

Signal Transduction

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Integrative Physiology and Pharmacology

Introduction to Physiology (0501110)

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Subject	Lecture No.	Lecturer	Pages in the 11 th edition. textbook	Pages in the 12 th edition textbook
Receptors: types and adaptation - Membrane or intracellular - Ion channels - G-protein - Enzyme linked - Intracellular - Second messengers - cAMP and cGMP, Phospholipid - Calcium calmodulin and IRS	1-2		910-915	886-891
Signal Transduction (Regulation of cellular machinery) Extracellular regulators: nervous, endocrine, paracrine and autocrine	3-4		934-936 962-963	910-912 940-941
Steroids: Their Signal Transduction And Mechanism Of Action, Thyroid hormones, Nitric Oxide	5		949 954	926-927 931

Textbook: Guyton Medical Textbook of Physiology By: Guyton and Hall 12th edition
 Book Chapter **Cell Signalling Biology** Michael J. Berridge

Objectives:

- Define first messenger (Hormones)
- List hormone types
- Describe receptor types
- Outline the hormone receptors interactions
- Describe second messenger mechanism of action
- List second messengers

Signaling Overview

1. Introduction

- A. Definitions
- B. Components involved in signaling
- C. Types of signaling

2. Types of Signaling Ligands - cell-surface vs. intracellular

3. Three Major Classes of Signaling Receptors

Ion Channel-linked

G protein-coupled receptors (GPRs)

Enzyme-linked receptors

 Tyrosine-Kinase Receptors

 Overview

 Mechanism of activation

 Different ways that TKRs can be activated

 TKs that are non-covalently linked with receptors

4. Second Messengers: cAMP, cGMP, IP3 and DAG, Ca²⁺, PIP3

5. Signaling Cascades

- A. Ras GTPase
- B. Adaptor proteins with SH2 and SH3 domains
- C. MAP kinase pathway
- D. 5 different kinases activated by different cascades
- E. JAK-STAT pathway

Signaling Overview

1. Introduction

A. Definitions

Signaling: Cell-cell communication via signals.

Signal transduction: Process of converting extracellular signals into intra-cellular responses.

Ligand: The signaling molecule.

Receptors: Bind specific ligands. Transmit signals to intracellular targets. Different receptors can respond differently to the same ligand.

B. Components involved in signaling:

Ligands

Receptors

Intracellular Signaling Proteins

Intermediary Proteins

Enzymes

Second Messengers

Target Proteins

Inactivating Proteins

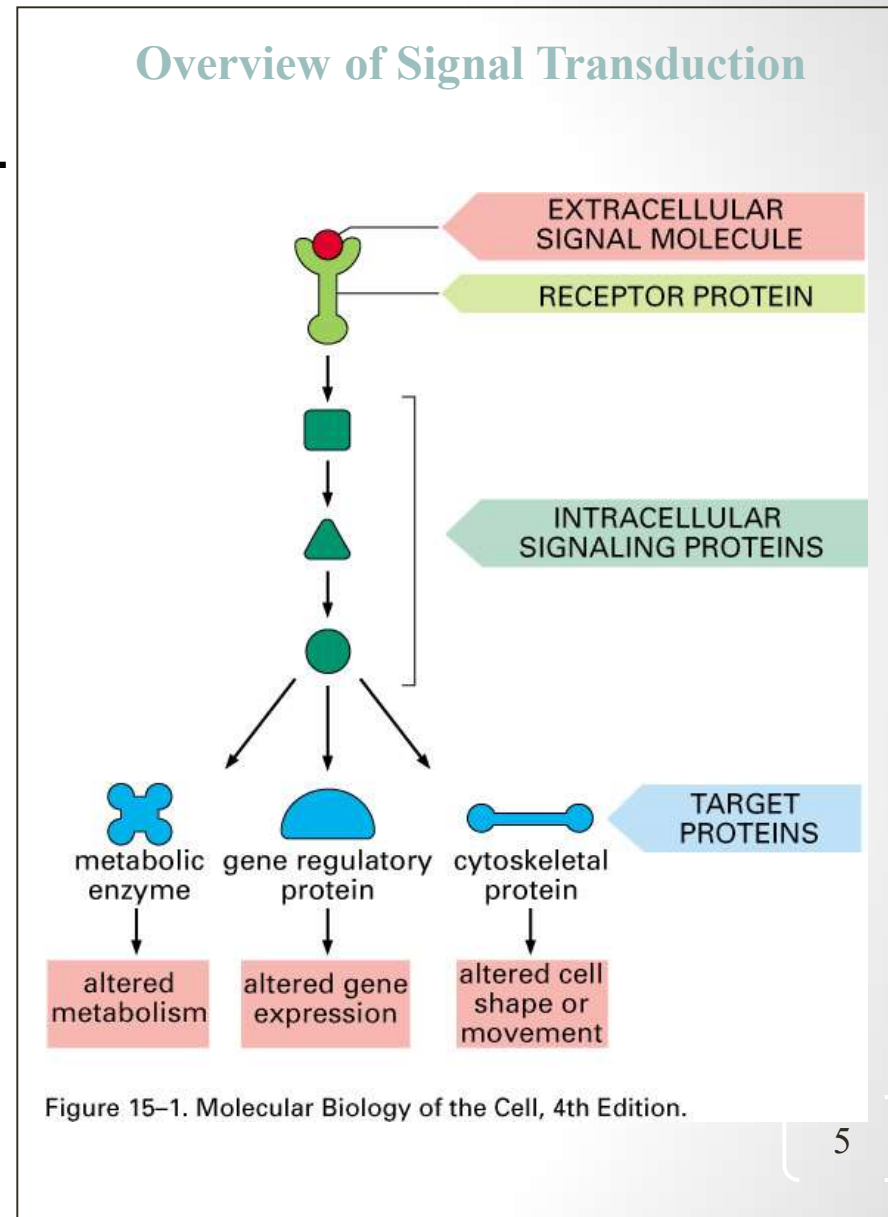
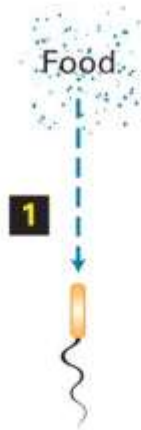
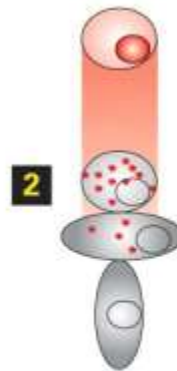


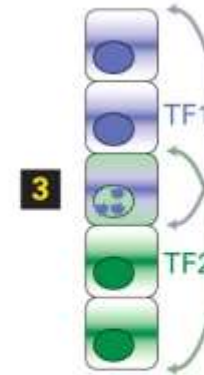
Figure 15-1. Molecular Biology of the Cell, 4th Edition.



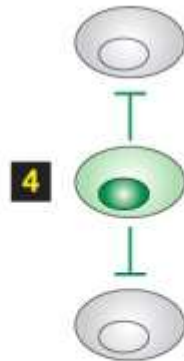
Cells adjust to their particular environmental inputs (e.g., oxygen, sugar, and temperature)



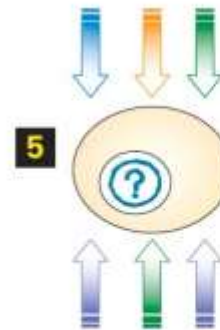
Graded signals create different cell types



Combined actions of transcription factors create different cell types



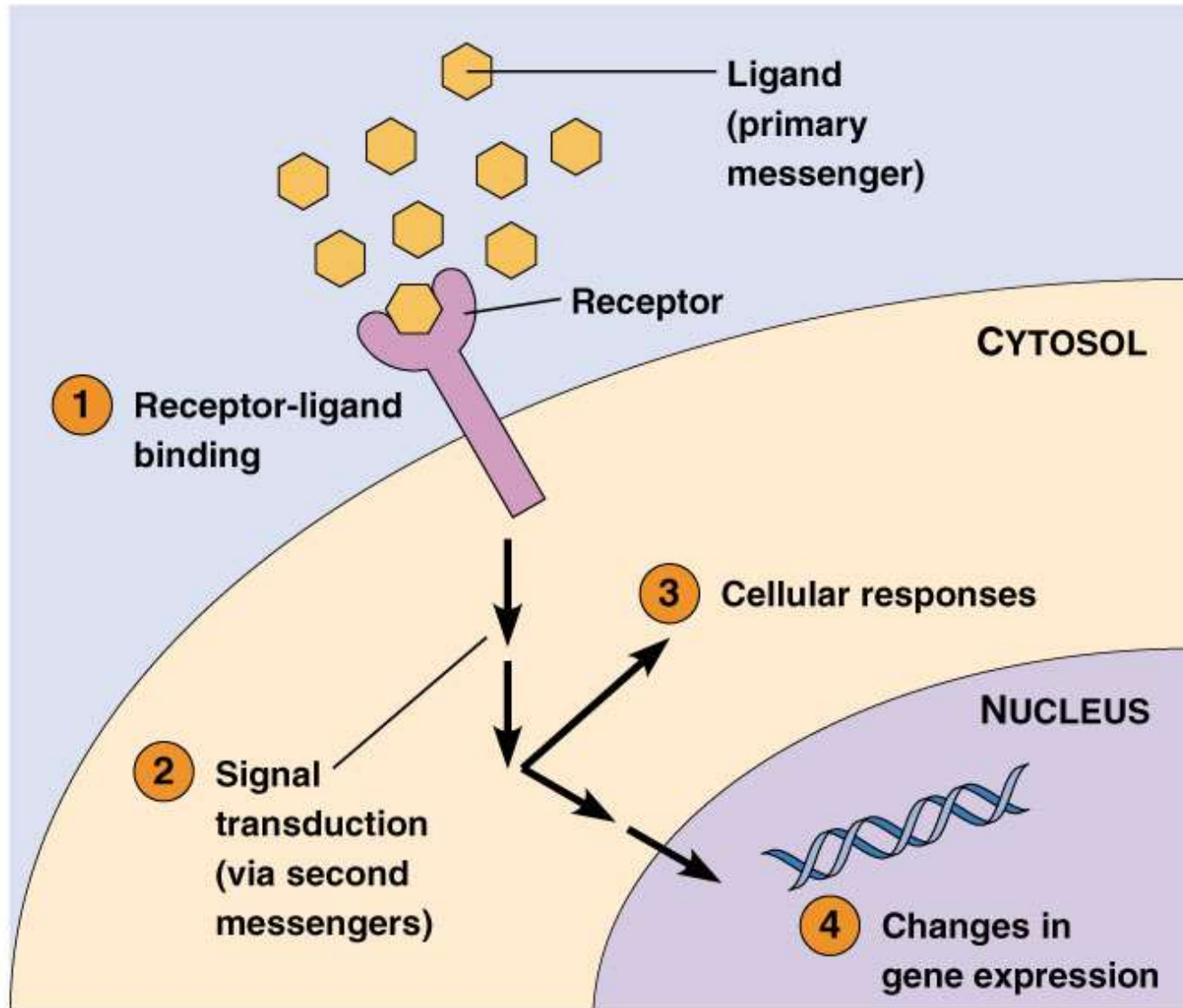
Lateral inhibition signals prevent duplication of unique cell types

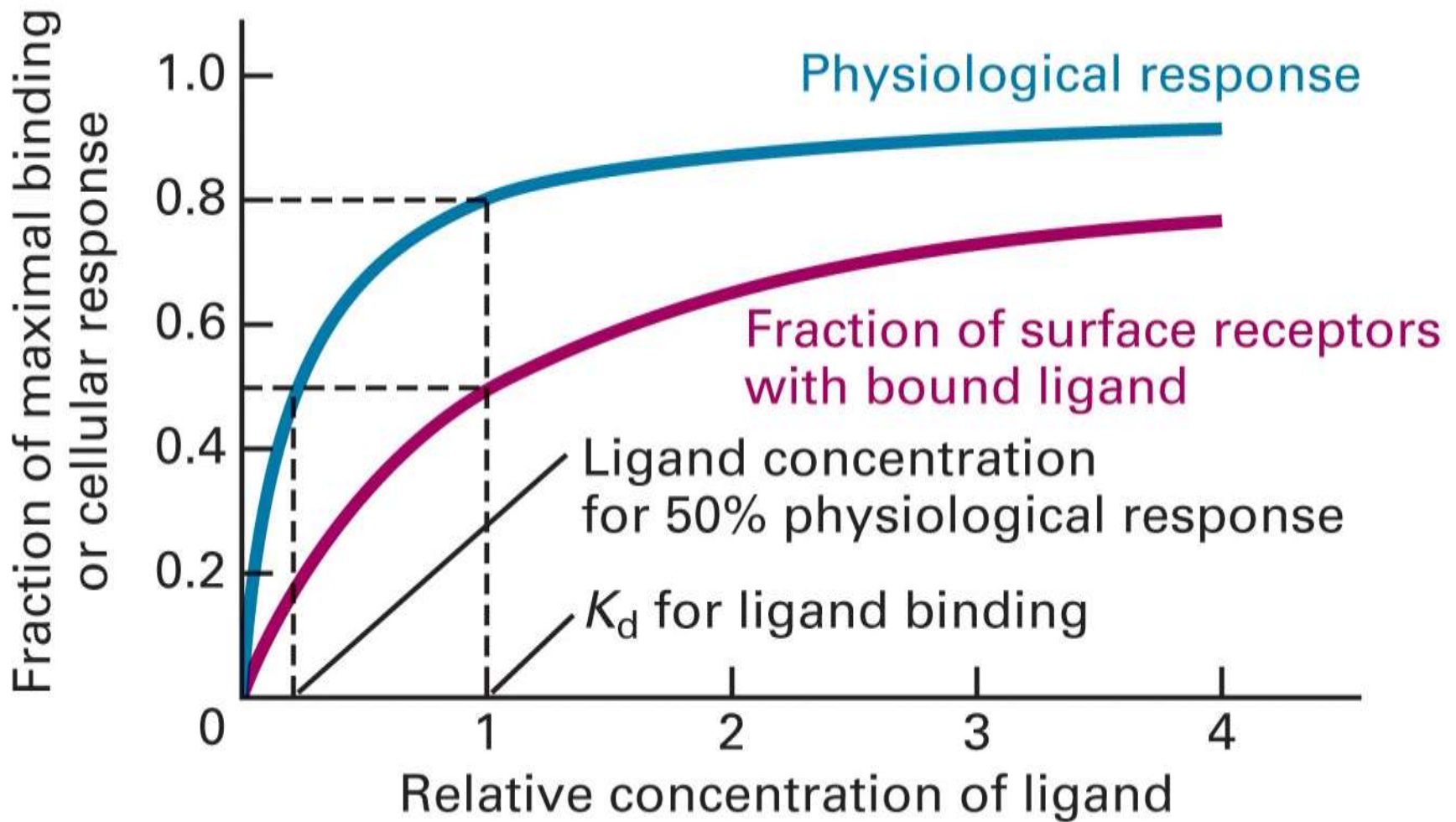


Integration of signals allows cells to adjust to their neighbors and to change with time

Signaling is responsible for how cells can respond to their environment and how they can differentiate or change over time

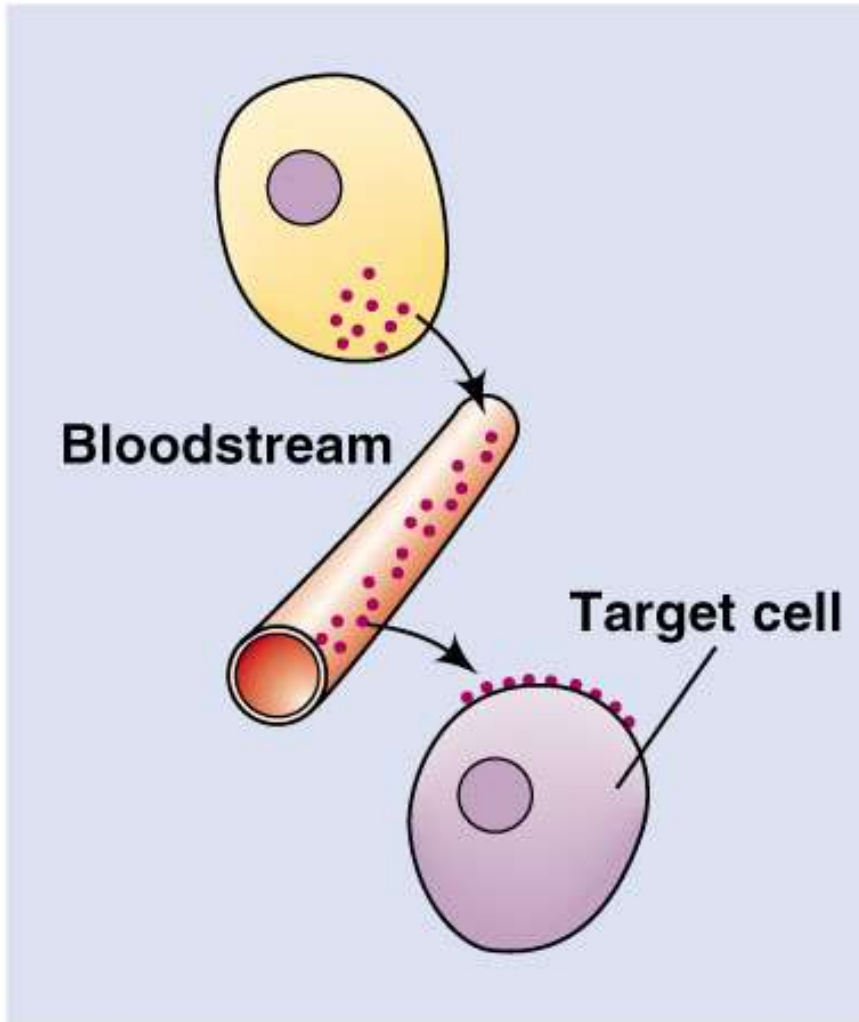
Signals get translated into cellular responses or changes in gene expression



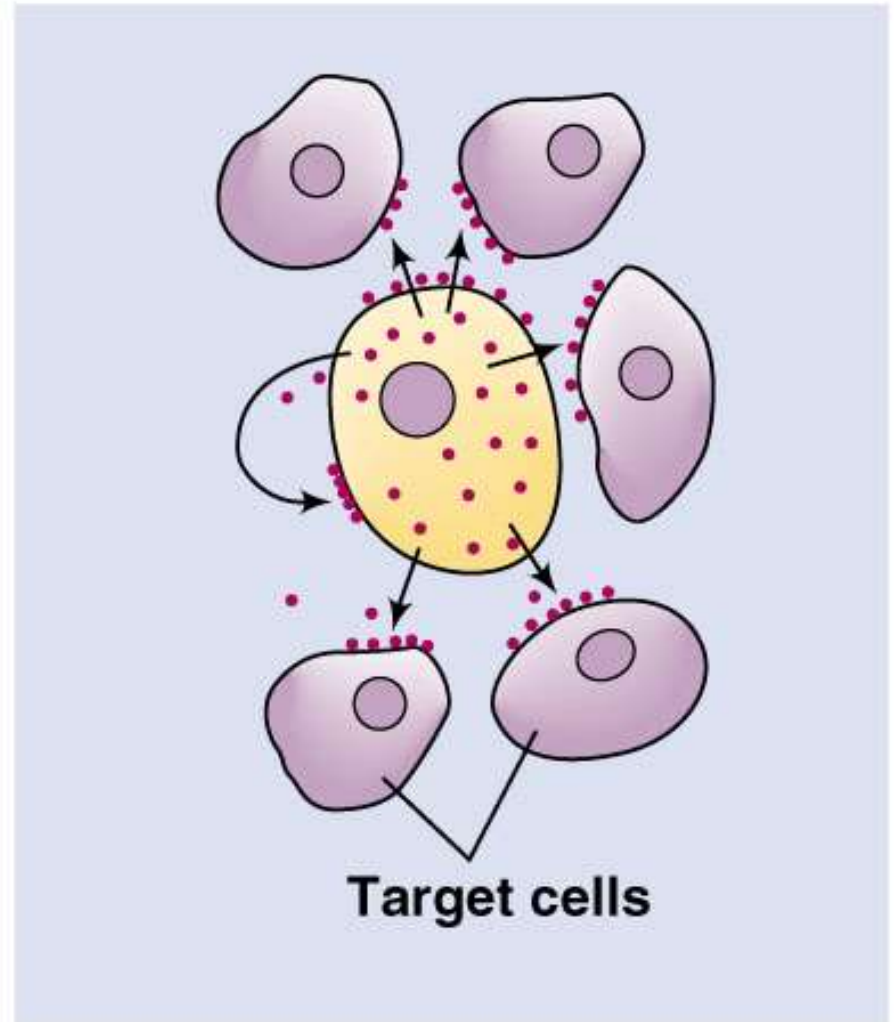


Not all of the receptor needs to be bound to induce a response

Signals can act locally or at a distance

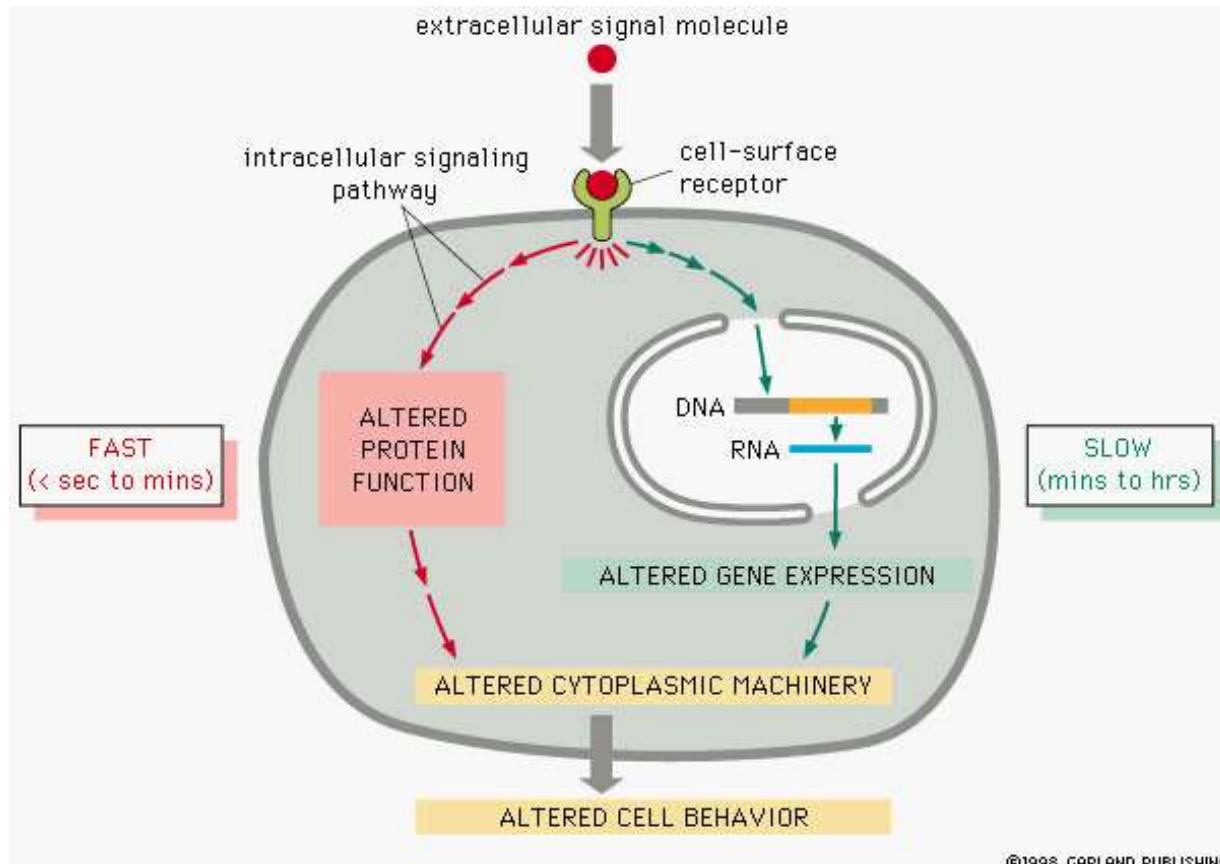


Hormones

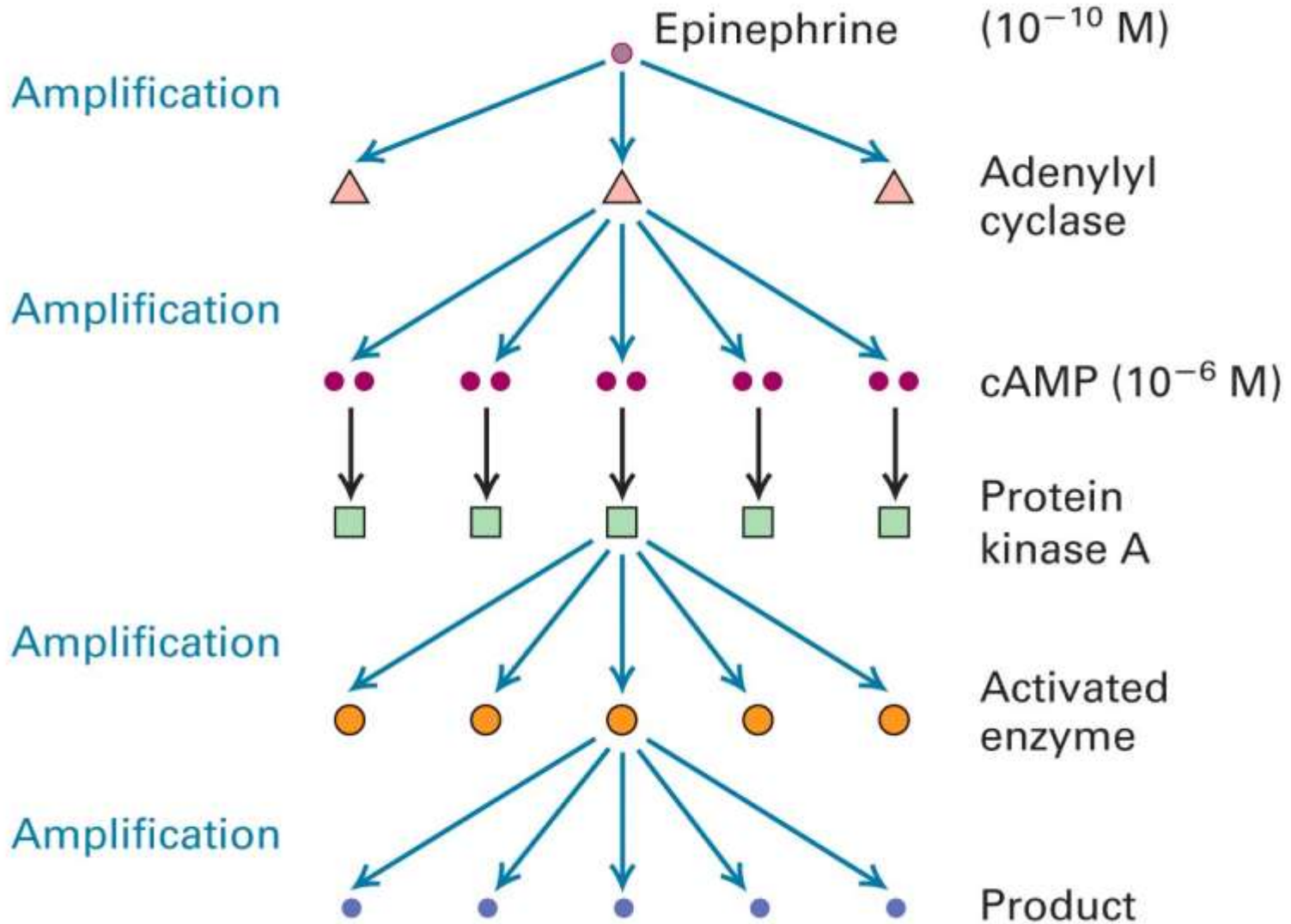


Local mediators

Responses can be fast or slow



Signals are amplified



Signaling Overview

1. Introduction

C. Types of signaling

i. Contact-dependent - via proteins in the PM:

ii. Via Secreted Signals:

- Autocrine - via growth factors, cell that releases the signal is also the target.
- Paracrine - via neurotransmitters and cytokines, action on adjacent target cells.
- Endocrine - via hormones, action on distant target cells.
- Synaptic - via neurotransmitters, action on post-synaptic cell in response to electrical stimuli

2. Types of Signaling Ligands:

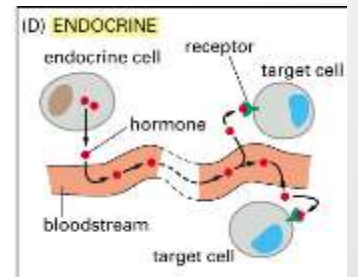
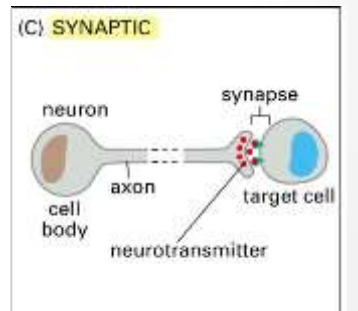
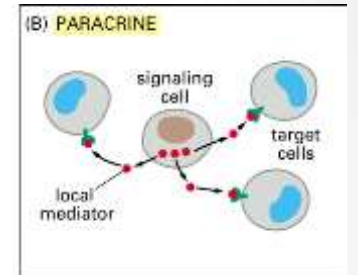
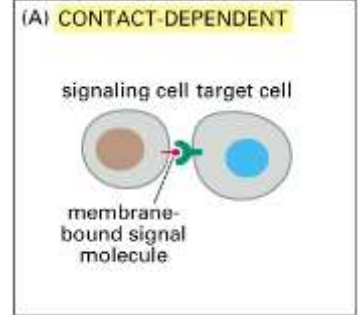
A. Ligands that bind to cell-surface receptors:

- Neurotransmitters (NT), i.e. norepinephrine, histamine - hydrophilic (charged, polar)
- Peptide hormones (P), i.e. insulin - can't cross membrane
- Growth factors (GF), i.e. NGF, EGF, PDGF
- Lipophilic signaling molecules, i.e. prostaglandins

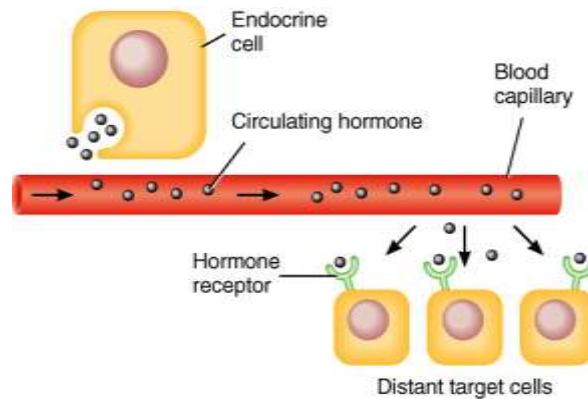
B. Ligands that bind to intracellular receptors:

lipid soluble hormones that diffuse across the plasma membrane and interact with receptors in the cytosol or nucleus. i.e. steroids, thyroxine, retinoic acid, nitric oxide.

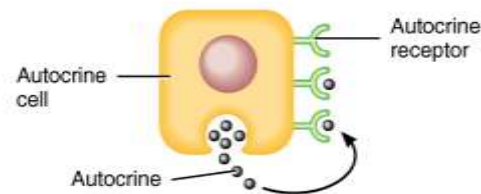
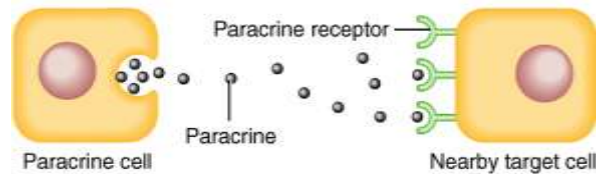
Types of Signaling



Local vs. Circulating hormones



(a) Circulating hormones



(b) Local hormones (paracrines and autocrines)

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Chemical classes of hormones

- ❑ Lipid-soluble hormones- use transport proteins in the **plasma**
 - ❑ Steroid: Lipids derived from cholesterol.
 - Are lipophilic hormones.
 - ❑ Testosterone.
 - ❑ Estradiol.
 - ❑ Cortisol.
 - ❑ Progesterone.
 - ❑ Thyroid (amine but lipid soluble)
 - ❑ Nitric oxide (NO)

Chemical classes of hormones

- ❑ Water-soluble – circulate in “free” form in the plasma
 - Amines:
 - ❑ Hormones derived from tyrosine and tryptophan.
 - Polypeptides and proteins:
 - ❑ Polypeptides:
 - Chains of < 100 amino acids in length.
 - ❑ ADH.
 - ❑ Protein hormones:
 - Polypeptide chains with > 100 amino acids.
 - Growth hormone.
- Eicosanoid (prostaglandins) derived from arachidonic acid (20 carbon 4 double bonds)

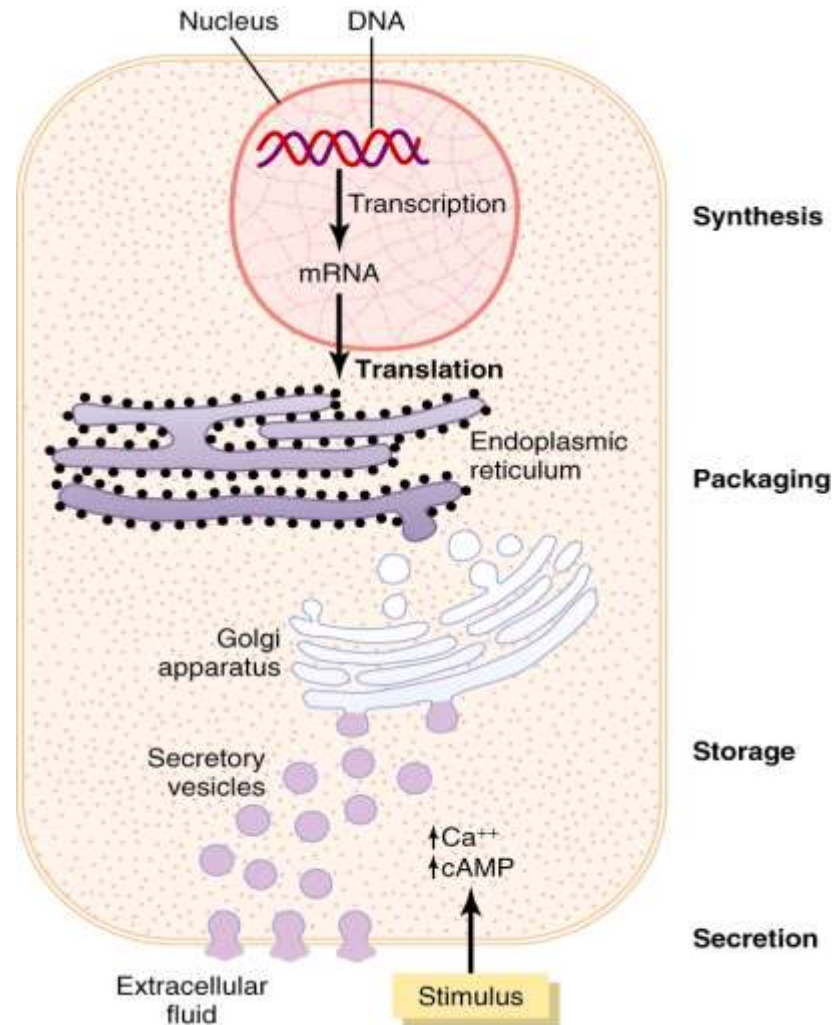
Chemical Classification of Hormones

- Glycoproteins:
 - Long polypeptides (>100) bound to 1 or more carbohydrate (CHO) groups.
 - FSH and LH, TSH and hCG (human chorionic gonadotropin)
They have α and β subunits (α is common and β is specific)
- Hormones can also be divided into:
 - Polar:
 - H₂O soluble.
 - Nonpolar (lipophilic):
 - H₂O insoluble.
 - Can gain entry into target cells.
 - Steroid hormones and T₄ (thyroxine –tetraiodothyronine))

Prohormones and Prehormones

- Prohormone:
 - Precursor is a longer chained polypeptide that is cut and spliced together to make the hormone.
 - Proinsulin – gives insulin
- Preprohormone:
 - Prohormone derived from larger precursor molecule.
 - Preproinsulin.
- Prehormone:
 - Molecules secreted by endocrine glands that are inactive until changed into hormones by target cells.
 - T_4 converted to T_3 (tri-iodothyronin).

Synthesis and secretion of peptide hormones



Chemical classification of hormones

Table 10-4 Chemical Classification and Function of Hormones

Chemical Classification	Examples	Regulated Function
Endocrine Hormones		
Amino acid derivatives	Epinephrine (adrenaline) and norepinephrine (both derived from tyrosine)	Stress responses; regulation of heart rate and blood pressure; release of glucose and fatty acids from storage sites
	Thyroxine (derived from tyrosine)	Regulation of metabolic rate
Peptides	Antidiuretic hormone (vasopressin)	Regulation of body water and blood pressure
	Hypothalamic hormones (releasing factors)	Regulation of tropic hormone release from pituitary gland
Proteins	Anterior pituitary hormones	Regulation of other endocrine systems
Steroids	Sex hormones (androgens and estrogens)	Development and control of reproductive capacity
	Corticosteroids	Stress responses; control of blood electrolytes
Paracrine Hormones		
Amino acid derivative	Histamine	Local responses to stress and injury
Arachidonic acid derivatives	Prostaglandins	Local responses to stress and injury

Peptide & Protein Hormones

Gland/Tissue

Hypothalamus

Hormones

- TRH, GnRH, CRH
GHRH, Somatostatin,

Anterior pituitary

- ACTH, TSH, FSH, LH,
PRL, GH

Posterior pituitary

- Oxytocin, ADH

Thyroid

- Calcitonin

Pancreas

- Insulin, Glucagon,
Somatostatin

Liver

- Somatomedin C (IGF-1)

Parathyroid

- PTH

Gland/Tissue

Placenta

Hormones

- HCG, HCS or HPL

Kidney

- Renin

Heart

- ANP

G.I. tract

- Gastrin, CCK,
Secretin, GIP,
Somatostatin

Adipocyte

- Leptin

Adrenal medulla

Amine Hormones

Gland/Tissue

Hormones

Hypothalamus

■ Dopamine

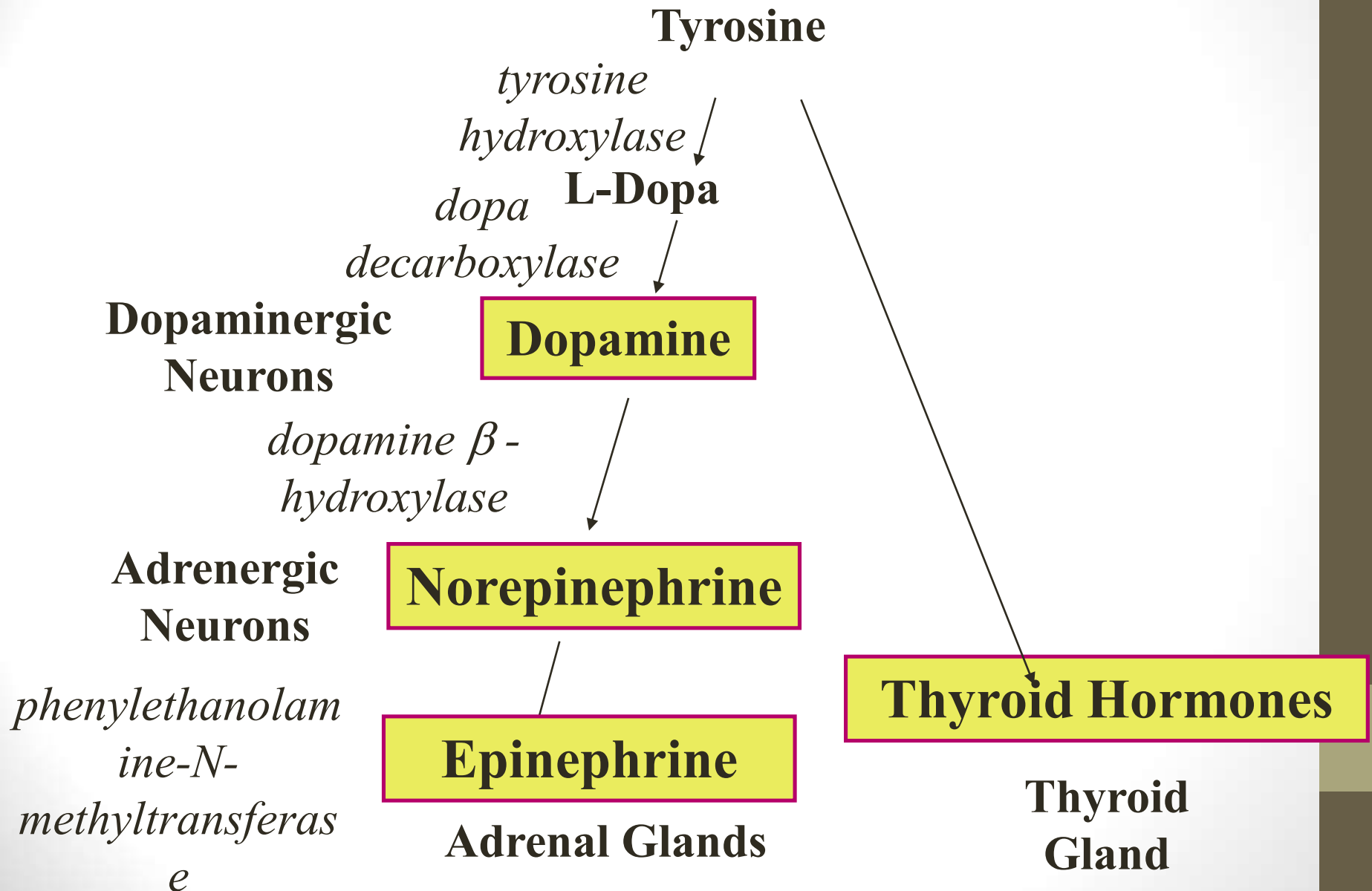
Thyroid

■ T₃, T₄

Adrenal medulla

■ Epinephrine and
Norepinephrine
(NE, EPI)

Synthesis of Amine Hormones



Steroid Hormones

Gland/Tissue

Adrenal Cortex

Testes

Ovaries

Corpus Luteum

Placenta

Kidney

Hormones

- Cortisol, Aldosterone, Androgens

- Testosterone

- Estrogens, Progesterone

- Estrogens, Progesterone

- Estrogens, Progesterone

- 1,25-Dihydroxycholecalciferol (calcitriol)

Hormone Activity

- Hormones affect only specific target tissues with specific receptors
- Receptors are dynamic and constantly synthesized and broken down
 - Down-regulation- decrease in receptor number or response
 - Up-regulation- increase in receptor number or activity

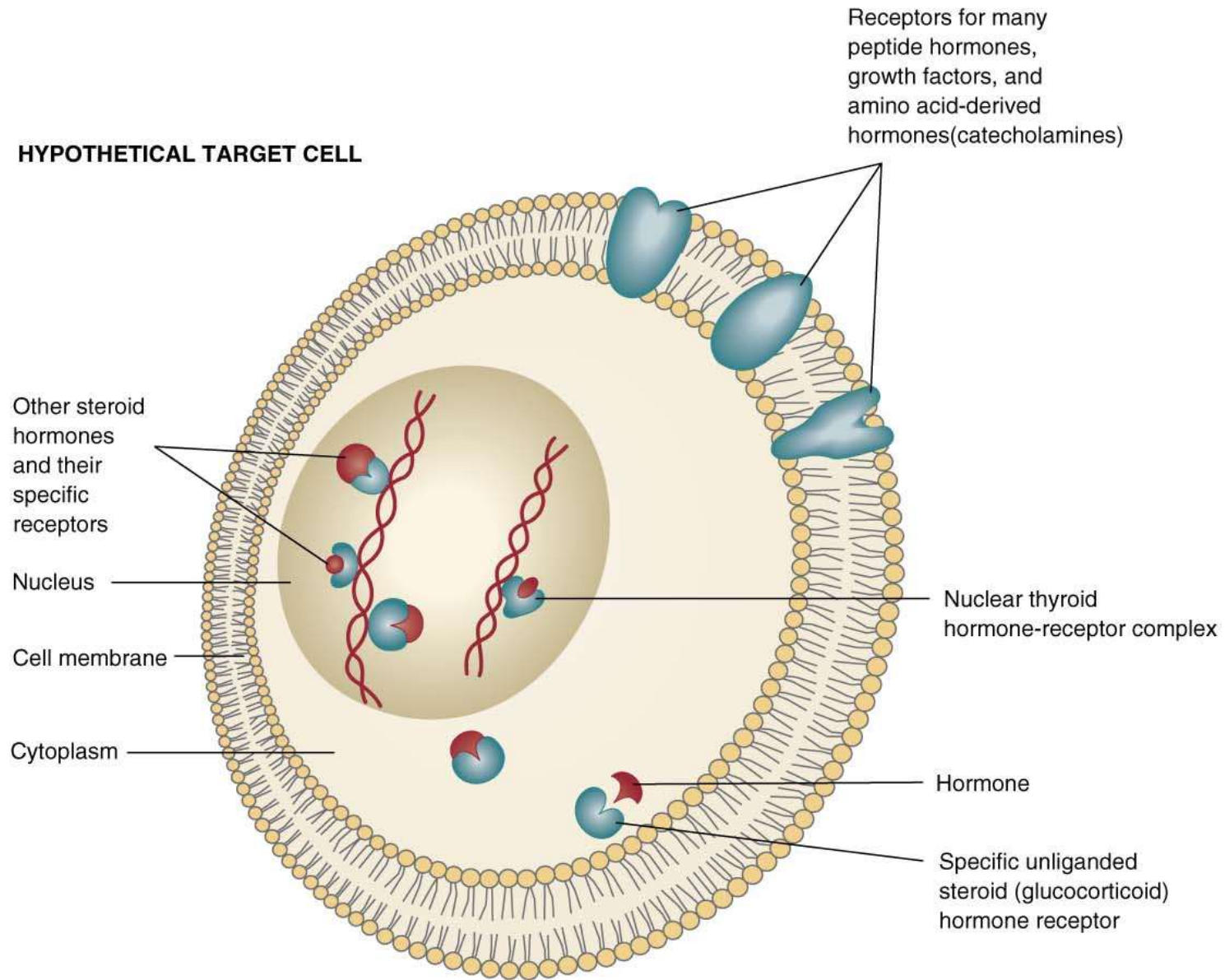
Effects of [Hormone] on Tissue Response

- Priming effect (upregulation):
 - Increase number of receptors formed on target cells in response to particular hormone.
 - Greater response by the target cell.
- Desensitization (downregulation):
 - Prolonged exposure to high [polypeptide hormone].
 - Subsequent exposure to the same [hormone] produces less response.
 - Decrease in number of receptors on target cells.
 - Insulin in adipose cells.
 - Pulsatile secretion may prevent downregulation.

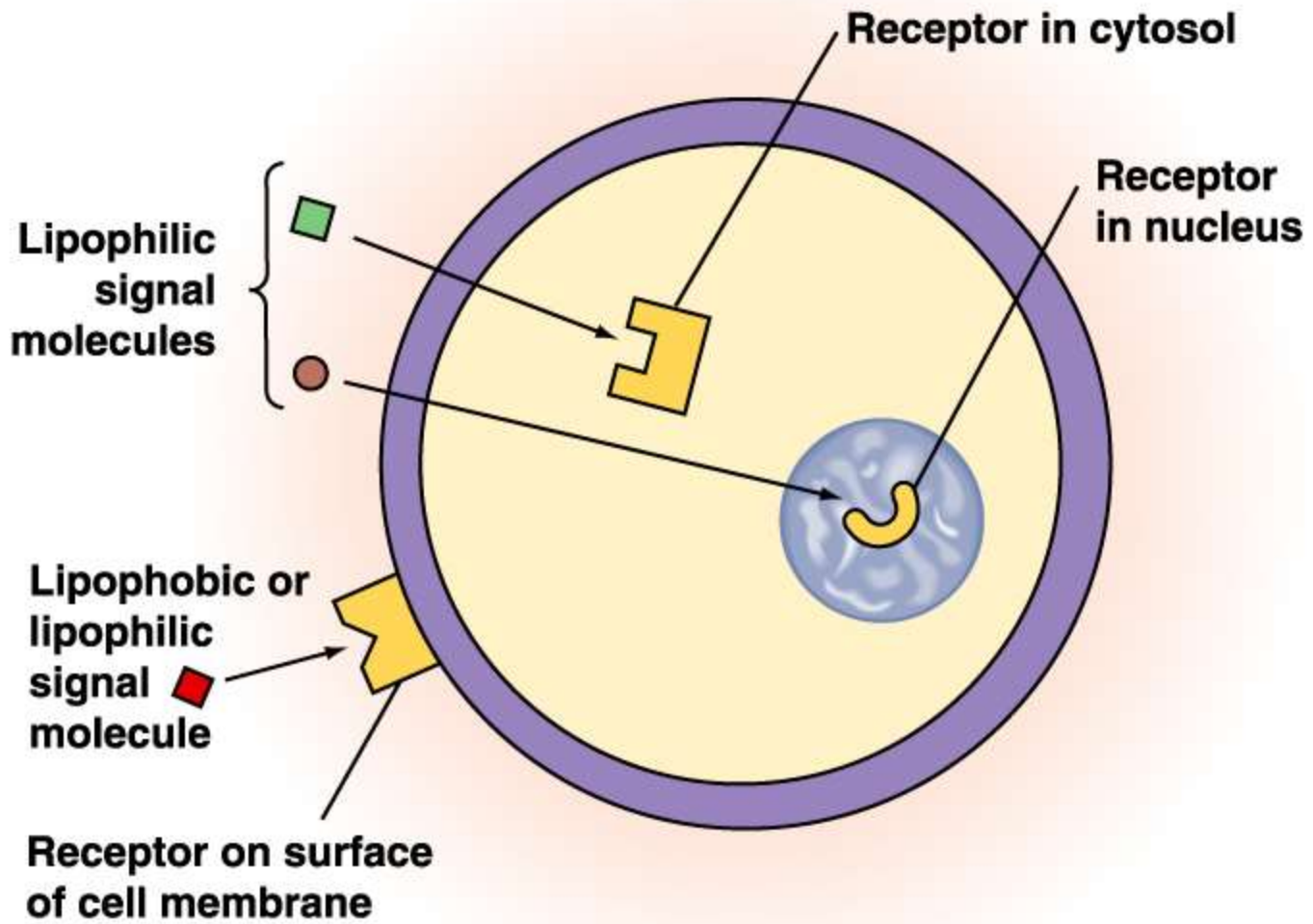
Effects of hormone concentration on Tissue Response

- [Hormone] in blood reflects the rate of secretion.
- Half-life:
 - Time required for the blood [hormone] to be reduced to $\frac{1}{2}$ reference level.
 - Minutes to days.
- Affinity of receptors to ligands, K_d
- Normal tissue responses are produced only when [hormone] are present within physiological range.
- Varying [hormone] within normal, physiological range can affect the responsiveness of target cells.

HYPOTHETICAL TARGET CELL



. Diagram showing the different locations of classes of hormone receptors expressed by a target cell.



Mechanisms of Hormone Action

- Hormones of same chemical class have similar mechanisms of action.
 - Similarities include:
 - Location of cellular receptor proteins depends on the chemical nature of the hormone.
 - Events that occur in the target cells.
- To respond to a hormone:
 - Target cell must have specific receptors for that hormone (specificity).
 - Hormones exhibit:
 - Affinity (bind to receptors with high bond strength).
 - Saturation (low capacity of receptors).

Mechanisms of Hormone Action

- ⊕ Response depends on both hormone and target cell
- ⊕ Lipid-soluble hormones bind to receptors inside target cells
- ⊕ Water-soluble hormones bind to receptors on the plasma membrane
 - ⊕ Activates second messenger system
 - ⊕ Amplification of original small signal
- ⊕ Responsiveness of target cell depends on
 - ⊕ Hormone's concentration
 - ⊕ Abundance of target cell receptors

Receptor

Receptors are specific membrane proteins, which are able to recognize and bind to corresponding ligand molecules, become activated, and transduce signal to next signaling molecules.

Glycoprotein or Lipoprotein

Receptors

- Specificity: The "specificity" of a ligand for a receptor is a description of how favorable the binding of the ligand for the receptor is compared with its possible binding to other types of receptors that may also be present.
- Affinity: "Affinity" simply refers to how strong the binding is (as judged by K association or K dissociation and ΔG_0). "High affinity" refers to very strong binding (large negative ΔG_0 and a very small K_d). The association or dissociation constant is often referred to as the "affinity" or "binding" constant.

ligand

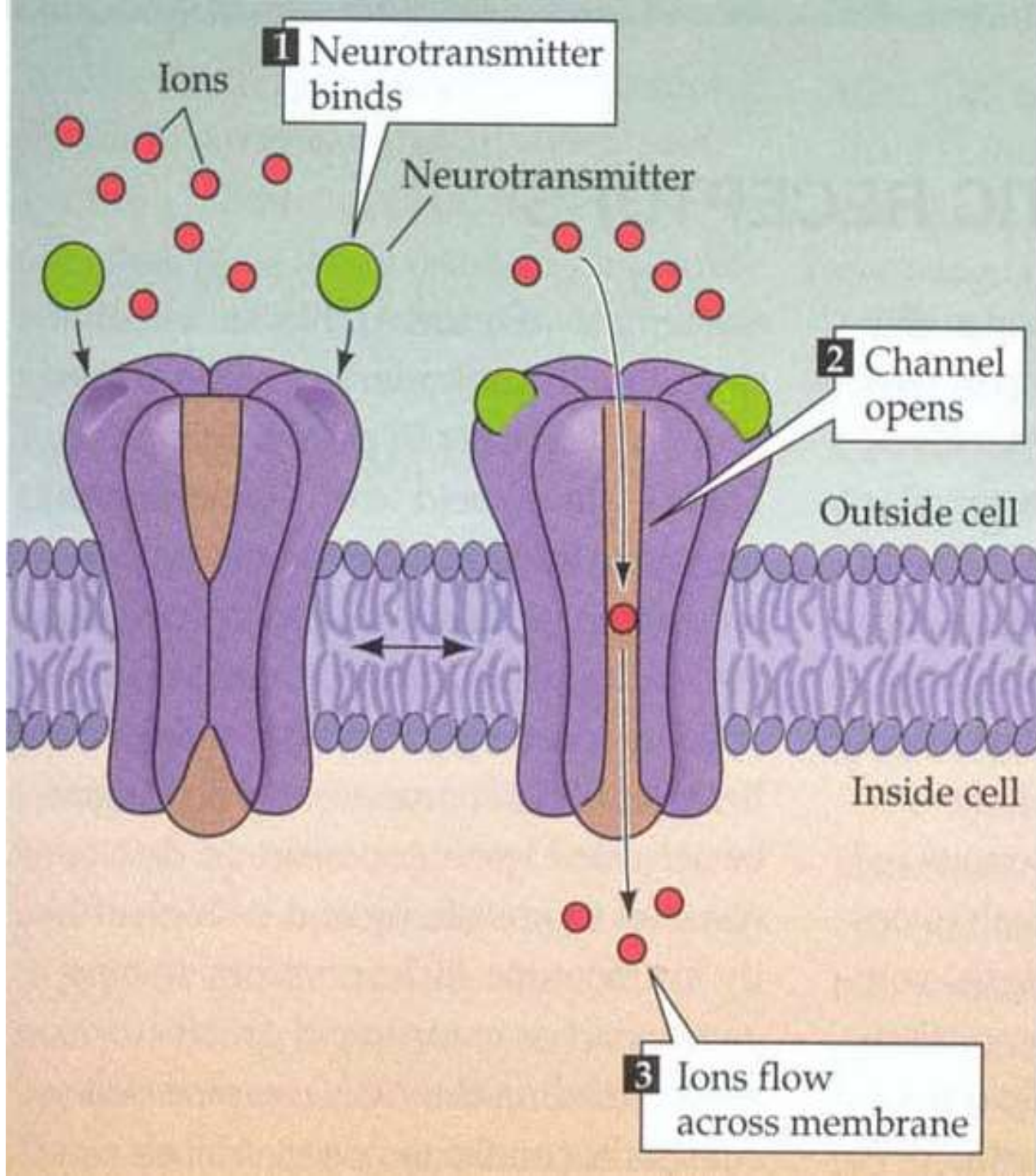
A small molecule that binds specifically to a larger one; for example, a hormone is the ligand for its specific protein receptor.

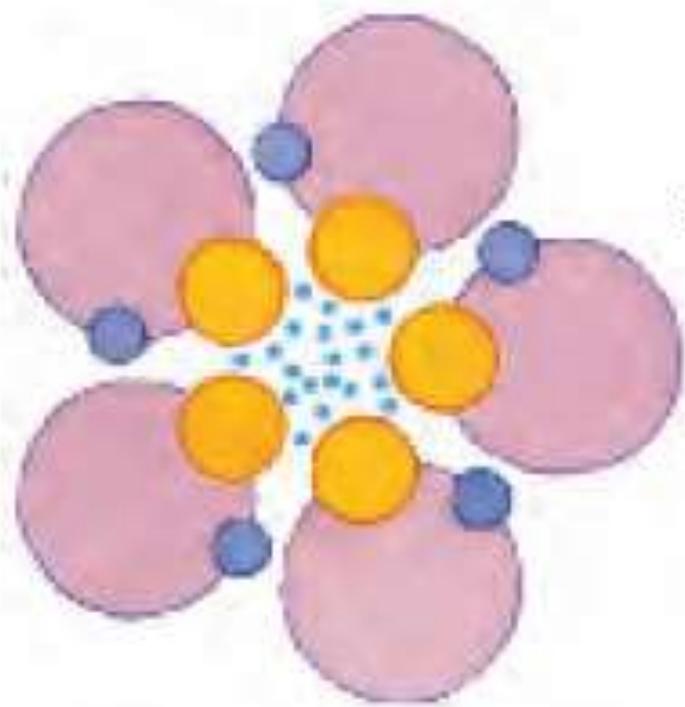
- **Membrane receptors**
Membrane Glycoprotein
- **Intracellular receptors**
Cytosol or nuclei
DNA binding protein

1. membrane receptors

1- Ligand-gate ion channels type (cyclic receptor)

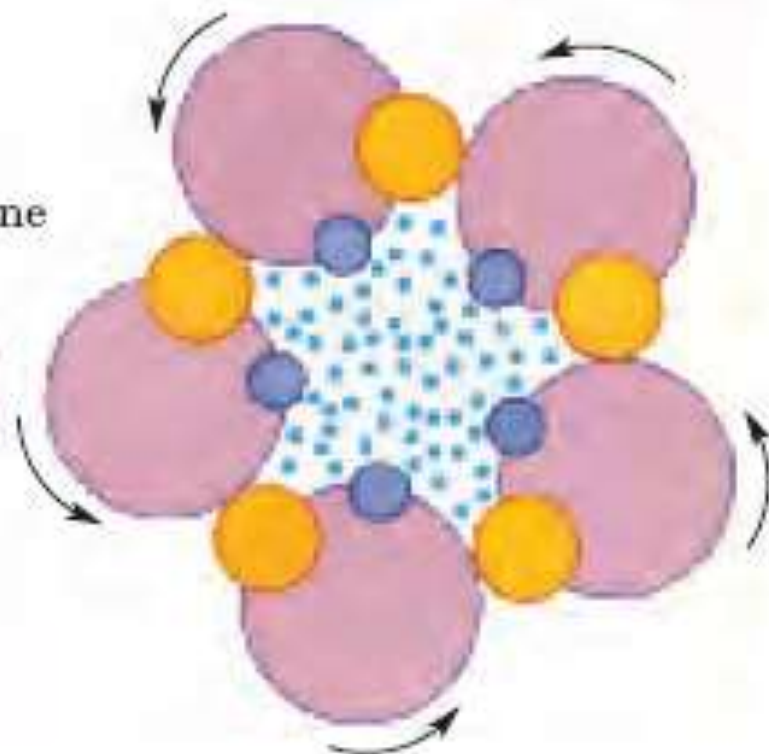
ligand → receptor → ion channel open or close



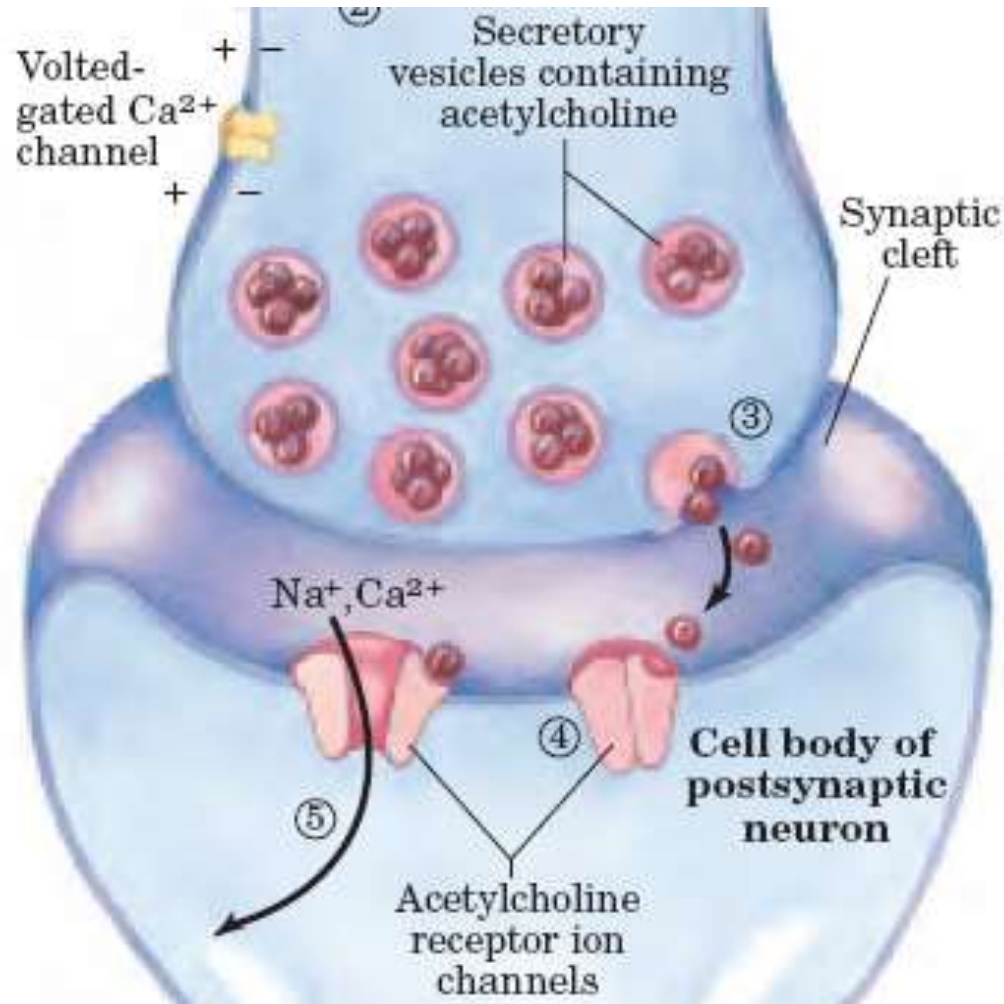


Closed

2 Acetylcholine

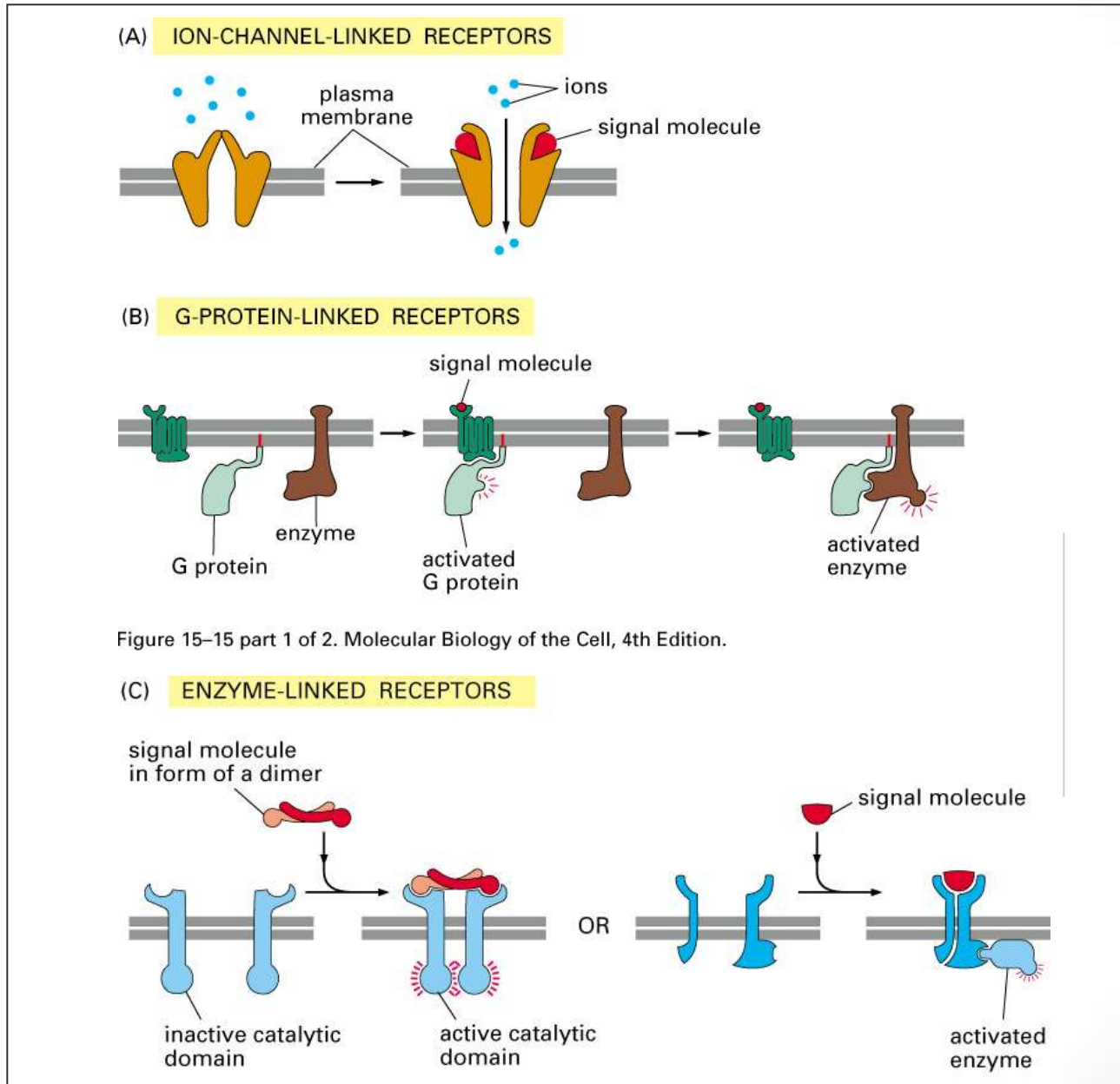


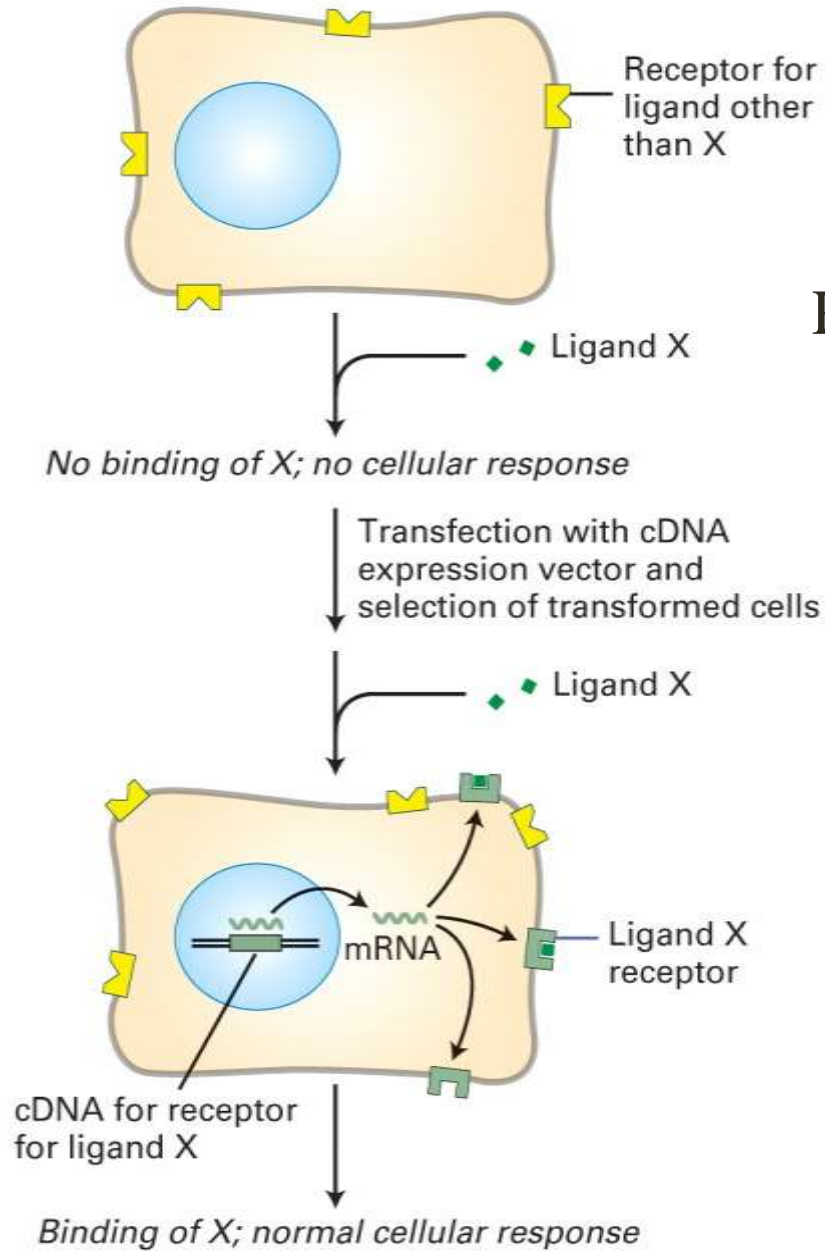
Open



Signaling Overview

3. Three major classes of surface receptors for signaling :





Receptors determine response

No receptor - no response

Signaling Overview

3. Three major classes of surface receptors for signaling, cont.:

A. Ion Channels:

B. **G protein-coupled receptors (GPRs):** largest family of cell surface receptors; present in all eukaryotes; ex: adrenergic receptors, opioid receptors.

1. Overview:

- a. 7 trans-membrane spanning domains
- b. Act as receptors for many different ligands including NT, H
- c. Large amount of receptor diversity, but common mechanism of action
- d. Transmit signals to intracellular targets via G proteins
- e. Targets are plasma membrane bound enzymes or ion channels

2. Mechanism of Activation of GPRs:

- a. Binding of ligand to extracellular domain of GPRs induces conformational change that allows cytosolic domain of the receptor to bind to inactive G protein at inner face of PM.
- b. This interaction activates the G protein, which dissociates from the receptor
- c. Activated G protein α subunit can now bind GTP instead of GDP, causing dissociation into activated α vs. $\beta\gamma$ subunits. Each of these can go on to activate target proteins.

C. Enzyme-linked receptors:

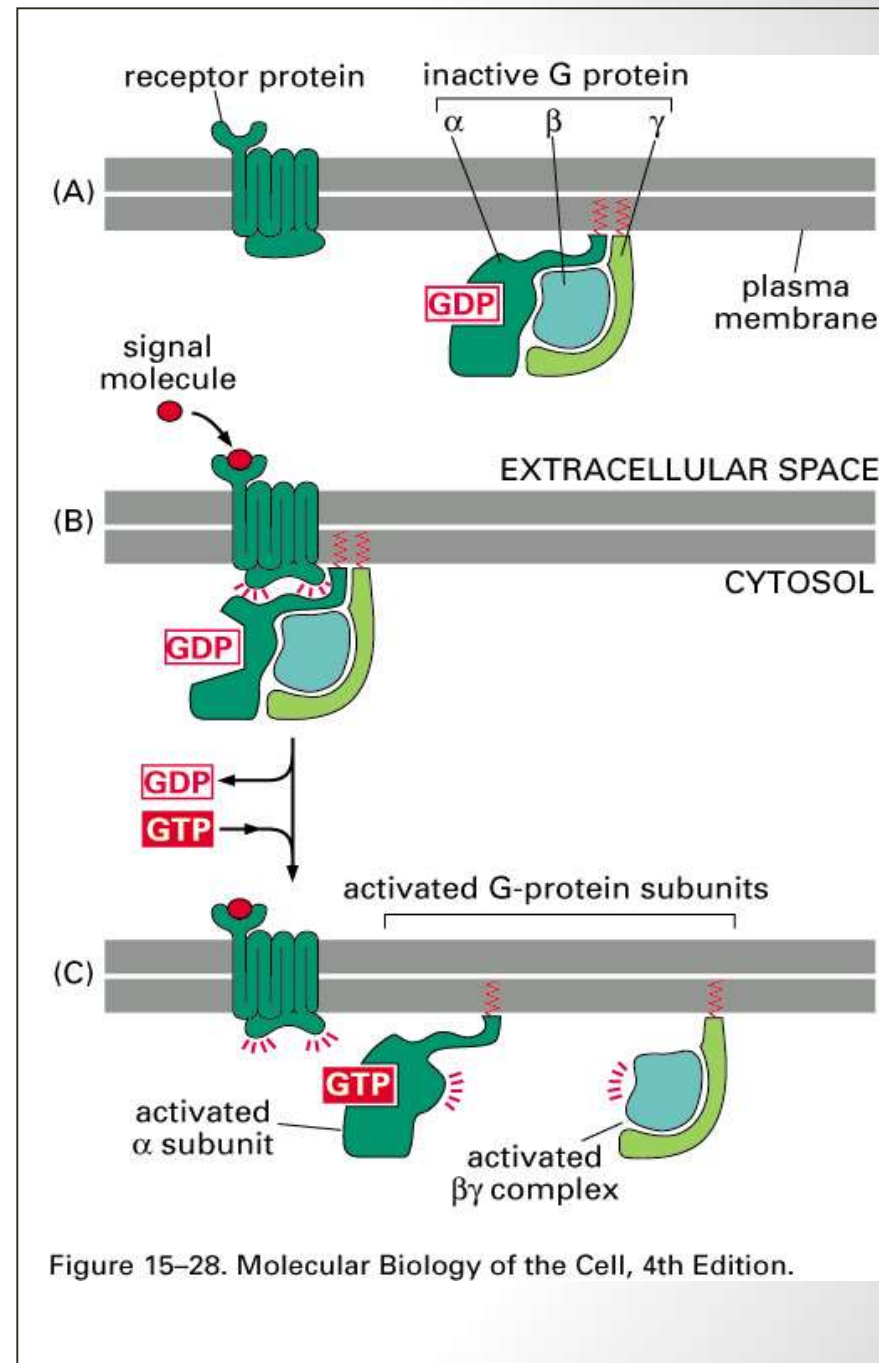


Figure 15-28. Molecular Biology of the Cell, 4th Edition.

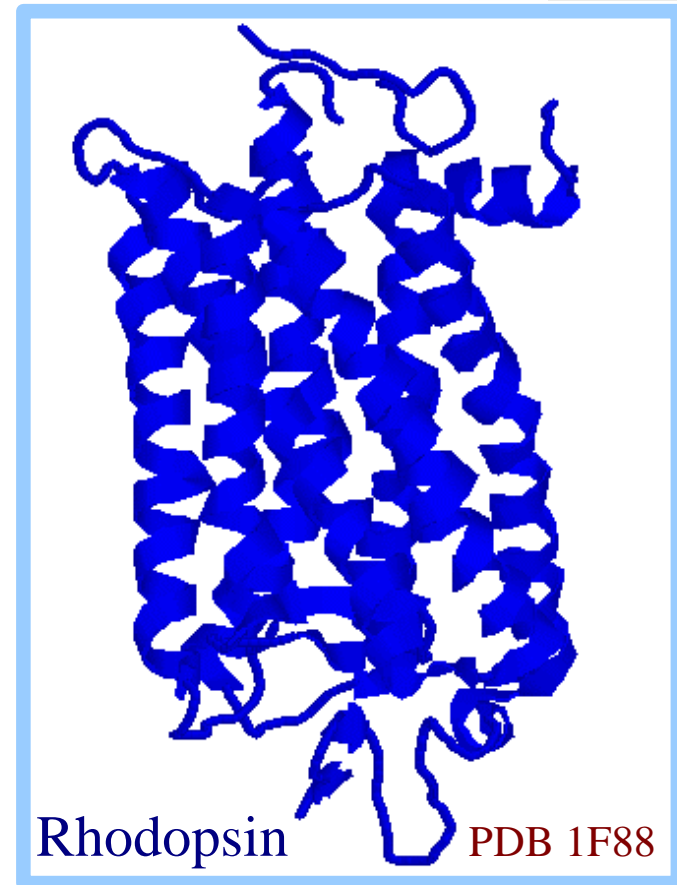
G Protein Signal Cascade

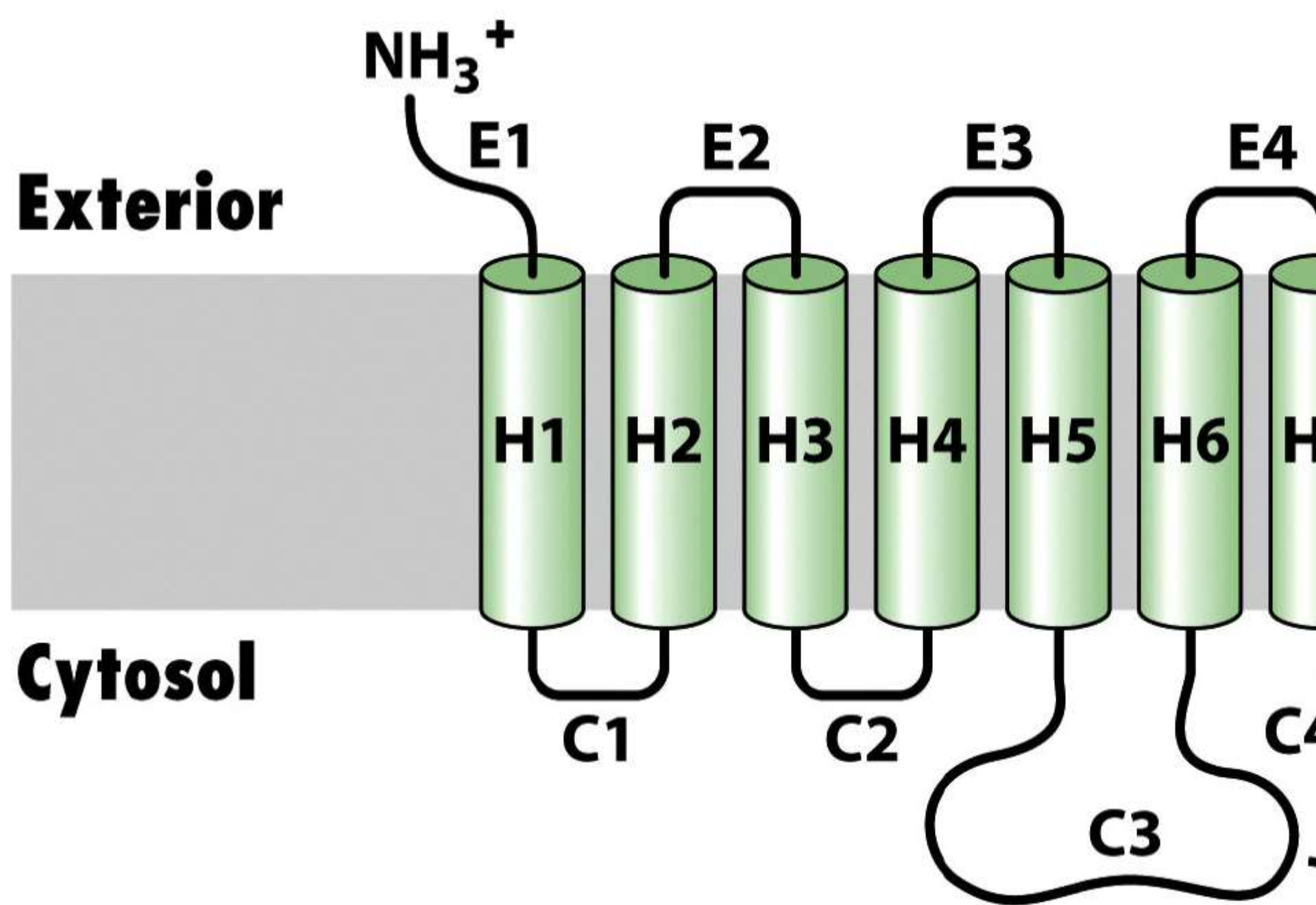
Most signal molecules targeted to a cell bind at the cell surface to **receptors** embedded in the **plasma membrane**.

Only signal molecules able to cross the plasma membrane (e.g., steroid hormones) interact with intracellular receptors.

A large family of **cell surface receptors** have a common structural motif, **7 transmembrane α -helices**.

Rhodopsin was the first of these to have its 7-helix **structure** confirmed by X-ray crystallography (in retina, low light vision).





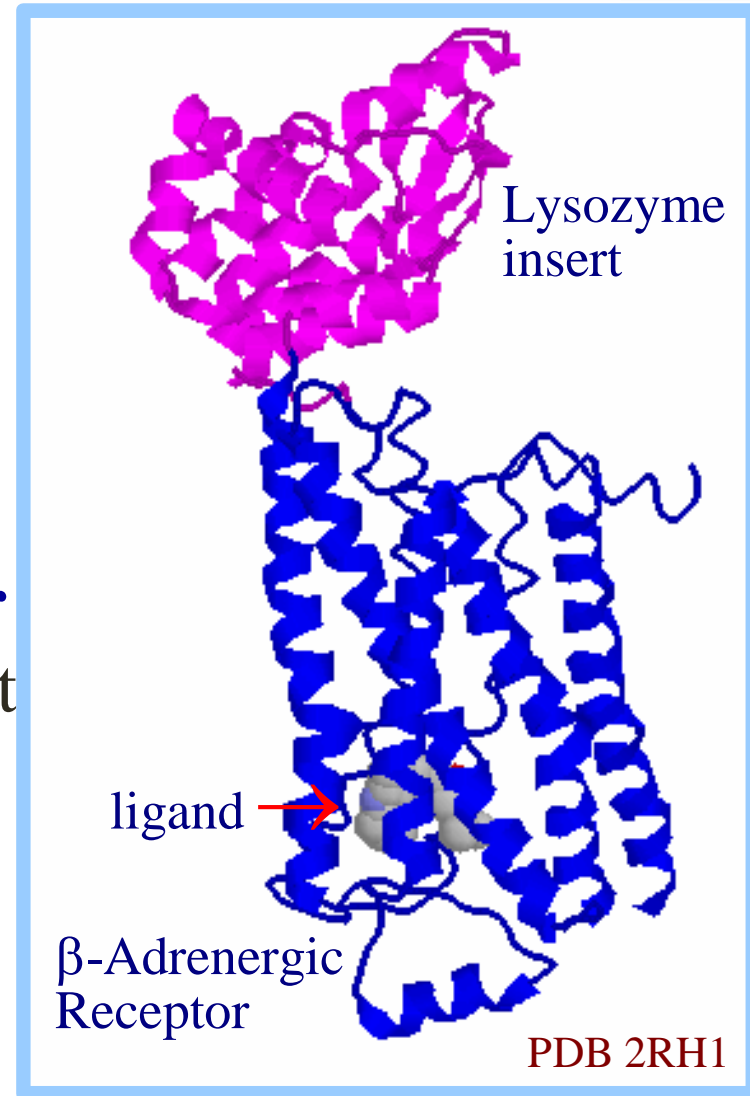
G Protein Signal Cascade

- ◆ **Rhodopsin** is unique.

It senses **light**, via a bound chromophore, retinal.

- ◆ **Most 7-helix receptors** have domains facing the **extracellular** side of the plasma membrane that recognize & bind **signal molecules (ligands)**.

E.g., the **β -adrenergic receptor** is activated by epinephrine & norepinephrine.



G Protein Signal Cascade

The **signal** is usually passed from a **7-helix receptor** to an intracellular **G-protein**.

- ◆ Seven-helix receptors are thus called **GPCR**, or **G-Protein-Coupled Receptors**.
- ◆ Approx. 800 different GPCRs are encoded in the human genome.

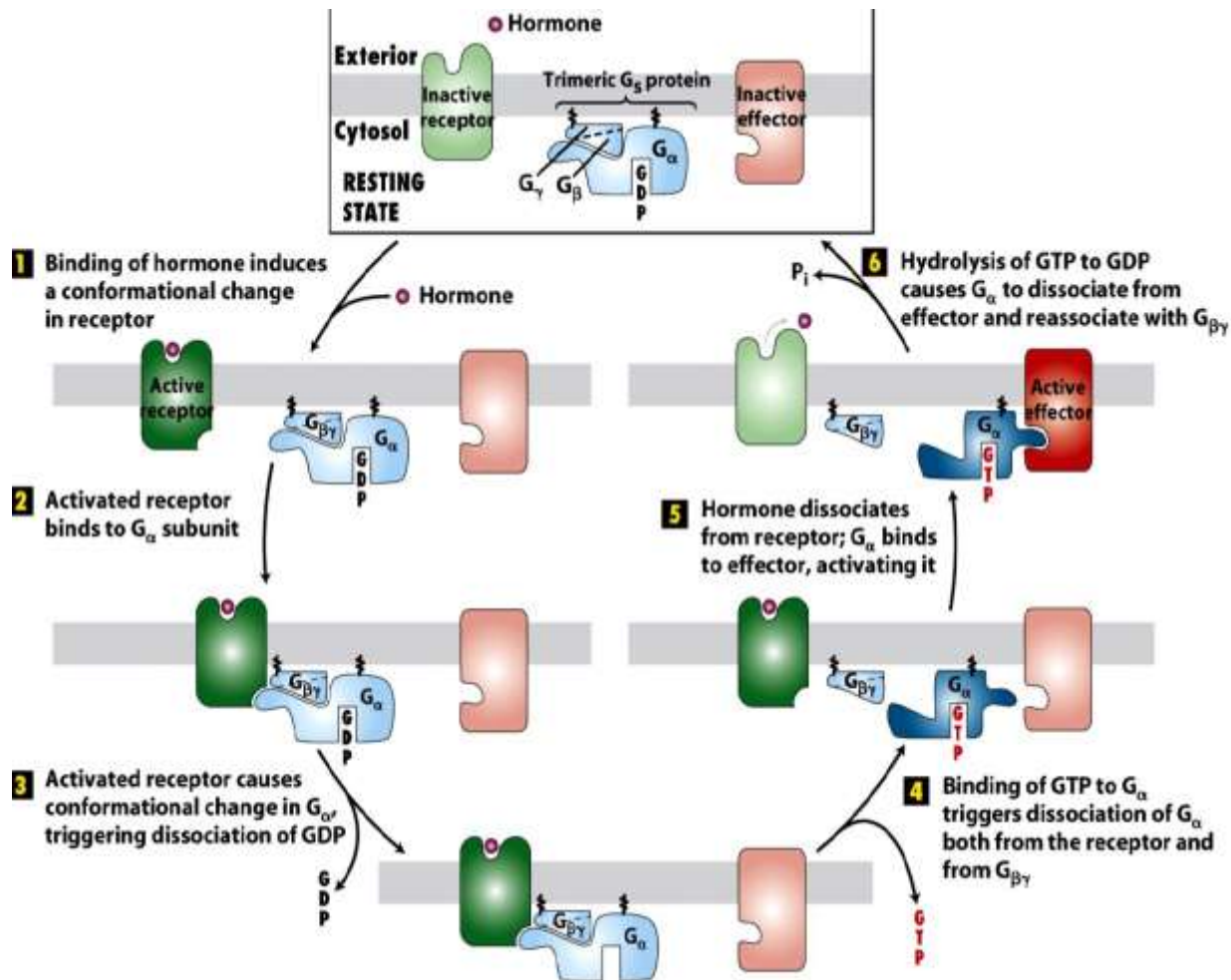


Figure 15-13

G Protein Signal Cascade

- ◆ **G-proteins** are **heterotrimeric**, with 3 subunits α , β , γ .
- ◆ A G-protein that activates cyclic-AMP formation within a cell is called a **stimulatory G-protein**, designated G_s with alpha subunit $G_{s\alpha}$.
- ◆ G_s is activated, e.g., by receptors for the hormones **epinephrine** and **glucagon**.

The **β -adrenergic receptor** is the **GPCR** for epinephrine.

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

G_{α} CLASS	ASSOCIATED EFFECTOR	2ND MESSENGER	RECEPTOR EXAMPLES
G_{α_s}	Adenylyl cyclase	cAMP (increased)	β -Adrenergic (epinephrine) receptor; receptors for glucagon, serotonin, vasopressin
G_{α_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_{α_q}	Phospholipase C	IP_3 , DAG (increased)	α_1 -Adrenergic receptor
G_{α_o}	Phospholipase C	IP_3 , DAG (increased)	Acetylcholine receptor in endothelial cells
G_{α_t}	cGMP phosphodiesterase	cGMP (decreased)	Rhodopsin (light receptor) in rod cells

*A given G_{α} subclass may be associated with more than one effector protein. To date, only one major G_{α_s} has been identified, but multiple G_{α_q} and G_{α_i} proteins have been described. Effector proteins commonly are regulated by G_{α} but in some cases by $G_{\beta\gamma}$ or the combined action of G_{α} and $G_{\beta\gamma}$.

IP_3 = inositol 1,4,5-trisphosphate; DAG = 1,2-diacylglycerol.

SOURCES: See L. Birnbaumer, 1992, *Cell* **71**:1069; Z. Farfel et al., 1999, *New Eng. J. Med.* **340**:1012; and K. Pierce et al., 2002, *Nature Rev. Mol. Cell Biol.* **3**:639.

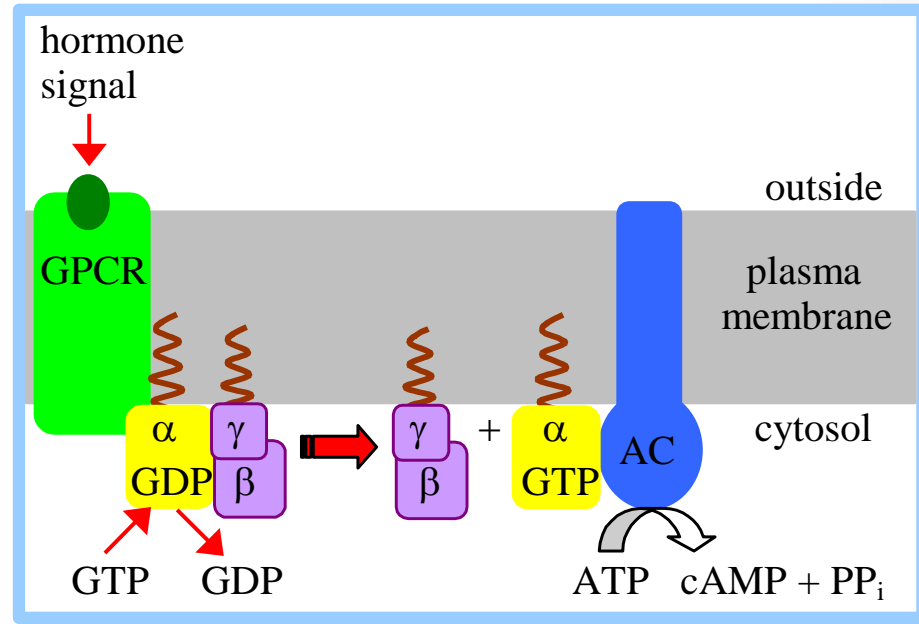
Table 15-1
Molecular Cell Biology, Sixth Edition

Summary of Hormones signaling pathways

IP ₃	cAMP	cGMP	Tyrosine kinase - intrinsic	Tyrosine kinase - receptor associated	Steroid
GnRH	FSH	ANP	Insulin	Prolactin	Glucocorticoid
Gastrin	LH	NO (EDRF)	IGF-1	Cytokines (IL-2,6,8)	Estrogen
Oxytocin	ACTH		FGF	GH	Progesterone
TRH	TSH		PDGF		Testosterone
ADH (V ₁)	CRH				Aldosterone
Histamine (H ₁)	hCG				Vitamin D
Angiotensin II	PTH				T ₃ /T ₄
	Calcitonin				Cortisol
	Glucagon				
	GHRH (can act via IP ₃ as well)				

G Protein Signal Cascade

- The α subunit of a G-protein (G_{α}) binds **GTP**, & can hydrolyze it to $GDP + P_i$.



- α & γ subunits have covalently attached **lipid anchors** that bind a G-protein to the plasma membrane cytosolic surface.
- Adenylate Cyclase** (AC) is a transmembrane protein, with cytosolic domains forming the catalytic site.

Adenylate Cyclase

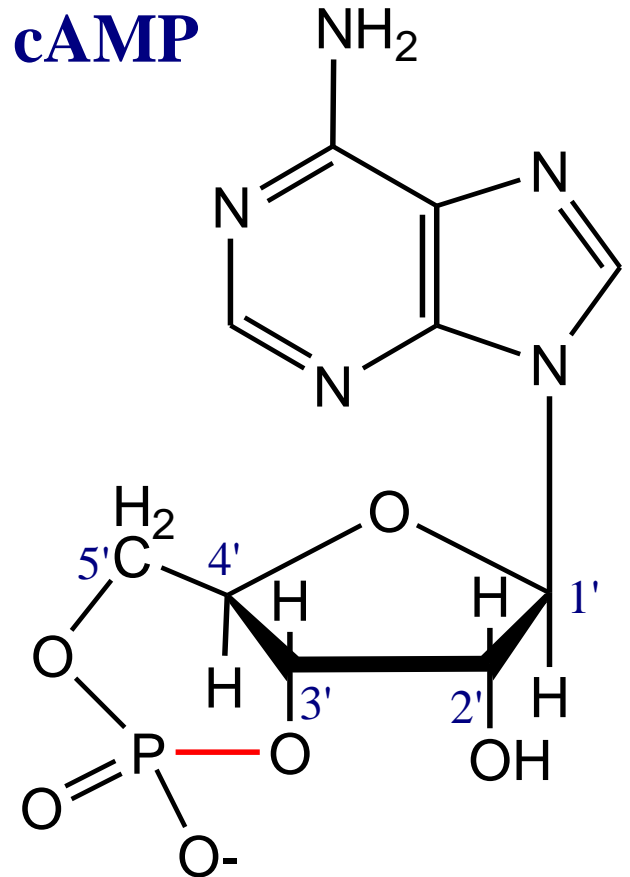
Adenylate Cyclase (Adenylyl Cyclase)



Binding of certain **hormones** (e.g., epinephrine) to the outer surface of a cell activates Adenylate Cyclase to form cAMP within the cell.

Cyclic AMP is thus considered to be a **messenger**.

cAMP

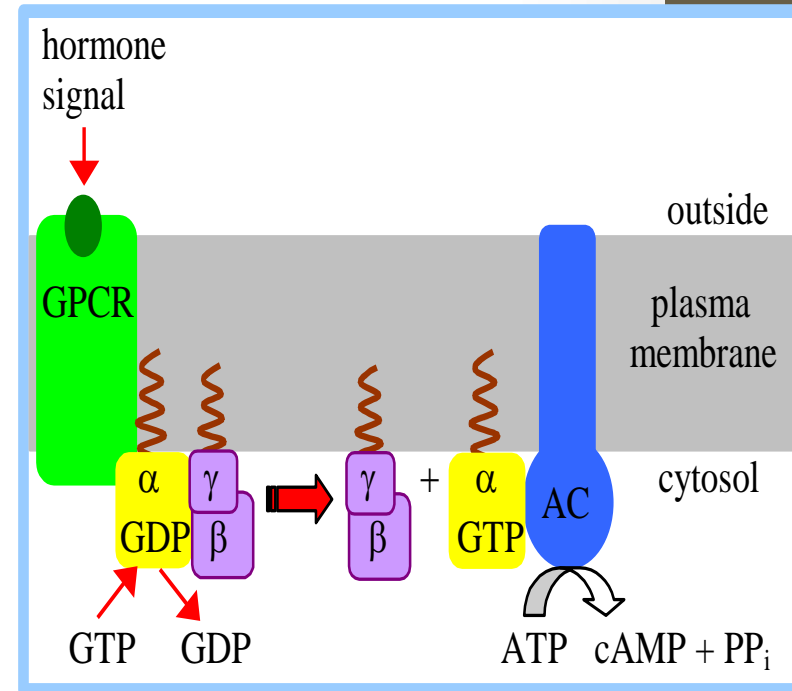


G Protein Signal Cascade

The **sequence of events** by which a hormone activates cAMP signaling:

1. Initially G_{α} has bound **GDP**, and α , β , & γ subunits are complexed together.

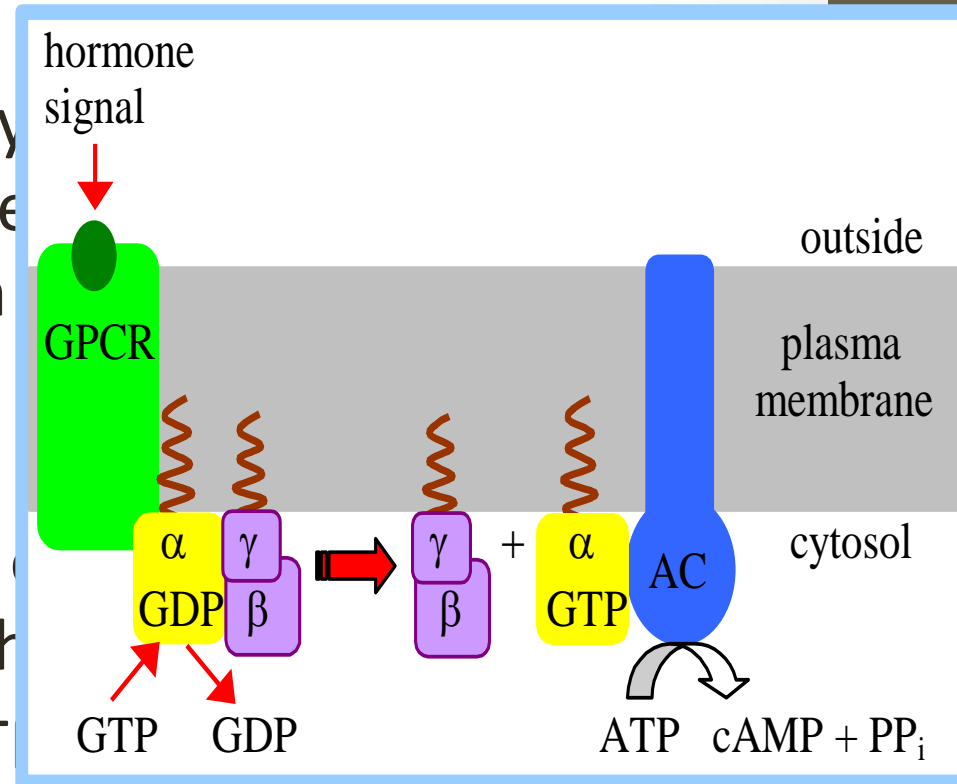
$G_{\beta,\gamma}$, the complex of β & γ subunits, **inhibits** G_{α} .



G Protein Signal Cascade

2. **Hormone binding**, usually domain of a 7-helix receptor undergoes a **conformational change** in transmitted to a **G-protein** side of the membrane.

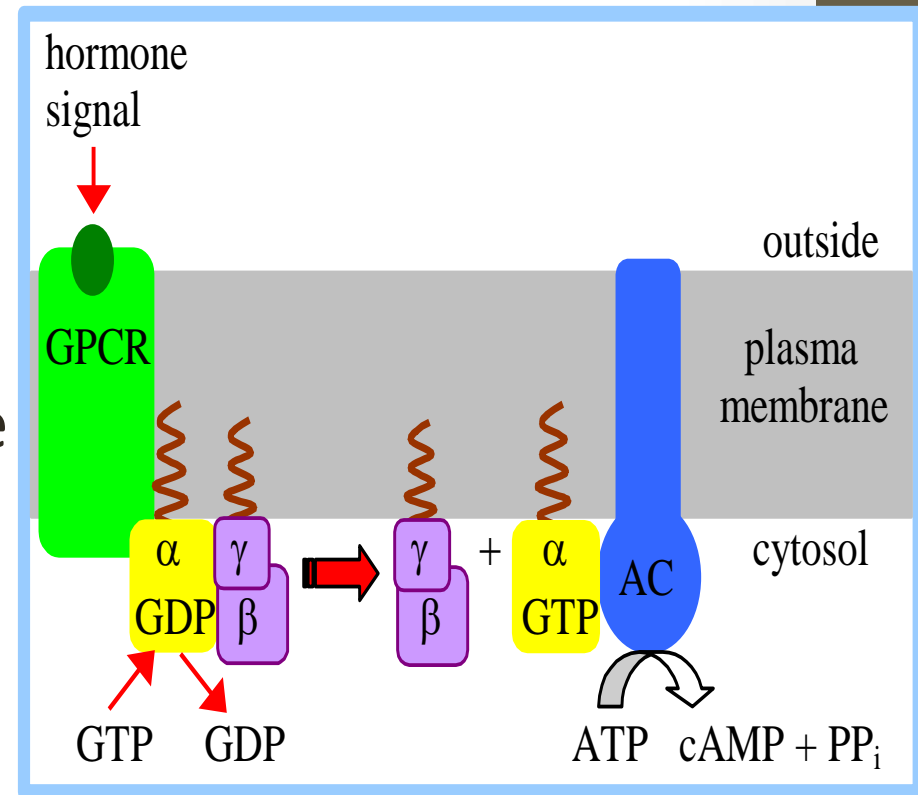
The nucleotide-binding site accessible to the cytosol, where G_{α} releases GDP & binds GTP



G Protein Signal Cascade

3. Substitution of **GTP** for GDP causes another conformational change in G_{α} .

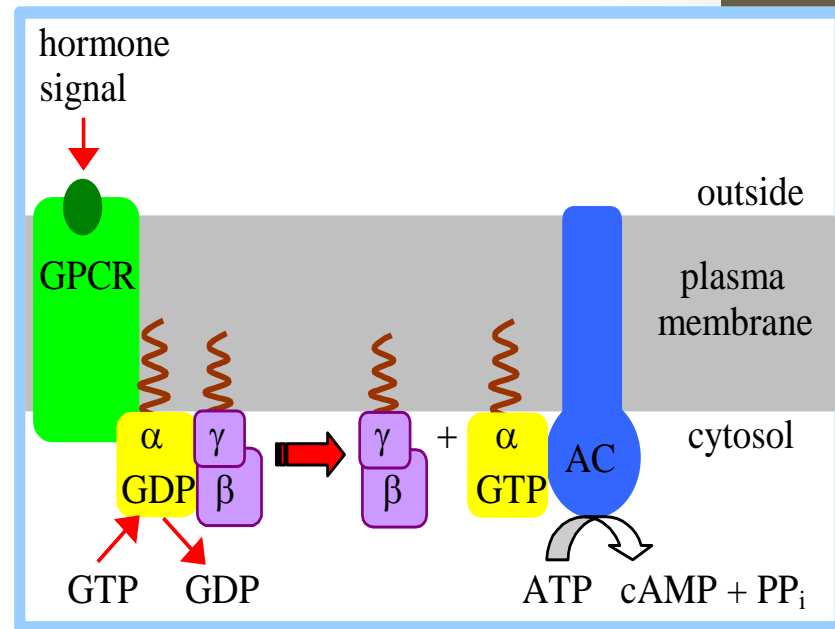
G_{α} -**GTP** dissociates from the inhibitory $\beta\gamma$ complex & can now bind to and activate **Adenylate Cyclase**.



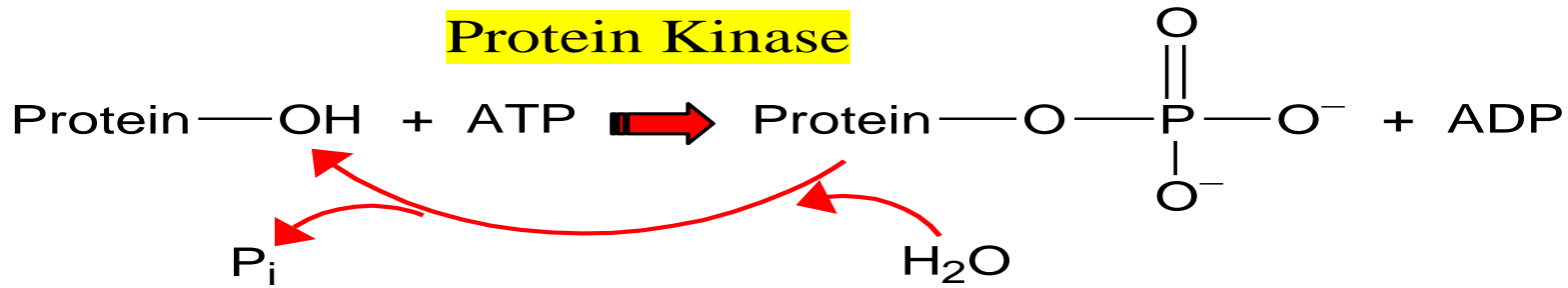
G Protein Signal Cascade

4. **Adenylate Cyclase**, activated by the stimulatory G_{α} -GTP, catalyzes synthesis of **cAMP**.

5. **Protein Kinase A** (cAMP Dependent Protein Kinase) catalyzes transfer of phosphate from ATP to serine or threonine residues of various cellular proteins, altering their activity.



Protein Kinase



Protein Phosphatase

Protein kinases and phosphatases are themselves regulated by complex signal cascades. For example:

- ◆ Some protein kinases are activated by **Ca⁺⁺-calmodulin**.
- ◆ **Protein Kinase A** is activated by **cyclic-AMP** (cAMP).

Protein Kinase A (cAMP-Dependent Protein Kinase) transfers P_i from ATP to OH of a Ser or Thr in a particular 5-amino acid sequence.

Protein Kinase A in the resting state is a complex of:

- 2 catalytic subunits (**C**)
- 2 regulatory subunits (**R**).

R₂C₂: When each (**R**) binds 2 cAMP, a conformational change causes (**R**) to release (**C**).

The catalytic subunits can then catalyze phosphorylation of Ser or Thr on target proteins.

PKIs, Protein Kinase Inhibitors, modulate activity of the catalytic subunits (**C**).

Turn off of the signal:

1. **G_α** hydrolyzes GTP to GDP + P_i. (**GTPase**).

The presence of **GDP** on G_α causes it to rebind to the inhibitory **βγ** complex.

Adenylate Cyclase is no longer activated.

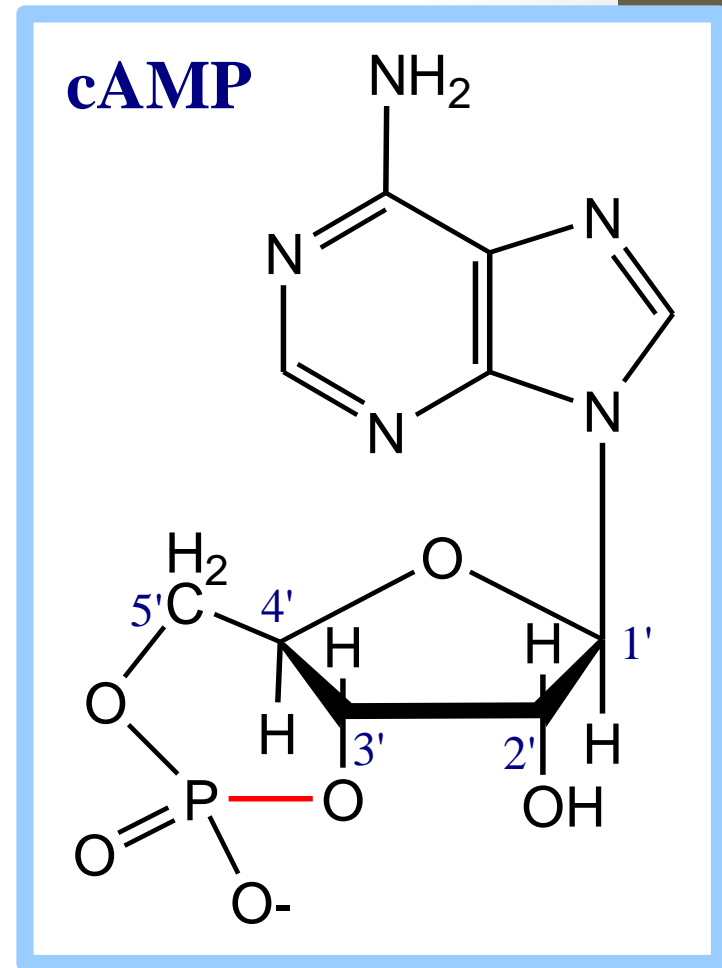
2. **Phosphodiesterases** catalyze hydrolysis of **cAMP → AMP**.

Phosphodiesterase enzymes catalyze:



The phosphodiesterase that cleaves cAMP is activated by phosphorylation catalyzed by Protein Kinase A.

Thus **cAMP stimulates its own degradation**, leading to rapid turnoff of a cAMP signal.



3. **Receptor desensitization** varies with the hormone.

- In some cases the **activated receptor** is **phosphorylated** via a G-protein Receptor Kinase.
- The phosphorylated receptor then may bind to a protein **β -arrestin**.
- **β -Arrestin** promotes **removal of the receptor** from the membrane by clathrin-mediated endocytosis.
- **β -Arrestin** may also bind a cytosolic **Phosphodiesterase**, bringing this enzyme close to where cAMP is being produced, contributing to signal turnoff.

4. **Protein Phosphatase** catalyzes removal by hydrolysis of phosphates that were attached to proteins via Protein Kinase A.

- ◆ **Different** isoforms of G_{α} have different signal roles. E.g.:
 - The **stimulatory** $G_{s\alpha}$, when it binds GTP, **activates** Adenylate cyclase.
 - An **inhibitory** $G_{i\alpha}$, when it binds GTP, **inhibits** Adenylate cyclase.

Different effectors & their receptors induce $G_{i\alpha}$ to exchange GDP for GTP than those that activate $G_{s\alpha}$.

- ◆ The complex of $G_{\beta,\gamma}$ that is released when G_{α} binds GTP is itself an effector that binds to and **activates or inhibits** several other proteins.
E.g., $G_{\beta,\gamma}$ **inhibits** one of several isoforms of **Adenylate Cyclase**, contributing to rapid signal turnoff in cells that express that enzyme.

Small GTP-binding proteins include (roles indicated):

- ◆ **initiation & elongation factors** (protein synthesis).
- ◆ **Ras** (growth factor signal cascades).
- ◆ **Rab** (vesicle targeting and fusion).
- ◆ **ARF** (forming vesicle coatomer coats).
- ◆ **Ran** (transport of proteins into & out of the nucleus).
- ◆ **Rho** (regulation of actin cytoskeleton)

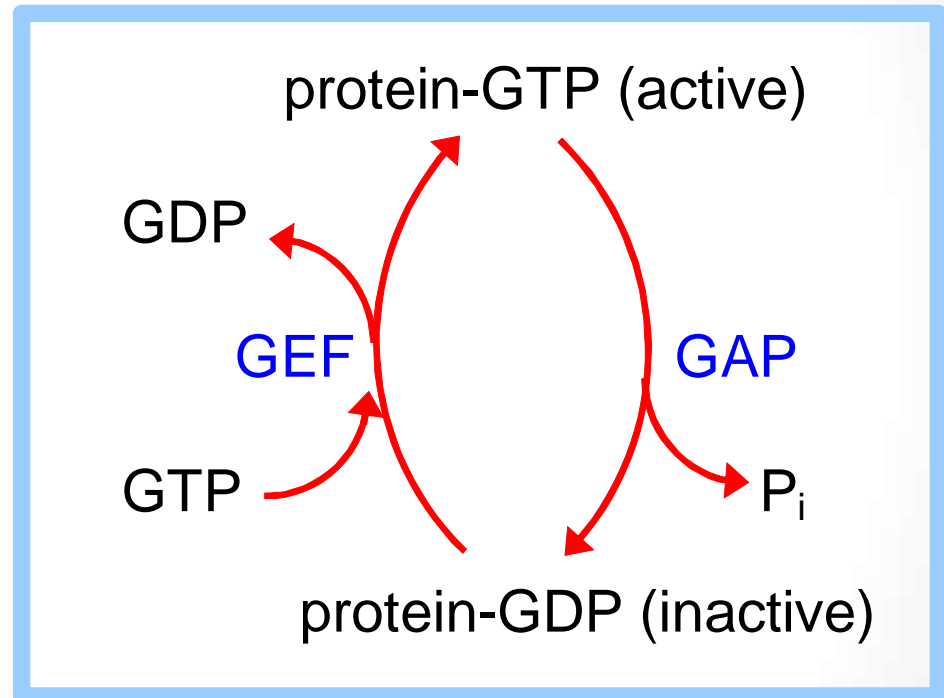
All GTP-binding proteins differ in conformation depending on whether GDP or GTP is present at their nucleotide binding site.

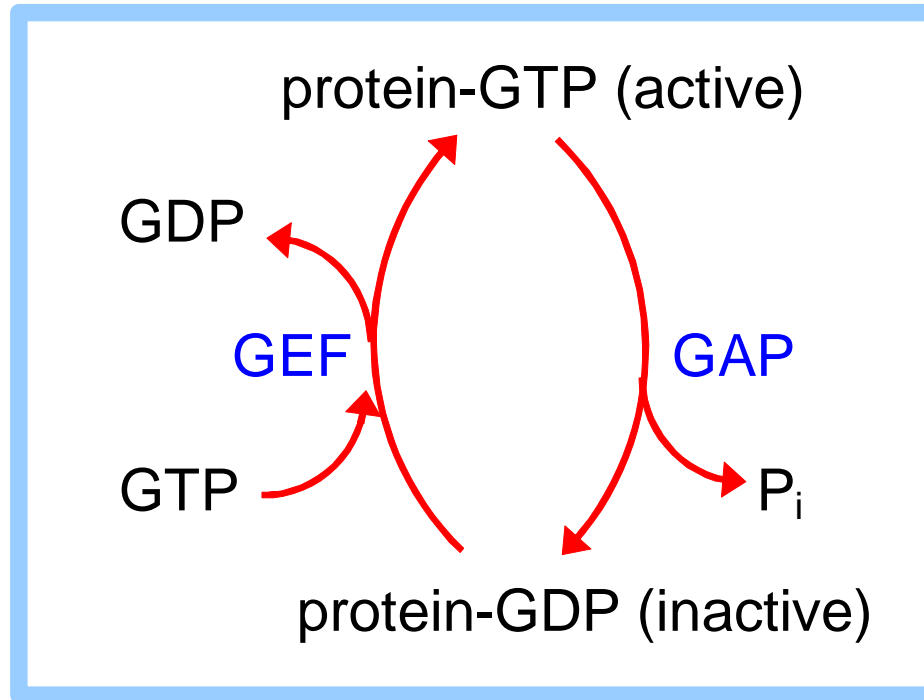
Generally, **GTP** binding induces the **active** state.

Most GTP-binding proteins depend on **helper proteins**:

GAPs,

GTPase Activating Proteins, promote GTP hydrolysis.

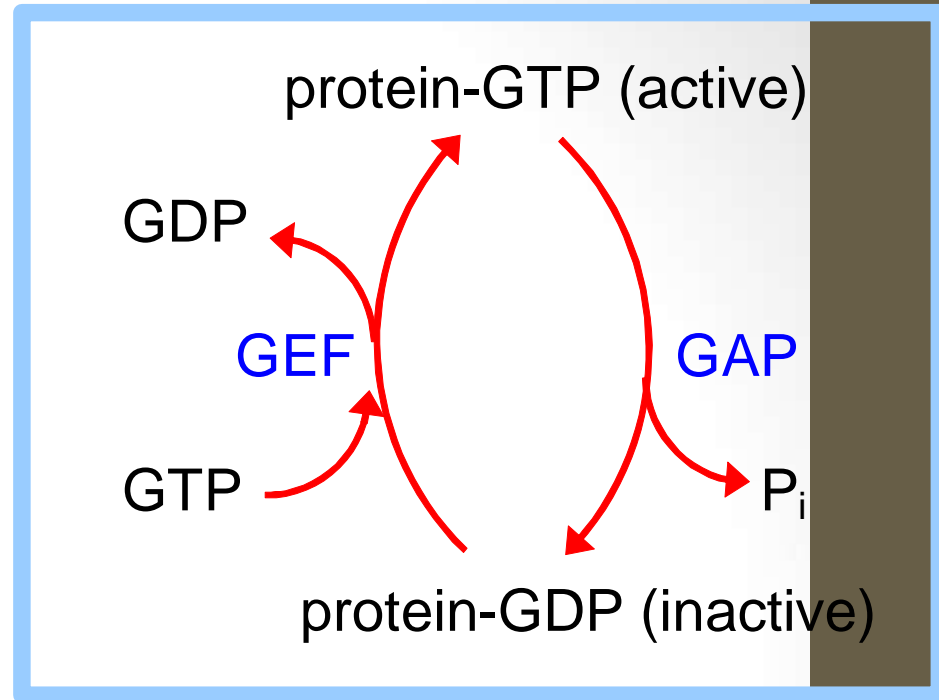




- ◆ **G_α** of a heterotrimeric G protein has innate capability for GTP hydrolysis.

It has the essential arginine residue normally provided by a GAP for small GTP-binding proteins.

GEFs, Guanine Nucleotide Exchange Factors, promote GDP/GTP exchange.



- ◆ An activated **receptor** (GPCR) normally serves as **GEF** for a heterotrimeric G-protein.
- ◆ Alternatively, **AGS** (Activator of G-protein Signaling) proteins may activate some heterotrimeric G-proteins, independent of a receptor.

Some AGS proteins have GEF activity.

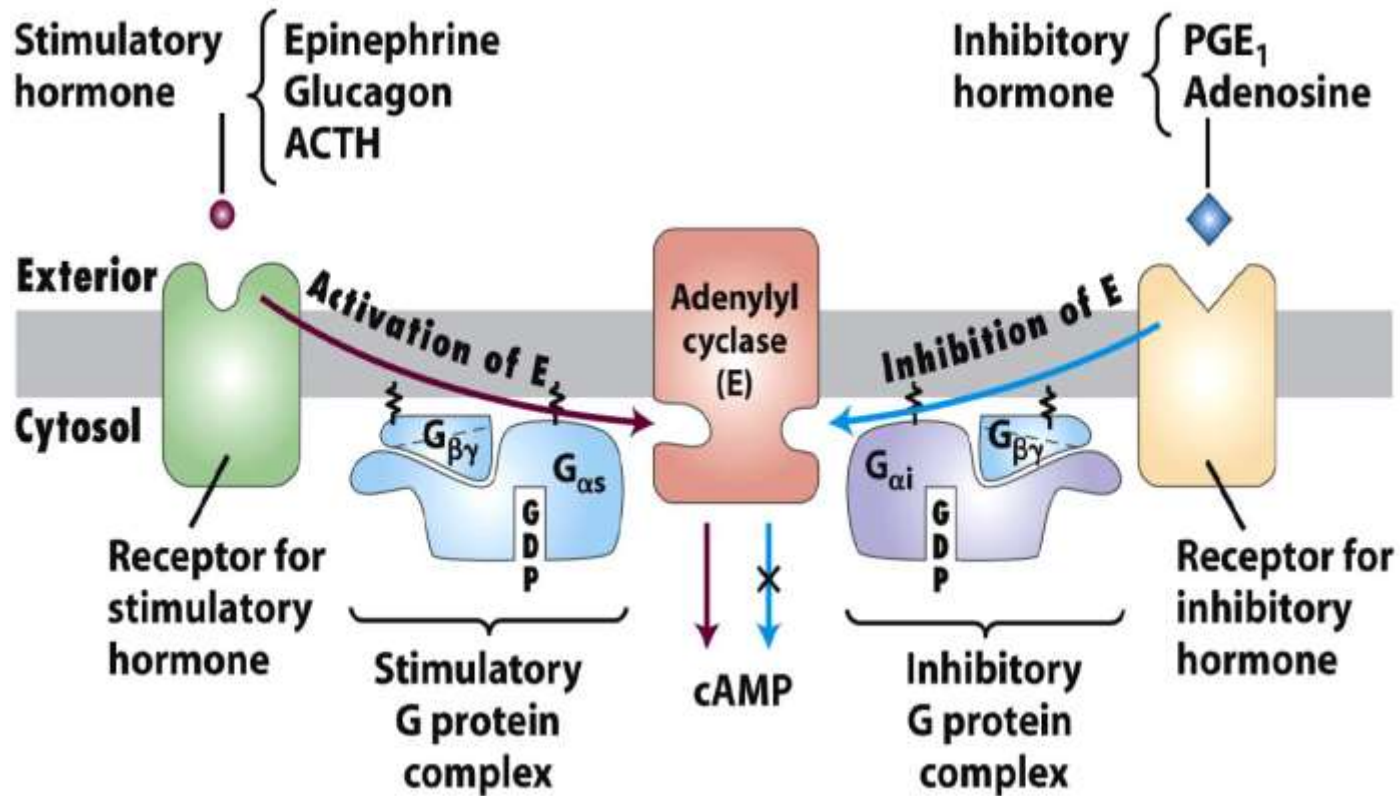


Figure 15-21

Signaling Overview

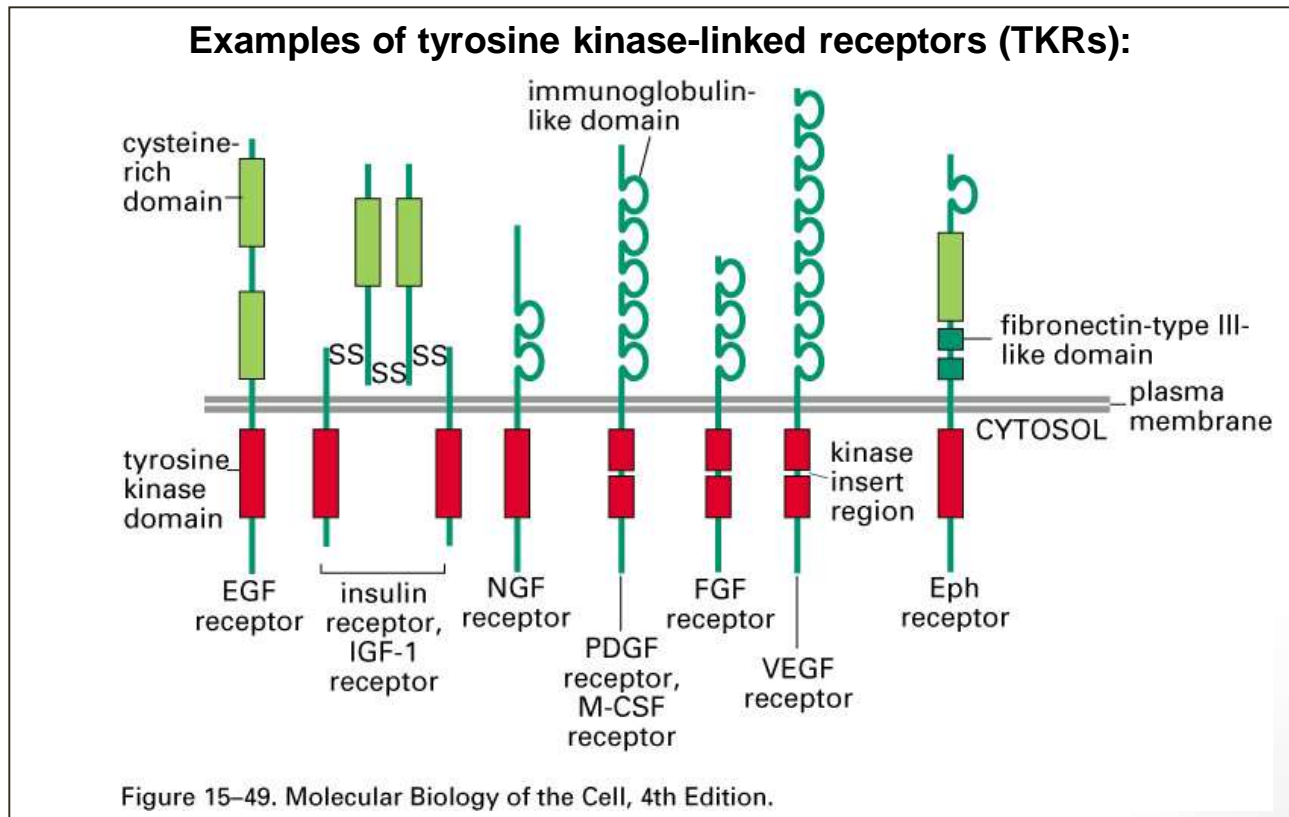
3. Three major classes of surface receptors for signaling, cont.:

C. Enzyme-linked receptors:

1. Tyrosine kinase-linked receptors (TKRs).

A. Overview of TKRs:

1. Cell surface receptors that are directly linked to intracellular enzymes (kinases).
2. Includes receptors for most growth factors (NGF, EGF, PDGF), insulin, and Src.
3. Common structure: N terminal extracellular ligand-binding domain, single TM domain, cytosolic C-terminal domain with tyrosine kinase activity.
4. Can be single polypeptide or dimer.



Signaling Overview

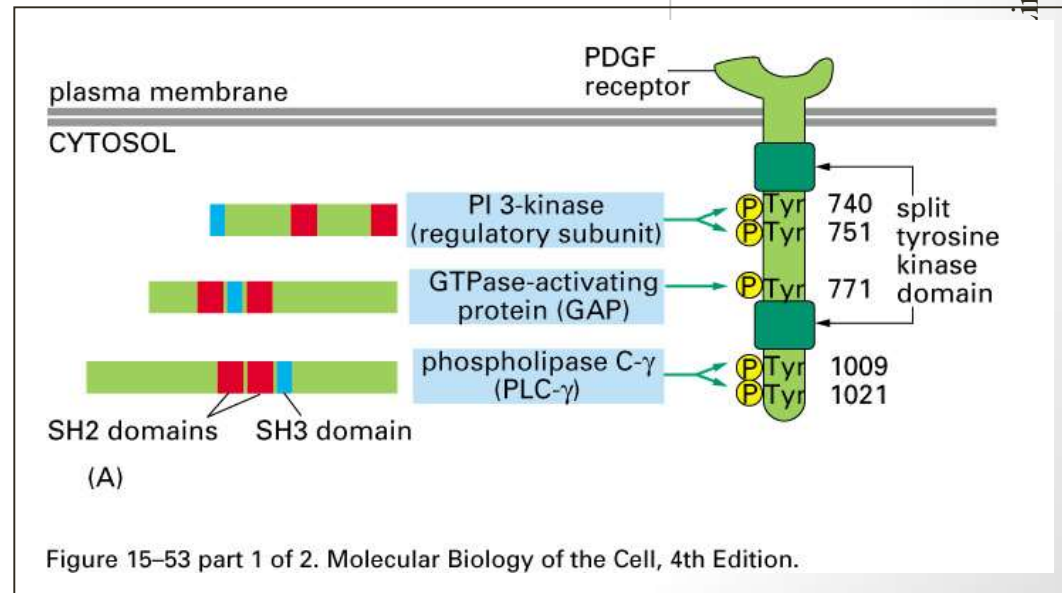
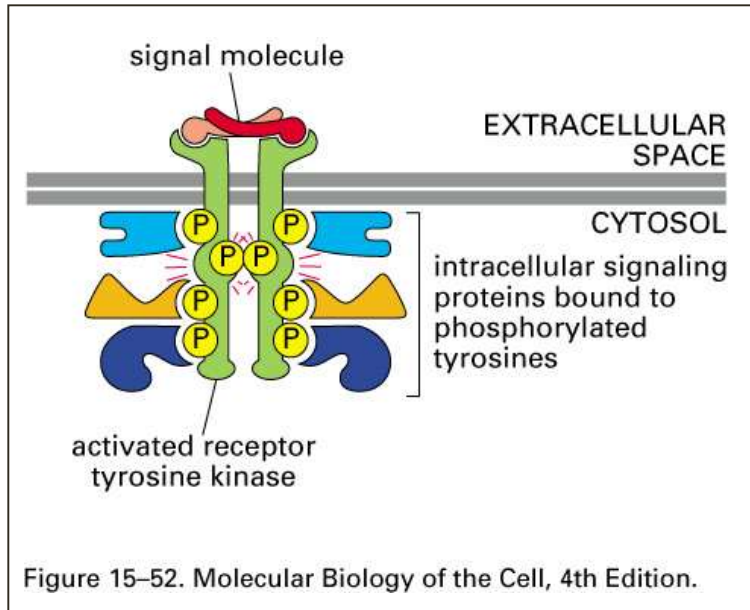
3. Three major classes of surface receptors for signaling, cont.:

C. Enzyme-linked receptors, cont.:

1. Tyrosine kinase-linked receptors (TKRs)

B. Mechanism of activation of TKRs:

- i.* ligand binding induces receptor dimerization (receptor crosslinking).
- ii.* dimerization leads to autophosphorylation of the receptor (cross-phosphorylation).
- iii.* phosphorylation increases kinase activity & also creates specific new binding sites.
- iv.* proteins that bind to these new binding sites transmit intracellular signals.



How receptor tyrosine kinases work together with monomeric GTPases:

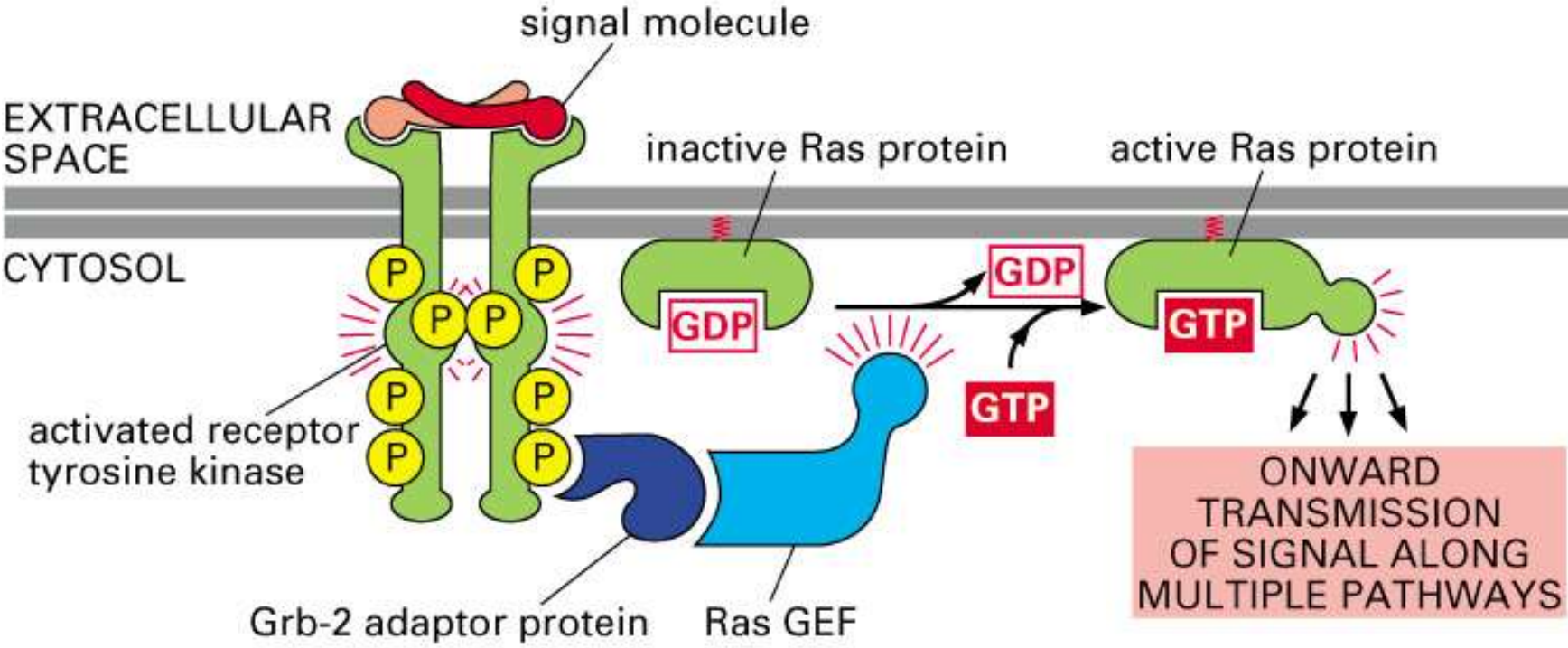


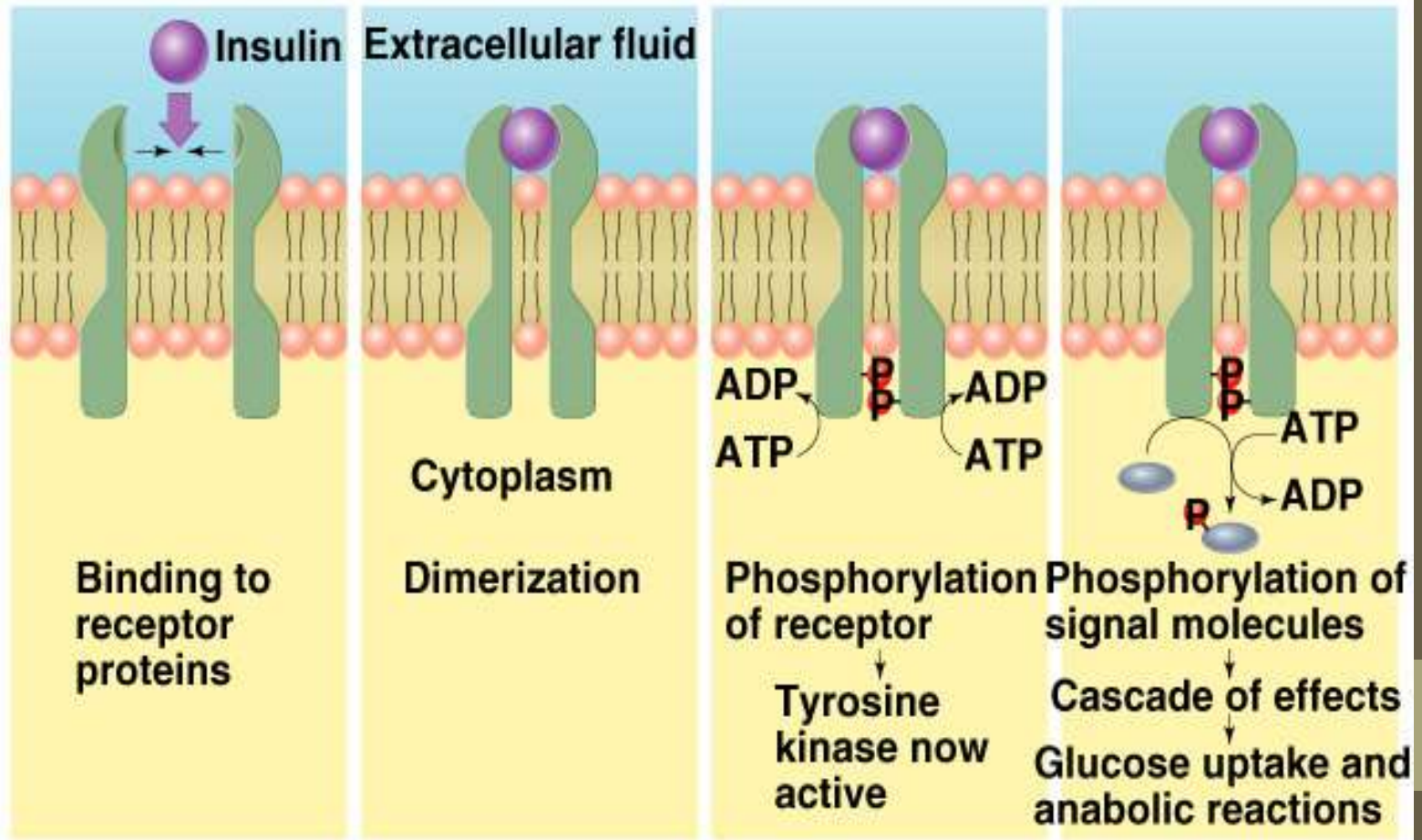
Figure 15-55. Molecular Biology of the Cell, 4th Edition.

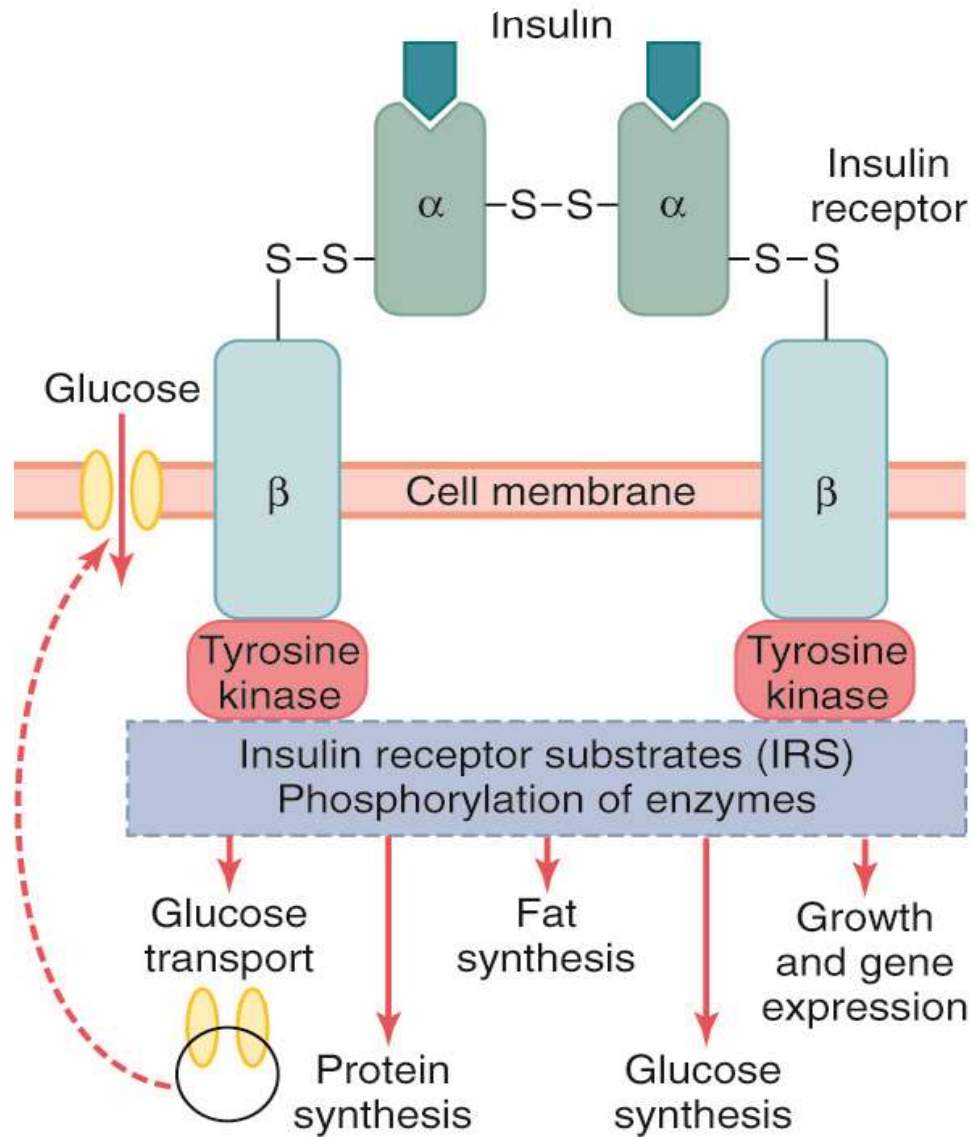
Tyrosine Kinase

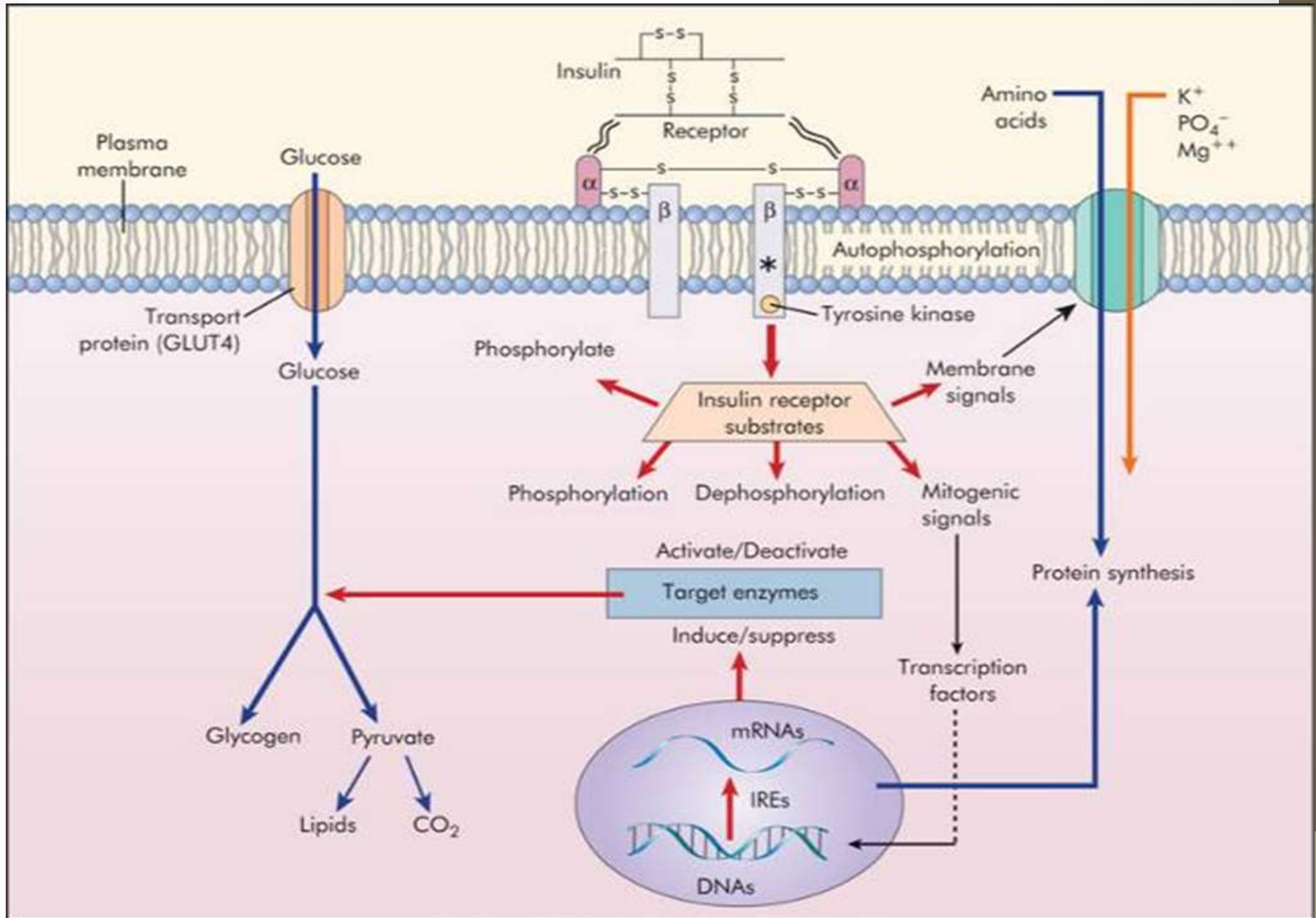
- Insulin receptor consists of 2 units that dimerize when they bind with insulin.
 - Insulin binds to ligand-binding site on plasma membrane, activating enzymatic site in the cytoplasm.
- Autophosphorylation occurs, increasing tyrosine kinase activity.
- Activates signaling molecules.
 - Stimulate glycogen, fat and protein synthesis.
 - Stimulate insertion of GLUT-4 carrier proteins.

Tyrosine Kinase

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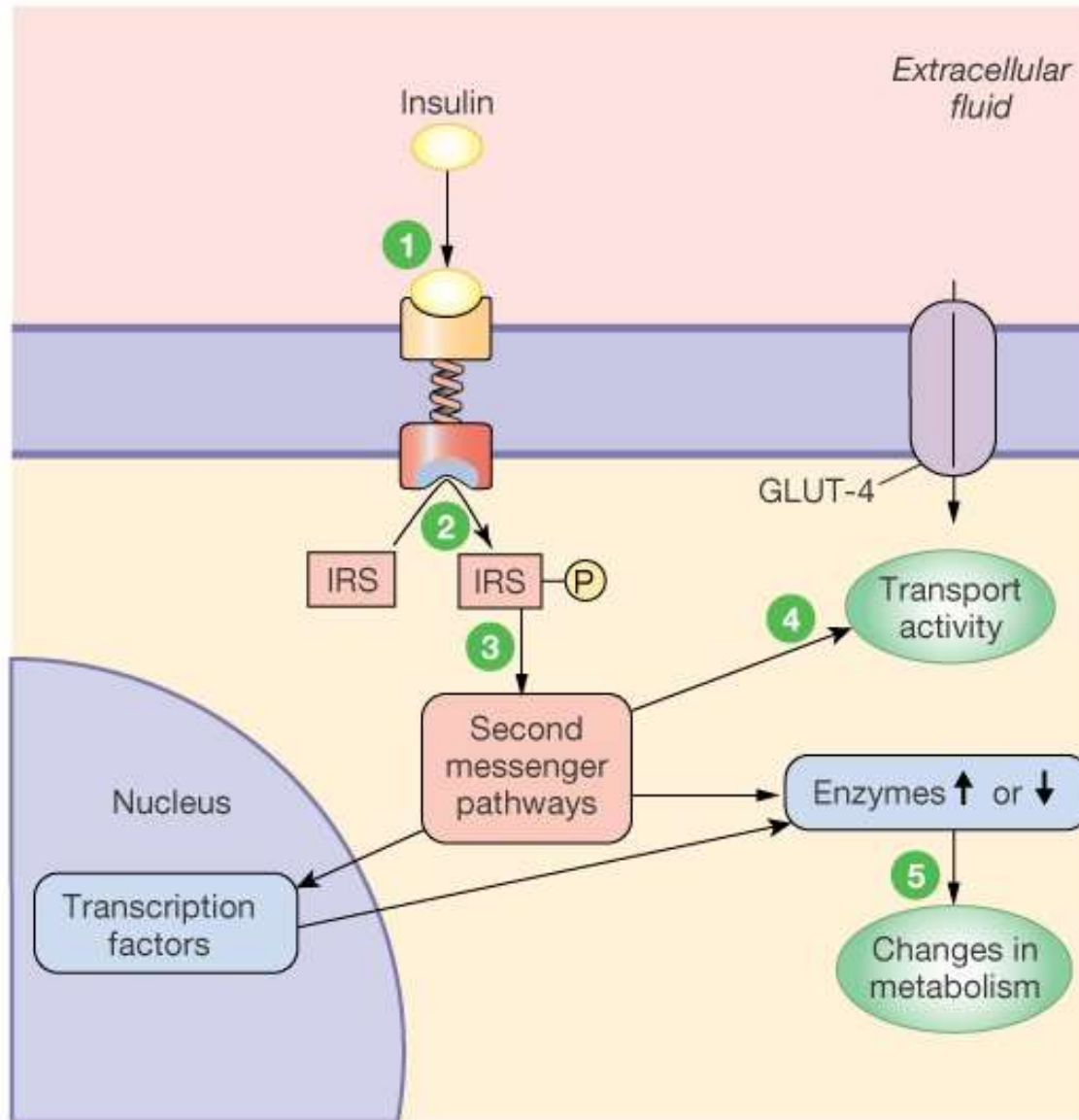






From Berne RM, Levy MN. *Principles of physiology*. ed 3, St Louis, 2000, Mosby.

Insulin Action on Cells:



- 1 Insulin binds to tyrosine kinase receptor.
- 2 Receptor phosphorylates insulin-receptor substrates (IRS).
- 3 Second messenger pathways alter protein synthesis and existing proteins.
- 4 Membrane transport is modified.
- 5 Cell metabolism is changed.

Signaling Overview

3. Three major classes of surface receptors for signaling, cont.:

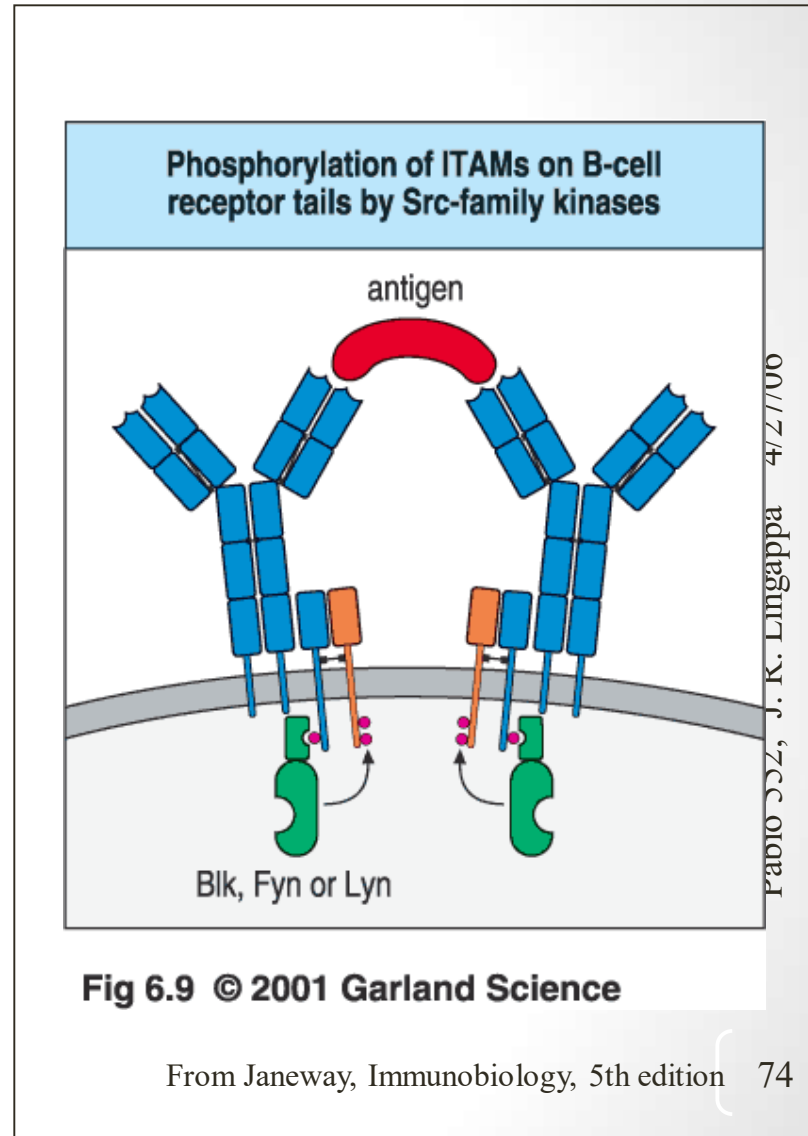
C. Enzyme-linked receptors, cont.:

2. TKs non-covalently associated with receptor (includes cytokine receptors, T & B cell receptors) = NRTKs

Cytokine receptors, as well as T and B cell receptors, stimulate tyrosine kinases that are non-covalently associated with receptor.

A. Overview

1. N-term. extracell. ligand-binding domain, transmembr α helix, C-term. cytosolic domain
2. Cytosolic domain has no catalytic (kinase) activity
3. Acts in conjunction with a non-receptor tyrosine kinase that is activated as a result of ligand binding.
4. Activation is similar to that of RTKs: ligand binding causes cross phosphorylation of associated tyrosine kinases that phosphorylate the receptor, providing phosphotyrosine binding sites for recruitment of proteins with SH2 domains.



Signaling Overview

3. Three major classes of surface receptors for signaling, cont.:

C. Enzyme-linked receptors, cont.:

B. Two kinds of kinases associate with NRTKs:

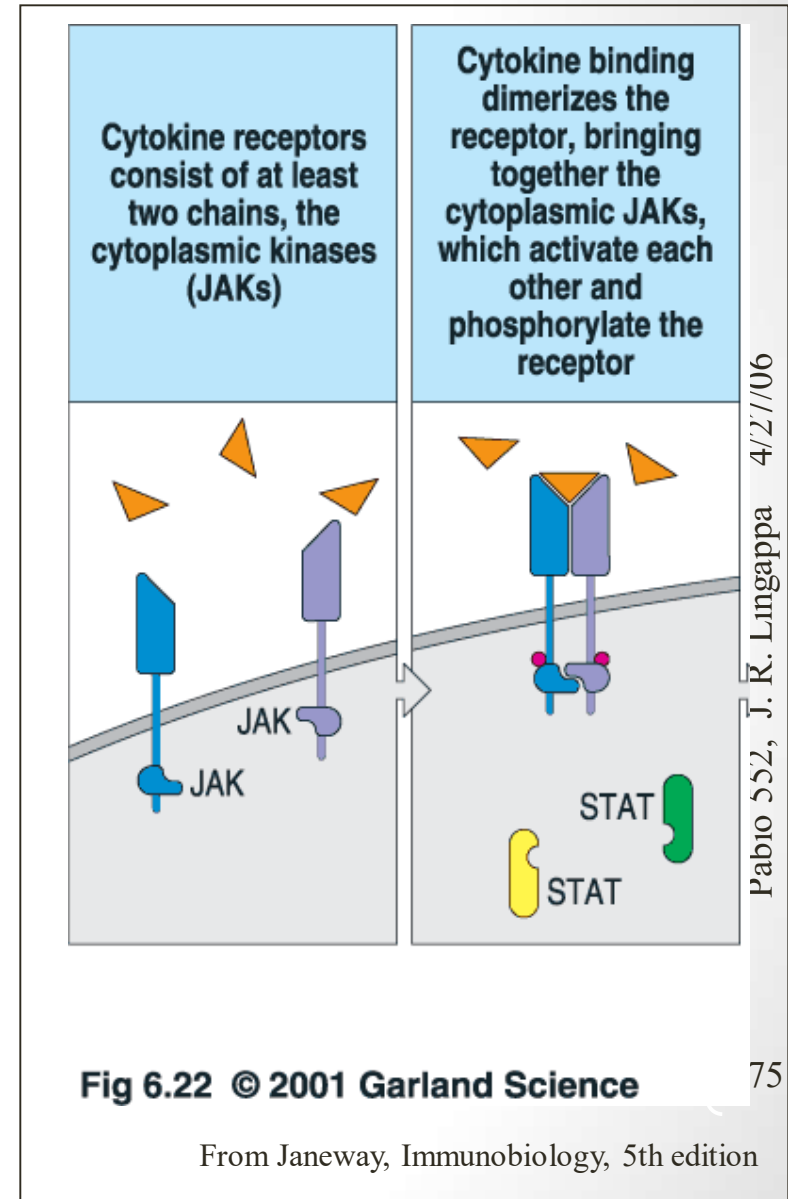
1. Src family protein kinases - important for B and T cell signaling
2. Janus kinases (JAK) - universally required for signaling from cytokine receptors.

C. Receptors can be linked to or associated with other enzymes, besides TKs, i.e.

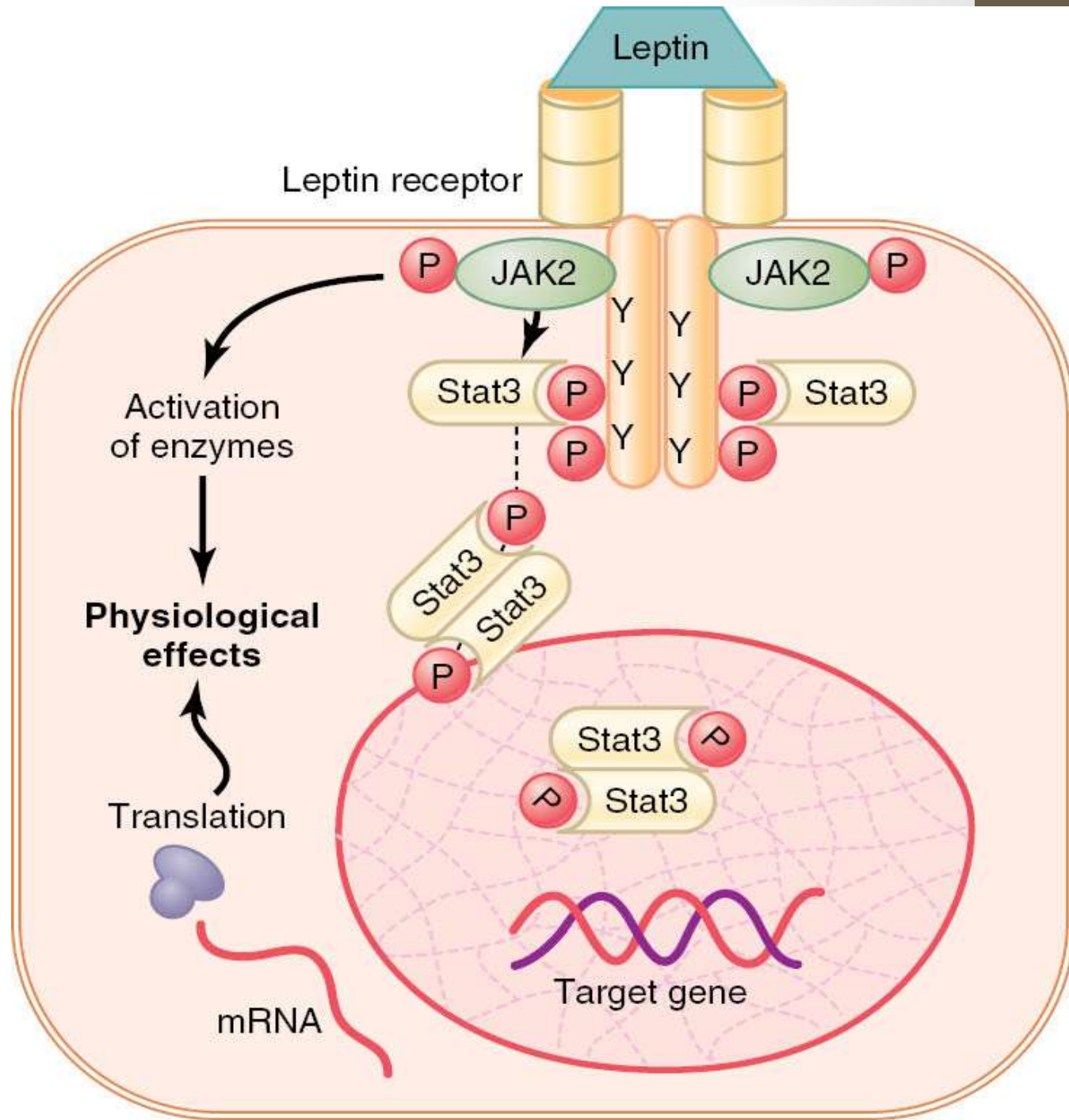
Protein-tyrosine phosphatases (remove phosphates, thereby terminate signals initiated by protein-tyrosine kinases).

Serine/ threonine kinases, i.e. TGF- β

Guanylyl cyclases



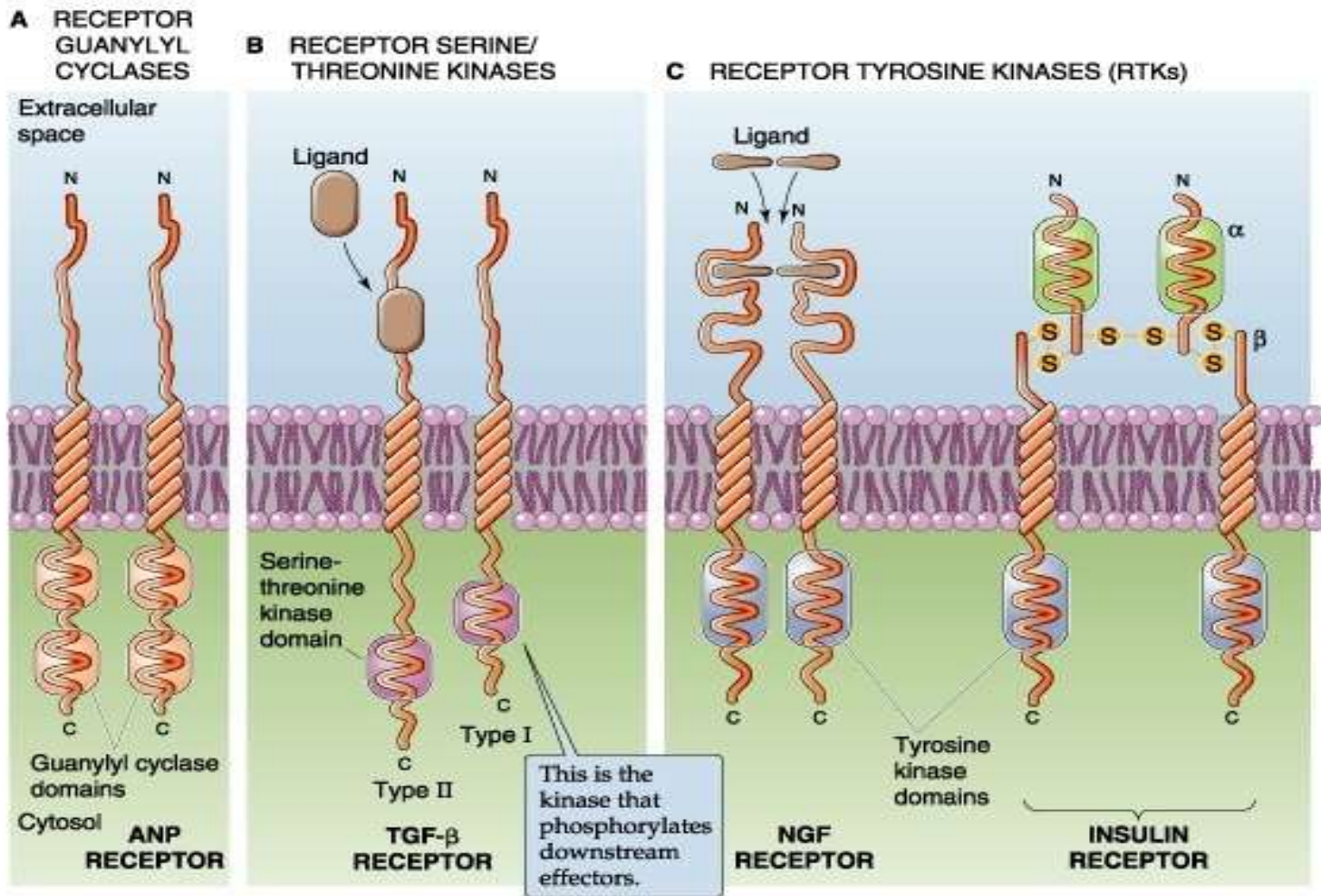
Enzyme-linked
Receptor (the
Leptin receptor)
JAK= Janus
Kinase
STAT= Signal
Transducer
and Activator
of Transcription



Enzyme-linked receptor (the leptin receptor)

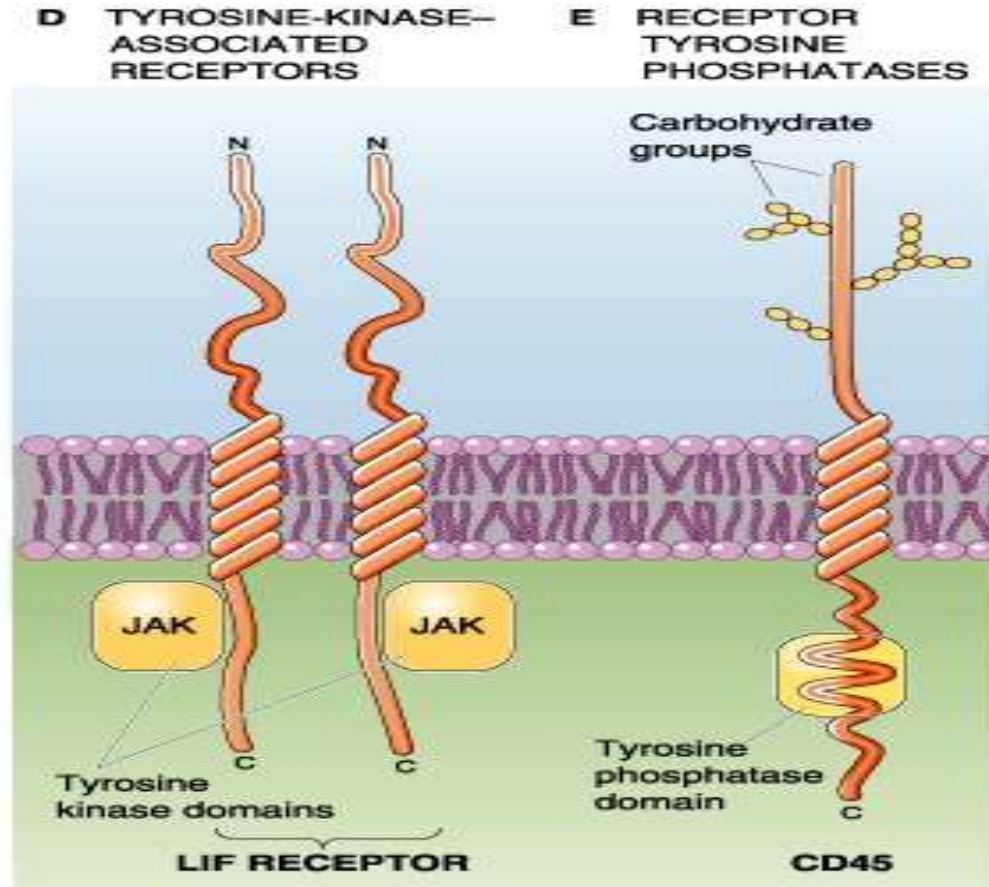
- The receptor exists as a homodimer (two identical parts)
- Leptin binds to the extracellular part of the receptor
- This causes activation of the intracellular associated janus kinas 2
- This causes phosphorylation of signal transducer and activator of transcription (STAT) proteins
- This then activates the transcription of target genes and synthesis of proteins
- JAK 2 phosphorylation also activates several other enzyme systems that mediate some of the more rapid effects of leptin

Enzyme linked receptors



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Tyrosine Kinase



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4. Second Messengers: for Hormones that can't cross PM

A. cAMP:

i. Production:

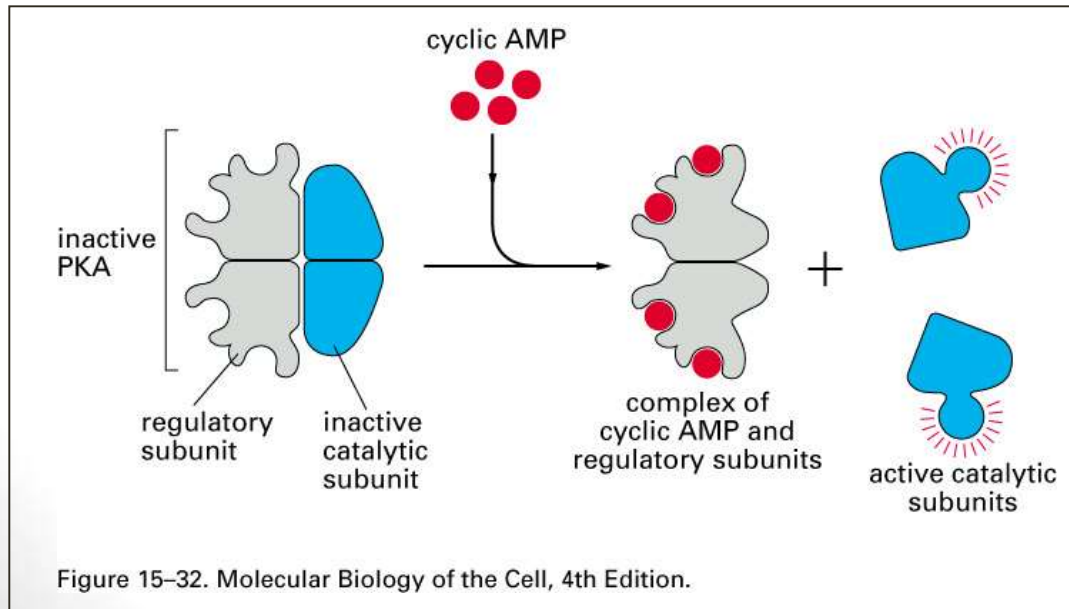
ATP converted to cAMP by adenylate cyclase (a large multipass TM protein)

Degraded by cAMP phosphodiesterase

ii. Action:

a. cAMP-dependent protein kinase (protein kinase A (PKA)).

PKA is a tetramer of catalytic and regulatory subunits
cAMP binding leads to dissociation of regulatory subunits and release of catalytic subunits which then phosphorylate target proteins in cytoplasm:



cAMP production:

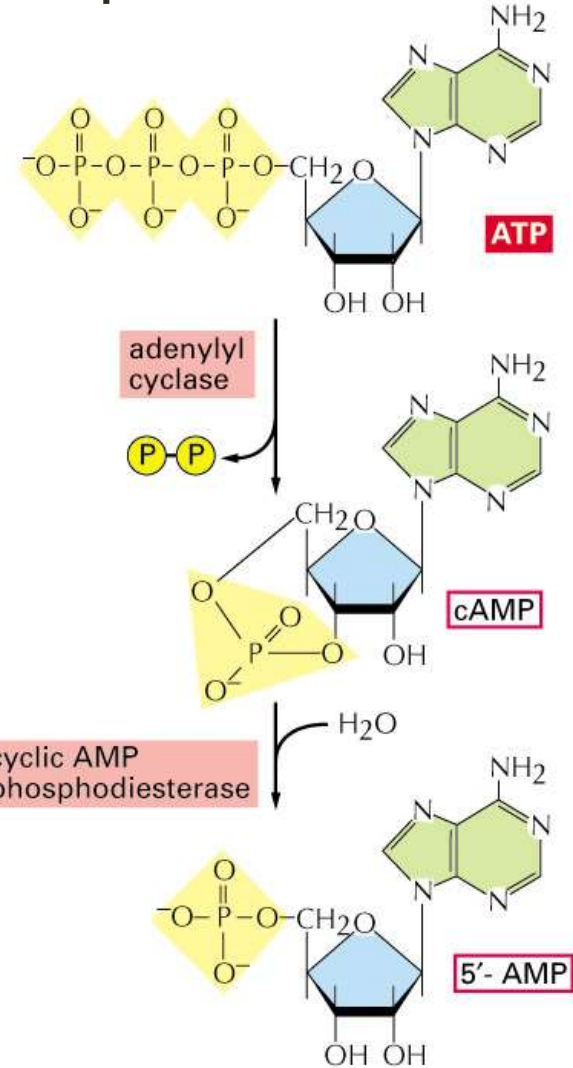


Figure 15-31. Molecular Biology of the Cell, 4th Edition.

4. Second Messengers, cont.:

A. cAMP, cont.

iii. Action:

b. PKA enters the nucleus and phosphorylates CREB (CRE binding protein), which binds to the cAMP response element (CRE), a regulatory DNA sequence associated with specific genes. This results in activation of transcription of those genes.

iv. Rapid turn on and rapid turn off of cAMP and activation by cAMP :

Question: what turns off proteins activated by protein kinases?

v. Amplification of signal at each step of signaling pathway - characteristic feature of signal transduction.

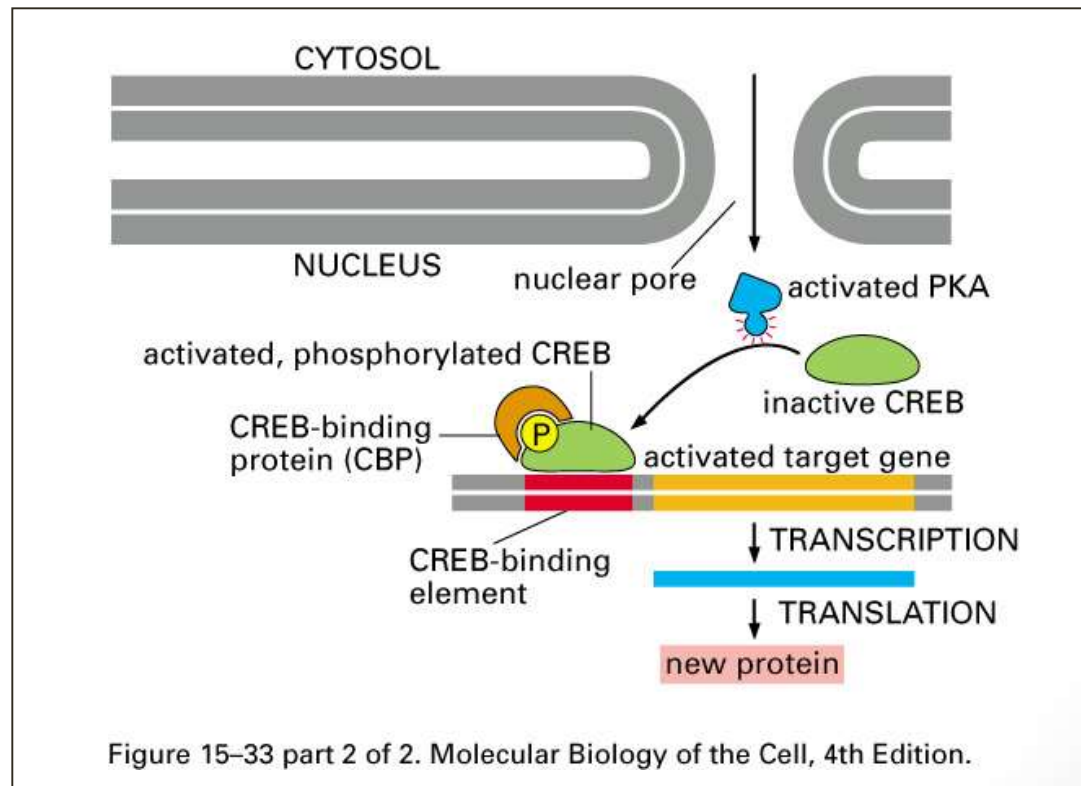


Figure 15-33 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

4. Second Messengers, cont.:

A. cAMP, cont.:

vi. Regulation of adenylate cyclase:

Receptors that cause increase in cAMP do so by activating G_s , a stimulatory protein that activates adenylate cyclase.

Adenylate cyclase is turned off by G_i , an inhibitory protein.

vii. Pathogens alter cAMP production:

Cholera toxin active subunit catalyzes transfer of ADP ribose from intracellular NAD to the α subunit of G_s , causing it to be continuously active, stimulating adenylate cyclase indefinitely. This causes ion channels that export chloride to produce a net efflux of Cl^- and water, leading to severe diarrhea characteristic of cholera.

B. cGMP:

1. produced from GTP by guanylyl cyclase;
2. activates cGMP-dependent kinases or other targets
3. example: G-prot. Coupled rhodopsin photoreceptor in rod cells of retina

Summary of how cAMP activates transcription:

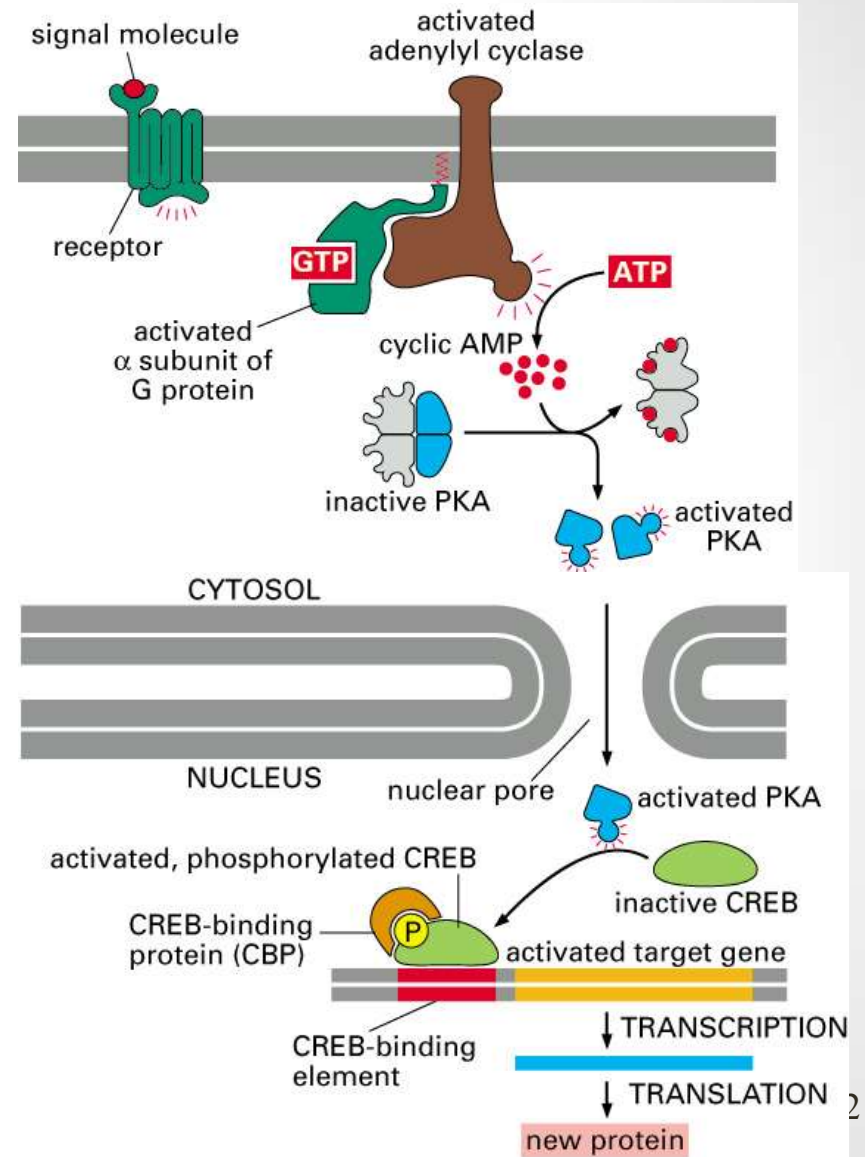


Figure 15-33 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Adenylate Cyclase-cAMP

- Phosphorylates enzymes within the cell to produce hormone's effects.
- Modulates activity of enzymes present in the cell.
- Alters metabolism of the cell.
- cAMP inactivated by phosphodiesterase.
 - Hydrolyzes cAMP to inactive fragments.

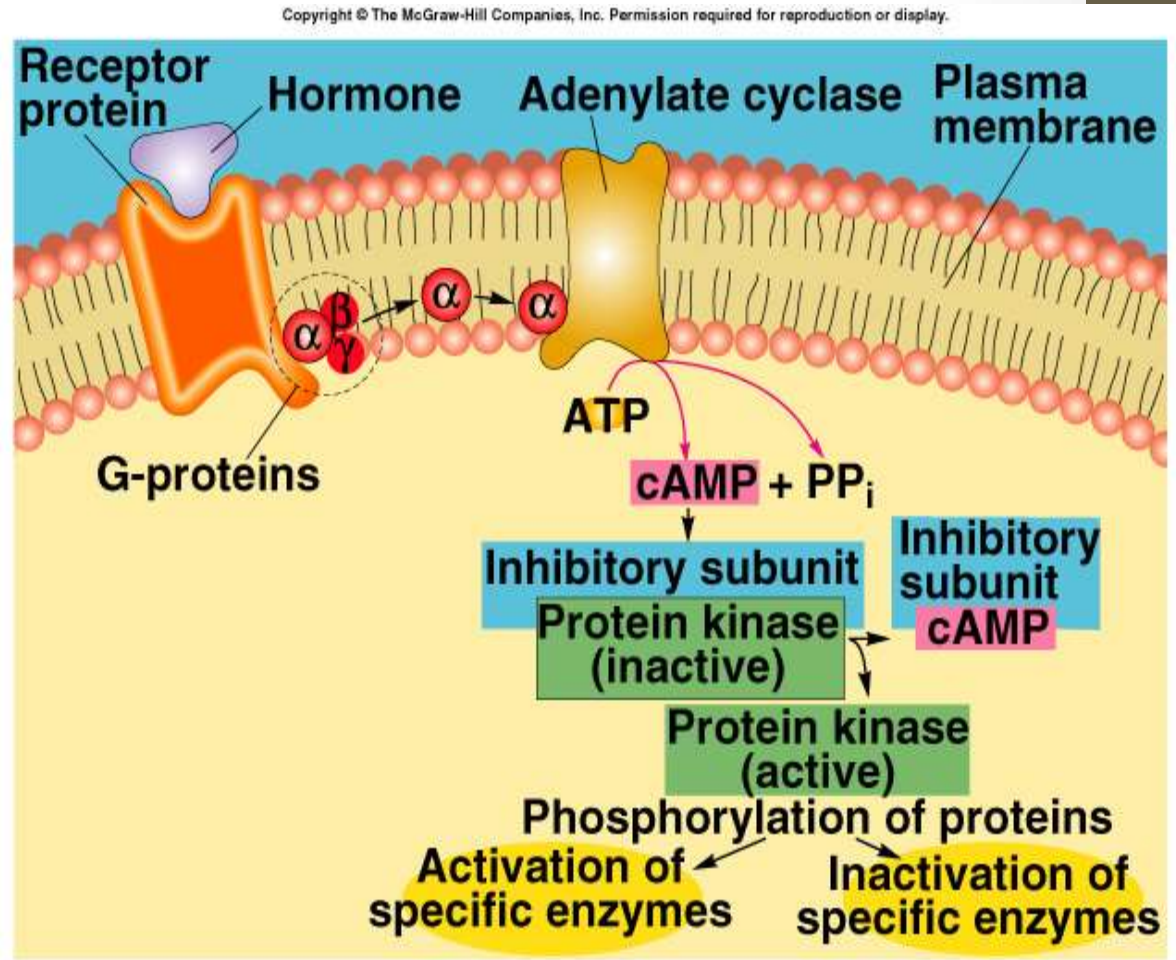
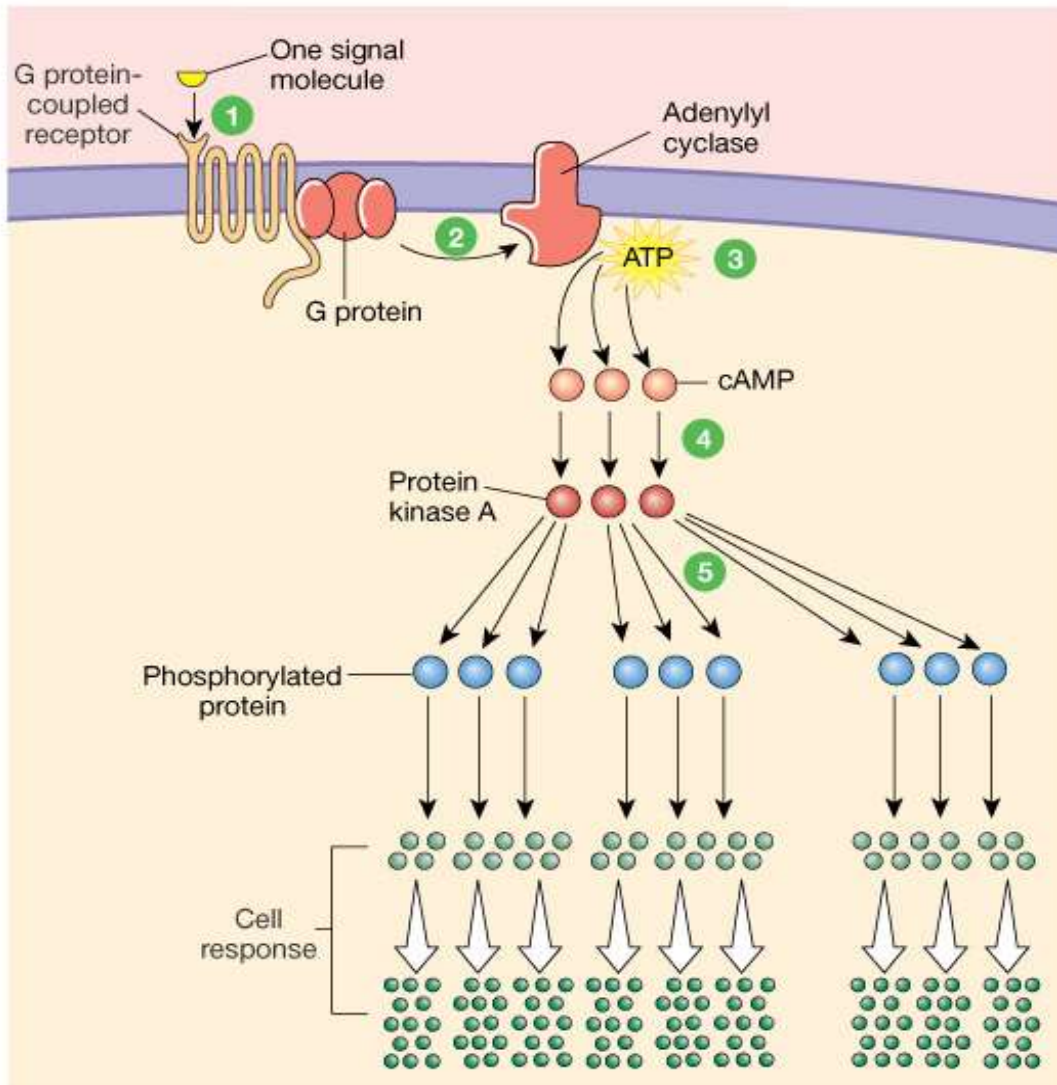


TABLE 20-3 Metabolic Responses to Hormone-Induced Rise in cAMP in Various Tissues

Tissue	Hormone Inducing Rise in cAMP	Metabolic 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 synthesis of glycogen; 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 cells	Epinephrine	Increase in contraction rate
Thyroid	TSH	Secretion of thyroxine
Bone cells	Parathyroid hormone	Increase in resorption of calcium from bone
Skeletal muscle	Epinephrine	Conversion of glycogen to glucose
Intestine	Epinephrine	Fluid secretion
Kidney	Vasopressin	Resorption of water
Blood platelets	Prostaglandin I	Inhibition of aggregation and secretion

G-Protein-coupled Receptors

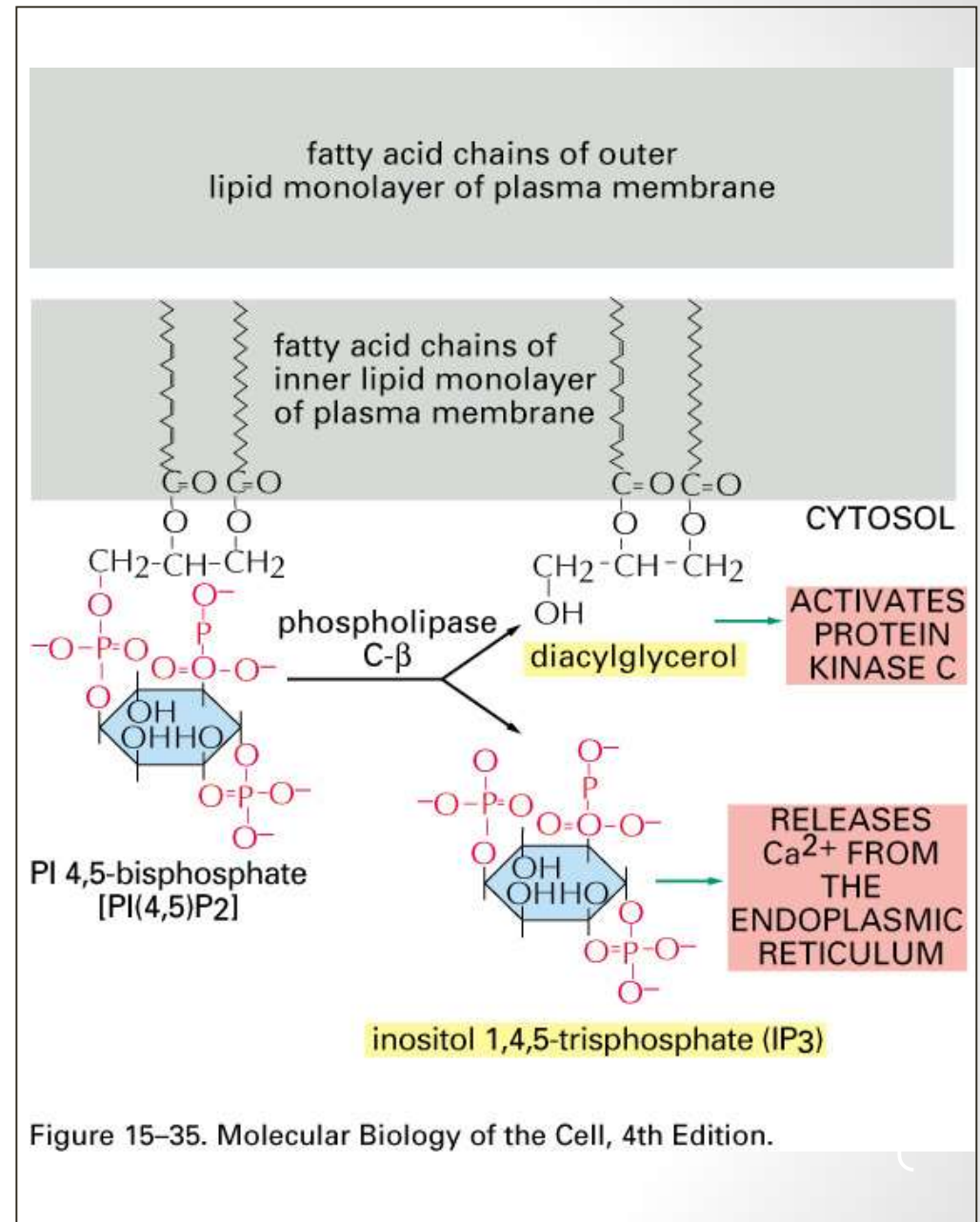


- 1** Signal molecule binds to G protein-linked receptor, which activates the G protein.
- 2** G protein turns on adenylyl cyclase, an amplifier enzyme.
- 3** Adenylyl cyclase converts ATP to cyclic AMP.
- 4** cAMP activates protein kinase A.
- 5** Protein kinase A phosphorylates other proteins, leading ultimately to a cellular response.

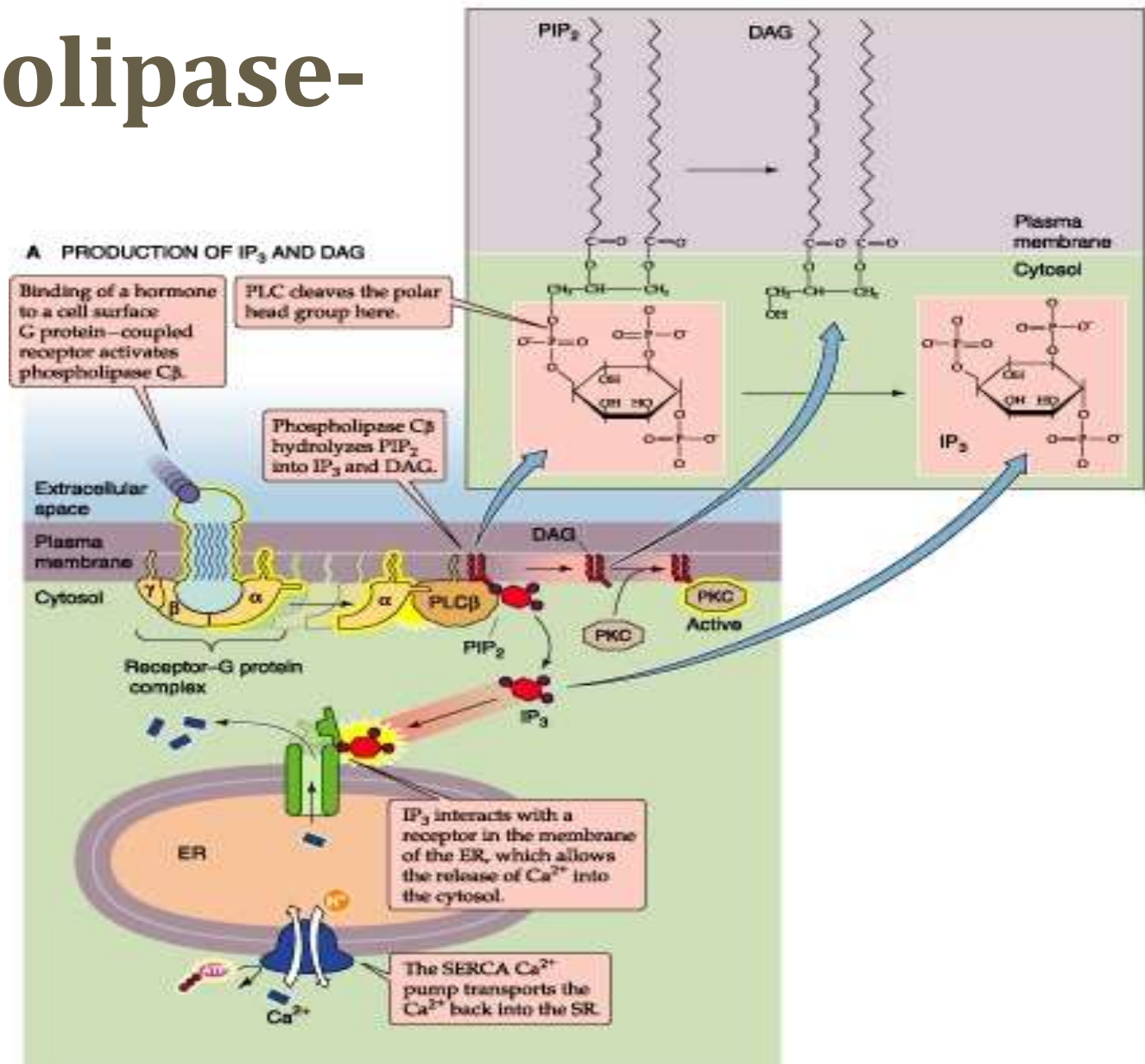
4. Second Messengers, cont.:

C. IP3 and DAG:

1. Overview: Phosphatidylinositol 4,5 bisphosphate (PIP2) triggers a 2-armed signaling pathway
 - a. PIP2 is a minor PL in inner leaflet of PM bilayer that is produced by phosphorylation of phosphatidylinositol and is involved in signaling
 - b. Ligand binding to certain receptors stimulates PIP2 hydrolysis by phospholipase C (PLC)
 - c. This produces diacylglycerol (DAG) and inositol 1,4,5-phosphate (IP3), both of which are 2nd messengers
 - d. PIP2 hydrolysis is activated by both GPRs and TKRs via different forms of PLC
 - e. PLC- β is stimulated by G_q proteins while PLC- γ has SH2 domains that allow binding to activated tyrosine kinases



Phospholipase-C-Ca²⁺



4. Second Messengers, cont.:

C. DAG and IP₃, cont.:

2. DAG: Remains associated with the PM

a. Stimulates the Ca²⁺-dependent protein kinase C signaling pathway, which activates other targets including the MAP kinase cascade (see below)

3. IP₃: Small polar molecule released into cytosol

a. Stimulates Ca²⁺ release from intracellular stores. *Question: where are these?*

b. Elevated Ca²⁺ alters activities of target proteins including kinases & phosphatases

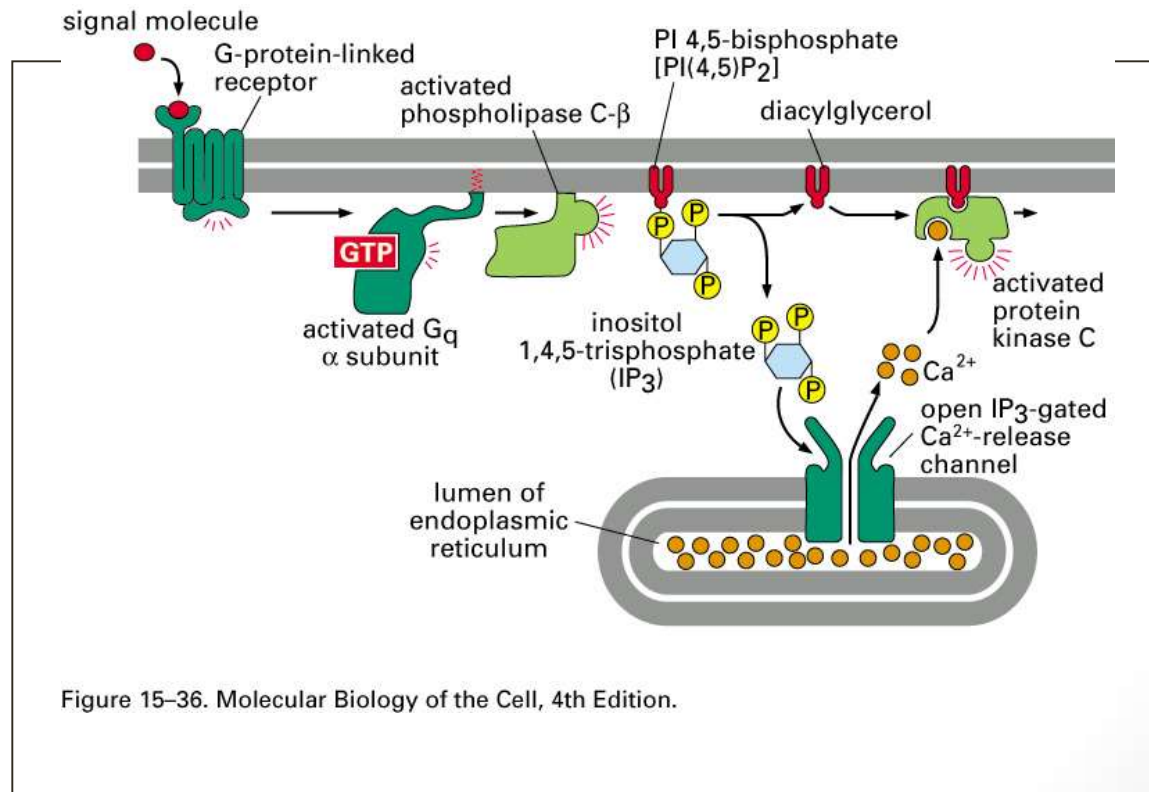
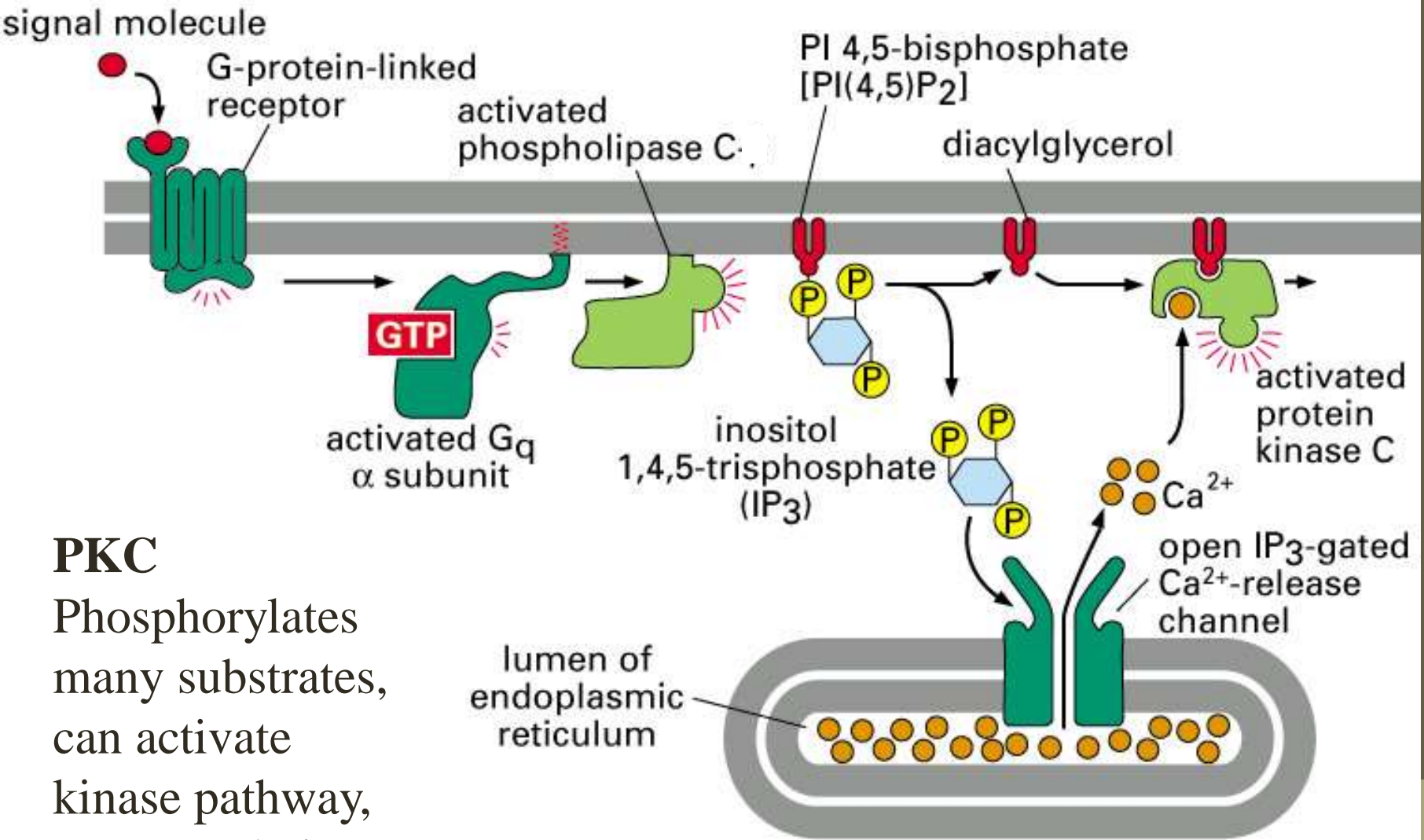


Figure 15-36. Molecular Biology of the Cell, 4th Edition.

PLC- signaling pathway



PKC

Phosphorylates many substrates, can activate kinase pathway, gene regulation

4. Second Messengers, cont.:

D. Ca^{+2} also acts as a second messenger

Ca^{+2} concentration kept low (10^{-7} M), rising locally due to transient signaling

Effects of intracellular Ca^{+2} are mediated by the Ca^{+2} binding protein calmodulin.

Ca^{+2} /calmodulin binds to target proteins, including protein kinases (Ca^{+2} calmodulin-dependent kinases; CaM-kinases), adenylyl cyclases, and phosphodiesterases, causing change in conformation and activation of these proteins.

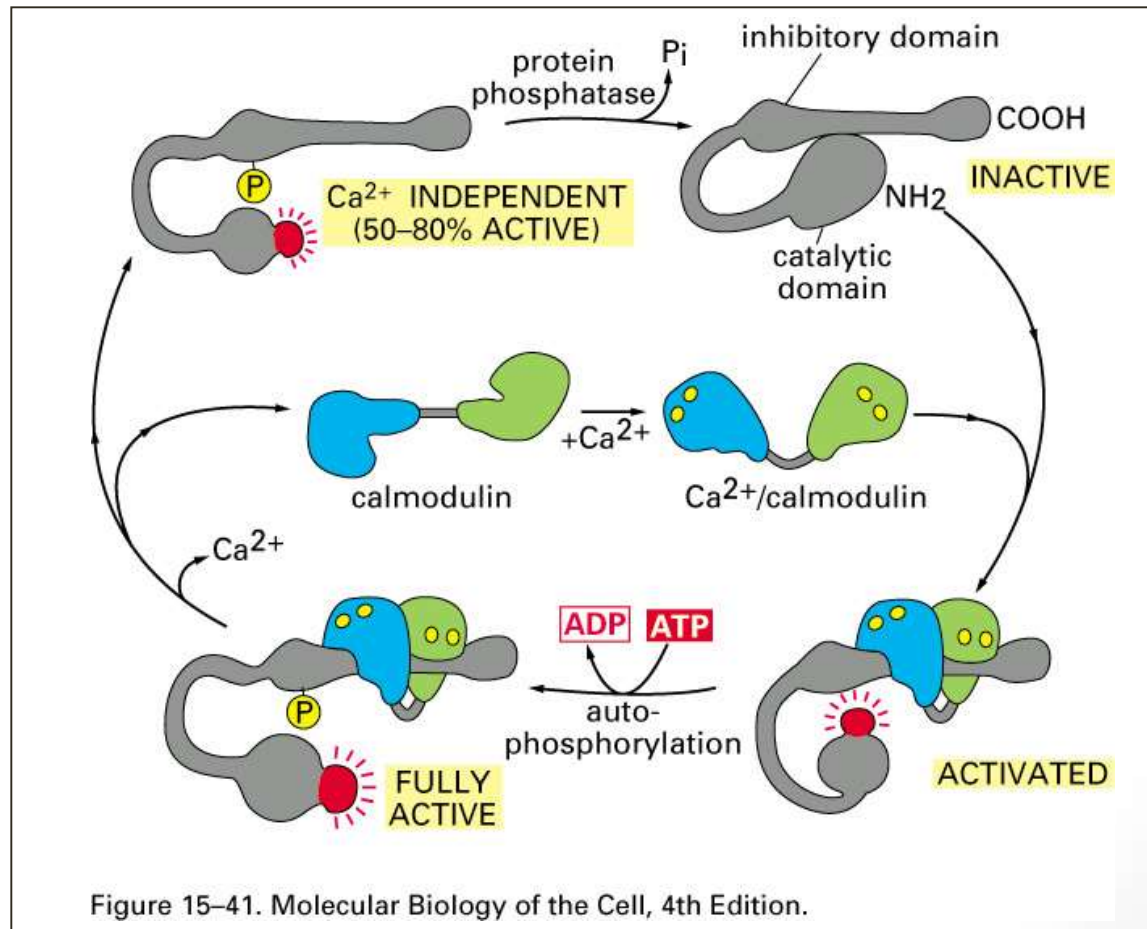
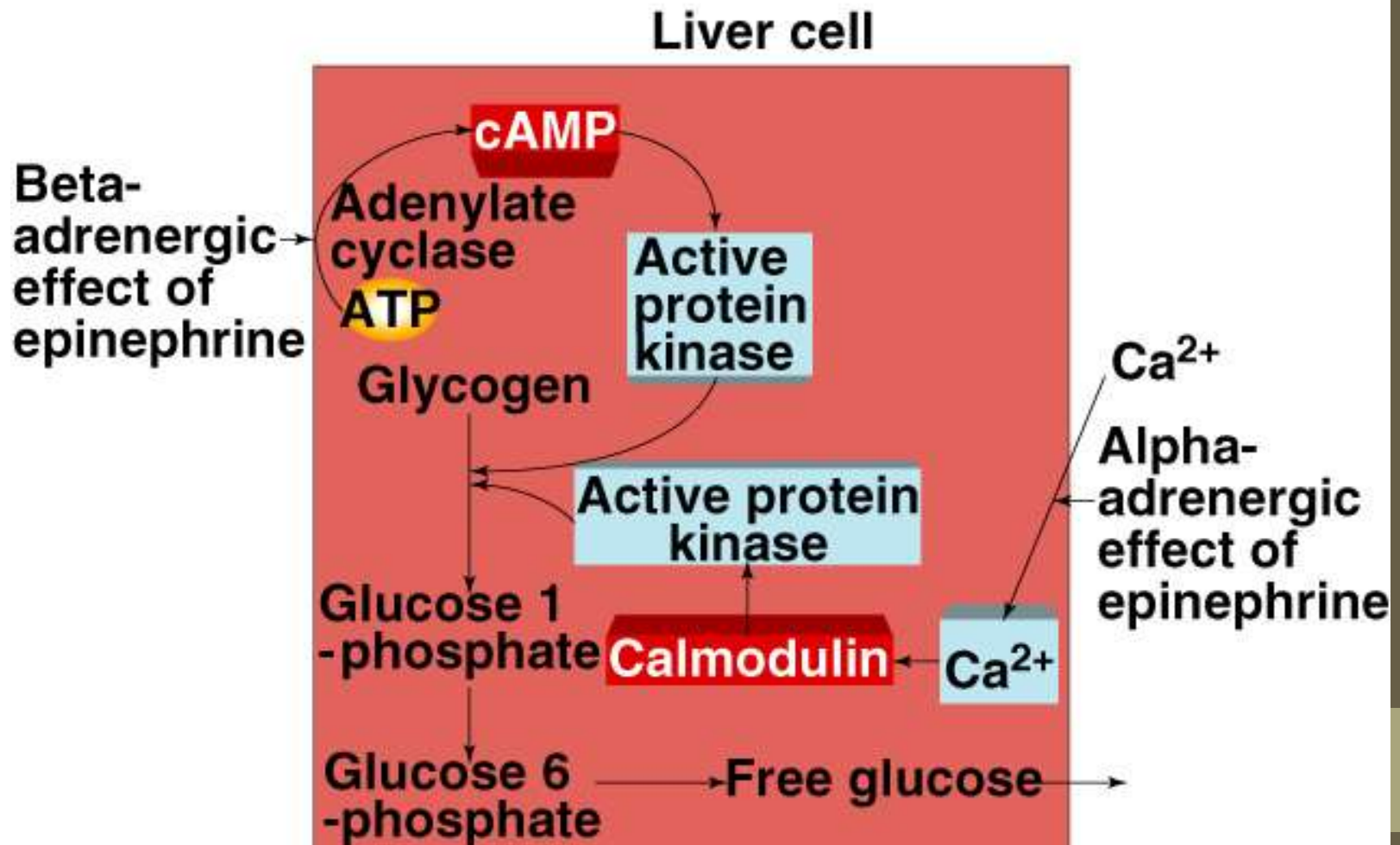


Figure 15–41. Molecular Biology of the Cell, 4th Edition.

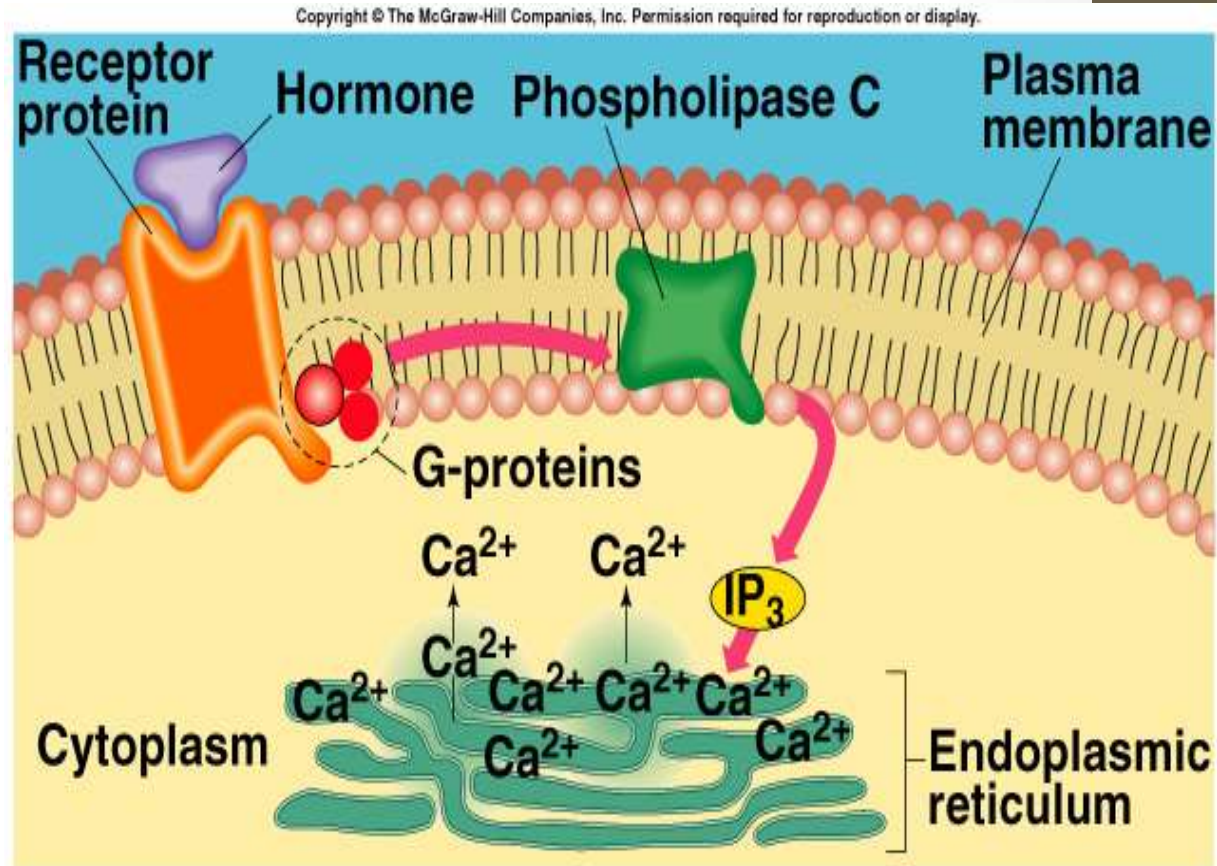
Epinephrine Can Act Through Two 2nd Messenger Systems

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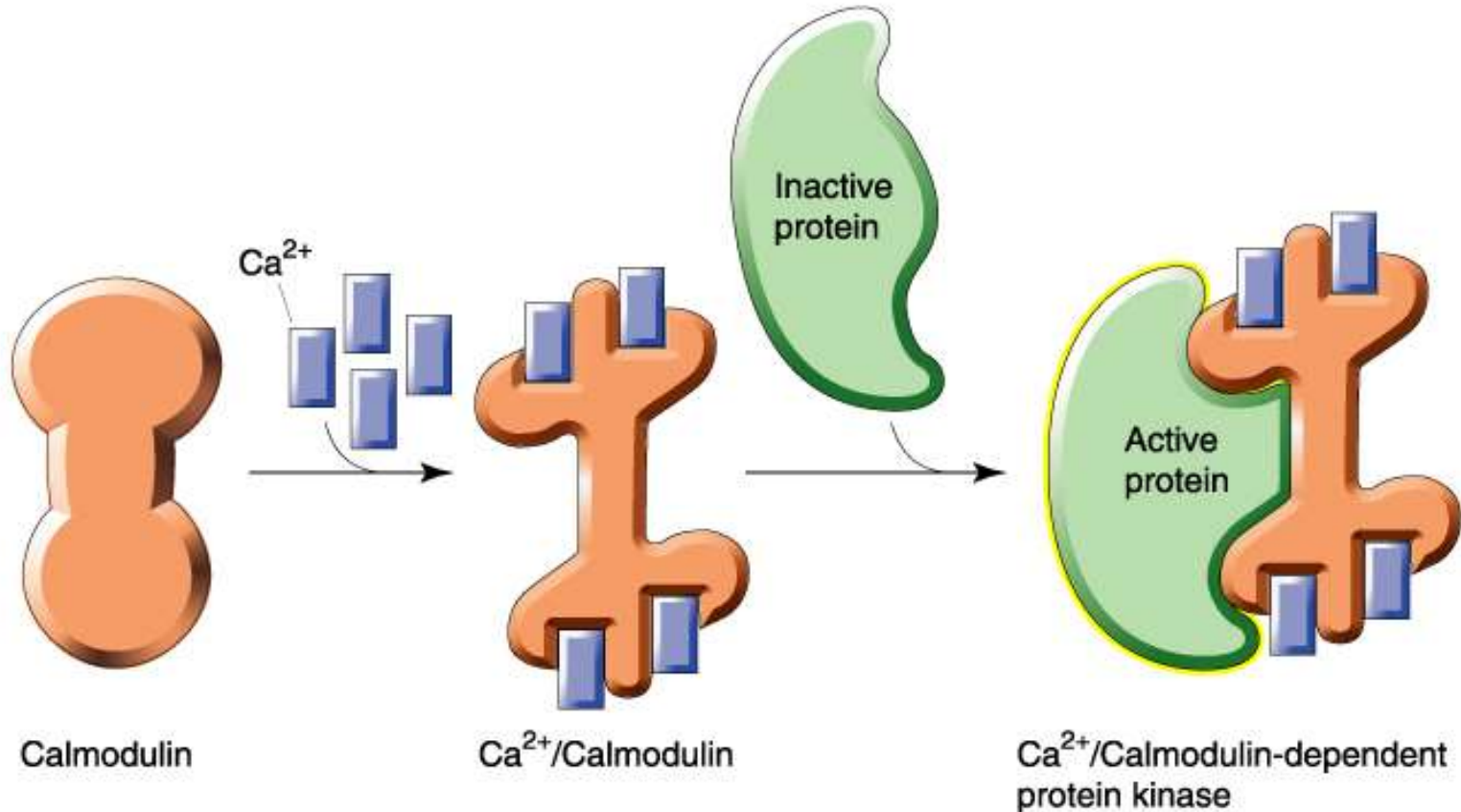


Ca²⁺- Calmodulin (continued)

- Ca²⁺ diffuses into the cytoplasm.
 - Ca²⁺ binds to calmodulin.
- Calmodulin activates specific protein kinase enzymes.
 - Alters the metabolism of the cell, producing the hormone's effects.



Ca²⁺- Calmodulin (continued)



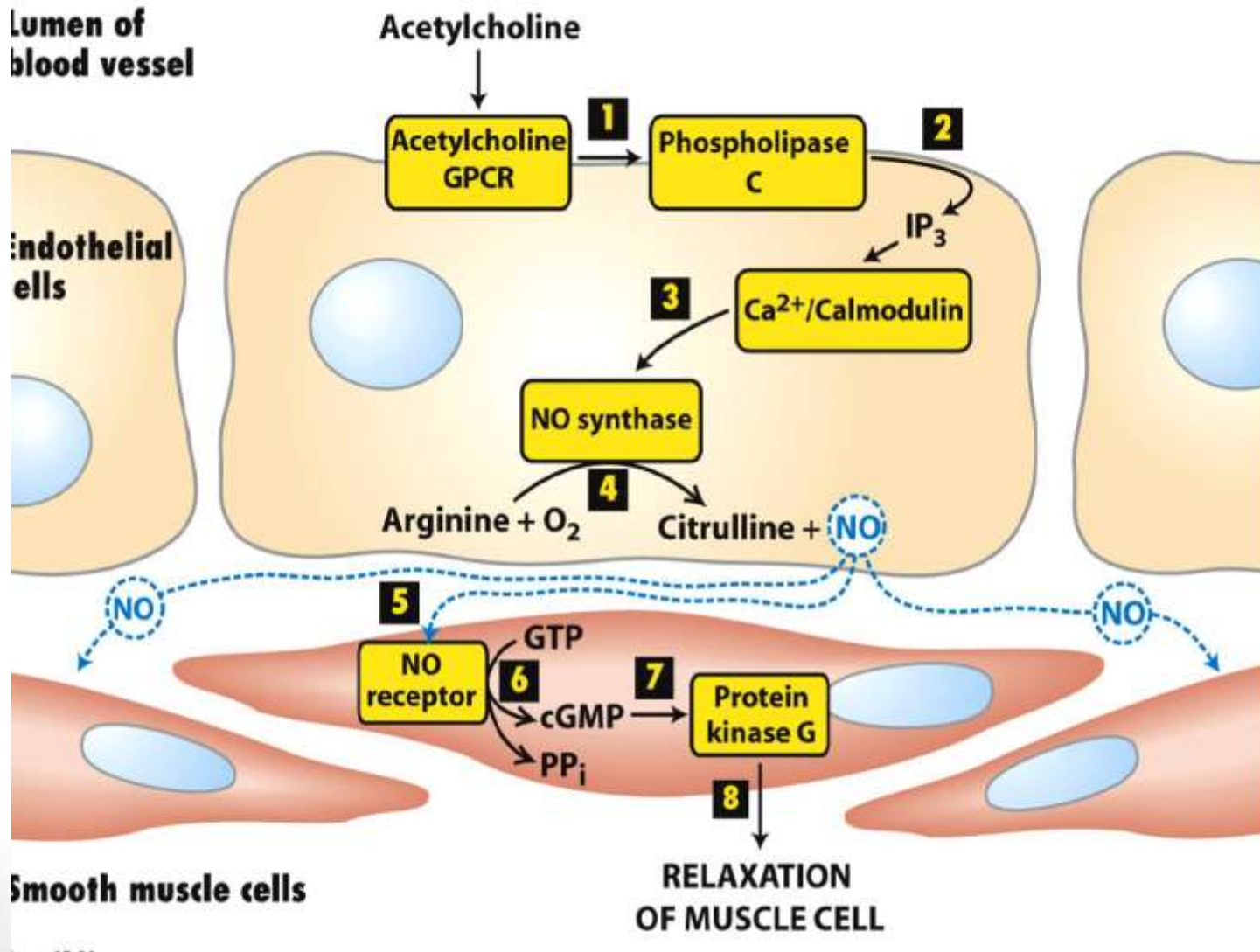
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Guanylate cyclase (GC) receptor

Membrane receptor – ANP

Soluble receptor – NO, CO

NO signaling



4. Second Messengers, cont.:

E. PIP3:

PIP2 phosphorylated by PI 3-kinase, resulting in PIP3, which is also a 2nd messenger.

PI 3-kinase can be activated by GPRs or TKRs.

One target of PIP3 is a protein-serine/threonine kinase called Akt, or protein kinase B, which becomes activated by a kinase called PDK1.

PIP3 binds to Akt at the pleckstrin homology domain.

Activation of Akt leads to regulation of target molecules, including BAD, which is pro-apoptotic and becomes inactivated by phosphorylation.

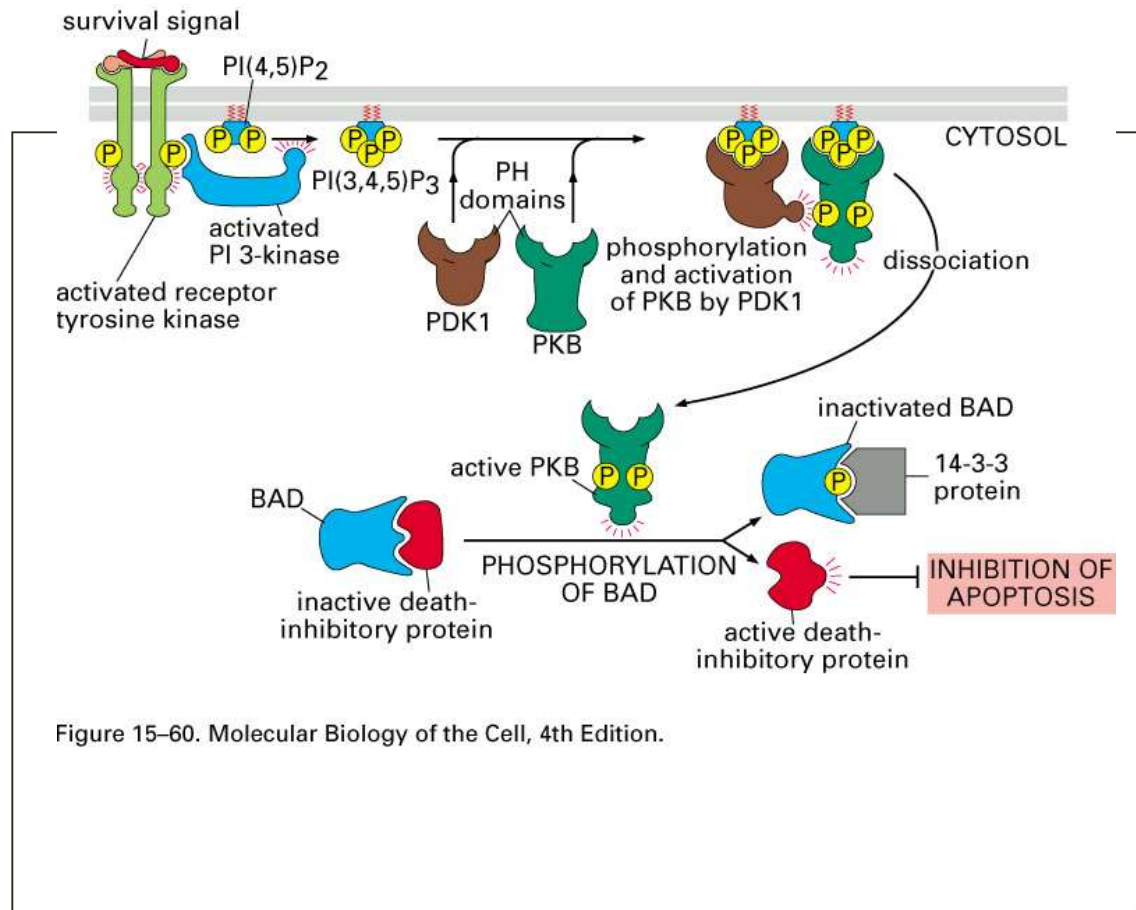


Figure 15-60. Molecular Biology of the Cell, 4th Edition.

5. Signaling Cascades, cont.:

5 downstream kinases activated by different signaling cascades

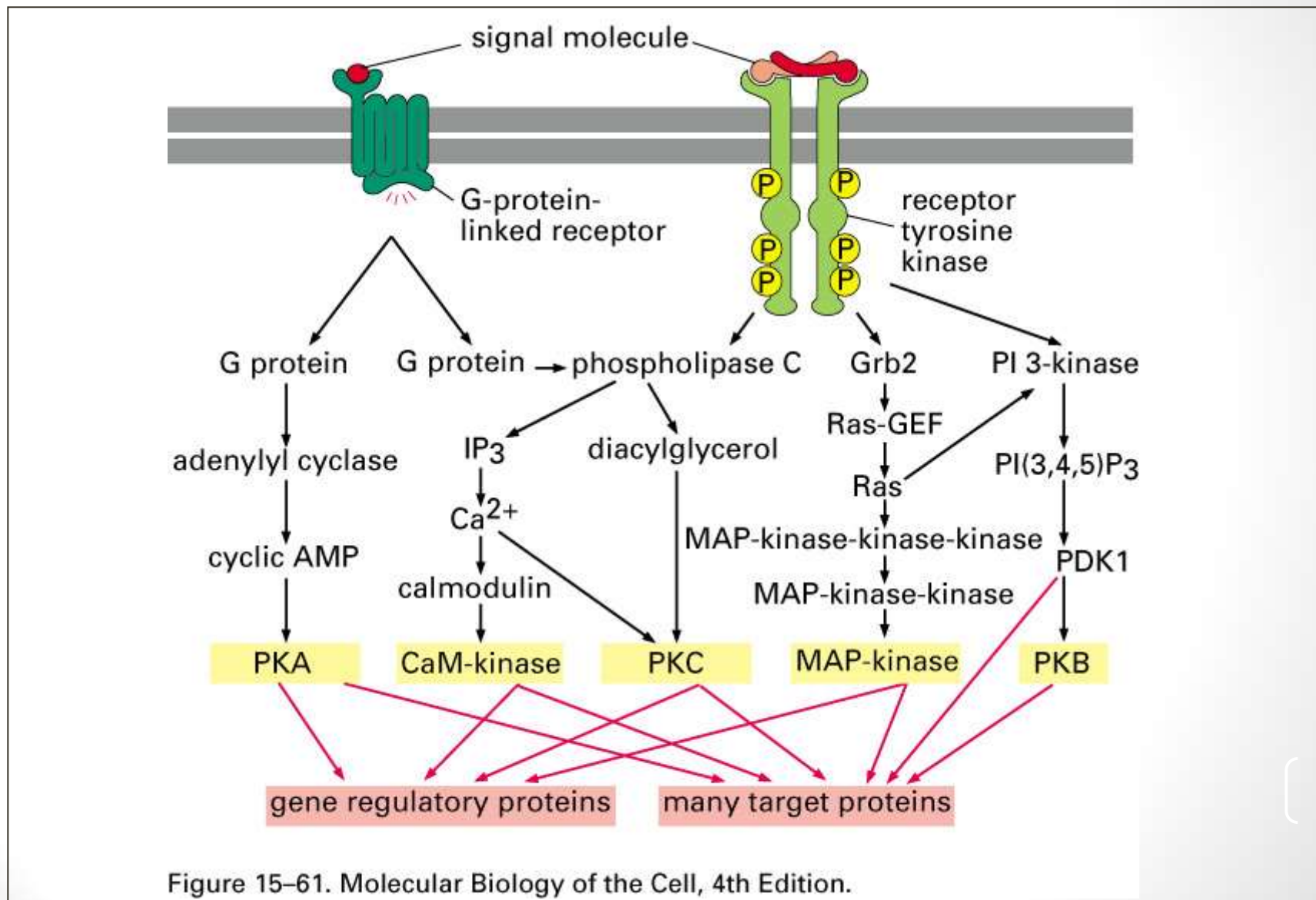
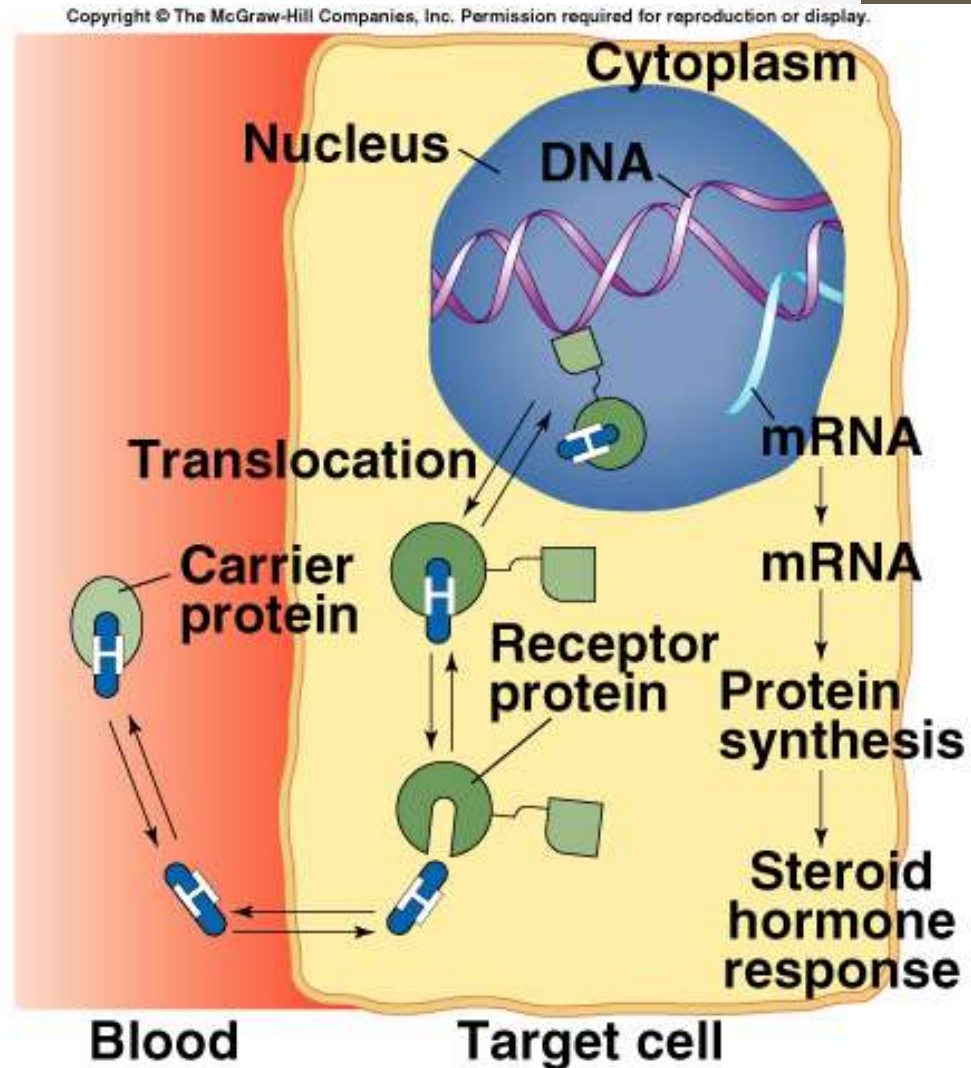


Figure 15-61. Molecular Biology of the Cell, 4th Edition.

Hormones That Bind to Nuclear Receptor Proteins

- Lipophilic steroid and thyroid hormones are attached to plasma carrier proteins.
 - Hormones dissociate from carrier proteins to pass through lipid component of the target plasma membrane.
- Receptors for the lipophilic hormones are known as nuclear hormone receptors.

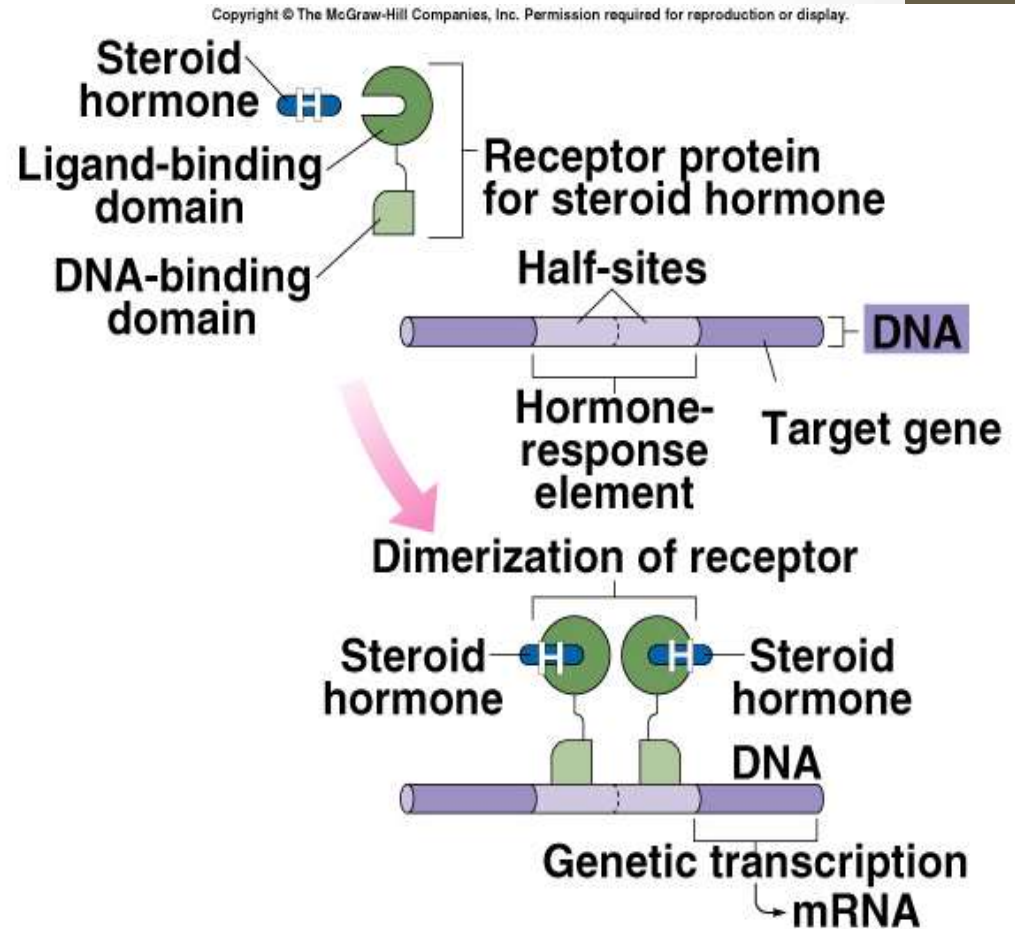


Nuclear Hormone Receptors

- Steroid receptors are located in cytoplasm and in the nucleus.
- Function within cell to activate genetic transcription.
 - Messenger RNA directs synthesis of specific enzyme proteins that change metabolism.
- Each nuclear hormone receptor has 2 regions:
 - A ligand (hormone)-binding domain.
 - DNA-binding domain.
- Receptor must be activated by binding to hormone before binding to specific region of DNA called HRE (hormone responsive element).
 - Located adjacent to gene that will be transcribed.

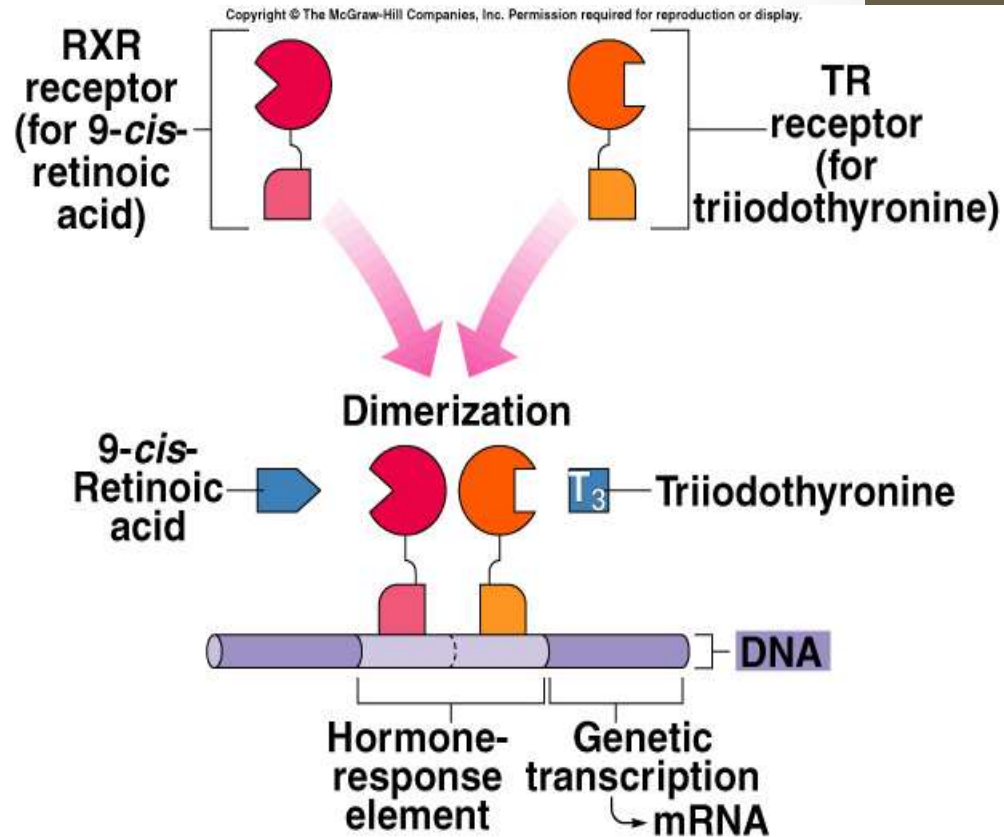
Mechanisms of Steroid Hormone Action

- Cytoplasmic receptor binds to steroid hormone.
- Translocates to nucleus.
- DNA-binding domain binds to specific HRE of the DNA.
- Dimerization occurs.
 - Process of 2 receptor units coming together at the 2 half-sites.
- Stimulates transcription of particular genes.

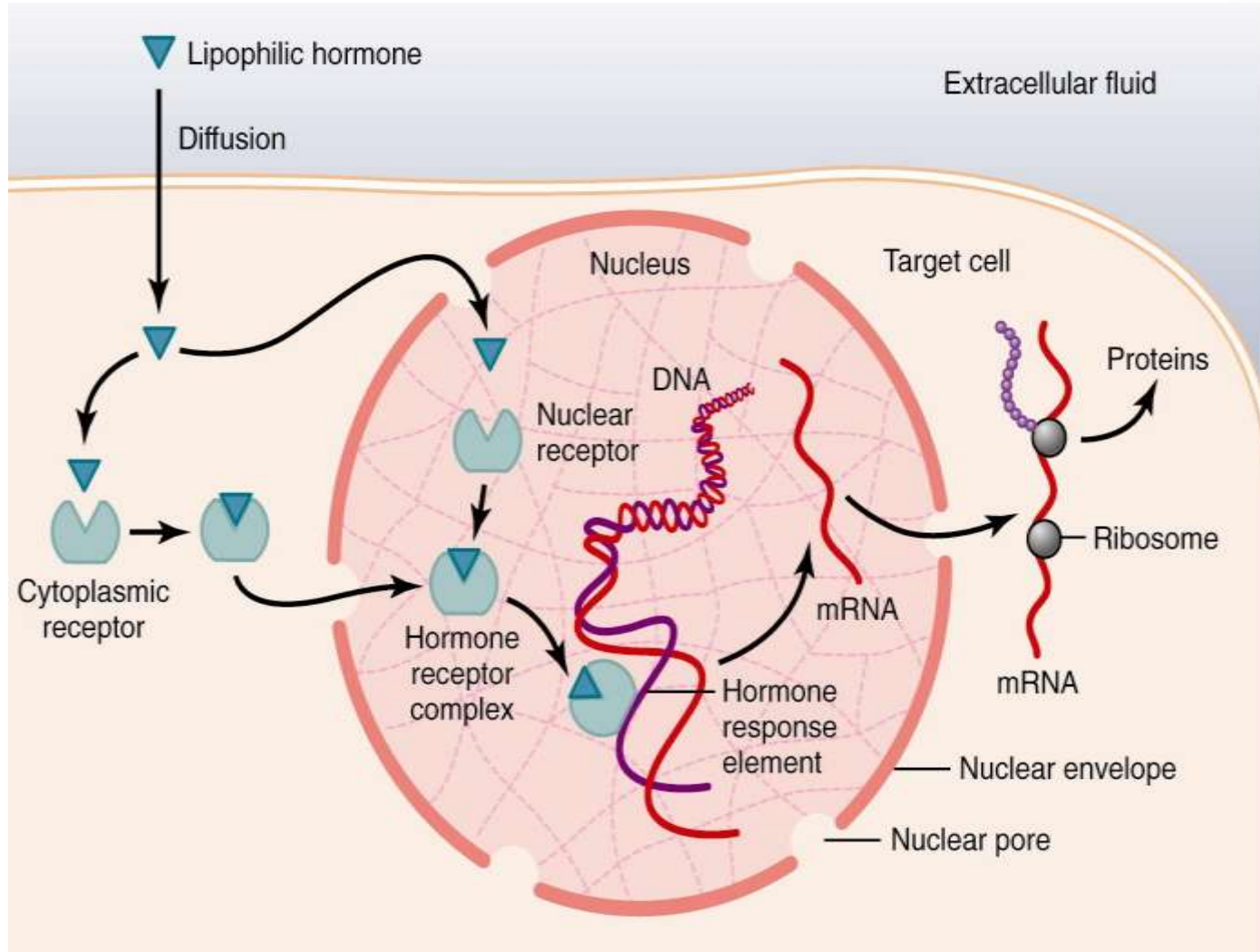


Mechanism of Thyroid Hormone Action

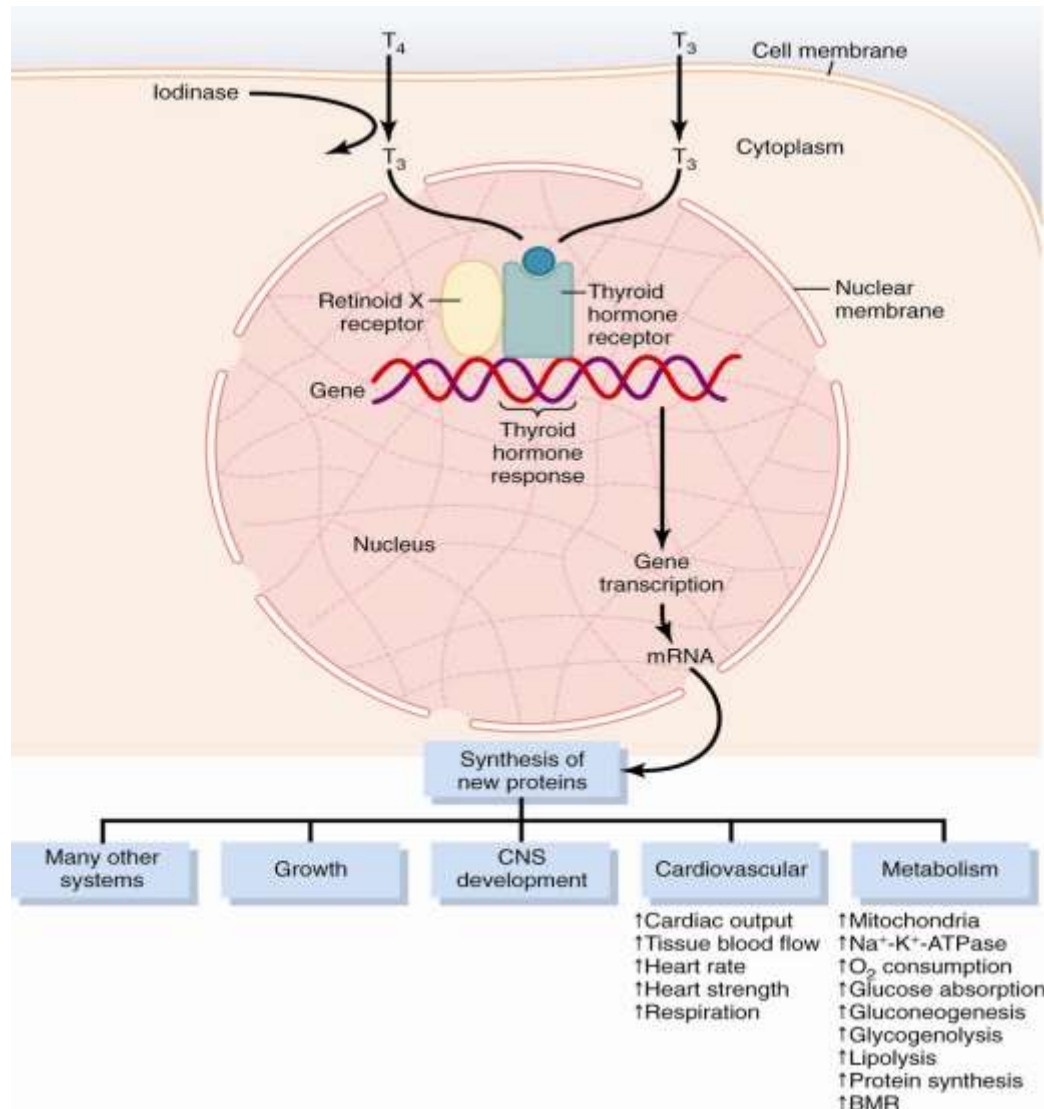
- T_4 passes into cytoplasm and is converted to T_3 .
- Receptor proteins located in nucleus.
 - T_3 binds to ligand-binding domain.
 - Other half-site is vitamin A derivative (9-cis-retinoic) acid.
 - DNA-binding domain can then bind to the half-site of the HRE.
 - Two partners can bind to the DNA to activate HRE.
 - Stimulate transcription of genes.



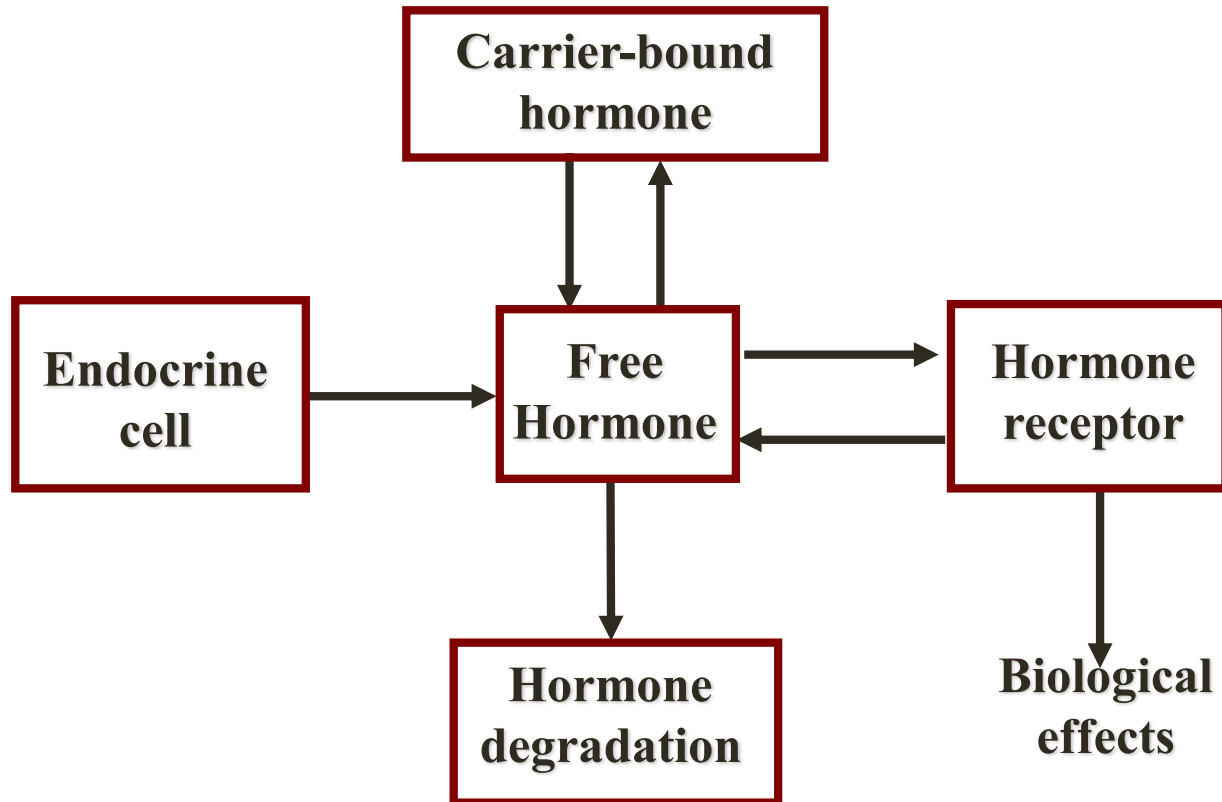
Steroid & Thyroid Hormones - Mechanism of Action



Actions of Thyroid Hormones



Determinants of Free Hormone Receptor Binding

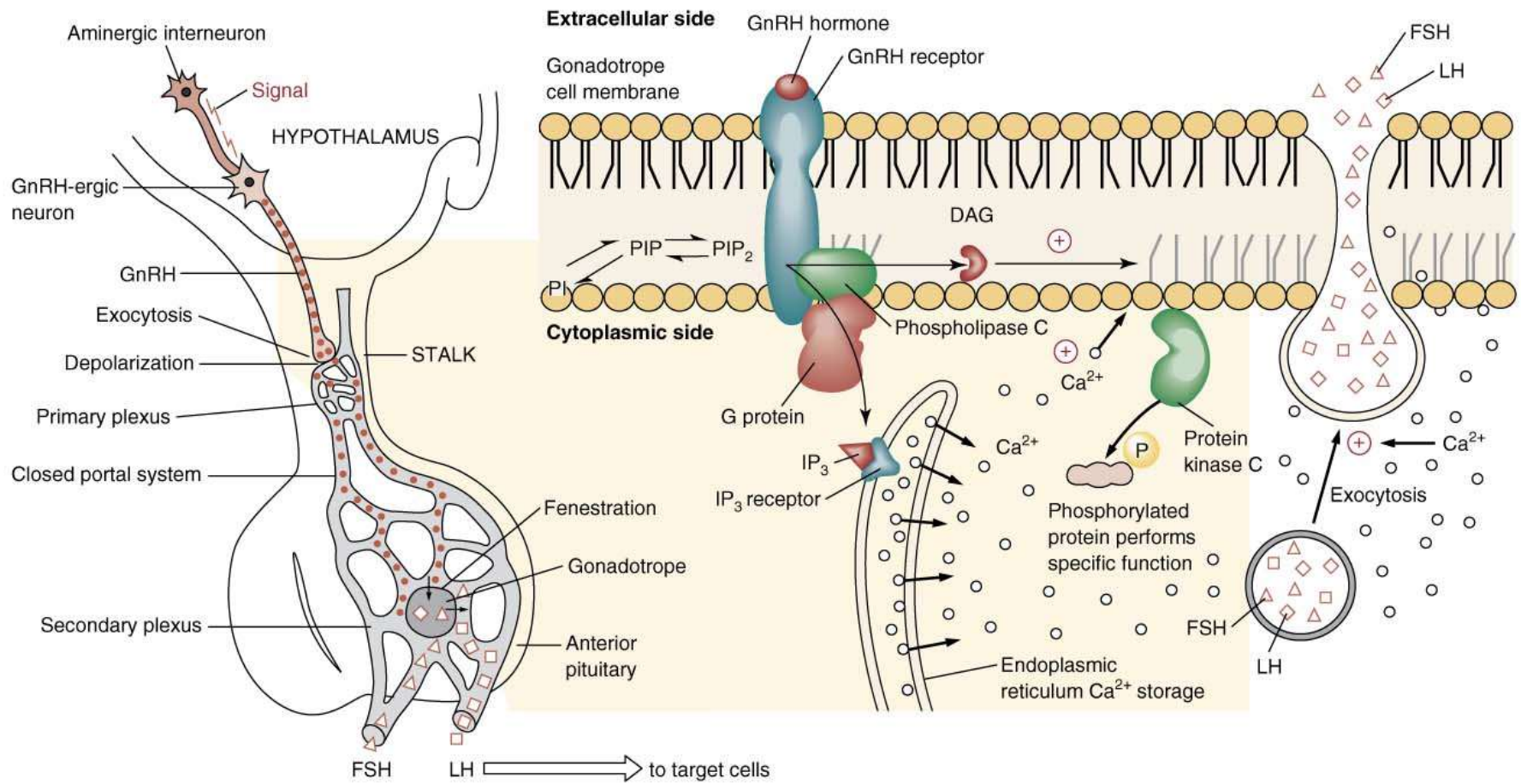


Correlation of Plasma Half-Life & Metabolic Clearance of Hormones with Degree of Protein Binding

Hormone	Protein binding (%)	Plasma half-life	Metabolic clearance (ml/minute)
Thyroid			
Thyroxine	99.97	6 days	0.7
Triiodothyronine	99.7	1 day	18
Steroids			
Cortisol	94	100 min	140
Testosterone	89	85 min	860
Aldosterone	15	25 min	1100
Proteins			
Thyrotropin	little	50 min	50
Insulin	little	8 min	800
Antidiuretic hormone	little	8 min	600

Circulating Transport Proteins

Transport Protein	Principle Hormone Transported
Specific	
Corticosteroid binding globulin (CBG, transcortin)	Cortisol, aldosterone
Thyroxine binding globulin (TBG)	Thyroxine, triiodothyronine
Sex hormone-binding globulin (SHBG)	Testosterone, estrogen
Nonspecific	
Albumin	Most steroids, thyroxine, triiodothyronine
Transthyretin (prealbumin)	Thyroxine, some steroids



Regulation of secretion of LH and FSH by protein kinase C.

Textbook of Biochemistry With Clinical Correlations, Sixth Edition, Edited by Thomas M. Devlin. Copyright © 2006 John Wiley & Sons, Inc.

Signaling molecule
(hormones)



Receptor of target cell



Intracellular molecule
(second messengers)



biological effect



**Signal
transduction**

Third messengers:

Third messengers are the molecules which transmit message from outside to inside of nucleus or from inside to outside of nucleus, also called DNA binding protein.

