Biosignaling

Principals of Biochemistry, Lehninger,
Chap 12
CBIO7100

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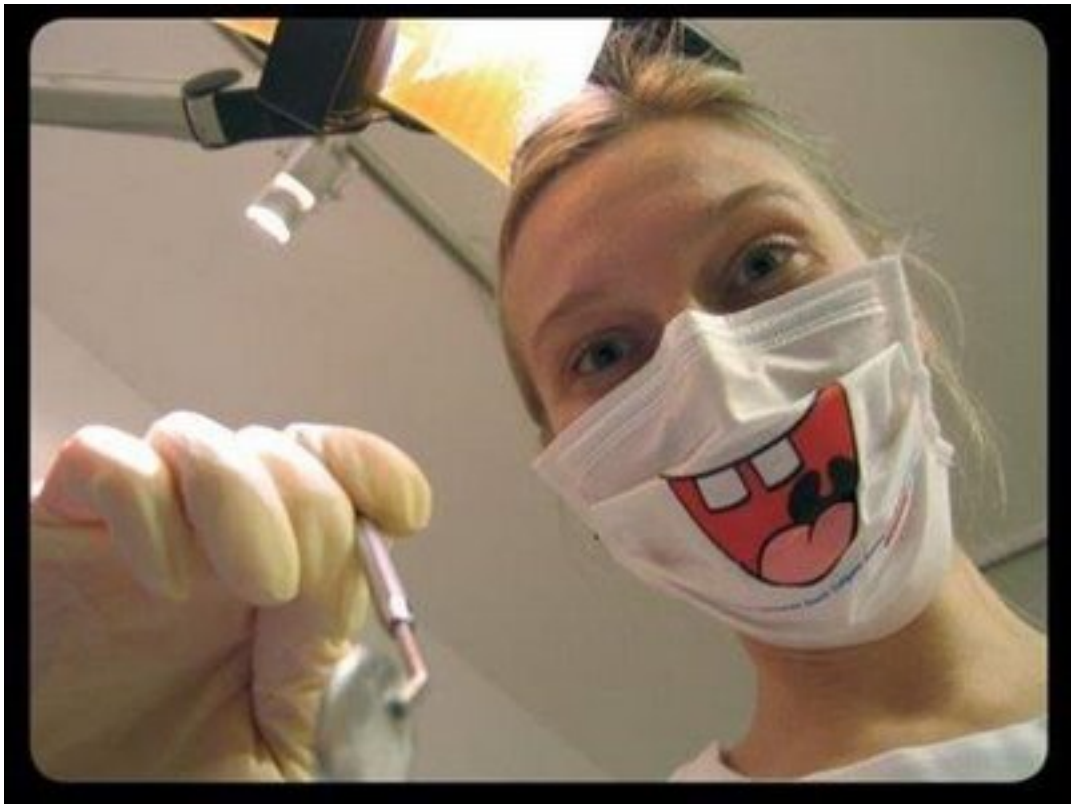
Lecture Outline

- Introduction: Importance of cell signaling to periodontal disease
- Principals of signal transduction, adaptors
- G protein coupled receptors, Chemokine receptors
- Receptor tyrosine kinases
- Inflammatory signaling: Toll like receptor signaling and the role of NfκB in periodontitis
- Receptor guanylyl kinases
- Hormone receptors

INTRODUCTION

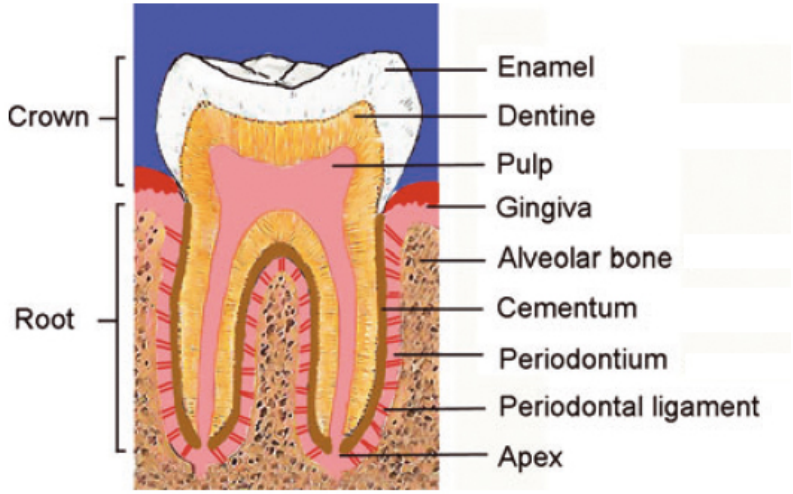
- **Why do I have to learn about signaling? I am going to be a dentist**

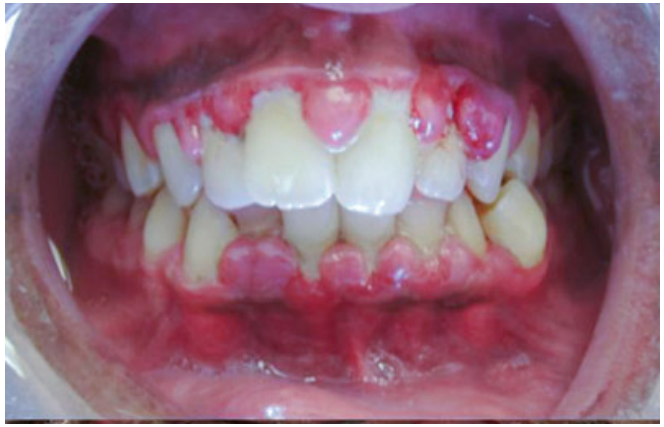


Signaling is central to teeth development and decay.

Signaling is important in periodontal disease.

Periodontal Disease (Gum Disease)





- Periodontal disease is inflammation and infection that destroys the tissues that support the teeth, including the gums, the periodontal ligaments, and the tooth sockets (alveolar bone).
- **Gingivitis:** inflammation of the gums due to bacteria. Signaling molecules produced by bacteria activate receptors on periodontal cells to ultimately destroy periodontal tissue.
- Although periodontal disease is initiated by bacteria that colonize the tooth surface and ginigva, the host signaling response is essential for breakdown of bone and connective tissue: Osteoclastogenesis and bone resorption.
- Periodontal infection requires expression of a number of signaling molecules: proinflammatory and antiinflammatory cytokines, growth factors, etc.

Cell signaling in periodontal disease

- **de Souza, et al. 2012. Modulation of host cell signaling pathways as a therapeutic approach in periodontal disease.** *Appl. Oral Sci.* 20:128-138.

  Pharmacological inhibitors of MAPK, NFκB and JAK/STAT pathways are being developed to manage periodontal disease.

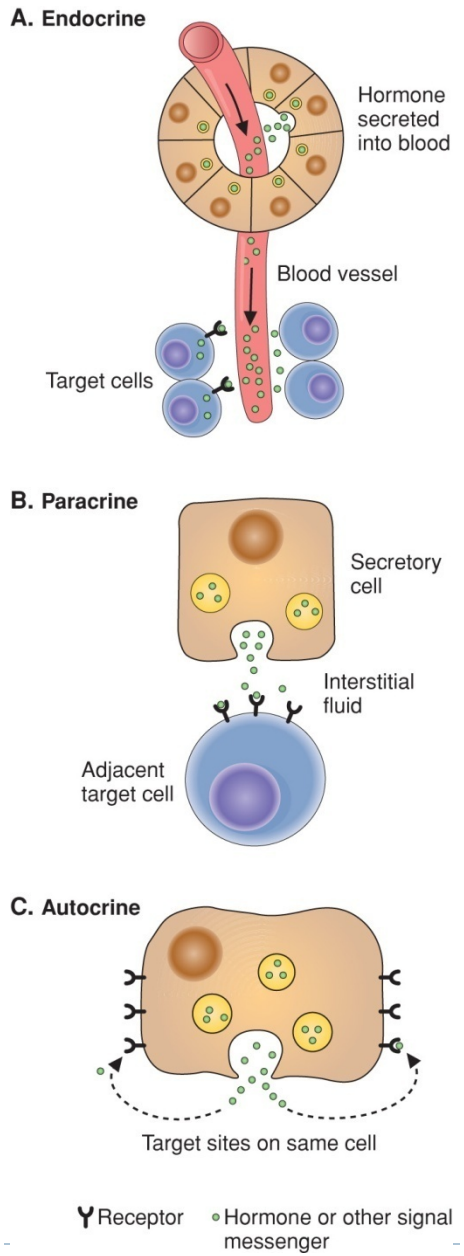
- **Kirkwood, et al., Novel host response therapeutic approaches to treat periodontal diseases.** *Periodontol 2000.* 2007 ; 43: 294–315.

  Periodontal disease initiation and progression occurs as a consequence of the host immune inflammatory response to oral pathogens.

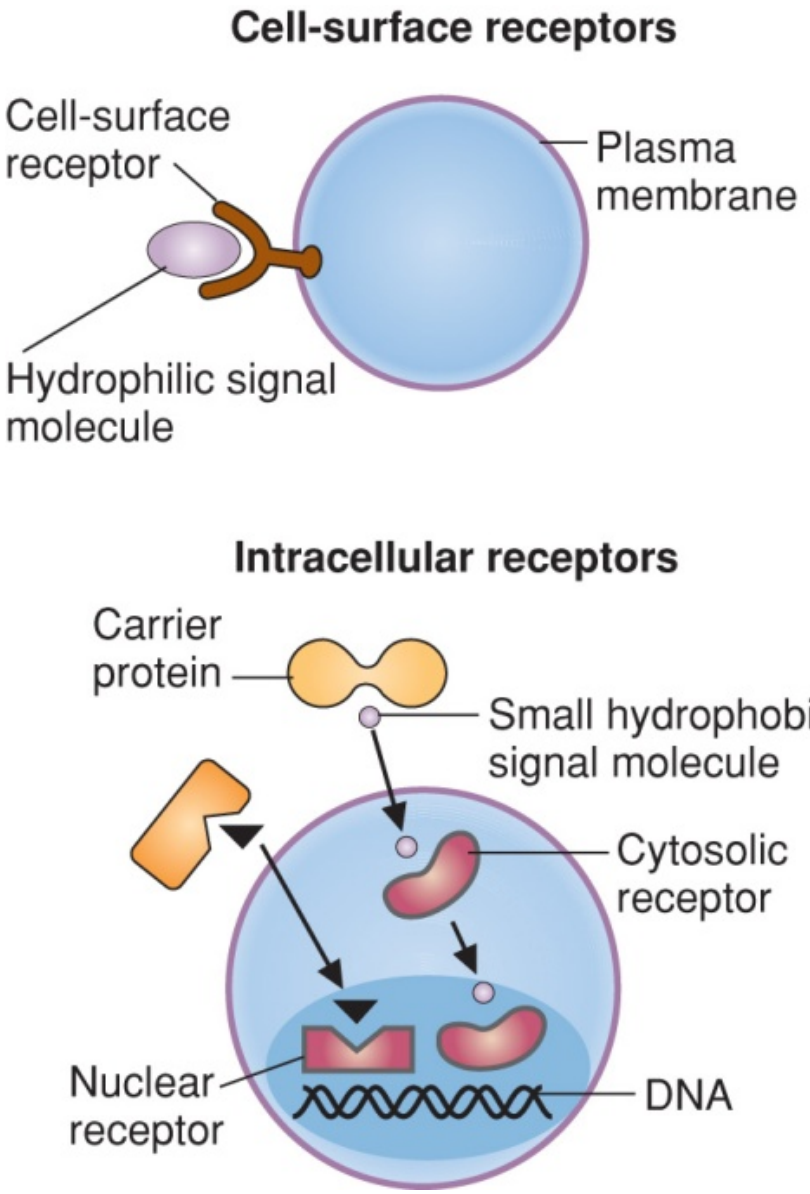
- **Li, et al. MAPK Usage in Periodontal Disease Progression.** *Journal of Signal Transduction*, Volume 2012, Article ID 308943.

  p38/MAPK-activated protein kinase signaling axis is needed for periodontal disease progression.

- **Graves,D. Cytkines that promote periodontal tissue destruction.** *Journal of Periodontology*. 2008, 1585-1591.



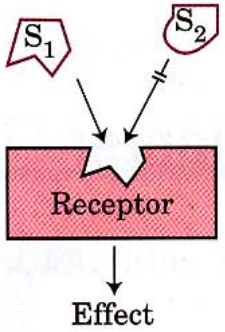
Chemical signals can be:
A.secreted from endocrine cells and act on distant target cells: **Endocrine** (Hormones)
B.Secreted and act on adjacent cells: **Paracrine**
C.Secreted and acts on same cell: **Autocrine**



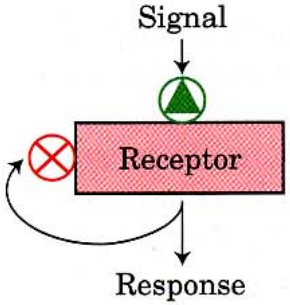
Chemical messengers can transduce signals without being metabolized by the cell

Properties of signal transducing systems

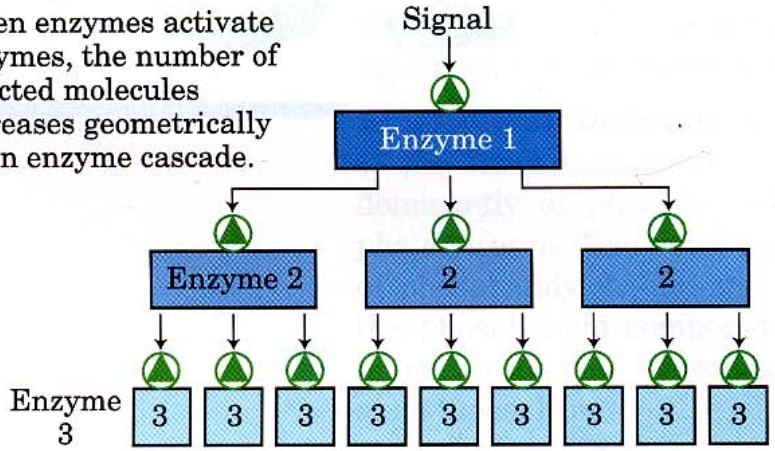
(a) Specificity
Signal molecule fits binding site on its complementary receptor; other signals do not fit.



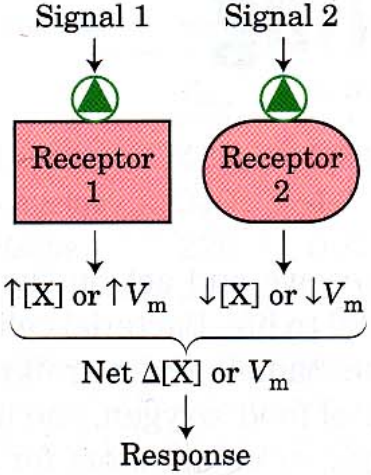
(c) Desensitization/Adaptation
Receptor activation triggers a feedback circuit that shuts off the receptor or removes it from the cell surface.



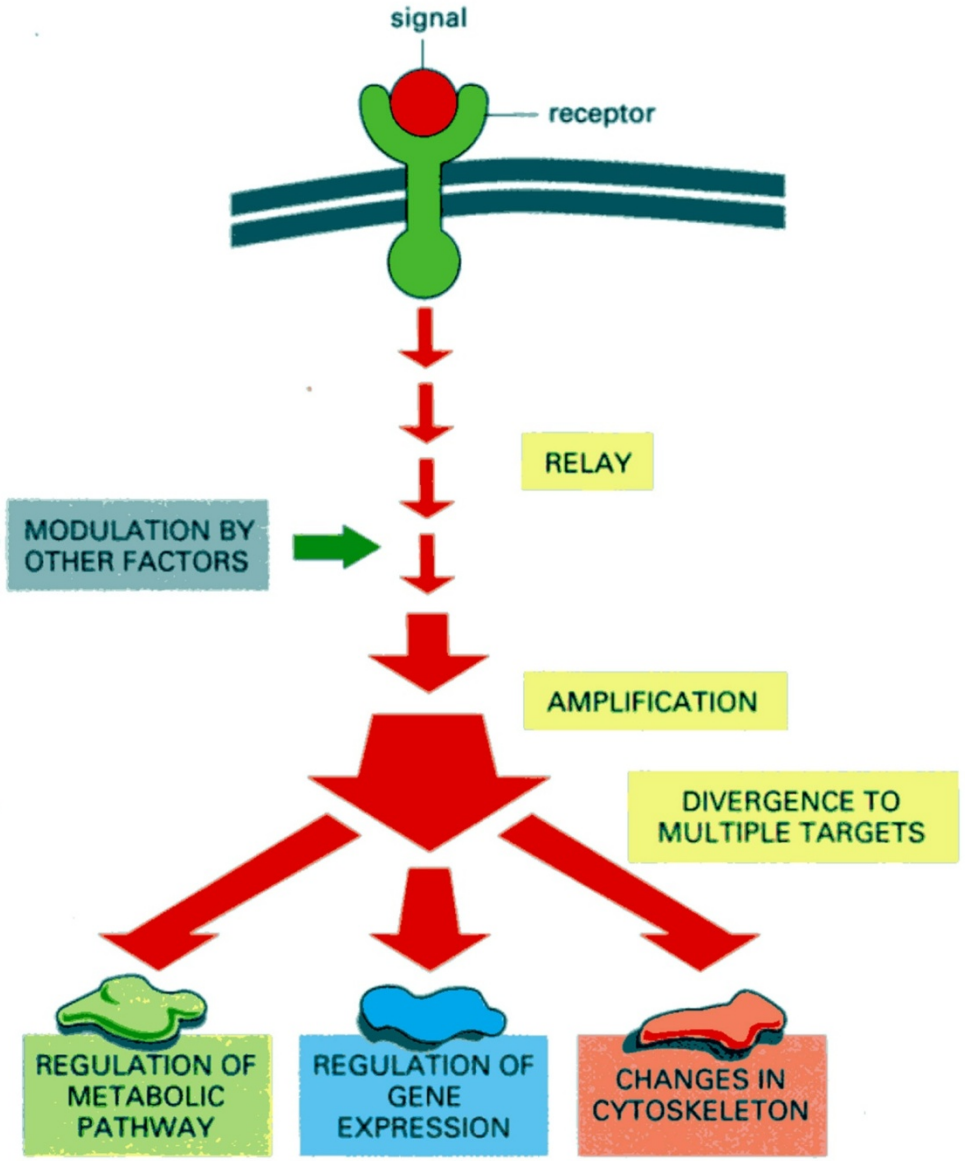
(b) Amplification
When enzymes activate enzymes, the number of affected molecules increases geometrically in an enzyme cascade.



(d) Integration
When two signals have opposite effects on a metabolic characteristic such as the concentration of a second messenger X, or the membrane potential V_m, the regulatory outcome results from the integrated input from both receptors.



Principals of Cell Signaling



How are signals transduced?

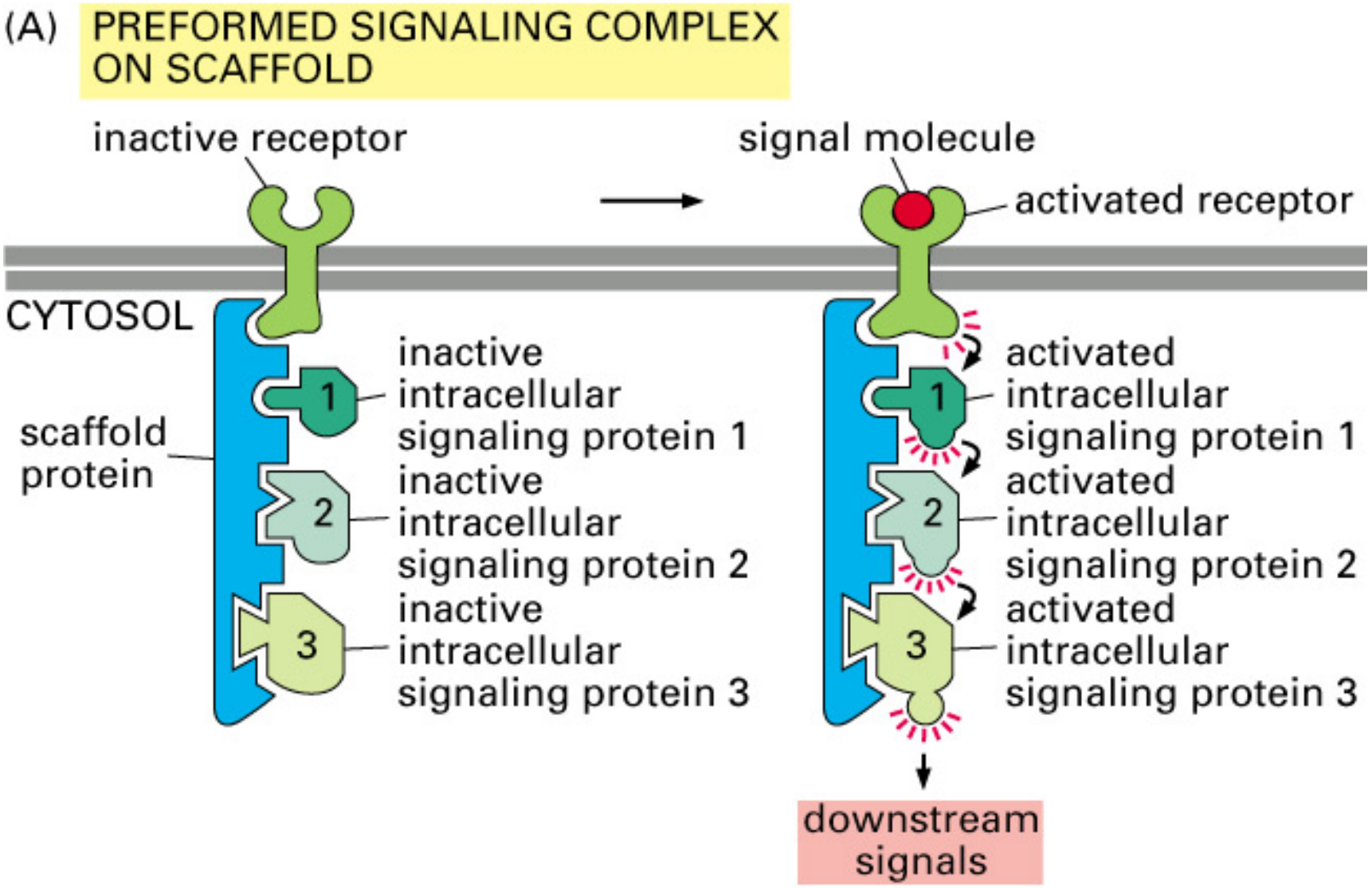


Figure 15–19 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

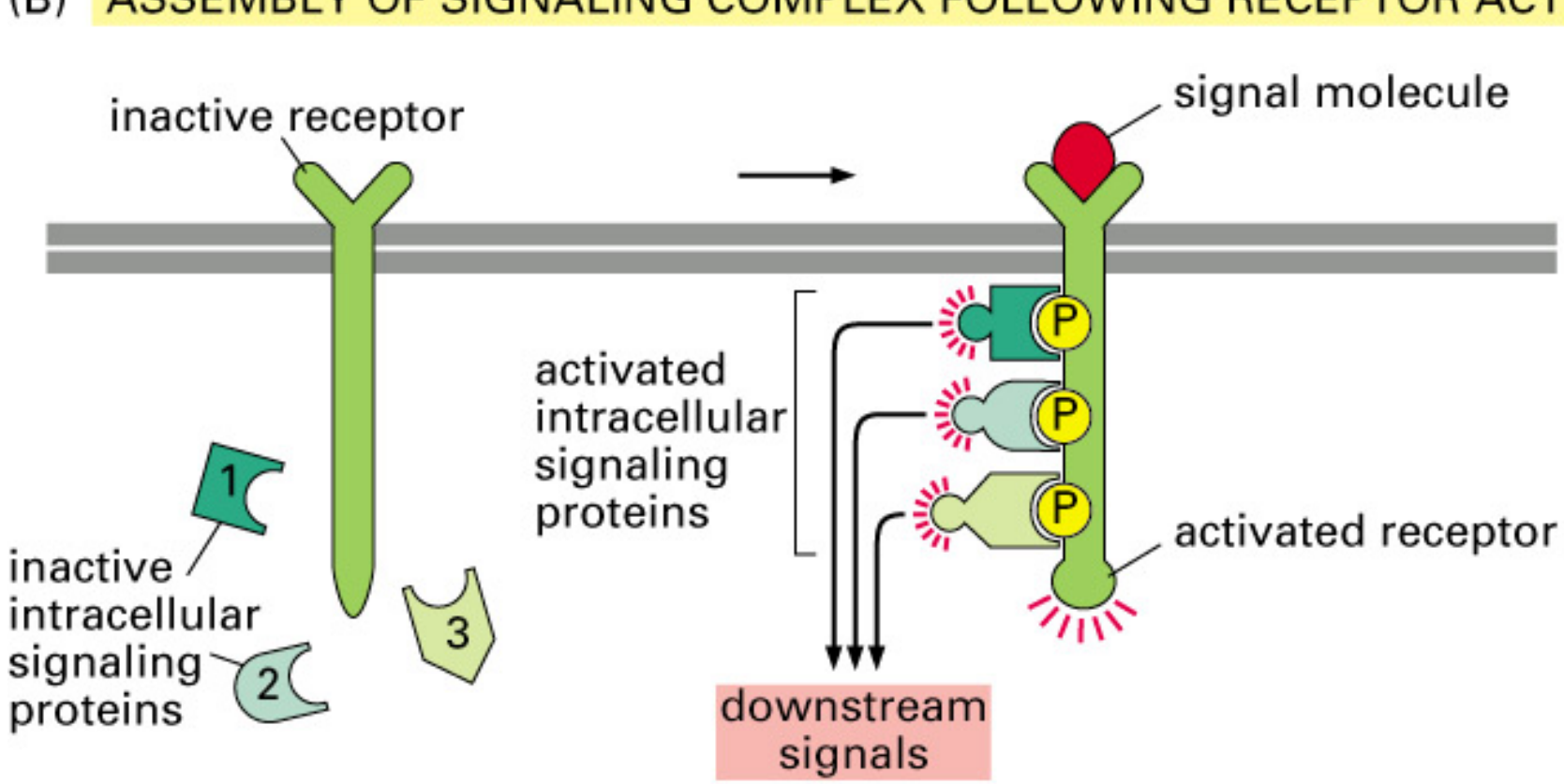
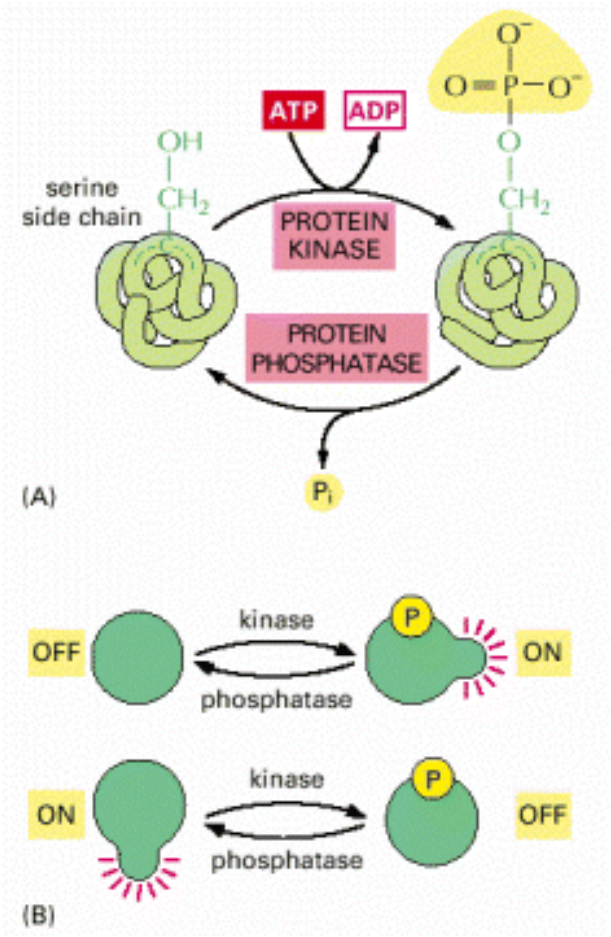


Figure 15–19 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Protein phosphorylation is a common modification to initiate signaling cascades

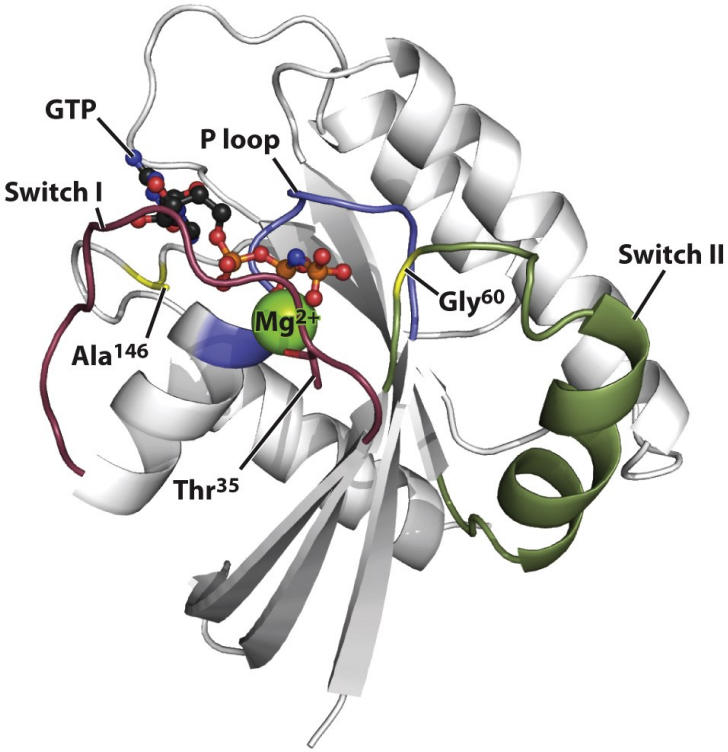
Serine, threonine, or tyrosine residues are phosphorylated by the addition of a PO4 from ATP



Serine, threonine, or tyrosine residues are phosphorylated by addition of a phosphate group to the hydroxyl group.

This change in charge and confirmation changes protein activity.

G-proteins are activated by the addition of a GTP molecule



Box 12-2 figure 2
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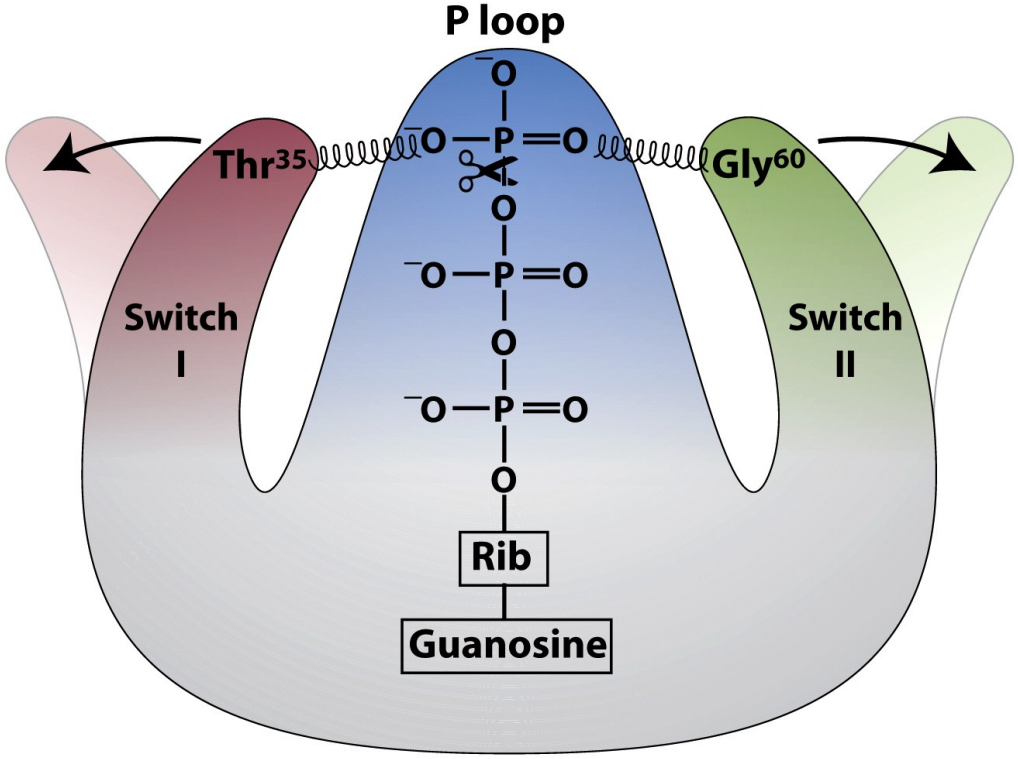

Box 12-2 figure 3
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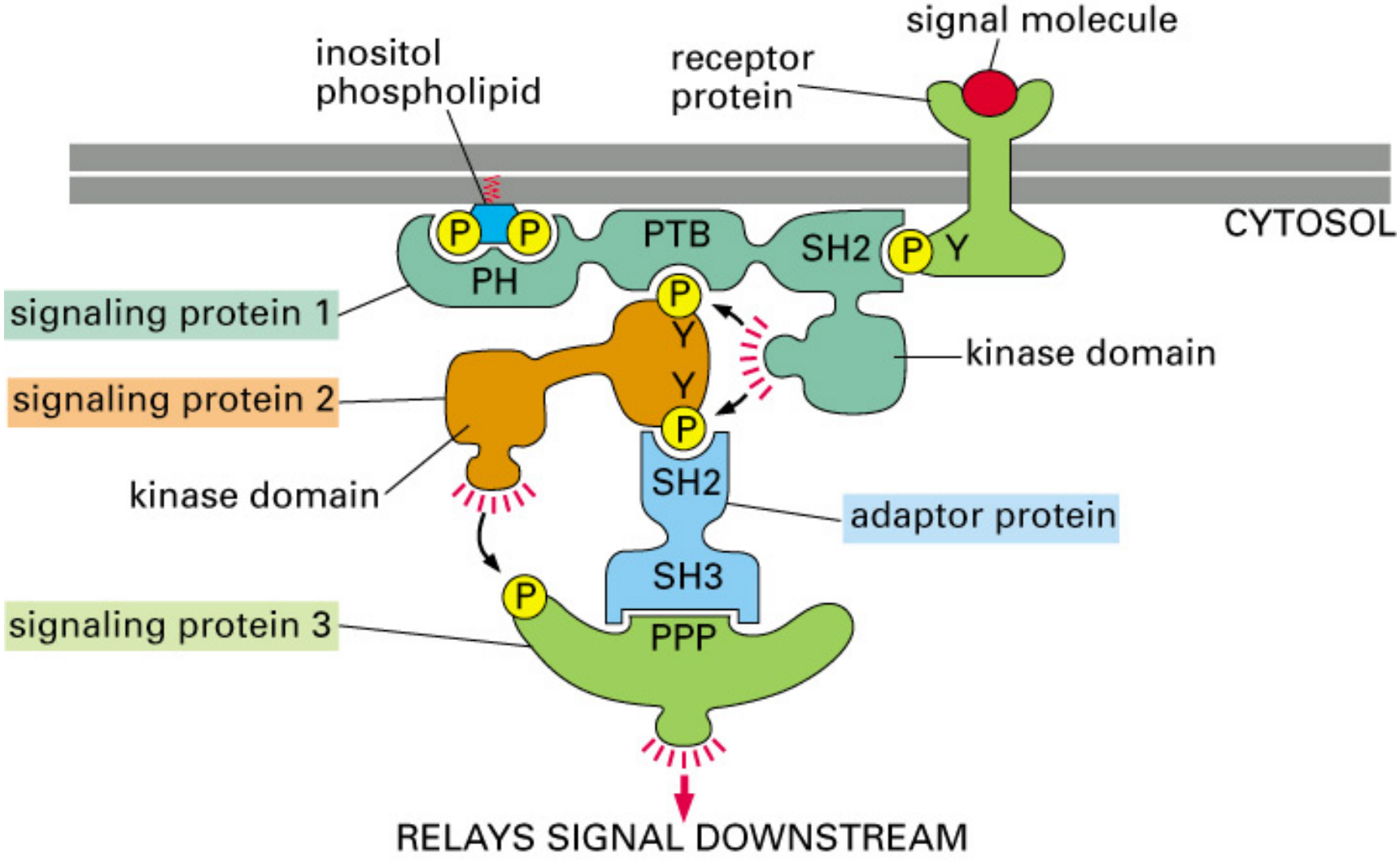
Signals are relayed by activated receptors signaling to downstream molecules via protein-protein interactions



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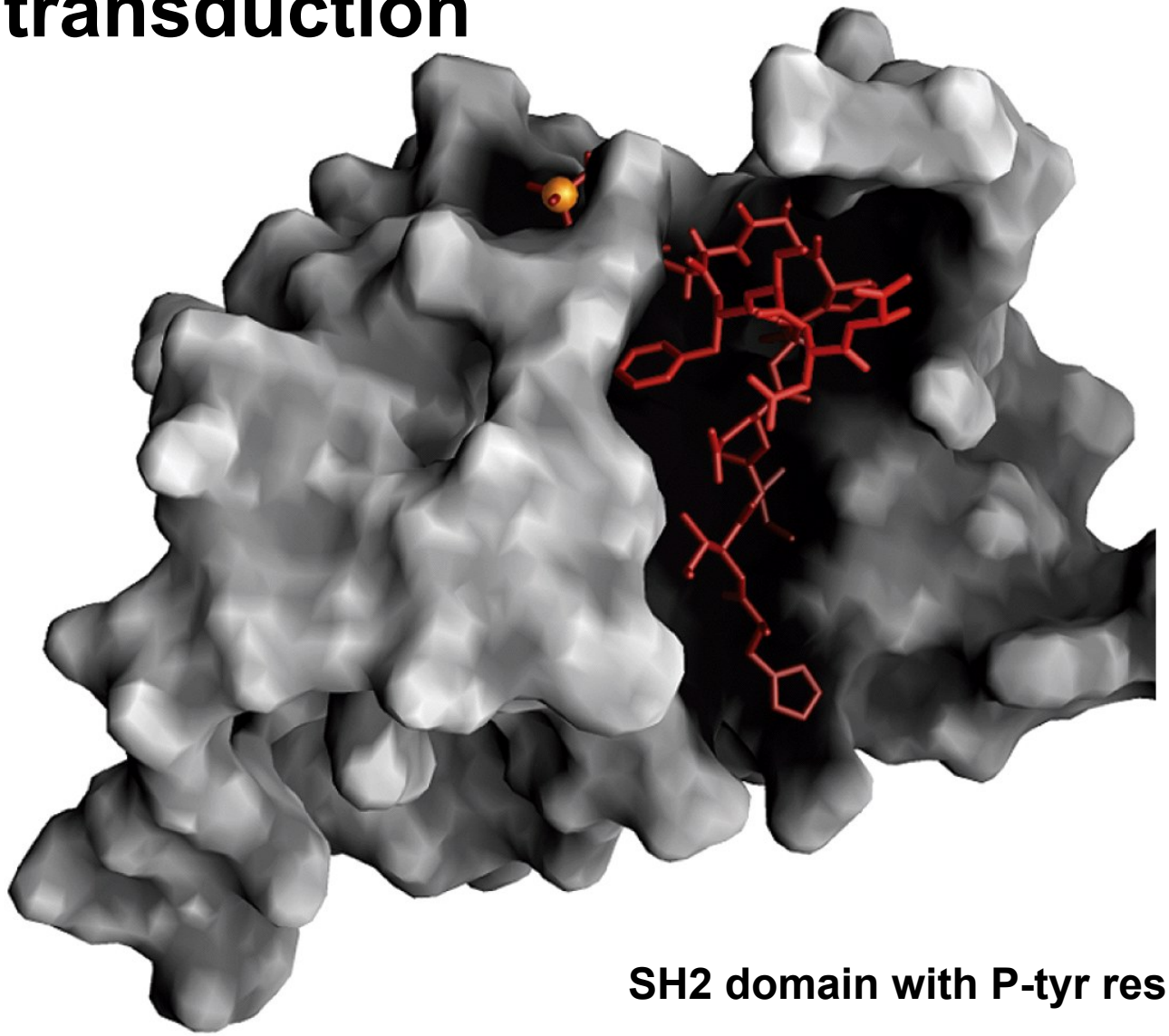
Protein-protein interactions mediate signal transduction



SH2 domain with P-tyr residue

Figure 12-21
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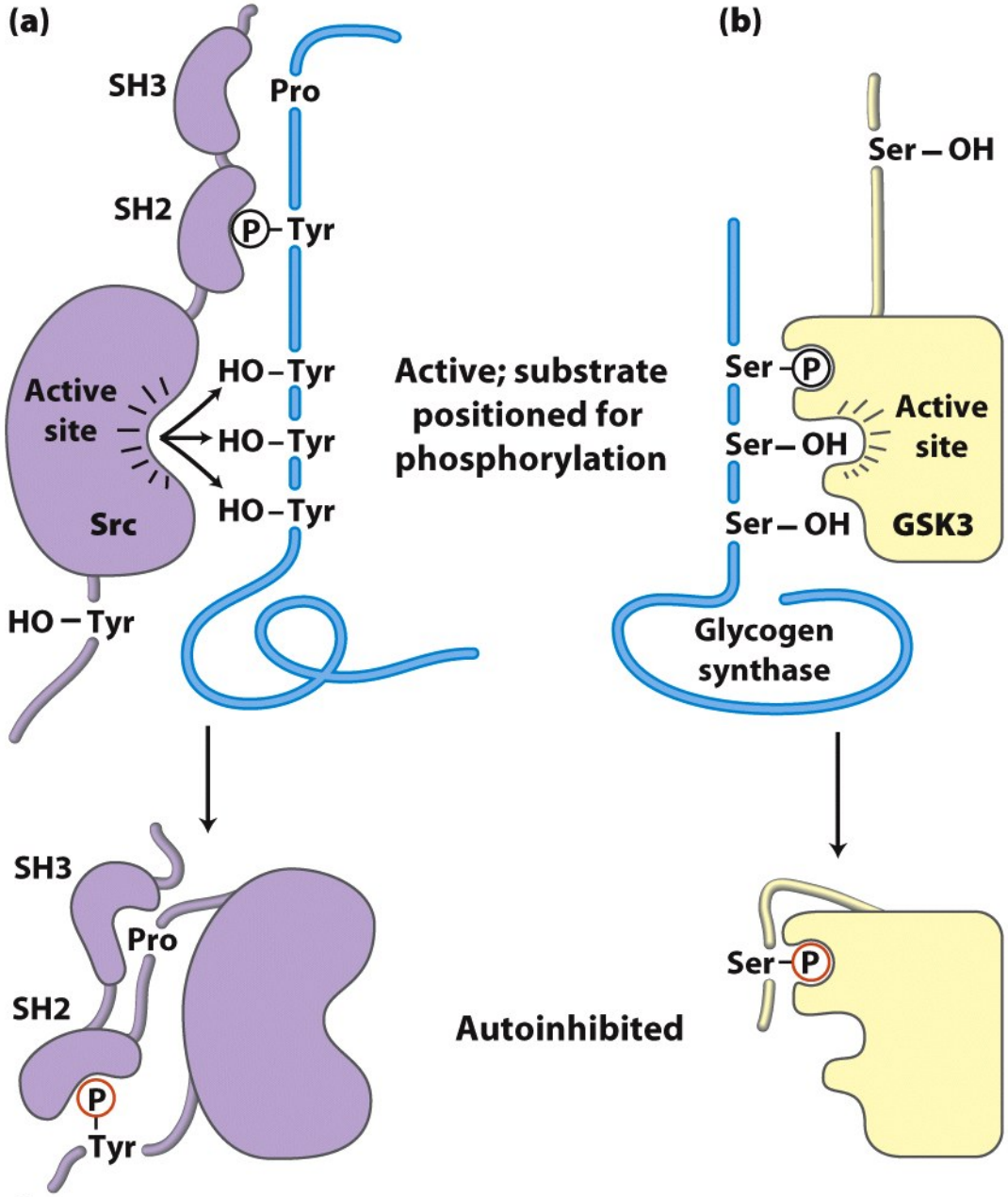

Figure 12-22
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Protein phosphorylation can activate or inactivate kinases

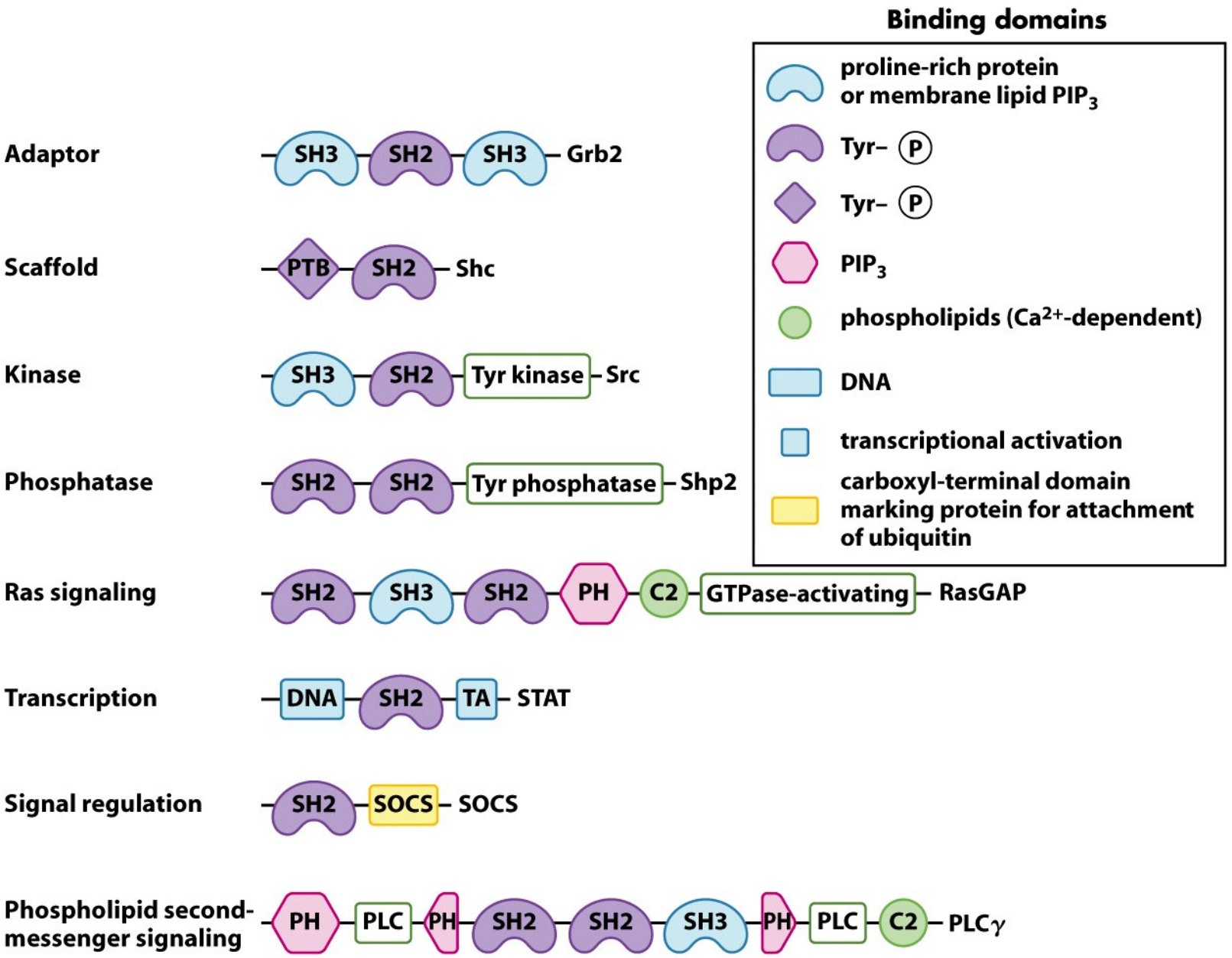

Figure 12-23

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Chemokines in Periodontal Disease

◆ Chemokines are a large family of chemotactic cytokines that stimulate the recruitment of inflammatory cells.

◆ Chemokines are produced by a number of cell types in the periodontium, such as fibroblasts, endothelial cells, macrophages, osteoclasts, epithelial cells, polymorphonuclear leukocytes, monocytes, lymphocytes, and mast cells.

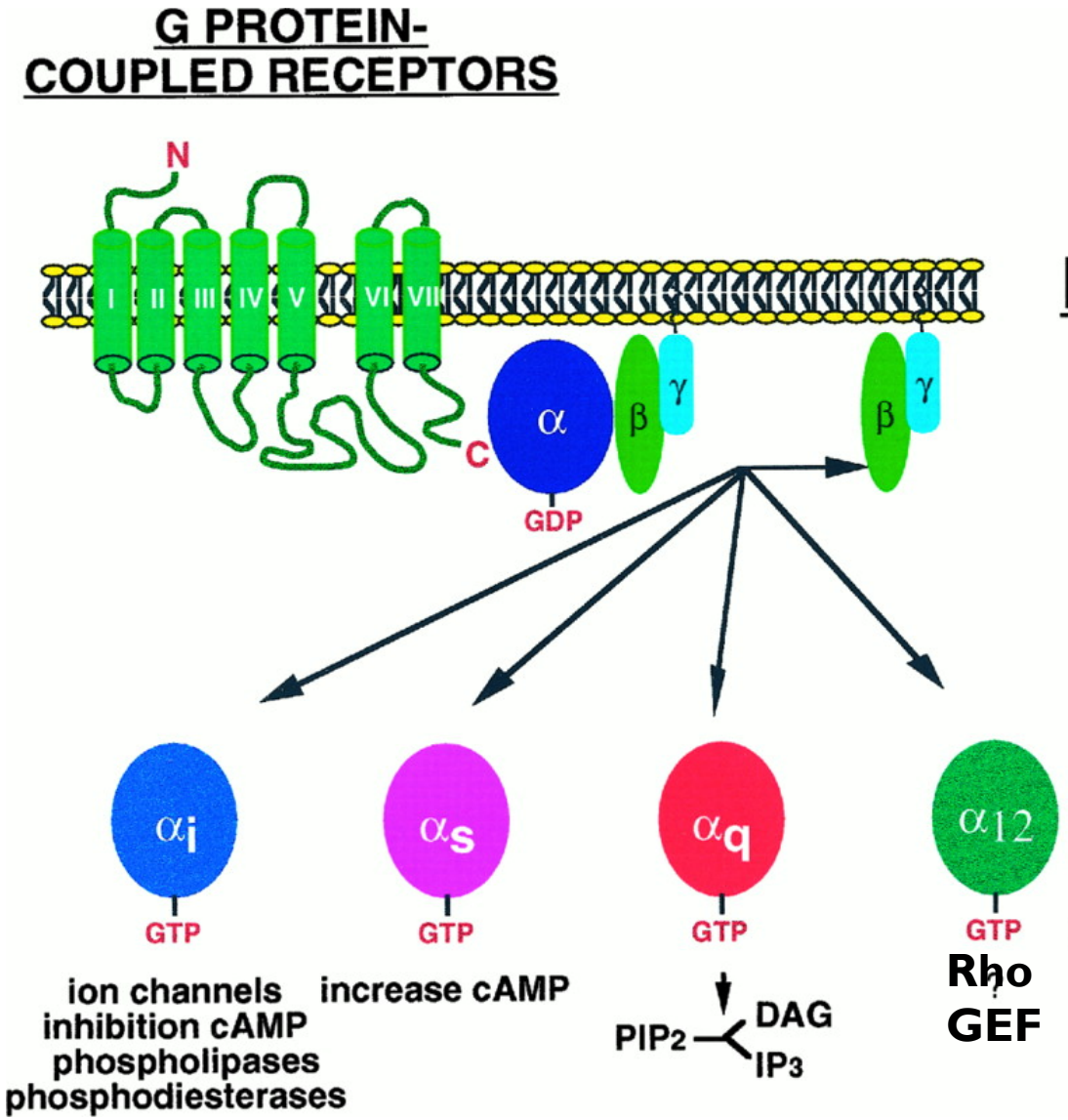
◆ Chemokines are divided into two major families based on the structure of the ligand; they are referred to as CC and CXC chemokines. Their receptors are: CC chemokine receptor (CCR) and CXC chemokine receptor (CXCR).

◆ Some chemokines contribute to inflammation-induced bone resorption and stimulate the recruitment, differentiation, or fusion of precursor cells to form osteoclasts or enhance osteoclast survival.

◆ Chemokines could also affect periodontal bone loss by their role in recruiting cells, such as neutrophils, that protect against bacterial invasion.

◆ **Chemokine receptors are G protein coupled receptors.**

Heptahelical (Serpentine) receptors

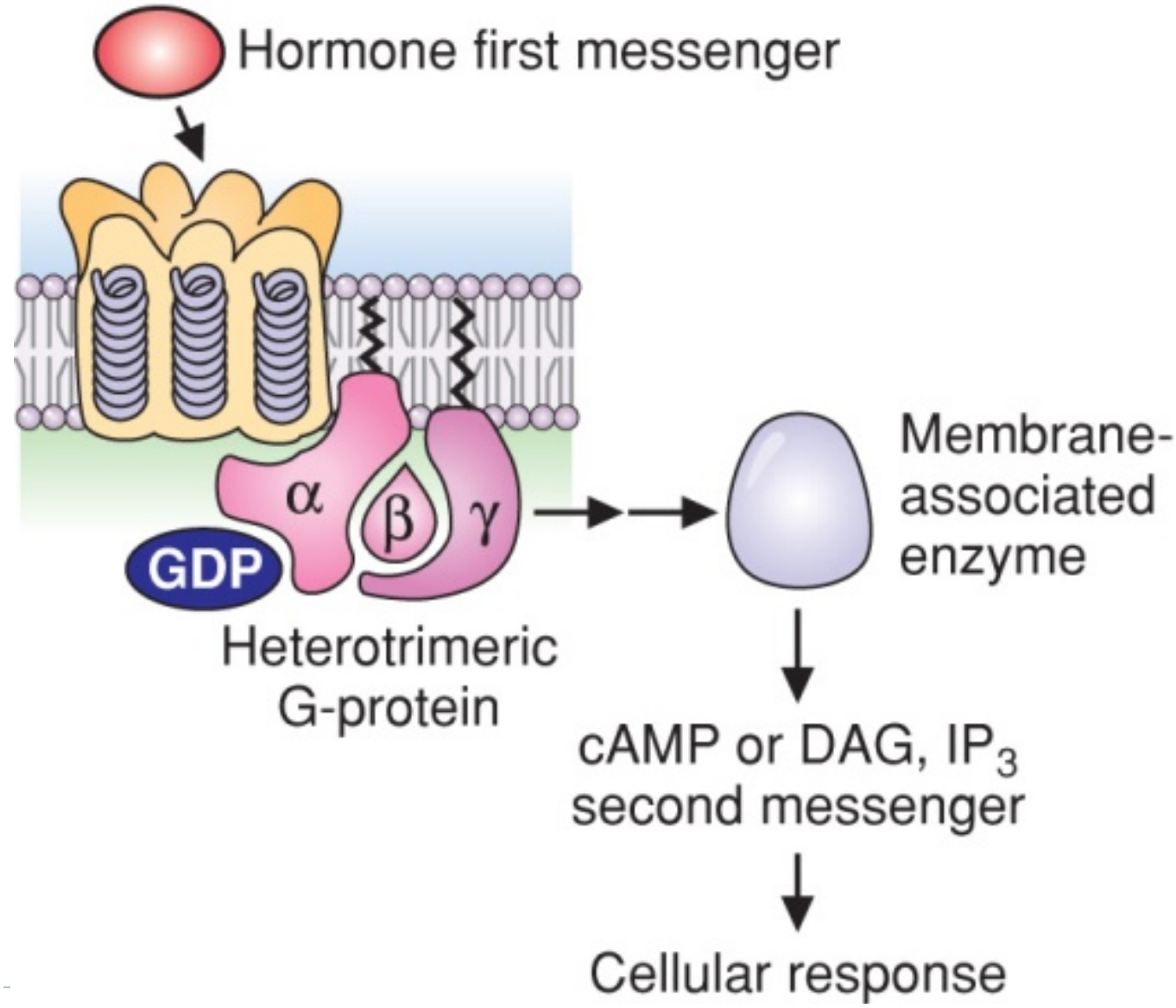


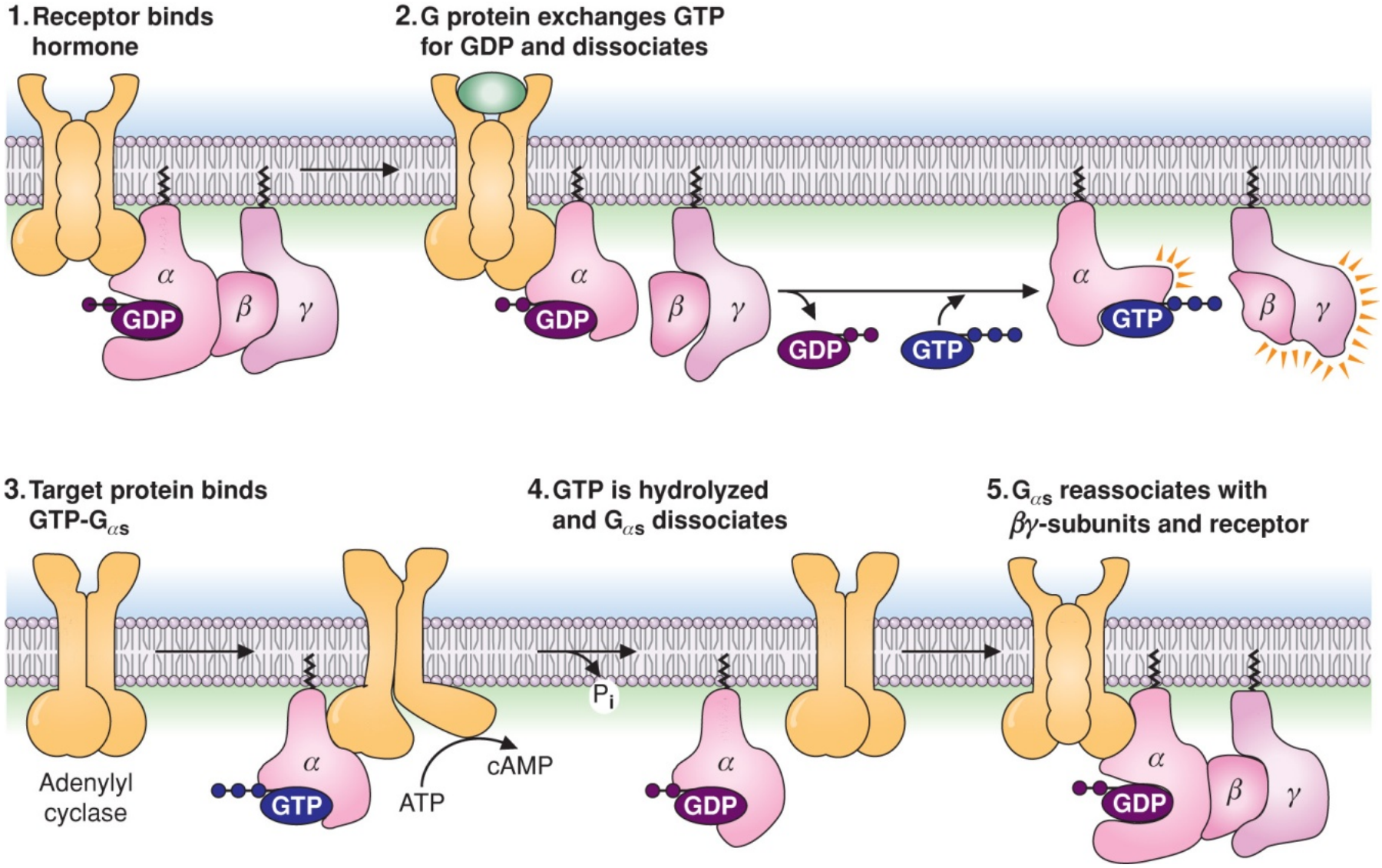
Biological functions

smell and taste
(~1000 types of receptors)
perception of light
neurotransmission
function of endocrine
and exocrine glands
chemotaxis
exocytosis
control of blood pressure
embryogenesis
development
cell growth and differentiation
HIV infection
oncogenesis

Gutkind, S. 1998. JBC 23:1839-1842.

Heptahelical receptors




1. Receptor binds hormone
2. G protein exchanges GTP for GDP and dissociates
α
β
γ
GDP
GTP
3. Target protein binds GTP-$G_{\alpha s}$
4. GTP is hydrolyzed and $G_{\alpha s}$ dissociates
5. $G_{\alpha s}$ reassociates with $\beta\gamma$-subunits and receptor
Adenylyl cyclase
ATP
cAMP
P_i

β-adrenergic receptor signaling to adenylyl cyclase

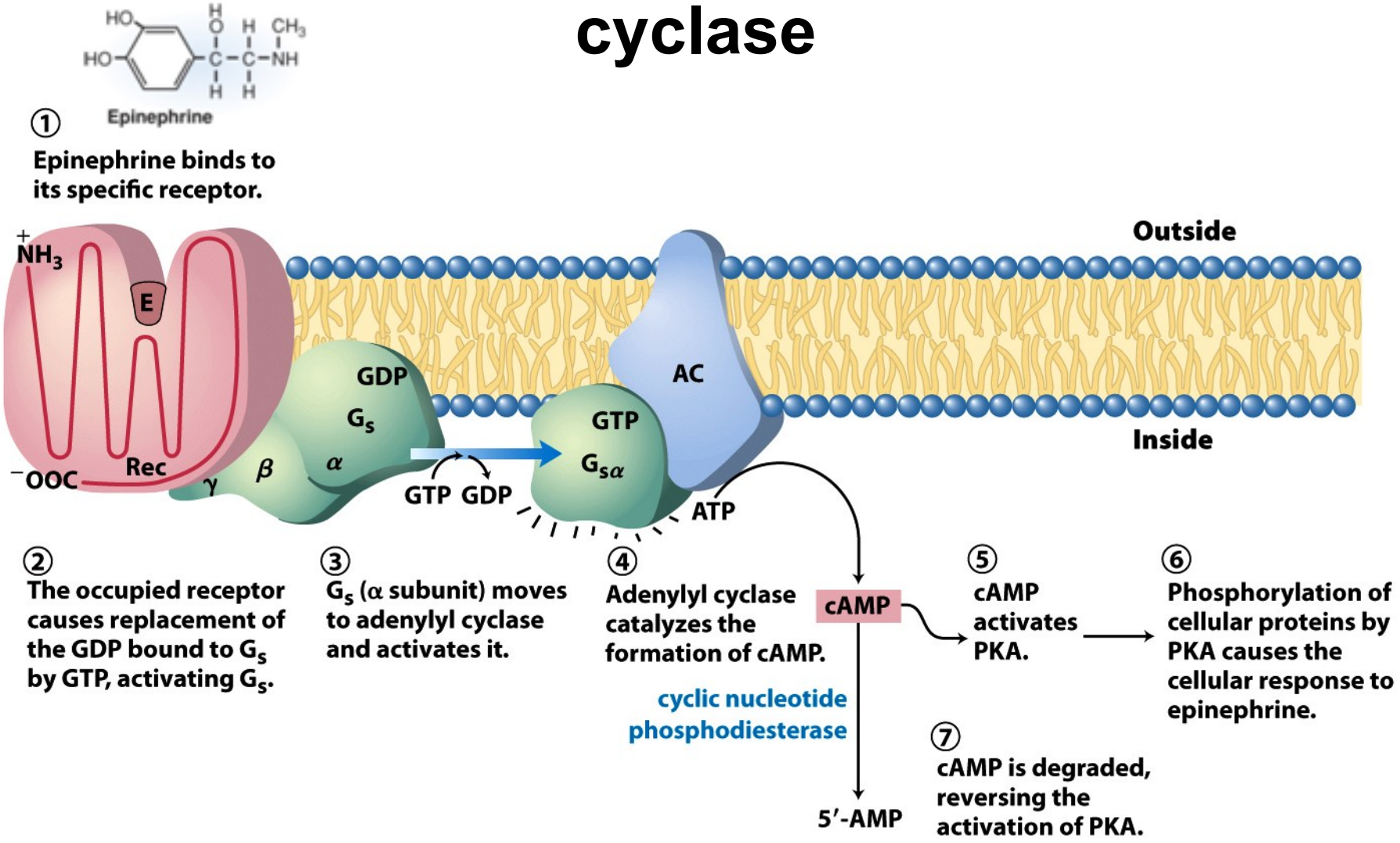


Figure 12-4a
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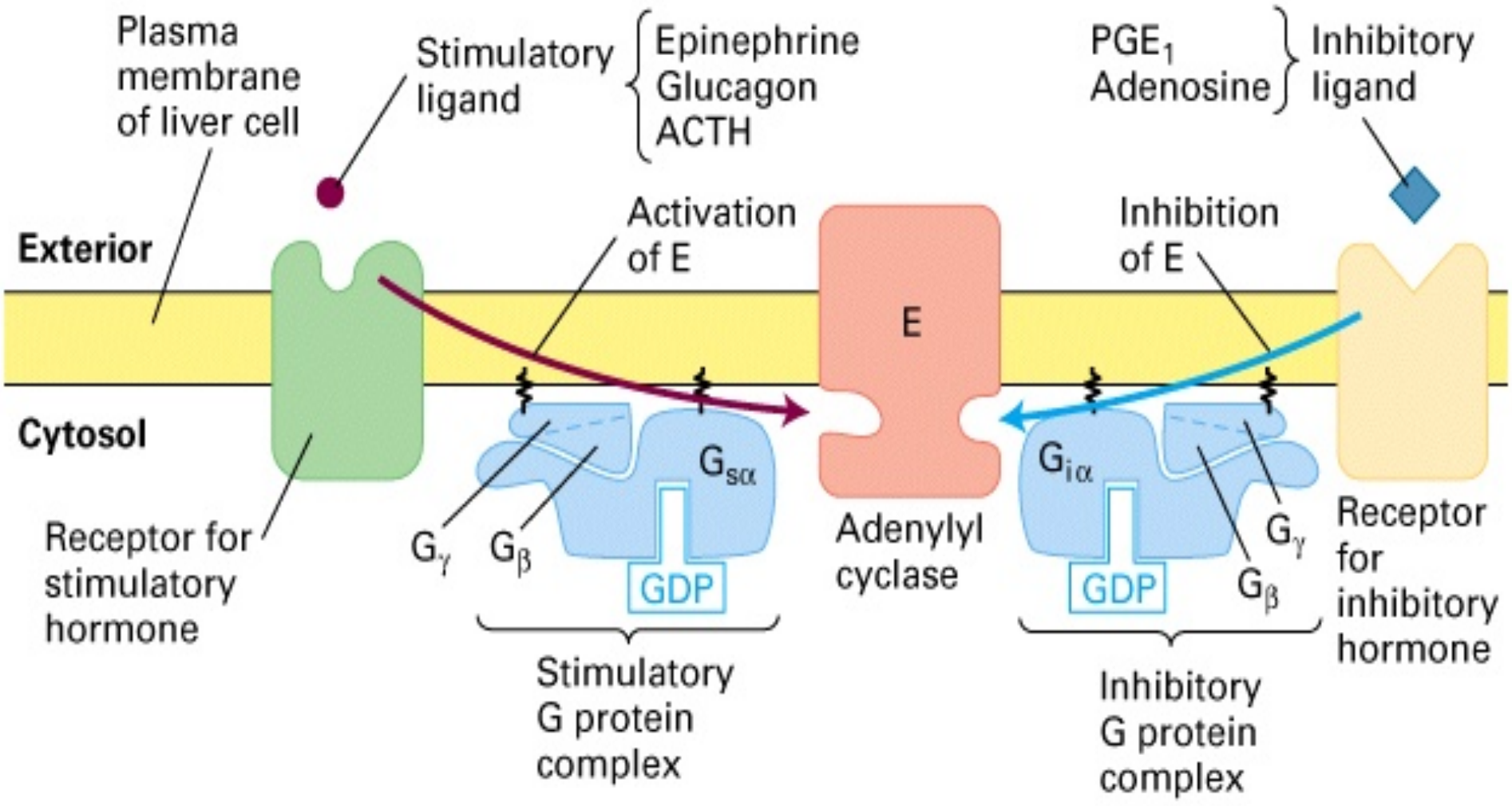
Some GPCRs stimulate Adenylyl cyclase through G_s
Other receptors inhibit Adenylyl cyclase through G_i



Activation of cAMP-dependent protein kinase (PKA)

AKAP, A kinase anchoring proteins

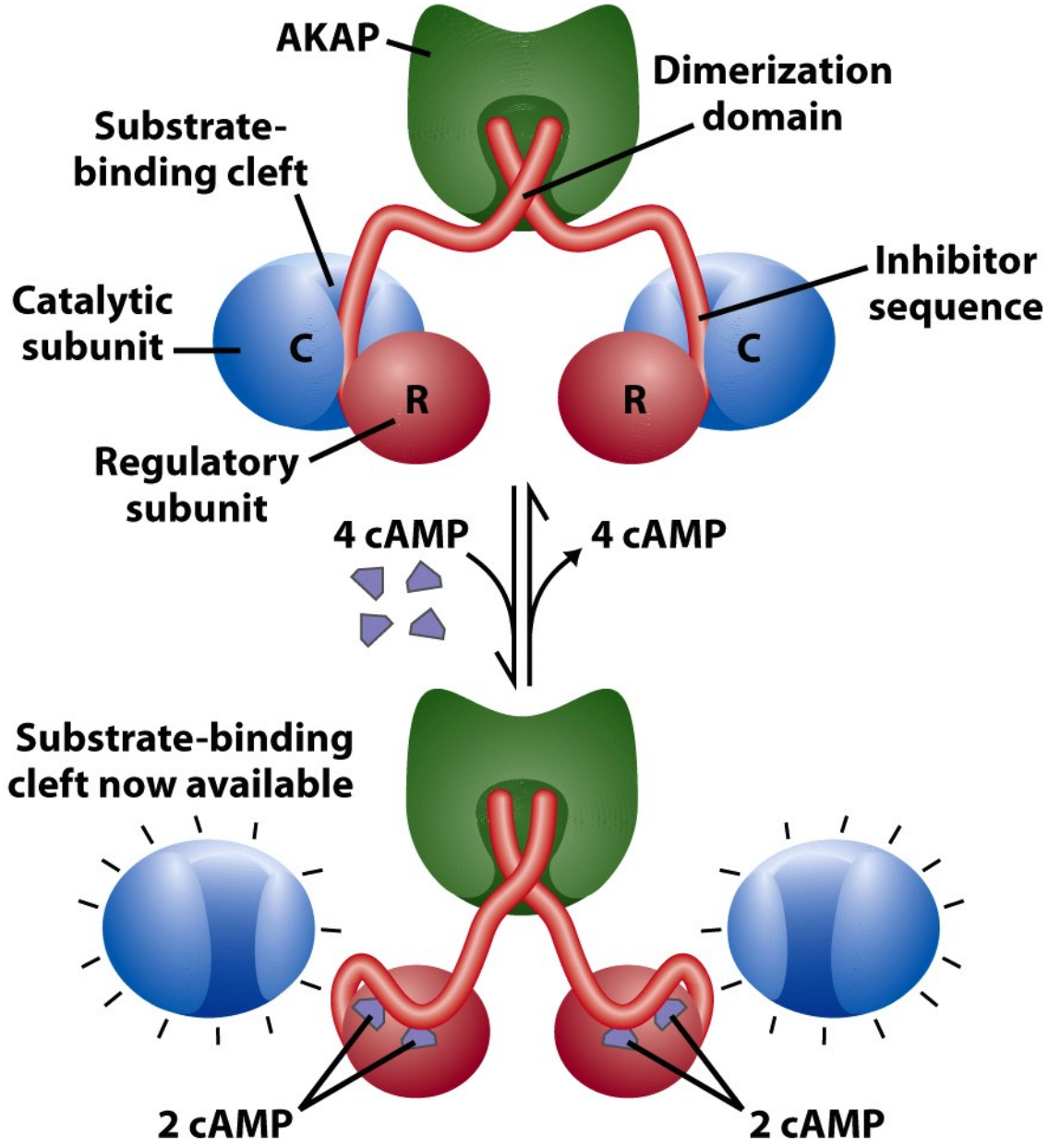


Figure 12-6a
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Activated PKA phosphorylates a number of proteins in serine and threonine residues

TABLE 12–2 Some Enzymes and Other Proteins Regulated by cAMP-Dependent Phosphorylation (by PKA)

Enzyme/protein	Sequence phosphorylated*	Pathway/process regulated
Glycogen synthase	RASCTSSS	Glycogen synthesis
Phosphorylase *b* kinase α subunit β subunit	 VEFRRLSI RTKRSGSV	Glycogen breakdown
Pyruvate kinase (rat liver)	GVLRRASVAZL	Glycolysis
Pyruvate dehydrogenase complex (type L)	GYLRRASV	Pyruvate to acetyl-CoA
Hormone-sensitive lipase	PMRRSV	Triacylglycerol mobilization and fatty acid oxidation
Phosphofructokinase-2/fructose 2,6-bisphosphatase	LQRRRGSSIPQ	Glycolysis/gluconeogenesis
Tyrosine hydroxylase	FIGRRQSL	Synthesis of L-dopa, dopamine, norepinephrine, and epinephrine
Histone H1	AKRKASGPPVS	DNA condensation
Histone H2B	KKAKASRKESYSVYVYK	DNA condensation
Cardiac phospholamban (cardiac pump regulator)	AIRRAST	Intracellular [Ca^{2+}]
Protein phosphatase-1 inhibitor-1	IRRRRPTP	Protein dephosphorylation
PKA consensus sequence†	xR[RK]x[ST]B	Many

*The phosphorylated S or T residue is shown in red. All residues are given as their one-letter abbreviations (see Table 3–1).

†x is any amino acid; B is any hydrophobic amino acid. See Box 3–3 for conventions used in displaying consensus sequences.

Table 12-2

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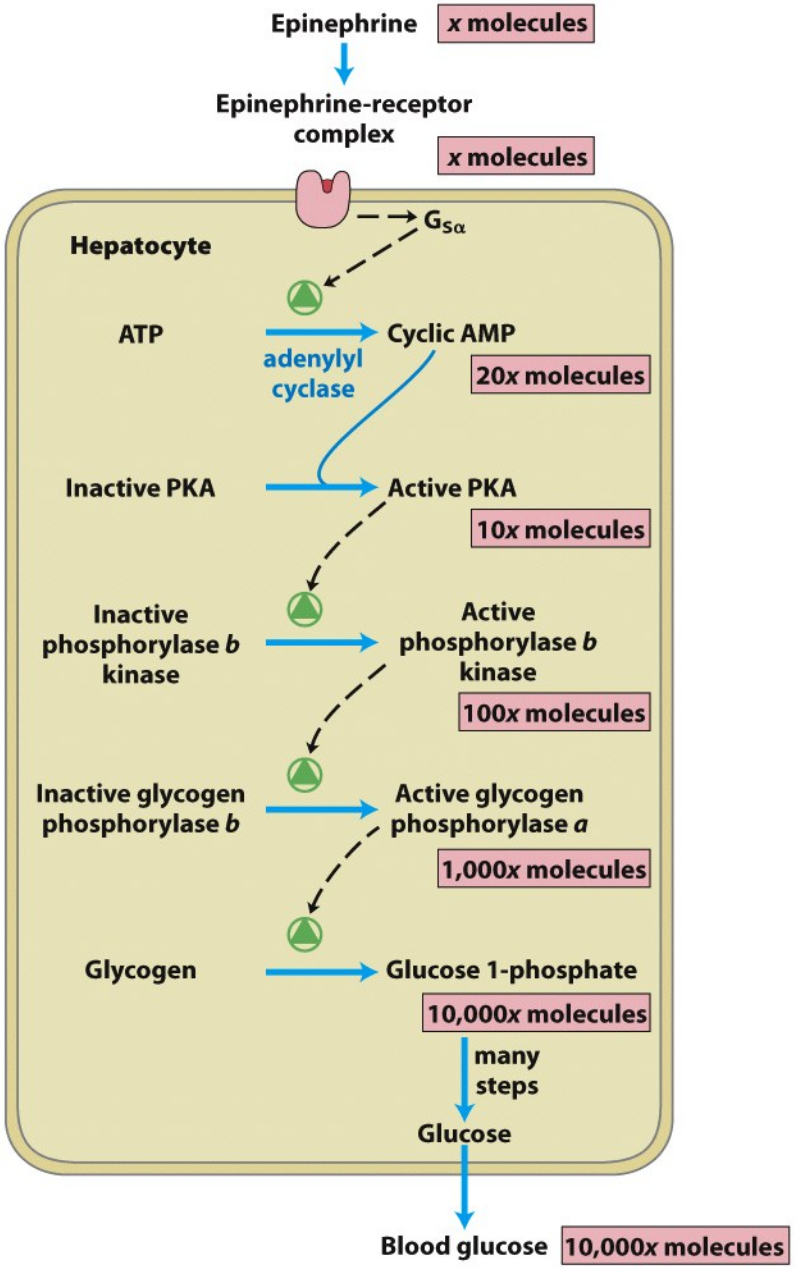

Elevation of blood glucose by epinephrine

Figure 12-7
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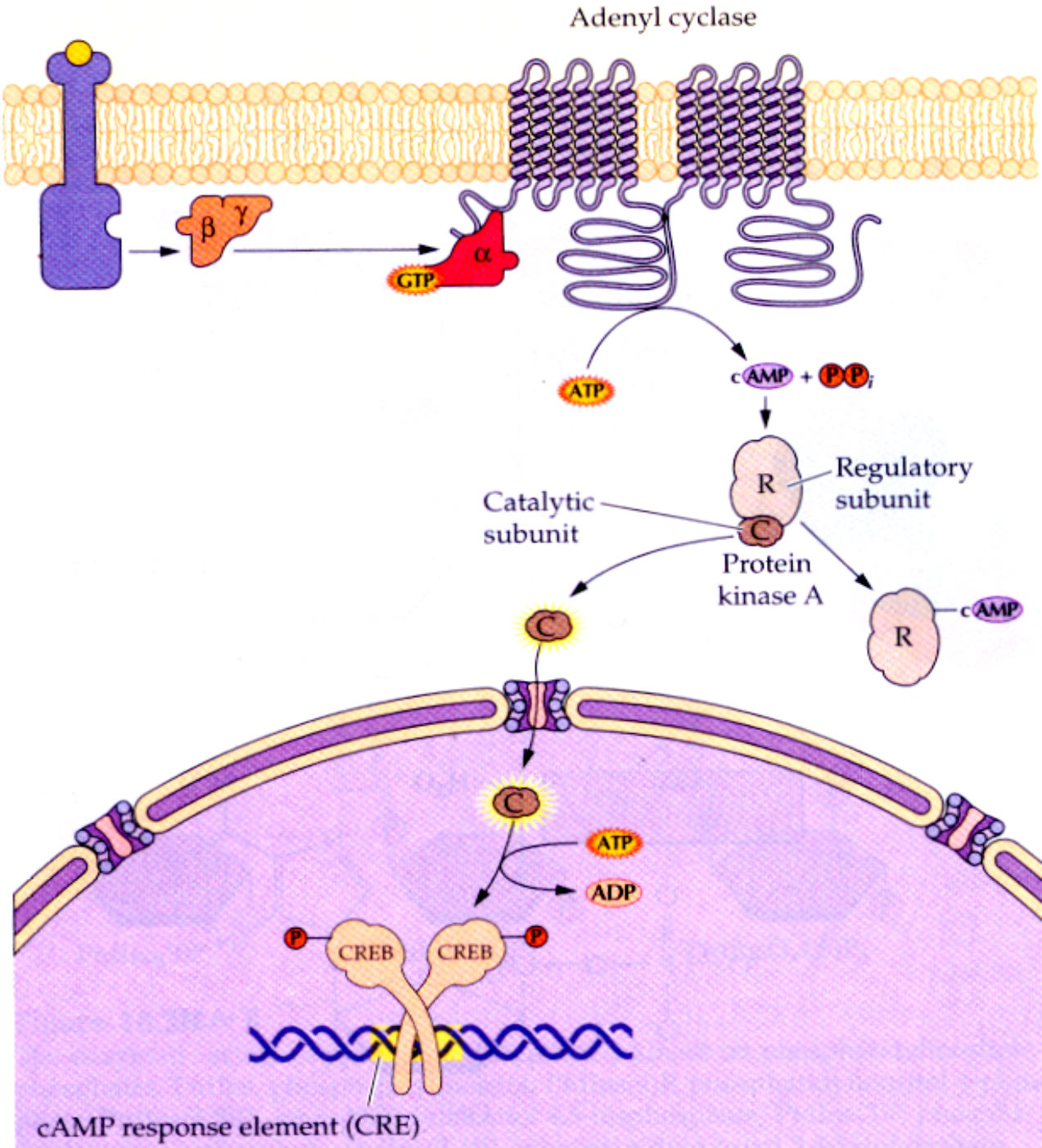
cAMP activates

Protein kinase A to phosphorylate

and activate cAMP response element binding (CREB) transcription factors.

cAMP response genes: c-fos, neurotrophin,

Somatostatin, corticotropin releasing hormone



β-adrenergic receptor is desensitized by phosphorylation and association with arrestin

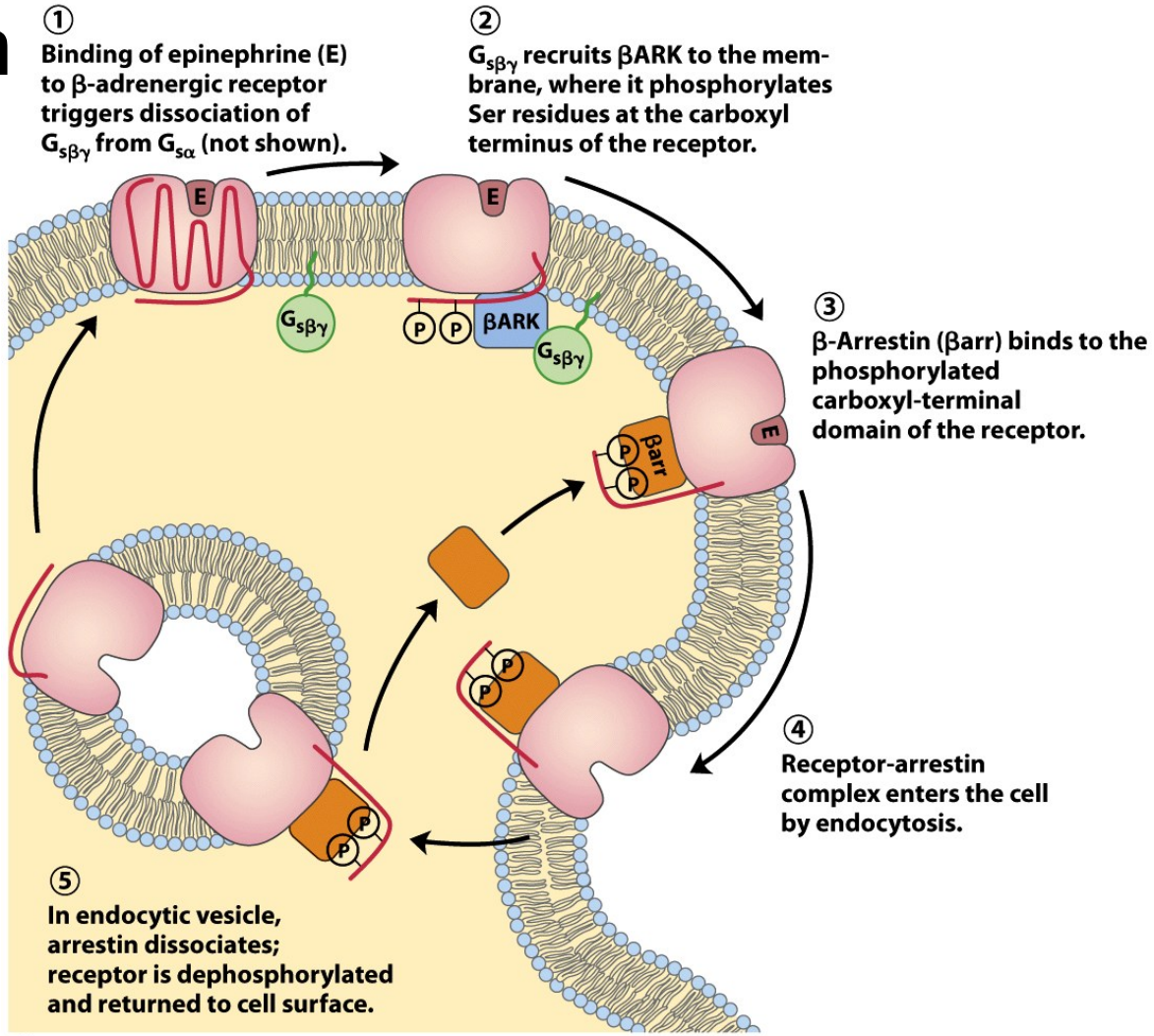


Figure 12-8
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cAMP synthesis and degradation

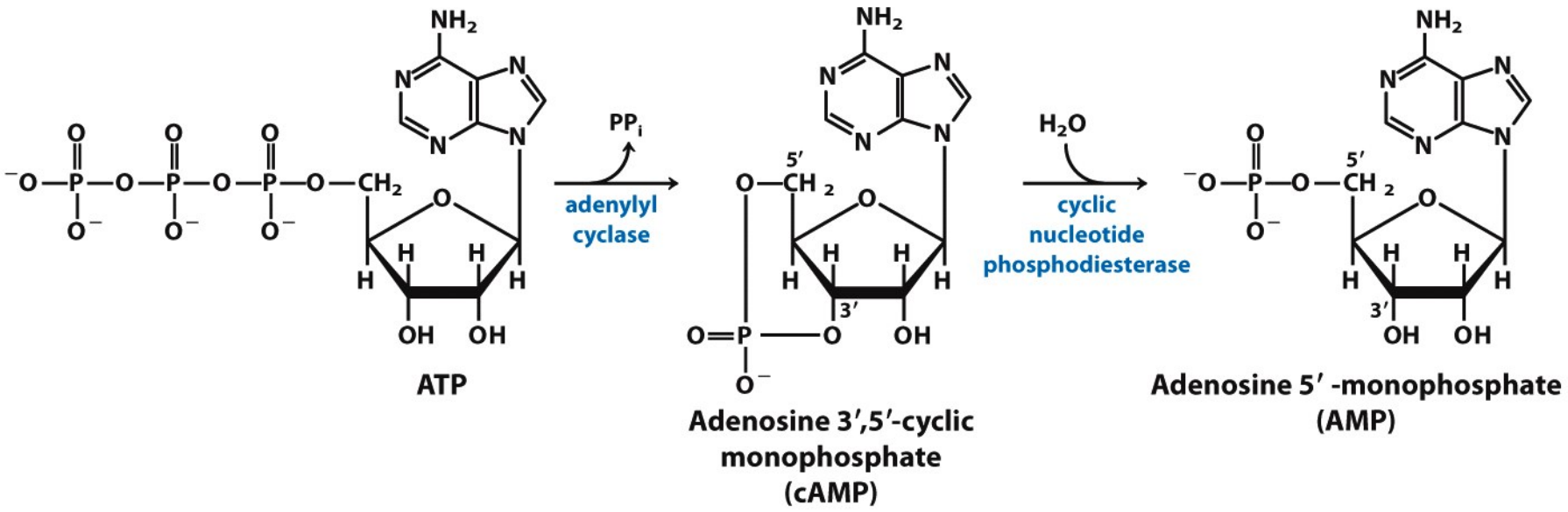

Figure 12-4b
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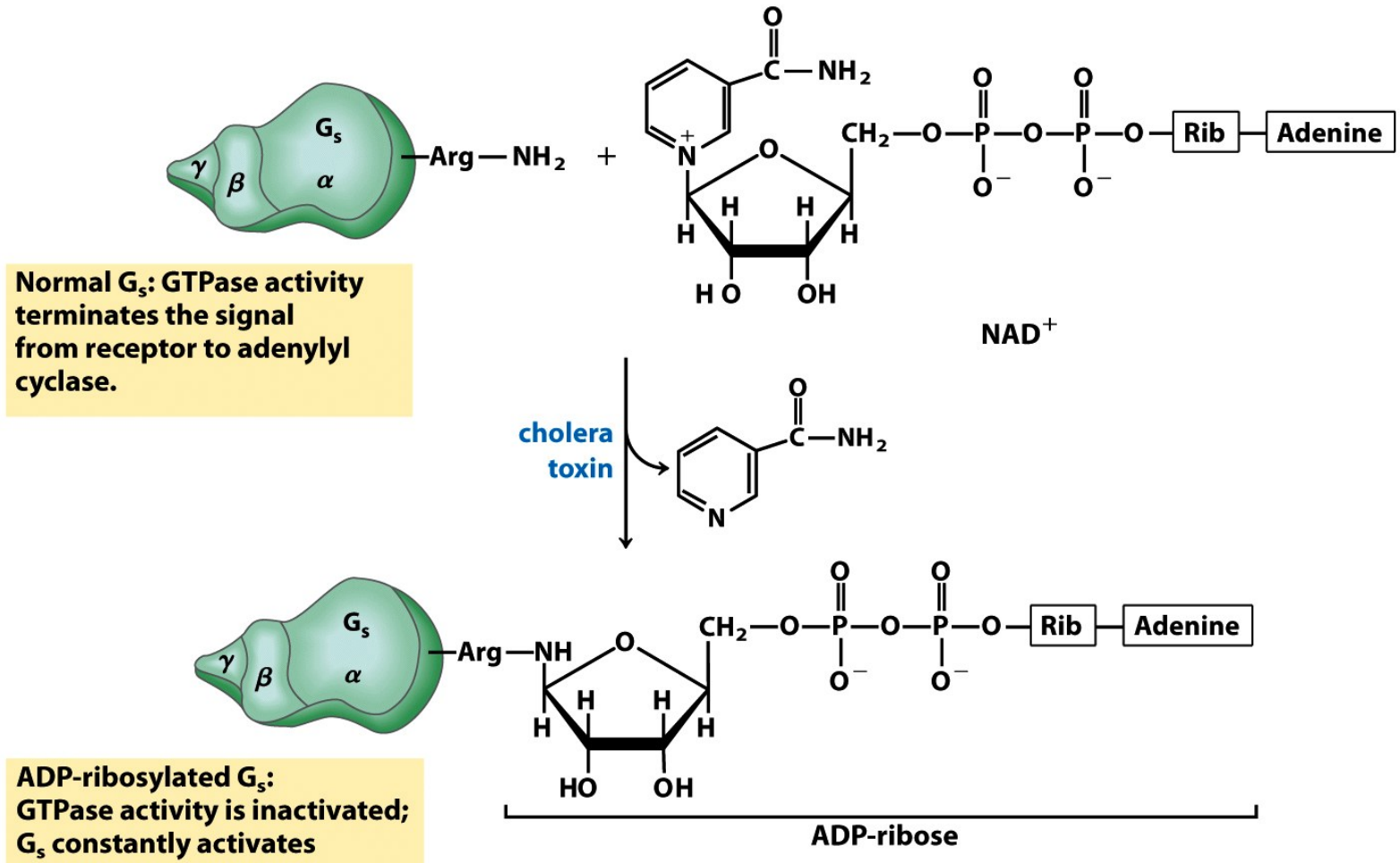
- cAMP is degraded by cAMP phosphodiesterase



Box 12-2 figure 5
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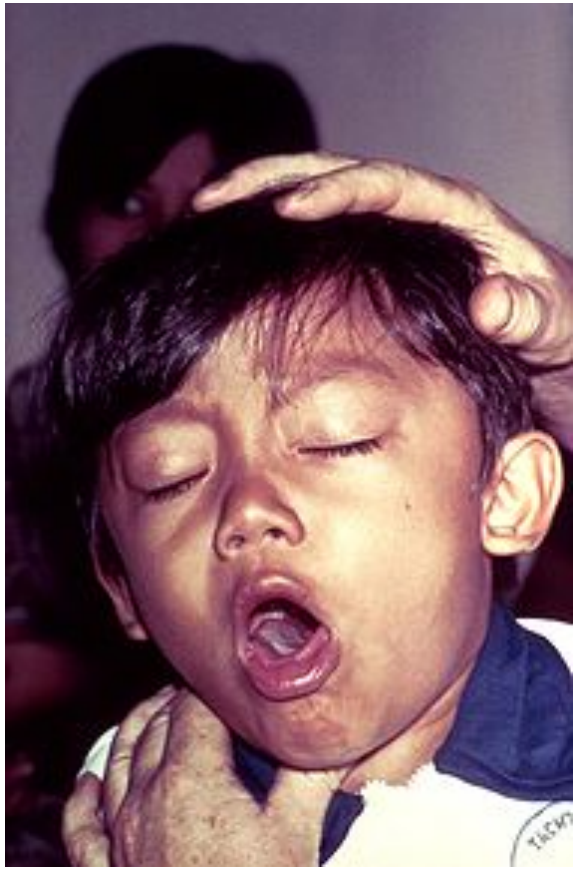
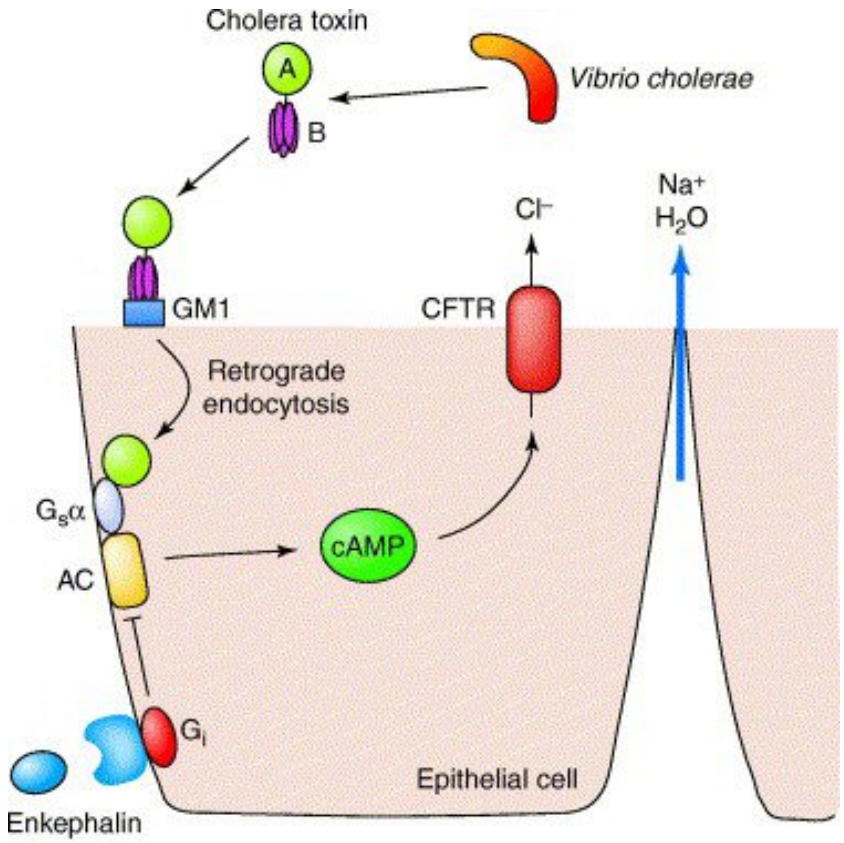
Bacterial toxins that cause cholera and whooping cough (pertussis) are enzymes that catalyze transfer of the ADP-ribose moiety of NAD+ to an Arg residue of Gs (cholera toxin) or Cys of Gi (pertussis toxin)

Whooping Cough



•**Pertussis**, also known as the **whooping cough**, is a highly contagious disease caused by the bacterium Borderella pertussis.
• It derived its name from the "whoop" sound made from the inspiration of air after a cough.
•**Pertussis toxin catalyzes ADP ribosylation of Gi subunit thus blocking the inhibition of adenylyl cyclase activity.**
•In an unclear mechanism, the ciliated epithelial cells in the respiratory tract that normally sweep away mucus are killed.
•Thus, vigorous coughing is needed to clear the track.
•Despite generally high coverage with the DTP and DTaP vaccines, pertussis is one of the leading causes of vaccine-preventable deaths world-wide.

Cholera Toxin



• Cholera toxin released from bacteria is absorbed into intestinal mucosal cells (enterocytes). Cholera toxin (A and B subunits) complexes with GM1 ganglioside receptor and gets internalized.

• Cholera toxin is a NAD-glycohydrolase that cleaves NAD and transfers the ADP ribose to Gαs to inhibit GTPase activity.

• Gαs is actively bound to AC (adenylyl cyclase) and increases cAMP production.

•The CFTR (cystic fibrosis transmembrane conductance regulator) channel is activated to result in secretion of Cl^- and Na^+ into the intestinal lumen.

•This ion secretion results in water loss leading to vomiting and diarrhea.

TABLE 12–3 Some Signals That Use cAMP as Second Messenger

Corticotropin (ACTH)
Corticotropin-releasing hormone (CRH)
Dopamine [D_1, D_2]
Epinephrine (β-adrenergic)
Follicle-stimulating hormone (FSH)
Glucagon
Histamine [H_2]
Luteinizing hormone (LH)
Melanocyte-stimulating hormone (MSH)
Odorants (many)
Parathyroid hormone
Prostaglandins E_1, E_2 (PGE_1, PGE_2)
Serotonin [5-HT-1a, 5-HT-2]
Somatostatin
Tastants (sweet, bitter)
Thyroid-stimulating hormone (TSH)

Note: Receptor subtypes in square brackets. Subtypes may have different transduction mechanisms. For example, serotonin is detected in some tissues by receptor subtypes 5-HT-1a and 5-HT-1b, which act through adenylyl cyclase and cAMP, and in other tissues by receptor subtype 5-HT-1c, acting through the phospholipase C–IP_3 mechanism (see Table 12–4).

Table 12-3

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Odorant receptors activate Adenylyl cyclase

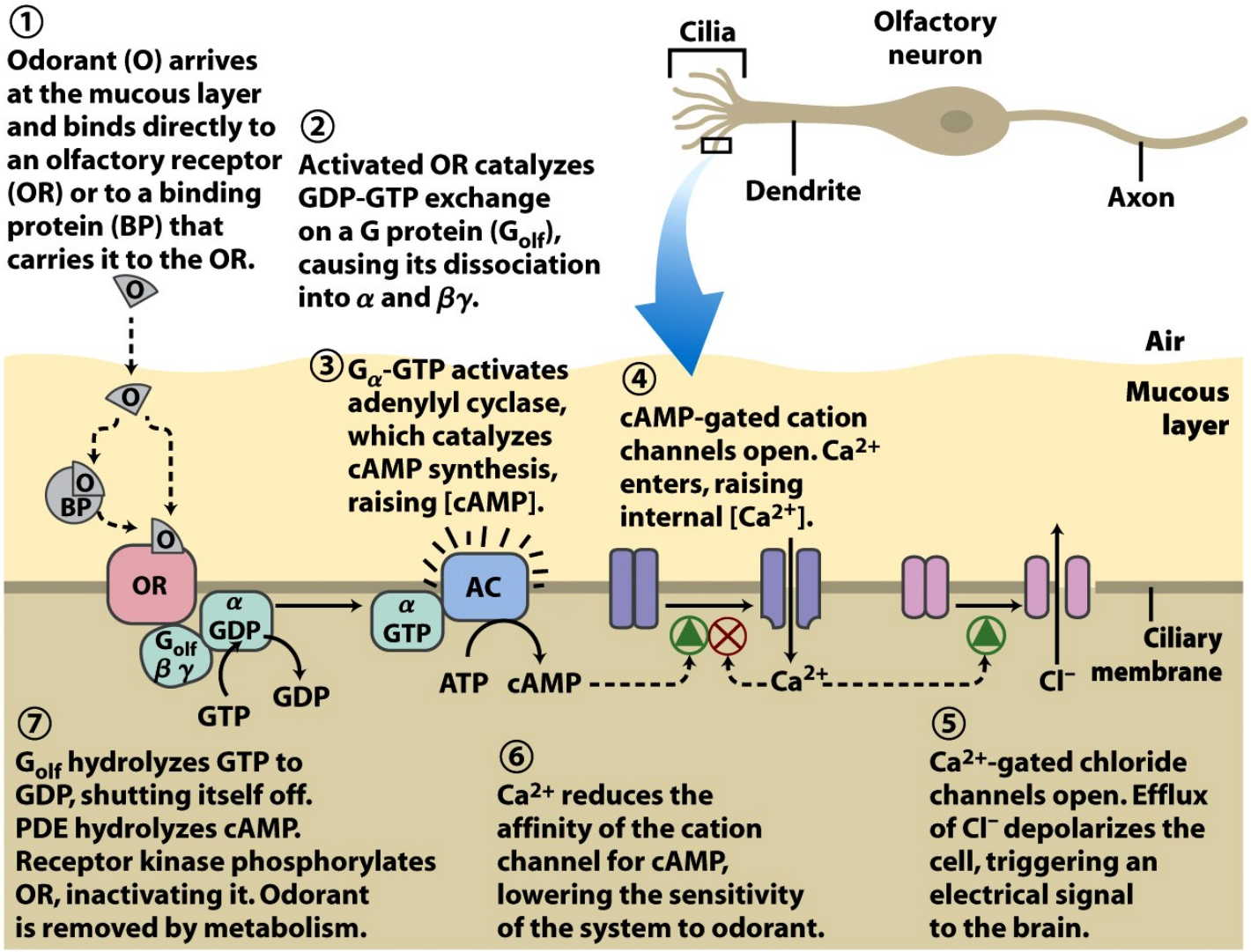

Figure 12-40
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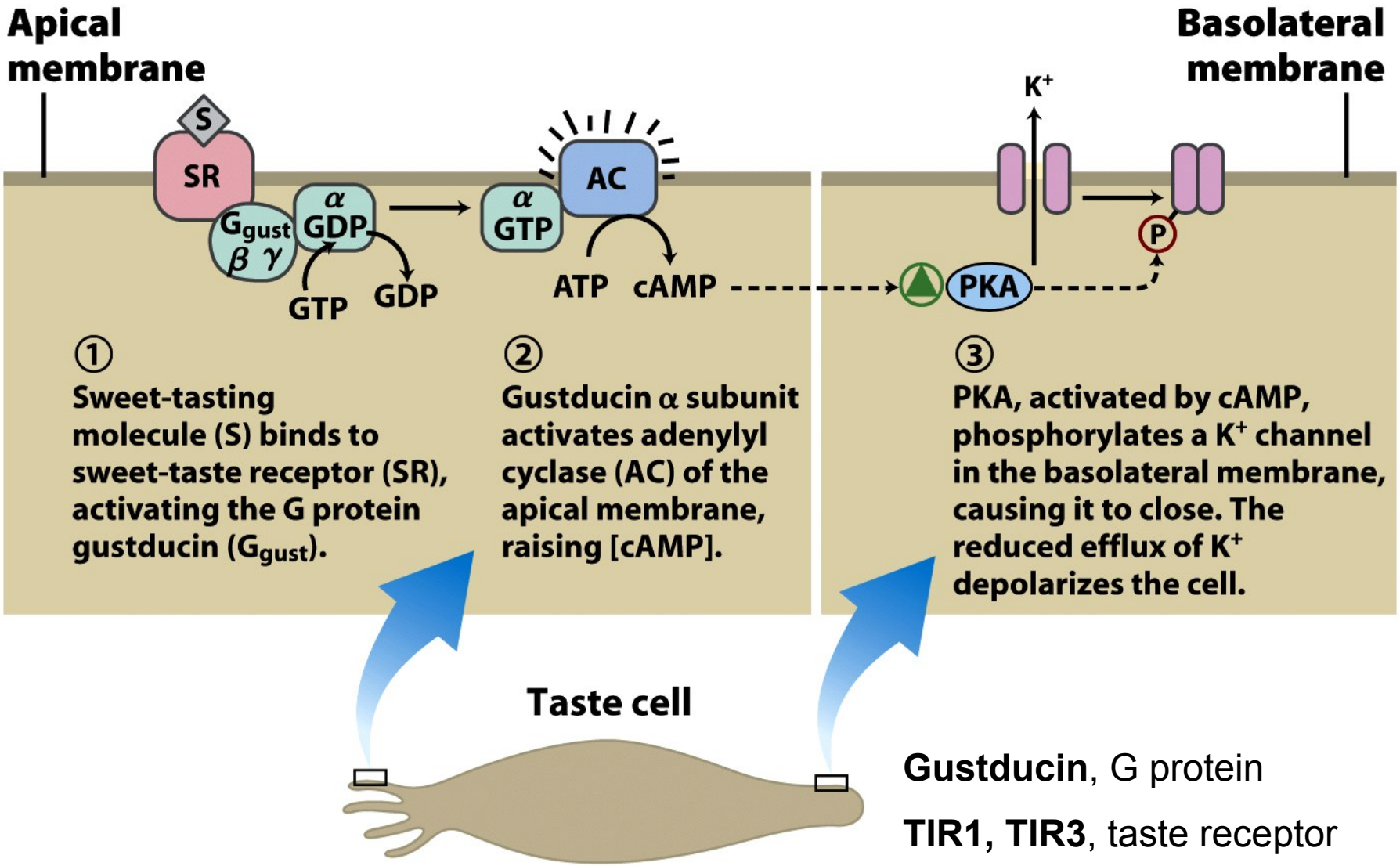
Smells are sensed by GPCR signaling

Taste receptors activate Adenylyl cyclase



Gustducin, G protein

TIR1, TIR3, taste receptor subunits

Figure 12-41
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Hormones, light, smells, and tastes are detected by GPCRs

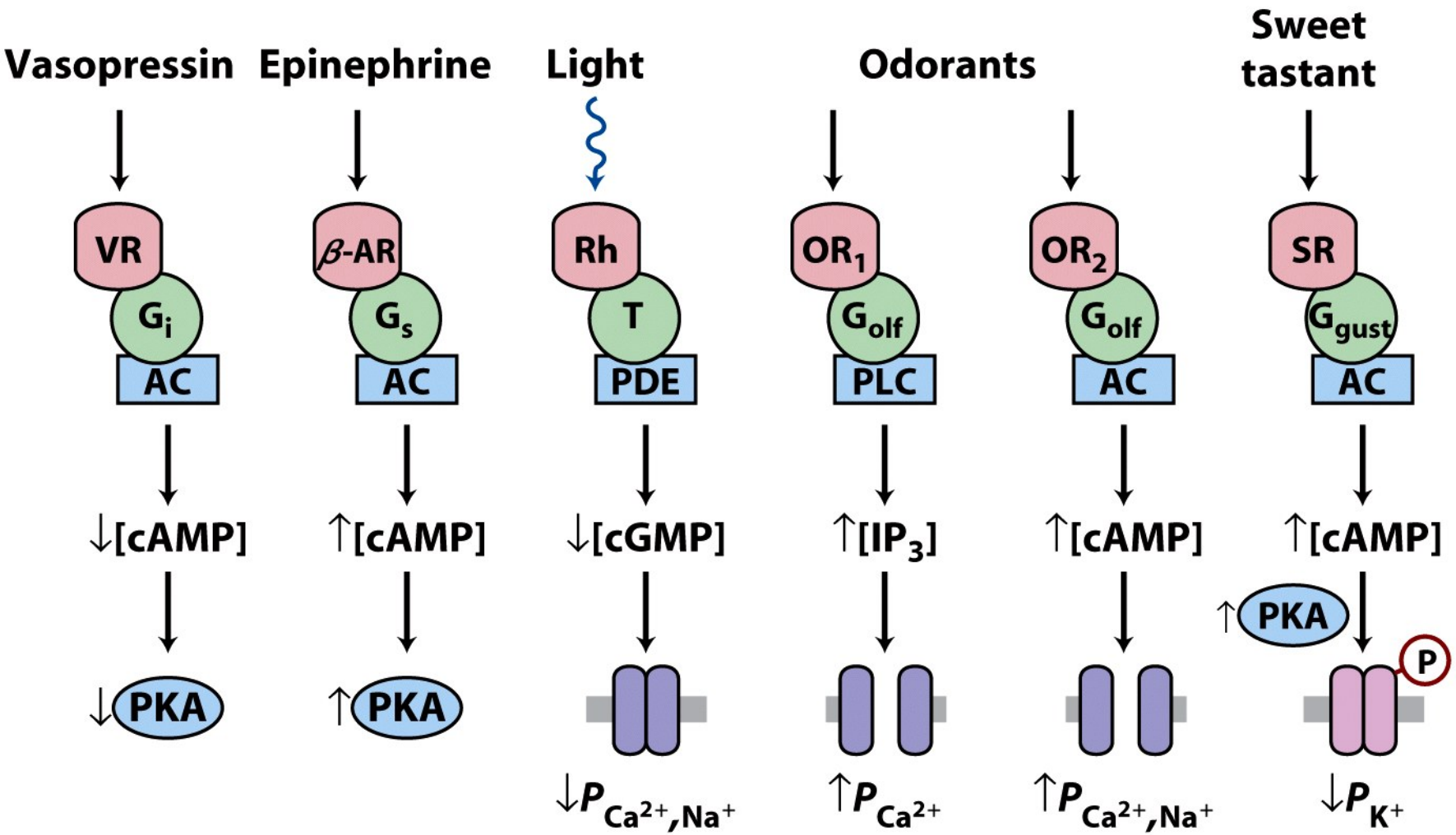

Figure 12-42
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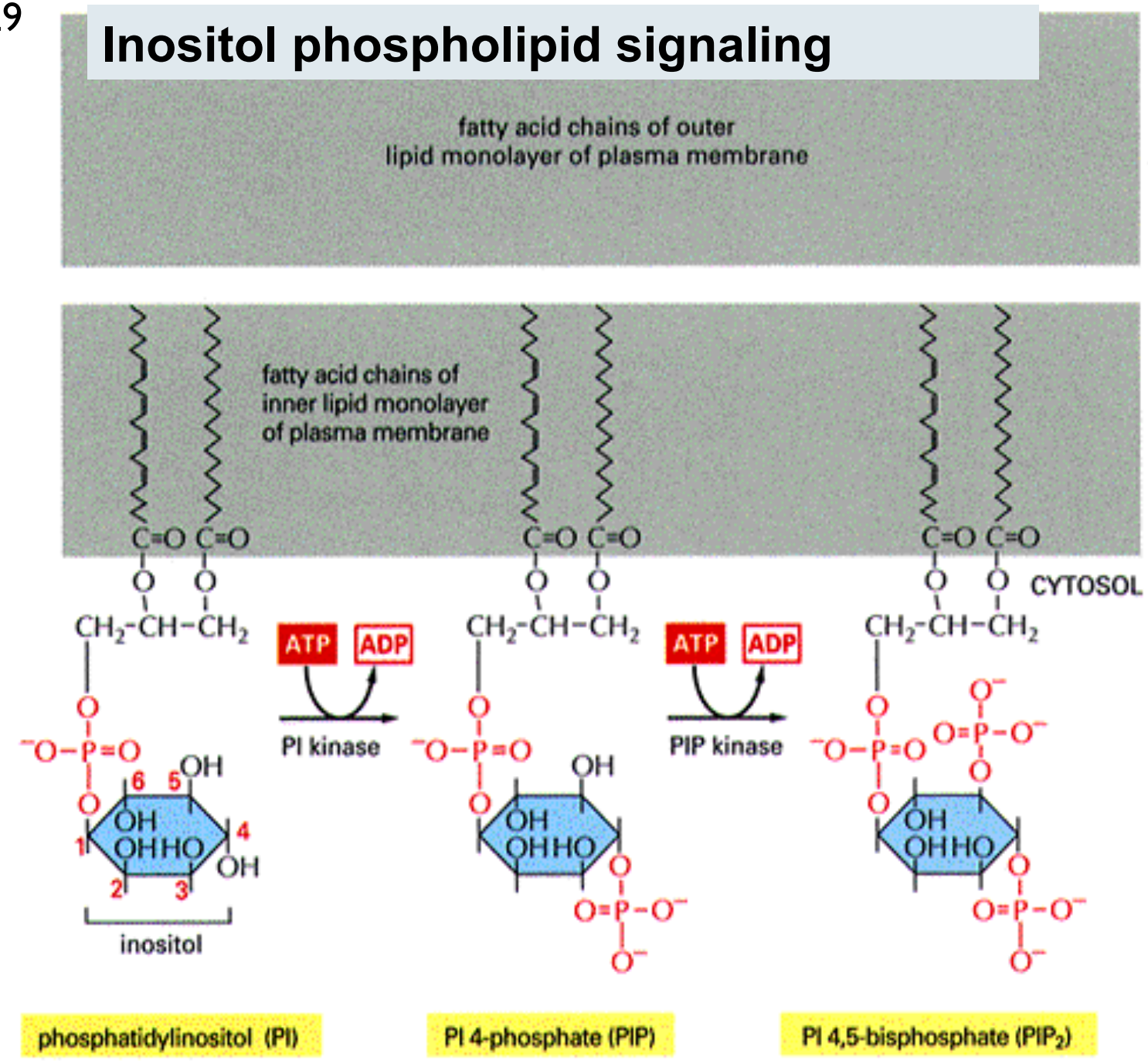
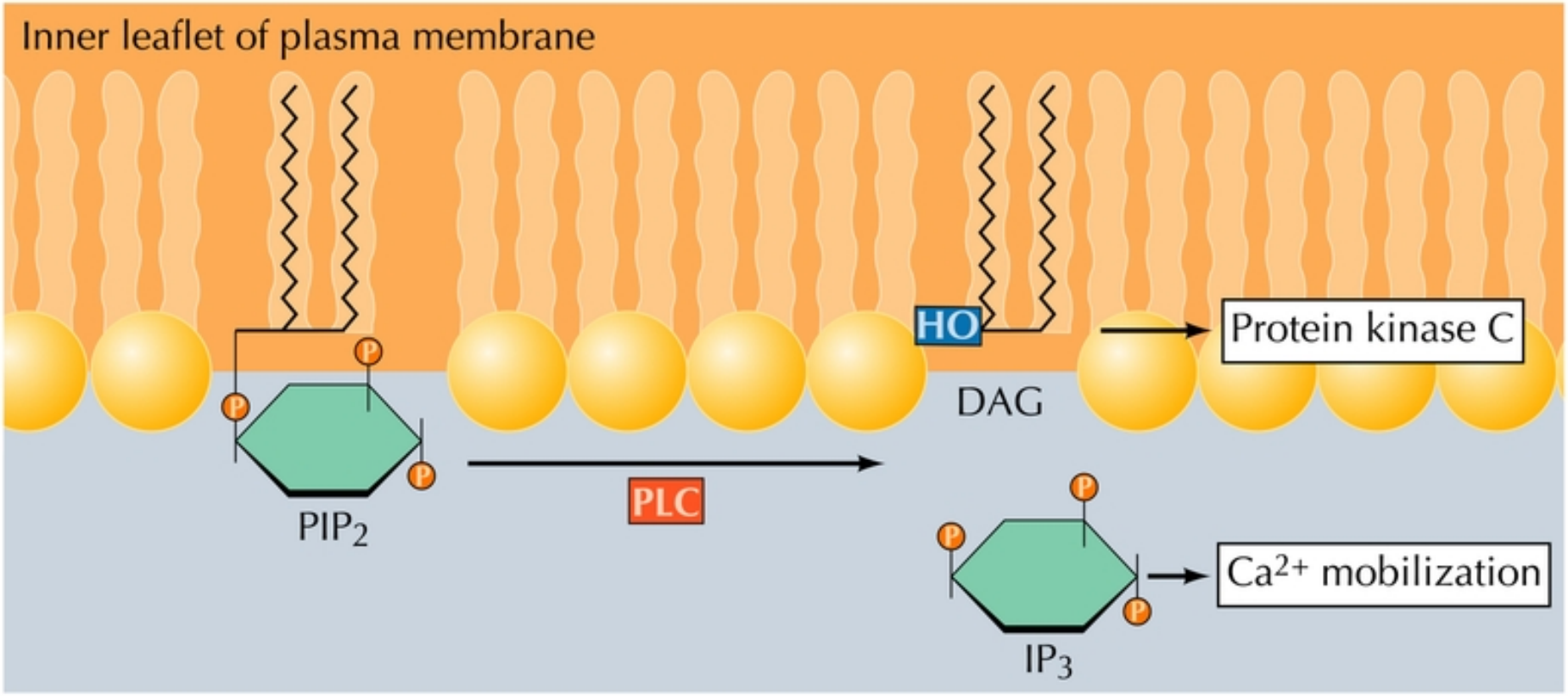
Fig. 15-29

Inositol phospholipid signaling



PI	Phosphoinositide
PIC	Phosphoinositidase C
PI3-Kinase (PI3K)	Phosphoinositide 3-kinase
PtdIns (PtdI, PI)	Phosphatidylinositol
PtdIns(3)P (PtInsP, PIP)	Phosphatidylinositol 3-phosphate
PtdIns(4,5)P_2 (PI(4,5)P_2, PIP_2)	Phosphatidylinositol (4,5)-bis phosphate
PtdIns(3,4,5) P_3 (PI(3,4,5)P_3, PIP_3)	Phosphatidylinositol (3,4,5)-phosphate
Ins(1,4)P_2 (IP_2)	Inositol (1,4) bisphosphate
Ins(1,4,5)P_3 (IP_3)	Inositol (1,4,5) trisphosphate
DAG	Diacylglycerol
PLC	Phospholipase C

Hydrolysis of PIP2



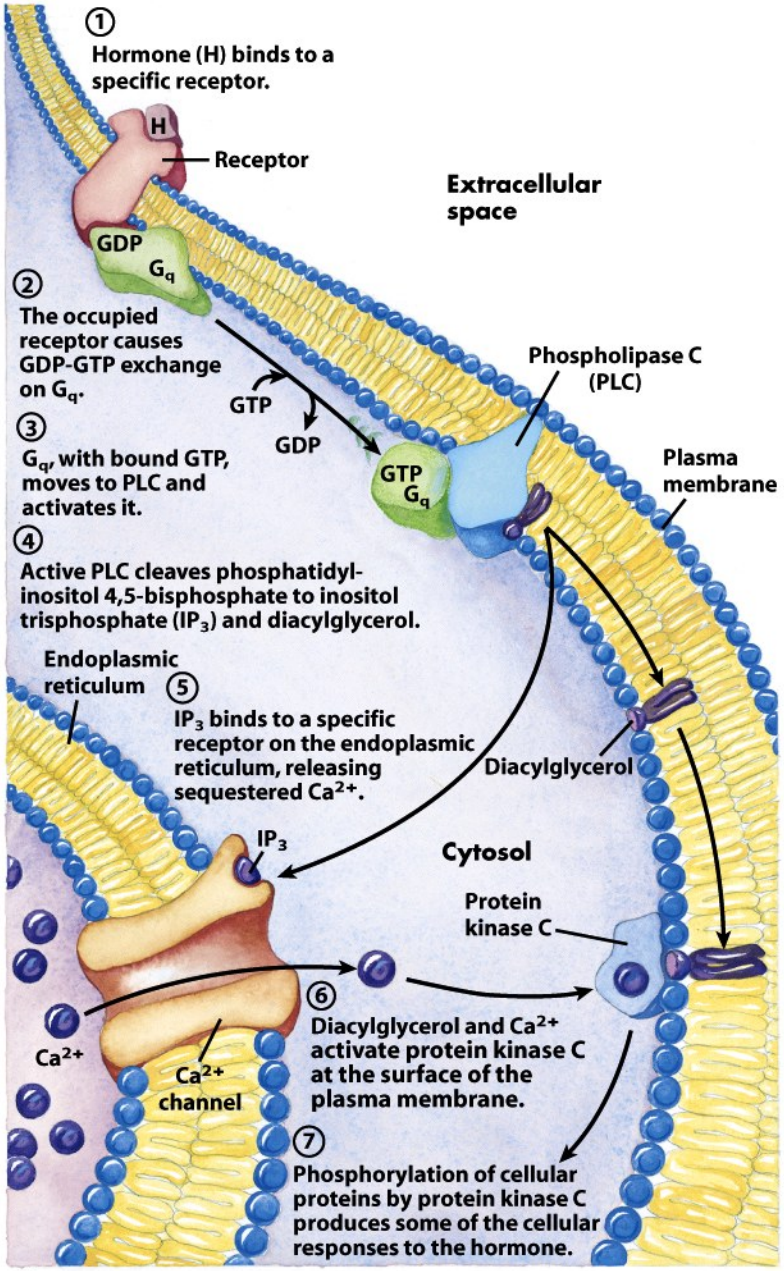


Figure 12-10

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Gq coupled receptors activate phospholipase C (PLC) the enzyme that cleaves PIP2 to IP3 and DAG.
This results in elevation of cytosolic Ca^{2+} and protein kianse C (PKC) activity.

TABLE 12–4 Some Signals That Act through Phospholipase C, IP_3, and Ca^{2+}

Acetylcholine [muscarinic M_1]	Gastrin-releasing peptide	Oxytocin
α_1-Adrenergic agonists	Glutamate	Platelet-derived growth factor (PDGF)
Angiogenin	Gonadotropin-releasing hormone (GRH)	Serotonin [5-HT-1c]
Angiotensin II	Histamine [H_1]	Thyrotropin-releasing hormone (TRH)
ATP [P_{2x}, P_{2y}]	Light (*Drosophila*)	Vasopressin
Auxin		

Note: Receptor subtypes are in square brackets; see footnote to Table 12–3.

Table 12-4
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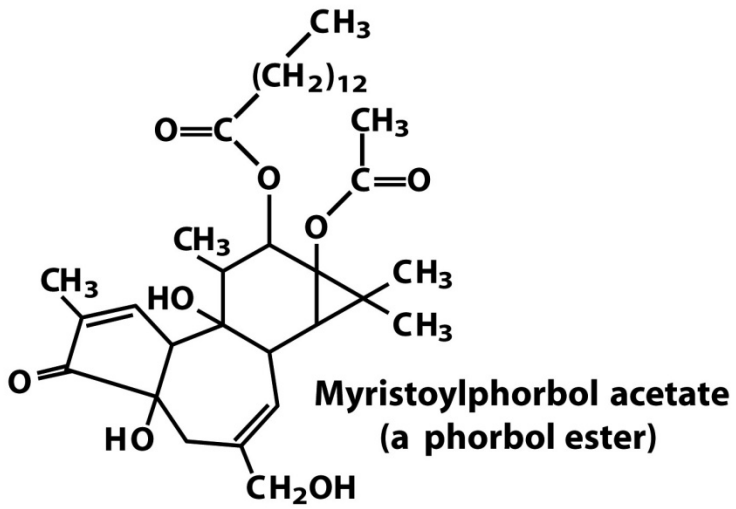

Myristoylphorbol acetate (a phorbol ester)

Phorbol ester (DAG analog) that can act as a tumor promoter

Signaling Through the PI System

- PLC hydrolyses phosphatidylinositol-4,5-bisphosphate (PIP_2) to form two second messengers: diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3)
- IP_3 diffuses to the cytosol and binds to IP_3 receptors on the membrane of a calcium store (endoplasmic reticulum)
- IP_3 binding results in Calcium release
- Increases in intracellular Calcium levels (up to 1 μM) are associated with changes in fusion of secretory granules to the cell membrane, microtubular aggregation and the function of contractile proteins

Calcium binds calmodulin and activates calmodulin-dependent protein kinases

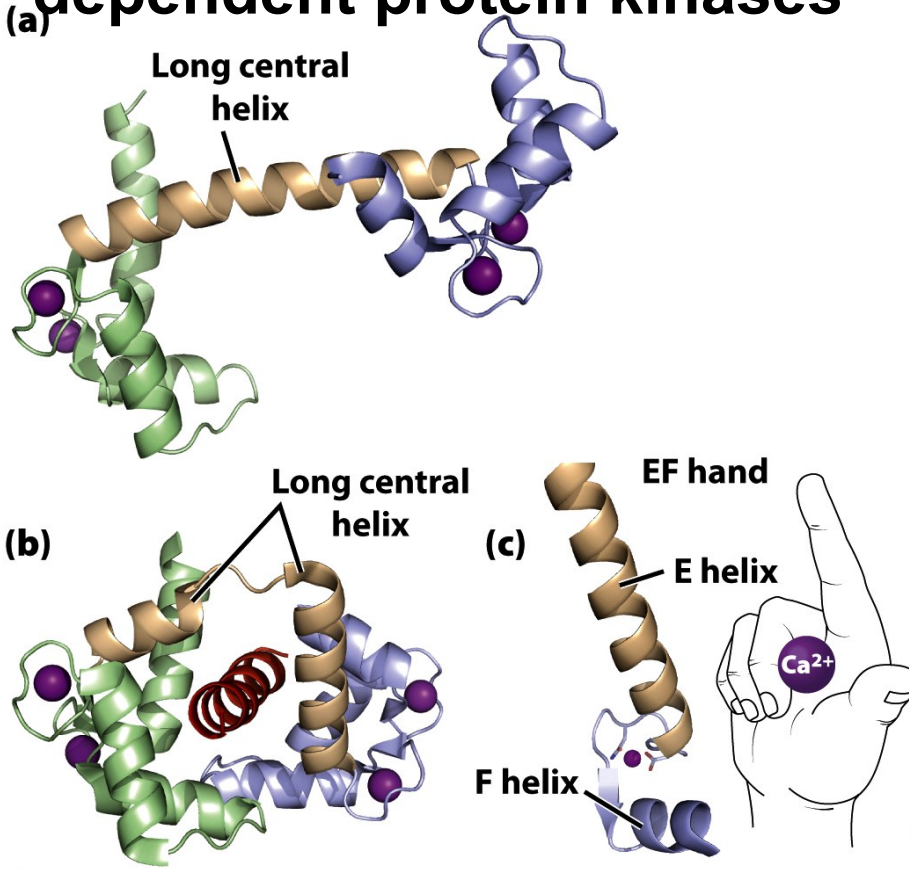

Figure 12-11
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TABLE 12–5	Some Proteins Regulated by Ca^{2+} and Calmodulin
Adenylyl cyclase (brain)	
Ca^{2+}/calmodulin-dependent protein kinases (CaM kinases I to IV)	
Ca^{2+}-dependent Na^{+} channel (*Paramecium*)	
Ca^{2+}-release channel of sarcoplasmic reticulum	
Calcineurin (phosphoprotein phosphatase 2B)	
cAMP phosphodiesterase	
cAMP-gated olfactory channel	
cGMP-gated Na^{+}, Ca^{2+} channels (rod and cone cells)	
Glutamate decarboxylase	
Myosin light chain kinases	
NAD^{+} kinase	
Nitric oxide synthase	
Phosphoinositide 3-kinase	
Plasma membrane Ca^{2+} ATPase (Ca^{2+} pump)	
RNA helicase (p68)	

Table 12-5
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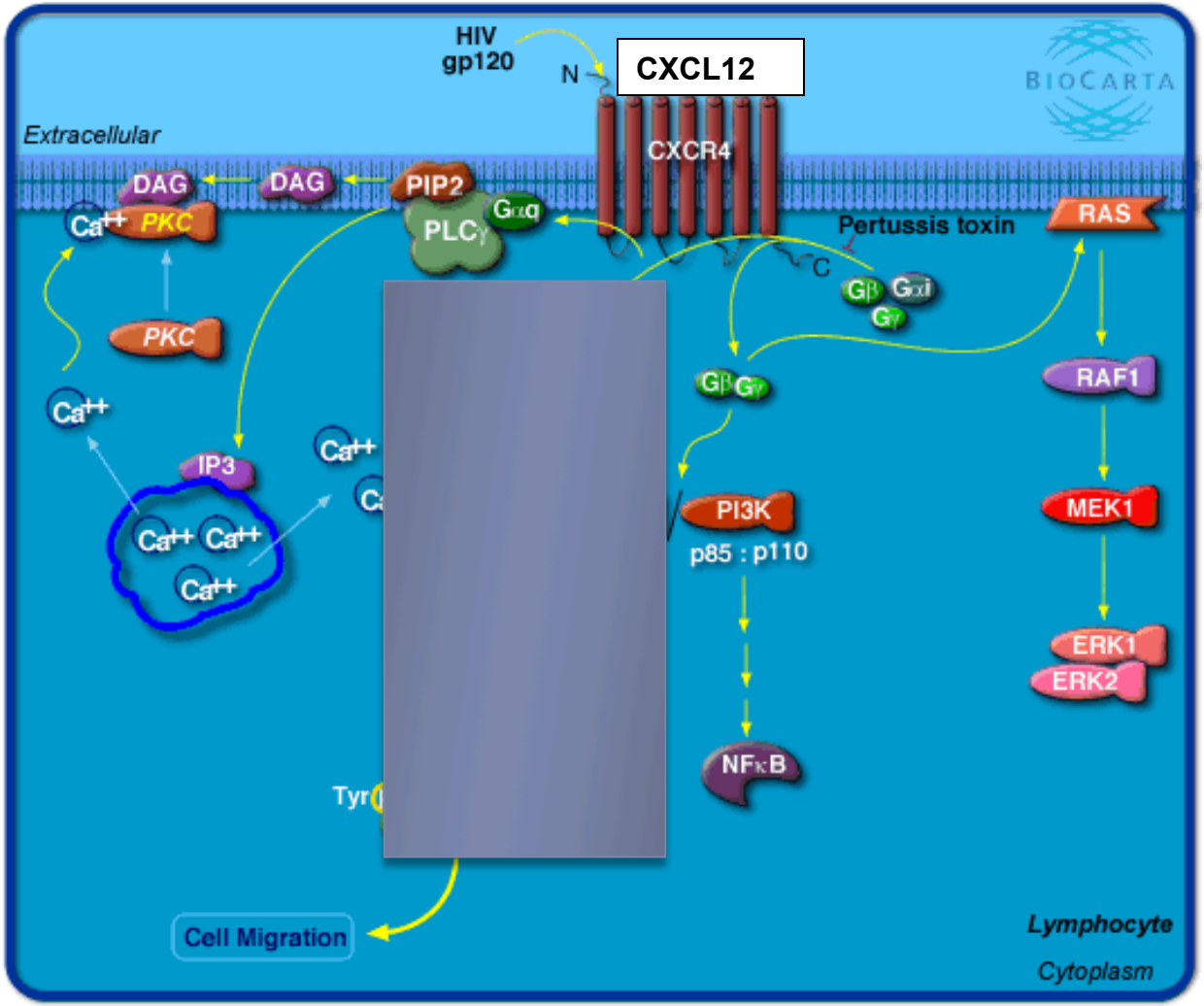
Calcium serves as a second messenger to regulate exocytosis, muscle contraction, cytoskeletal rearrangements, fertilization, etc.

Diacylglycerol Activates Protein Kinase C Pathway

- DAG activates a serine/threonine Ca^{+2}-dependent protein kinase called Protein Kinase C (PKC)
- High intracellular Ca^{+2} levels alter PKC so that it translocates to the cell membrane
- Once translocated, it is activated by DAG, Ca^{+2} and phosphatidylserine (PS)
- Once activated, PKC phosphorylates specific proteins in the cytosol, or in some cases, the plasma membrane

CXCL12 and CXCR4 are expressed by human gingival fibroblasts in periodontal disease



◆ CXCL12 and the receptor CXCR4 are expressed in both normal and periodontal diseased gingival tissues.

◆ CXCL12 levels are enhanced by stimulation with tumour necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and transforming growth factor-β (TGF–β).

◆ CXCR4 signaling activates migration of neutrophils and lymphocytes into effector sites.

Receptor Tyrosine kinases in periodontal disease

◆ Polypeptide growth factors are a class of potent natural biologic mediators that regulate many of the activities of wound healing including cell proliferation, migration, and metabolism.

◆ Platelet-derived growth factor (PDGF) and insulin-like growth factor-I (IGF-I) regulate DNA and protein synthesis in bone cells and interact synergistically to enhance soft tissue wound healing.

◆ PDGF-B and IGF-I can significantly enhance the formation of the periodontal attachment apparatus during the early phases of wound healing following surgery.

◆ In patients with bilateral osseous periodontal lesions, PDGF/IGF-1 resulted in a significant promotion in bone and periodontal regeneration.

Receptor Tyrosine kinases

TABLE 13.1 Representative Peptide Hormones, Neuropeptides, and Growth Factors

Signaling molecule	Size[a]	Activities[b]
Peptide hormones		
Insulin	A = 21, B = 30	Regulation of glucose uptake; stimulation of cell proliferation
Glucagon	29	Stimulation of glucose synthesis
Growth hormone	191	General stimulation of growth
Follicle-stimulating hormone (FSH)	α = 92, β = 118	Stimulation of the growth of oocytes and ovarian follicles
Prolactin	198	Stimulation of milk production
Neuropeptides and neurohormones		
Substance P	11	Sensory synaptic transmission
Oxytocin	9	Stimulation of smooth muscle contraction
Vasopressin	9	Stimulation of water reabsorption in the kidney
Enkephalins	5	Analgesics
β-Endorphin	31	Analgesic
Growth factors		
Nerve growth factor (NGF)	118	Differentiation and survival of neurons
Epidermal growth factor (EGF)	53	Proliferation of many types of cells
Platelet-derived growth factor (PDGF)	A = 125, B = 109	Proliferation of fibroblasts and other cell types
Interleukin-2	133	Proliferation of T lymphocytes
Erythropoietin	166	Development of red blood cells

[a] Size is indicated in number of amino acids. Some hormones and growth factors consist of two different polypeptide chains, which are designated either A and B or α and β.

[b] Most of these hormones and growth factors possess other activities in addition to those indicated.

Six subfamilies of receptor tyrosine kinases

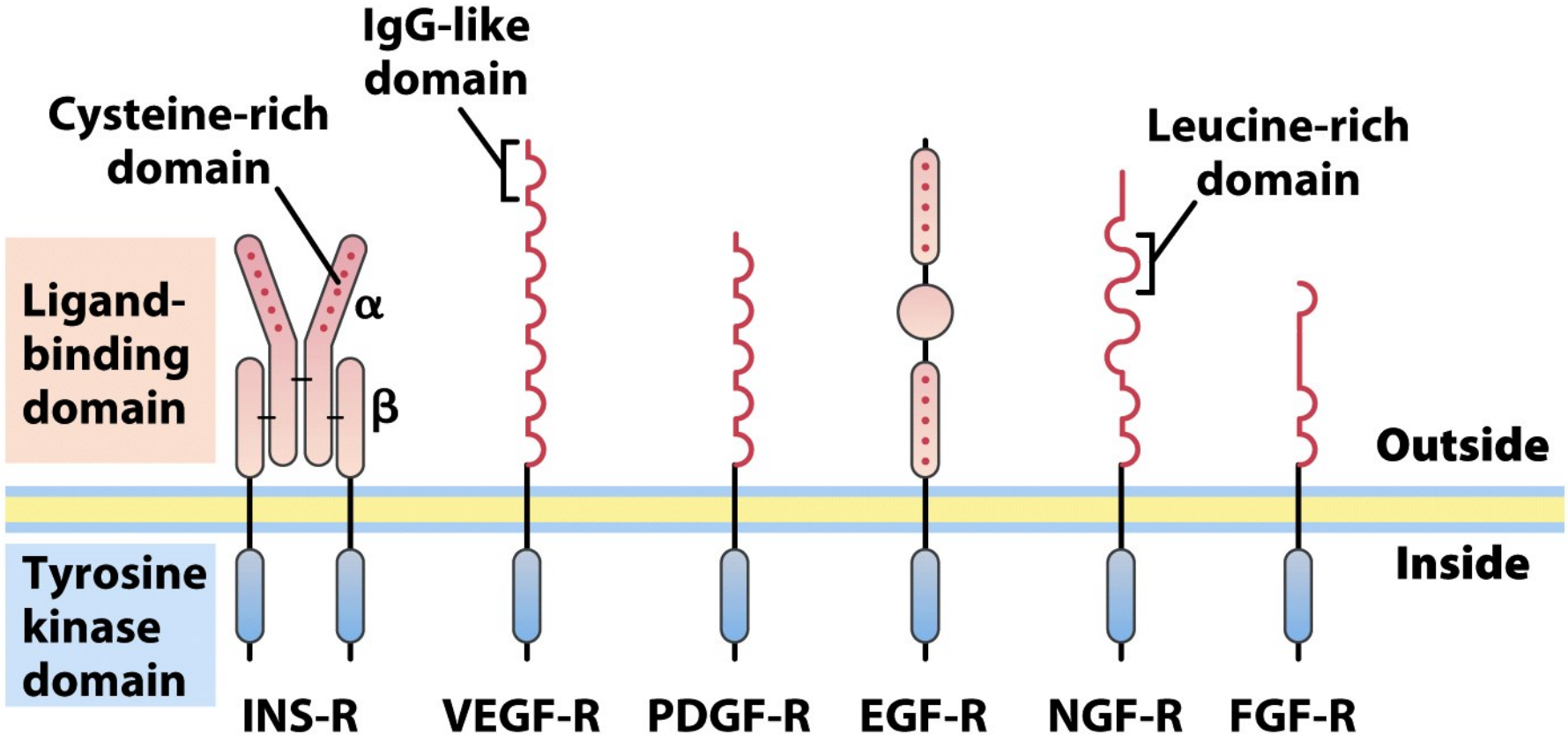


Figure 12-17
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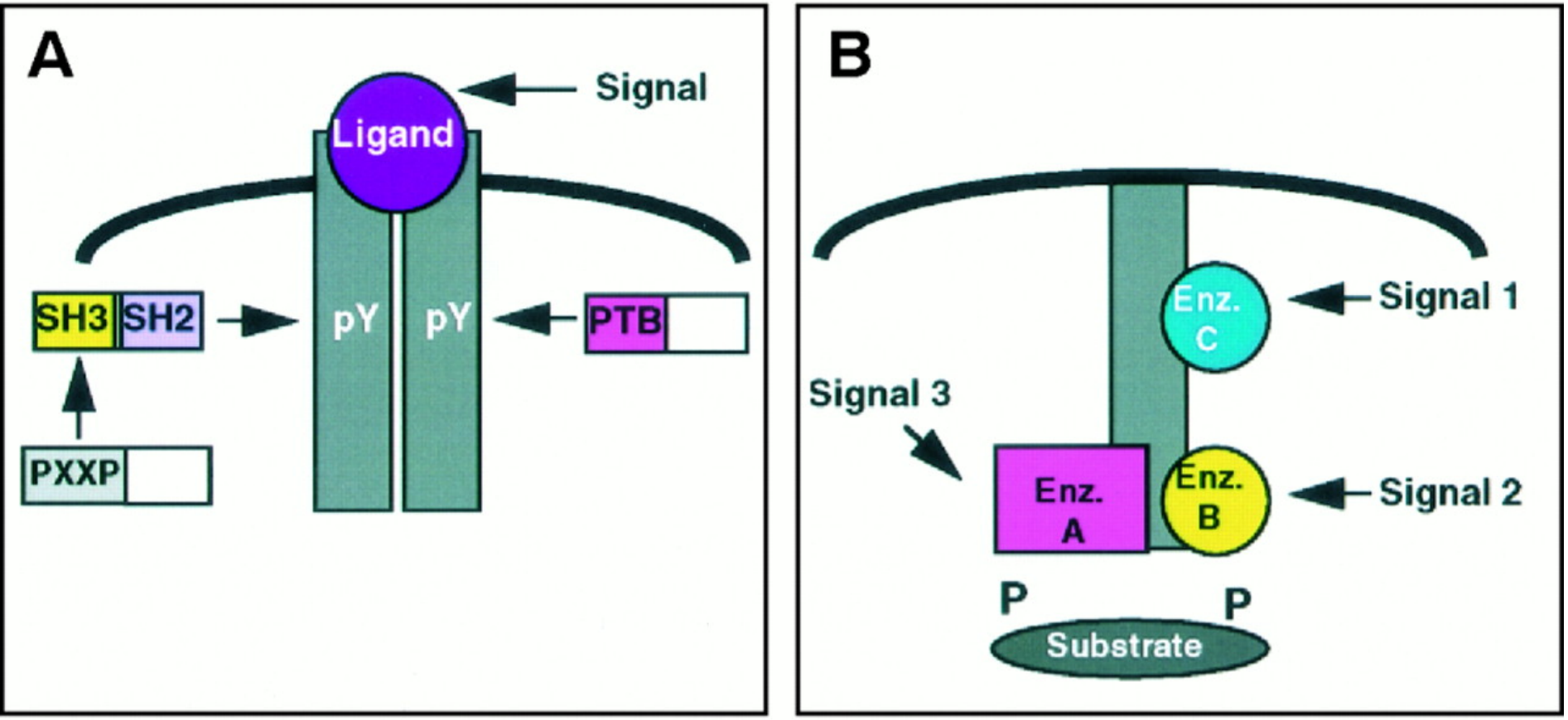
The most studied growth factors for periodontal regeneration are platelet-derived growth factor (PDGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), and insulin like growth factor (IGF).

Autophopshorylation by activated tyrosine kinase receptors recruit downstream effector proteins (adaptors)



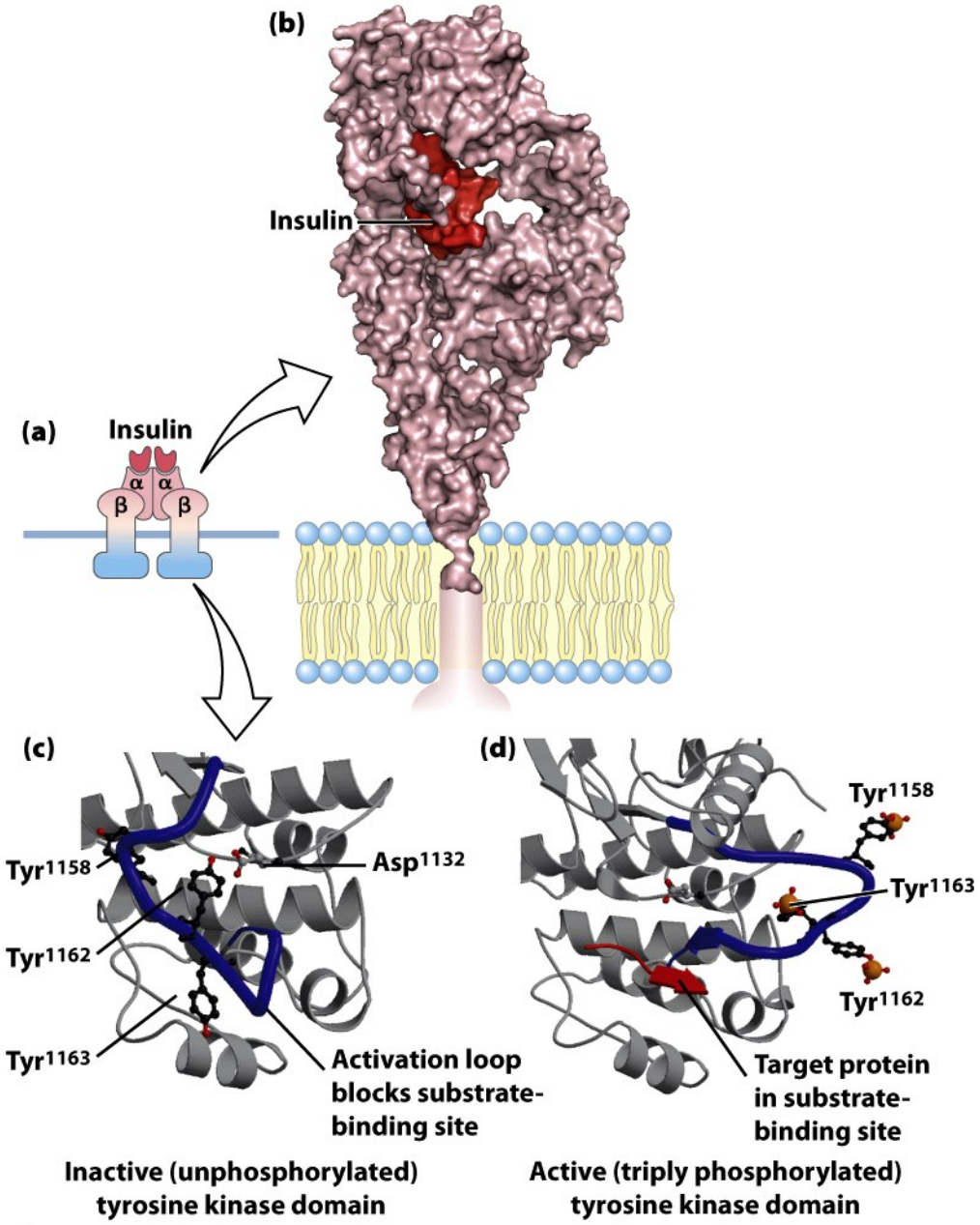


Figure 12-14

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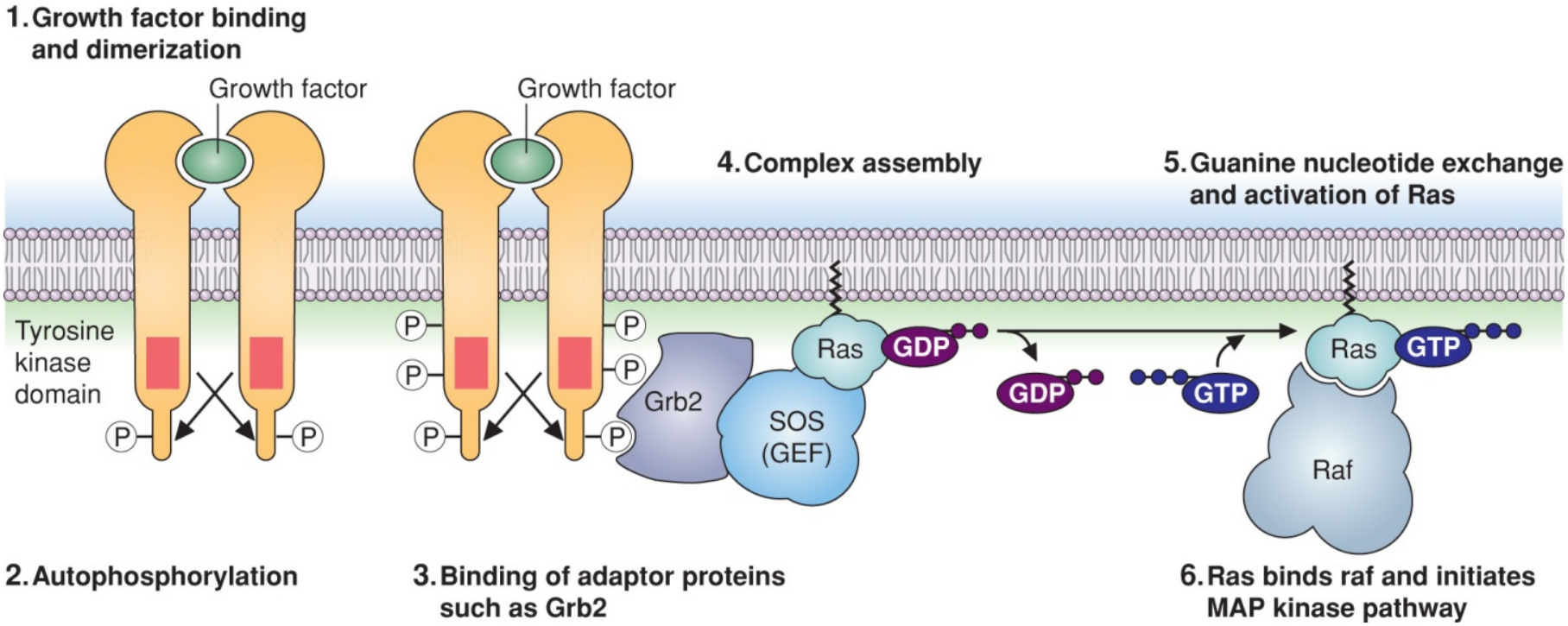
Receptor Tyrosine Kinases



Activation of heterotrimeric G proteins

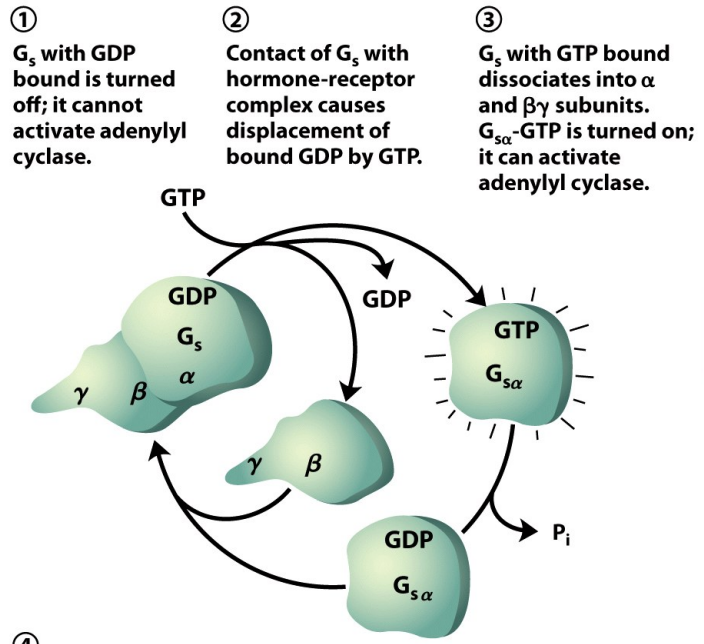


Figure 12-5
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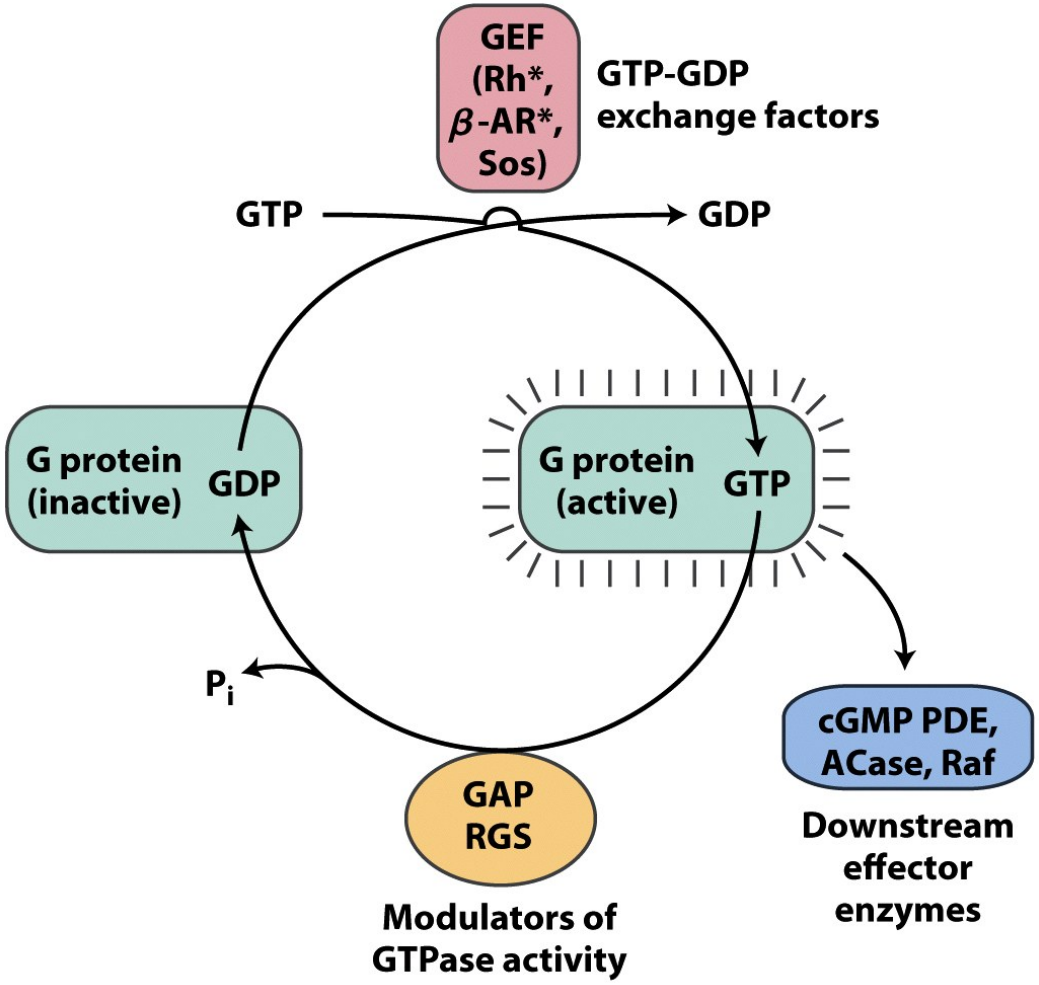
Activation of small G proteins (Ras superfamily)



Box 12-2 figure 4
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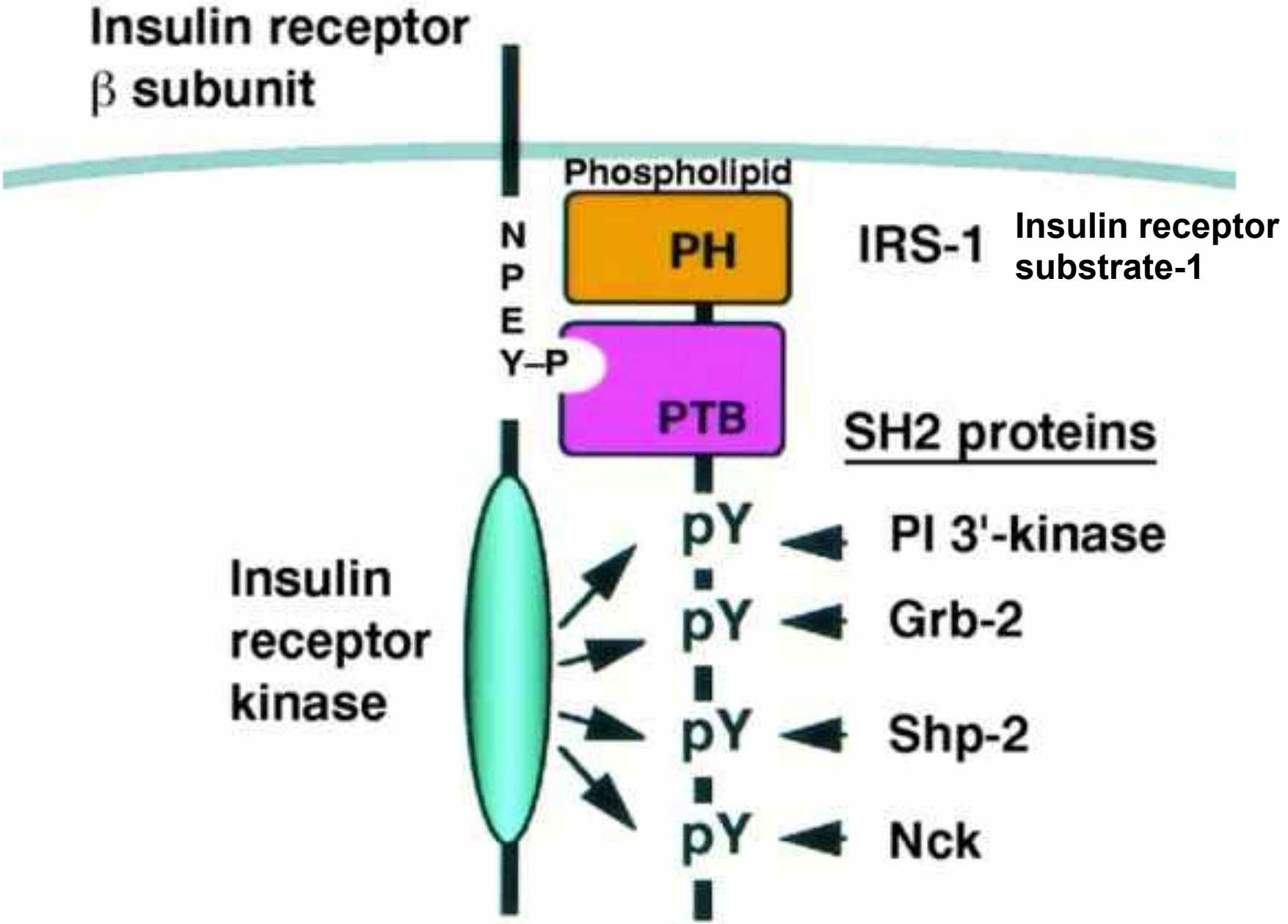
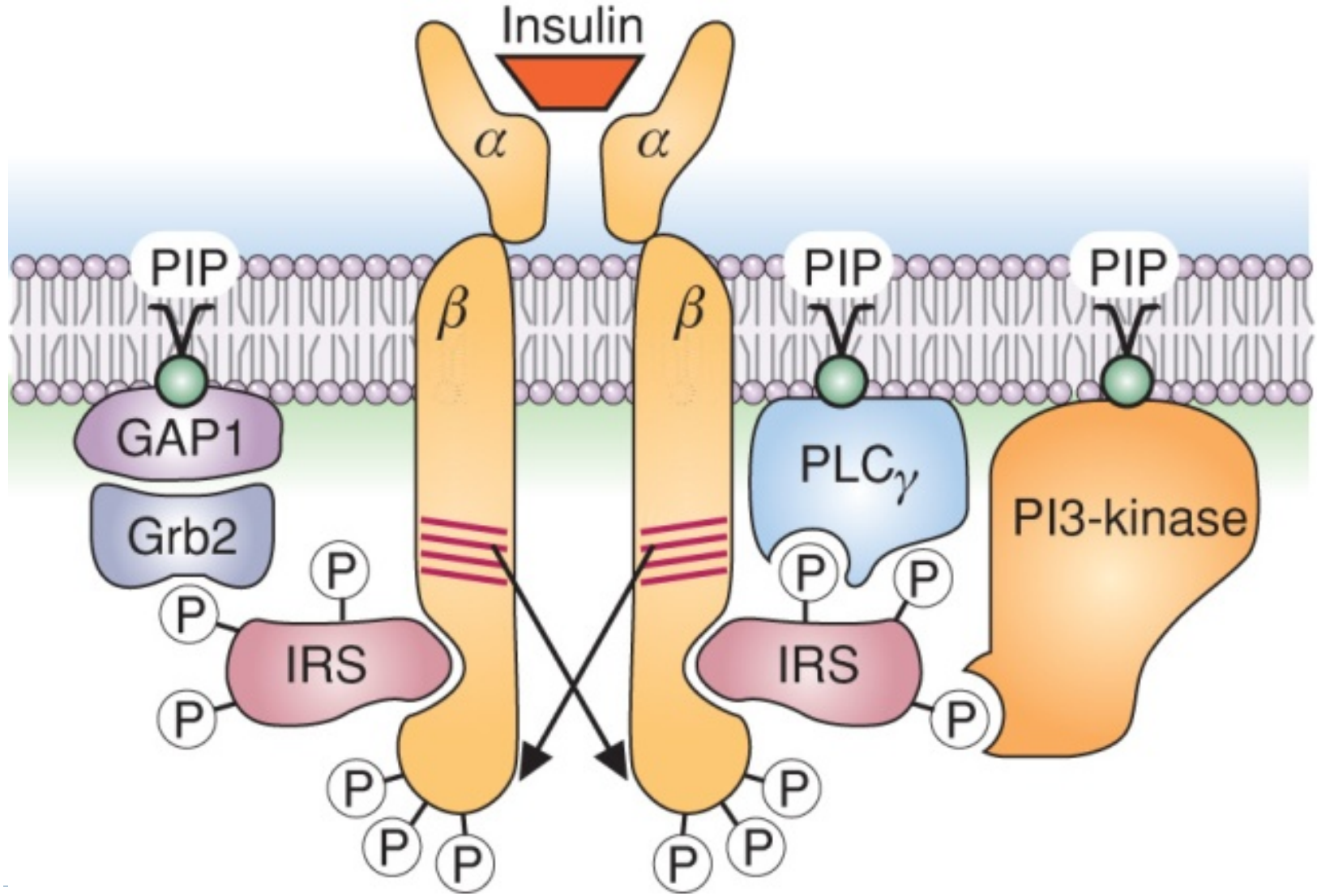

Insulin receptor
β subunit
Phospholipid
PH
IRS-1
Insulin receptor substrate-1
NPEY–P
PTB
SH2 proteins
pY
PI 3'-kinase
Insulin receptor kinase
pY
Grb-2
pY
Shp-2
pY
Nck

Insulin Receptor



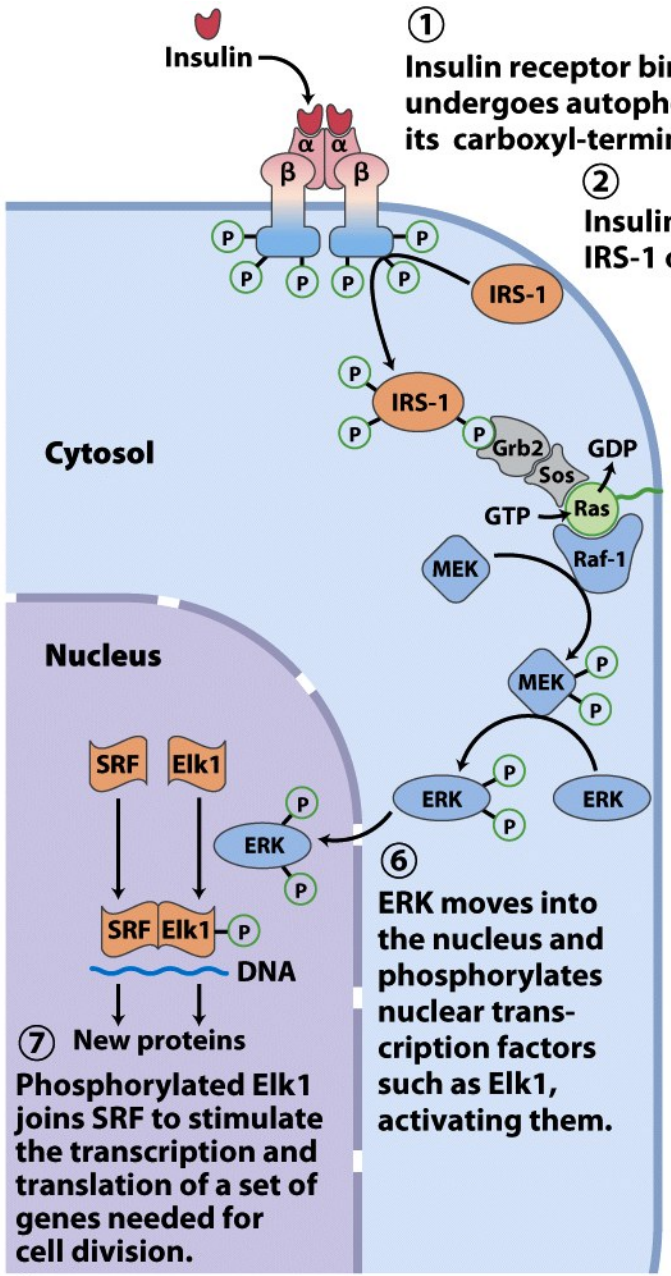


Figure 12-15
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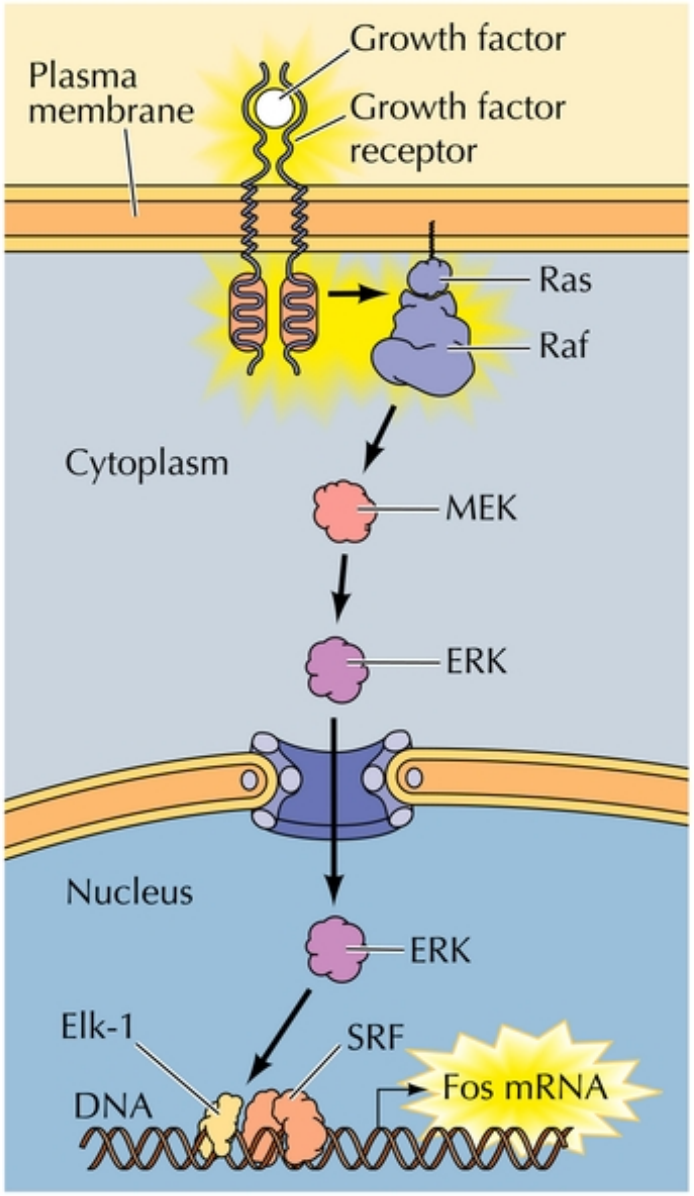
Regulation of gene expression by insulin through a MAP (mitogen activated protein) kinase cascade:

MEK=MAP kinase kinase

Extracellular regulated kinase (ERK) is a MAPK.

Phsophorylated ERK enters the nucleus to phosphorylate (S/Thr)and activate transcription factors.

Growth factor and hormone signaling leads to activation of transcription factors that upregulate cell proliferation



Ras/MAPK Pathway Proteins that Regulate Cell Proliferation

Signaling Molecule	Activity	Function/Location
Insulin receptor substrate (IRS-1) or Src homology 2 (SH2) protein (Shc)	Adaptor proteins that bind phospho tyrosine residues in tyrosine kinase receptors	Transmit signal from membrane receptors to Ras. Cytosolic location, close to plasmamembrane.
Grb2	No known catalytic activity	Binds and recruits Sos to the membrane via SH2 domains that bind phosphorylated tyrosines on the receptor or adaptor protein. Cytosolic location.
Son of sevenless (Sos)	Guanine nucleotide exchange factor Regulates c-Ras by exchanging a GDP for a GTP	Recruited to the membrane by Grb2 to activate Ras. Cytosolic location.
c-Ras	GDP/GTP binding GTPase	Mediates signal transduction from receptors to mitogen-activated protein kinases. Anchored to the plasmamembrane on cytoplasmic side by farnesylation.
c-Raf (MAP kinase kinase kinase)	Ser/Thr kinase	Growth-dependent regulation of Ser/Thr Phosphorylation of MEKK. Cytoplasmic.
MEK (MAP kinase kinase)	Dual-specificity kinase (Ser/Thr/Tyr)	Growth-dependent regulation of Ser/Thr/Tyr residues. Phosphorylates MAPK on Thr and Tyr. Cytoplasmic.
MAP kinase (MAPK), Extracellular signal-regulated kinase (ERK)	Dual-specificity kinase (Ser/Thr/Tyr)	Autophosphorylates on Tyr at regulatory site. Phosphorylates (Ser/Thr) and activates transcription factors. Cytoplasmic and nuclear.
Elk-1 and SRF (serum response factor)	Transcription factors	Regulates serum response element (SRE)-mediated transcription. Nuclear.
c-Fos	Transcription factor	Regulates AP-1 and SRE-mediated transcription. Induces Cyclin expression. Nuclear.

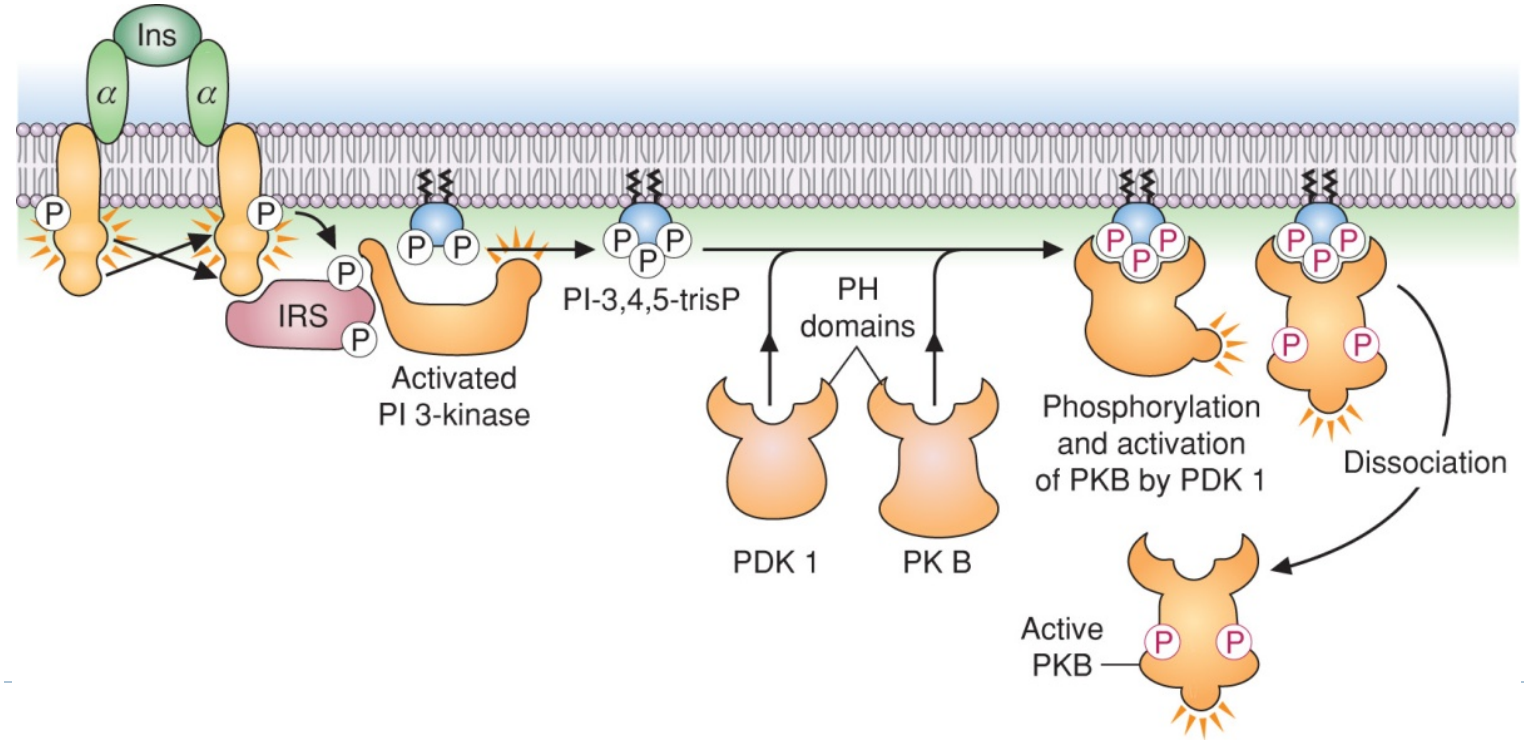
Insulin signaling:

• activates cell growth and survival via MAP kinase, Akt/PKB (protein kinase B) and PLCγ activities

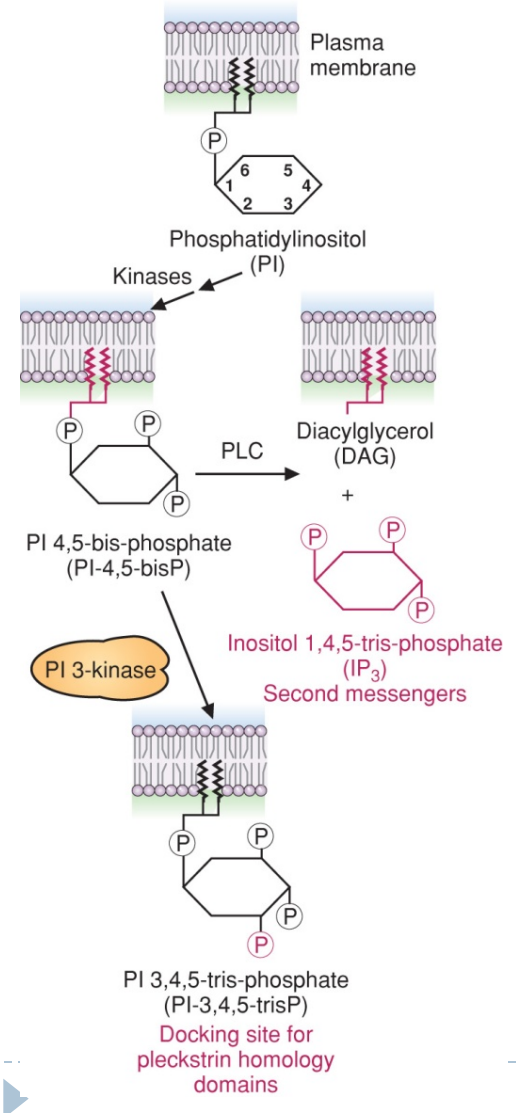
• increases protein synthesis

• regulates nutrient availability and storage

• glucose transport into skeletal muscle and glycogen synthesis



Phosphatidyl inositol phosphates in signal transduction

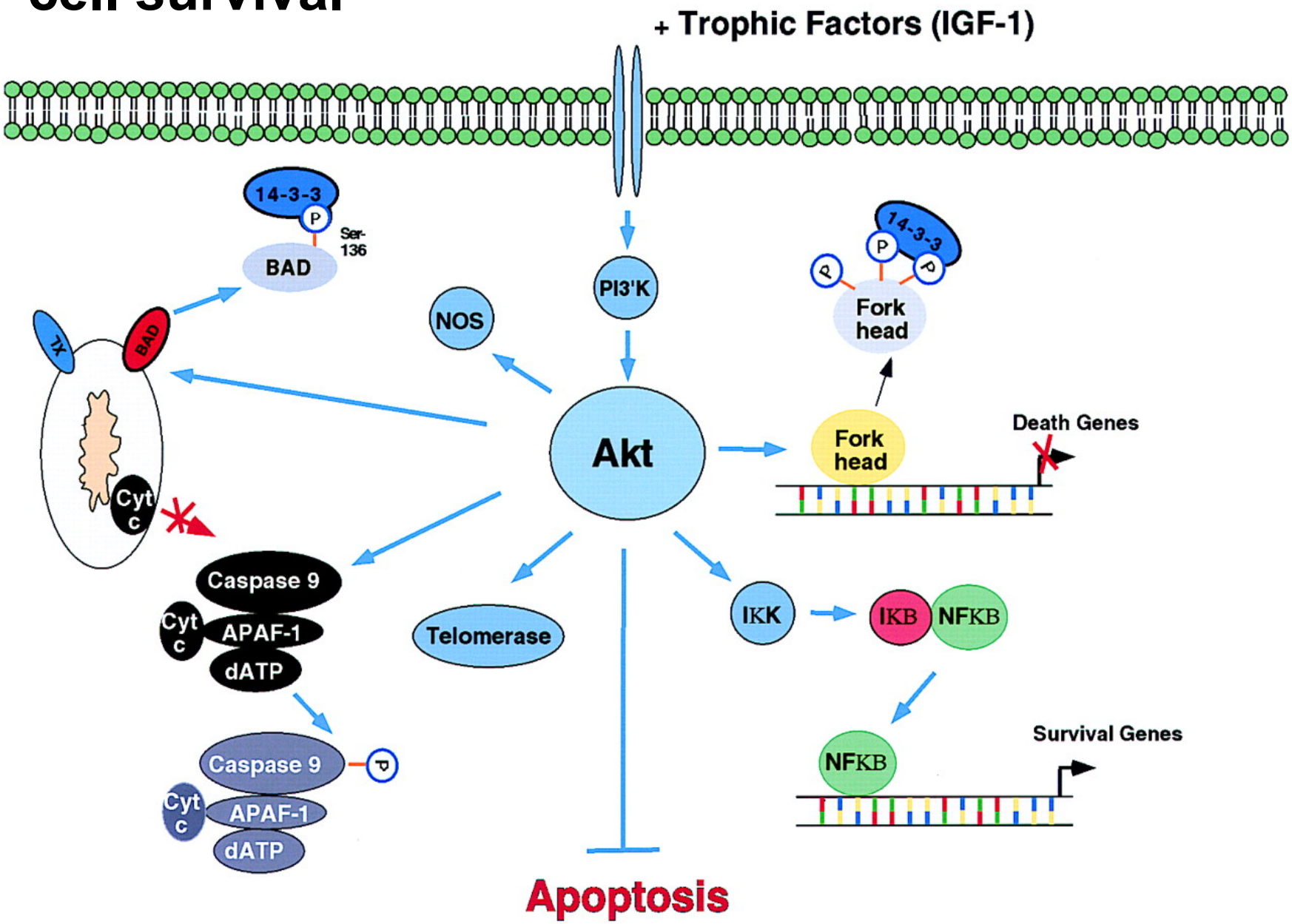


- PIPs are activated by both tyrosine kinase receptors and heptahelical receptors.

- Cleavage of PIP2 by phospholipase C leads to generation of IP3 and DAG that activate protein kinase C.

- Phosphorylation of PIP2 by PI3 kinase leads to PIP3 formation that activates Akt/protein kinase B.

Activated Akt /protein kinase B (PKB) signals to cell survival



Activation of glycogen synthase by insulin

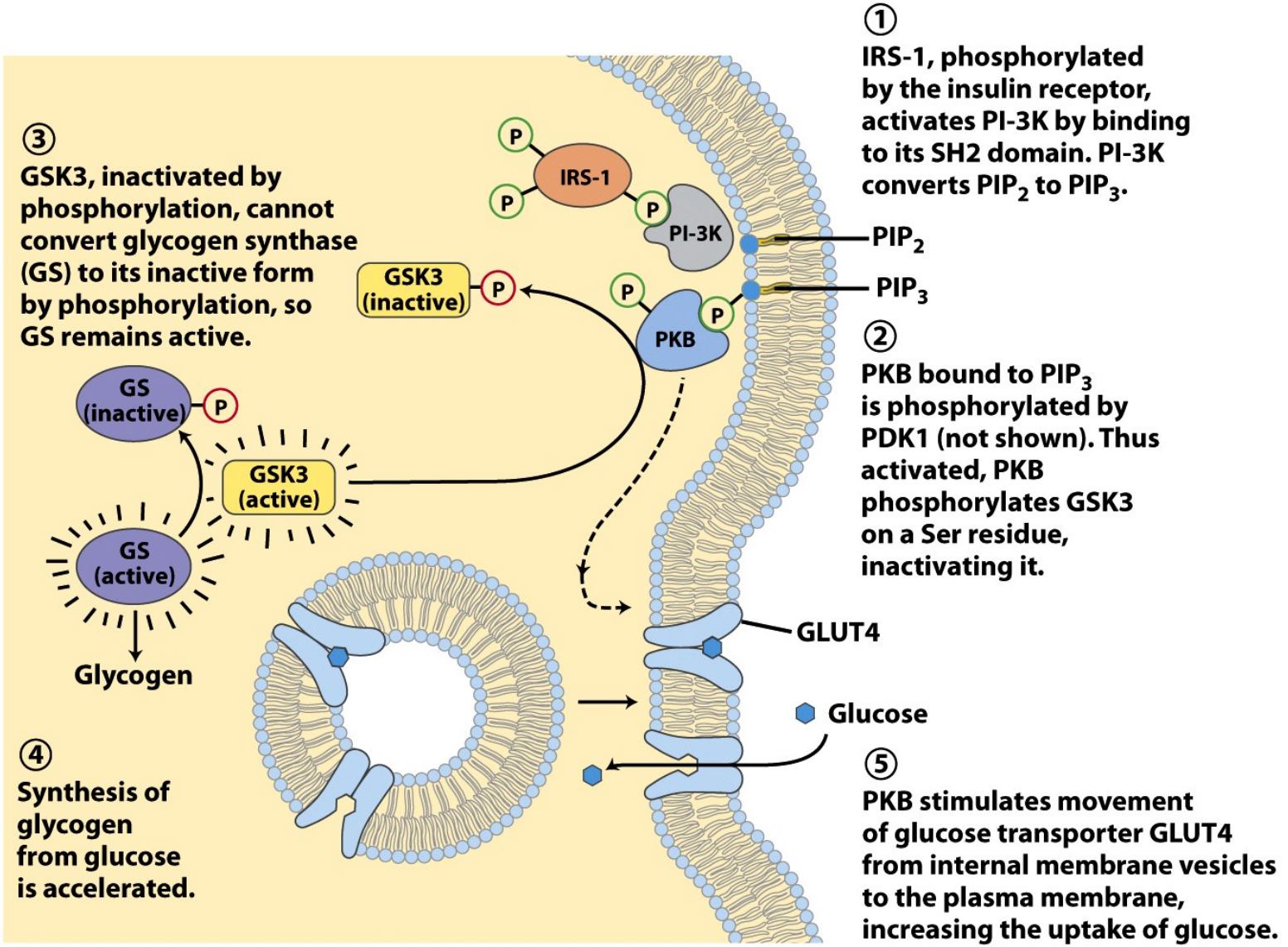


Figure 12-16
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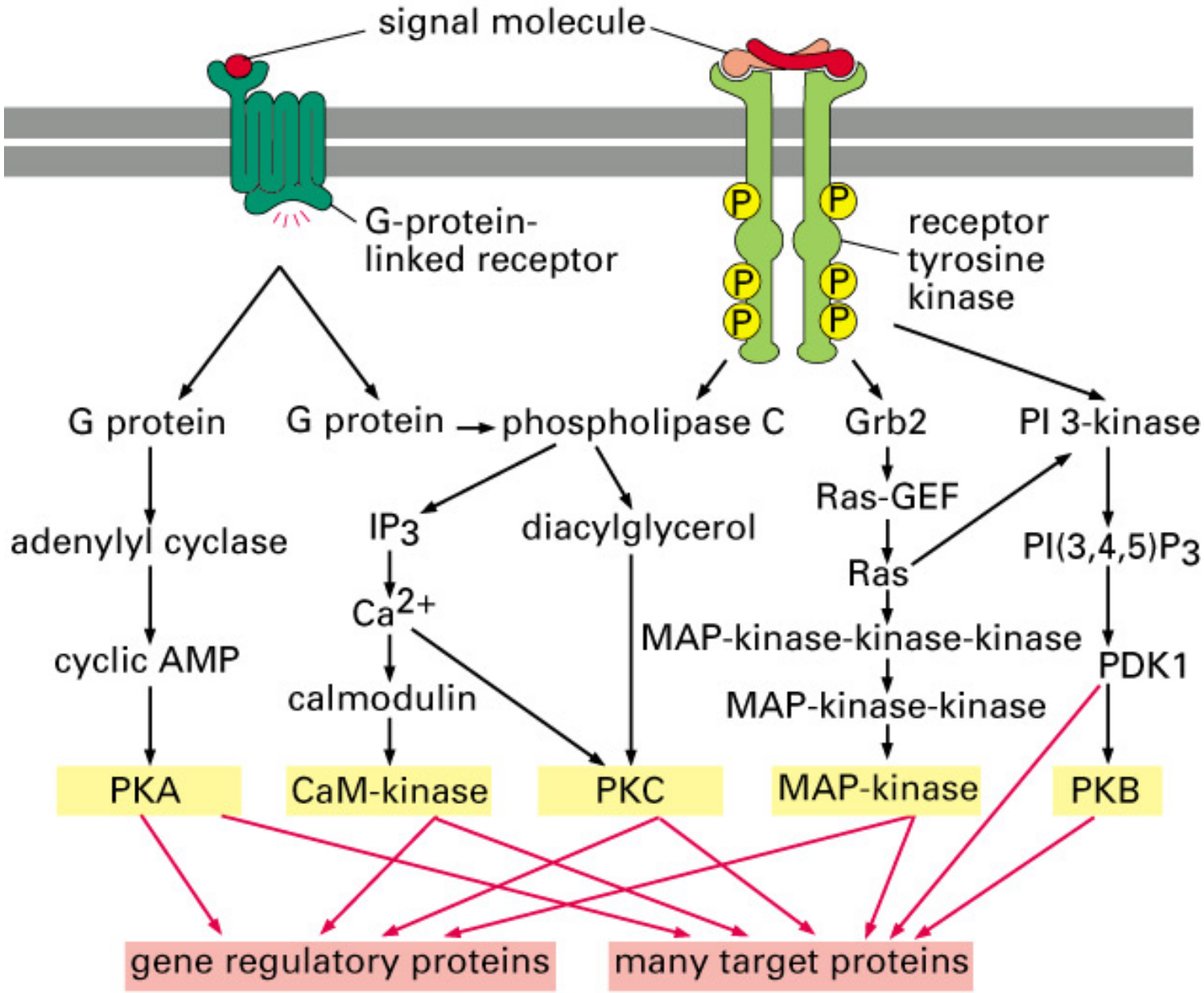


Figure 15–61. Molecular Biology of the Cell, 4th Edition.

Inflammatory signaling in periodontal disease

◆ Periodontal tissue destruction is initiated by pathogens: anaerobic bacteria and spirochetes.

◆ These microorganisms produce harmful byproducts and enzymes, such as collagenases and proteases, which degrade the extracellular matrix to produce nutrients for their growth.

◆ Resultant tissue damage creates pathways for invasion, followed by irritation and inflammation of host tissues, eliciting a host immune response.

◆The local immune response in gingival tissues involves recruitment of inflammatory cells, generation of cytokines and lytic enzymes, and osteoclast activation.

◆ The inflamed periodontium contain periodontal ligament cells, fibroblasts, osteoblasts, osteoclasts, neutrophils, antigen presenting cells such as dendritic cells, macrophages, T cells, and B cells.

◆ These cells recognize and interact with bacterial constituents (e.g., cytoplasmic membranes, peptidoglycans, outer membrane proteins, lipopolysaccharide, capsules, and cell-surface fimbriae) to generate proinflammatory mediators, such as interleukin (IL-1), tumor necrosis factor (TNF)-α, IL-6, and prostaglandin E2 (PGE2).

Toll-Like Receptor (TLR) Signaling

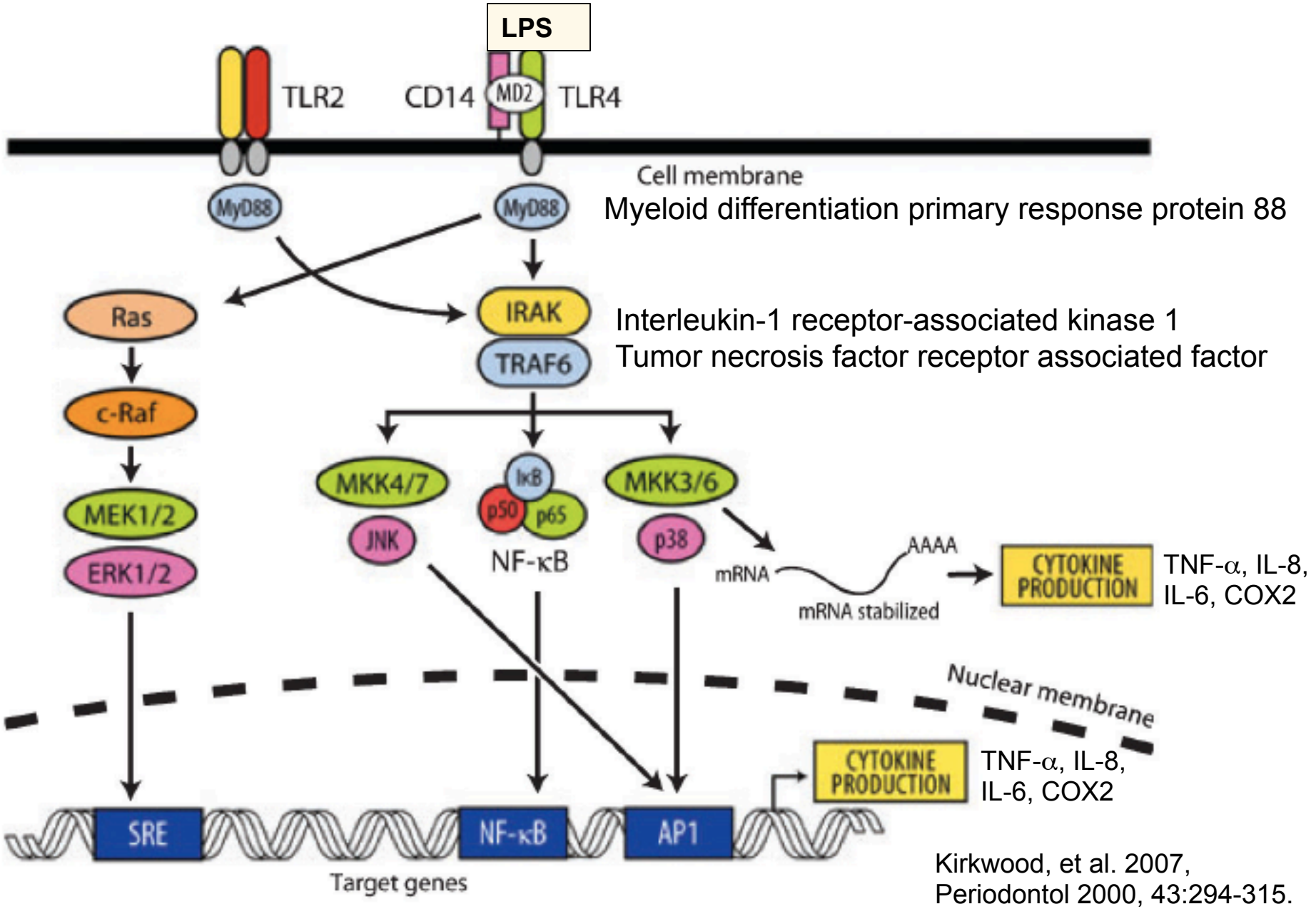
◆ Bacterial (e.g. *Porphoyromonas gingivalis*) constituents lipopolysaccharide (LPS), peptidoglycans, lipoprtoeins, flagellin, bacterial DNA are ligands for **Toll Like Receptors (TLR)** on periodontal cells (gingival epithelial cells, gingival fibroblasts, endothelium, osteoblasts, osteoclasts, periodontal ligament fibroblasts).

◆ TLR signaling activates MAPKs: Extracellular regulated kinase (ERK)
P38 MAPK
Jun kinase (JNK)

◆ Signaling via MAPKs activate transcription factors: induce inflammatory cytokine and chemokine expression (IL-6, -11, CXCL, tumor necrosis factor (TNF−α)).

◆ Nuclear factor kB (NfκB) is a transcription factor that induces transcription of interleukins that promote the inflammatory response.

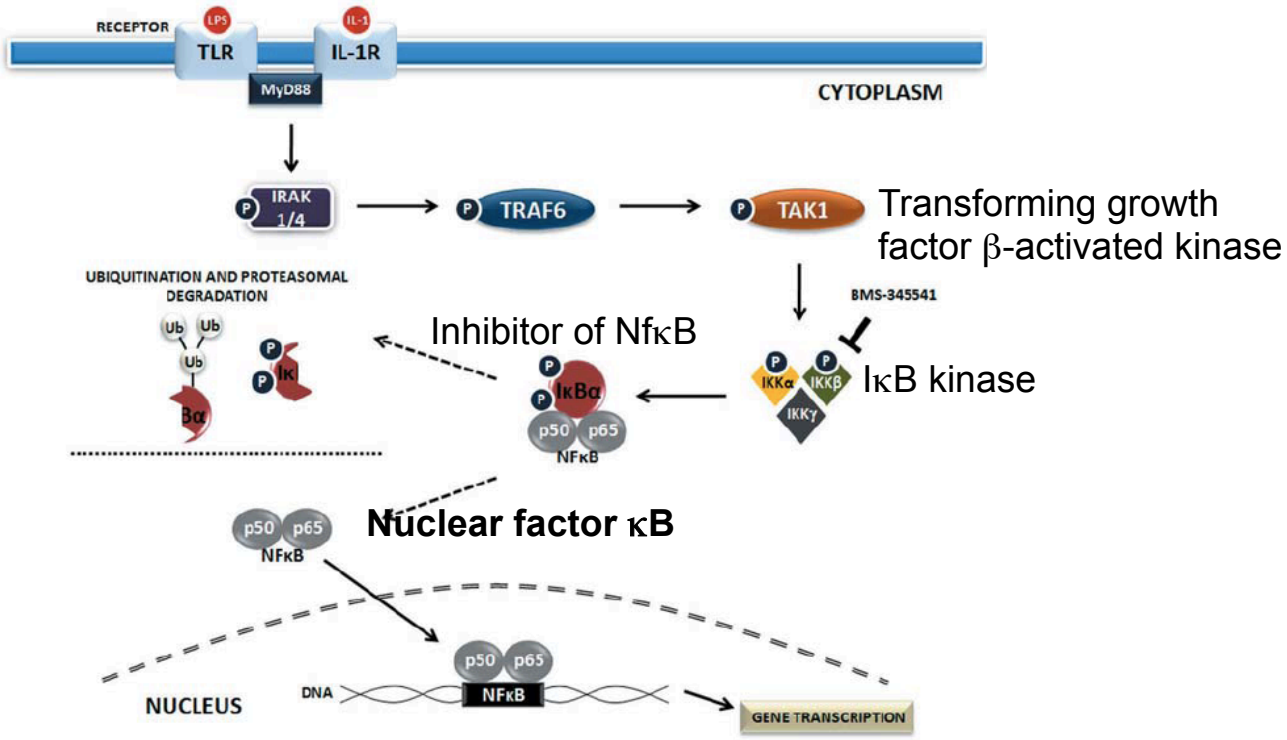
◆TLR signaling leads to osteoclastogenesis and increased net bone resorption in the local periodontal environment. Therefore, p38 *MAPK inhibitors can reduce the progression of PD by protecting against periodontal inflammation.*



Kirkwood, et al. 2007, Periodontol 2000, 43:294-315.

- Pro-inflammatory cytokines: IL-6, IL-11, IL-1, TNF-α, inerferon γ (IFN-γ), RANKL

NfkB is activated by TLR/interleukin receptor (IL-R) and tumor necrosis factor receptors (TNFR)



De Souza, et al., 2012, J Appl. Oral Sci., 20:128-38.

NfkB activates osteoclast differentiation (Osteoclastogenesis) and enzymes that degrade the connective tissue (matrix metalloproteinases). This results in alveolar bone resorption leading to tooth loss.

Receptor Activator of Nuclear Factor κ B (RANK) is expressed on osteoclasts and precursors (monocytes, macrophages) and is activated by RANKL found on stromal cells (lymphocytes) and osteoblasts.

JAK/STAT Pathway

- Cytokine signaling via janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway

- Many cytokines (Interleukins, interferons) and growth factors activate JAK/STAT signaling.

- Affects expression of genes with both pro-and anti-inflammatory activities.

- JAK/STAT pathway is associated with periodontitis.

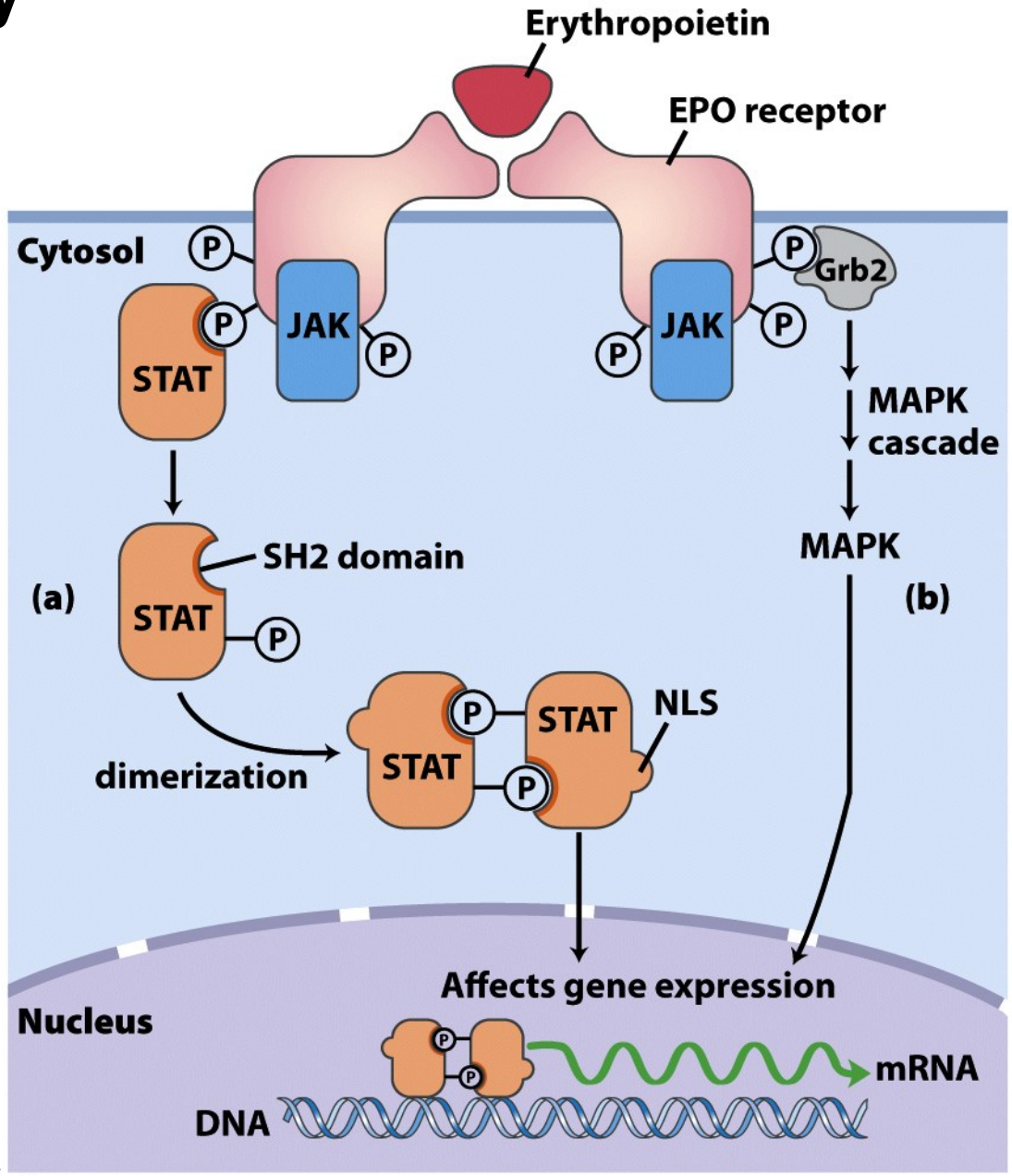


Figure 12-18
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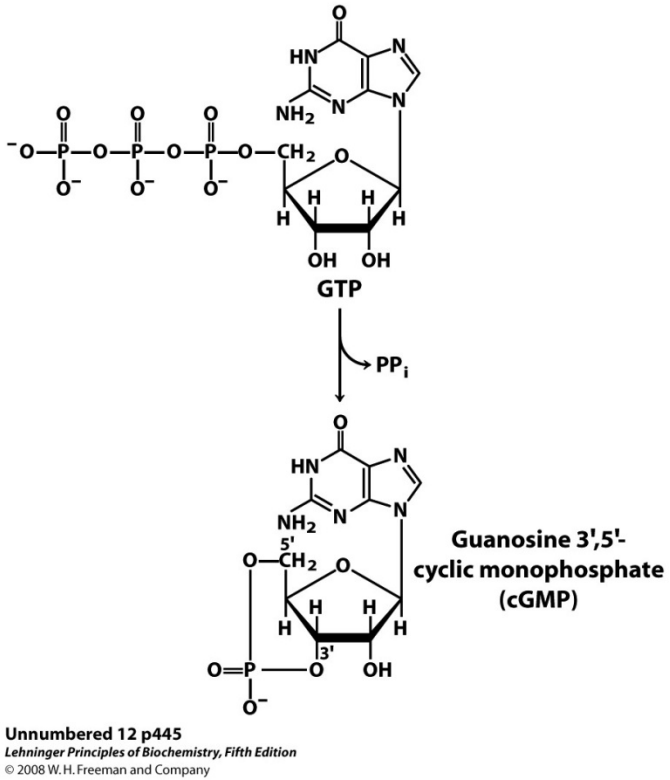
Guanylyl cyclases and signaling by cyclic GMP

- Guanylyl cyclases are a family of enzymes that catalyze the conversion of GTP to cGMP.
- The family comprises both **membrane-bound and soluble isoforms** that are expressed in nearly all cell types.
- They are regulated by diverse extracellular agonists that include peptide hormones, bacterial toxins, and free radicals, as well as intracellular molecules, such as calcium and adenine nucleotides.
- Stimulation of guanylyl cyclases and the resultant accumulation of cGMP activates **Protein Kinas G** (**cGMP-dependent protein kinases)**, **cGMP-regulated phosphodiesterases, and cyclic nucleotide-gated ion channels.**
- Guanylyl cyclases and cGMP-mediated signaling cascades play a central role in the regulation of diverse (patho)physiological processes, including vascular smooth muscle motility, intestinal fluid and electrolyte homeostasis, and retinal phototransduction.

Receptor guanylyl kinases:
Two types of Gyanylyl Cyclases

Atrial naturiuretic factor



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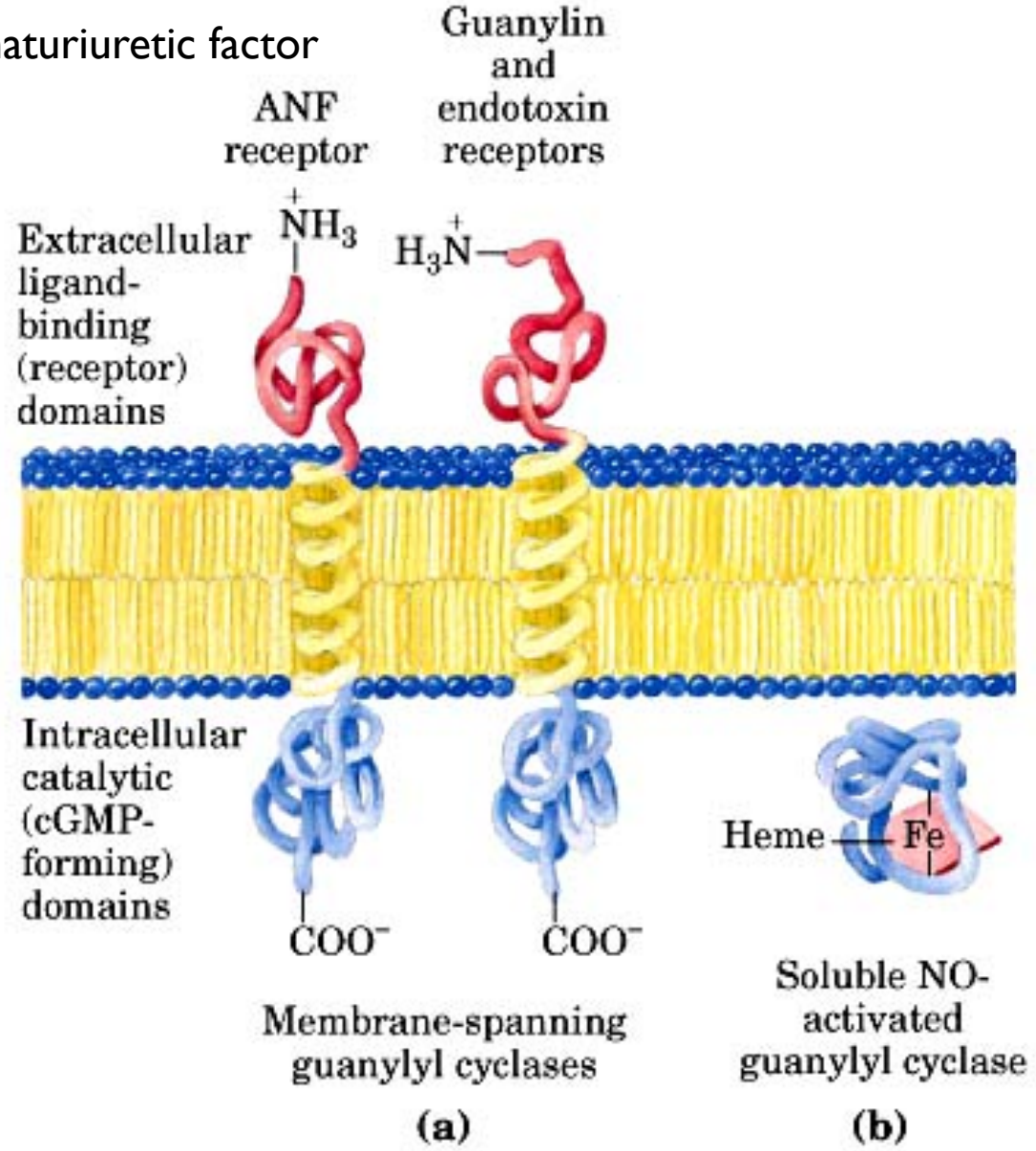
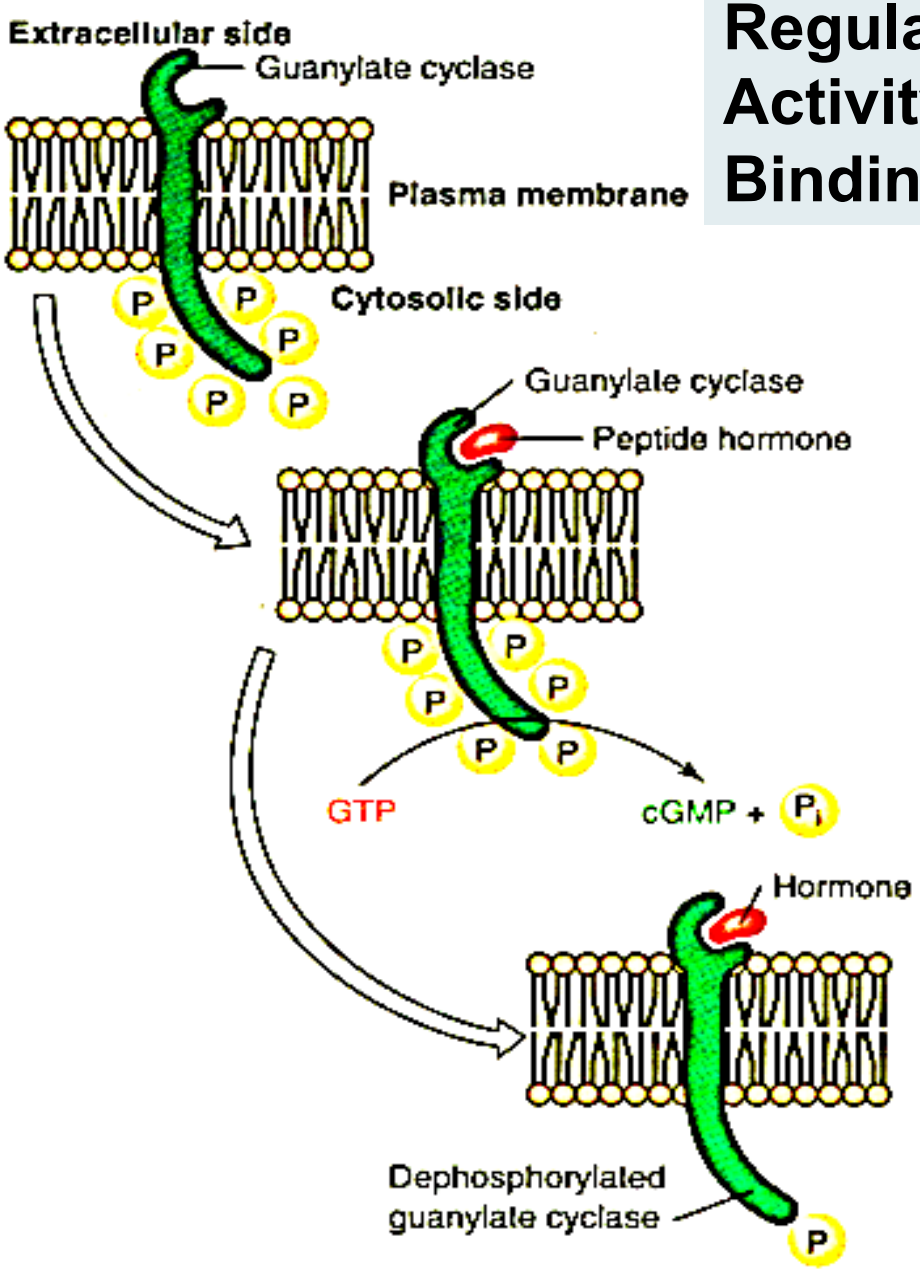


Regulation of Guanylyl Cyclase Activity after Peptide Hormone Binding



- The enzyme exists in a highly phosphorylated state under normal conditions.

- Binding of hormone (ANF, guanylin) enhances enzyme activity, followed by a rapid dephospho-rylation of GC and a return of activity to basal state despite continued presence of peptide.

- cGMP activates Protein Kinase G, which in turn phosphorylates cellular proteins.

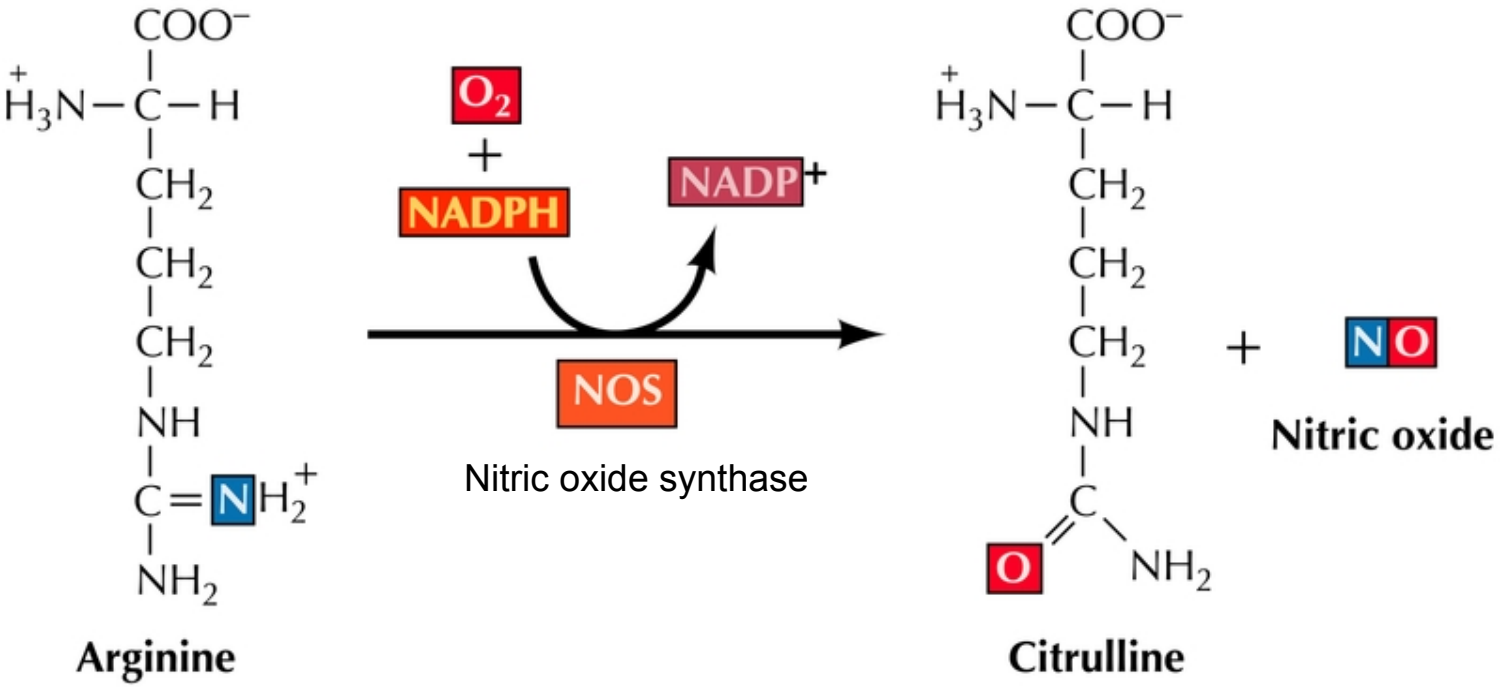
Protein Kinase G

- cGMP-dependent protein kinase or Protein Kinase G (PKG) is a **serine/threonine**-specific protein kinase that is activated by cGMP.
- PKG phosphorylates a number of biologically important targets and is implicated in the regulation of smooth muscle relaxation, platelet function, sperm metabolism, cell division, and nucleic acid synthesis.
- Two PKG genes, coding for PKG type I (PKG-I) and type II (PKG-II), have been identified in mammals.

What is Nitric Oxide?

- First described in 1979 as a potent relaxant of peripheral vascular smooth muscle.
- Used by the body as a signaling molecule.
- Serves different functions depending on body system. i.e. neurotransmitter, vasodilator, bactericide.
- Environmental Pollutant
- First gas known to act as a biological messenger

Nitric Oxide Synthase (NOS) activates soluble guanylyl cyclase

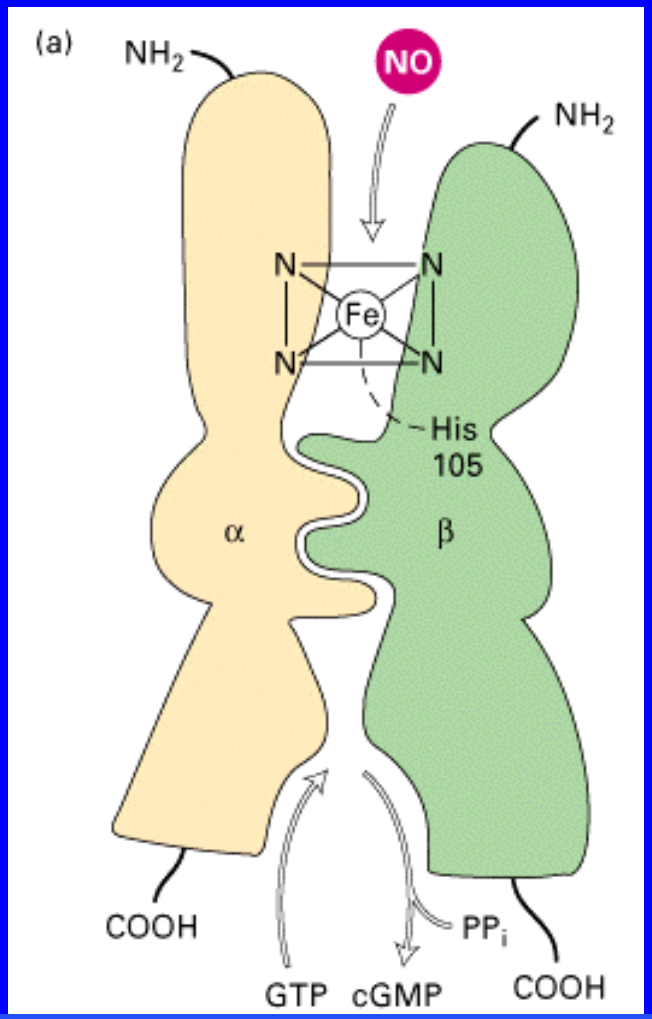


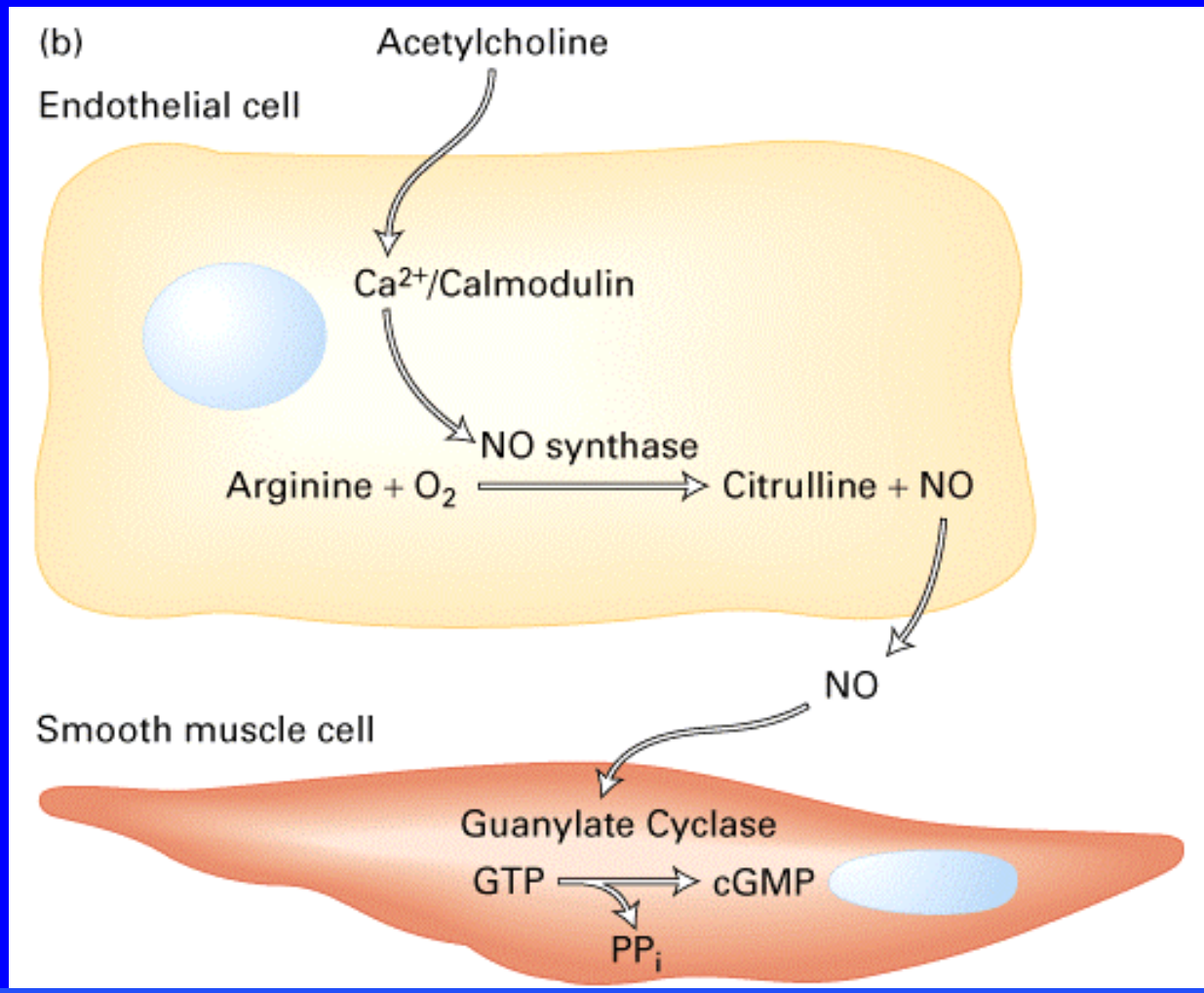
◆ NO can be an inflammatory mediator in bone resorption and **periodontic disease**

◆ NOS is found in vascular endothelial cells and activates guanylyl cyclase

◆ NOS can be constitutive (e-NOS) or induced (i-NOS). i-NOS expression is increased in periodontal disease.

Regulation of contractility of arterial smooth muscle by NO and cGMP





NO activates the soluble GC to produce cGMP.
NO is a short-lived messenger.
NO analogs are useful for relaxation of cardiac muscle.

Protein Kinase G

- Receptor guanylyl cyclase (GC) or NO-mediated GC activity increases cGMP levels.
- Protein Kinase G is stimulated by the elevation of cytosolic cGMP.
- Protein kinase G is a serine/threonine kinase that regulates smooth muscle relaxation, platelet function, sperm metabolism, cell division, and nucleic acid synthesis.

Summary for Protein Kinases

- Many amino acid-derived hormones or polypeptide hormones bind to cell membrane receptors and transmit their signal by:

1) ⇑ cAMP and transmission through the PKA pathway;

2) triggering PIP_2 hydrolysis and stimulation of PKC and IP_3-Ca^{+2} pathways;

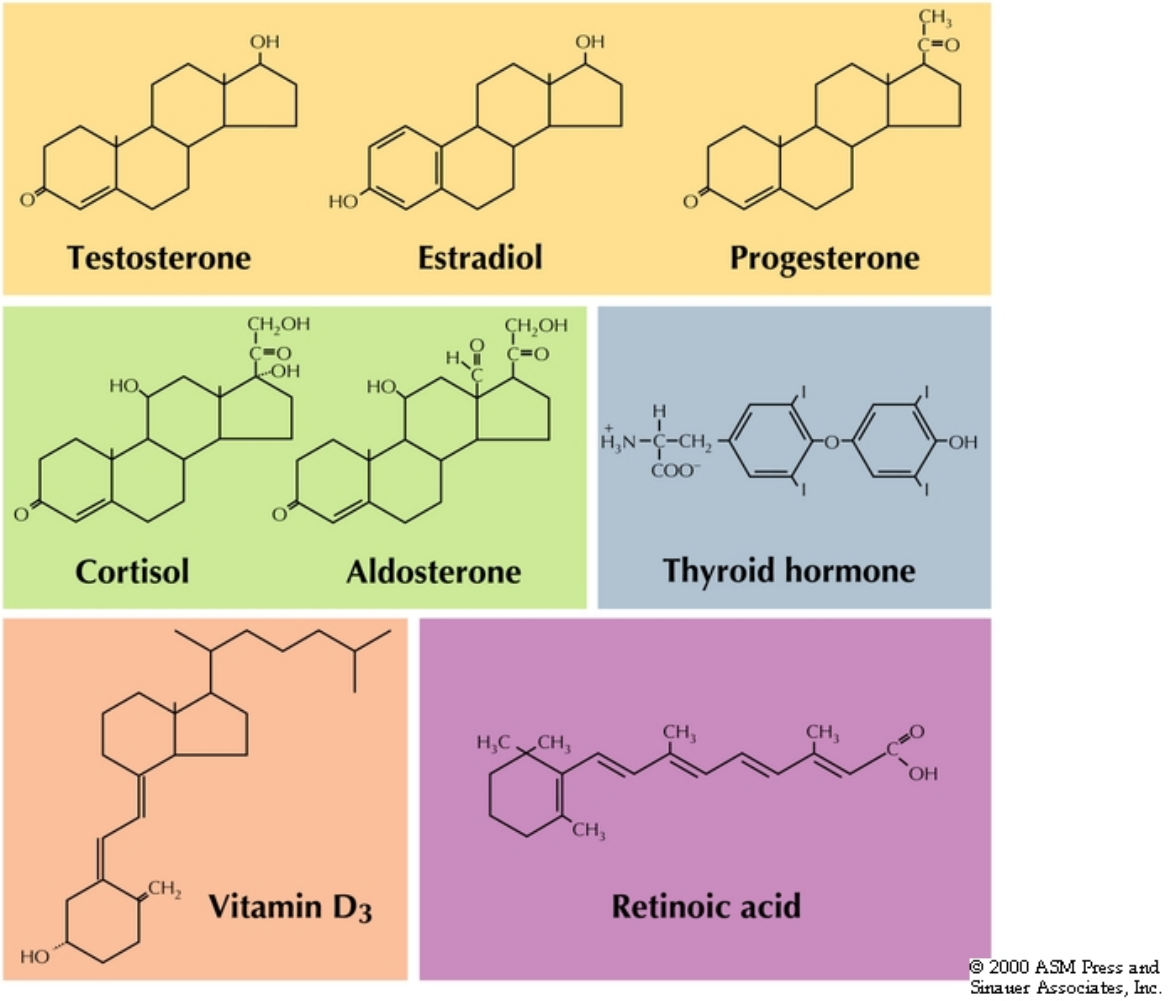
3) ⇑ cGMP levels and activation of protein kinase G (PKG) pathway

- PKA, PKC and PKG phosphorylate proteins on *serine or threonine* residues while other kinases (receptor TKs or cytoplasmic) phosphorylate *tyrosine* residues

Nuclear Receptor Superfamily

- large family of structurally related ligand-inducible transcription factors, including:
  - steroid receptors (SRs),
  - thyroid/retinoids receptors (TR, RARs and RXRs), vitamin D receptors (VDR),
  - estrogen receptors (ERα and ERβ),
  - and orphan receptors for which no ligand has been yet identified.
- While having in common a modular structure, they are activated by distinct lipophilic (hydrophobic) small molecules such as glucocorticoids, progesterone, estrogens, retinoids, and fatty acid derivatives.
- **estrogen and progesterone receptors play a role in the development of some forms of gingival or periodontal disease**

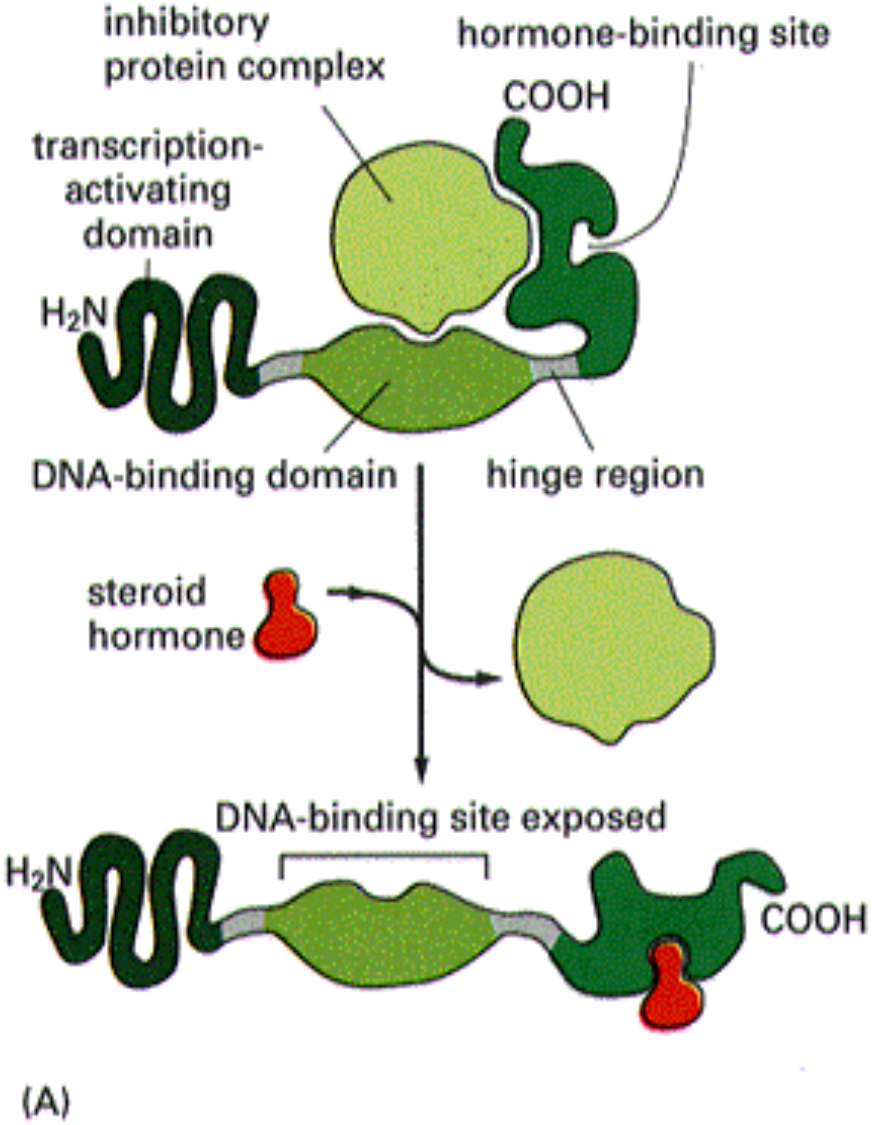
Steroid Hormones: Critical for development, differentiation, and disease

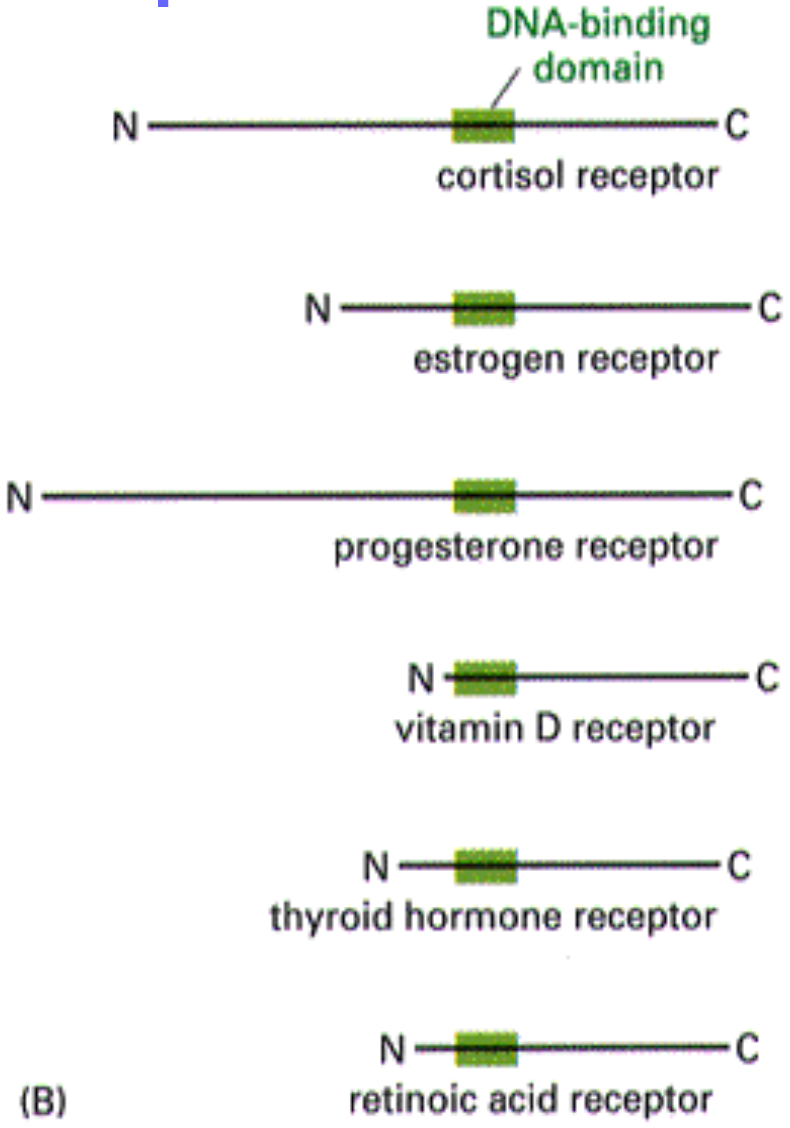


- Steroid hormones are synthesized from cholesterol in the gonads and adrenal glands.

Fig.15-12

Steroid hormone receptors



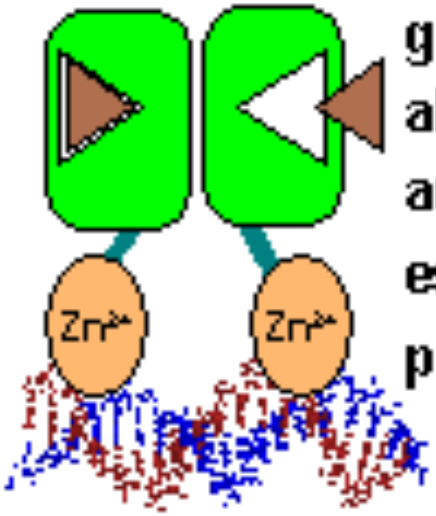


Steroid hormone receptors can act as transcription factors

Nuclear Receptor Superfamily

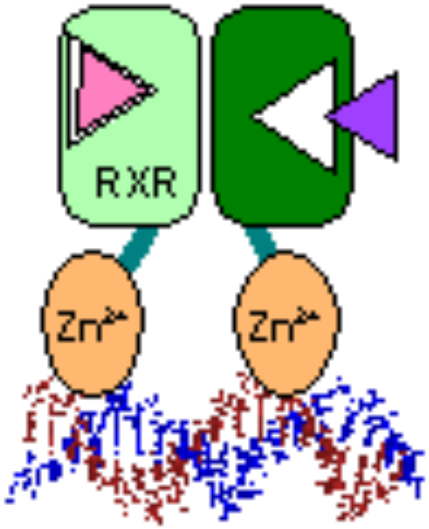
structural and functional organization of the multidomain nuclear receptors

steroid receptor homodimers



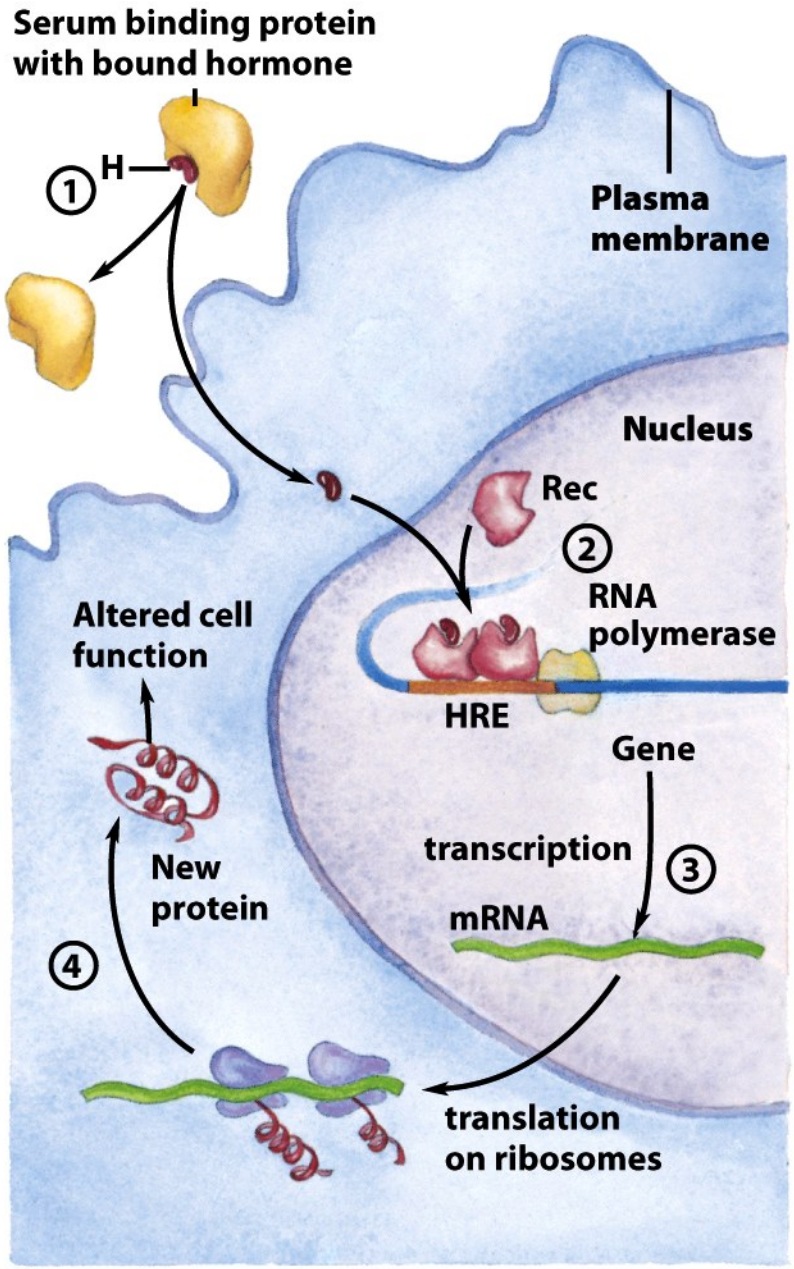
glucocorticoid
aldosterone
androgen
estradiol
progesterone

9-retinoic acid receptor (RXR) heterodimers



thyroid hormone
all-*trans* retinoic acid
dihydroxy-D_3
eicosanoids
fatty acids
bile acids

modified from J. Olefsky (2001) J Biol Chem 276:36863



1. Hormone (H), carried to the target tissue on serum binding proteins, diffuses across the plasma membrane and binds to its specific receptor protein (Rec) in the nucleus.

2. Hormone binding changes the conformation of Rec; it forms homo- or heterodimers with other hormone-receptor complexes and binds to specific regulatory regions called hormone response elements (HREs) in the DNA adjacent to specific genes.

3. Binding regulates transcription of the adjacent gene(s), increasing or decreasing the rate of mRNA formation.

4. Altered levels of the hormone-regulated gene product produce the cellular response to the hormone.

Figure 12-29

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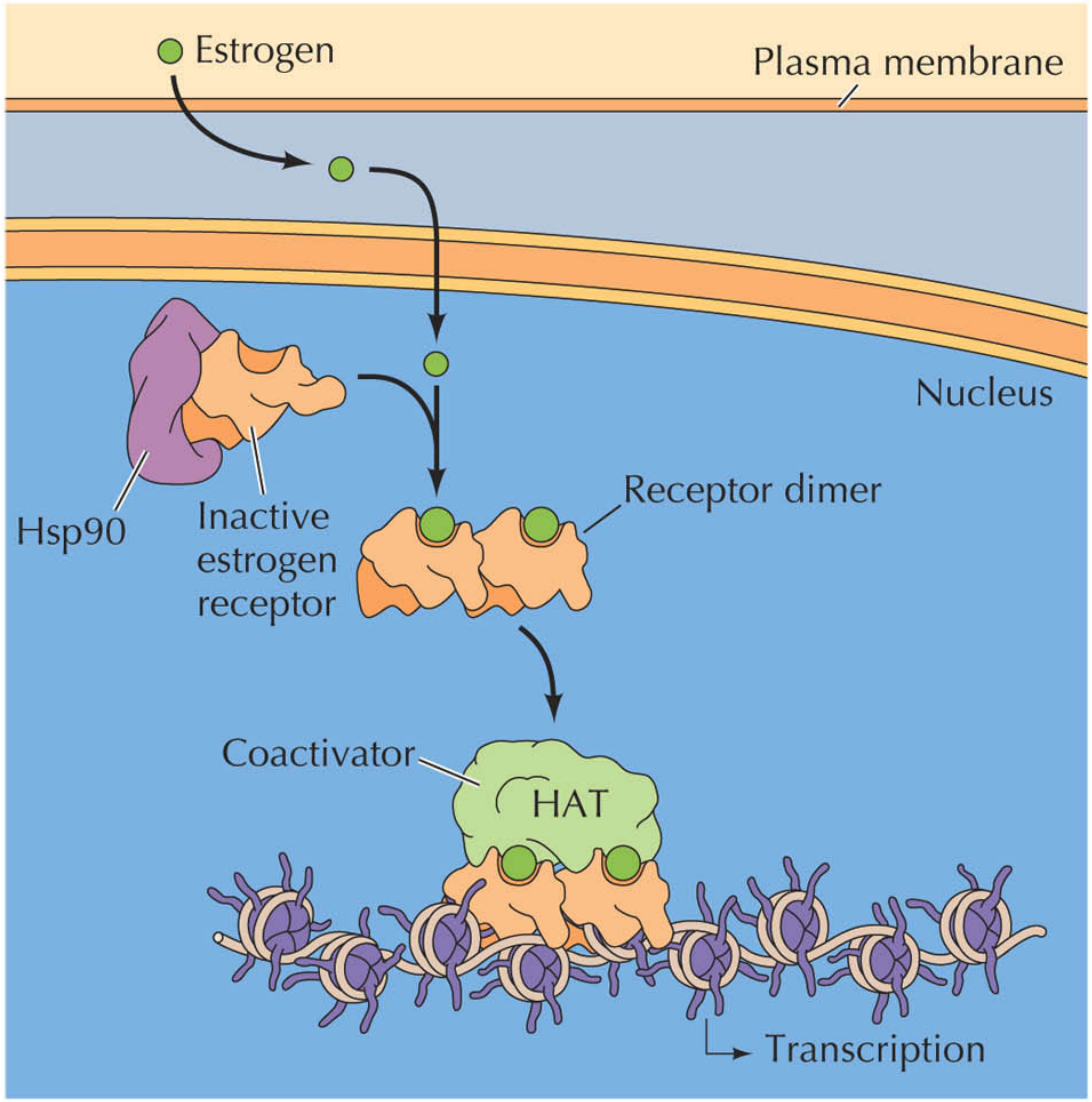
Estrogen Signaling



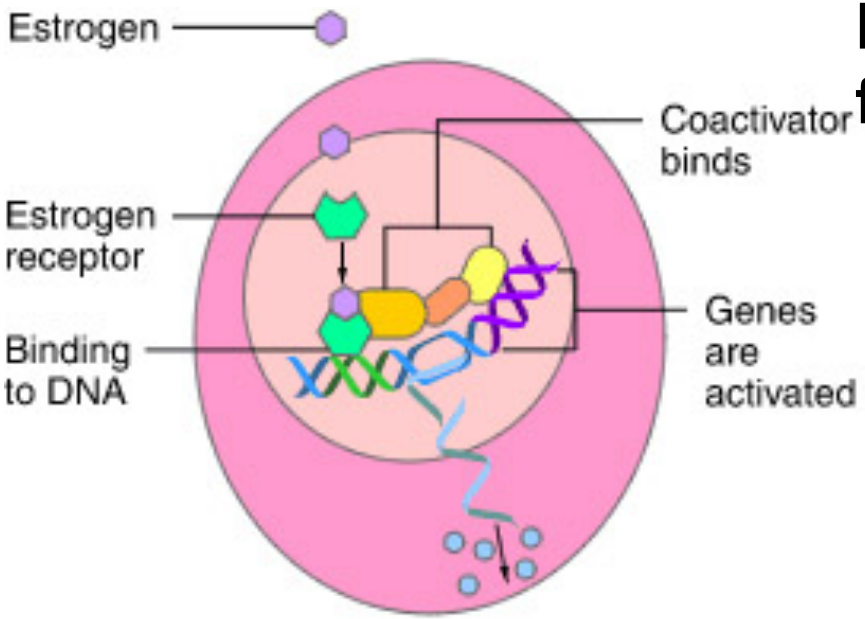
THE CELL, Third Edition, Figure 13.3 ASM Press and Sinauer Associates, Inc.

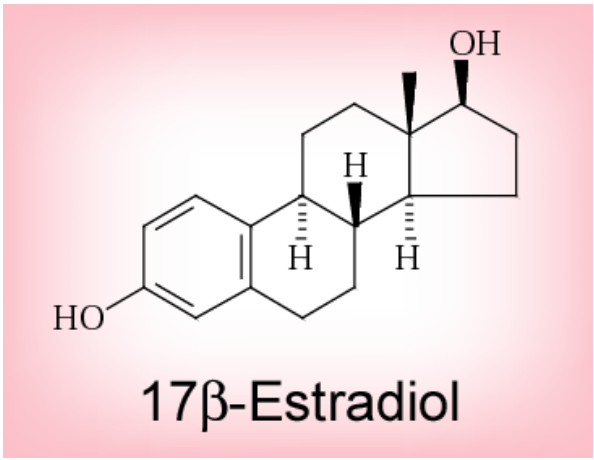
Estrogen diffuses across the pm and binds estrogen receptor (ER).

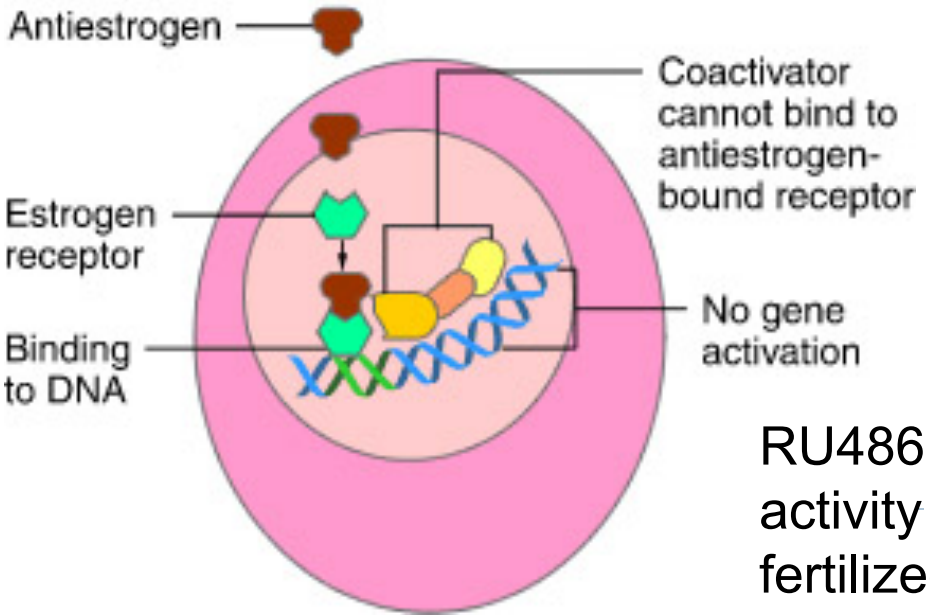
Estrogen binding displaces Heat shock protein Hsp90 from ER.

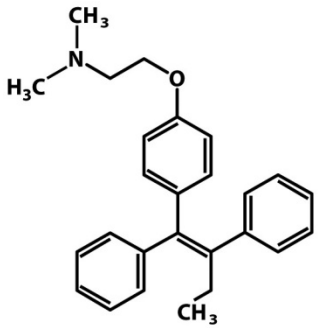
Receptor dimers bind coactivators (Histone acetyltransferase) to promote transcription of estrogen responsive genes.

Estrogen mimetics can be used for breast cancer therapy

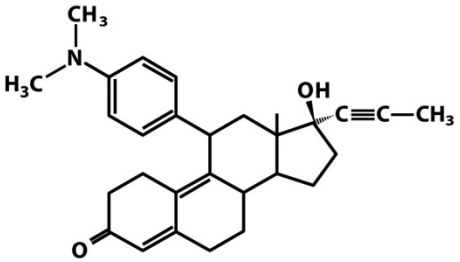








Tamoxifen



Mifepristone (RU486)

Unnumbered 12 p456
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RU486 blocks progesterone receptor activity and blocks implantation of the fertilized ovum in the uterus