosphorylate themselves



How a mutant RTK acts as dominant negative manner

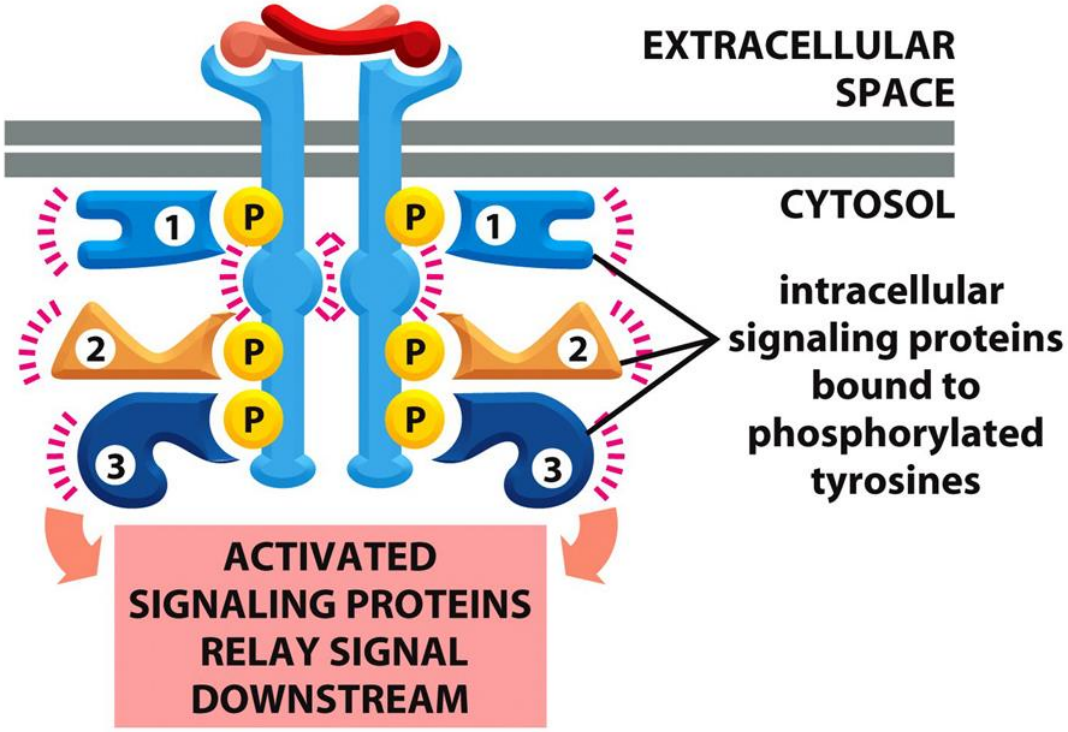
Dominant negative: a mutant protein exhibits an inhibitory manner for wild type protein.





1.2 Phosphorylation on RTK has dual roles

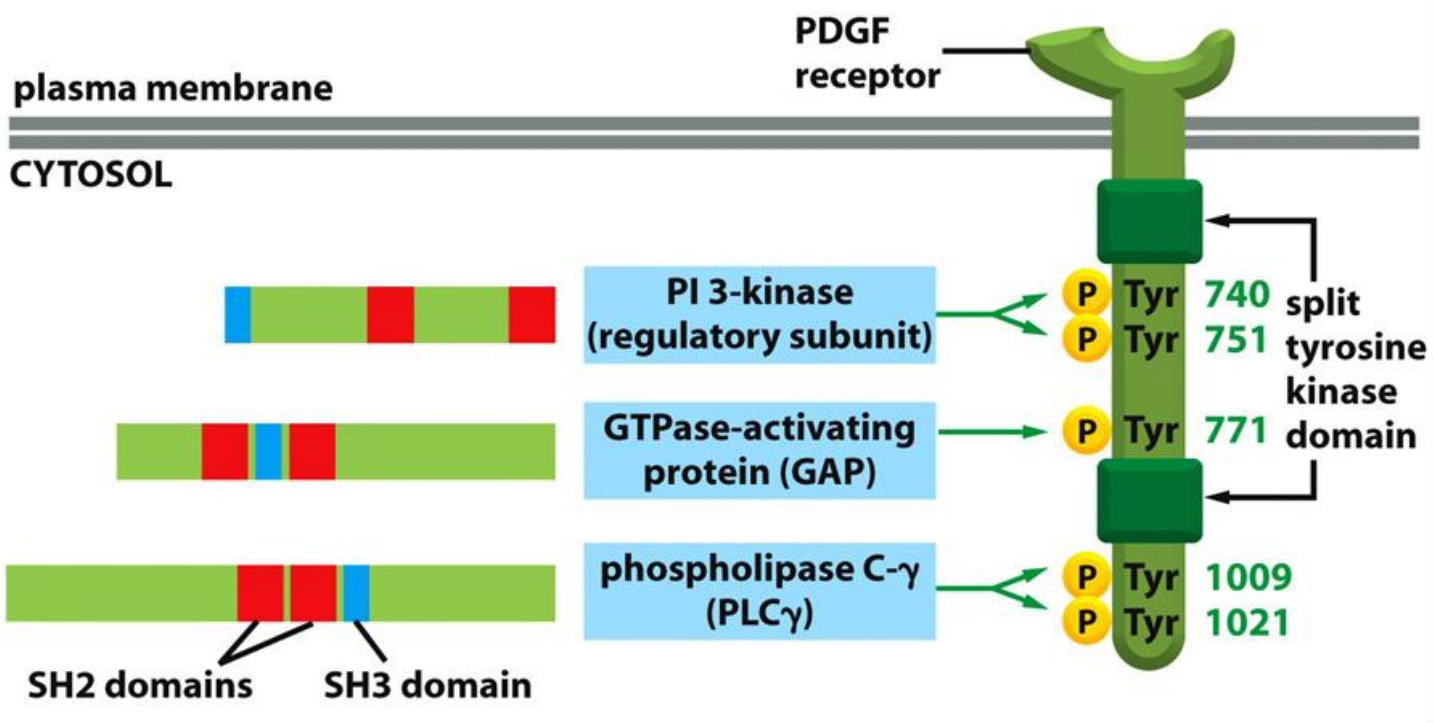
- Activates RTK kinase activity
- Introduce phospho-Tyr that can recruit other protein factors to relay signals



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

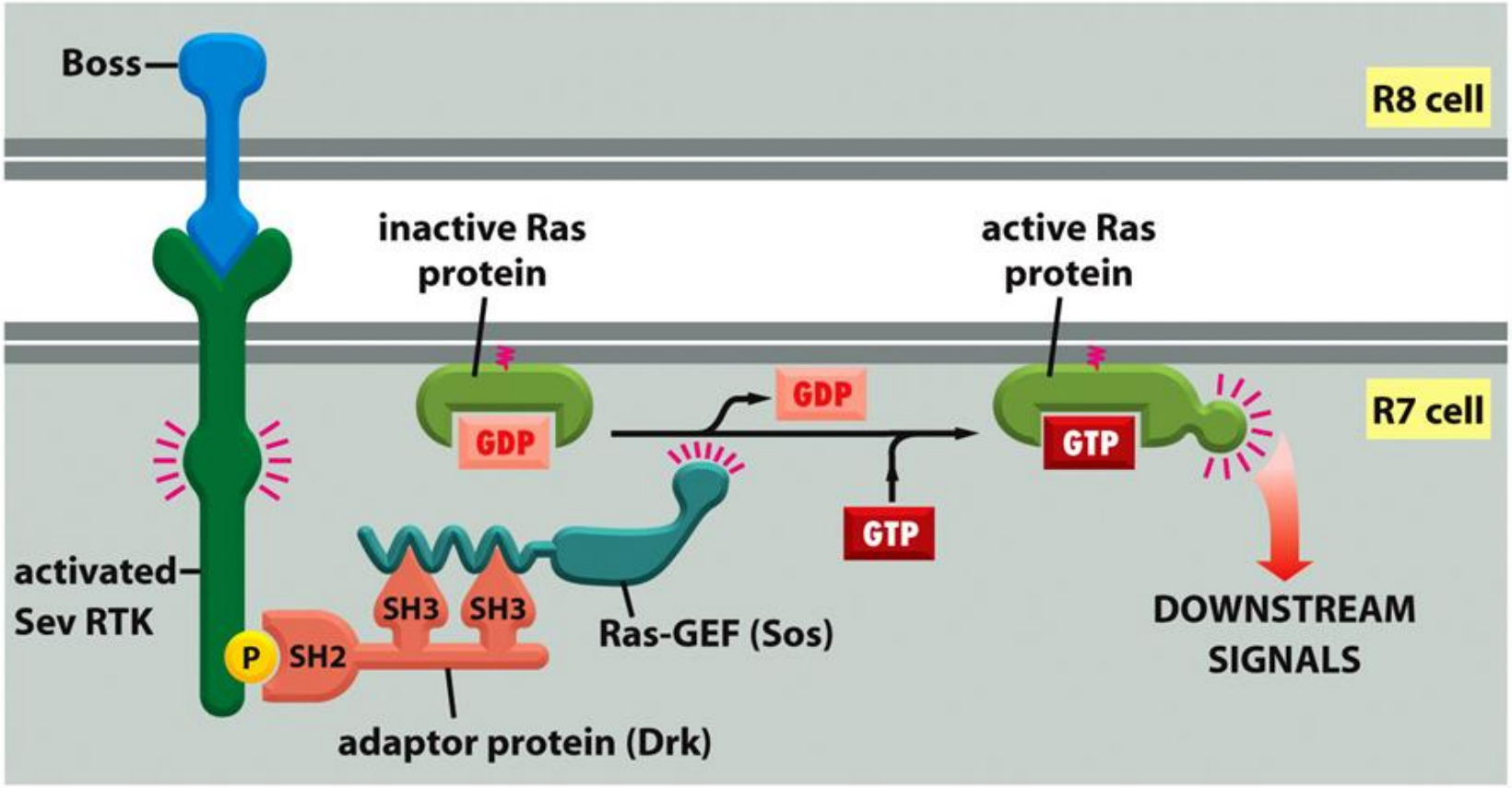
- Ras --- name derived from *Rat sarcoma virus* , found by RSV infection.
- Small monomeric GTPase, ~20KD, weak GTP hydrolysis activity.
- Highly mutated in human cancers,~ 30%, in pancreatic cancer, mutation rate 95%.
- Pivotal roles in cell proliferation, survival, motility, etc.
- tethered on lipid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

Table 15–5 The Ras Superfamily of Monomeric GTPases

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

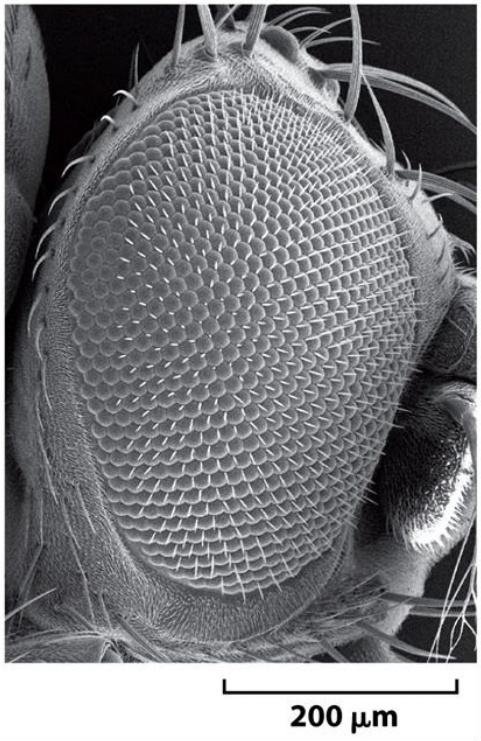
Deficiency of *bride of sevenless (Boss)* causes this failure, too

Sev- RTK receptor

Boss- Sev's ligand

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*

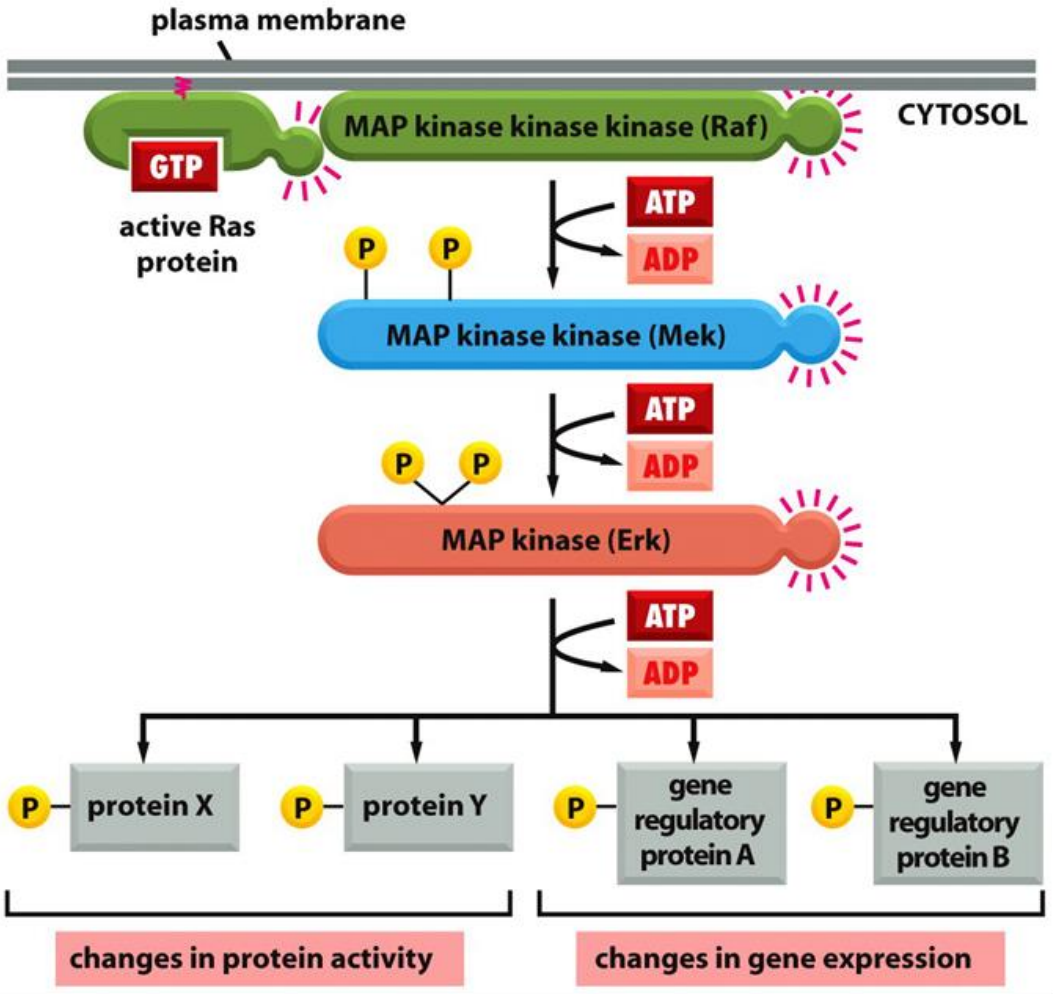
Ras locates downstream of *Sev*



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

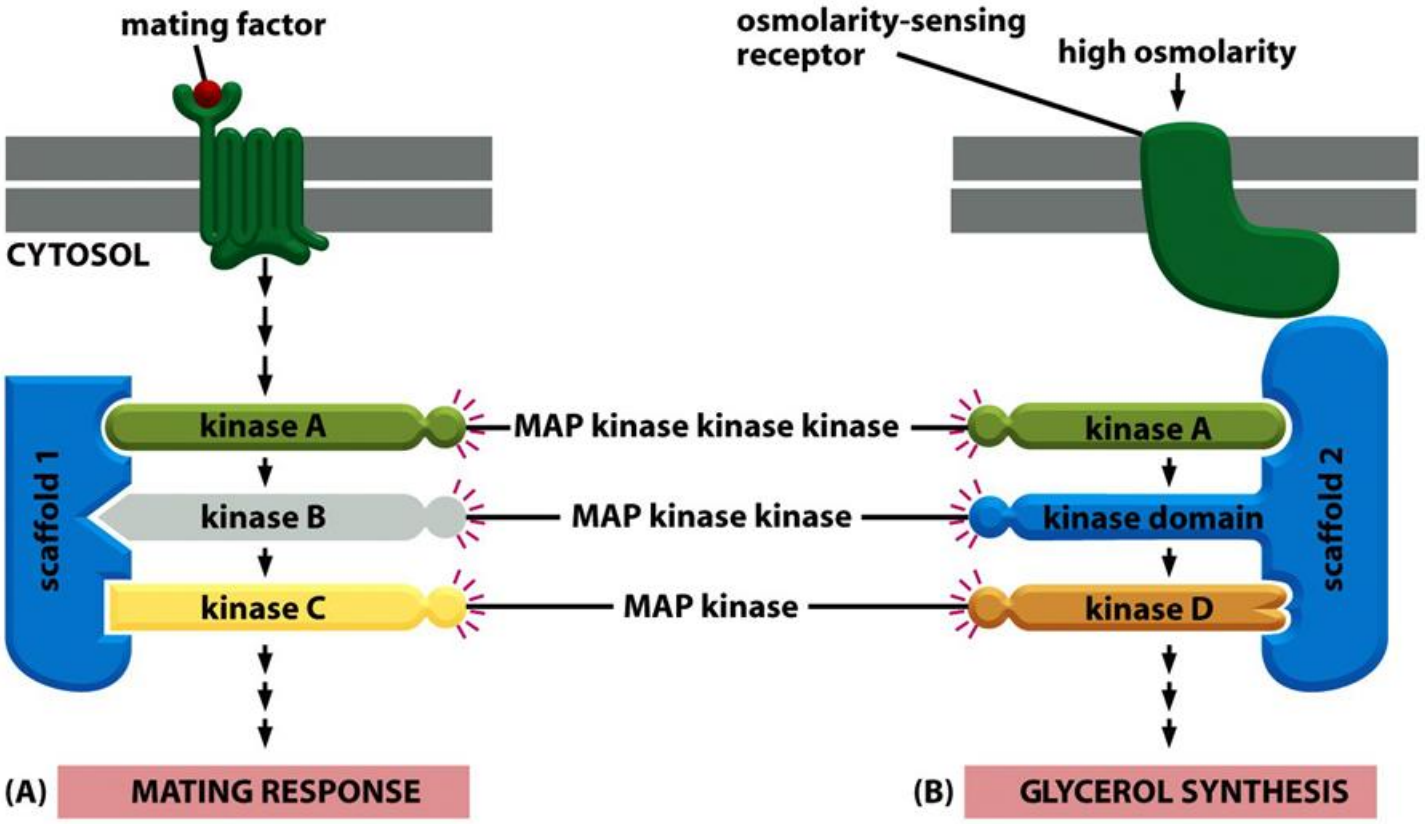
分裂素

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



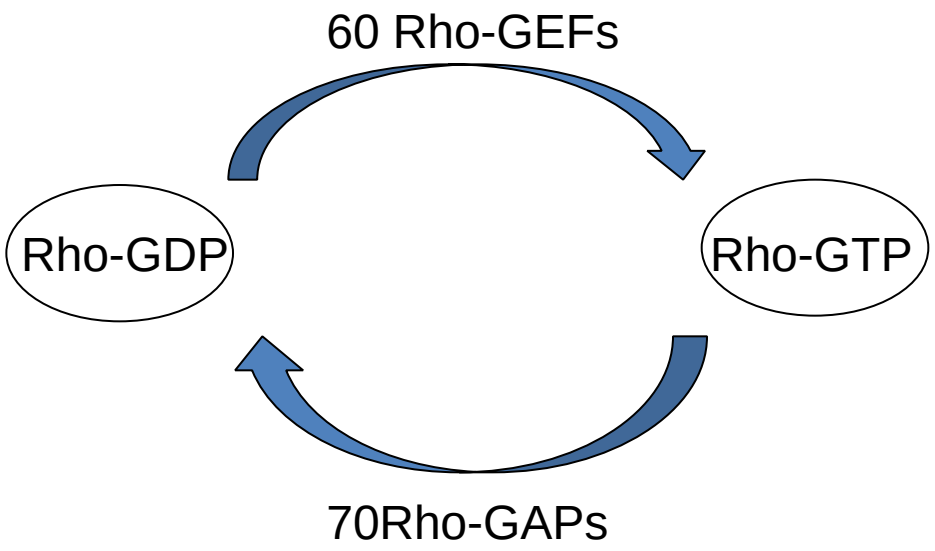
MAP kinase pathway controls both protein activity and gene transcription

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



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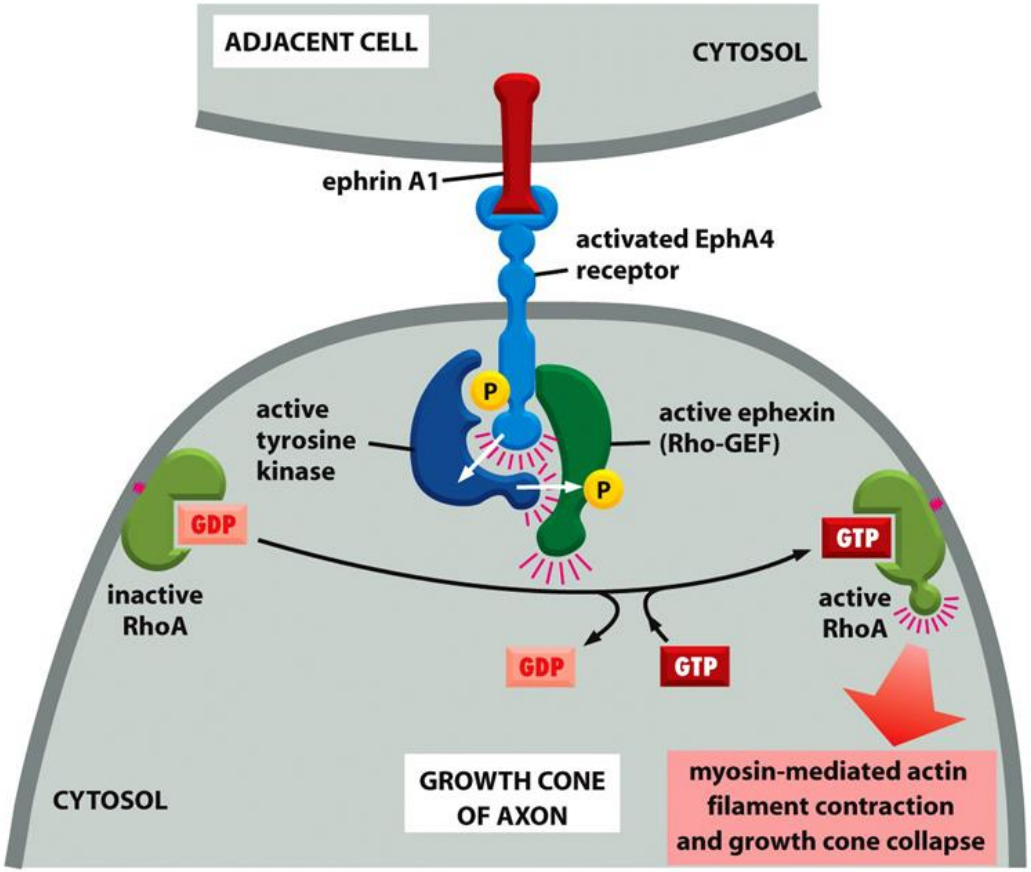
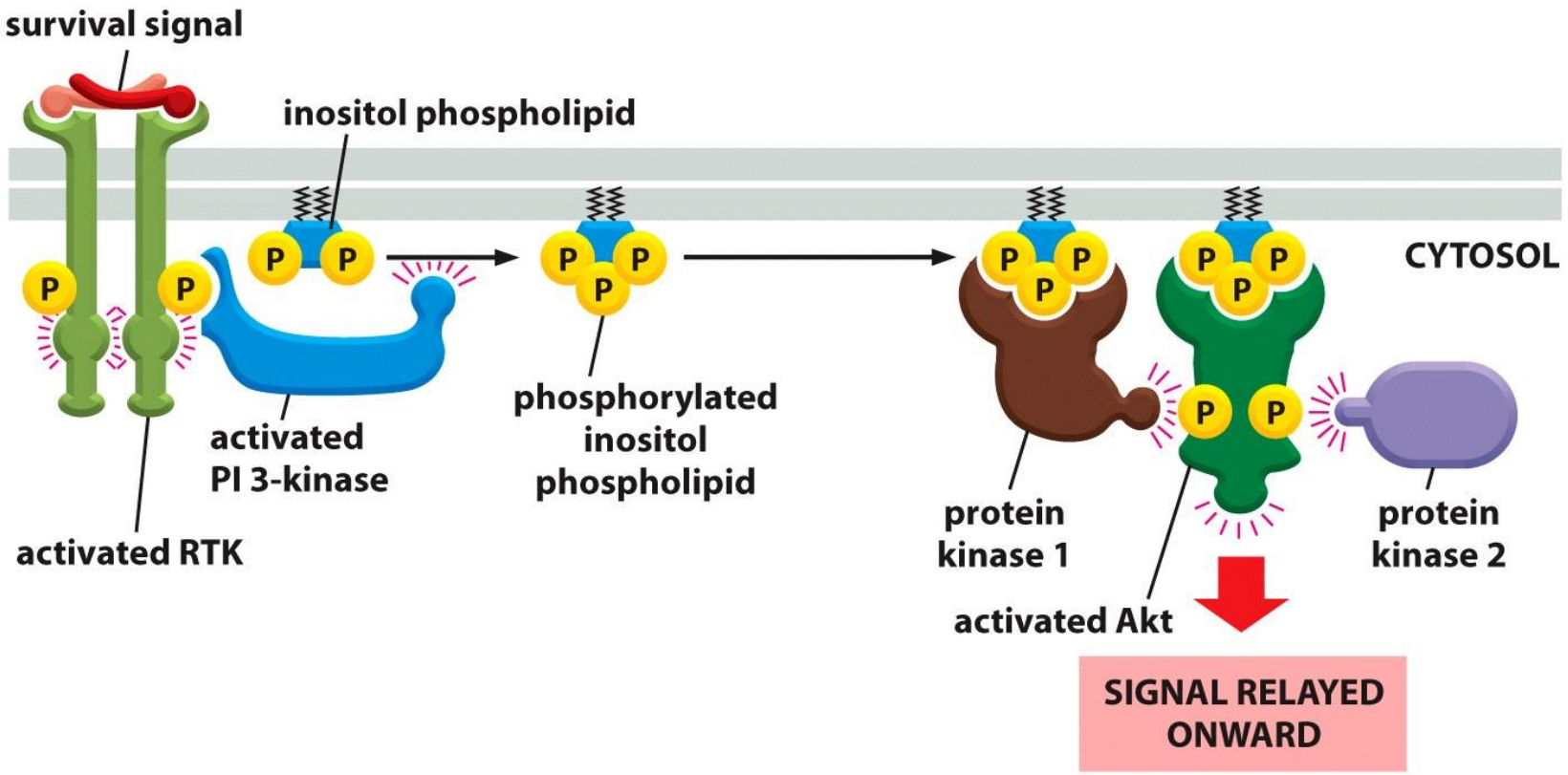
For example: ephrin induces growth cone collapse



1.3.3. PI3K pathway promotes cell growth and survival



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PI3K and PTEN in controlling PIP3

- PTEN: Phosphatase and tensin homolog

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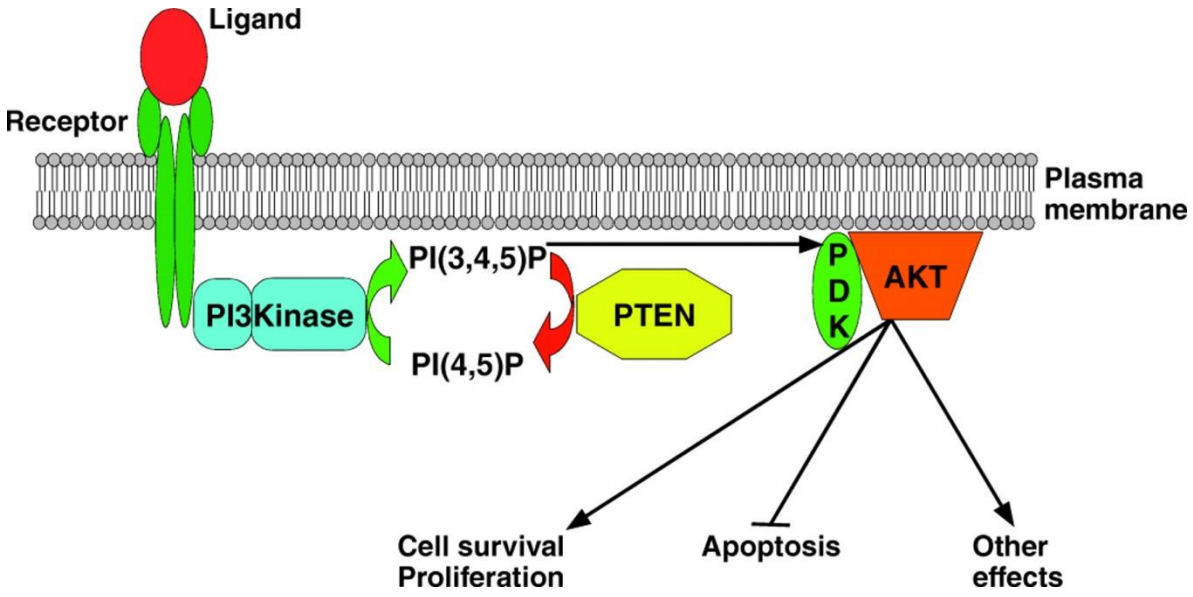


PI3K hyperactivation and PTEN loss of function frequently occur in human cancers

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

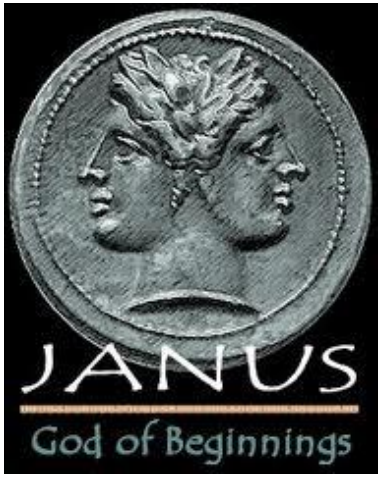


Table 15–6 Some Extracellular Signal Proteins That Act Through Cytokine Receptors and the JAK–STAT Signaling Pathway

SIGNAL PROTEIN	RECEPTOR-ASSOCIATED JAKs	STATS ACTIVATED	SOME RESPONSES
γ-interferon	JAK1 and JAK2	STAT1	activates macrophages
α-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?

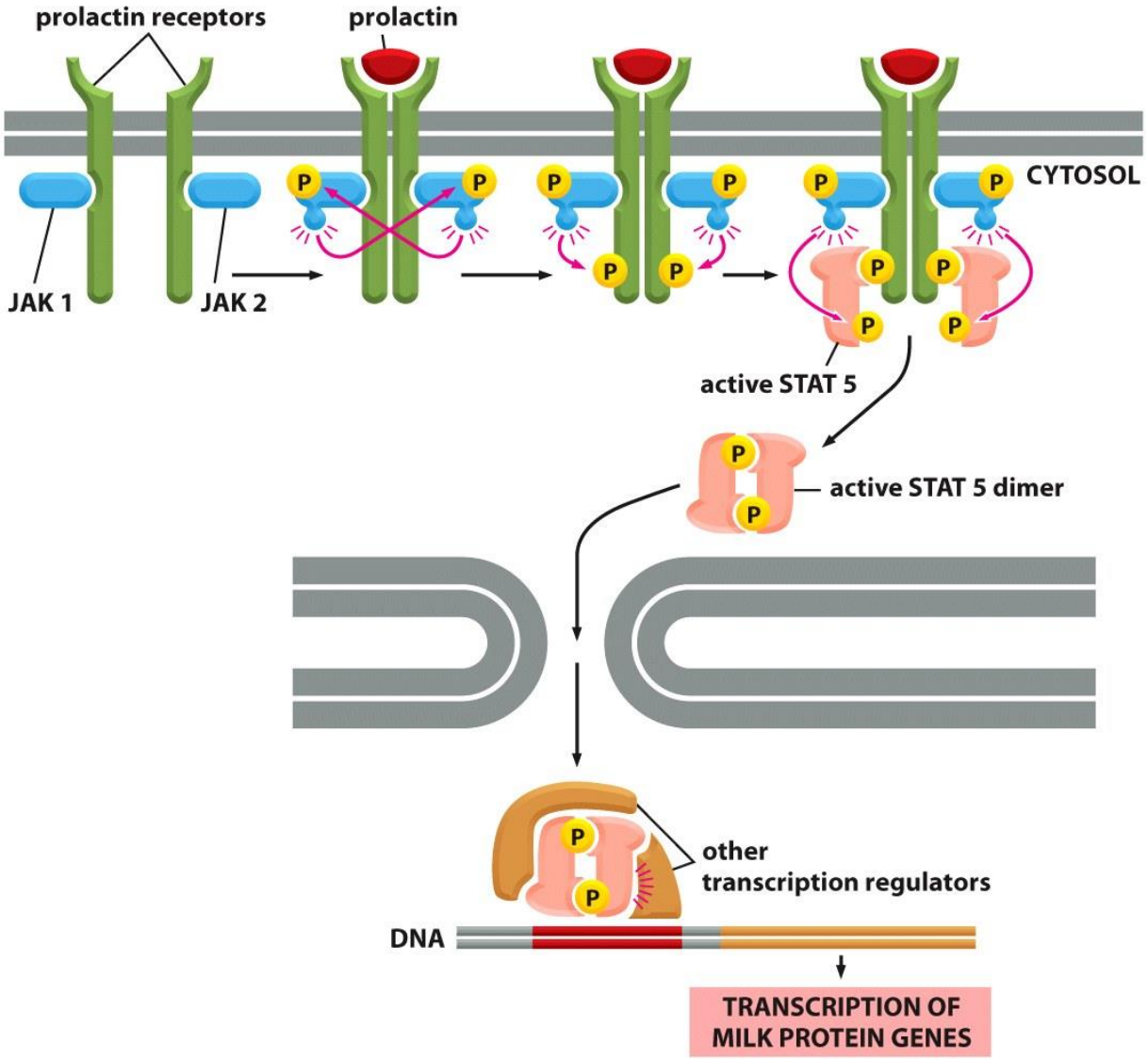


Figure 16-39 Essential Cell Biology 3/e (© Garland Science 2010)

2.2.2. Src tyrosine kinase family

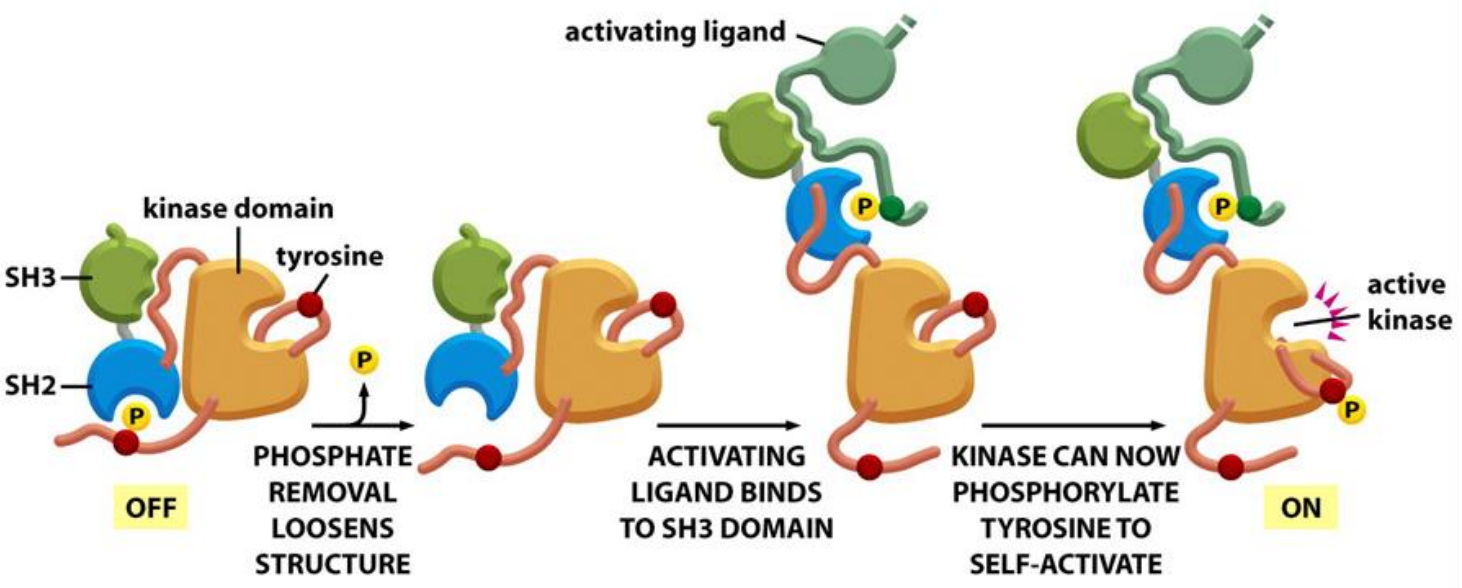
♦The largest family of cytoplasmic tyrosine kinases

♦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

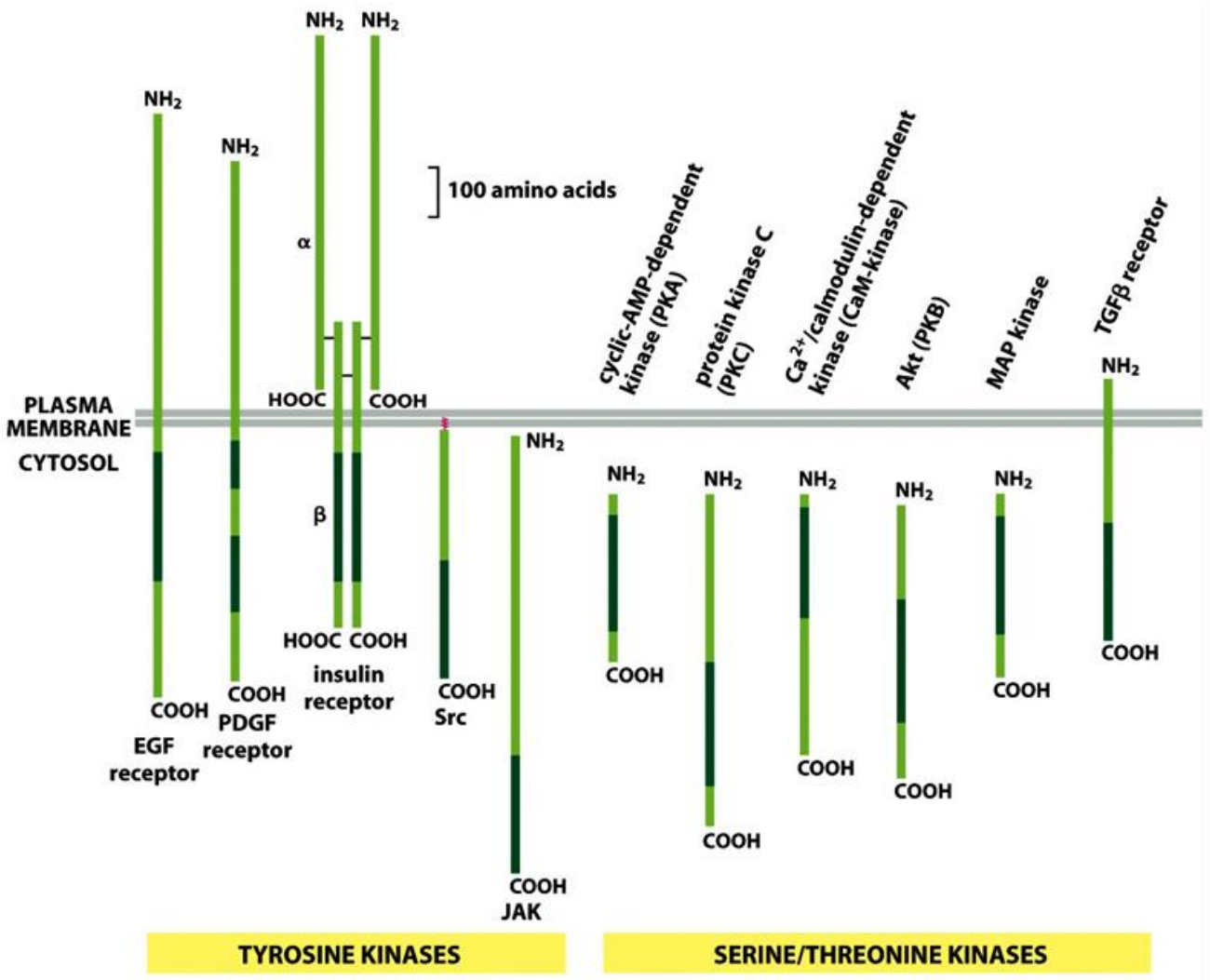
3. Serine/Threonine kinase receptor

- 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 β superfamily (derived from Tumor growth factor), Secreted and dimeric proteins, ~30-40 members for human, two categories:

  TGFβ/ativin family

  bone morphogenetic protein (BMP) family
- Control diverse activity in differentiation, proliferation, cell death, development, etc.

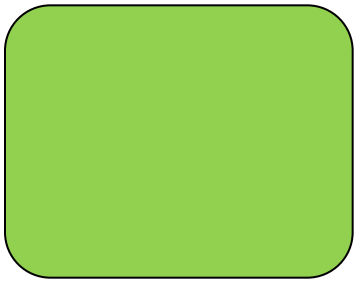
A summary and comparison for Tyrosine kinase and Ser/Thr kinase



4. Histidine-kinase-associated receptors

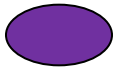
Mediates Bacterial chemotaxis response

Sugars
Amino acids
Peptides,



Attractants

bacteria



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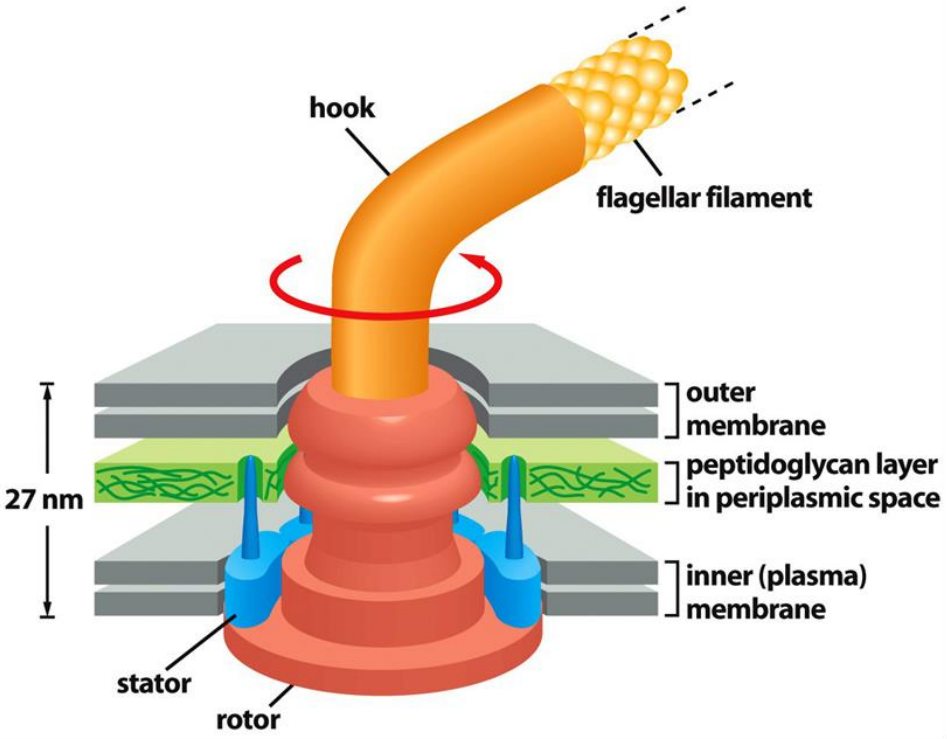
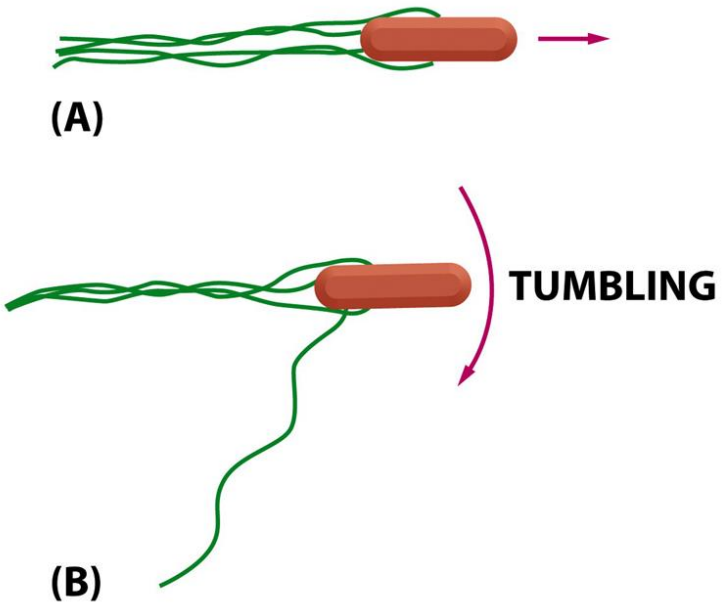
Noxious chemicals

repellents

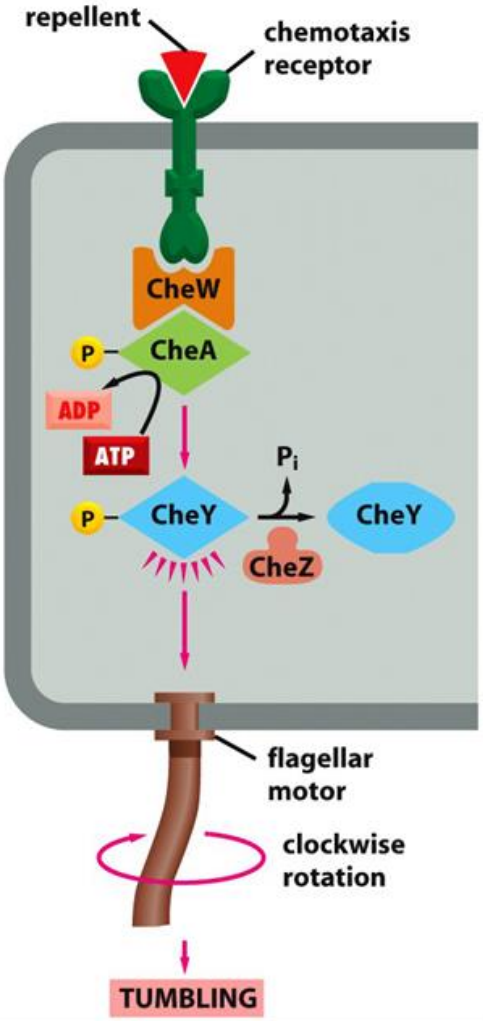
Bacteria movement through flagella

proton pumps couple proton gradient to motor rotation
Normally all motor rotate counterclockwise.
Every sec or two, some motor rotate clockwise---tumbling



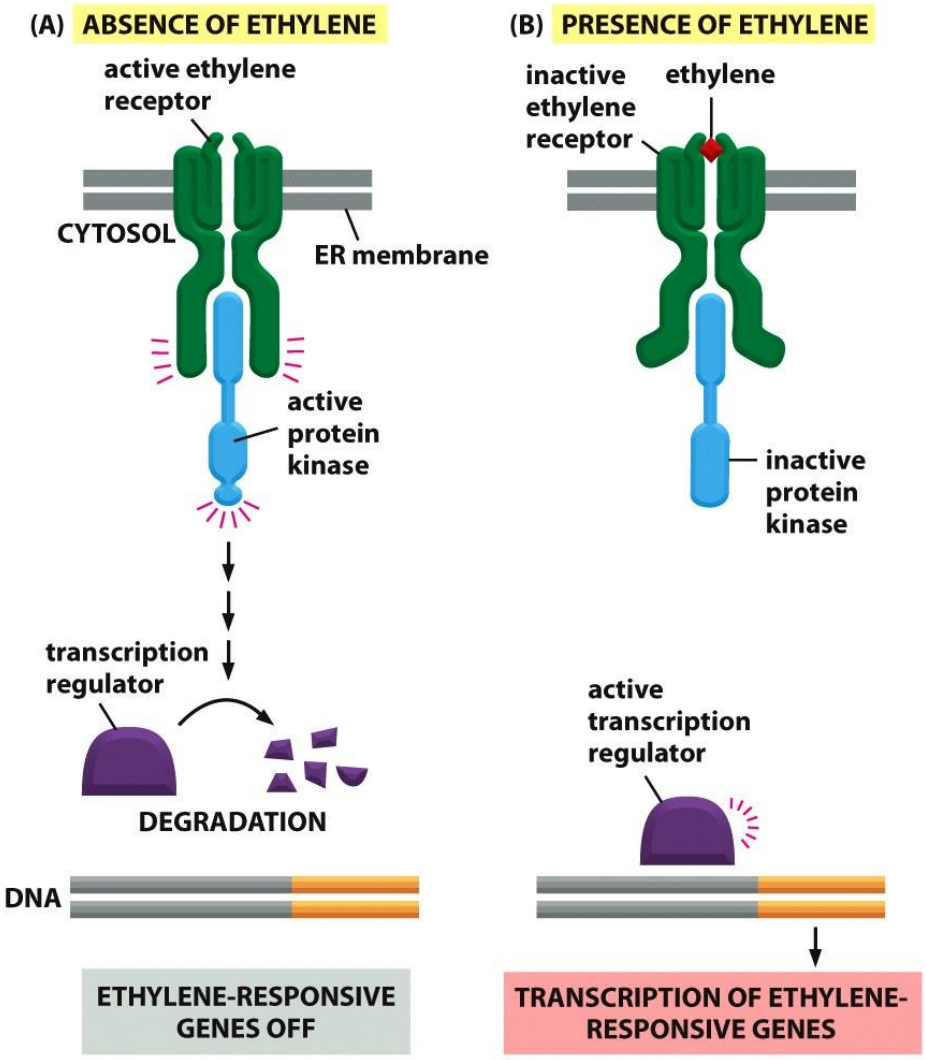


Mechanism for chemotaxis through Chemotaxis receptors---histidine-kinase-associated receptors



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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