Chapter 2

General Mechanism of Hormone Action

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Contents

1. Cellular receptors and hormone action

1) Hormone receptors bind specific hormones

- Each type of **receptor** is capable of binding only one specific hormone, or at most, a small number of closely related hormones.
- **Ligand**: agonist vs. antagonist
- Ligand binding specificity
- Affinity

- $R + H \underset{k_{off}}{\overset{k_{on}}{\rightleftarrows}} RH$

- $K_a = \frac{1}{K_d} = \frac{[RH]}{[R][H]}$

[R] = concentration of free (unbound) receptor
[H] = concentration of free (unbound) hormone
[RH] = conc. of bound receptor-hormone complex
K_a = association constant
K_d = dissociation constant; the higher the affinity of a receptor for hormone, the lower is the K_d value (typically 10^{-11}~10^{-8} M)

2) Tissue responses to a hormone are determined by the presence of specific receptors

- Insulin → increase glucose uptake by hepatocytes, fat cells, and certain muscle cells, and interacts with many other cell types
- Parathyroid hormone (PTH) → elevates serum Ca^{2+} levels by releasing Ca^{2+} from bone, stimulating Ca^{2+} uptake from the gut and preventing Ca^{2+} loss from the kidney.

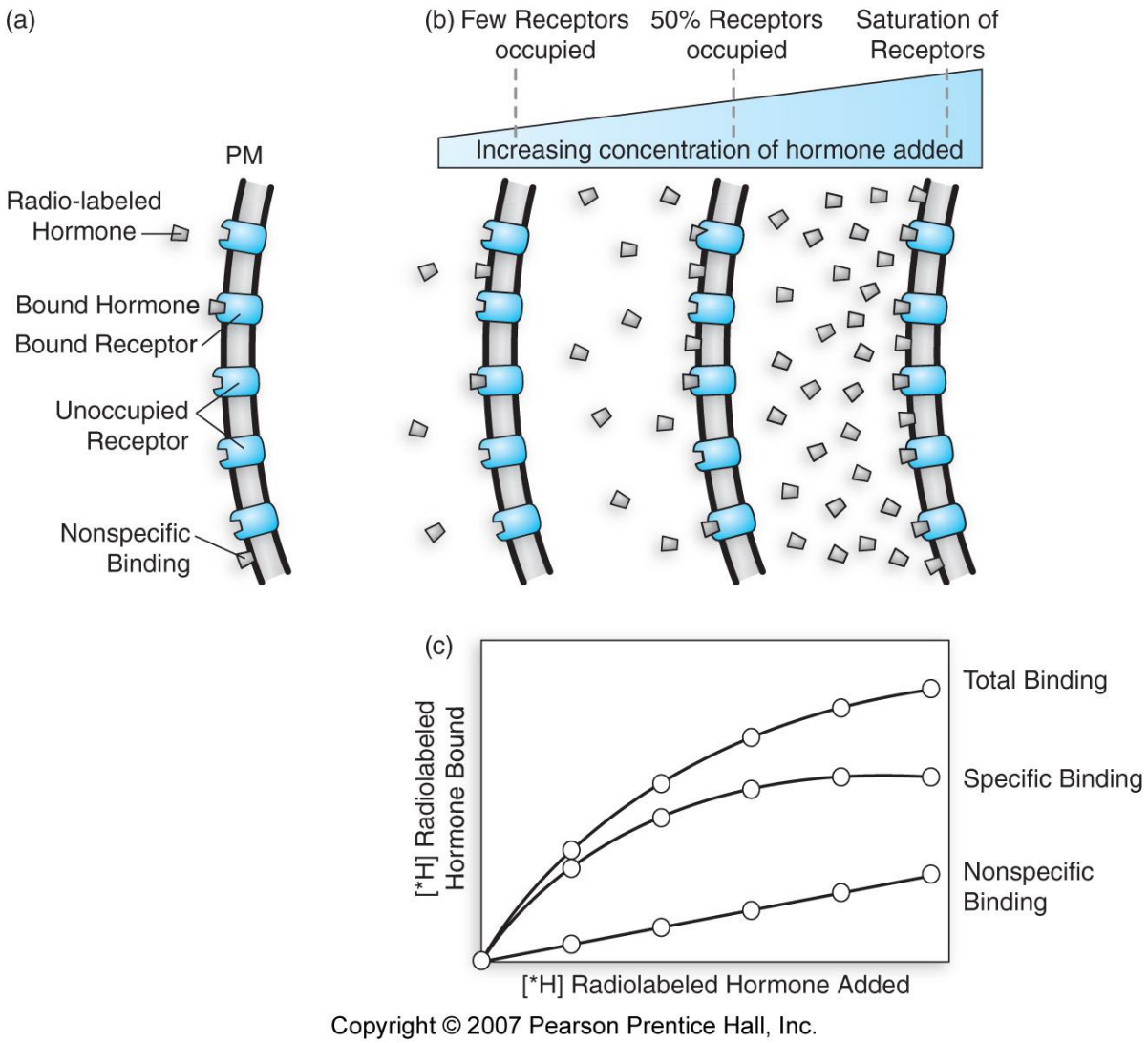
3) Some hormones can activate multiple receptor isoforms

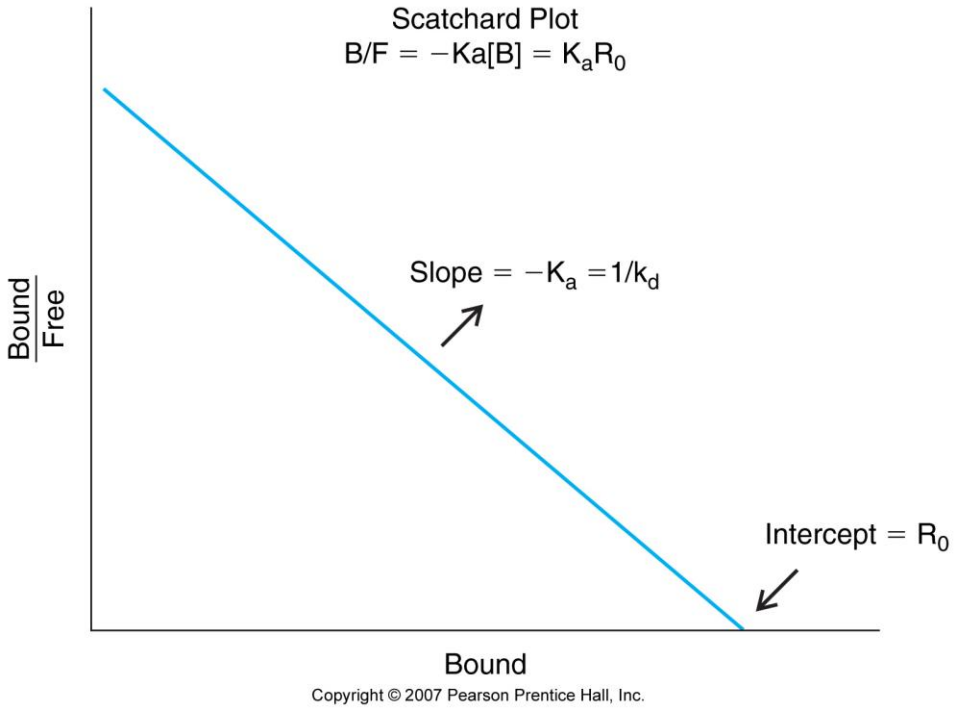
- Estrogen: estrogen receptor alpha (ERα) and beta (ERβ) → evoke different responses in different tissues, or even different responses in the same tissues, depending upon the relative abundance of each of these two receptor isoforms in the target cells
- i.e., in some mammary gland cells, estrogen stimulates cell proliferation by activating ERα, while activation of ERβ in other cells can inhibit cell growth.

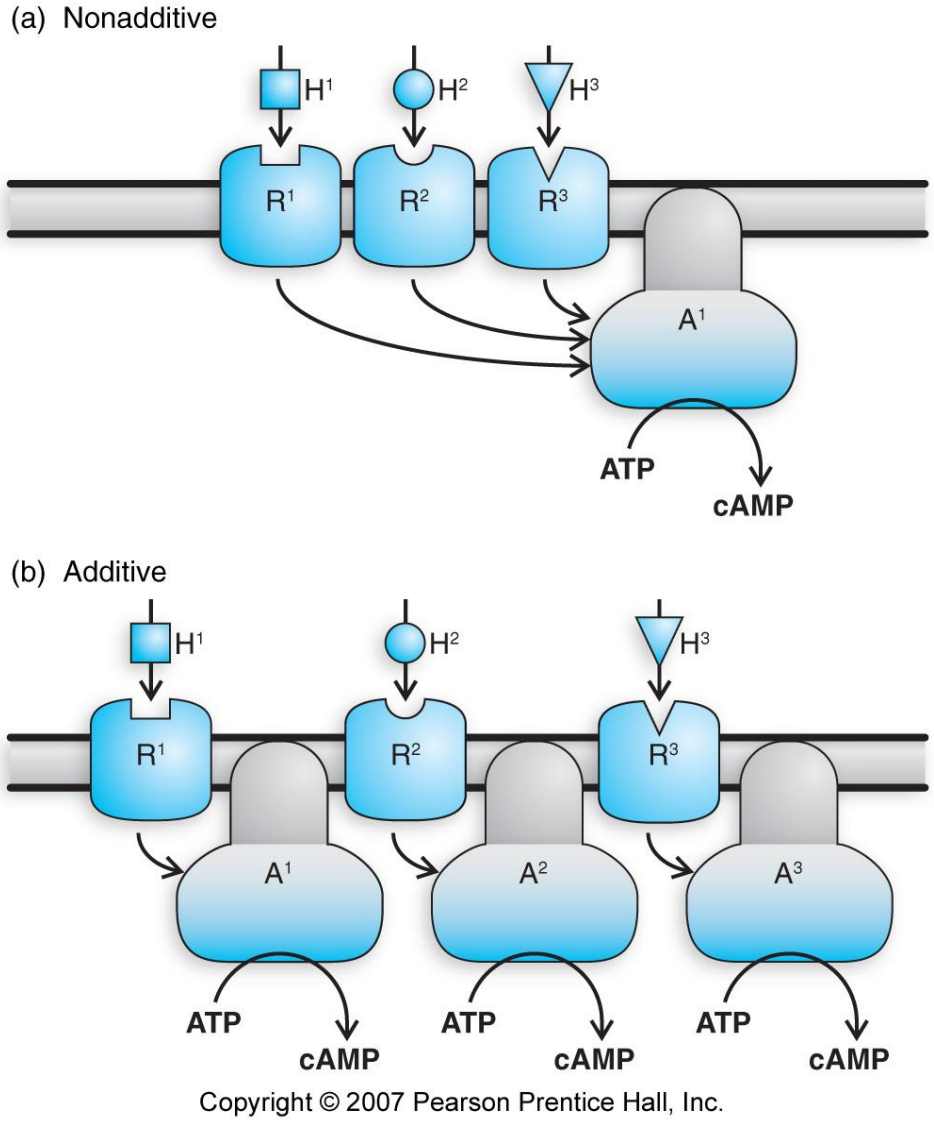
2. Characteristics of physiological receptors

- High affinity
- Specificity
- Saturability: Under normal conditions, a cell generally produces between 2,000 and 100,000 receptor molecules.
- Reversible nature
- Associated with physiological response in the target cell

Fig. 3.1. Schematic representation of (a) hormone molecules binding to plasma membrane receptors, (b) hormone molecules present in increasing concentrations and corresponding increases in receptor binding, as in a typical saturation receptor binding assay, and (c) saturation receptor binding curves in a typical saturation binding assay.

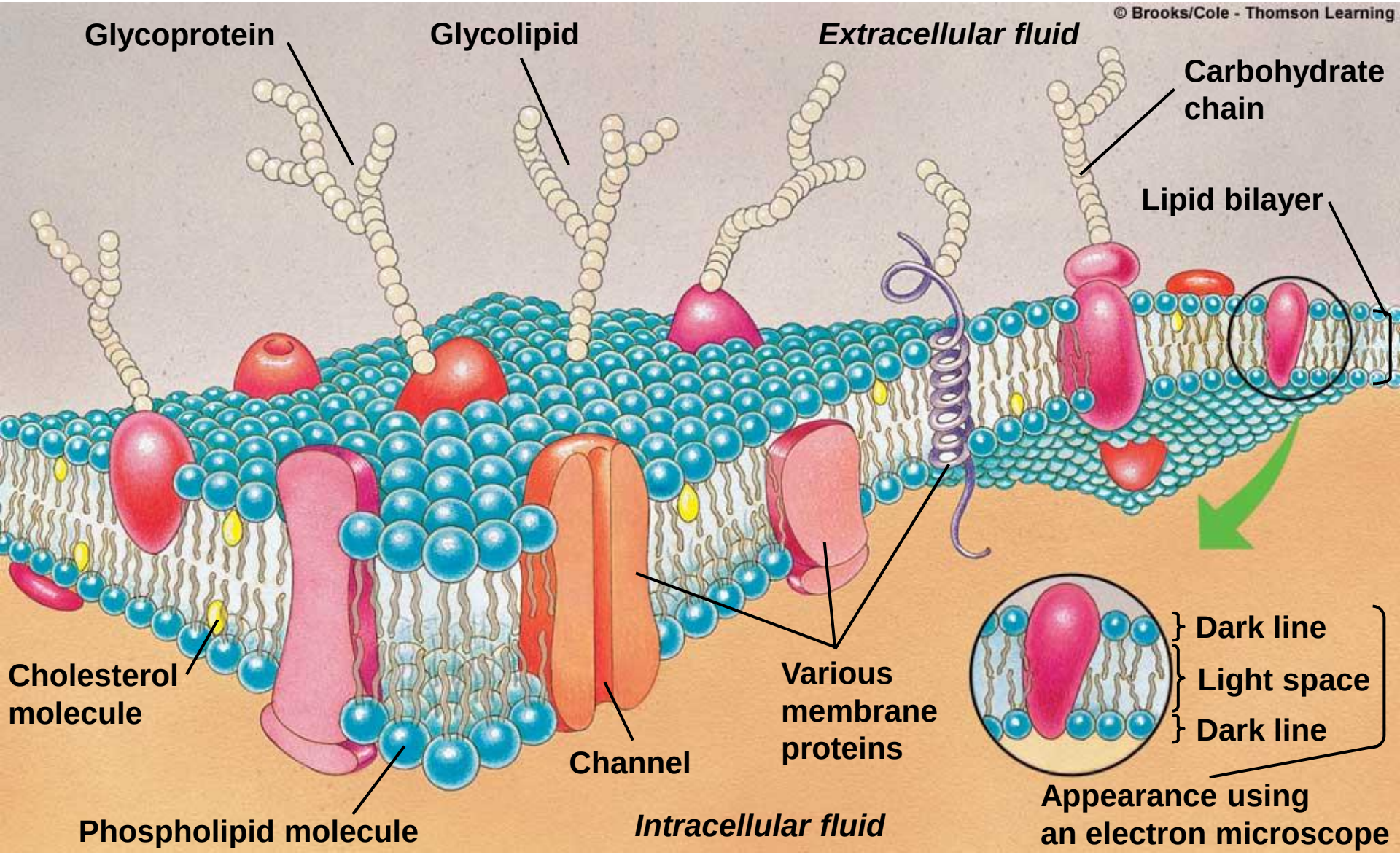






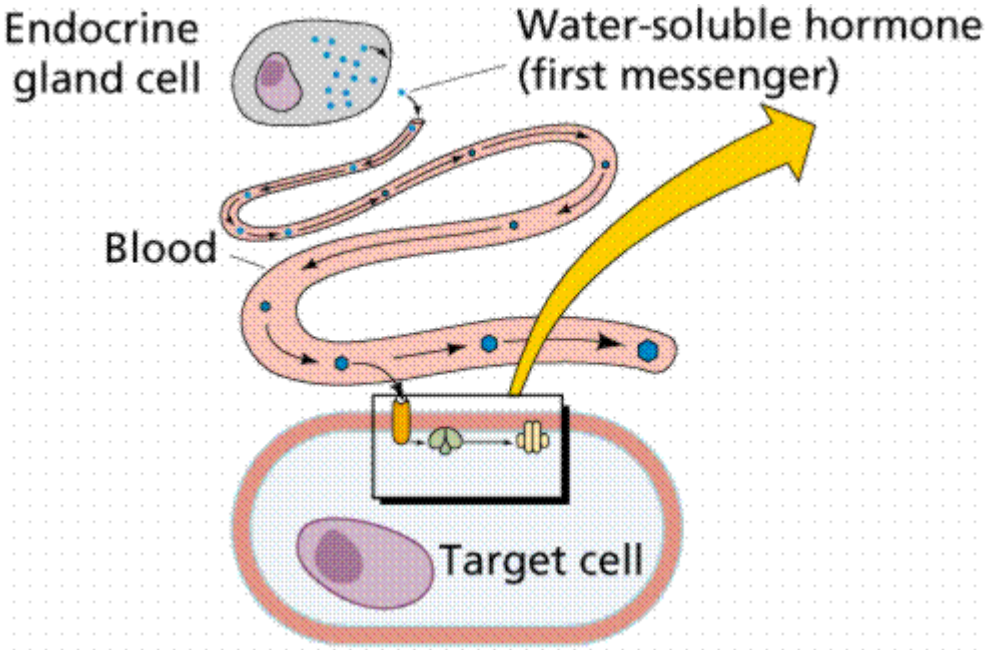
Mechanism of Hormone Action

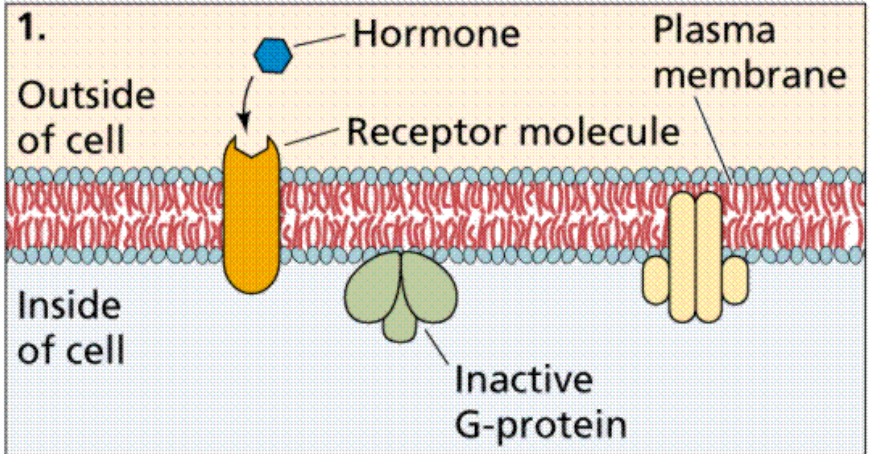
- Binding to receptor
  - Cell surface receptors
  - Intracellular receptors : Cytoplasmic
    Nuclear
    Mitochondrial
- Activation of postreceptor messengers
- Cellular answer

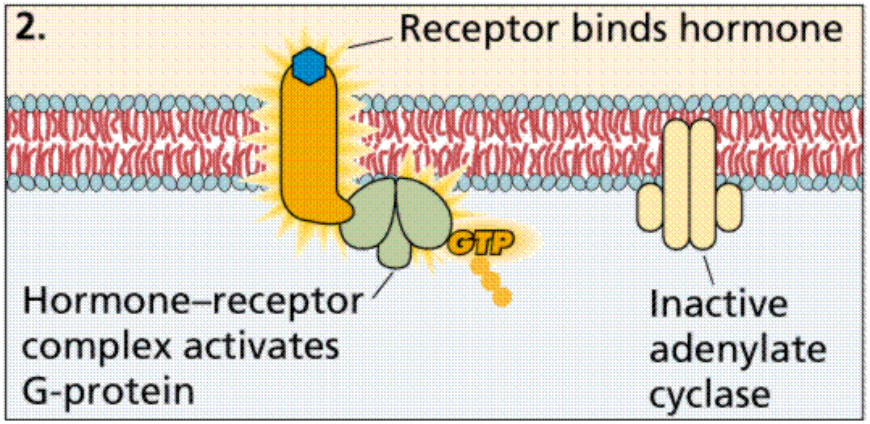


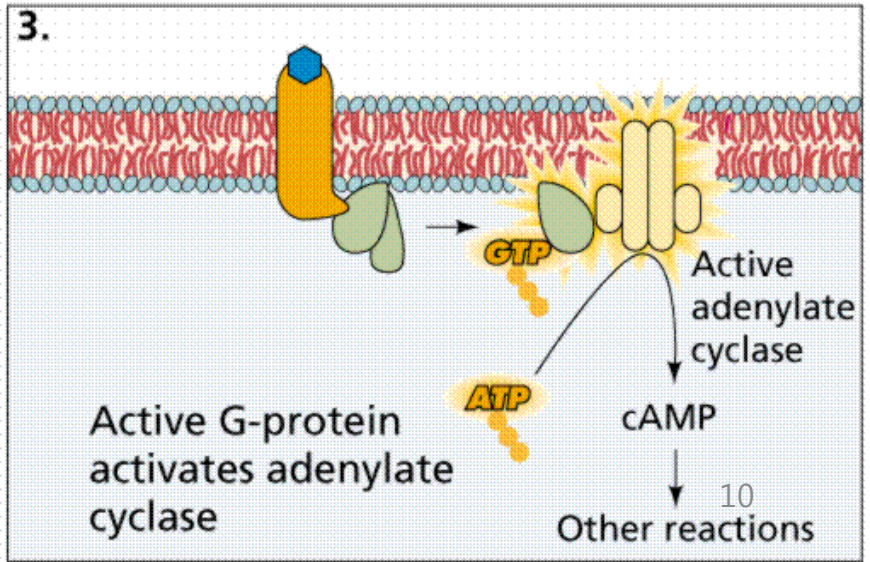
Fluid mosaic model of plasma membrane structure.

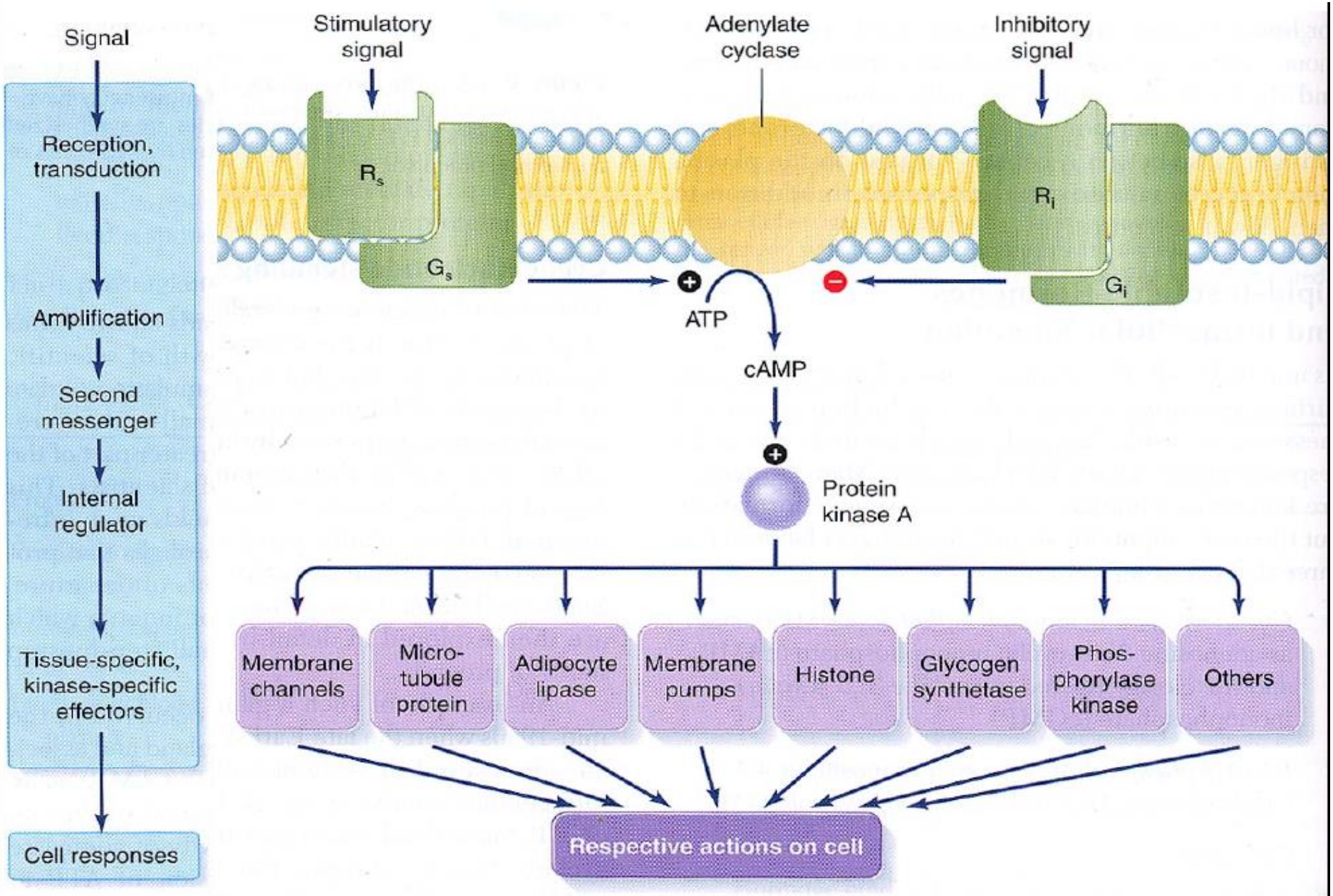
Water soluble hormone and Cell surface receptors










Signal
Reception, transduction
Amplification
Second messenger
Internal regulator
Tissue-specific, kinase-specific effectors
Cell responses
Stimulatory signal
Adenylate cyclase
Inhibitory signal
R_s
G_s
R_i
G_i
ATP
cAMP
Protein kinase A
Membrane channels
Micro-tubule protein
Adipocyte lipase
Membrane pumps
Histone
Glycogen synthetase
Phos-phorylase kinase
Others
Respective actions on cell

3. Plasma membrane hormone receptors

1) **Membrane receptor families** have characteristic structures and functional properties

A. Four main classes of membrane-bound receptors:

a. Class I: A superfamily of receptors coupled to G (GTP-binding) proteins (G-protein-coupled receptors, GPCRs); Receptors for ACTH, LH, FSH, hCG, TSH, glucagon, katecholamines, muscarine, serotonine, dopamine, histamine

b. Class II: Receptors that are also enzymes (tyrosine protein kinases, serine/threonine kinases, or guanylate cyclase); Receptors for insulin, growth factors (tyrosine kinase), ANP(guanylyl cyclase), TGF-β (serine-threonine kinase). Receptors for GH, cytokines, interferones.

c. Class III: Receptors that are associated with enzymes (cytokine receptors associated with tyrosine kinases)

d. Class IV: Receptors coupled to ion channels; Receptor for Ach.

Class I of Protein receptor

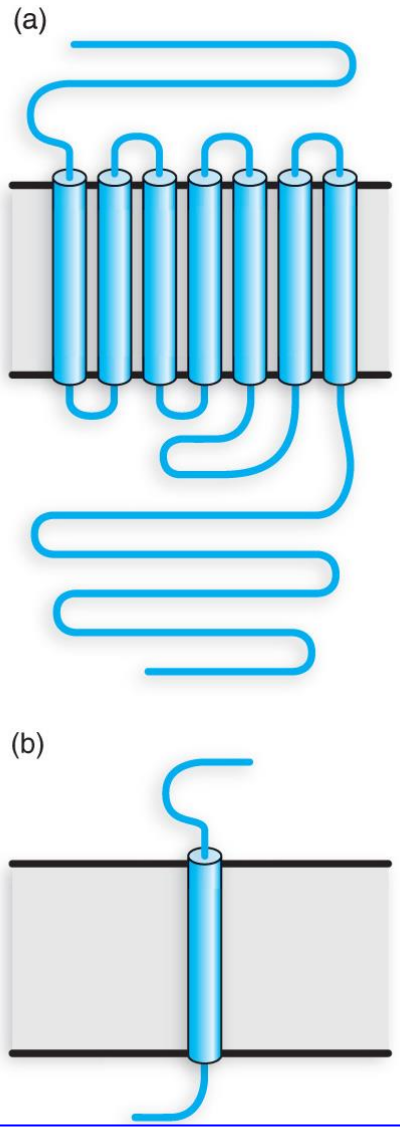


Fig. 3.4. Schematic representation of (a) multi- and (b) single-transmembrane receptors

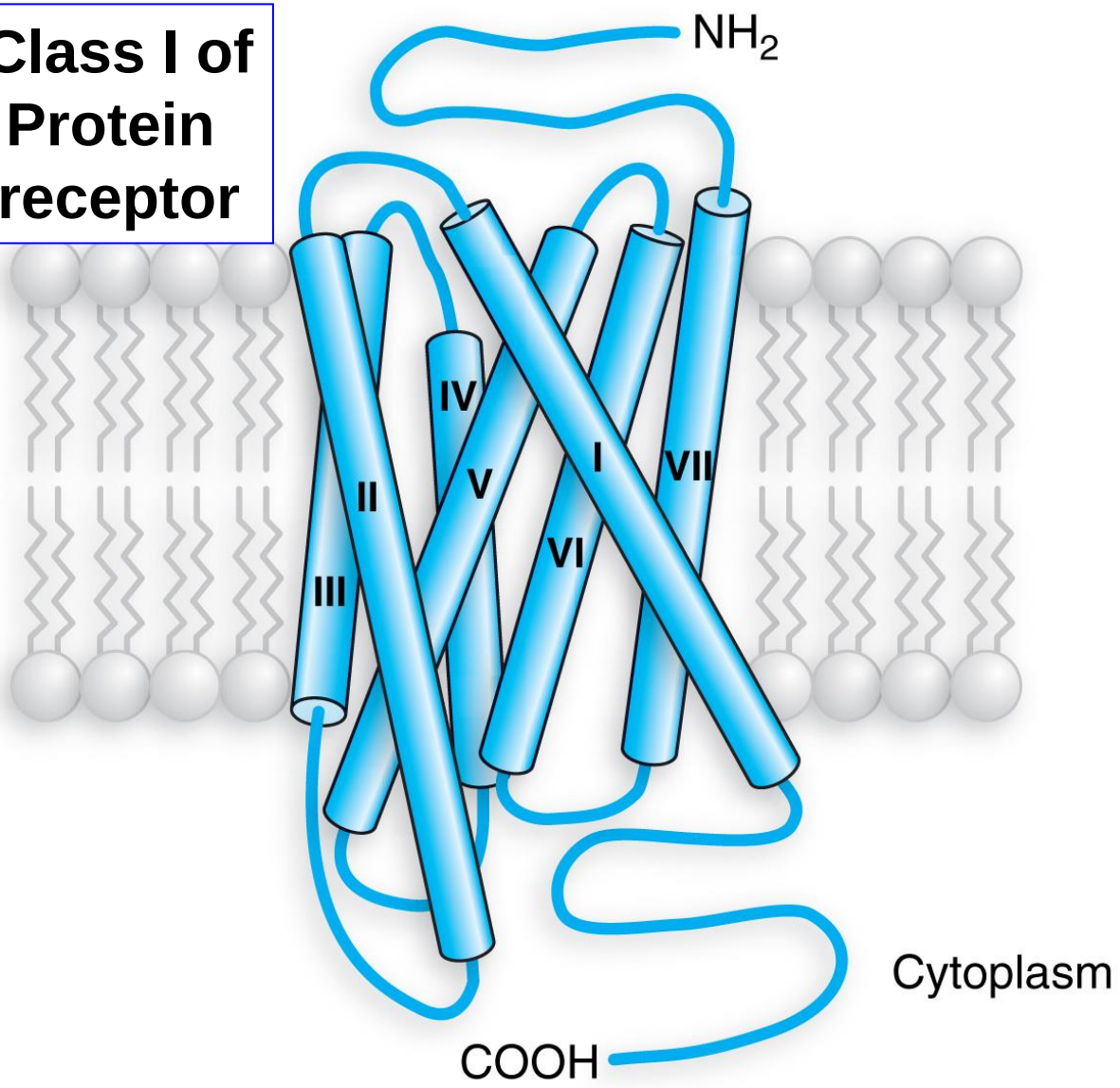
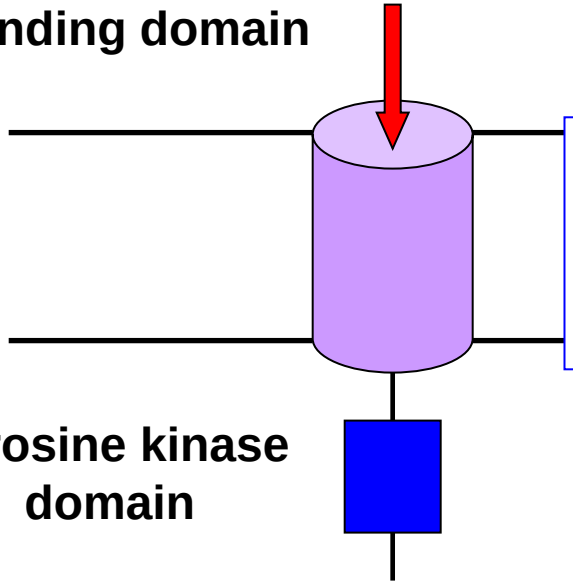


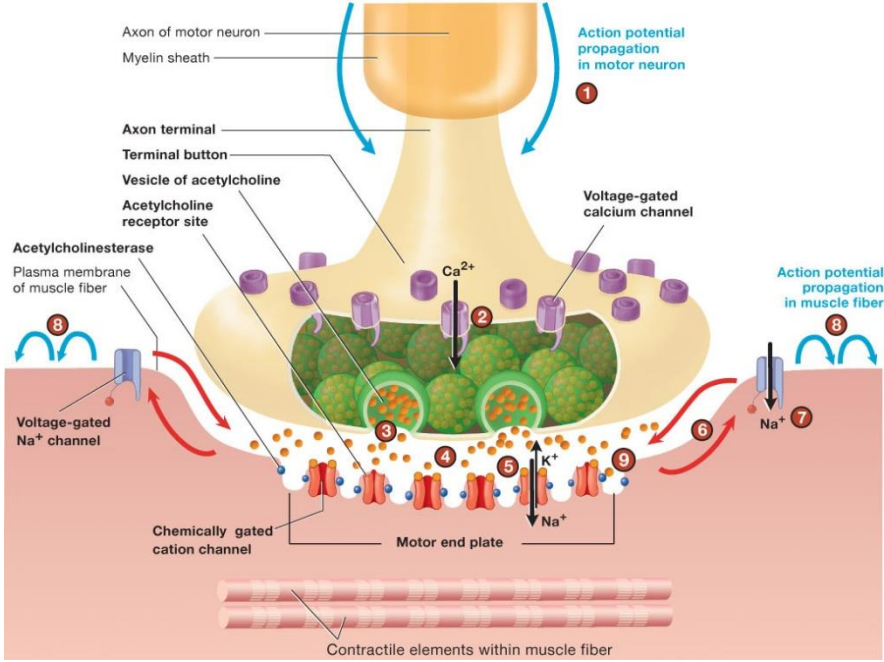
Fig. 3.5. Schematic model of insertion of **G-protein**-coupled receptors in the plasma membrane.

Binding domain



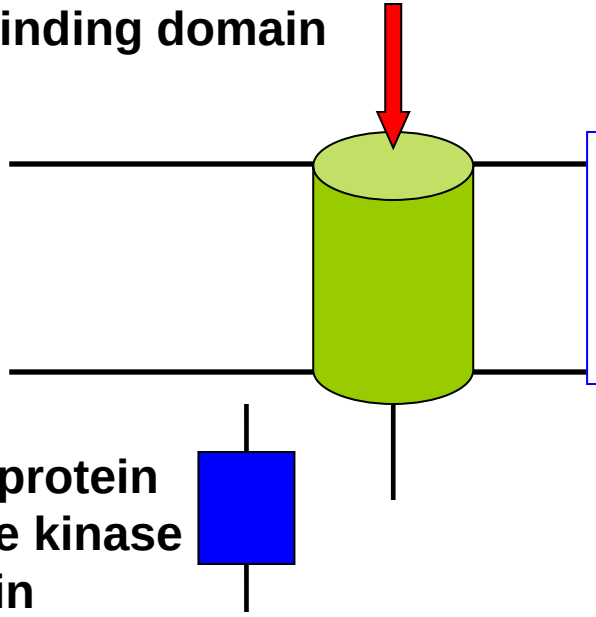
Tyrosine kinase domain

Class II of Protein receptor



Class IV of Protein receptor

Binding domain



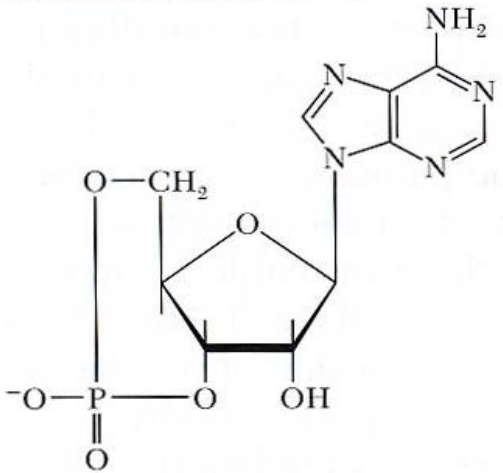
Accessory protein With tyrosine kinase domain

Class III of Protein receptor

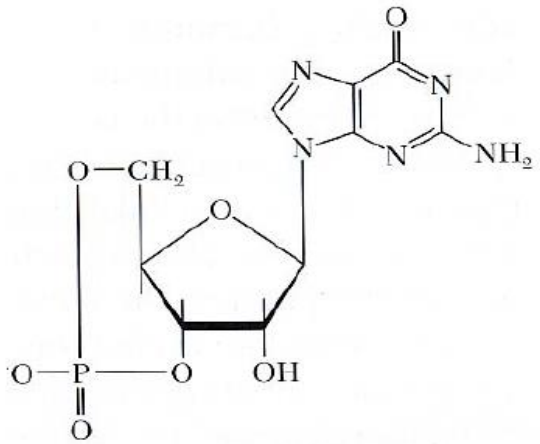
4. Second messengers of hormone action

Second messengers (stimulated by lipid insoluble hormones):

CYCLIC NUCLEOTIDES

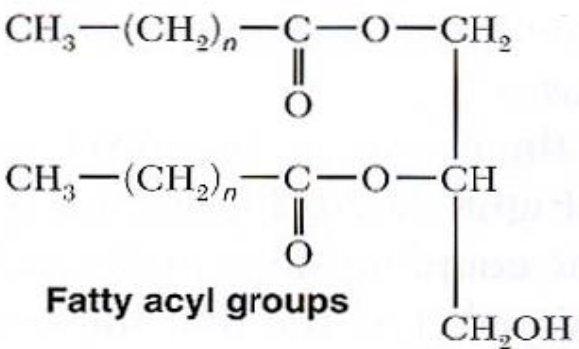


3′,5′-Cyclic AMP
(cAMP)



3′,5′-Cyclic GMP
(cGMP)

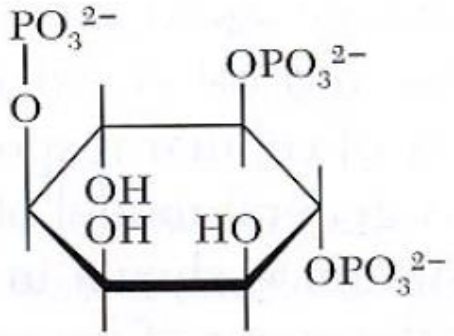
INOSITOL PHOSPHOLIPIDS



Fatty acyl groups

Glycerol

1,2-Diacylglycerol
(DAG)



Inositol
1,4,5-trisphosphate
(IP_3)

CALCIUM ION

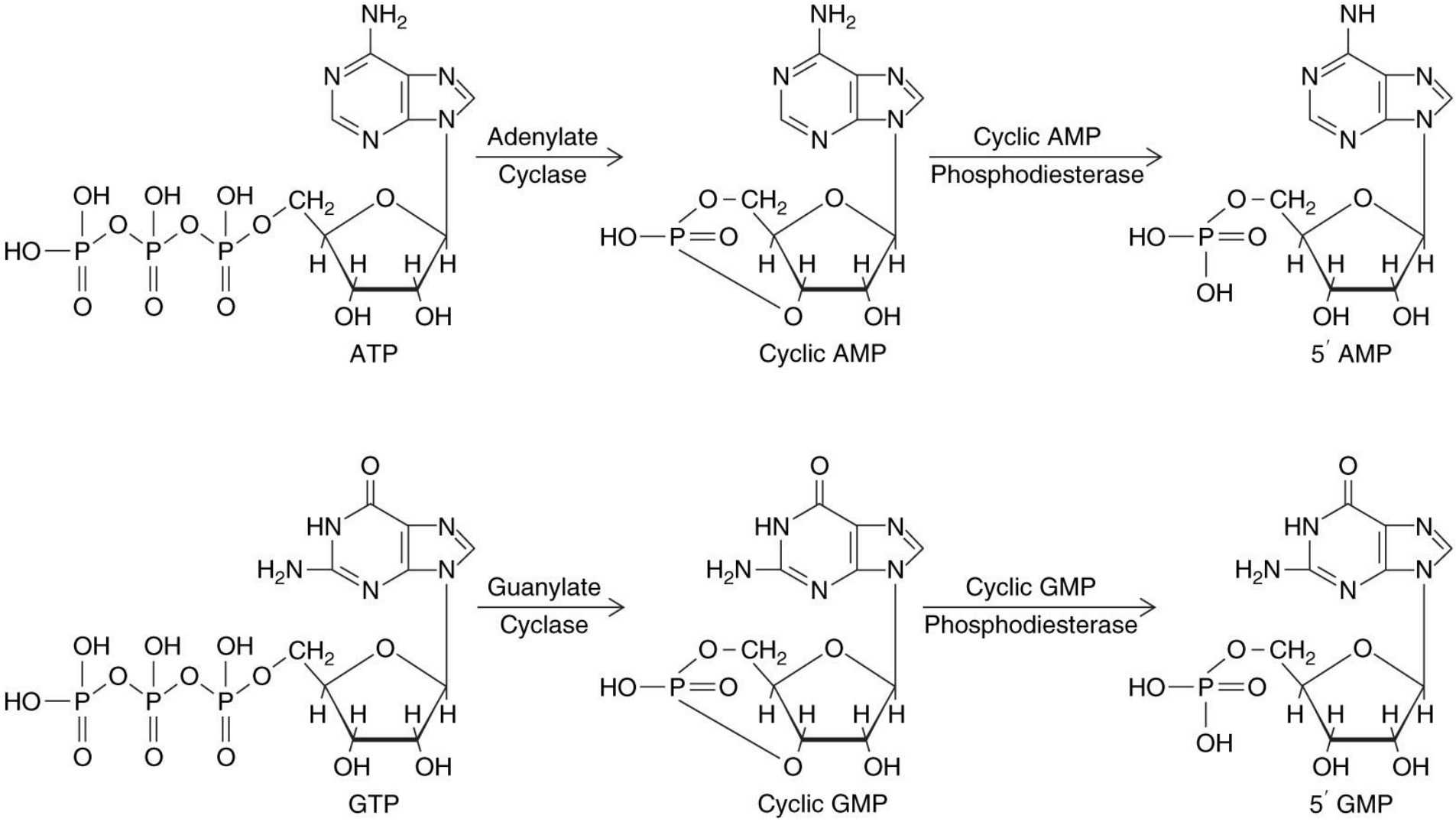


Fig. 3.6. Cyclic nucleotide synthesis and inactivation

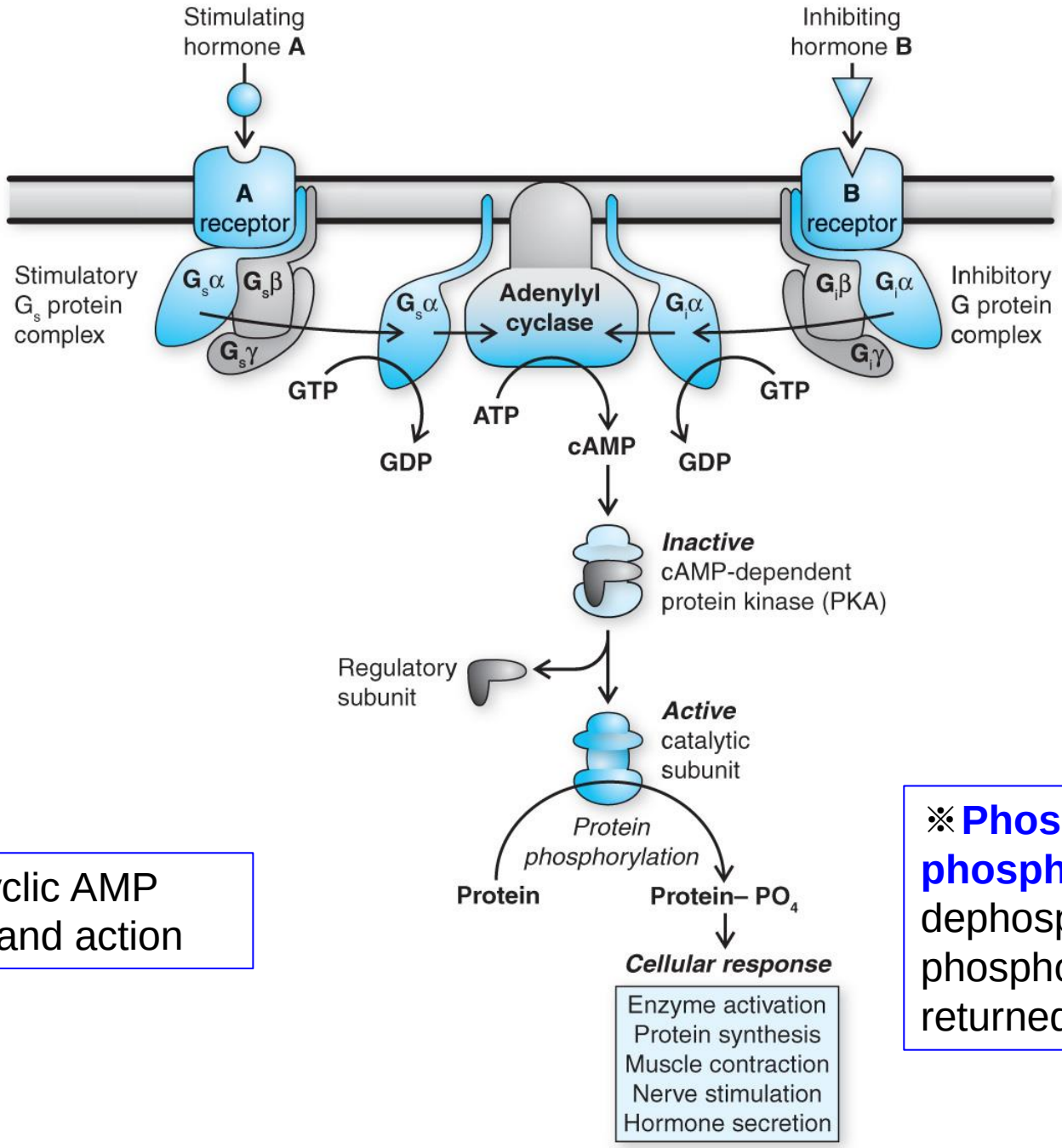
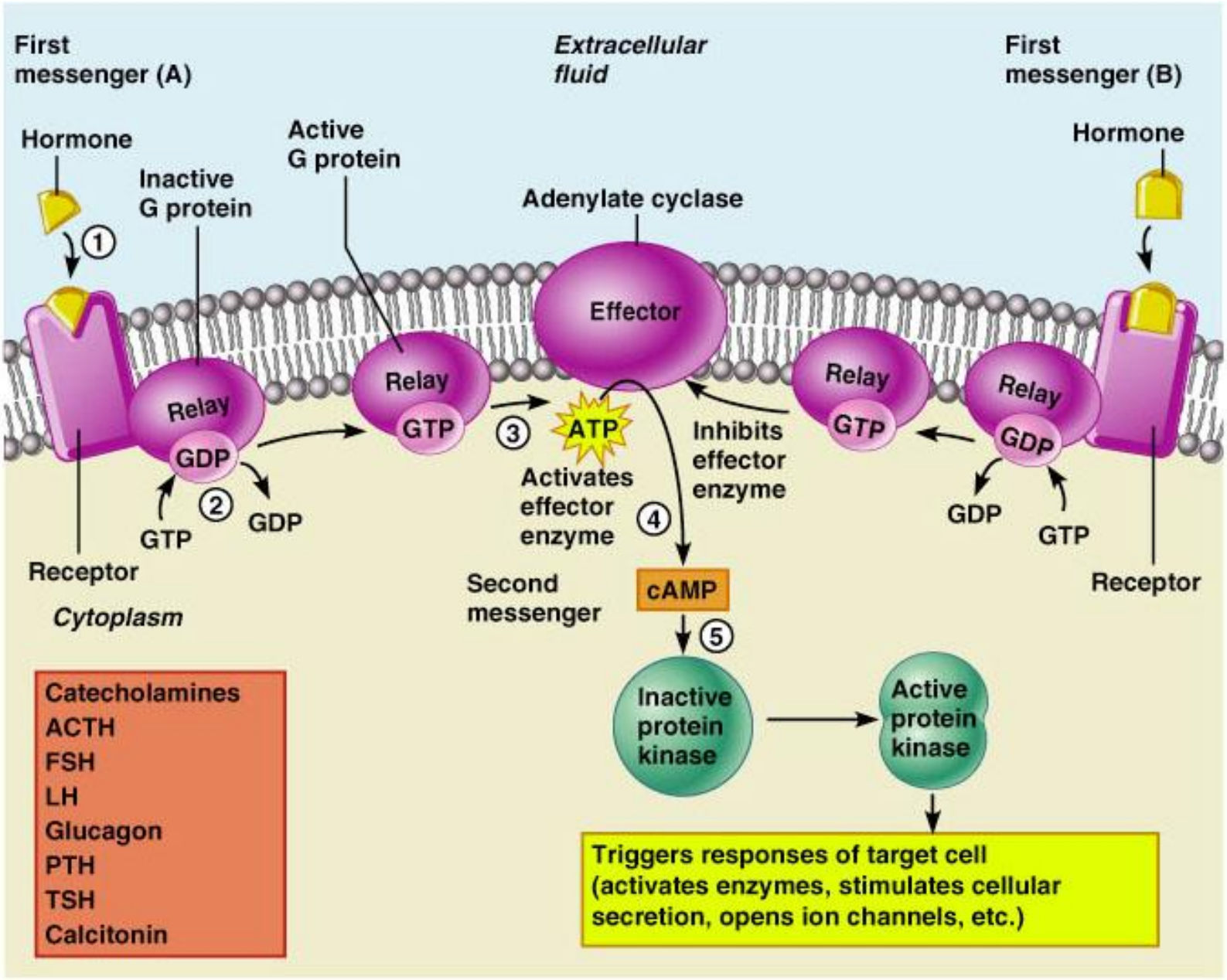


Fig. 3.7. Cyclic AMP production and action

※**Phosphoprotein phosphatases**: dephosphorylate the phospho-protein → returned to basal level


First messenger (A)
Extracellular fluid
First messenger (B)
Hormone
Inactive G protein
Active G protein
Adenylate cyclase
Hormone
1
Effector
Relay
GDP
Relay
GTP
Relay
GTP
Relay
GDP
2
GTP
GDP
3
ATP
Activates effector enzyme
Inhibits effector enzyme
GDP
GTP
Receptor
Cytoplasm
4
Second messenger
cAMP
Receptor
5
Catecholamines
ACTH
FSH
LH
Glucagon
PTH
TSH
Calcitonin
Inactive protein kinase
Active protein kinase
Triggers responses of target cell (activates enzymes, stimulates cellular secretion, opens ion channels, etc.)

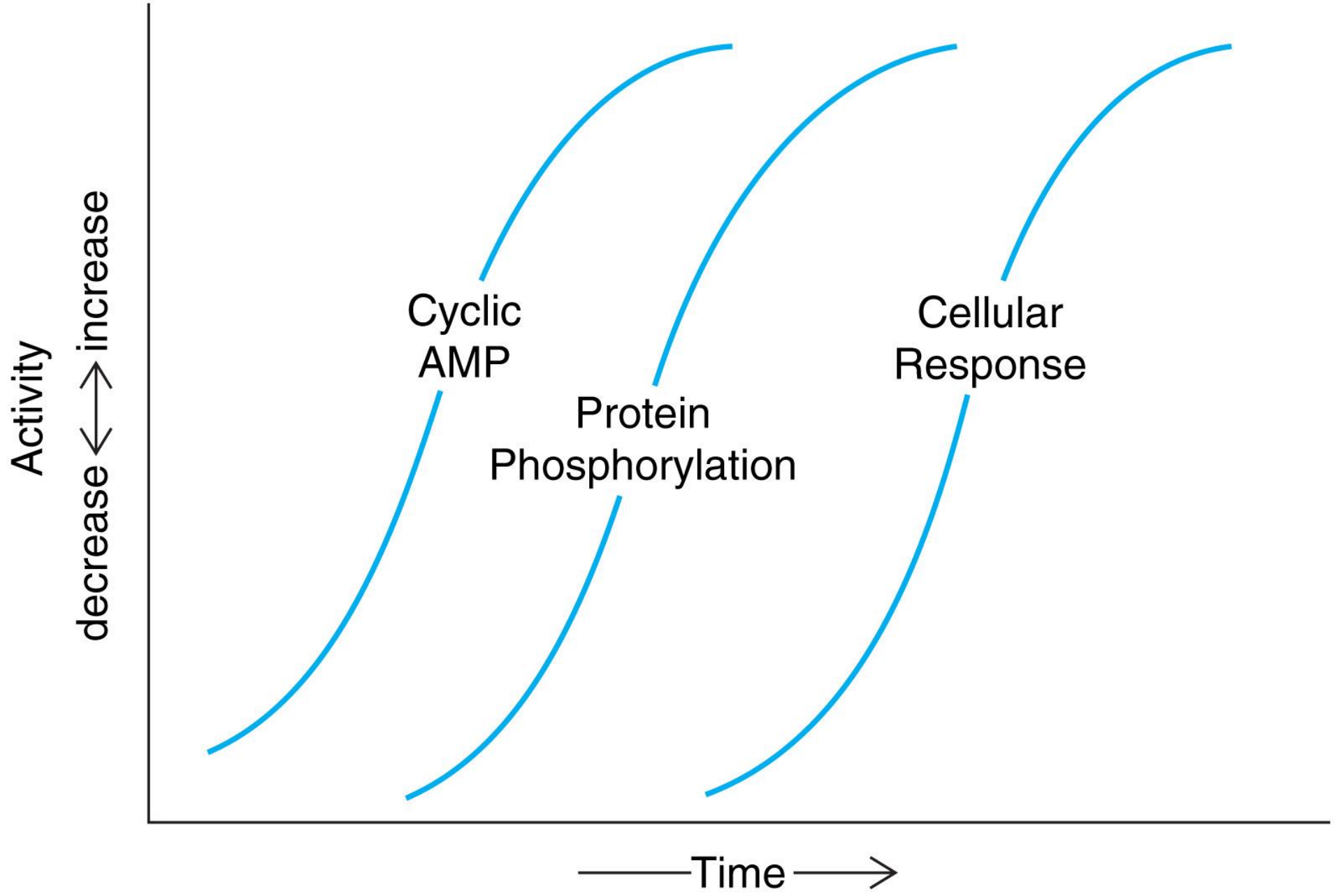
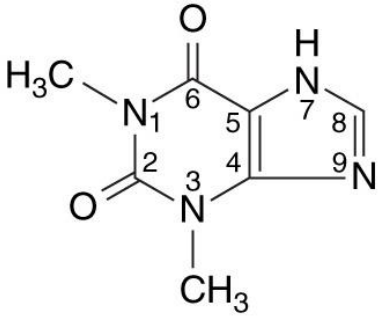


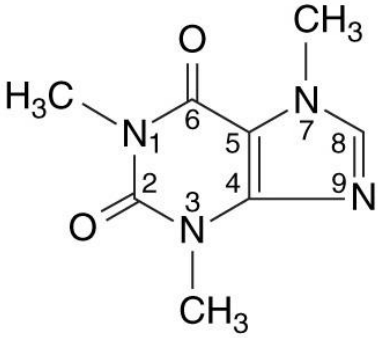
Fig. 3.8. Temporal cellular events in hormone-mediated cAMP production and action

Inhibitors of cAMP- and cGMP-dependent phosphodiesterase activity

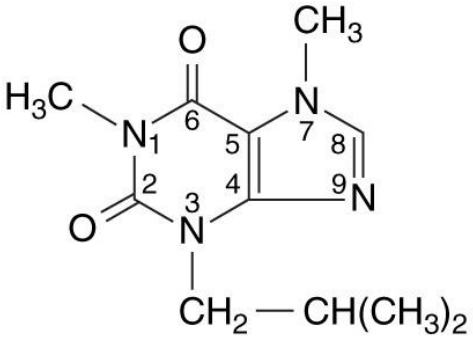
- Caffeine, theophylline, and theobromine are methylxanthines derived from coffee, tea, and cocoa, respectively.
- Theophylline: the most potent of the three
- Adenylate cyclase is always active.



Theophylline



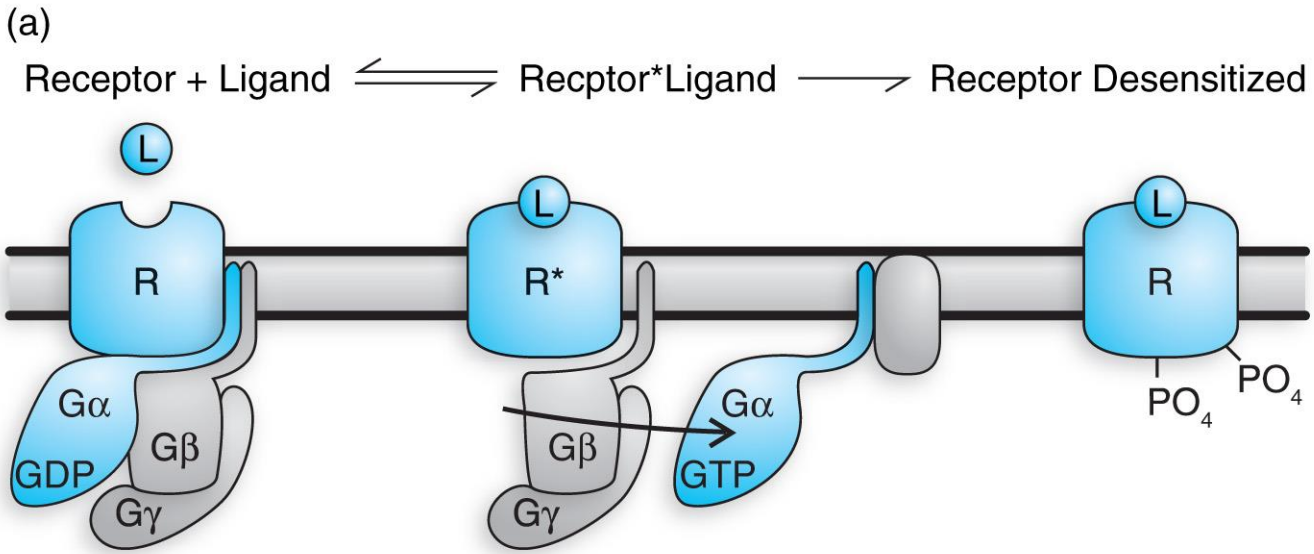
Caffeine

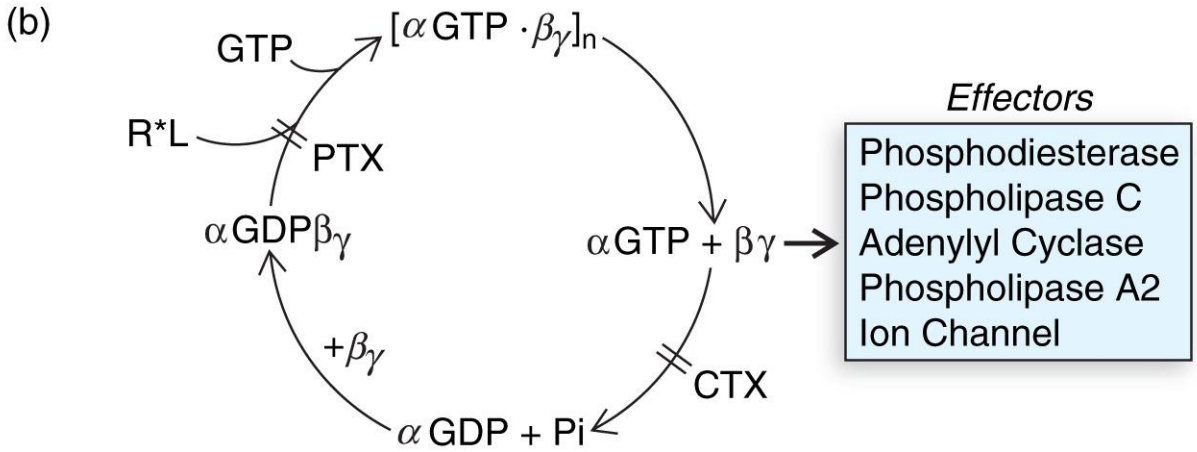


I-Methyl-3-Isobutylxanthine

Fig. 3.9. Methylxanthine structures: theophylline, caffeine, and 1-methyl-3-isobutylxanthine (a synthetic xanthine analog)

5. Membrane receptor signal transduction

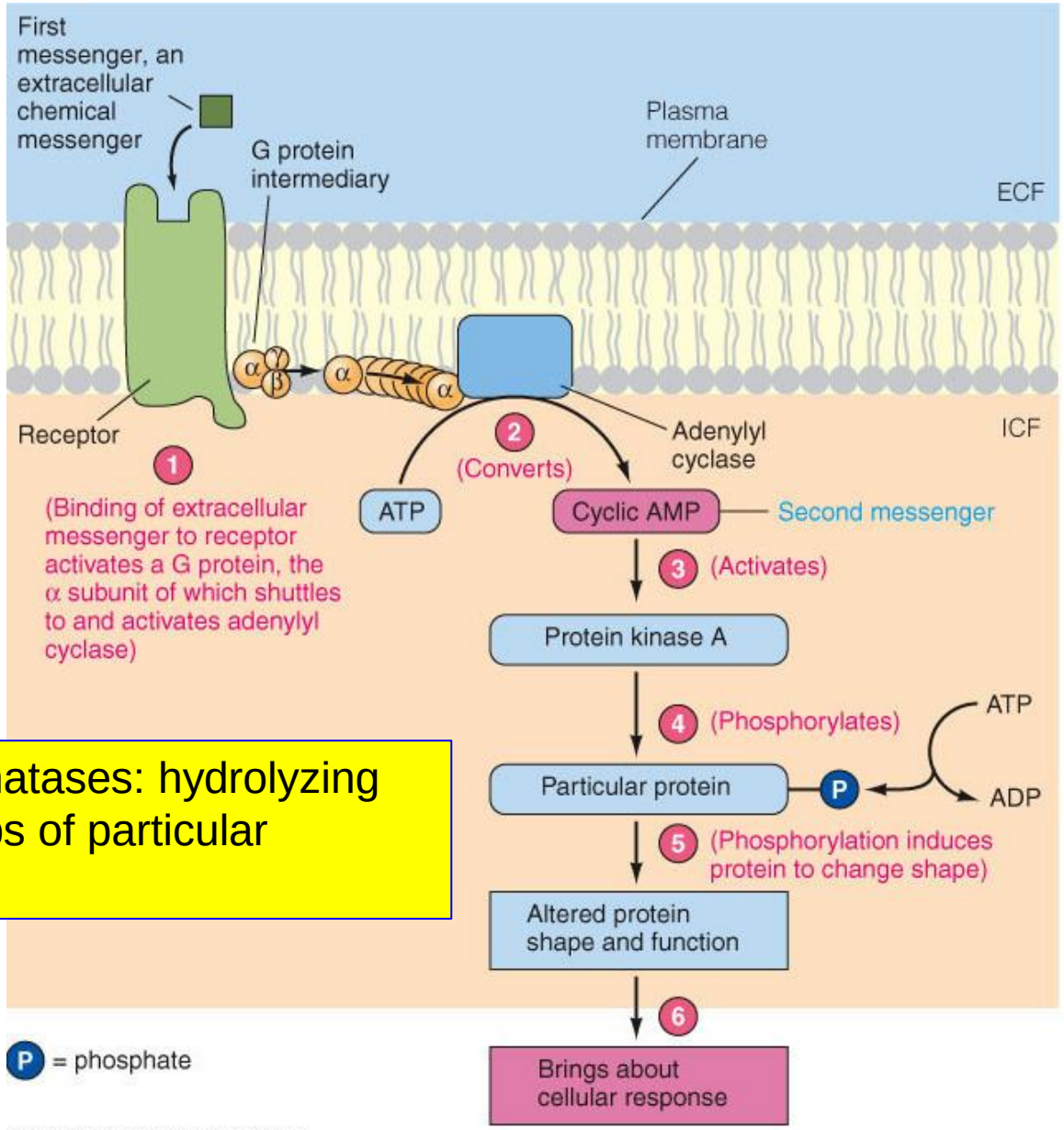




- PTX (pertussis toxin) → blocks the catalysis of GTP exchange by the receptor
- CTX (cholera toxin) → inhibitor of GTPase

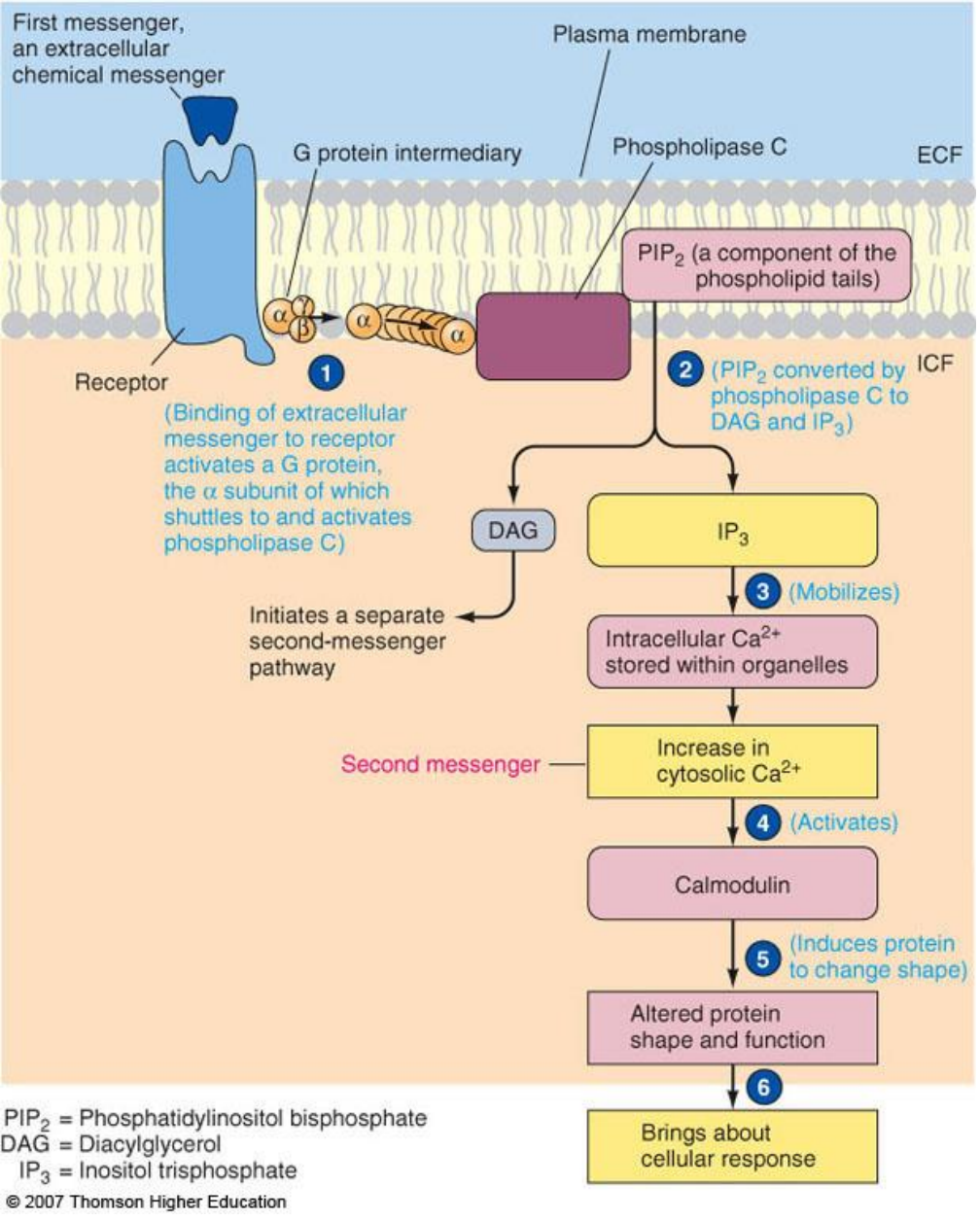
Fig. 3.11. Receptor-G-protein-mediated signal transduction

6. Phosphorylated proteins as physiological effectors



※Protein phosphatases: hydrolyzing phosphoryl groups of particular proteins

7. Multiple membrane messengers



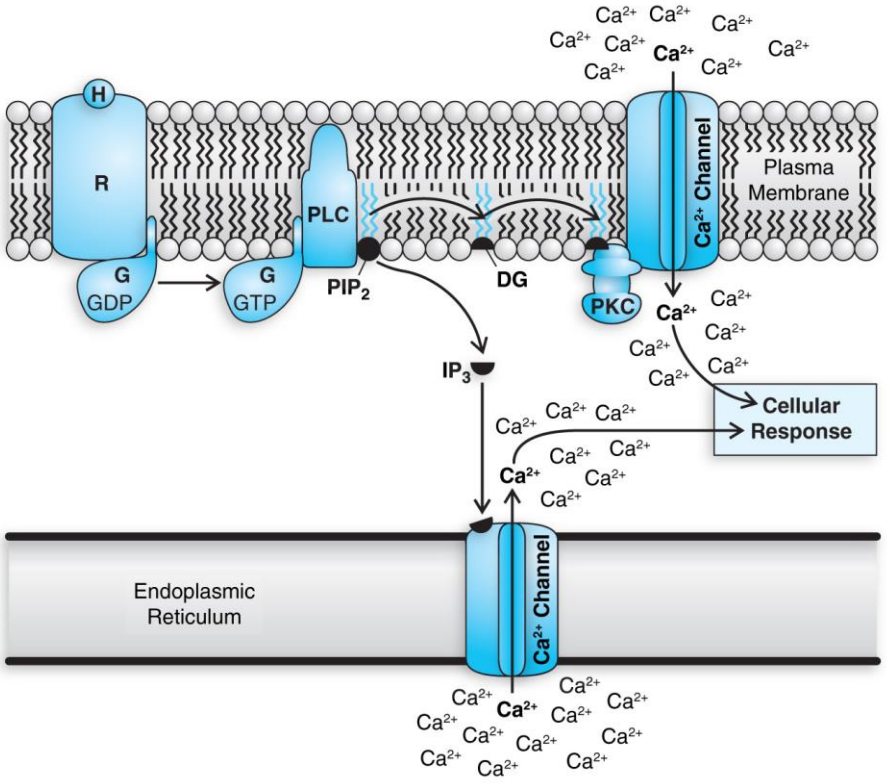


Fig. 3.12. Inositol phosphate and receptor signal transduction

※DAG = DG, diacylglycerol

8. Eicosanoids and hormone action

- In 1930s, human semen and extracts of seminal vesicles from animals caused uterine tissue to contract or relax → "prostaglandin (PG)"
- PGs belong to a family of chemically related substances, *eicosanoids*.
- Nobel Prize in Physiology/Medicine for 1982: Bergström, Samuelsson, and Vane for their "discoveries concerning prostaglandins and biologically related substances".

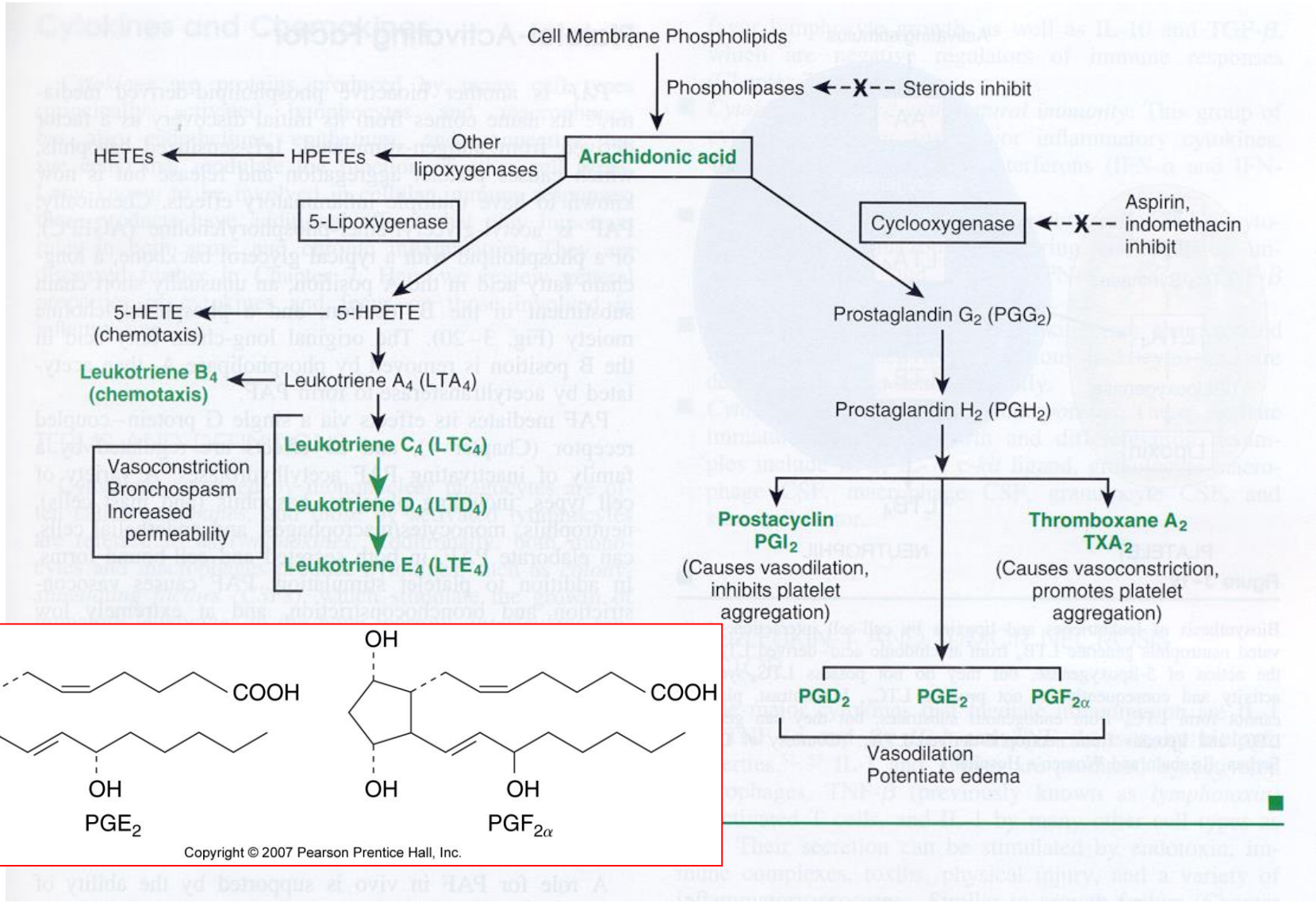
Sune K. Bergström

Bengt I. Samuelsson

John R. Vane

The Nobel Prize in Physiology or Medicine 1982 was awarded jointly to Sune K. Bergström, Bengt I. Samuelsson and John R. Vane *"for their discoveries concerning prostaglandins and related biologically active substances"*.

Arachidonic Acid Metabolites



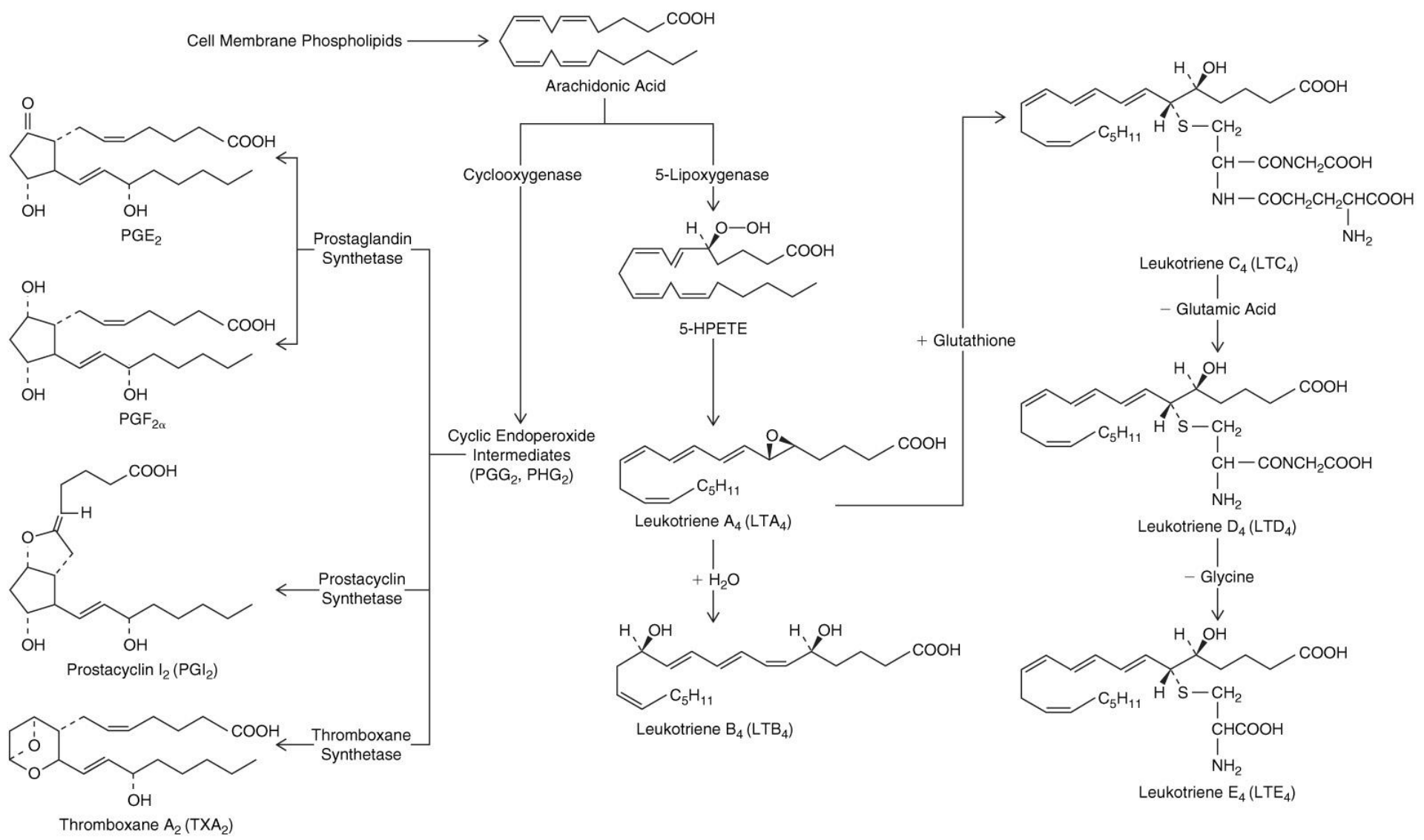


Fig. 3.13. General scheme for prostaglandin, prostacyclin, thromboxane, and leukotriene biosynthesis.

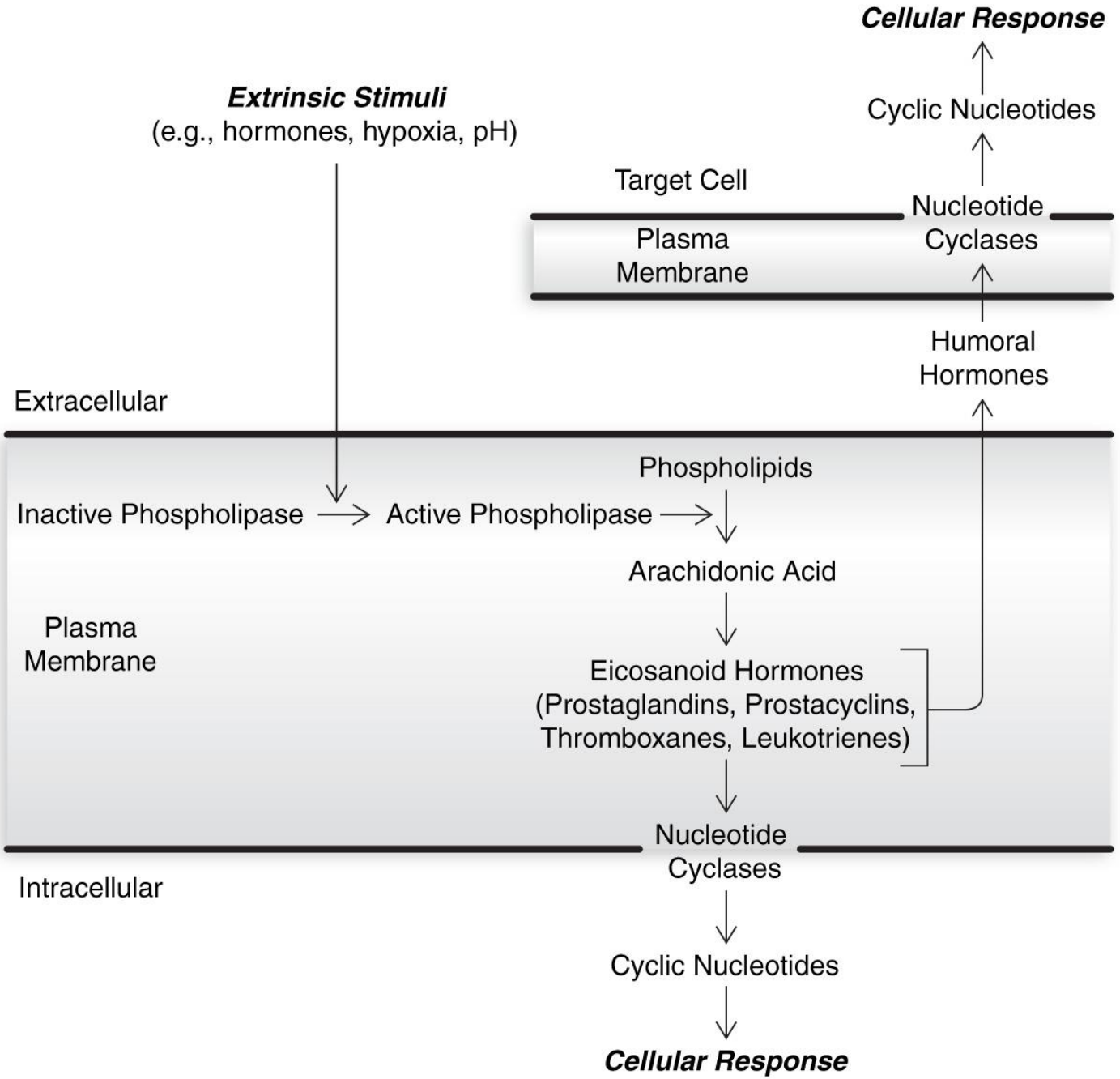


Fig. 3.14. General scheme of eicosanoid biosynthesis and mechanisms of action.

TABLE 20-3

Actions of Prostaglandins

BODY SYSTEM ACTIVITY	ACTIONS OF PROSTAGLANDINS
Reproductive System	Promote sperm transport by action on smooth muscle in the male and female reproductive tracts
	Play a role in ovulation
	Play important role in menstruation
	Contribute to preparation of the maternal portion of the placenta
	Contribute to parturition
Respiratory System	Some promote bronchodilation, others bronchoconstriction
Urinary System	Increase the renal blood flow
	Increase excretion of water and salt
Digestive System	Inhibit HCl secretion by the stomach
	Stimulate intestinal motility
Nervous System	Influence neurotransmitter release and action
	Act at the hypothalamic "thermostat" to increase body temperature
	Exacerbate sensation of pain
Endocrine System	Enhance cortisol secretion
	Influence tissue responsiveness to hormones in many instances
Circulatory System	Influence platelet aggregation
Fat Metabolism	Inhibit fat breakdown
Defense System	Promote many aspects of inflammation, including development of fever

Inflammatory Actions of Eicosanoids

Action	Metabolite
• Vasoconstriction	• Thromboxane A_2, leukotrienes C_4, D_4, E_4
• Vasodilation	• PGI_2, PGE_1, PGE_2, PGD_2
• Increased vascular permeability	• Leukotrienes C_4, D_4, E_4
• Chemotaxis, leukocyte adhesion	• Leukotriene B_4, HETE, lipoxins

The following is a comparison of different types of prostaglandin, prostaglandin I_2 (PGI_2), prostaglandin E_2 (PGE_2), and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$).

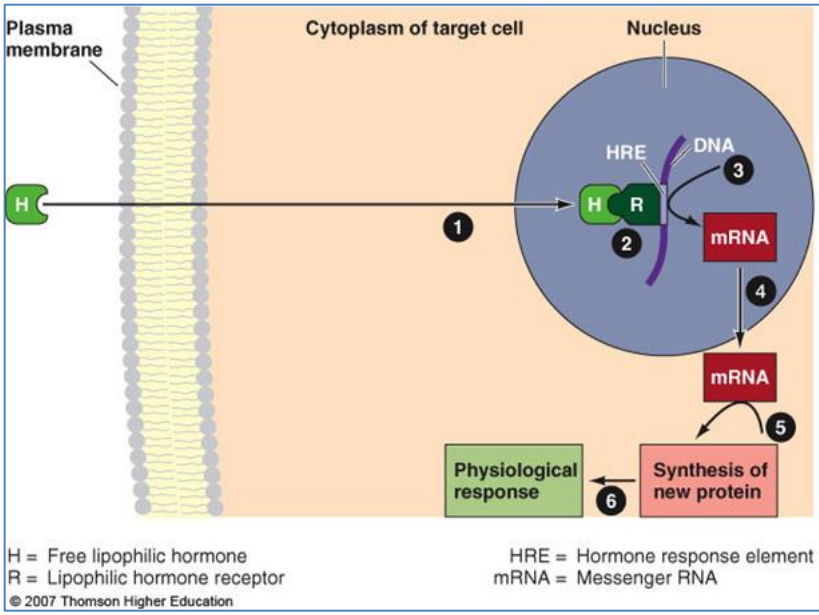
Type	Receptor	Function
PGI_2	IP	•vasodilation •inhibit platelet aggregation •bronchodilatation
PGE_2	EP_1	•bronchoconstriction •GI tract smooth muscle contraction
	EP_2	•bronchodilatation •GI tract smooth muscle relaxation •vasodilatation
	EP_3	•↓ gastric acid secretion •↑ gastric mucus secretion •uterus contraction (when pregnant) •GI tract smooth muscle contraction • lipolysis inhibition •↑ autonomic neurotransmitters •↑ platelet response to their agonists and ↑ atherothrombosis in vivo
	Unspecified	•Hyperalgesia •Pyrogenic
$PGF_{2\alpha}$	FP	•uterus contraction •bronchoconstriction

9. Nuclear hormone receptors

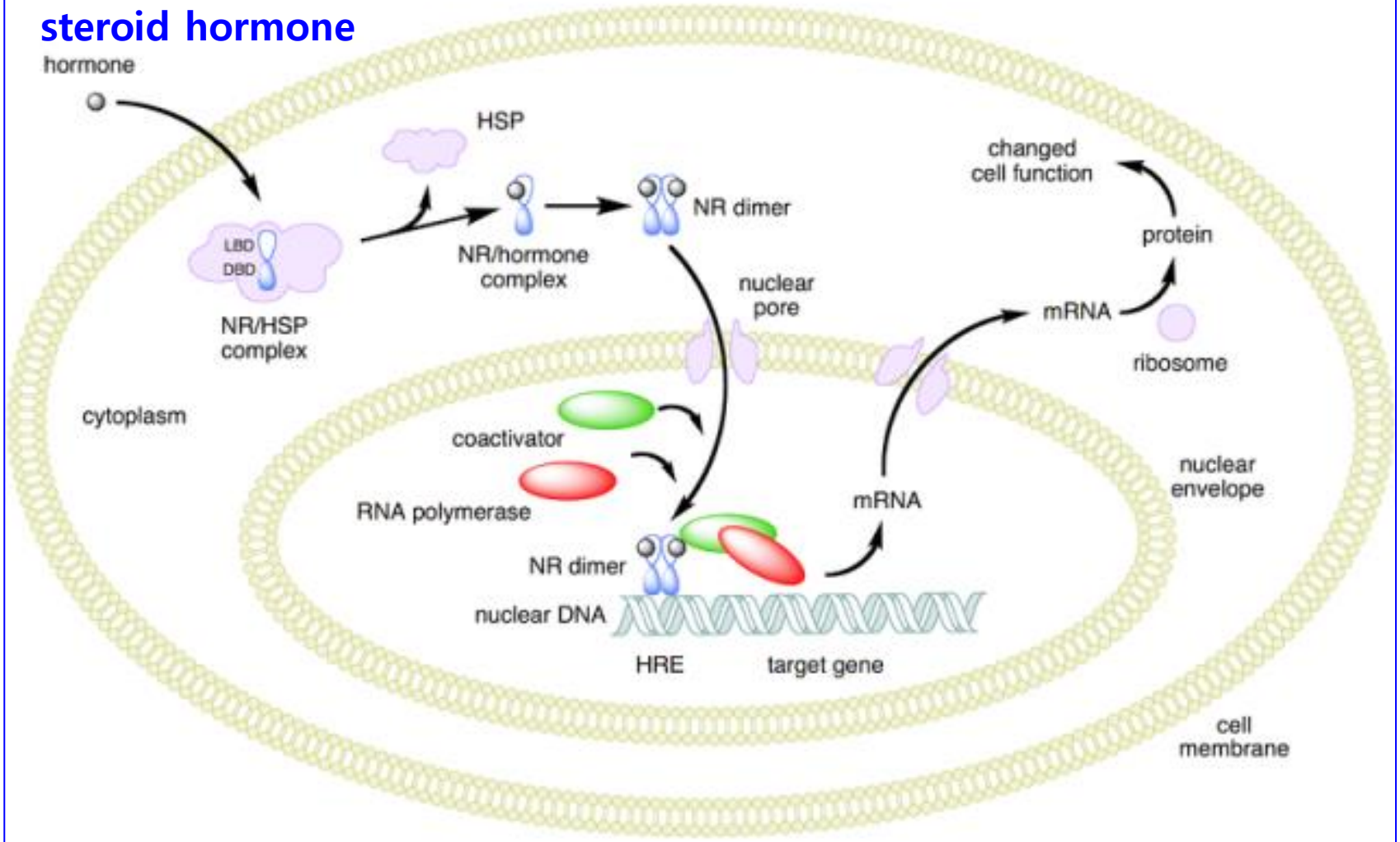
- Nuclear receptors are ligand-regulated transcription factor that control gene expression by binding to target genes usually in the region near their promoters.

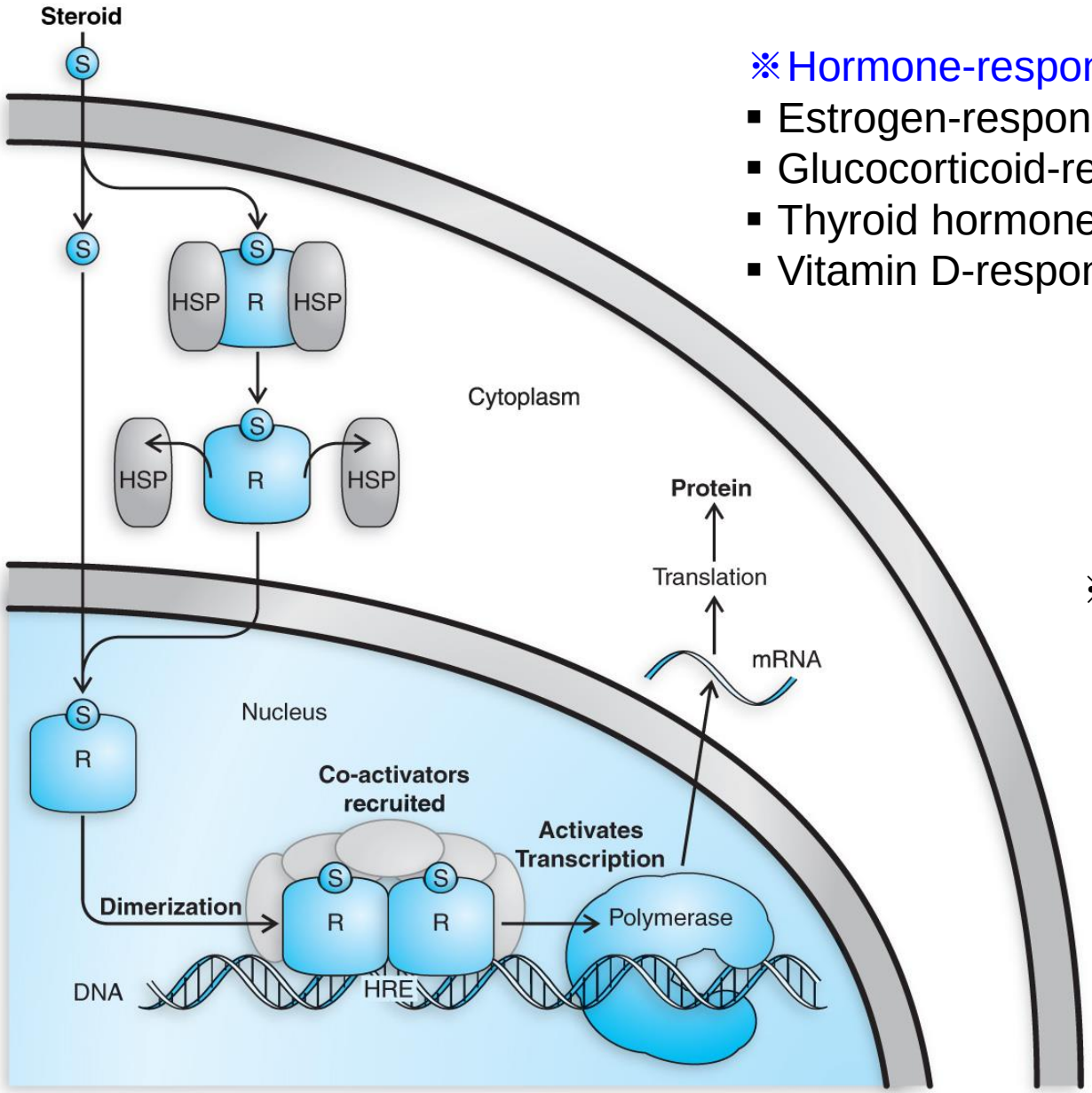
1) Class I: steroid hormone. Are present in either the cytosol or the nucleus. Ligand binding promotes dissociation of certain proteins and formation of receptor homodimers that bind to specific DNA element (HREs)

2) Class II: thyroid hormone, retinoid, vitamin D, PPAR. Receptors already present in the nucleus in the unliganded state. They are commonly active in the absence of hormone.



steroid hormone





※Hormone-responsive elements (HRE)

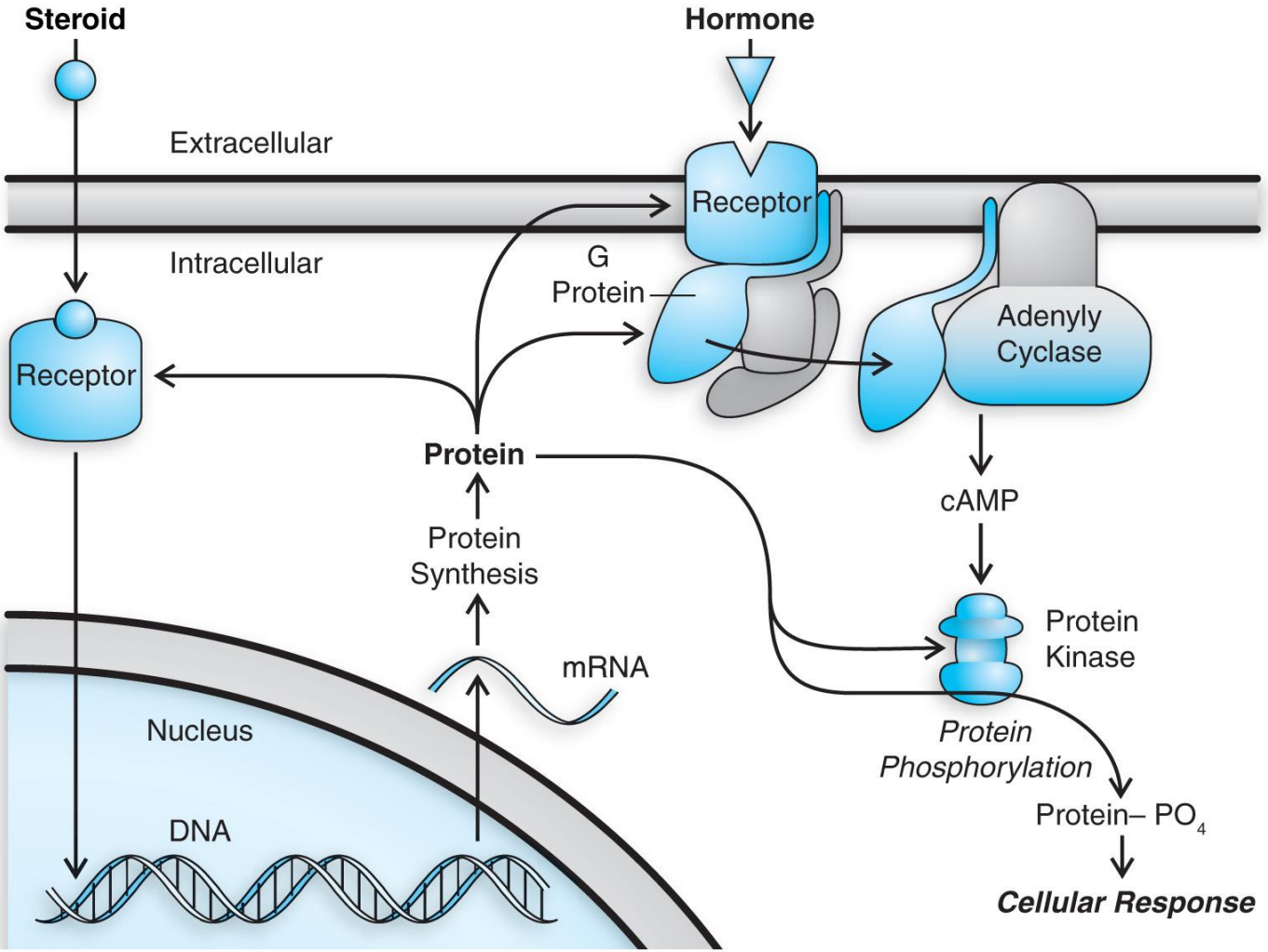
- Estrogen-response element (ERE)
- Glucocorticoid-responsive element (GRE)
- Thyroid hormone-responsive element (T_3RE)
- Vitamin D-responsive element (VDRE)

※HSP, Heat shock protein

Fig. 3.15. Mechanisms of steroid hormone action.

10. Permissive, additive, and synergistic actions of hormones

- Permissive actions of steroid hormones



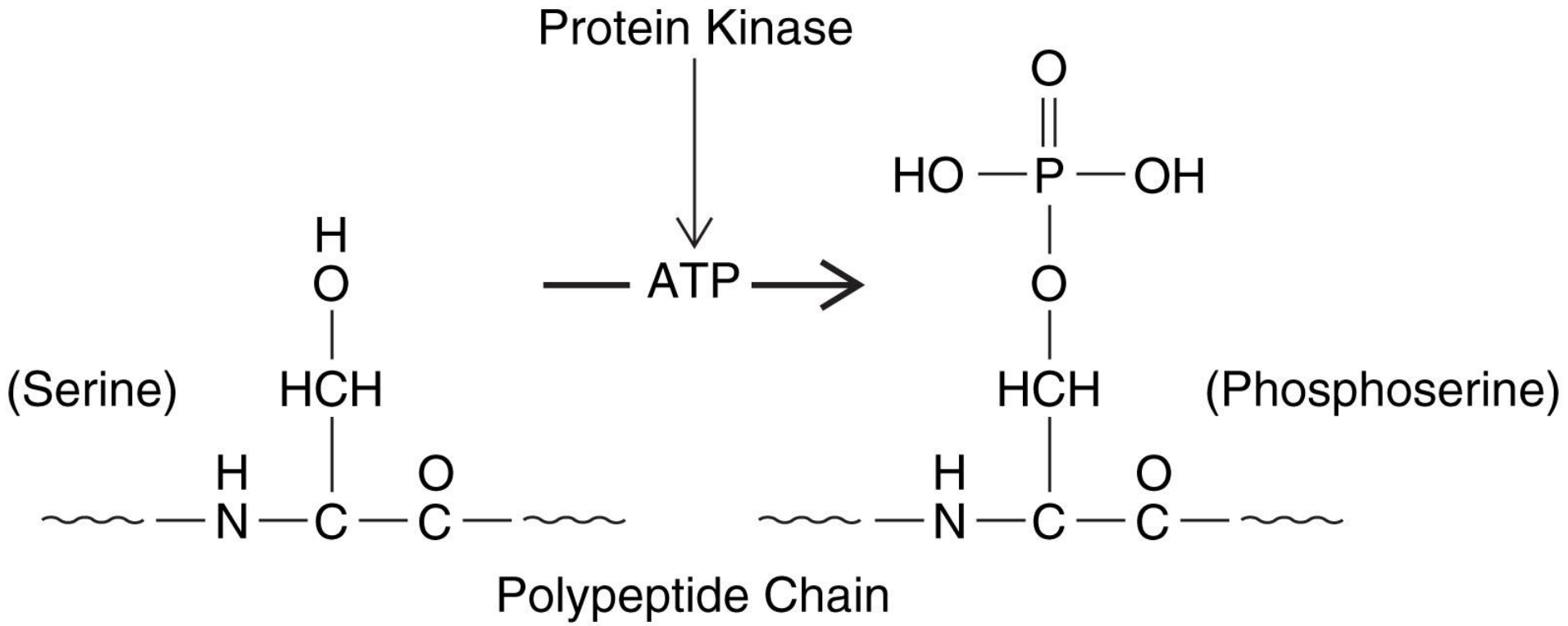


Fig. 3.17. Protein kinase phosphorylation of a protein substrate.

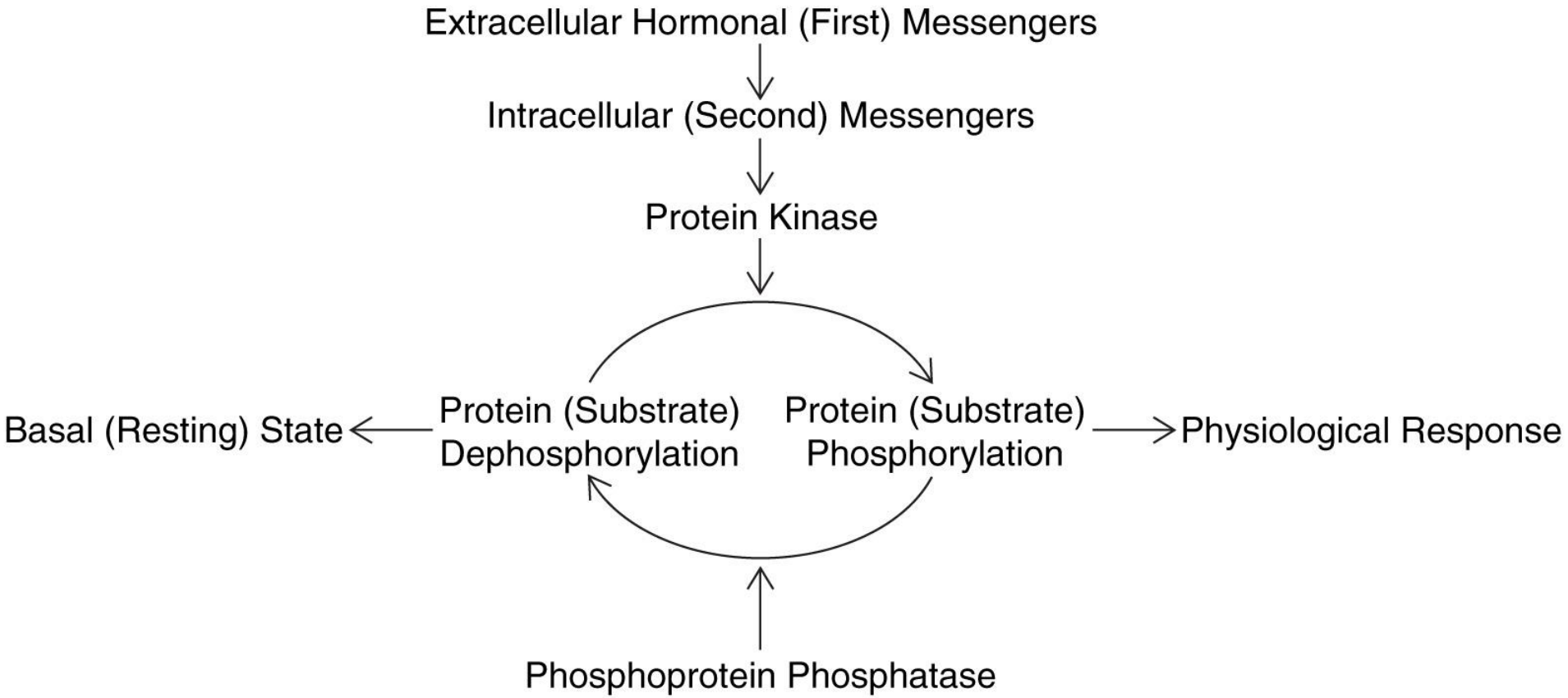
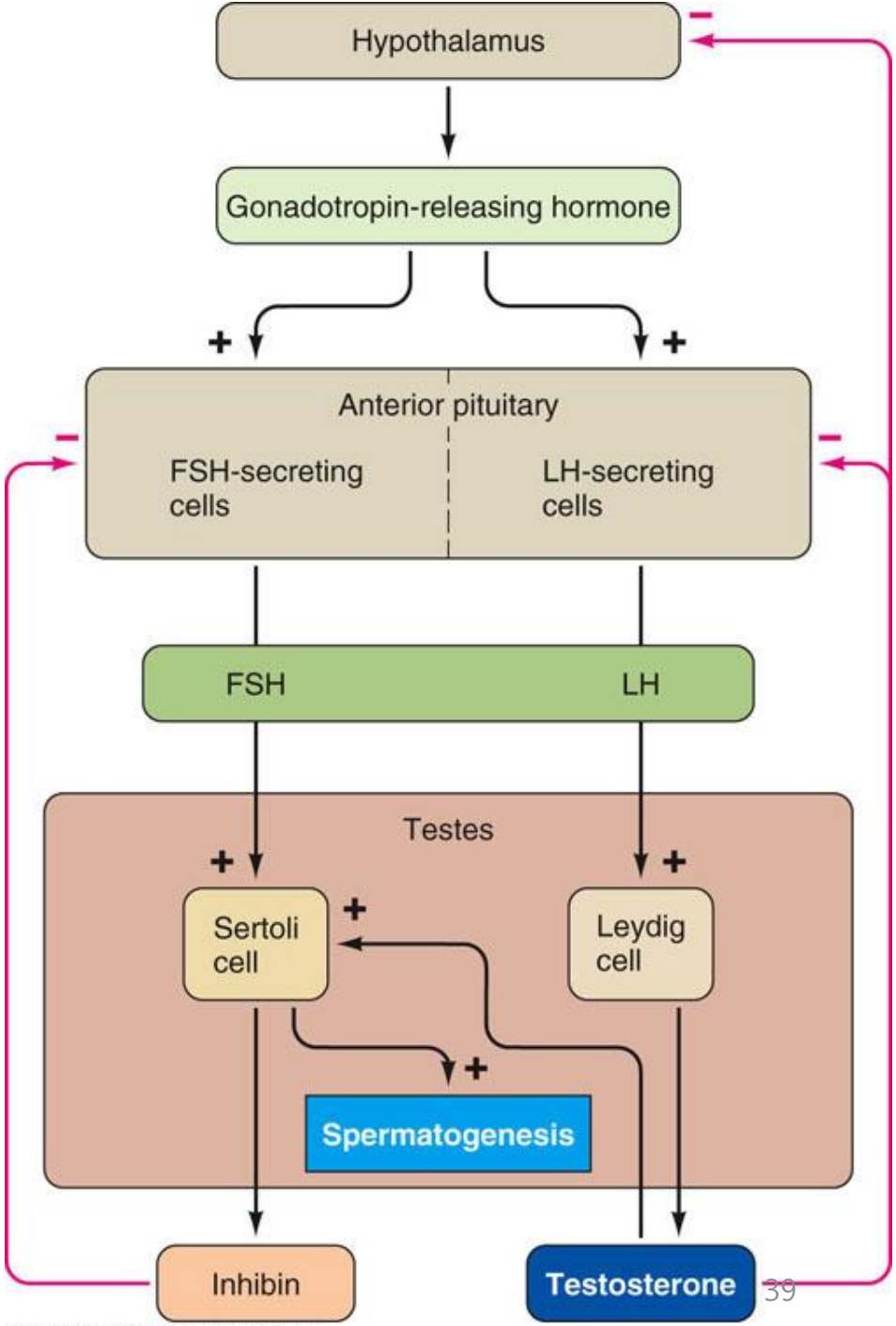


Fig. 3.18. Role of protein kinases and phosphoprotein phosphatases in hormone action.

- Additive effects of hormones
  - epinephrine and glucagon: stimulate glycogenolysis and the release of glucose by liver cells
- Synergism: FSH and LH

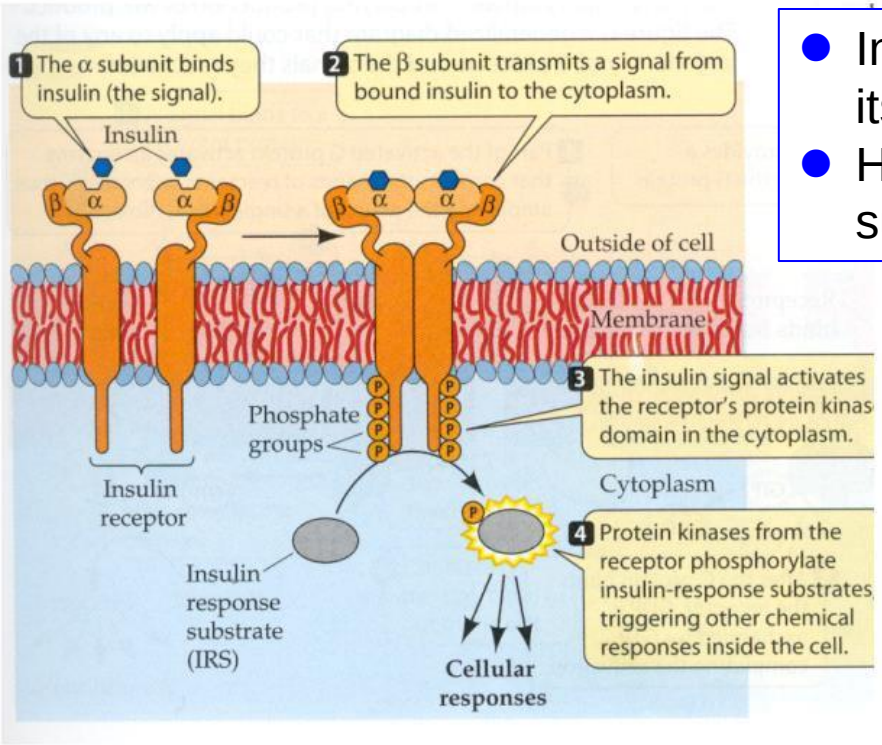
- Synergism: FSH and LH
  LH and FSH from the anterior pituitary control testosterone secretion and spermatogenesis

- GnRH: gonadotropin-releasing hormone
- LH: luteinizing hormone
- FSH: follicle-stimulating hormone
- Inhibin



11. Receptor regulation

- Negative or “down” regulation: decrease numbers of receptors
- Positive or “up” regulation: increase numbers of receptors



- Interaction of insulin and its receptor
- Heterodimer: α and β subunits

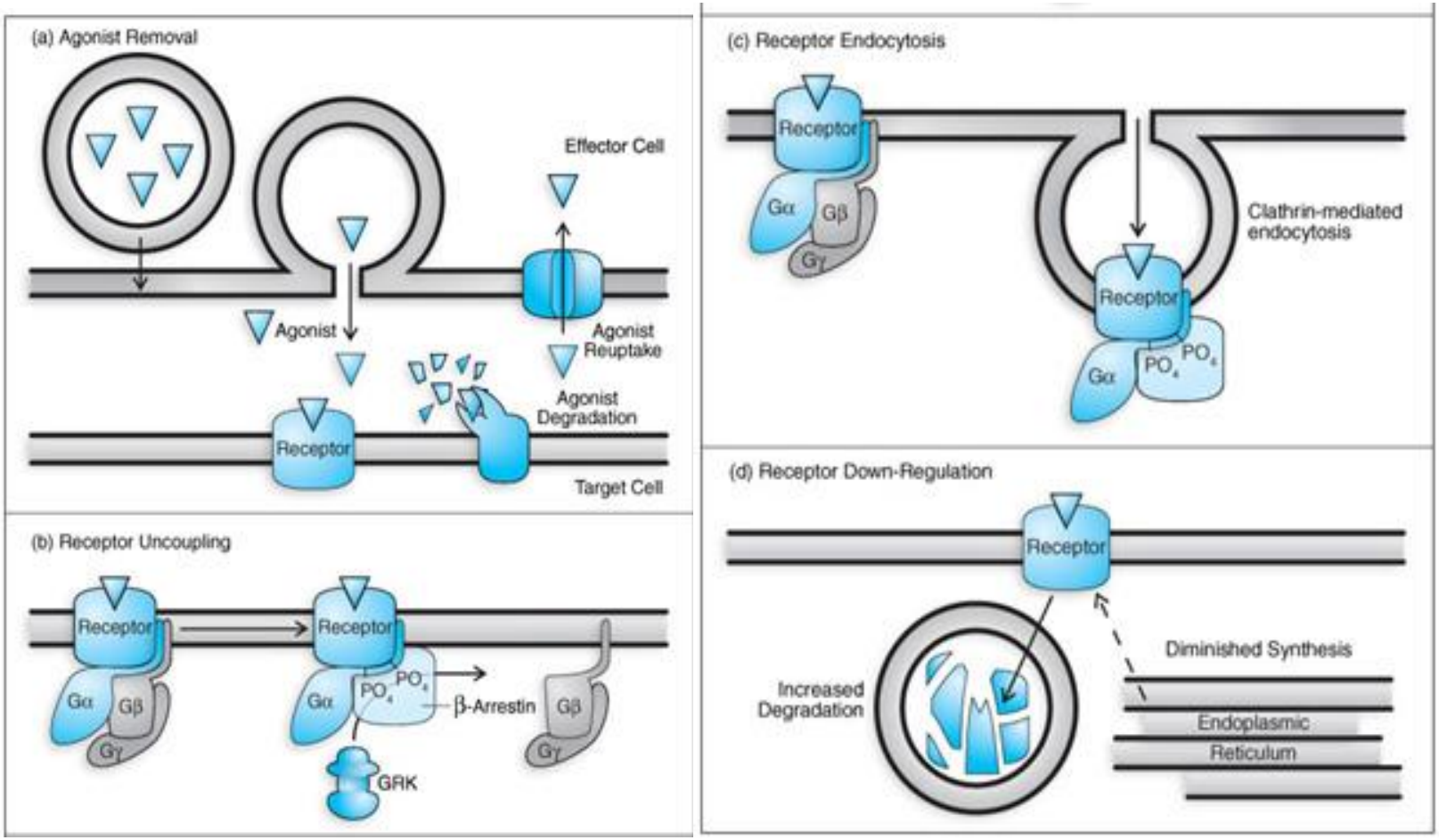
12. Termination of hormone action

a. Agonist removal

b. Receptor uncoupling

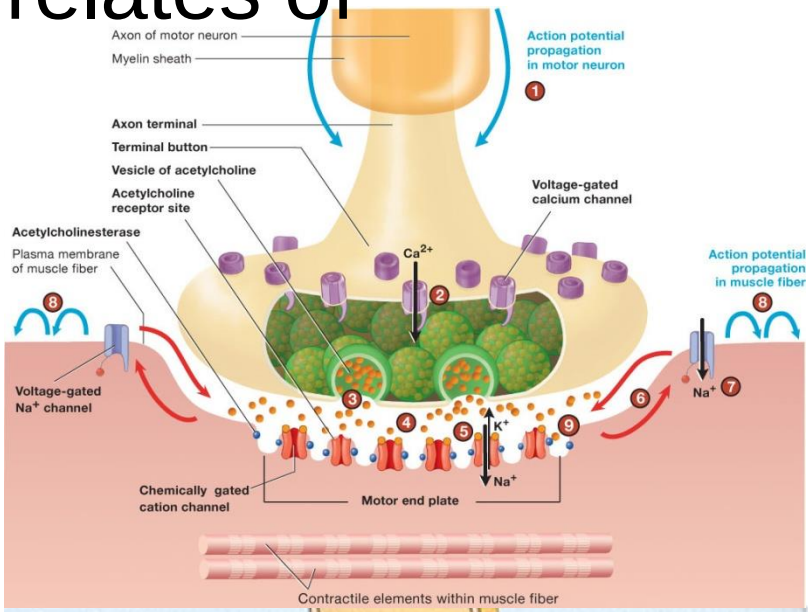
c. Receptor endocytosis

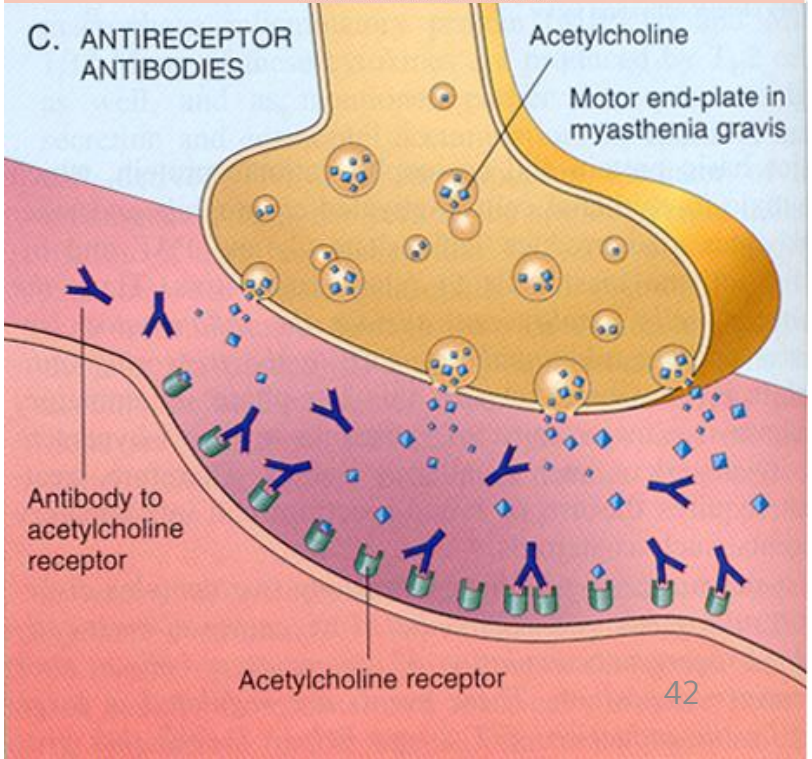
d. Receptor down-regulation



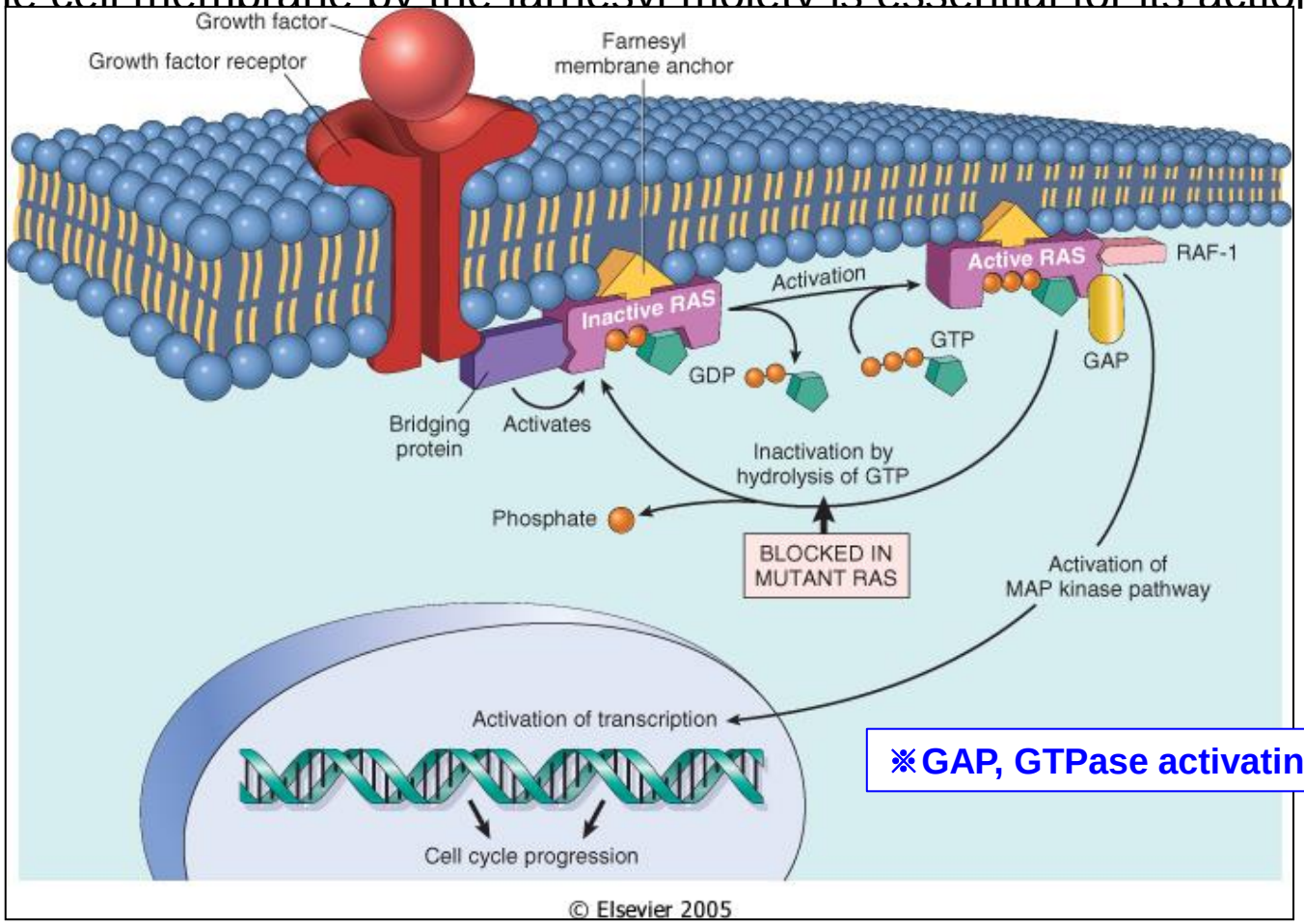
13. Pathophysiological correlates of hormone action

- Myasthenia gravis inactivates Ach receptor sites.
  - Muscular weakness
  - Autoimmune disease
  - Antireceptor antibodies erroneously bind to acethylcholine receptor and give false signals.
  - AChE destroys much of the Ach.
  - Treatment: neostigmine that inhibits AChE temporarily, prolongs the action of Ach at the neuromuscular junction.

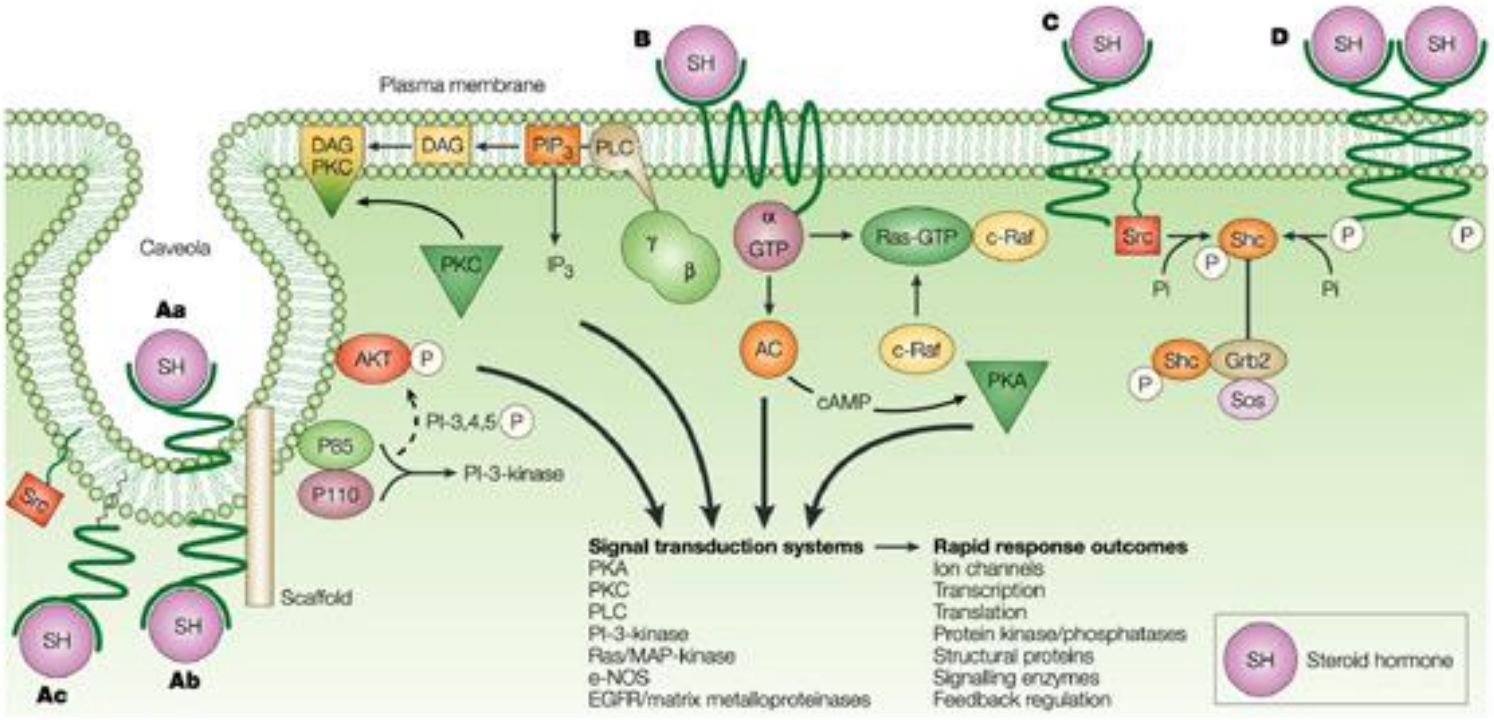




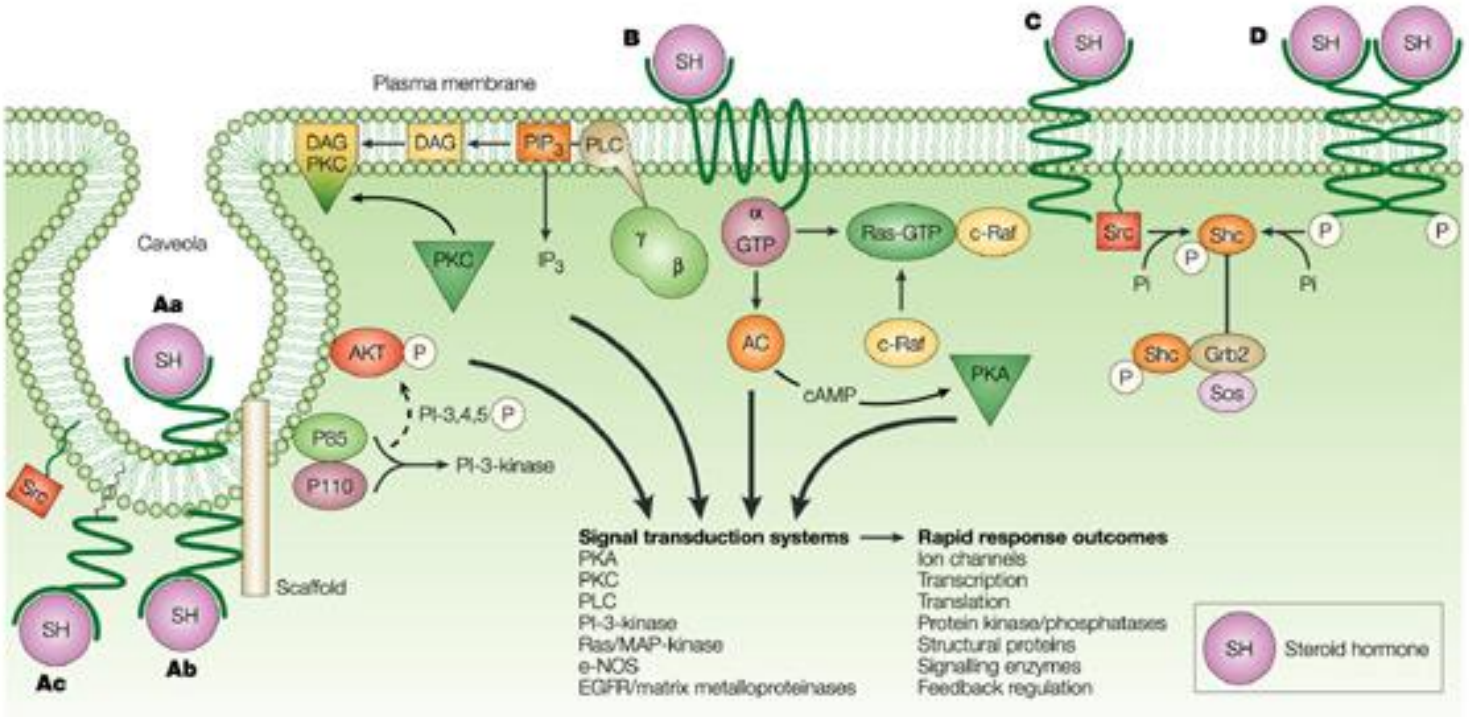
●Mutation of G protein: Model for action of RAS genes. When a normal cell is stimulated through a growth factor receptor, inactive (GDP-bound) RAS is activated to a GTP-bound state. Activated RAS recruits RAF and stimulates the MAP-kinase pathway to transmit growth-promoting signals to the nucleus. The mutant RAS protein is permanently activated because of inability to hydrolyze GTP, leading to continuous stimulation of cells without any external trigger (becoming **cancer cells**). The anchoring of RAS to the cell membrane by the farnesyl moiety is essential for its action.



※GAP, GTPase activating protein



- A | Three classes of membrane receptor are shown illustrating **the classic nuclear steroid-hormone receptor associated with a caveola**.
  - Aa | The receptor is technically outside the cell and is associated with the outer surface of the plasma membrane in the flask of the caveola.
  - Ab | The receptor is tethered by a scaffolding protein to the plasma membrane on the inner surface of a caveola.
  - Ac | The receptor is tethered to the caveolae by a palmitic acid molecule that is esterified to a receptor Ser or Thr with the fatty-acid side chain 'inserted' into the membrane (palmitoylation).
- B | A **G-protein-coupled receptor** with its ligand-binding domain on the outside of the cell and a seven-membrane spanning peptide transition followed by an intracellular peptide domain that can bind G, and proteins.



C | A **single-spanning membrane receptor** with intrinsic kinase activity that might be functional as a monomer.

D | **Same as C except a homodimer**. Caveolae are flask-shaped membrane invaginations present in the outer cell membrane of many cells; they are believed to serve as a 'platform' to accumulate or 'dock' signal-transduction-related molecules.The signal-transduction systems are listed as candidates for mediating rapid responses to steroid hormones and are based on published data. The details remain to be defined on the basis of careful experimentation. The two ovals with Ras-GTP and c-Raf 'touching' are to suggest that c-Raf was recruited to the complex. AC, adenylyl cyclase; DAG, diacylglycerol; EGFR, epidermal growth factor receptor; e-NOS, endothelial nitric oxide synthase; IP_3, inositol triphosphate; MAP, mitogen-activated protein; PI3K, phosphatidylinositol 3-kinase; PIP_3, phosphatidylinositol triphosphate; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase