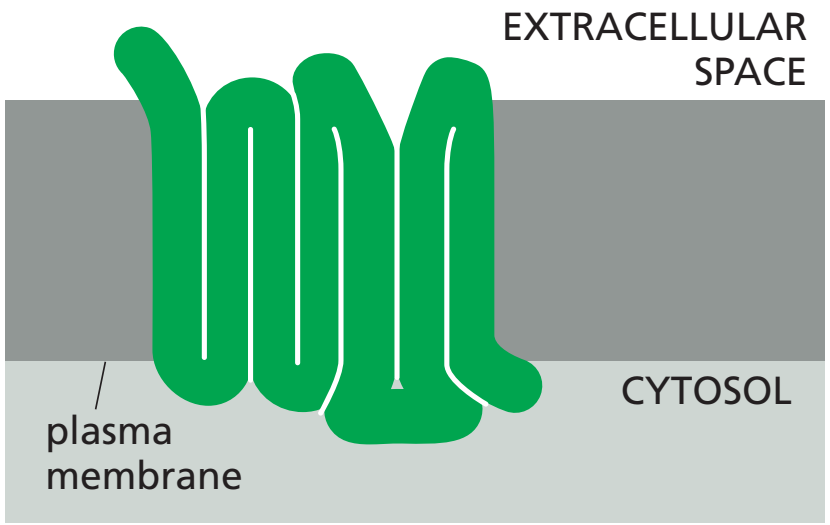
CHAPTER

15

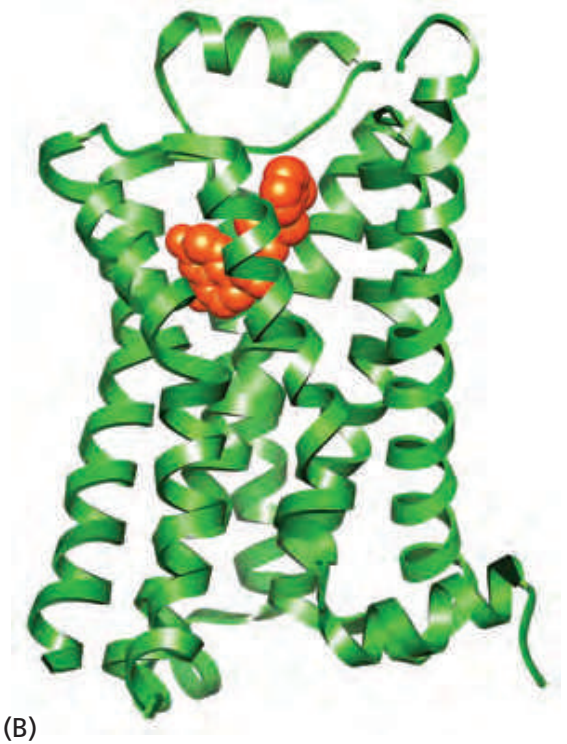
Cell Signaling

IN THIS CHAPTER

A G-protein-coupled receptor (GPCR)



(A)



(B)

Figure 15–21 A G-protein-coupled receptor (GPCR). (A) GPCRs that bind small ligands such as adrenaline have small extracellular domains, and the ligand usually binds deep within the plane of the membrane to a site that is formed by amino acids from several transmembrane segments. GPCRs that bind protein ligands have a large extracellular domain (not shown here) that contributes to ligand binding. (B) The structure of the β_2-adrenergic receptor, a receptor for the neurotransmitter adrenaline, illustrates the typical cylindrical arrangement of the seven transmembrane helices in a GPCR. The ligand *(orange)* binds in a pocket between the helices, resulting in conformational changes on the cytoplasmic surface of the receptor that promote G-protein activation (not shown). (PDB code: 3P0G.)

GPCR have seven transmembrane domains, composed of alpha helices

They have N-terminal extracellular region and a C-terminal intracellular region

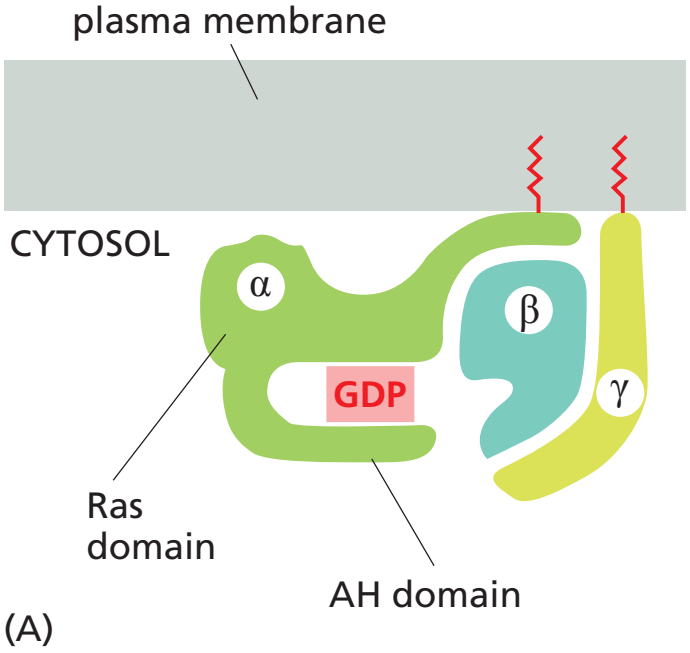
These seven transmembrane domains make 6 loops

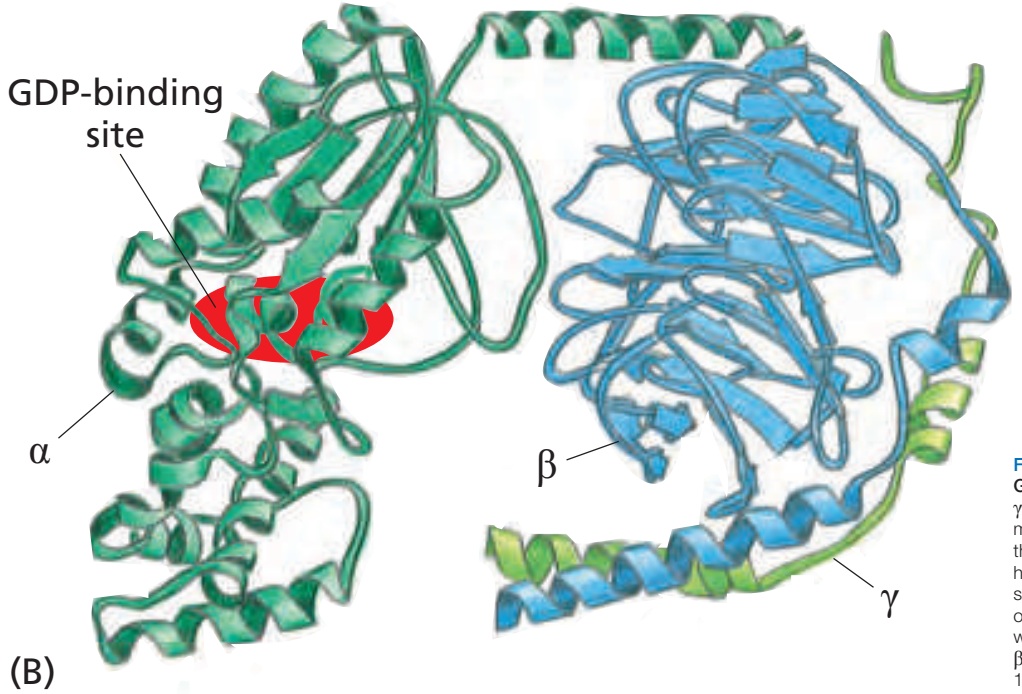
three extracellular loops interacting with ligand molecules, three intracellular loops interacting with G proteins

More than 700 GPCRs in humans

Ligands are diverse: proteins, peptides, fatty acids, molecules we smell or taste,photons

The structure of an inactive heterotrimeric G protein





GPCRs use trimeric G proteins to signal.
Activation of a GPCR triggers the release of GDP from the α subunit, allowing GTP binding
G proteins are a family of related proteins.
There exist at least 20 different kinds G_α subunits
And there are several B and Y variations. So there are various different G protein complexes!

Figure 15–22 The structure of an inactive G protein. (A) Note that both the α and the γ subunits have covalently attached lipid molecules *(red tails)* that help bind them to the plasma membrane, and the α subunit has GDP bound. (B) The three-dimensional structure of the inactive, GDP-bound form of a G protein called G_s, which interacts with numerous GPCRs, including the β_2-adrenergic receptor shown in Figures 15–21 and 15–23. The α subunit contains the GTPase domain and binds to one side of the β subunit. The γ subunit binds to the opposite side of the β subunit, and the β and γ subunits together form a single functional unit. The GTPase domain of the α subunit contains two major subdomains: the "Ras" domain, which is related to other GTPases and provides one face of the nucleotide-binding pocket; and the alpha-helical or "AH" domain, which clamps the nucleotide in place. (B, based on D.G. Lombright et al., *Nature* 379:311–319, 1996. With permission from Macmillan Publishers Ltd.)

Activation of a G protein by an activated GPCR

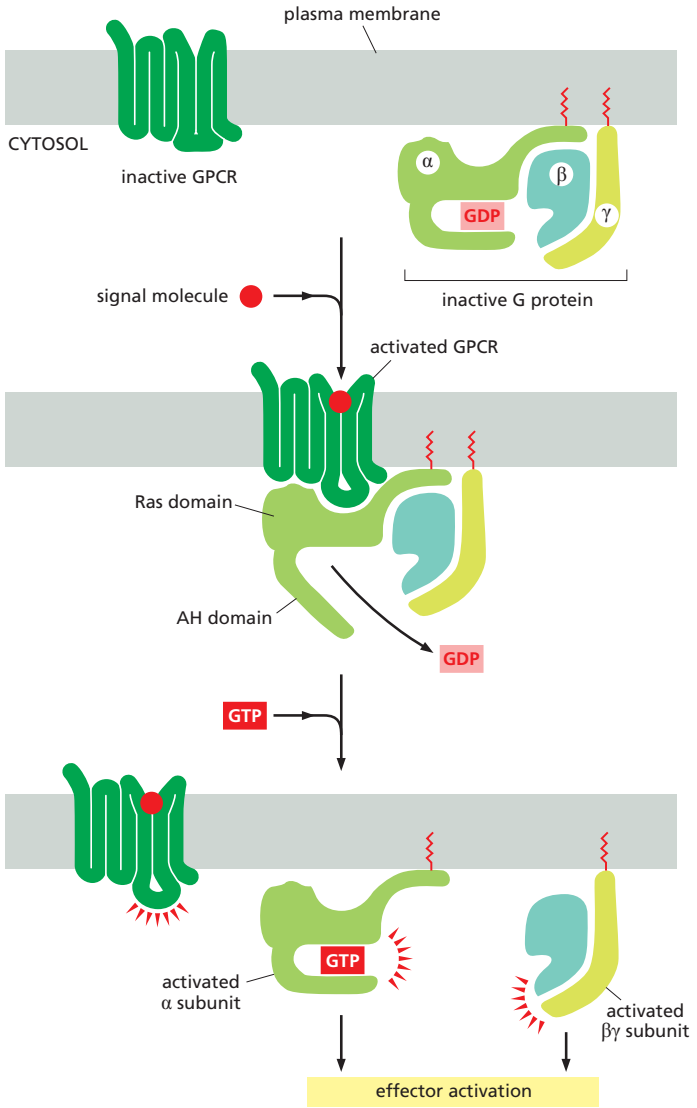


Figure 15–23 Activation of a G protein by an activated GPCR. Binding of an extracellular signal molecule to a GPCR changes the conformation of the receptor, which allows the receptor to bind and alter the conformation of a trimeric G protein. The AH domain of the G protein α subunit moves outward to open the nucleotide-binding site, thereby promoting dissociation of GDP. GTP binding then promotes closure of the nucleotide-binding site, triggering conformational changes that cause 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 (not shown). 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 (Movie 15.1).

G protein can activate their target when bound to GTP

The main targets are:

- Adenylate (adenylyl) cyclase, to increase or decrease [cAMP]
- Channels
- Phospholipase C, to generate IP3 and diacylglycerol

The G protein α-subunit is a GTPase

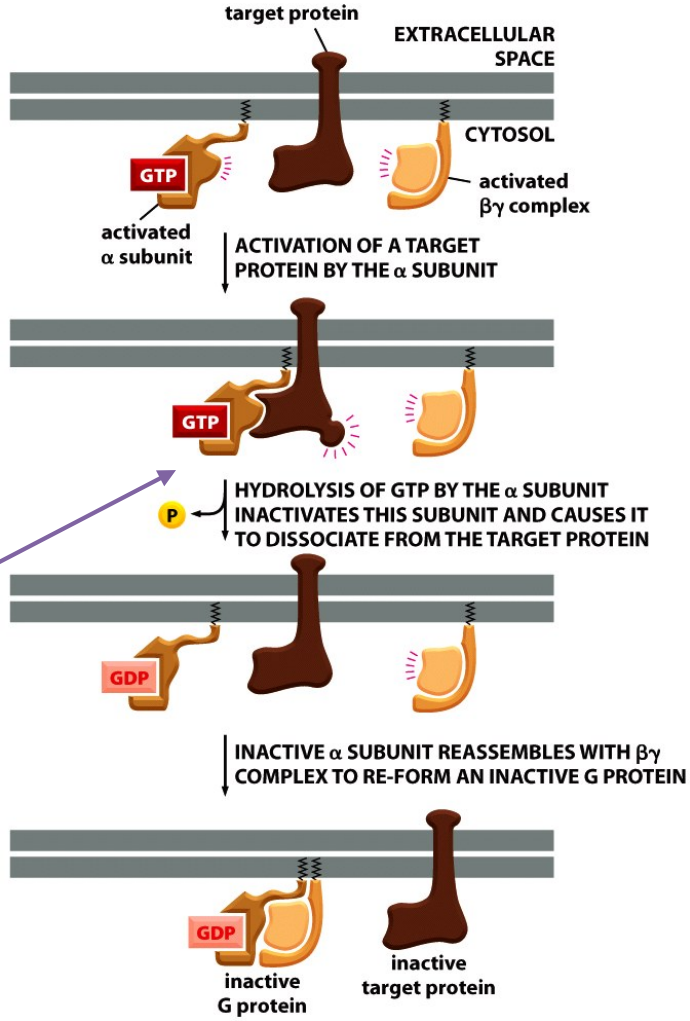


Figure 16-18 *Essential Cell Biology* (© Garland Science 2010)

Four Major Families of Trimeric G Proteins* with different functions

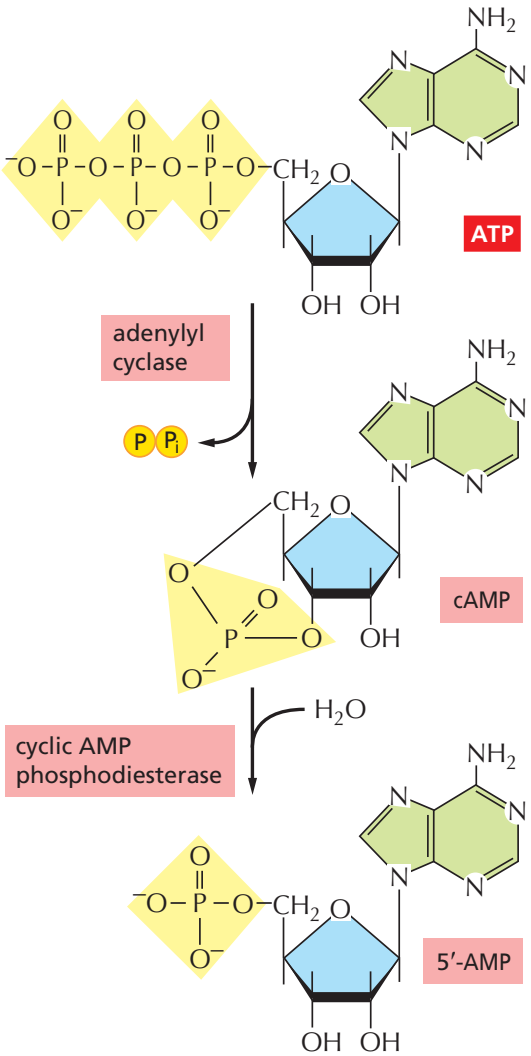
TABLE 15–3 Four Major Families of Trimeric G Proteins*

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.

GPCR signaling through Cyclic AMP

What is cyclic AMP ?



Derived from ATP

Two phophates removed & remaining phosphate is covalently bound to the sugar group. Enzyme: Adenylyl cyclase

cAMP is shortlived and “uncycled” to 5’-AMP. Enzyme: cAMP phosphodiesterase

The short-lived nature makes it an excellent signaling molecule

Figure 15–25 The synthesis and degradation of cyclic AMP. In a reaction catalyzed by the enzyme adenylyl cyclase, cyclic AMP (cAMP) is synthesized from ATP through a cyclization reaction that removes two phosphate groups as pyrophosphate (PP_i); a pyrophosphatase drives this synthesis by hydrolyzing the released pyrophosphate to phosphate (not shown). Cyclic AMP is short-lived (unstable) in the cell because it is hydrolyzed by specific phosphodiesterases to form 5′-AMP, as indicated.

cAMP activates cyclic- AMP-dependent protein kinase (PKA)

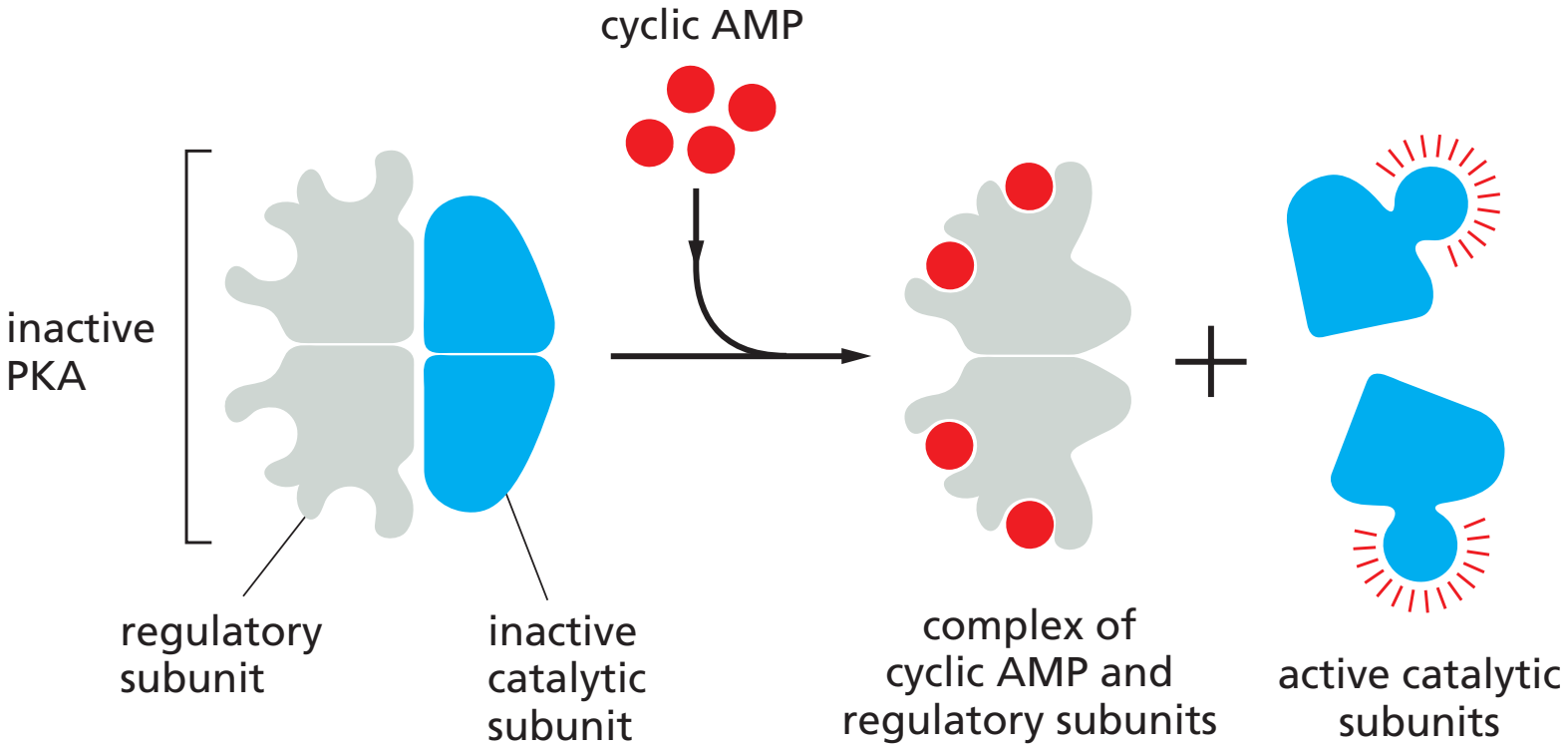
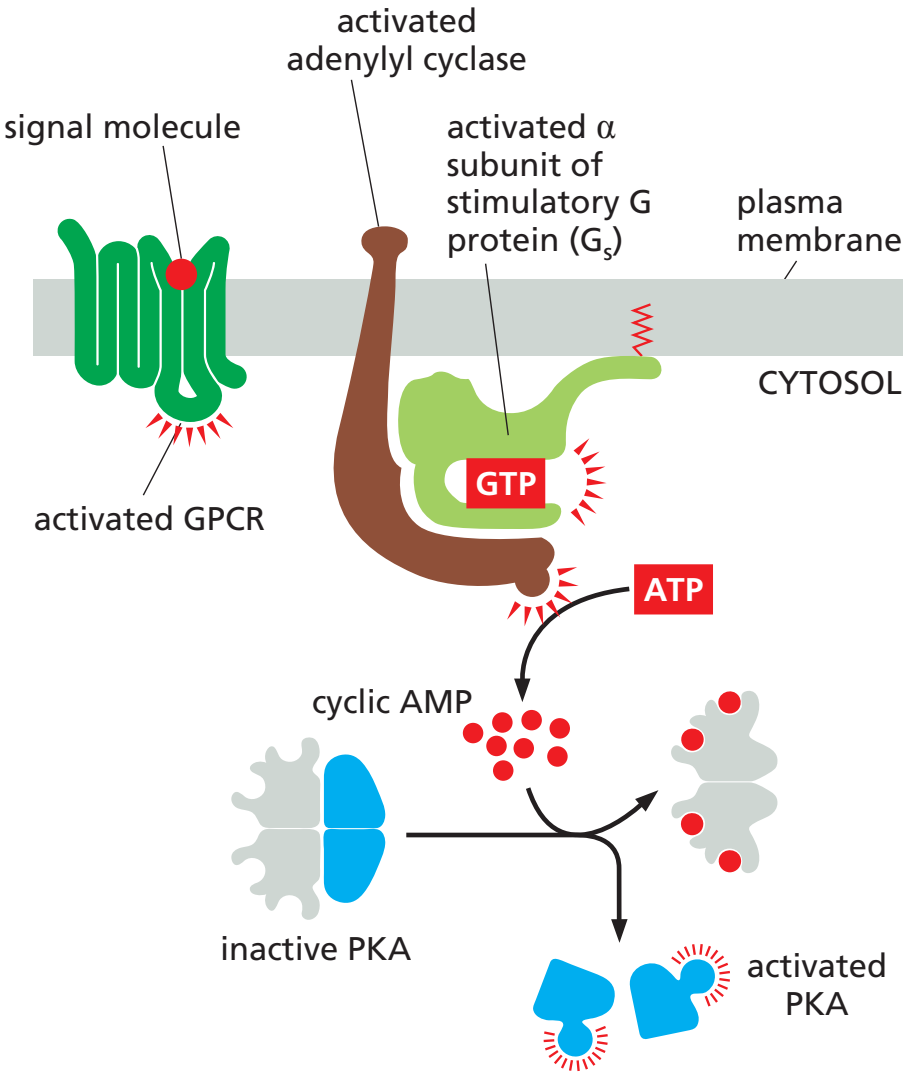


Figure 15–26 The activation of cyclic-AMP-dependent protein kinase (PKA). The binding of cAMP to the regulatory subunits of the PKA tetramer induces a conformational change, causing these subunits to dissociate from the catalytic subunits, thereby activating the kinase activity of the catalytic subunits. The release of the catalytic subunits requires the binding of more than two cAMP molecules to the regulatory subunits in the tetramer. This requirement greatly sharpens the response of the kinase to changes in cAMP concentration, as discussed earlier (see Figure 15–16). Mammalian cells have at least two types of PKAs: type I is mainly in the cytosol, whereas type II is bound via its regulatory subunits and special anchoring proteins to the plasma membrane, nuclear membrane, mitochondrial outer membrane, and microtubules. In both types, once the catalytic subunits are freed and active, they can migrate into the nucleus (where they can phosphorylate transcription regulatory proteins), while the regulatory subunits remain in the cytoplasm.

GPRC signaling with cAMP and PKA



GPCR gets activated

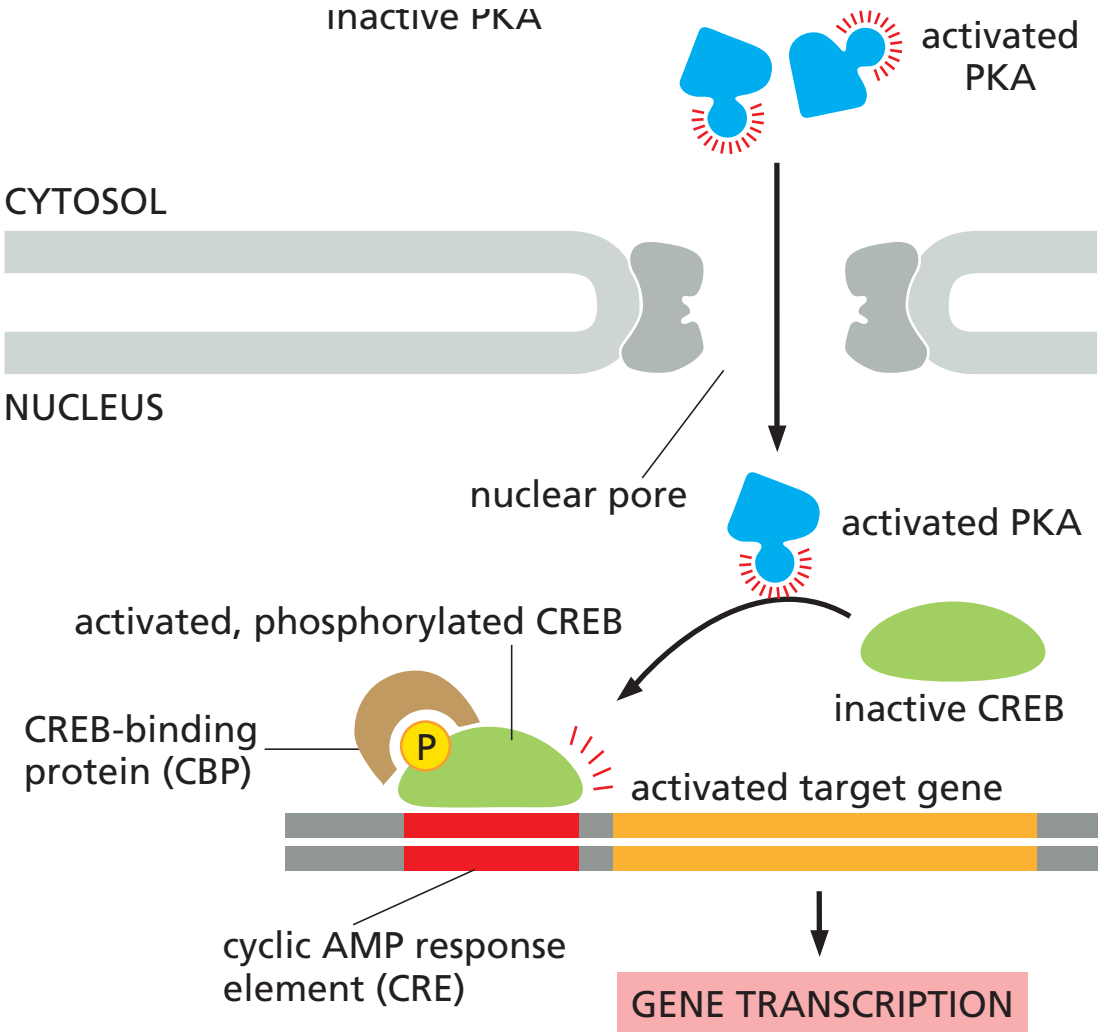
a-subunit activates adenylyl cyclase

ATP is converted to cAMP

cAMP binds to complex inactived PKA multiple times

PKA is released from the complex and is active

Active PKA translocates to the nucleus to activate CREB



Active PKA translocates to the nucleus

PKA activates a transcription factor CREB by phosphorylation

CREB can interact with CBP and activate transcription

How a rise in intracellular cyclic AMP concentration can alter gene transcription

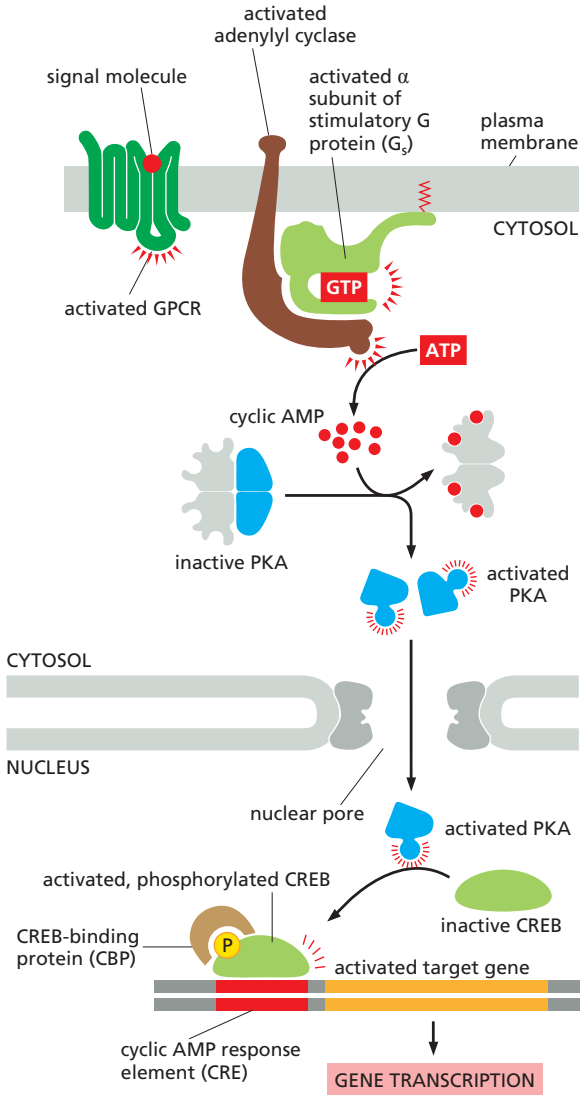


Figure 15–27 How a rise in intracellular cyclic AMP concentration can alter gene transcription. The binding of an extracellular signal molecule to its GPCR activates adenylyl cyclase via G_s and thereby increases cAMP concentration in the cytosol. This rise activates PKA, and the released catalytic subunits of PKA can then enter the nucleus, where they phosphorylate the transcription regulatory protein CREB. Once phosphorylated, CREB recruits the coactivator CBP, which stimulates gene transcription. In some cases, at least, the inactive CREB protein is bound to the cyclic AMP response element (CRE) in DNA before it is phosphorylated (not shown). See Movie 15.2.

An increase in cyclic AMP in response to an extracellular signal

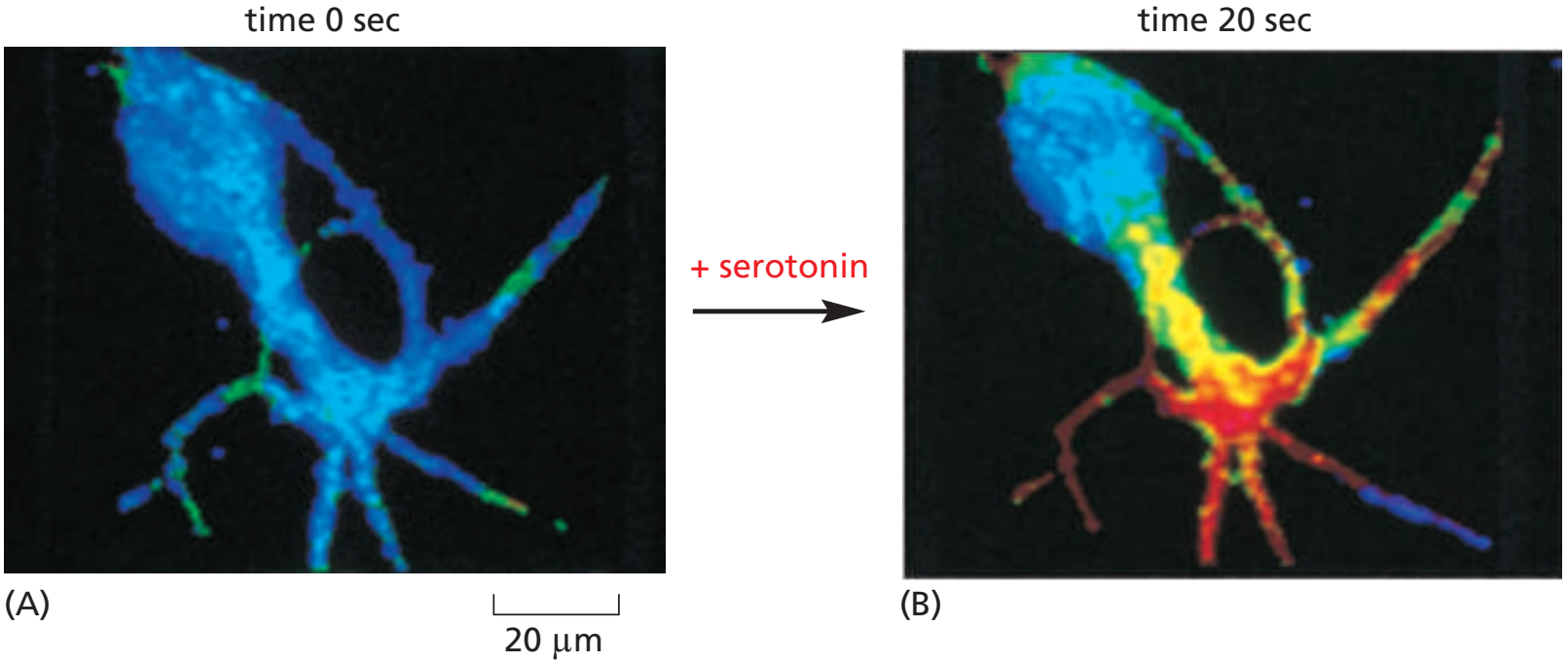


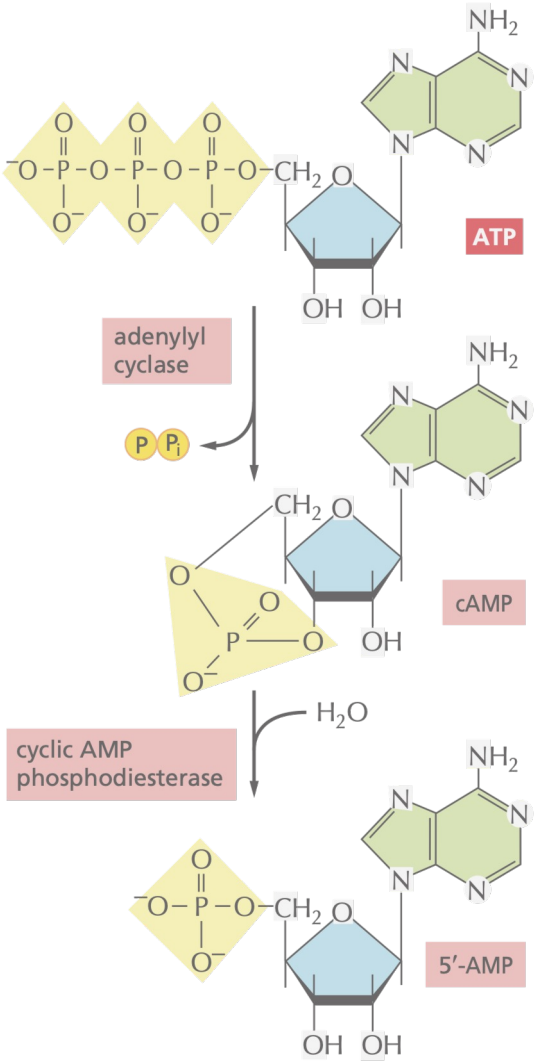
Figure 15–24 An increase in cyclic AMP in response to an extracellular signal. This nerve cell in culture is responding to the neurotransmitter serotonin, which acts through a GPCR to cause a rapid rise in the intracellular concentration of cyclic AMP. To monitor the cyclic AMP level, the cell has been loaded with a fluorescent protein that changes its fluorescence when it binds cyclic AMP. *Blue* indicates a low level of cyclic AMP, *yellow* an intermediate level, and *red* a high level. (A) In the resting cell, the cyclic AMP level is about 5×10^{-8} M. (B) Twenty seconds after the addition of serotonin to the culture medium, the intracellular level of cyclic AMP has increased to more than 10^{-6} M in the relevant parts of the cell, an increase of more than twentyfold. (From B.J. Bacskai et al., *Science* 260:222–226, 1993. With permission from AAAS.)

Some Hormone-induced Cell Responses Mediated by Cyclic AMP

TABLE 15–1 Some Hormone-induced Cell Responses Mediated by Cyclic AMP		
Target tissue	Hormone	Major response
Thyroid gland	Thyroid-stimulating hormone (TSH)	Thyroid hormone synthesis and secretion
Adrenal cortex	Adrenocorticotrophic hormone (ACTH)	Cortisol secretion
Ovary	Luteinizing hormone (LH)	Progesterone secretion
Muscle	Adrenaline	Glycogen breakdown
Bone	Parathormone	Bone resorption
Heart	Adrenaline	Increase in heart rate and force of contraction
Liver	Glucagon	Glycogen breakdown
Kidney	Vasopressin	Water resorption
Fat	Adrenaline, ACTH, glucagon, TSH	Triglyceride breakdown

Vasopressin is also a “love” hormone, when you are in love GPRC signaling is active!

In some cases not cyclic AMP is made but cyclic GMP upon GPCR actication!



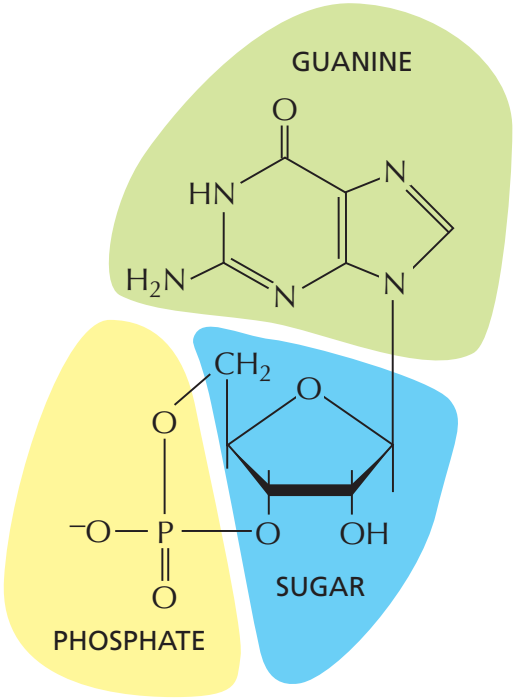


Figure 15–37 Cyclic GMP.

Guanylate cyclase is the enzyme responsible!

The response of a rod photoreceptor cell to light

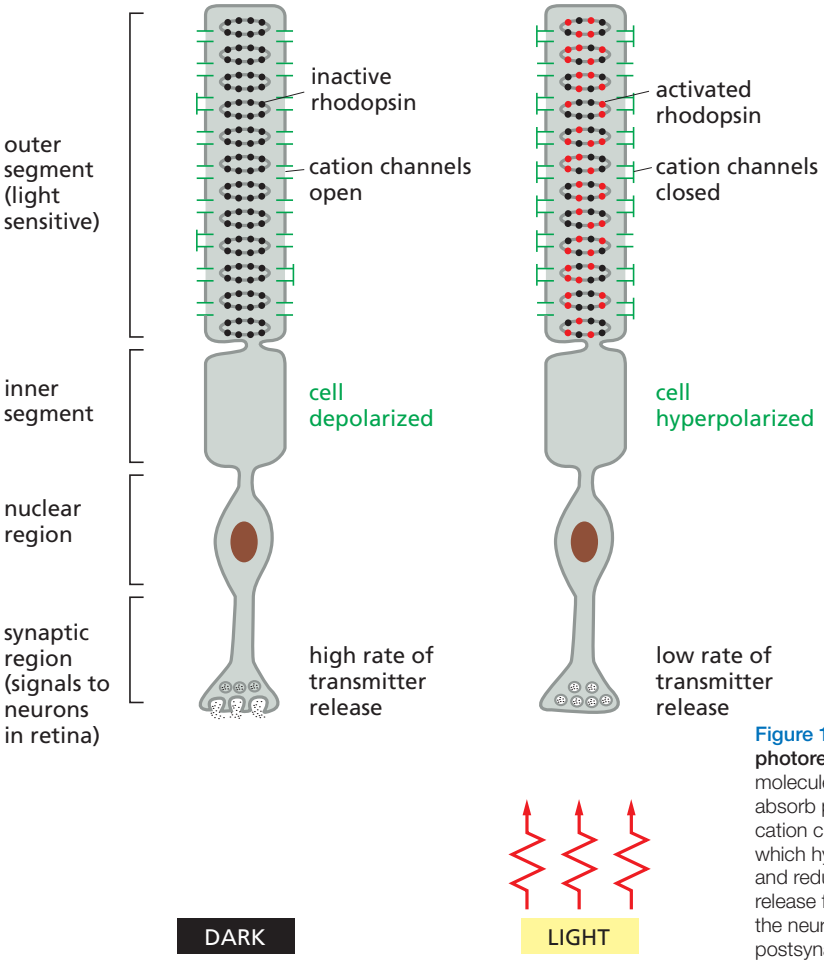


Figure 15–39 The response of a rod photoreceptor cell to light. Rhodopsin molecules in the outer-segment discs absorb photons. Photon absorption closes cation channels in the plasma membrane, which hyperpolarizes the membrane and reduces the rate of neurotransmitter release from the synaptic region. Because the neurotransmitter inhibits many of the postsynaptic retinal neurons, illumination serves to free the neurons from inhibition and thus, in effect, excites them. The neural connections of the retina lie between the light source and the outer segment, and so the light must pass through the synapses and rod cell nucleus to reach the light sensors.

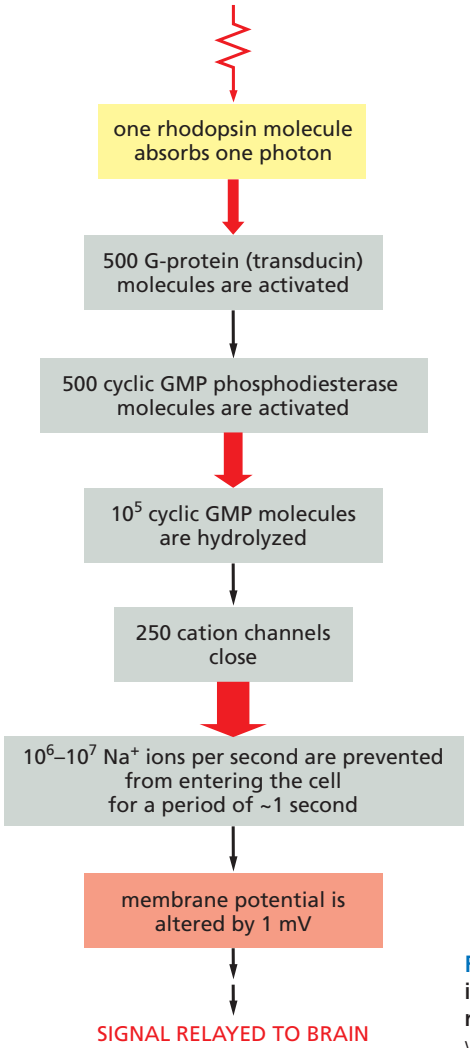


Figure 15–41 Amplification in the light-induced catalytic cascade in vertebrate rods. The *red arrows* indicate the steps where amplification occurs, with the thickness of the arrow roughly indicating the magnitude of the amplification.

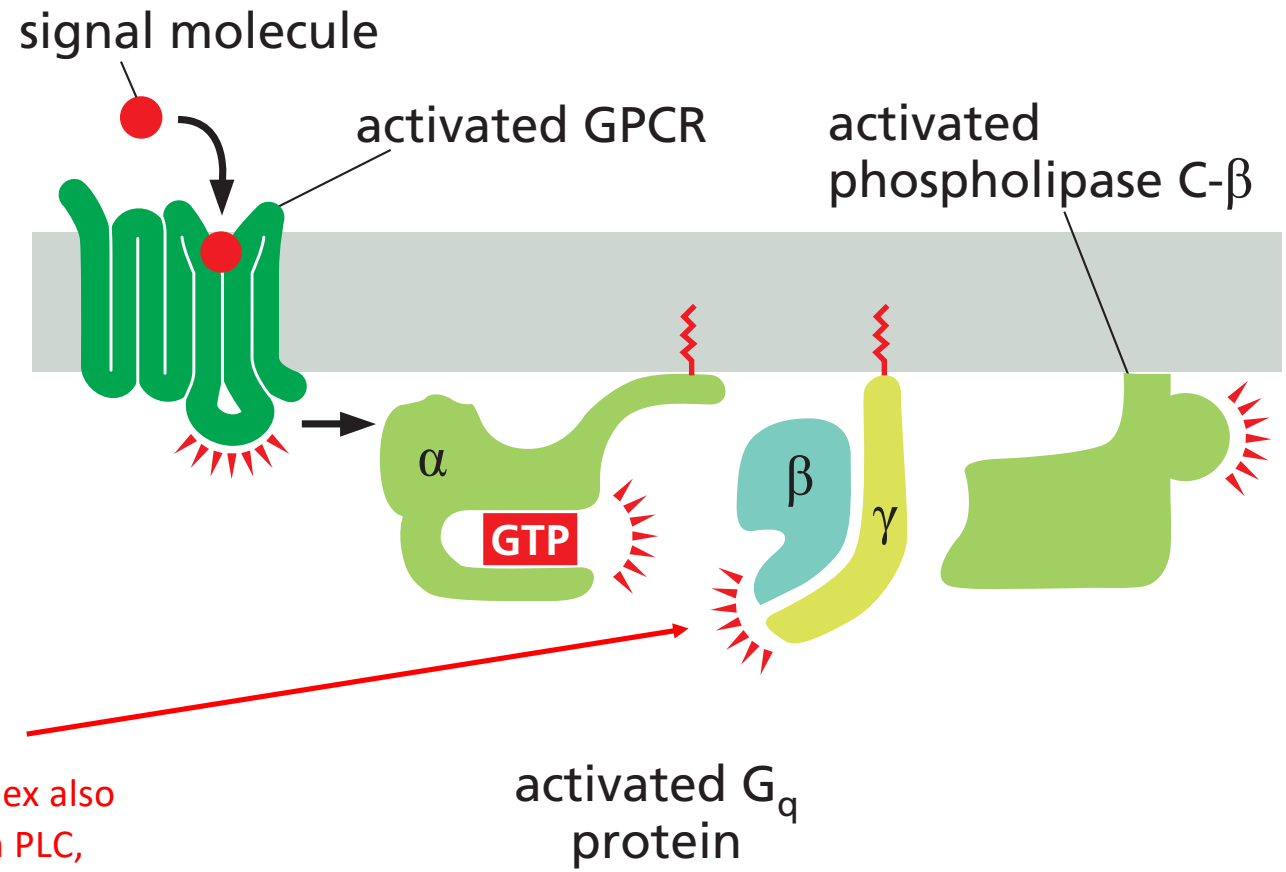
GPCR signaling through phospholipase C

Some Cell Responses in Which GPCRs Activate PlCβ

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

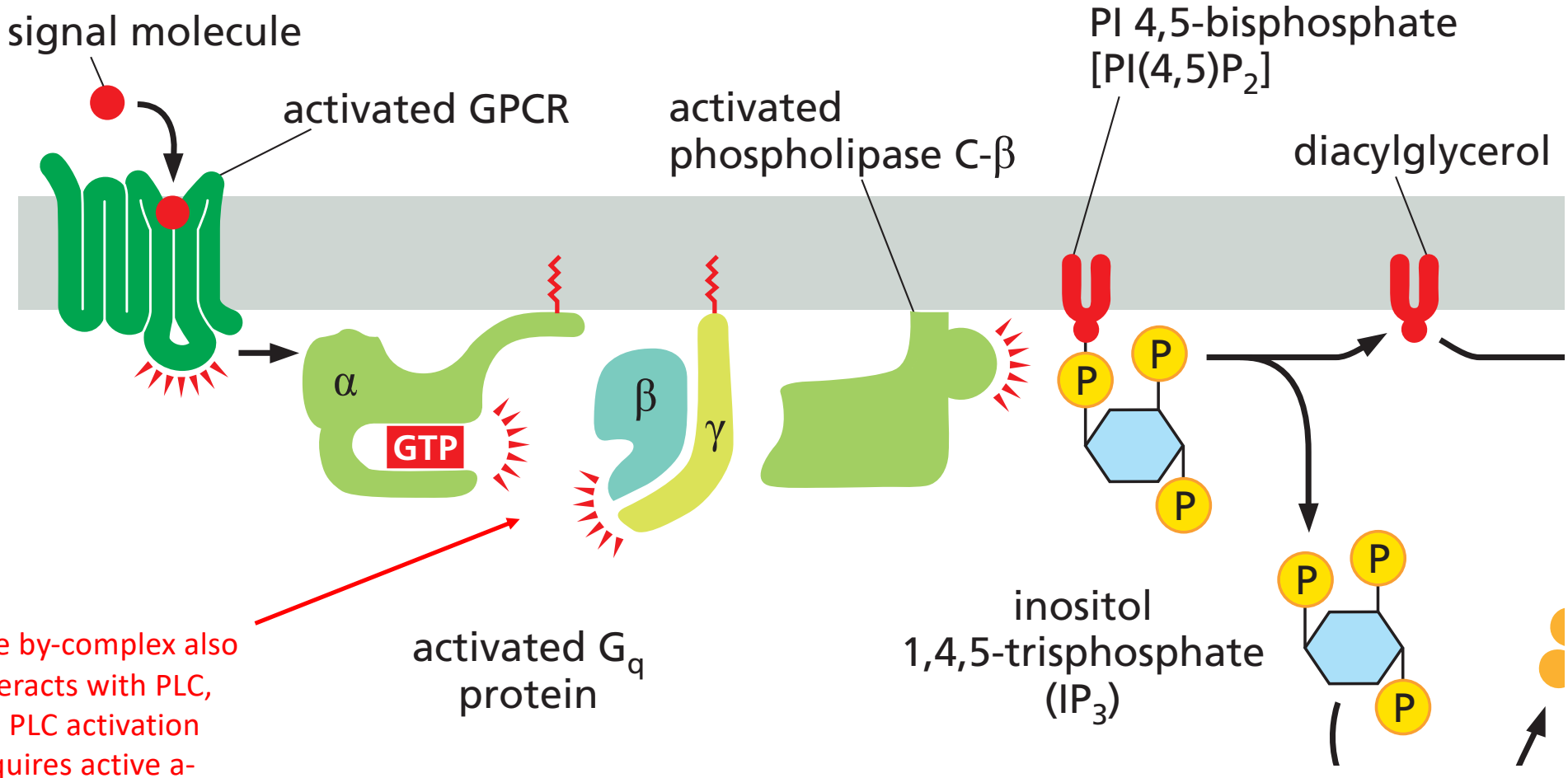
Vasopressin again!?!

How GPCRs increase cytosolic Ca2+ and activate protein kinase C

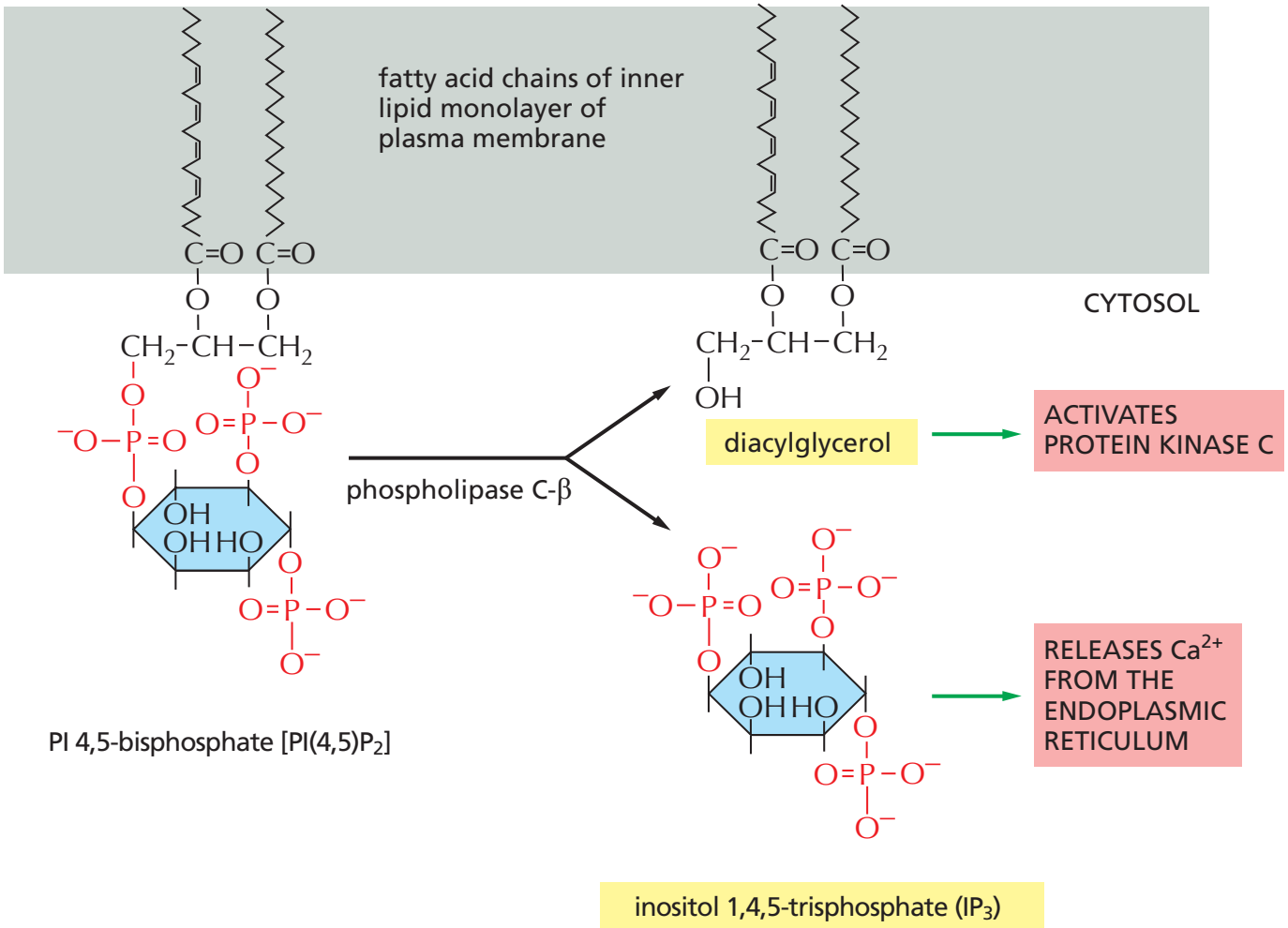


The by-complex also interacts with PLC, i.e. PLC activation requires active a-unit and by-complex

The target molecule of PLC is PI 4,5-biphosphate



The hydrolysis of PI(4,5) P2 by phospholipase C-β



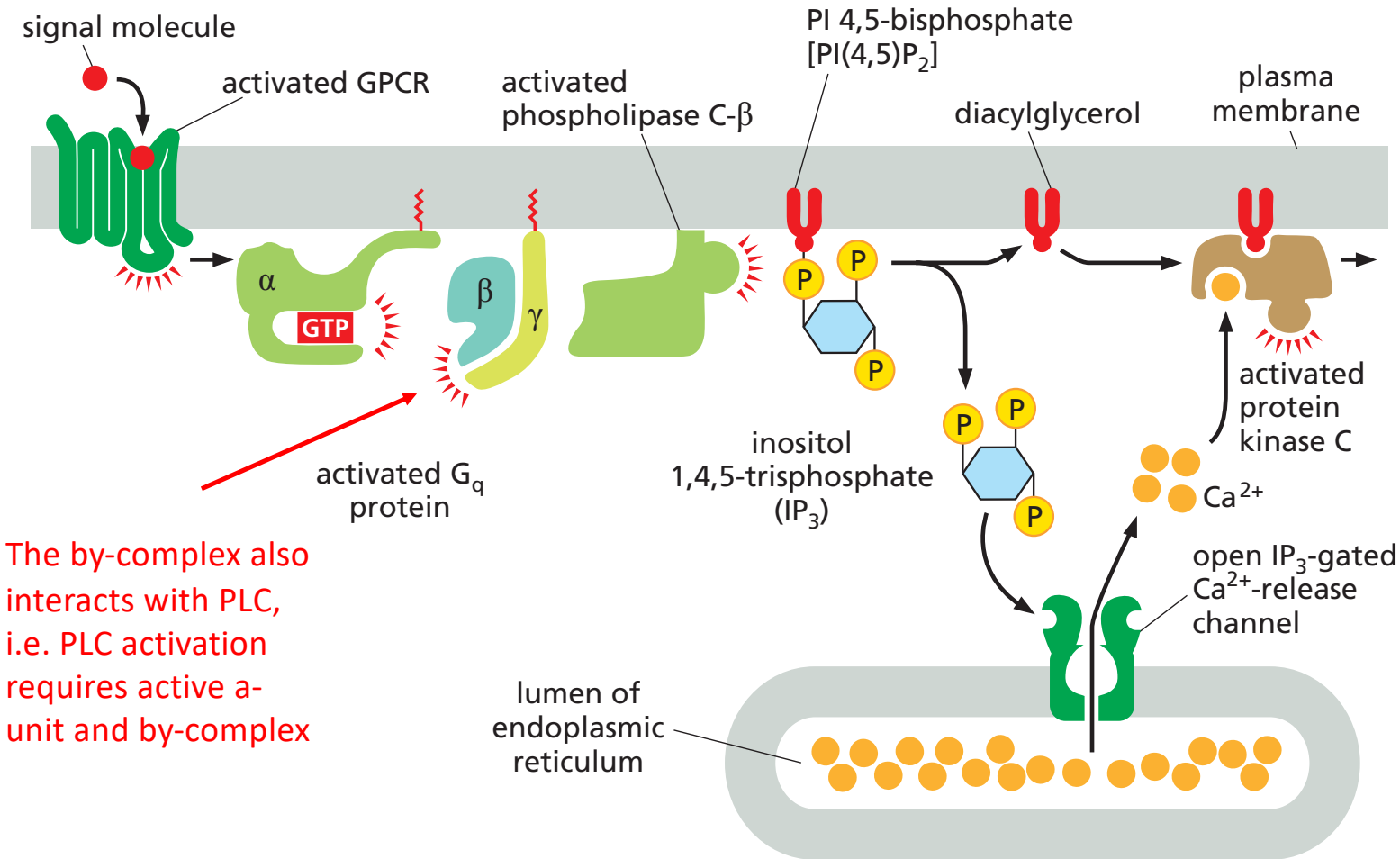
Two important products

Diacylglycerol

Inositol 1,4,5-triphosphate

Figure 15–28 The hydrolysis of PI(4,5) P_2 by phospholipase C-β. Two second messengers are produced directly from the hydrolysis of PI(4,5)P_2: inositol 1,4,5-trisphosphate (IP_3), which diffuses through the cytosol and releases Ca^{2+} from the endoplasmic reticulum, and diacylglycerol, which remains in the membrane and helps to activate protein kinase C (PKC; see Figure 15–29). There are several classes of phospholipase C: these include the β class, which is activated by GPCRs; as we see later, the γ class is activated by a class of enzyme-coupled receptors called receptor tyrosine kinases (RTKs).

How GPCRs increase cytosolic Ca2+ and activate protein kinase C



The by-complex also interacts with PLC, i.e. PLC activation requires active a-unit and by-complex

Figure 15–29 How 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. The α subunit and βγ complex of G_q are both involved in this activation. Two second messengers are produced when $PI(4,5)P_2$ is hydrolyzed by activated PLCβ. Inositol 1,4,5-trisphosphate (IP_3) diffuses through the cytosol and releases Ca^{2+} from the ER by binding to and opening IP_3-gated Ca^{2+}-release channels (IP_3 receptors) in the ER membrane. The large electrochemical gradient for Ca^{2+} across this membrane causes Ca^{2+} to escape into the cytosol when the release channels are opened. Diacylglycerol remains in the plasma membrane and, together with phosphatidylserine (not shown) and Ca^{2+}, helps to activate protein kinase C (PKC), which is recruited from the cytosol to the cytosolic face of the plasma membrane. Of the 10 or more distinct isoforms of PKC in humans, at least 4 are activated by diacylglycerol (Movie 15.3).

Positive and negative feedback produce Ca2+ waves and oscillations

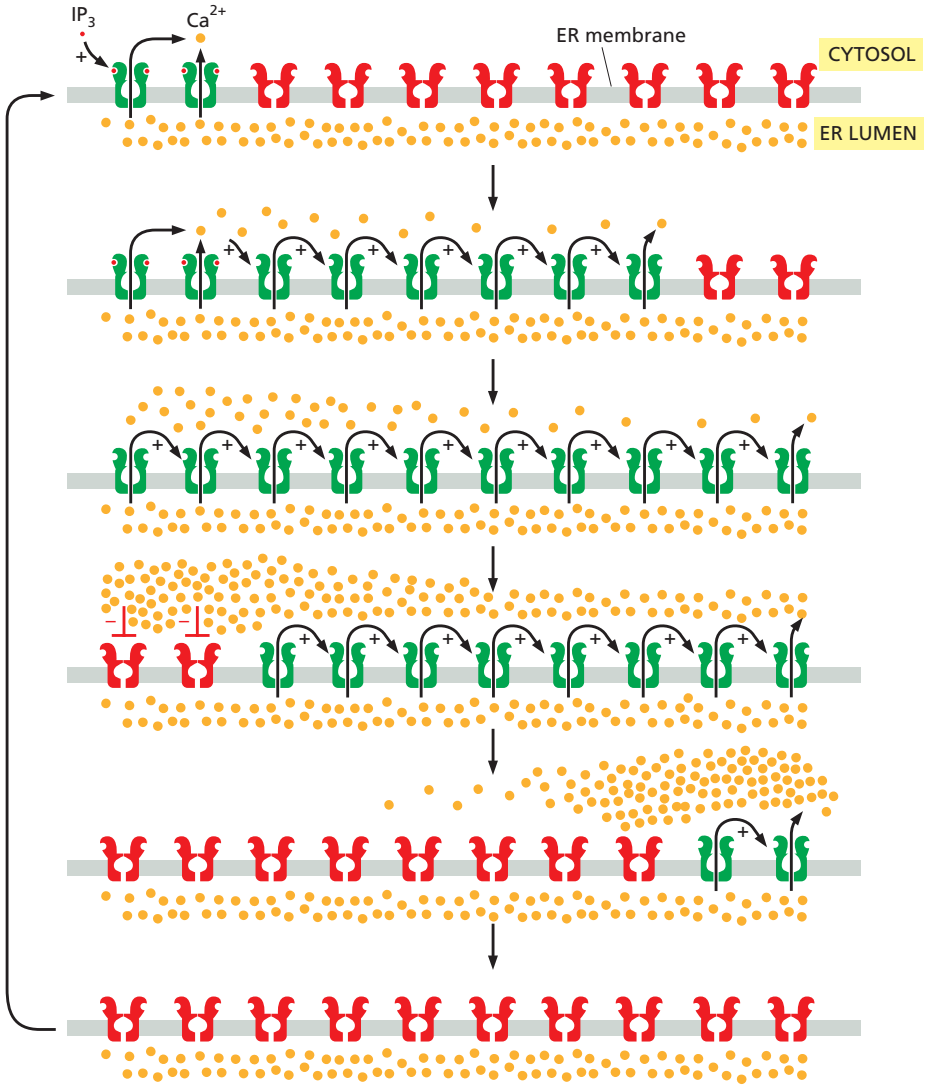


Figure 15–31 Positive and negative feedback produce Ca^{2+} waves and oscillations. This diagram shows IP_3 receptors and ryanodine receptors on a portion of the ER membrane: active receptors are in *green*; inactive receptors are in *red*. When a small amount of cytosolic IP_3 activates a cluster of IP_3 receptors at one site on the ER membrane (*top*), the local release of Ca^{2+} promotes the opening of nearby IP_3 and ryanodine receptors, resulting in more Ca^{2+} release. This positive feedback (indicated by *positive* signs) produces a regenerative wave of Ca^{2+} release that spreads across the cell (see Figure 15–30). These waves of Ca^{2+} release move more quickly across the cell than would be possible by simple diffusion. Also, unlike a diffusing burst of Ca^{2+} ions, which will become more dilute as it spreads, the regenerative wave produces a high Ca^{2+} concentration across the entire cell. Eventually, the local Ca^{2+} concentration inactivates IP_3 receptors and ryanodine receptors (*middle*; indicated by *red* negative signs), shutting down the Ca^{2+} release. Ca^{2+}-pumps reduce the local cytosolic Ca^{2+} concentration to its normal low levels. The result is a Ca^{2+} spike: 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 *(bottom)*. Eventually, however, the negative feedback wears off, allowing IP_3 to trigger another Ca^{2+} wave. The end result is repeated Ca^{2+} oscillations (see Figure 15–32). Under some conditions, these oscillations can be seen as repeating narrow waves of Ca^{2+} moving across the cell.

The fertilization of an egg by a sperm triggers a wave of cytosolic Ca2+

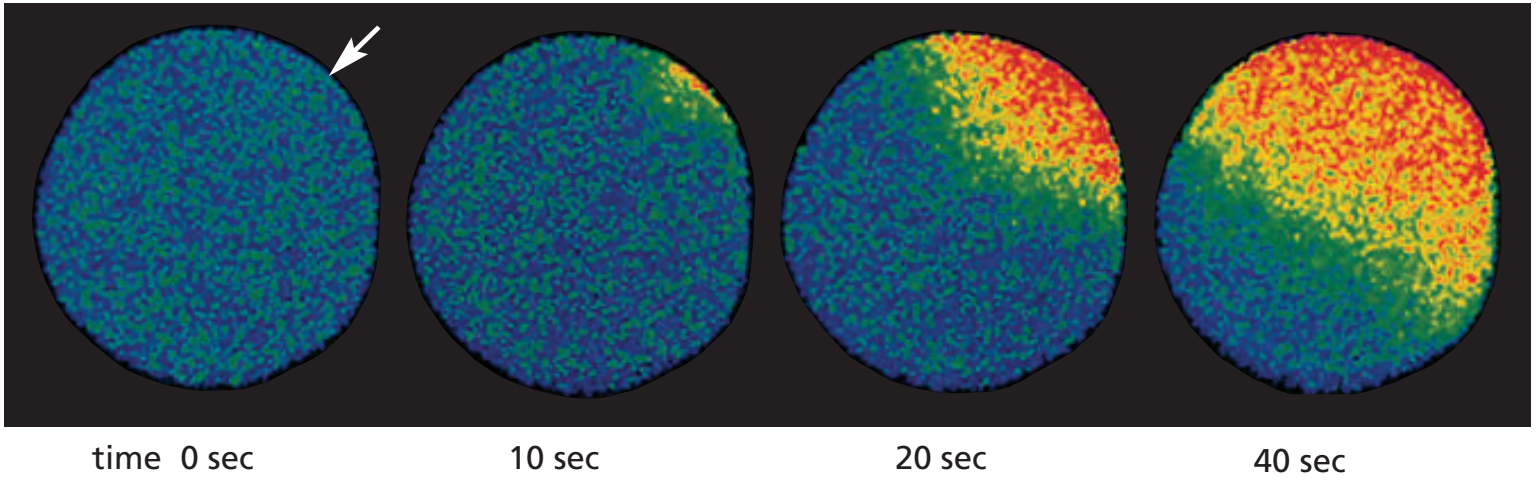


Figure 15–30 The fertilization of an egg by a sperm triggers a wave of cytosolic Ca^{2+}. This starfish egg was injected with a Ca^{2+}-sensitive fluorescent dye before it was fertilized. A wave of cytosolic Ca^{2+} *(red)*, released from the ER, sweeps across the egg from the site of sperm entry *(arrow).* This Ca^{2+} wave changes the egg cell surface, preventing the entry of other sperm, and it also initiates embryonic development (**Movie 15.5**). The initial increase in Ca^{2+} is thought to be caused by a sperm-specific form of PLC (PLCζ) that the sperm brings into the egg cytoplasm when it fuses with the egg; the PLCζ cleaves $PI(4,5)P_2$ to produce IP_3, which releases Ca^{2+} from the egg ER. The released Ca^{2+} stimulates further Ca^{2+} release from the ER, producing the spreading wave, as we explain in Figure 15–31. (Courtesy of Stephen A. Stricker.)

Calcium binds to Ca2+/calmodulin which relays the signal to target proteins

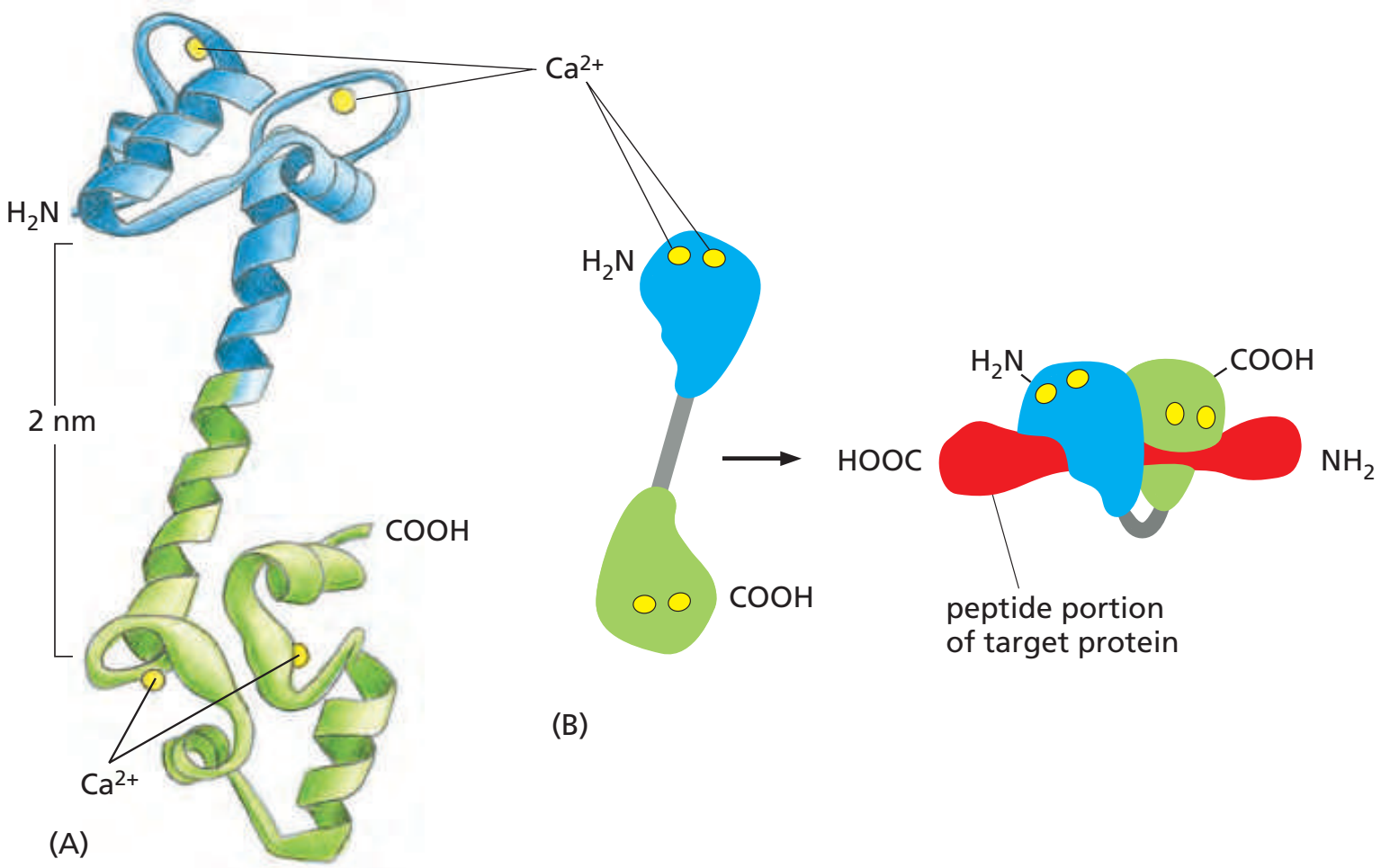
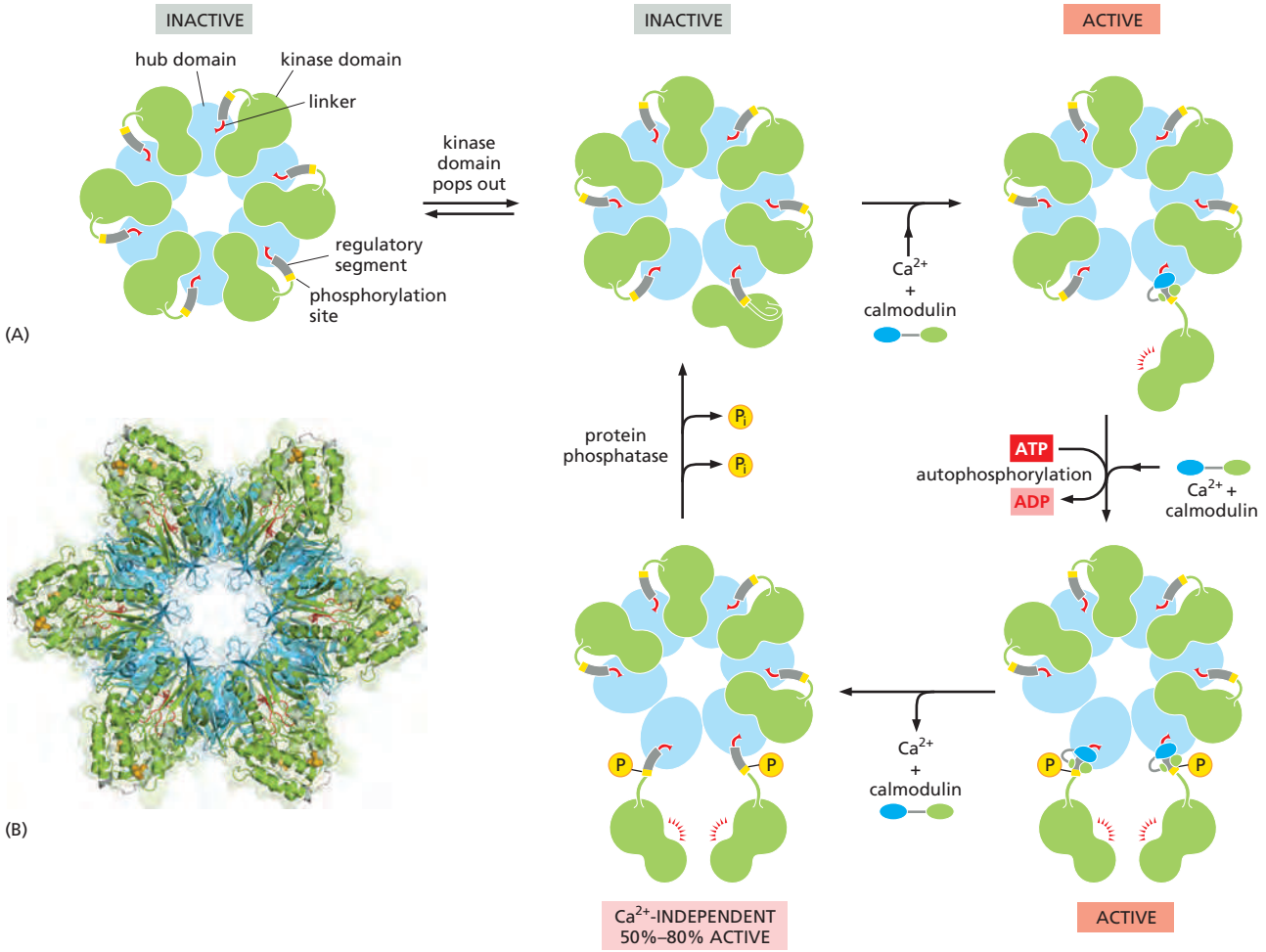


Figure 15–33 The structure of Ca^{2+}/calmodulin. (A) The molecule has a dumbbell shape, with two globular ends, which can bind to many target proteins. The globular ends are connected by a long, exposed α helix, which allows the protein to adopt a number of different conformations, depending on the target protein it interacts with. Each globular head has two Ca^{2+}-binding sites (Movie 15.6). (B) Shown is the major structural change that occurs in Ca^{2+}/calmodulin when it binds to a target protein (in this example, a peptide that consists of the Ca^{2+}/calmodulin-binding domain of a Ca^{2+}/calmodulin-dependent protein kinase). Note that the Ca^{2+}/calmodulin has "jack-knifed" to surround the peptide. When it binds to other targets, it can adopt different conformations. (A, based on x-ray crystallographic data from Y.S. Babu et al., *Nature* 315:37–40, 1985. With permission from Macmillan Publishers Ltd; B, based on x-ray crystallographic data from W.E. Meador, A.R. Means, and F.A. Quiocho, *Science* 257:1251–1255, 1992, and on nuclear magnetic resonance (NMR) spectroscopy data from M. Ikura et al., *Science* 256:632–638, 1992.)

CaM-kinase II is an important Kinase regulated by Ca2+ can calmodulin



6 CaMKII form a ring

Kinase domain pops in and out naturally

Calmodulin can bind the popped-out domain in the presence of Ca2+

And CamKII gets phosphorylated

Calmodulin can be released and p-CaMKII stays active (but less so!)

Once dephosphorylated CaMKII goes back to the inactive state

The 6 proteins mean it can be more or less active i.e. 1 to 6 CaMKII can be activated depending on cytosolic Ca2+ levels and Calmodulin!

CaM-kinase II as a frequency decoder of Ca2+ oscillations due to its nature

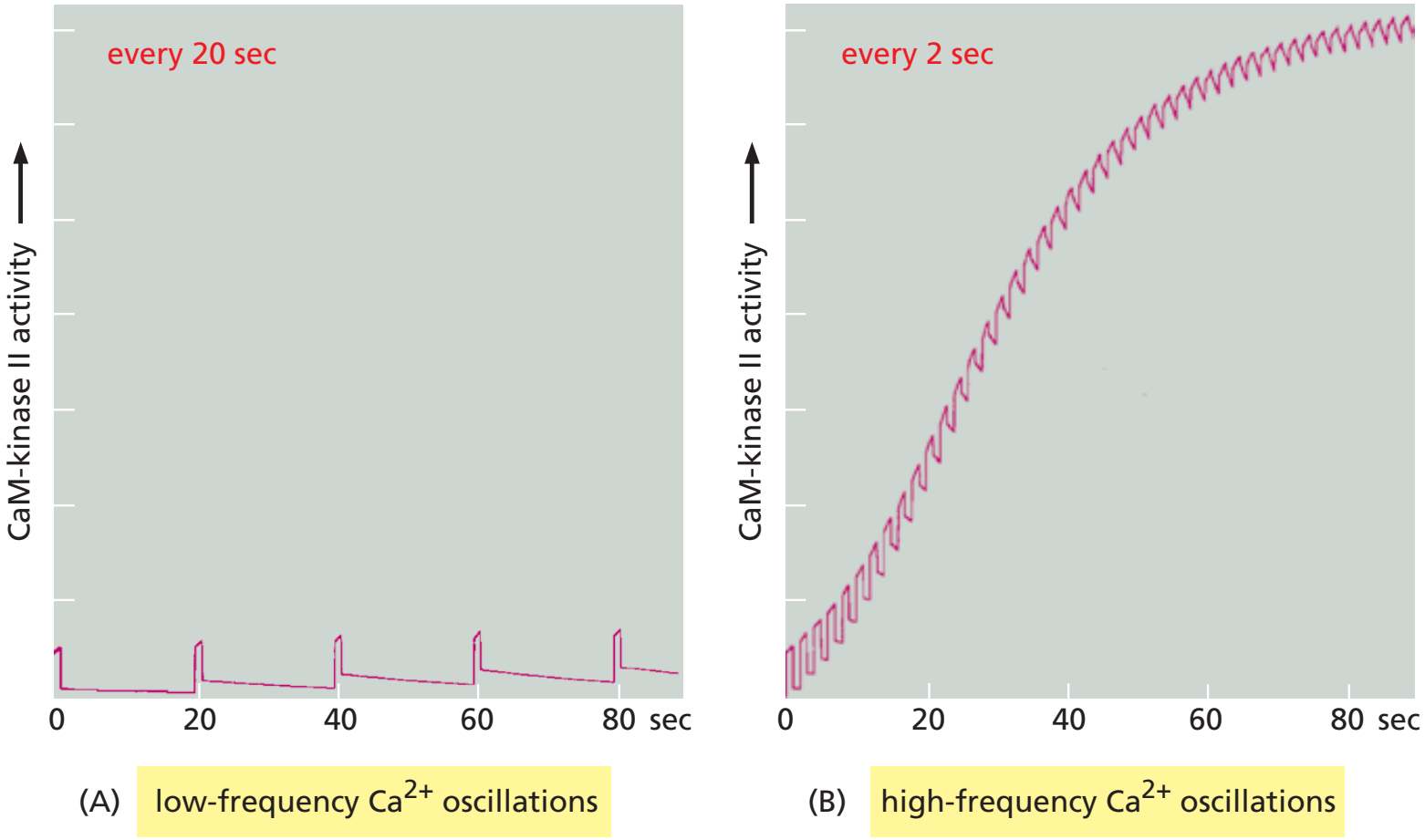
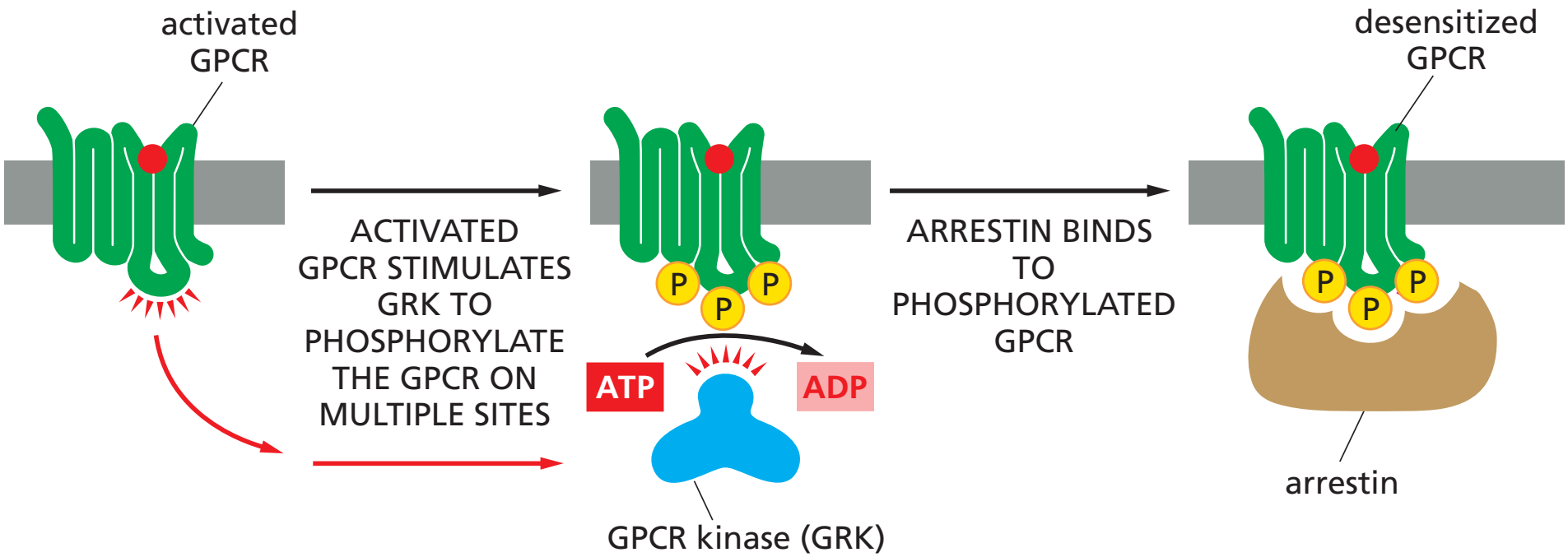


Figure 15–35 CaM-kinase II as a frequency decoder of Ca^{2+} oscillations. (A) At low frequencies of Ca^{2+} spikes, the enzyme becomes inactive after each spike, as the autophosphorylation induced by Ca^{2+}/calmodulin binding does not maintain the enzyme's activity long enough for the enzyme to remain active until the next Ca^{2+} spike arrives. (B) At higher spike frequencies, however, the enzyme fails to inactivate completely between Ca^{2+} spikes, so its activity ratchets up with each spike. If the spike frequency is high enough, this progressive increase in enzyme activity will continue until the enzyme is autophosphorylated on all subunits and is therefore maximally activated. Although not shown, once enough of its subunits are autophosphorylated, the enzyme can be maintained in a highly active state even with a relatively low frequency of Ca^{2+} spikes (a form of cell memory). The binding of Ca^{2+}/calmodulin to the enzyme is enhanced by the CaM-kinase II autophosphorylation (an additional form of positive feedback), helping to generate a more switchlike response to repeated Ca^{2+} spikes. (From P.I. Hanson, T. Meyer, L. Stryer, and H. Schulman, *Neuron* 12:943–956, 1994. With permission from Elsevier.)

Stopping GPCR signaling: GPCR kinases (GRKs) and arrestins in GPCR desensitization



GRK's are a negative regulators of the GPCR

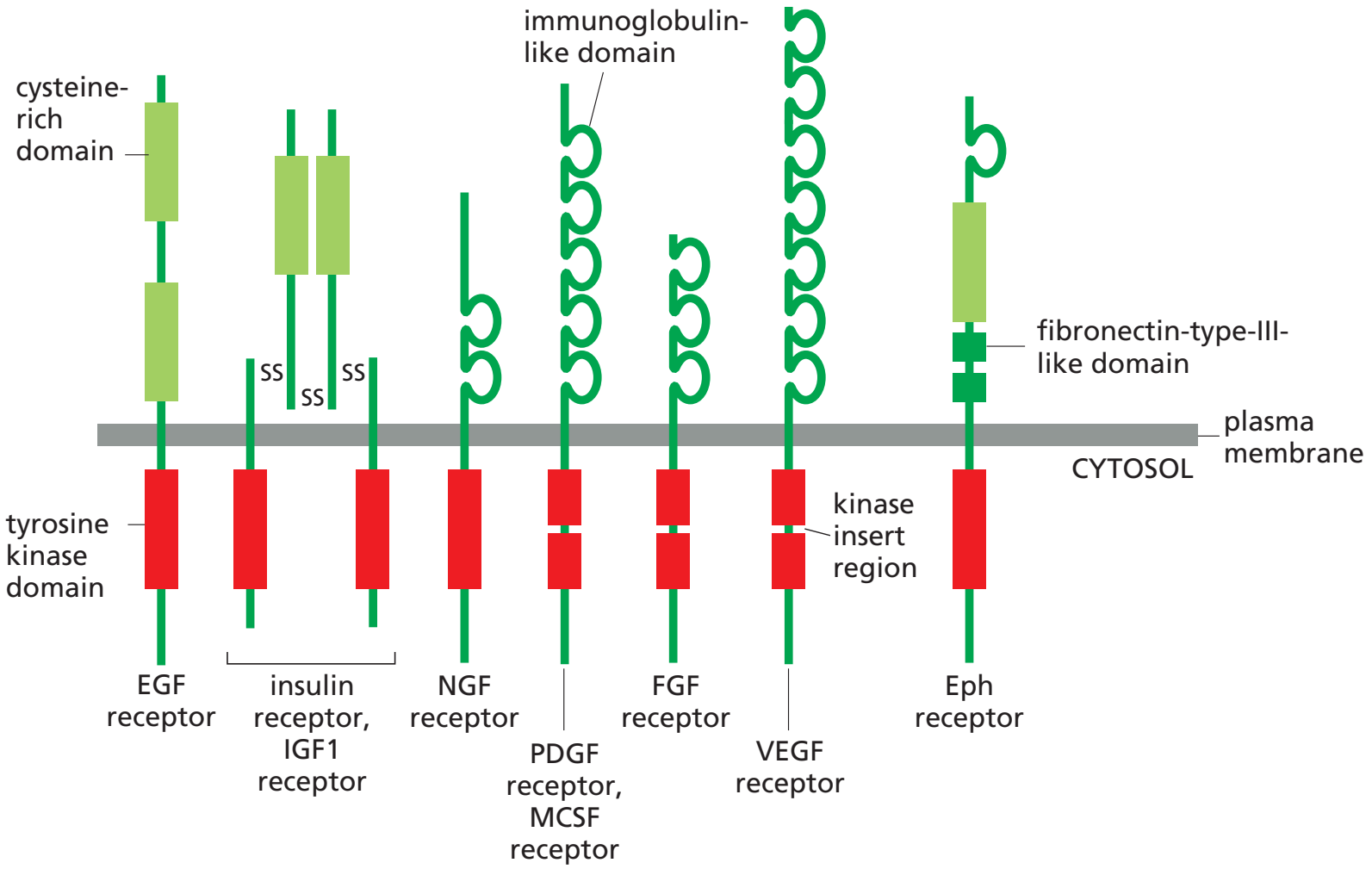
Figure 15–42 The roles of GPCR kinases (GRKs) and arrestins in GPCR desensitization. A GRK phosphorylates only activated receptors because it is the activated GPCR that activates the GRK. The binding of an arrestin to the phosphorylated receptor prevents the receptor from binding to its G protein and also directs its endocytosis (not shown). Mice that are deficient in one form of arrestin fail to desensitize in response to morphine, for example, attesting to the importance of arrestins for desensitization.

Receptor Tyrosine Kinase (RTK) signaling

RTKs a large group of signaling receptors

TABLE 15–4 Some 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

RTKs are similar on the intracellular domain

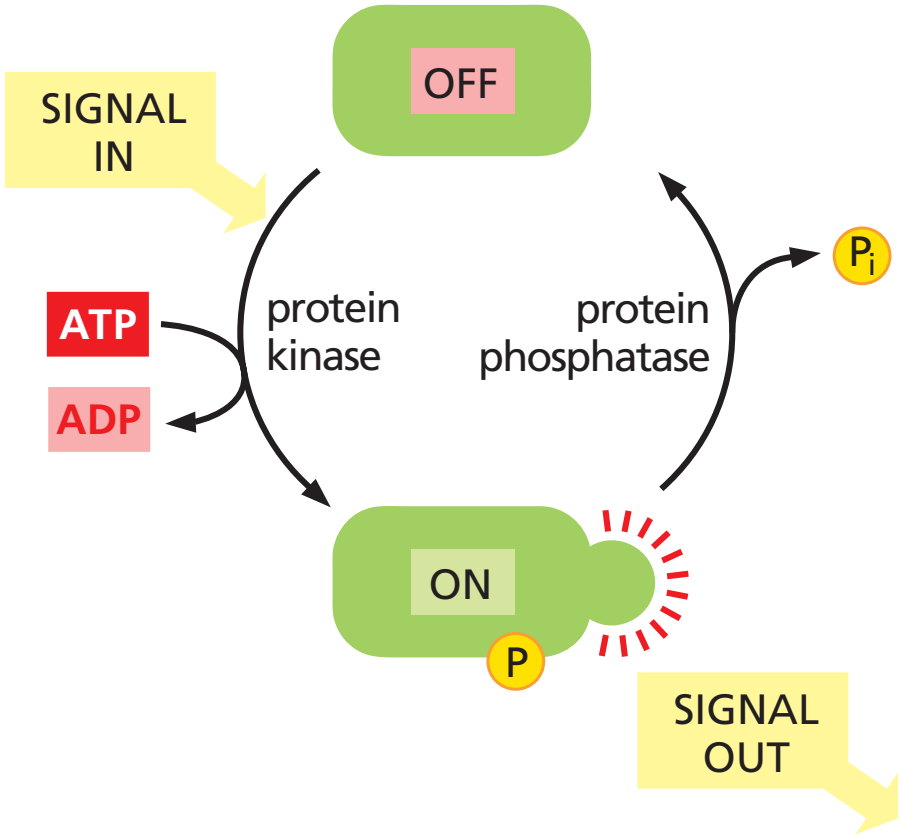


The receptors (R) have an intracellular Kinase (K) domain that can phosphorylates a Tyrosine (T), often in in their own protein sequence

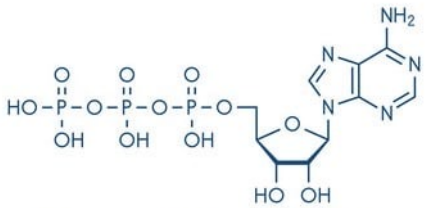
The extracellular domain is variable

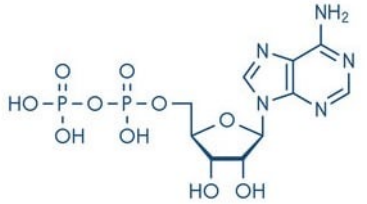
Kinase insert region = extra segment emerging from the folded kinase domain, important for interactions with other proteins.

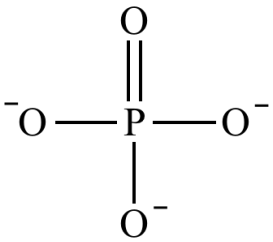
Two types of intracellular signaling proteins that act as molecular switches



(A) SIGNALING BY PHOSPHORYLATION







Phosphate group is covalently bound to a protein by a special enzyme class called a kinase.

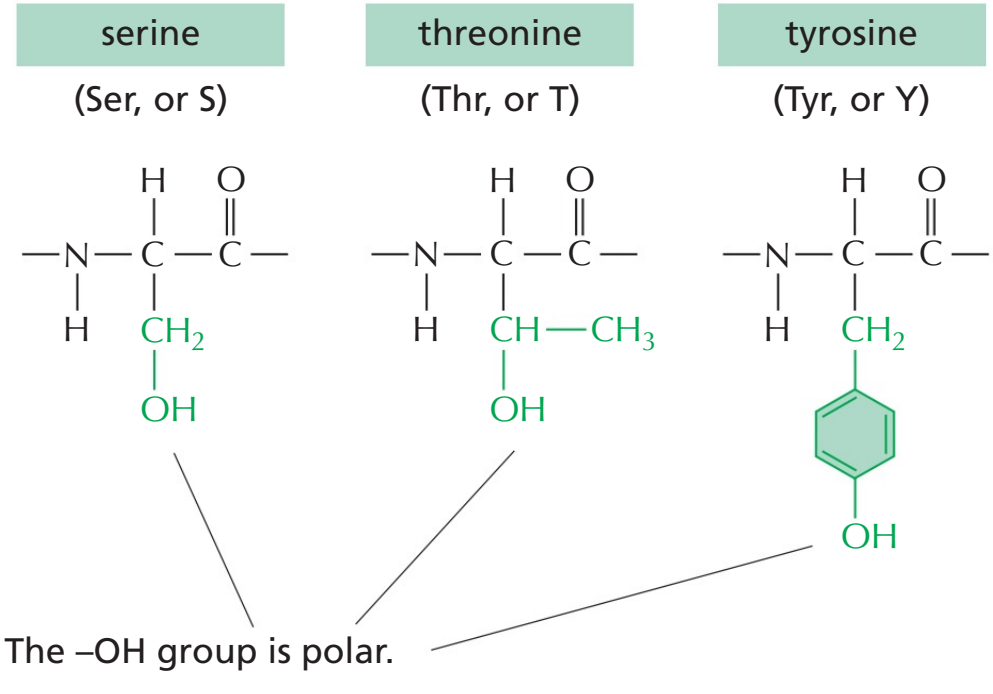
The group is added to the side chain of Tyrosine, Threonine or Serine

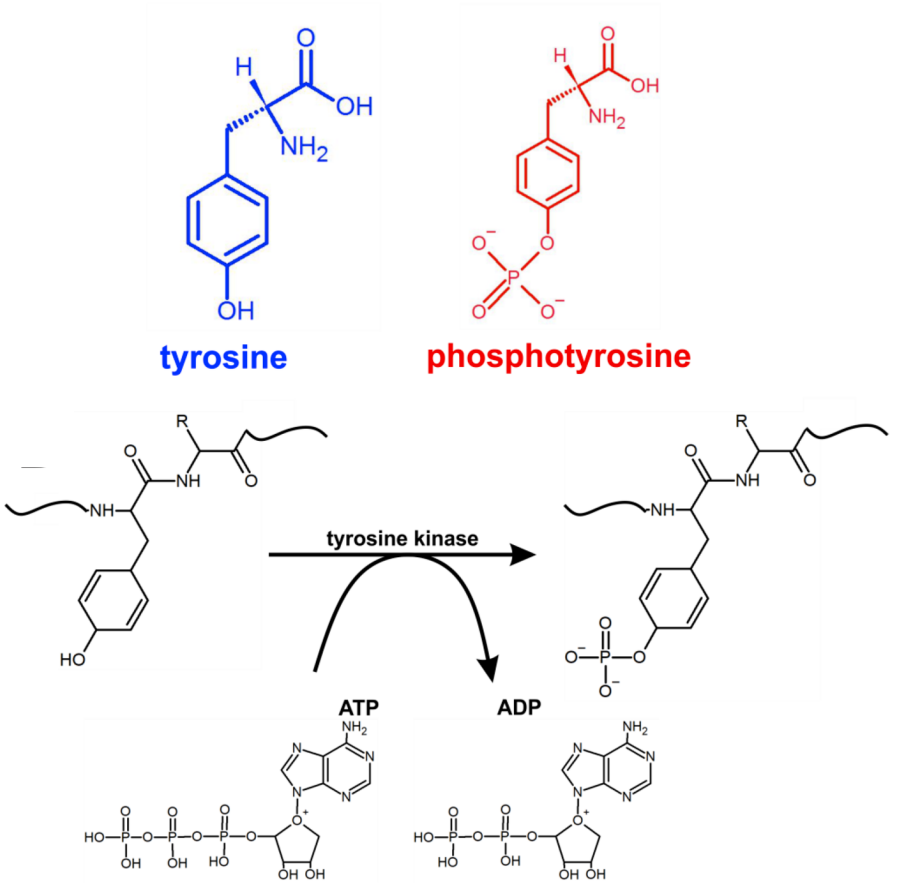
Phosphatases remove the group

Phosphorylation can lead to changes is conformation, allow different interactions with new proteins and many more

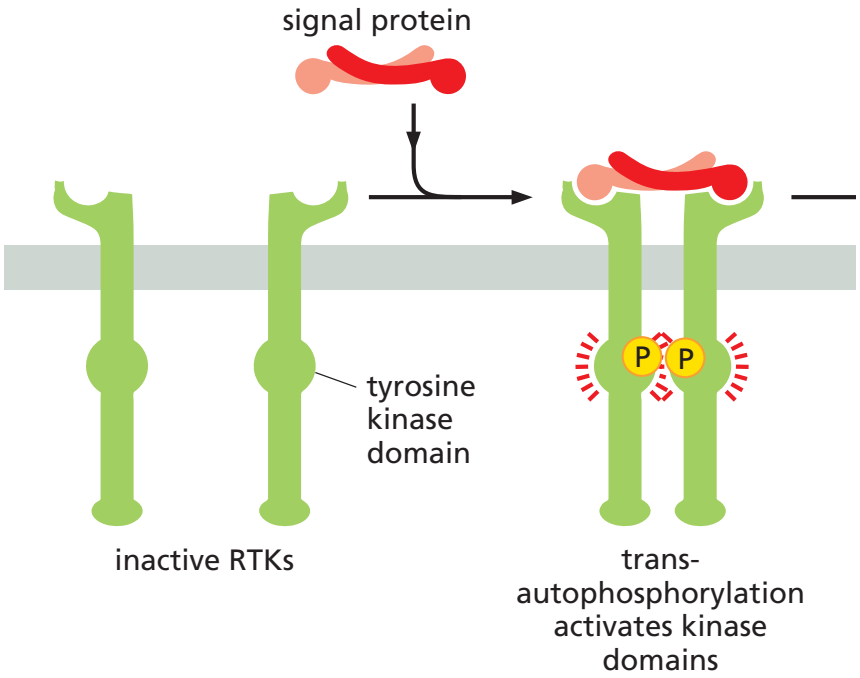
In some cases, dephosphorylation is the activating signal!

Three amino acids can be phosphorylated on their side chain





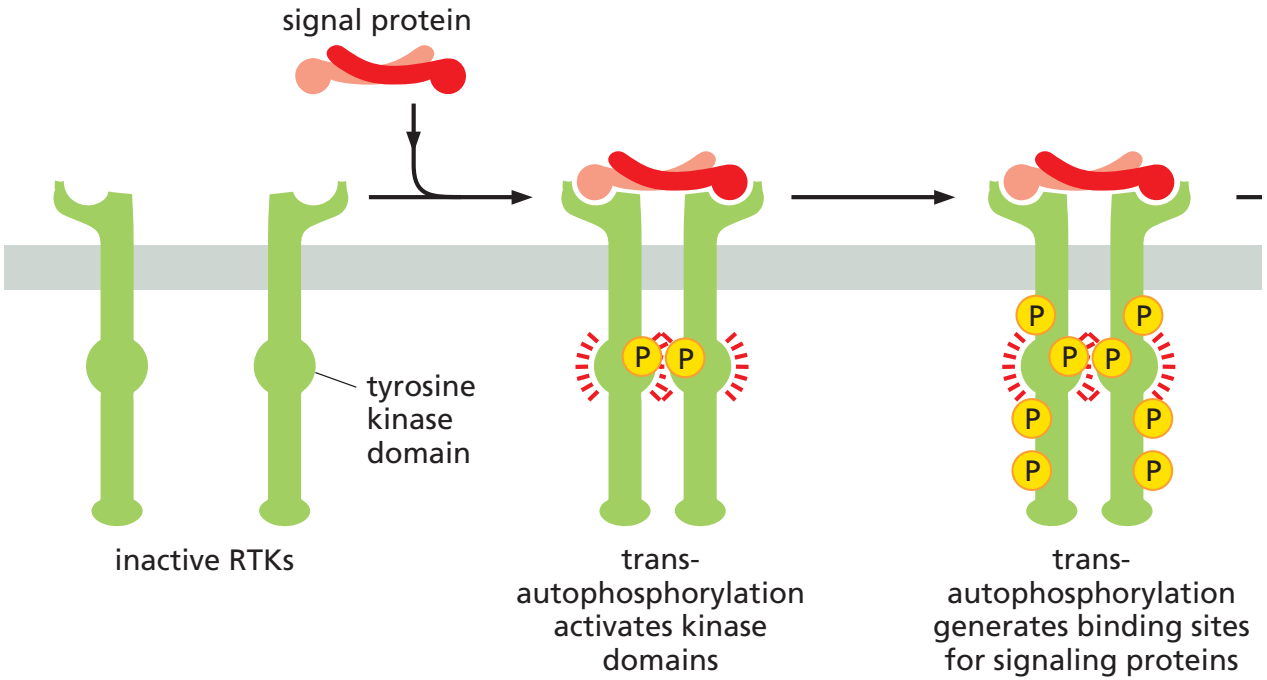
Activation of RTKs by dimerization



Two RTK are bound by a ligand, it does not have to be one ligand binding to two RTKs as shown in the example!

The RTK dimerize and make an initial Tyrosine autophosphorylation
auto = “self”

Activation of RTKs by dimerization

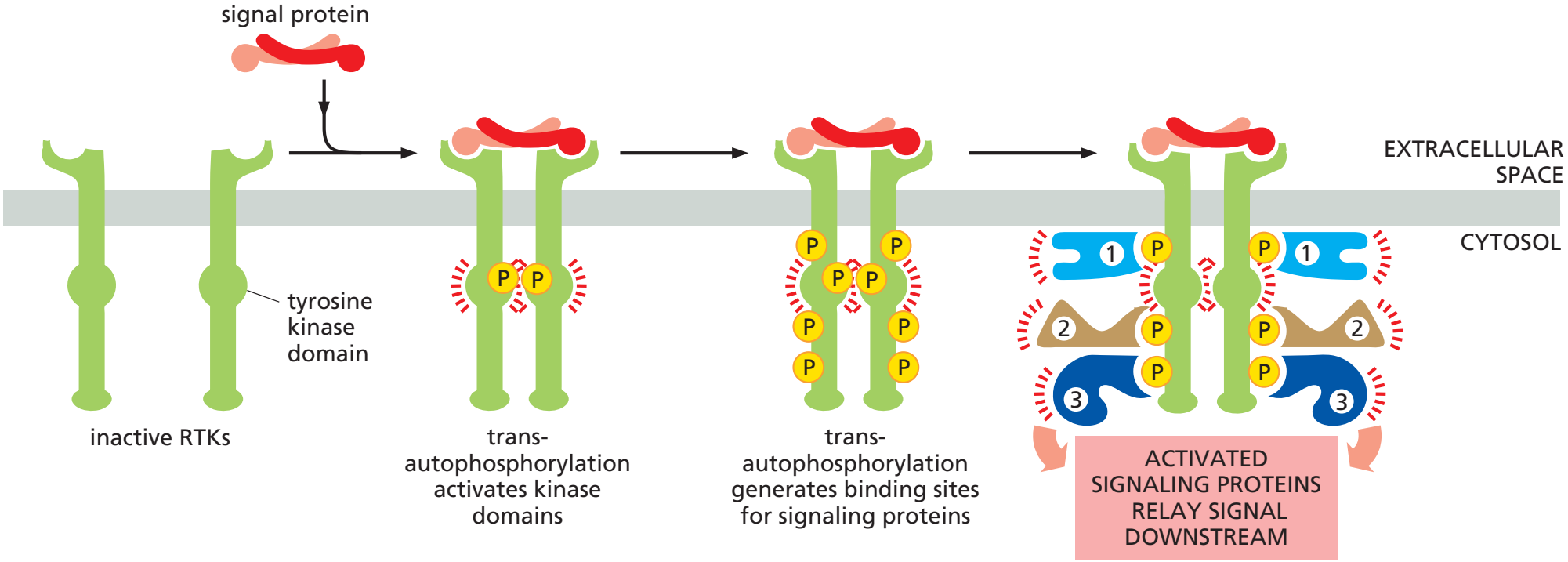


Two RTK are bound by a ligand, it does not have to be one ligand binding to two RTKs as shown in the example!

The RTK dimerize and make an initial Tyrosine autophosphorylation
auto = “self”

first phosphorylation initiates trans-autophosphorylation of several tyrosines

Activation of RTKs by dimerization



Two RTK are bound by a ligand, it (Does not have to be dimerized ligand binding to two RTKs as shown in the example!)

The RTK dimerize and make an initial Tyrosine autophosphorylation auto = “self”

first phosphorylation initiates trans-autophosphorylation of several tyrosines

Phospho-Tyrosine sites recruit and/or activate downstream signaling proteins

Activation of RTKs by dimerization

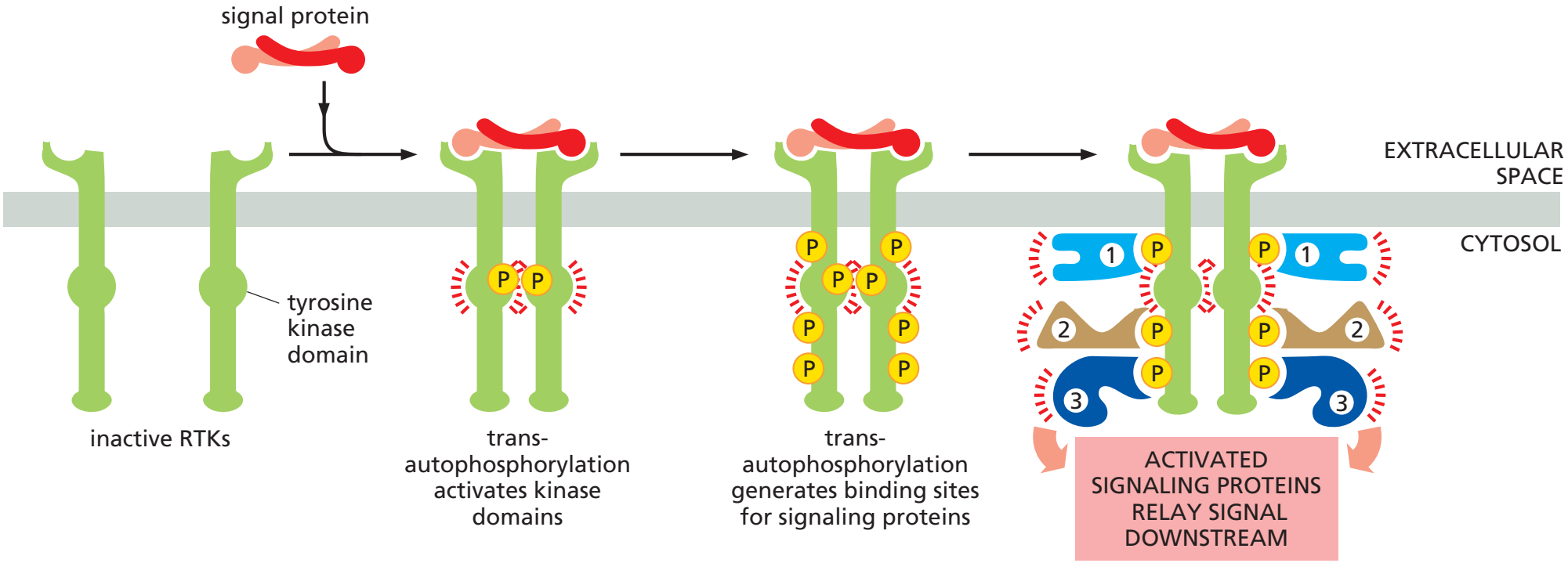


Figure 15–44 Activation of RTKs by dimerization. In the absence of extracellular signals, most RTKs exist as monomers in which the internal kinase domain is inactive. Binding of ligand brings two monomers together to form a dimer. In most cases, the close proximity in the dimer leads the two kinase domains to phosphorylate each other, which has two effects. First, phosphorylation at some tyrosines in the kinase domains promotes the complete activation of the domains. Second, phosphorylation at tyrosines in other parts of the receptors generates docking sites for intracellular signaling proteins, resulting in the formation of large signaling complexes that can then broadcast signals along multiple signaling pathways.

Mechanisms of dimerization vary widely among different RTK family members. In some cases, as shown here, the ligand itself is a dimer and brings two receptors together by binding them simultaneously. In other cases, a monomeric ligand can interact with two receptors simultaneously to bring them together, or two ligands can bind independently on two receptors to promote dimerization. In some RTKs—notably those in the insulin receptor family—the receptor is always a dimer (see Figure 15–43), and ligand binding causes a conformational change that brings the two internal kinase domains closer together. Although many RTKs are activated by transautophosphorylation as shown here, there are some important exceptions, including the EGF receptor illustrated in Figure 15–45.

Important RTK exception: Activation of the EGF receptor kinase

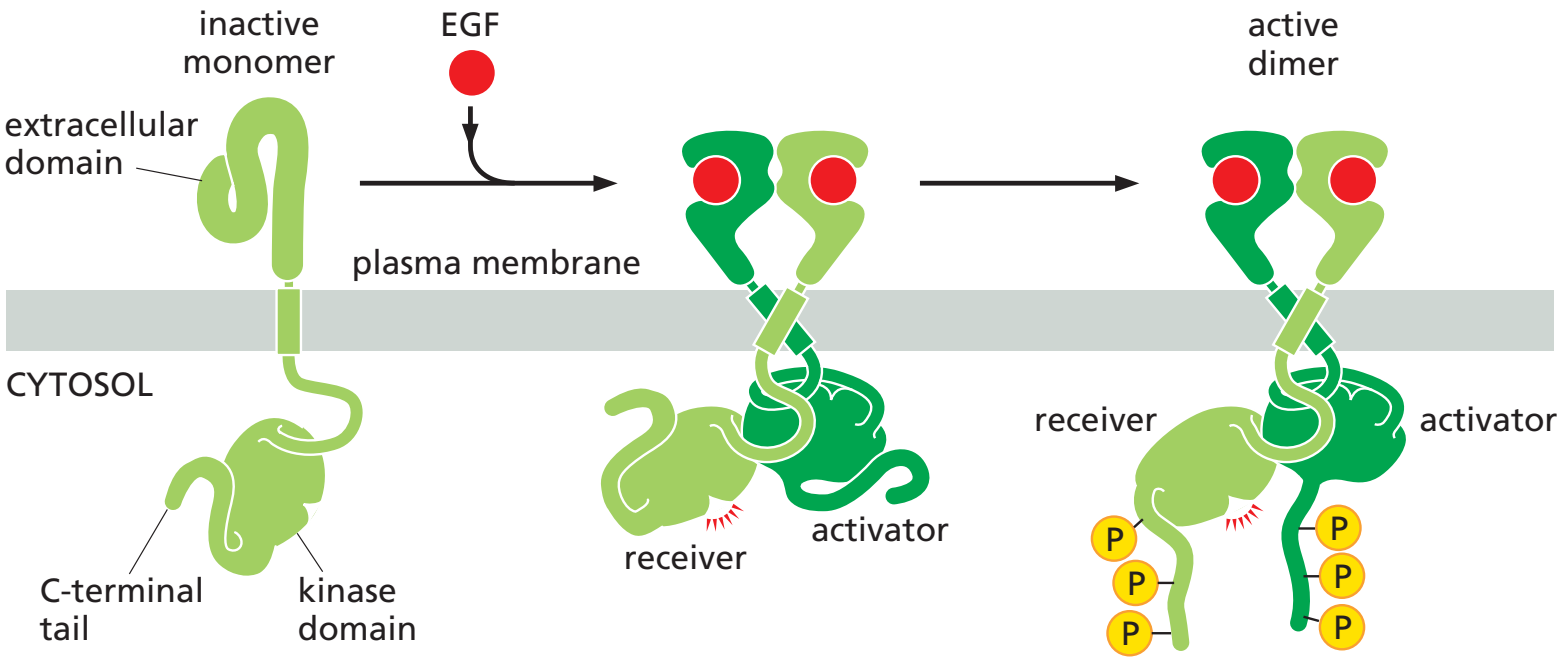
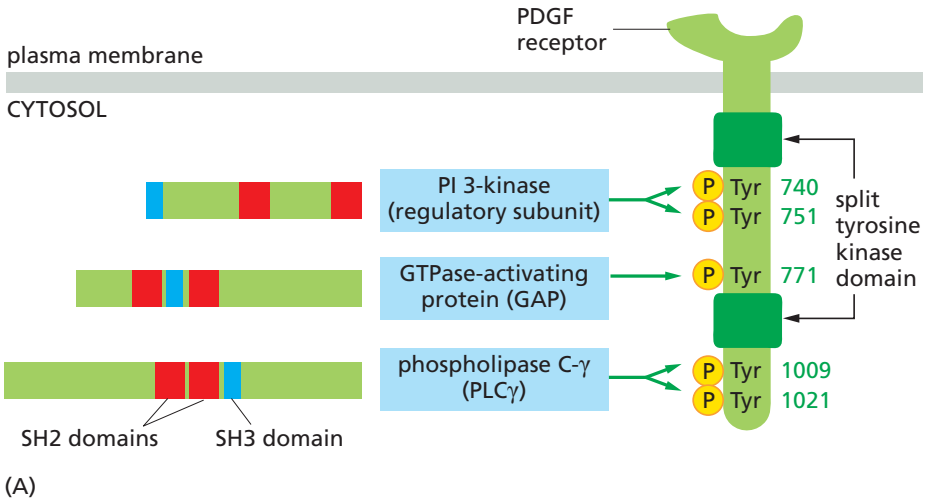


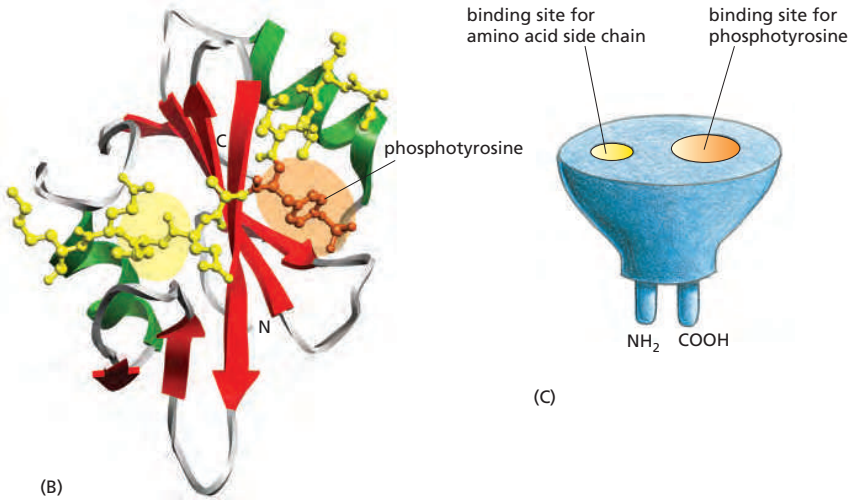
Figure 15–45 Activation of the EGF receptor kinase. In the absence of ligand, the EGF receptor exists primarily as an inactive monomer. EGF binding results in a conformational change that promotes dimerization of the external domains. The receptor kinase domain, unlike that of many RTKs, is not activated by transautophosphorylation. Instead, dimerization orients the internal kinase domains into an asymmetric dimer, in which one kinase domain (the "activator") pushes against the other kinase domain (the "receiver"), thereby causing an activating conformational change in the receiver. The active receiver domain then phosphorylates multiple tyrosines in the C-terminal tails of both receptors, generating docking sites for intracellular signaling proteins (see Figure 15–44).

Instead of symmetric phosphorylation there is an activator and receiver protein
Eventhough these are identical receptor proteins!

The kinase domain of the activator pushes against the receiver. The receiver changes conformation and the receiver kinase domain phosphorylates tyrosines on both receptors

The binding of SH2- containing intracellular signaling proteins to an activated RTK





SH2 domain in 3D structure and animation

The phospho-tyrosines are docking sites for proteins containing SH2 (Src Homology) domains or PTB (PhosphoTyrosine Binding)

The multitude of phospho-tyrosines present allow for different proteins to interact with the receptor as well as activate various different signaling pathways

Thee important ones:

PI3 Kinase

RAS signaling (via the GAP)

PLC (also a target of GPRCs!)

Very important in various cancertypes!

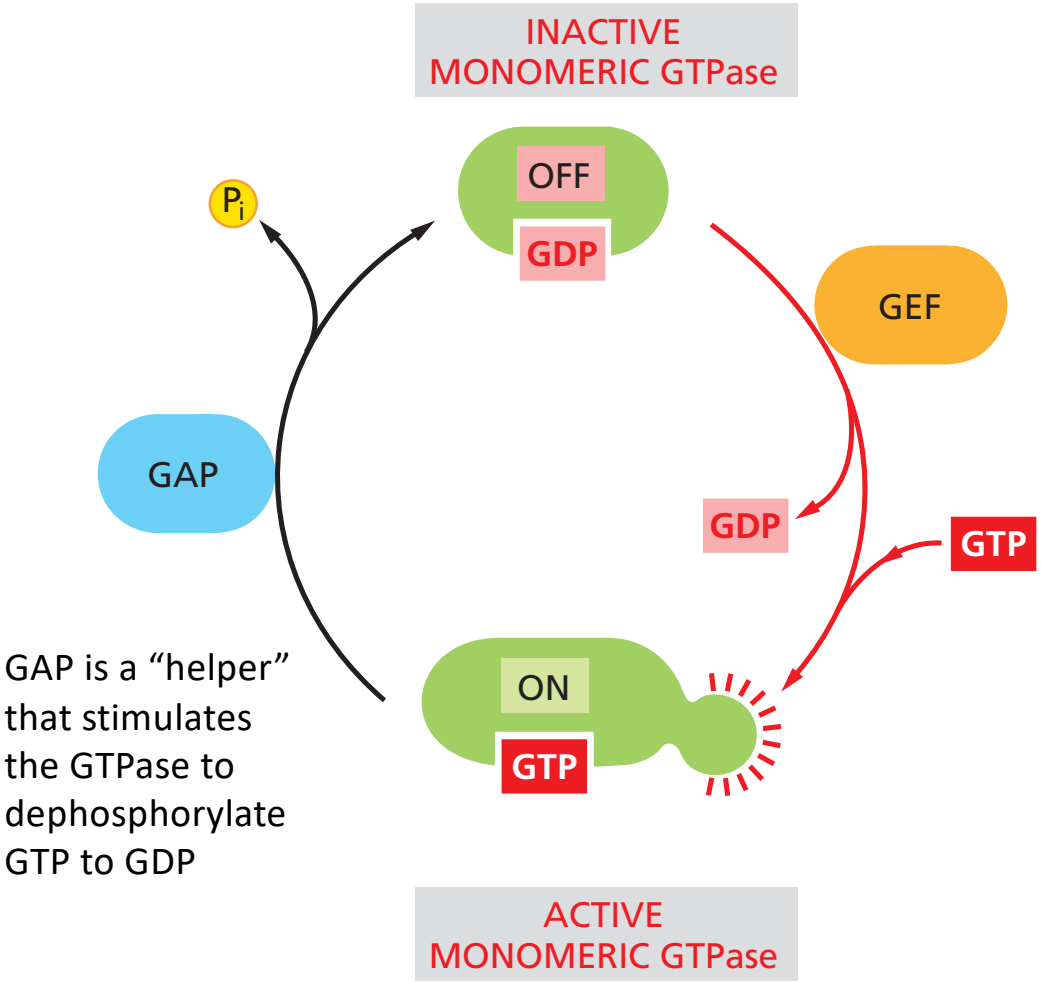
RAS signaling

The Ras Superfamily of Monomeric GTPases

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 3–67.		

RAS is a monomeric GTPase

The regulation of a monomeric GTPase



GAP is a "helper" that stimulates the GTPase to dephosphorylate GTP to GDP

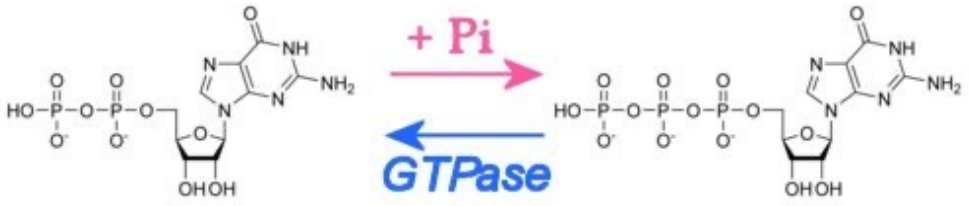
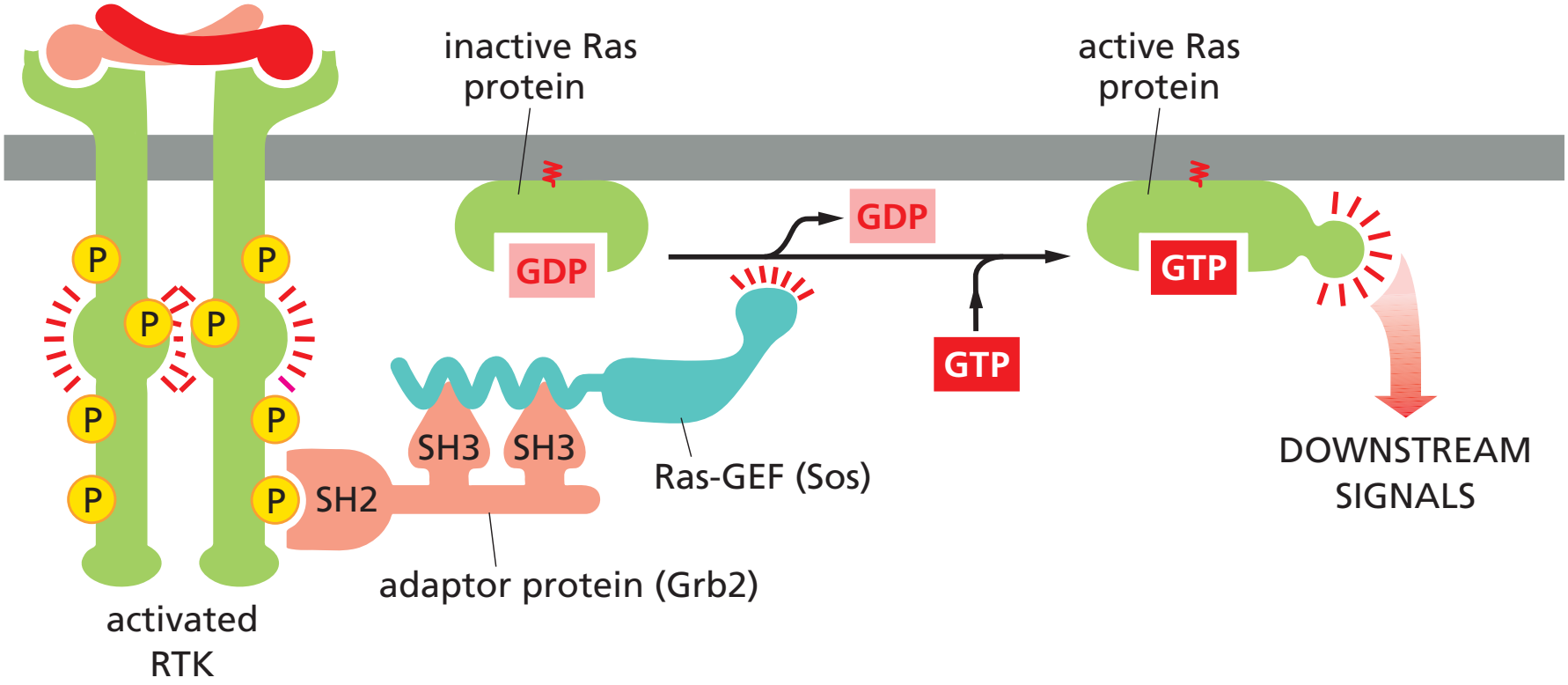


Figure 15–8 The regulation of a monomeric GTPase. GTPase-activating proteins (GAPs) inactivate the protein by stimulating it to hydrolyze its bound GTP to GDP, which remains tightly bound to the inactivated GTPase. Guanine nucleotide exchange factors (GEFs) activate the inactive protein by stimulating it to release its GDP; because the concentration of GTP in the cytosol is 10 times greater than the concentration of GDP, the protein rapidly binds GTP and is thereby activated.

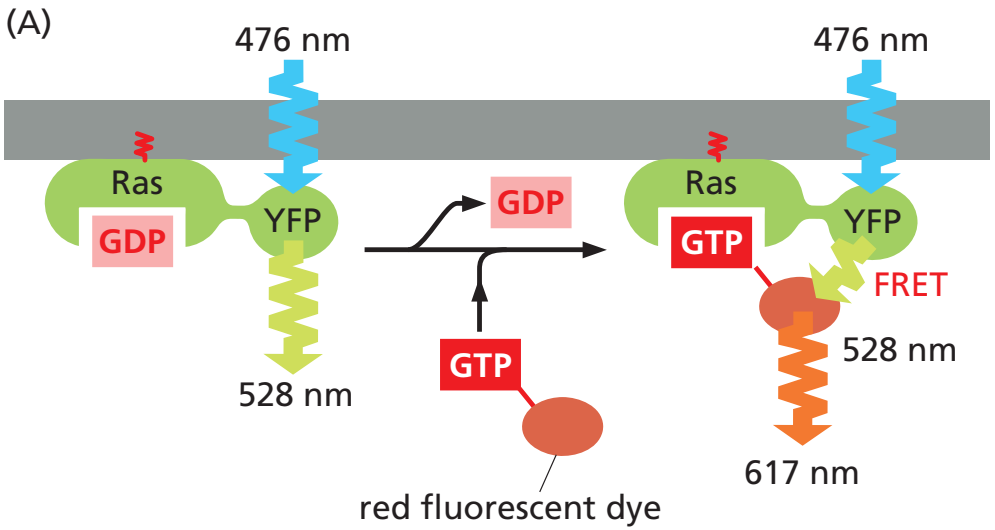
How an RTK activates Ras

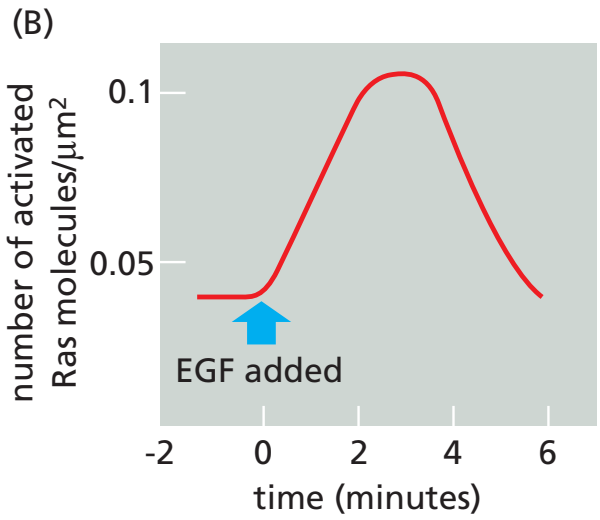


an adaptor protein Grb2 interacts with Ras-GEF (Sos) with facilitates the exchange of GDP to GTP in RAS resulting in activated RAS

RAS is anchored to the membrane with a lipid modification

Transient activation of Ras revealed by single-molecule fluorescence resonance energy transfer (FRET)





Using a FRET experiment we can visualize active RAS signaling

Figure 15–48 Transient activation of Ras revealed by single-molecule fluorescence resonance energy transfer (FRET). (A) Schematic drawing of the experimental strategy. Cells of a human cancer cell line are genetically engineered to express a Ras protein that is covalently linked to yellow fluorescent protein (YFP). GTP that is labeled with a red fluorescent dye is microinjected into some of the cells. The cells are then stimulated with the extracellular signal protein EGF, and single fluorescent molecules of Ras-YFP at the inner surface of the plasma membrane are followed by video fluorescence microscopy in individual cells. When a fluorescent Ras-YFP molecule becomes activated, it exchanges unlabeled GDP for fluorescently labeled GTP; the energy emitted by the YFP now activates the fluorescent GTP to emit red light (called fluorescence resonance energy transfer, or FRET; see Figure 9–26). Thus, the activation of single Ras molecules can be followed by the emission of red fluorescence from a previously yellow-green fluorescent spot at the plasma membrane. As shown in (B), activated Ras molecules can be detected after about 30 seconds of EGF stimulation. The red signal peaks at about 3 minutes and then decreases to baseline by 6 minutes. As Ras-GAP is found to be recruited to the same spots at the plasma membrane as Ras, it presumably plays a major part in rapidly shutting off the Ras signal. (Modified from H. Murakoshi et al., *Proc. Natl Acad. Sci. USA* 101:7317–7322, 2004. With permission from National Academy of Sciences.)

The MAP kinase module activated by Ras

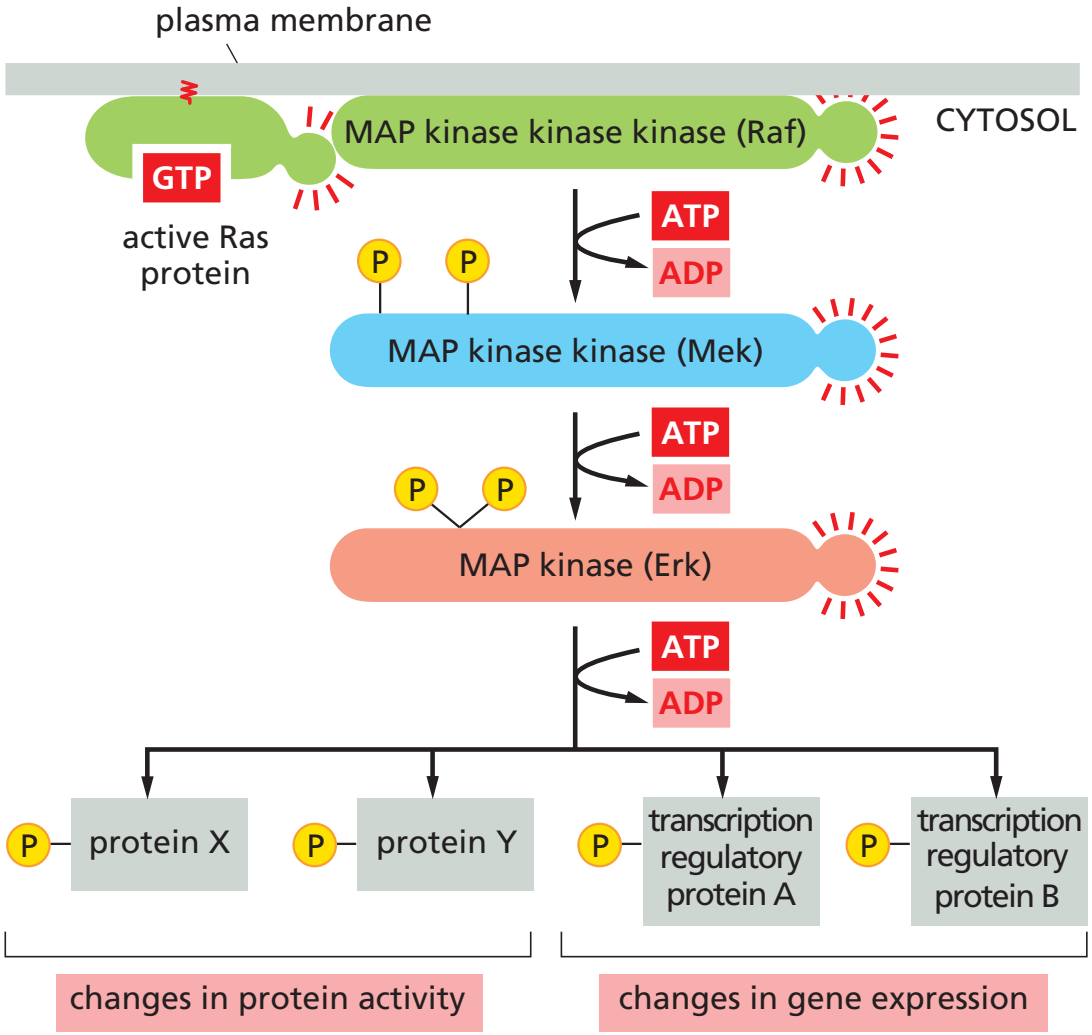


Figure 15–49 The MAP kinase module activated by Ras. The three-component module begins with a MAP kinase kinase kinase called *Raf.* Ras recruits Raf to the plasma membrane and helps activate it. Raf then activates the MAP kinase kinase *Mek*, which then activates the MAP kinase *Erk.* Erk in turn phosphorylates a variety of downstream proteins, including other protein kinases, as well as transcription regulators in the nucleus. The resulting changes in protein activities and gene expression cause complex changes in cell behavior.

Mutations in Ras are often found in cancer

Three most famous types of RAS proteins

K-RAS
N-RAS
H-RAS

Tissue	H-Ras	K-Ras	N-Ras
Adrenal gland	1%	0%	5%
Biliary tract	0%	32%	1%
Bone	2%	1%	0%
Breast	1%	5%	1%
Central nervous system	0%	1%	2%
Cervix	9%	8%	1%
Endometrium	1%	14%	0%
Eye	0%	4%	1%
Gastrointestinal tract (site indeterminate)	0%	19%	0%
Haematopoietic and lymphoid tissue	0%	5%	12%
Kidney	0%	1%	0%
Large intestine	0%	32%	3%
Liver	0%	7%	4%
Lung	1%	17%	1%
Meninges	0%	0%	0%
Oesophagus	1%	4%	0%
Ovary	0%	15%	4%
Pancreas	0%	60%	2%
Parathyroid	0%	0%	0%
Peritoneum	0%	6%	ND
Pituitary	2%	0%	0%
Placenta	0%	0%	0%
Pleura	0%	0%	0%
Prostate	6%	8%	1%
Salivary gland	16%	4%	0%
Skin	5%	2%	19%
Small intestine	0%	20%	25%
Stomach	4%	6%	2%
Testis	0%	5%	4%
Thymus	0%	15%	0%
Thyroid	4%	3%	7%
Upper aerodigestive tract	9%	4%	3%
Urinary tract	12%	4%	3%

Nat Rev Mol Cell Biol. 2008; 9(7):517-31

Mutations occur at the amino acids:

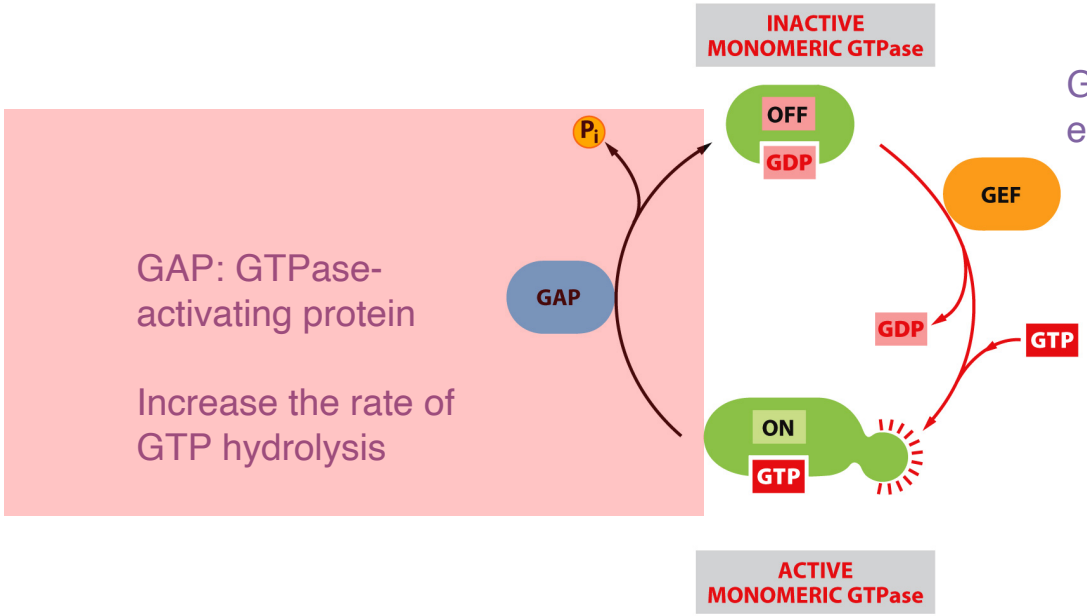
- G12
- G13
- Q61

Mutants exhibit impaired GTPase activity, hence conferring a gain-of-function

Mutant Ras probably participate in tumor initiation in a lot of cases

In mice, mutant Ras can be engineered to be expressed in a single tissue
- With this strategy, mouse models of several human cancers (pancreatic, lung, ovarian…) have been generated

Ras activity is regulated by GAP and GEF



GAP: GTPase-activating protein

Increase the rate of GTP hydrolysis

GEF: guanine nucleotide exchange factor

Figure 15-8 Molecular Biology of the Cell 6e (© Garland Science 2015)

Oncogenic mutant forms of Ras become resistant to GAP-mediated GTPase stimulation, becoming locked in the GTP-bound state

PI3K signaling

The generation of phosphoinositide docking sites by PI 3-kinase

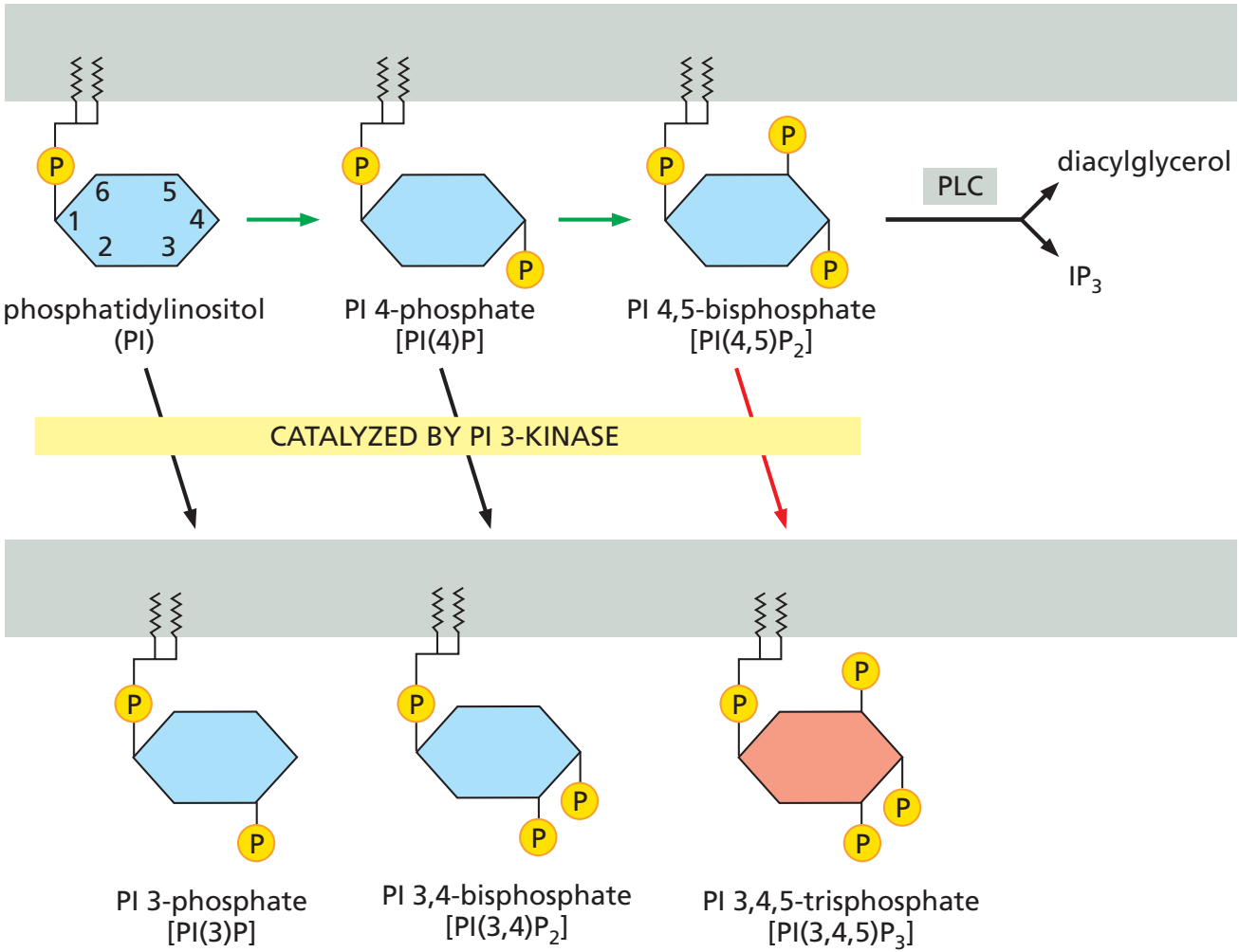
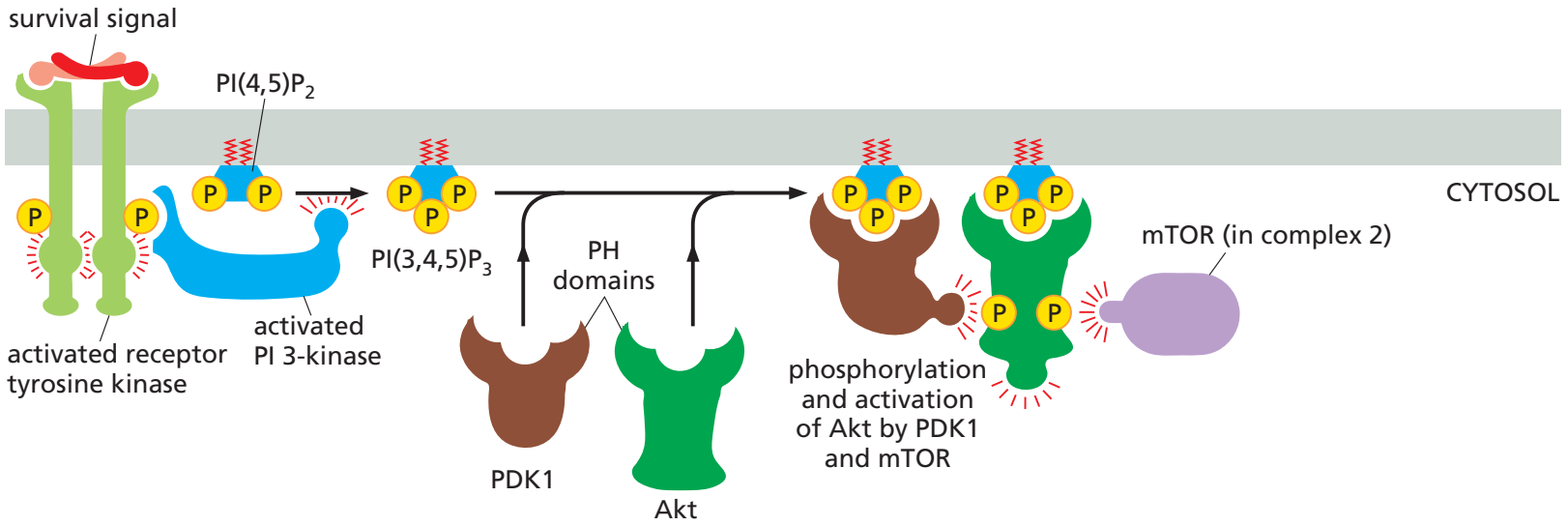


Figure 15–52 The generation of phosphoinositide docking sites by PI 3-kinase. PI 3-kinase phosphorylates the inositol ring on carbon atom 3 to generate the phosphoinositides shown at the bottom of the figure (diverting them away from the pathway leading to IP_3 and diacylglycerol; see Figure 15–28). The most important phosphorylation (indicated in *red*) is of $PI(4,5)P_2$ to $PI(3,4,5)P_3$, which can serve as a docking site for signaling proteins with $PI(3,4,5)P_3$-binding PH domains. Other inositol phospholipid kinases (not shown) catalyze the phosphorylations indicated by the *green arrows.*

PI 3-kinase activates AKT



PI3K is recruited by an RTK
PI3K creates docking sites where proteins with a PH domain can bind
PDK1 activates AKT by phosphorylation

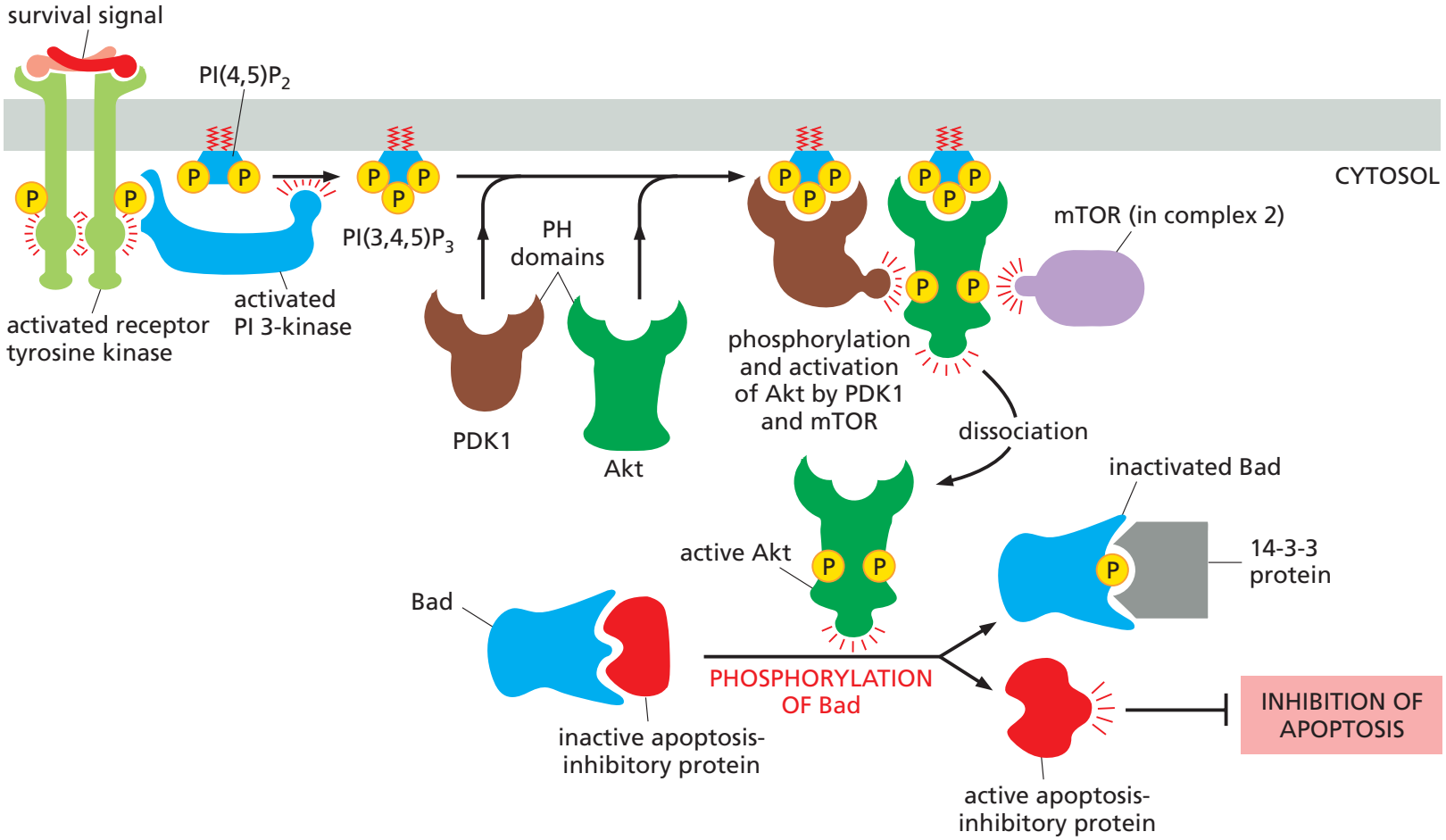
p-AKT activates many cellular programs including

Cell growth
Anti-apoptosis

A phosphatase PTEN removes phosphate groups from PI(3,4,5)P3

PTEN is a negative regulator of PI3K signaling

One way in which signaling through PI 3-kinase promotes cell survival



Activation growth: mTOR activation by the PI-3-kinase–Akt signaling pathway

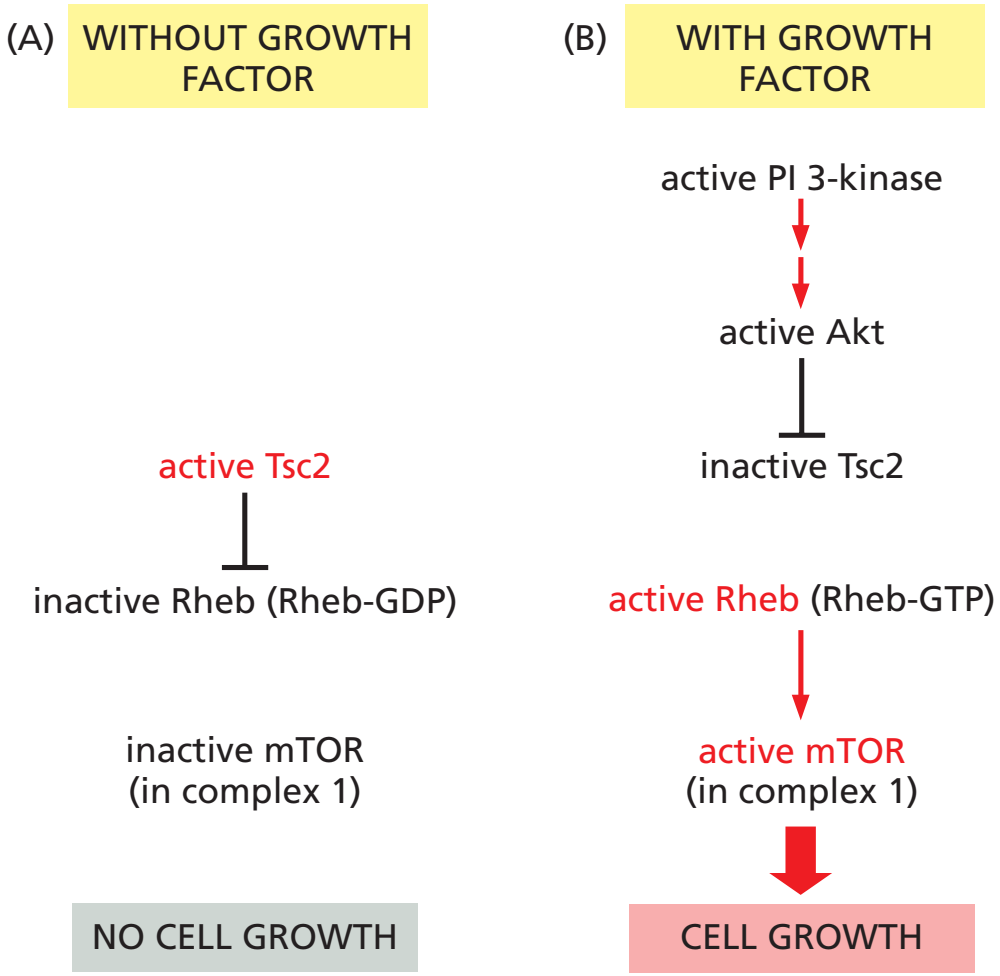
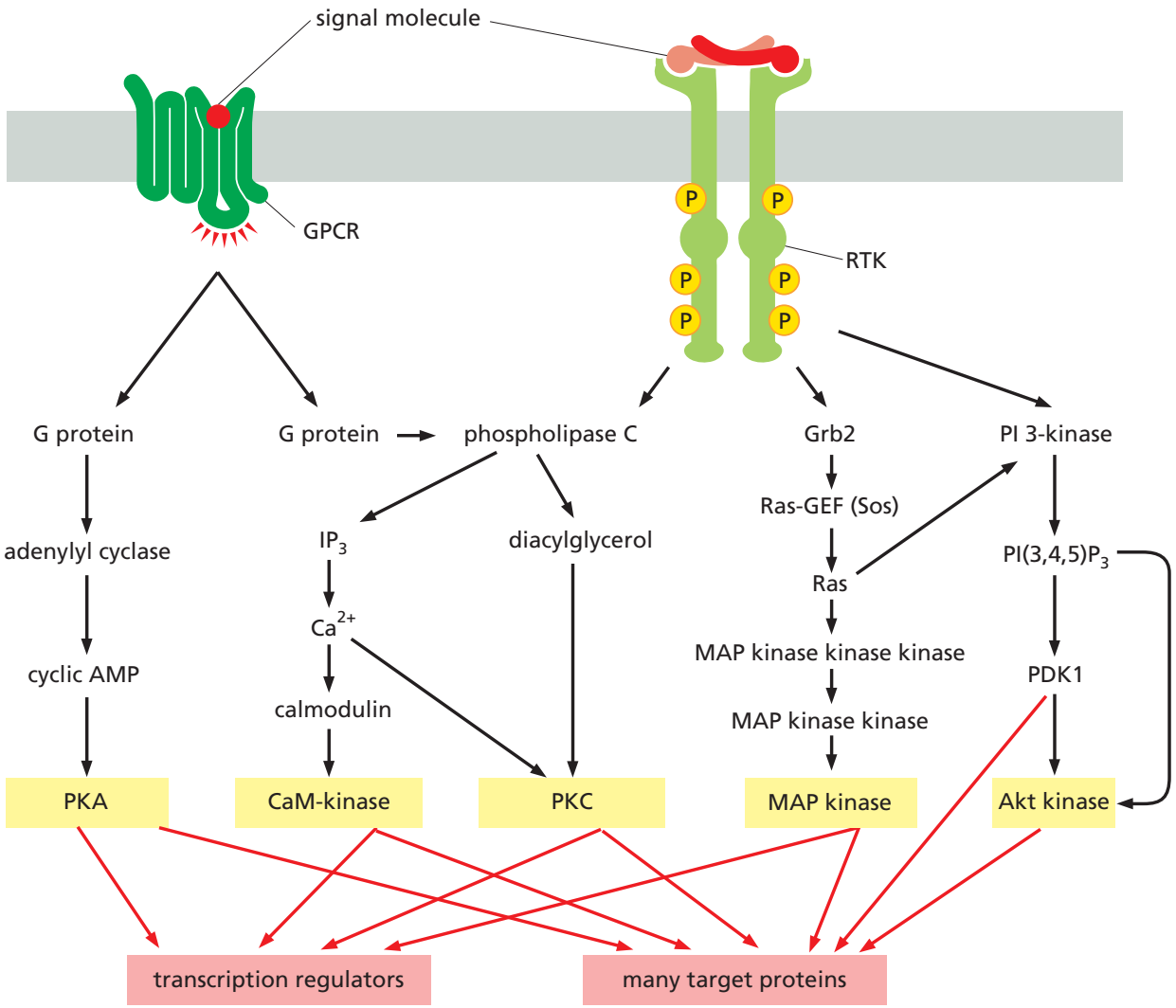


Figure 15–54 Activation of mTOR by the PI-3-kinase–Akt signaling pathway. (A) In the absence of extracellular growth factors, Tsc2 (a Rheb-GAP) keeps Rheb inactive; mTOR in complex 1 is inactive, and there is no cell growth. (B) In the presence of growth factors, activated Akt phosphorylates and inhibits Tsc2, thereby promoting the activation of Rheb. Activated Rheb (Rheb-GTP) helps activate mTOR in complex 1, which in turn stimulates cell growth. Figure 15–53 shows how growth factors (or survival signals) activate Akt. The Erk MAP kinase (see Figure 15–49) can also phosphorylate and inhibit Tsc2 and thereby activate mTOR. Thus, both the PI-3-kinase–Akt and Ras–MAP-kinase signaling pathways converge on mTOR in complex 1 to stimulate cell growth.

Tsc2 is short for *tuberous sclerosis protein 2,* and it is one component of a heterodimer composed of Tsc1 and Tsc2 (not shown); these proteins are so called because mutations in either gene encoding them cause the genetic disease *tuberous sclerosis,* which is associated with benign tumors that contain abnormally large cells.

Five parallel intracellular signaling pathways activated by GPCRs, RTKs, or both

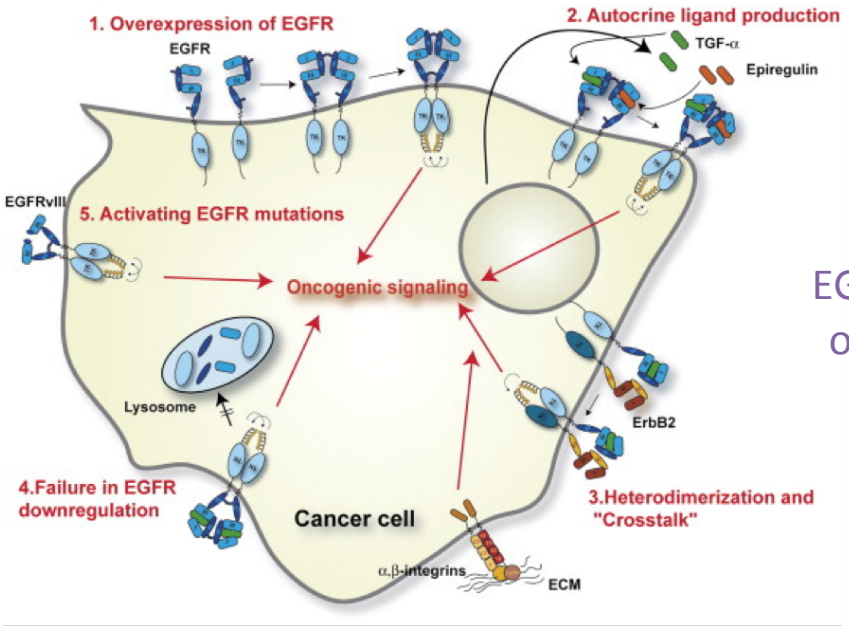


PLC is PLCb by GPCR and PLCy by RTK, the effect is similar!

Figure 15–55 Five parallel intracellular signaling pathways activated by GPCRs, RTKs, or both. In this simplified example, the five kinases (shaded *yellow*) at the end of each signaling pathway phosphorylate target proteins (shaded *red*), many of which are phosphorylated by more than one of the kinases. The phospholipase C activated by the two types of receptors is different: GPCRs activate PLCβ, whereas RTKs activate PLCγ (not shown). Although not shown, some GPCRs can also activate Ras, but they do so independently of Grb2, via a Ras-GEF that is activated by Ca^{2+} and diacylglycerol.

Activation of EGFR in cancer

Aberrant EGF-R signaling in cancer

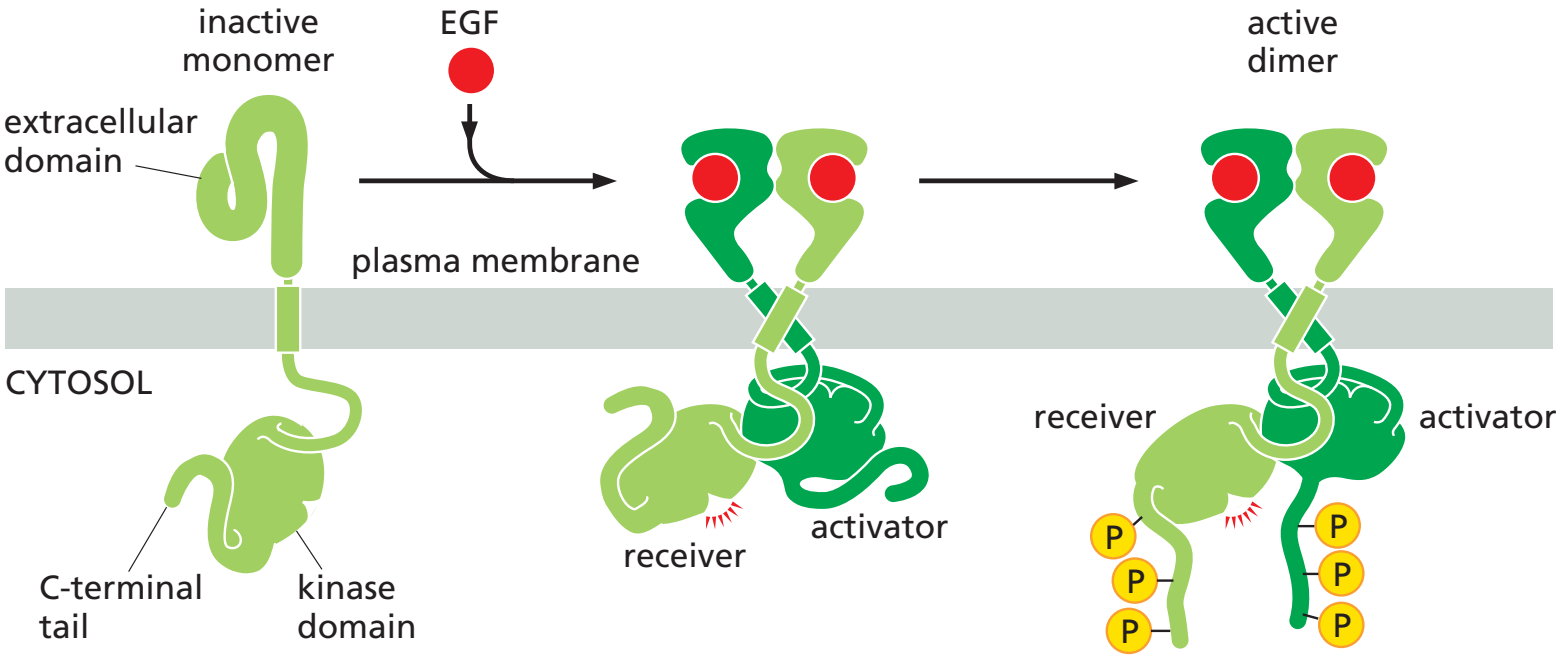


EGF-R can become over-activated in cancer

- gene amplification (40% glioblastomas)
- mutations in the kinase domain (10% lung adenocarcinoma)

targeted therapies against mutant EGF-R:
Gefitinib (Iressa®)
Erlotinib (Tarceva®)

Activation of the EGF receptor kinase

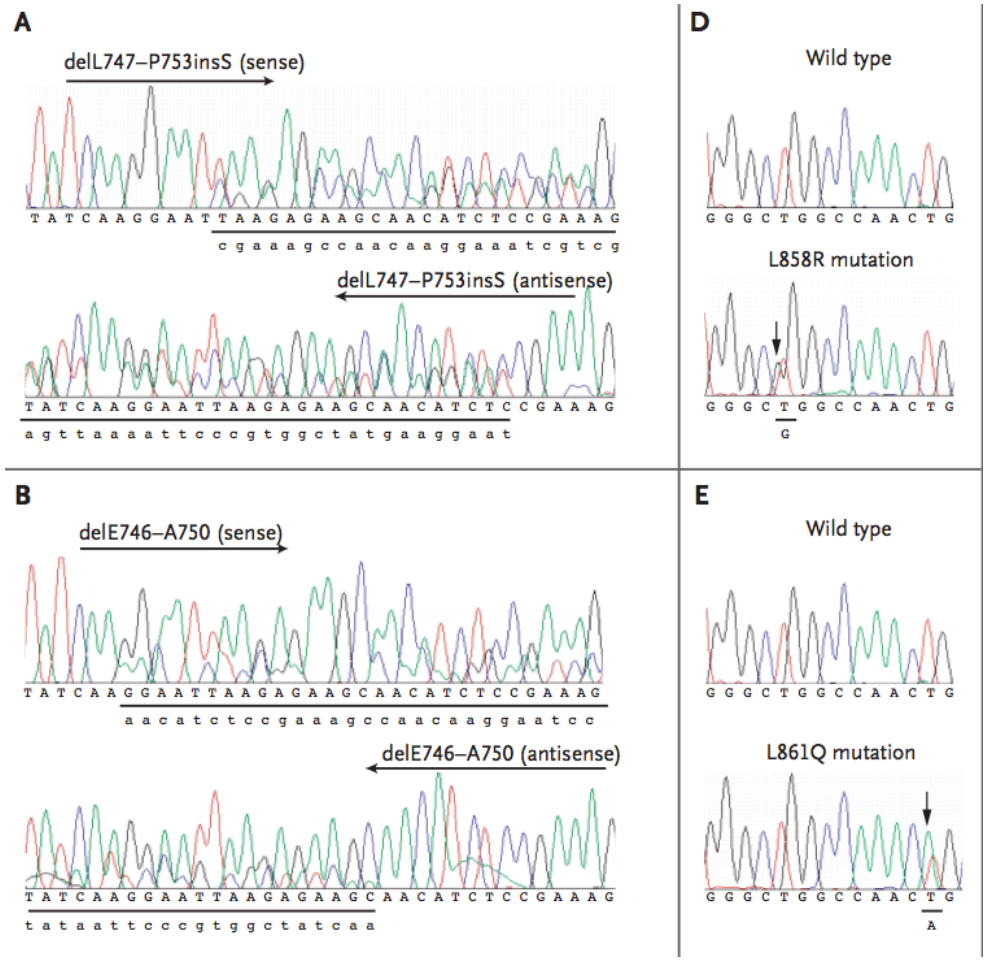


Mutations occur in the kinase domain of the receptor -> the mutant kinase domain is permanently active! Thus RAS-MAPK and Pi3K are always active!
Uncontrolled cell growth

Mutations found in EGFR

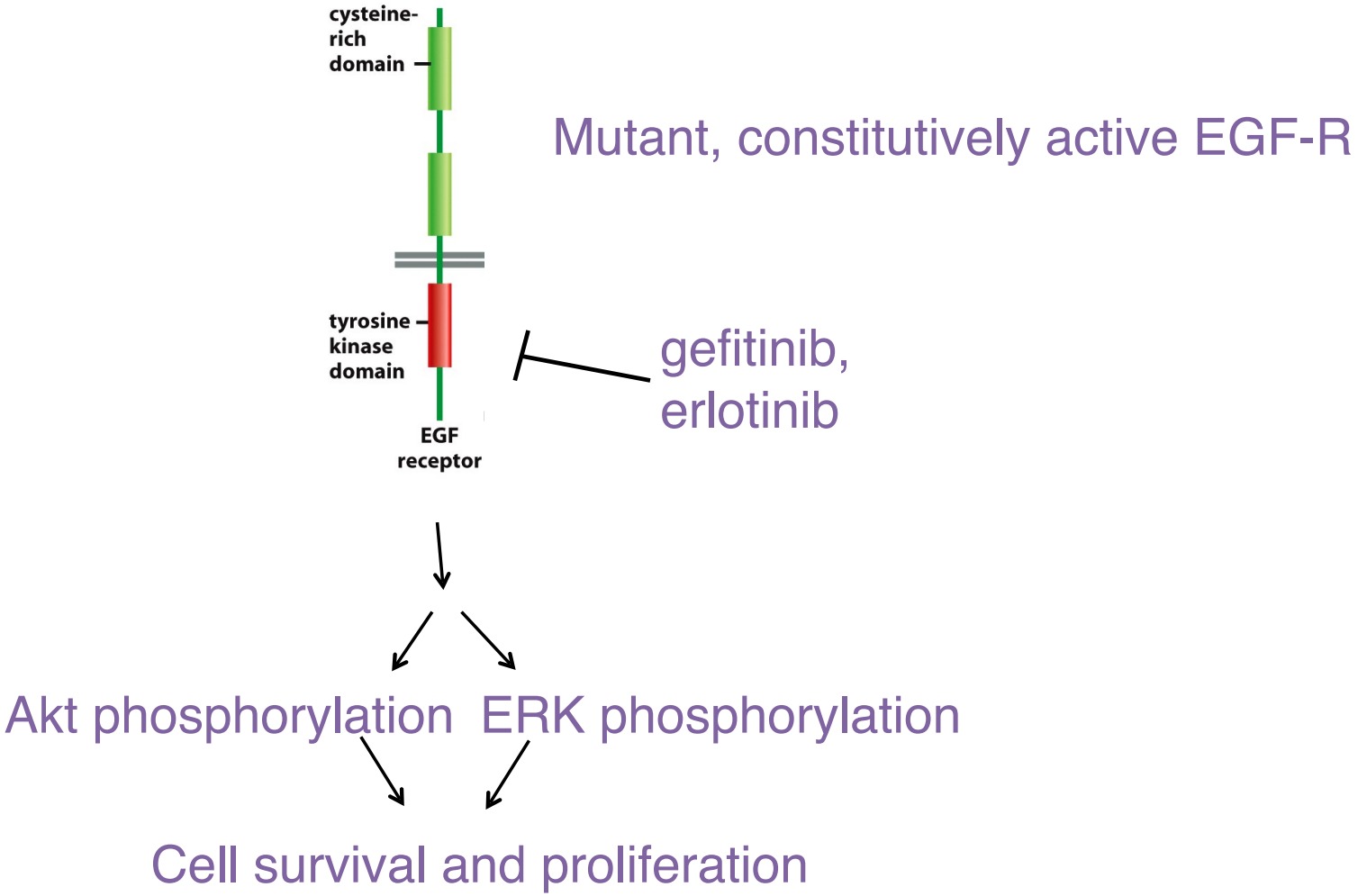
Sanger sequencing

Deletions keeping the reading frame (A & B), or missense mutations (D & E)



The NEW ENGLAND JOURNAL of MEDICINE
ESTABLISHED IN 1812 MAY 20, 2004 VOL. 350 NO. 21

Inhibitors can target constitutively active EGFR



Summary

GPCR signaling and downstream signaling events

RTK signaling and downstream signaling events

Understand there is a lot of overlap in downstream signaling targets

Understand how mutations can hyperactivate signaling