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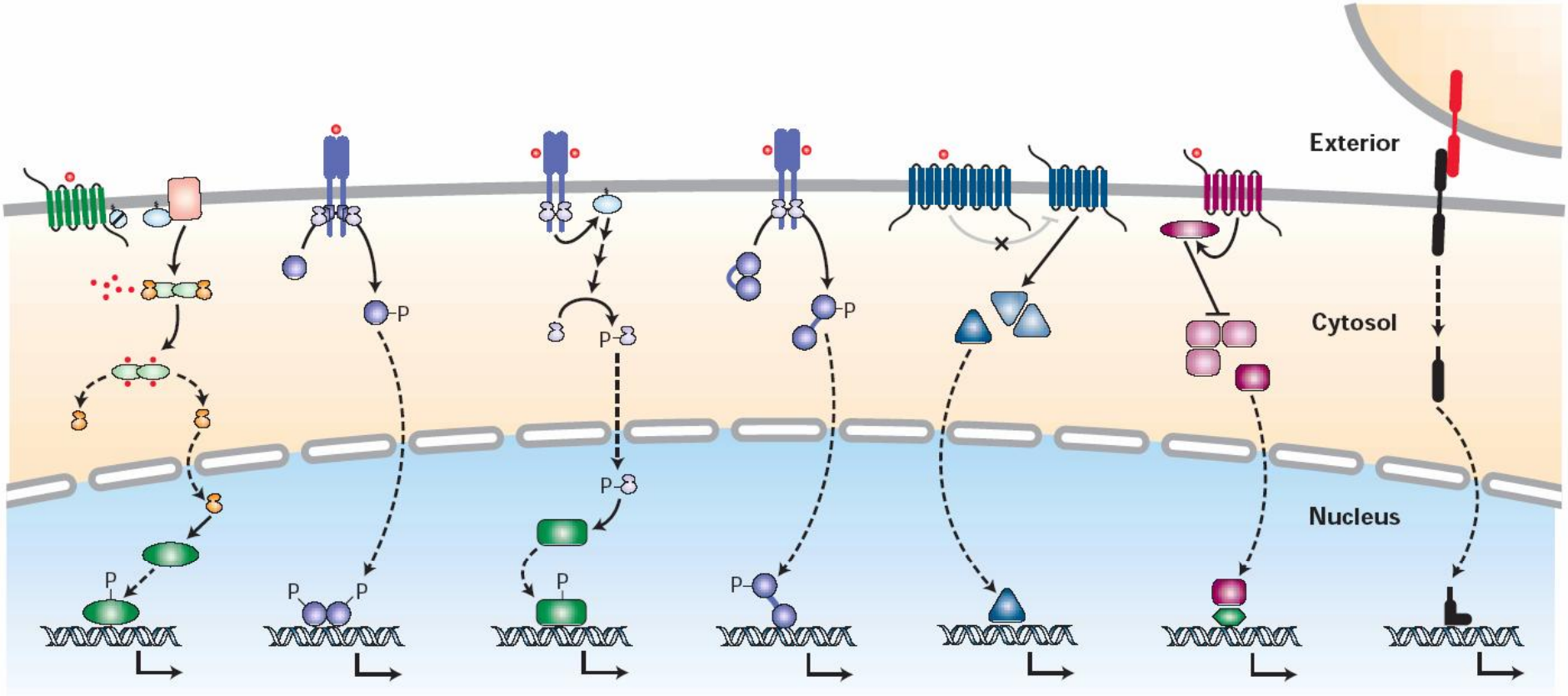
Molecular Cell Biology

Fifth Edition

Chapter 14:
Signaling Pathways That Control Gene Activity

SIGNALING AT THE CELL SURFACE



G protein-coupled receptors	Cytokine receptors	Receptor tyrosine kinases	TGFβ receptors	Hedgehog (Hh) receptors	Wnt receptors	Notch receptor
Linked to a trimeric G protein that controls the activity of an effector protein (here adenylyl cyclase)	Associated with cytosolic JAK kinases	Cytosolic domain with tyrosine kinase activity	Cytosolic domain with serine/threonine kinase activity	Hh ligand tethered to membrane of signaling cell by cholesterol anchor	Palmitoylated Wnt ligand binds seven transmembrane protein receptor complex	Ligand, Delta, is a transmembrane protein on signaling cell
Activate cytosolic or nuclear transcription factors via several pathways (here one involving protein kinase A)	Activate cytosolic STAT transcription factors by phosphorylation	Activate cytosolic kinases (here MAP kinase) that translocate to the nucleus and activate nuclear transcription factors by phosphorylation	Activate Smad transcription factors in the cytosol by phosphorylation	Control processing of transcription factor by proteolysis; Hh binding causes release from cytosolic complex	Release an activated transcription factor from a multiprotein complex in the cytosol	Cytosolic domain of Notch released by proteolysis acts in association with nuclear transcription factors

A typical mammalian cell often express cell-surface receptors for more than 100 different types of extracellular signaling molecules that function primarily to regulate the activity of transcription factors.

TABLE 14-1 Overview of Major Receptor Classes and Signaling Pathways

Receptor Class/Pathway*	Distinguishing Characteristics
RECEPTORS LINKED TO TRIMERIC G PROTEINS	
G protein–coupled receptors (13)	*Ligands:* Epinephrine, glucagon, serotonin, vasopressin, ACTH, adenosine, and many others (mammals); odorant molecules, light; mating factors (yeast) *Receptors:* Seven transmembrane α helices; cytosolic domain associated with a membrane-tethered trimeric G protein *Signal transduction:* (1) Second-messenger pathways involving cAMP or IP_3/DAG; (2) linked ion channels; (3) MAP kinase pathway

TABLE 14-1 Overview of Major Receptor Classes and Signaling Pathways

Receptor Class/Pathway*	Distinguishing Characteristics
RECEPTORS WITH INTRINSIC OR ASSOCIATED ENZYMATIC ACTIVITY	
TGFβ receptors (14, 15)	*Ligands:* Transforming growth factor β superfamily (TGFβ, BMPs), activin, inhibins (mammals); Dpp (*Drosophila*) *Receptors:* Intrinsic protein serine/threonine kinase activity in cytosolic domain (type I and II) *Signal transduction:* Direct activation of cytosolic Smad transcription factors
Cytokine receptors (14, 15)	*Ligands:* Interferons, erythropoietin, growth hormone, some interleukins (IL-2, IL-4), other cytokines *Receptors:* Single transmembrane α helix; conserved multi-β strand fold in extracellular domain; JAK kinase associated with intracellular domain *Signal transduction:* (1) Direct activation of cytosolic STAT transcription factors; (2) PI-3 kinase pathway; (3) IP_3/DAG pathway; (4) Ras-MAP kinase pathway
Receptor tyrosine kinases (14)	*Ligands:* Insulin, epidermal growth factor (EGF), fibroblast growth factor (FGF), neurotrophins, other growth factors *Receptor:* Single transmembrane α helix; intrinsic protein tyrosine kinase activity in cytosolic domain *Signal transduction:* (1) Ras–MAP kinase pathway; (2) IP_3/DAG pathway; (3) PI-3 kinase pathway
Receptor guanylyl cyclases (13)	*Ligands:* Atrial natriuretic factor and related peptide hormones *Receptor:* Single transmembrane α helix; intrinsic guanylate cyclase activity in cytosolic domain *Signal transduction:* Generation of cGMP
Receptor phosphotyrosine phosphatases	*Ligands:* Pleiotrophins, other protein hormones *Receptors:* Intrinsic phosphotyrosine phosphatase activity in cytosolic domain inhibited by ligand binding *Signal transduction:* Hydrolysis of activating phosphotyrosine residue on cytosolic protein tyrosine kinases
T-cell receptors	*Ligands:* Small peptides associated with major histocompatability (MHC) proteins in the plasma membrane of macrophages and other antigen-presenting cells *Receptors:* Single transmembrane α helix; several protein kinases associated with cytosolic domain; found only on T lymphocytes *Signal transduction:* (1) Activation of cytosolic protein tyrosine kinases; (2) PI-3 kinase pathway; (3) IP_3/DAG pathway; (4) Ras–MAP kinase pathway

TABLE 14-1 Overview of Major Receptor Classes and Signaling Pathways

Receptor Class/Pathway*	Distinguishing Characteristics
RECEPTORS THAT ARE ION CHANNELS	
Ligand-gated ion channels (7, 13)	*Ligands:* Neurotransmitters (e.g., acetylcholine, glutamate), cGMP, physical stimuli (e.g., touch, stretching), IP_3 (receptor in ER membrane) *Receptors:* Four or five subunits with a homologous segment in each subunit lining the ion channel *Signal transduction:* (1) Localized change in membrane potential due to ion influx, (2) elevation of cytosolic Ca^{2+}

TABLE 14-1 Overview of Major Receptor Classes and Signaling Pathways

Receptor Class/Pathway*	Distinguishing Characteristics
PATHWAYS INVOLVING PROTEOLYSIS	
Wnt pathway (15)	*Ligands:* Secreted Wnt (mammals); Wg (*Drosophila*) *Receptors:* Frizzled (Fz) with seven transmembrane α helices; associated membrane-bound LDL receptor–related protein (Lrp) required for receptor activity *Signal transduction:* Assembly of multiprotein complex at membrane that inhibits the proteasome-mediated proteolysis of cytosolic β-catenin transcription factor, resulting in its accumulation
Hedgehog (Hh) pathway (15)	*Ligands:* Cell-tethered Hedgehog *Receptors:* Binding of Hh to Patched (Ptc), which has 12 transmembrane α helices; activation of signaling from Smoothened (Smo), with 7 transmembrane α helices *Signal transduction:* Proteolytic release of a transcriptional activator from multiprotein complex in the cytosol
Notch/Delta pathway (14, 15)	*Ligands:* Membrane-bound Delta or Serrate protein *Receptors:* Extracellular subunit of Notch receptor noncovalently associated with transmembrane-cytosolic subunit *Signal transduction:* Intramembrane proteolytic cleavage of receptor transmembrane domain with release of cytosolic segment that functions as co-activator for nuclear trascription factors
NF-κB pathways (14, 15)	*Ligands:* Tumor necrosis factor α (TNF-α), interleukin 1 (mammals); Spätzle (*Drosophila*) *Receptors:* Various in mammals; Toll and Toll-like receptors in *Drosophila* *Signal transduction:* Phosphorylation-dependent degradation of inhibitor protein with release of active NF-κB transcription factor (Dorsal in *Drosophila*) in the cytosol

TABLE 14-1 Overview of Major Receptor Classes and Signaling Pathways

Receptor Class/Pathway*	Distinguishing Characteristics
INTRACELLULAR RECEPTORS PATHWAYS	
Nitric oxide pathway (13)	*Ligands:* Nitric oxide (NO) *Receptor:* Cytosolic guanylyl cyclase *Signal transduction:* Generation of cGMP
Nuclear receptor pathways (11)	*Ligands:* Lipophilic molecules including steroid hormones, thyroxine, retinoids, and fatty acids in mammals and ecdysone in *Drosophila* *Receptors:* Highly conserved DNA-binding domain, somewhat conserved hormone-binding domain, and a variable domain; located within nucleus or cytosol *Signal transduction:* Activation of receptor's transcription factor activity by ligand binding

*Unless indicated otherwise, receptors are located in the plasma membrane. Numbers in parentheses indicate chapters in which a receptor/pathway is discussed in depth.

SOURCES: J. Gerhart, 1999, *Teratology* **60**:226, and A. Brivanlou and J. E. Darnell, 2002, *Science* **295**:813.

Receptor Protein Tyrosine Kinases

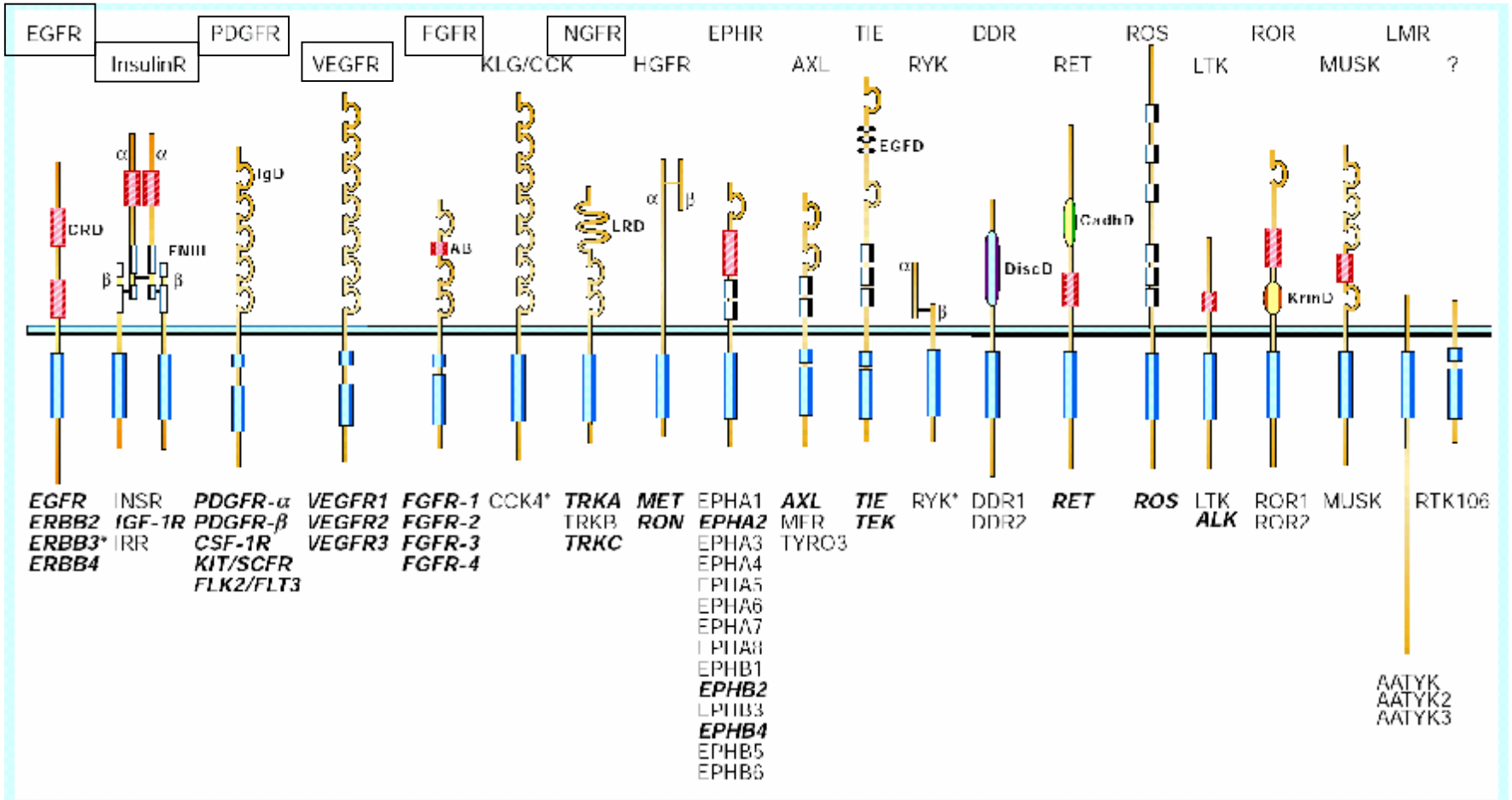


Figure 1 Human receptor protein–tyrosine kinases. The prototypic receptor for each family is indicated above the receptor, and the known members are listed below. Abbreviations of the prototypic receptors: EGFR, epidermal growth factor receptor; InsR, insulin receptor; PDGFR, platelet-derived growth factor receptor; VEGFR, vascular endothelial growth factor receptor; FGFR, fibroblast growth factor receptor; KLG/CCK, colon carcinoma kinase; NGFR, nerve growth factor receptor; HGFR, hepatocyte growth factor receptor; EphR, ephrin receptor; Axl, a Tyro3 PTK; Tie, tyrosine kinase receptor in endothelial cells; RYK, receptor related to tyrosine kinases; DDR, discoidin domain receptor; Ret, rearranged during transfection; ROS, RPTK expressed in some epithelial cell types; LTK, leukocyte tyrosine kinase; ROR, receptor orphan; MuSK, muscle-specific kinase; LMR, Lemur. Other abbreviations: AB, acidic box; CadhD, cadherin-like domain; CRD, cysteine-rich domain; DiscD, discoidin-like domain; EGFD, epidermal growth factor-like domain; FNIII, fibronectin type III-like domain; IgD, immunoglobulin-like domain; KrinD, kringle-like domain; LRD, leucine-rich domain. The symbols α and β denote distinct RPTK subunits. RPTK members in bold and italic type are implicated in human malignancies (see Table 1). An asterisk indicates that the member is devoid of intrinsic kinase activity.

Nature 411, 355, 2001

Cytoplasmic protein-tyrosine kinases

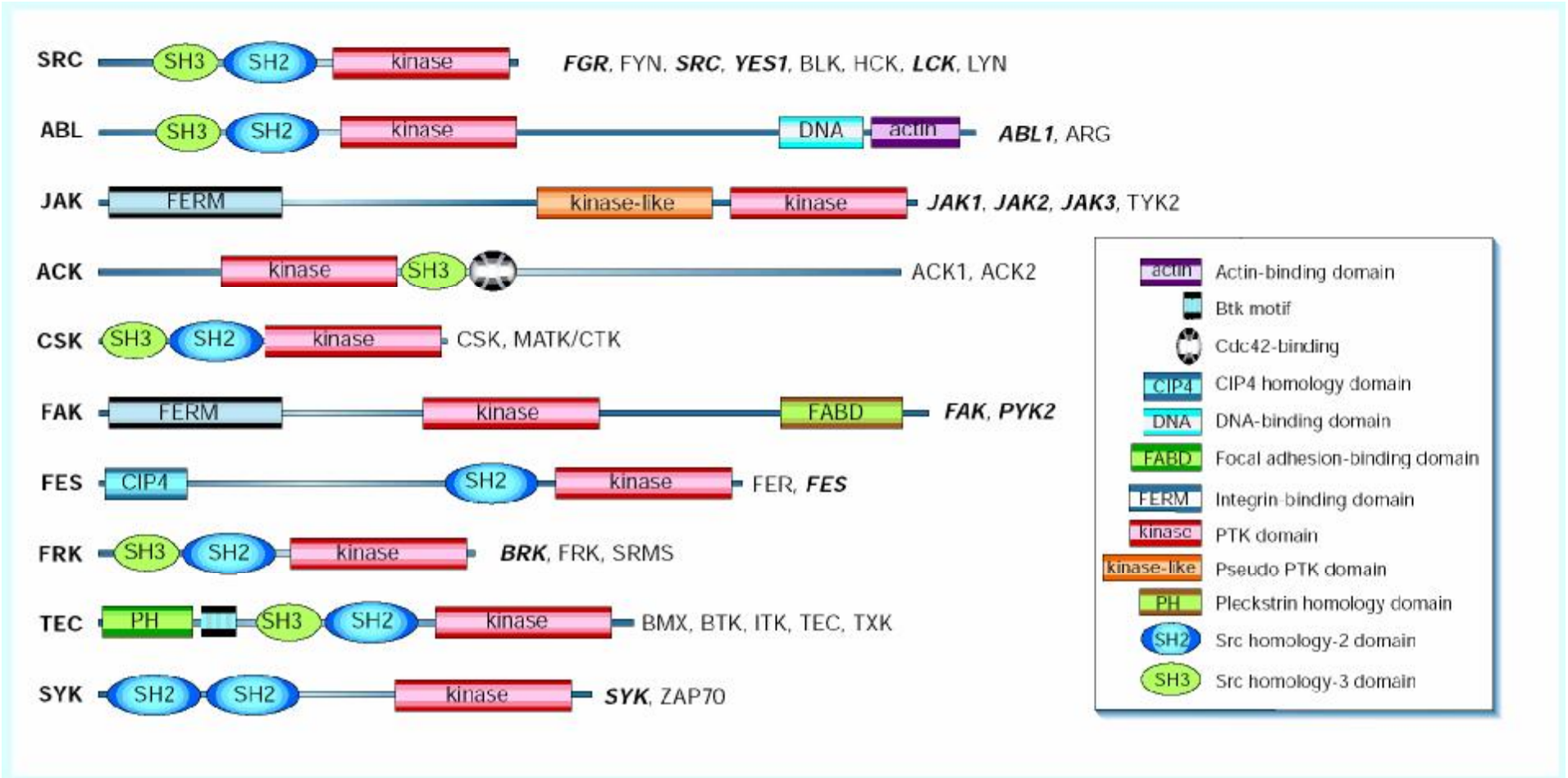


Figure 2 Human cytoplasmic protein-tyrosine kinases. The family members are indicated to the right and the family name to the left of each PTK. The PTK members in bold and italic type are implicated in human malignancies (see Table 1).

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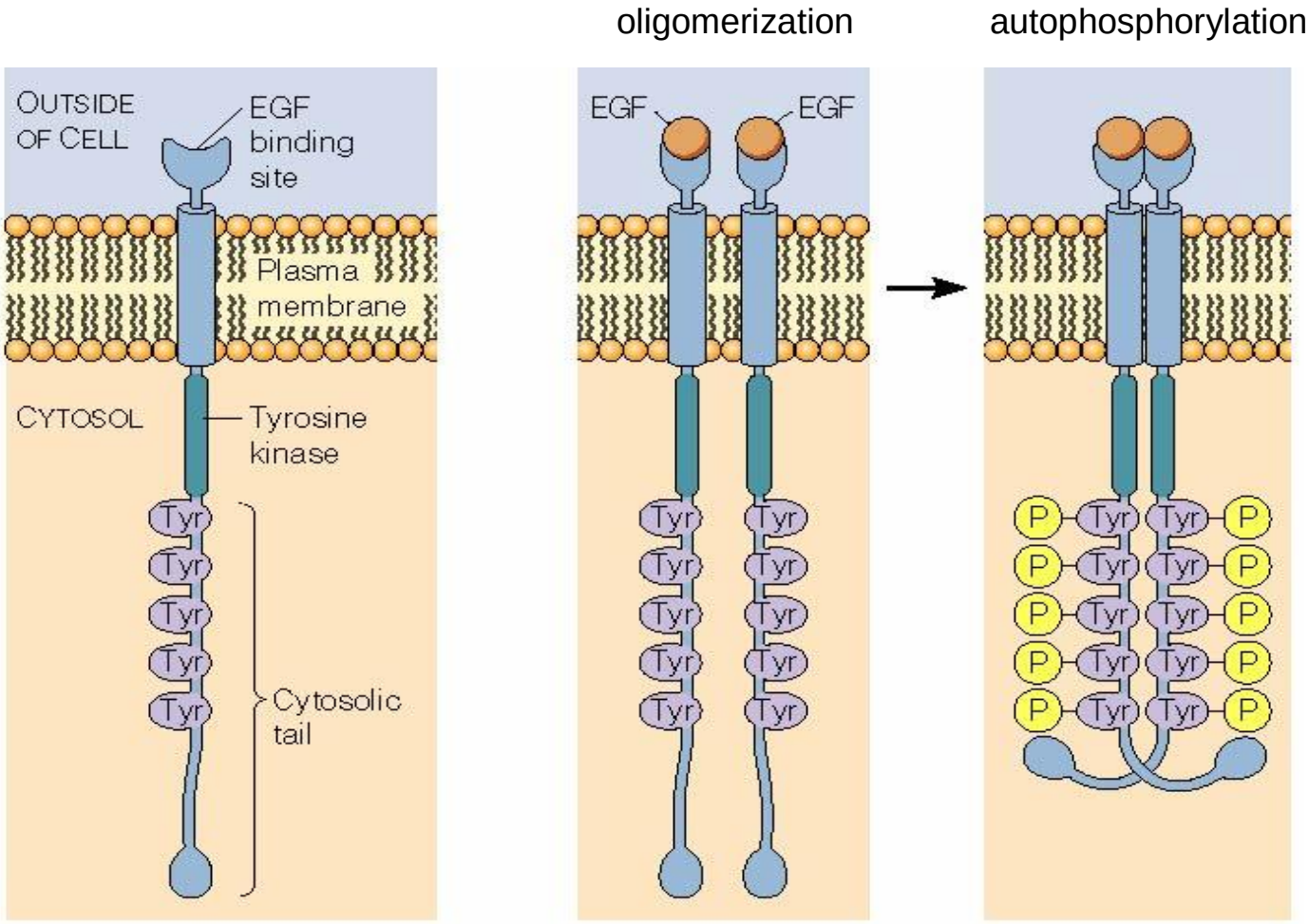
Figure 14-17 The Structure and Activation of a Receptor Tyrosine Kinase (RTK)



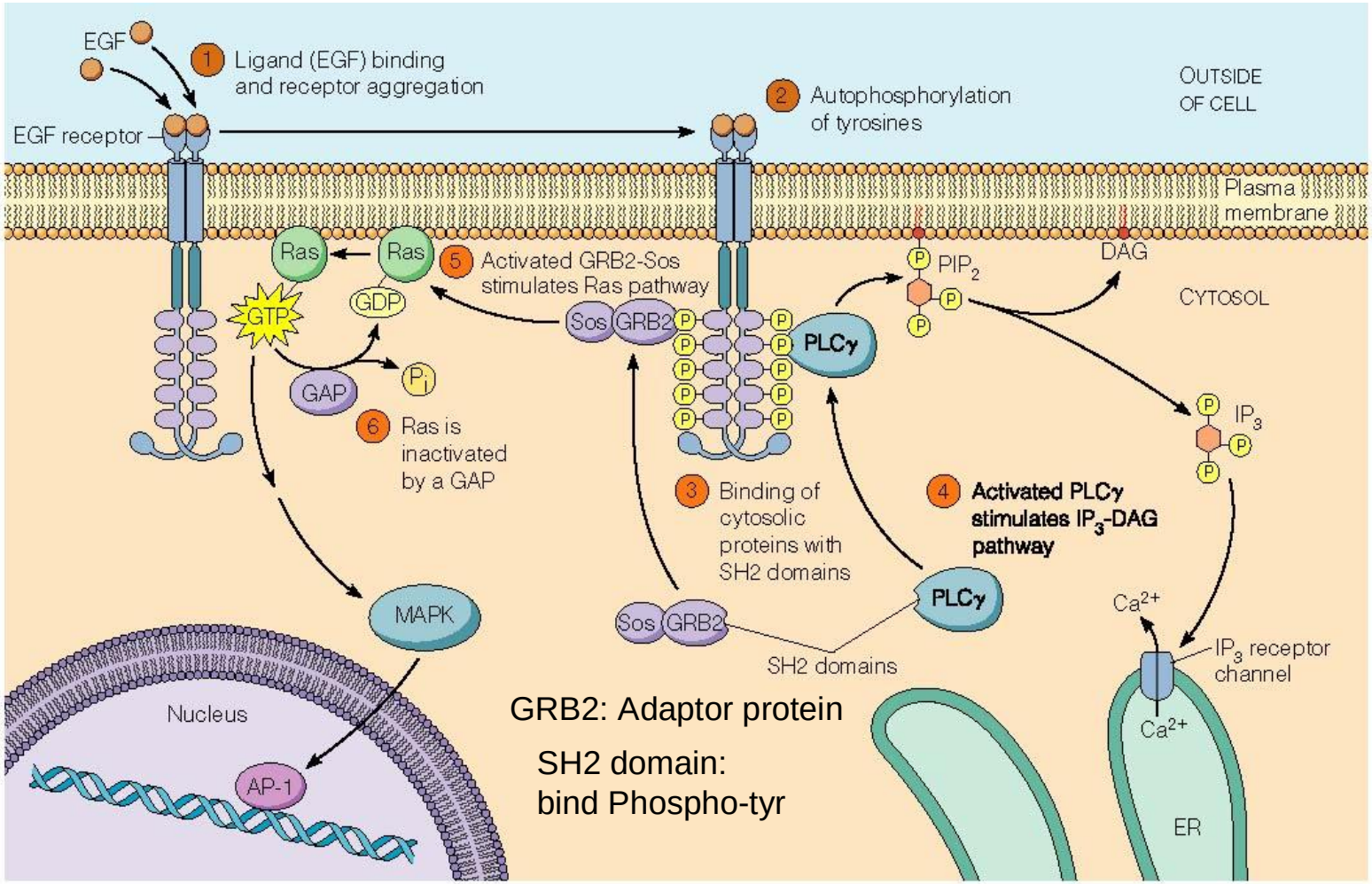
(a) Structure of the epidermal growth factor (EGF) receptor

(b) Activation of the EGF receptor

Figure 14-18 Signal Transduction Through Receptor Tyrosine Kinases

Ras: monomeric G protein

Sos is a GEF



GRB2: Adaptor protein

SH2 domain:
bind Phospho-tyr

The Ras protein cycle

- Ras, likes G_α, belongs to the GTPase superfamily of intracellular switch proteins, however, Ras is not directly linked to RTKs.
- Ras activation is accelerated by guanine-nucleotide exchange factor (GEF), which binds to the Ras-GDP, causing dissociation of the bound GDP.
- GTP then binds spontaneously to the empty Ras ([GTP] > [GDP]).
- GTPase-activating protein (GAP) binds to Ras-GTP and accelerates its intrinsic GTPase activity by a hundredfold.
- The average lifetime of a GTP bound to Ras is about 1 minute.
- The mutant Ras proteins are found in many types of human cancer. These Ras mutants bind but cannot hydrolyze GTP.

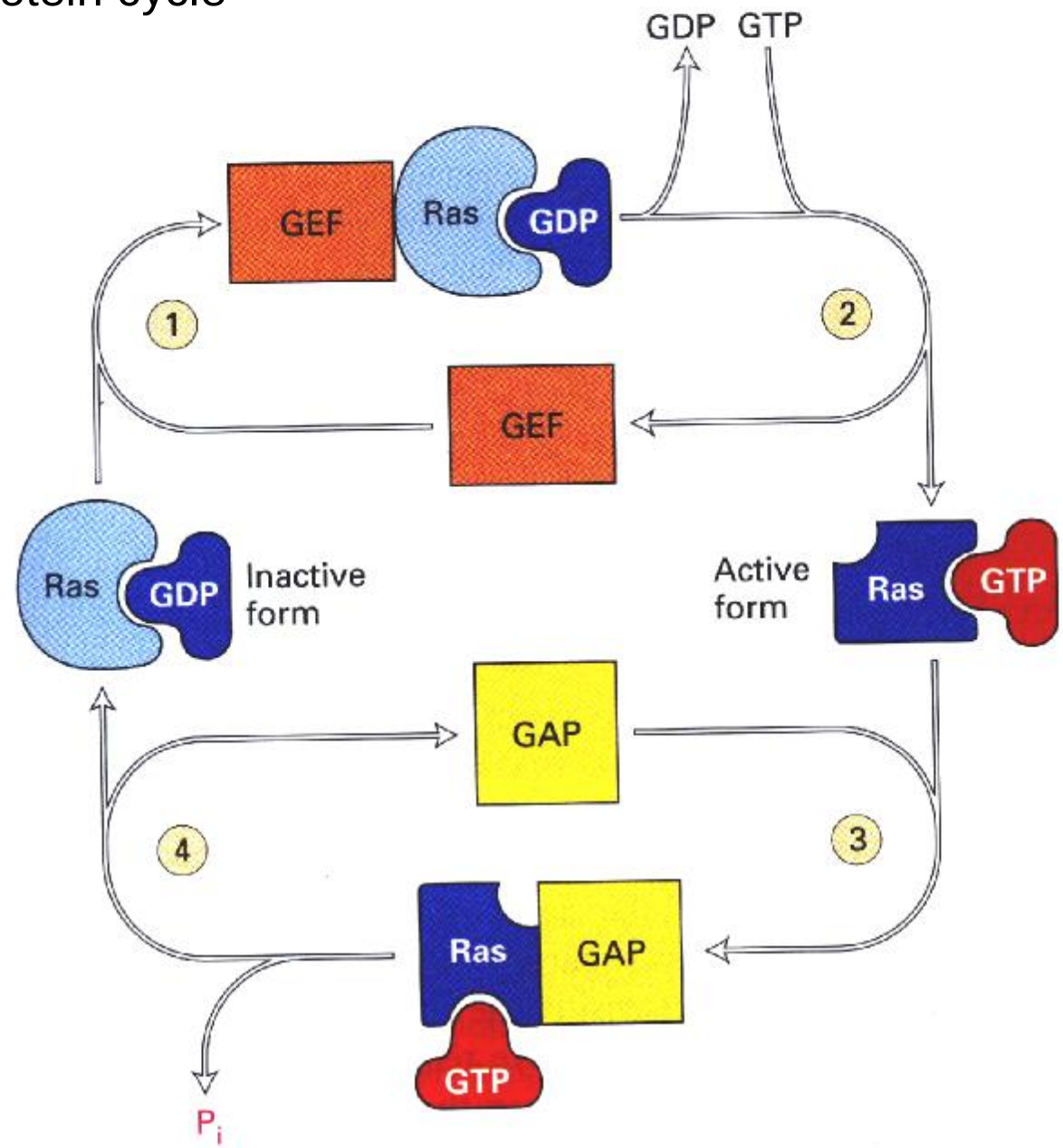


Fig. 20-22 Lodish et al. MOLECULAR CELL BIOLOGY, 4th Edition

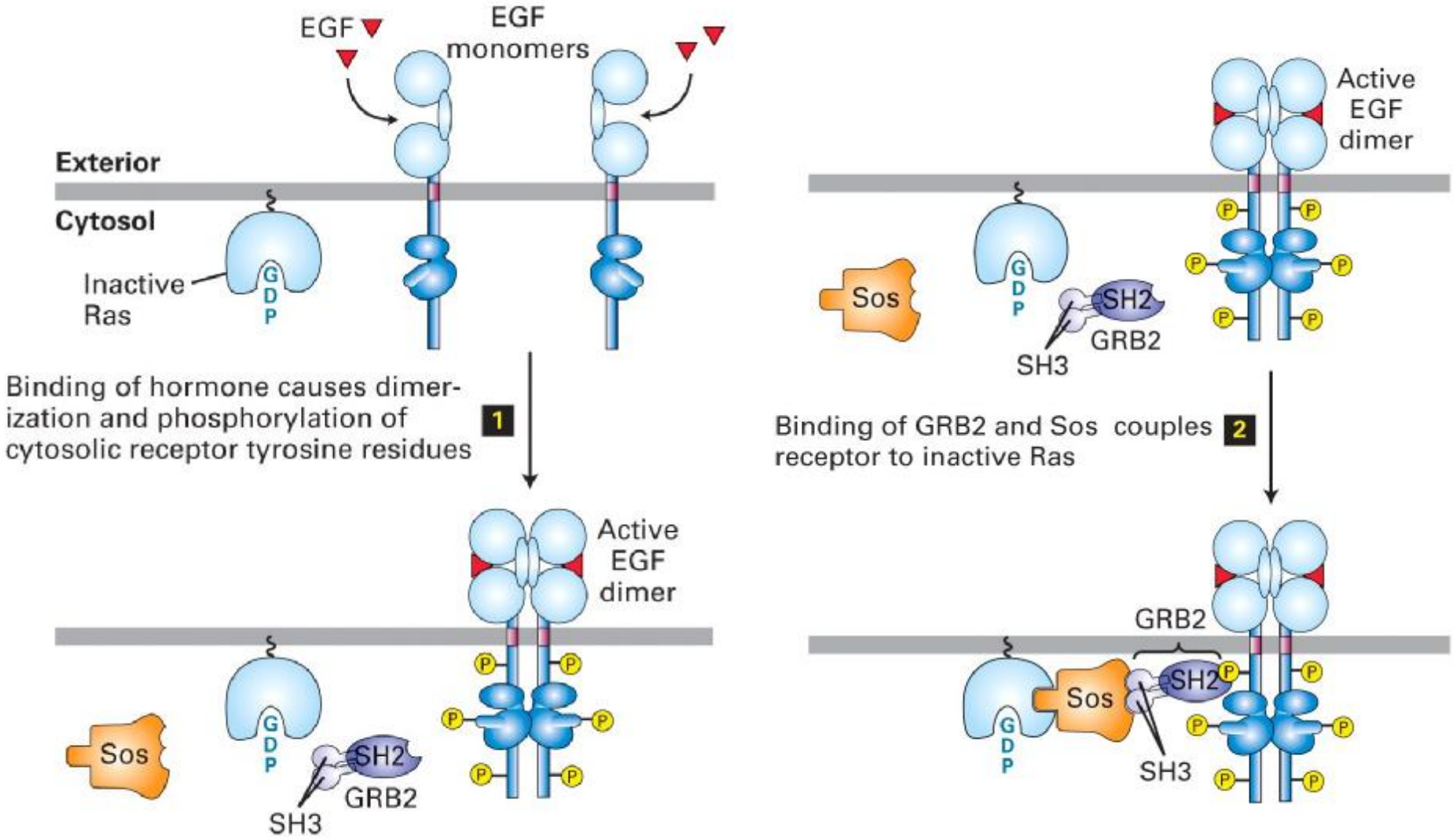
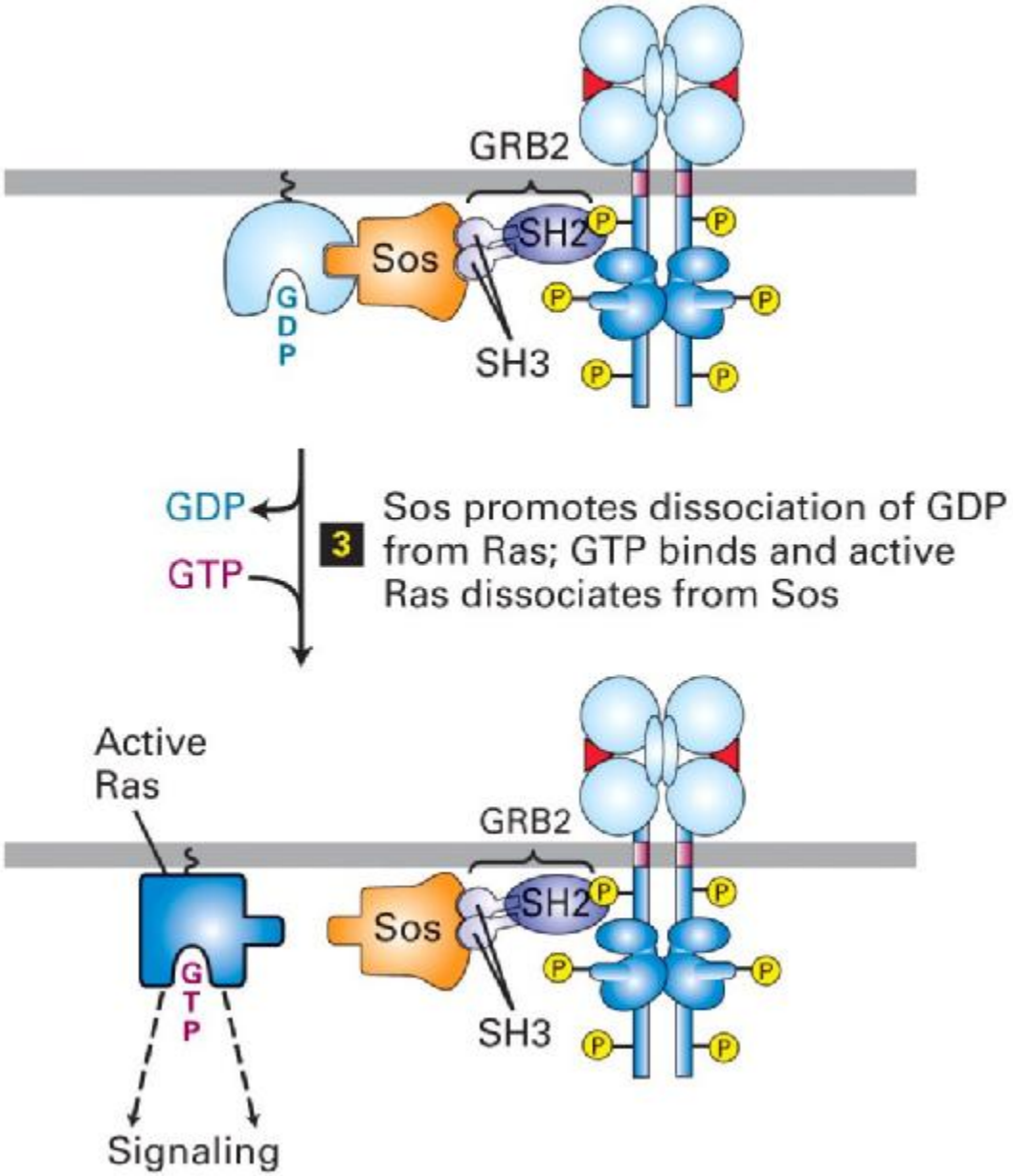
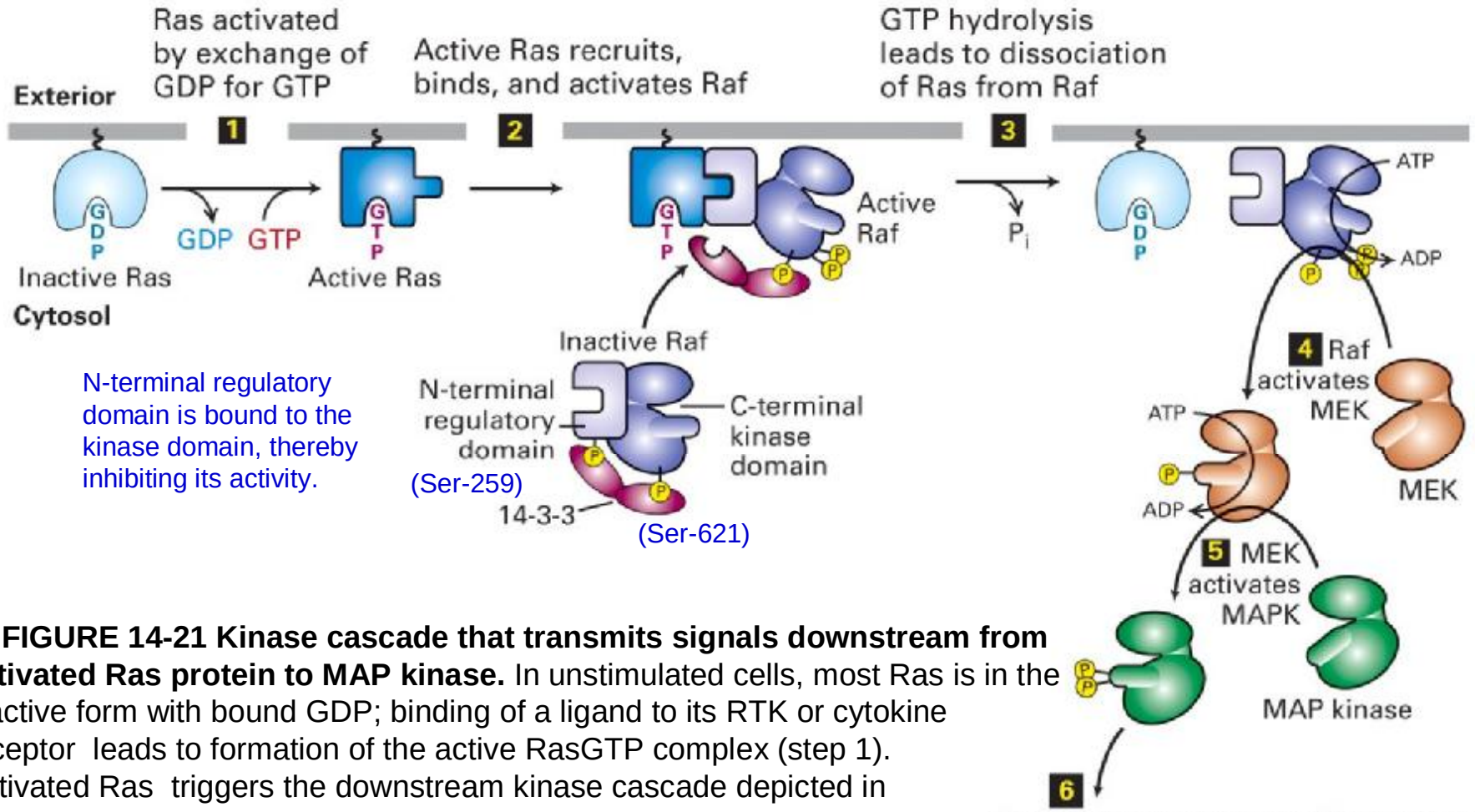


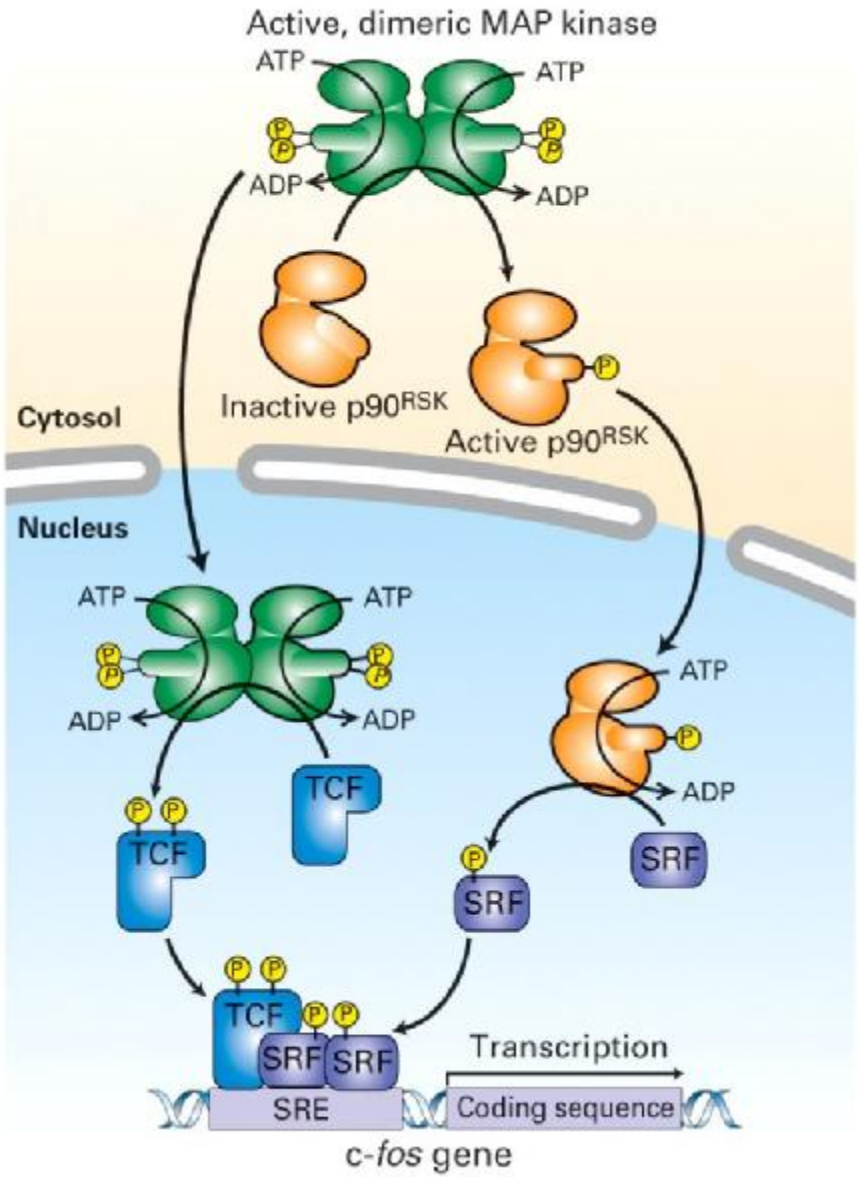
FIGURE 14-16 Activation of Ras following ligand binding to receptor tyrosine kinases (RTKs). The receptors for epidermal growth factor (EGF) and many other growth factors are RTKs. The cytosolic adapter protein GRB2 binds to a specific phosphotyrosine on an activated, ligand-bound receptor and to the cytosolic Sos protein, bringing it near its substrate, the inactive RasGDP. The guanine nucleotide–exchange factor (GEF) activity of Sos then promotes formation of active RasGTP. Note that Ras is tethered to the membrane by a hydrophobic farnesyl anchor (see Figure 5-15). [See J. Schlessinger, 2000, *Cell* **103**:211, and M. A. Simon, 2000, *Cell* **103**:13.]



- GRB2 contains a SH2 (Src homology 2) domain which binds to a specific phosphotyrosine residue and two SH3 (Src homology 3) domains, which bind to and activate Sos. Thus, GRB2 acts as adapter protein for the EGF receptor, and Sos functions as a GEF.
- RTKs also can recruit signal molecules through the PTB (phosphotyrosine binding) domain.
- SH2-binding specificity is determined by residues C-terminal to the phosphotyrosine, while PTB-binding specificity is determined by residues N-terminal to the phosphotyrosine.
- SH3 domains selectively bind to proline-rich sequences in Sos and other proteins.
- Ras is tethered to the membrane by a farnesyl anchor.

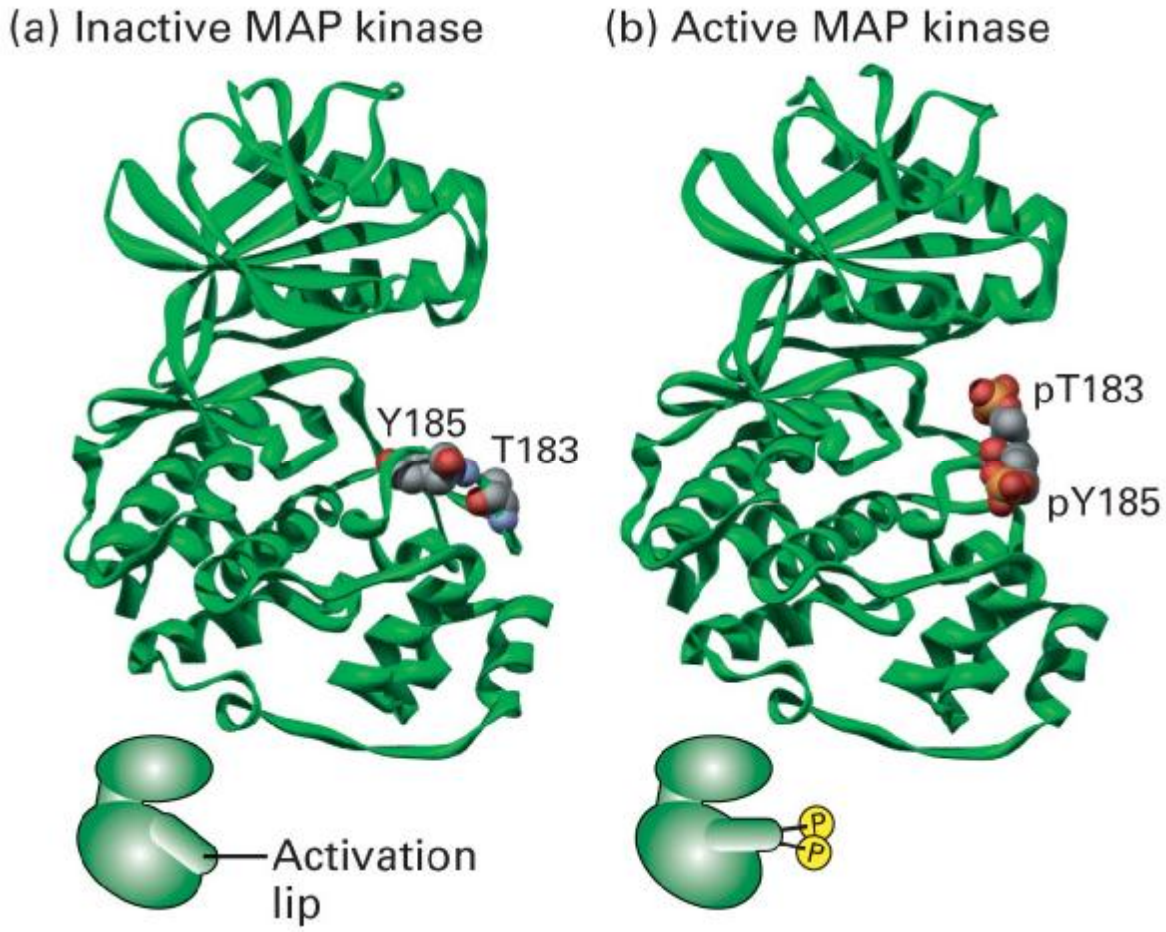


▲ FIGURE 14-21 Kinase cascade that transmits signals downstream from activated Ras protein to MAP kinase. In unstimulated cells, most Ras is in the inactive form with bound GDP; binding of a ligand to its RTK or cytokine receptor leads to formation of the active RasGTP complex (step 1). Activated Ras triggers the downstream kinase cascade depicted in steps 2 – 6, culminating in activation of MAP kinase (MAPK). In unstimulated cells, binding of the 14-3-3 protein to Raf stabilizes it in an inactive conformation. Interaction of the Raf N-terminal regulatory domain with RasGTP relieves this inhibition, results in dephosphorylation of one of the serines that bind Raf to 14-3-3, and leads to activation of Raf kinase activity (steps 2 and 3). Note that in contrast to many other protein kinases, activation of Raf does not depend on phosphorylation of the activation lip. After inactive RasGDP dissociates from Raf, it presumably can be reactivated by signals from activated receptors, thereby recruiting additional Raf molecules to the membrane. See the text for details. [See E. Kerkhoff and U. Rapp, 2001, *Adv. Enzyme Regul.* **41**:261; J. Avruch et al., 2001, *Recent Prog. Hormone Res.* **56**:127; and M. Yip-Schneider et al., 2000, *Biochem. J.* **351**:151.]

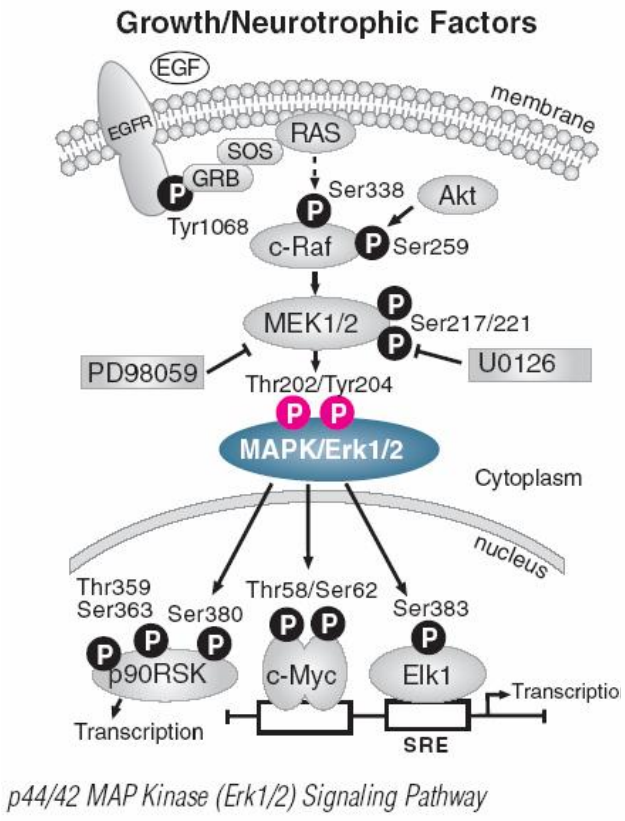


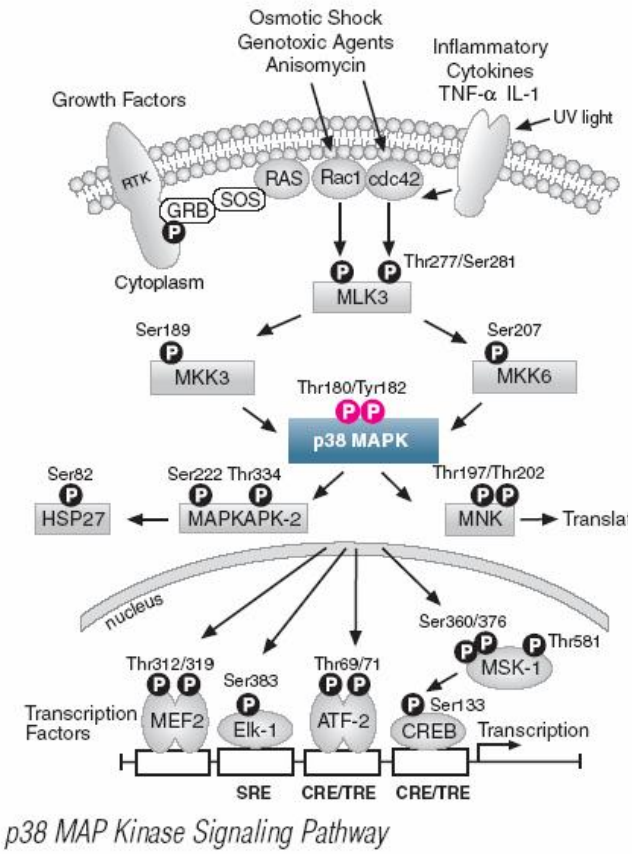
▲ **FIGURE 14-23 Induction of gene transcription by activated MAP kinase.** In the cytosol, MAP kinase phosphorylates and activates the kinase $p90^{RSK}$, which then moves into the nucleus and phosphorylates the SRF transcription factor. After translocating into the nucleus, MAP kinase directly phosphorylates the transcription factor TCF. Together, these phosphorylation events stimulate transcription of genes (e.g., c-*fos*) that contain an SRE sequence in their promoter. See the text for details. [See R. Marais et al., 1993, *Cell* **73**:381, and V. M. Rivera et al., 1993, *Mol. Cell Biol.* **13**:6260.]

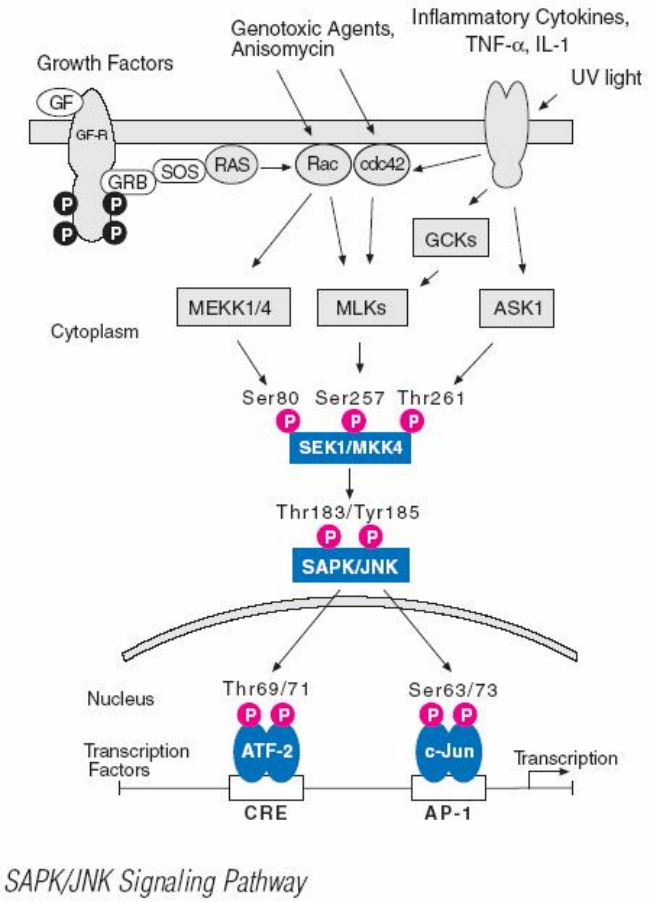
Early-response genes: *c-Fos*, *c-Jun*
SRE: serum-response element
TCF: ternary complex factor
SRF: serum response factor



▲ **EXPERIMENTAL FIGURE 14-22 Molecular structures of MAP kinase in its inactive, unphosphorylated form (a) and active, phosphorylated form (b).** Phosphorylation of MAP kinase by MEK at tyrosine-185 (Y185) and threonine-183 (T183) leads to a marked conformational change in the activation lip. This change promotes dimerization of MAP kinase and binding of its substrates, ATP and certain proteins. A similar phosphorylationdependent mechanism activates JAK kinases, the intrinsic kinase activity of RTKs, and MEK. [After B. J. Canagarajah et al., 1997, *Cell* **90**:859.]





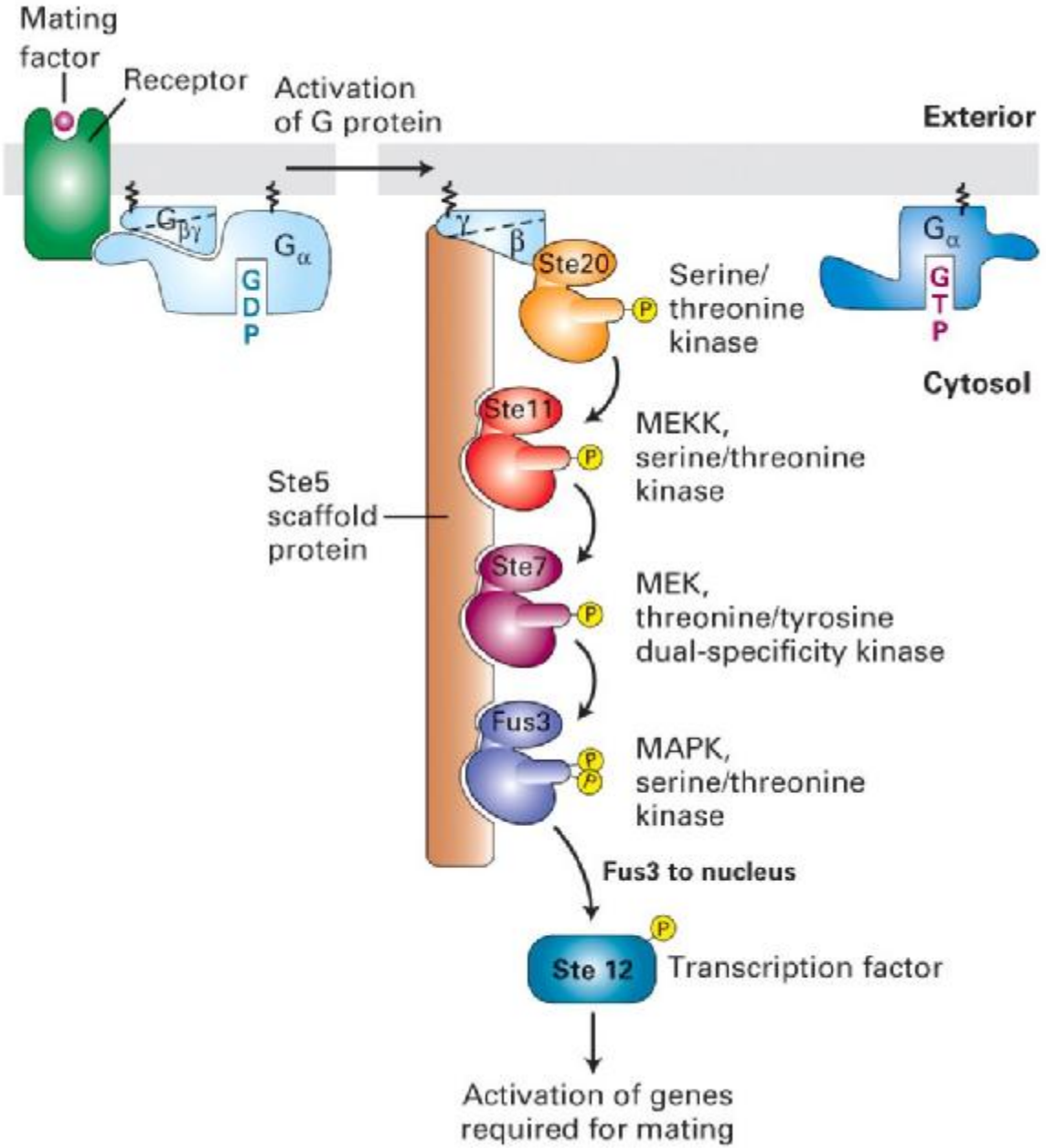


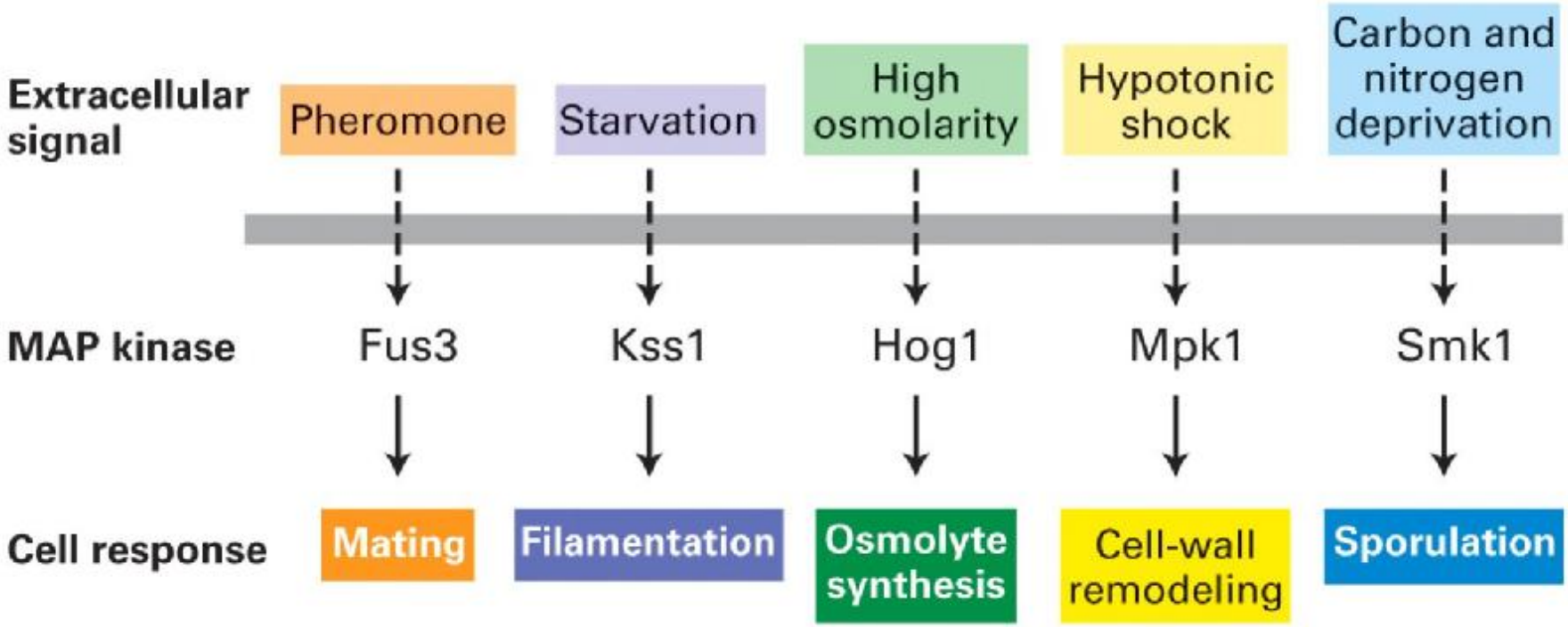
Proliferation
differentiation

Inflammation
apoptosis

apoptosis

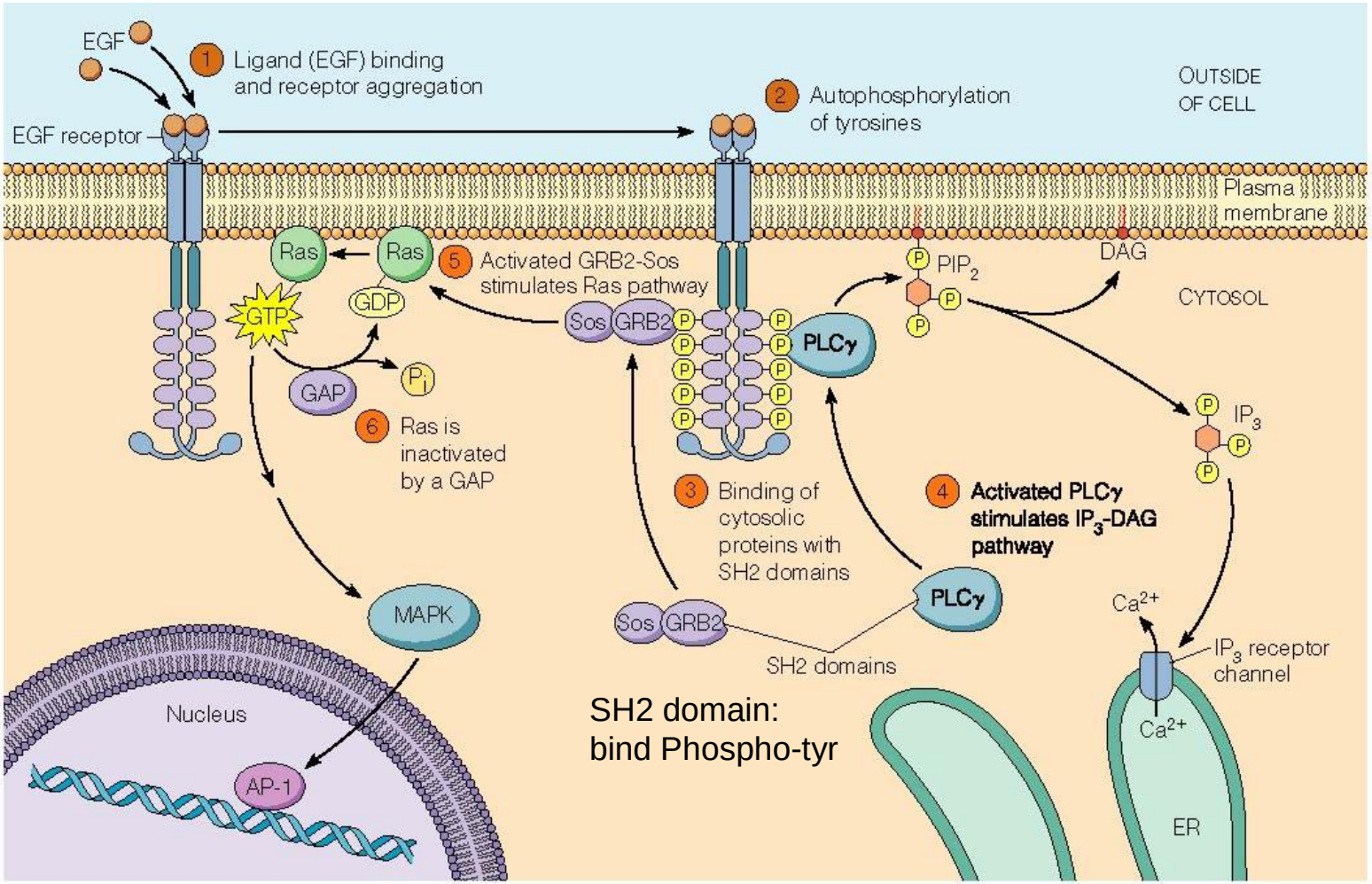
FIGURE 14-24 Kinase cascade that transmits signals downstream from mating factor receptors in *S. cerevisiae.* The receptors for yeast **a** and α mating factors are coupled to the same trimeric G protein. Ligand binding leads to activation and dissociation of the G protein (see Figure 13-10). In the yeast mating pathway, the dissociated Gβγ activates a protein kinase cascade analogous to the cascade downstream of Ras that leads to activation of MAP kinase (see Figure 14-21). The final component, Fus3, is functionally equivalent to MAP kinase (MAPK) in higher eukaryotes. Association of several kinases with the Ste5 scaffold contributes to specificity of the signaling pathway by preventing phosphorylation of other substrates. [See A. Whitmarsh and R. Davis, 1998, *Trends Biochem. Sci*. **23**:481, and H. Dohlman and J. Thorner, 2001, *Ann. Rev. Biochem.* **70**:703.]

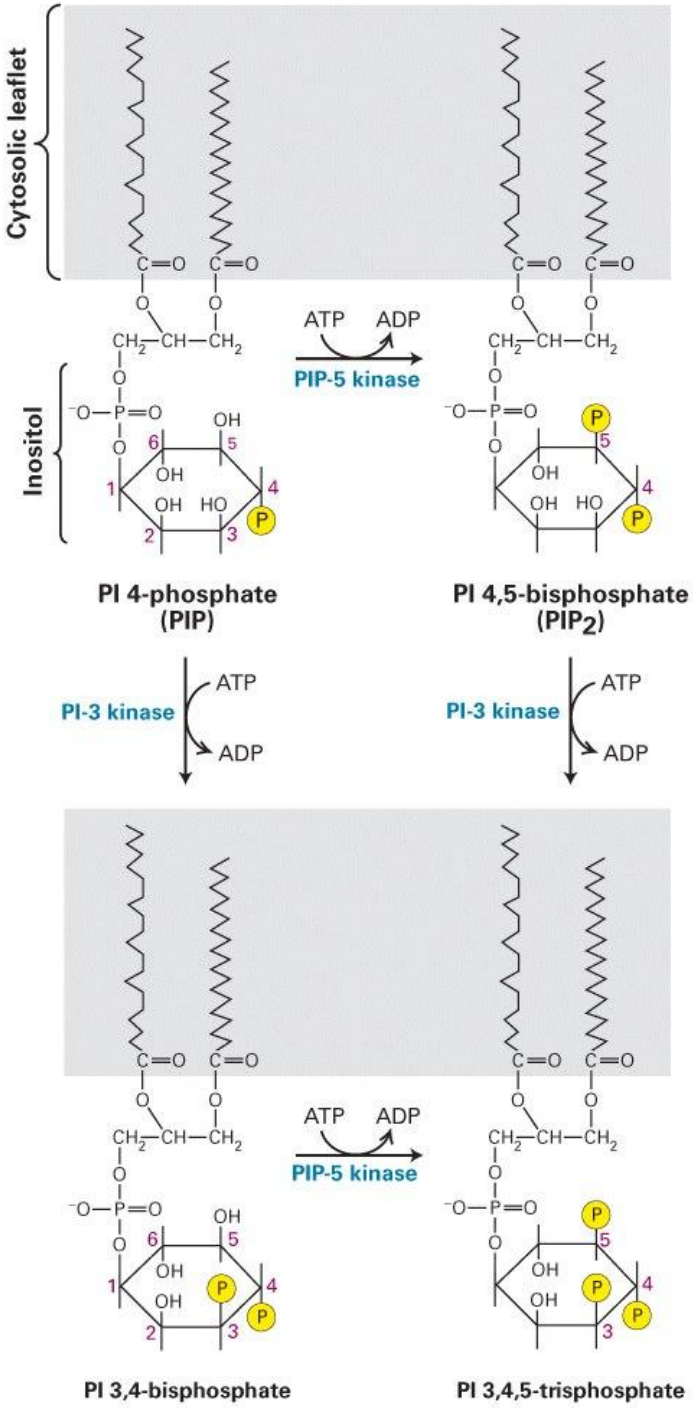




▲ **FIGURE 14-25 Overview of five MAP kinase pathways in *S. cerevisiae.*** Each pathway is triggered by a specific extracellular signal and leads to activation of a single different MAP kinase, which mediates characteristic cellular responses. Formation of pathway-specific complexes of MAP kinases and scaffold proteins prevents "cross talk" between pathways containing a common component such as the MEKK Ste11, which occurs in the mating, filamentation, and osmoregulatory pathways (see Figure 14-24). [Adapted from H. D. Madhani and G. R. Fink, 1998, *Trends Genet.* **14**(4):152.]

Phospholipase Cγ is activated by some RTKs and cytokine recepors





In addition to initiating the IP3/DAG pathway, some activated RTKs and cytokine receptors can initiate another phosphoinositide pathway, the PI-3 kinase pathway, by recruiting the enzyme phosphatidylinositol-3 kinase to the membrane. This pathway is important in certain signaling pathways essential for cell proliferation or for the prevention of apoptosis.

▲ **FIGURE 14-26 Generation of phosphatidylinositol 3-phosphates.** The enzyme phosphatidylinositol-3 kinase (PI-3 kinase) is recruited to the membrane by many activated receptor tyrosine kinases (RTKs) and cytokine receptors. The 3-phosphate added by this enzyme is a binding site for various signal transduction proteins. [See L. Rameh and L. C. Cantley, 1999, *J. Biol. Chem.* **274**:8347.]

→ Both PI-3 phosphates act as docking sites for signal-transducing proteins: PKB

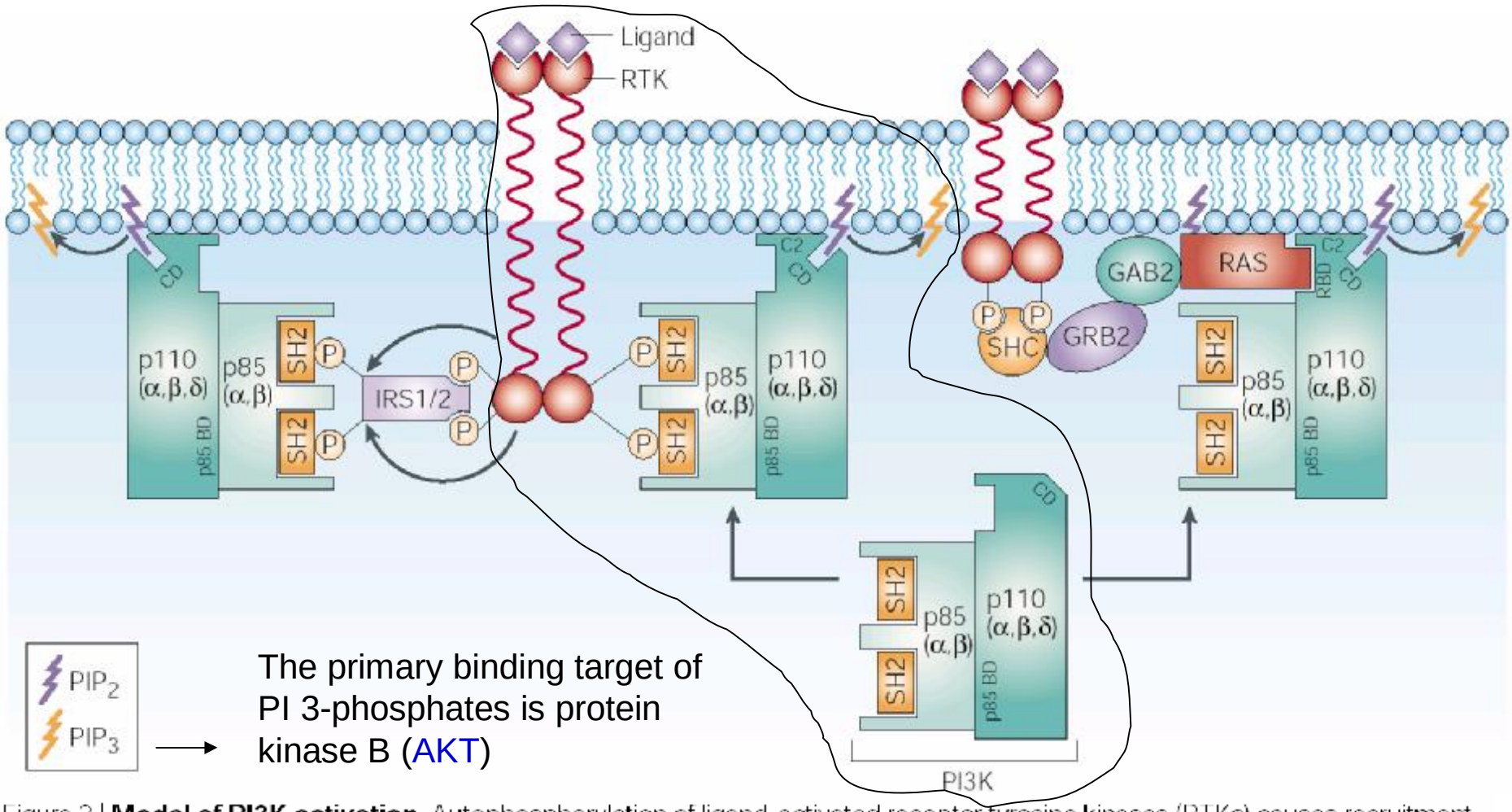
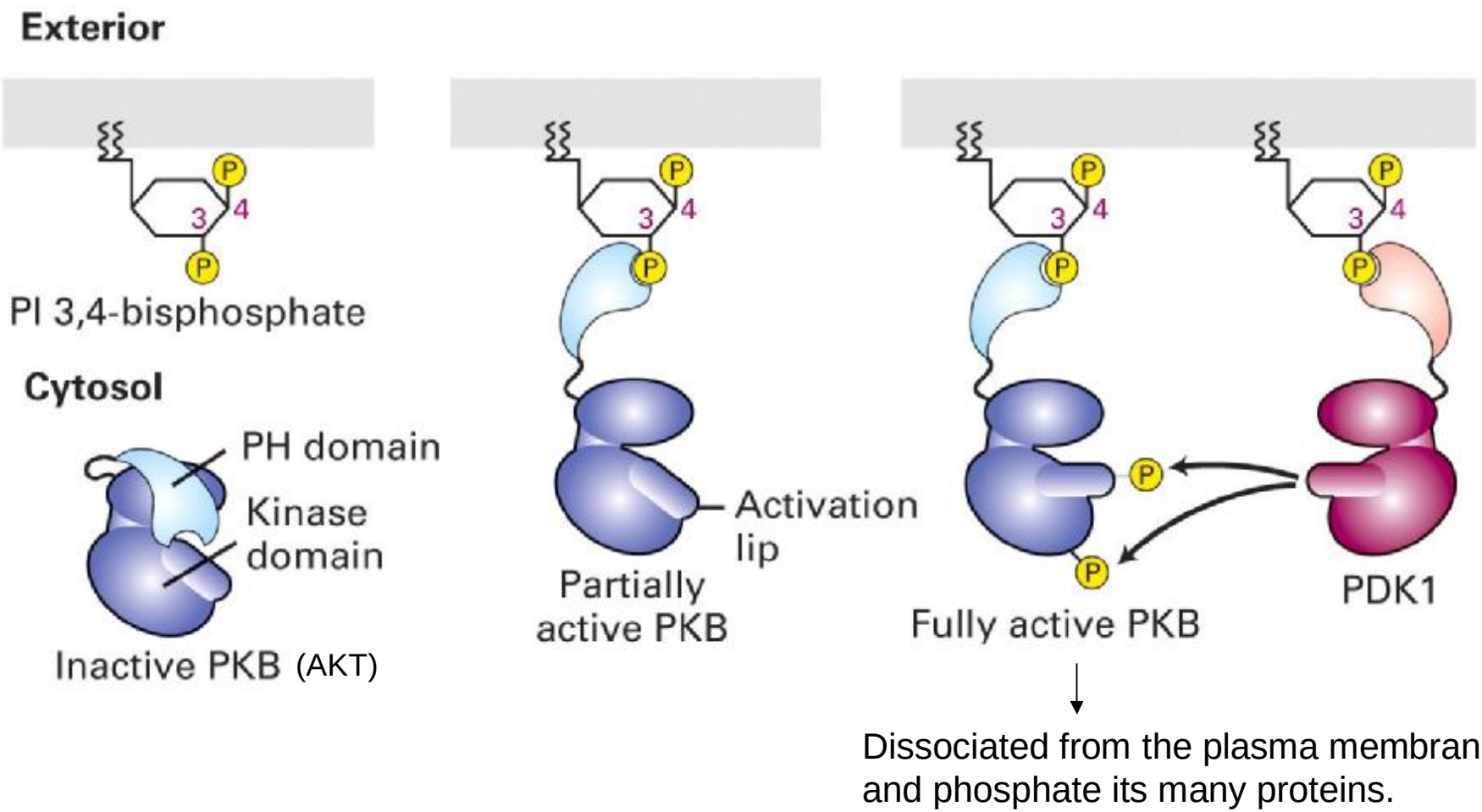
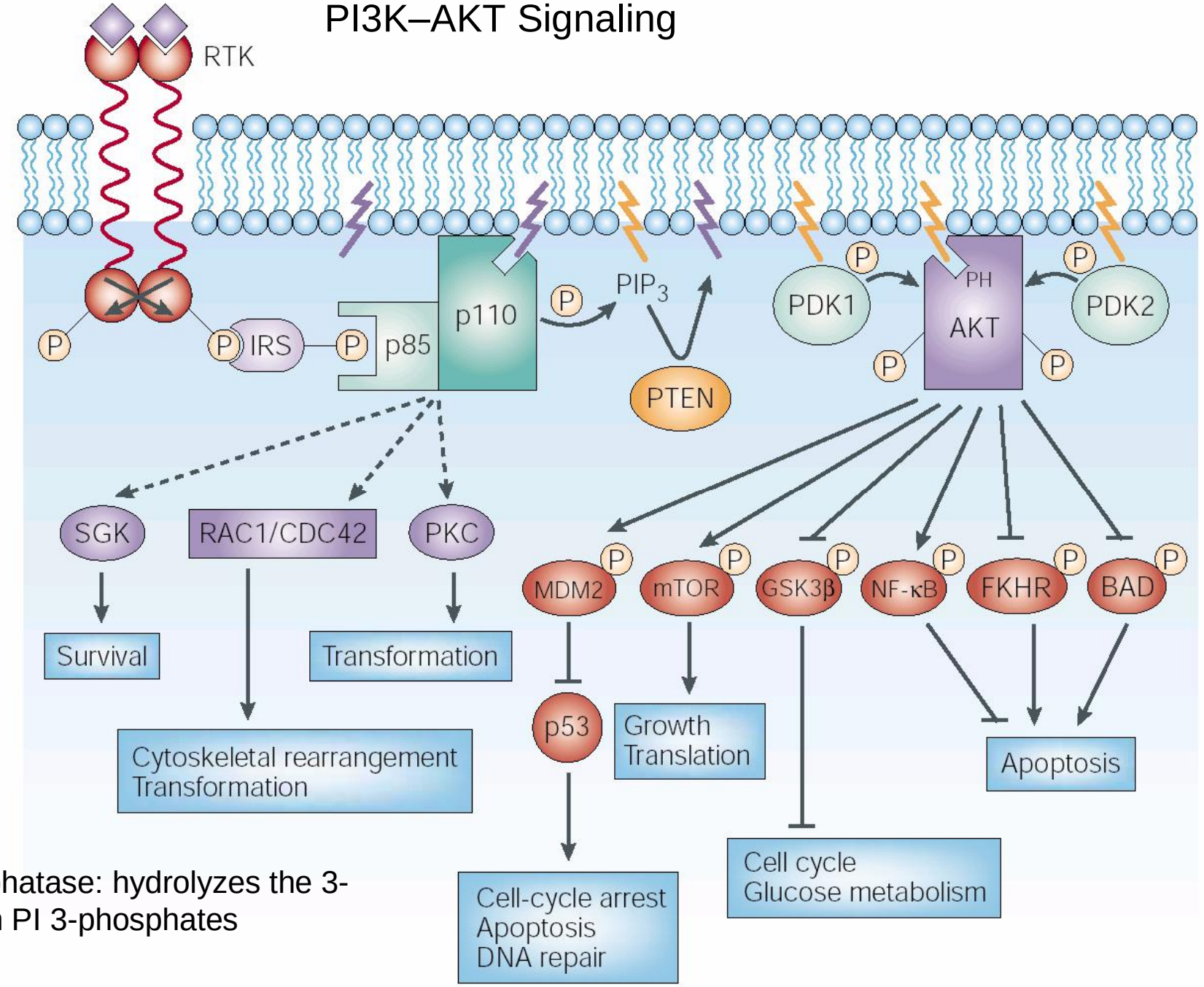


Figure 2 | **Model of PI3K activation.** Autophosphorylation of ligand-activated receptor tyrosine kinases (RTKs) causes recruitment of inactive heterodimeric class IA phosphatidylinositol 3-kinases (PI3Ks) through the interaction of phosphotyrosine residues on the receptor and SRC-homology 2 (SH2) domains on the PI3K p85 regulatory subunit, or the adaptor proteins IRS1 and IRS2. IRS1 and IRS2 are phosphorylated by the activated receptor, generating docking sites for the SH2 domains of p85 and inducing proper assembly of the signalling complex. These SH2–phosphotyrosine interactions bring PI3K in close proximity to its substrate at the plasma membrane and relieve the inhibitory action of p85 on the p110 catalytic subunit, which is then free to convert PtdIns(4,5)P_2 (PIP_2) into PtdIns(3,4,5)P_3 (PIP_3). Alternatively, binding of PI3K to activated RAS can also stabilize its membrane localization and activate the catalytic domain. This occurs by recruitment of the adaptor proteins SHC, GRB2 and GAB2 to activated RTKs. C2, C2 domain; CD, catalytic domain; p85 BD, p85-binding domain; RBD, RAS-binding domain.



▲ **FIGURE 14-27 Recruitment and activation of protein kinase B (PKB) in PI-3 kinase pathways.** In unstimulated cells, PKB is in the cytosol with its PH domain bound to the catalytic domain, inhibiting its activity. Hormone stimulation leads to activation of PI-3 kinase and subsequent formation of phosphatidylinositol (PI) 3-phosphates (see Figure 14-26). The 3-phosphate groups serve as docking sites on the plasma membrane for the PH domain of PKB and another kinase, PDK1. Full activation of PKB requires phosphorylation both in the activation lip and at the C-terminus by PDK1. [Adapted from A. Toker and A. Newton, 2000, *Cell* **103**:185, and M. Scheid et al., 2002, *Mol. Cell Biol.* **22**:6247.]

PI3K–AKT Signaling



PTEN phosphatase: hydrolyzes the 3-phosphate in PI 3-phosphates

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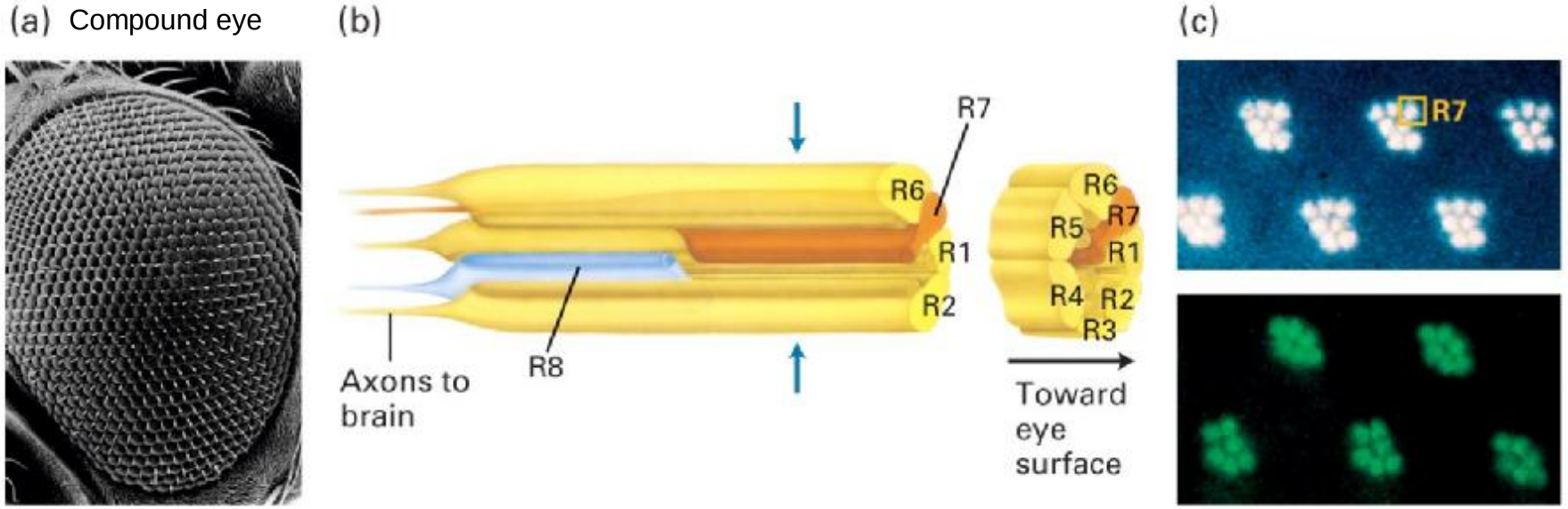
Table 14-2 Examples of Growth Factor Families

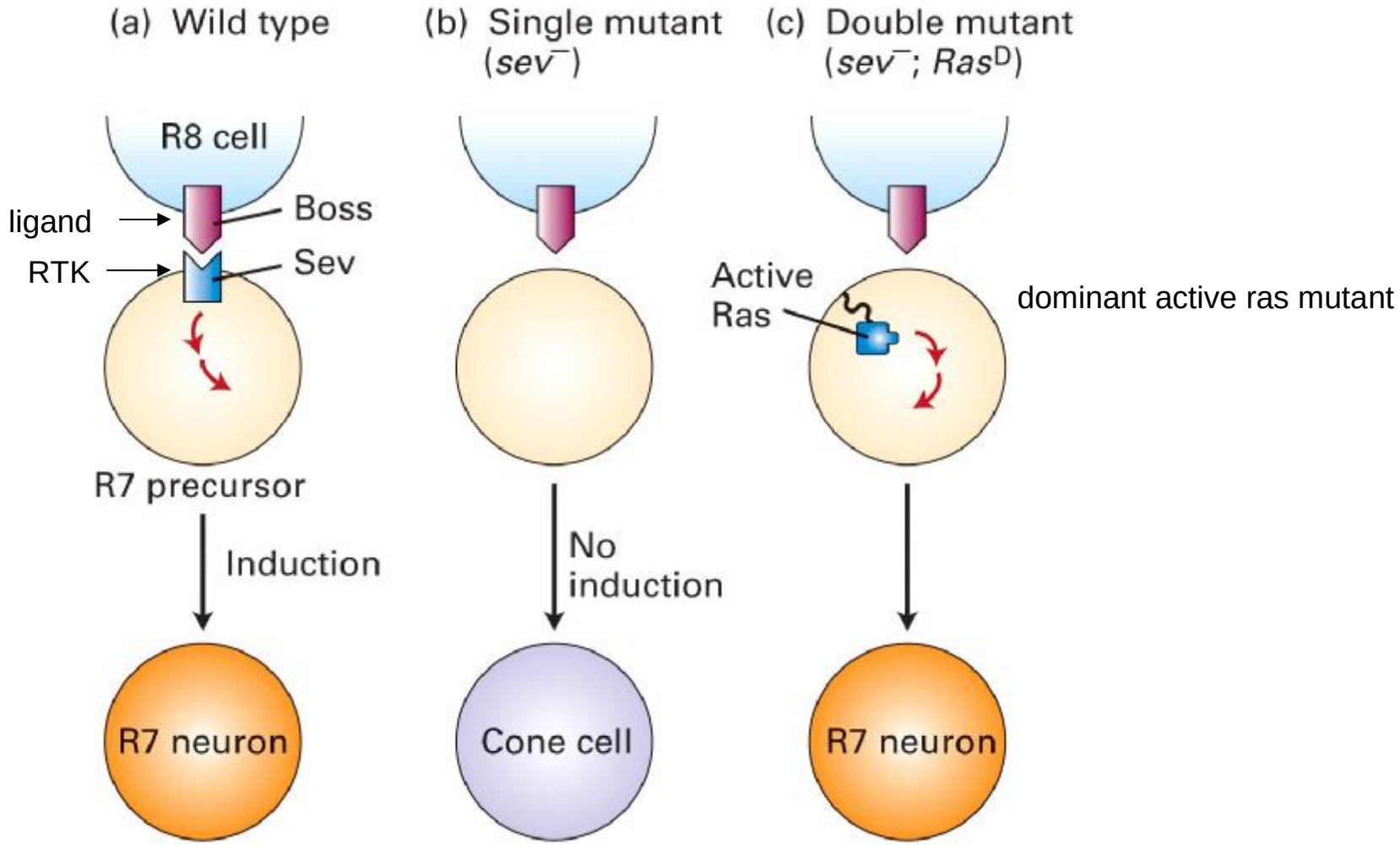
Table 14-2 Examples of Growth Factor Families

Growth Factor	Target Cells	Type of Receptor Complex
Epidermal growth factor (EGF)	Wide variety of epithelial and mesenchymal cells	Tyrosine kinase
Transforming growth factor-α (TGFα)	Same as EGF	Tyrosine kinase
Platelet-derived growth factor (PDGF)	Mesenchyme, smooth muscle, trophoblast	Tyrosine kinase
Transforming growth factor-β (TGFβ)	Fibroblastic cells	Serine-threonine kinase
Fibroblast growth factor (FGF)	Mesenchyme, fibroblasts, many other cell types	Tyrosine kinase
Interleukin-2 (IL-2)	Cytotoxic T lymphocytes	Complex of three subunits
Colony stimulating factor-1 (CSF-1)	Macrophage precursors	Tyrosine kinase
Wnts	Many types of embryonic cells	Seven-pass protein



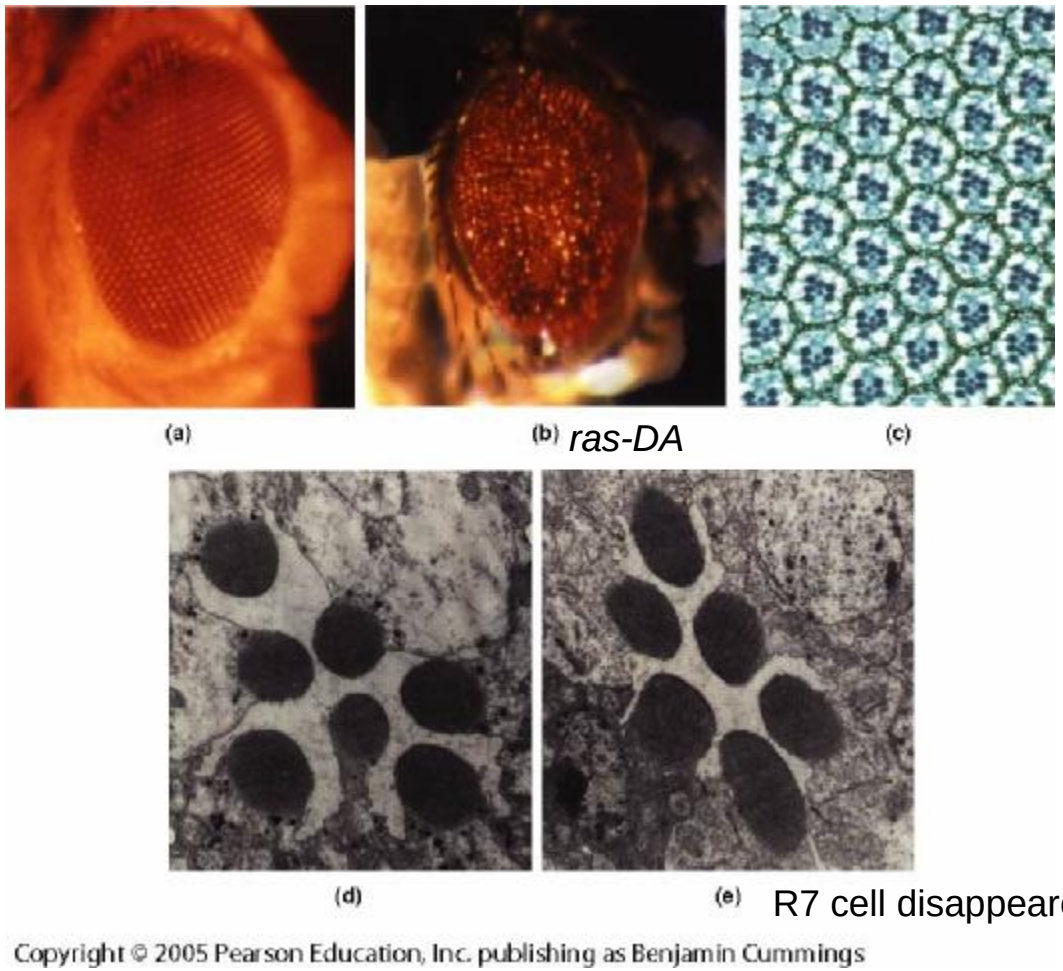
Same 800 individual eyes called ommatidia
22 cells/each ommatidium, 8 of which are photosensitive neurons called retinula (R cells), R1-R8
R7 cell development is specifically regulated by *Sevenless* (*Sev*) RTK
R7 photoreceptor is necessary for flies to see UV light

▲ **FIGURE 14-17 The compound eye of *Drosophila melanogaster.*** (a) Scanning electron micrograph showing individual ommatidia that compose the fruit fly eye. (b) Longitudinal and cutaway views of a single ommatidium. Each of these tubular structures contains eight photoreceptors, designated R1–R8, which are long, cylindrically shaped light-sensitive cells. R1–R6 (yellow) extend throughout the depth of the retina, whereas R7 (brown) is located toward the surface of the eye, and R8 (blue) toward the backside, where the axons exit. (c) Comparison of eyes from wild-type and *sevenless* mutant flies viewed by a special technique that can distinguish the photoreceptors in an ommatidium. The plane of sectioning is indicated by the blue arrows in (b), and the R8 cell is out of the plane of these images. The seven photoreceptors in this plane are easily seen in the wild-type ommatidia *(top)*, whereas only six are visible in the mutant ommatidia *(bottom)*. Flies with the *sevenless* mutation lack the R7 cell in their eyes. [Part (a) from E. Hafen and K. Basler, 1991, *Development* **1** (suppl.):123; part (b) adapted from R. Reinke and S. L. Zipursky, 1988, *Cell* **55**:321; part (c) courtesy of U. Banerjee.]



▲ **EXPERIMENTAL FIGURE 14-18 Genetic studies reveal that activation of Ras induces development of R7 photoreceptors in the *Drosophila eye.*** (a) During larval development of wild-type flies, the R8 cell in each developing ommatidium expresses a cell-surface protein, called *Boss*, that binds to the Sev RTK on the surface of its neighboring R7 precursor cell. This interaction induces changes in gene expression that result in differentiation of the precursor cell into a functional R7 neuron. (b) In fly embryos with a mutation in the *sevenless (sev)* gene, R7 precursor cells cannot bind Boss and therefore do not differentiate normally into R7 cells. Rather the precursor cell enters an alternative developmental pathway and eventually becomes a cone cell. (c) Double-mutant larvae *(sev; Ras*D*)* express a constitutively active Ras (RasD) in the R7 precursor cell, which induces differentiation of R7 precursor cells in the absence of the Boss-mediated signal. This finding shows that activated Ras is sufficient to mediate induction of an R7 cell. [See M. A. Simon et al., 1991, *Cell* **67**:701, and M. E. Fortini et al., 1992, *Nature* **355**:559.]

Figure 14A-1 The compound eye of *Drosophila*

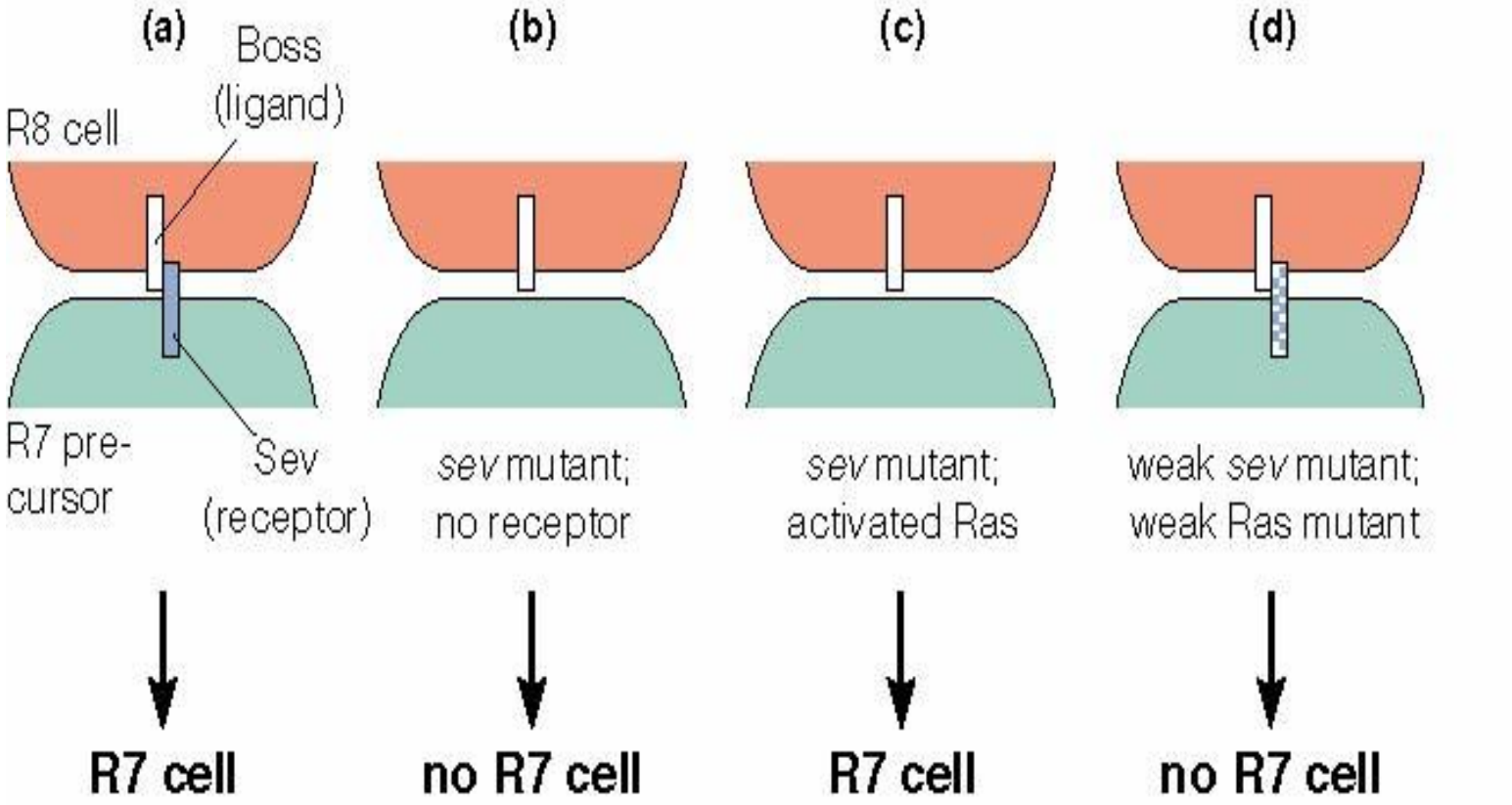


ras-DA

DA: dominant active

R7 cell disappeared

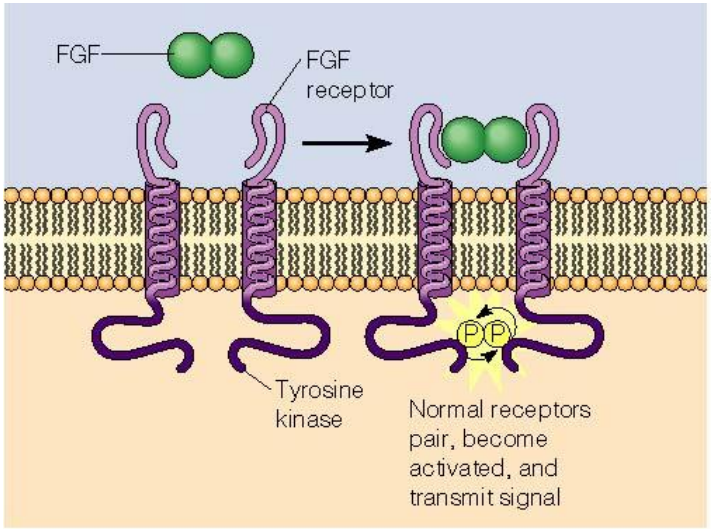
Figure 14A-2 Mutations in the Ras pathway can either bypass or enhance defects in RTK signaling



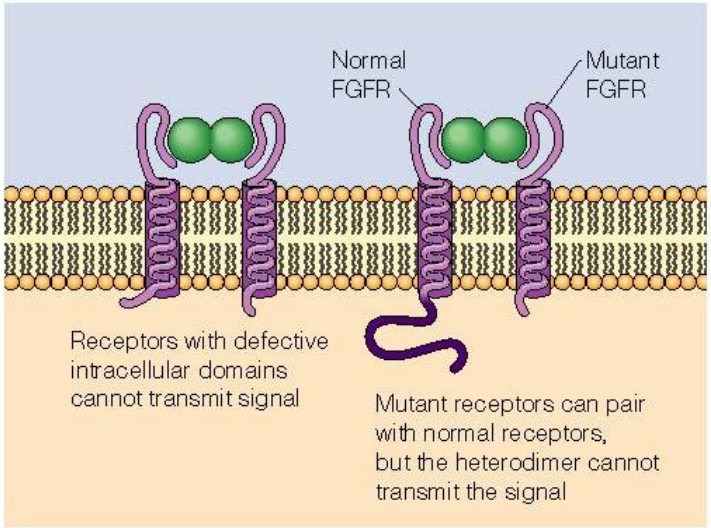
SOS: son of ???? ⟶ Son of bitch? No way ⟶ Right, it's son of sevenless

Boss ⟶ Bride of sevenless

Figure 14-19 Dominant Negative Disruption of FGF Receptor (FGFR) Function

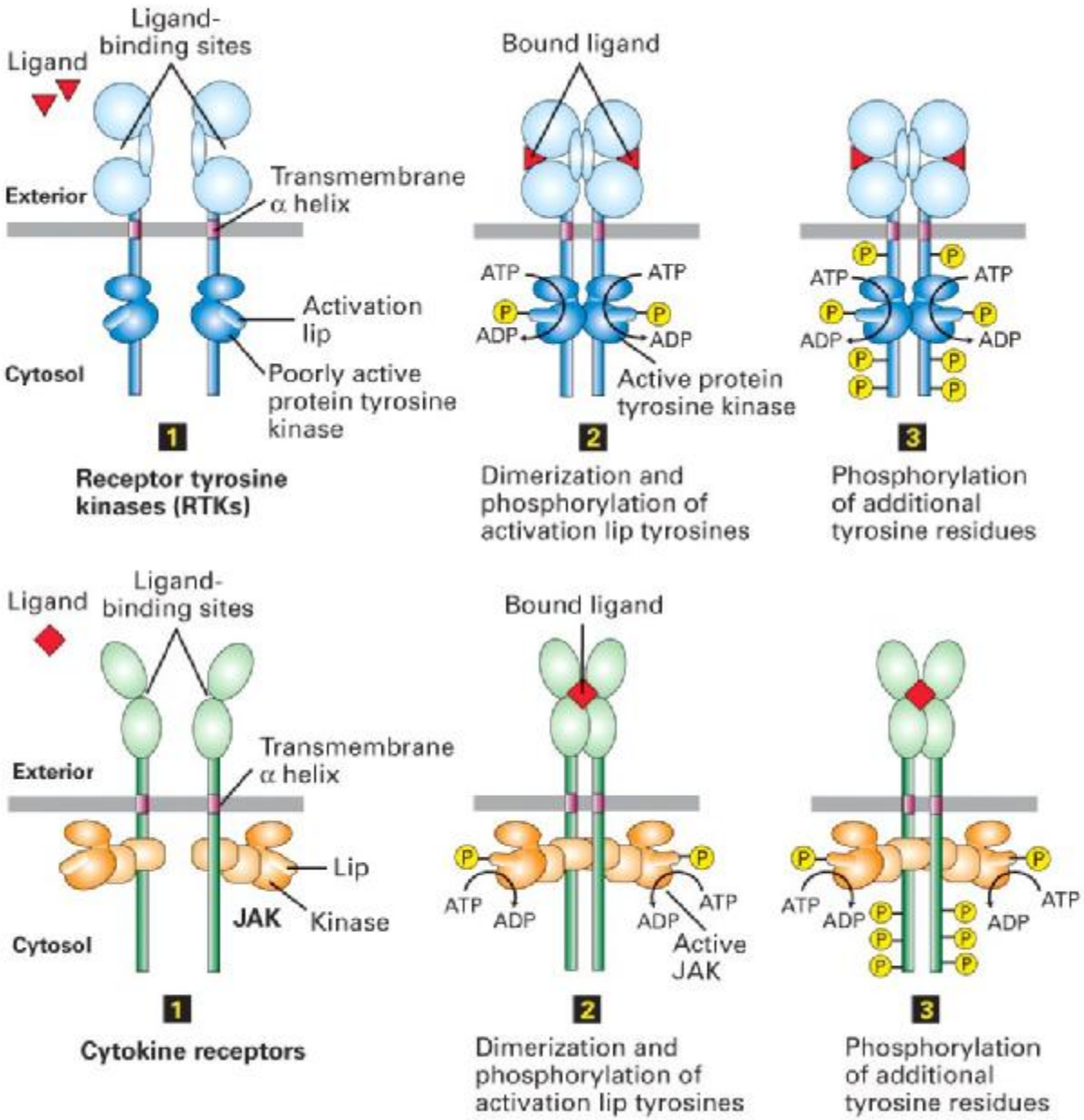


(a) Normal FGFR: FGF binds; FGFRs form a dimer

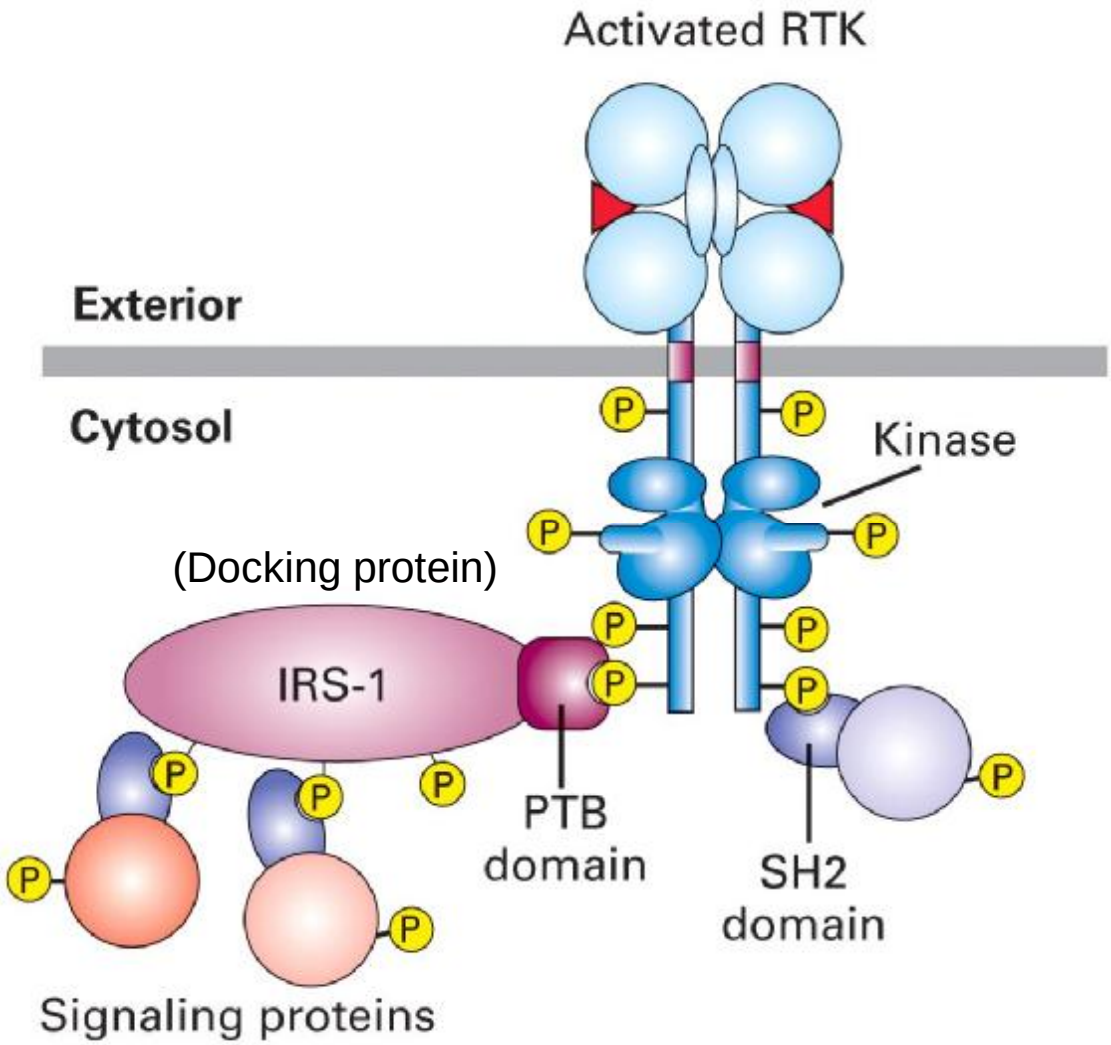


(b) Dominant negative mutation in FGFR

Figure 14-20 Fibroblast Growth Factor Signaling is Essential for Mesoderm Production in Embryos



▲ FIGURE 14-5 General structure and ligand-induced activation of receptor tyrosine kinases (RTKs) and cytokine receptors. The cytosolic domain of RTKs contains a protein tyrosine kinase catalytic site, whereas the cytosolic domain of cytokine receptors associates with a separate JAK kinase (step 1). In both types of receptor, ligand binding causes a conformational change that promotes formation of a functional dimeric receptor, bringing together two intrinsic or associated kinases, which then phosphorylate each other on a tyrosine residue in the activation lip (step 2). Phosphorylation causes the lip to move out of the kinase catalytic site, thus allowing ATP or a protein substrate to bind. The activated kinase then phosphorylates other tyrosine residues in the receptor's cytosolic domain (step 3). The resulting phosphotyrosines function as docking sites for various signal-transduction proteins (see Figure 14-6).



▲ FIGURE 14-6 Recruitment of signal-transduction proteins to the cell membrane by binding to phosphotyrosine residues in activated receptors. Cytosolic proteins with SH2 (purple) or PTB (maroon) domains can bind to specific phosphotyrosine residues in activated RTKs (shown here) or cytokine receptors. In some cases, these signal-transduction proteins then are phosphorylated by the receptor's intrinsic or associated protein tyrosine kinase, enhancing their activity. Certain RTKs and cytokine receptors utilize multidocking proteins such as IRS-1 to increase the number of signaling proteins that are recruited and activated. Subsequent phosphorylation of the IRS-1 by receptor kinase activity creates additional docking sites for SH2-containing signaling proteins.

SH2 (Src homology 2) domain: binds to a specific phosphotyrosine residue. SH2-binding specificity is determined by residues C-terminal to the phosphotyrosine.

PTB (phosphotyrosine binding) domain: PTB-binding specificity is determined by residues N-terminal to the phosphotyrosine.

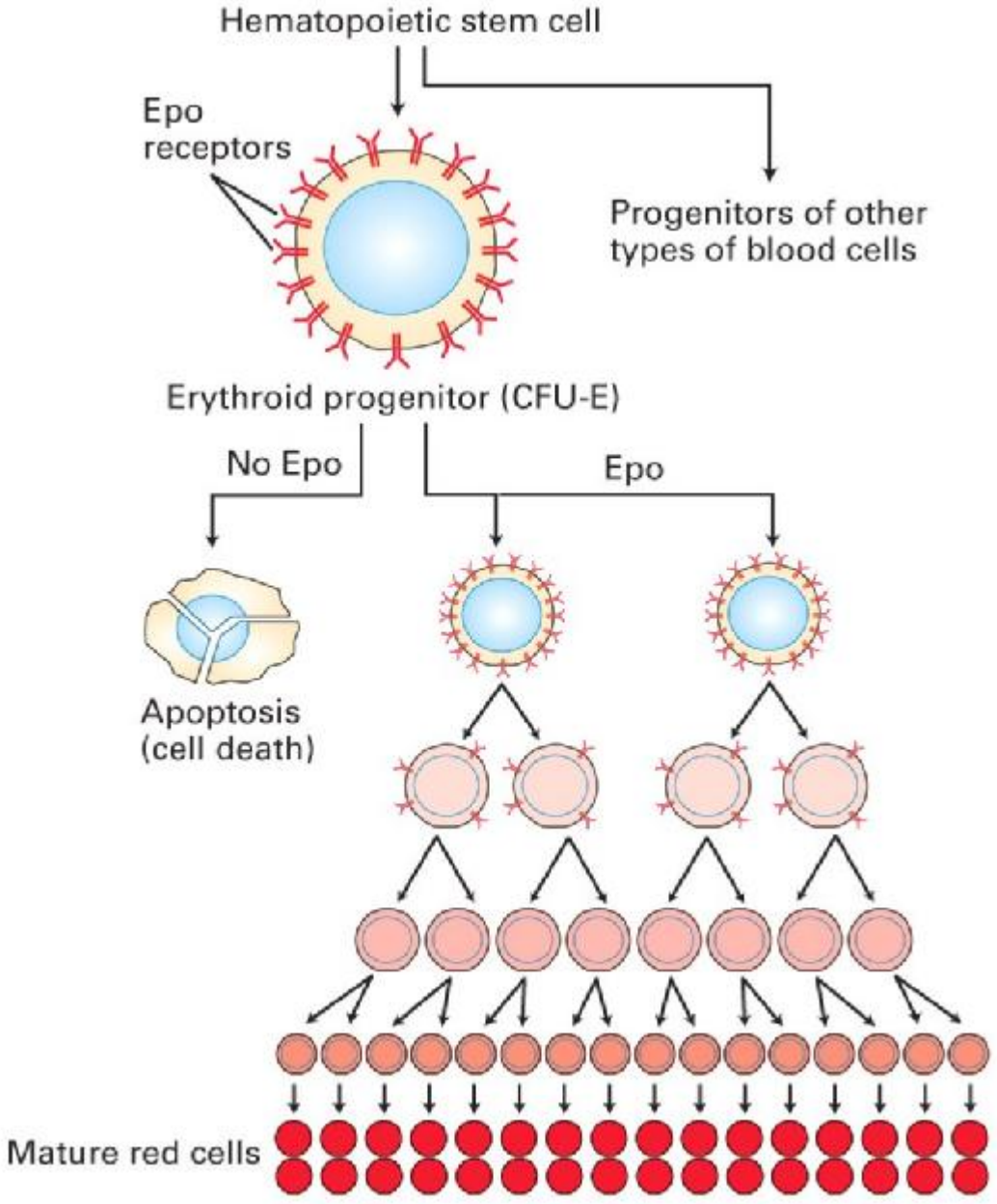
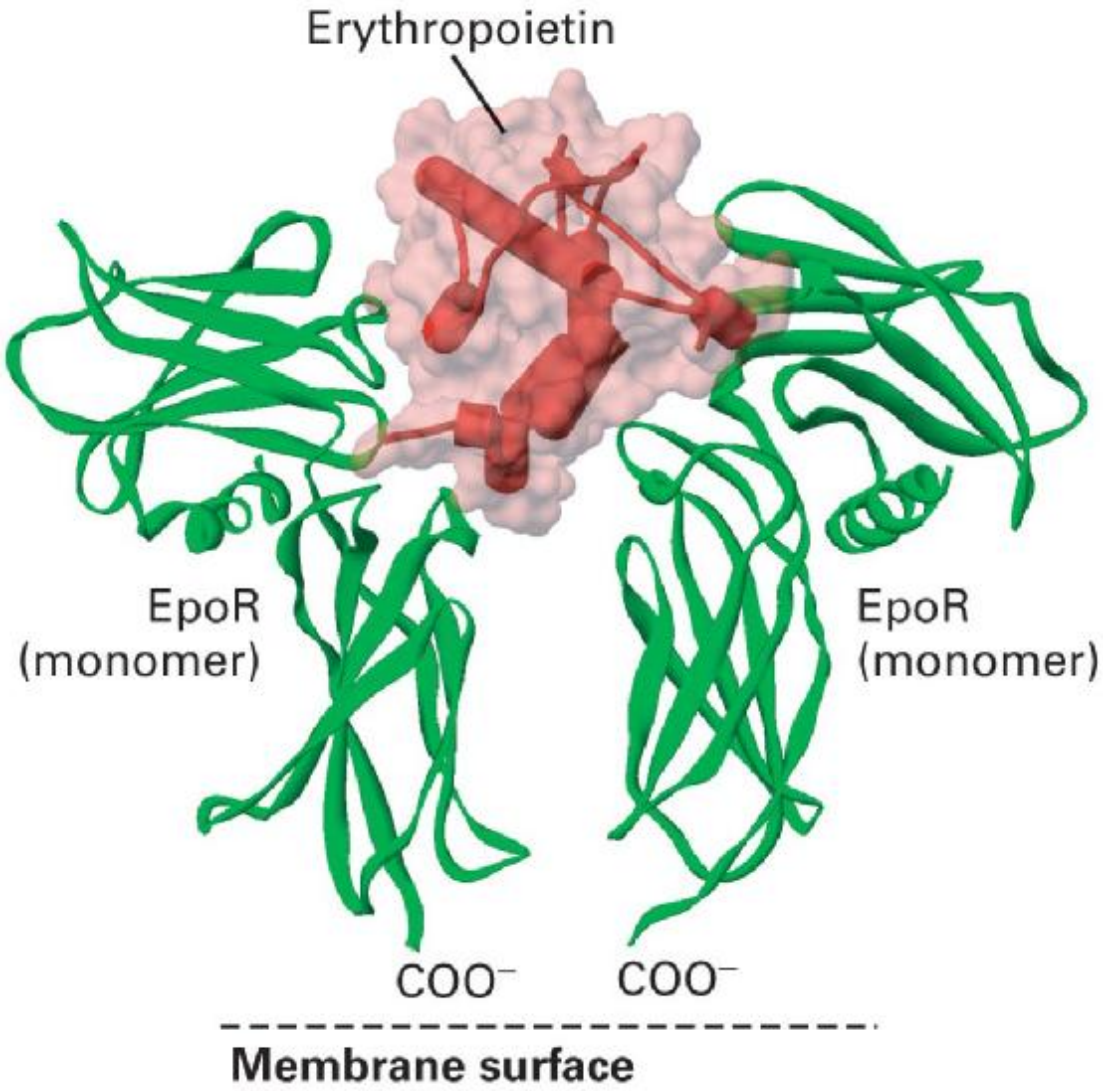


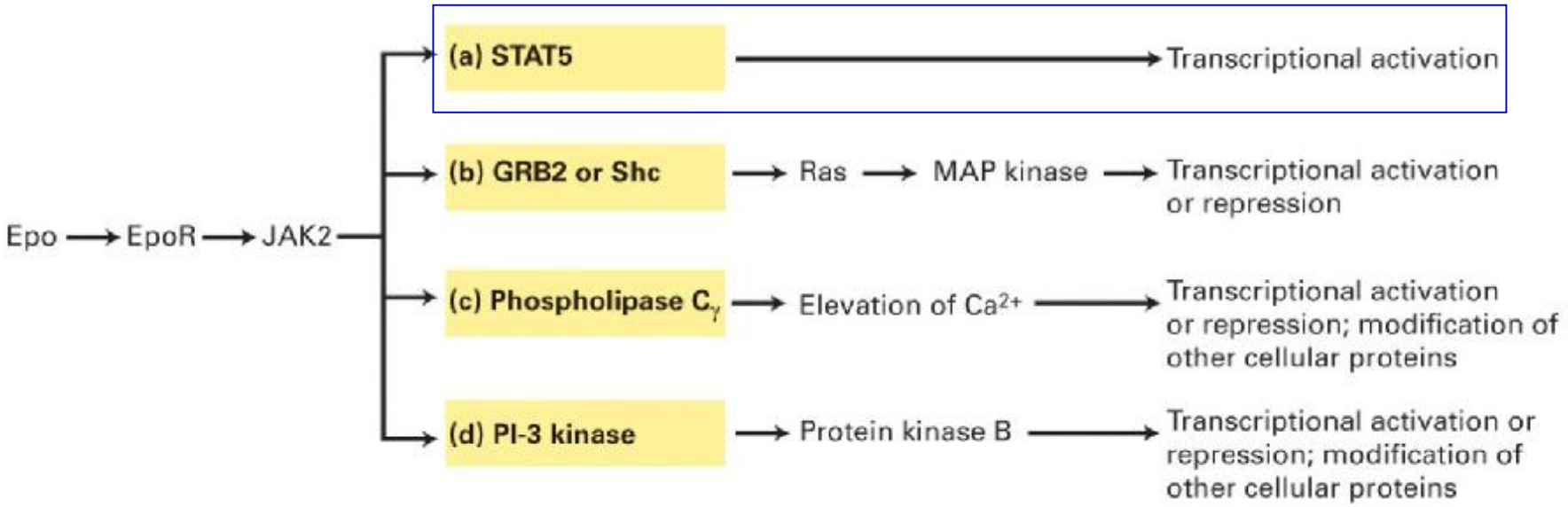
FIGURE 14-7 Role of erythropoietin in formation of red blood cells (erythrocytes). Erythroid progenitor cells, called *colony-forming units erythroid (CFU-E)*, are derived from hematopoietic stem cells, which also give rise to progenitors of other blood cell types. In the absence of erythropoietin (Epo), CFU-E cells undergo apoptosis. Binding of erythropoietin to its receptors on a CFU-E induces transcription of several genes whose encoded proteins prevent programmed cell death (apoptosis), allowing the cell to survive and undergo a program of three to five terminal cell divisions. Epo stimulation also induces expression of erythrocyte-specific proteins such as the globins, which form hemoglobin, and the membrane proteins glycophorin and anion-exchange protein. The Epo receptor and other membrane proteins are lost from these cells as they undergo differentiation. If CFU-E cells are cultured with erythropoietin in a semisolid medium (e.g., containing methylcellulose), daughter cells cannot move away, and thus each CFU-E produces a colony of 30–100 erythroid cells, hence its name. [See M. Socolovsky et al., 2001, *Blood* **98**:3261.]

Many cytokines induce formation of important types of blood cells
IL, IF, G-CSF, Thrombopoitin, erythropoitin (Epo), …..

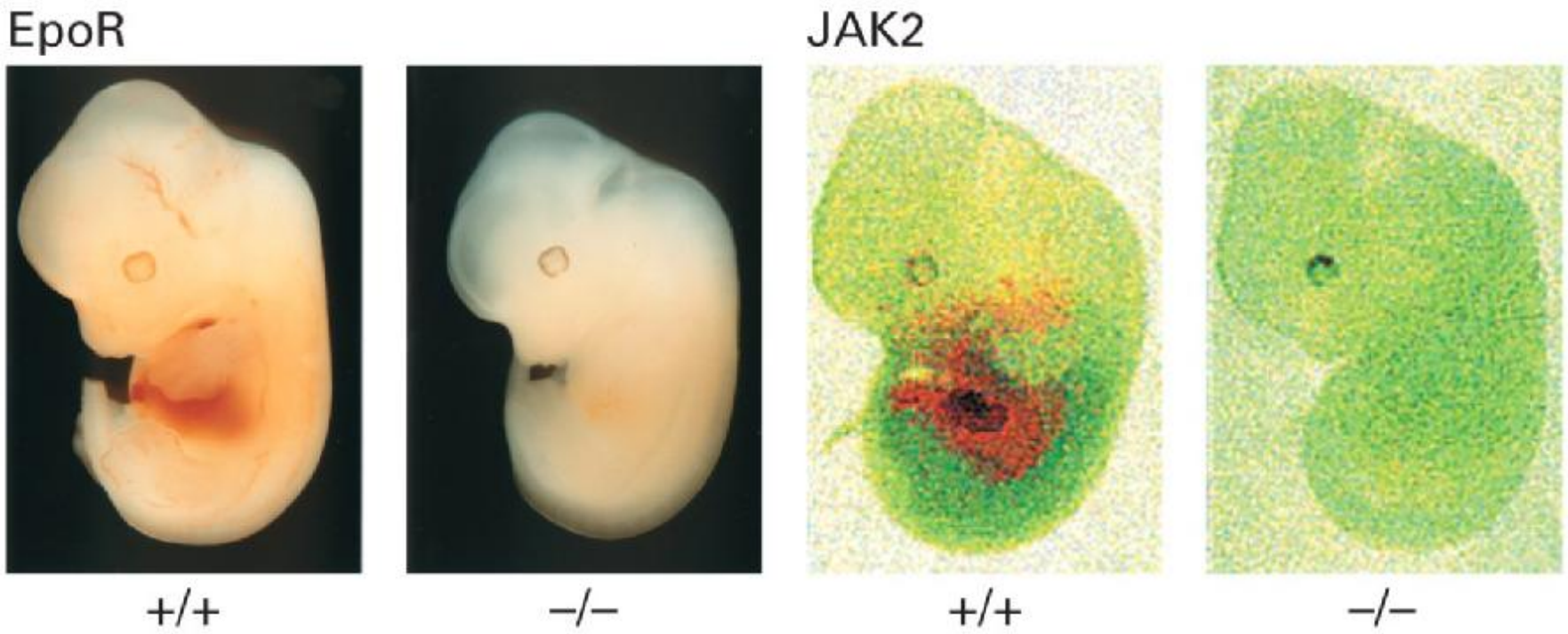


▲ **FIGURE 14-8 Structure of erythropoietin bound to the extracellular domains of a dimeric erythropoietin receptor (EpoR).** Erythropoietin contains four conserved long helices that are folded in a particular arrangement. The extracellular domain of an EpoR monomer is constructed of two subdomains, each of which contains seven conserved strands folded in a characteristic fashion. Side chains of residues on two of the helices in erythropoietin contact loops on one EpoR monomer, while residues on the two other Epo helices bind to the same loop segments in a second receptor monomer, thereby stabilizing the dimeric receptor. The structures of other cytokines and their receptors are similar to erythropoietin and EpoR. [Adapted from R. S. Syed et al., 1998, *Nature* **395**:511.]

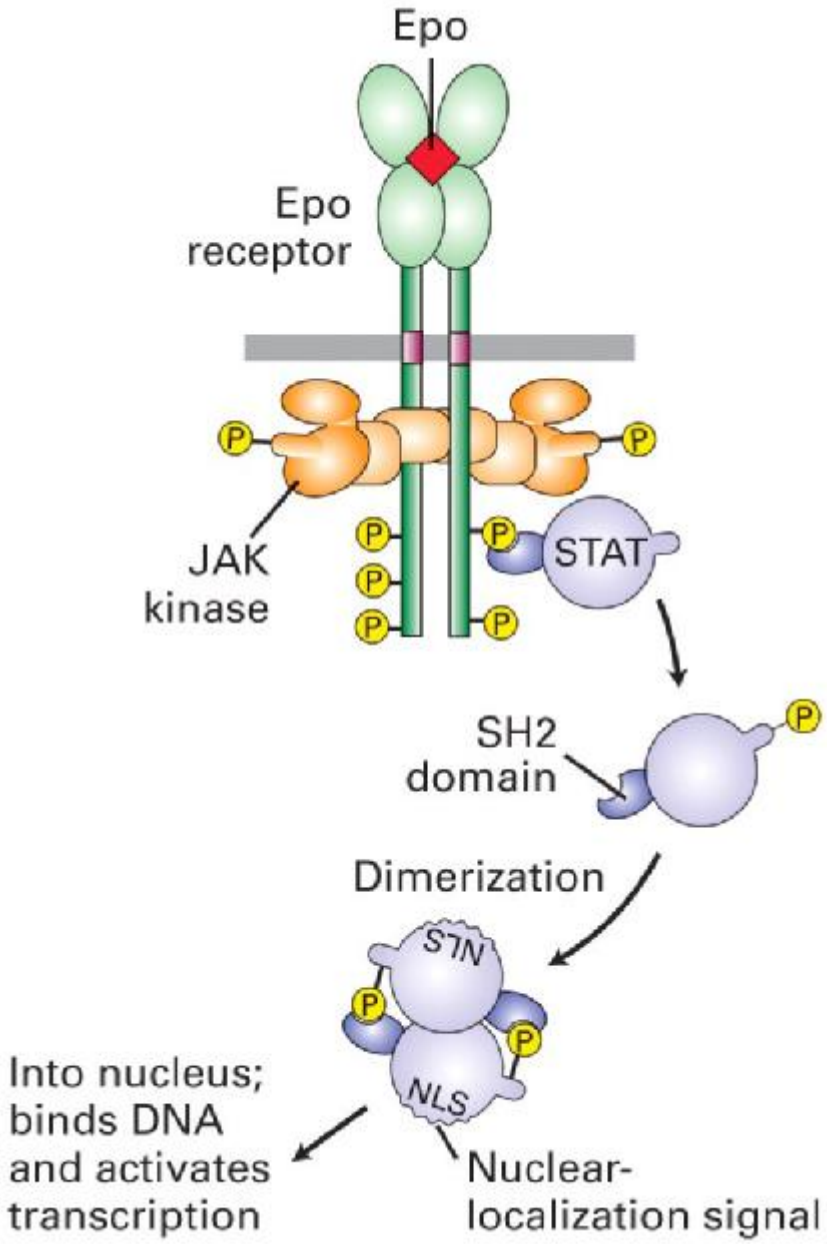
All cytokines have a similar tertiary structure, consisting of four long conserved α helices folded together in a specific orientation. Similarly, the structures of all cytokine receptors are quite similar, with their extracellular domains constructed of two subdomains, each of which contains seven conserved β strands folded together in a characteristic fashion.



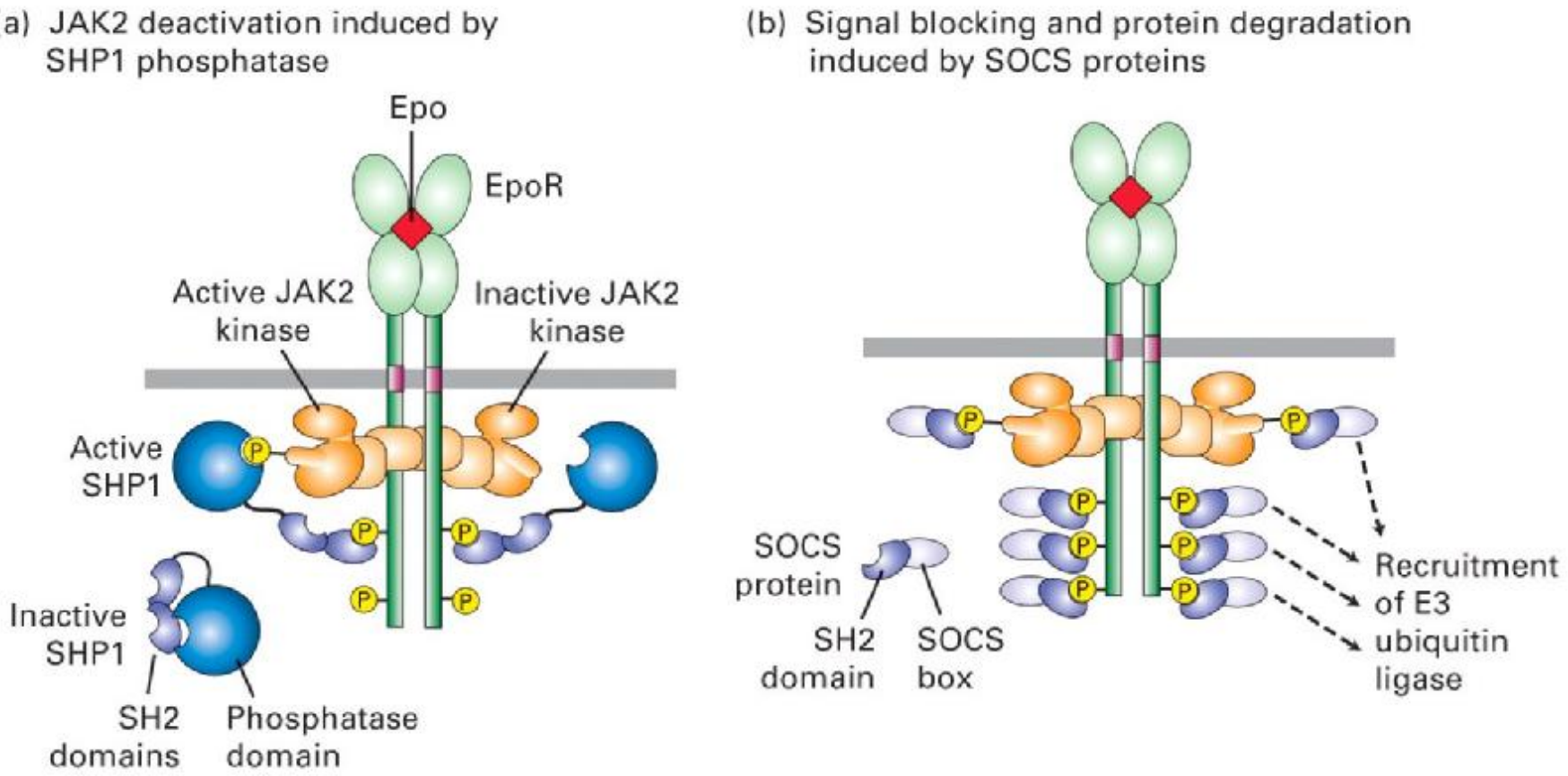
▲ **FIGURE 14-9 Overview of signal-transduction pathways triggered by ligand binding to the erythropoietin receptor (EpoR), a typical cytokine receptor.** Four major pathways can transduce a signal from the activated, phosphorylated EpoR-JAK complex (see Figure 14-5, *bottom*). Each pathway ultimately regulates transcription of different sets of genes. (a) In the most direct pathway, the transcription factor STAT5 is phosphorylatedand activated directly in the cytosol. (b) Binding of linker proteins (GRB2 or Shc) to an activated EpoR leads to activation of the Ras–MAP kinase pathway. (c, d) Two phosphoinositide pathways are triggered by recruitment of phospholipase C and PI-3 kinase to the membrane following activation of EpoR. Elevated levels of Ca2 and activated protein kinase B also modulate the activity of cytosolic proteins that are not involved in control of transcription.



▲ **EXPERIMENTAL FIGURE 14-11 Studies with mutant mice reveal that both the erythropoietin receptor (EpoR) and JAK2 are essential for development of erythrocytes.** Mice in which both alleles of the EpoR or JAK2 gene are “knocked out” develop normally until embryonic day 13, at which time they begin to die of anemia due to the lack of erythrocyte-mediated transport of oxygen to the fetal organs. The red organ in the wild-type embryos (+/+) is the fetal liver, the major site of erythrocyte production at this developmental stage. The absence of color in the mutant embryos (-/-) indicates the absence of erythrocytes containing hemoglobin. Otherwise the mutant embryos appear normal, indicating that the main function of the EpoR and JAK2 in early mouse development is to support production of erythrocytes. [EpoR images from H. Wu et al., 1995, *Cell* **83**:59; JAK2 images from H. Neubauer et al., 1998, *Cell* **93**:307.]

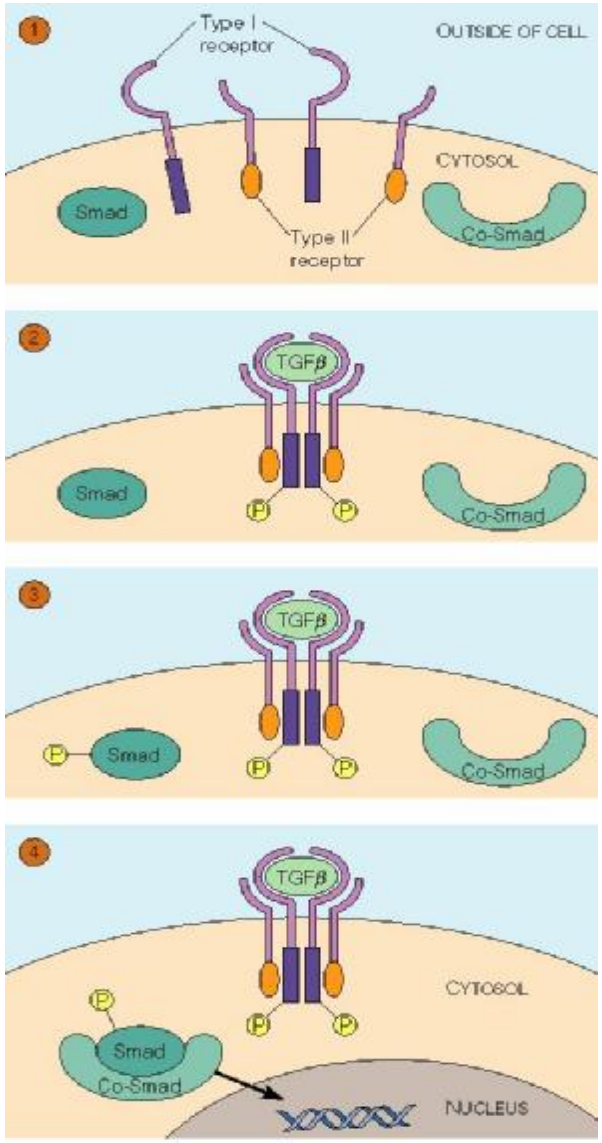


▲ **FIGURE 14-12 JAK-STAT signaling pathway.** Following ligand binding to a cytokine receptor and activation of an associated JAK kinase, JAK phosphorylates several tyrosine residues on the receptor's cytosolic domain (see Figure 14-5, *bottom*). After an inactive monomeric STAT transcription factor binds to a phosphotyrosine in the receptor, it is phosphorylated by active JAK. Phosphorylated STATs spontaneously dissociate from the receptor and spontaneously dimerize. Because the STAT homodimer has two phosphotyrosine–SH2 domain interactions, whereas the receptor-STAT complex is stabilized by only one such interaction, phosphorylated STATs tend not to rebind to the receptor. The STAT dimer, which has two exposed nuclear-localization signals (NLS), moves into the nucleus, where it can bind to promoter sequences and activate transcription of target genes.



▲ **FIGURE 14-14 Two mechanisms for terminating signal transduction from the erythropoietin receptor (EpoR).** (a) SHP1, a protein tyrosine phosphatase, is present in an inactive form in unstimulated cells. Binding of an SH2 domain in SHP1 to a particular phosphotyrosine in the activated receptor unmasks its phosphatase catalytic site and positions it near the phosphorylated tyrosine in the lip region of JAK2. Removal of the phosphate from this tyrosine inactivates the JAK kinase. (b) SOCS proteins, whose expression is induced in erythropoietin-stimulated erythroid cells, inhibit or permanently terminate signaling over longer time periods. Binding of SOCS to phosphotyrosine residues on the EpoR or JAK2 blocks binding of other signaling proteins *(left).* The SOCS box can also target proteins such as JAK2 for degradation by the ubiquitinproteasome pathway *(right).* Similar mechanisms regulate signaling from other cytokine receptors. [Part (a) adapted from S. Constantinescu et al., 1999, *Trends Endocrin. Metabol.* **10**:18; part (b) adapted from B. T. Kile and W. S. Alexander, 2001, *Cell. Mol. Life Sci.* **58**:1.]

Figure 14-21 Signal Transduction by TGFβ Receptor Family Proteins (Serine/Threonine kinase receptor)



TGF-β superfamily: transforming growth factor β

↓

Anti-proliferation
Differentiation

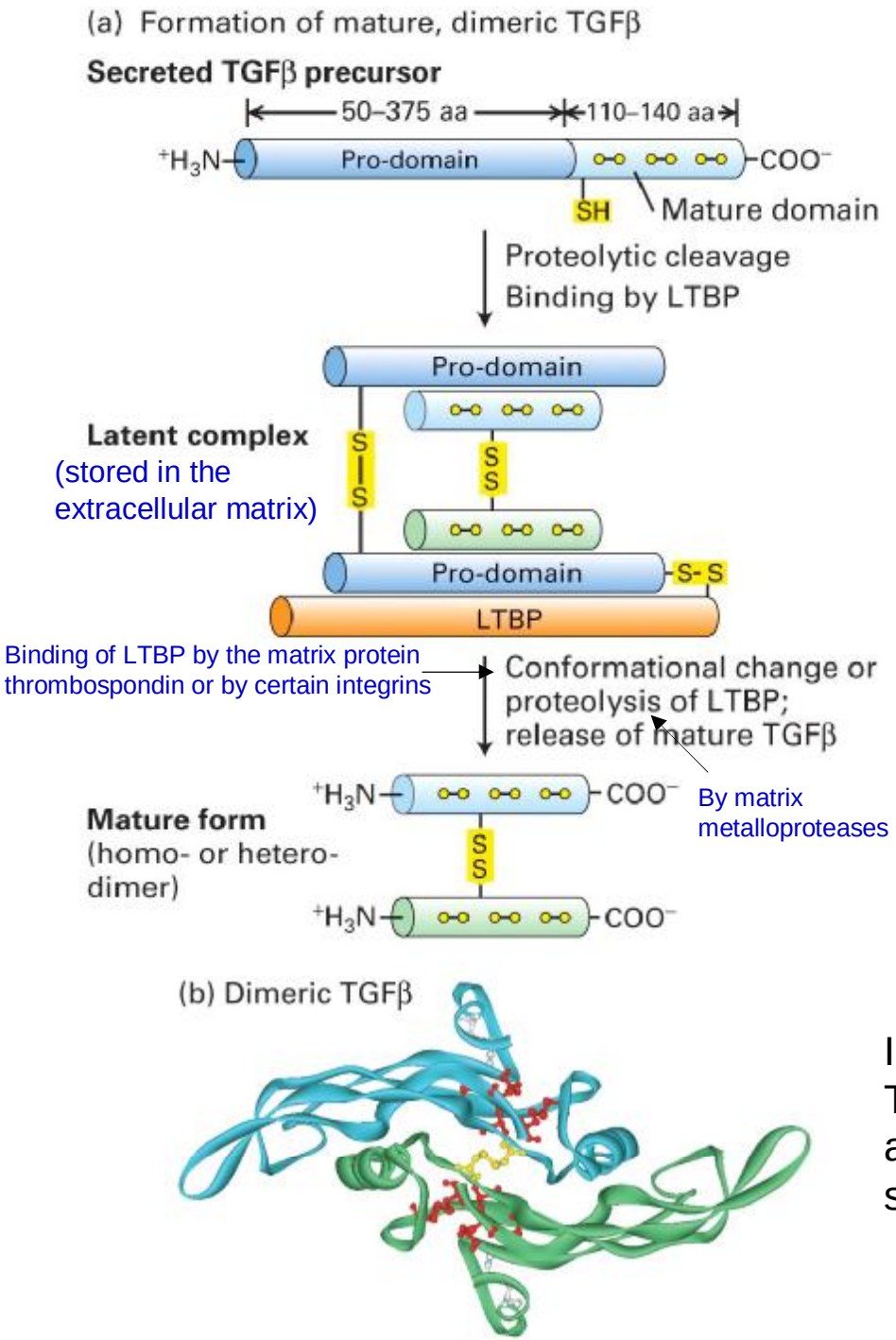


FIGURE 14-1 Formation and structure of TGF superfamily of signaling molecules. (a) TGFβ precursors are cleaved soon after being secreted. The pro-domain and mature domain are stored in the extracellular matrix in a complex that also contains latent TGFβ–binding protein (LTBP). The mature domain contains six conserved cysteine residues (yellow circles), which form three intrachain disulfide bonds and also a single disulfide bond connecting two monomers. Following proteolysis or a conformational change in LTBP, the active homo- or heterodimeric protein is released. (b) In this ribbon diagram of mature TGFβ dimer, the two subunits are shown in green and blue. Disulfide-linked cysteine residues are shown in ball-and-stick form. The three intrachain disulfide linkages (red) in each monomer form a cystine-knot domain, which is resistant to degradation. [Part (a) see J. Massagué and Y.-G. Chen, 2000, *Genes and Devel.* **14**:627; part (b) from S. Daopin et al., 1992, *Science* **257:**369.]

In human TGFβ consists of three protein isoforms, TGFβ-1, TGFβ-2, and TGFβ-3, each encoded by a unique gene and expressed in both a tissue-specific and developmentally regulated fashion.

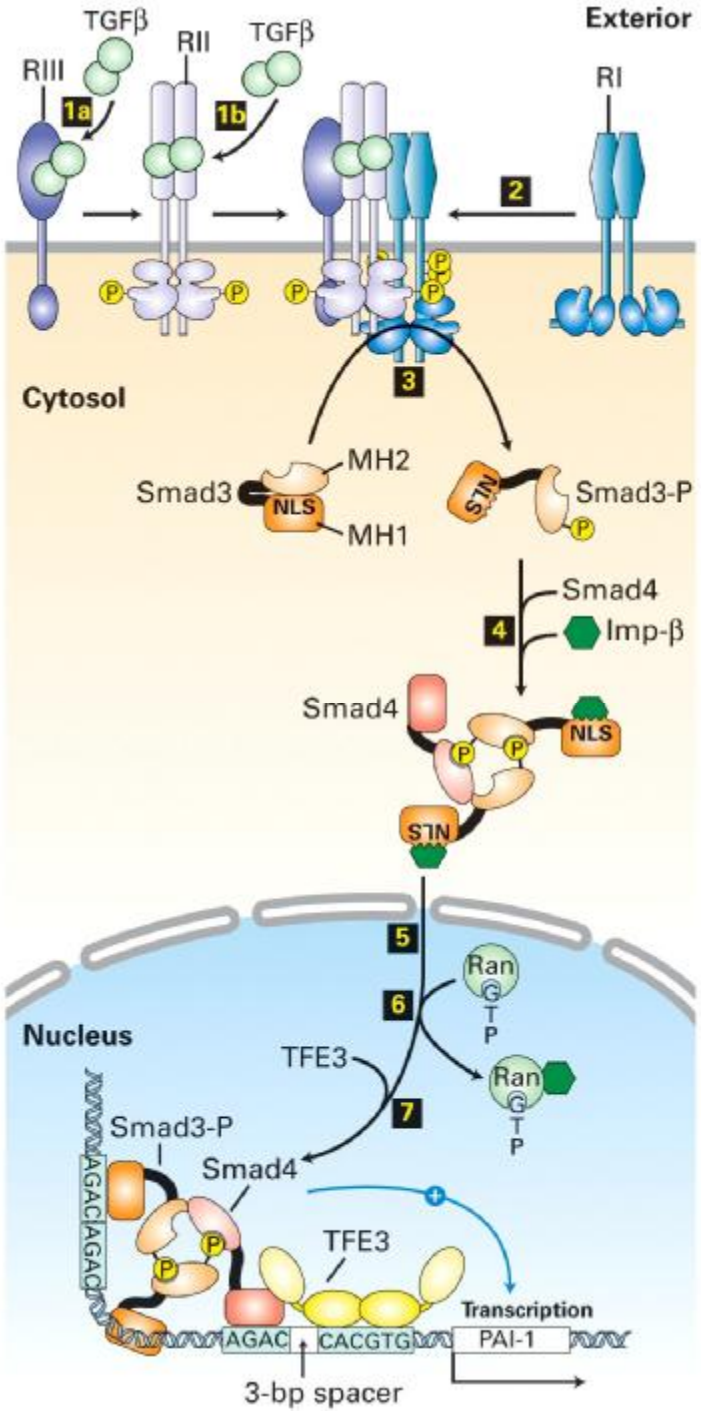
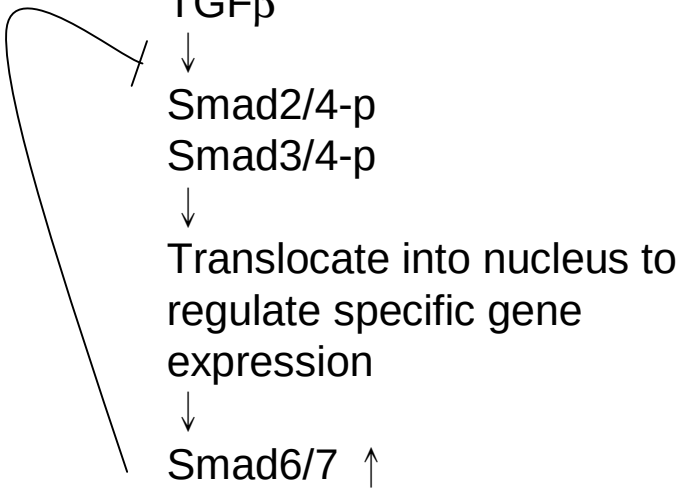


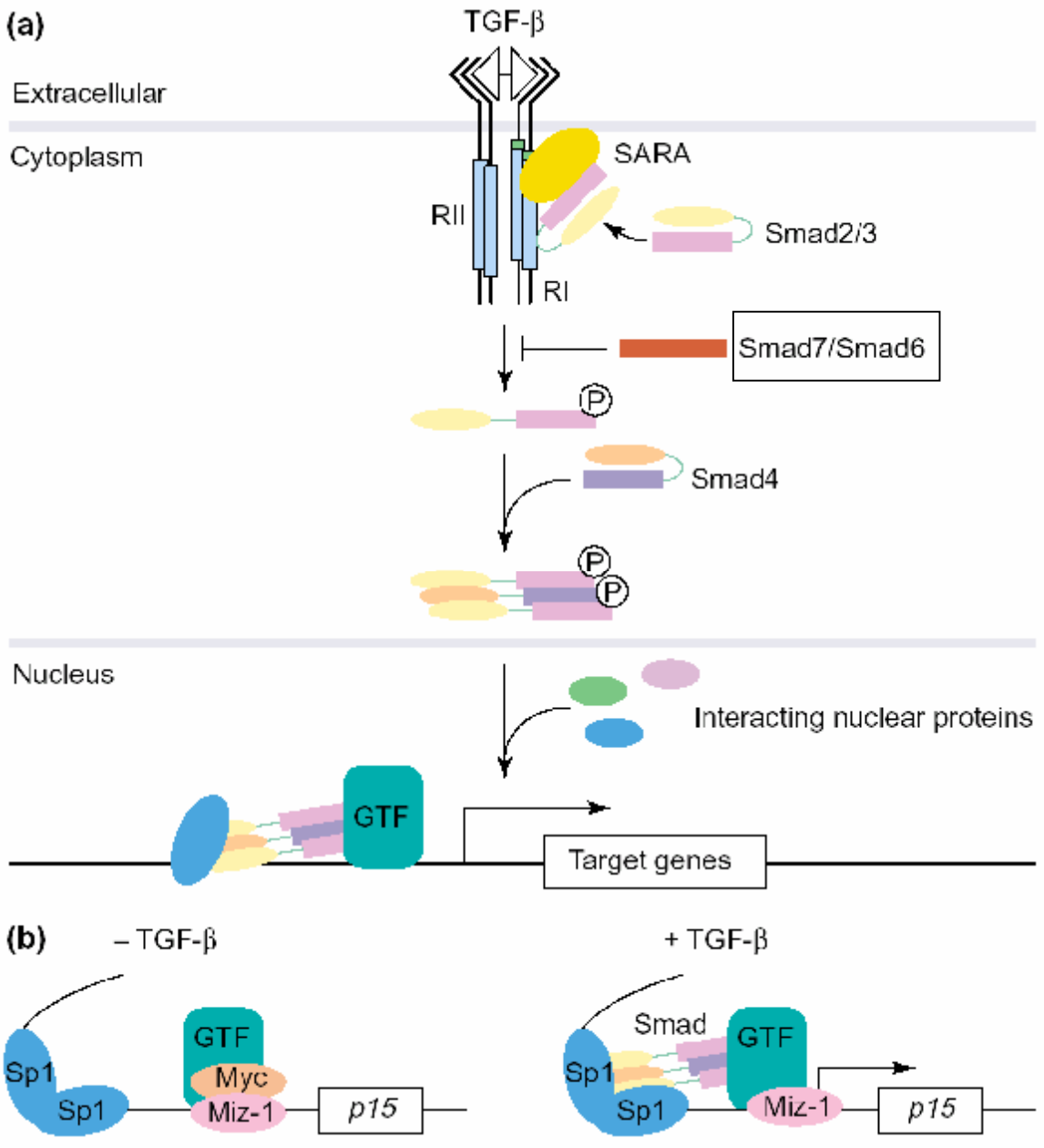
FIGURE 14-2 TGF-Smad signaling pathway. Step 1a : In some cells, TGFβ binds to the type III TGFβ receptor (RIII), which presents it to the type II receptor (RII). Step 1b: In other cells, TGFβ binds directly to RII, a constitutively phosphorylated and active kinase. Step 2: Ligand-bound RII recruits and phosphorylates the juxtamembrane segment of the type I receptor (RI), which does not directly bind TGFβ. This releases the inhibition of RI kinase activity that otherwise is imposed by the segment of RI between the membrane and kinase domain. Step 3: Activated RI then phosphorylates Smad3 (shown here) or another R-Smad, causing a conformational change that unmasks its nuclear-localization signal (NLS). Step 4: Two phosphorylated molecules of Smad3 interact with a co-Smad (Smad4), which is not phosphorylated, and with importin (Imp-β), forming a large cytosolic complex. Steps 5 and 6: After the entire complex translocates into the nucleus, RanGTP causes dissociation of Imp-β. Step 7: A nuclear transcription factor (e.g., TFE3) then associates with the Smad3/Smad4 complex, forming an activation complex that cooperatively binds in a precise geometry to regulatory sequences of a target gene. Shown at the bottom is the activation complex for the gene encoding plasminogen activator inhibitor (PAI-1). See the text for additional details. [See Z. Xiao et al., 2000, *J. Biol. Chem.* **275**:23425; J. Massagué and D. Wotton, 2000, *EMBO J.* **19**:1745; X. Hua et al., 1999, *Proc. Nat'l. Acad. Sci. USA* **96**:13130; and A. Moustakas and C.-H. Heldin, 2002, *Genes Devel.* **16**:1867.]

R-Smads (Recetor-regulated): Smad1, Smad2, Smad3
Co-Smads: Smad4
I-Smads (inhibitory): Smad6, Smad7

Down-regulation of the TGF-β signaling pathway

1. Negative feedback loop

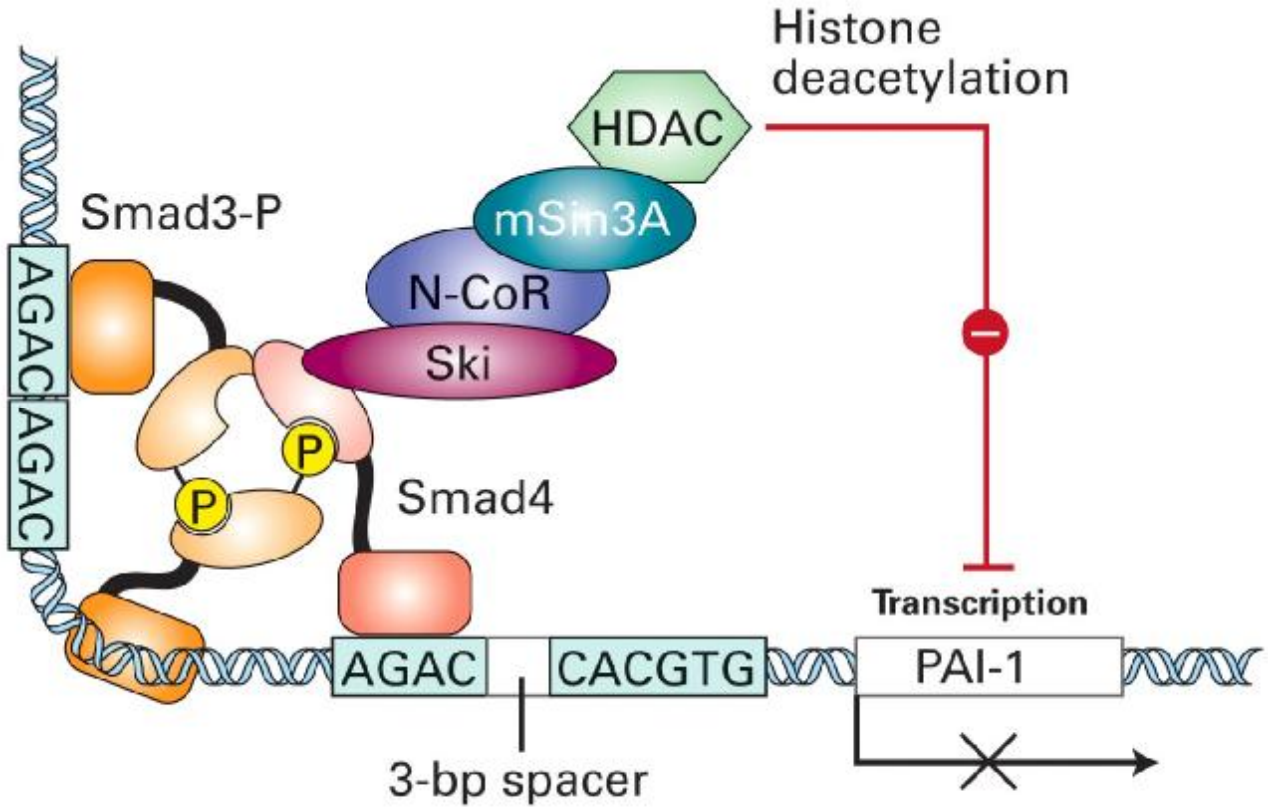




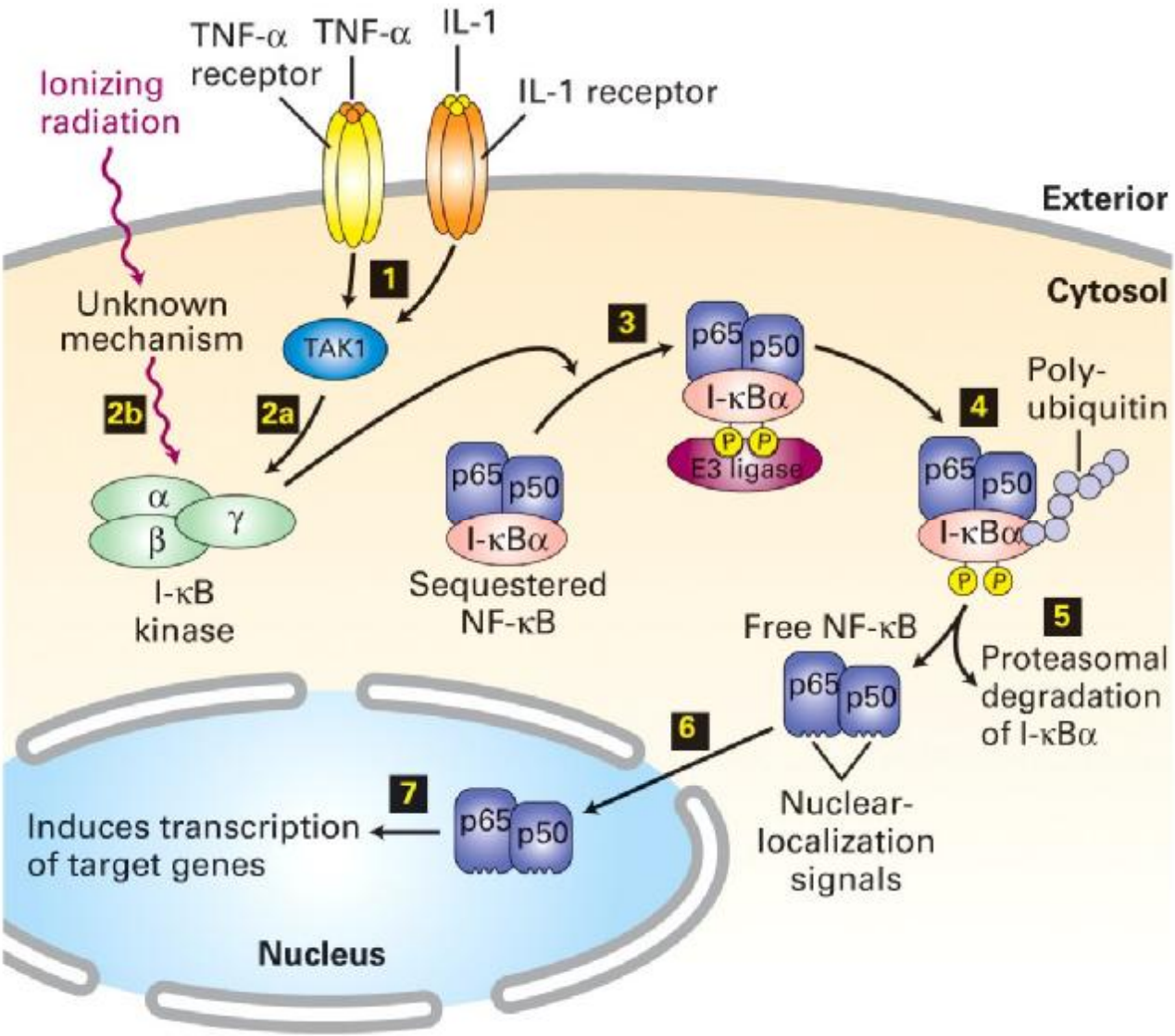
TRENDS in Cell Biology 11, S44, 2001

2. Ski-mediated down-regulation

TGFβ
↓
Rapid degradation of
Ski and SnoN
↓
After a few hours, expression
of both Ski and SnoN ↑ ↑
↓
Dampen long-term signaling
effects due to continued
exposure to TGFβ



▲ FIGURE 14-3 Schematic model of Ski-mediated down-regulation of the response to TGFb stimulation. Ski binds to Smad4 in Smad3/Smad4 or Smad2/Smad4 (not shown) signaling complexes and may partially disrupt interactions between the Smad proteins. Ski also recruits a protein termed *N-CoR* that binds directly to mSin3A, which in turn interacts with histone deacetylase (HDAC), an enzyme that promotes histone deacetylation (Chapter 11). As a result, transcription activation induced by TGFβ and mediated by Smad complexes is shut down. [See S. Stroschein et al., 1999, *Science* **286**:771; X. Liu et al., 2001, *Cytokine and Growth Factor Rev.* **12**:1; and J.-W. Wu et al., 2002, *Cell* **111**:357.]



Anti-apoptosis
inflammation

FIGURE 14-28 NF-kB signaling pathway. In resting cells, the dimeric transcription factor NF–κB, composed of p50 and p65, is sequestered in the cytosol, bound to the inhibitor I–κBα. Stimulation by TNF-α or IL-1 induces activation of TAK1 kinase (step1), leading to activation of the trimeric I–κB kinase (step 2a). Ionizing radiation and other stresses can directly activate I–κB kinase by an unknown mechanism (step 2b). Following phosphorylation of I–κBα by I–κB kinase and binding of E3 ubiquitin ligase (step 3), polyubiquitination of I–κBα (step 4) targets it for degradation by proteasomes (step 5). The removal of I–κBα unmasks the nuclear-localization signals (NLS) in both subunits of NF–κB, allowing their translocation to the nucleus (step 6). Here NF–κB activates transcription of numerous target genes (step 7), including the gene encoding the subunit of I–κBα, which acts to terminate signaling. [See M. Karin and Y. Ben-Neriah, 2000, *Ann. Rev. Immunol.* **18**:621, and R. Khush, F. Leulier, and B. Lemaitre, 2001, *Trends Immunol.* **22**:260.]