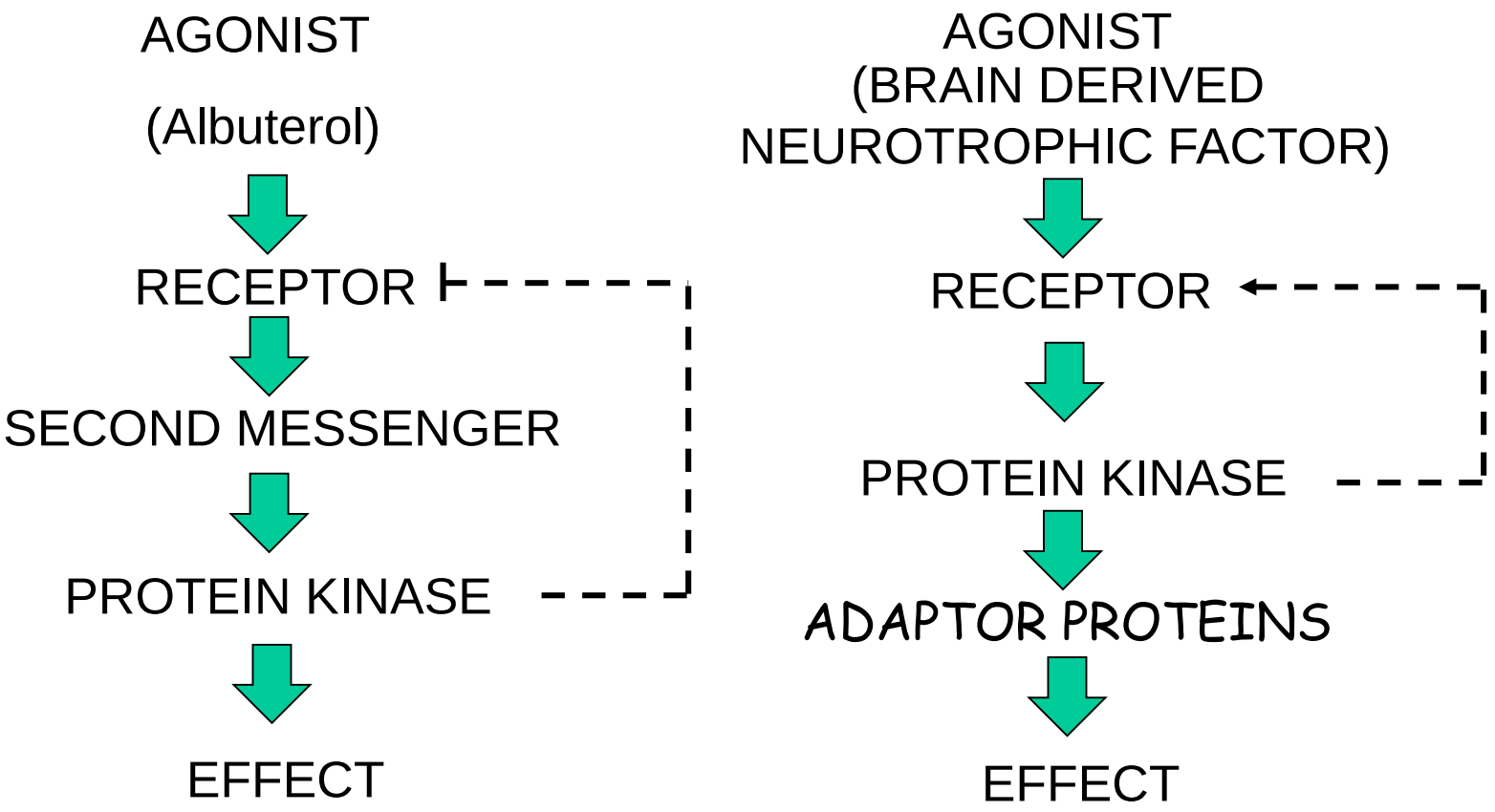
September 25, 2020

Vic Ramkumar, Ph.D.

Intracellular Signaling & Signal Propagation

CELL SIGNALING CYCLE



G Protein-Coupled Receptor

Receptor Tyrosine Kinase

Section 1

What is Signal Transduction?

- Transmission of a signal from a hormone or drug to produce some cellular function.
- Signals are generally detected by receptor proteins present on the cell surface.
- Cell surface receptors discriminate the signals and channel these to specific cellular effectors to produce a function.
- Drugs mimic (agonists) or antagonize (antagonists) the effects of endogenous chemicals on receptors.

EXTRACELLULAR SIGNALLING

Relevant Concepts

Types of signalling processes: Endocrine, paracrine, autocrine synaptic, plasma membrane attached protein

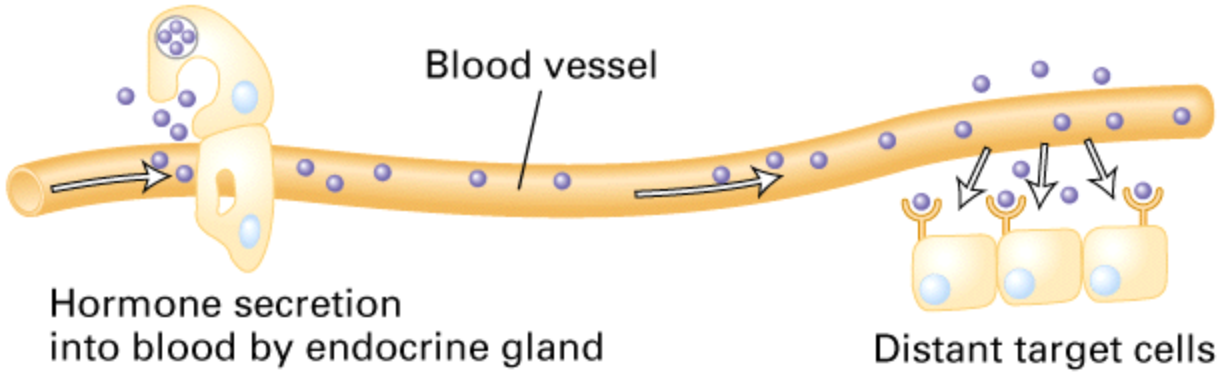
Ligand

Second messengers

Receptors - G protein coupled receptors, ion channel receptor, tyrosine kinase-linked receptor, receptors with enzymatic activity

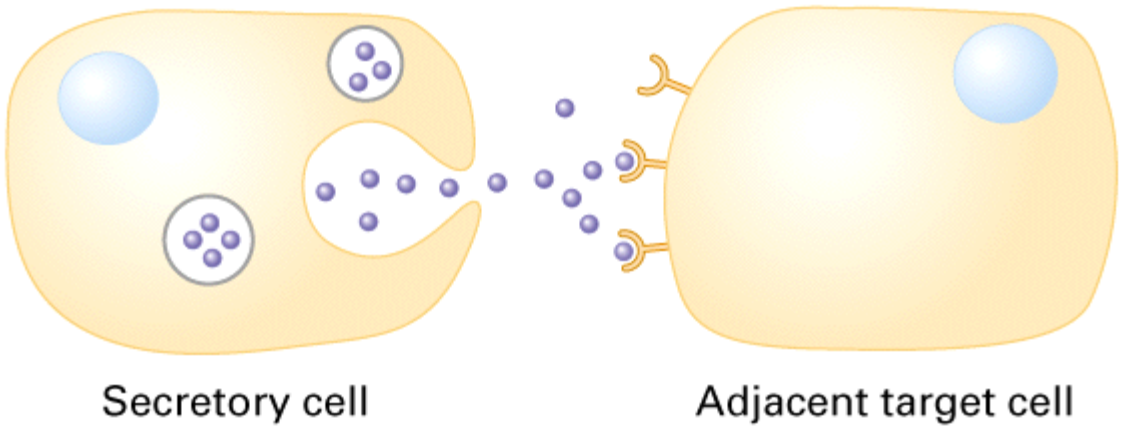
Distance Communication

EPINEPHRINE, GLUCAGON
ACTH, INSULIN

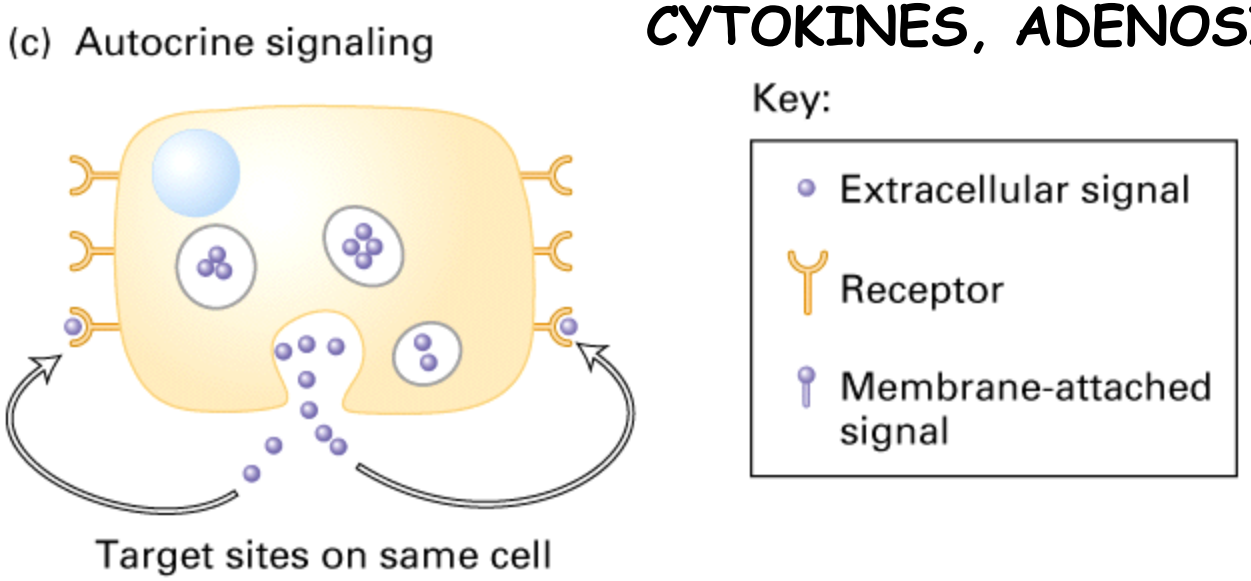


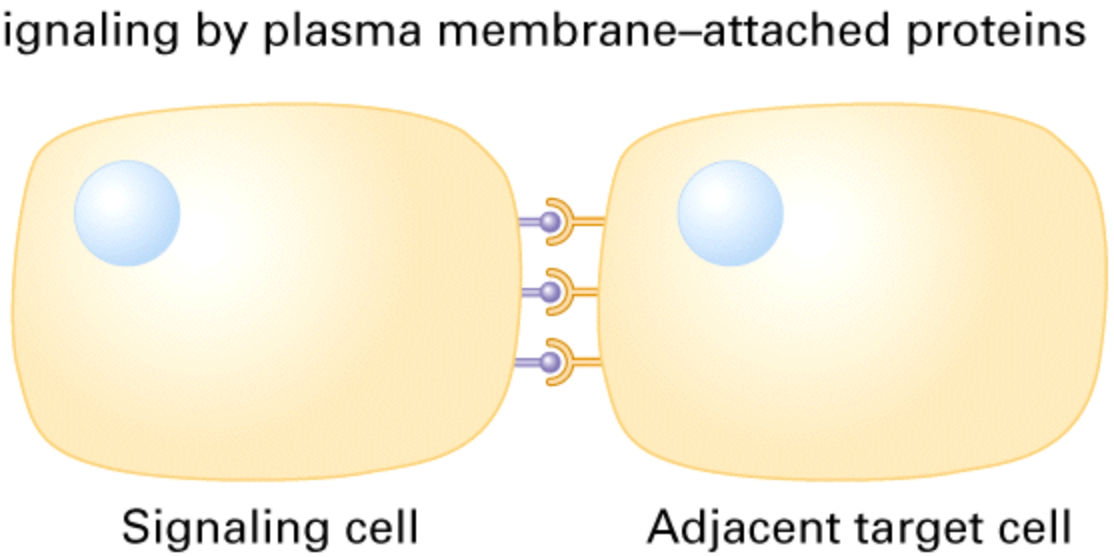
Local Communication

CYTOKINES (IL-2), ADENOSINE, ADP



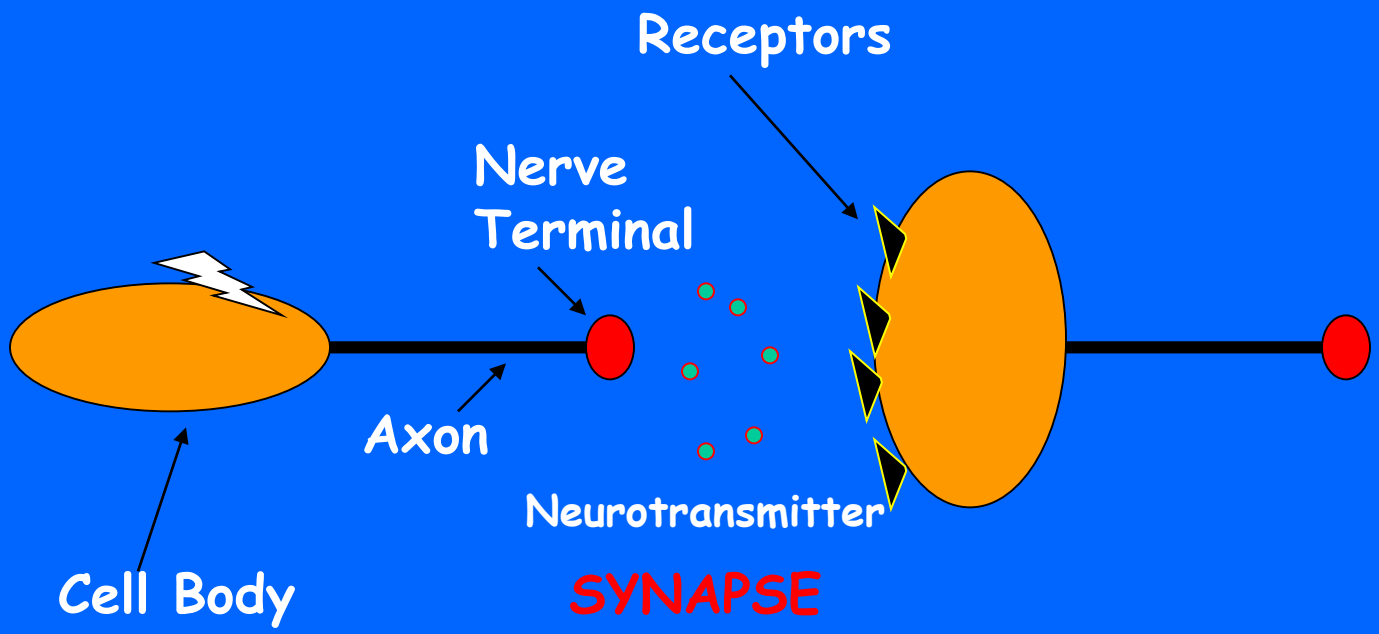
CYTOKINES, ADENOSINE



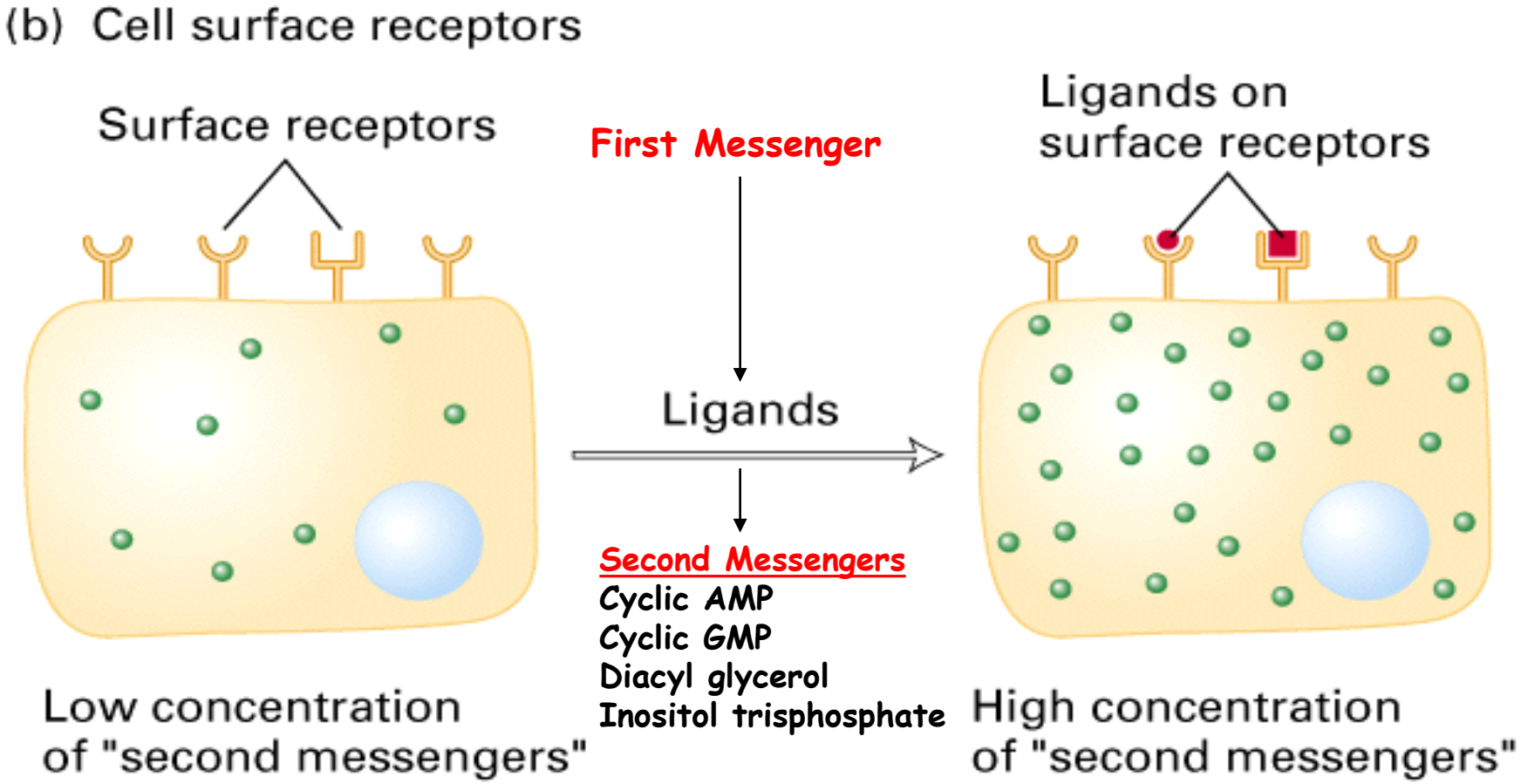


T CELL

Model of Synaptic Transmission



- Agonists impart their information by increasing the generation of second messengers



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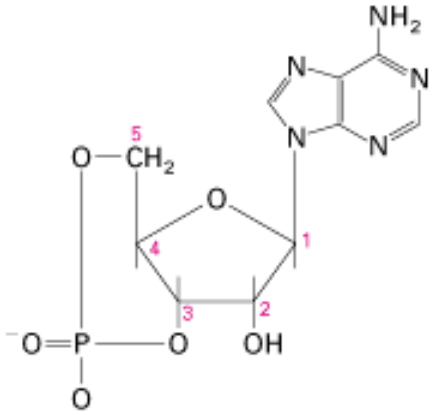
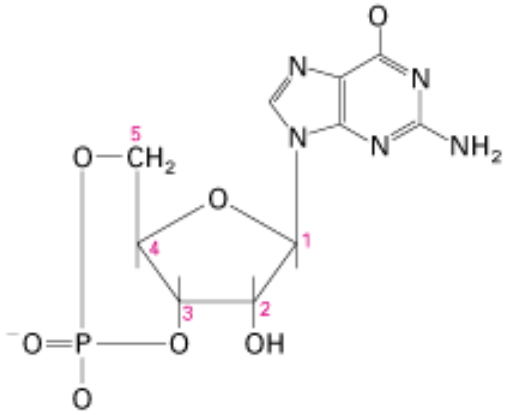
SECOND MESSENGERS

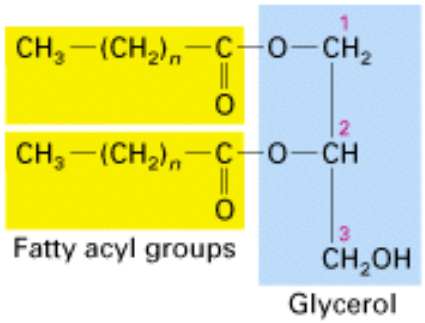
- Second messenger convey the signal from receptor and effector to intracellular targets



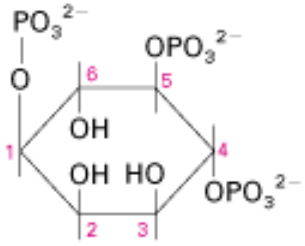
3′,5′-Cyclic AMP
(cAMP)



3′,5′-Cyclic GMP
(cGMP)



1,2-Diacylglycerol
(DAG)



Inositol
1,4,5-trisphosphate
(IP_3)

Adenylyl cyclase ← (ATP)

(Earle Sutherland, Nobel Prize)

Guanylate cyclase ← (GTP)

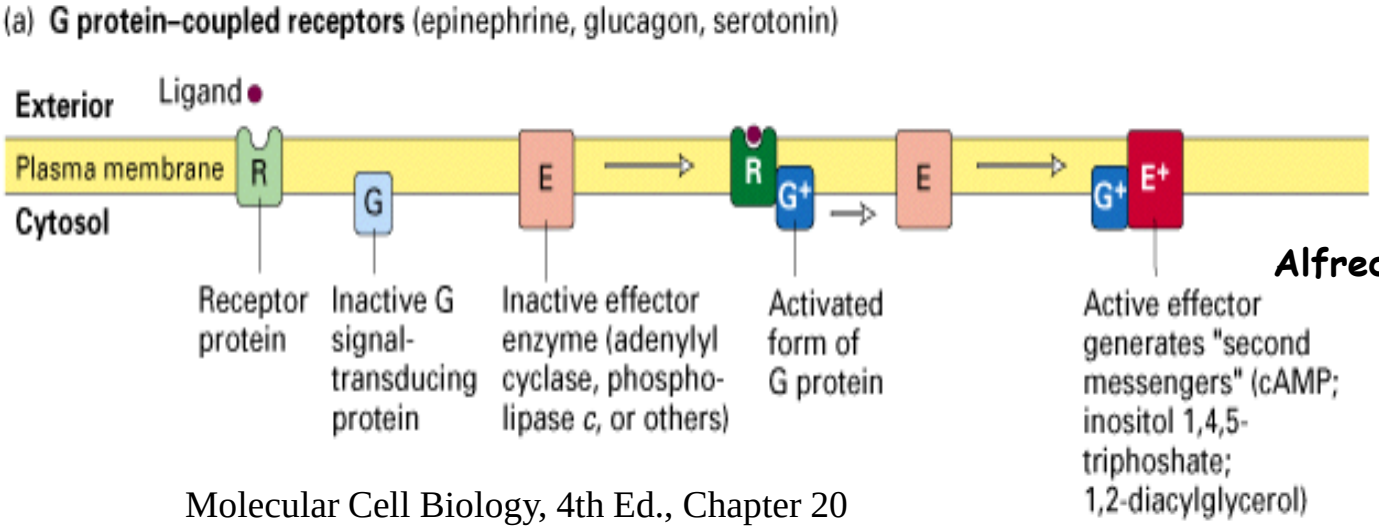
Phospholipase C ← (Phosphatidyl inositol bisphosphate)

Section 2: Relevant Concepts

- G protein-coupled receptor
- G proteins – heterotrimeric, monomeric
- Cholera toxin, pertussis toxins
- Adenylyl cyclase
- β–adrenergic receptors
- Inositol 1,4,5 trisphosphate (IP_3)
- Diacyl glycerol
- Phosphodiesterases

RECEPTOR CLASSIFICATION

A. G-Protein Coupled Receptors – transmit signals from Hormones and drugs through G proteins



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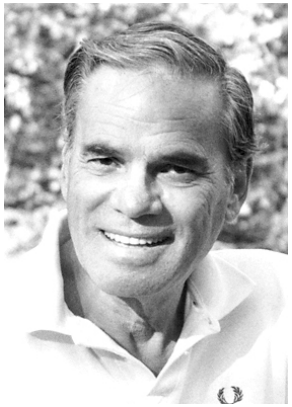
Alfred Gilman, MD, Ph.D.



Martin Rodbell, Ph.D.

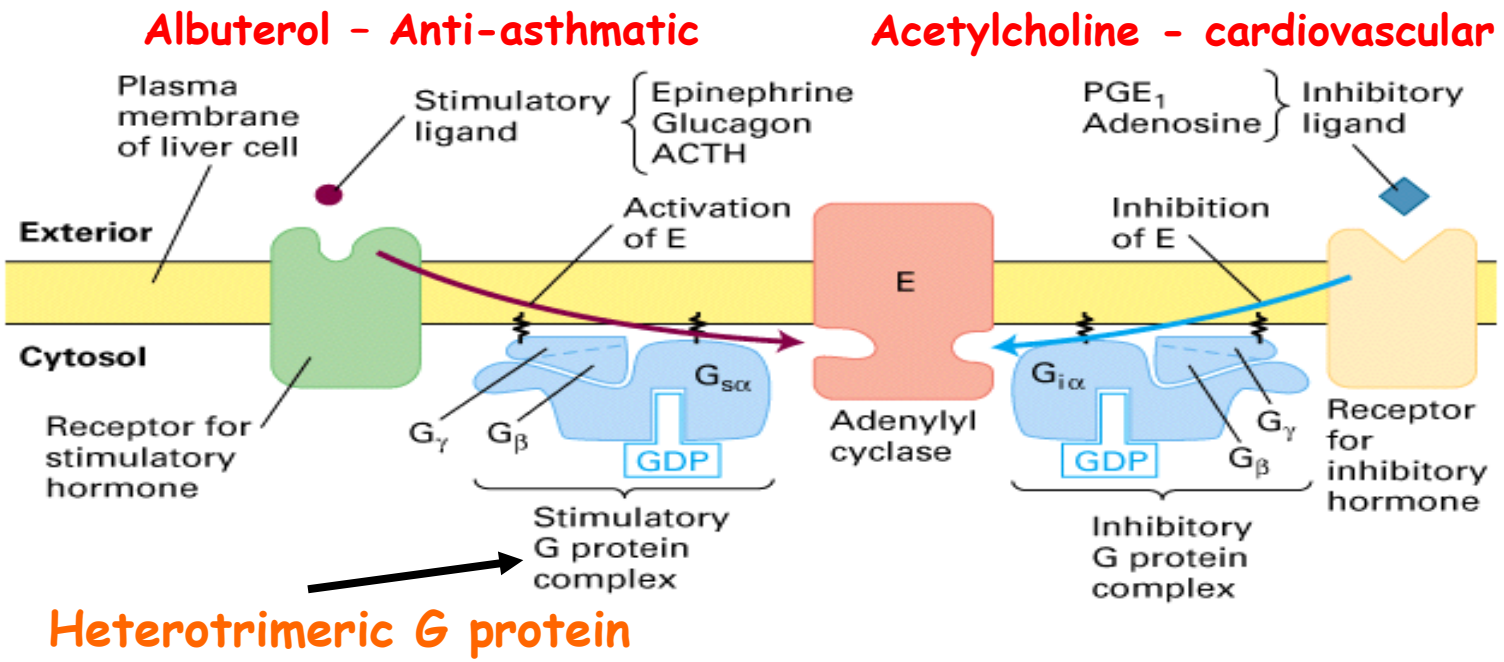
<u>Associated Diseases:</u> congestive heart failure, Parkinson's disease, migraine, asthma, hypertension, pulmonary edema. GPCRs serve as the targets of most prescribed drugs.

Drugs Regulating G-Protein Coupled Receptors Contribute to a High Percentage of Prescribed Drugs

- Vasodilation (β_2 adrenergic receptor agonists)
- Vasoconstriction (α_1 adrenergic receptor agonists)
- Positive inotropic/chronotropic (β_1 adrenergic receptor agonists)
- Bronchodilator (β_2 adrenergic receptor agonists)
- Parkinson's disease (dopamine D2 agonists)
- Alzheimer's disease (muscarinic receptor agonists)
- Migraines (serotonin receptor)

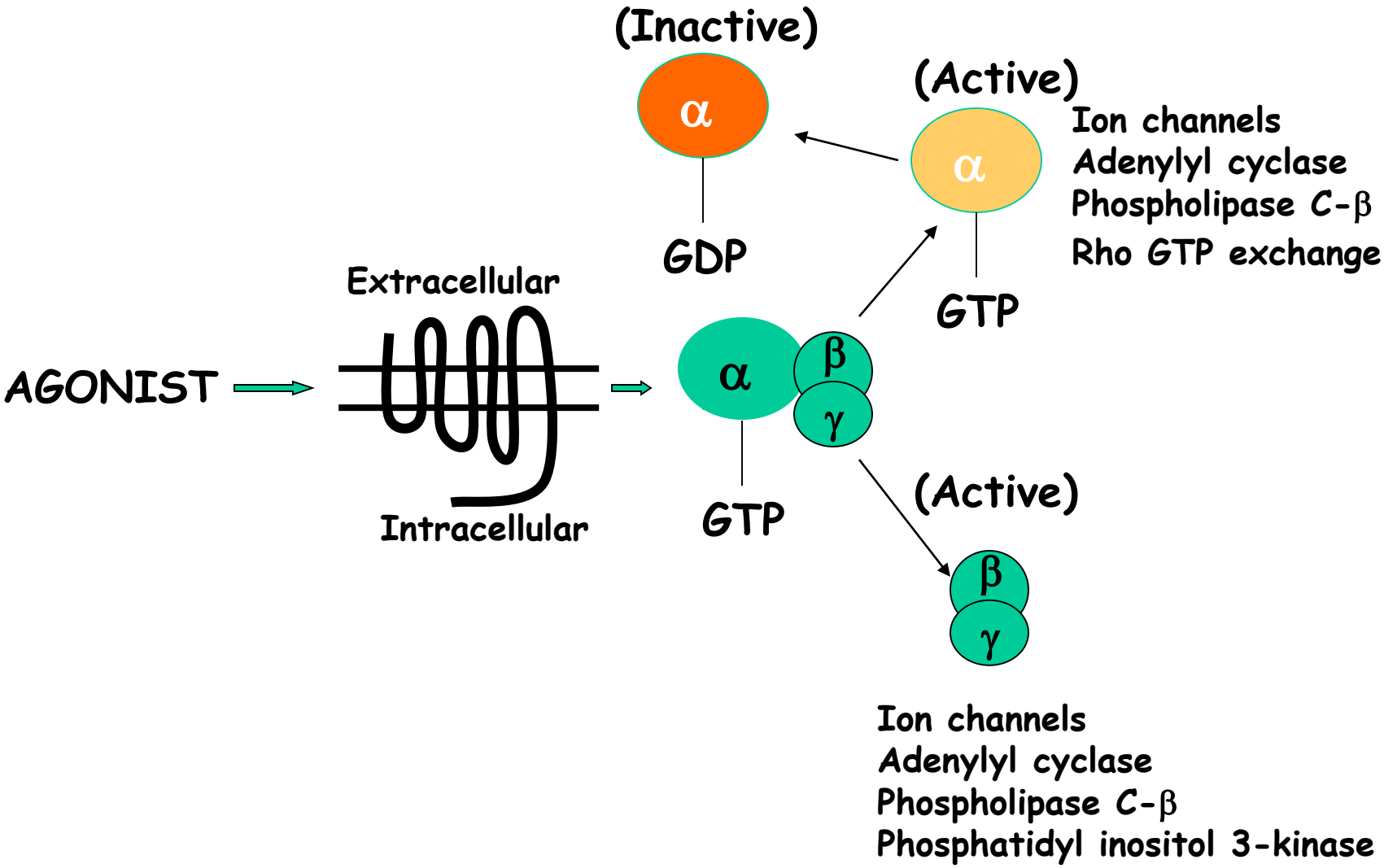
A. G-Protein Coupled Receptors (~1000 identified)

RECEPTOR CLASSIFICATION

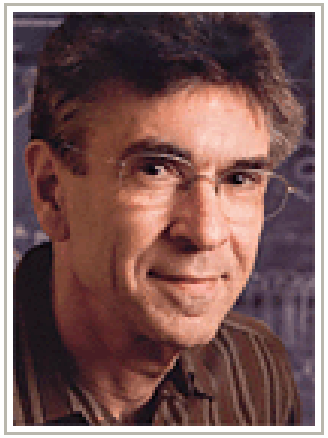


Molecular Cell Biology, 4th Ed., Chapter 20, Fig. 20-16

G Protein Activation and Functions

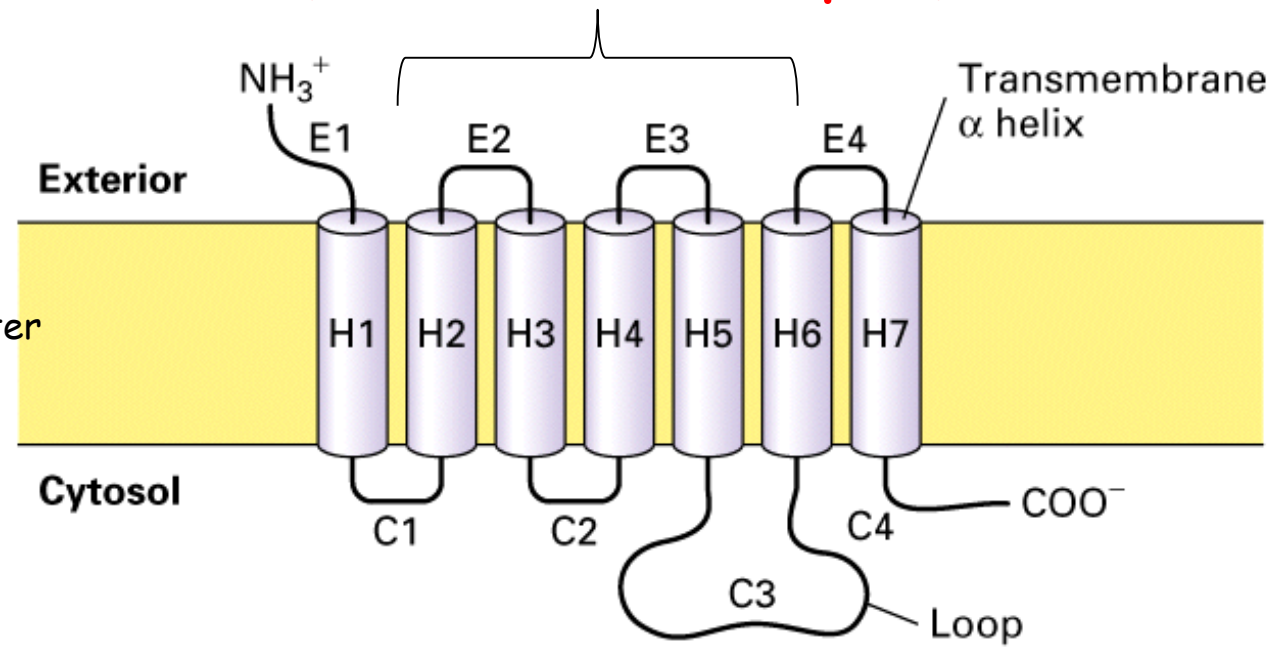


Model of a G-protein Coupled Receptor



Robert Lefkowitz, MD
Duke University Med Center
Nobel Prize 2012

(Ligand Binding)
(7-transmembrane spans)



Beta Adrenergic Receptor

(G protein interaction, Regulation)

Michio Ui

Pertussis toxin – ADP ribosylation of G_i

Cholera toxin – ADP ribosylation of G_s

Site of action of cholera toxin

Model of Heterotrimeric G Protein Activation

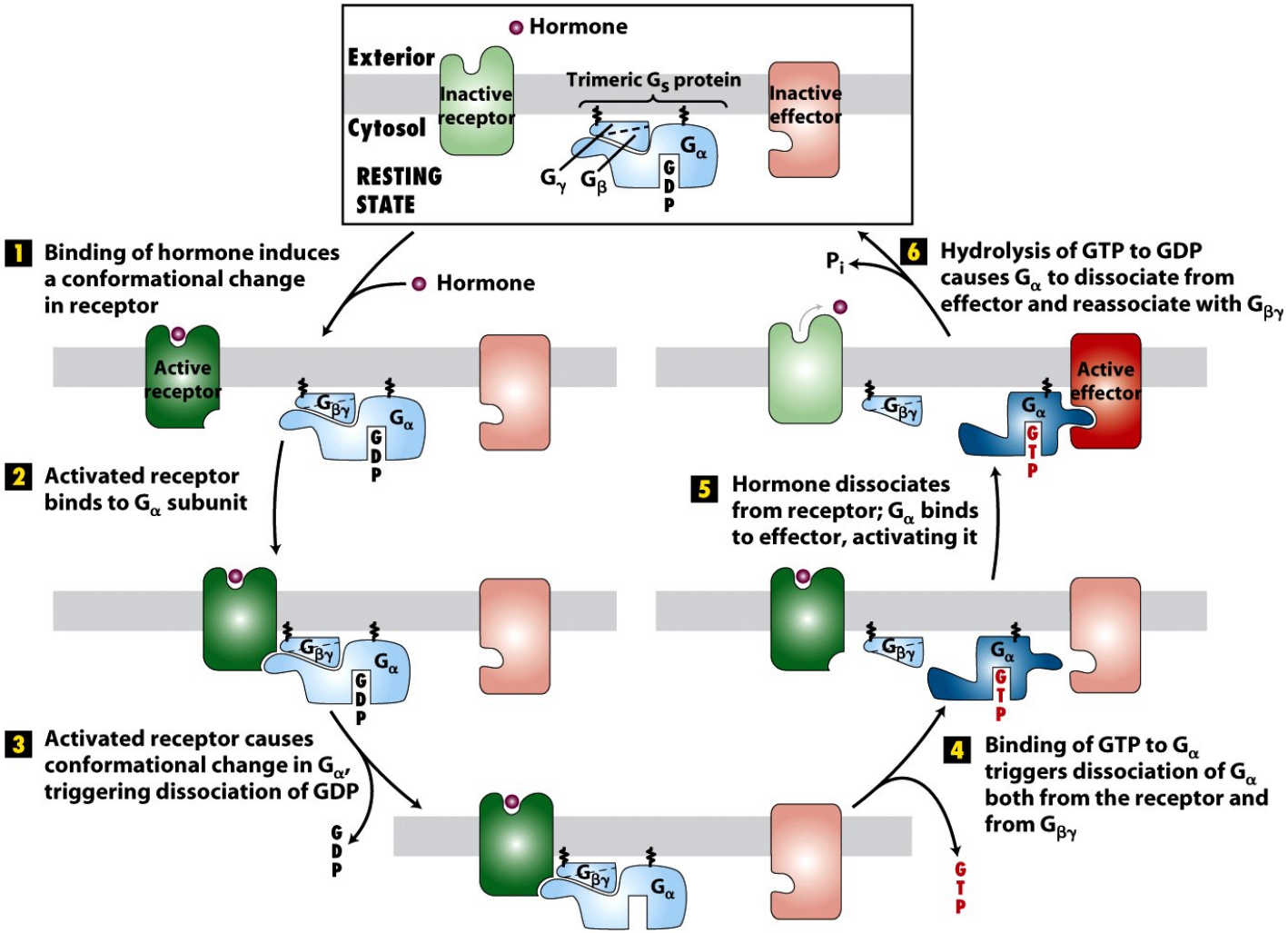


Figure 15-13
Molecular Cell Biology, Sixth Edition

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

*A given G_α subclass may be associated with more than one effector protein. To date, only one major $G_{\alpha s}$ has been identified, but multiple $G_{\alpha q}$ and $G_{\alpha i}$ 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

Coupling of G Protein to K+ Channel Activation

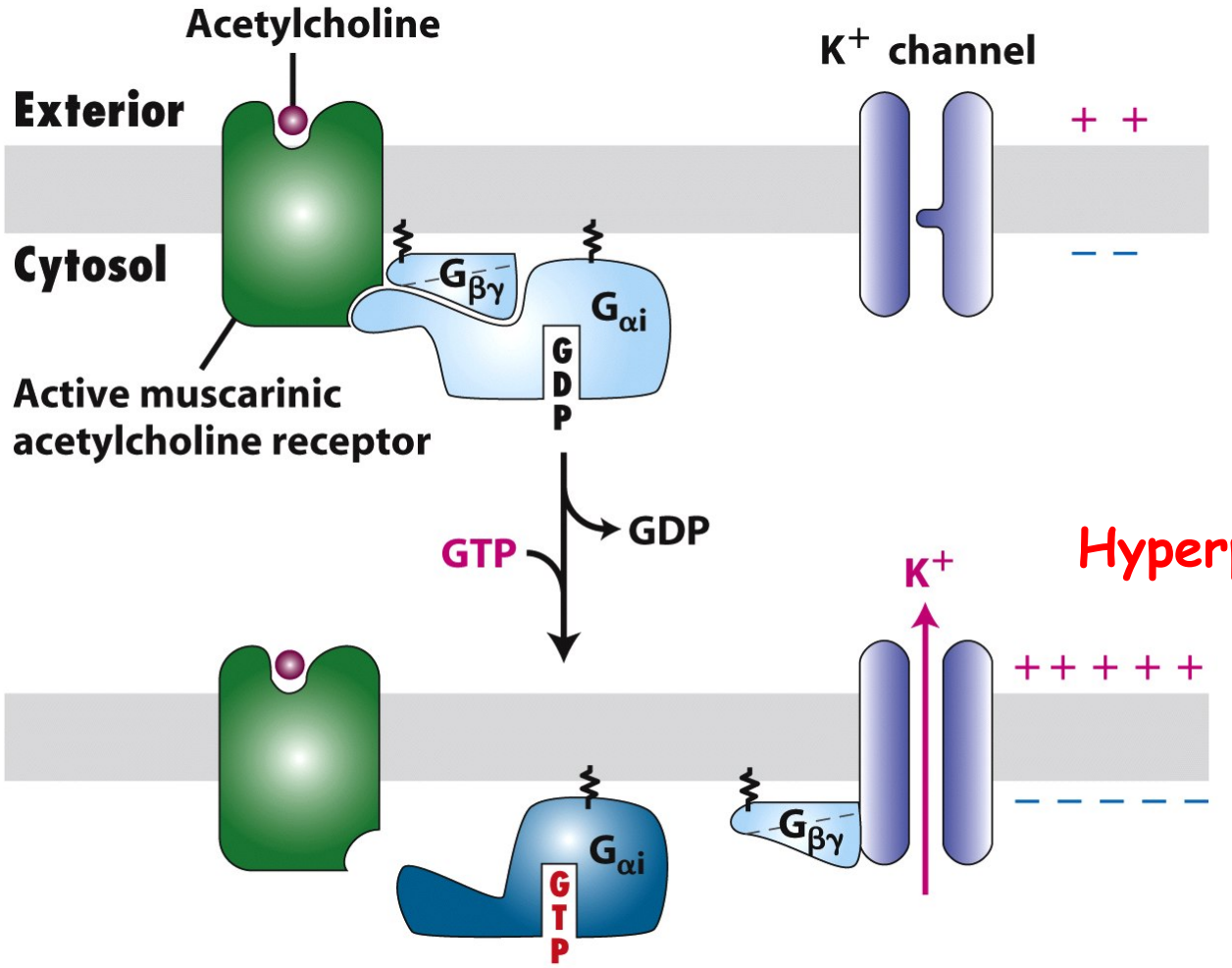


Figure 15-15
Molecular Cell Biology, Sixth Edition

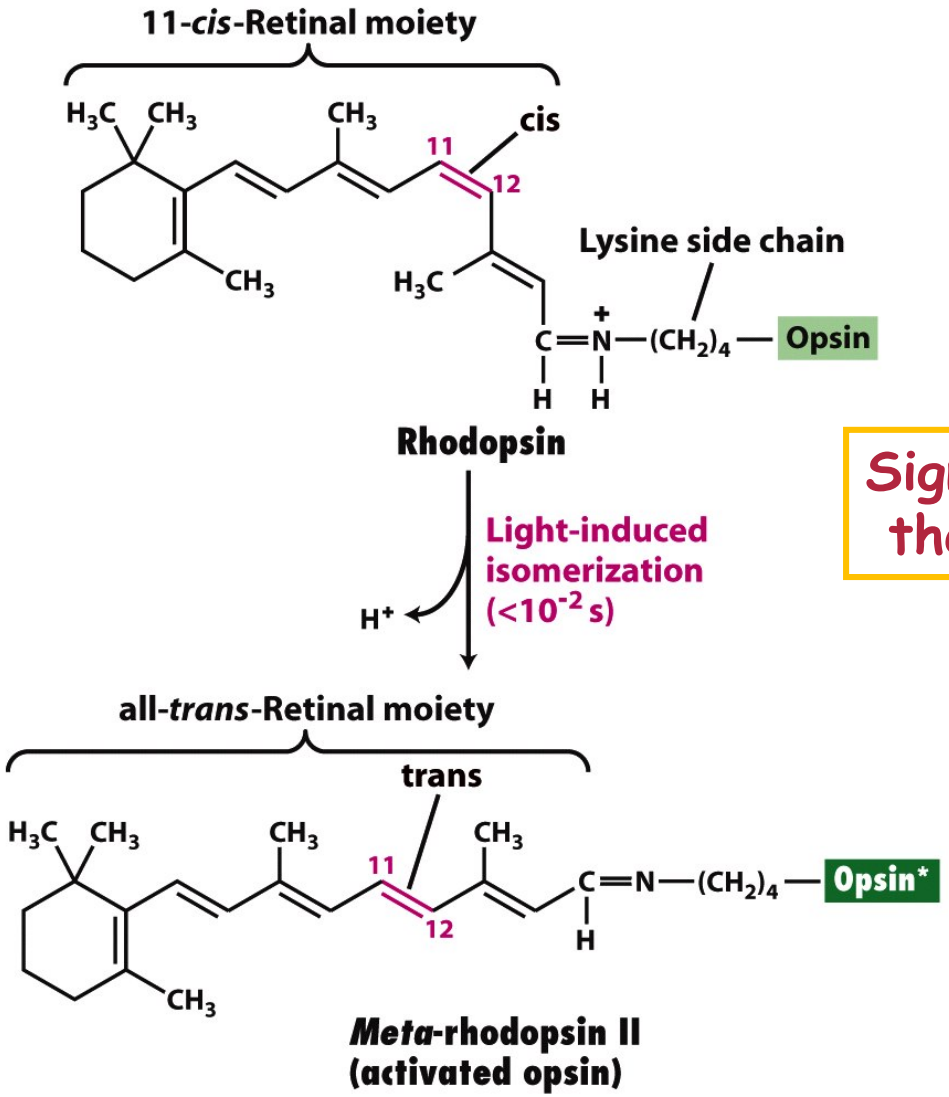
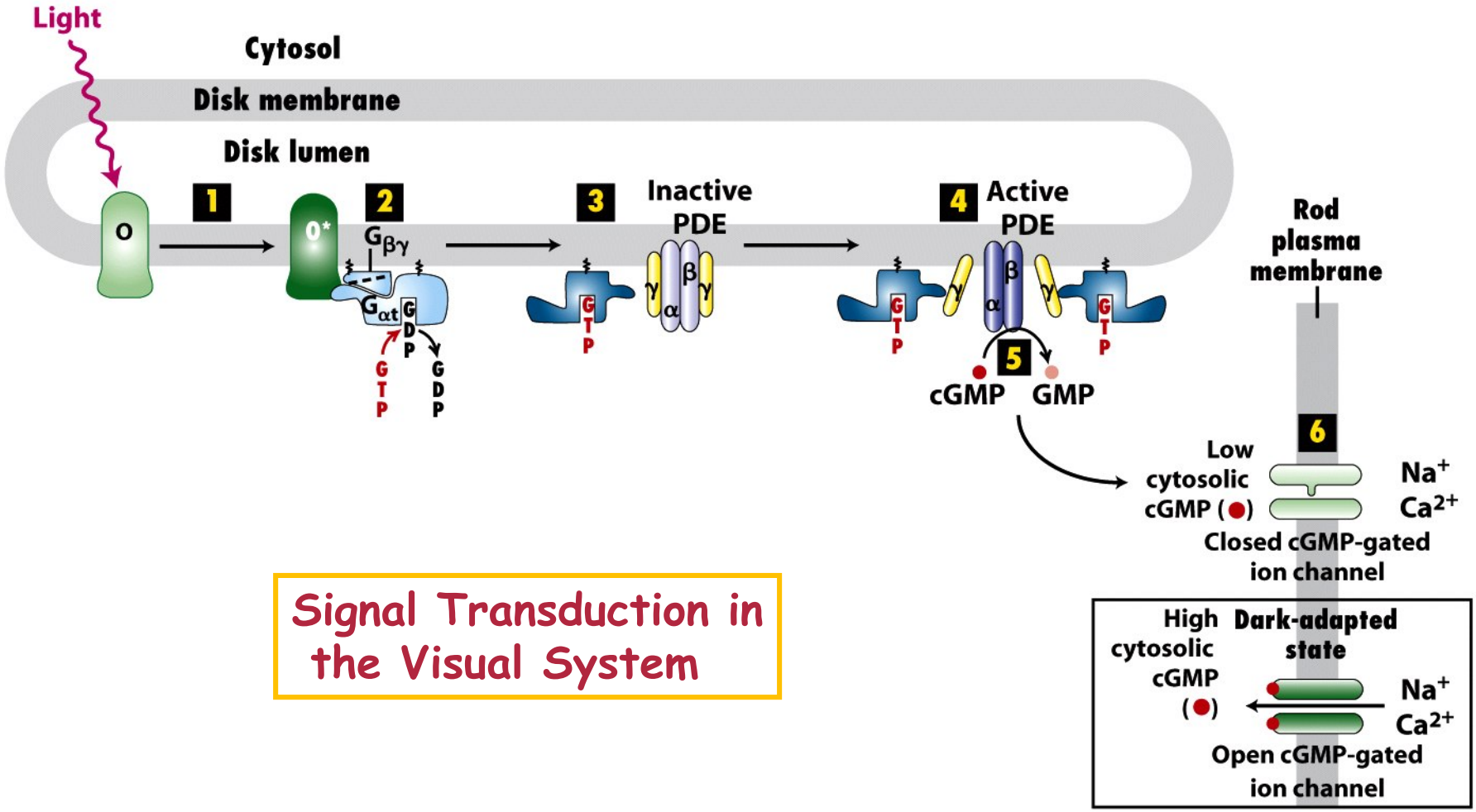


Figure 15-17
Molecular Cell Biology, Sixth Edition

Signal Transduction in the Visual System



Signal Transduction in the Visual System

Figure 15-18
Molecular Cell Biology, Sixth Edition

Signal Transduction in the Visual System

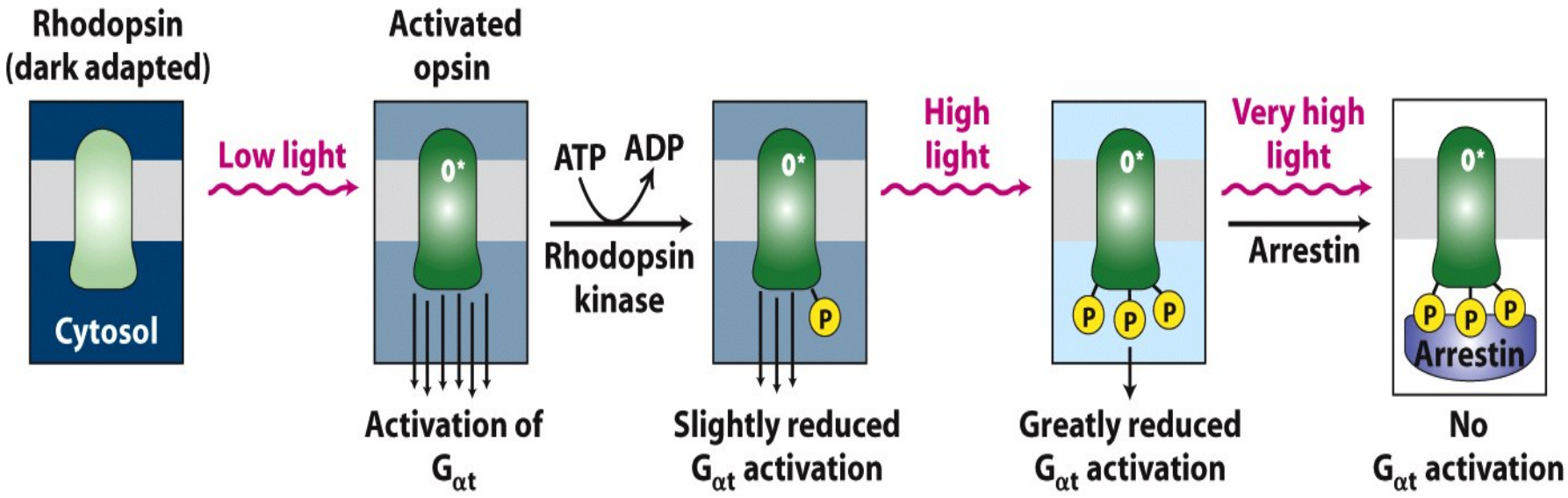
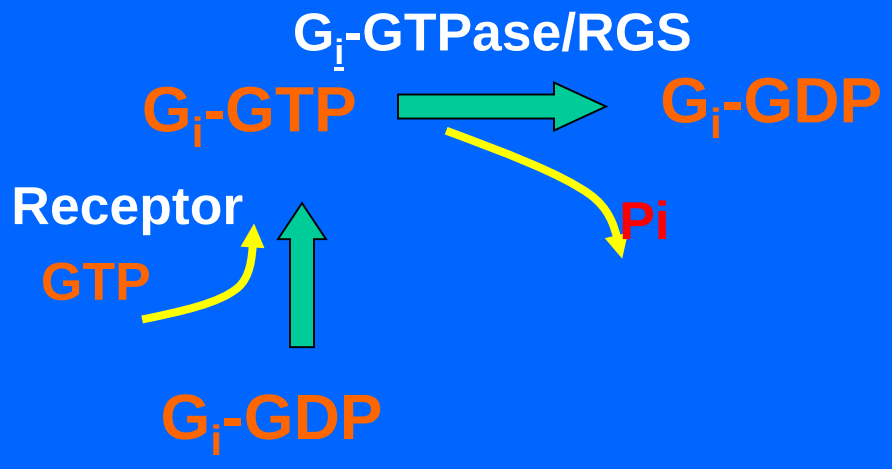


Figure 15-20
Molecular Cell Biology, Sixth Edition

Regulators of G Protein Signalling (RGS)

- Terminating the signal is essential to limit the response
- RGS are GTPase activating proteins (GAPs) for G_i and, Gq, G_o



- RGS inhibited signalling mediated through G_i, Gq and G_o

- RGS does not act as a guanine nucleotide dissociation inhibitor (GDI)or a guanine nucleotide dissociation stimulator (GDS) and does not act on G_s

Section 3: Relevant Concepts

Cyclic AMP

Cyclic AMP dependent protein kinase

Phosphoprotein phosphatase

Amplification

PI hydrolysis

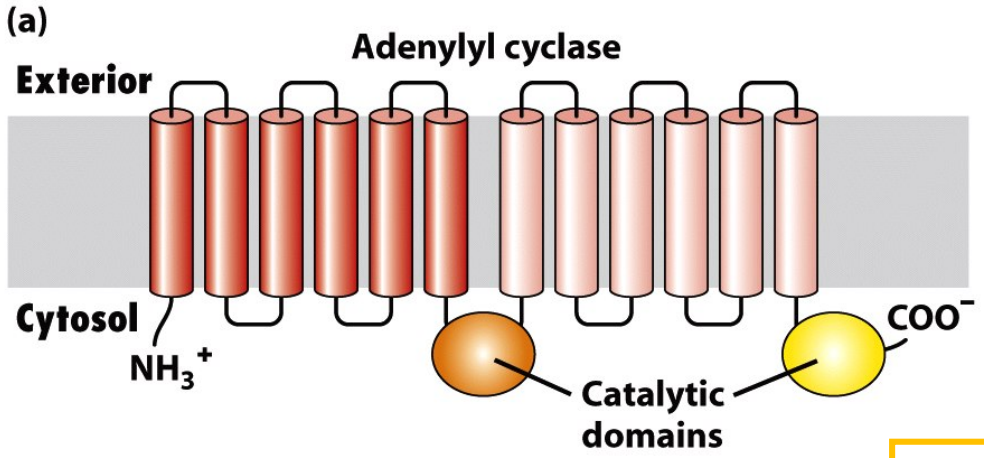
Calmodulin

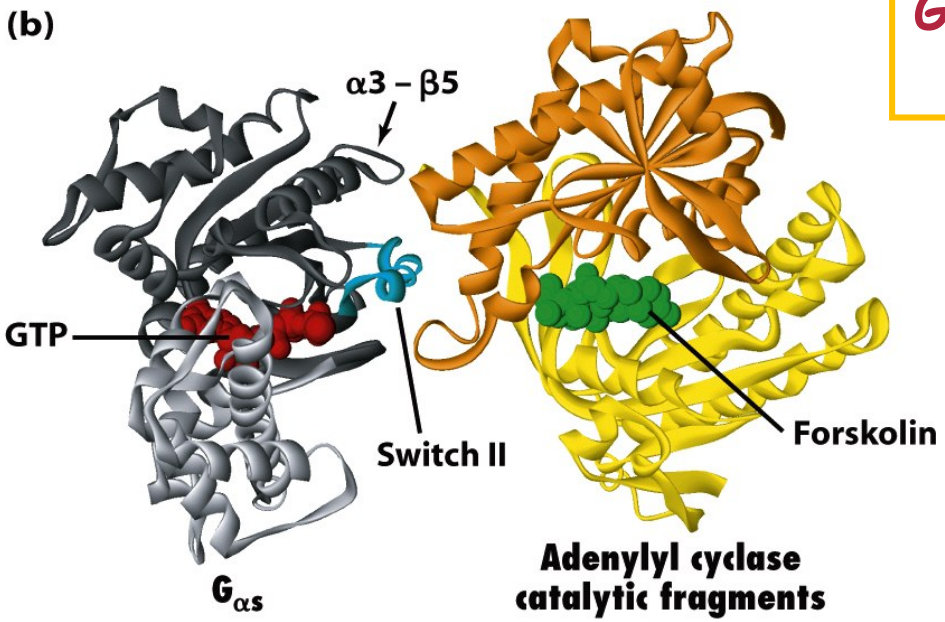
Protein kinase C

Store-operated Ca^{2+} channel

PI-3 kinase

Phospholipase C

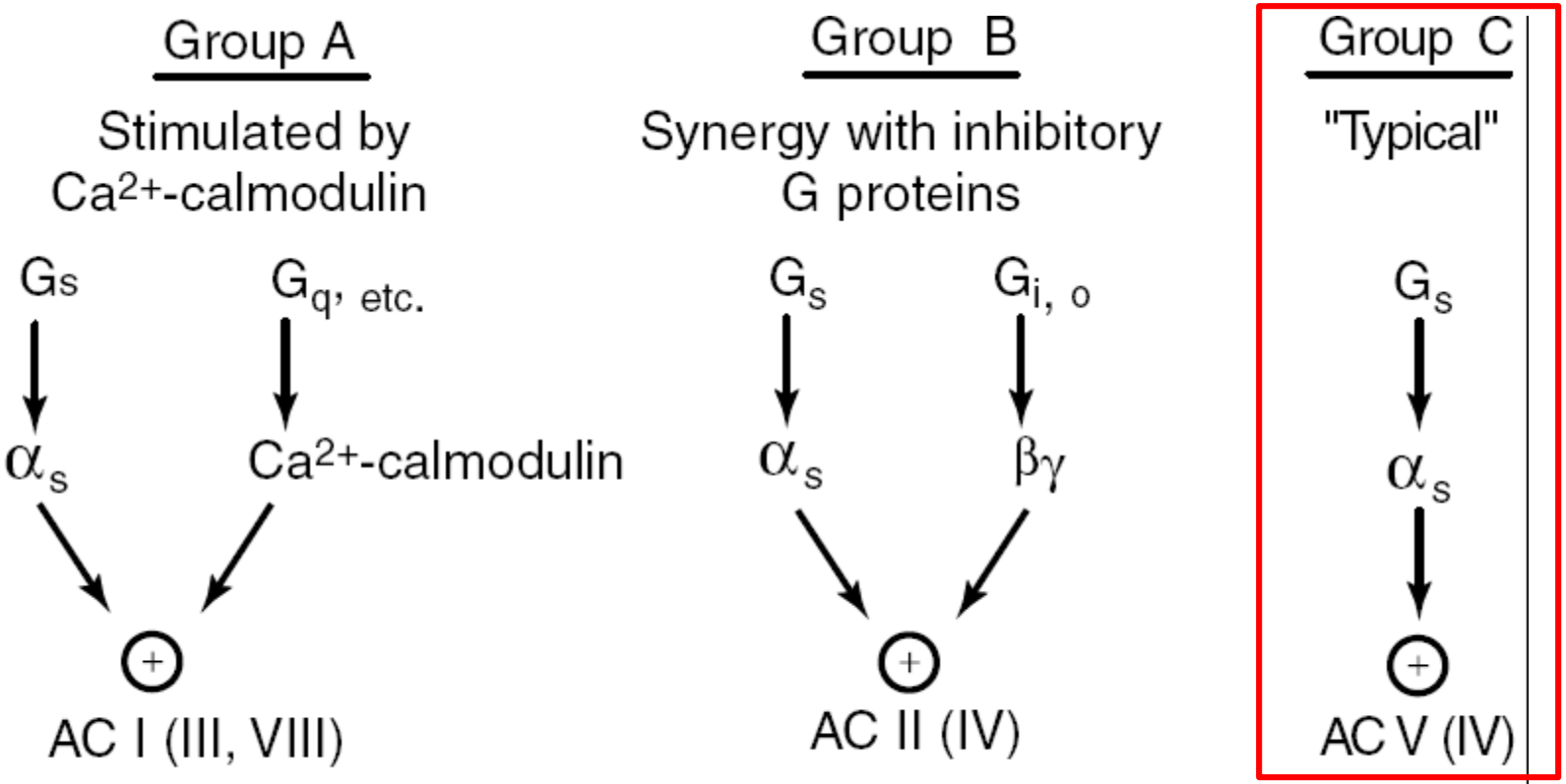




G_s – Adenylyl Cyclase Interaction

Figure 15-22
Molecular Cell Biology, Sixth Edition

Adenylyl Cyclase Isoforms



Activation of Protein Kinase A

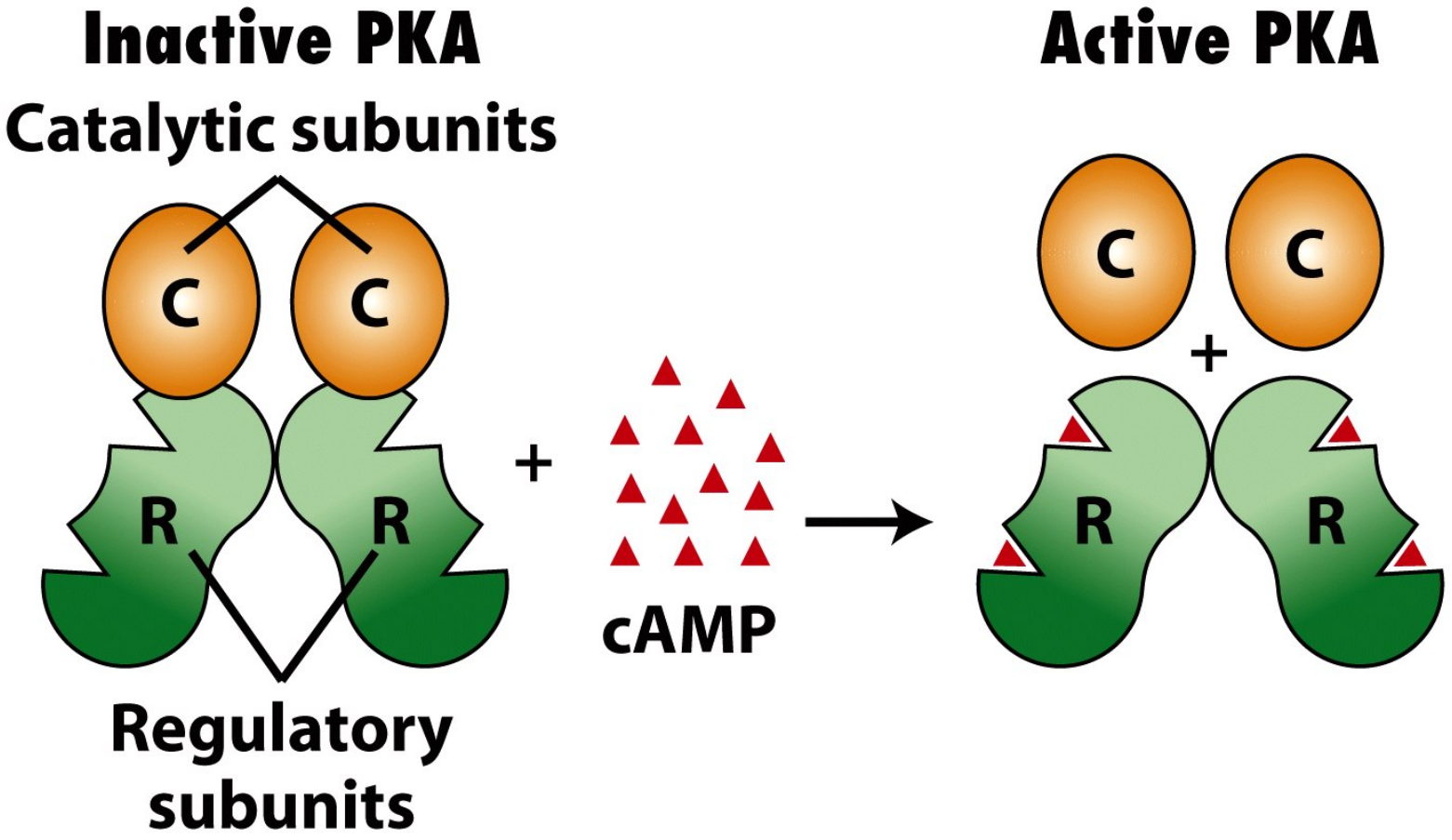


Figure 15-23a
Molecular Cell Biology, Sixth Edition

Activation of Protein Kinase A by Cyclic AMP

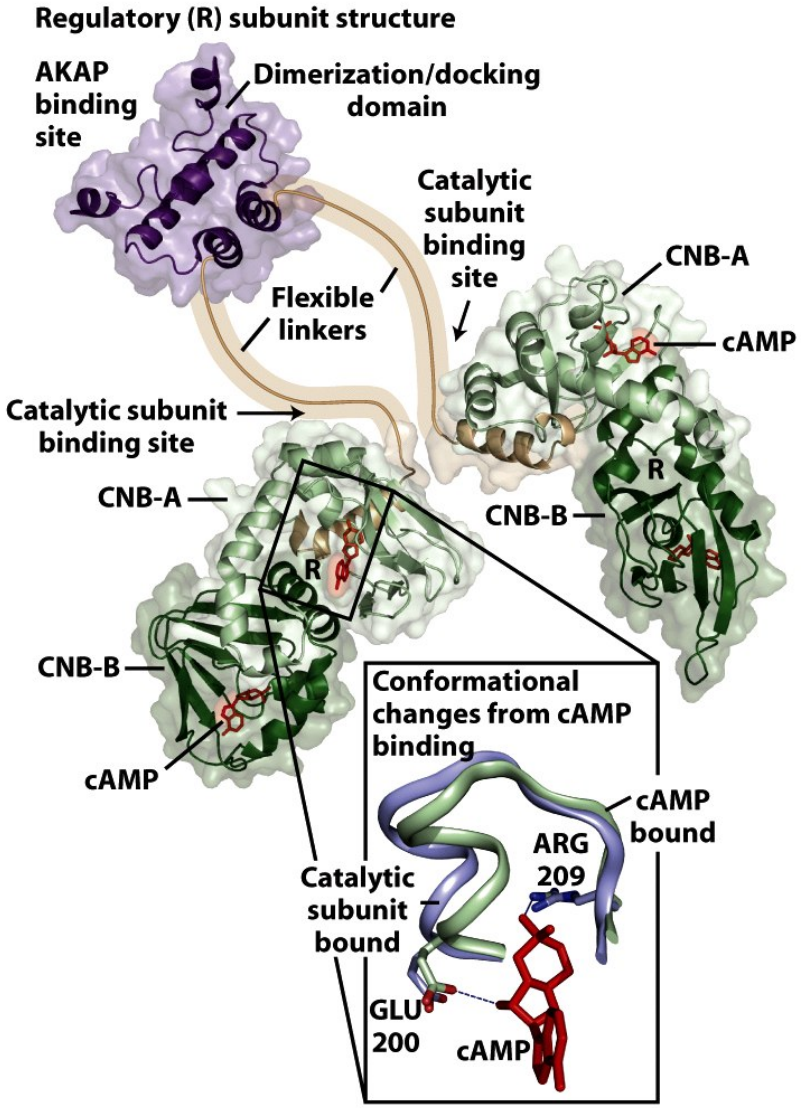


Figure 15-23b
Molecular Cell Biology, Sixth Edition

- AKAP = A kinase associated proteins –localizes PKA to specific regions of the cell and target these sites for cyclic AMP responses
- CNB-A/CNB-B are two cyclic AMP binding sites on the regulatory subunit of PKA
- Cyclic AMP binding changes the conformation of the regylatory subunits allowing dissociation of the catalytic subunits
- Cyclic AMP interacts with Glu200 and Arg209 of the regulatory subunit

Regulation of Glycogen Synthesis and Metabolism

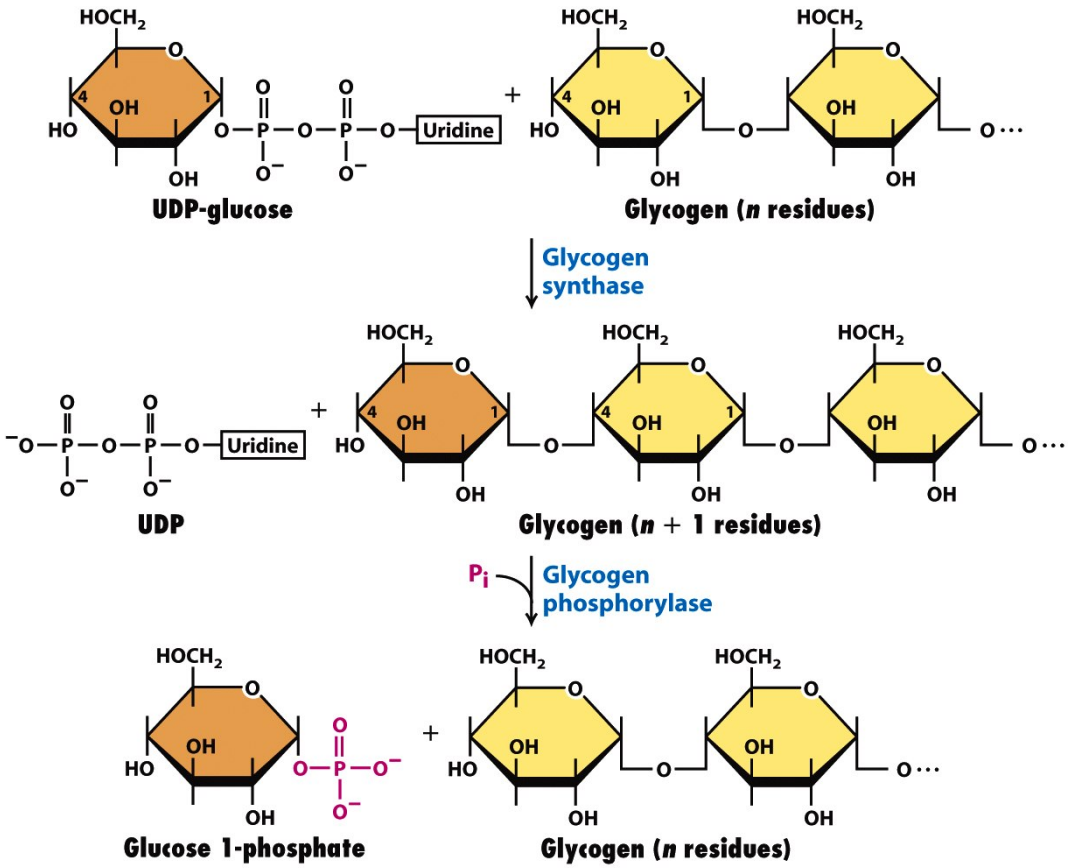
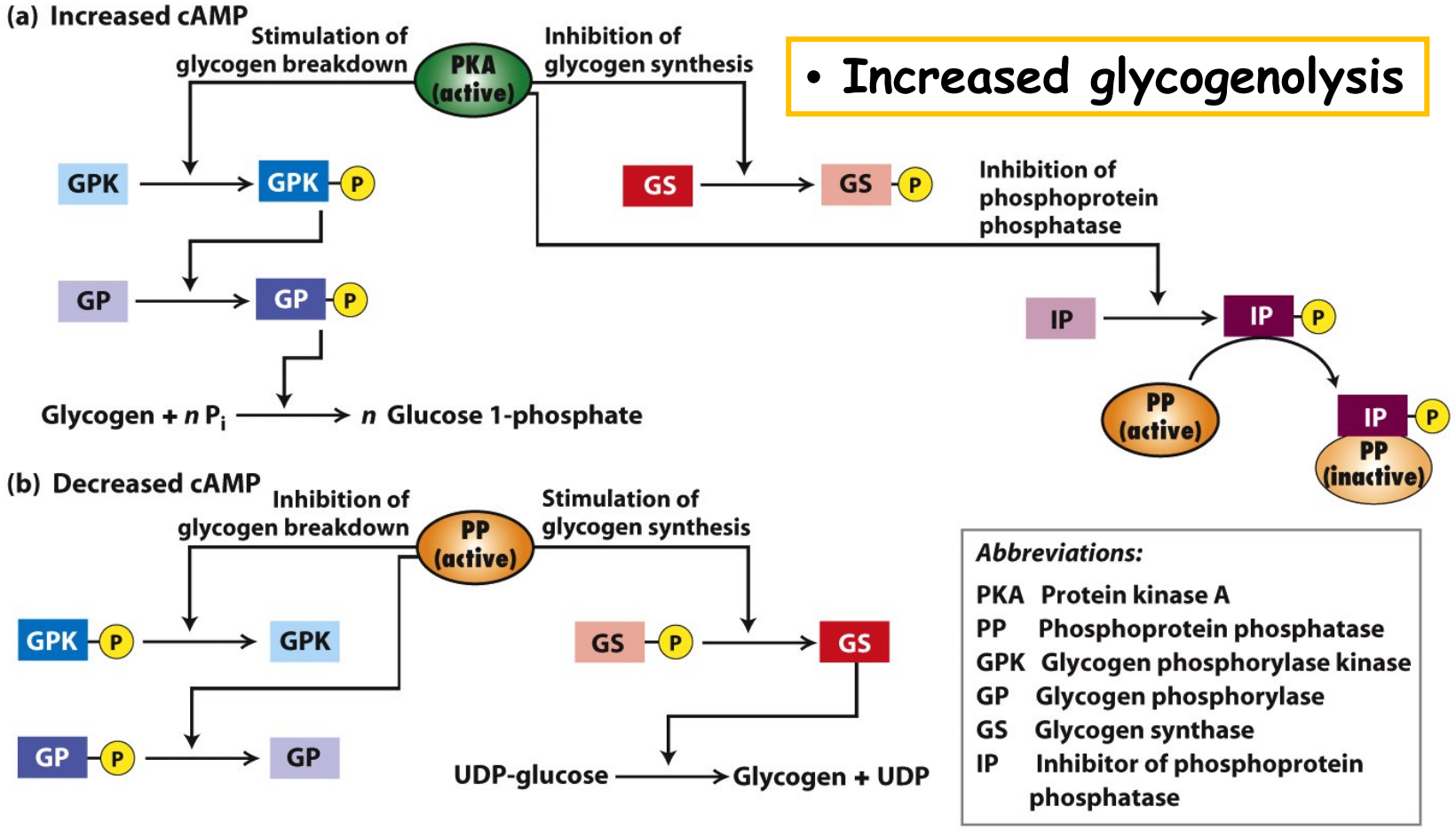


Figure 15-24
Molecular Cell Biology, Sixth Edition

Gerty & Carl Cori
Washington Univ. St. Louis, MO (Nobel Prize)

Protein Kinase A Regulates Glycogen Synthesis and Metabolism in the Liver and Skeletal Muscle



- Increased glycogenolysis

- Increased glycogen synthesis

Figure 15-25
Molecular Cell Biology, Sixth Edition

Section 4: Receptor Regulation

Relevant Concepts

Endocytosis

Beta adrenergic receptor kinase

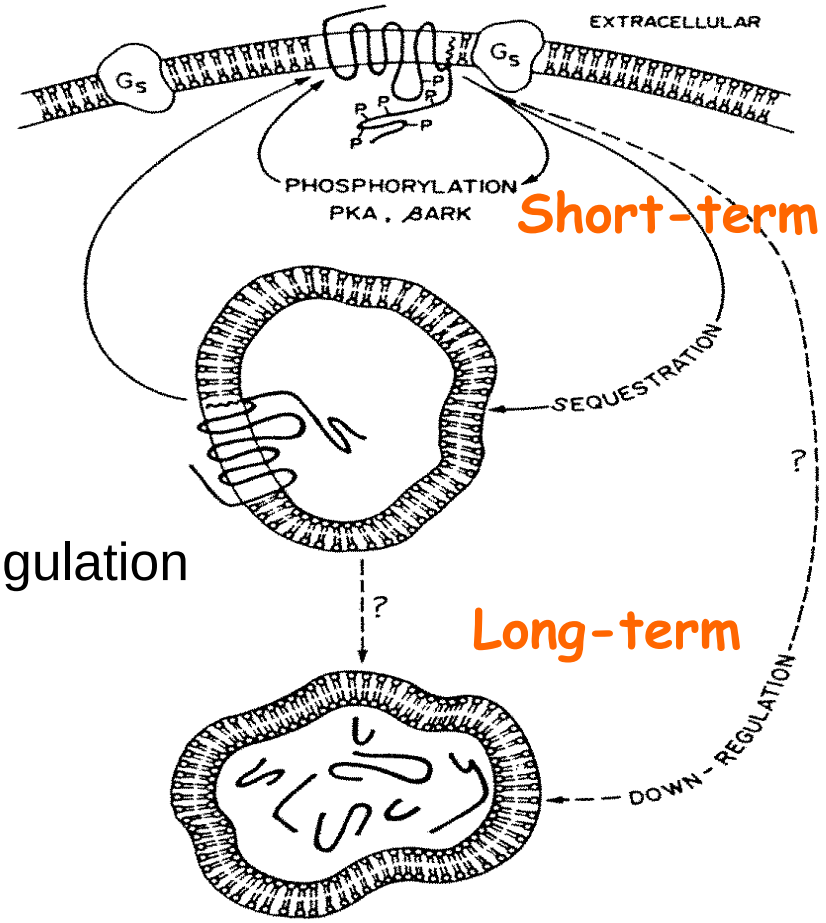
Arrestin

Homologous and heterologous desensitization

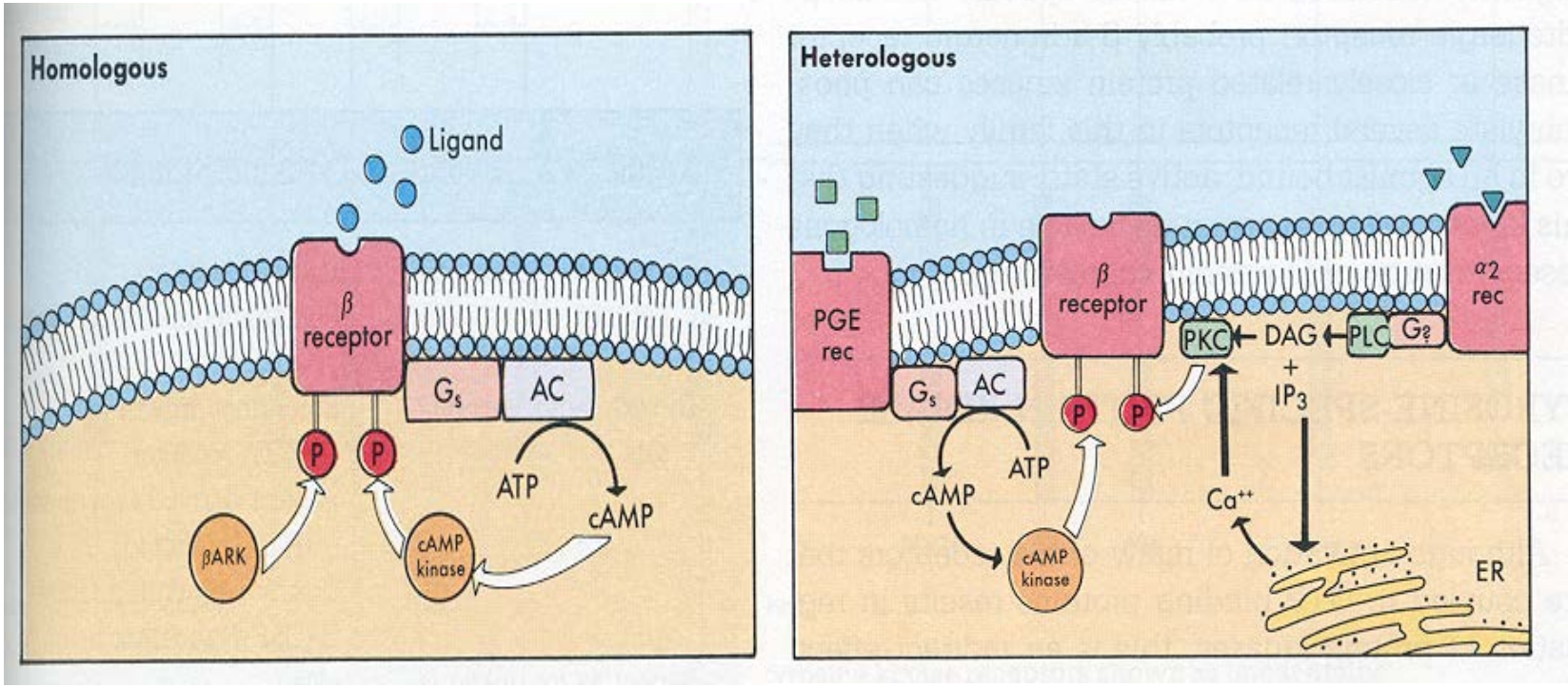
B-arrestin

TERMINATING THE SIGNAL: Time-dependent transit of β-receptor upon agonist exposure

- PKA dependent phosphorylation of the β-adrenergic receptor
- β-adrenergic receptor kinase mediated phosphorylation of the β-adrenergic receptor
- Sequestration
- Receptor degradation and down-regulation



Agonist-induced desensitization of the β_2-adrenergic receptor



- Homologous desensitization - requires activation by agonist for that receptor (PKA, β-ARK)
- Heterologous desensitization – involves activation of a different receptor (PKA)

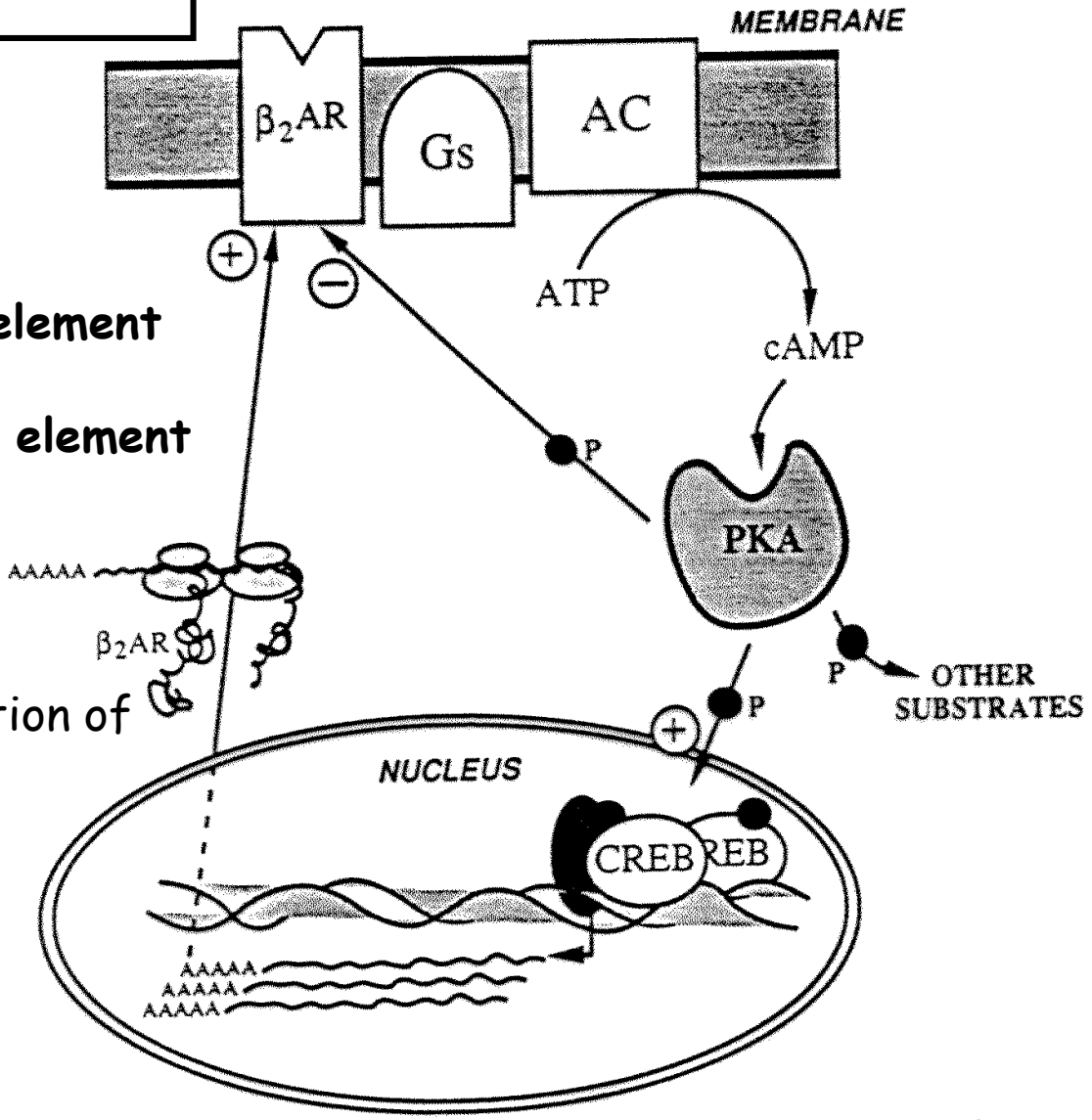
β-adrenergic receptor kinase (βARK)

RECOVERY: β_2AR-stimulated gene transcription

CRE = cyclic AMP response element

CREB = cyclic AMP response element binding protein

- PKA-dependent phosphorylation of CREB promotes DNA binding activity and transcription



Ca^{2+} Mobilizing Receptors

Receptor ⟶ Rise in Intracellular Ca^{2+}

<u>Relevant Receptors (G_q-coupled)</u>

- $Alpha_1$ adrenergic receptor (vasoconstriction)
- Angiotensin 1
- Bradykinin – (B_1 and B_2 receptors) - pain/inflammation
- Muscarinic acetylcholine (M_1) – cerebral cortex (memory)
- Serotonin receptor ($5HT_{2A}$) – migraine, depression
- Histamine (H_1) receptor (bronchoconstriction, vasodilation)

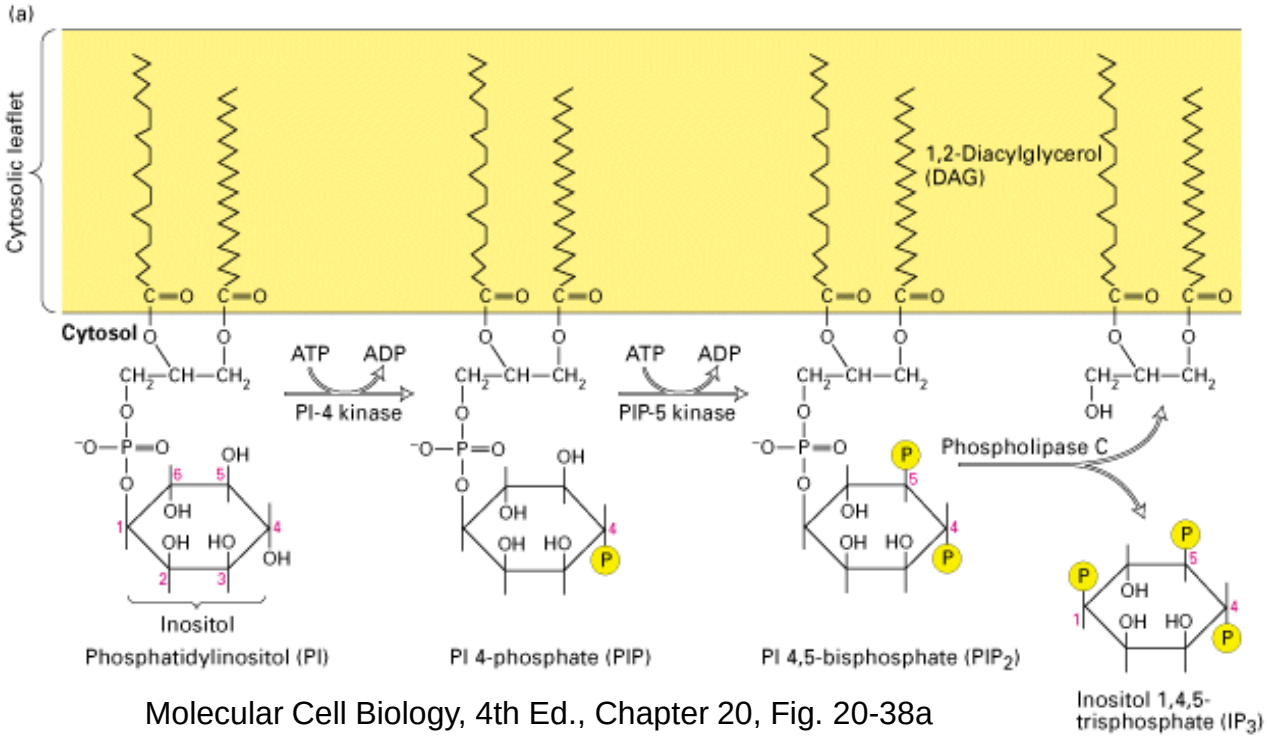
➢ Receptors coupled to the Gq protein

Phospholipase C Generates IP_3 and Diacylglycerol

PI ⇨ PIP ⇨ PIP_2 ⇨ IP_3 + DAG ⇨ Response

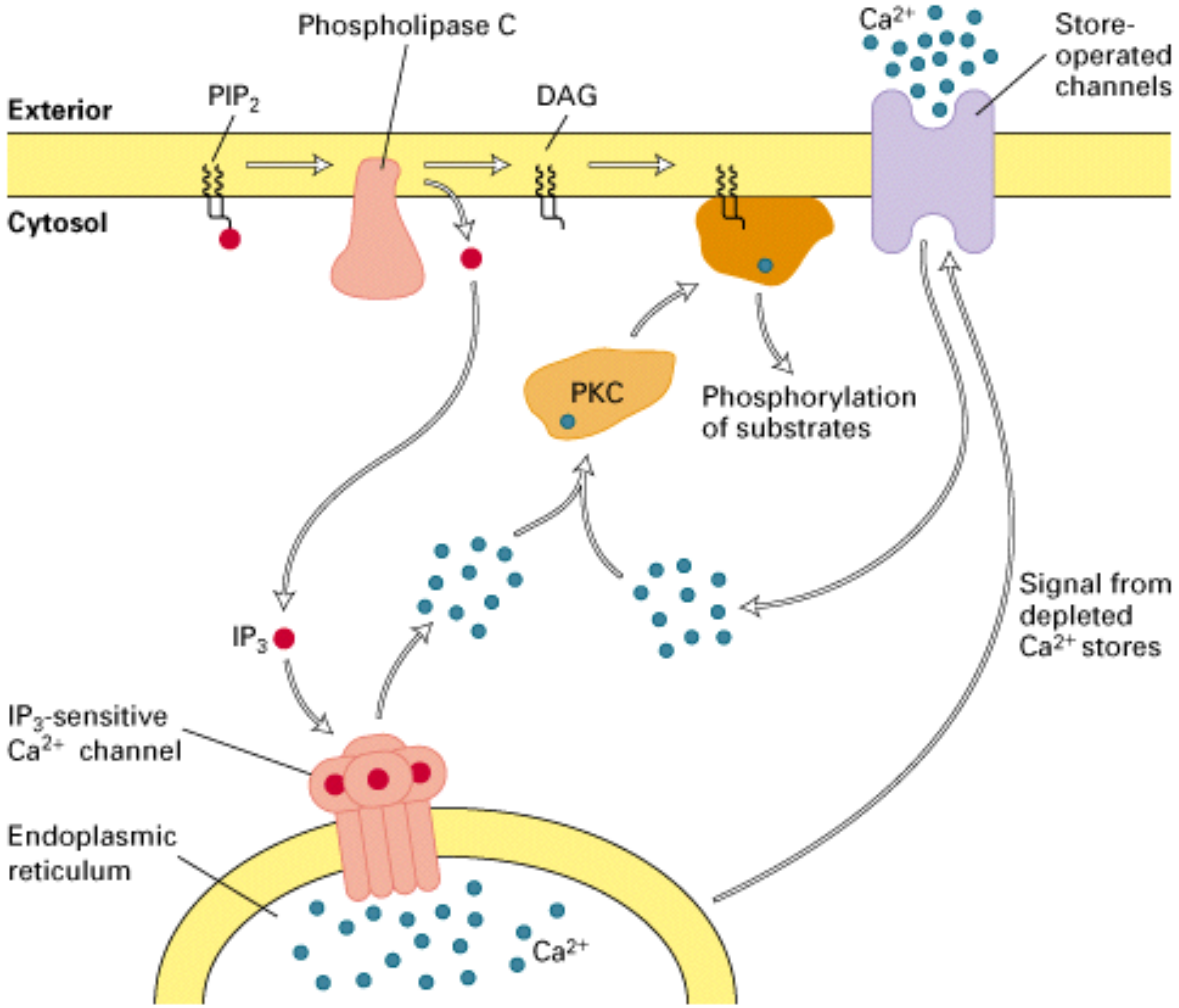
(Michael Berridge, 1983)

Michel Berridge



Molecular Cell Biology, 4th Ed., Chapter 20, Fig. 20-38a

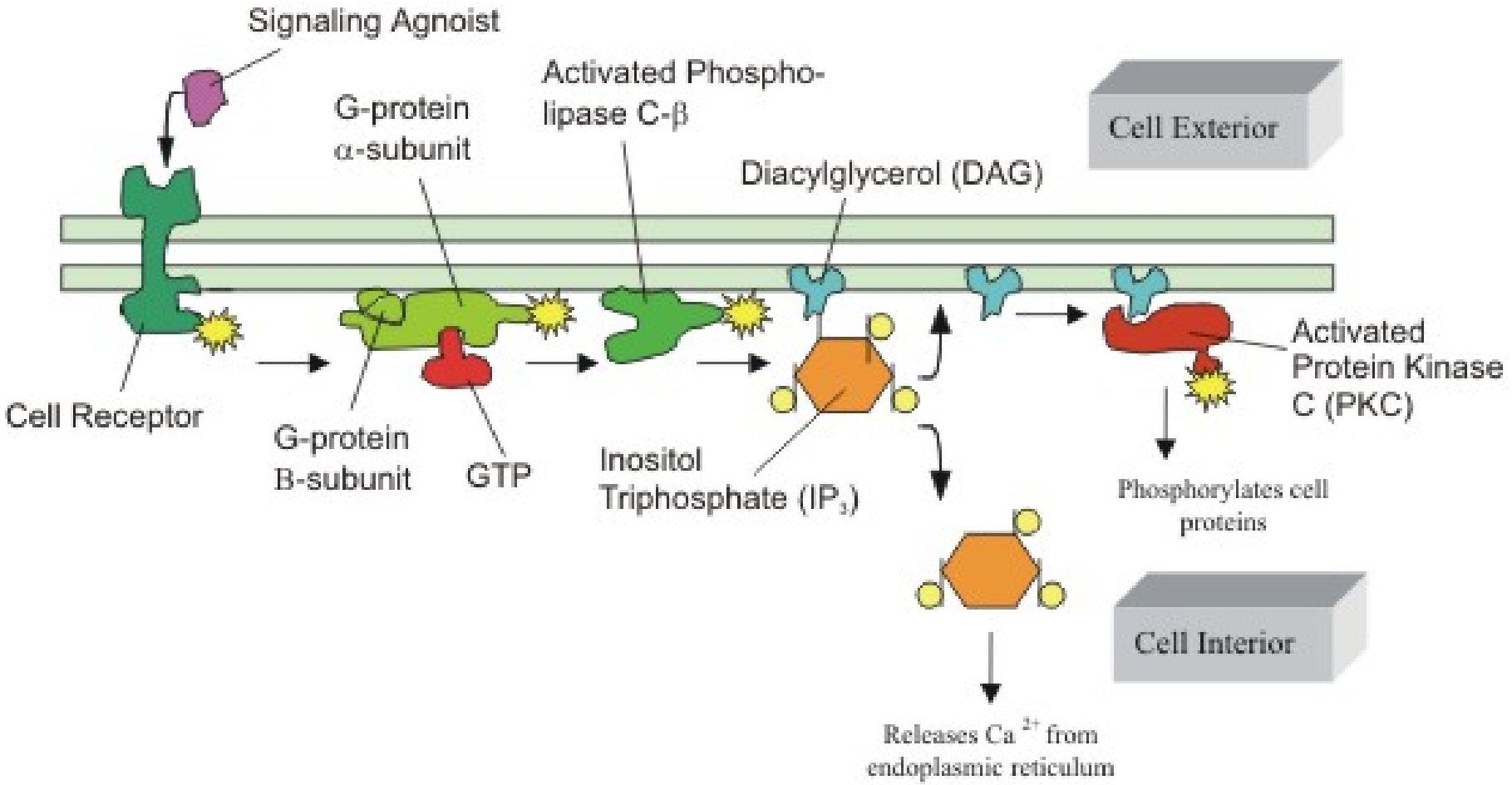
- Phospholipase C cleaves PIP-2 to generate DAG and IP3



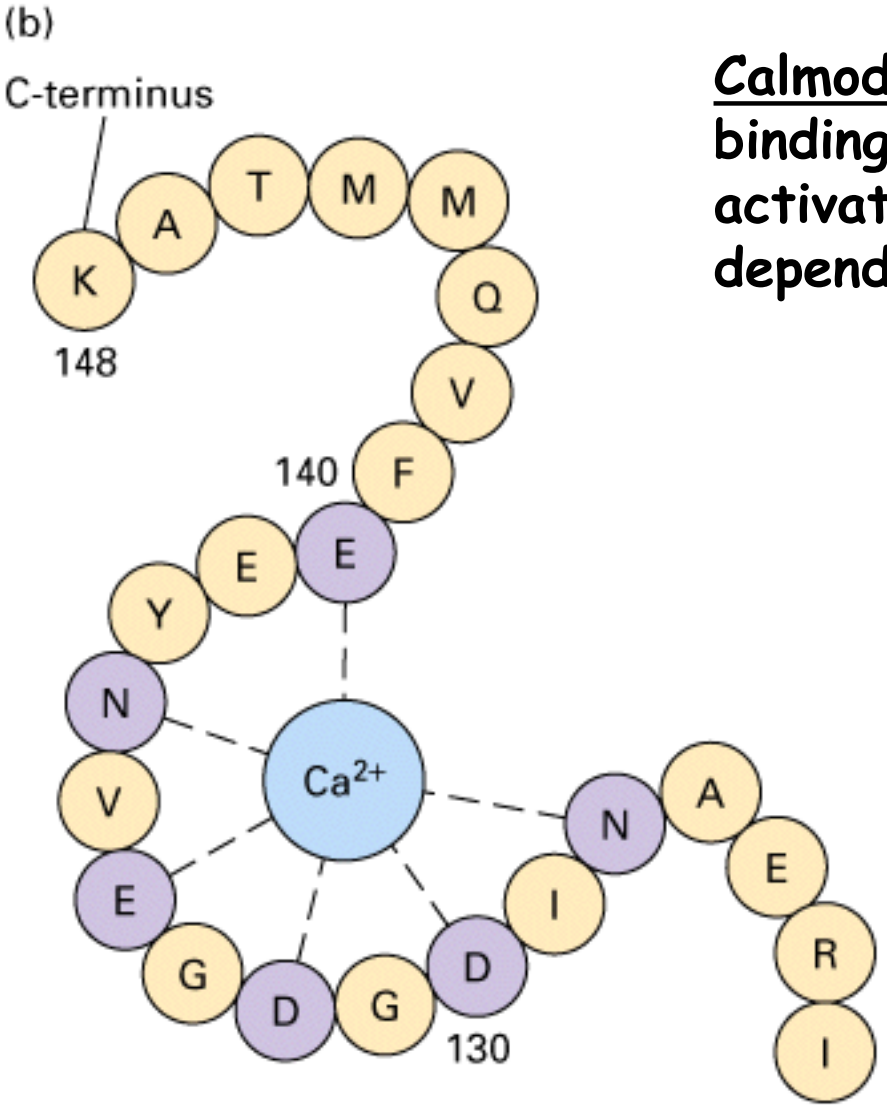
- IP3 increases cytosolic Ca^{2+} release from the endoplasmic reticulum
- DAG and Ca^{2+} activates protein kinase C
- Store operated channels replenishes intracellular Ca^{2+}

Molecular Cell Biology, 4th Ed., Chapter 20, Fig. 20-39

Protein Kinase C Activation



Additional role of Ca^{2+}:Binding to Calmodulin

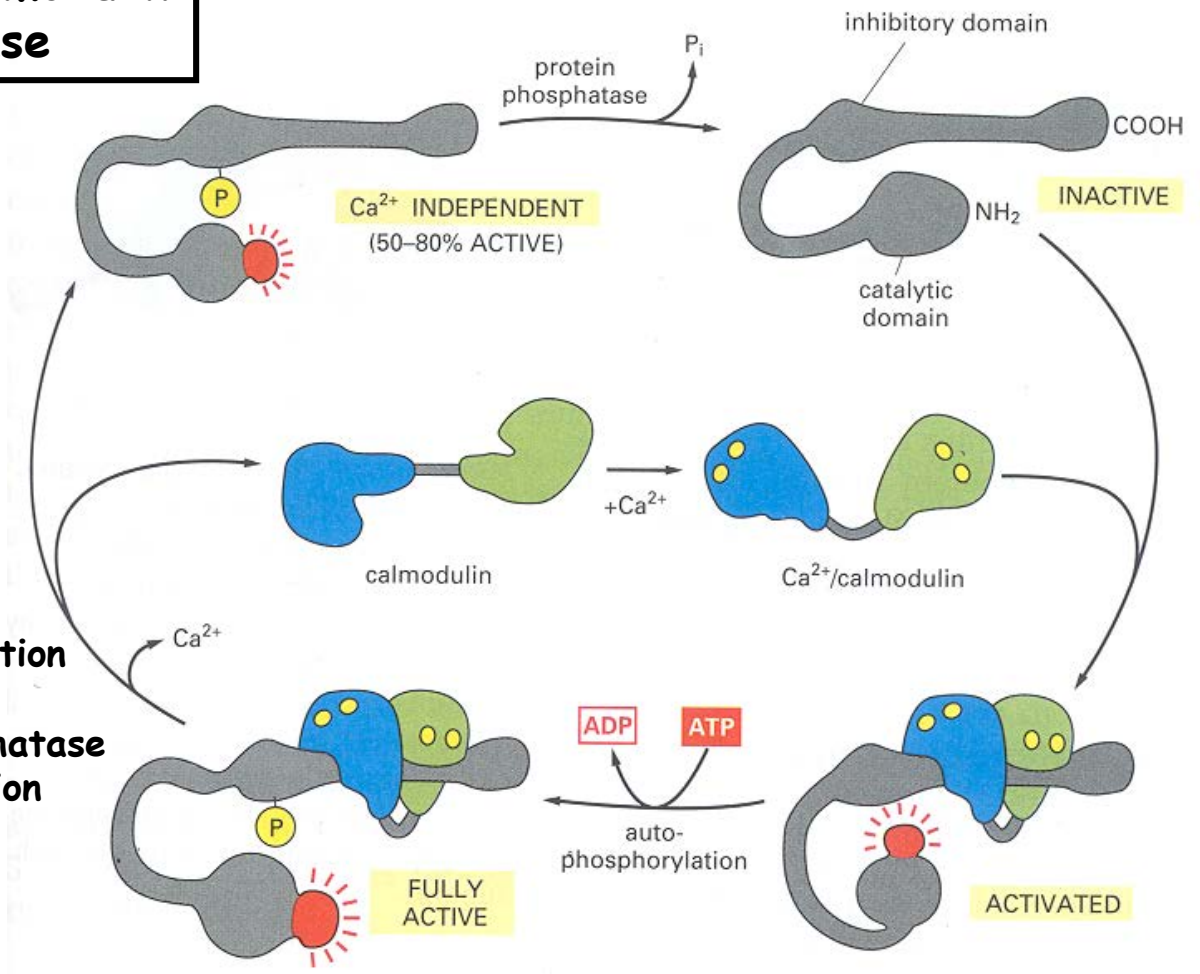


Calmodulin – a major Ca^{2+} binding protein in the cell. It activates a Ca^{2+} -calmodulin dependent protein kinase.

Molecular Cell Biology, 4th Ed., Chapter 20, Fig. 20-41b

Activation of Ca^{2+} -Calmodulin Dependent Protein kinase

- Activated by Ca2+/calmodulin
- Activated by autophosphorylation
- Ca2+ independent activation
- Inactivated by protein phosphatase
- Implicated in memory formation



Alberts et al., Eds., Molecular Biology of THE CELL, 3rd Ed., 1994, Fig. 15-35

Ca^{2+} Calmodulin-Dependent Activation of Endothelial Nitric Oxide Synthase

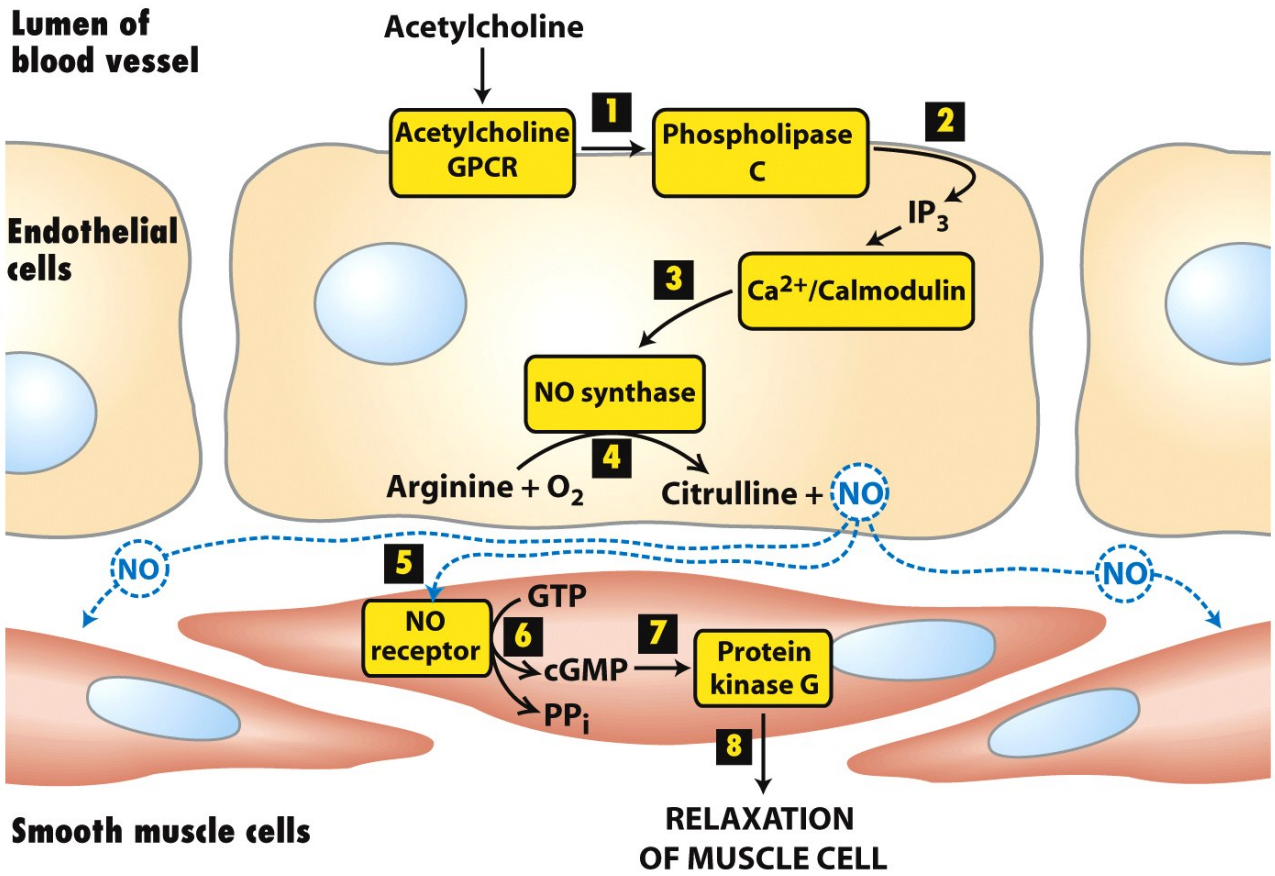


Figure 15-31
Molecular Cell Biology, Sixth Edition

- Acetylcholine stimulates intracellular Ca^{2+} release via muscarinic receptors
- Ca^{2+}/calmodulin couples GPCR to activation of nitric oxide synthase
- Nitric oxide synthase converts arginine to citrulline and NO
- NO mediates relaxation of vascular smooth muscle via increasing cyclic GMP and PKG

RELEVANT CONCEPTS

Receptor dimerization

Autophosphorylation

Guanine nucleotide exchange factor (GEF)

GTPase activating protein (GAP)

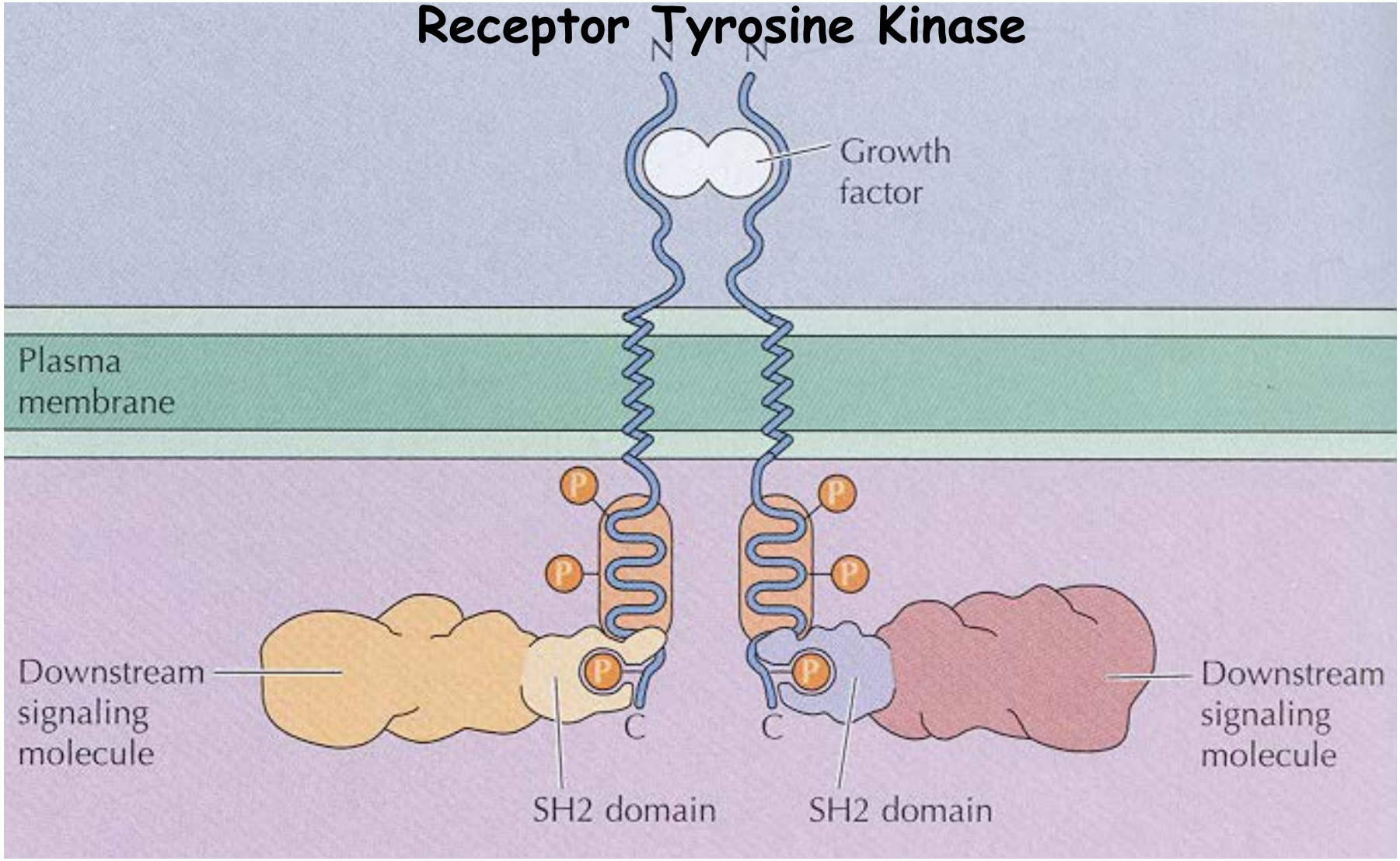
Adaptor proteins (GRB2, Sos)

Growth factors stimulate Ras activation

Monomeric G proteins

- Ras proteins
- Rac, Rho, cdc42
- Signaling of growth factor receptors
- SH2 domains

Receptor Tyrosine Kinase



SH2 = Src homology domain 2

Cooper, G.M., The Cell: A Molecular Approach, 1997, Chap. 13, Fig. 13.15

RAS Protein Activation

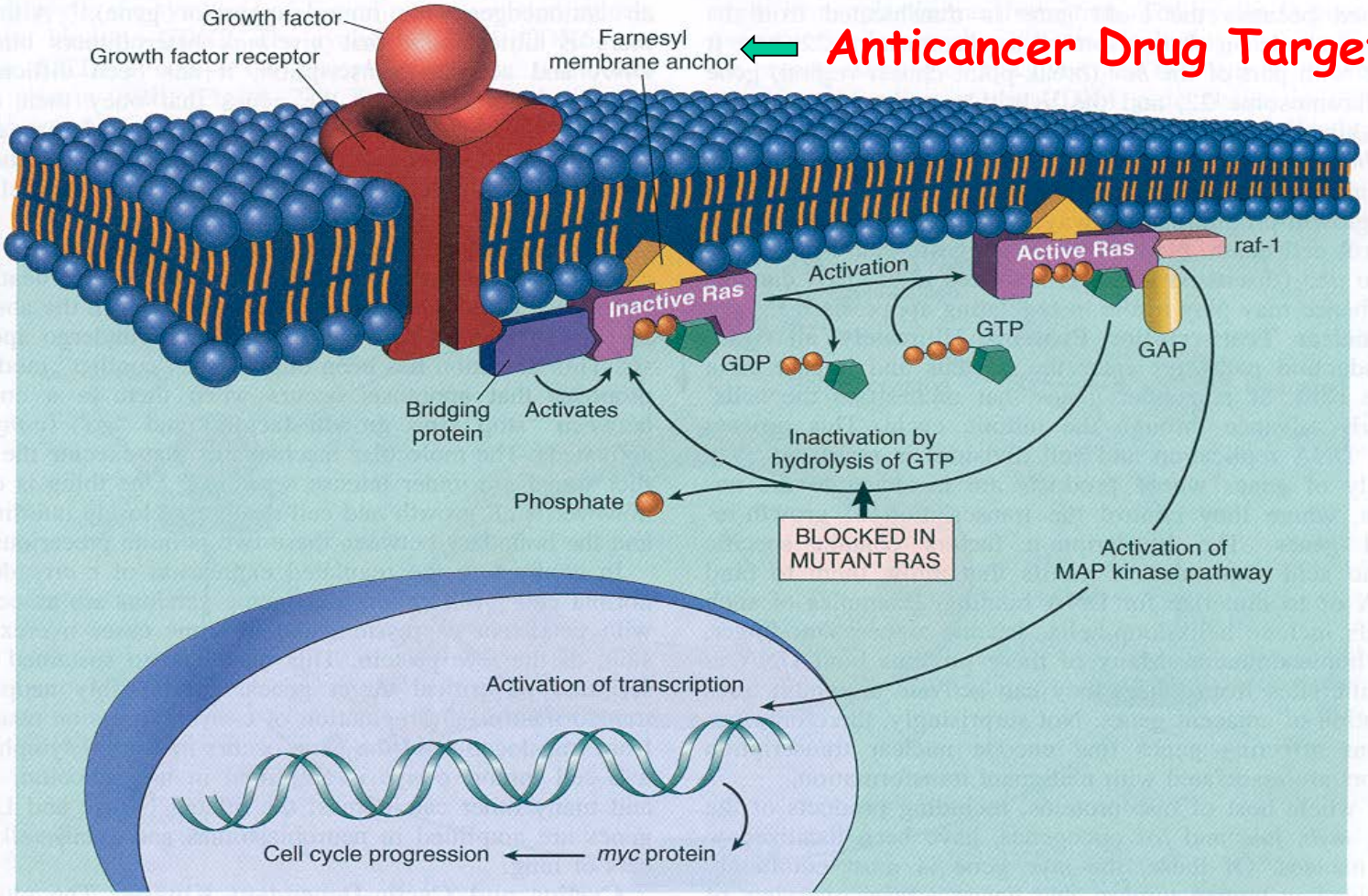
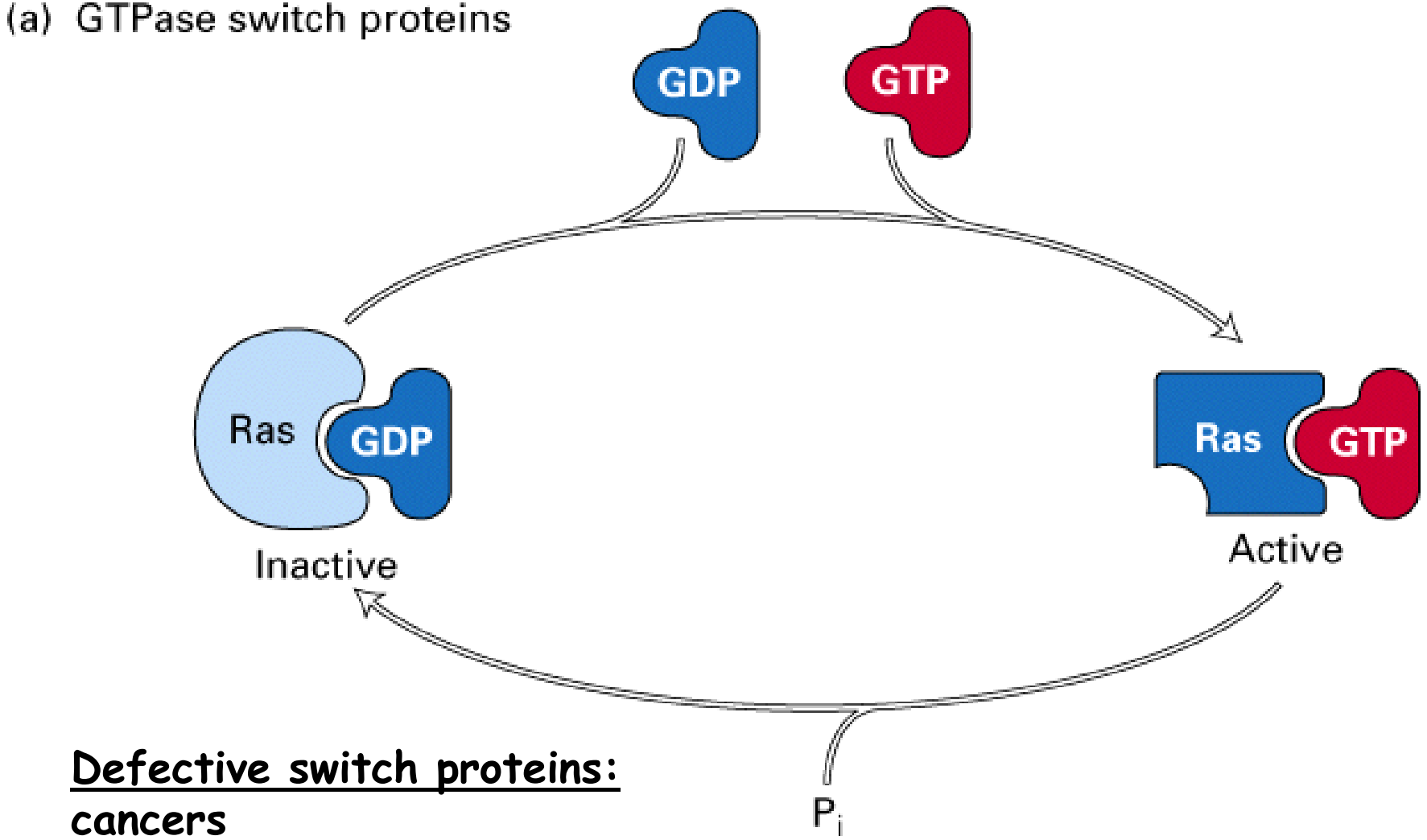


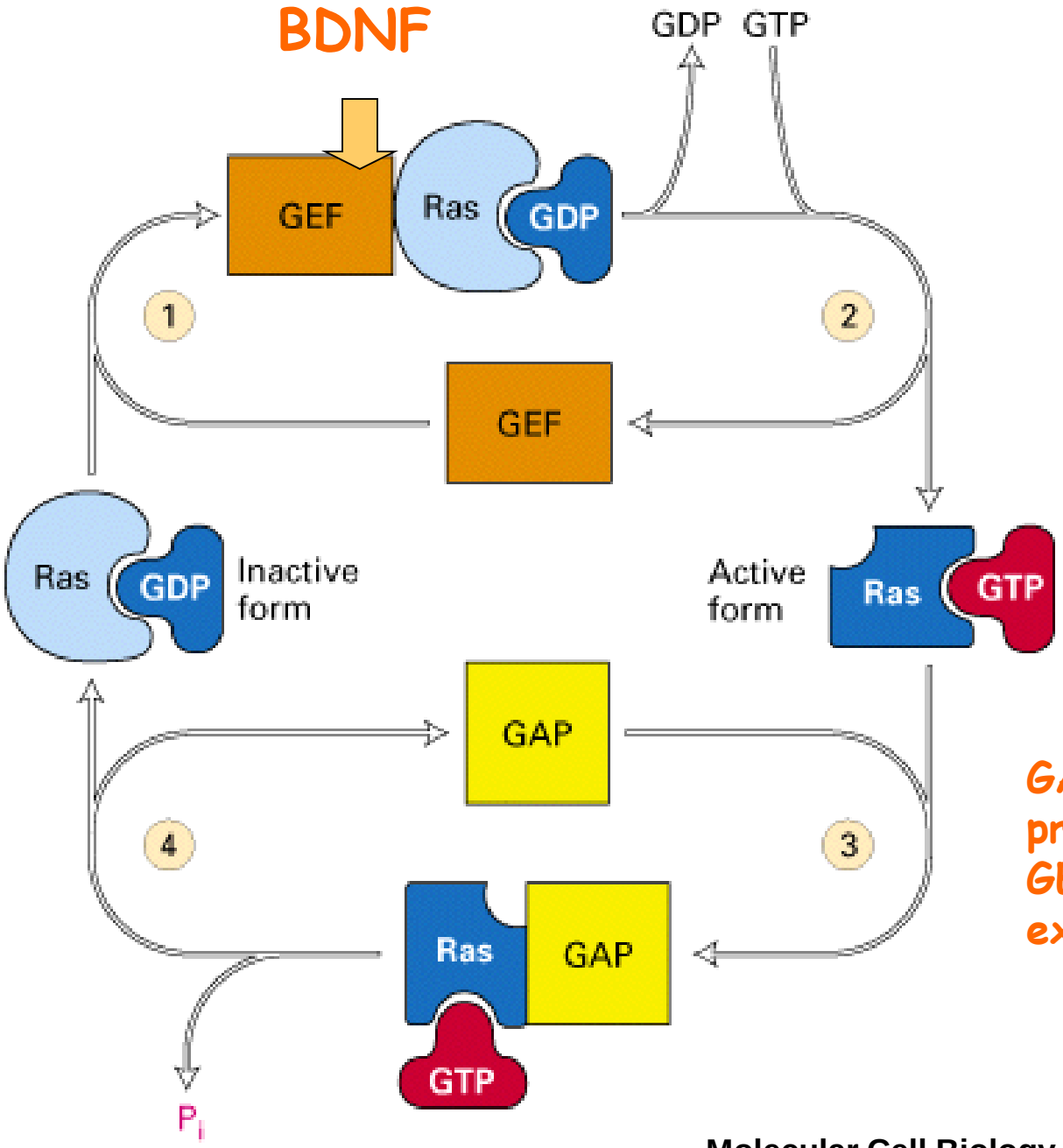
Figure 8–25

Curr Opin Drug Discov Devel 2004 Jul;7(4):478-86.
Farnesyltransferase inhibitors as anticancer agents: critical crossroads.
Doll RJ, Kirschmeier P, Bishop WR



Defective switch proteins:
cancers

GEF-Ras-GAP Interaction



GAP = GTPase activating protein
GEF = guanine nucleotide exchange factor

Molecular Cell Biology, 4th Ed., Chapter 20, Fig. 20-22

SECOND MESSENGER - TRANSCRIPTION

Relevant Concepts

Early response genes i.e. *fos, jun*

Ternary complex factor

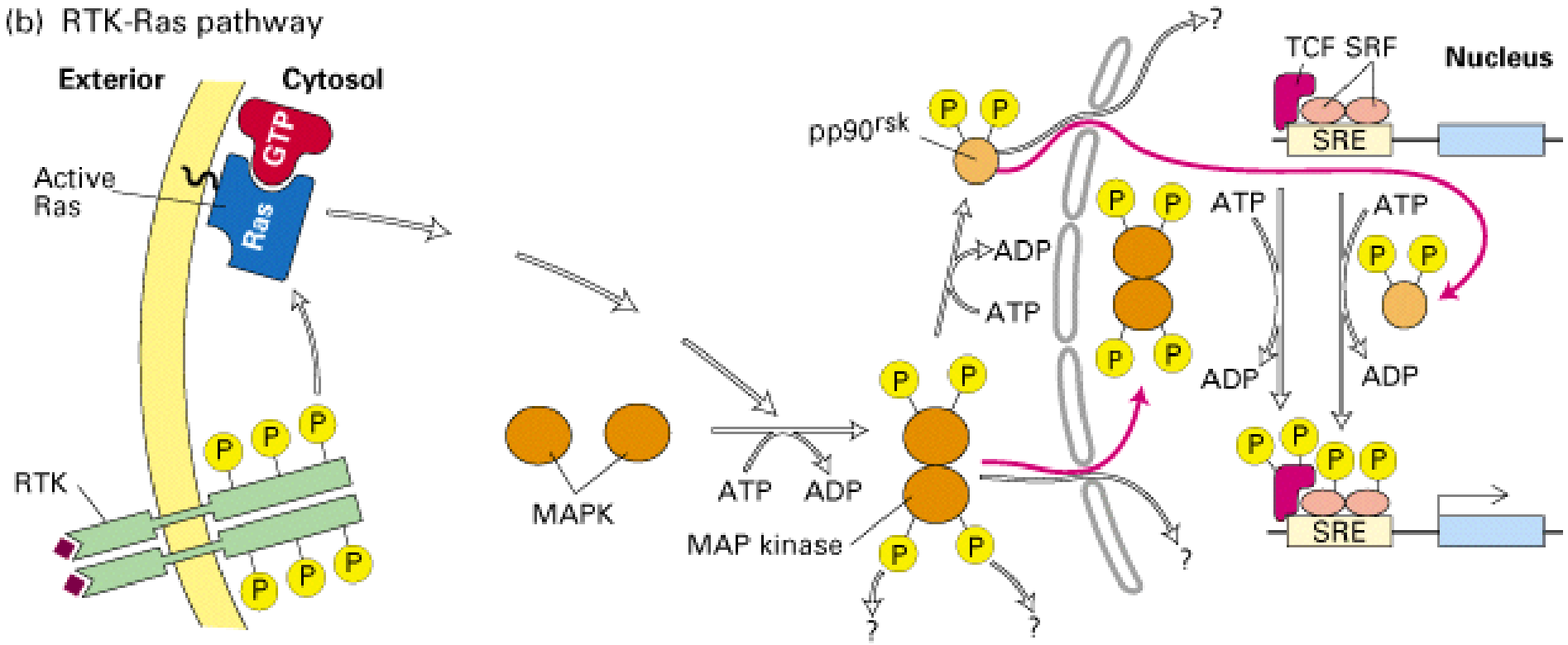
Serum response element

Protein phosphatases

Calcineurin

DARPP-32

Receptor Tyrosine Kinase – Gene Transcription Pathway

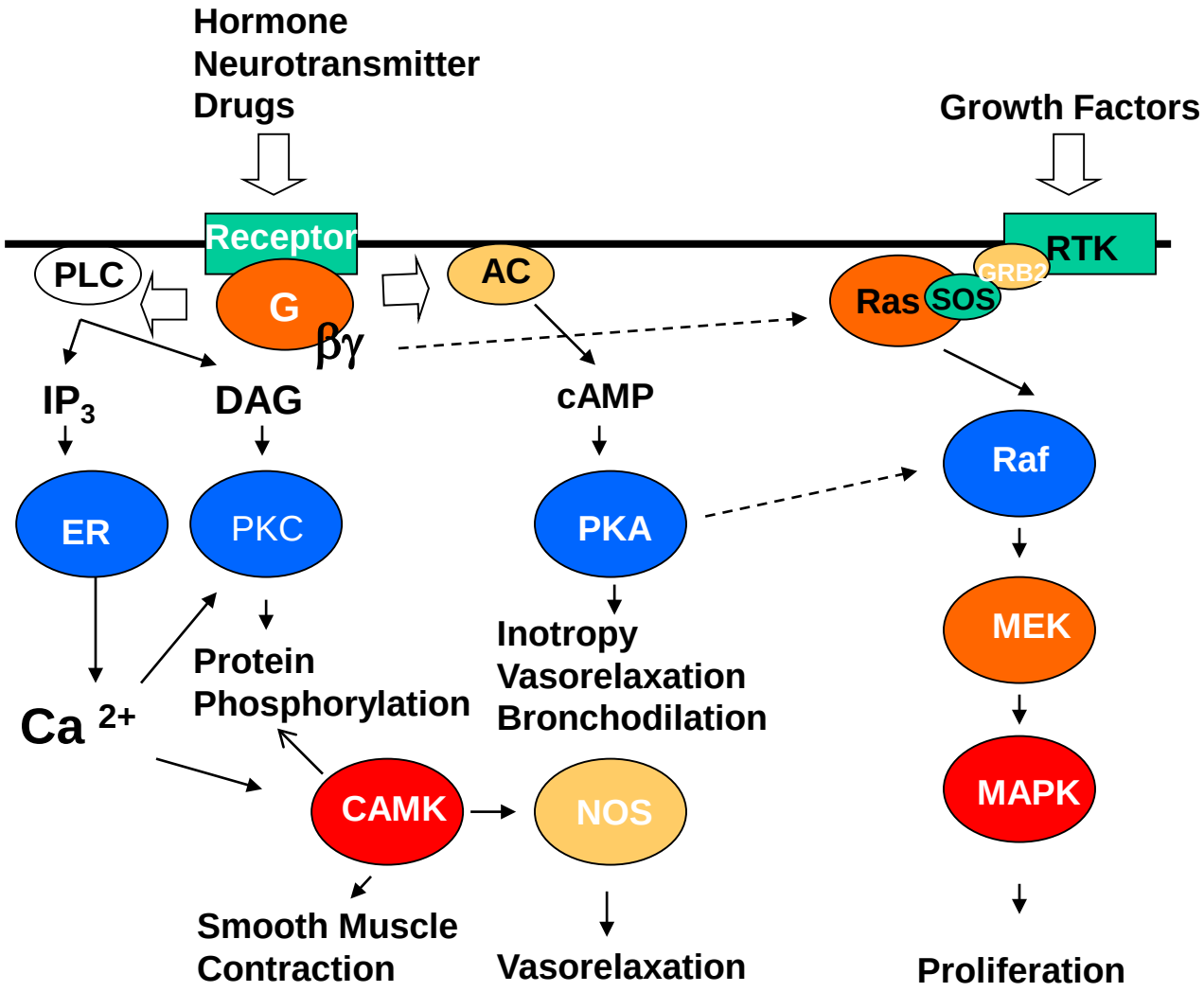


TCF = ternary complex factor
SRE = serum response element
SRF = serum response factor

Example: c-fos gene

Molecular Cell Biology, 4th Ed., Chapter 20, Fig. 20-48b

Drugs Signaling Pathways



Nobel Prizes Awarded for G Protein Signaling

- Cyclic AMP – Earl Sutherland (1971)
- Protein kinases - Edmund Fischer and Erwin Krebs (1992)
- G protein – Alfred Gilman and Martin Rodbell (1994)
- NO/cyclic GMP – Robert Furchgott, Louis Ignarro, Ferid Murad (1998)
- Signal transduction in the nervous system – Arvid Carlsson, Paul Greengard, Eric Kandel (2000)
- Adrenergic Receptors – Bob Lefkowitz and Brian Kobilka (2012)