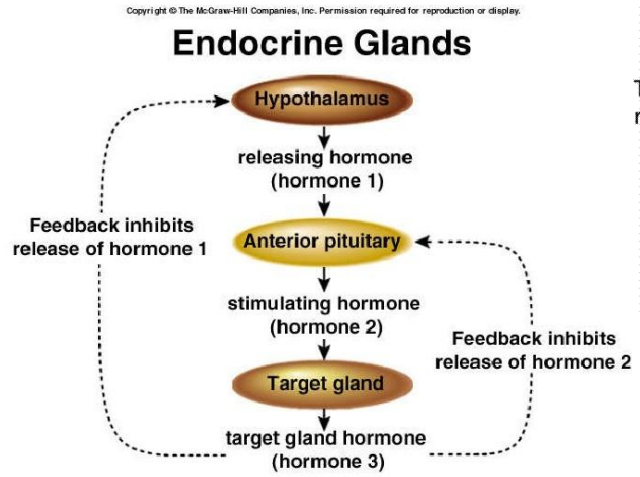
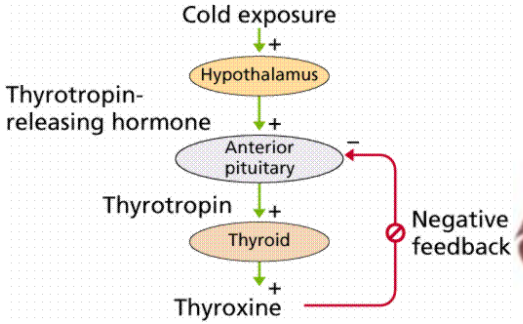
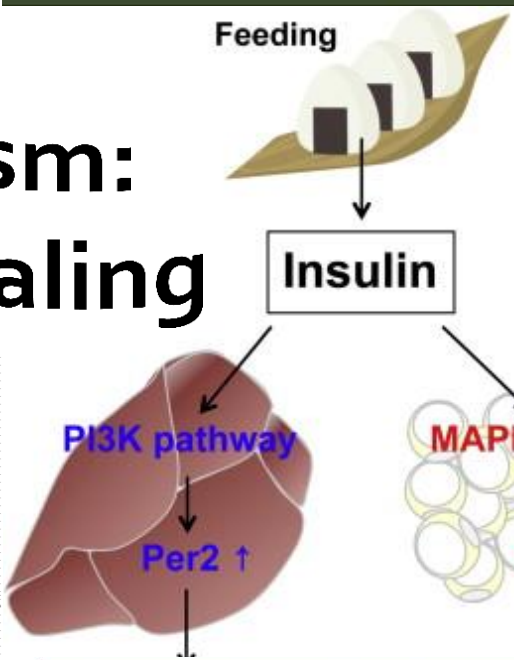
Nafith Abu Tarboush
DDS, MSc, PhD
natarboush@ju.edu.jo
www.facebook.com/natarboush

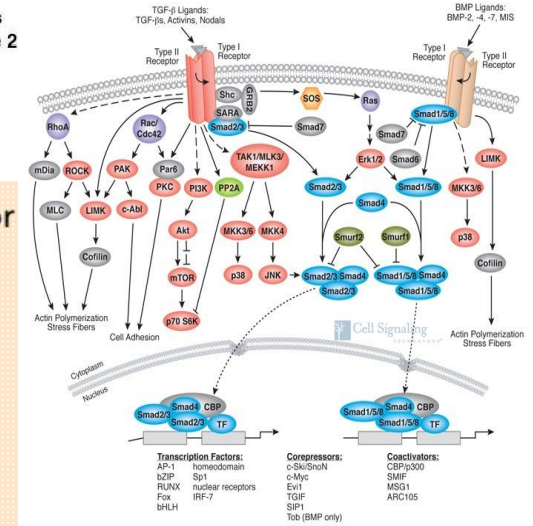
THE UNIVERSITY OF JORDAN

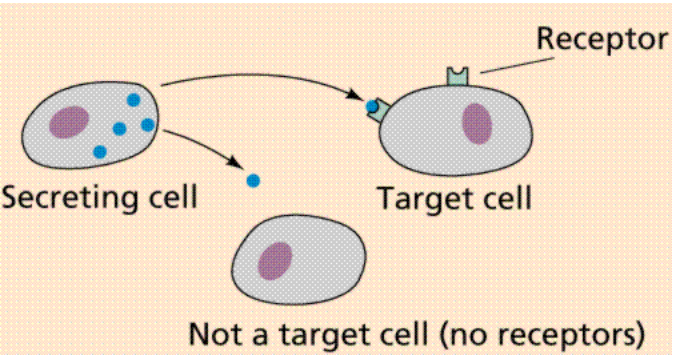
Integration of Metabolism: hormones & Cellular Signaling



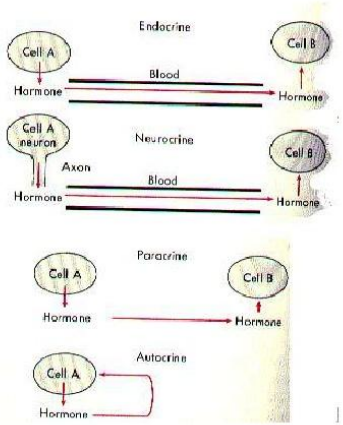








Types of cell-to-cell signaling



Endocrine Hormones: travel via bloodstream to target cells

Neurocrine hormones: released from nerve terminals

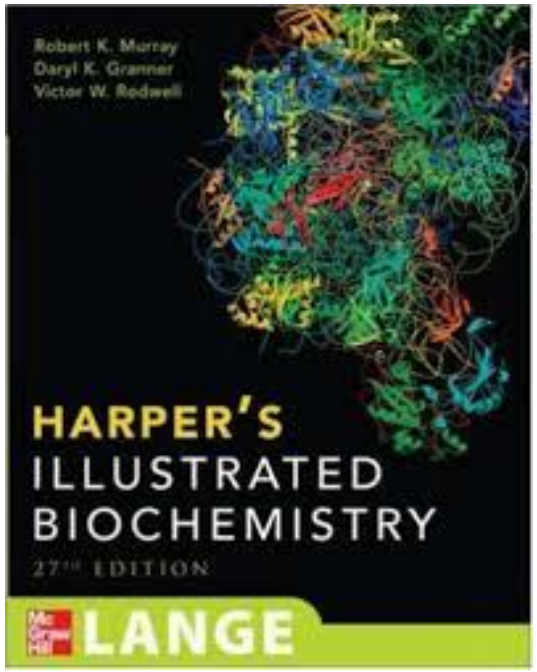
Paracrine hormones: act on adjacent cells

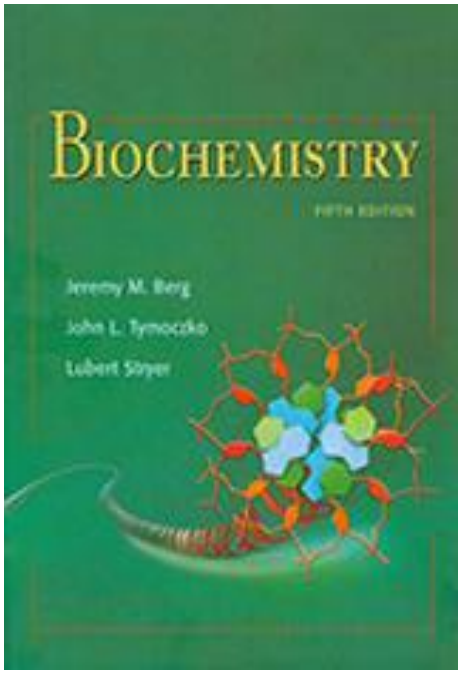
Autocrine hormones: Released and act on the cell that secreted them.

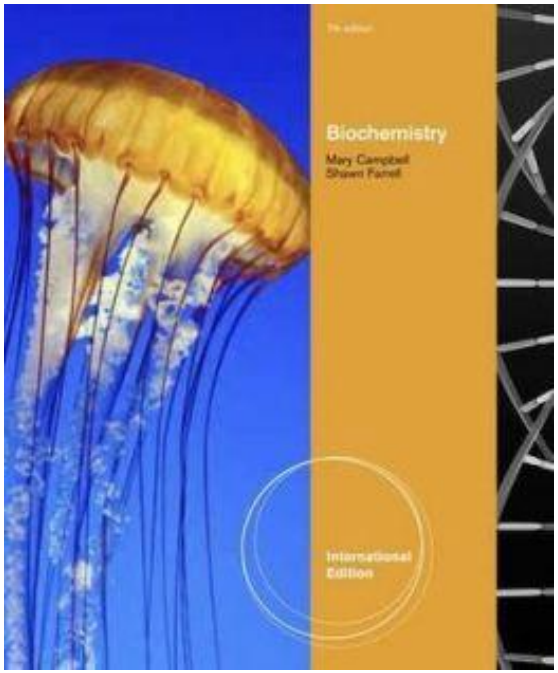
Intracrine Hormones: act within the cell that produces them.

Resources for the 3 lectures

- Harper's Illustrated Biochemistry
- Stryer's Biochemistry
- Campbell's Biochemistry

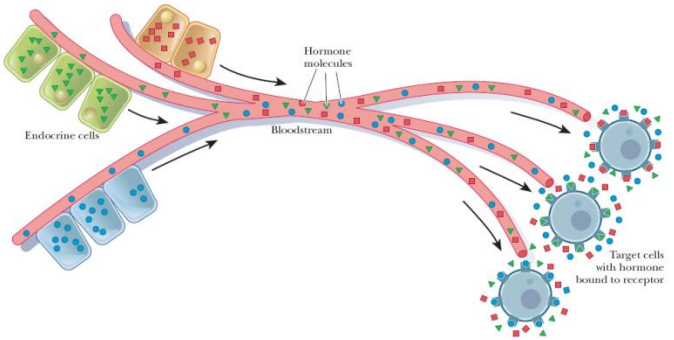




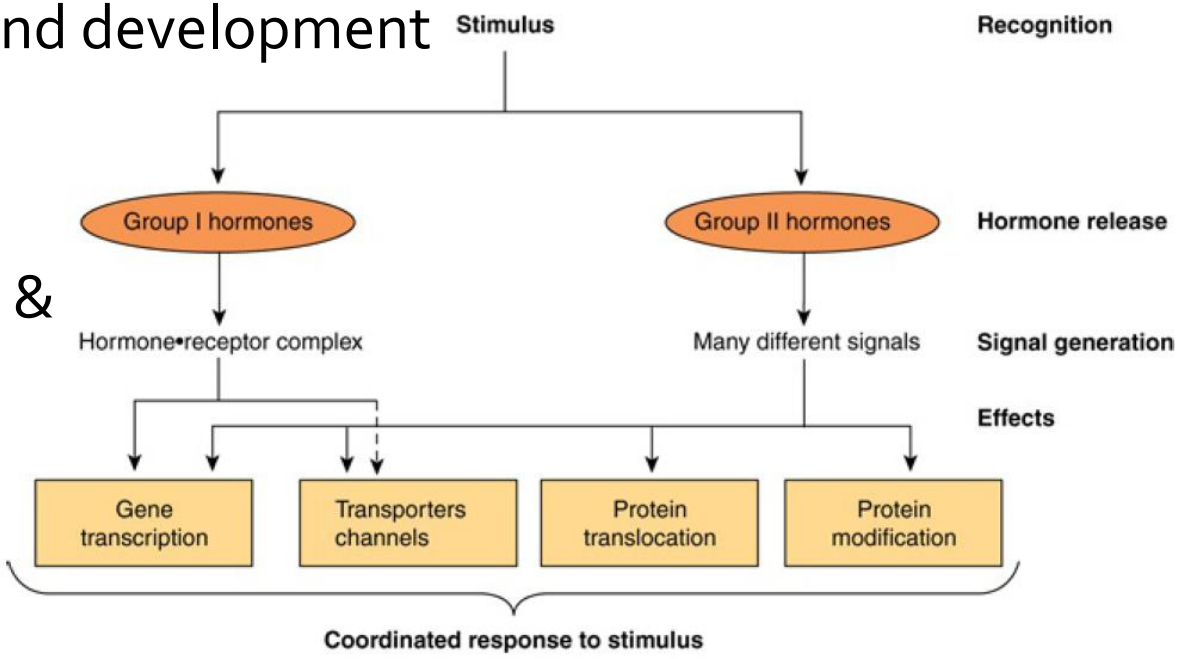


Hormones: The Remote Controllers

- What are hormones? Organic, blood, low amounts, source & target
- Functions:
  - They help maintain homeostasis
  - Mediate responses to external stimuli
  - Play roles in growth and development

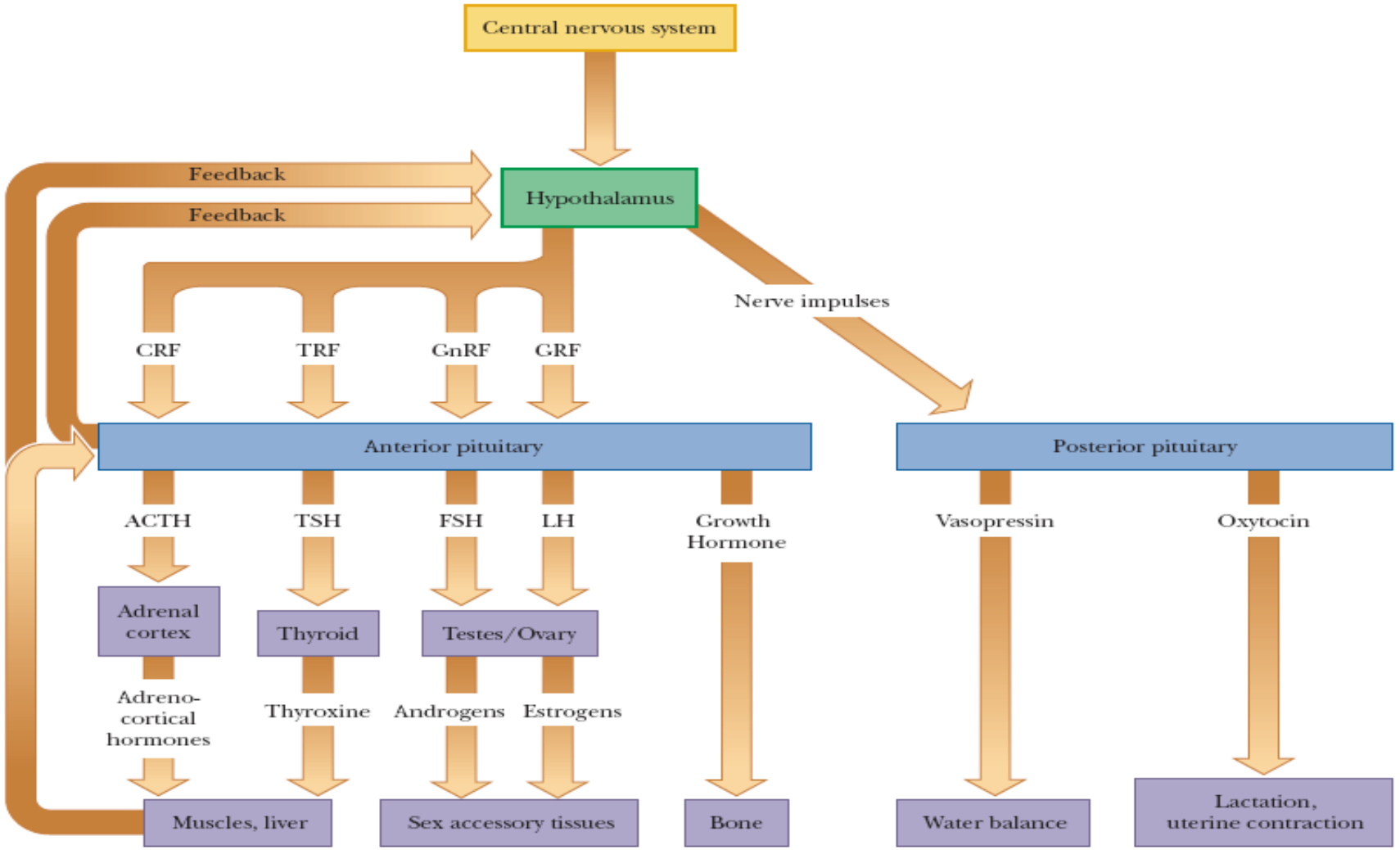


- Classes:
  - Endocrine hormones
    - Distance; stability; & concentration
  - Paracrine hormones
  - Autocrine hormones



Nervous vs./& Endocrine

Two systems act individually and together in regulating the human physiology



THE TARGET CELL CONCEPT

- 200 types of differentiated cells in humans
- Only a few produce hormones! (<50 known hormones)
- All of the 75 trillion cells in a human are targets to one or more

- One hormone → several cell types
- One cell type → several hormones
- One hormone → several effects

- The definition of a target has been expanded to include any cell in which the hormone (ligand) binds to its receptor, regardless of the action

THE TARGET CELL CONCEPT

- Several factors determine the response of a target cell to a hormone:

Factors affect the concentration of the hormone at the target cell
✓ The rate of synthesis and secretion of the hormone
✓ The proximity of the target cell to the hormone source (dilution)
✓ The $\mathbf{K_d}$ of the hormone – receptor complex
✓ The rate of conversion of inactive form to the fully active form
✓ The rate of clearance from the plasma

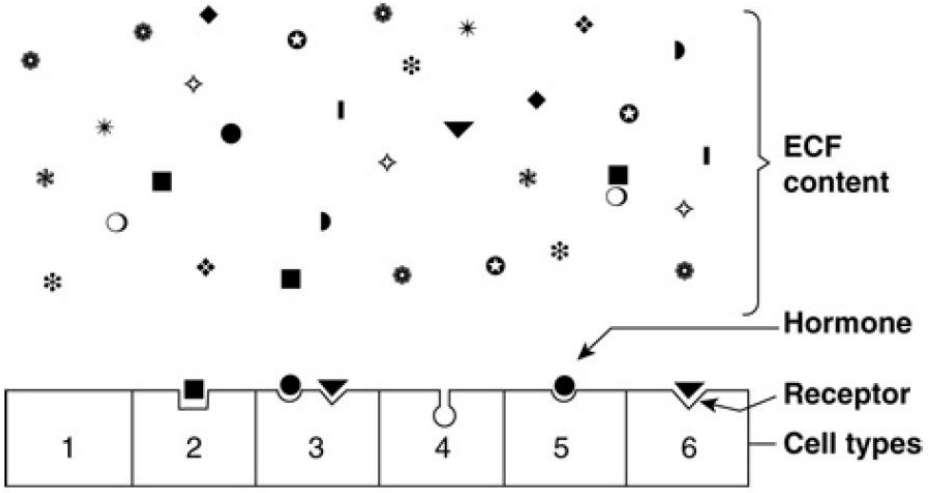
THE TARGET CELL CONCEPT

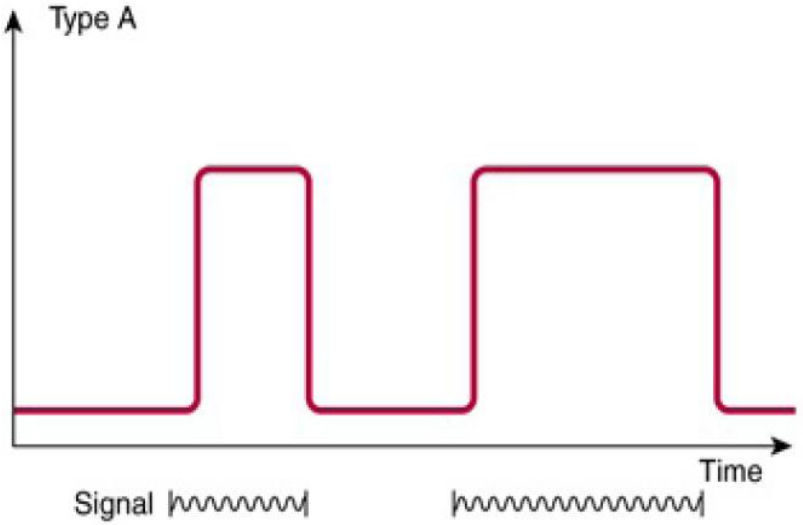
- Several factors determine the response of a target cell to a hormone:

Factors affecting the target cell response
✓ The number, relative activity, and state of occupancy of receptors
✓ The metabolism (activation / inactivation) of the hormone in the target cell
✓ The presence of factors within target cell necessary for the response
✓ Up- or down-regulation of the receptors upon interaction with ligand ✓ Post-receptor desensitization of the cell

Receptors Discriminate Precisely Receptors Follow Type A Response

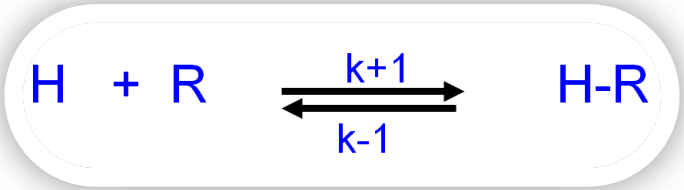
- Major challenge:
  - Atto- to nano-molar range (10^{-15} to 10^{-9} mol/L) vs. Structurally similar molecules (sterols, amino acids, peptides, and proteins): micro- to milli-molar (10^{-6} to 10^{-3} mol/L) range

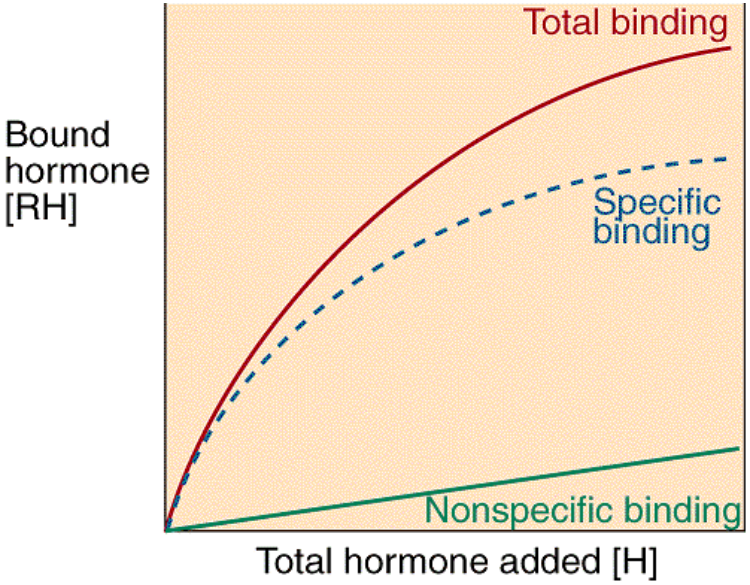




Accordingly; Hormone-Receptor Interactions

- Should be specific: displaceable by agonist or antagonist
- Should be saturable
- Should occur within the concentration range provided



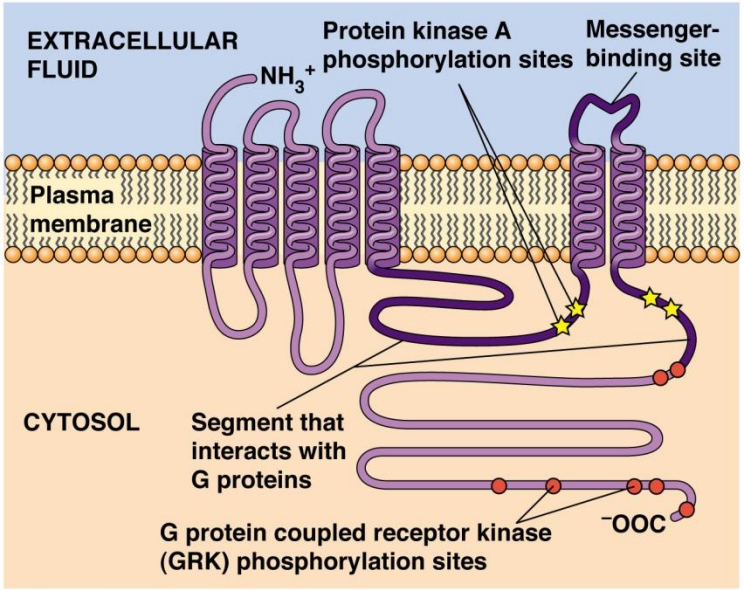


- Association constant K_a
- Dissociation constant K_d

- $K_a = [H\text{-}R] / \{[H] \times [R]\}$
- $K_d = \{[H] \times [R]\} / [H\text{-}R]$

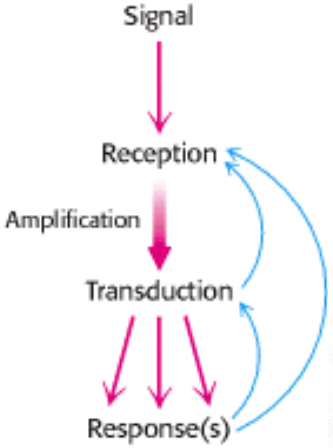
- 20X dissociation constant is enough to saturate the receptor
- $\mathbf{K_d}$ values for many hormone range from 10^{-9} to 10^{-11} M

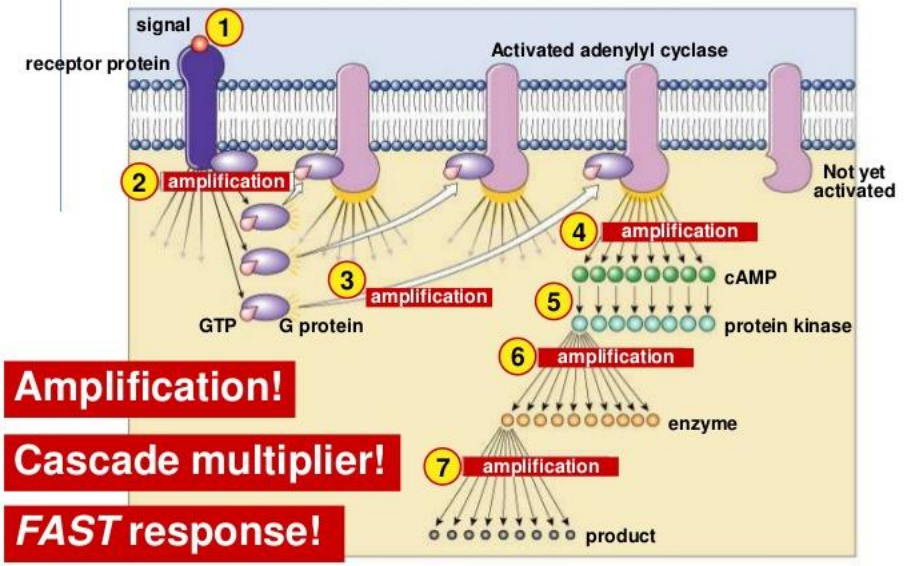
Receptor Domains

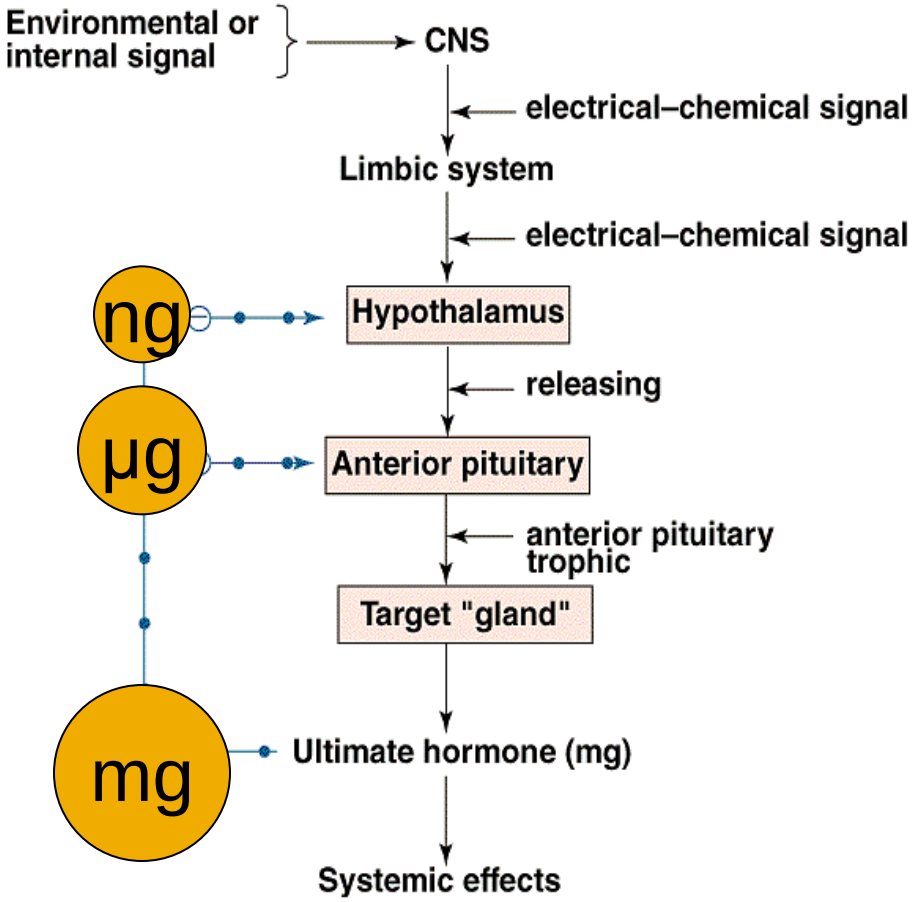


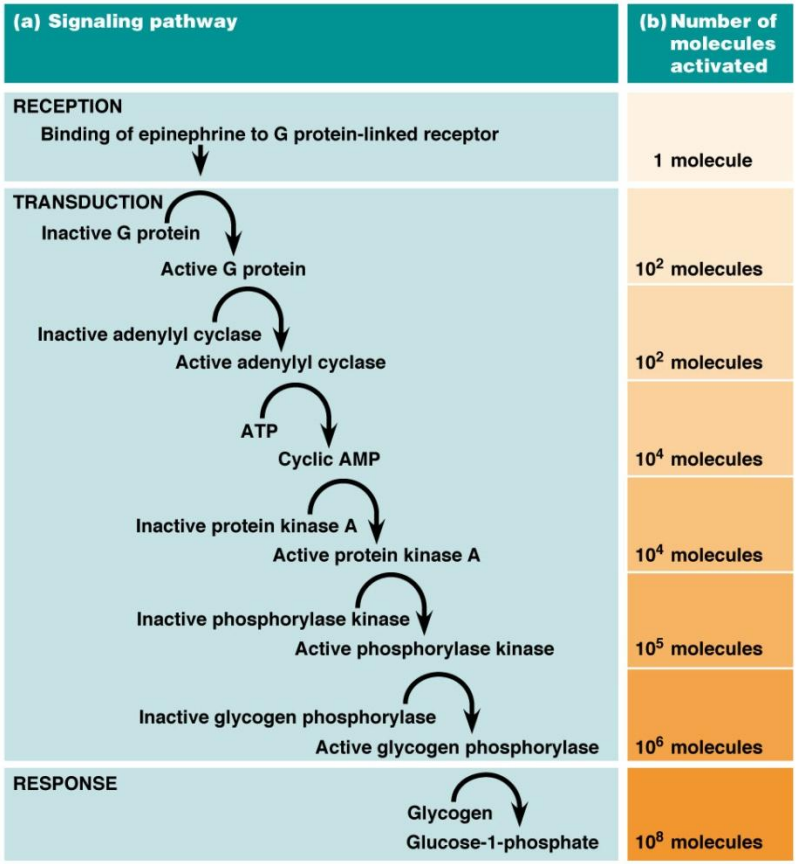
- All receptors have at least two functional domains:
  - Recognition domain
  - Coupling or signal transduction domain
- Coupling occurs in two general ways:
  - Changing the activity of an enzyme (Polypeptide & catecholamines, plasma membrane)
  - Direct (steroids, retinoids, and thyroid hormones, intracellular)
- Steroid, thyroid, and retinoid hormone receptors:
  - Hormone binding site ; DNA binding site; co-regulator proteins binding site, cellular trafficking proteins binding site
- Receptor–effector coupling— provides the first step in amplification

Signal Amplification



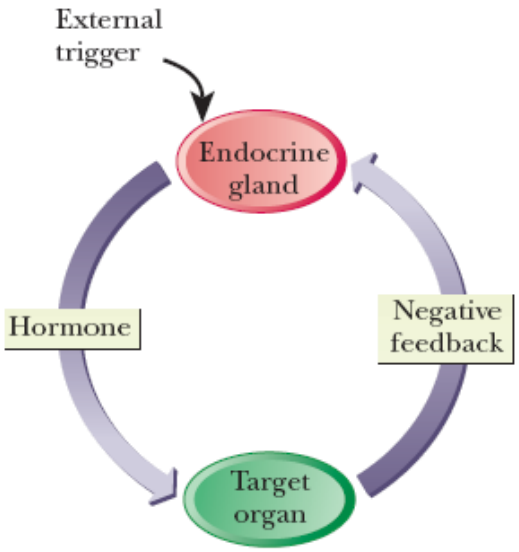


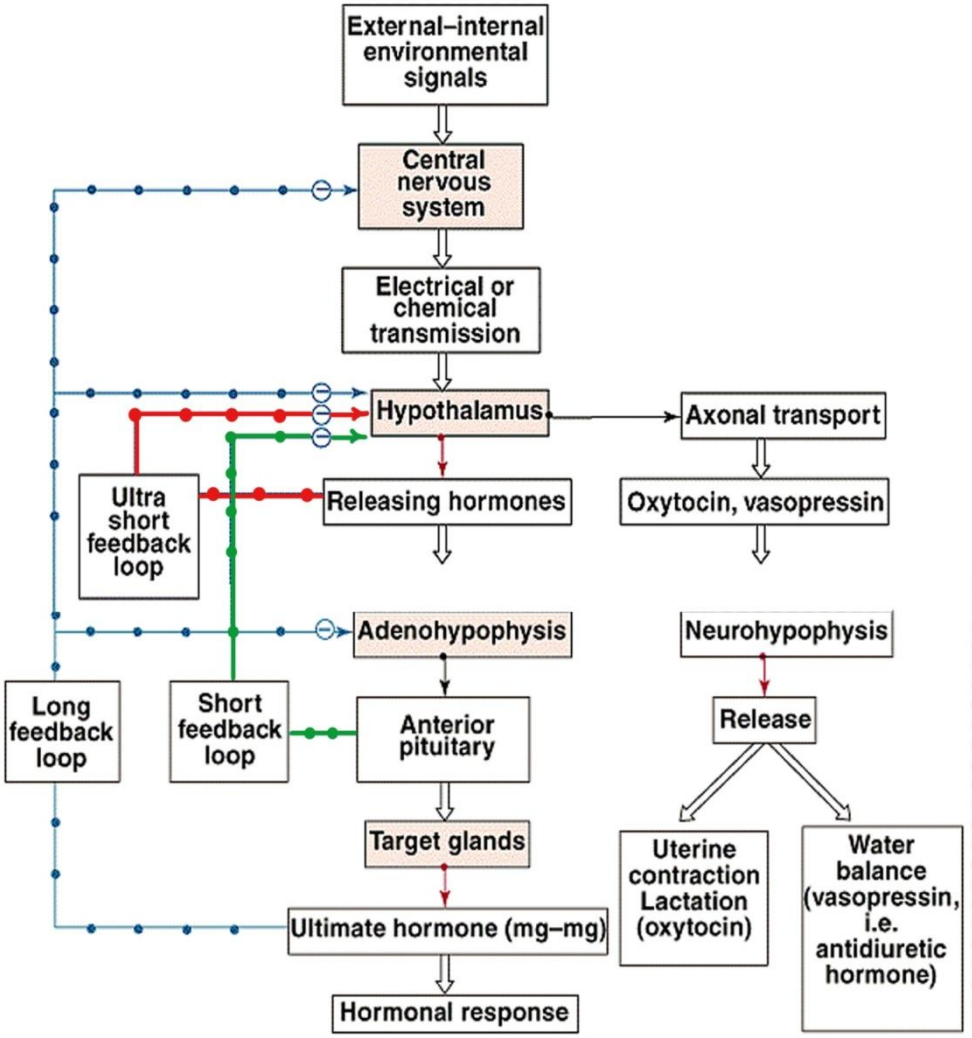




How the release is controlled?

- Feedback inhibition
  - Ultrashort loop
  - Short loop
  - Long loop





Classification of Hormones Chemical Structure

- Chemical composition; solubility; location of receptors; nature of the signal used to mediate hormonal action

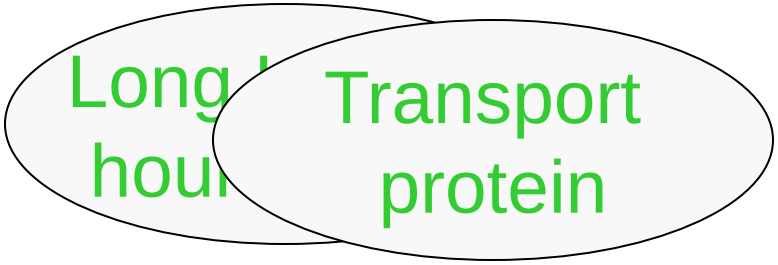
✓ **Polypeptides**: Pituitary hormones; Hypothalamic releasing hormones; Insulin, Growth factors…

✓ **Amino acid derivatives**: Adrenalin, Thyroid hormones

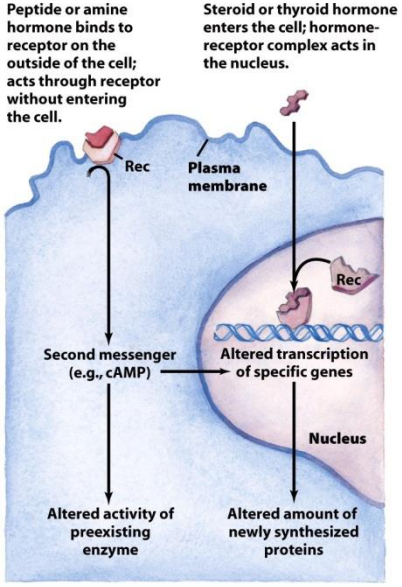
✓ **Steroids**

Classification of Hormones Mechanism of Action

- Hormones that bind to intracellular receptors
  - Steroids
  - Thyroid hormones
  - Calcitriol, retinoic acid

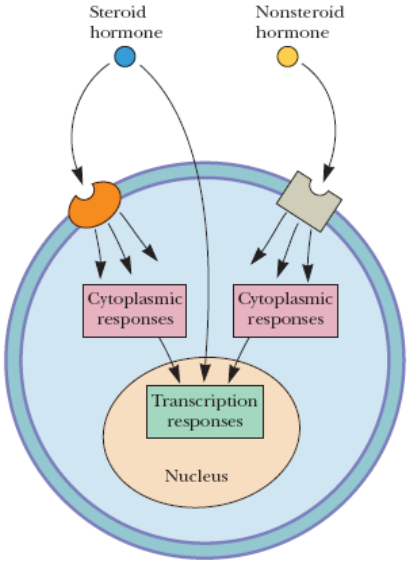


- Hormones that bind to cell surface receptors (According to second messenger):
  - cAMP (β adrenergic factor, glucagon, ACTH)
  - cGMP (atrial natriuretic factor, Nitric oxide)
  - Calcium or phosphatidyl inositol (oxytocin, TRH)
  - Kinase or phosphatase cascade (insulin, GH)



General Features of Hormone Classes

	Group I	Group II
Types	Steroids, iodothyronines, calcitriol, retinoids	Polypeptides, proteins, glycoproteins, catecholamines
Action	Slow	Fast
Solubility	Lipophilic	Hydrophilic
Transport proteins	Yes	No
Plasma $t_{1/2}$	Long (hrs - days)	Short (minutes)
Receptor	Intracellular	Plasma membrane
Mediator	Receptor-hormone complex	cAMP, cGMP, Ca^{2+}, kinase cascades, metabolites of phosphoinositols



Hormones Classes
Steroid hormones

A. Sex hormones - are divided into 3 groups
   1. Male sex hormones or Androgens
   2. Female sex hormones or Estrogens
   3. Pregnancy hormones or Progestines

B. Hormones of Adrenal Cortex
   1. Mineralocorticoids: aldosterone. ...
   2. Glucocorticoids: cortisol. ...
   3. Adrenal androgens: male sex hormones mainly dehydroepiandrosterone (DHEA) and testosterone

Hormones Classes
Non steroid hormones

A. Peptide and protein hormones

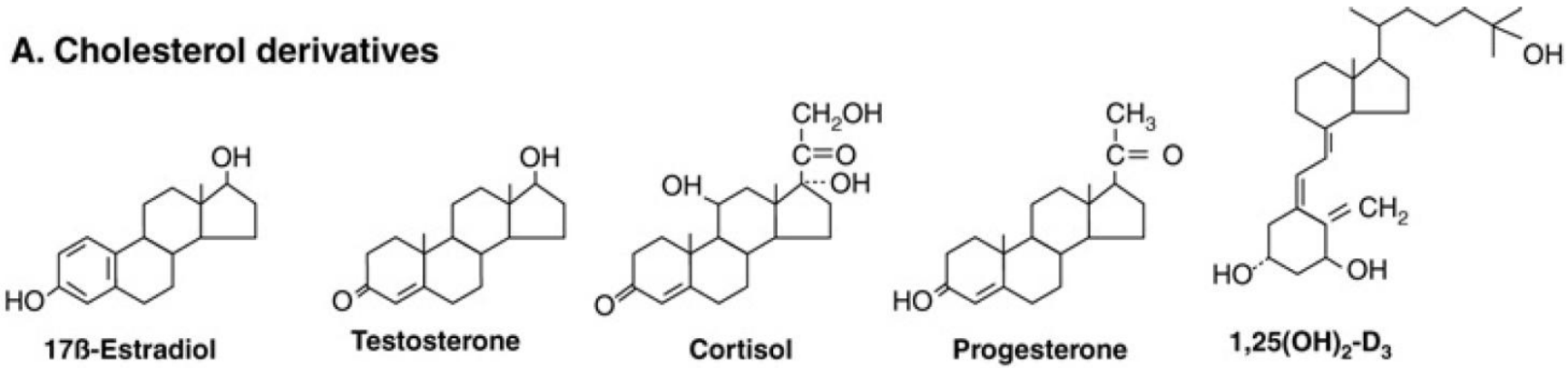
- ✓ All hypothalamic, pituitary, digestive hormones
- ❖ All pituitary hormones are made from single polypeptide chains **EXCEPT**: TSH; FSH; LH (homodimers) – glycoproteins (≈ 25 kDa)

B. Amino acid derivatives

- ✓ Amines - derived from tyrosine or tryptophan
  TH, dopamine, epinephrine, melatonin

Structure of Hormones

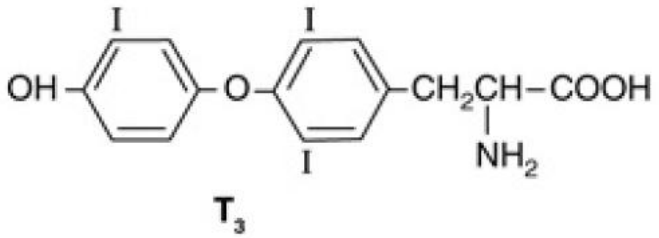
- Lipid – soluble hormones:



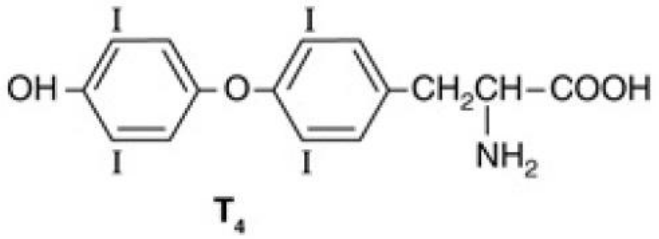
Structure of Hormones

- Amino Acid-Derived Hormones

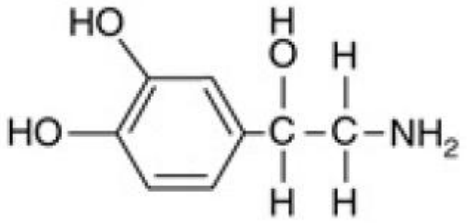
Tyrosine derivatives



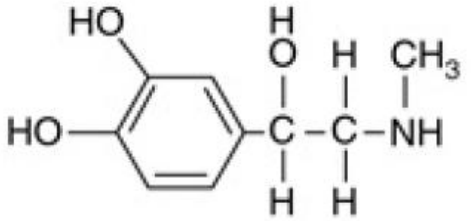
T_3



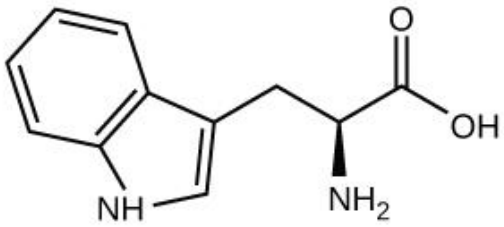
T_4



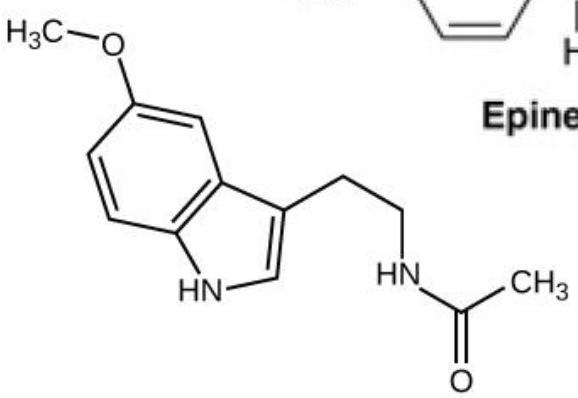
Norepinephrine



Epinephrine



Tryptophan

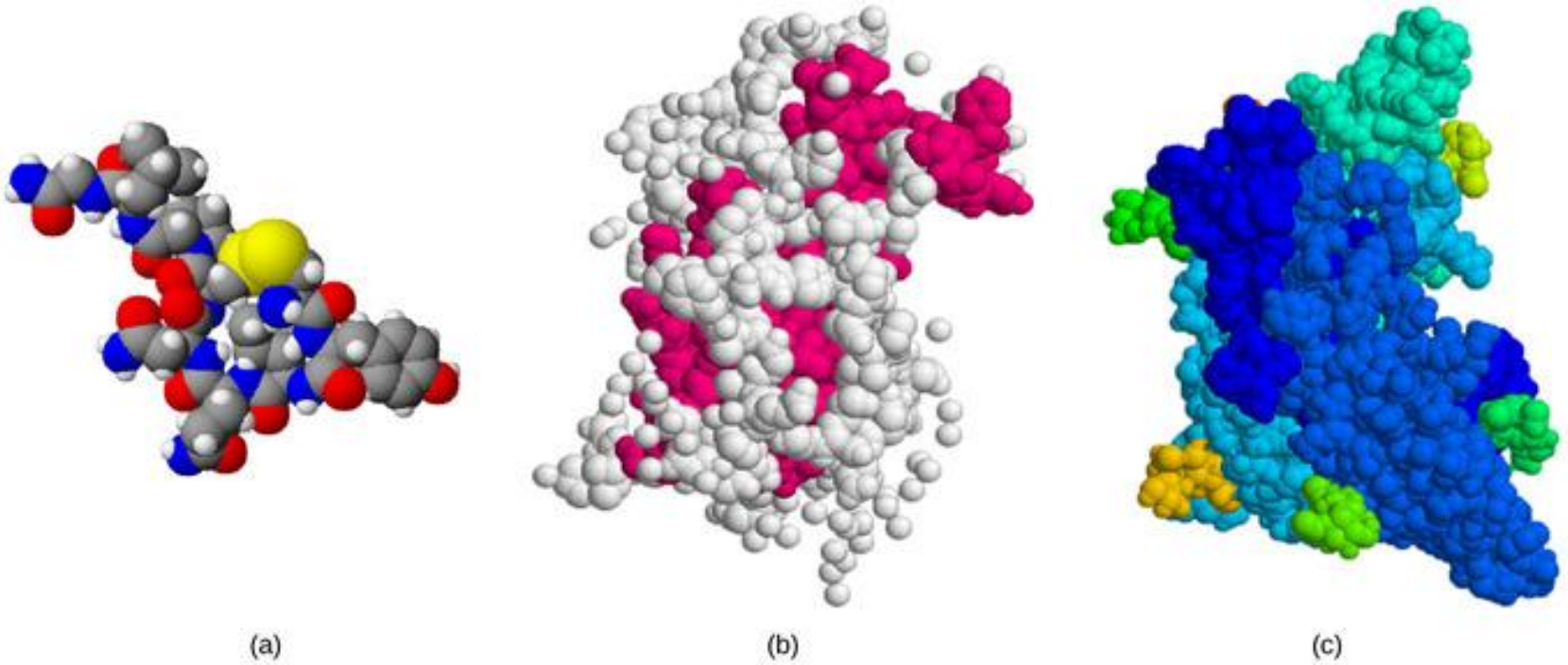


Melatonin

Structure of Hormones

- Peptide & Protein Hormones

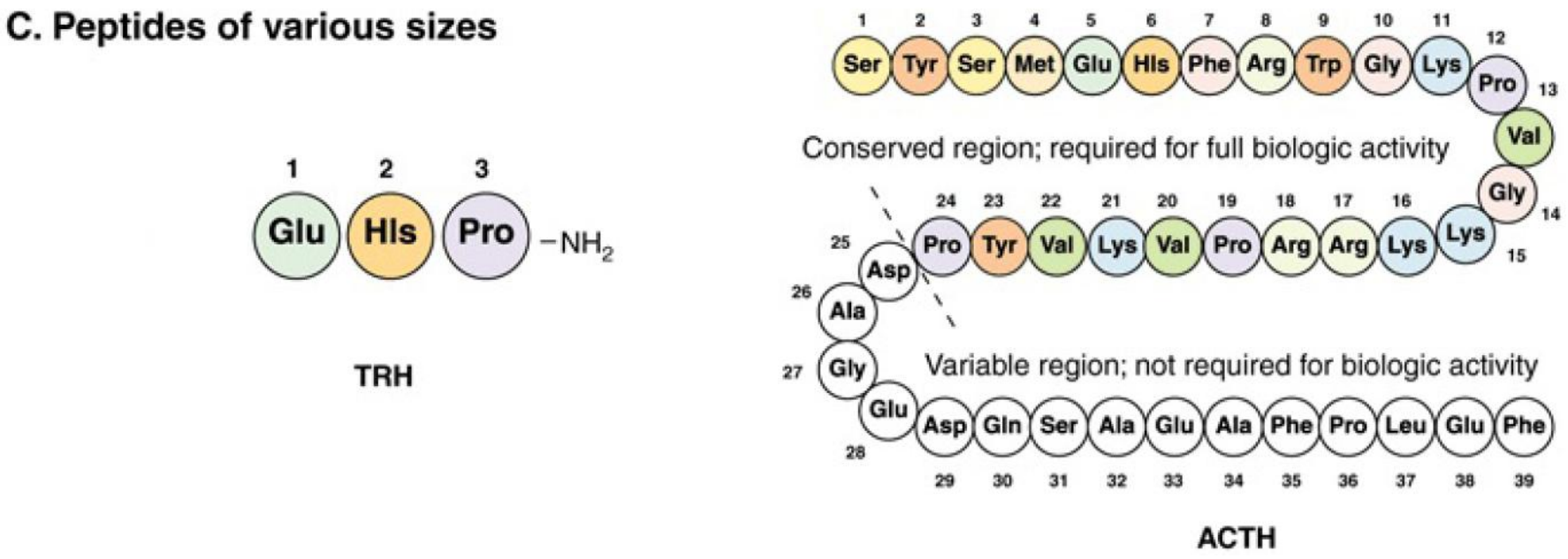
Oxytocin GH FSH



(a) (b) (c)

Structure of Hormones

- Peptide & Protein Hormones



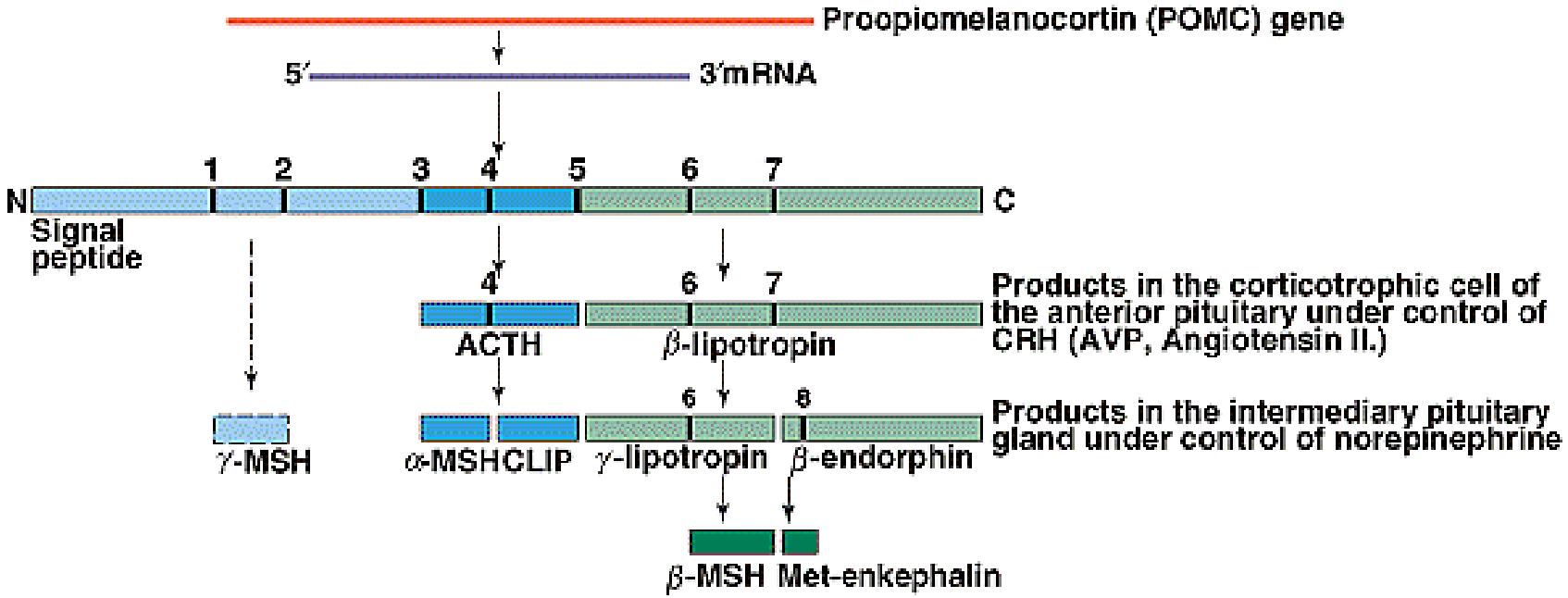
Structure of Hormones

Peptide & Protein Hormones

Hormone	Structure
GHRH	44
TRH	3
GnRH	10
CRH	41
ADH	9
Vasopressin	9
Angiotensin I	10
Angiotensin II	8
Insulin	51
Glucagon	29

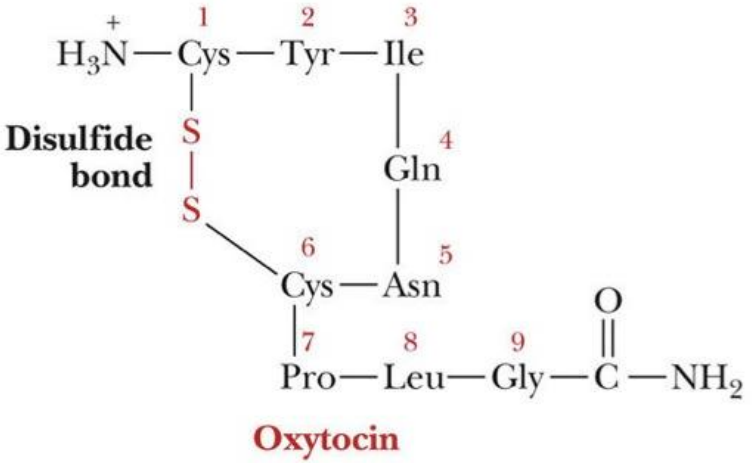
Synthesis of Peptide Hormones

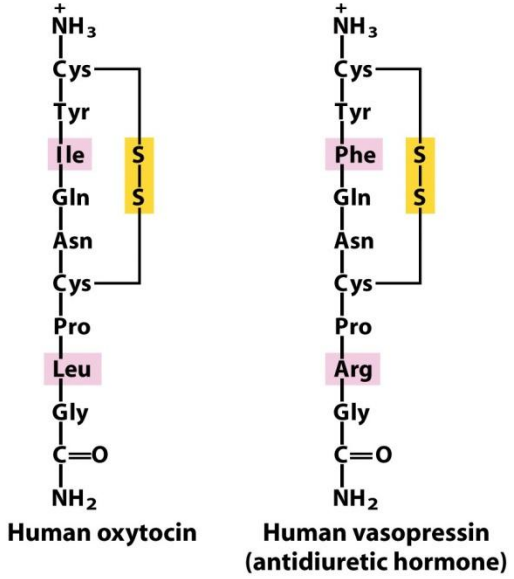
- From precursor polypeptides
  - One gene may code more than one hormone (POMC)
  - The cleavage depends on specific enzymes

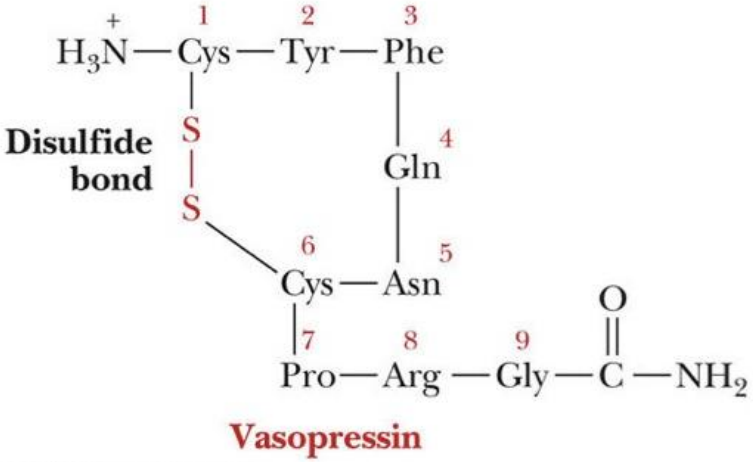


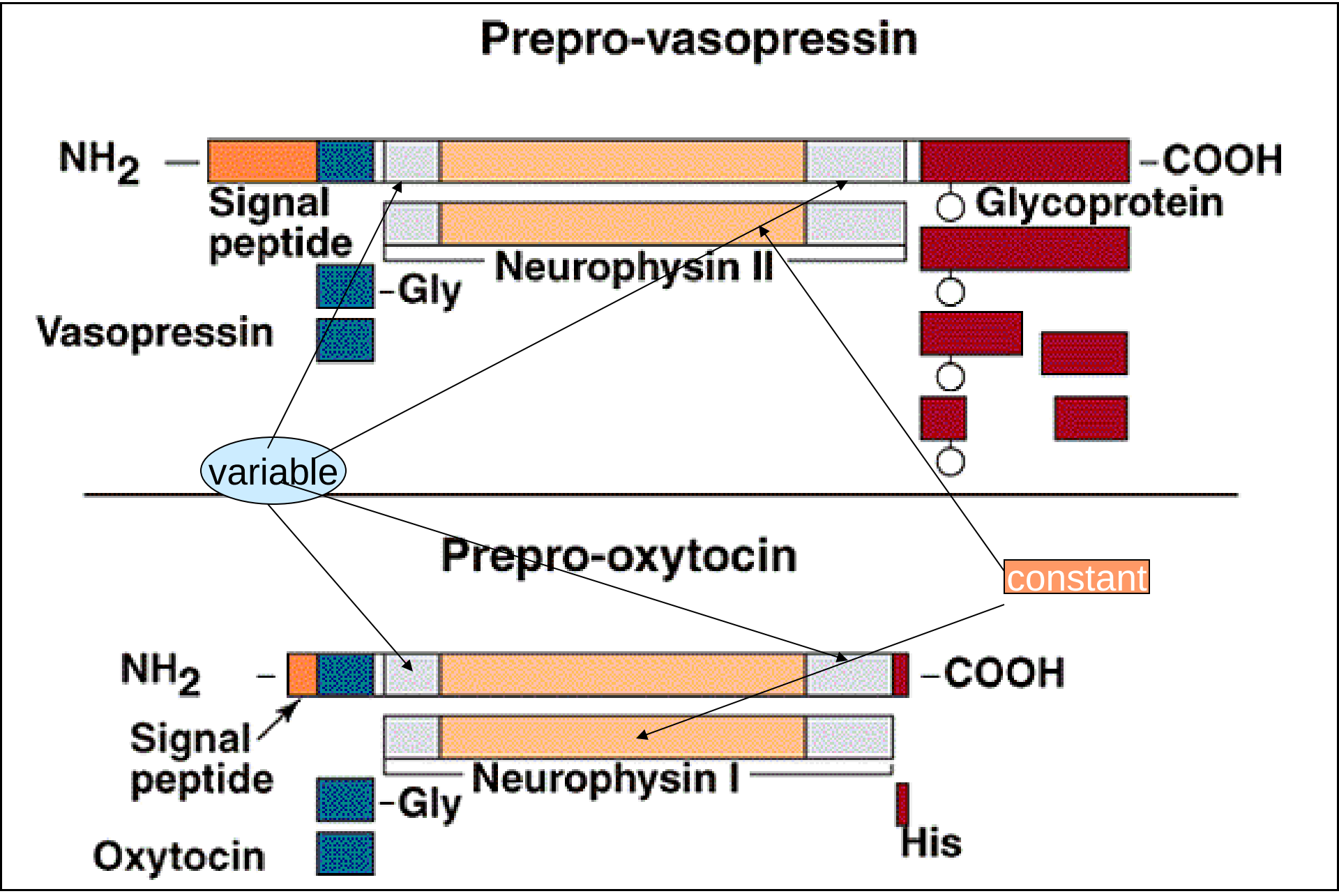
Synthesis of Peptide Hormones

- From precursor polypeptides
  - Vasopressin and oxytocin
  - Synthesis in separate cell bodies of hypothalamic neurons

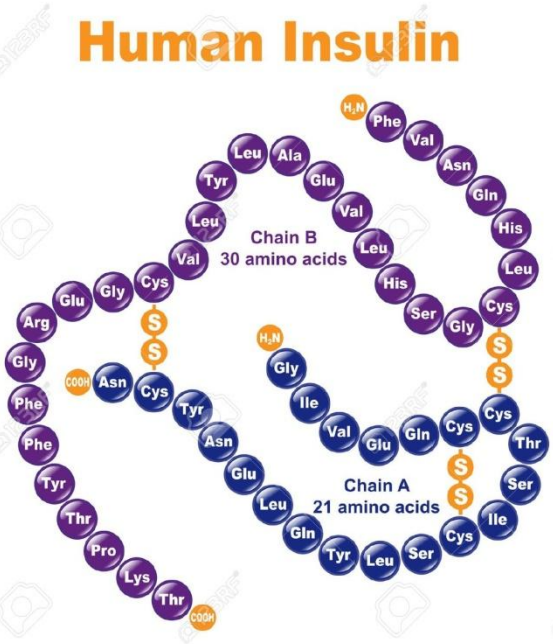







Prepro-vasopressin
NH2
COOH
Signal peptide
Glycoprotein
Neurophysin II
Gly
Vasopressin
variable
Prepro-oxytocin
constant
NH2
COOH
Signal peptide
Neurophysin I
Gly
Oxytocin
His

Synthesis of Peptide Hormones

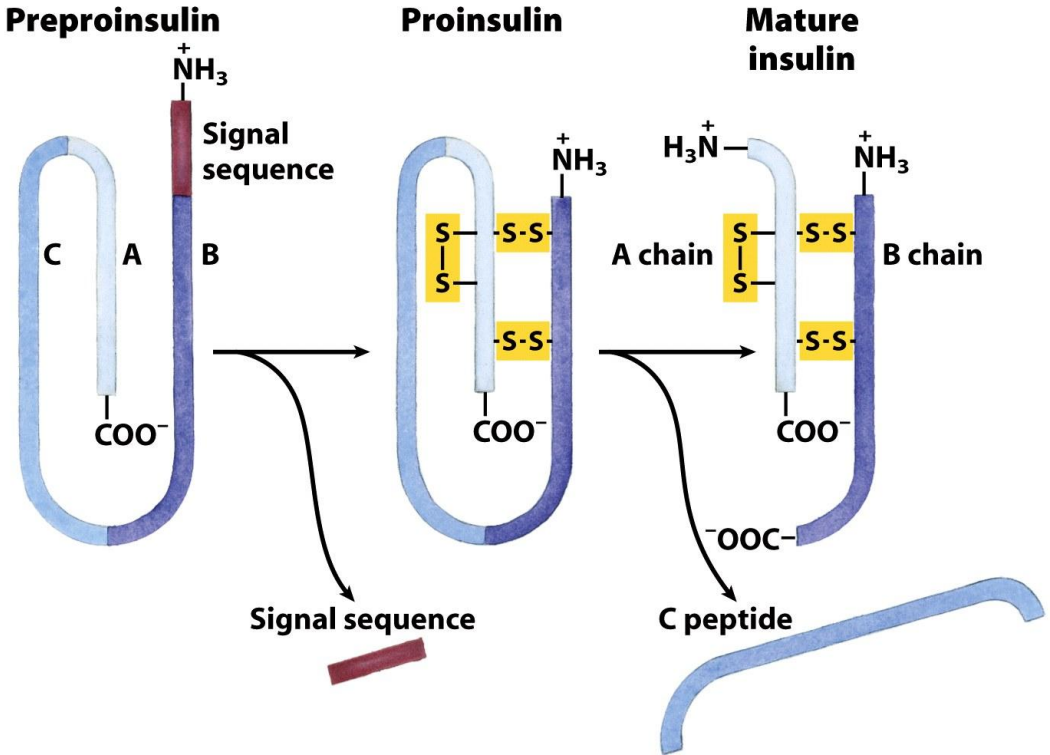


- Peptide & Protein Hormones
- From Pre-pro-hormones

- A larger precursor preproinsulin
  - 23 aa signal sequence
  - 3 disulfide bonds

- Proinsulin
  - Remove the C peptide

- Mature insulin
  - A and B chains



Target cells interactive effects

1. **Permissive effects** – one hormone enhances the effect of a later hormone
   - ✓ **Estrogen up-regulates progesterone receptors** in uterus
   - ✓ **Thyroid hormone increases the effect of epinephrine** on breakdown of triglycerides in adipocytes

2. **Integrative effects –** hormones produce complementary effects on different tissues
   - ✓ **PTH and calcitriol** increase ECF **calcium**

Target cells interactive effects

3. **Synergistic effects:**
   - ✓ Both **FSH and estrogen** necessary for **normal oocyte development**
   - ✓ **FSH and testosterone** together increase **spermatogenesis**

4. **Antagonistic effects:**
   - ✓ **Insulin and glucagon**

Detection, and generation of cellular response

Transduction of hormone signal

Signal Transduction

- Transduction: conversion of one form of a signal to another so as cells can produce many kinds of responses in different ways
- Amplification is a MUST
- Signal (polar, large) should bind receptors:
  - Intrinsic
  - Transmembrane
  - Intra- & extracellular domains
- Is that enough? The need for 2nd messenger
  - Few in number
  - Restricted movement

Second messengers

- Ability to diffuse to other cellular compartments
- Amplification of the signal
  - Enzyme activation
  - Membrane channels
- Some second messengers are common in multiple signaling pathways (≈ 30 hormones uses cAMP!!!)
  - Permits fine tuning but can pose problems
- Types of 2nd messengers:
  - Small molecules: cAMP, cGMP, Ca^{+2}
  - Phosphorylation through kinases

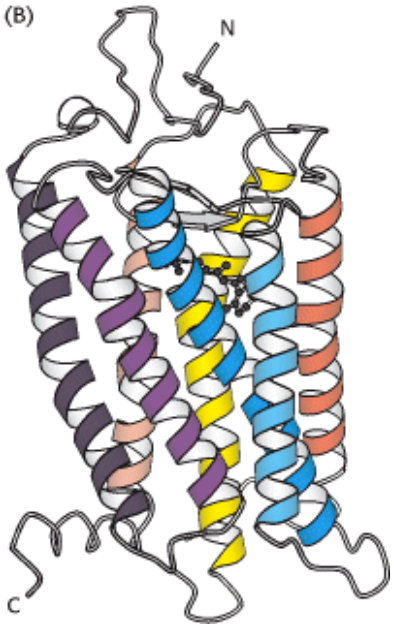
Signal Termination

- Is it important?
  - Keeps cells responsive to new signals
  - Failure of termination may cause problem e.g GH & cancer

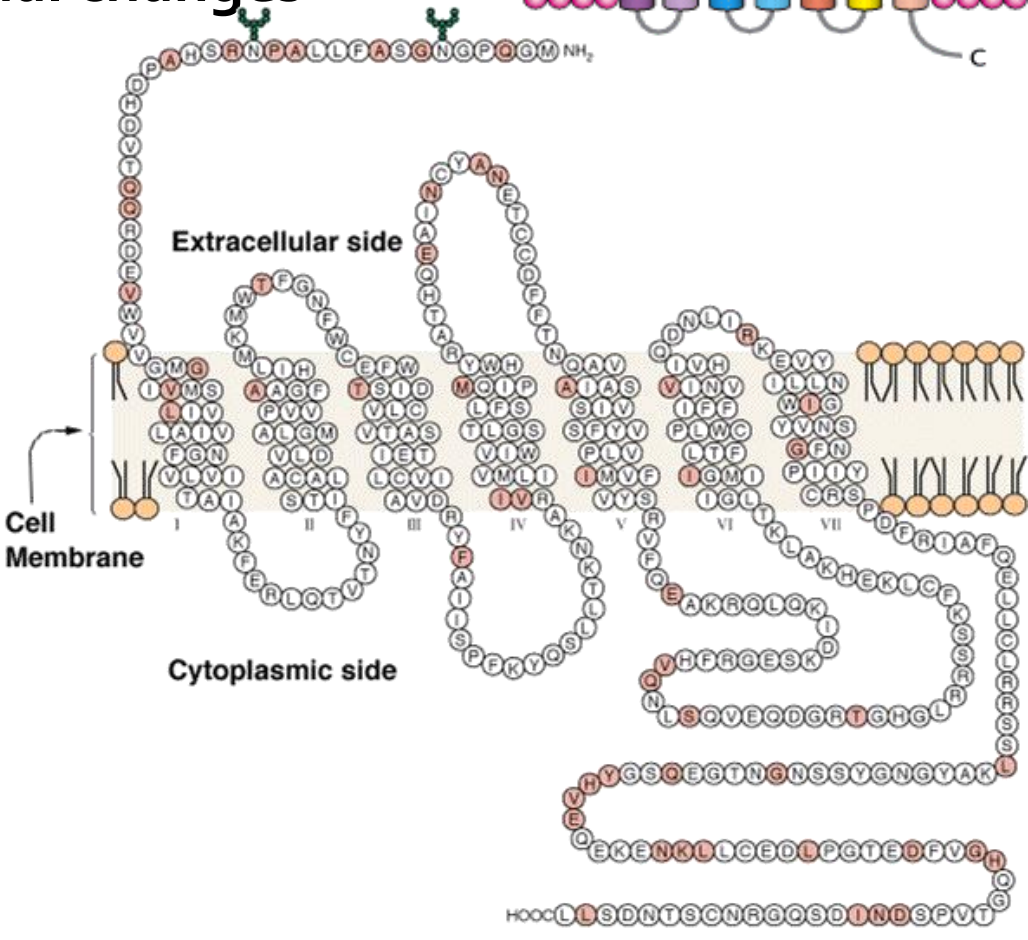
- How it is achieved?
  - Degradation of the second messenger
  - Dephosphorylation by hydrolysis

Membrane Associated Receptors
7-Transmembrane Helix Receptors (7TM)

- 7 α-helices: H-bonding, rigid, hydrophobic
- Signal induces conformational changes
- Is it enough?



Rhodopsin receptor



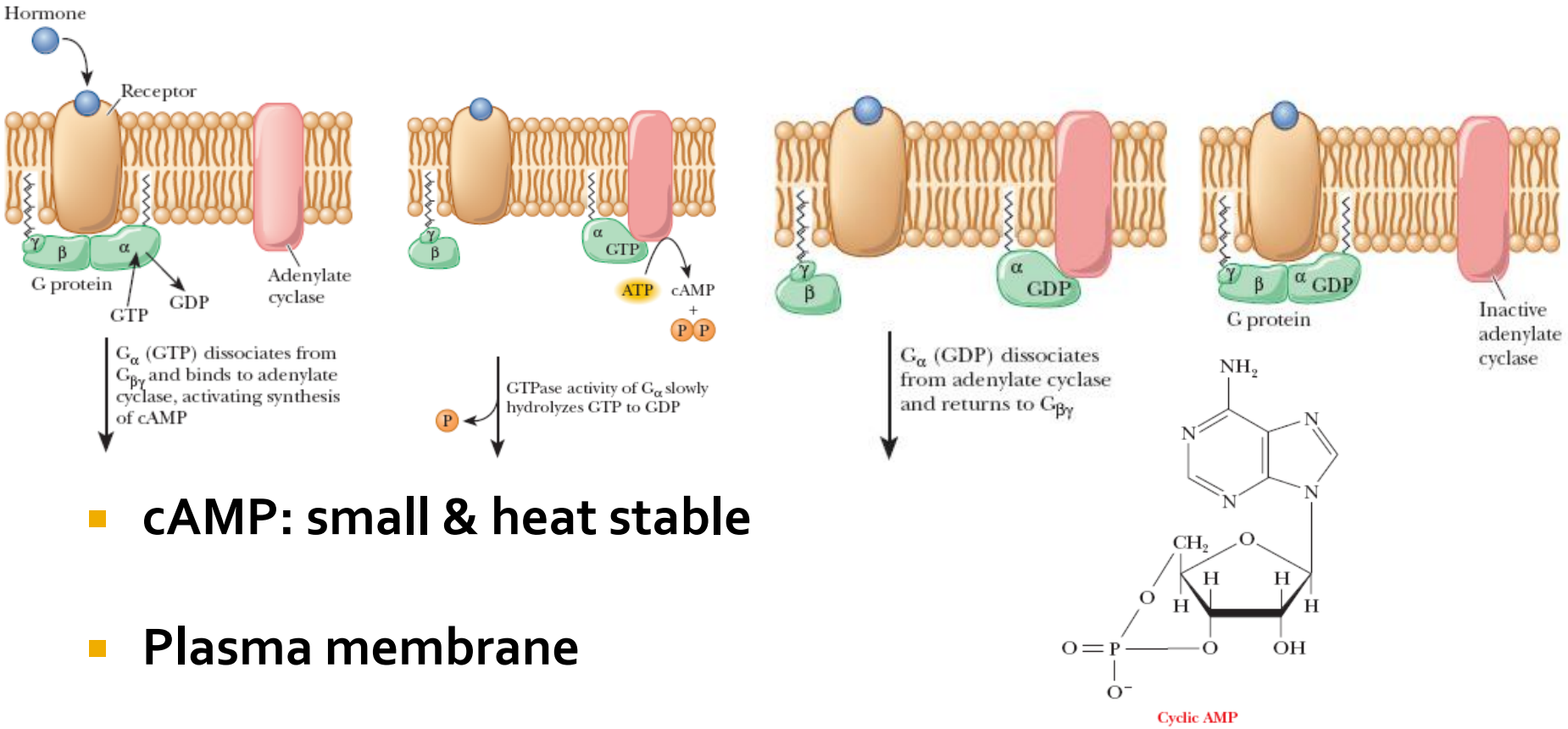
- Many Ser & Thr residues

Biological Functions Mediated by 7TM

- Smell, Taste, Vision
- Neurotransmission
- Hormone Secretion
- Chemotaxis
- Exocytosis
- Cell Growth, Development
- Viral Infection

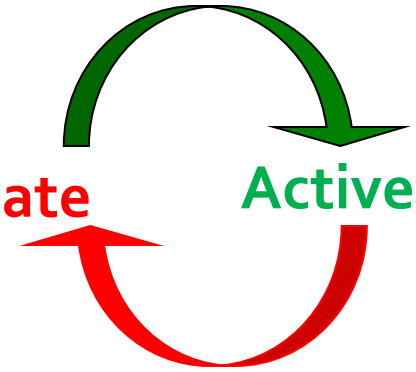
All these receptors share the same basic structure; however, they differ in their specificity and effects

G-proteins & cAMP



- **cAMP: small & heat stable**
- **Plasma membrane**
- **Hormone → Specific receptor (β1- or β2-adrenergic receptor) → G protein → Adenylate cyclase → cAMP → protein kinase A → phosphorylation**

G Protein cycles between two forms



Inactive State

Active State

Bound to GDP

Bound to GTP

Heterotrimer αβγ

Dissociated α + βγ dimer

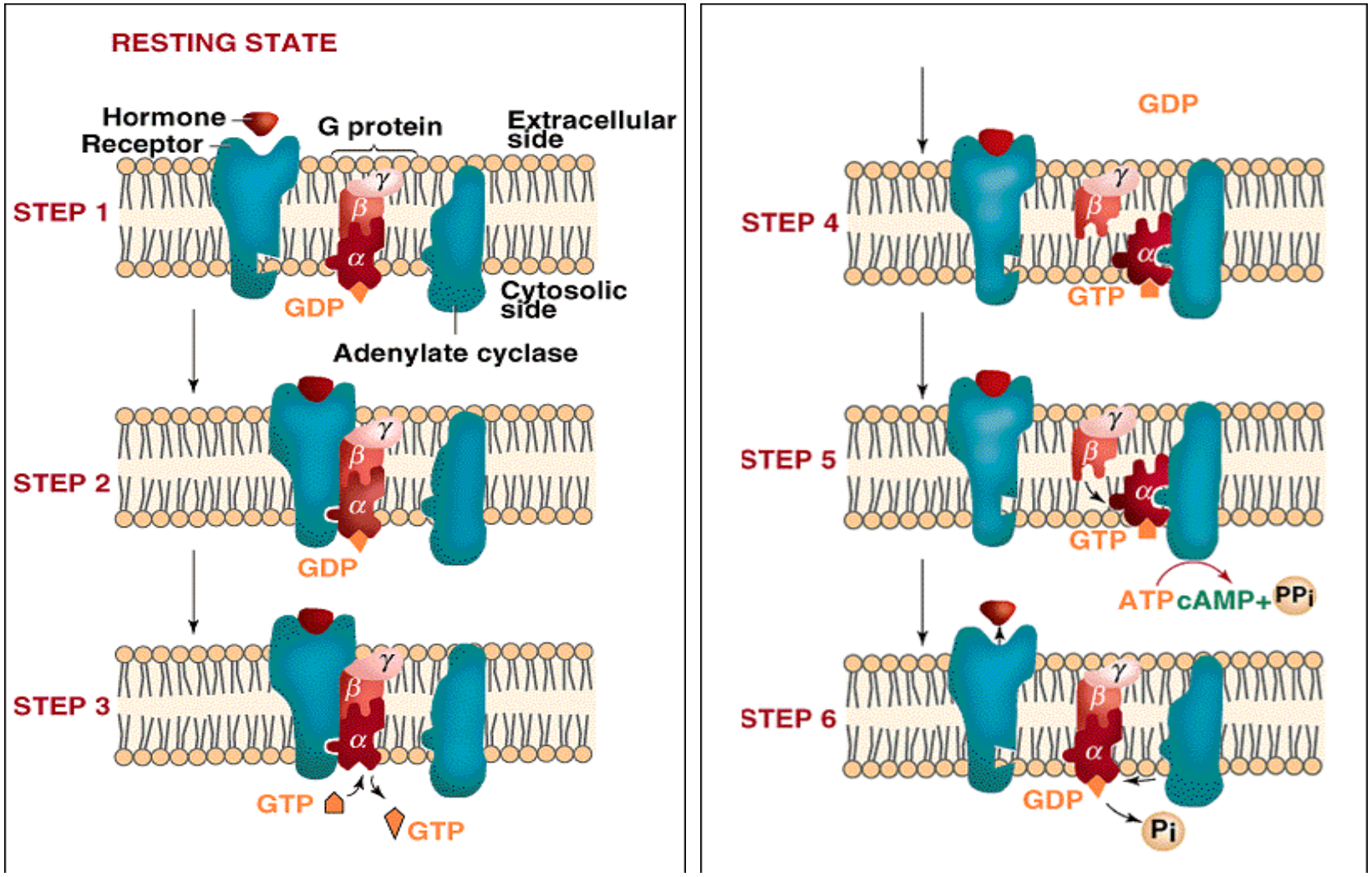
α subunit has GTPase Activity

Activated Receptor

↑ Adenylate Cyclase

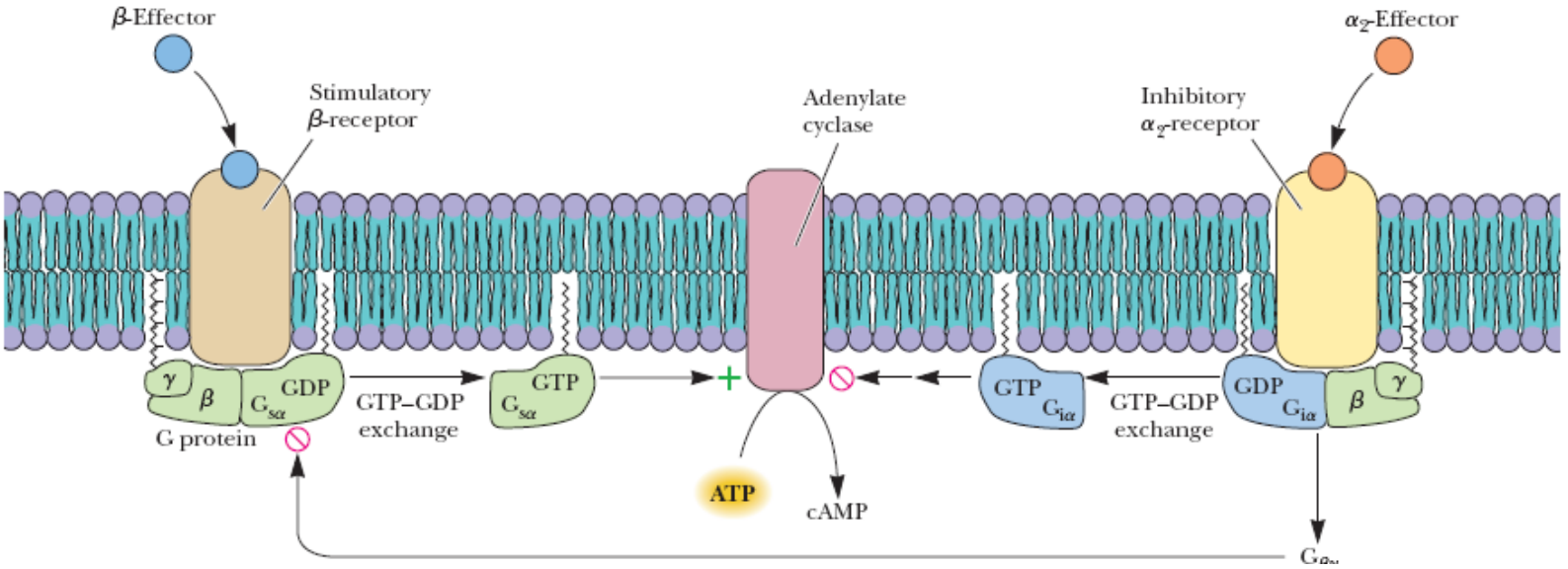
↑ cAMP

Biochemical Response



α subunit has GTPase Activity

G protein: stimulatory or inhibitory?



- **Cyclic AMP & G Proteins:**
  - Hormone → receptor (α2-receptor) → G protein → inhibits adenylate cyclase

G Proteins

- G proteins:
  - More than 100 known G protein–coupled receptors and more than 20 known G proteins
  - Can be activated by combinations of hormones
    - Epinephrine & glucagon act via a stimulatory G protein in liver cells
  - Other than cAMP:
    - Stimulating phospholipase C
    - Opening or closing membrane ion channels

G_α subunit transduce many activities

G_s	↑ Adenylate Cyclase
G_{olf}	↑ Adenylate Cyclase
Transducin	↑ cGMP Phosphodiesterase
G_i	↓ Adenylate Cyclase
G_o	Ca^{2+} Channels
Gq	↑ Phospholipase C

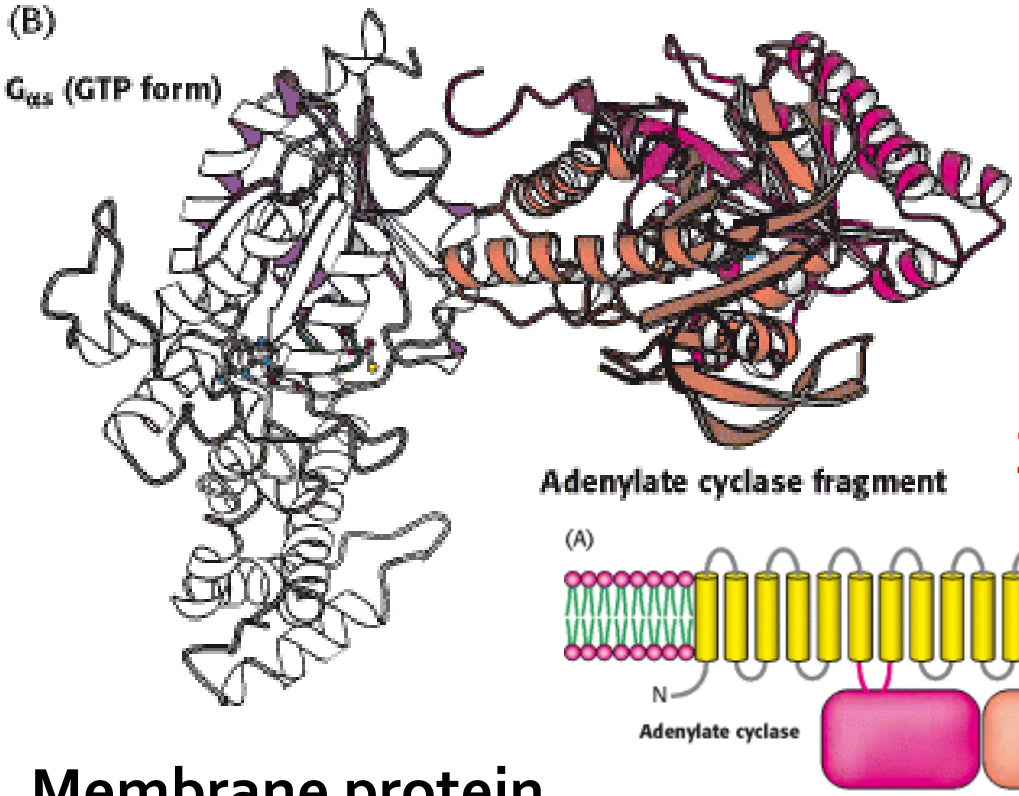
G Proteins (cont.)

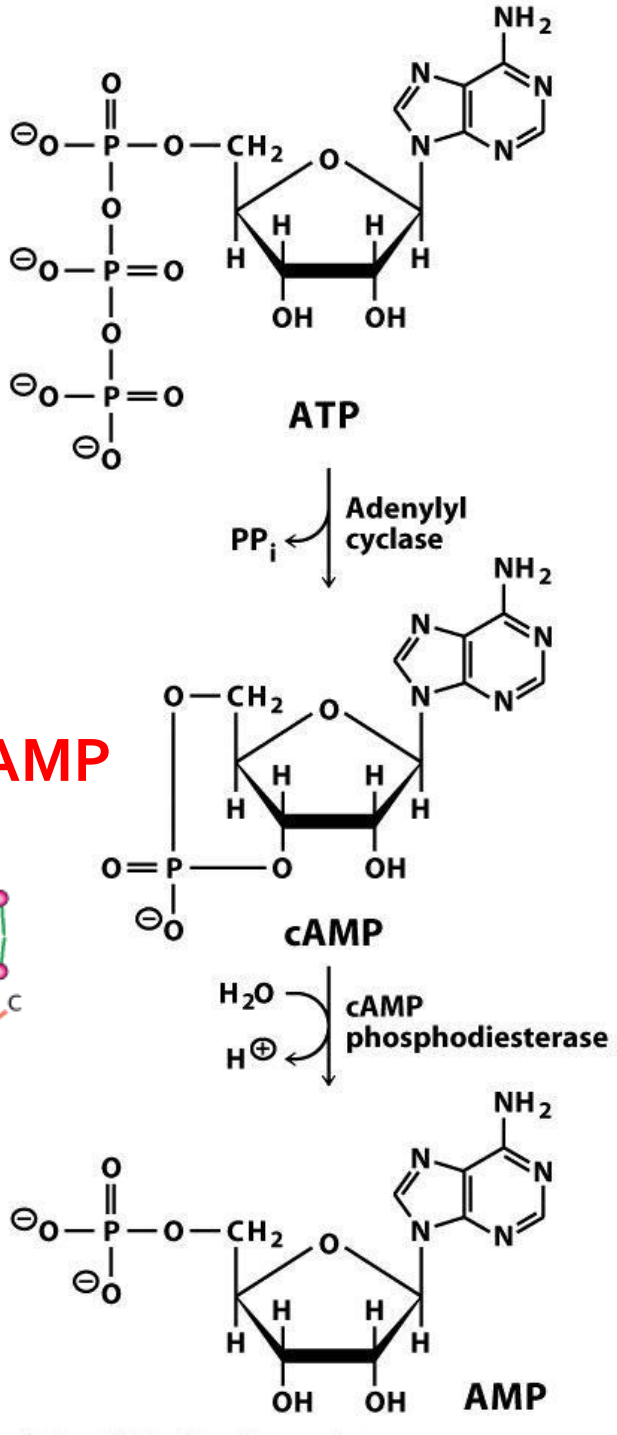
- α and γ Subunits have covalently attached fatty acid
- α and βγ can interact with other proteins
- All 7TM receptors appear to be coupled to G proteins

GPCRs

- Amplification: receptor → 100's of G protein → 100's of adenylate cyclase → 100's X 1000's molecules/sec of cAMP

Adenylate Cyclase



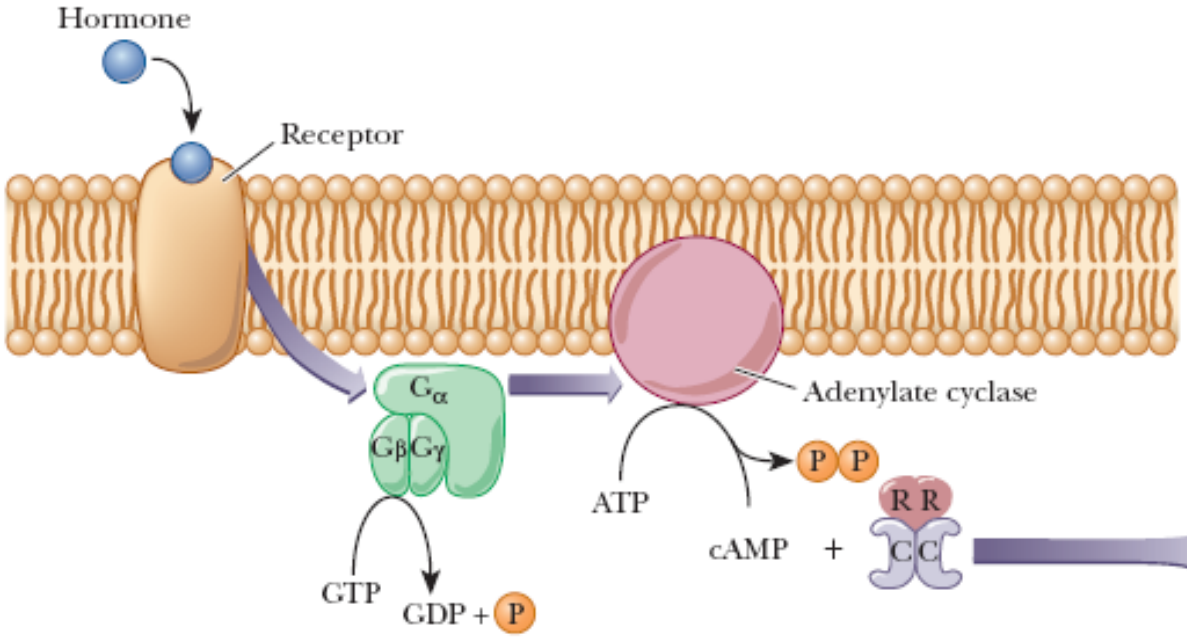


- Membrane protein
- 12 helices
- Two large intracellular domains
- Activated by G protein

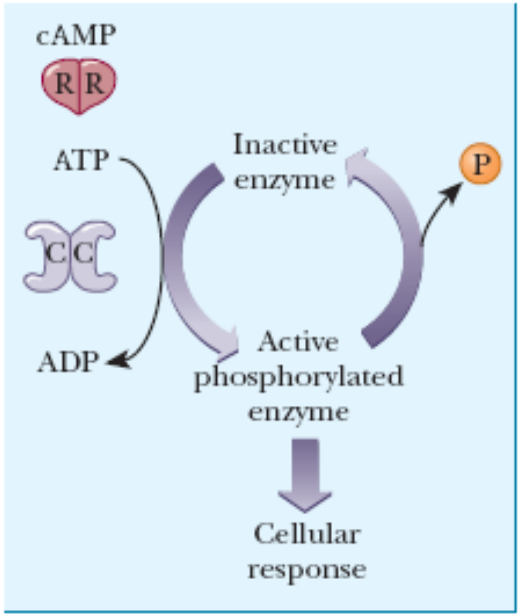
cAMP can affect a wide range of cellular processes

- ↑ degradation of storage fuels
- ↑ secretion of acid by gastric mucosa
- Dispersion of melanin pigment granules
- ↓ aggregation of blood plateletes
- Opening of chloride channels

Then what?



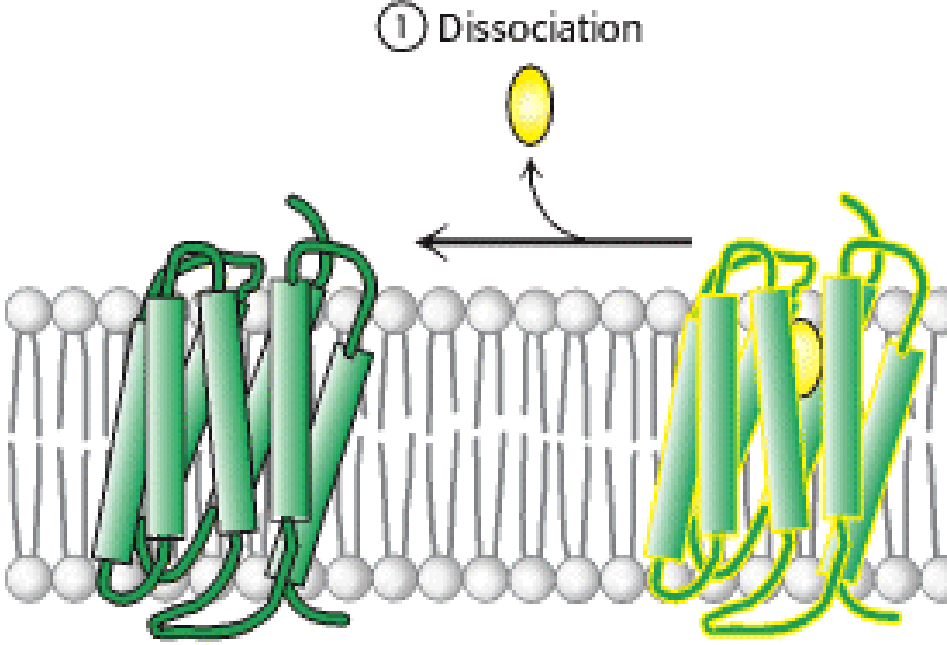
Usually:
Ser or Thr

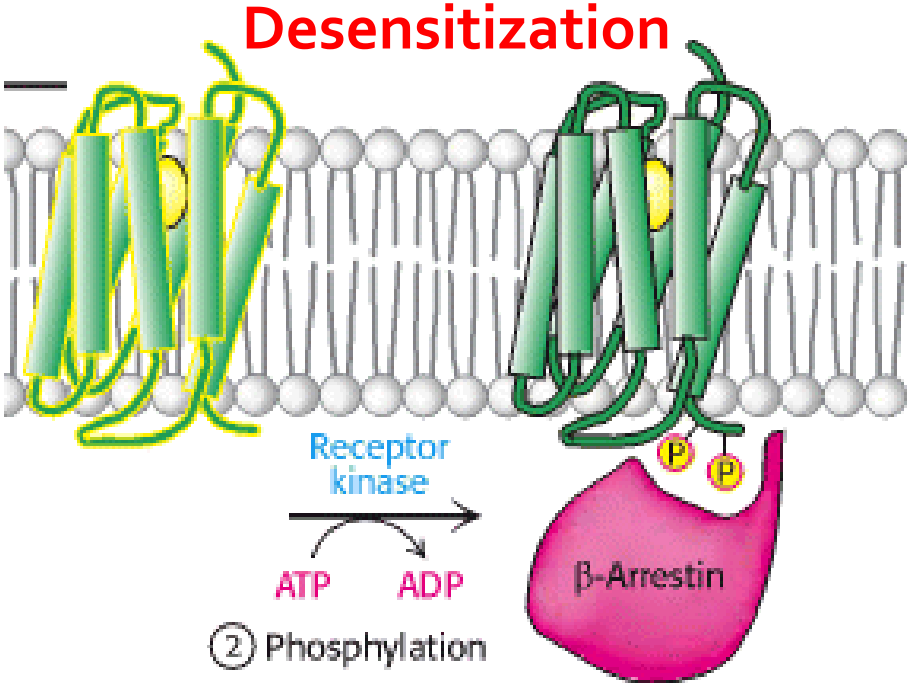


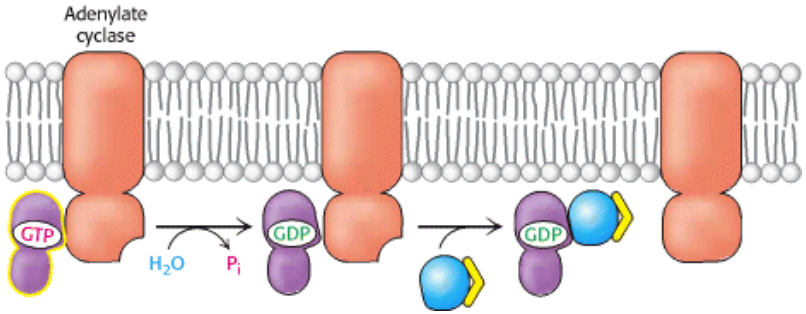
Glycogen Synthase!!

Switching off the signal

- Dissociation of the hormone
- GTPase activity of Gα subunit
- Hydrolysis of cAMP (phosphodiesterase)
- Phosphorylation of the hormone bound-receptor followed by binding to β-Arrestin







Cholera

- **Cholera toxin → unregulated activity of adenylate cyclase in epithelial cells → Excessive cAMP in epithelial cells stimulates active transport of Na^+ → large flow of Na^+ and water from the mucosa → diarrhea**