Hormone mechanism of action

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Cellular Mechanism of Action

- two major types of hormone action at target tissues
  - the action of tropic hormones (peptide and glycoprotein hormones) with receptors at the cell membrane level
  - the smaller steroid hormones enter cells readily, and the basic mechanism of action involves specific receptor molecules within the cells

Intracellular Receptors : estrogen, thyroid hormone

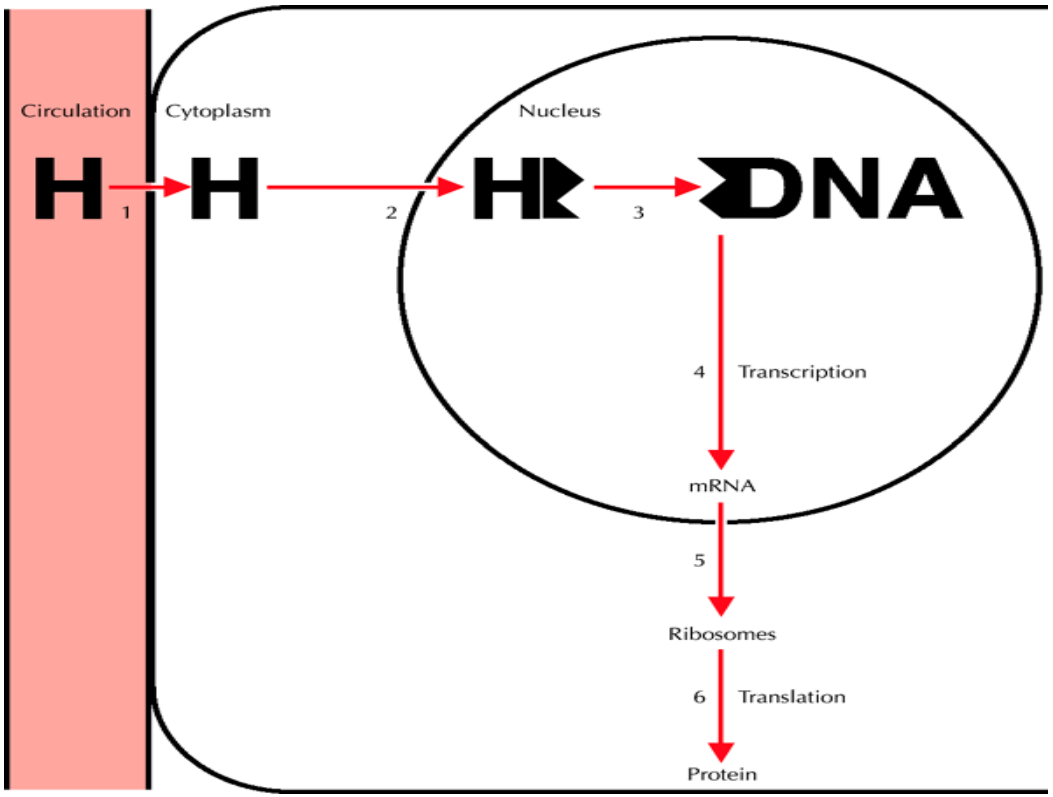
G Protein Receptors : tropic hormones, PG, odors

Ion Gate Channels: acetylcholine receptors

Receptors with Intrinsic Enzyme Activity: insulin & growth factors

Other Receptors

Mechanism of Action for Steroid Hormones



(1)steroid hormone diffusion across the cell membrane
(2)steroid hormone binding to receptor protein
(3)interaction of a hormone-receptor complex with nuclear DNA,
(4)synthesis of messenger RNA (mRNA)
(5)transport of the mRNA to the ribosomes
(6)protein synthesis in the cytoplasm that results in specific cellular activity

The hormone-receptor complex binds to specific DNA sites (hormone-responsive elements) that are located upstream of the gene. The specific binding of the hormone-receptor complex with DNA results in RNA polymerase initiation of transcription

Mechanism of Action for Steroid Hormones

Duration of exposure to a hormone is as important as dose

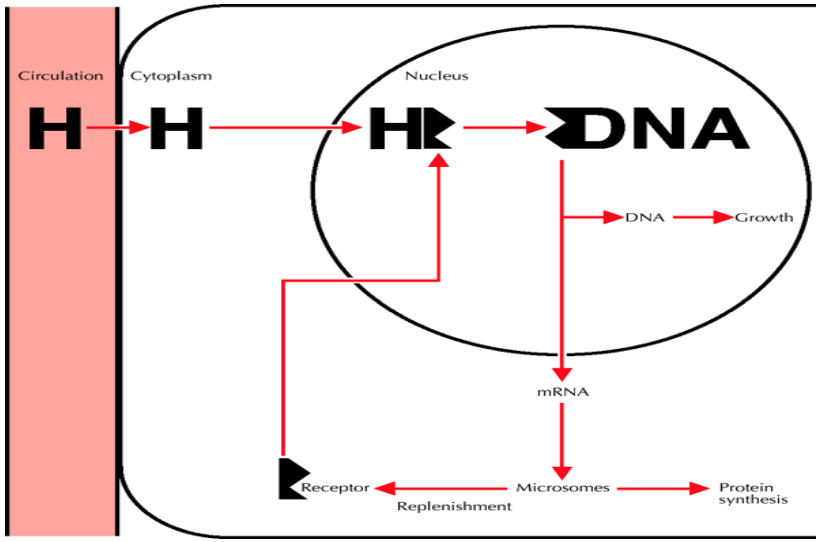
- Biologic activity is maintained only while the nuclear site is occupied with the hormone-receptor complex.

- a major factor in the potency differences among the various estrogens (estradiol, estrone, estriol) is the length of time the estrogen-receptor complex occupies the nucleus

- important action of estrogen is the modification of its own and other steroid hormone activity by affecting receptor concentrations.
  - Estrogen increases target tissue responsiveness to itself and to progestins and androgens by increasing the concentration of its own receptor and that of the intracellular progestin and androgen receptors
  - Progesterone and clomiphene limit tissue response to estrogen by blocking this mechanism, thus decreasing over time the concentration of estrogen receptors

Mechanism of Action for Steroid Hormones

- Estrogen and progestin receptors exit continuously from the nucleus to the cytoplasm and are actively transported back to the nucleus



Hormone-receptor processing

- Estrogen processing involves the rapid degradation of receptors unbound with estrogen
- Estriol vs estradiol
- depletion of estrogen receptors in the endometrium by progestational agents is the fundamental reason for adding progestins to estrogen treatment programs

Mechanism of Action for Tropic Hormones

- Tropic Horthe releasing hormones originating in the hypothalamus and a variety of peptides and glycoproteins released by the anterior pituitary gland and placenta
- The specificity of the tropic hormone depends on the presence of a receptor in the cell membrane of the target tissue
- do not enter the cell to stimulate physiologic events but unite with a receptor on the surface of the cell

The Cyclic AMP Mechanism

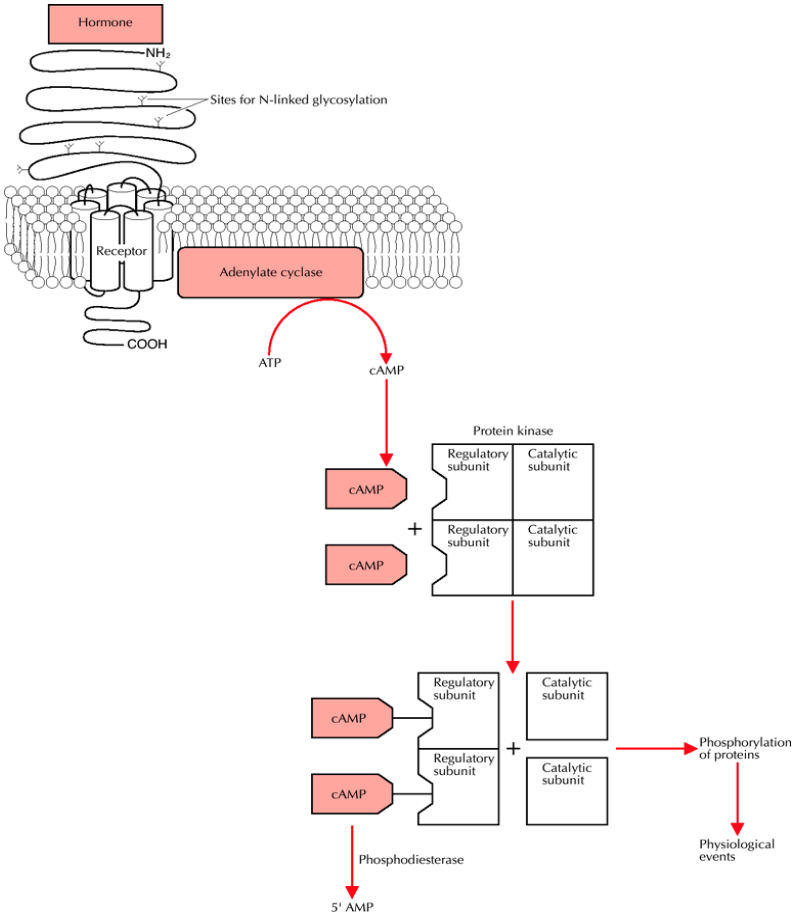
The Calcium Messenger System

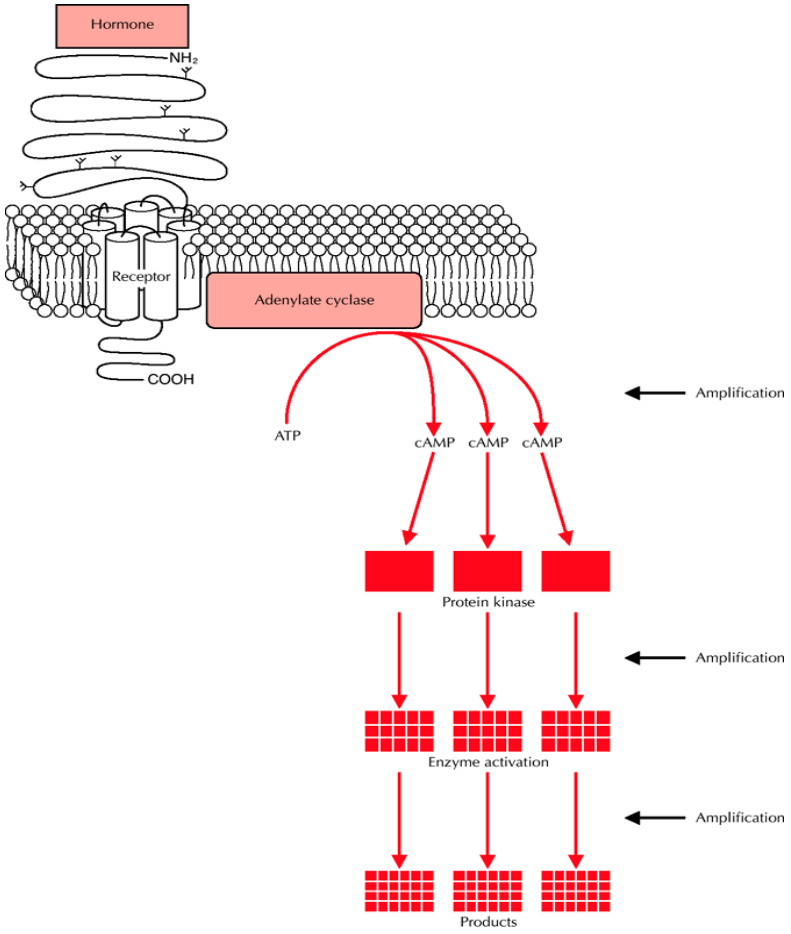
Kinase Receptors

Mechanism of Action for Tropic Hormones

The Cyclic AMP Mechanism

- intracellular messenger for FSH, LH, human chorionic gonadotropin (hCG), thyroid-stimulating hormone (TSH), and ACTH

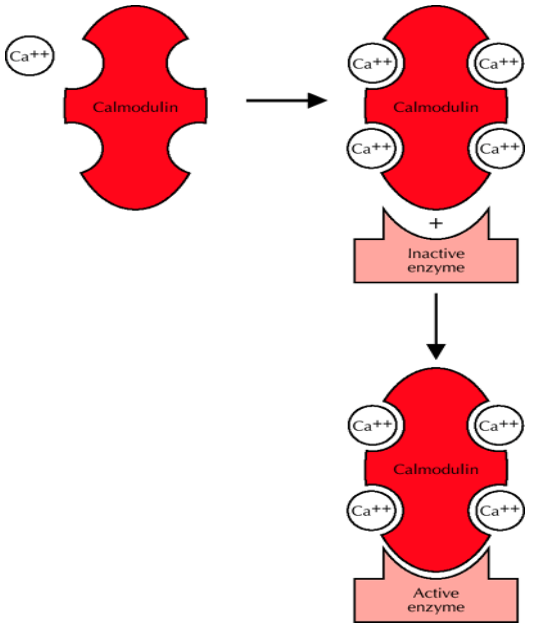




Mechanism of Action for Tropic Hormones

The Calcium Messenger System

- Intracellular calcium concentration is a regulator of both cyclic AMP and cyclic GMP levels
- Activation of the surface receptor either opens a channel in the cell membrane that lets calcium ions into the cell, or calcium is released from internal stores
- calcium flux is an important intracellular mediator of response to hormones, functioning itself as a second messenger in the nervous system and in muscle
- phospholipase C:
  - inositol triphosphate (IP_3) ➡ cellular responses
  - diacylglycerol (DAG) ➡ calmodulin



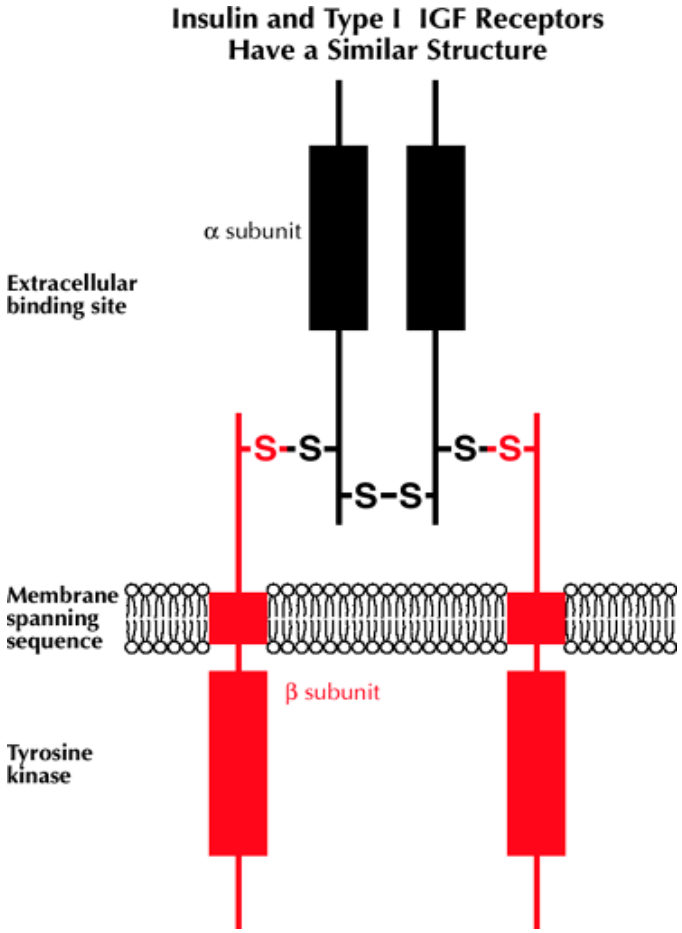
Mechanism of Action for Tropic Hormones

Kinase Receptors

- cell membrane receptors of insulin, insulin-like growth factor, epidermal growth factor, platelet-derived growth factor, and fibroblast growth factor

- All tyrosine kinase receptors have a similar structure : an extracellular domain for ligand binding, a single transmembrane domain, and a cytoplasmic domain

- The cytoplasmic domains respond to ligand binding by undergoing conformational changes and autophosphorylation



Regulation of Tropic Hormones

- Modulation of the peptide hormone mechanism is an important biologic system for enhancing or reducing target tissue response

4 major components of the regulation of tropic hormone

- Autocrine and paracrine regulation factors
- Heterogeneity of tropic hormones
- Up- and down-regulation of receptors
- Regulation of adenylate cyclase

Regulation of Tropic Hormones

Autocrine and paracrine regulation factors

- Growth factors
- polypeptides are autocrine and paracrine regulators operate by binding to cell membrane receptors with an intracellular component of tyrosine kinase activity
- involved in mitogenesis, tissue and cellular differentiation, chemotactic actions, and angiogenesis
- activin, inhibin, IGF-I, IGF-II, TGF-β, FGF, EGF

- Activin & Inhibin
- disulfide-linked dimers composed of peptide subunits
- similar structure but antagonist actions in some system (FSH secretion)
- activin activity is regulated by follistatin

- TGF-β
- stimulate/inhibit growth and differentiation
- ovary: promotes granulosa cell differentiation by enhancing FSH action and antagonizing down-regulation of FSH receptors
- maintaining normal bone mass with IGF

Regulation of Tropic Hormones

Autocrine and paracrine regulation factors

- EGF
- structural analog of TGF-α and is involved in mitogenesis
- ovary: important for granulosa cell proliferation

- FGF
- most potent mitogens , secreted by the granulosa cell
- modulation of enzyme activity involved in the physical act of ovulation
- angiogenic function during the development of the corpus luteum

Regulation of Tropic Hormones

Autocrine and paracrine regulation factors

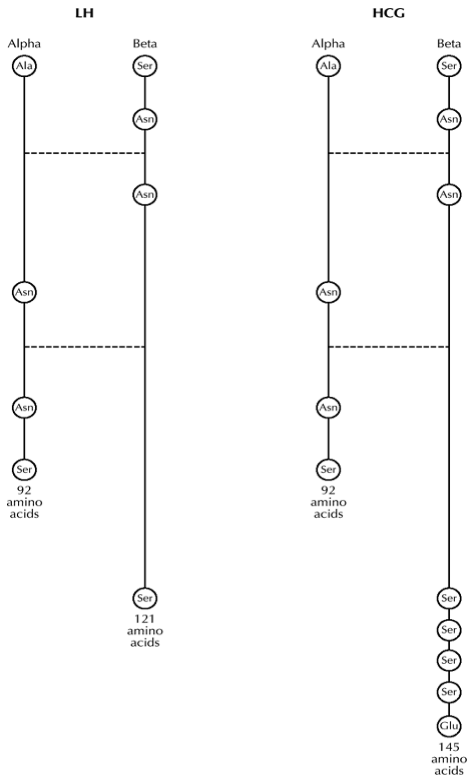
- Insulin-Like Growth Factors
- single-chain polypeptides that resemble insulin in structure and function
- IGF-I amplifies the action of gonadotropins and coordinates the functions of theca and granulosa cells
- In the theca: increases steroidogenesis
- In the granulosa: formation and increase in numbers of FSH and LH receptors, steroidogenesis, secretion of inhibin, and oocyte maturation
- Insulin may share IGF receptor and modulate ovarian cellular function

- Biologic potency and availability of IGF: six different IGF-binding protein various IGFBPs differ in their actions and individual expression, depending on the specific cell type and tissue

Regulation of Tropic Hormones

Heterogeneity of tropic hormones

- Glycoproteins (FSH and LH) are not single proteins but should be viewed as a family of heterogeneous forms of varying immunologic and biologic activity
- Various forms (isoforms) arise in various ways (20–30 isoforms of both FSH and LH in the bloodstream throughout menstrual cycle)
- **The overall activity of a glycoprotein, therefore, is due to the effects of the mixture of forms that reach and bind to the target tissue**



- FSH, LH, TSH, and hCG) share a common α chain
- β chains differ in both amino acid and carbohydrate content

Regulation of Tropic Hormones

Heterogeneity of tropic hormones

- The gene for the α subunit shared by FSH, LH, hCG, and TSH is located on chromosome 6q12.21
- Protein kinase regulation of the α promoter is a principal part of the overall mechanism

- The gene for the FSH β subunit is on chromosome 11p13, and in the pituitary, it is markedly influenced by activin
- The genes that encode for the β subunits of LH, hCG, and TSH are located in a cluster on chromosome 19q13.32
- DNA sequences of the β-hCG genes and the β-LH gene are 96% identical
- only primates and horses have been demonstrated to have genes for the β subunit of chorionic gonadotropin

- Although the β subunit specifies the biologic activity of an individual glycoprotein, the combination of the α and β subunits is necessary for full hormonal expression
- α subunit also plays an important role in accomplishing normal receptor binding and activation

Regulation of Tropic Hormones

Heterogeneity of tropic hormones: Variation in Carbohydrate

- The glycopeptide hormones can be found in the pituitary existing in a variety of forms, differing in their carbohydrate (oligosaccharides) makeup

- The isoform mixture of gonadotropins is influenced both quantitatively and qualitatively by GnRH and the feedback of the steroid hormones, producing posttranslational carbohydrate modifications

- modulates half-lives and bioactivity

- Half life:
- circulating half-life of a gonadotropin is mainly proportional to the amount of sialic acid present
- higher content of sialic acid in FSH compared with LH accounts for the more rapid clearance of LH from the circulation

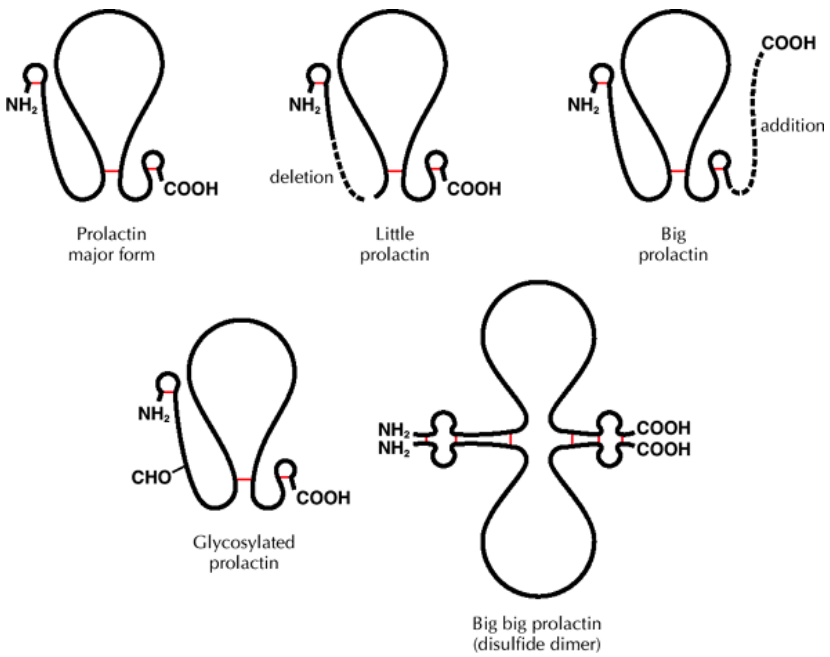
- Bioactivity
- binding and **activation**
- carbohydrate component affects the biologic activity of the hormone-receptor complex after binding

Regulation of Tropic Hormones

Heterogeneity of tropic hormones: Prolactin

- In most mammalian species, prolactin is a single-chain polypeptide of 199 amino acids, 40% similar in structure to growth hormone and placental lactogen
- differences in prolactin were observed based on size, glycosylation, phosphorylation, and variations in binding and charge
- encoded by a single gene on chromosome 6, producing a molecule that in its major form is maintained in three loops by disulfide bonds
- result of posttranslational modifications

- big prolactins account for 10% ~ 25% of hyperprolactinemia



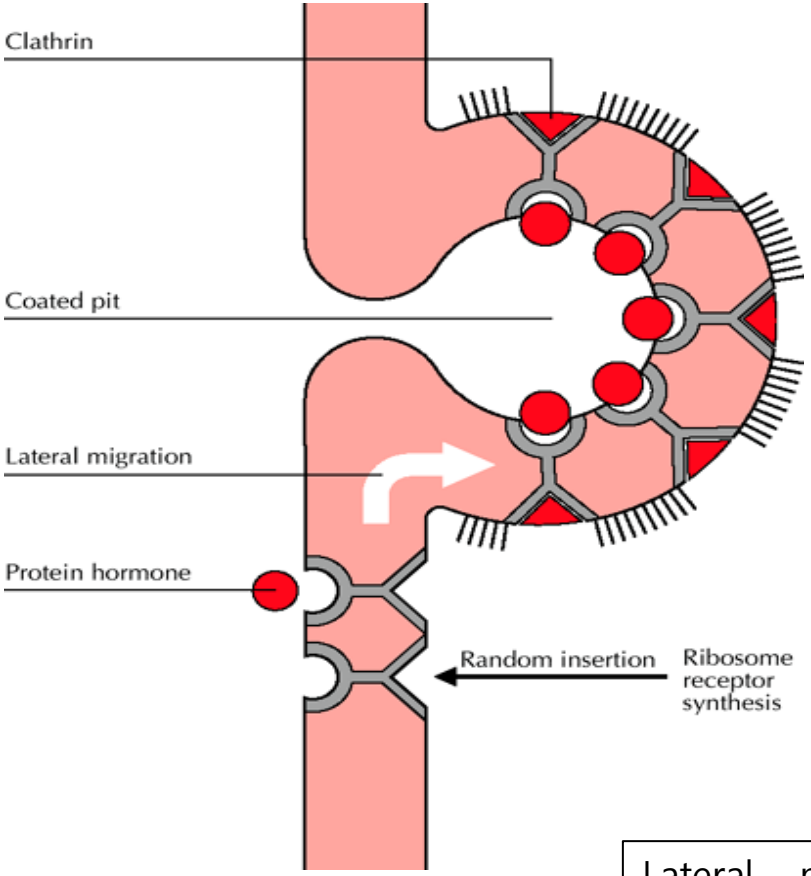
Regulation of Tropic Hormones

Up- and down-regulation of receptors

- Positive or negative modulation of receptors by homologous hormones

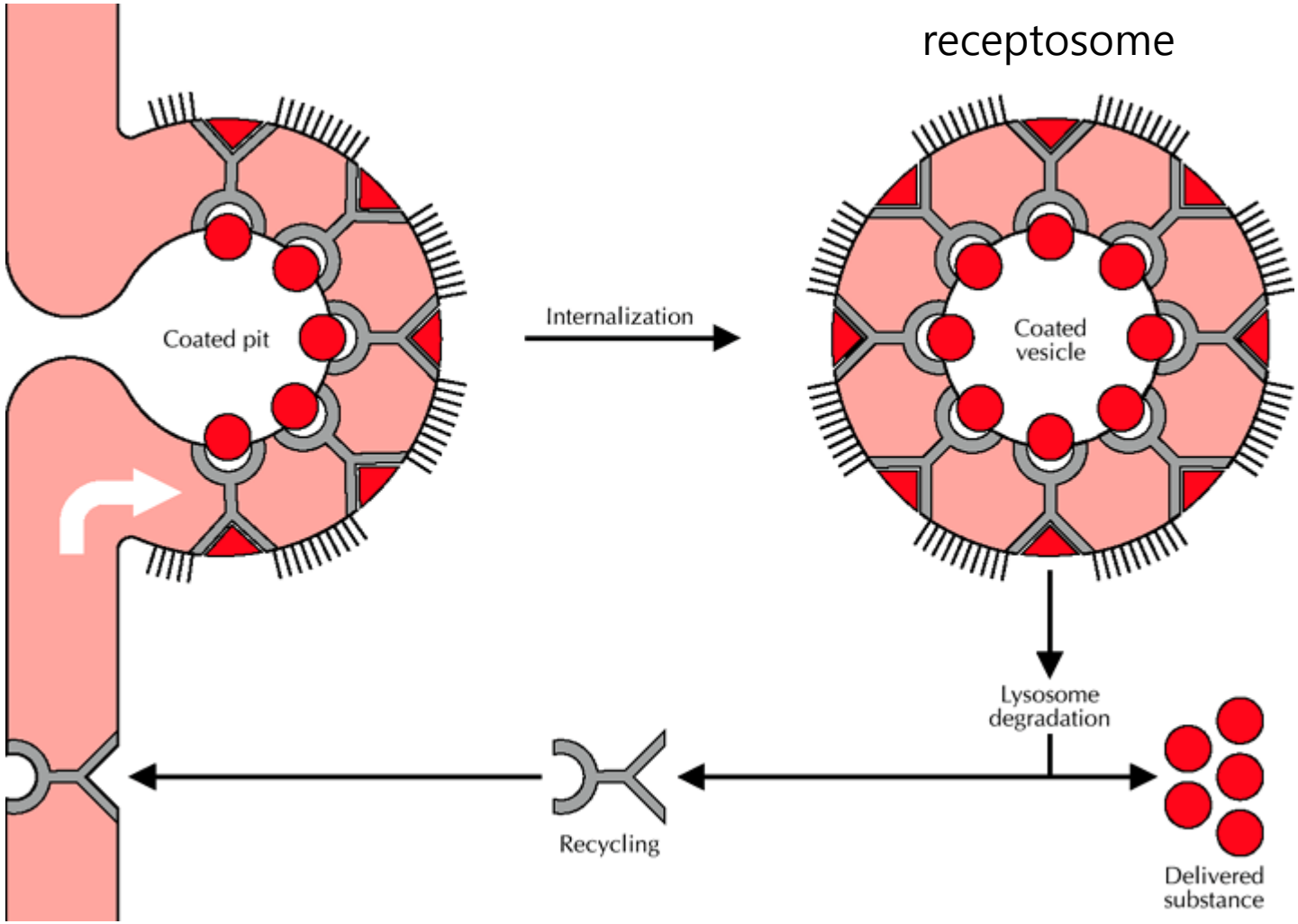
Process of internalization

- deactivation of the hormone-receptor complex could be accomplished by dissociation of the complex or loss of receptors from the cell, either by shedding (externally) or by internalization of the receptors into the cell
- an excess concentration of a tropic hormone, such as LH or GnRH, will stimulate the process of internalization, leading to a loss of receptors in the cell membrane and a decrease in biologic response
- reason for episodic (pulsatile) secretion: avoid down-regulation and to maintain, its receptors



- receptors are **randomly inserted** into the cell membrane after intracellular synthesis
- an external binding site-specific for a polypeptide hormone,
  transmembrane region
  internal site -plays a role in the process of internalization
- receptor is bound to a polypeptide hormone and high concentrations of the hormone in the circulation, the hormone-receptor complex moves through the cell membrane in a process called **lateral migration**
- Lateral migration carries the complex to a specialized region of the cell membrane, the **coated pit**

Lateral migration, thus, concentrates hormone-receptor complexes in the coated pit (**clustering**), allowing increased internalization of the complex via the special mechanism of receptor-mediated endocytosis



When fully occupied, the coated pit invaginates, pinches off, and enters the cell as a coated vesicle also called a receptosome

Regulation of Tropic Hormones

Up- and down-regulation of receptors

- **Potocytosis**: utilizes cholesterol-rich membrane invaginations called caveolae (far fewer in number and smaller in structure than the clathrin coated pits) for the internalization of small molecules and ions
- caveolin: major protein structural component of caveolae, interact with NO

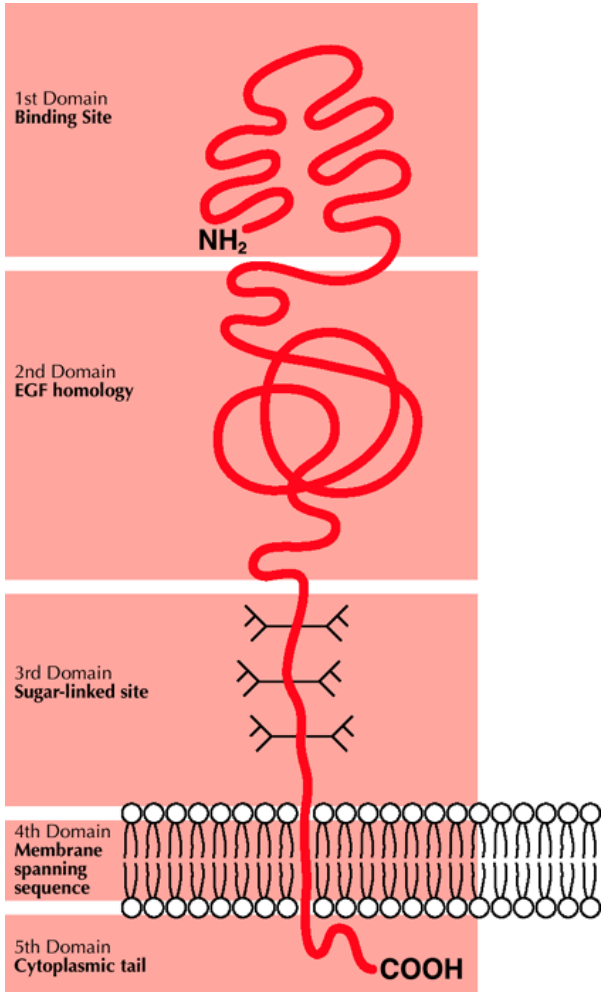
- FSH, LH, hCG, GnRH, TSH, TRH, and insulin
- Cell membrane receptors can be randomly distributed in the cell membrane and transmit information to modify cell behavior
- Internalization ➡ a method for down-regulation by degradation in lysosomes
- the coated pit can be viewed as a trap to immobilize hormone-receptor complexes
  - lysosome for degradation
  - recycled back to the cell surface as a means of transporting (hGC into materno-fetal circulations)

Regulation of Tropic Hormones

Up- and down-regulation of receptors

- LDL and its receptor
- the principal messenger delivering the cholesterol to steroid producing cells
- LDL receptor :"mosaic protein"
- synthesized as a precursor of 860 AAs.

1. NH_2-terminal of 292 amino acids, composed of a sequence of 40 amino acids repeated with some variation seven times. This domain is the binding site for LDL and is located on the external surface of the cell membrane.
2. Approximately 400 amino acids 35% homologous to epidermal growth factor precursor.
3. The sugar-linked site.
4. 22 Hydrophobic amino acids that cross the cell membrane. Deletion of the transmembrane signal sequence (found in a naturally occurring mutation) results in an LDL receptor that is secreted from the cell instead of being inserted into the membrane.
5. Cytoplasmic tail of 50 amino acids that is located internally and serves to cluster LDL receptors in coated pits.



Regulation of Tropic Hormones

Up- and down-regulation of receptors

- LDL and its receptor
- when the coated pit is fully occupied with LDL, a coated vesicle is delivered into the cell in the process called endocytosis
- vesicle moves to the Golgi system to the lysosomes in which the structure undergoes degradation, releasing cholesterol esters and the receptor
- receptor can be recycled or degraded

- intracellular level of free cholesterol
  - the rate-limiting enzyme for cholesterol synthesis
  - reesterification of excess cholesterol for storage as lipid droplets
  - synthesis of LDL receptors

- cholesterol derived from the LDL transport process
  - utilization in the mitochondria for steroidogenesis
  - reesterification for storage
  - use in membrane structures
  - excretion

Regulation of Tropic Hormones

Regulation of adenylate cyclase

- The G Protein System

- Adenylate cyclase composed of 3 units: a receptor, **a guanyl nucleotide regulatory unit**, a catalytic unit

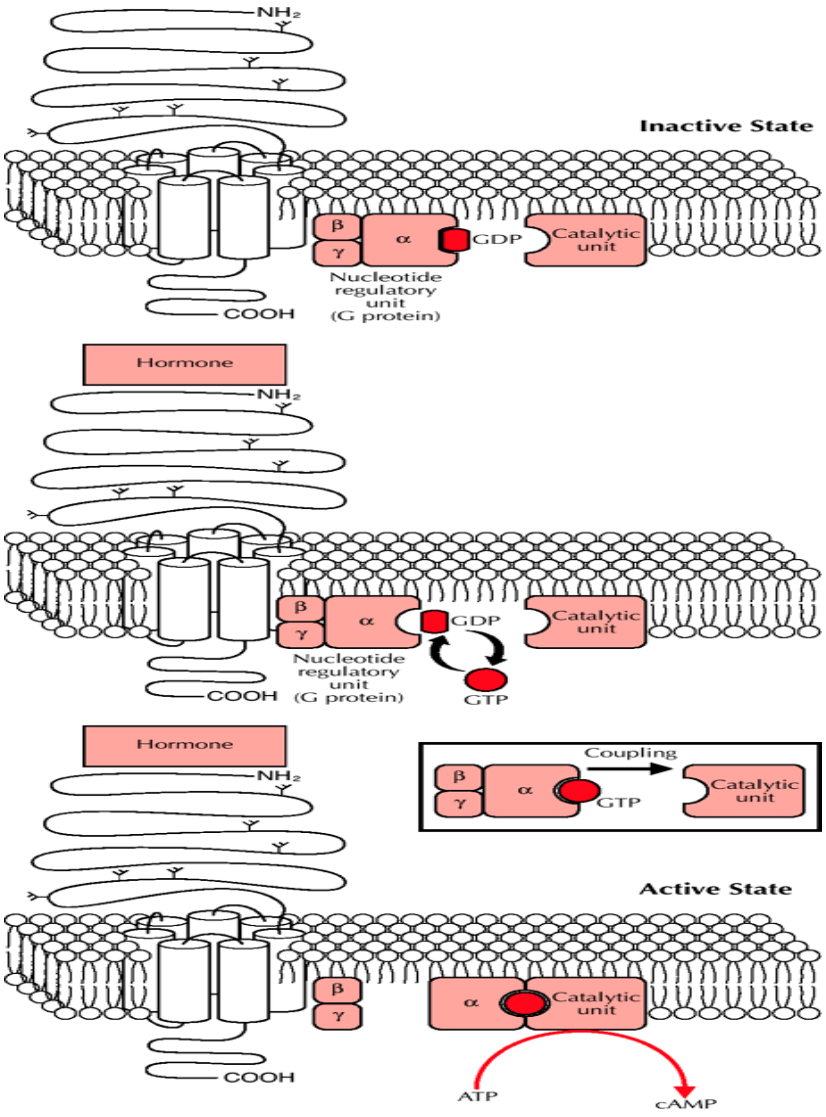
- Upon binding, the complex of hormone, receptor and nucleotide regulatory unit is activated leading to an uptake of GTP by the regulatory unit
  ⟹ activation and uptake of GTP result in an active enzyme that can convert ATP to cyclic AMP.

- couples receptors to active ligands, playing roles in signal transduction, intracellular transport, and exocytosis

- hormone-receptor complex to work through a common messenger (cyclic AMP) with both and inhibitory nucleotide regulatory G proteins

Regulation of Tropic Hormones

Regulation of adenylate cyclase



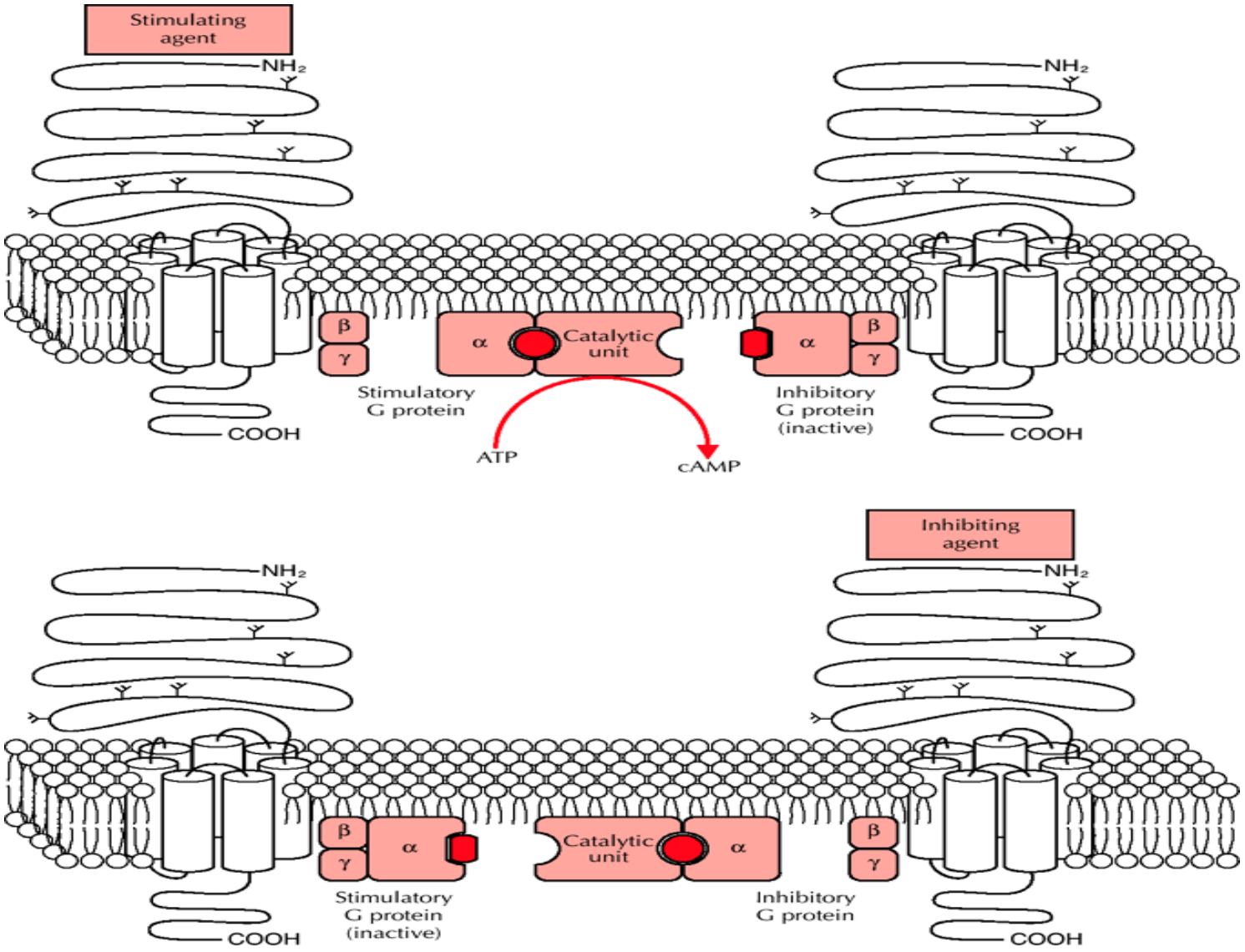
- GDP is bound to the α subunit
- Hormone-receptor interaction and binding change the α subunit conformation

- GTP replaces GDP on the α subunit, freeing the β and γ subunits

- allows the GTP α subunit to bind to the catalytic unit of adenylate cyclase, forming the active enzyme
- Intrinsic GTPase activity quickly hydrolyzes the GTP-α to GDP-α, which leads to reassociation with the β and γ subunits, reforming the G protein complex for further activation
- **The functional specificity is due to the α subunit, which differs for each G protein**

Regulation of Tropic Hormones

Regulation of adenylate cyclase



Regulation of Tropic Hormones

Regulation of adenylate cyclase

- Mutations in the G Protein System
- Loss of function mutations of a G protein or a given receptor will result in hormone deficiency syndromes

Some Genetic Diseases Due to Specific G Protein System Mutations

Mutation	Disorder
Activating LH receptor	Precocious puberty in boys
Inactivating LH receptor	Male pseudohermaphroditism
Inactivating FSH receptor	Premature ovarian failure
$G_s\alpha$ (stimulatory)	McCune-Albright syndrome
$G_i\alpha$ (inhibitory)	Hypothyroidism
Rhodopsin	Retinitis pigmentosa
Vasopressin	Diabetes insipidus

-sexual precocity, polyostotic fibrous dysplasia, café-au-lait skin pigmentation, and autonomous functioning of various endocrine glands
-unregulated activity (gain in function) of the adenylate cyclase system because of a mutation in the $G_s\alpha$ gene

-$G_s\alpha$ protein mutations : adrenal and ovarian tumors, growth hormone-secreting pituitary adenomas, and thyroid adenomas

Regulation of Tropic Hormones

Regulation of adenylate cyclase

- Coupling and Uncoupling
- LH stimulates steroidogenesis in corpus luteum through the coupling of stimulatory regulatory units to the catalytic units of adenylate cyclase
- Prostaglandin $F_2\alpha$ is directly luteolytic, inhibiting luteal steroidogenesis luteolytic action: exerted via an inhibitory regulatory unit that leads to uncoupling with the catalytic unit, thus interfering with gonadotropin action

- Desensitization
- increasing concentrations of tropic hormones directly associated with desensitization of adenylate cyclase independent of the internalization of receptors
- rapid, acute change without loss of receptors
- involves receptor phosphorylation
- decreased gonadotropin secretion in the presence of prolonged continuous GnRH stimulation

- Alterations in Regulatory Proteins
- activation of the mitogen-activated protein kinase (MAPK) system increases levels of SF-1, which in turn attenuates StAR expression

Regulation of Tropic Hormones

- Summary of Down Regulation

- Down regulation is a decrease in response of continuous stimulation

1. Desensitization by autophosphorylation of the cytoplasmic segment of the receptor

2. Loss of receptors by internalization, a relatively slow mechanism

3. Uncoupling of the regulatory and catalytic subunits of the adenylate cyclase enzyme

4. Alterations in key intracellular regulatory proteins

"Sorry I fired you, Maggie. I got my husband's testosterone patches mixed up with my estrogen patches."