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Programme: M.Sc., Biochemistry

Course Title : Biochemistry of Signal Transduction
Course Code : BC203CR

Unit-2
Cell Surface Receptors and Signaling

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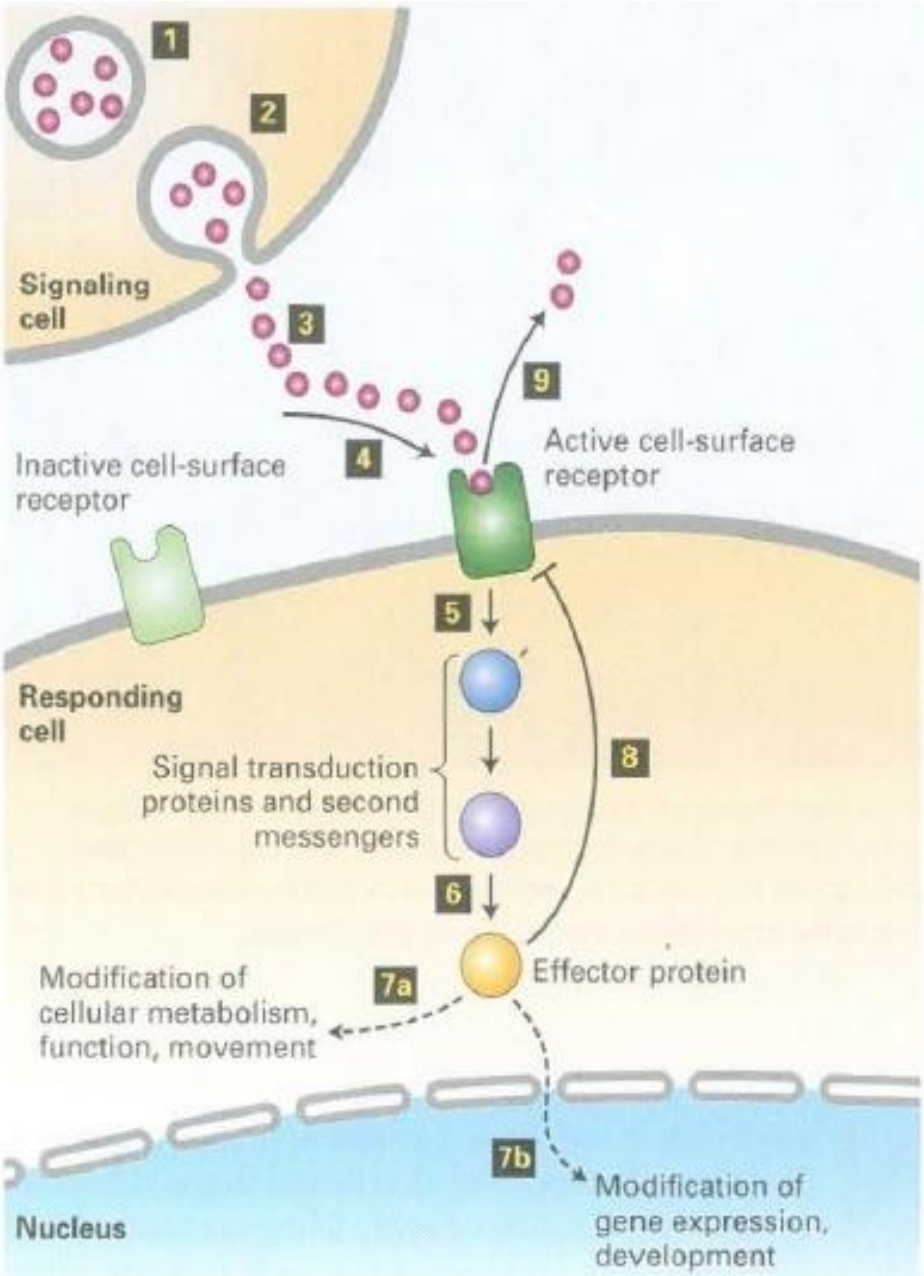
Common Receptor Proteins

- G-protein coupled receptors
- Receptor tyrosine-kinases
- Ion channel receptors

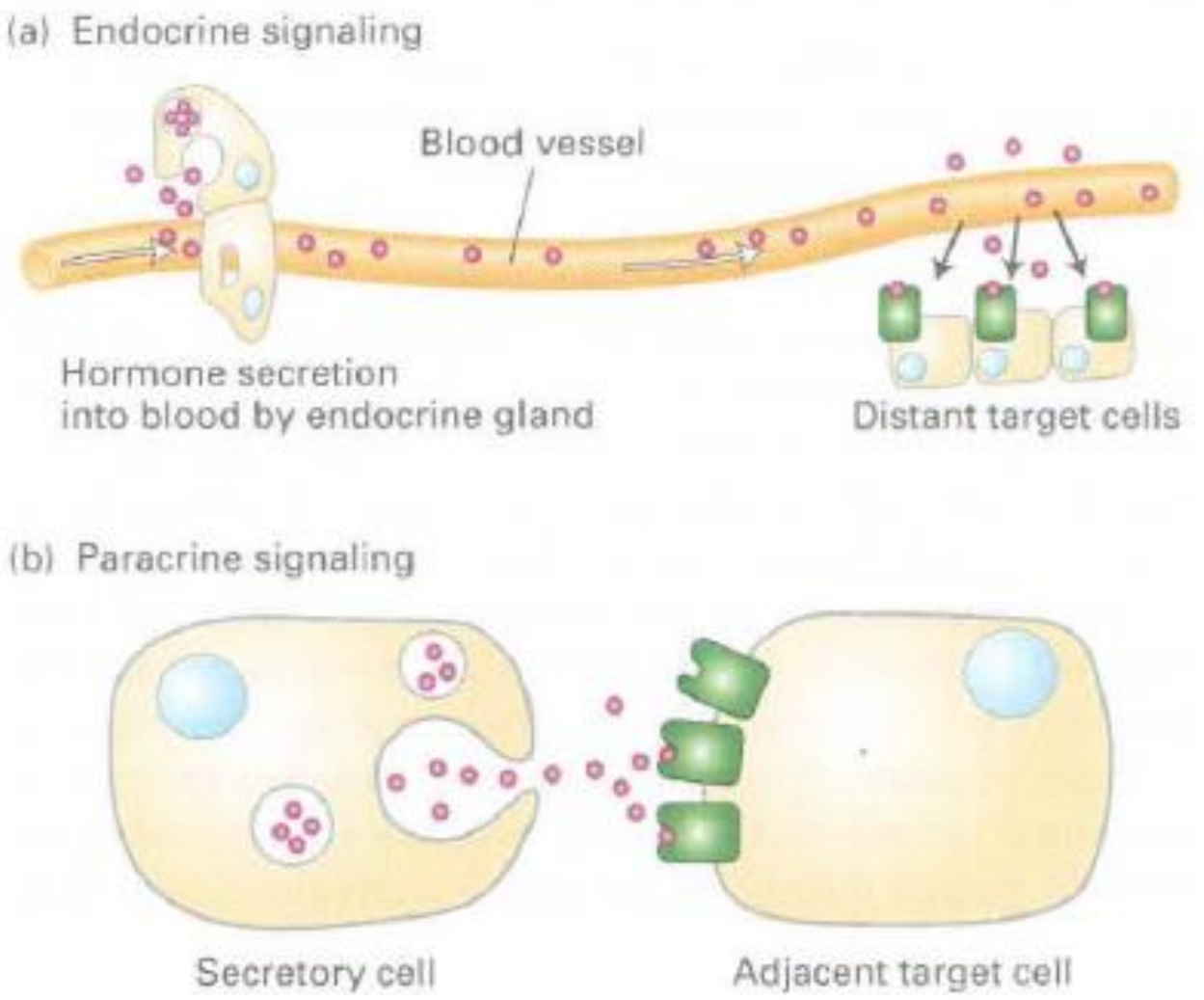
Signaling by cell-surface receptors



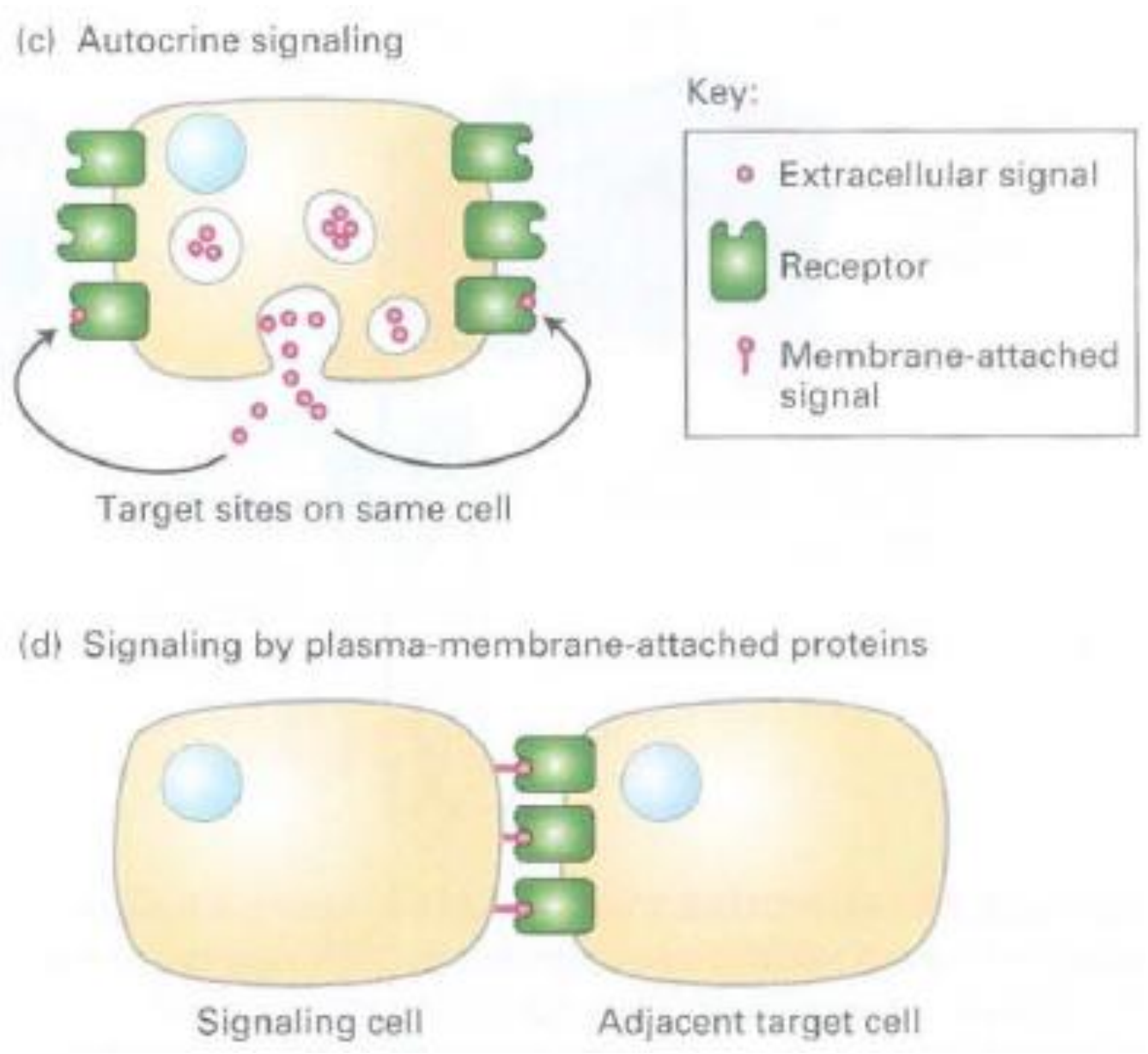
1. Synthesis of the signaling molecule by the signaling cell and its incorporation into small intracellular vesicles
2. Release into the extracellular space by exocytosis
3. Transport of the signal to the target cell
4. Binding of the signaling molecule to a specific cell-surface receptor protein triggers a conformational change in the receptor, thus activating it
5. Activated receptor then activates one or more downstream signal transduction proteins or small-molecule second messengers
6. Activation of one or more effector proteins
7. Short-term change in cellular function, metabolism, or movement or a long-term change in gene expression or development
8. Termination or down-modulation of the cellular response is caused by negative feedback from intracellular signaling molecules
9. By removal of the extracellular signal

- The overall process of converting extracellular signals into intracellular responses, as well as the individual steps in this process, is termed signal transduction
- External signals include
  1. Membrane-anchored and secreted proteins or peptides (e.g., vasopressin and insulin)
  2. Small hydrophobic molecules (e.g., steroid hormones and thyroxine)
  3. Small hydrophilic molecules (e.g., epinephrine)
  4. Gases (e.g.,0_2, nitric oxide)
  5. Physical stimuli (e.g., light).

Types of extracellular signaling

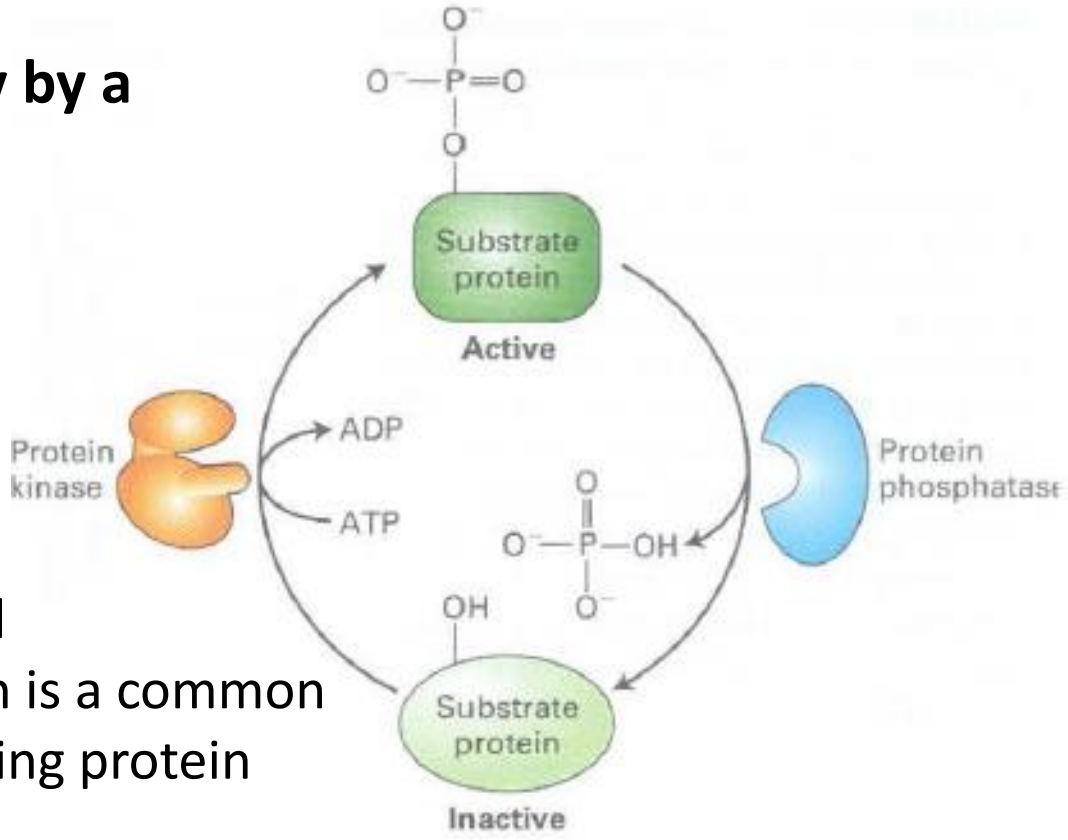


Cell-to-cell signaling by extracellular chemicals occurs over distances from a few micrometers in autocrine and paracrine signaling to several meters in endocrine signaling.



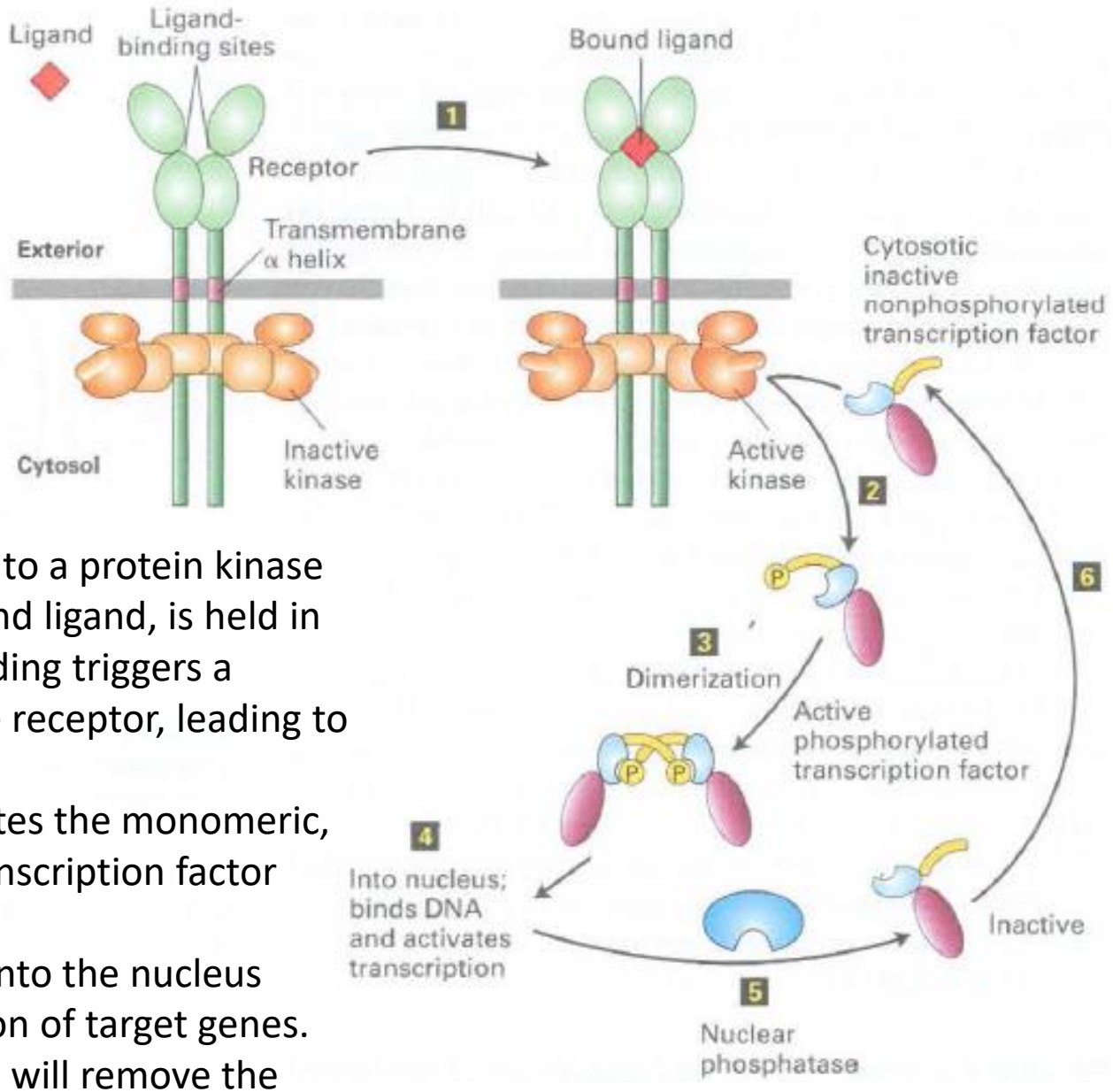
Proteins attached to the plasma membrane of one cell can interact directly with cell-surface receptors on adjacent cells.

Regulation of protein activity by a kinase/phosphatase switch.



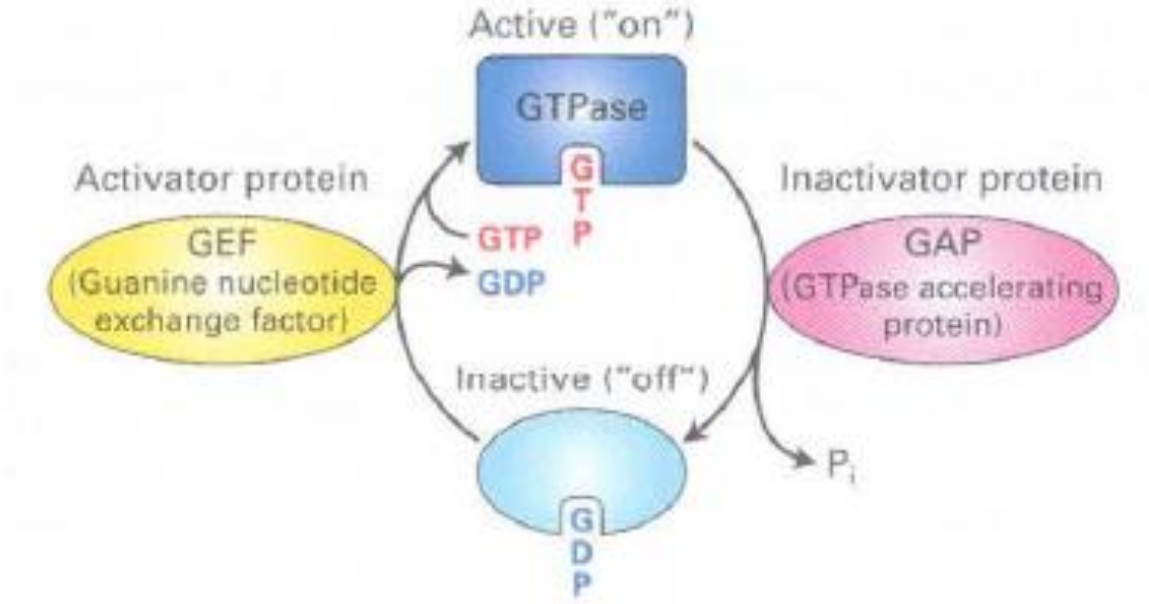
1. The cyclic phosphorylation and dephosphorylation of a protein is a common cellular mechanism for regulating protein activity.
2. In this example, the target, or substrate, protein is inactive(light green) when not phosphorylated and active (dark green) when phosphorylated; some proteins have the opposite pattern.
3. Both the protein kinase and the phosphatase act only on specific target proteins, and their activities are usually highly regulated.

A simple signal transduction pathway involving one kinase and one target protein

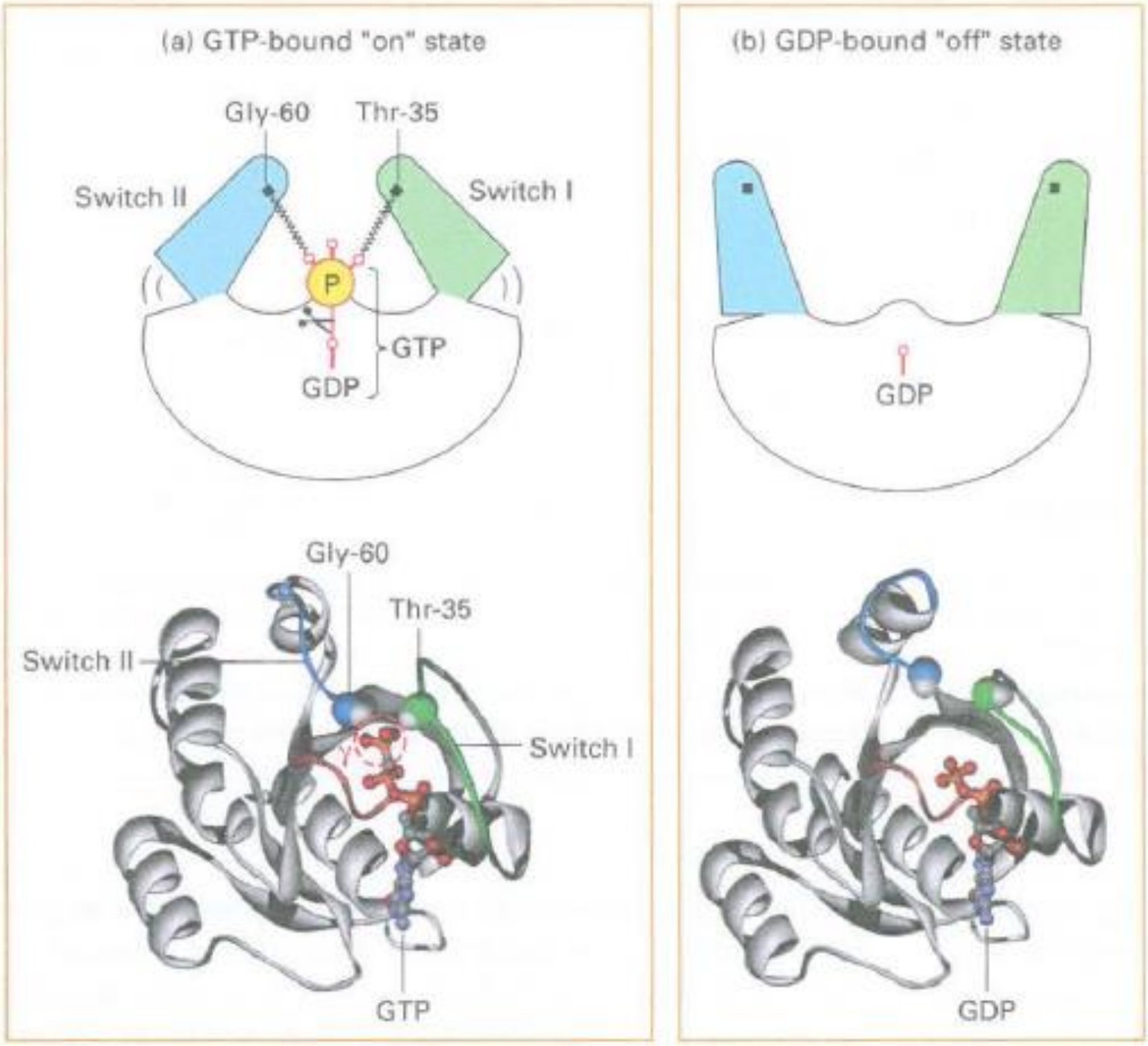


1. The receptor is tightly bound to a protein kinase that, in the absence of a bound ligand, is held in the inactive state. Ligand binding triggers a conformational change in the receptor, leading to activation of the kinase.
2. The kinase then phosphorylates the monomeric, inactive form of a specific transcription factor
3. leading to its dimerization
4. movement from the cytosol into the nucleus where it activates transcription of target genes.
5. A phosphatase in the nucleus will remove the phosphate group from the transcription factor
6. causing it to form the inactive monomer and then move back into the cytosol.

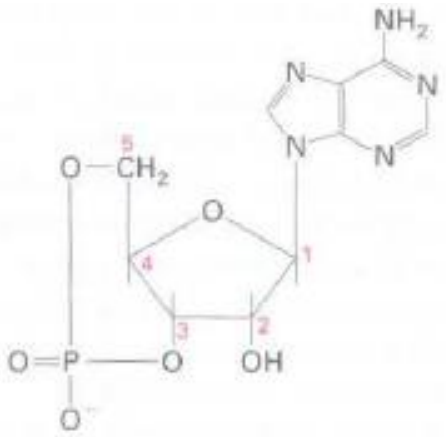
GTPase switch proteins cycle between active and inactive forms



- The switch protein is active when it has bound GTP and inactive when it has bound GDP.
- Conversion of the active into the inactive form by hydrolysis of the bound GTP is accelerated by GAPs(GTPase-accelerating proteins) and other proteins.
- Reactivation is promoted by GEFs (guanine nucleotide exchange factors) that catalyze the dissociation of the bound GDP and its replacement by GTP.

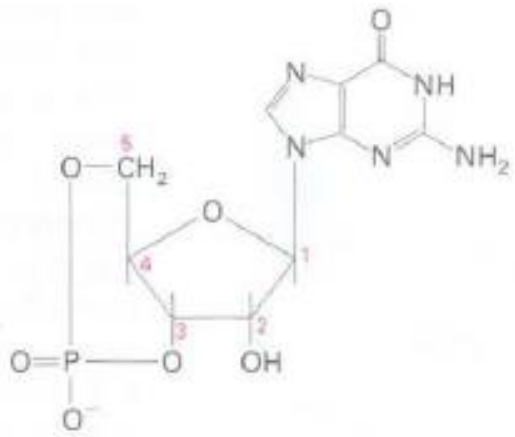

(a) GTP-bound "on" state
Gly-60
Thr-35
Switch II
Switch I
P
GTP
GDP
(b) GDP-bound "off" state
GDP
Gly-60
Thr-35
Switch II
Switch I
GTP
GDP

Intracellular "Second Messengers" Transmit and Amplify Signals from Many Receptors



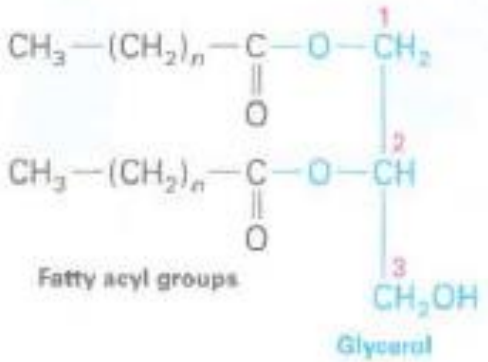
3',5'-Cyclic AMP
(cAMP)

Activates protein kinase A (PKA)



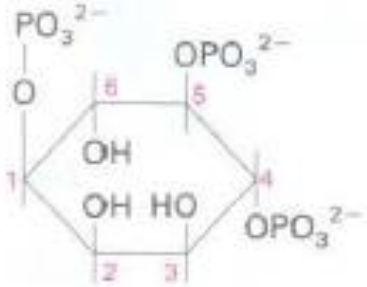
3',5'-Cyclic GMP
(cGMP)

Activates protein kinase G (PKG) and opens cation channels in rod cells



1,2-Diacylglycerol
(DAG)

Activates protein kinase C (PKC)

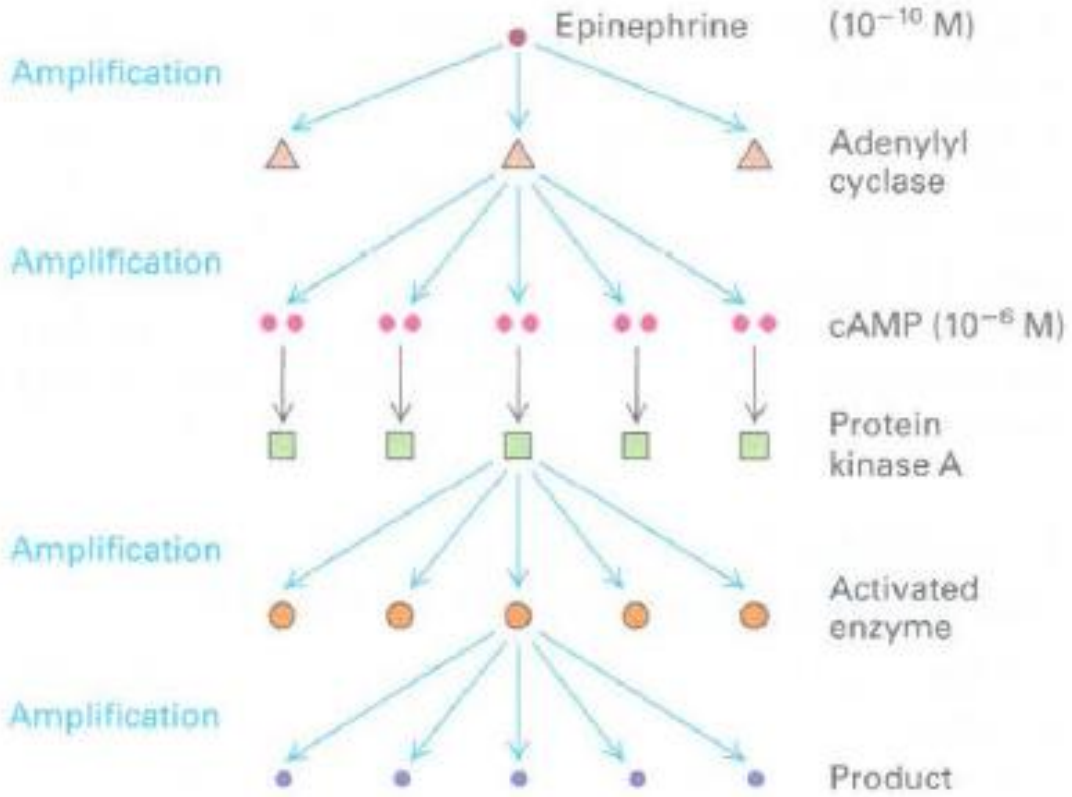


Inositol
1,4,5-trisphosphate
(IP_3)

Opens Ca^{2+} channels in the endoplasmic reticulum

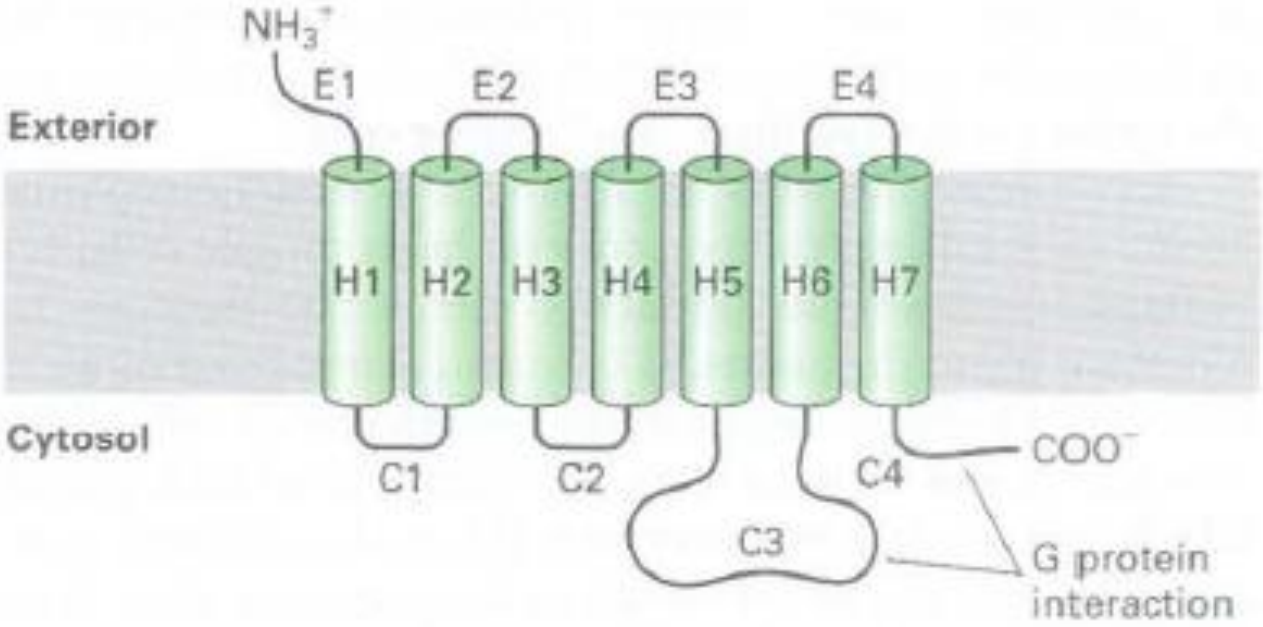
- $Ca2^{+}$, cAMP, and other nonprotein, low-molecular-weight intracellular molecules act as "second messengers”,relaying and often amplifying the signal of the"first messenger," that is, the ligand.
- Binding of ligand to cell-surface receptors often results in a rapid increase (or, occasionally, decrease) in the intracellular concentration of these ions or molecules.

Amplification of an extracellular signal



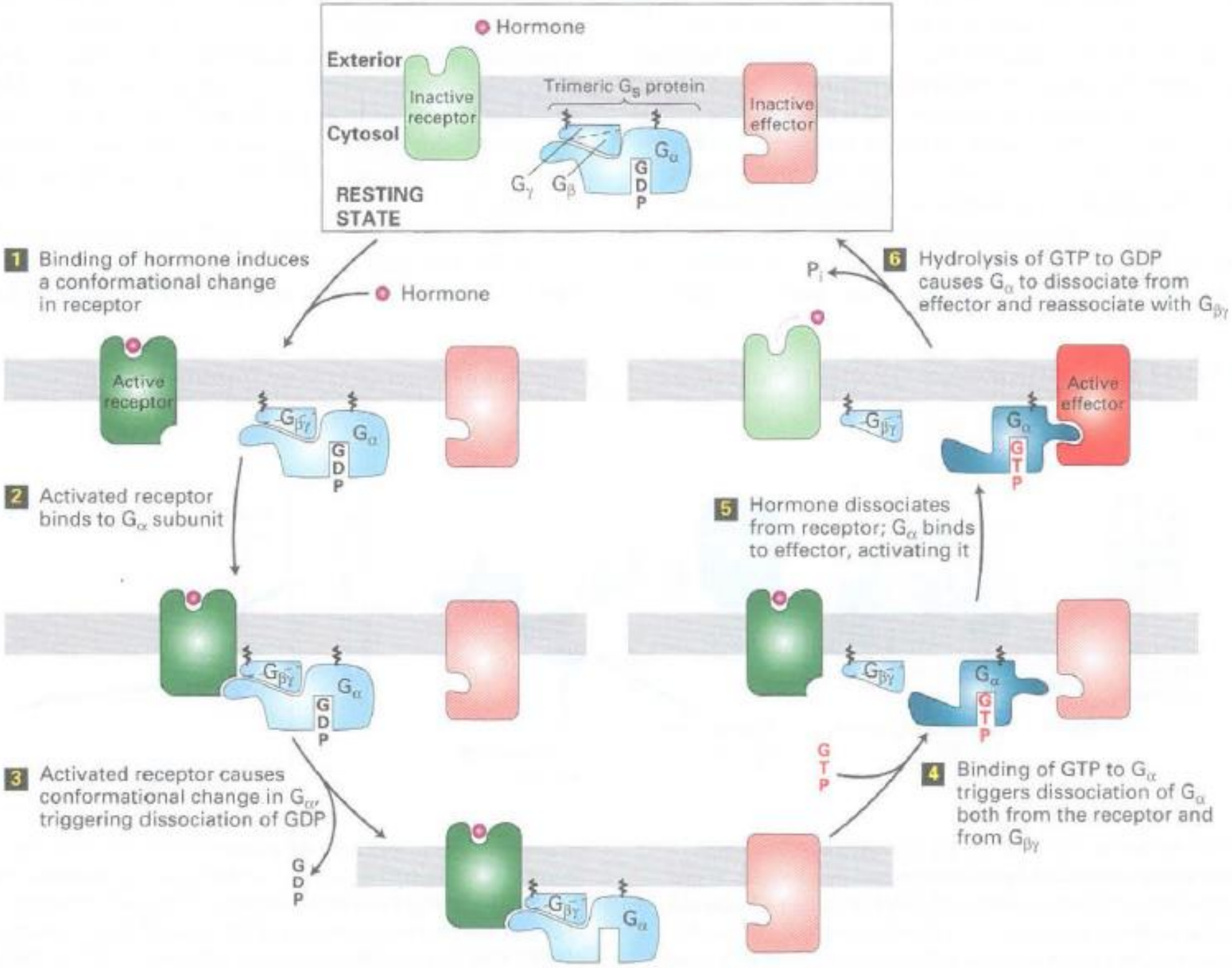
1. Binding of a single epinephrine molecule to one G protein coupled receptor molecule induces activation of several molecules of adenylyl cyclase, the enzyme that catalyzes the synthesis of cyclic AMP
2. Each of these enzyme synthesizes a large number of cAMP molecules, the first level of amplification. Two molecules of cAMP activate one molecule of protein kinase A (PKA)
3. Each activated PKA phosphorylates and activates multiple target proteins. This second level of amplification may involve several sequential reactions in which the product of one reaction activates the enzyme catalyzing the next reaction.
4. The more steps in such a cascade, the greater the signal amplification possible.

G Protein-Coupled Receptors: Structure and Mechanism



All receptors of this type have the same orientation in the membrane and contain

1. Seven transmembrane α-helical regions (Hl-H7)
2. Four extracellular segments (E l-E4)
3. Four cytosolic segments (Cl-C4) -The carboxyl-terminal segment (C4), the C3 loop, and, in some receptors, also the C2 loop are involved in interactions with a coupled trimeric G protein.

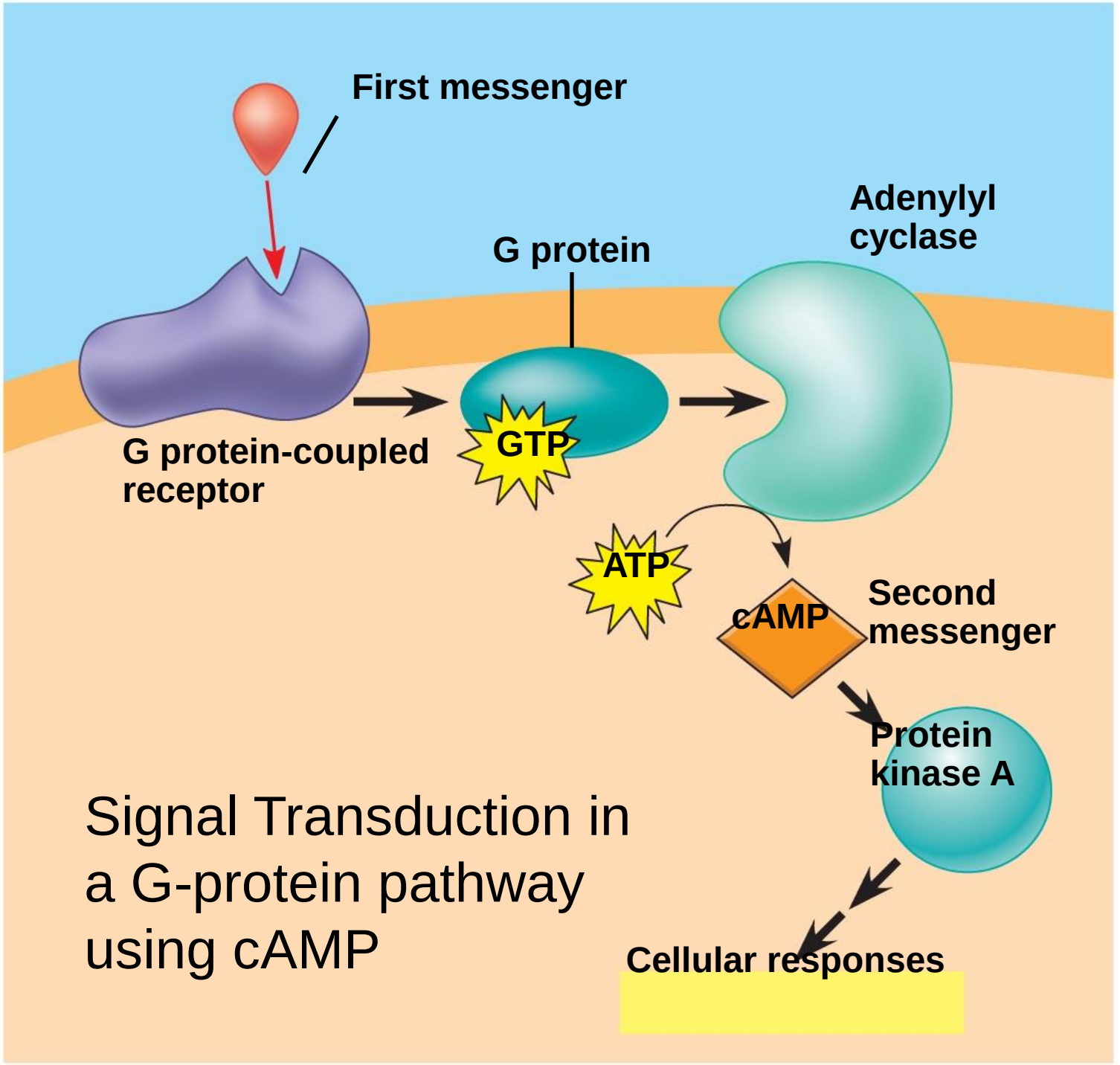

Hormone
Exterior
Cytosol
Inactive receptor
Trimeric G_s protein
Inactive effector
G_γ
G_β
G_α
G D P
RESTING STATE
1 Binding of hormone induces a conformational change in receptor
Hormone
Active receptor
$G_{\beta\gamma}$
G_α
G D P
2 Activated receptor binds to G_α subunit
3 Activated receptor causes conformational change in G_α, triggering dissociation of GDP
G D P
4 Binding of GTP to G_α triggers dissociation of G_α both from the receptor and from $G_{\beta\gamma}$
G T P
5 Hormone dissociates from receptor; G_α binds to effector, activating it
Active effector
G T P
6 Hydrolysis of GTP to GDP causes G_α to dissociate from effector and reassociate with $G_{\beta\gamma}$
P_i

1. The Gα and Gβγ subunits of trimeric G proteins are tethered to the membrane by covalently attached lipid molecules
2. Following ligand binding, exchange of GDP with GTP, and dissociation of the G protein subunits occur
3. The free Gα-GTP binds to and activates an effector protein.
4. Hydrolysis of GTP terminates signaling and leads to reassembly of the trimeric G protein, returning the system to the resting state.
5. Binding of another ligand molecule causes repetition of the cycle.
6. In some pathways, the effector protein is activated by the free Gβγ subunit.

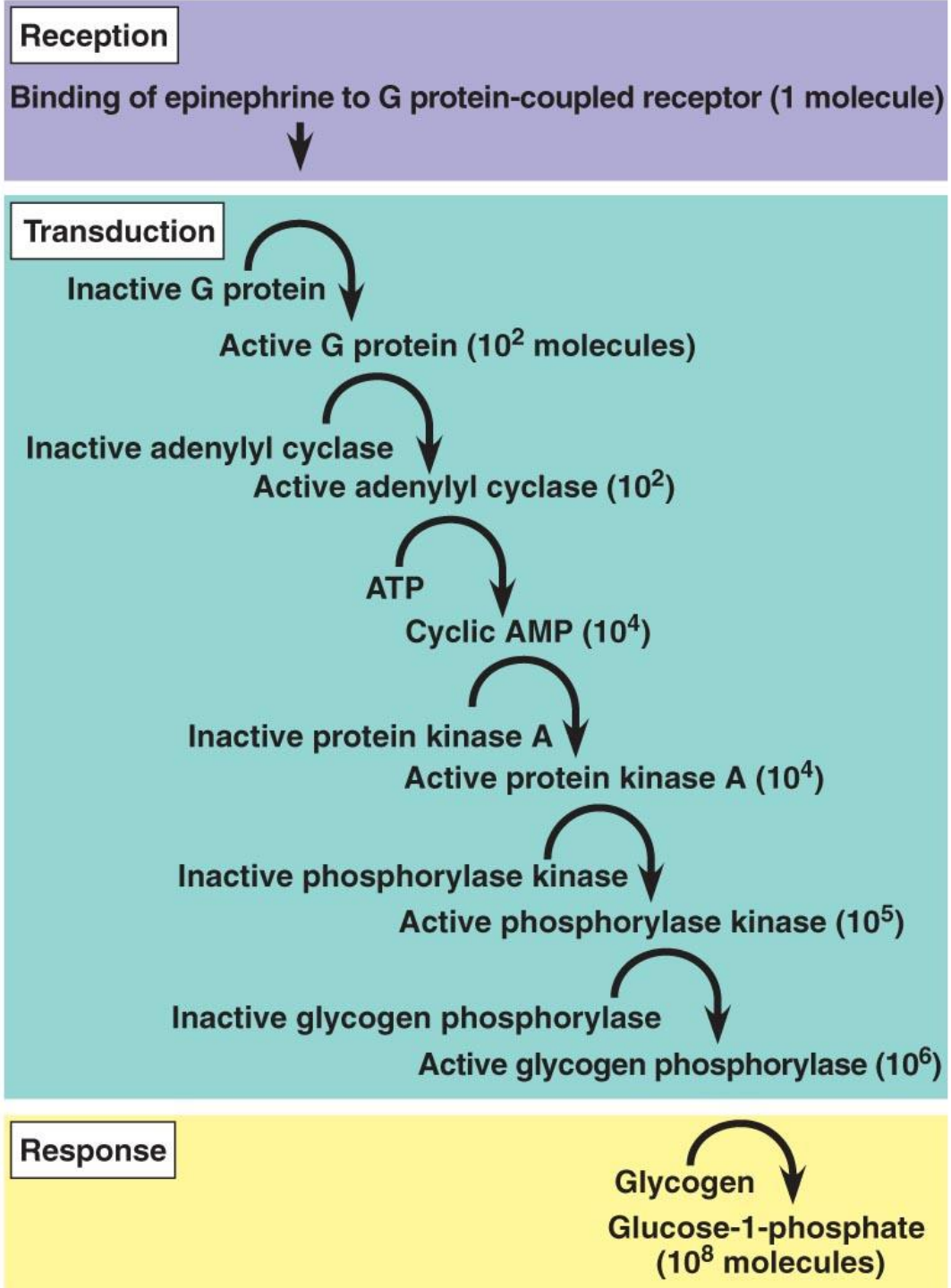
Different G Proteins Are Activated by Different GPCRs and In Turn Regulate Different Effector Proteins

TABLE 15-1 Major Classes of Mammalian Trimeric G Proteins and Their Effectors

G_α Class	Associated Effector	2nd Messenger	Receptor Examples
$G_{\alpha s}$	Adenylyl cyclase	cAMP (increased)	β-Adrenergic (epinephrine) receptor; receptors for glucagon, serotonin, vasopressin
$G_{\alpha i}$	Adenylyl cyclase K^+ channel ($G_{\beta\gamma}$ activates effector)	cAMP (decreased) Change in membrane potential	α_2-Adrenergic receptor Muscarinic acetylcholine receptor
$G_{\alpha olf}$	Adenylyl cyclase	cAMP (increased)	Odorant receptors in nose
$G_{\alpha q}$	Phospholipase C	IP_3, DAG (increased)	α_1-Adrenergic receptor
$G_{\alpha o}$	Phospholipase C	IP_3, DAG (increased)	Acetylcholine receptor in endothelial cells
$G_{\alpha t}$	cGMP phosphodiesterase	cGMP (decreased)	Rhodopsin (light receptor) in rod cells



Signal Transduction in a G-protein pathway using cAMP

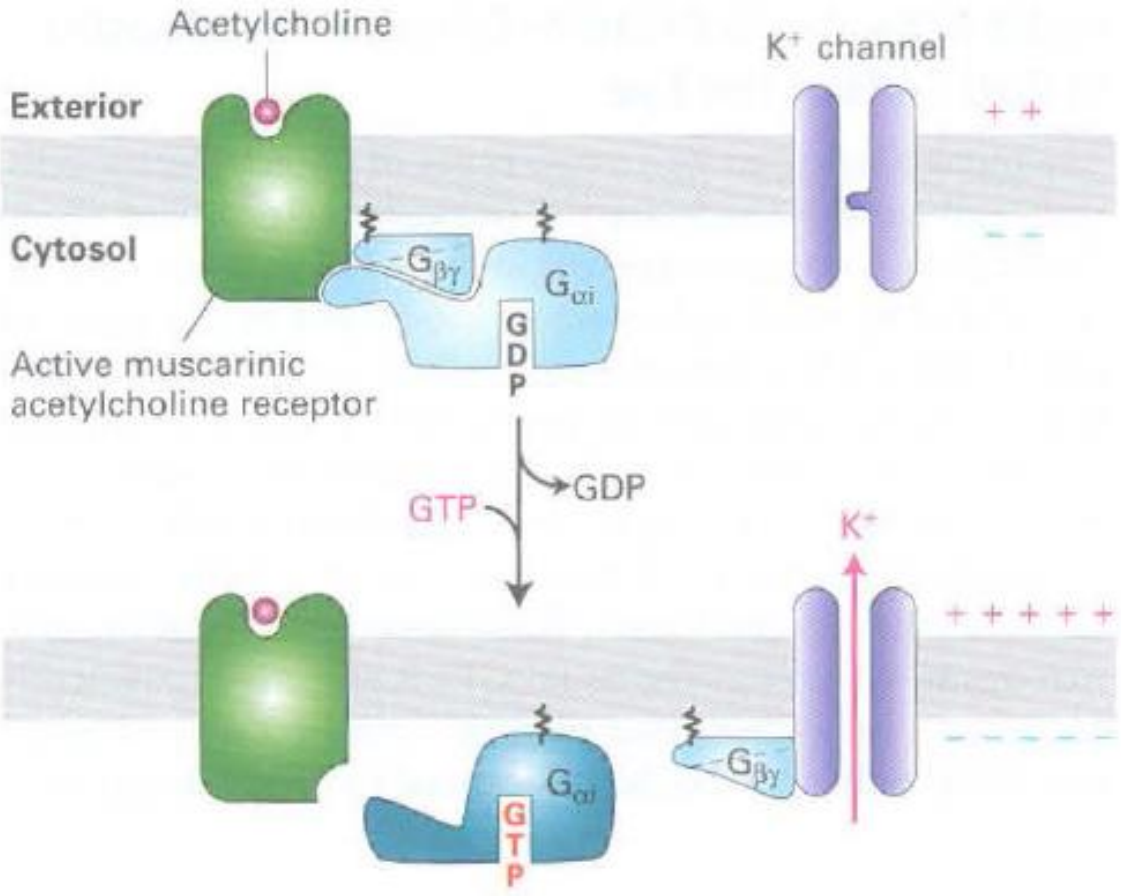

Reception
Binding of epinephrine to G protein-coupled receptor (1 molecule)
Transduction
Inactive G protein
Active G protein (10^2 molecules)
Inactive adenylyl cyclase
Active adenylyl cyclase (10^2)
ATP
Cyclic AMP (10^4)
Inactive protein kinase A
Active protein kinase A (10^4)
Inactive phosphorylase kinase
Active phosphorylase kinase (10^5)
Inactive glycogen phosphorylase
Active glycogen phosphorylase (10^6)
Response
Glycogen
Glucose-1-phosphate
(10^8 molecules)

Acetylcholine Receptors in the Heart Muscle Activate a G Protein That Opens K+ Channels

- *Muscarinic acetylcholine receptors are a type of GPCR* found in cardiac muscle.
- When activated, these receptors *slow the rate of heart muscle contraction*
- Receptor is coupled to a Gαi subunit, and ligand binding leads to opening of associated K+ channels (the effector protein) in the plasma membrane

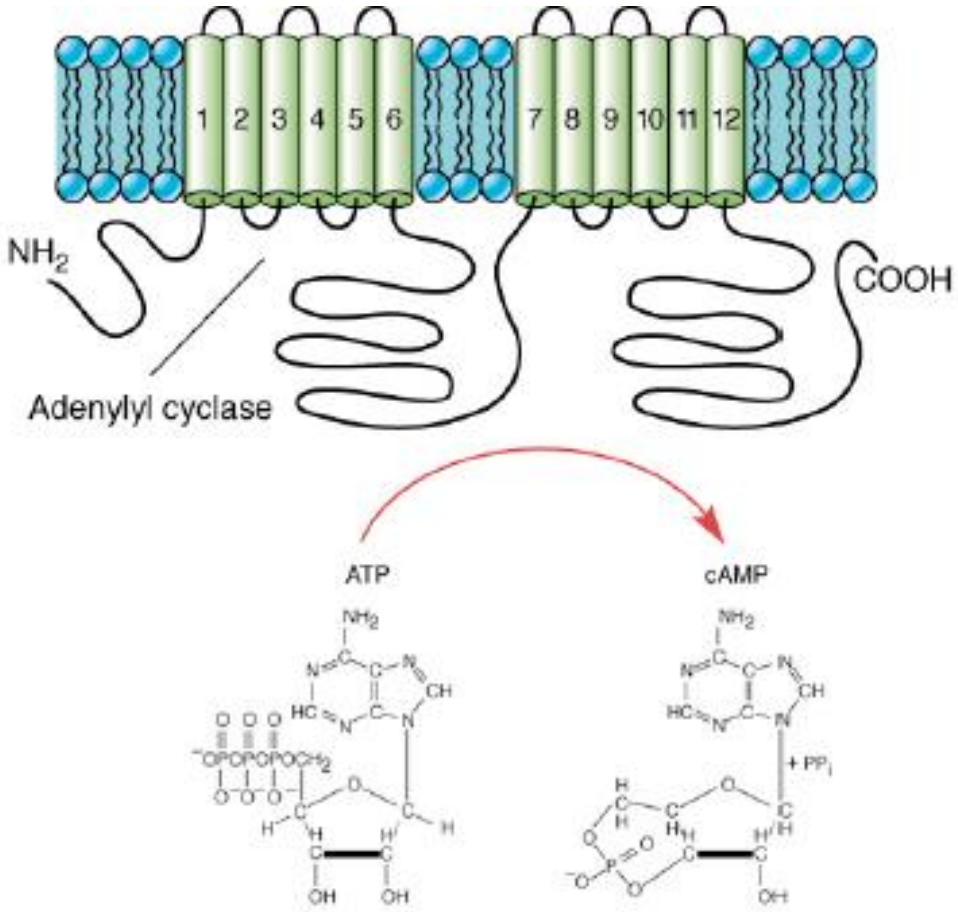
Activation of the muscarinic acetylcholine receptor and its effector K+ channel in heart muscle

1. Binding of acetylcholine triggers activation of the $G_{\alpha i}$ subunit and its dissociation from the $G_{\beta\gamma}$ subunit in the usual way
2. The released $G_{\beta\gamma}$ subunit (rather than $G_{\alpha i}$-GTP) binds to and opens the associated effector protein, a K channel.
3. The increase in K permeability hyperpolarizes the membrane, which reduces the frequency of heart muscle contraction.



Second Messenger cAMP

- A second messenger is a substance that is released in the cytoplasm following activation of a receptor.
- It is non-specific and can generate a variety of responses in the cell.
- cAMP is synthesized by an integral membrane protein, adenylyl cyclase, using ATP as a substrate.



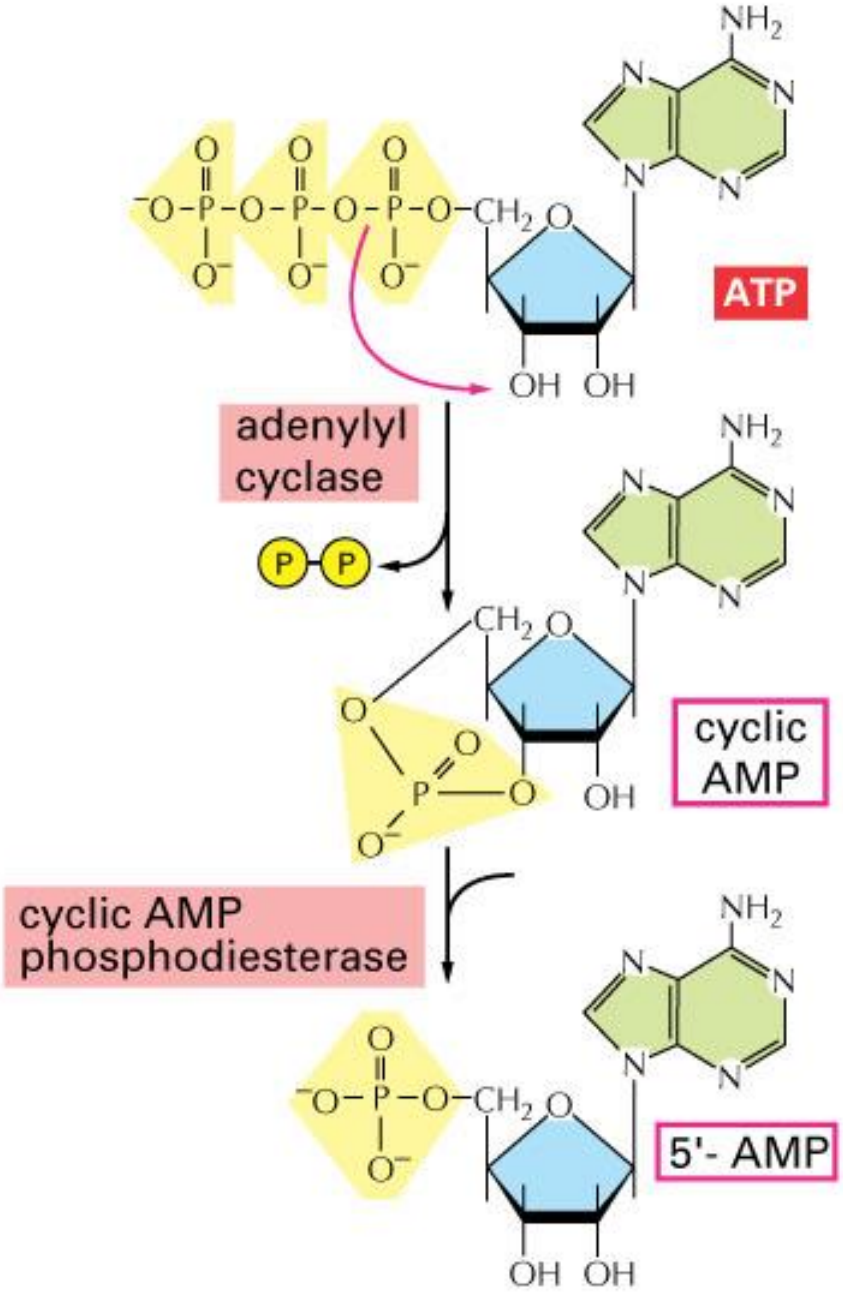
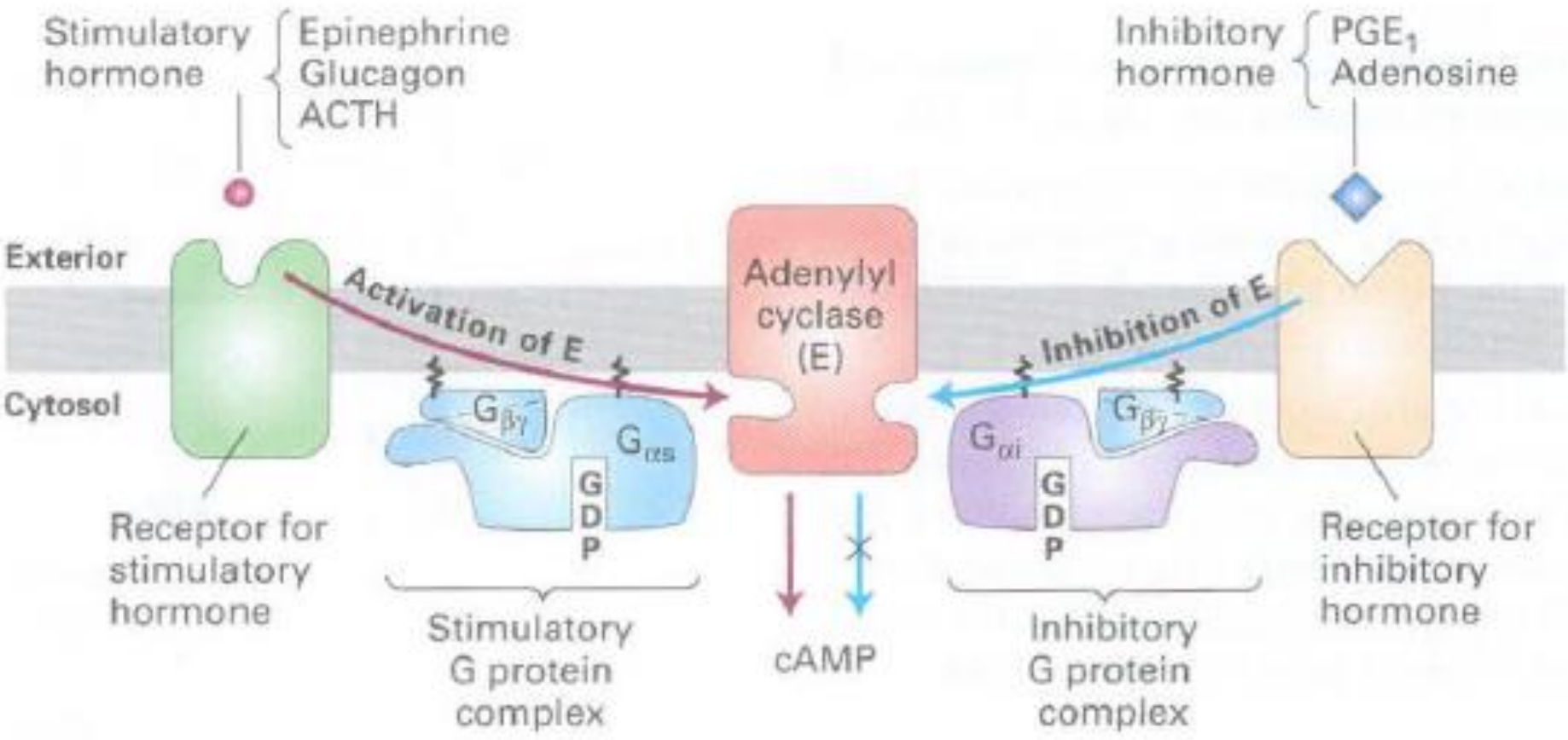


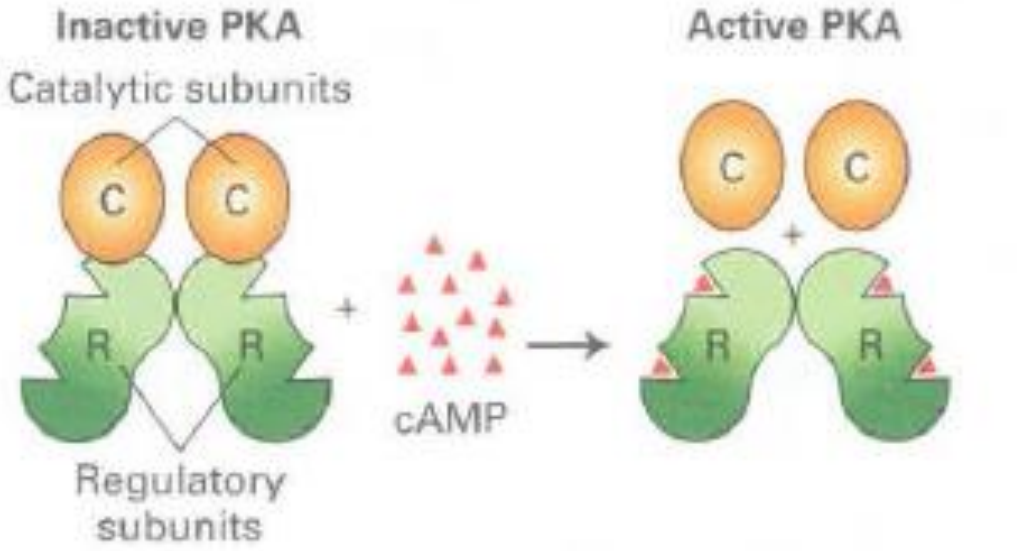
Figure 16-21 Essential Cell Biology, 2/e. (© 2004 Garland Science)

G Protein- Coupled Receptors That Activate or Inhibit Adenylyl Cyclase

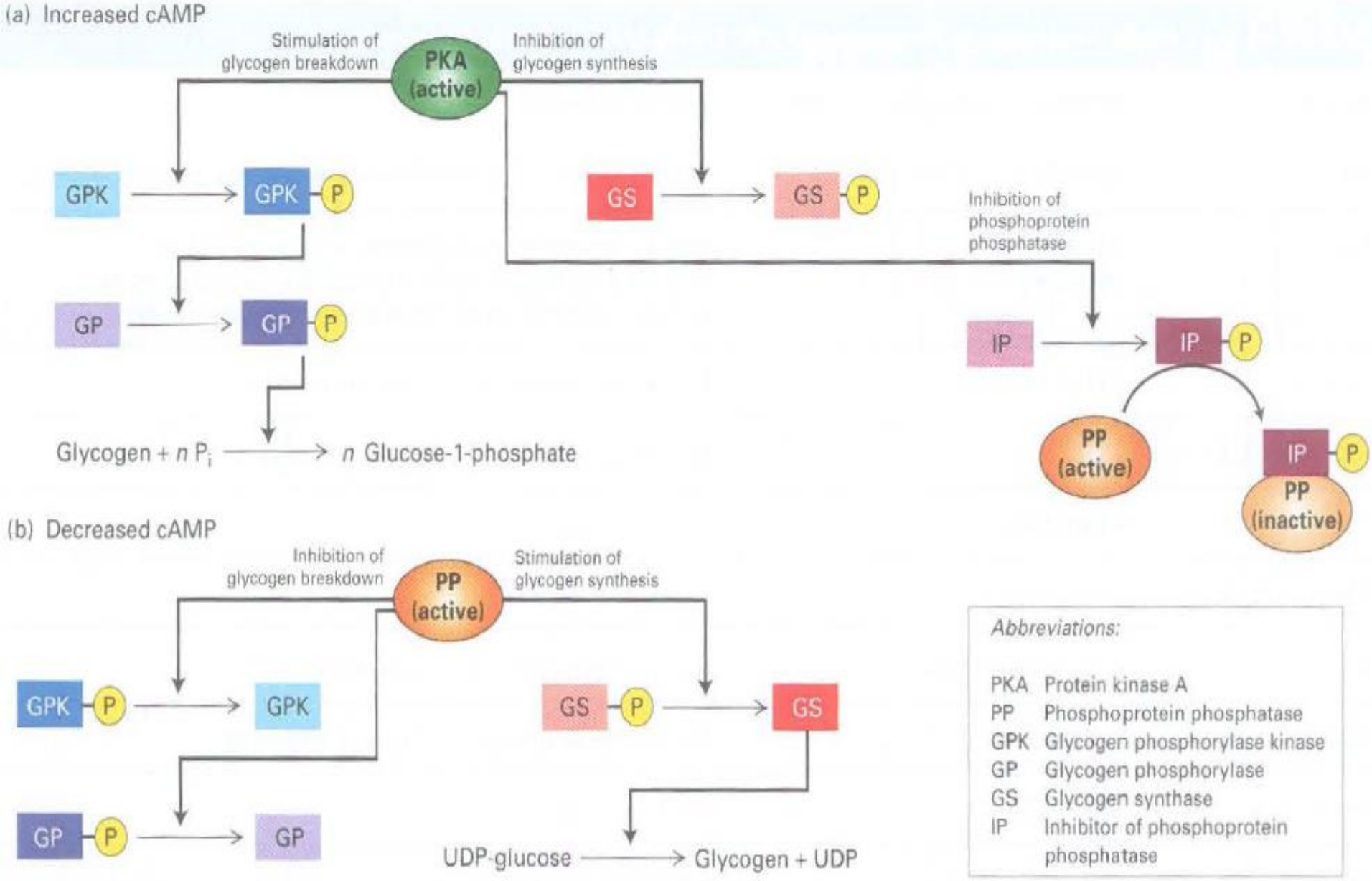


Hormone-induced activation and inhibition of adenylyl cyclase in adipose cells - Ligand binding to Gαs-coupled receptors causes activation of adenylyl cyclase, whereas ligand binding to Gαi-coupled receptors causes inhibition of the enzyme. The Gβγ subunit in both stimulatory and inhibitory G proteins is identical; the Ga subunits and their corresponding receptors differ.

cAMP Activates Protein Kinase A by Releasing Inhibitory Subunits



Protein kinase A (PKA) consists of two regulatory (R) subunits (green) and two catalytic (C) subunits.
When cAMP (red triangle) binds to the regulatory subunit, the catalytic subunit is released, thus activating PKA.


(a) Increased cAMP
Stimulation of glycogen breakdown
PKA (active)
Inhibition of glycogen synthesis
GPK
GPK P
GS
GS P
Inhibition of phosphoprotein phosphatase
GP
GP P
IP
IP P
Glycogen + n Pi
n Glucose-1-phosphate
PP (active)
IP P
PP (inactive)
(b) Decreased cAMP
Inhibition of glycogen breakdown
PP (active)
Stimulation of glycogen synthesis
GPK P
GPK
GS P
GS
GP P
GP
UDP-glucose
Glycogen + UDP
Abbreviations:
PKA Protein kinase A
PP Phosphoprotein phosphatase
GPK Glycogen phosphorylase kinase
GP Glycogen phosphorylase
GS Glycogen synthase
IP Inhibitor of phosphoprotein phosphatase

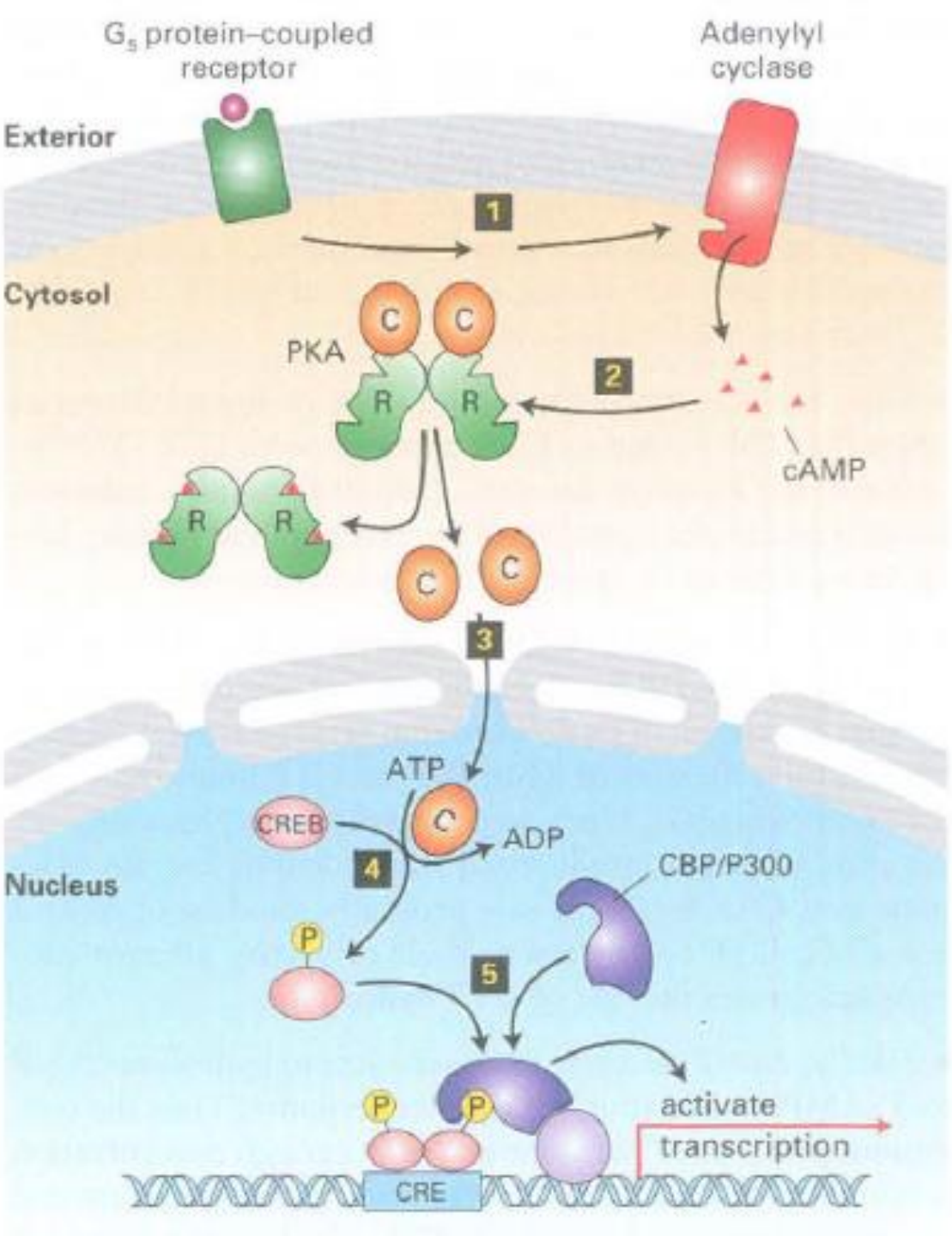
cAMP-Mediated Activation of Protein Kinase A Produces Diverse Responses in Different Cell Types

TABLE 15-2 Cellular Responses to Hormone-Induced Rise in cAMP in Various Tissues*

Tissue	Hormone Inducing Rise in cAMP	Cellular Response
Adipose	Epinephrine; ACTH; glucagon	Increase in hydrolysis of triglyceride; decrease in amino acid uptake
Liver	Epinephrine; norepinephrine; glucagon	Increase in conversion of glycogen to glucose; inhibition of glycogen synthesis; increase in amino acid uptake; increase in gluconeogenesis (synthesis of glucose from amino acids)
Ovarian follicle	FSH; LH	Increase in synthesis of estrogen, progesterone
Adrenal cortex	ACTH	Increase in synthesis of aldosterone, cortisol
Cardiac muscle	Epinephrine	Increase in contraction rate
Thyroid gland	TSH	Secretion of thyroxine
Bone	Parathyroid hormone	Increase in resorption of calcium from bone
Skeletal muscle	Epinephrine	Conversion of glycogen to glucose-1-phosphate
Intestine	Epinephrine	Fluid secretion
Kidney	Vasopressin	Resorption of water
Blood platelets	Prostaglandin I	Inhibition of aggregation and secretion

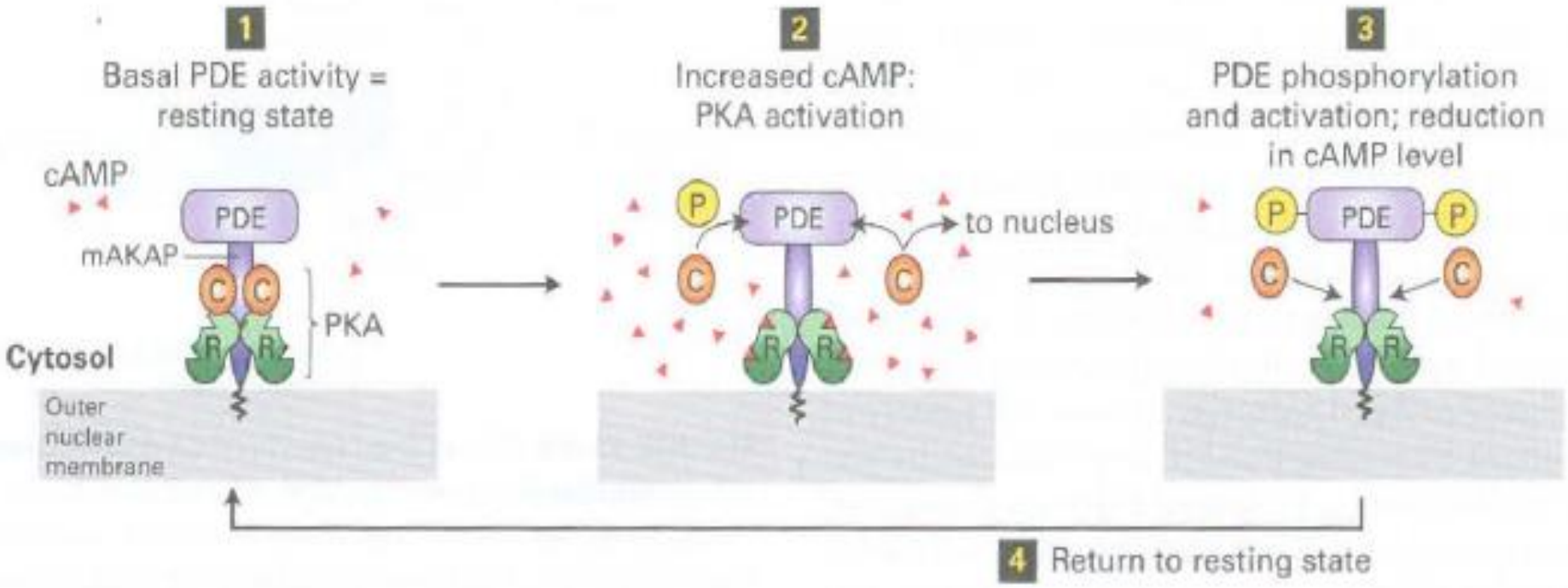
Activation of CREB transcription factor following ligand binding to G_s protein-coupled receptors

- Receptor stimulation leads to activation of protein kinase A (PKA)
- Catalytic subunits of PKA translocate to the nucleus and there phosphorylate and activate the CREB transcription factor.
- Phosphorylated CREB associates with the co-activator CBP/P300 and other proteins to stimulate transcription of the various target genes controlled by the CRE regulatory element


G_s protein–coupled receptor
Adenylyl cyclase
Exterior
Cytosol
PKA
C
C
R
R
1
2
cAMP
R
R
C
C
3
ATP
C
CREB
ADP
4
CBP/P300
Nucleus
P
5
P
P
activate transcription
CRE

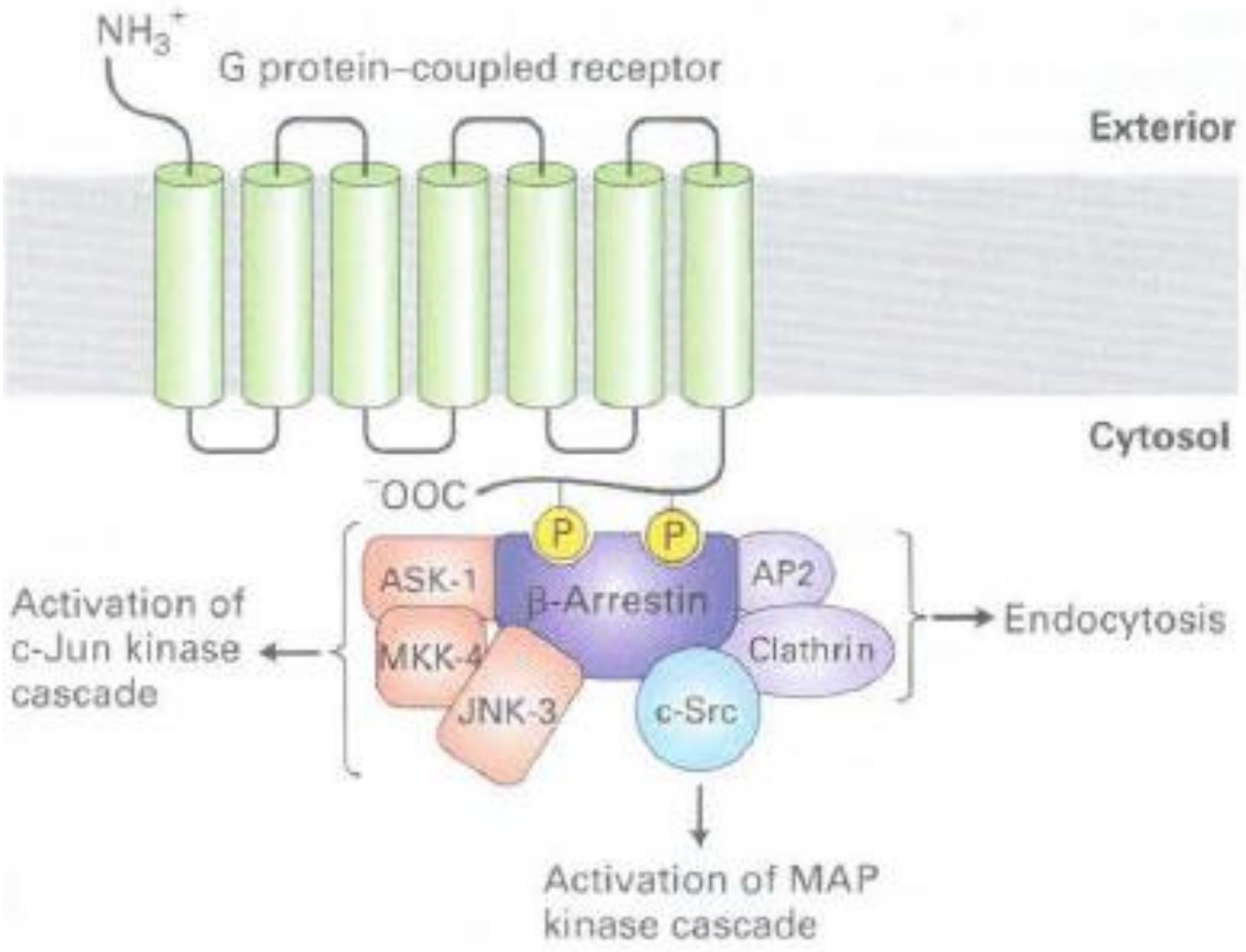
Localization of protein kinase A (PKA) to the nuclear membrane in heart muscle by an A-kinase associated protein (AKAP)

- This member of the AKAP family, designated mAKAP, anchors both cAMP phosphodiesterase (PDE) and the regulatory subunit of PKA to the nuclear membrane.
- Maintain them in a negative feedback loop that provides local control of the cAMP level and PKA activity.



1. The basal level of PDE activity in the absence of hormone (resting state) keeps cAMP levels below those necessary for PKA activation
2. Activation of β-adrenergic receptors causes an increase in cAMP level .
3. The resulting binding of cAMP to the regulatory (R) subunits of PKA releases the active catalytic (C) subunits into the cytosol.
4. Some C subunits enter into the nucleus, where they phosphorylate and thus activate certain transcription factors .
5. Other C subunits phosphorylate PDE, stimulating its catalytic activity.
6. Active PDE hydrolyzes cAMP, thereby driving cAMP levels back to basal levels and causing re-formation of the inactive PKA-R complex.

Role of β-arrestin in GPCR desensitization and signal transduction

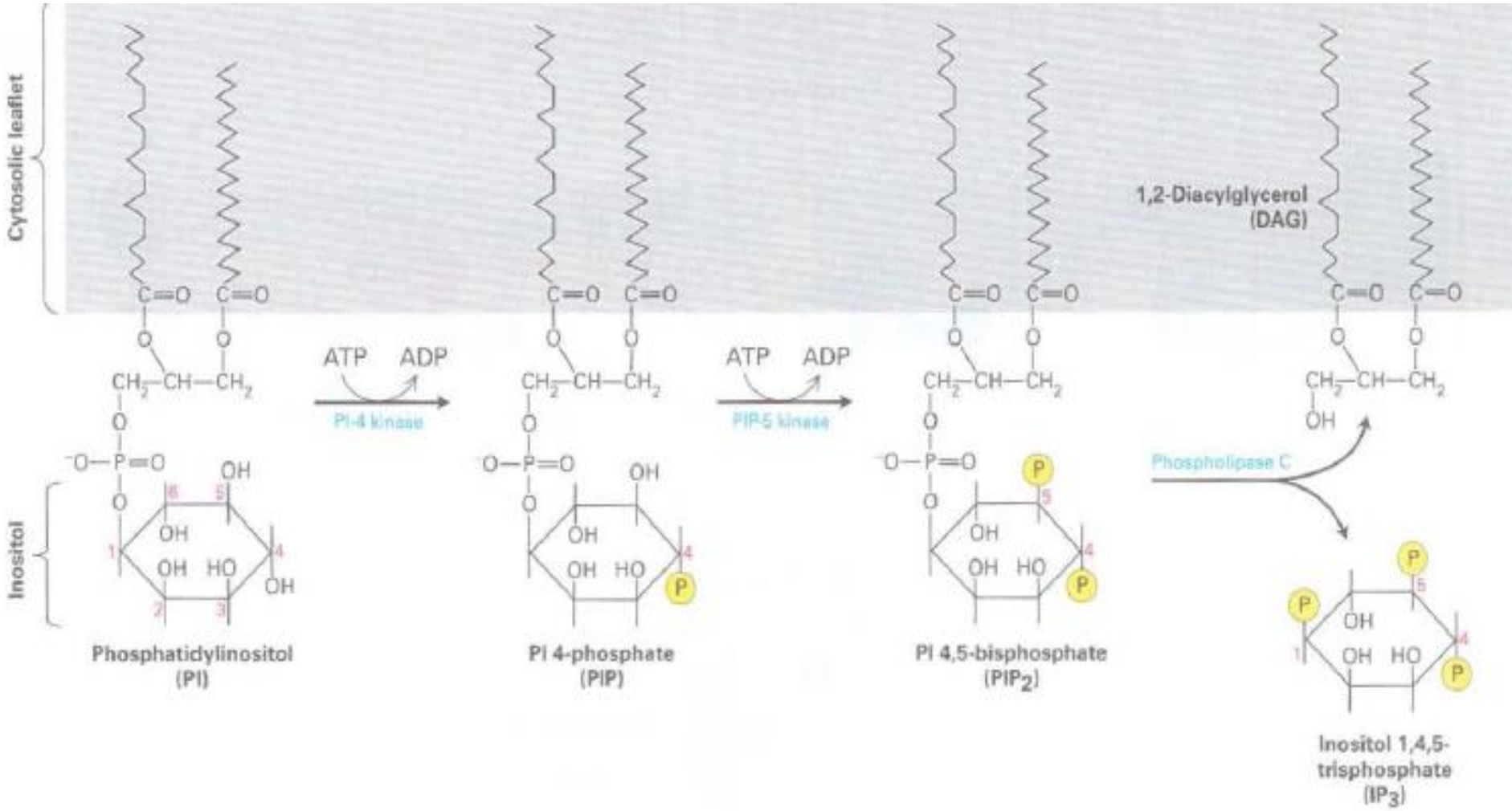


1. β-Arrestin binds to **phosphorylated serine and threonine** residues in the (-terminal segment of G protein-coupled receptors (GPCRs).
2. Clathrin and AP2, two other proteins bound by β-arrestin, promote endocytosis of the receptor

3. β-arrestin also functions in transducing signals from activated receptors by binding to and activating several cytosolic protein kinases**,** **c-Src** activates the MAP kinase pathway, leading to phosphorylation of key transcription factors

4. Interaction of β-arrestin with three other proteins, including JNK-3 (a Jun N-terminal kinase), results in phosphorylation and activation of another transcription factor**,** **c-Jun**

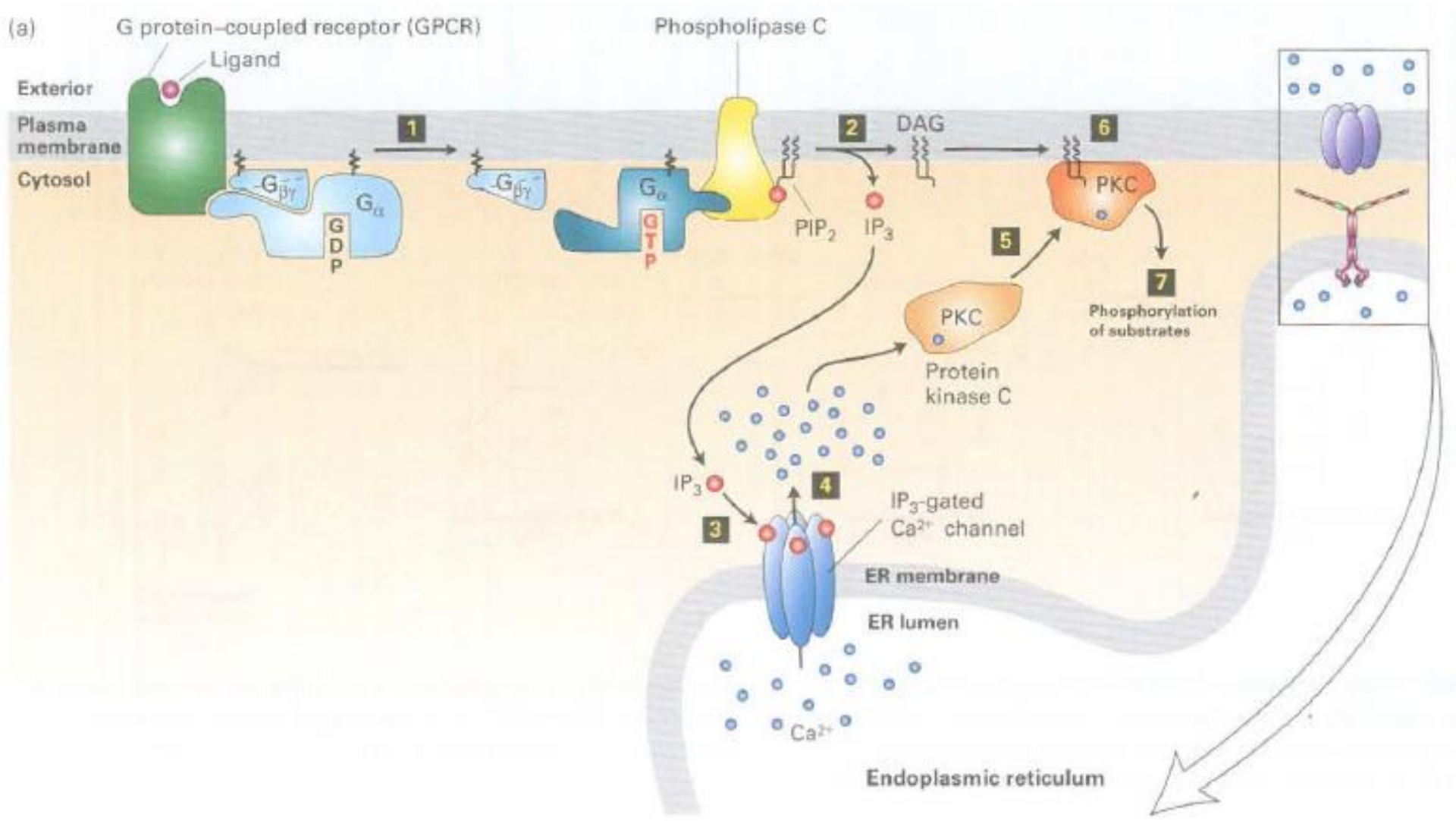
Activated Phospholipase C Generates Two Key Second Messengers Derived from the Membrane Lipid Phosphatidylinositol



Each membrane-bound PI kinase places a phosphate (yellow circles) on a specific hydroxyl group on the inositol ring, producing the phosphorylated derivatives PIP and PIP2

Cleavage of PIP2 by phospholipase C yields the two important second messengers **DAG** and **IP3**

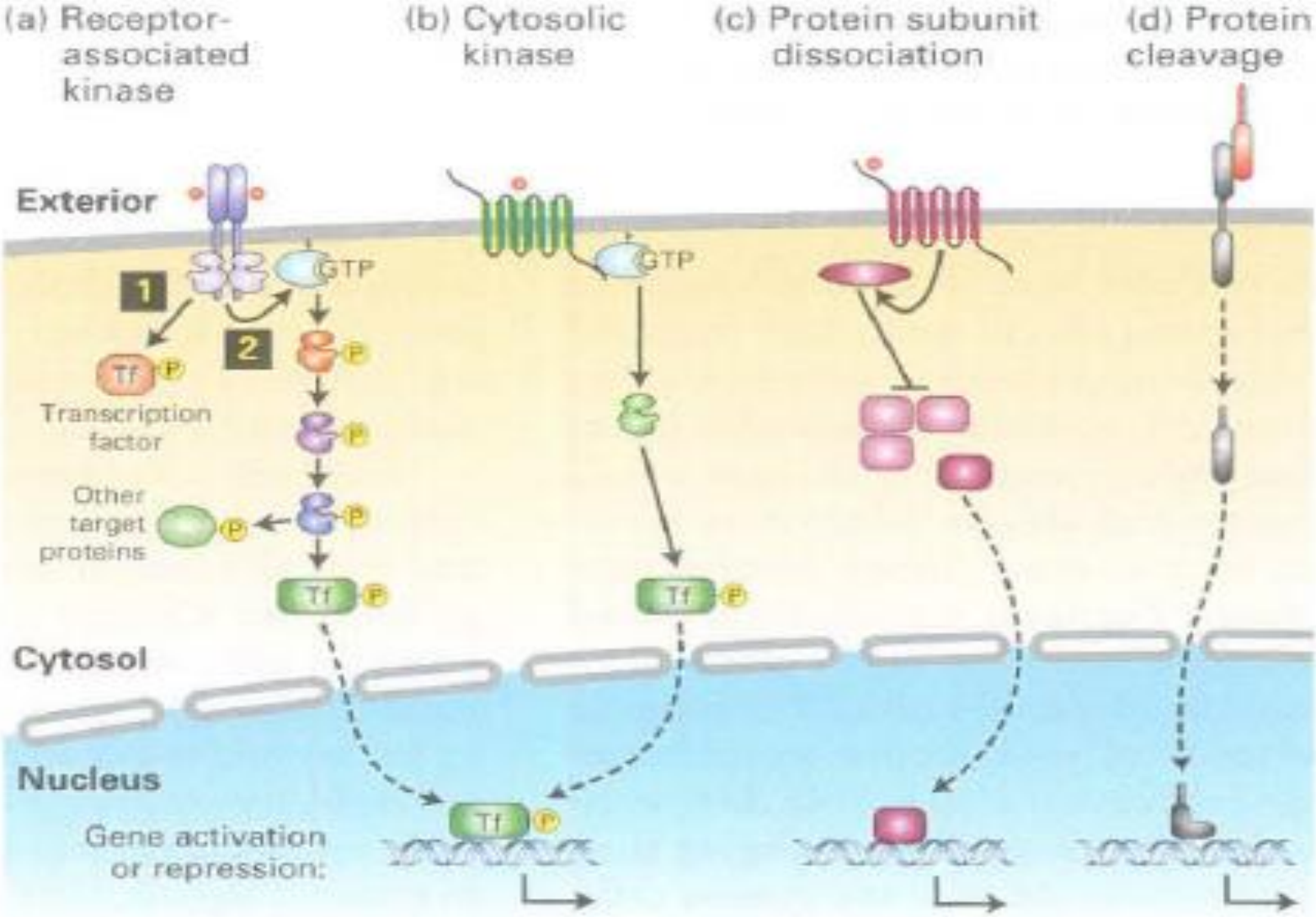
IP3/DAG pathway and the elevation of cytosolic Ca^{2+}



Opening of endoplasmic reticulum Ca^{2+} *channels.* *This* pathway can be triggered by ligand binding to GPCRs that activate the G alpha subunit leading to activation of phospholipase C

Receptors That Activate Protein Tyrosine Kinases

Based on the sequence of intracellular events.



Representative receptors and *pathways*	RTKs	GPCRs	*Wnt*	*Notch/Delta*
	TGF-β receptors	*cAMP/PKA/CREB*	*Hedgehog*	
	Cytokine receptors		*NF-kB*	
	JAK-STAT			
	Ras/MAP Kinase			

Receptor associated kinase

- The cytosolic domains of many receptors contain protein kinase domains or are tightly associated with a cytosolic kinase;
- Commonly the kinases are activated by ligand binding followed by receptor dimerization.
- Some of these kinases directly phosphorylate and activate transcription factors
- Many of these receptors also activate small GTP-binding "switch" proteins such as Ras
- Eg: RTKs, TGF-P receptors, Cytokine receptors, *JAK-STAT, Ras/MAP Kinase*

Cytosolic kinase

- Other receptors, mainly the seven-spanning receptors, activate the larger GTP-binding Gα proteins, which in turn activate specific kinases or other signaling proteins
- eg: GPCRs, *cAMP/PKA/CREB*

Protein subunit dissociation

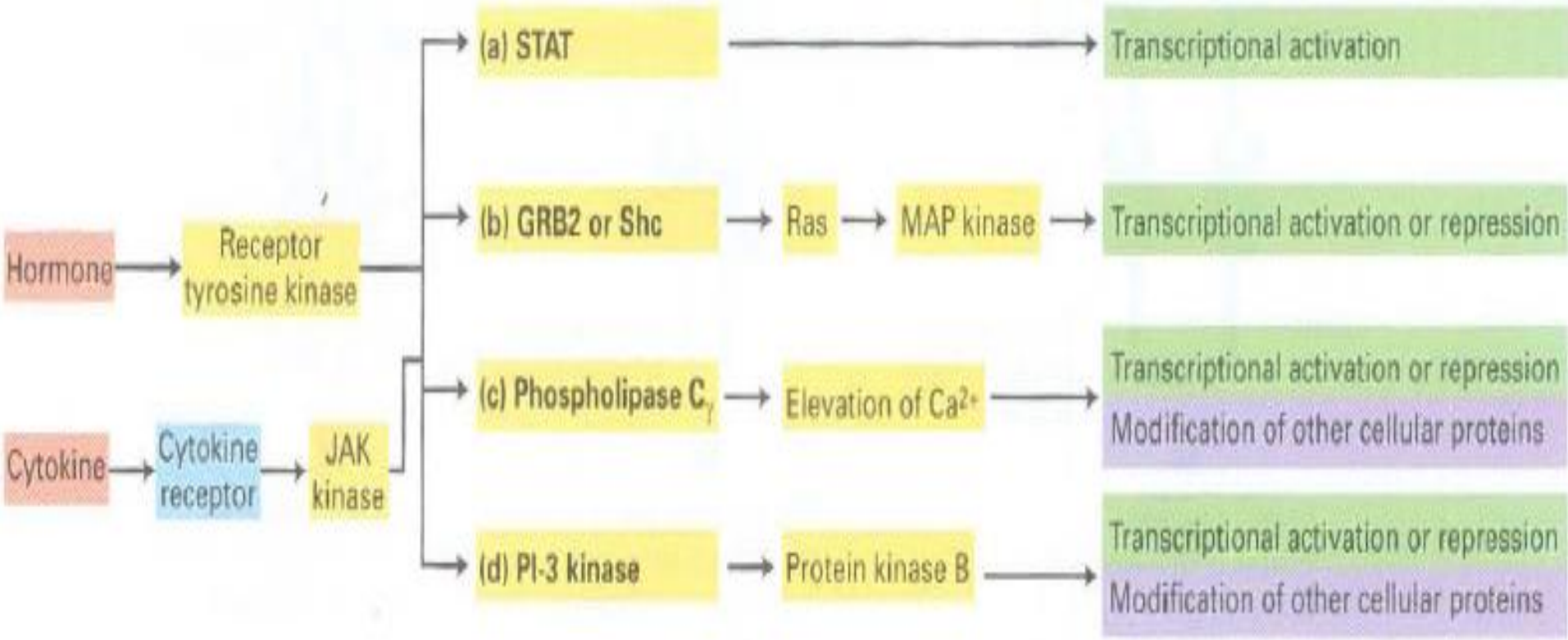
- Several signaling pathways involve disassembly of a multiprotein complex in the cytosol, releasing a transcription factor that then translocates into the nucleus
- *eg: Wnt, Hedgehog, NF-kB*

Protein cleavage

- Some signaling pathways are irreversible; in many cases proteolytic cleavage of a receptor releases an active transcription factor
- *eg: Notch/Delta*

- There are two broad categories of receptors that activate tyrosine kinases:
  - Those in which the tyrosine kinase enzyme is an intrinsic part of the receptor's polypeptide chain called the receptor tyrosine kinases (RTKs)
  - Cytokine receptors, in which the receptor and kinase (*JAK kinase)*are encoded by different genes yet bound tightly together
- Both classes of receptors activate similar intracellular signal transduction pathways

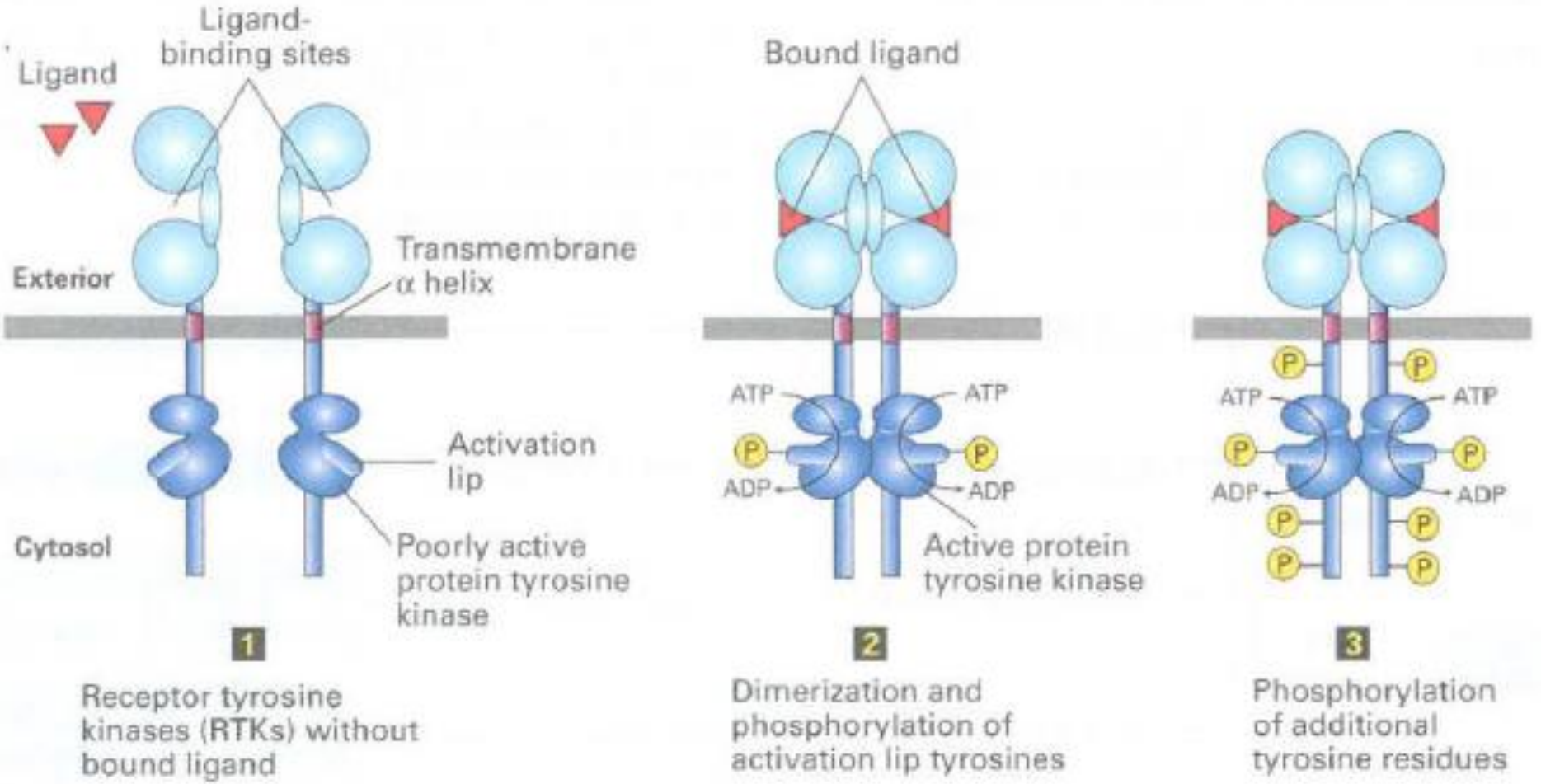
Overview of signal transduction pathways triggered by receptors that activate protein tyrosine kinases



a. In the most direct pathway, mainly employed by cytokine receptors, a STAT transcription factor binds to the activated receptor, becomes phosphorylated, moves to the nucleus, and directly activates transcription
b. Binding of one type of adapter protein (GRB2 or Shc) to an activated receptor leads to activation of the Ras/MAP kinase pathway

- Two phosphoinositide pathways are triggered by recruitment of phospholipase C and PI-3 kinase to the membrane
- Elevated levels of Ca^{2+} and activated protein kinase B modulate the activity of transcription factors as well as of cytosolic proteins that are involved in metabolic pathways or cell movement or shape

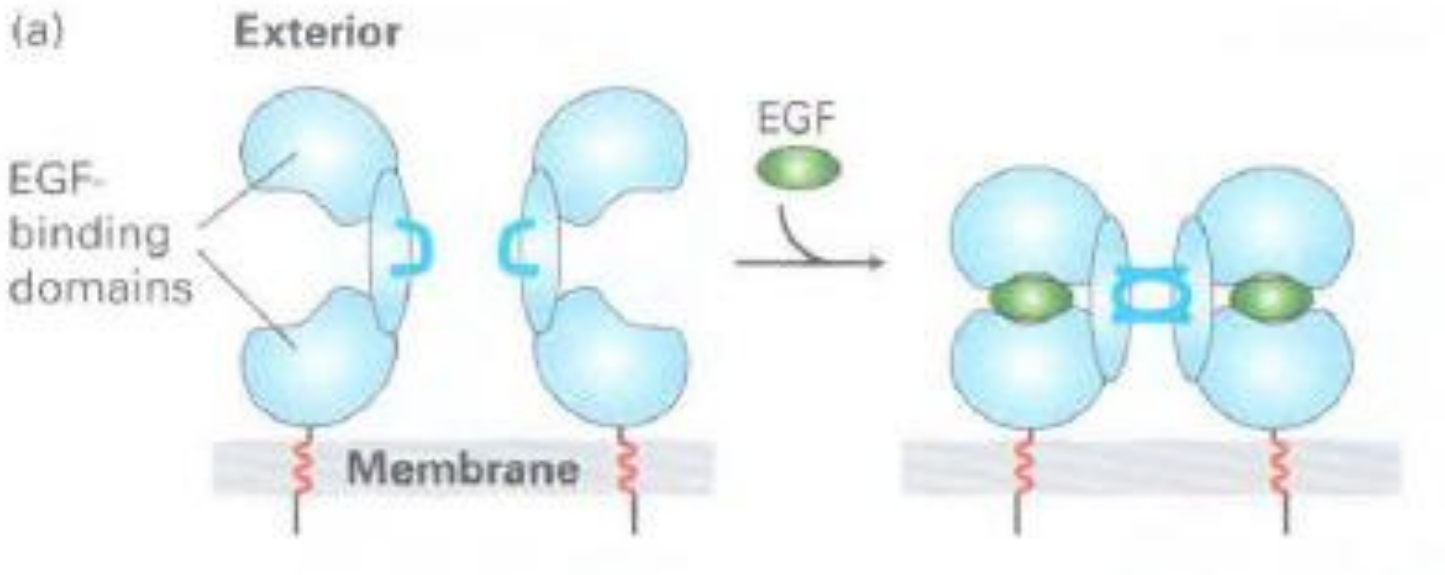
General structure and activation of receptor tyrosine kinases (RTKs)



- The cytosolic domain of RTKs contains an intrinsic protein tyrosine kinase catalytic site.
- In the absence of ligand, RTKs generally exist as monomers with poorly active kinases.
- Ligand binding causes a conformational change that promotes formation of a functional dimeric receptor, bringing together two poorly active kinases and phosphorylate each other on a tyrosine residue in the activation lip

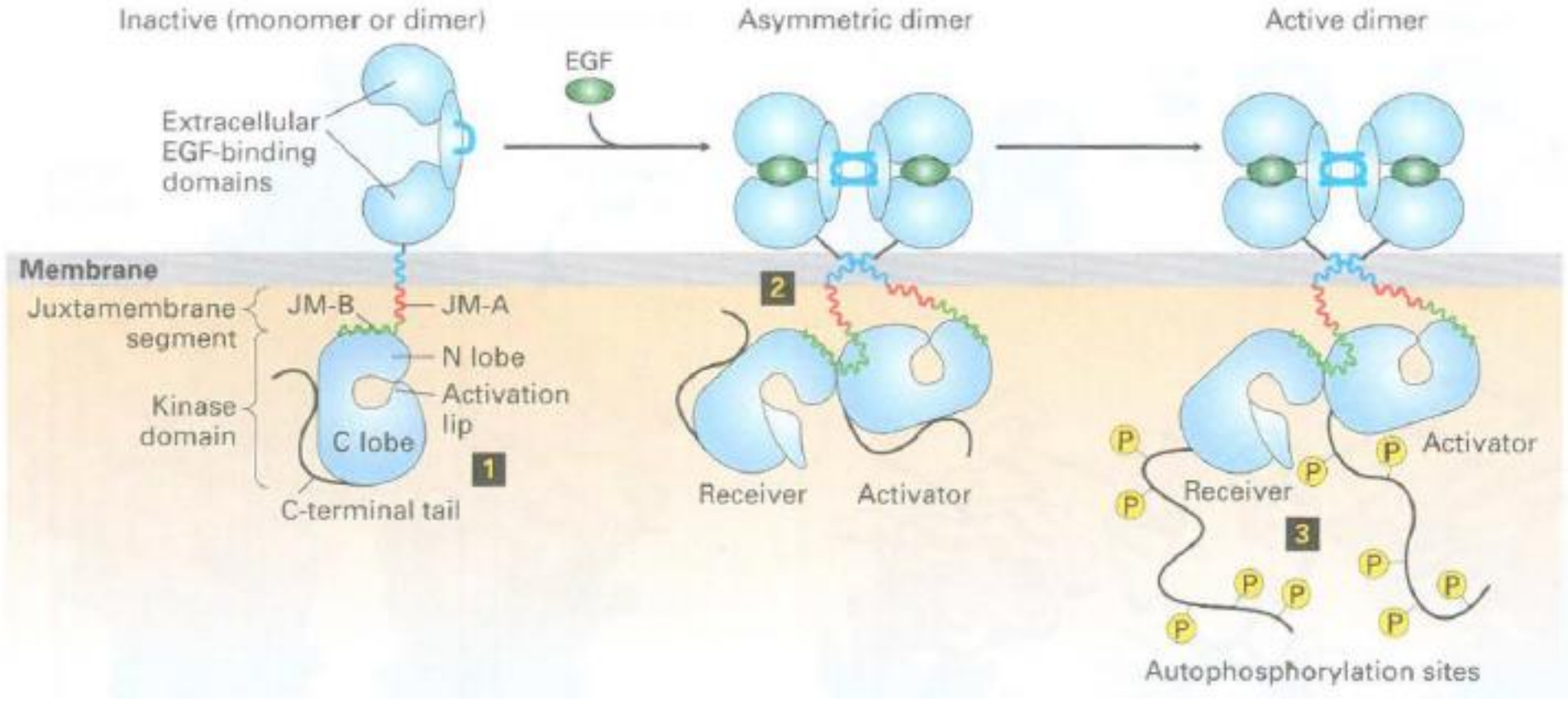
- Phosphorylation causes the lip to move out of the kinase catalytic site, thus increasing the ability of ATP and the protein substrate to bind.
- The activated kinase then phosphorylates several tyrosine residues in the receptor's cytosolic domain
- The resulting phosphotyrosines function as docking sites for various signal transduction proteins

Ligand-induced dimerization of HER1, a human receptor for epidermal growth factor (EGF)



Extracellular and transmembrane domains of HER1, which is a receptor tyrosine kinase - Binding of one EGF molecule to a monomeric receptor causes an alteration in the structure of a loop between the two EGF-binding domains

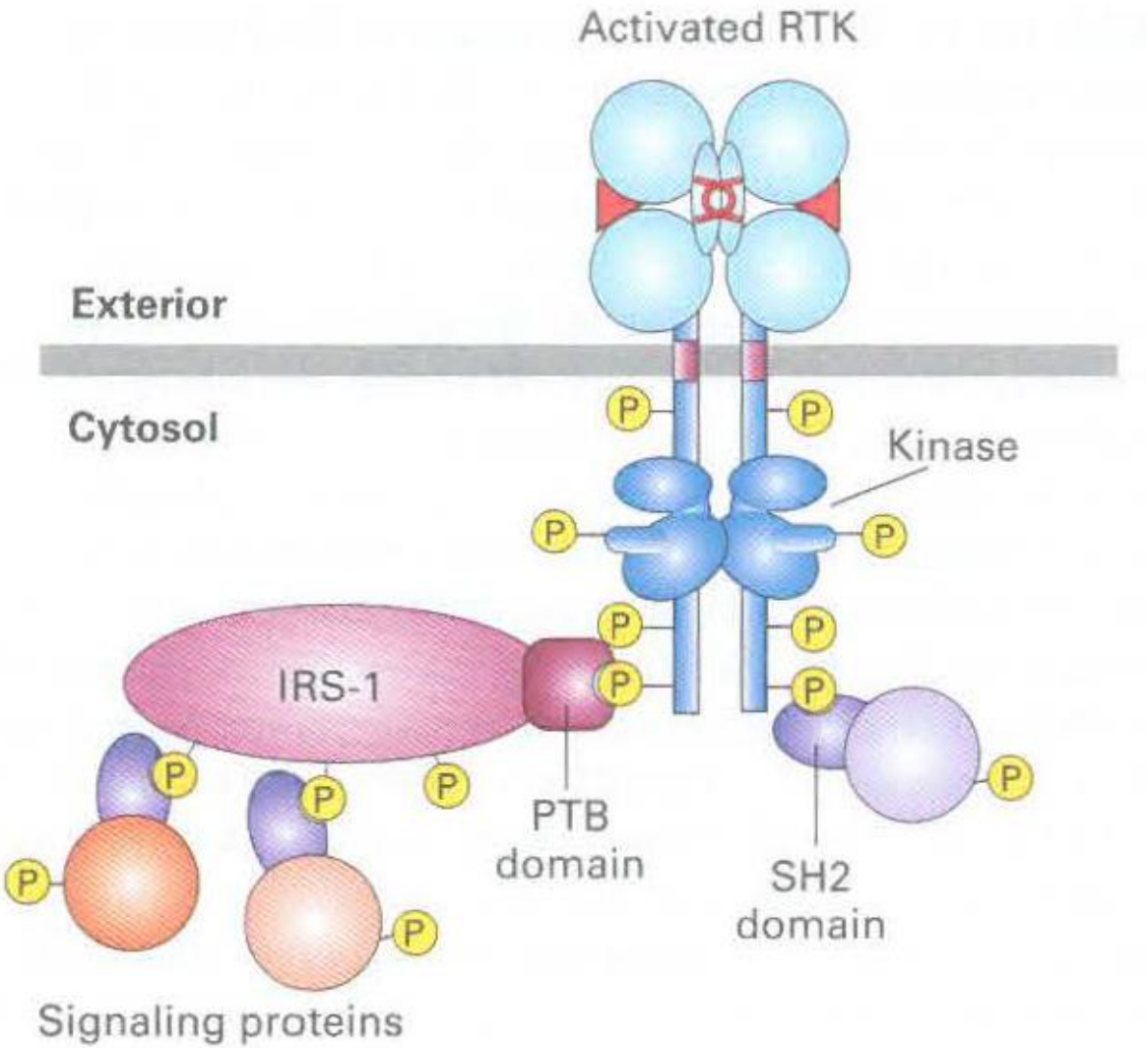
Activation of EGF receptor by EGF results in the formation of an asymmetric kinase domain dimer



- In the inactive monomeric state, the unstructured segment of the juxtamembrane domain(JM-B) binds to the upper, or N lobe of the kinase domain, causing a conformational change that positions the activation lip in the kinase active site and thus inhibits kinase activation
- Receptor dimerization generates an asymmetric kinase dimer

- Activator kinase binds the juxtamembrane segment of the receiver kinase, causing a conformational change that removes the activation lip from the kinase site of the receiver kinase, activating its kinase activity
- The active kinase then phosphorylates tyrosine residues in the C-terminal segments of the receptor cytosolic domain

Recruitment of intracellular signal transduction proteins to the cell membrane by binding to phosphotyrosine residues in receptors or receptor-associated proteins



- Cytosolic proteins with SH2 or PTB domains can bind to specific phosphotyrosine residues in activated RTKs or cytokine receptors.
- 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 a receptor-bound IRS-1 by the receptor kinase creates additional docking sites for SH2-containing signaling proteins.

The Ras/MAP Kinase Pathway

The Ras/MAP Kinase Pathway

- The Ras protein, a monomeric (small) G protein, belongs to the GTPase superfamily of intracellular switch proteins
- Activated Ras promotes formation of signal transduction complexes containing protein kinases.
- This *kinase cascade* culminates in activation of certain members of the MAP kinase family, which can translocate into the nucleus and phosphorylate many different proteins

- The target proteins for MAP kinase are transcription factors that regulate expression of proteins with important roles in the cell cycle and in differentiation
- An activating mutation in a RTK, Ras, or a protein in the MAP kinase cascade is found in almost all types of human tumours

Ras, a GTPase Switch Protein, Operates Downstream of Most RTKs and Cytokine Receptors

- Monomeric G protein known as Ras alternates between an active "on" state with a bound GTP and an inactive "off" state with a bound GDP
- Unlike trimeric G proteins, Ras is not directly linked to cell-surface receptors
- Ras activation is accelerated by a *guanine nucleotide exchange factor (GEF), which binds to the Ras·GDP complex,* causing dissociation of the bound GDP

- GTPase-activating protein (GAP) domain is not present in Ras
- Most oncogenic, constitutively active Ras proteins (Ras^{D}) contain a mutation at position 12.
- Replacement of the normal glycine-12 with any other amino acid (except proline) blocks the functional binding of GAP and "locks" Ras in the active GTP-bound state

Receptor Tyrosine Kinases and JAK Kinases Are Linked to Ras by Adapter Proteins

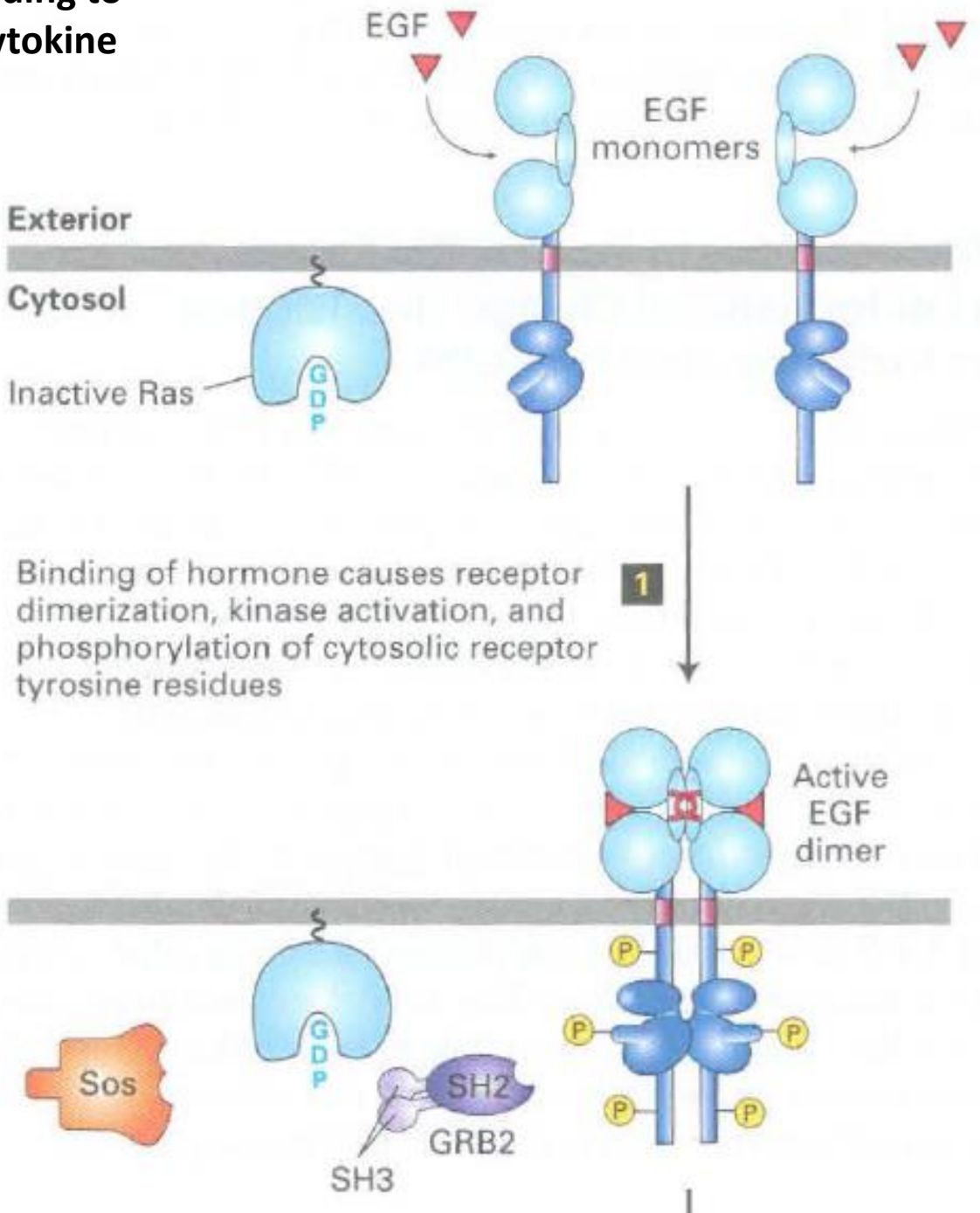
- In order for activated RTKs and cytokine receptors to activate Ras, two cytosolic proteins- GRB2 and Sos-must first be recruited to provide a link between the receptor and Ras
- GRB2 is an *adapter protein*- has no enzymatic activity and serves as a link, or scaffold, between two other proteins-in this case between the activated receptor and Sos

- GRB2 is able to serve as an adapter protein because of its SH2 domain, which binds to a specific phosphotyrosine residue in the activated RTK (or cytokine receptor)
- GRB2 adapter protein contains two *SH3 domains, which bind to Sos*
- Sos is a guanine nucleotide exchange protein (GEF), which catalyzes conversion of inactive GDP bound Ras to the active GTP-bound form

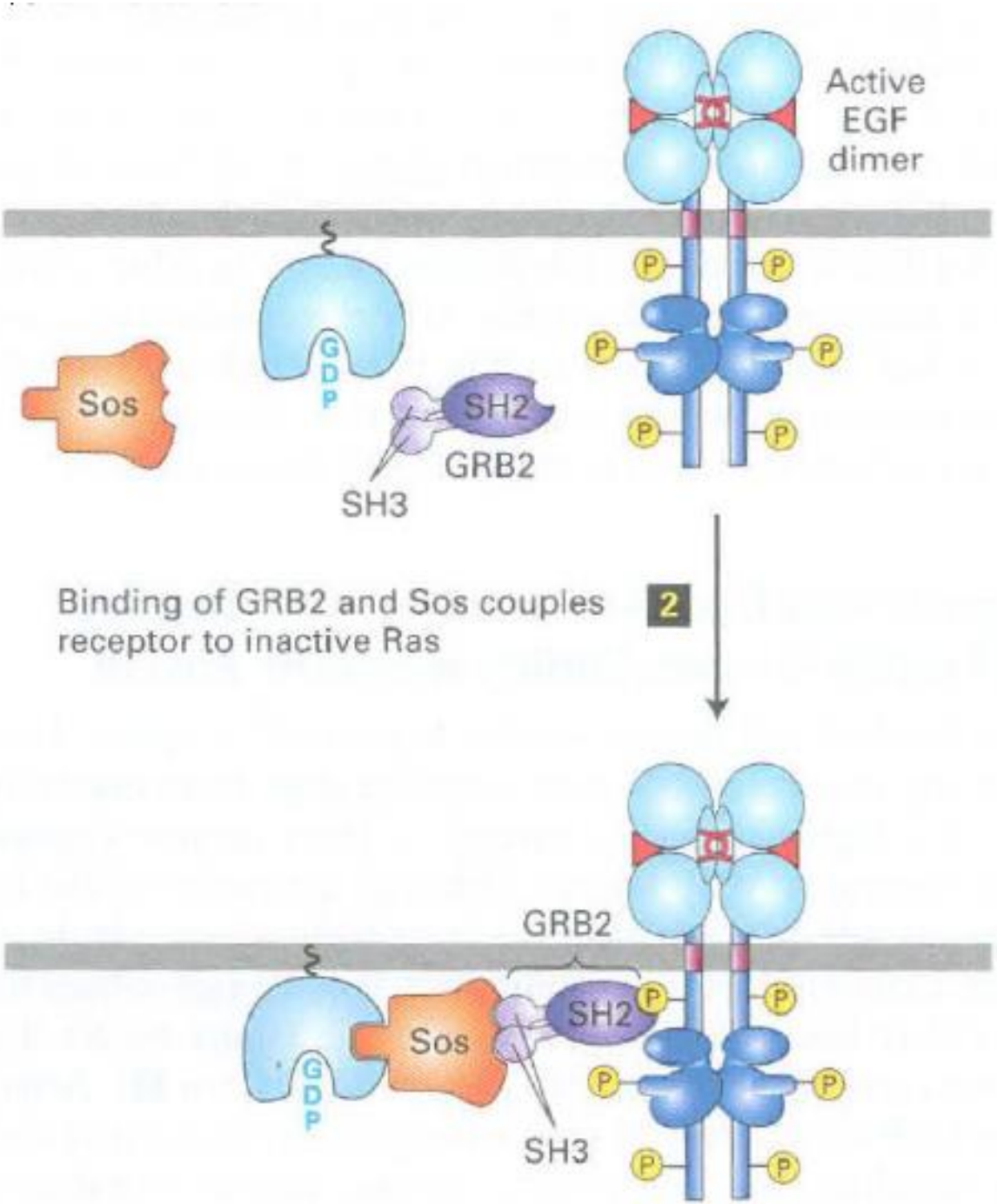
Activation of Ras following ligand binding to receptor tyrosine kinases (RTKs) or cytokine receptors.

The receptors for epidermal growth factor (EGF) and many other growth factors are RTKs

Ras is tethered to the cytosolic surface of the plasma membrane by a hydrophobic farnesyl anchor

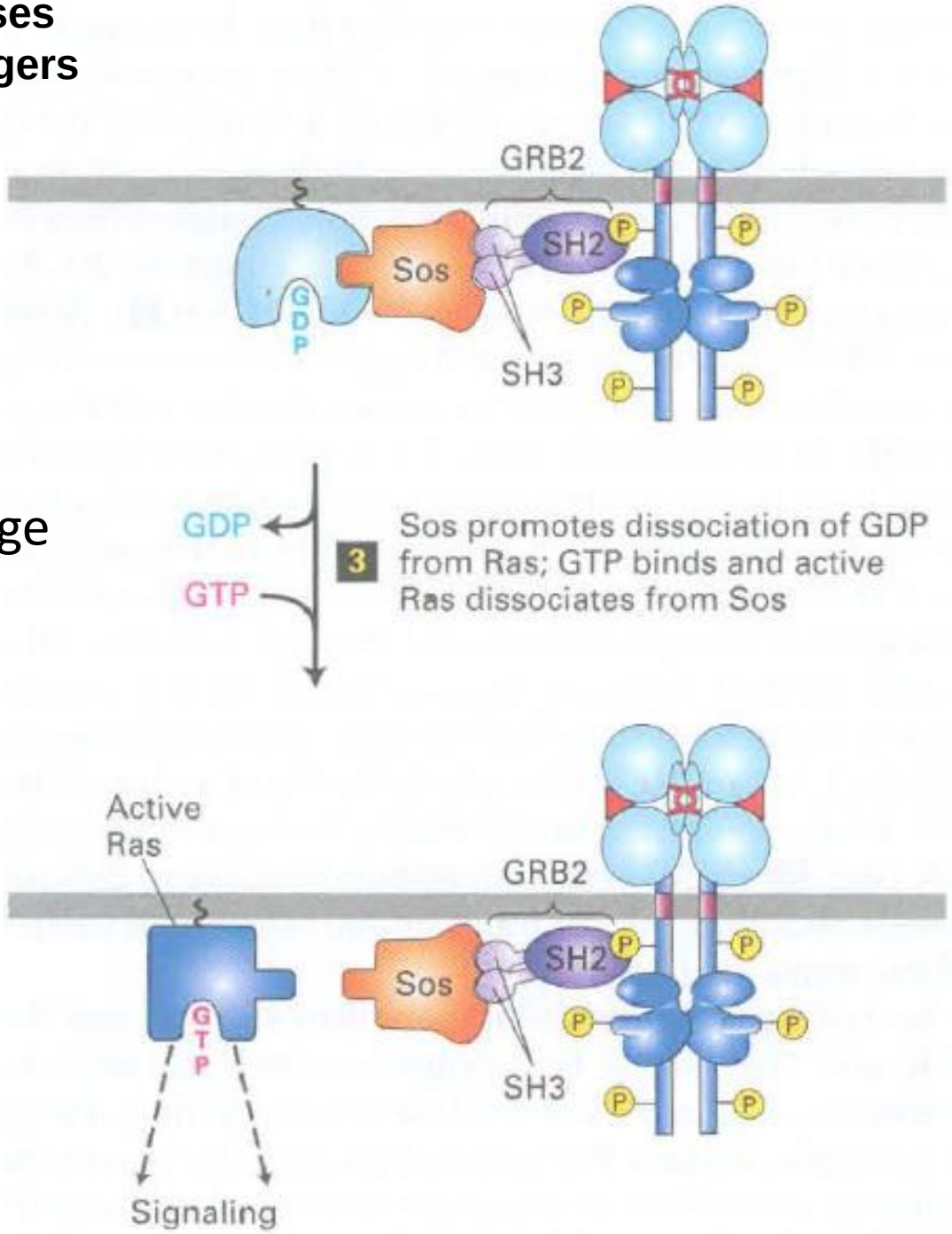


The cytosolic adapter protein GRB2 binds to a specific phosphotyrosine on an activated, ligand-bound receptor and to the cytosolic Sos protein, bringing it near the plasma membrane and to its substrate, the inactive Ras·GDP



Binding of Sos to Inactive Ras Causes a Conformational Change That Triggers an Exchange of GTP for GDP

The guanine nucleotide exchange factor (GEF) activity of Sos then promotes formation of active Ras·GTP.



Ras/MAP kinase pathway

- 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 Ras·GTP complex
- Activated Ras triggers the downstream kinase cascade culminating in activation of MAP kinase (MAPK)
- In unstimulated cells, binding of a dimer of the **14-3-3** protein to Raf stabilizes it in an inactive conformation
- The 14-3-3 protein binds phosphoserine residues in a number of important signaling proteins.

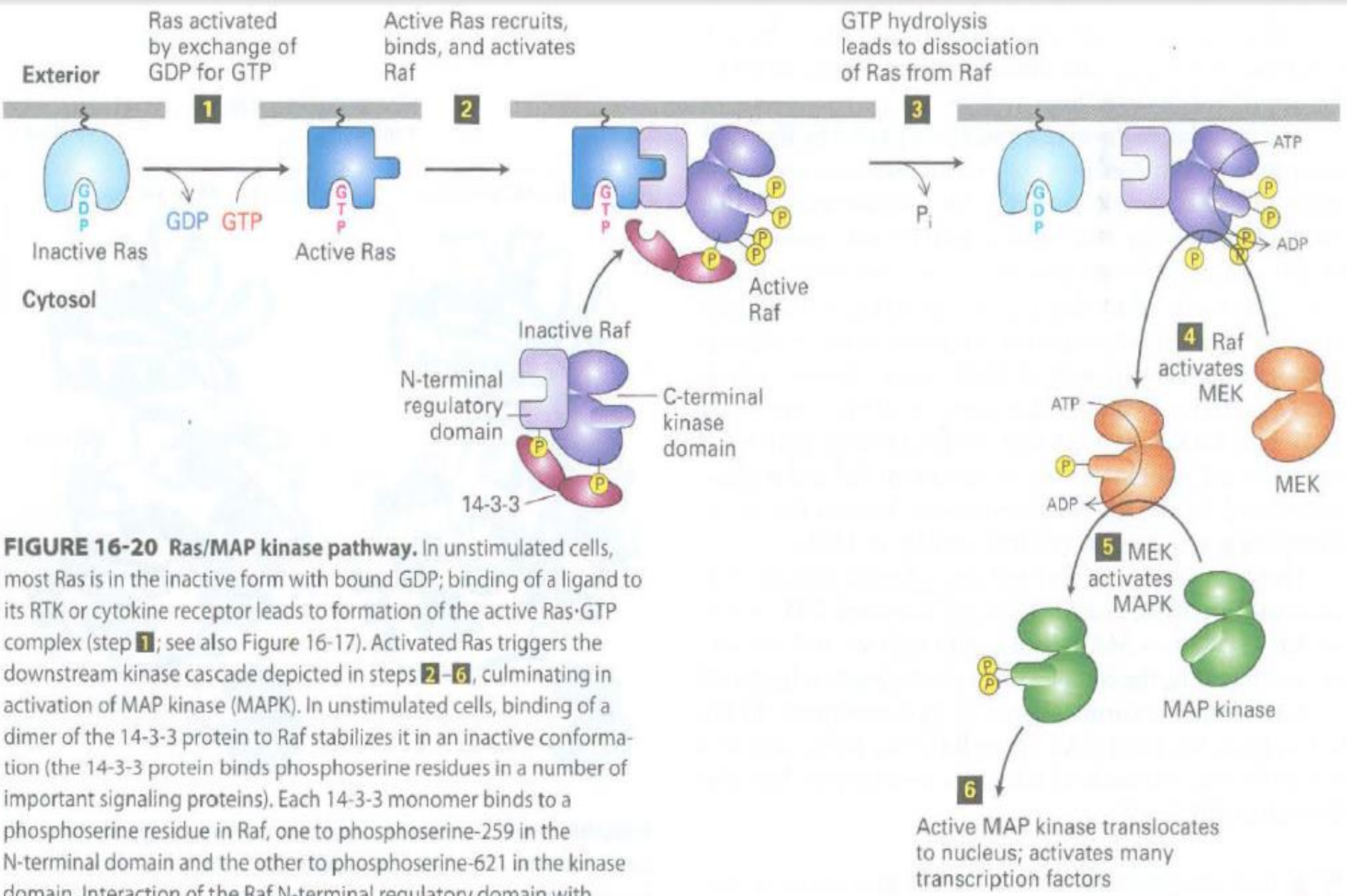


FIGURE 16-20 Ras/MAP kinase pathway. In unstimulated cells, most Ras is in the inactive form with bound GDP; binding of a ligand to its RTK or cytokine receptor leads to formation of the active Ras·GTP complex (step 1; see also Figure 16-17). Activated Ras triggers the downstream kinase cascade depicted in steps 2–6, culminating in activation of MAP kinase (MAPK). In unstimulated cells, binding of a dimer of the 14-3-3 protein to Raf stabilizes it in an inactive conformation (the 14-3-3 protein binds phosphoserine residues in a number of important signaling proteins). Each 14-3-3 monomer binds to a phosphoserine residue in Raf, one to phosphoserine-259 in the N-terminal domain and the other to phosphoserine-621 in the kinase domain. Interaction of the Raf N-terminal regulatory domain with Ras·GTP results in dephosphorylation of one of the serines that bind Raf to 14-3-3, phosphorylation of other residues, and activation of Raf

- Each 14-3-3 monomer binds to a phosphoserine residue in Raf, one to phosphoserine-259 in the N-terminal domain and the other to phosphoserine-621 in the kinase domain.

- Interaction of the Raf N-terminal regulatory domain with Ras·GTP results in dephosphorylation of one of the serines that bind Raf to 14-3-3, phosphorylation of other residues, and activation of Raf kinase activity.

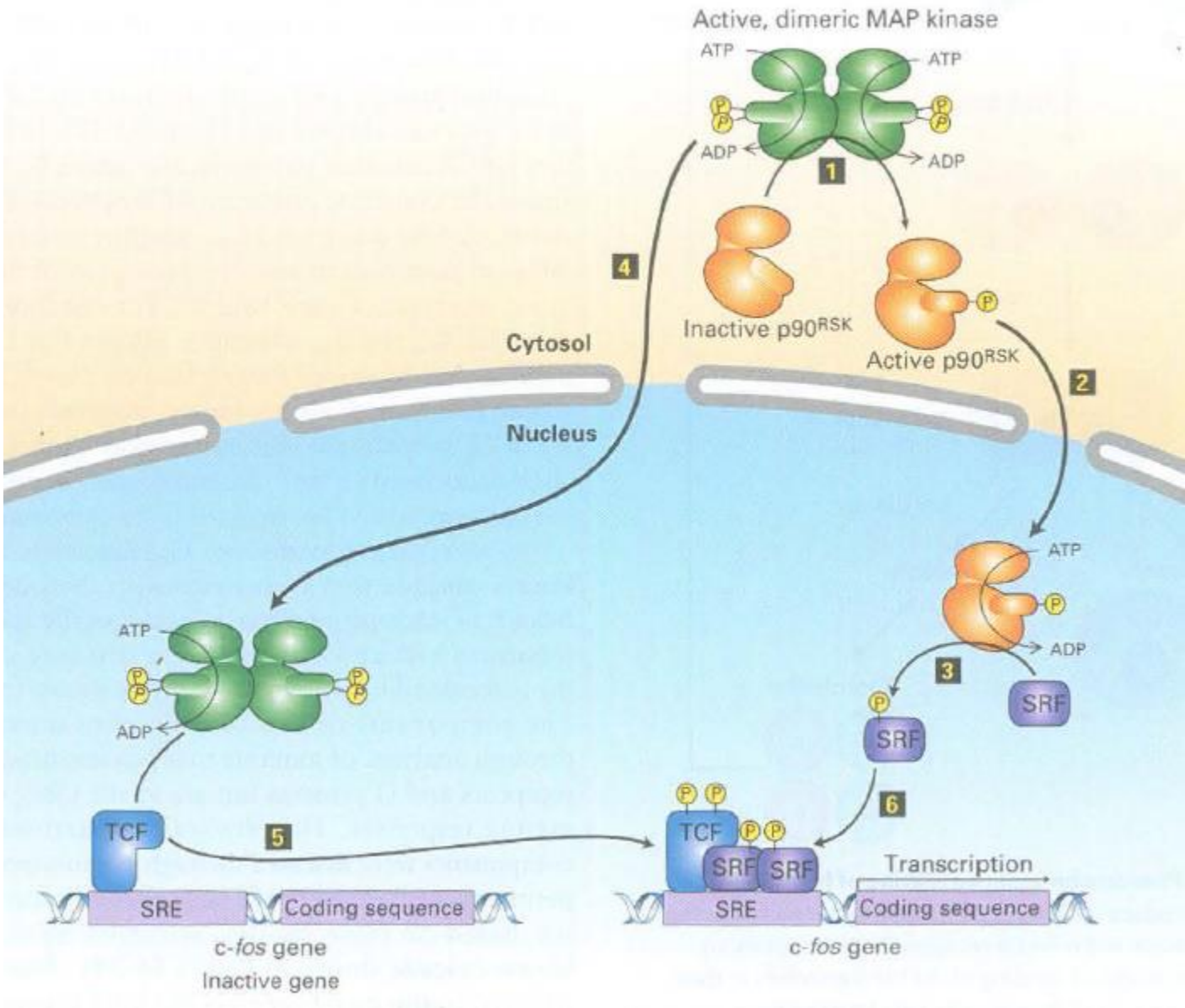
- Raf subsequently phosphorylates and thereby activates MEK
- Active MEK then phosphorylates and activates MAP kinase, another serine/threonine kinase also known as ERK
- MAP kinase phosphorylates many different proteins, including nuclear transcription factors that mediate cellular responses

- The phosphotyrosine residue (**pYl85**) also plays a key role in binding specific substrate proteins to the surface of MAP kinase
- Phosphorylation promotes not only the catalytic activity of MAP kinase but also its dimerization.
- The dimeric form of MAP kinase is translocated to the nucleus, where it regulates the activity of many nuclear transcription factors.

MAP Kinase Regulates the Activity of Many Transcription Factors Controlling Early Response Genes

- RTKs that bind growth factors utilize the MAP kinase pathway to activate genes encoding proteins such as c-Fos, which in turn propel the cell through the cell cycle.
- The enhancer that regulates the *c-fos gene contains a serum response element (SRE), so named because it is activated* by many growth factors in serum
- By direct activation of one transcription factor, *ternary complex factor (TCF)*
- *Indirect activation* of another, *serum response factor (SRF).*

Induction of gene transcription by 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 that is already bound to the promoter of the *c-fos gene.*
- Phosphorylated TCF and SRF act together to stimulate transcription of genes (e.g., *c-fos) that contain an SRE sequence in their* promoter