Cell Signaling

Chapter 15

General Overview of Signaling

1 – Incorporation of signaling molecule into vesicles

2- Release by exocytosis

3- Transport to target cell

4 – Signaling molecule binds to cell-surface receptor or intracellular receptors

5- Activated receptor triggers one or more signal transduction pathways

6 a – short-term changes

6b – long-term changes

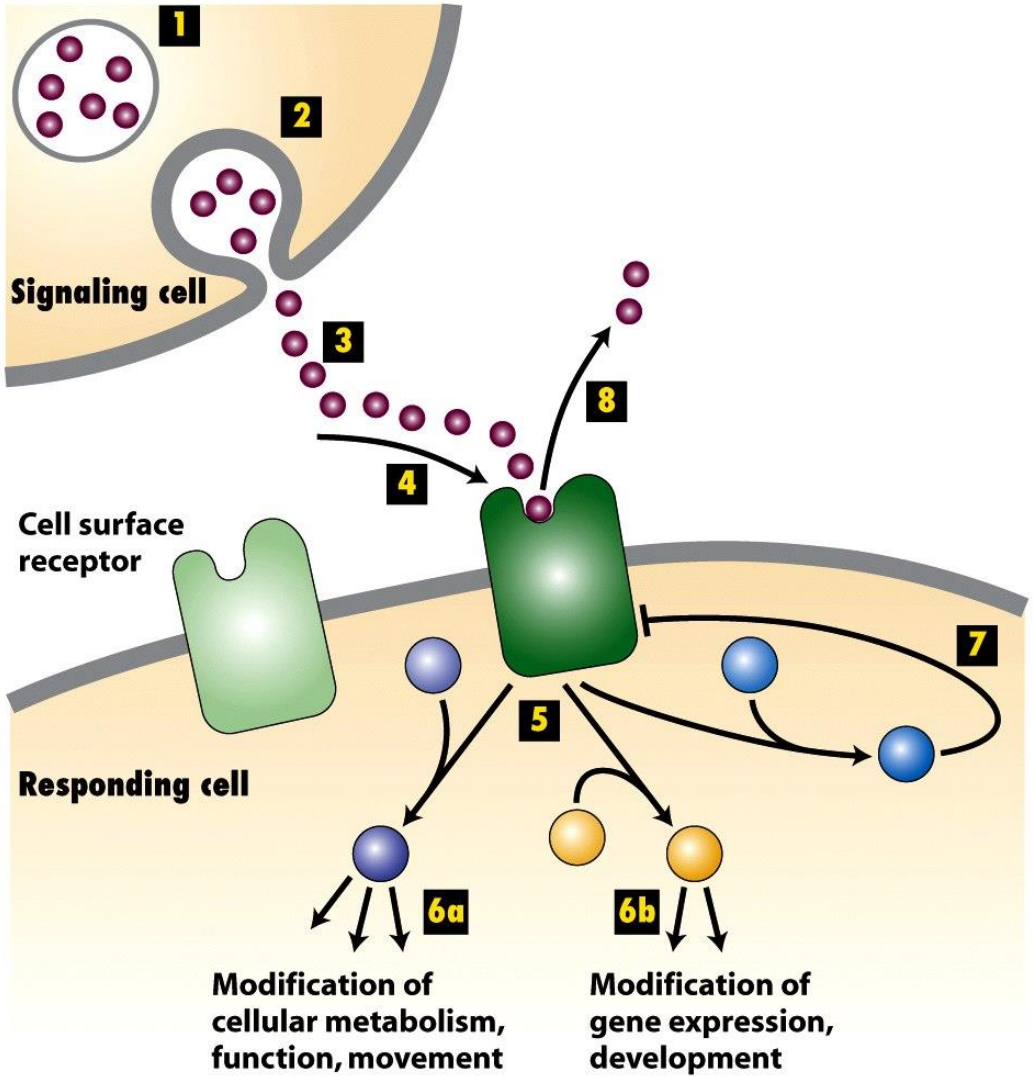

Figure 15-1
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Production and release of signaling molecules

- Signaling cells produce signaling molecules - **SYNTHESIS**
- Signaling molecules can be small molecules (catecholamines) or large peptide molecules
- Small Signaling molecules are made in the cytosol and then transported in vesicles
- Large peptide and protein hormones are synthesized and processed via the secretory pathway
- Local increase in calcium ion concentration causes the vesicles to fuse with the plasma membrane – **RELEASE OF THE SIGNAL**
- Released hormones or peptides are in the blood for a very short time – maybe minutes before they are degraded by proteases in blood and tissue - **TRANSPORT**
- These signaling molecules can be taken up by transporters or internalized after they bind to receptors – **RECEPTOR-LIGAND INTERACTION**
- **INTRACELLULAR SIGNALLING PATHWAYS**
- Short-term responses – these signaling molecules are terminated by their own degradation
- Long-term response

Types of Signaling

- Endocrine – Hormones travel through the circulatory system before they encounter their target
- Paracrine – signaling molecules that act on the cells in close proximity, example – neurotransmitters
- Autocrine – Cells respond to their own signaling molecules eg. tumor cells
- Integral membrane protein signaling molecules function as ligands for receptors on adjacent cells. In other cases, proteolytic cleavage of the extracellular domain releases a soluble molecule eg. EGF

Signaling molecules can be short or long range

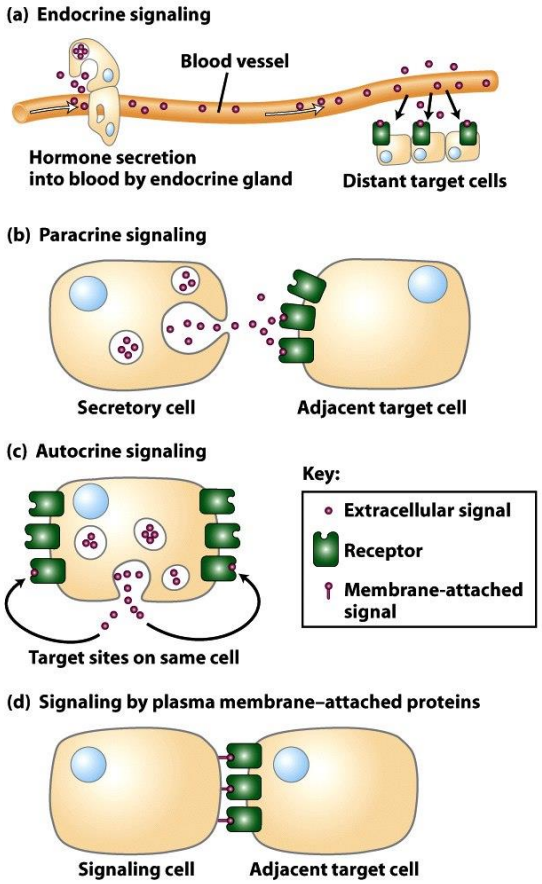

Figure 15-2
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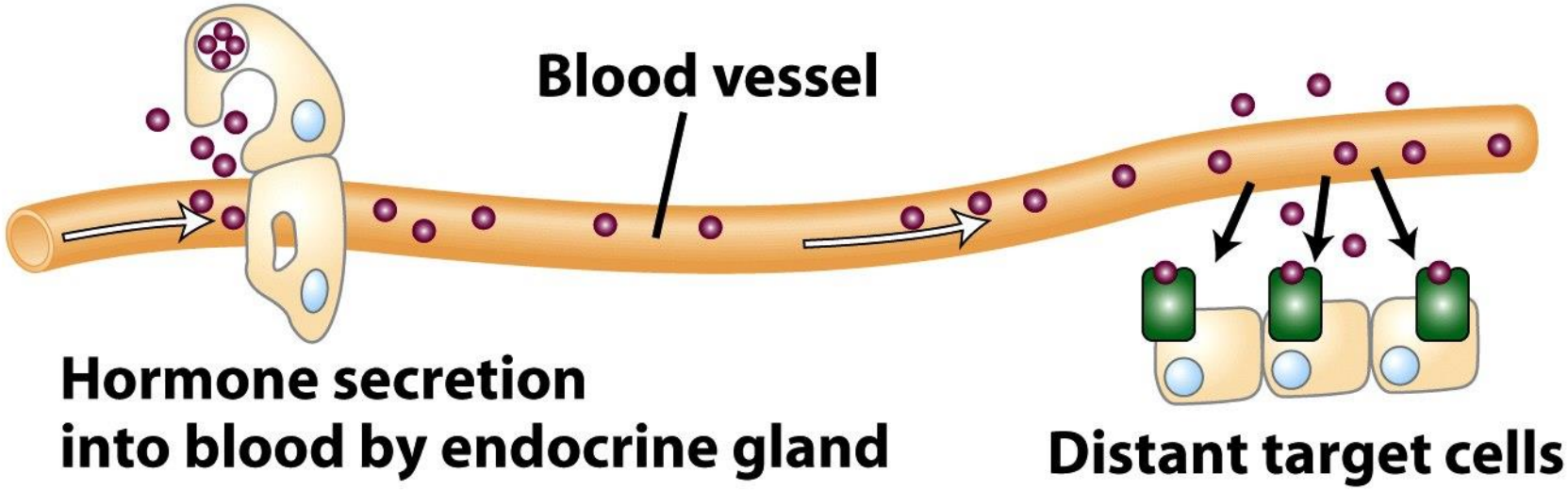
Endocrine signaling



Key:

- Extracellular signal
- Receptor
- Membrane-attached signal

Figure 15-2a
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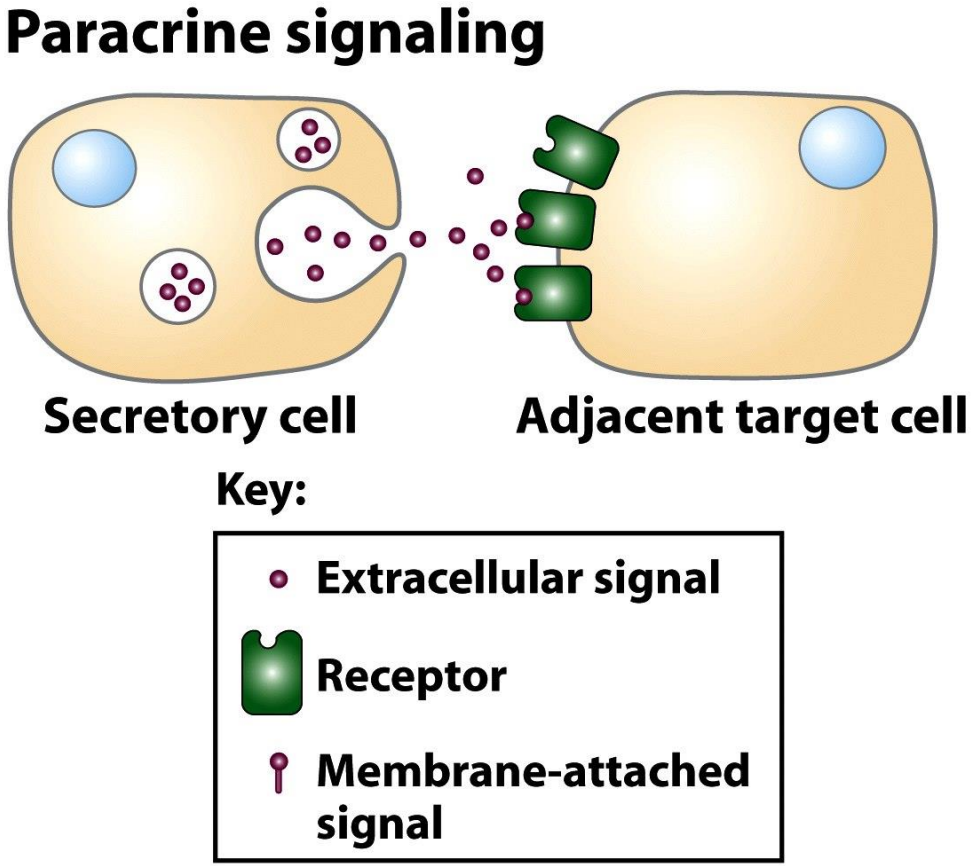

Figure 15-2b
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Some hormones like epinephrine are involved in short range (paracrine signaling - neurotransmitter) and long range (endocrine signaling – hormone)

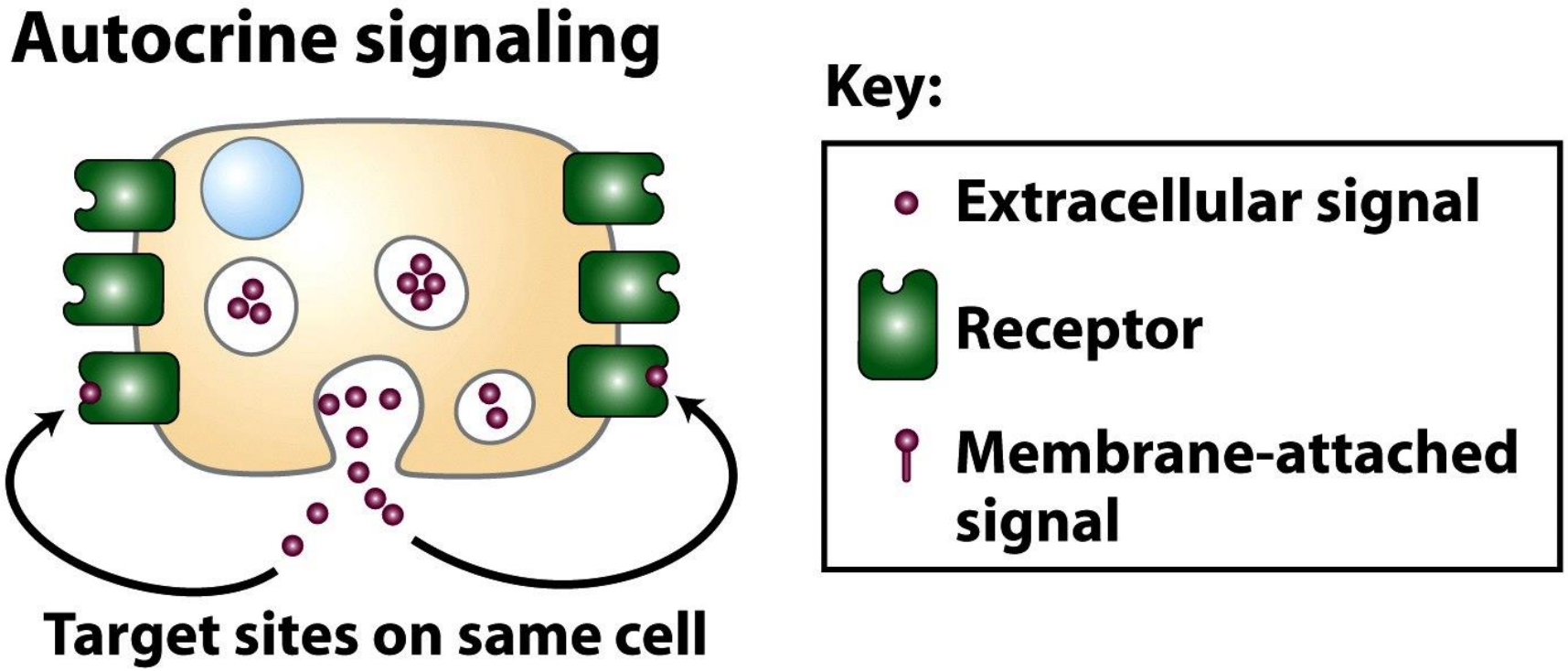

Figure 15-2c
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Signaling by plasma membrane–attached proteins

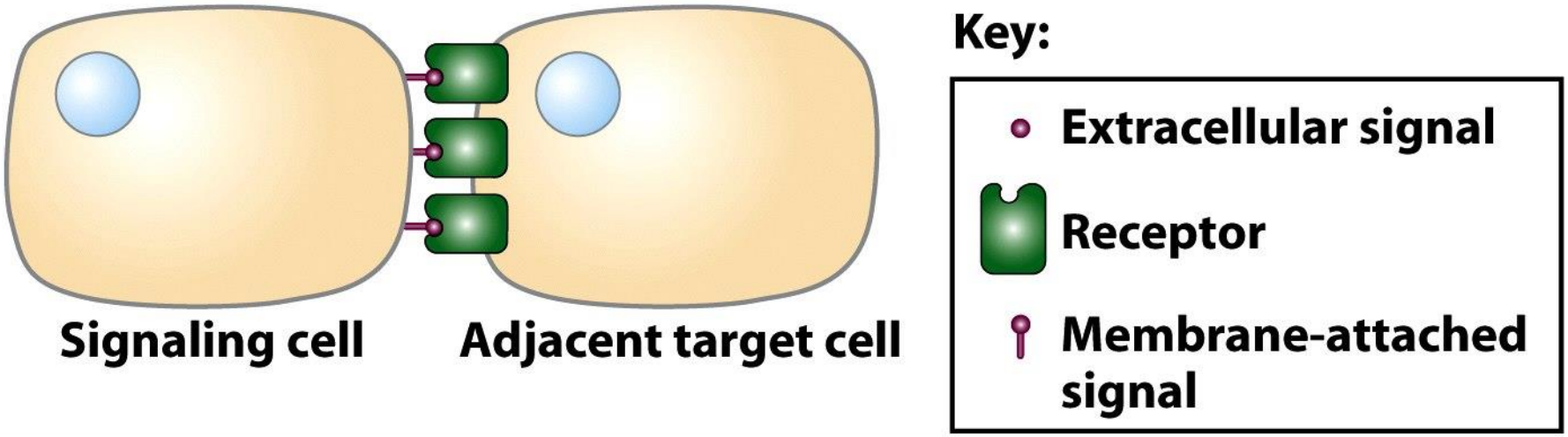

Figure 15-2d
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EGF is synthesized as an integral membrane protein. Cleavage by a matrix Protease releases an extracellular form that can signal in an autocrine or a paracrine manner.

How small patches of amino acids are important for specific binding to a ligand?

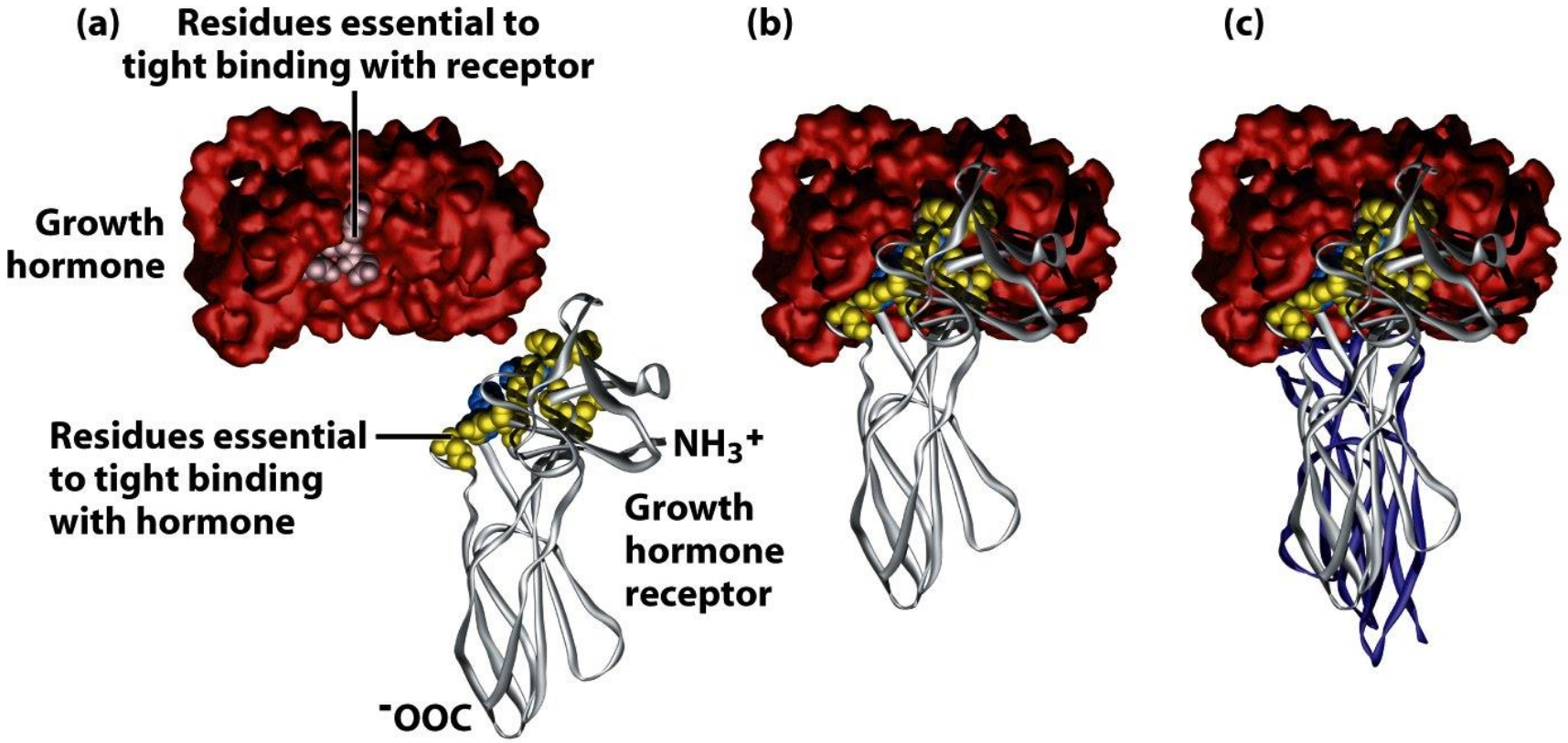

Figure 15-3
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Hormone induced receptor dimerization

Mutational studies involving Key residues in hormone-receptor binding will be discussed in lecture.
Role of single , double and triple mutations will be discussed.

There will be at least one home work problem based on this concept.

Where are the receptors located and what does ligand binding do to them?

- Receptors are located on the surface of the target cells
- Binding of the signaling molecule causes the receptor to undergo a conformational change and initiates a sequence of reactions leading to specific cellular responses
- **Diversity** - different cell types have different receptors for the same ligand and induce different responses.

  Same receptor in various cell types bind the same ligand but produces different responses in the cell.

Diversity and functionality of G protein coupled receptors

- G protein coupled receptors for epinephrine that are found in different types of mammalian cells
- In liver and adipose tissue, binding of epinephrine to beta-adrenergic receptors causes release of glucose and fatty acids
- Epinephrine when it binds to heart muscle cells increase rate of contraction (beta-adrenergic receptors)
- When Epinephrine binds to smooth muscle cells of the intestine, it causes relaxation (beta-adrenergic receptors)
- Epinephrine bound to alpha adrenergic receptors on the the smooth muscle cells (lining the blood vessels) of the intestines, skin and kidney causes arteries to constrict

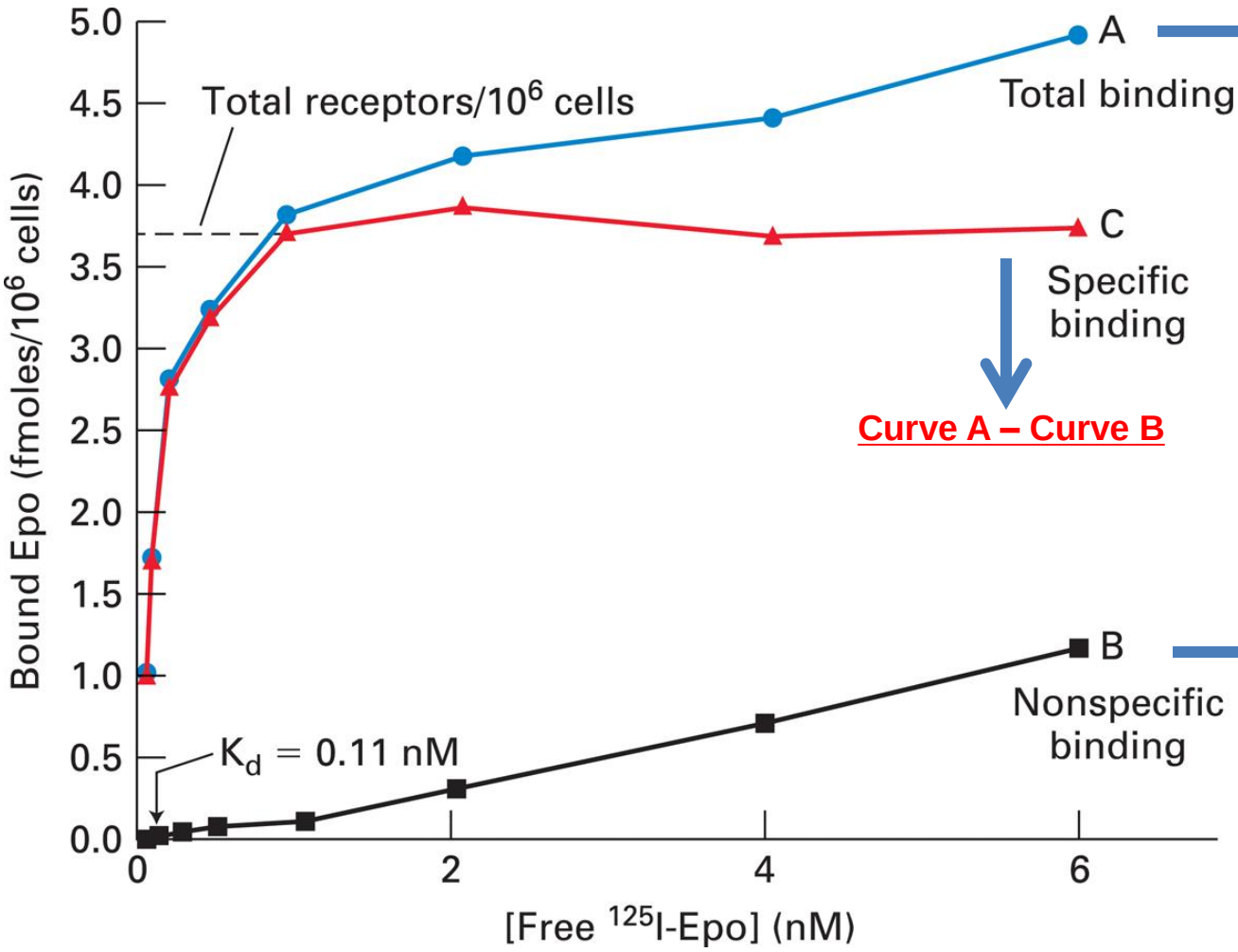
Cell Surface receptors

- 1000 to 50,000 copies per cell and there are 10^6 proteins on the plasma membrane. Purification and isolation is difficult
- Binding specificity of a receptor refers to its ability to distinguish closely related substances eg. Insulin receptor binds insulin and IGF-1
- Ligand binding depends on weak, multiple noncovalent forces (Ionic, van der waals and hydrophobic interactions)
- Effector specificity – the receptor-ligand complex produces a specific cellular response eg. Acetylcholine-receptor complexes in skeletal muscle triggers contraction, in heart muscle slows the rate of contraction, in pancreatic cells triggers a rise in calcium concentration and causes release of enzymes
- Measure of affinity of a receptor for its ligand is given by the disocciation constant, K_d

Kd = [R][L]/[RL]

$K_d = K_{off}/K_{on}$

Binding assays can determine the number of receptors per cell and disocciation constant (K_d)



Cells transfected with Epo receptor gene. Gene is expressed and the receptor protein is present on the cell surface – Here total binding is **ligand binding to the receptor** and **ligand binding non-specifically to the cell surface**

Control cells with **no Epo receptor on the cell surface** – so all binding is non specific

Competition assays

- Competition assays - Unlabeled low affinity ligand (competitor) is added to cell assay system which has a constant amount of radiolabeled high affinity ligand
- Used to detect weak binding of ligand to its receptor.
- The concentration of the competitor required to inhibit binding of half the radioactive ligand approximates the K_d value for the competitor binding to the receptor

COMPETITION ASSAYS

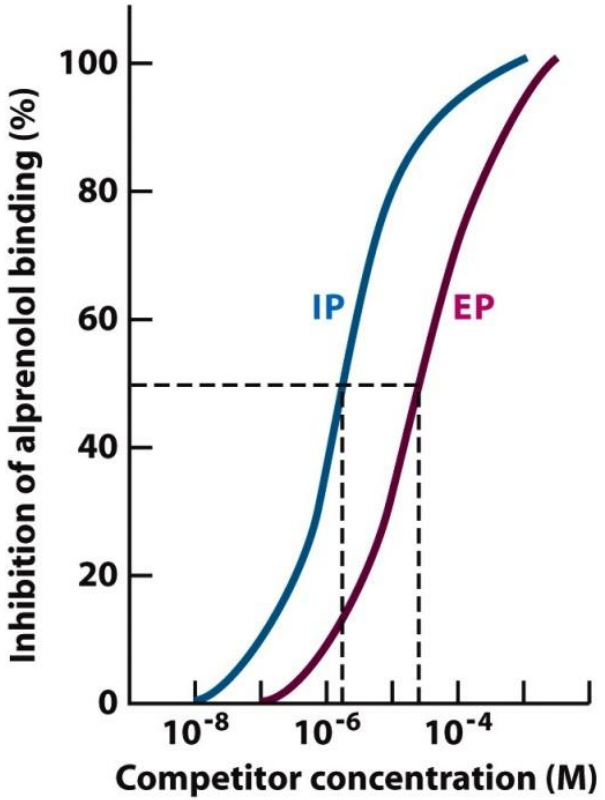


Figure 15-5
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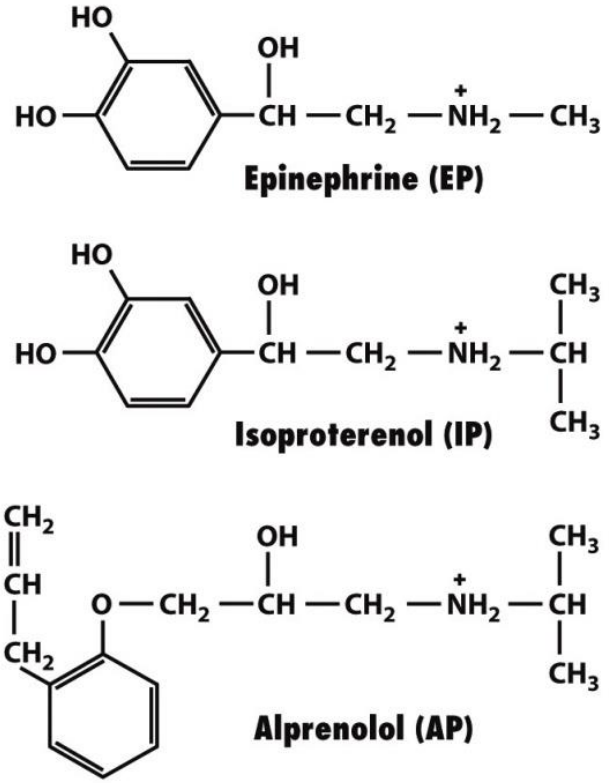

High affinity ligand – Alprenolol
Low affinity ligands – natural hormone epinephrine and synthetic ligand Isoproterenol

Agonists and Antagonists

- Agonist – Analogs that mimic function of a natural signaling molecule by binding to its receptor and inducing the normal response

  Example – Isoproterenol is an agonist of epinephrine on bronchial smooth muscle cells

- Antagonists – Bind to receptor but induce no response. They hinder the binding of the normal ligand to its receptor and thus reduce usual physiological response.

  Example – Alprenolol is an example of an antoagonist of epinephrine's action on cardiac muscle cells

Maximum cellular response

- The K_d value or binding affinity of a receptor to its ligand must be greater than the normal (unstimulated) level of that ligand in extracellular fluids
- The normal concentration of the ligand must be well below the K_d value. So, a rise in ligand concentration can cause an increase in the number of receptor-ligand interactions
- Maximum cellular response to a particular ligand is induced when less than 100% of the receptors are bound with ligand

Maximum physiological response to signal occurs when only a fraction of the receptors are bound by ligand

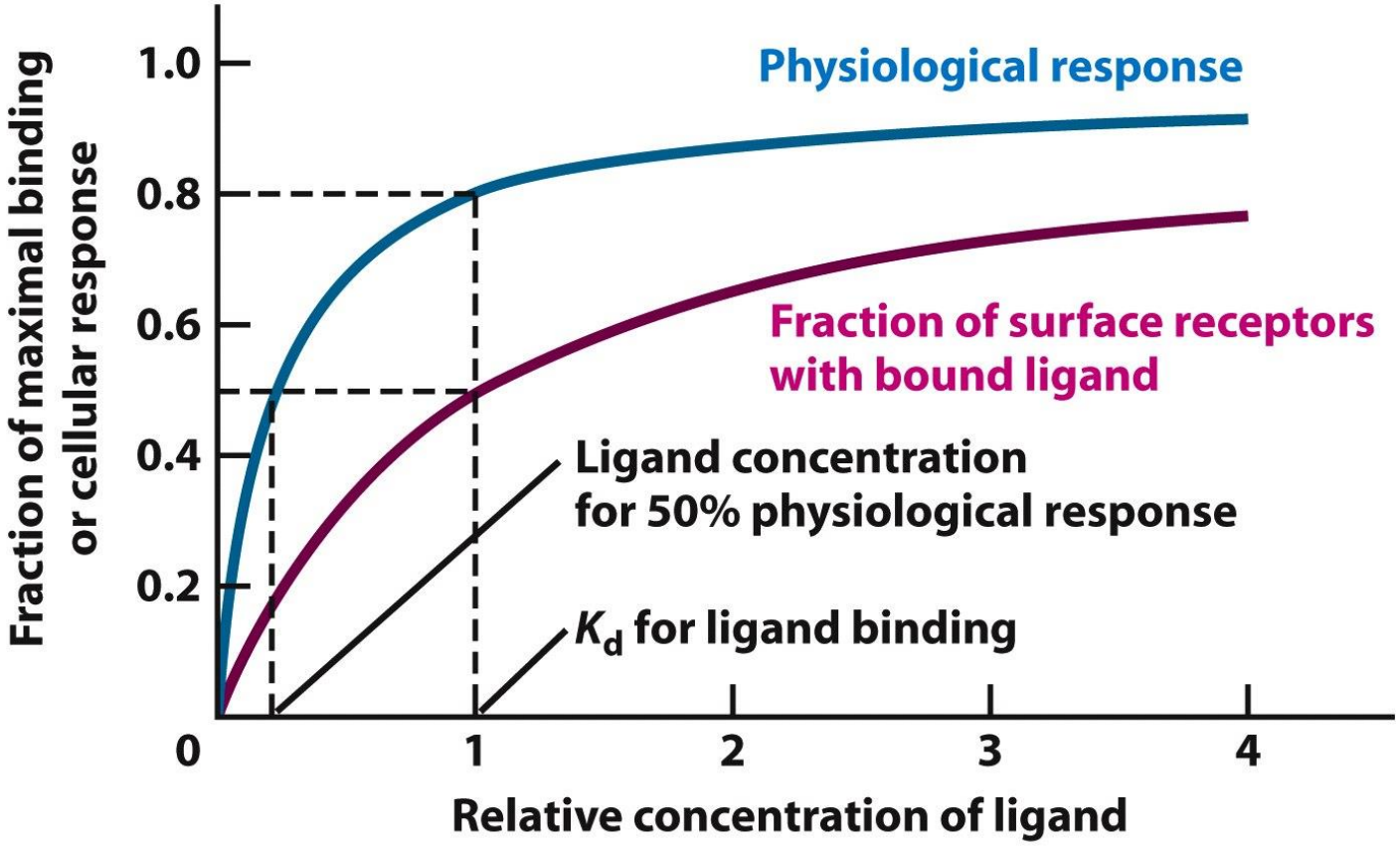

Figure 15-6
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Receptor Purification

- Affinity labeling – cell are mixed with a radiolabeled ligand for the receptor of interest. Unbound ligand is washed away the cells are treated with a crosslinker to bind the ligand and the receptor.
- Affinity chromatography – A ligand to a receptor of interest is chemically linked to the beads of a column. Receptor binds to the column and is washed off the beads with an excess of soluble ligand
- Cloning and expression

Functional Expression assay for a cell-surface receptor

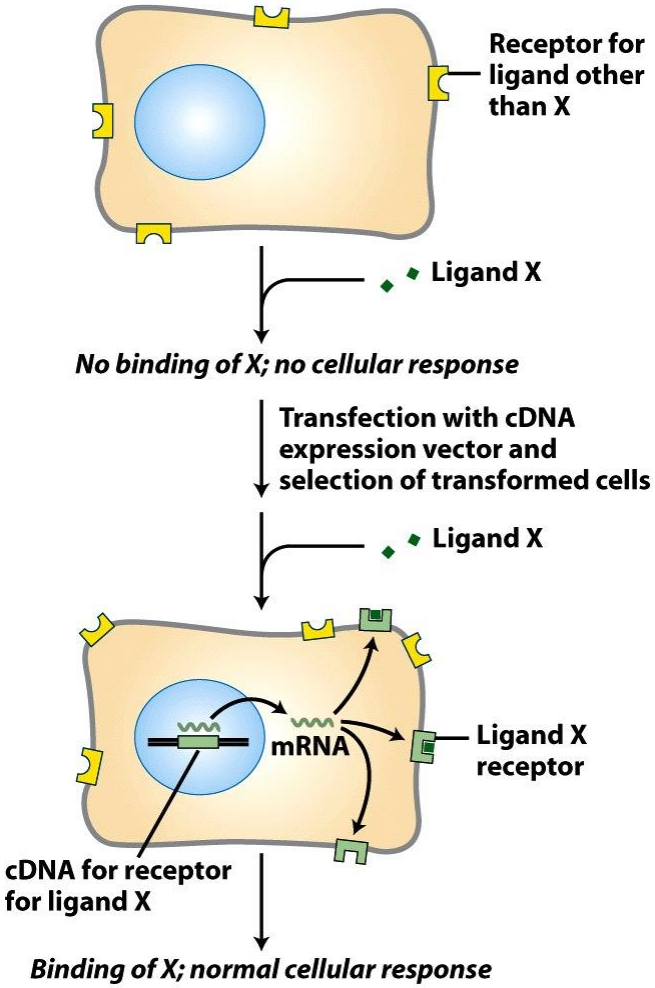

Figure 15-7
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SENSITIZATION OF CELLS TO EXTERNAL SIGNALS

- Sensitization of receptors to ligand – depends on the number of receptors on the cell for a particular ligand and the affinity for the ligand
- Increasing the number of receptors would increase the cellular response only if an increasing amount of ligand is present
- EGF and HER2 receptor
  - 25% of breast cancer have an elevated expression of HER2
  - This overproduction makes the cells hypersensitive to ambient levels of EGF that are normally too low to stimulate cell proliferation
  - Reduction in sensitivity to external ligands is called desensitization

Intracellular Signal-Transduction

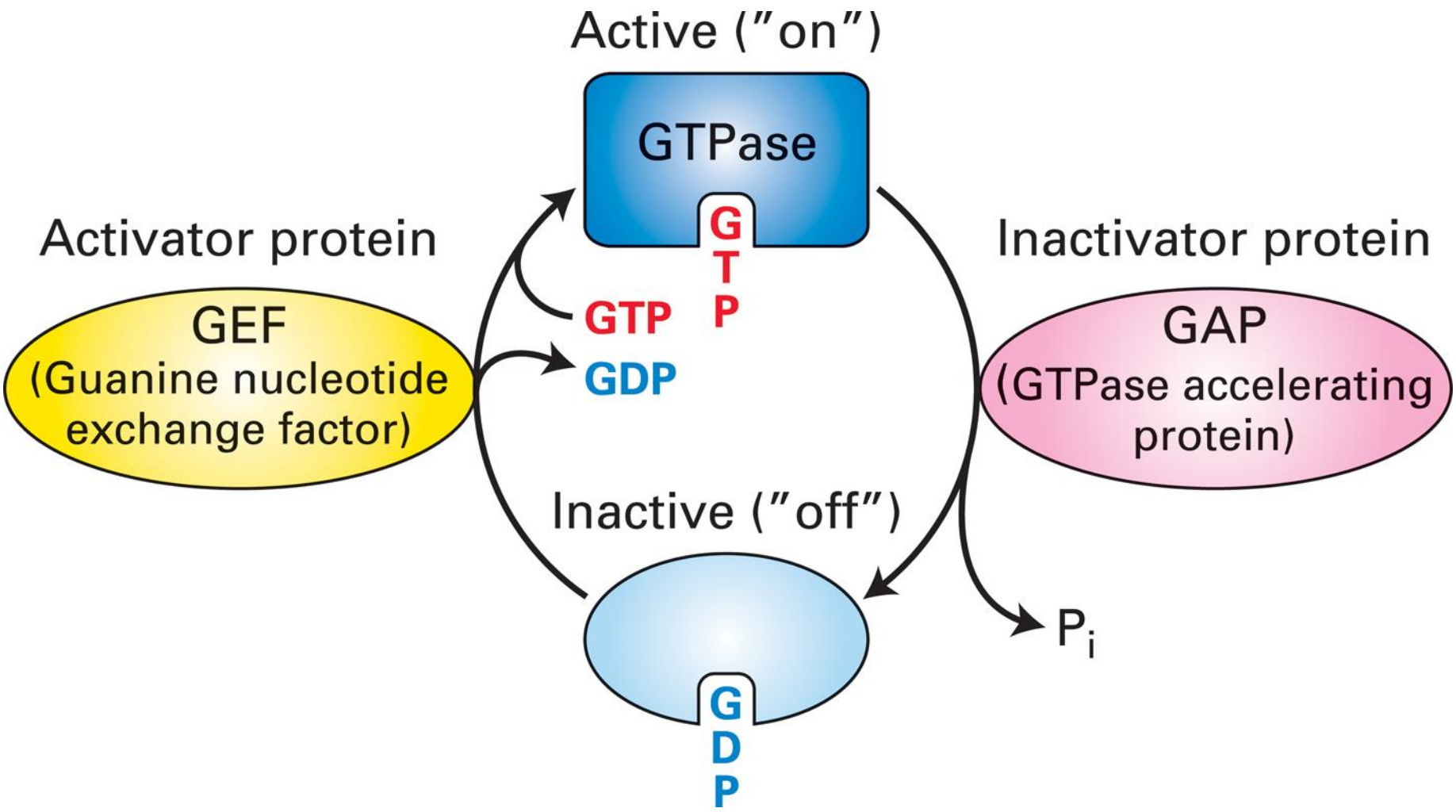
- Changes in activity or function of pre-existing proteins

- Changes in the gene expression induced by modification of transcription factors

Highly conserved components of intracellular signaling

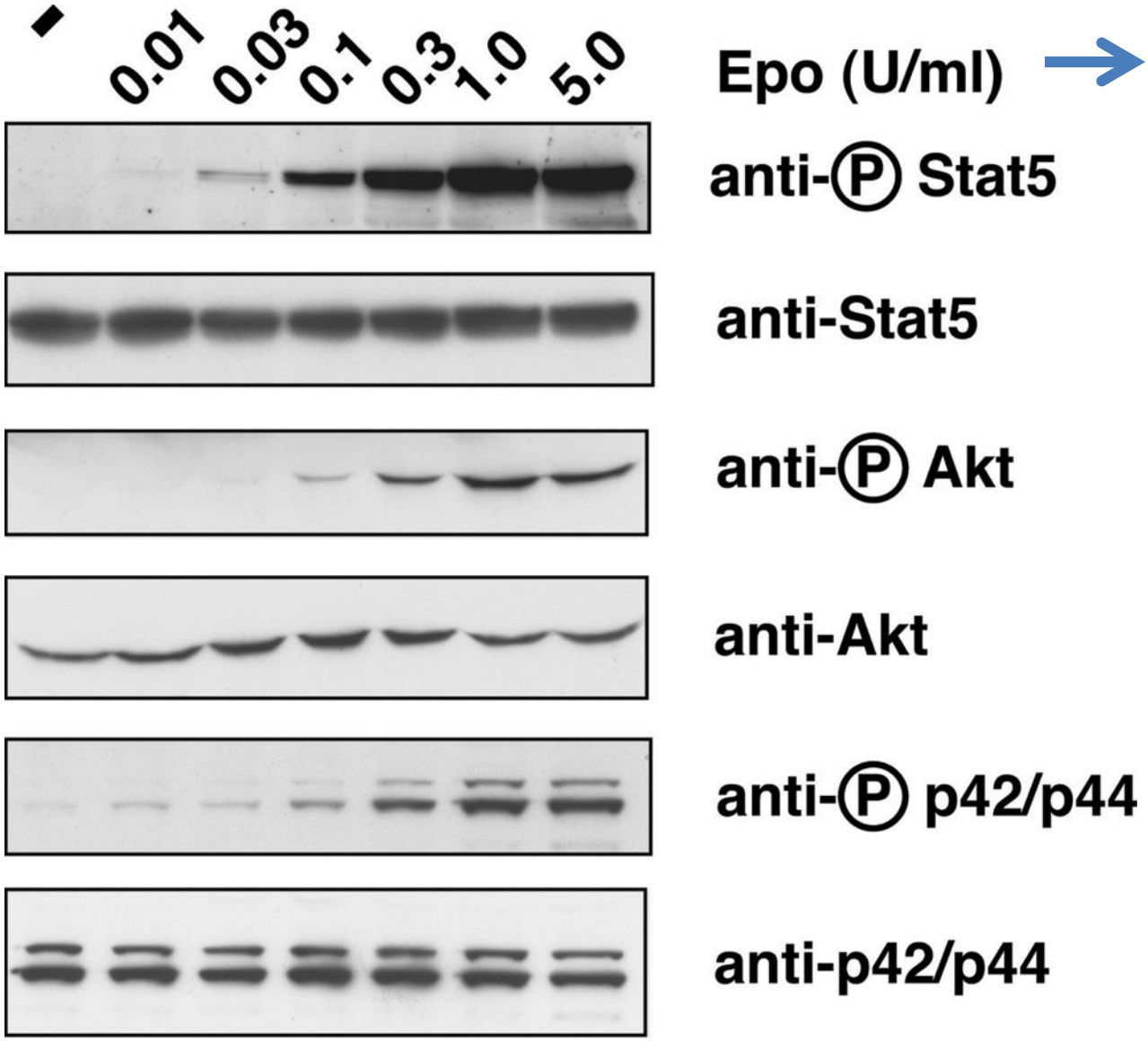
- GTP- binding proteins or G-proteins
- Protein kinases or phosphatases
- Second messengers
  - cAMP
  - DAG and IP_3
  - Ca^{2+}

GTP binding Proteins



Activation of three signal transduction proteins by phosphorylation – An example.

These three proteins undergo phosphorylation when Epo (ligand) binds to its receptor. The way you can monitor the signal being tranduced inside is to look for phosphorylated forms of the same proteins. This will fall under short term changes associated with signal transduction.

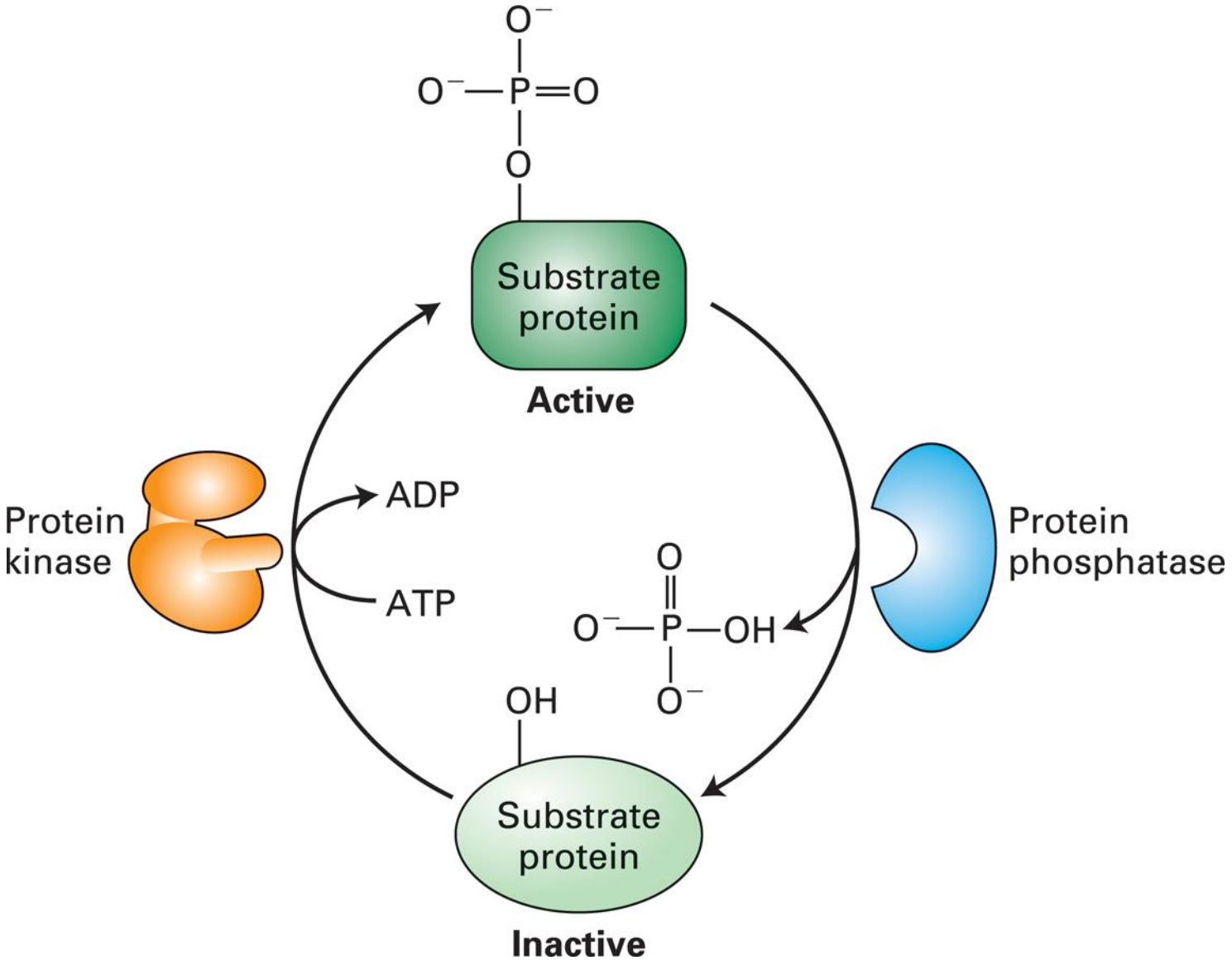


With increasing concentrations of ligand, you see an increase in the phosphorylated forms of the three proteins

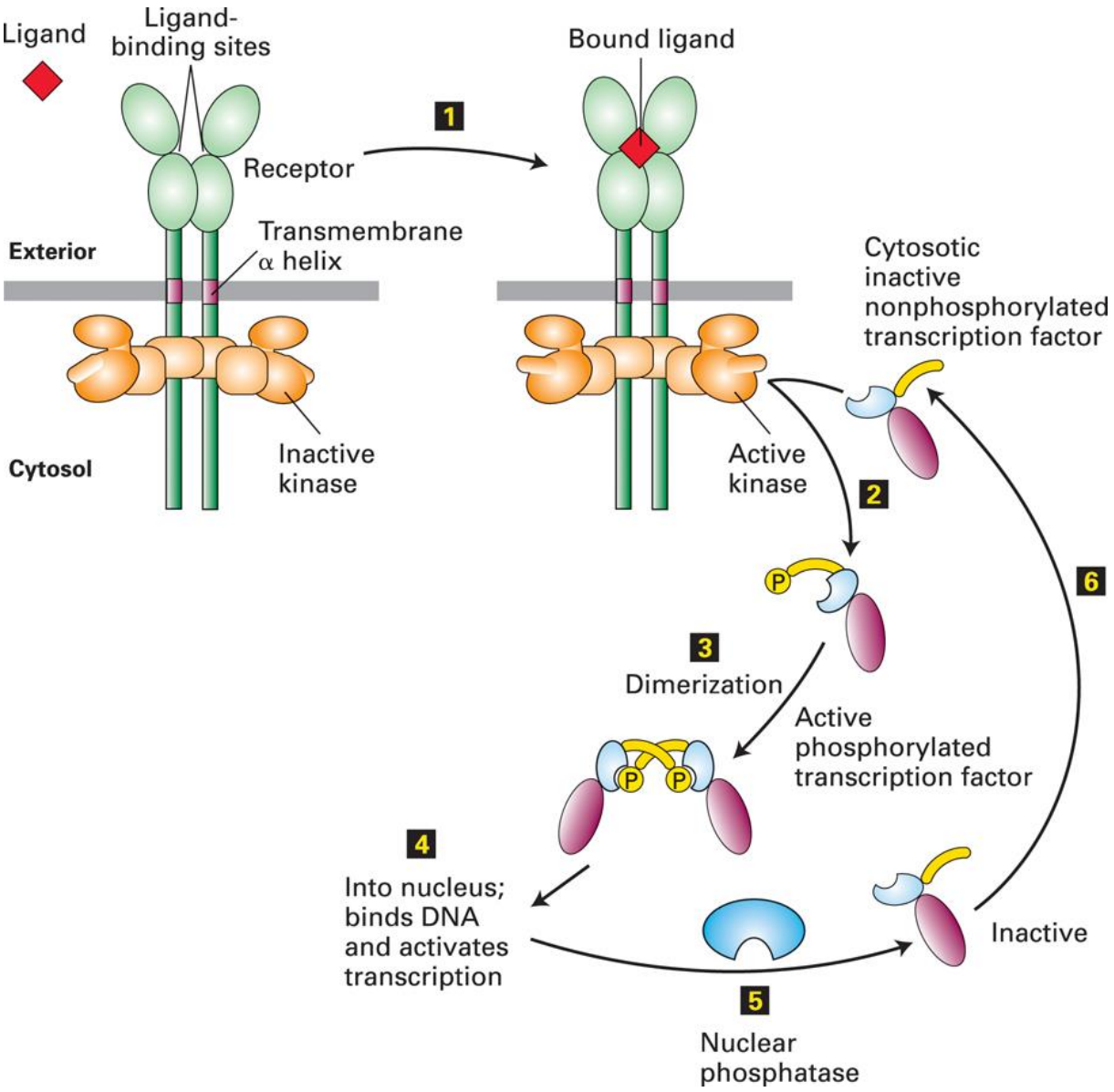
Kinases

- Kinases add a phosphate group
  - Addition of phosphate group to –OH group of tyrosine
  - Addition of phosphate group to the hydroxyl group of serine or threonine or both
  - Protein Kinases are modulated themselves by other kinases
  - Receptors possess intrinsic kinase or phosphatase activities or they interact with proteins in the cytosol that possess these activities

PROTEIN KINASES AND PHOSPHATASES



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



G proteins - Switching mechanism for G proteins

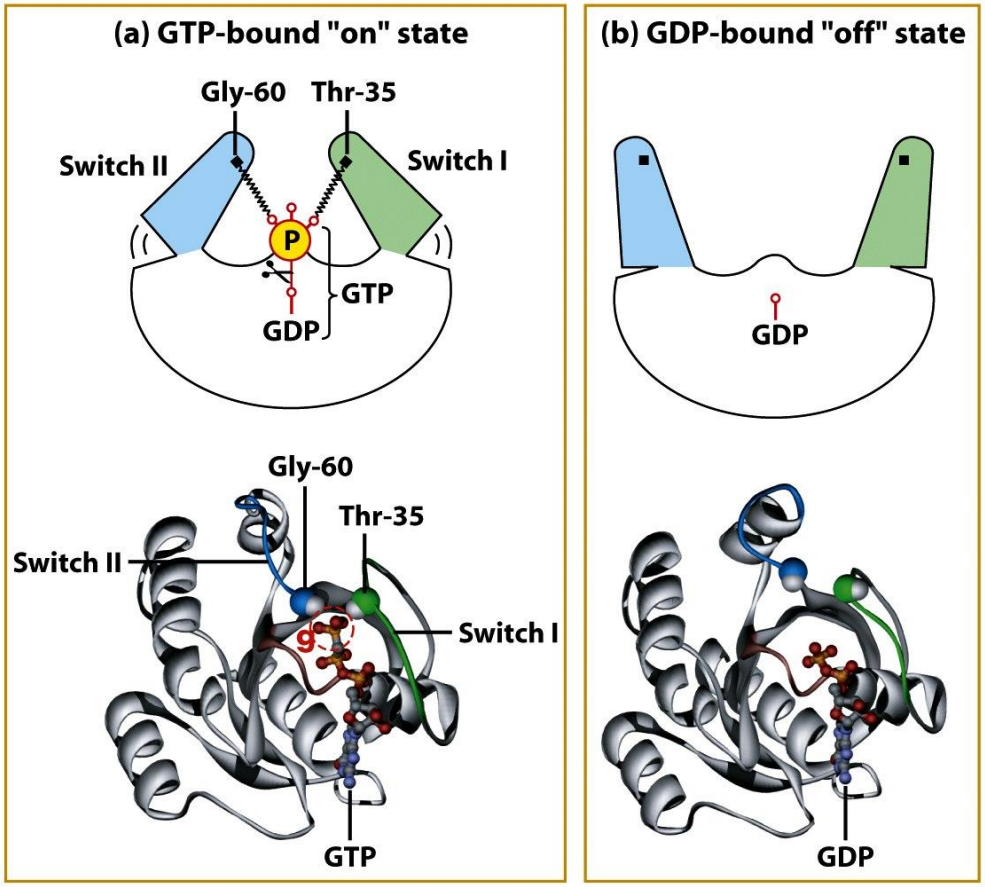


Figure 15-8
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G proteins

- Guanine nucleotide exchange factor (GEF) causes the release of GDP from the switch protein
- The G protein has intrinsic GTPase activity
- Rate of GTP hydrolysis determines how long the switch is on
- Regulation of G protein activity – GTPase activating protein (GAP) and by regulator of G protein signaling (RGS) regulate GTP hydrolysis
- GTP binds to the G-alpha subunit
- Two classes of switch proteins – trimeric (large) G proteins and monomeric (small) G protein (Ras)

G protein coupled receptor signaling

- General structure of G protein coupled receptor
- Most receptors are G-protein coupled receptors

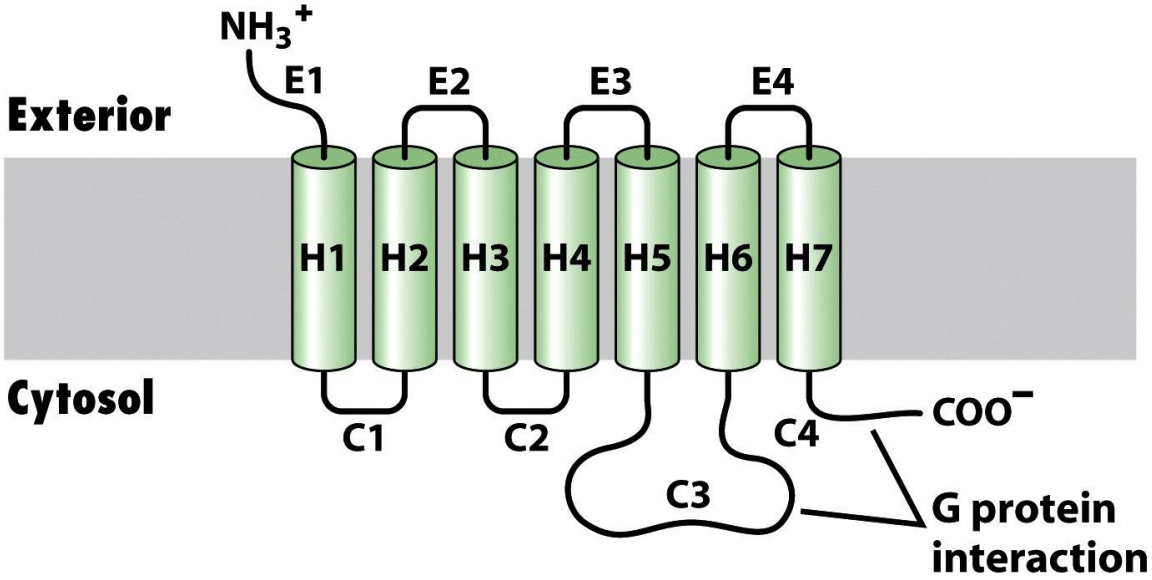

Figure 15-10
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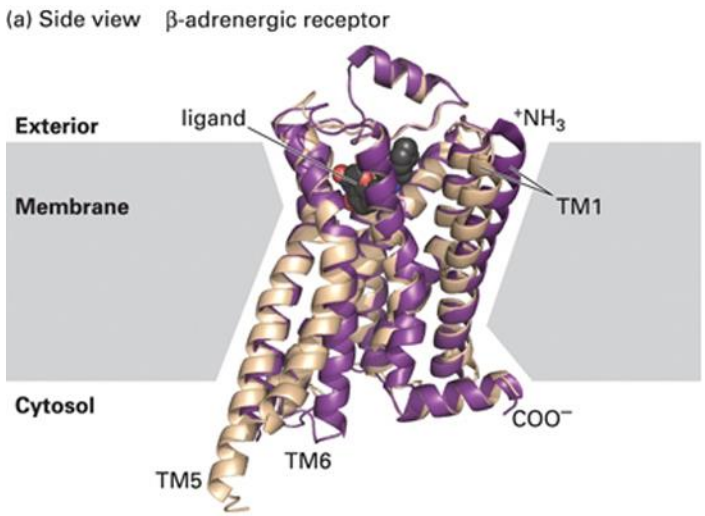
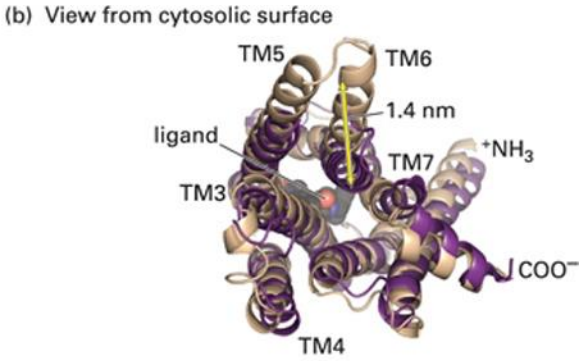
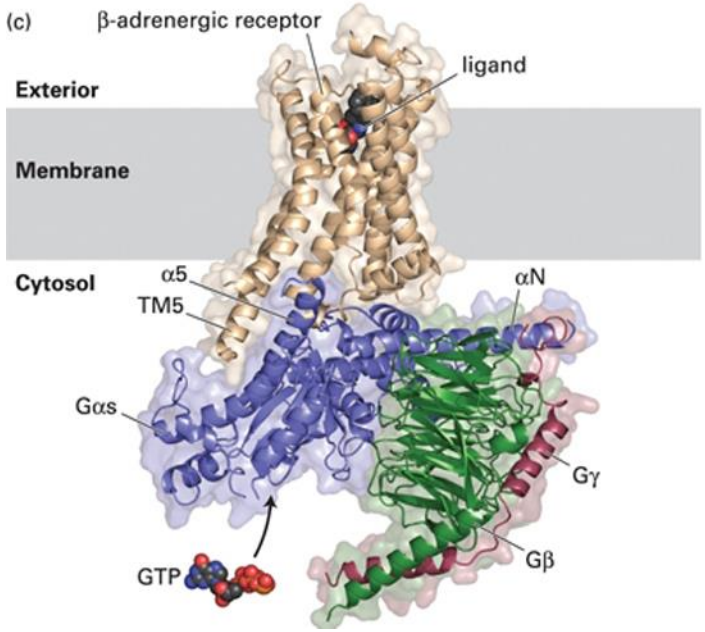
Common features of G protein coupled signaling receptors

- A receptor that contains 7 membrane spanning domains
- Coupled trimeric G protein which functions as a switch by cycling between active and inactive forms
- A membrane- bound effector protein
- Feedback regulation and desensitization of the signaling pathway
- A second messenger is involved

Structure of the b-adrenergic receptor in the inactive and active states and with its associated trimeric G protein, G_{as}.







Chimeric experiments with adrenergic receptors

Recombinant DNA contsructs of different regions of different receptors and their effect on receptor function

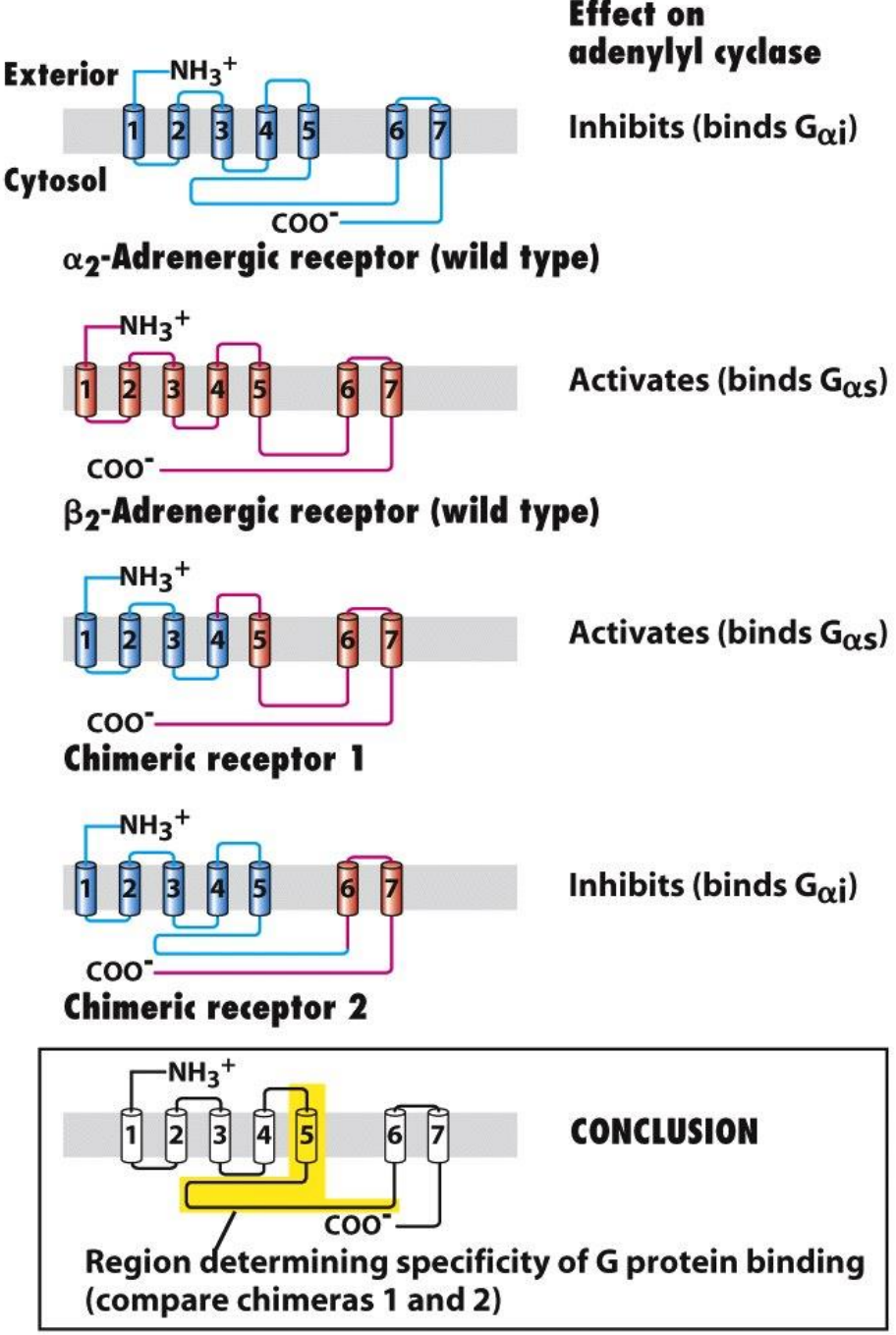


Figure 15-12
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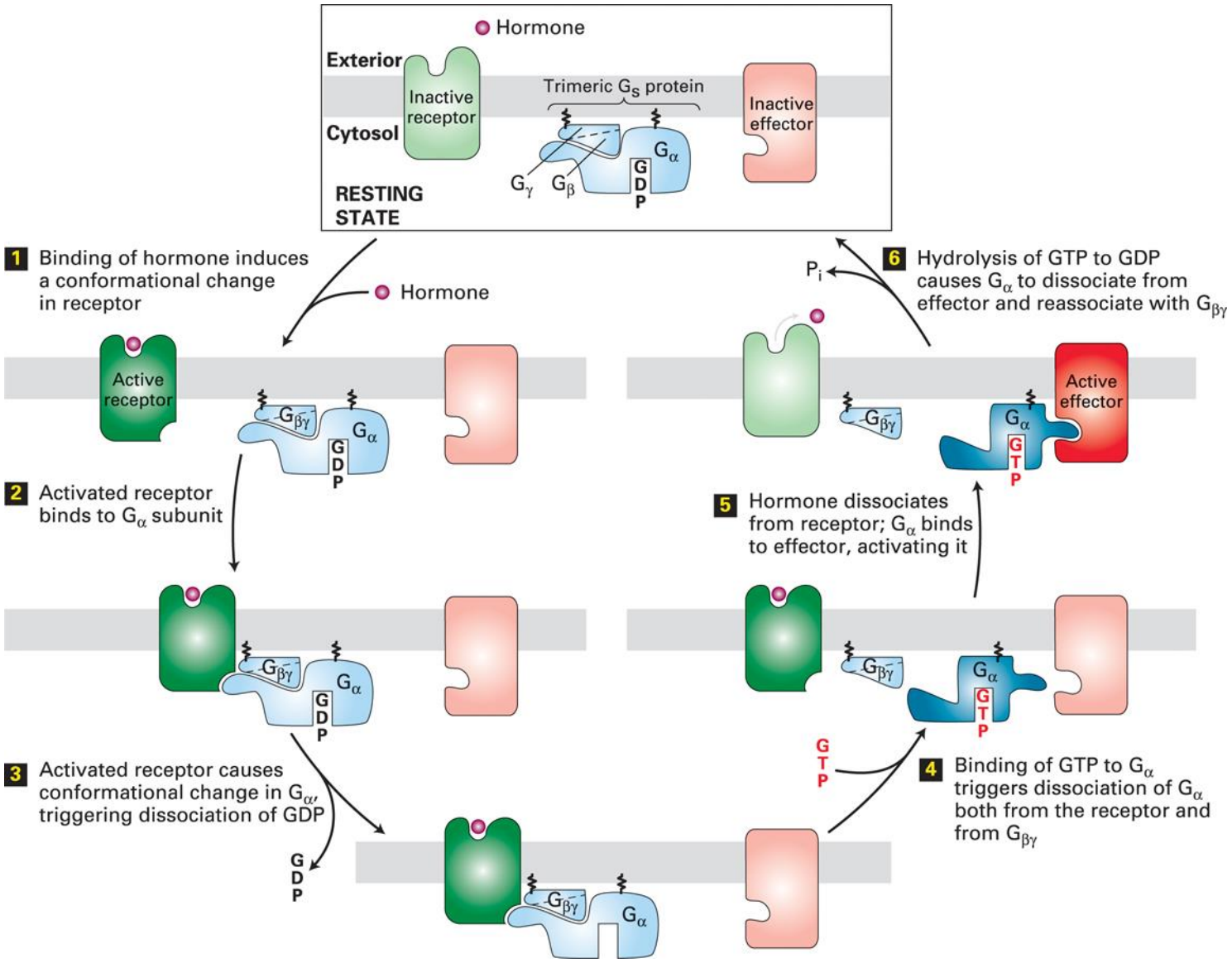
Mechanism of G protein coupled receptor signaling



How fast is G protein activation and which subunits dissociate?

FRET – Fluorescence Resonance Energy Transfer

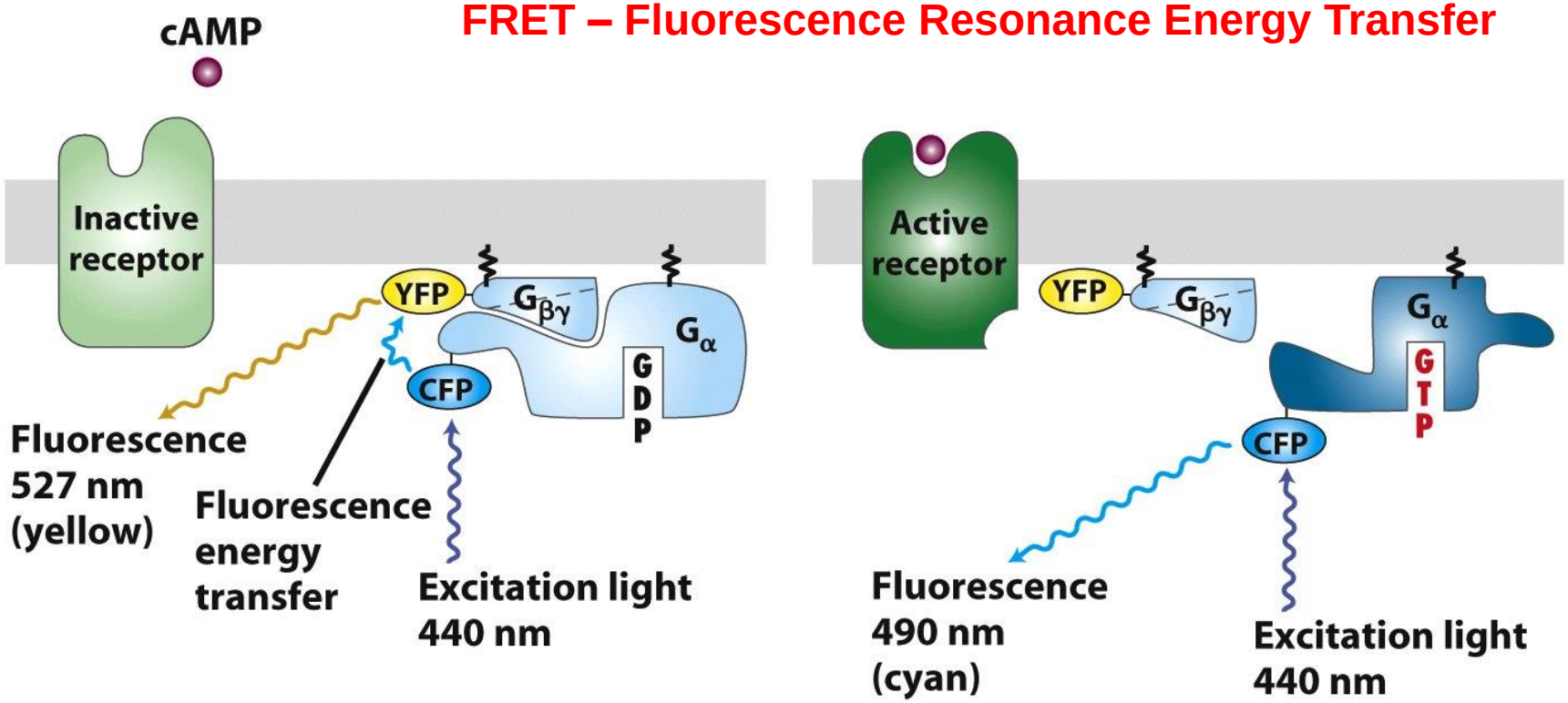


Figure 15-14a
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Result: Activation of G protein occurs within seconds of ligand binding in Dictyostelium discoideum cells

Decrease in fluorescence of yellow light with time

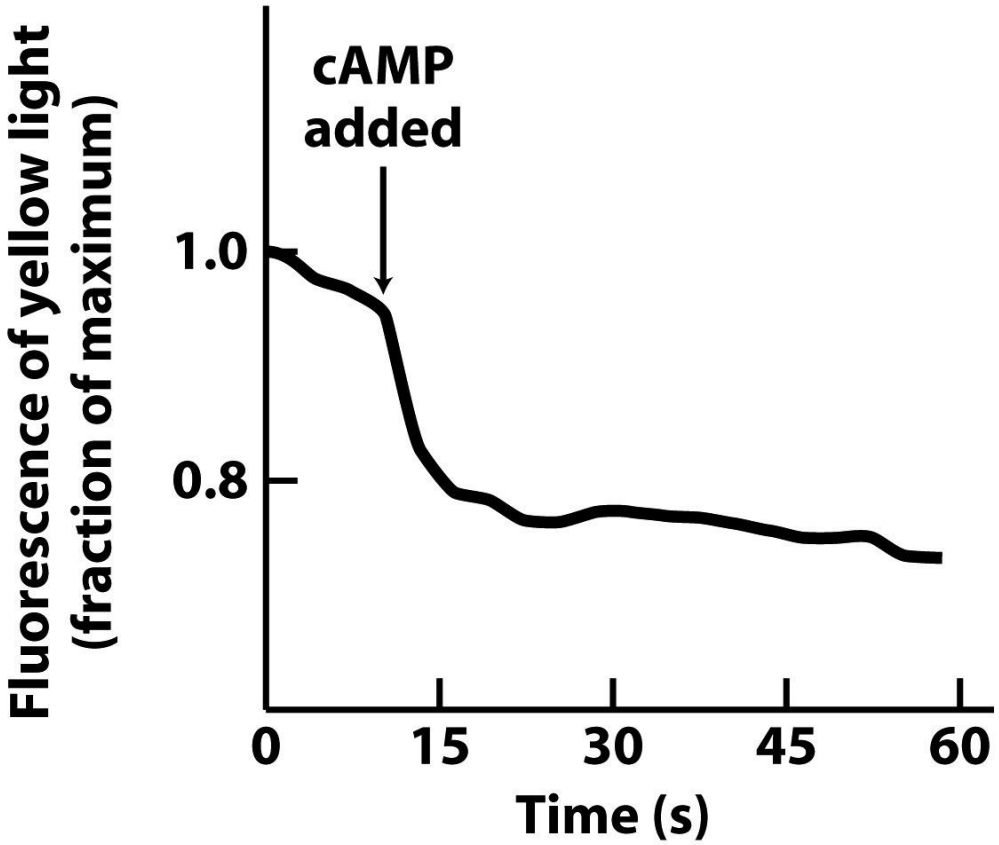

Figure 15-14b
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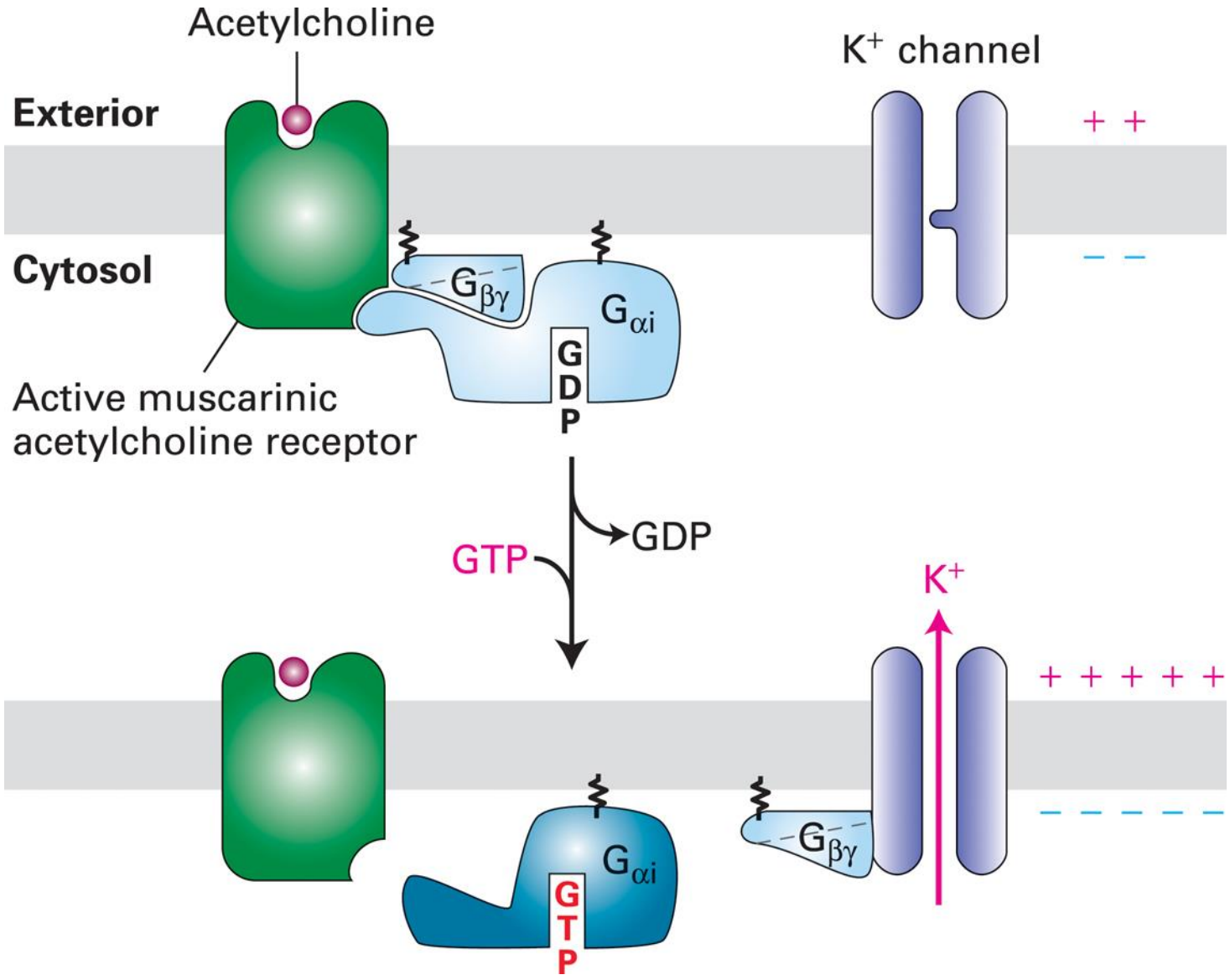
G protein receptors linked to ion channels

- The effector proteins are Na^+ or K^+ channels
- Muscarinic acetylcholine receptor slows the contraction of the heart
- Coupled to $G_{\alpha i}$ protein and binding of ligand leads to opening of K^+ channels
- It is the $G_{\beta\gamma}$ subunit that binds the effector protein
- Purified $G_{\beta\gamma}$ subunits when added to heart muscles plasma membrane cause K^+ channels to open . This indicates that the $G_{\beta\gamma}$ subunits are the ones that interact with the K^+ channel

Muscarinic Acetylcholine receptor (in heart muscle



Different GPCRS activate different G proteins which in turn effect different signaling pathways

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
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21 different alpha subunits encoded by 16 genes – alternative splicing
6 beta subunits
12 gamma subunits

Second messengers

- Low molecular weight intracellular signaling molecules
- Calcium ions
  - In muscle, increase in calcium results in contraction
  - In endocrine cells, the same increase causes exocytosis of secretory vesicles
  - In nerve cells, increase in calcium causes exocytosis of neurotransmitters

Second messengers (cont’d)

- Cyclic AMP - rise in cAMP triggers activation of protein kinases (protein kinase A), regulates ion channels
- DAG activates protein Kinase C
- Inositol trisphosphate opens calcium channels in the endoplasmic reticulum
- cGMP activates protein kinase G

INTRACELLULAR SIGNALING PATHWAY – RHODOPSIN, a G-protein Coupled receptor on rod cells

Human Rod Cell

Human retina consists of rods and cones.

Rods are stimulated by weak light over a range of wavelengths and cones are involved in color vision.

Rhodopsin = Opsin + light absorbing pigment 11-cis-retinal
Human rod cell contains $4X10^7$ molecules of rhodopsin

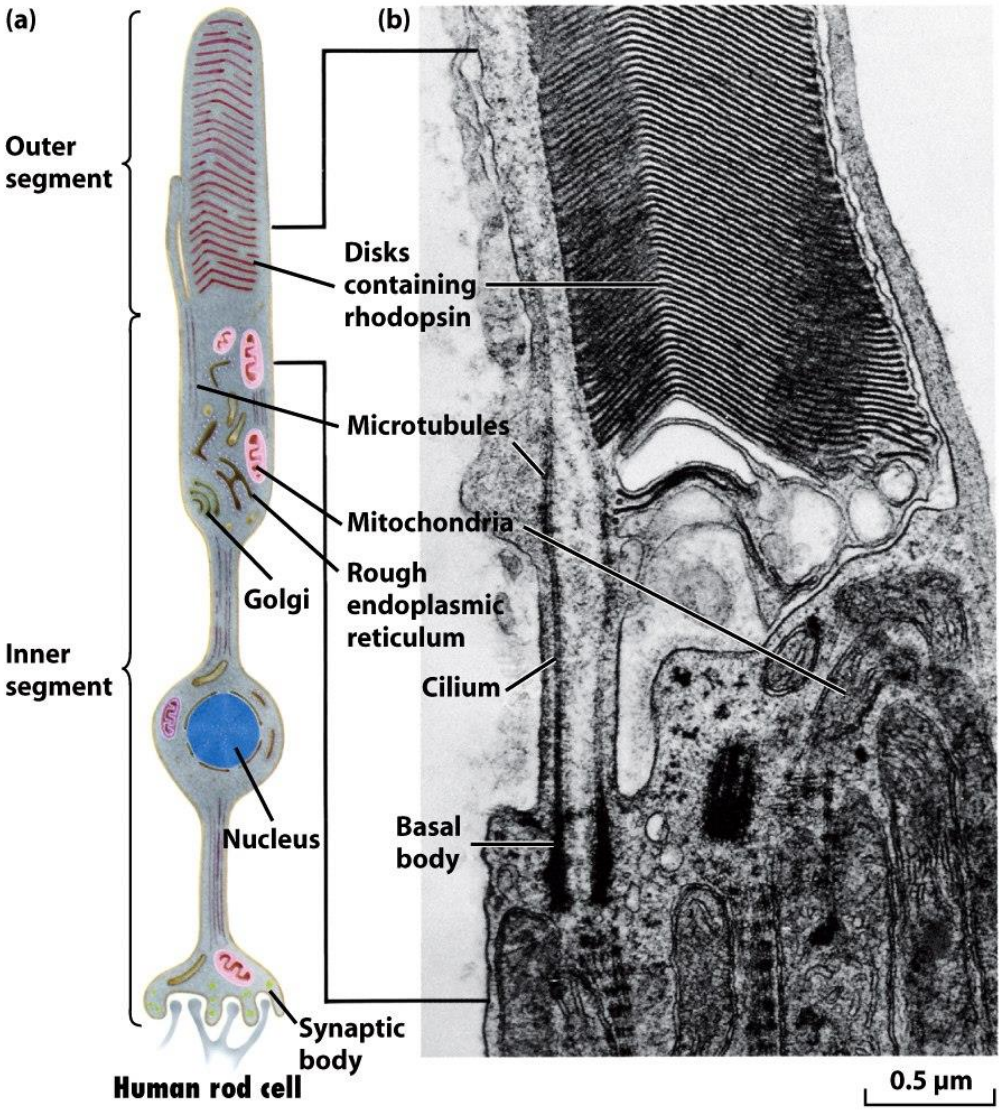

Figure 15-16
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G protein coupled Rhodopsins

- The retinal moiety in rhodopsin is converted from a cis to a all-trans isomer upon absorption of a photon
- This activated rhodopsin binds and activates a G protein, $G_{\alpha t}$.
- Activated opsin is unstable and disocciates into opsin and retinal(trans form)
- In the dark all-trans retinal is converted back to cis-retinal which can bind opsin to form rhodopsin

Light- triggered step in vision

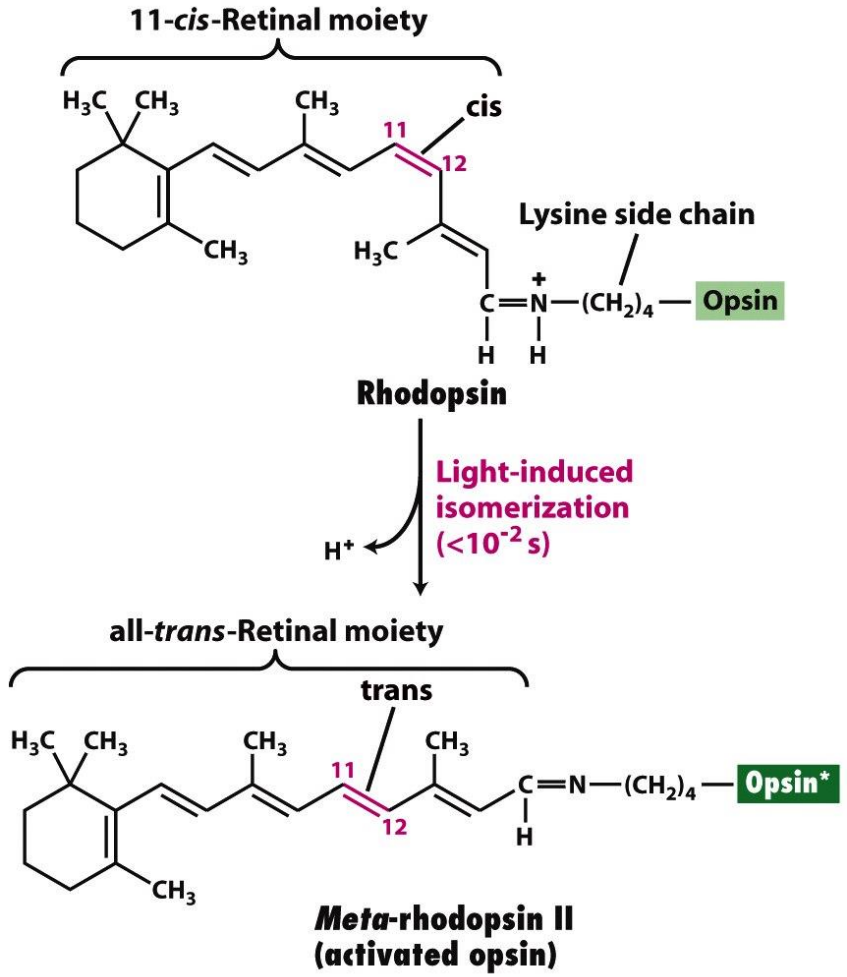

Figure 15-17
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RHODOPSIN AND ITS G PROTEIN

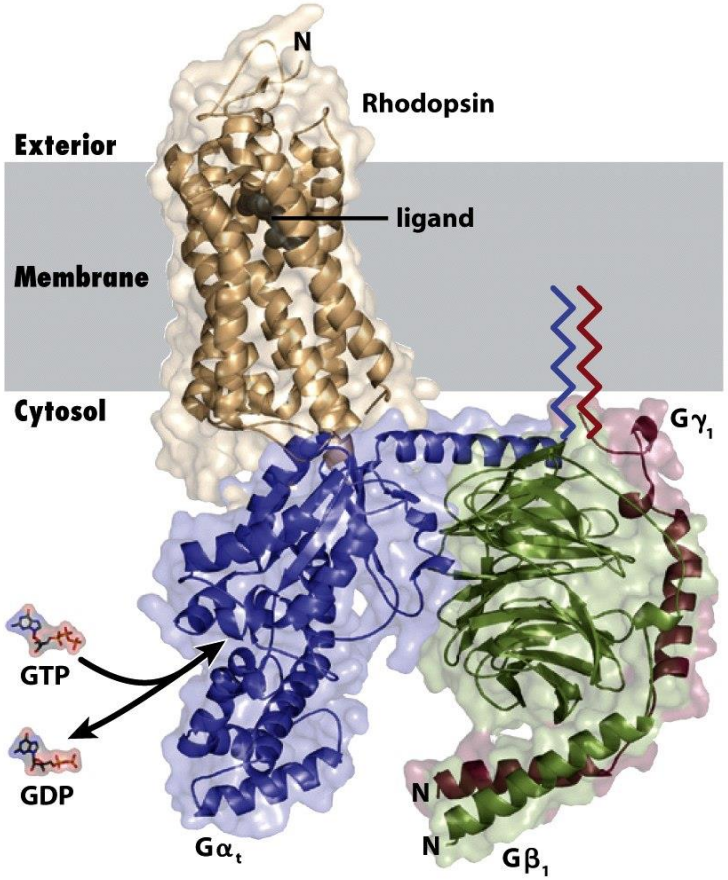

Figure 15-19
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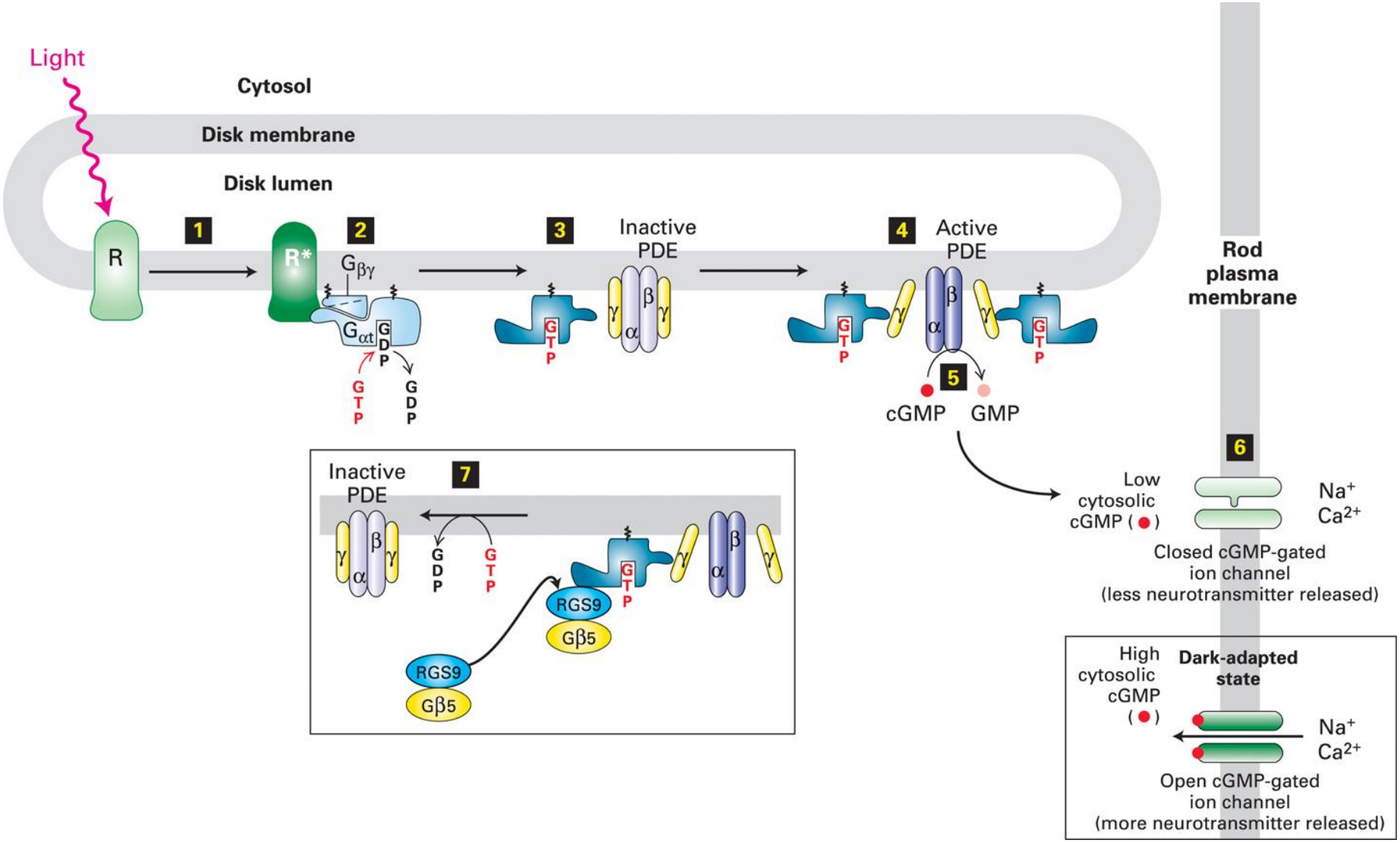
G_γ directly contacts G_β
But not G_α.

RHODOPSIN and its G PROTEIN



Active G_{α}.GTP is converted to inactive G_{α}.GDP by a specific GTPase activating protein

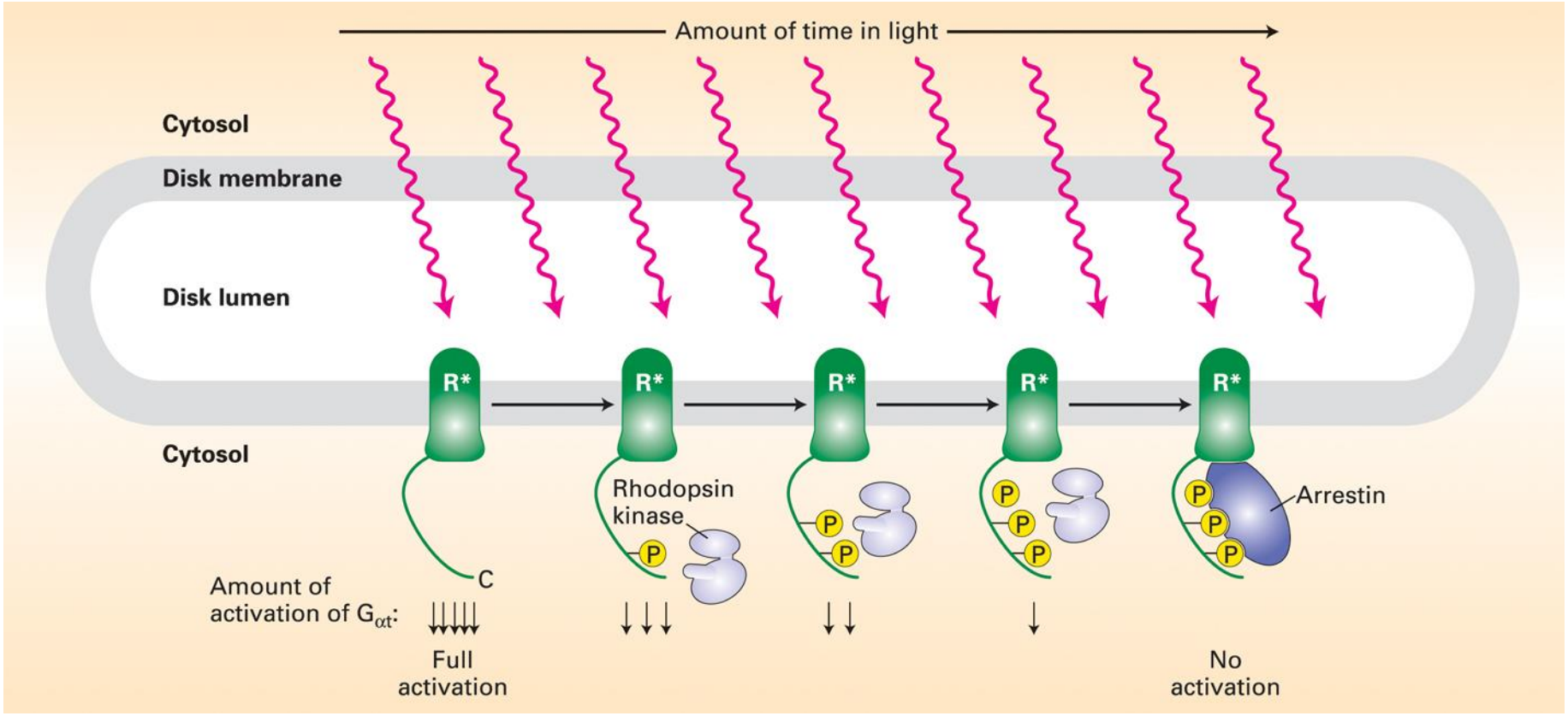
In the presence of light :

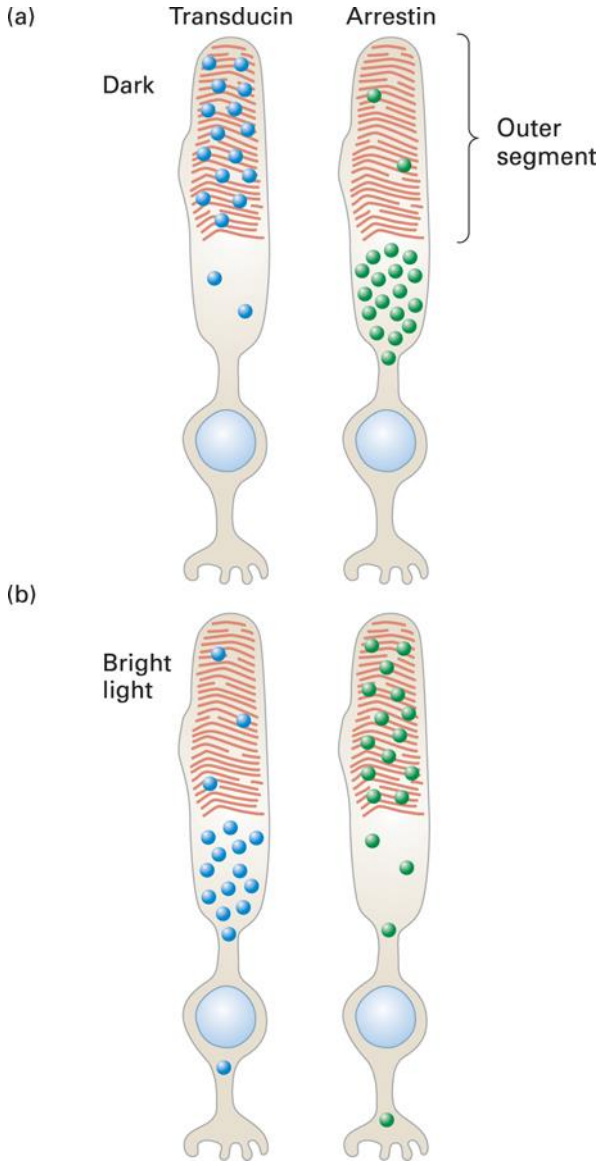
The more photons are absorbed by rhodopsin, the more channels are closed, fewer sodium and calcium ions cross the membrane from outside and the membrane potential becomes more negative. So less neurotransmitter is released. This change is transmitted to the brain where it is perceived as light.

In the dark:

In the dark, the membrane potential of a rod cell is -30mV. This state of the membrane is called depolarization.
This causes the rod cells to constantly secrete neurotransmitters and so the neurons they synapse are continually stimulated.
Depolarization is due to open sodium, calcium and potassium channels. Once light is absorbed, this causes the channels to close and the inside becomes more negative.

Inhibition of rhodopsin signaling by rhodopsin kinase.




(a)
Transducin
Arrestin
Dark
Outer segment
(b)
Bright light

Visual adaptation by Opsin

Rod cells are inhibited at high light level and cone cells are insensitive to low levels of light.
Visual adaptation

Rhodopsin Kinase
Three serine phosphorylation sites on the cytosol facing surface
The more sites are phosphorylated the less opsin is able to activate Gxt
Rhodopsin is desensitized by bright light

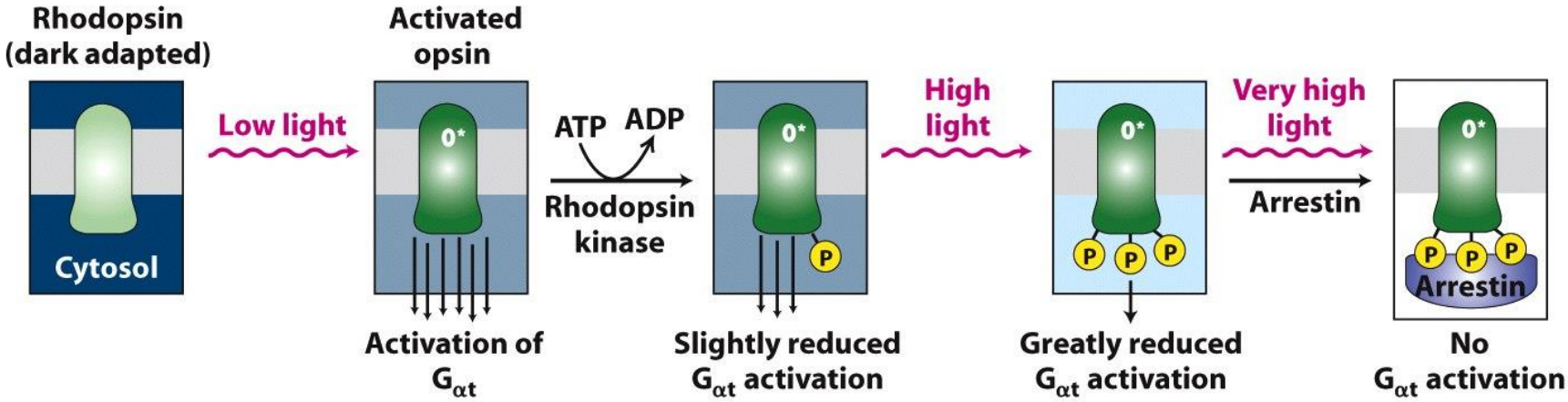


Figure 15-20
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