Chemical Signaling

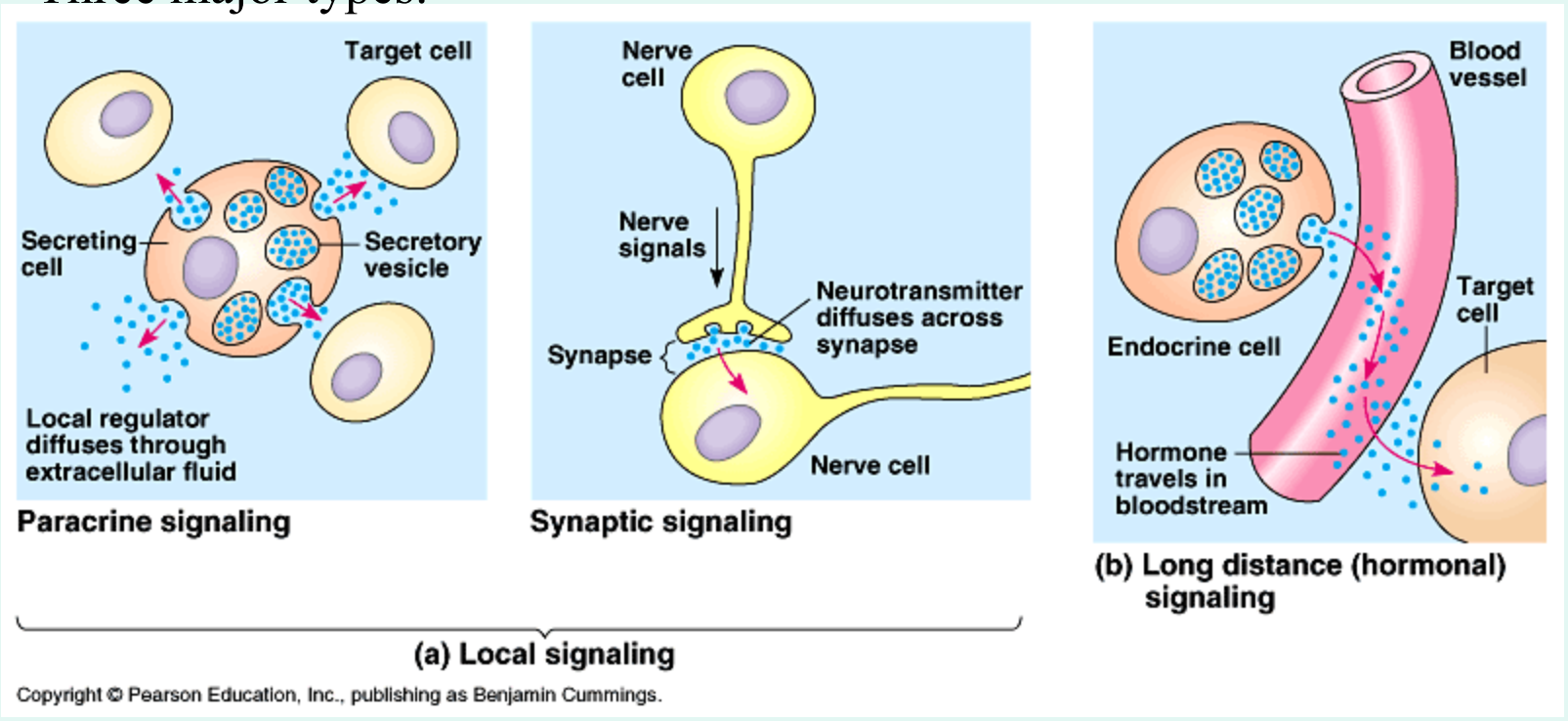
Hormonal Signal Transduction Pathways

Types of Chemical Signaling

Chemical signaling between cells is one of the most important ways that activities of tissues and organs are coordinated.

The nervous system is the other major coordinating system in animals, but even here chemical signaling is used between adjacent neurons.

Three major types:

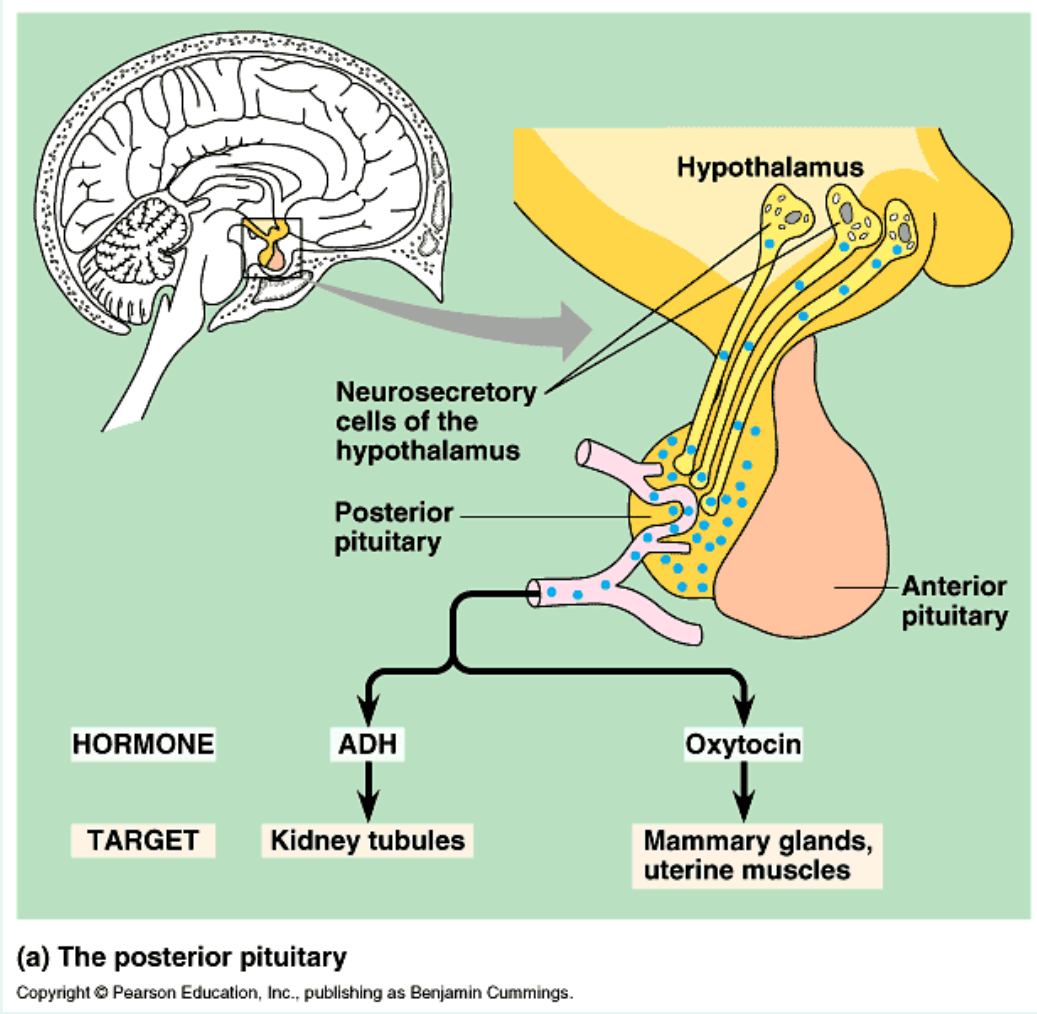


Sites of Hormone Release

More commonly, especially in the vertebrates, the sources of hormones are specialized glands called endocrine glands.

Even here the source of the hormone is often modified nerve cells or neurosecretory cells.

Some organs that have other functions also release hormones - for example, many hormones are released by different organs of the digestive system.



(a) The posterior pituitary

Types of Responses Controlled by Hormones

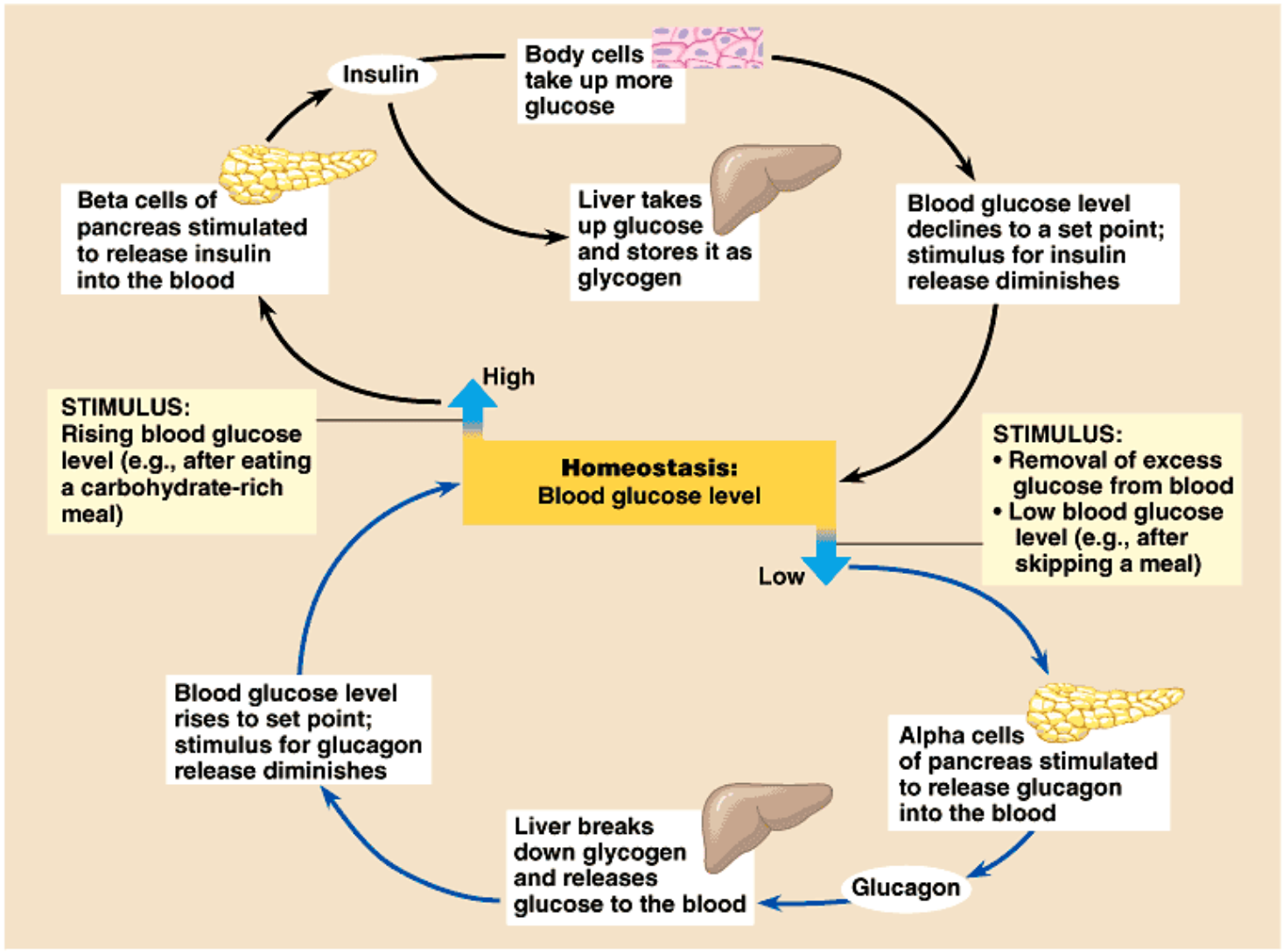
Hormones are not only used for long-distance signaling around the body, but they also provide for more prolonged regulatory responses.

Prolonged responses can persist for minutes, hours, or even many days.

Examples include maintenance of blood osmolarity, blood glucose, metabolic rate of specific tissues, control of reproductive cycles.

The nervous system is used for more rapid responses of shorter duration.

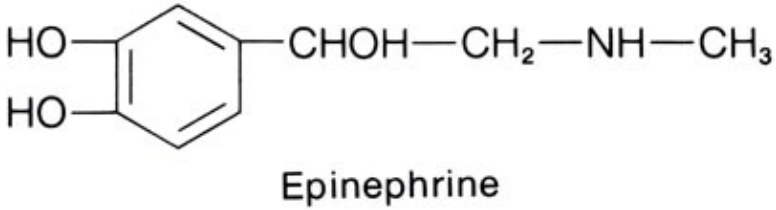
Example of Regulation of Blood Glucose



Types of Hormones

1) Amines:

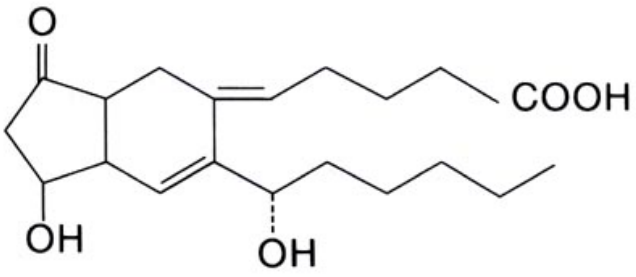
Catecholamines, epinephrine and norepinephrine, derived from amino acids



Epinephrine

2) Prostaglandins:

Cyclic unsaturated fatty acids

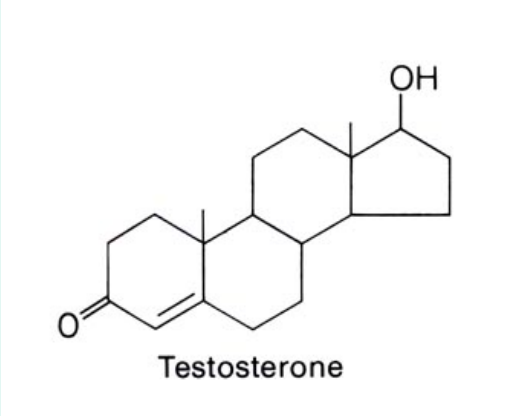


Prostaglandin PGE_2

Types of Hormones

3) Steroid hormones:

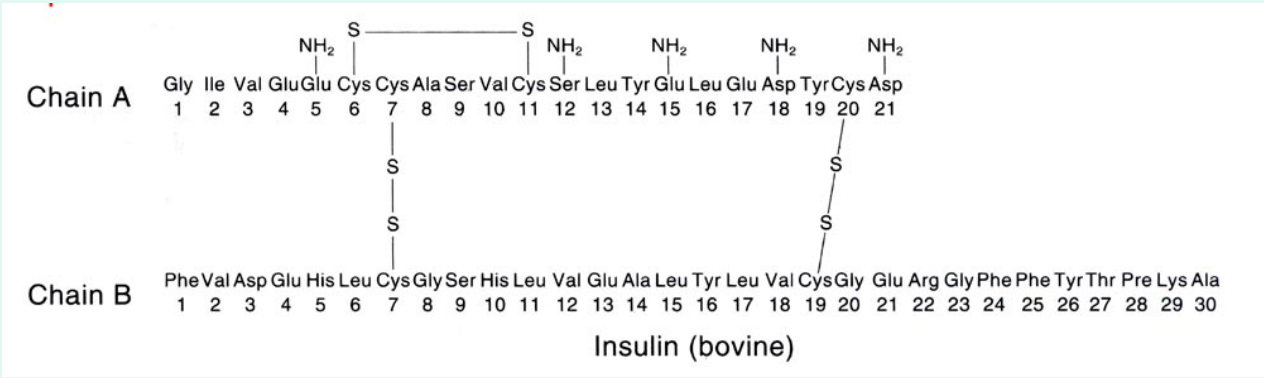
Include testosterone and estrogen, cyclic hydrocarbons derived from cholesterol.



Testosterone

4) Peptides and proteins:

Include insulin

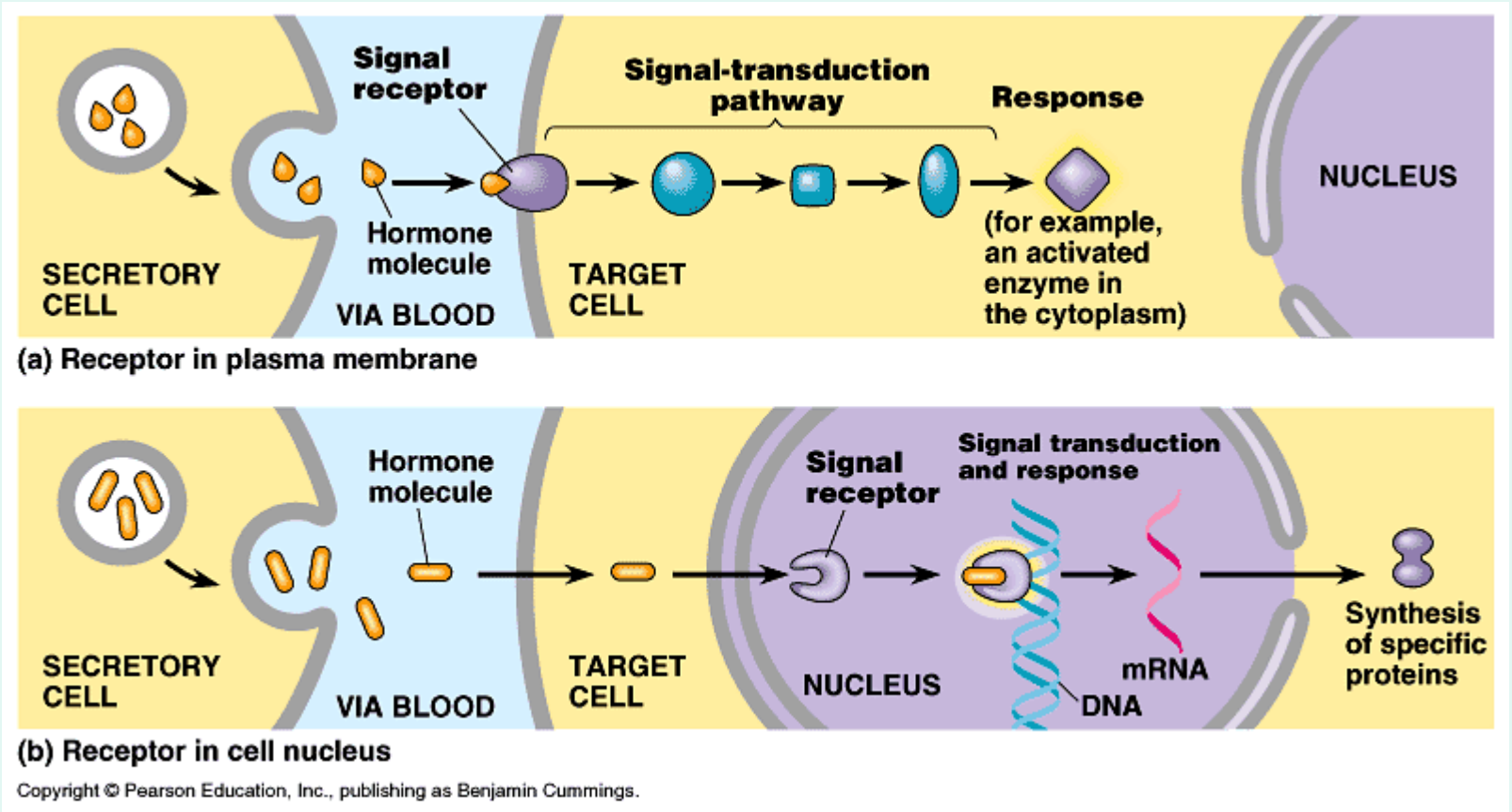


Insulin (bovine)

Three Steps in Cell Signaling

Target organ specificity is the result of specific receptor molecules for the hormone, either on the plasma membrane surface, or in some cases in the cytoplasm, of cells in the target organ.

1) Reception **2) Transduction** **3) Response**



G-Protein-Linked Receptors

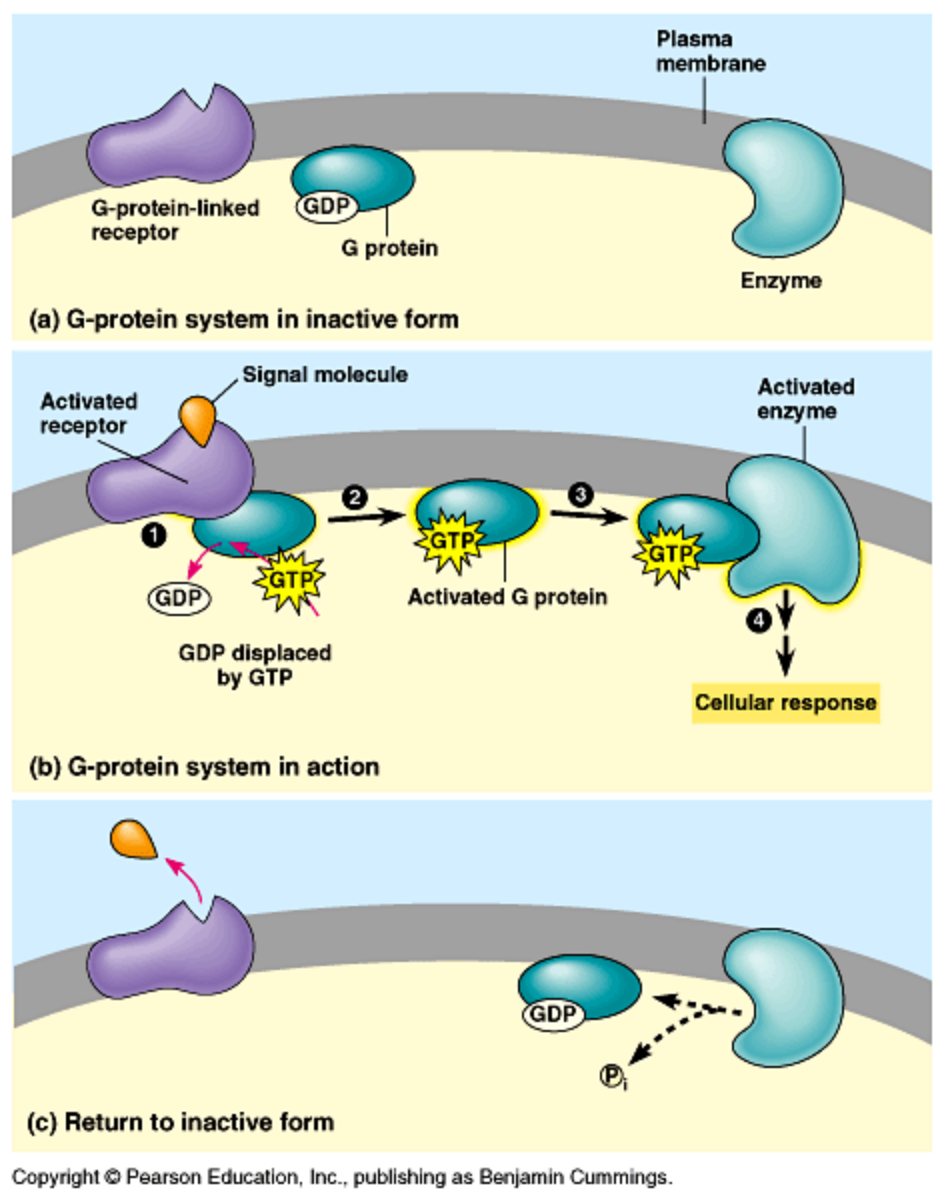
Many hormone receptors on the cell surface function with the help of a G protein.

The G protein is in an active form when it binds GTP and is in an inactive form when GDP is bound.

When a signal molecule (hormone) binds to the receptor in the membrane, GTP binds to the G protein, activating it.

The activated G protein then moves along the membrane to an inactive enzyme, binds to it and activates the enzyme.

The G protein then hydrolyzes GTP to GDP + P_i and both dissociate from the enzyme leaving the G protein inactivated but ready for re-use.

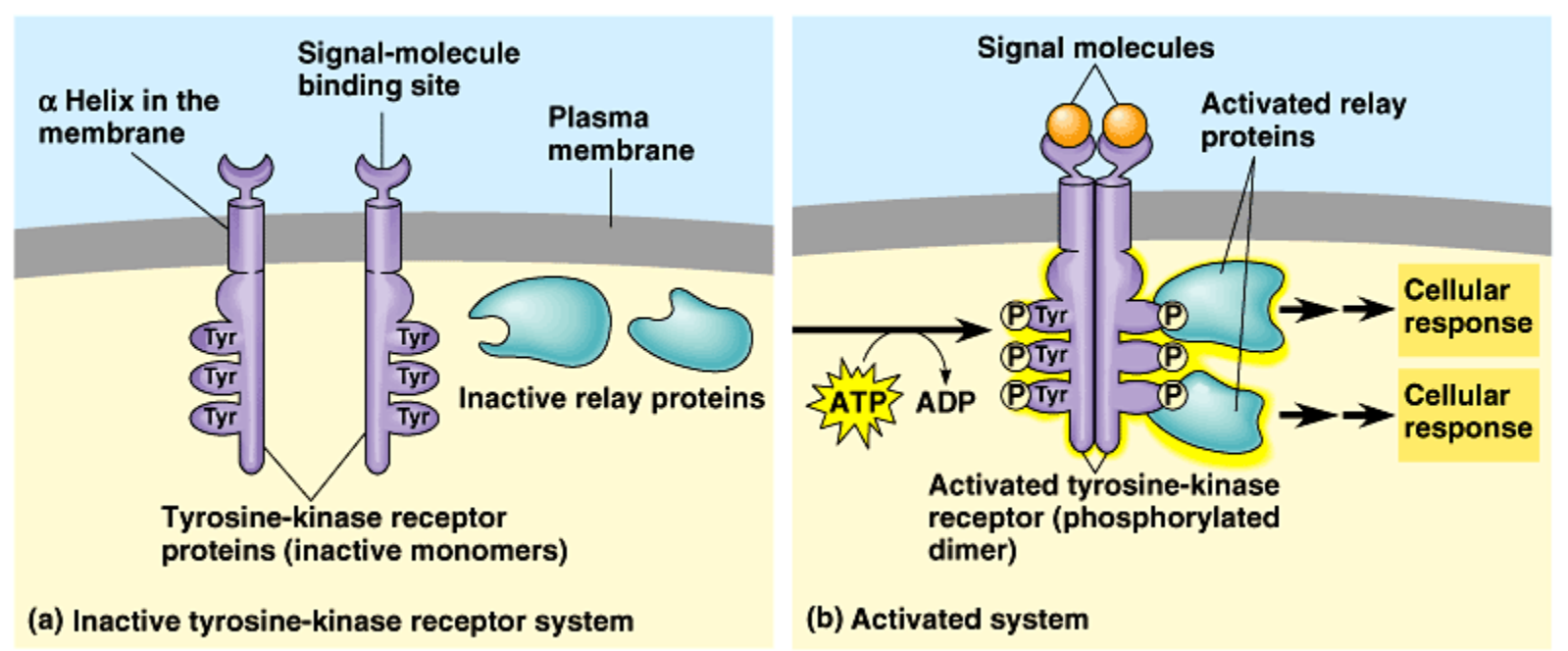


Tyrosine-Kinase Receptors

Binding of signal molecules to inactive tyrosine-kinase receptor monomers causes them to aggregate into dimers and phosphorylate the tyrosines on each other's tails.

Inactive relay proteins then bind to a specific phosphorylated tyrosine on the activated receptor and become activated relay proteins. As many as 10 different relay proteins can be simultaneously activated by a single activated tyrosine-kinase receptor.

Each activated relay protein can trigger a separate transduction pathway leading to multiple end-results in the cell.



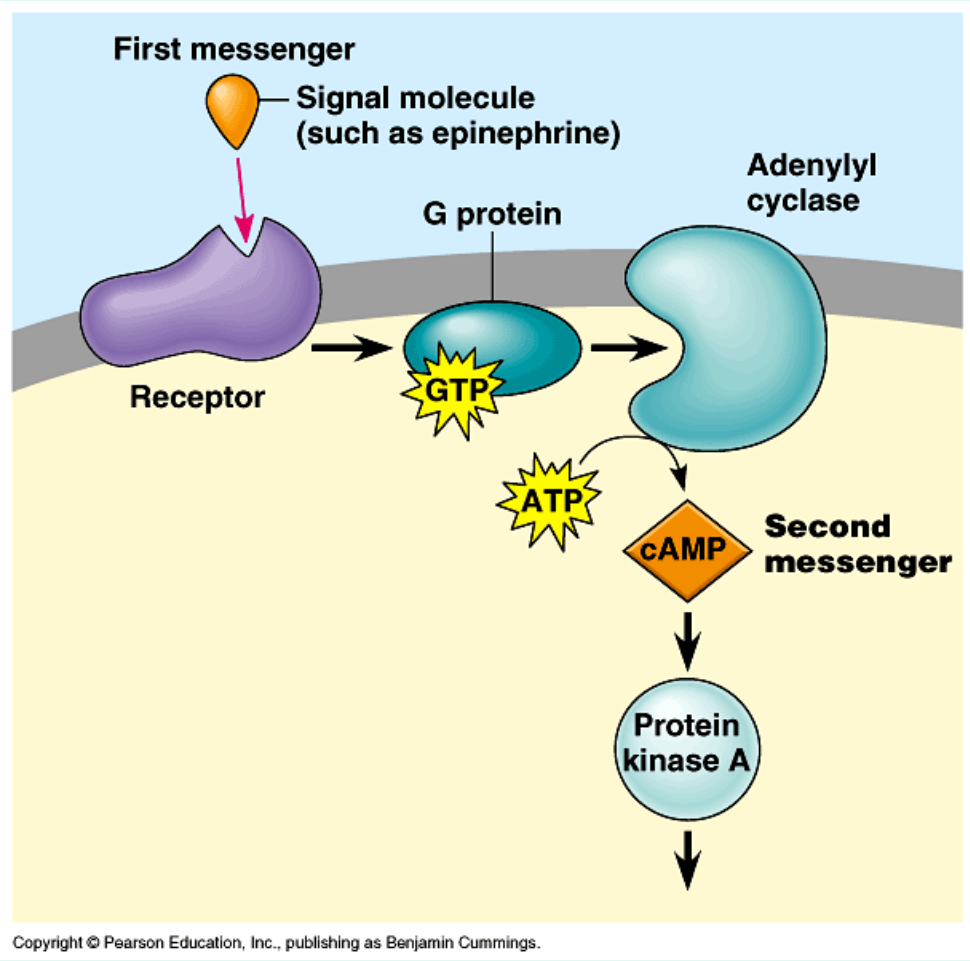
Cyclic AMP as a Second Messenger

Most water-soluble hormones do not readily enter the target cell - they bind to a surface receptor.

In many cases, a G protein is activated which then activates an enzyme, adenylyl cyclase which converts ATP to cyclic AMP (cAMP).

cAMP then serves as a second messenger which activates another enzyme in the cell, often a protein kinase (an enzyme that phophorylates a protein, activating it).

cAMP initiates a chain of events (the signal transduction pathway) that results in some specific response of the cell to the first messenger (hormone).



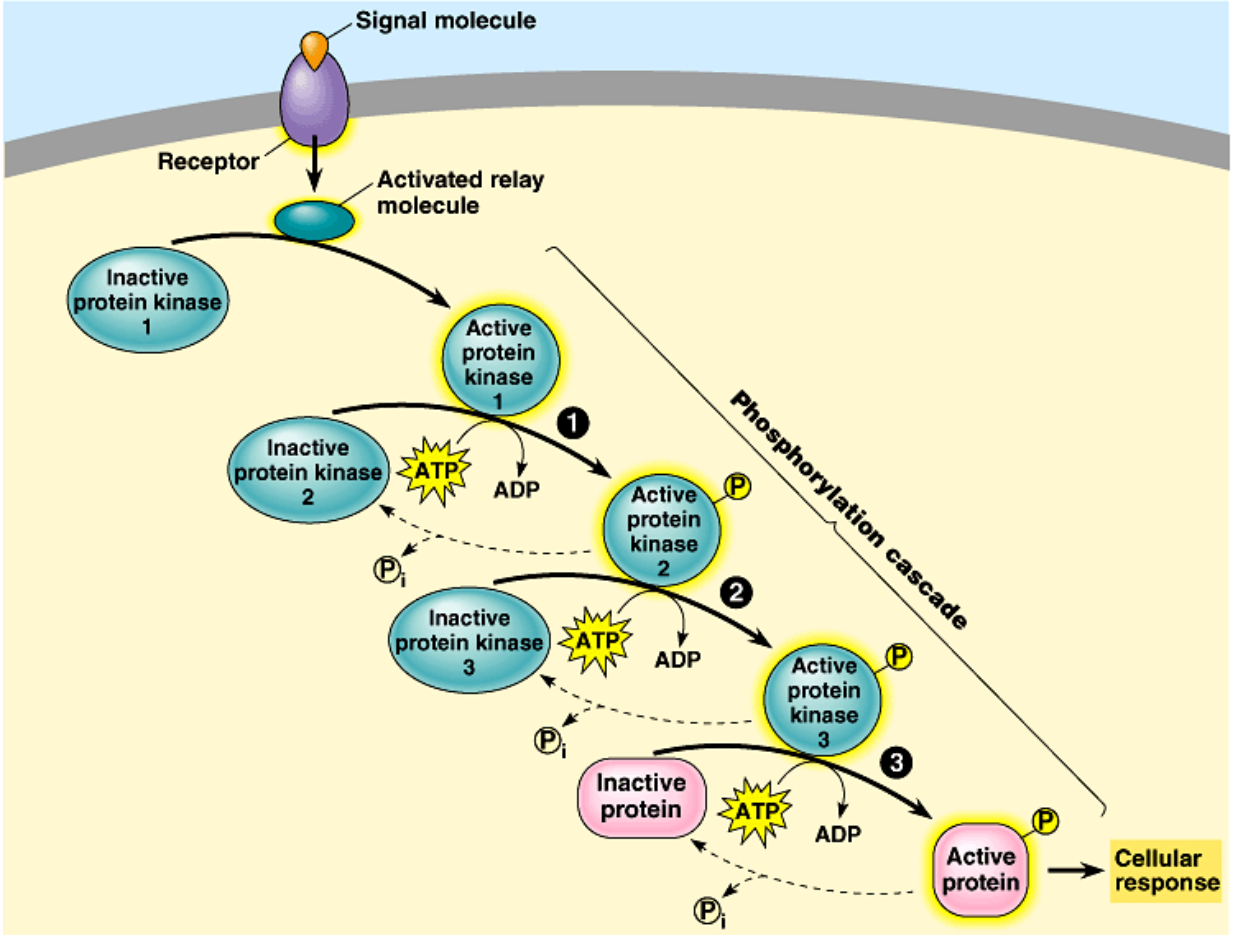
Phosphorylation Cascade

Signal transduction pathways often involve an enzyme phosphorylation cascade.

A series of protein kinases each activate the next in a series by phosphorylating the inactive precursor.

Because each protein kinase is an enzyme it catalyzes activation of a number of the inactive kinases of the next member in the series.

The result is a large amplification of the original signal, binding of a hormone to the external receptor.

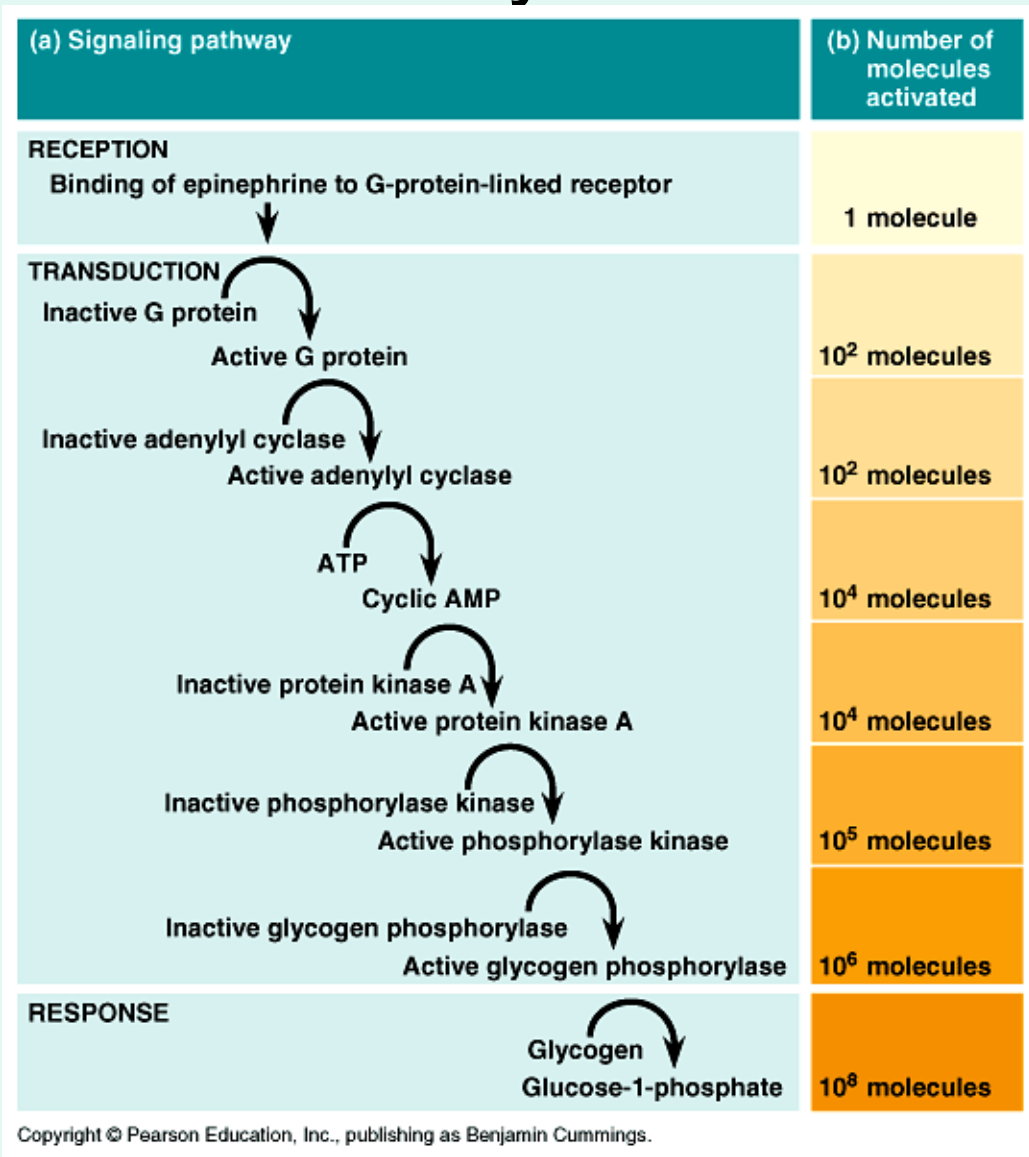


Activation of Glycogen Breakdown by Epinephrine

Epinephrine (adrenaline) triggers a large increase in the rate of glycogen breakdown into glucose-1-P units that then feed into glycolysis.

Binding of one epinephrine molecule activates about 100 G protein molecules which then activate 100 adenylyl cyclase molecules.

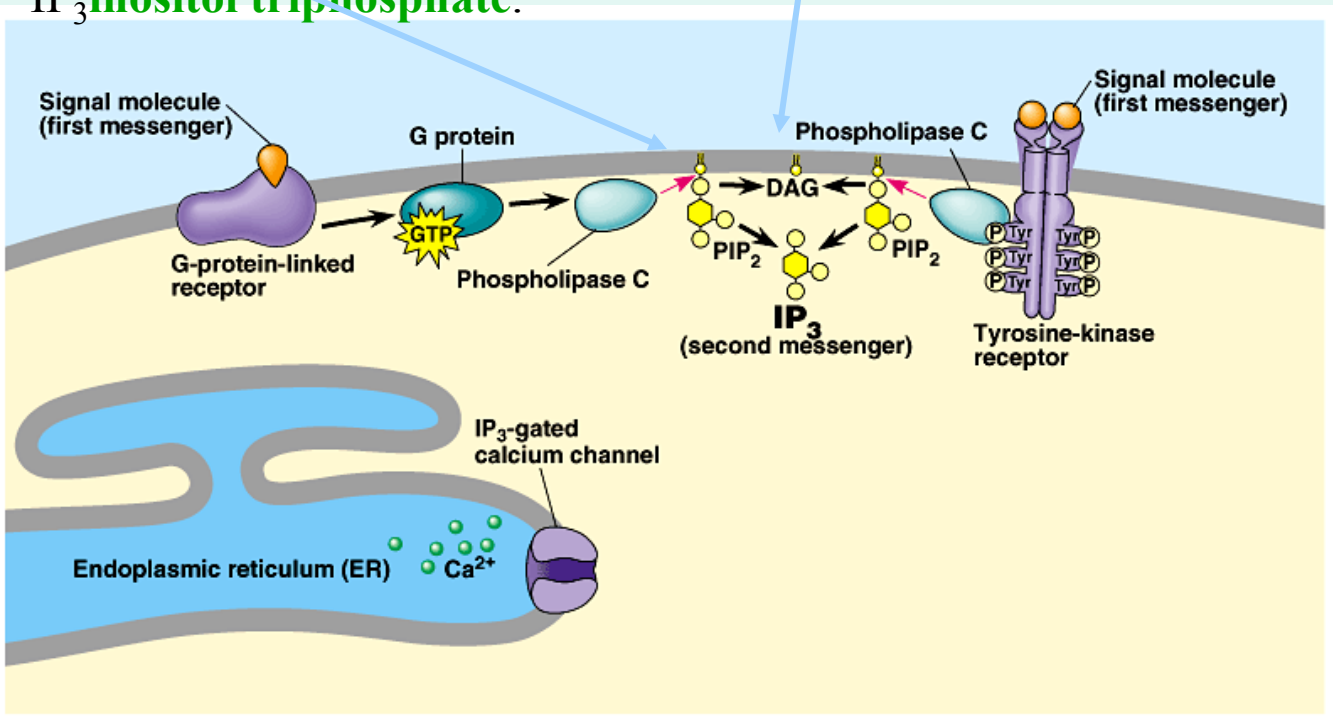
Each adenylyl cyclase generates about 100 cAMP producing 10,000 cAMP molecules each of which can activate an inactive protein kinase A.



Ca^{2+} and IP_3 second messengers

Phospholipase C cleaves a membrane phospholipid

PIP_2 (Phosphatidylinositol bisphosphate) into two second messengers, DAG **diacylglycerol** and IP_3**inositol triphosphate**.

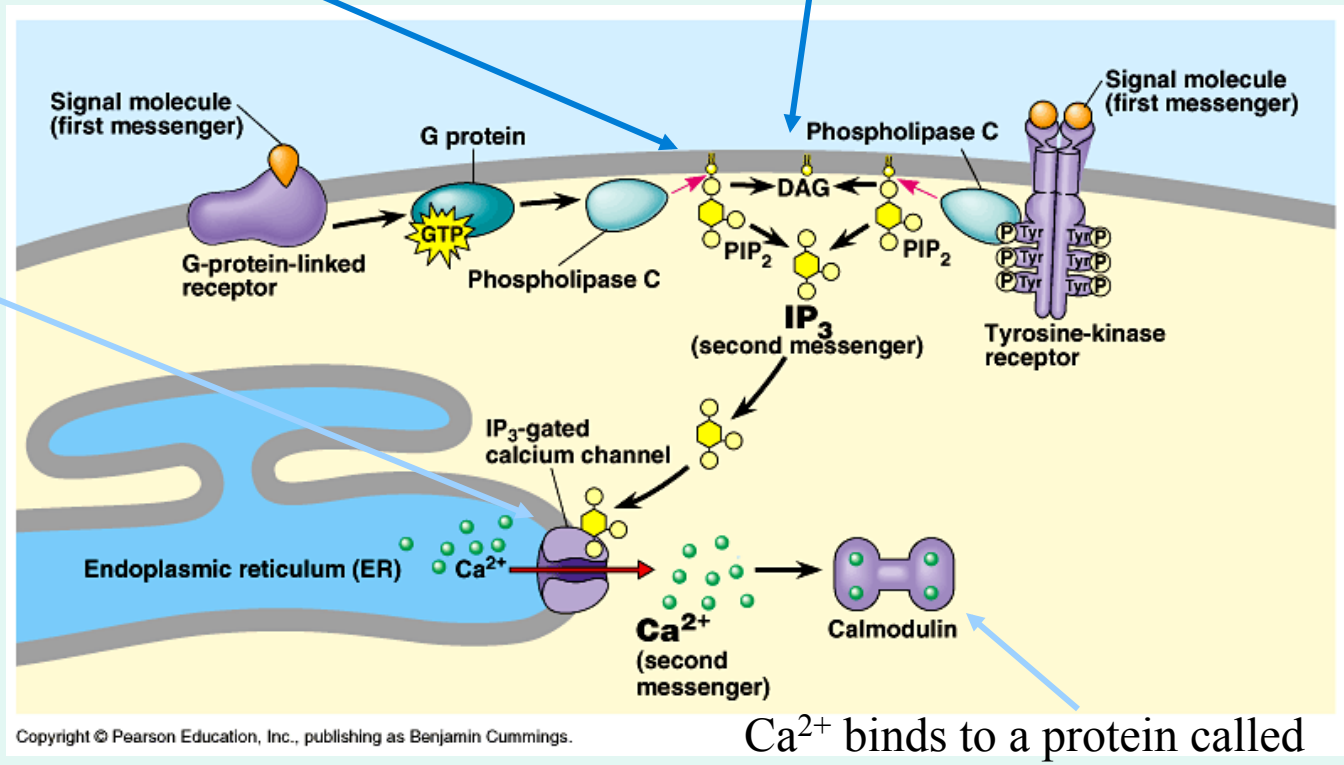


Ca^{2+} and IP_3 second messengers

Phospholipase C cleaves a membrane phospholipid PIP_2 into two second messengers, DAG (diacylglycerol) and IP_3 (inositol triphosphate).

IP_3 diffuses through the cytosol and binds to an IP_3-gated Ca^{2+} channel in the ER membrane.

Ca^{2+} flows out of ER raising Ca^{2+} level in the cytosol.



Ca^{2+} binds to a protein called calmodulin

Ca^{2+} and IP_3 second messengers

Phospholipase C cleaves a membrane phospholipid PIP_2 into two second messengers, DAG and IP_3.

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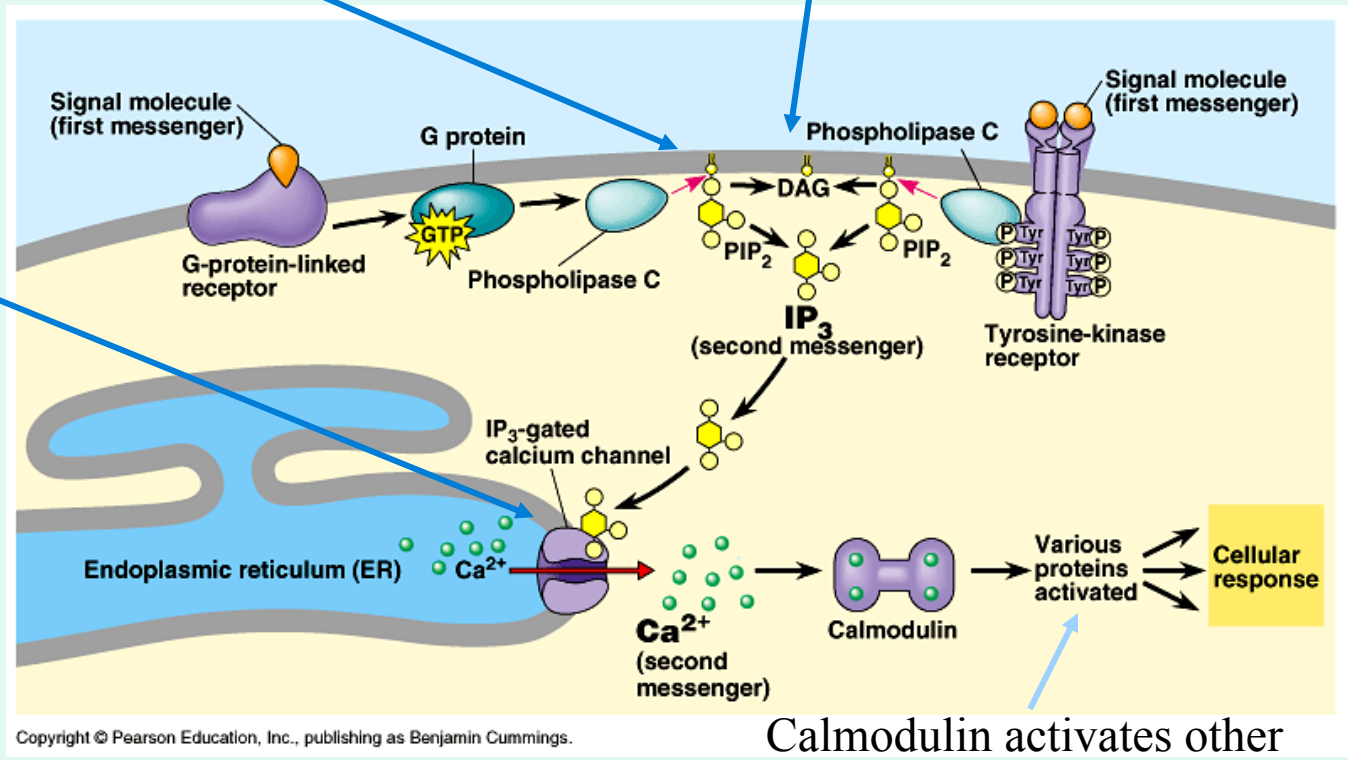
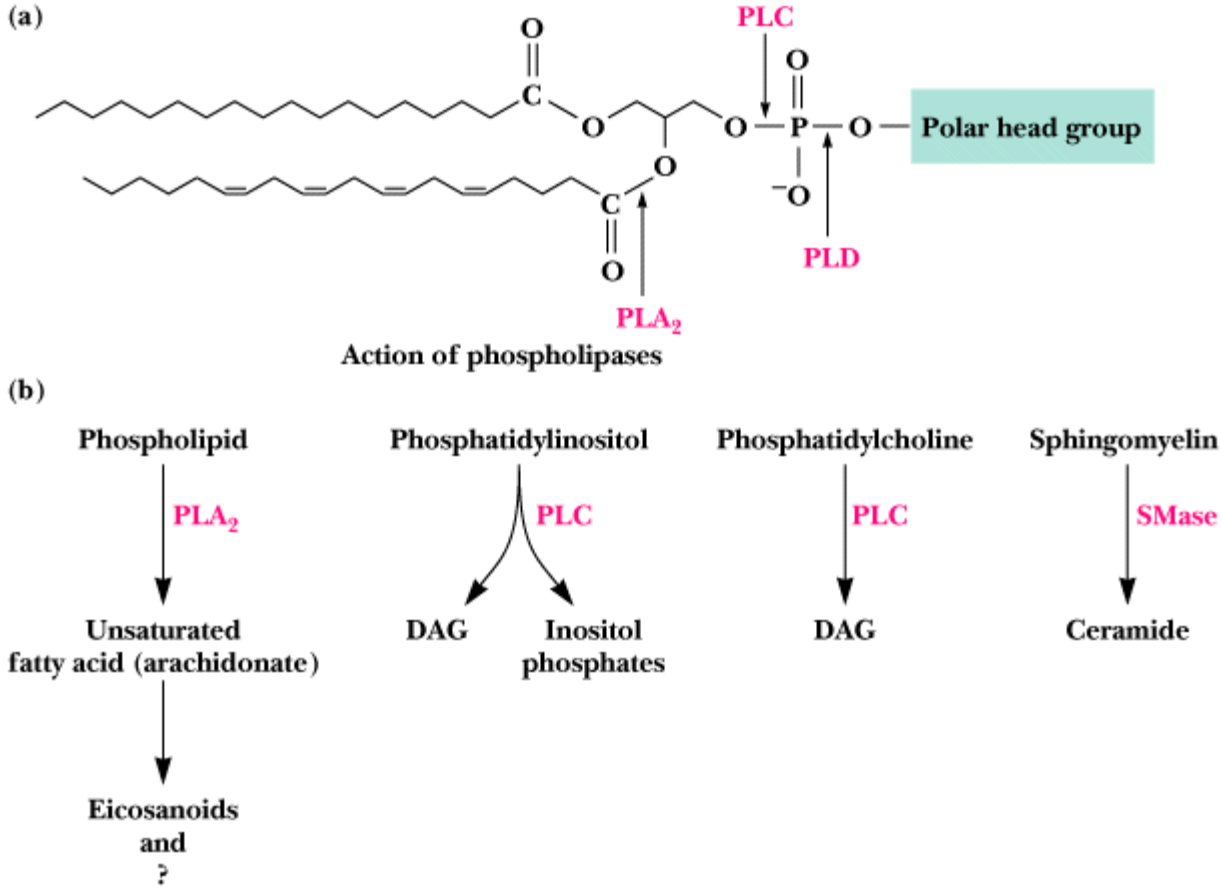


Calmodulin activates other cellular proteins

Garrett & Grisham: Biochemistry, 2/e
Figure 34.11



Steroid Hormones

Steroid hormones are soluble in the plasma membrane and readily enter the cytosol.

Steroids bind to a mobile receptor in the cytosol

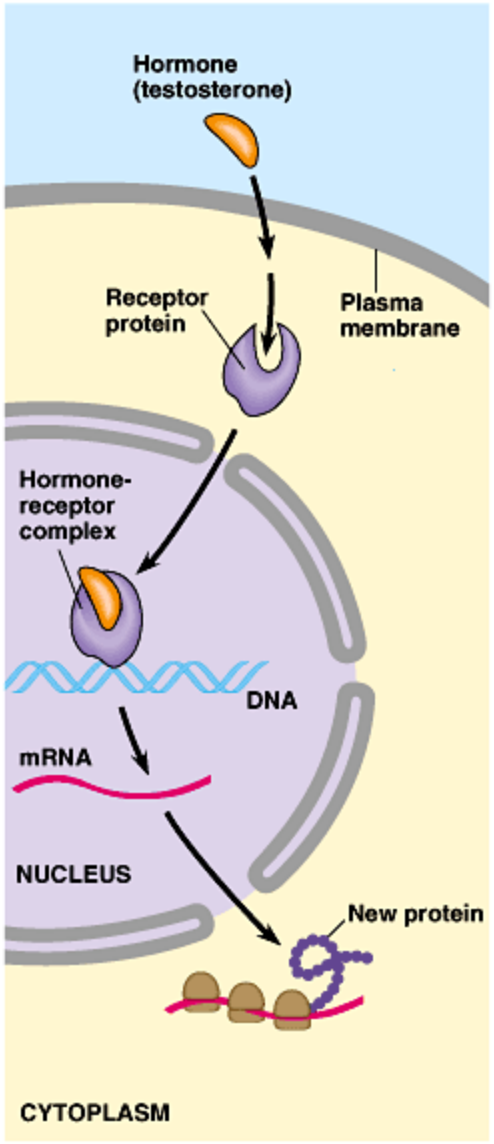
The hormone-receptor complex enters the nucleus and binds to specific genes.

The hormone-receptor complex acts as a transcription factor which turns on the genes.

Messenger RNA is transcribed, leaves the nucleus, and is translated into a specific protein by ribosomes.

The specific proteins then carry out functions (if they are enzymes) or produce structures in the target cell.

Because steroid hormones initiate protein synthesis, their effects are produced more slowly, but are more long-lasting than those produced by other hormones.

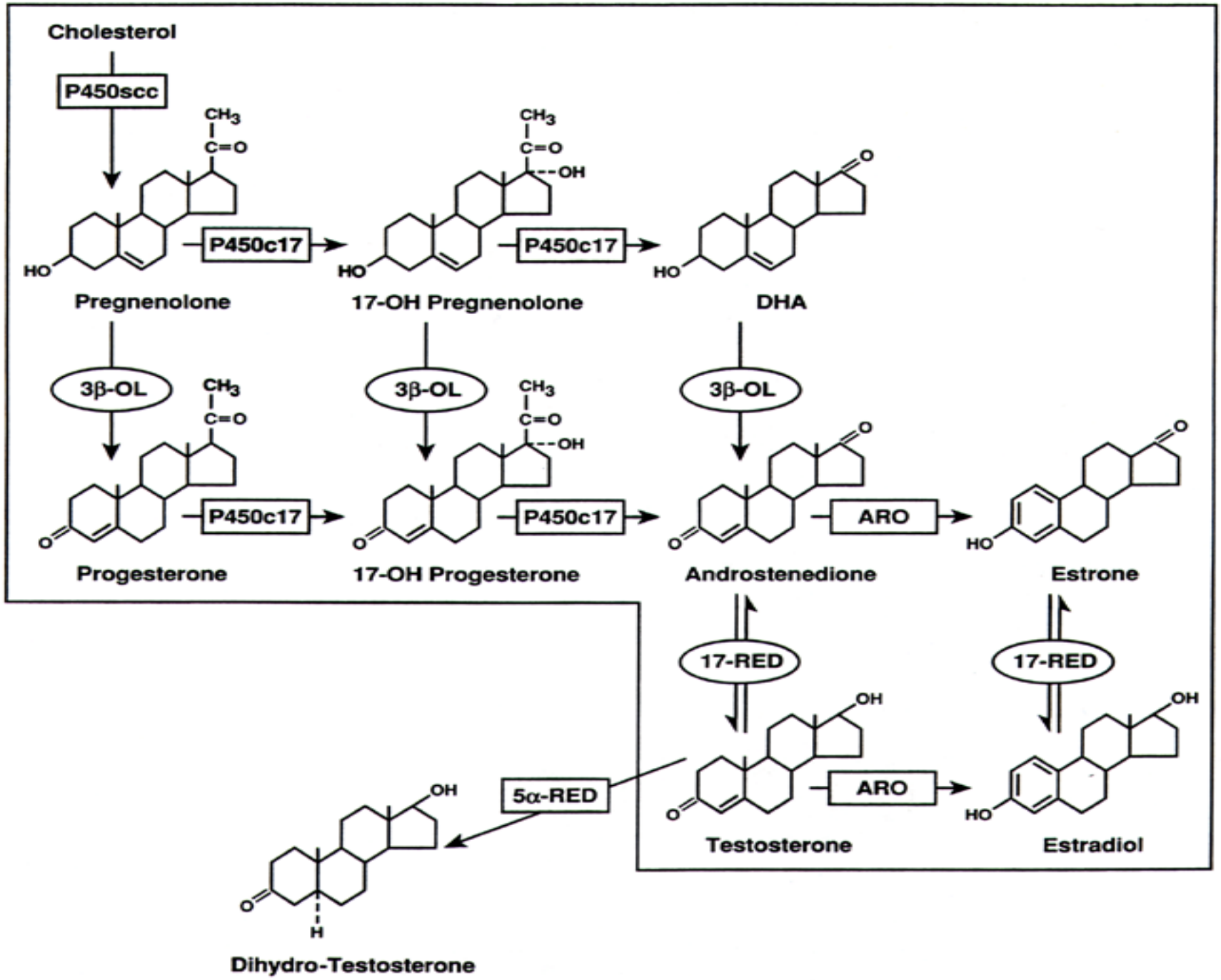


Classes of Hormones

- Steroids vs. Peptide Hormones

Hormones fall into 2 general classes based on their molecular structure and synthesis.

- All steroid hormones are made initially from the precursor (precursor = first step in biosynthetic pathway) cholesterol.


Cholesterol
P450scc
P450c17
P450c17
Pregnenolone
17-OH Pregnenolone
DHA
3β-OL
3β-OL
3β-OL
P450c17
P450c17
ARO
Progesterone
17-OH Progesterone
Androstenedione
Estrone
17-RED
17-RED
5α-RED
ARO
Testosterone
Estradiol
Dihydro-Testosterone

Steroid Hormones

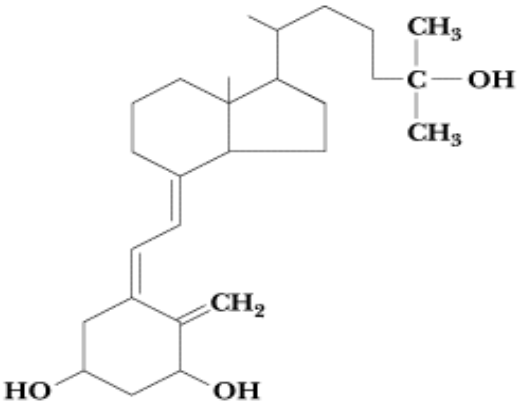
- Steroid hormones are produced by the gonads and adrenal cortex.
- Thyroid hormones are not steroids, but will be categorized with steroids for simplicity.
- Steroid hormones are made from cholesterol in the smooth endoplasmic reticulum and mitochondria of endocrine cells.

Steroid Hormones

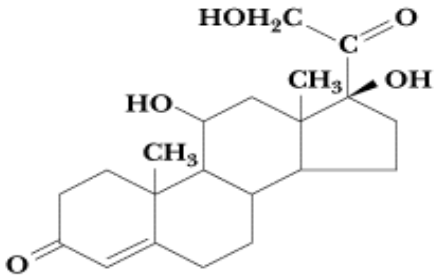
- Steroid hormones cannot be stored in vesicles in the endocrine cells that produce them. As soon as steroid hormones are produced, they diffuse out of the endocrine cell and enter the bloodstream.
- Steroid hormones are lipid soluble and their receptors are located inside their target cell.

Alcuni ormoni steroidei

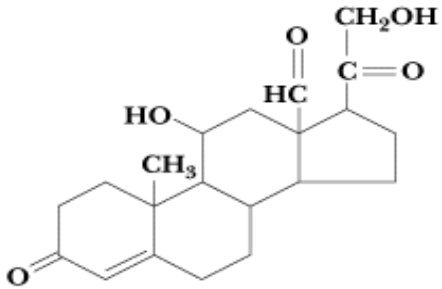
Garrett & Grisham: Biochemistry, 2/e
Figure 34.41



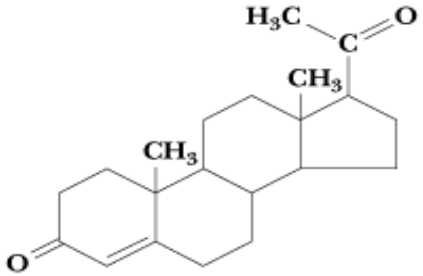
1,25-Dihydroxyvitamin D_3



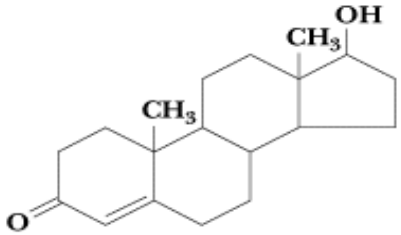
Cortisol



Aldosterone

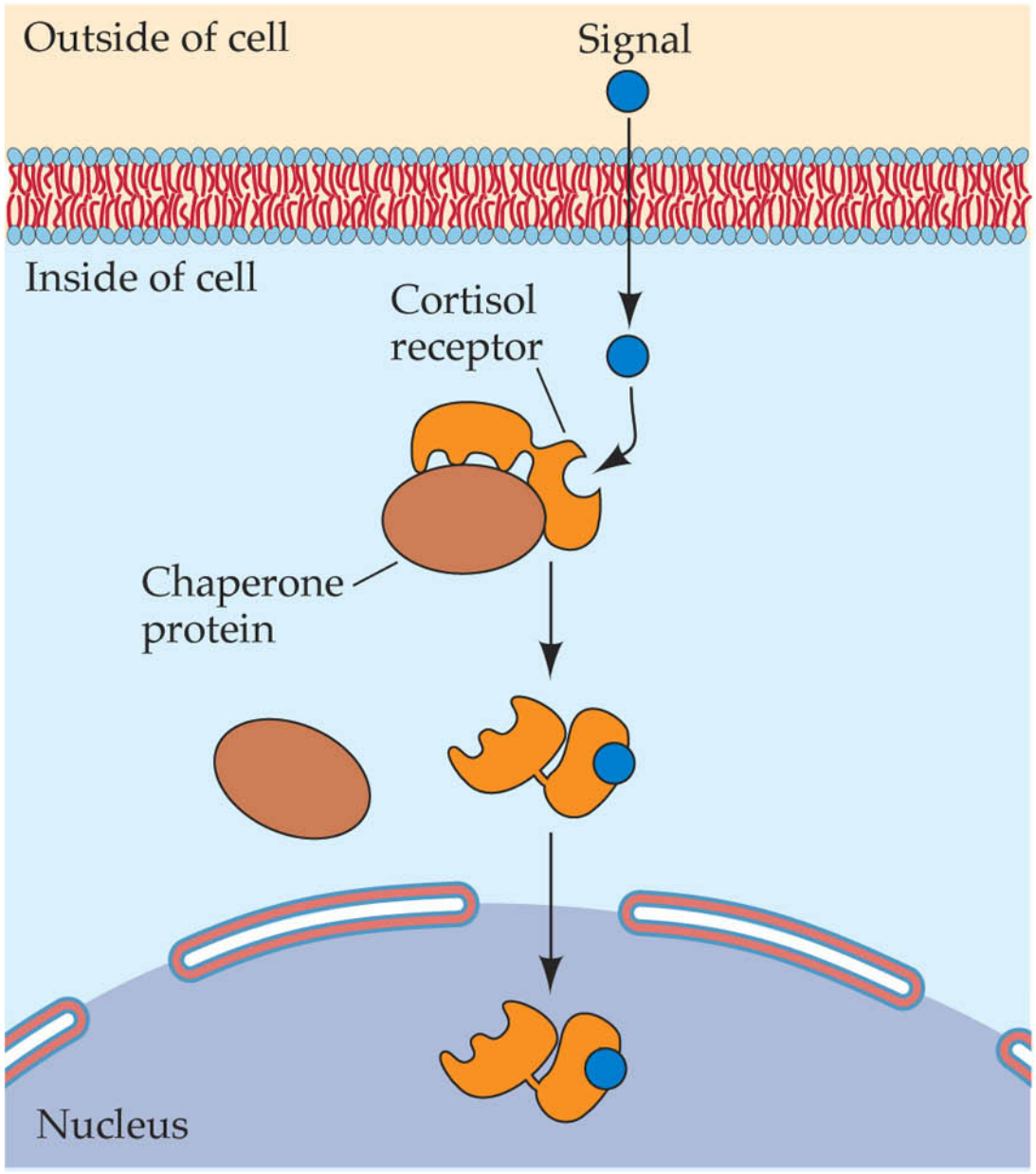


Progesterone

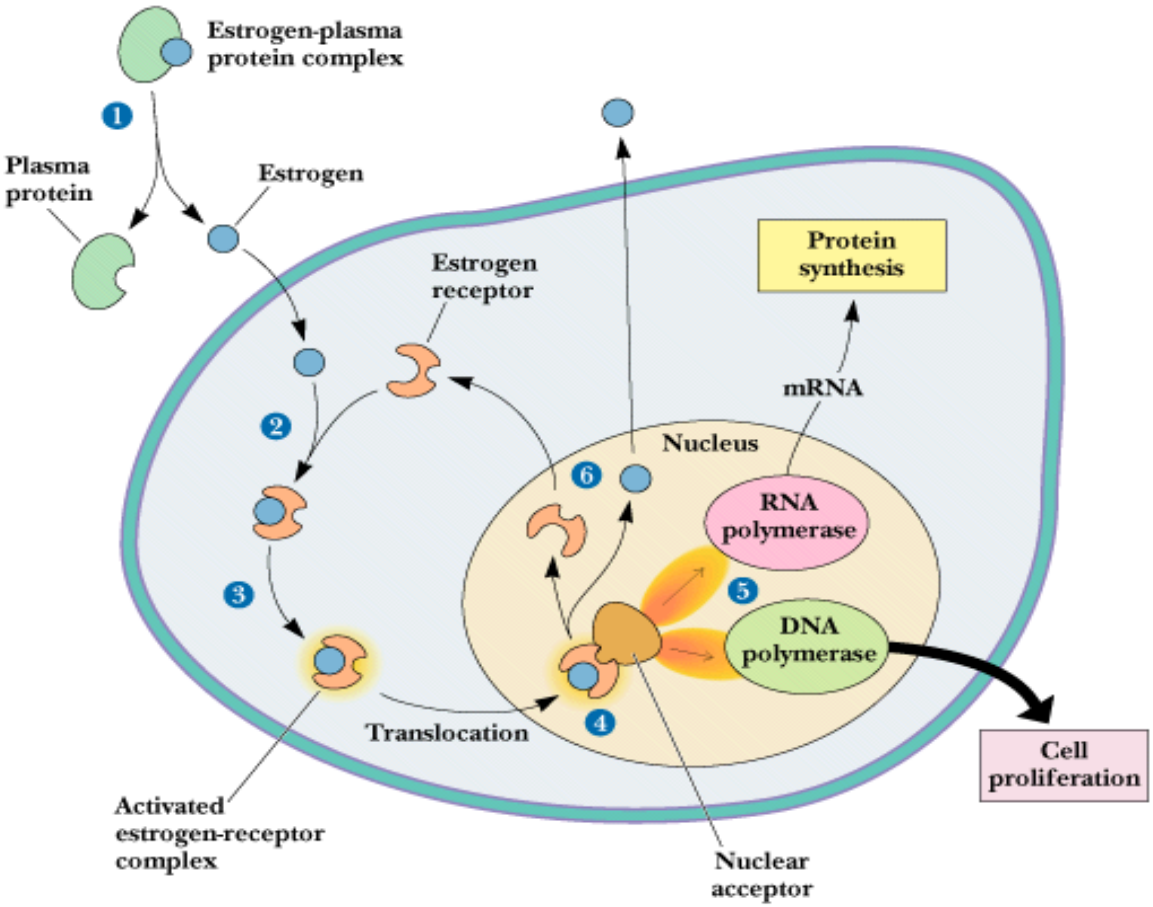


Testosterone

Saunders College Publishing



LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, **Figure 15.8 A Cytoplasmic Receptor**



L'H (es. estrogeno) si dissocia dalle proteine della membrana plasmatica, diffonde nella cellula e si lega alle proteine recettoriali. Il complesso attivo formato dall'H-R migra nel nucleo, dove interag con il DNA o con dei fattori di trascrizione o entrambi

HORMONES WHICH ACT VIA NUCLEAR RECEPTORS (NRs)

- **While most regulators act via membranal receptors, some (mostly hormones) have *intracellular* receptors.**
- **The ligands of such receptors are small hydrophobic molecules, and binding of the appropriate ligand converts them into active transcription factors, namely, proteins which stimulate, or reduce, the rate of gene transcription.**

HORMONES WITH NUCLEAR RECEPTORS

- **Thus these receptors, collectively named "nuclear receptors" (NRs), function as "ligand-activated transcription factors".**
- **The steroid hormones have nuclear receptors, and so do the thyroid hormones.**
- **In addition, derivatives of vitamin D and vitamin A (retinoids), which are synthesized from precursors consumed as food, also have NRs.**

MECHANISM OF RECEPTOR ACTIVATION

- **The *inactive* receptors of four of the steroids: cortisol, aldosterone, progesterone and testosterone reside in the *cytoplasm*. A single receptor molecule forms a complex with several proteins, most of them heat-shock proteins (HSPs).**
- **HSPs are known to be induced by various cellular stresses, and to act as chaperons, namely, proteins that act to preserve the conformation of other proteins.**

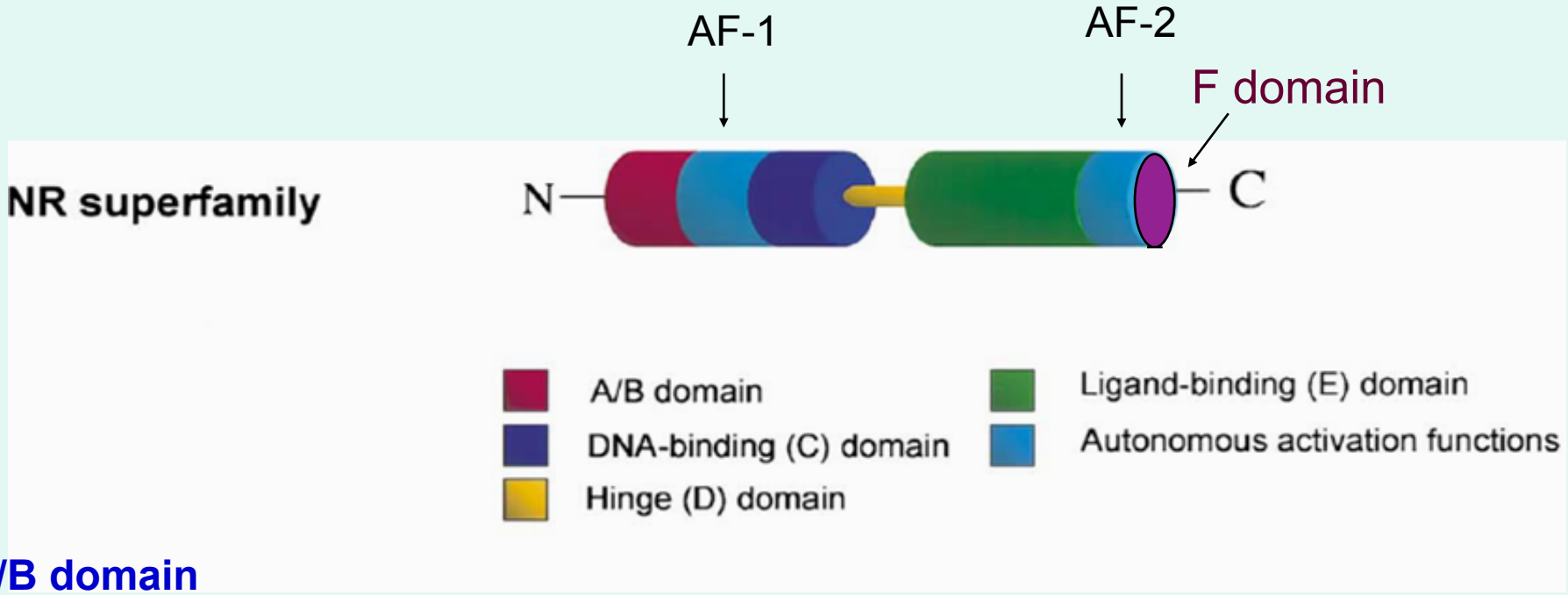
RECEPTOR ACTIVATION (Continued)

- **When one of the four steroid hormones binds to its receptor, the complex dissociates; two activated receptor molecules form a *homodimer*, which enters the nucleus and functions as a transcription factor.**

STRUCTURE OF NUCLEAR RECEPTORS

- **Nuclear receptors are homologous.**
- **It is customary to divide the receptor molecule into five domains: *A/B, C, D, E, and F.***
- **Domain C, the DNA-binding domain, shows the highest degree of homology among the receptors.**
- **Domain E, the ligand-binding domain, shows lower degree of homology. Greater homology is observed when the ligands are similar.**

NRs are highly structurally related



A/B domain

Variable in sequence and length

Activation function-1 (AF-1)

Ligand independent

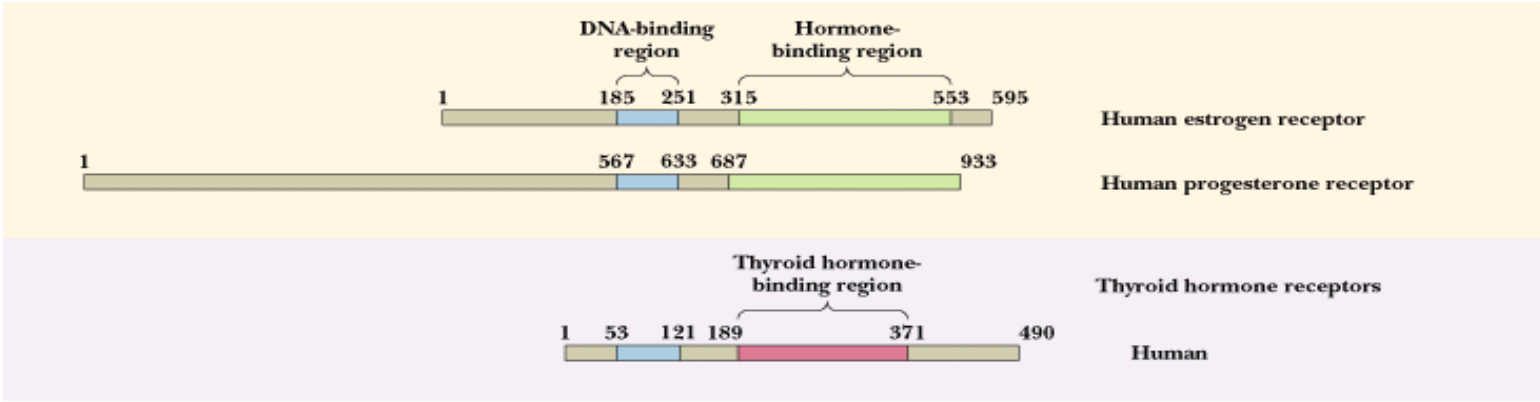
Tissue specific

F domain

Hypervariable in sequence and length

Tutti recettori nucleari per gli steroidi sono caratterizzati da una organizzazione strutturale simile (superfamiglia genica)

Garrett & Grisham: Biochemistry, 2/e
Figure 34.43



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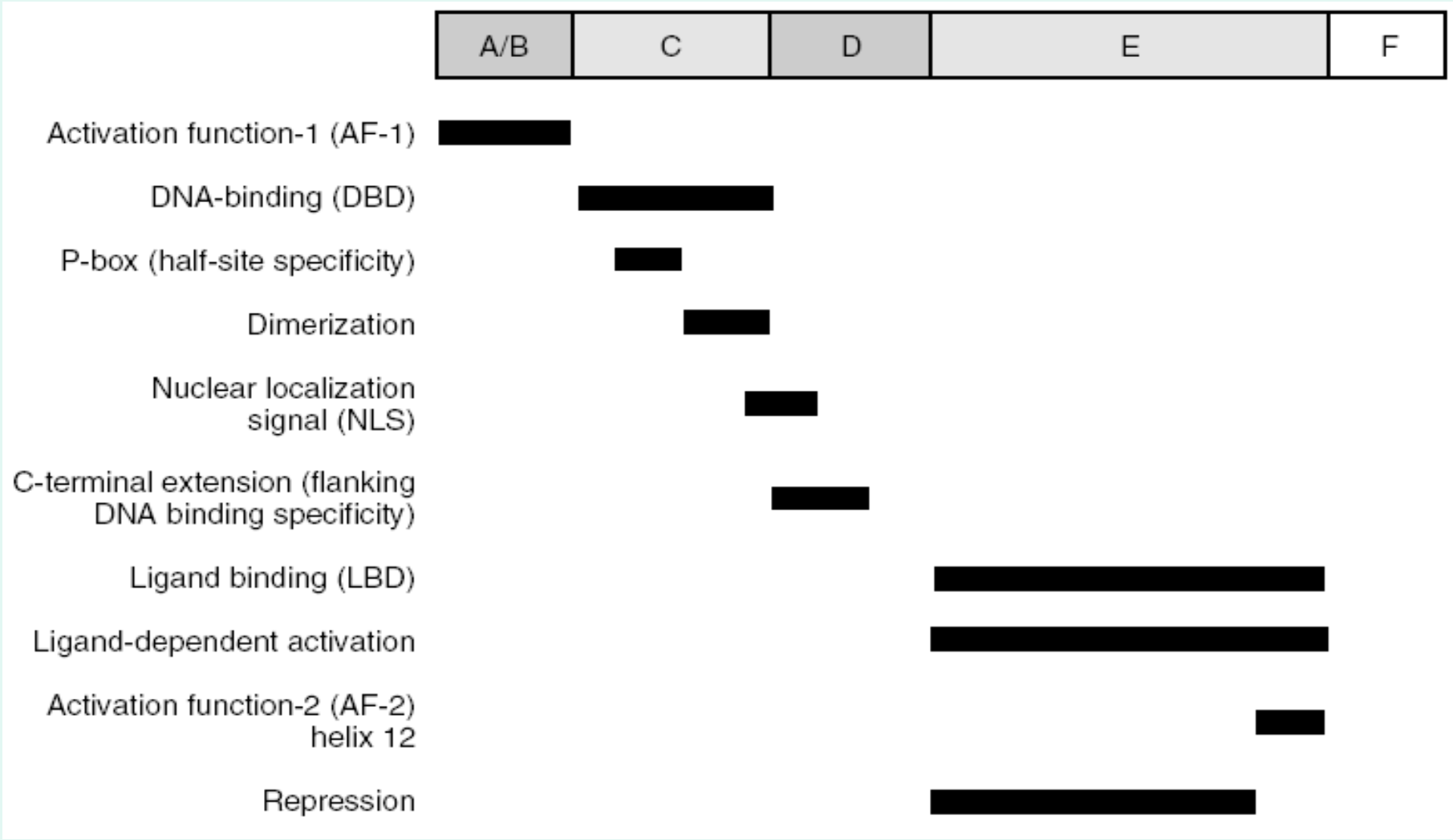
Domain structure of nuclear receptor



STRUCTURE OF NUCLEAR RECEPTORS (NRs)

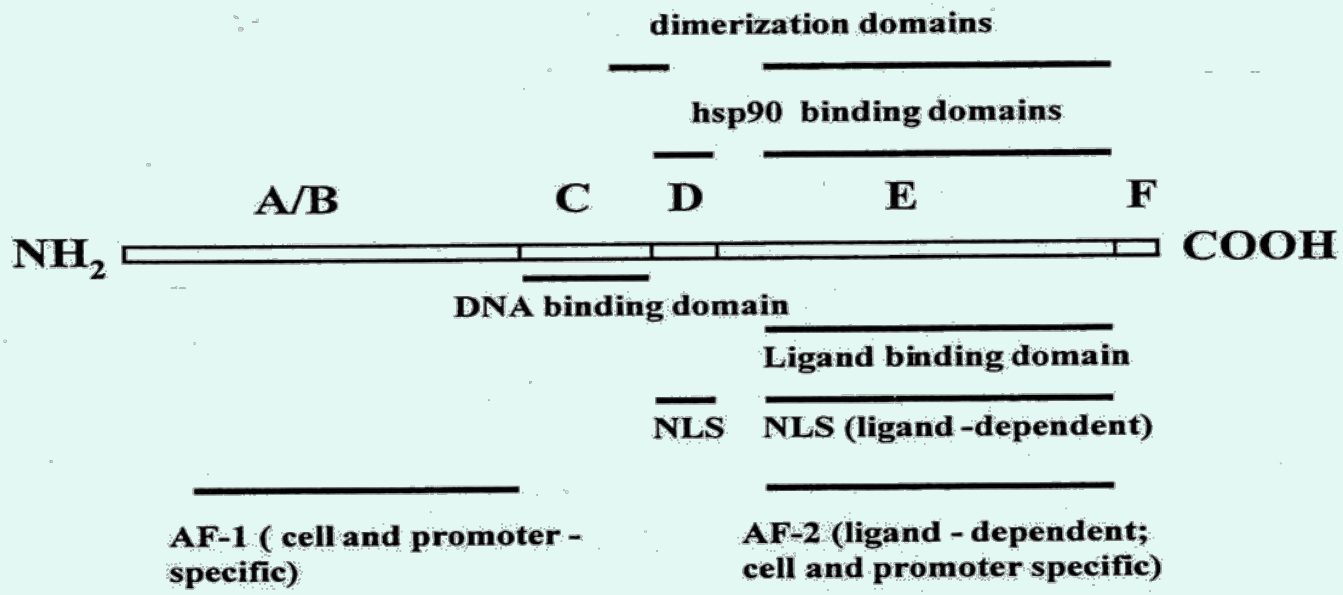


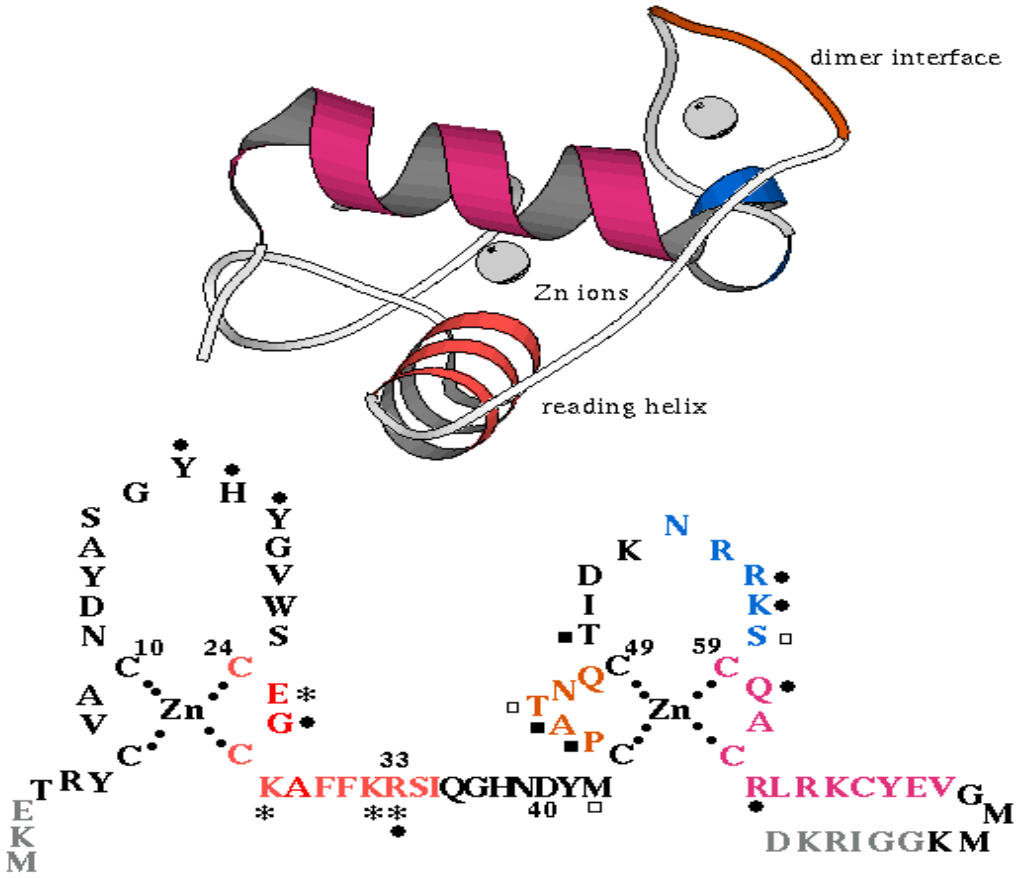
FIGURE 10–2. Modular structure of NRs. Based on sequence homologies and on functional studies, the NR molecule contains six segments, A through F, with an ill-defined A/B frontier. The C and E domains are best conserved across different species, in contrast with the poorly conserved A/B domain. The F domain is absent in many receptors and has no known function. The functional domains identified in the different receptors are indicated in the figure. NLS are nuclear localization sequences; AF-1, AF-2 are the transcription transactivating functions 1 and 2, respectively.

I domini che legano il DNA hanno un alto grado di omologia

- La loro caratteristica piu' saliente e' la disposizione conservata di 9 Cys (presenti in tutti i recettori per gli steroidi)

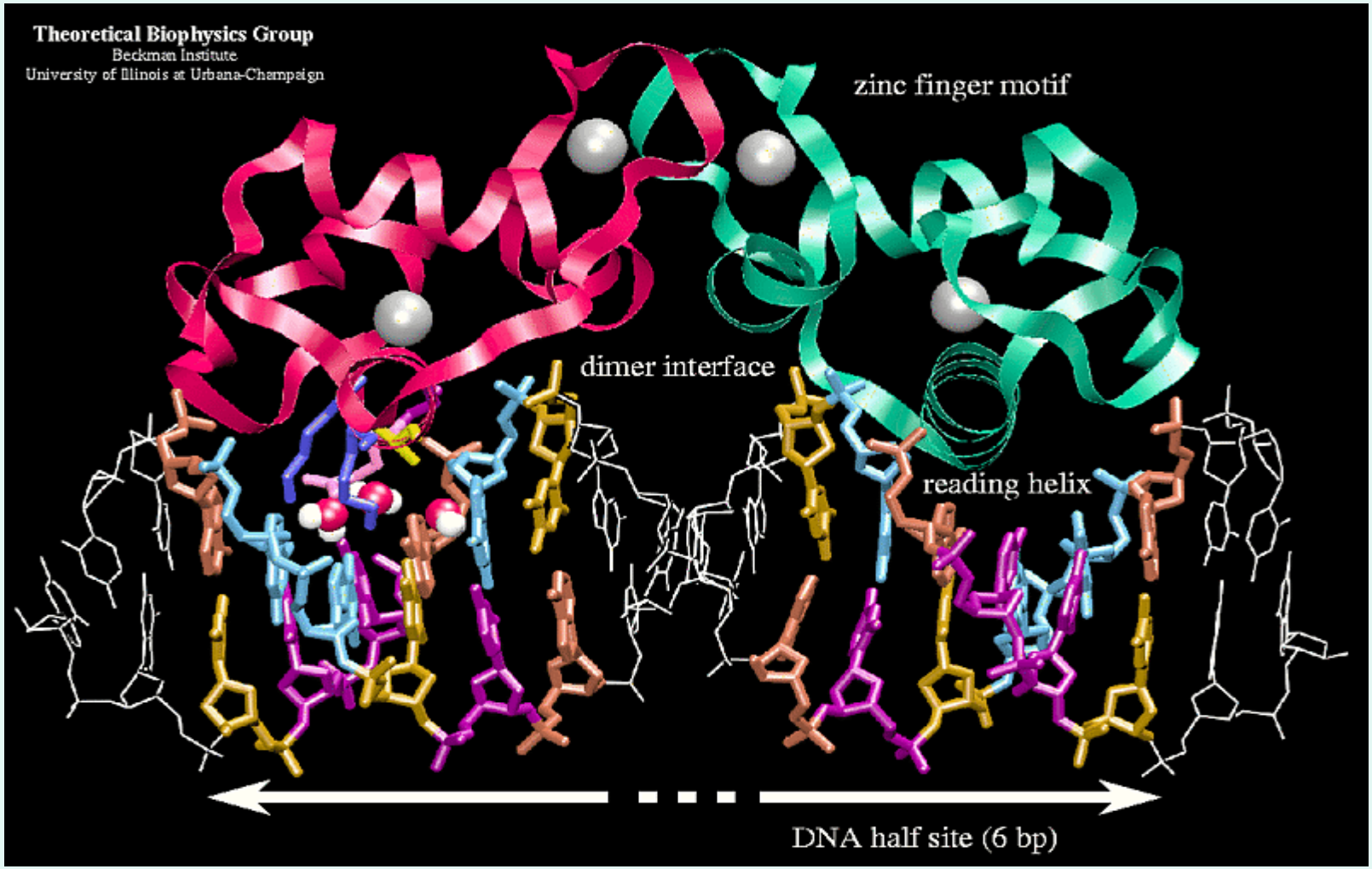
- Tre coppie di queste Cys sono organizzate in sequenze Cys-X-X-Cys, che si trovano comunemente nelle proteine *zinc finger* che legano il DNA

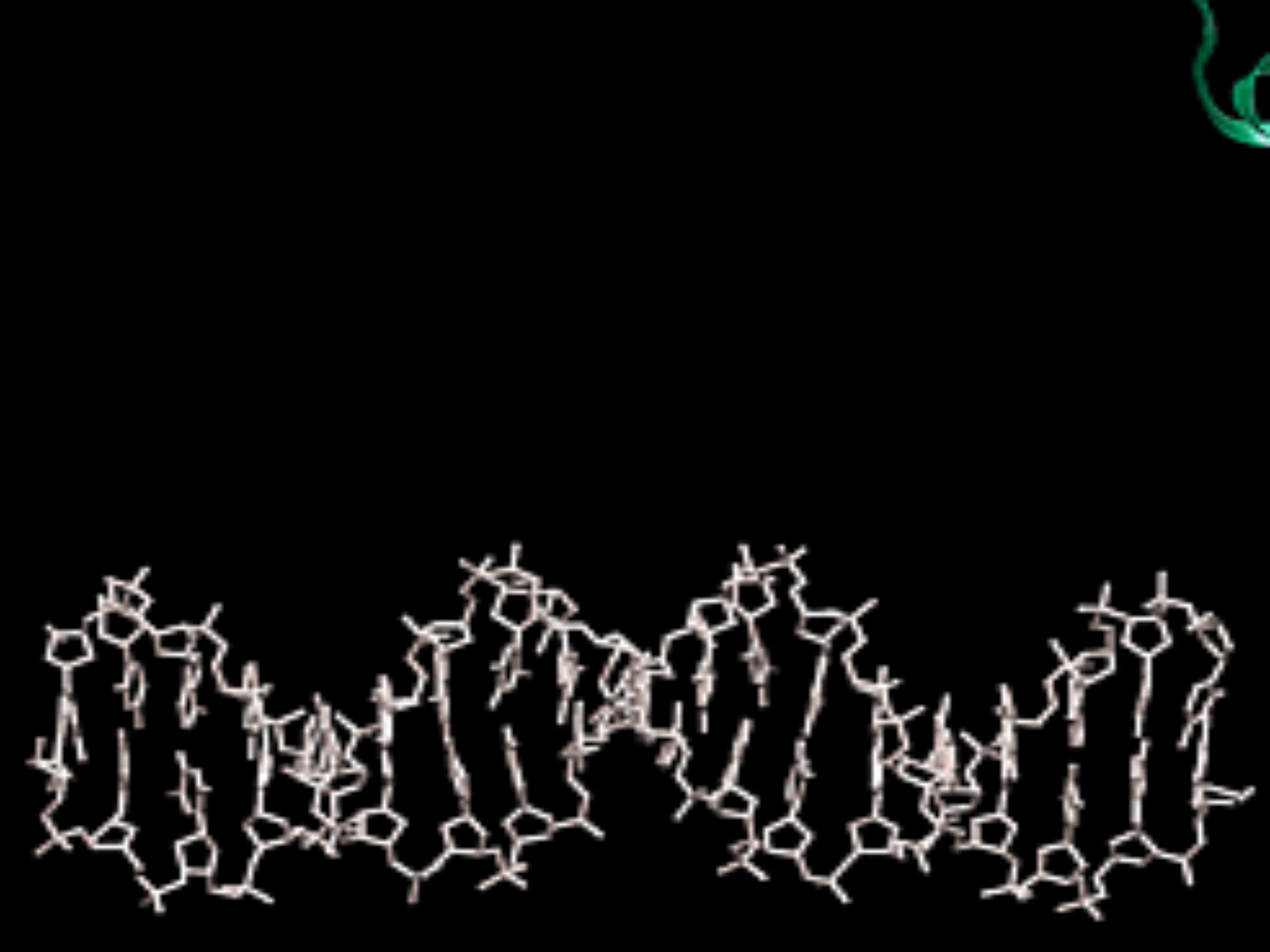
DNA binding domain of the Estrogen Receptor



Theoretical Biophysics Group, UIUC

Estrogen receptor DNA binding domain





RESPONSE ELEMENTS OF NRs

- **Dimers of NRs bind to response elements comprised of two half-sites; each half-site binds one monomer.**

- **One type of response element is a *palindrome* (inverted repeat), in which the half-sites, one a mirror image of the other regarding the sequence of bases, are found in each DNA strand. This is the most common response element for steroid hormone receptor homodimers.**

Palindromi

I nasi sani

Madam, I'm Adam

E la sete sale

Roma tibi subito motibus ibit amor

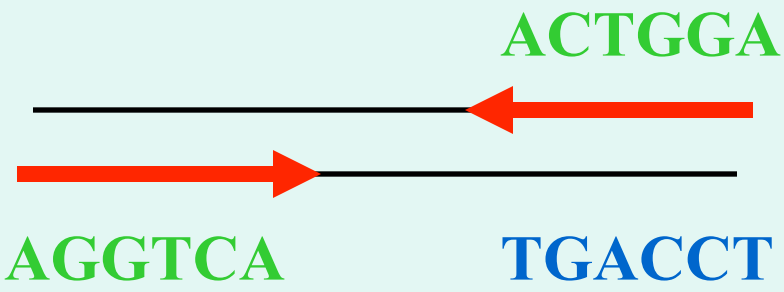
Ora baro

Aerea

Ingegni

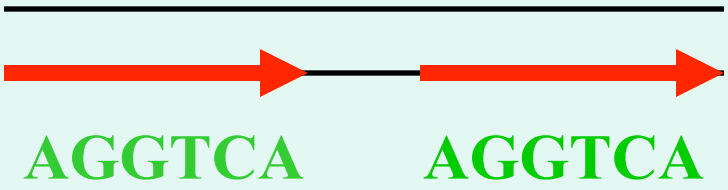
Eran i modi di dominare

RESPONSE ELEMENTS FOR THE STEROID HORMONE RECEPTORS



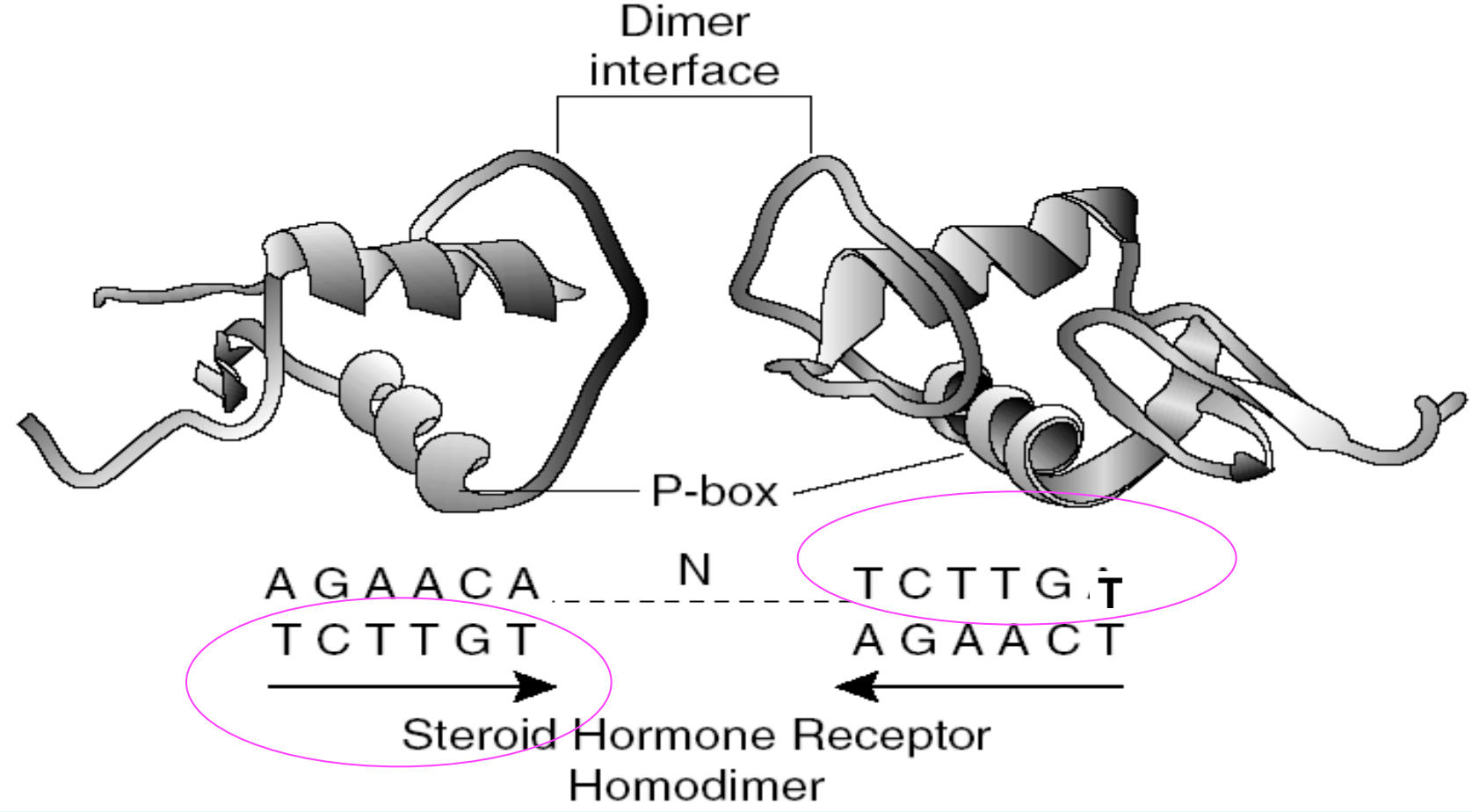
PALINDROME

(INVERTED REPEAT)



DIRECT REPEAT

Structure basis for Steroid hormone receptors DNA binding specificity

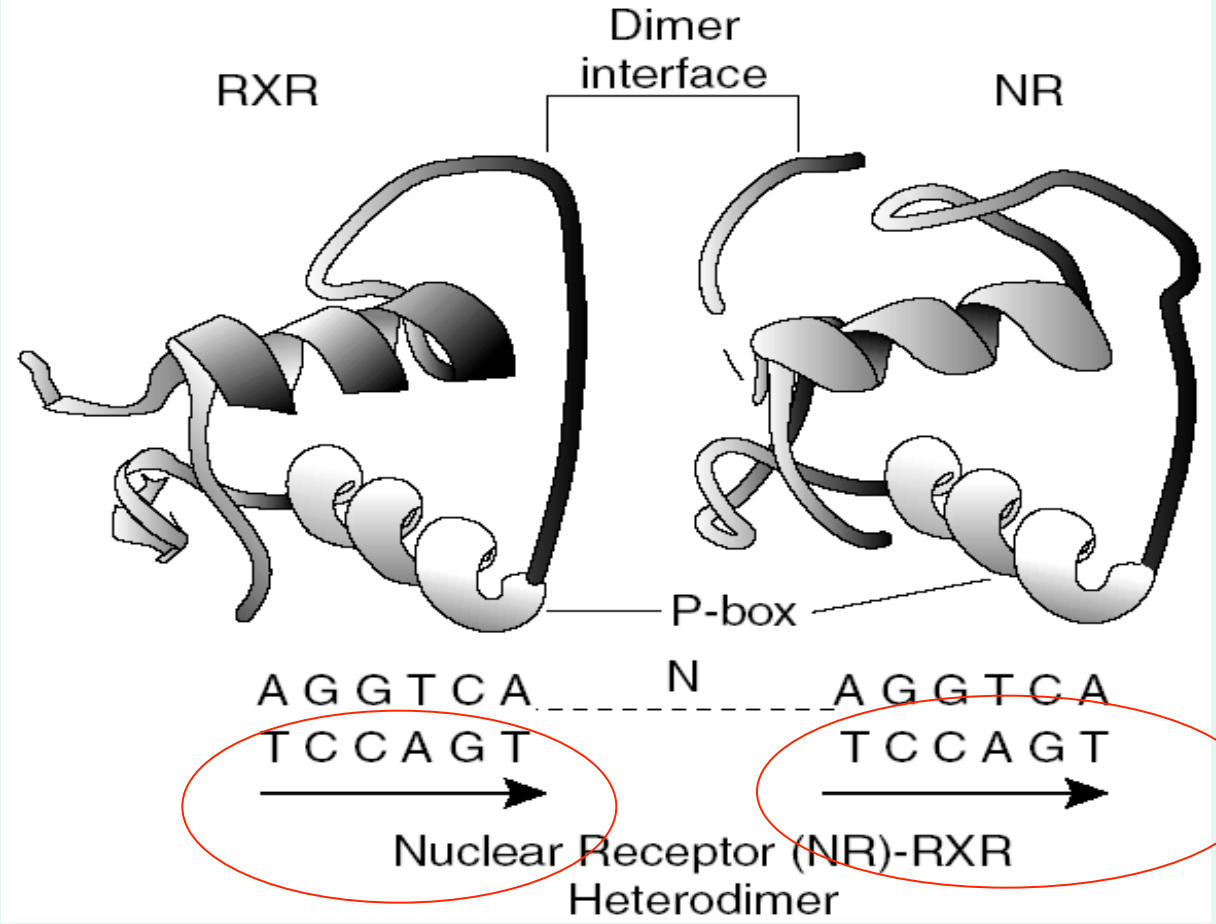


RESPONSE ELEMENTS OF NRs (continued)

- **The other type of response element is a *direct repeat*, in which the same base sequence appears twice in a row on the same DNA strand.**

- The specificity of a response element for a receptor is determined not only by the base sequence but also by the spacing between the two half-sites.

Direct repeats



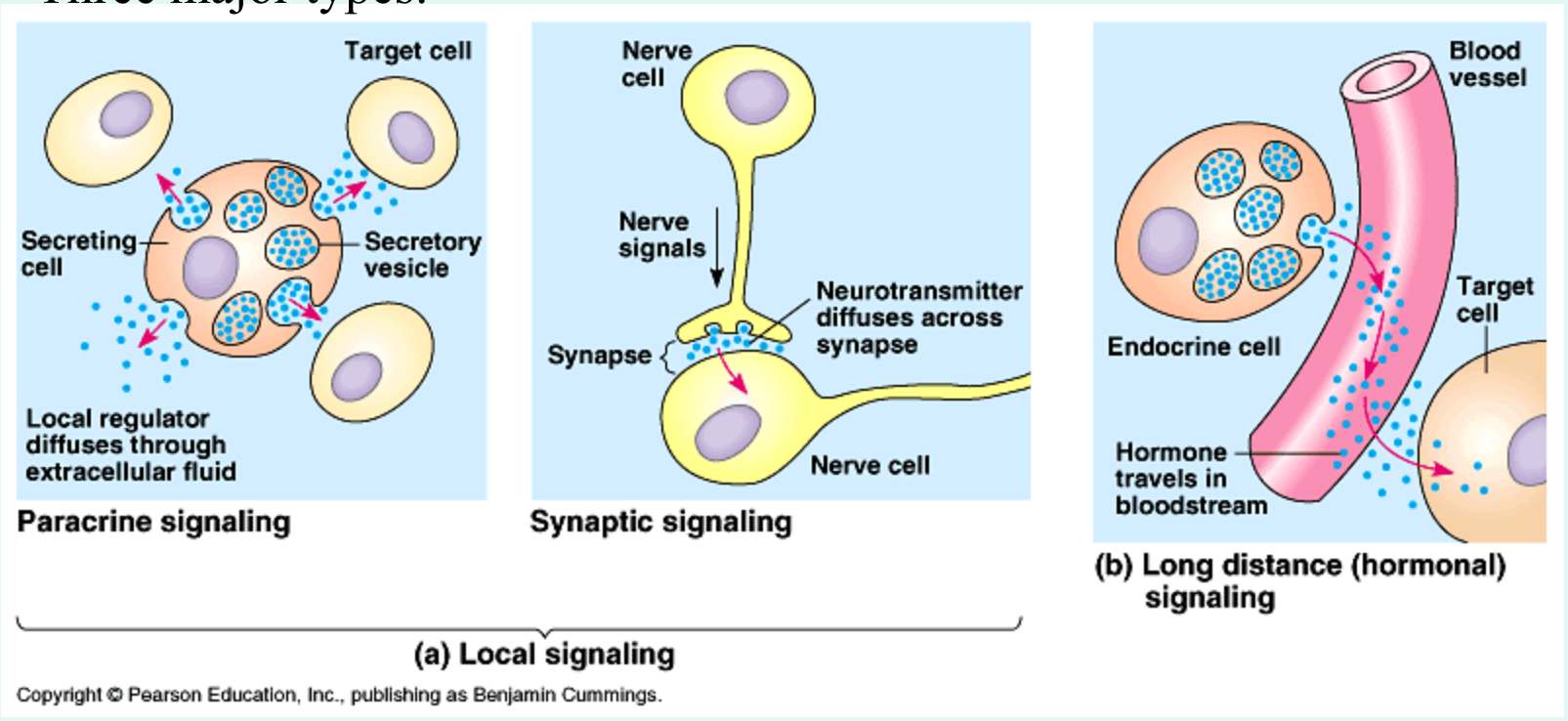
- NR= VDR, RAR, TR, or PPAR

Types of Chemical Signaling

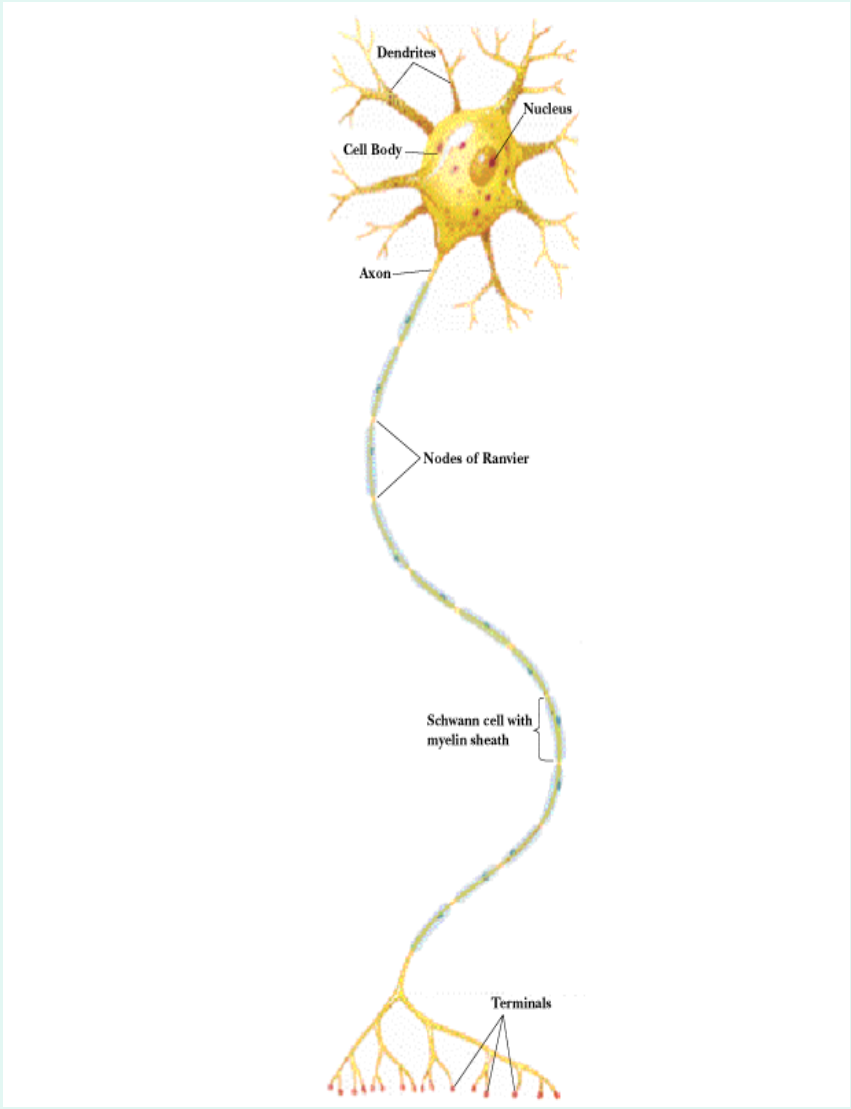
Chemical signaling between cells is one of the most important ways that activities of tissues and organs are coordinated.

The nervous system is the other major coordinating system in animals, but even here chemical signaling is used between adjacent neurons.

Three major types:



Come passano le informazioni nervose da un neurone all'altro?



Motoneurone di mammifero

L'assone trasmette segnali da questa cellula ad altre per mezzo delle sinapsi

Il numero di sinapsi che un dato neurone puo' ricevere e' molto variabile: da 1 a molte migliaia.

Tipicamente un motoneurone spinale piu' di 10,000 sinapsi.

Il numero di sinapsi per neurone del telencefalo umano e' di ca 40,000 (!)

Communication at the Synapse

A crucial feature of neurotransmission

- Chemical synapses are different from electrical
- Neurotransmitters facilitate cell-cell communication at the synapse

- Un potenziale d’azione sulla membrana presinaptica causa il rilascio di una sostanza chimica (neurotrasmettitore).

- Questa si lega ai recettori presenti sulla membrana post-sinaptica innescando un nuovo potenziale d’azione

The Cholinergic Synapse

A model for many others

- Synaptic vesicles in synaptic knobs contain acetylcholine (10,000 molecules per vesicle)
- Arriving action potential depolarizes membrane, opening Ca++ channels and causing vesicles to fuse with plasma membrane
- Acetylcholine spills into cleft, migrates to adjacent cells and binds to receptors

- Il rilascio di AcetilColina puo’ essere alterato da numerose tossine:
- Toxin effects: botulism toxin inhibits Ac-choline release, black widow's latrotoxin protein overstimulates

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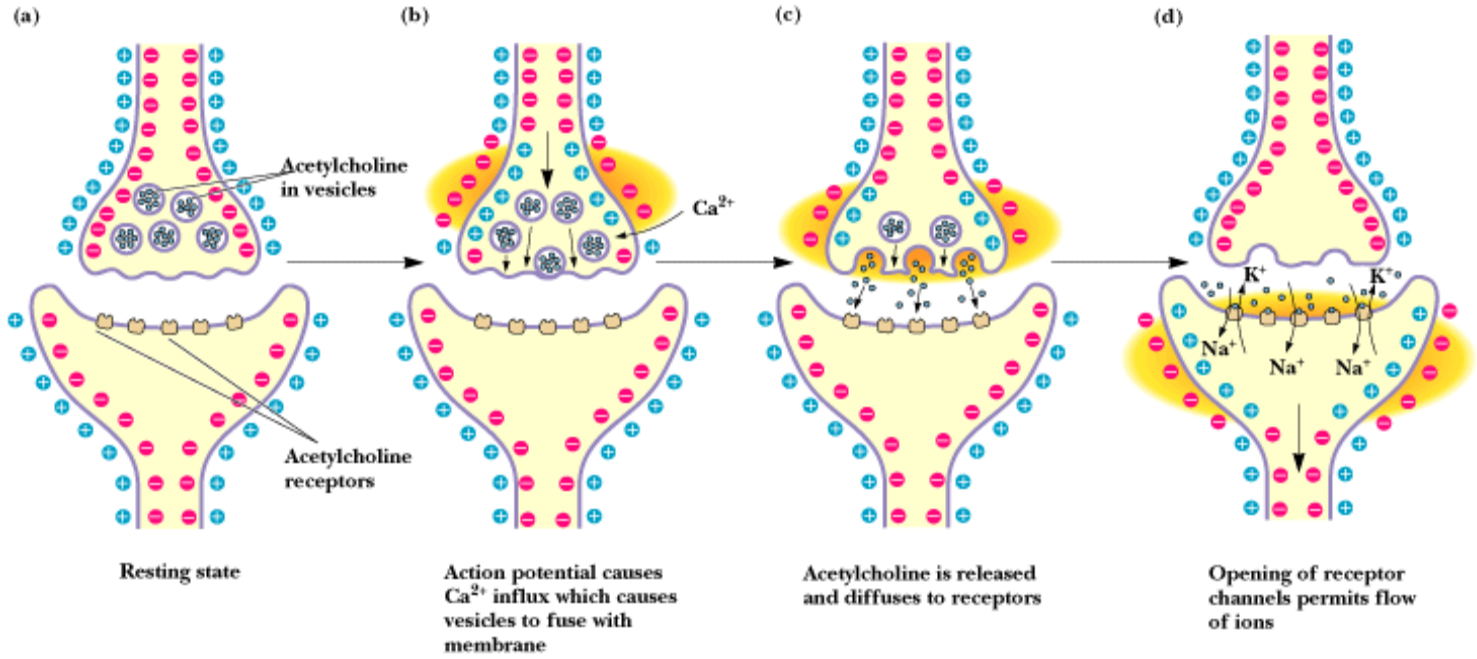
Figure 34.57



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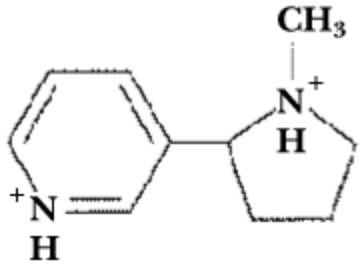
Two Classes of Ac-Ch Receptor

Nicotinic and muscarinic

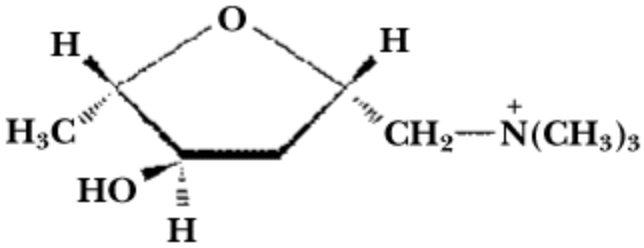
- As always, toxic agents have helped to identify and purify hard-to-find biomolecules
- Nicotinic Ac-Ch receptors are ion channels
- Muscarinic Ac-Ch receptors are transmembrane proteins that interact with G proteins
- Acetylcholinesterase degrades Ac-Ch in cleft
- Transport proteins and V-type H^+-ATPases return Ac-Ch to vesicles - called reuptake

Nicotiana tabacum



Nicotine

Amanita muscaria



Muscarine

NICOTINIC RECEPTOR

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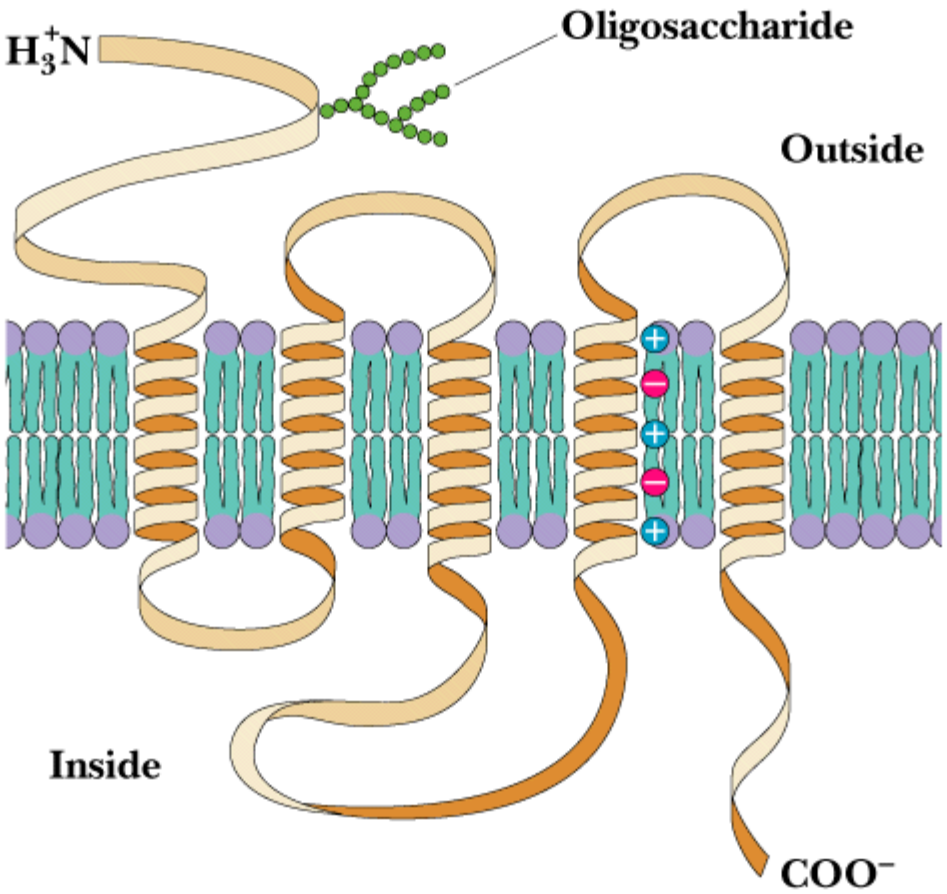
Figure 34.59



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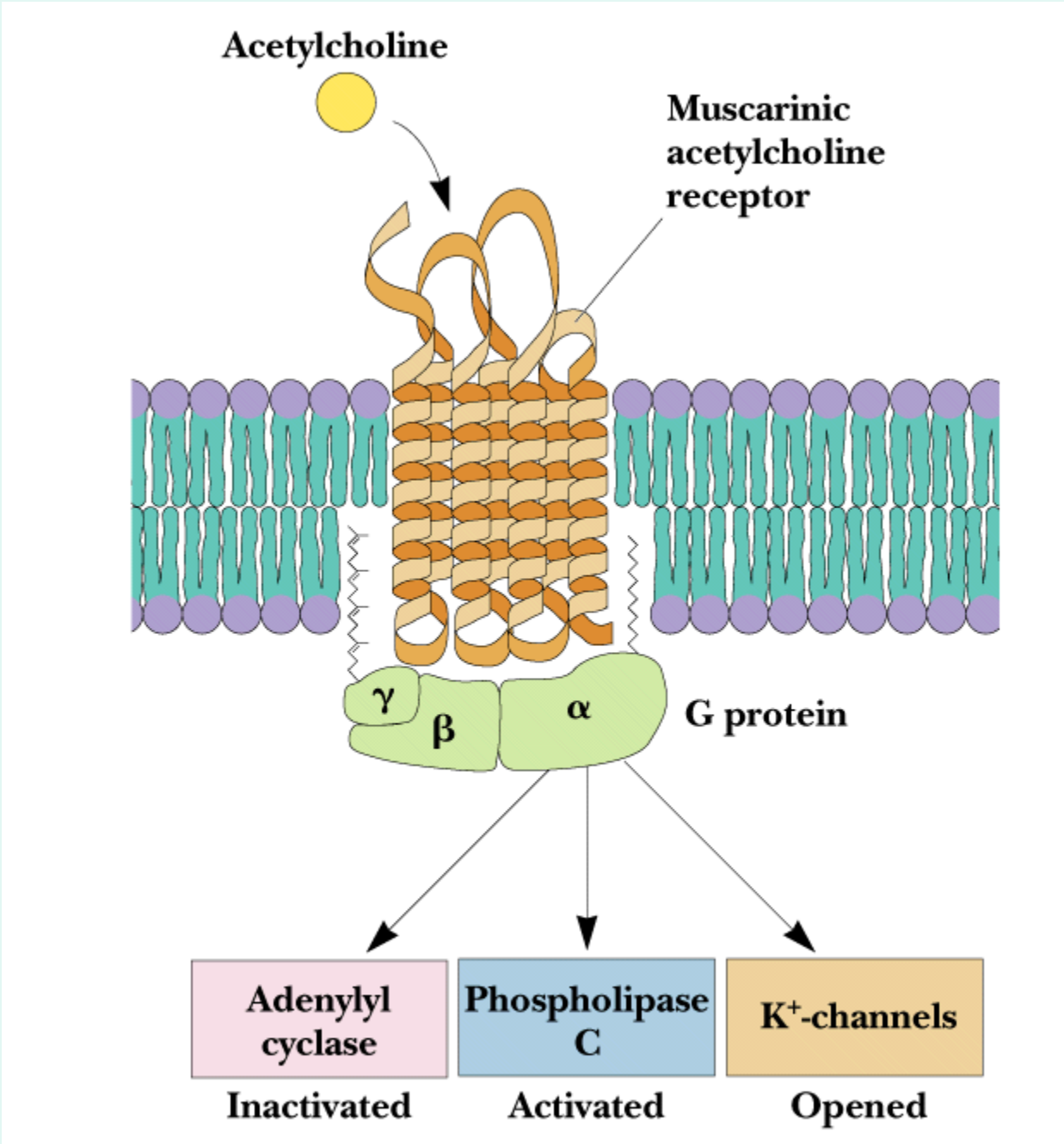
Acetylcholine
Muscarinic acetylcholine receptor
γ
β
α
G protein
Adenylyl cyclase
Phospholipase C
K+-channels
Inactivated
Activated
Opened

Gli antagonisti dei recettori nicotinico e muscarinico

- Sono neurotossine potenti
- Si legano al recettore e ostacolano l'apertura del canale ionico:
- D-tubocurarina
- Cobratossina
- Alfa-bungarotossina
- Atropina (anti-muscarinico)

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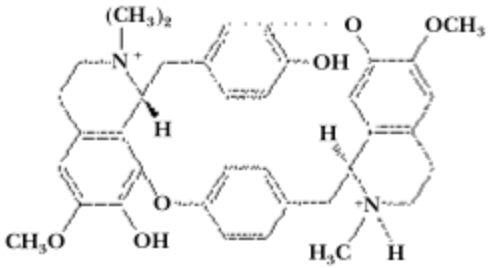
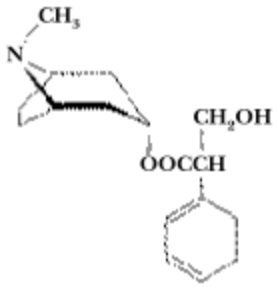
Figure 34.63

Chondrodendron



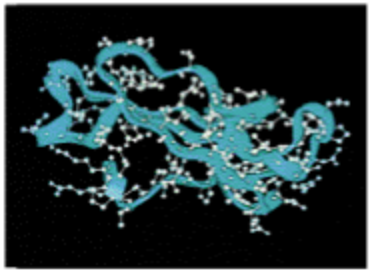
Tubocurarine

Deadly nightshade
(*Atropa belladonna*)



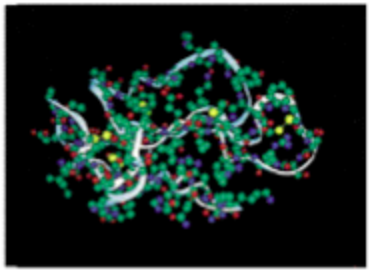
Atropine

Indian cobra
(*Naja naja*)



Cobratoxin

Bungarus multicinctus



α-Bungarotoxin

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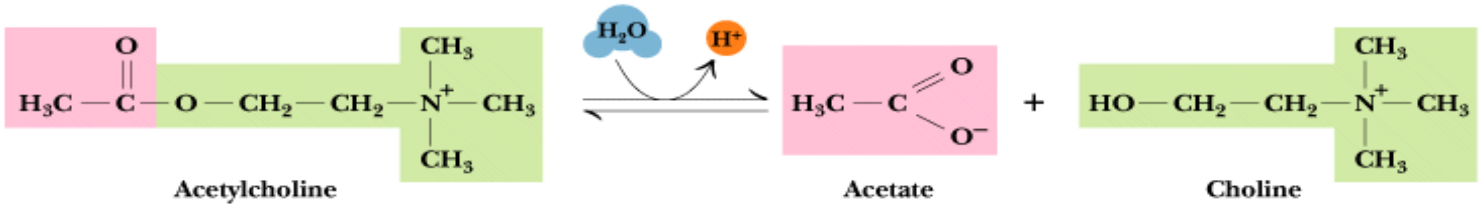
Figure 34.61



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L'acetilcolinesterasi degrada l'AcCh in acetato e colina nello spazio intersinaptico

Inibitori dell'acetilcolinesterasi

- E' una **Ser esterasi** (Ser al sito attivo)
- Malathion e parathion (insetticidi)
- Sarin e tabun (gas nervini guerre chimiche)
- Fisiostigmina e neostigmina (+ blandi, farmaci contro la miastenia gravis)

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Figure 34.66