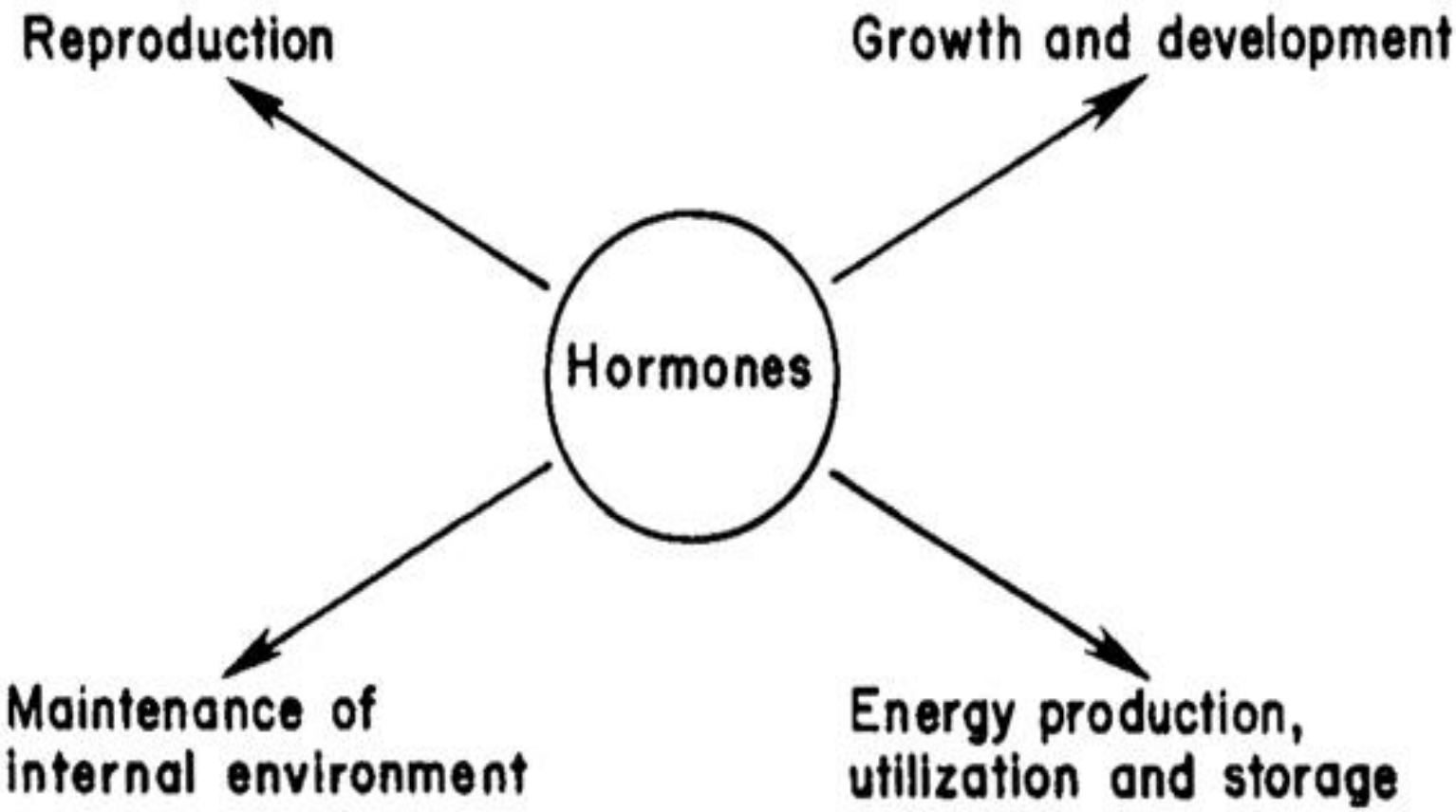
# Estrogens and Progestins

ANSC 630 Reproductive Biology I

# Hormone Functions



# Physiological Roles of Hormones

- Neuromodulation
- Reproductive Processes
- Metabolism (anabolic/catabolic)
- Cellular proliferation and growth
- Excretion and readsorption
- Behavior
- Immune system
- *More being discovered every day* !

# Classical Definition of a Hormone:

**Physiological organic substance produced by specialized cells and released into circulating blood or lymph for transport to target tissues in distant organs to exert specific actions.**

**Classical hormones are cell signaling molecules that:**

- **are synthesized by endocrine cells, e.g., gonadotrophs**
- **are secreted into the circulation (blood or lymph)**
- **interact with proteins called receptors on target cells (e.g., theca cells of ovarian follicle)**
- **have specific effects on target cells (e.g., stimulate theca cells to produce androgens such as testosterone)**

# Modern Definition of Hormone

- **Hormone**
  - **Substance released by one cell to regulate another cell. Synonymous with chemical messenger.**
  - **Delivered through endocrine, neuroendocrine, neurocrine, paracrine, autocrine, lactocrine or pheromonal systems**
- **Chemical Nature of Hormones:**
  - **Amino Acids (norepinephrine, epinephrine, dopamine from tyrosine; thyroid hormones Triiodothyronine (T3) and Thryoxin (T4) from two iodinated tyrosines**
  - **Peptides (e.g., oxytocin) and Proteins (e.g., Follicle Stimulating Hormone and Luteinizing Hormone)**
  - **Steroid Hormones**
    - **Intact steroid nucleus (cortisol, estrogen, progesterone)**
    - **Broken steroid nucleus (Vitamin D and metabolites)**

# Different Categories of Hormones

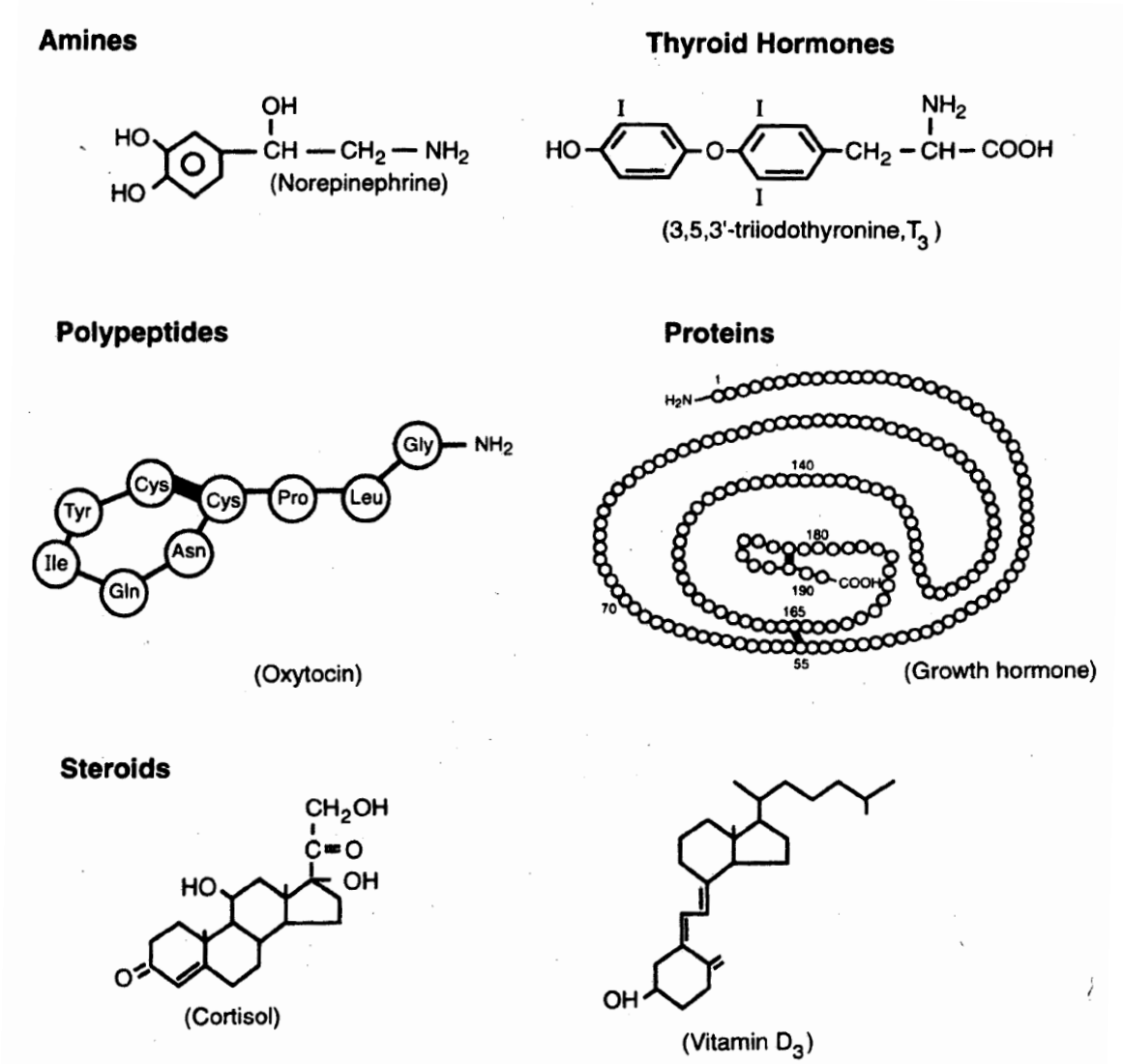
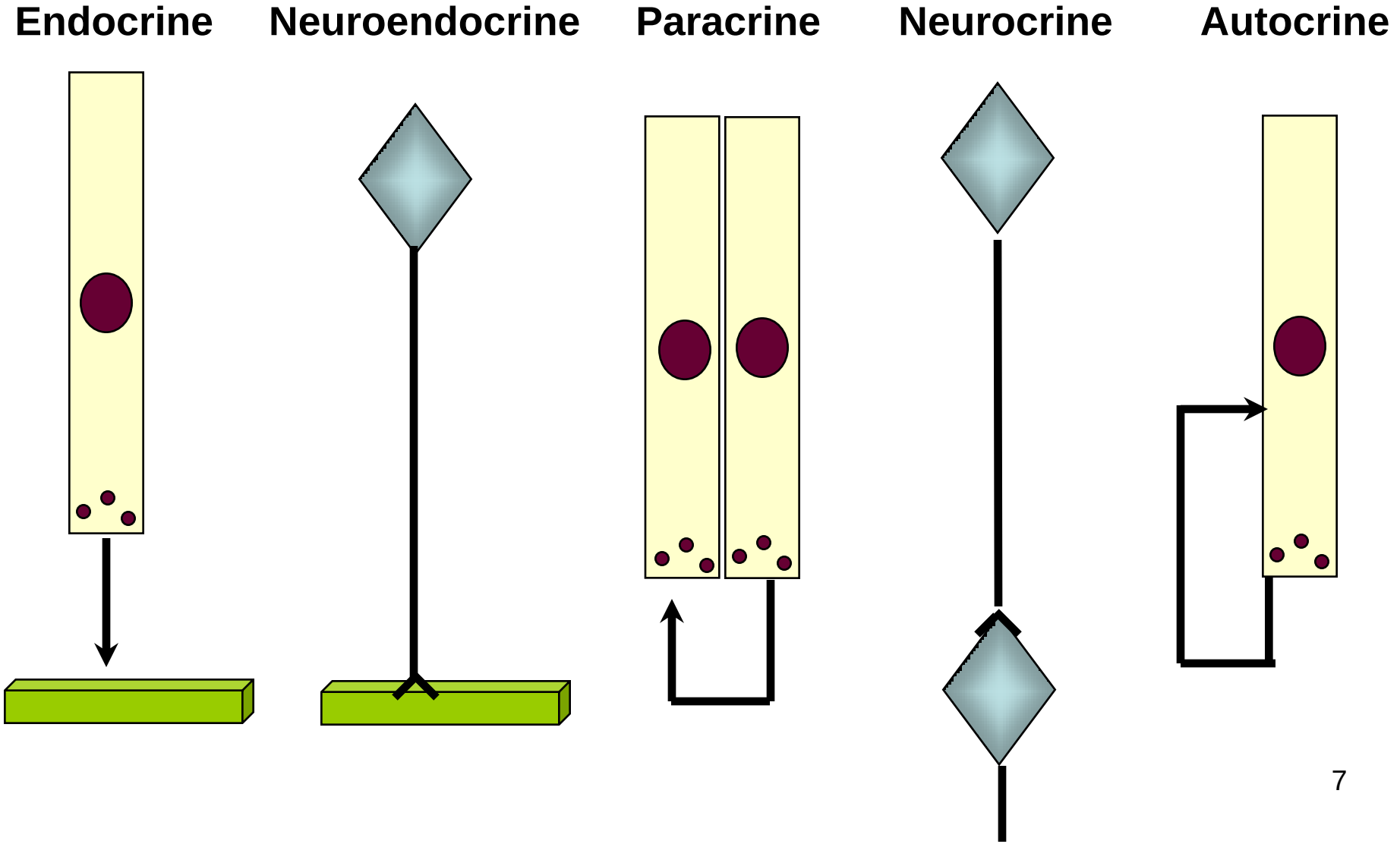


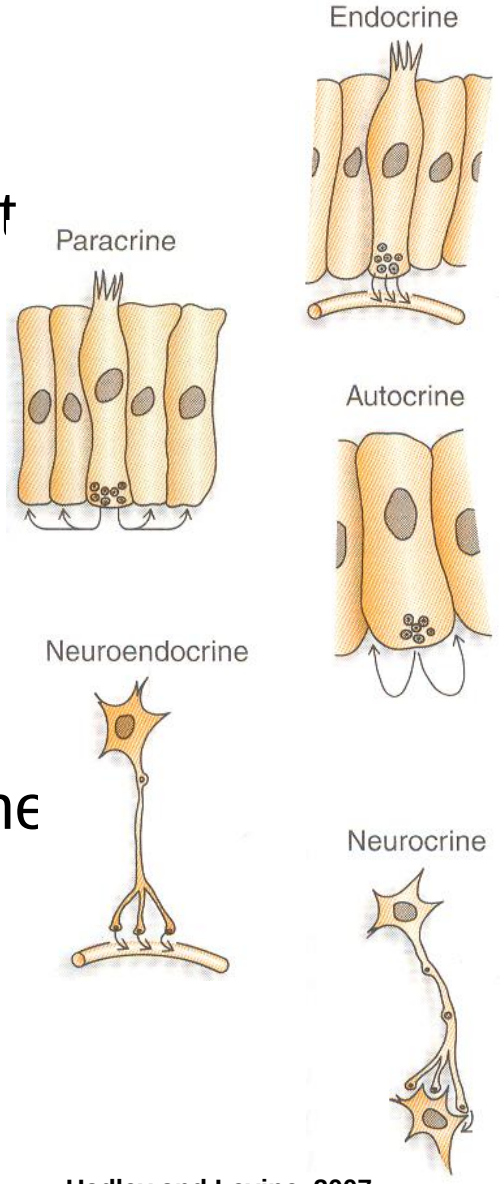

**Fig. 1-1** Examples of different categories of hormones. In the case of the protein hormone, each circle represents an amino acid, as shown for the polypeptide hormone.

# Methods of Hormone Delivery



# Modes of hormone action

- **Endocrine:** the hormone is bloodborne
- **Paracrine:** hormone diffuses to adjacent cells through the extracellular space
- **Autocrine:** hormone feed back on cell of origin
- **Neuroendocrine:** hormone is released by a nerve cell into the bloodstream
- **Neurocrine:** neuron terminal contacts the target cell and release neurohormone in specialized sites
- **Lumonal:** hormone is released into the lumen (gut, uterus, cerebral ventricles)




Hadley and Levine, 2007

# LACTOCRINE

THE TRANSFER OF BIOLOGICALLY ACTIVE MOLECULES FROM MOTHER TO NEONATE VIA COLOSTRUM OR FIRST MILK; FOR EXAMPLE, RELAXIN

# Classical Endocrine Glands and Their Hormones

**Table 1-1** Classical Endocrine Glands and Their Hormones

| Gland | | Hormone |
|---|---|---|
| Pituitary | Anterior lobe | Luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin (PRL), growth hormone (GH), adrenocorticotropin (ACTH), β-lipotropin, β-endorphin, thyroid-stimulating hormone (TSH) |
| | Intermediate lobe | Melanocyte-stimulating hormone (MSH), β-endorphin |
| | Posterior lobe | Vasopressin (AVP) or antidiuretic hormone (ADH), oxytocin |
| Thyroid | | Thyroxine ($T_4$), 3,5,3′-triiodothyronine ($T_3$), calcitonin |
| Parathyroid | | Parathyroid hormone (PTH) |
| Adrenal | Cortex | Cortisol, aldosterone, dehydroepiandrosterone, androstenedione |
| | Medulla | Epinephrine, norepinephrine |
| Gonads | Testis | Testosterone, estradiol, androstenedione, inhibin, activin, müllerian-inhibiting substance |
| | Ovary | Estradiol, progesterone, testosterone, androstenedione, inhibin, activin, FSH-releasing peptide, relaxin, follistatin |
| Placenta | | Human chorionic gonadotropin (hCG), human placental lactogen (hPL), progesterone, estrogen |
| Pancreas | | Insulin, glucagon, somatostatin, pancreatic polypeptide, gastrin, vasoactive intestinal peptide (VIP) |
| Pineal | | Melatonin, biogenic amines, several peptides |

# Patterns of Hormone Secretion

- Circhoral (frequency of 1 h)
- Ultradian (frequency of 1-24 h)
- Circadian (periodicity is 1 day)
- Quotidian (recurs every day)
- Circannual or Seasonal (relation to seasonal phases of the year)

# Endocrine System: General Considerations

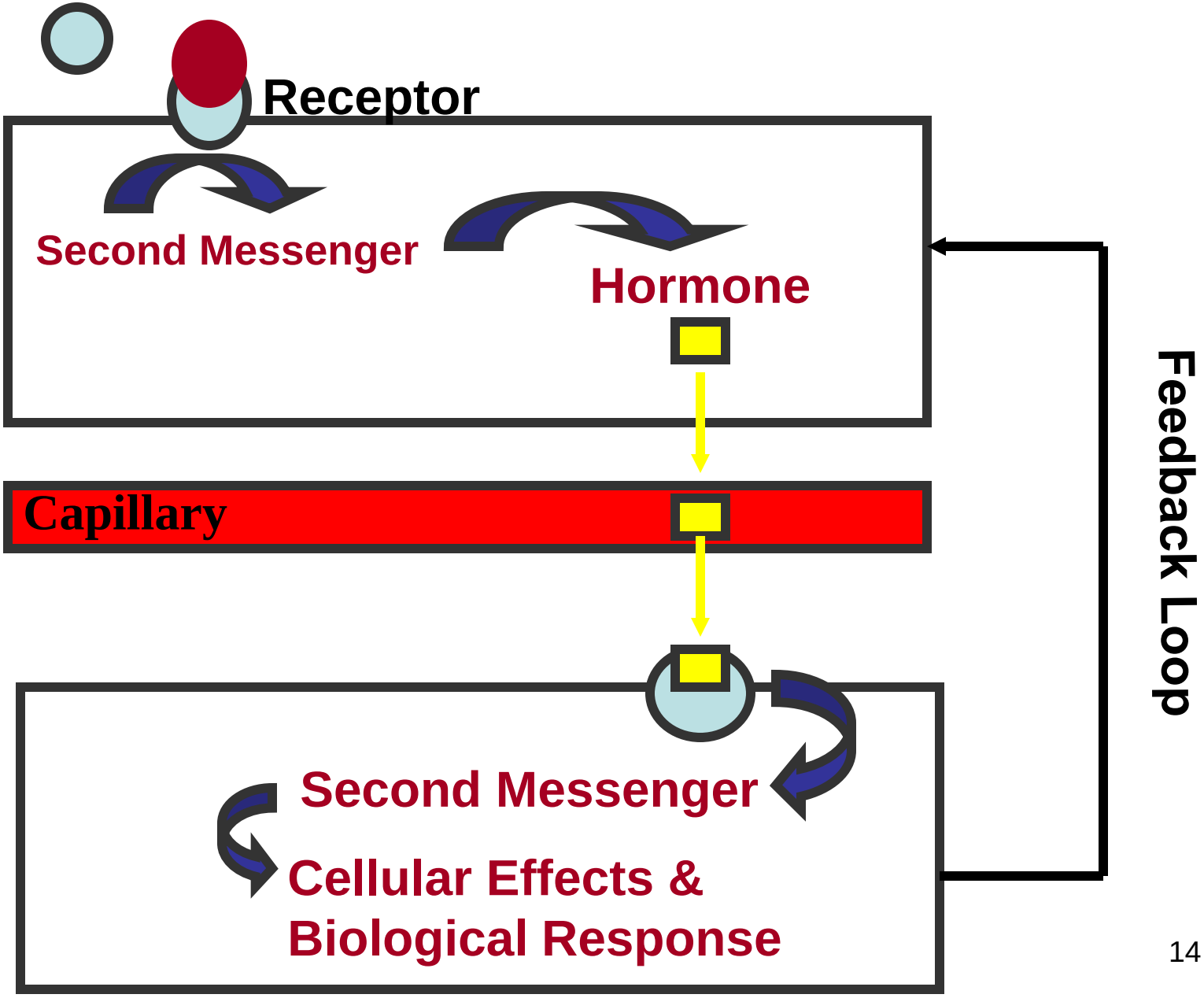
- **Synthesis of hormones**
  - Regulated at several levels: gene, mRNA,enzyme
- **Distribution network**
  - Blood/cerebral fluid/other body fluids
- **Site of action (target cell/organ)**
  - Transduced through hormone specific receptor and 2nd messengers
- **Loss of hormone action**
  - Mechanism to metabolize or degrade the hormone
- **Feedback**
  - Negative and positive feedback, but also cooperative effects as estrogen increases receptors for progesterone

# Control of hormone release

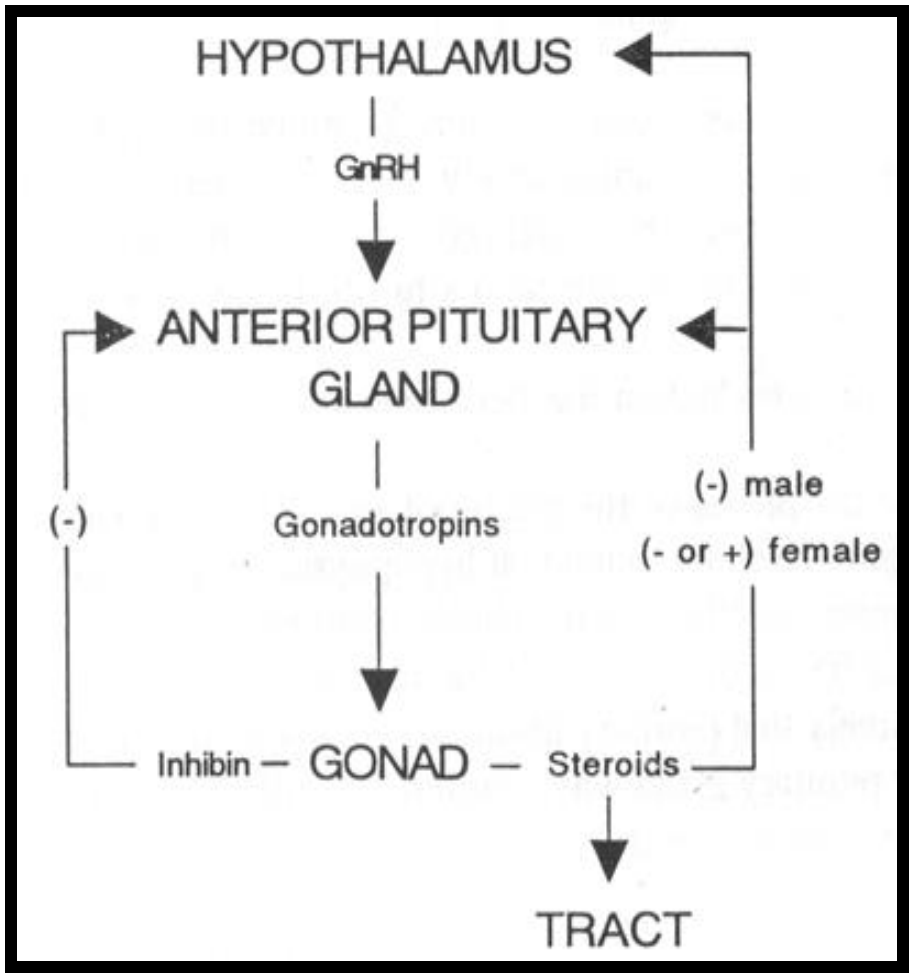
- **Regulated (Small Luteal Cell vs. Constitutive (Large Luteal Cell**

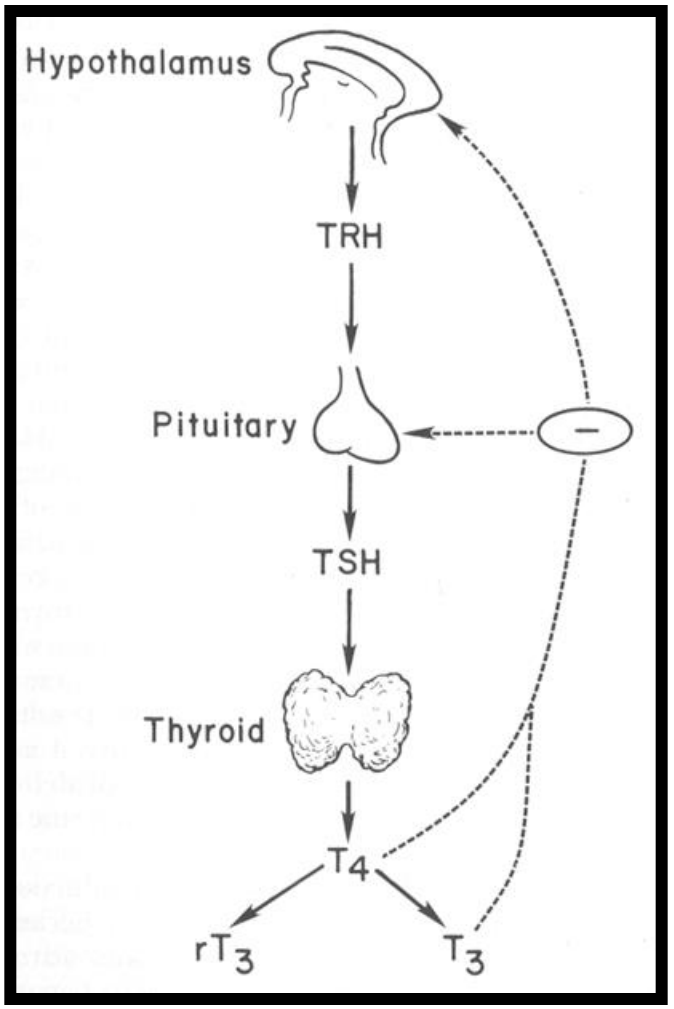
- Peptide and protein hormones
  - Exocytosis such as oxytocin and neurophysin
  - Triggered by stimulatory signal such as releasing factors such as GnRH causes release of FSH and LH or $Ca^{2+}$ induced release of oxytocin and neurophysin 1
- Steroid and thyroid hormones
  - Limited storage within fat droplets
  - Diffusion according to concentration gradient
  - Concentrations in blood and tissues depend on synthesis

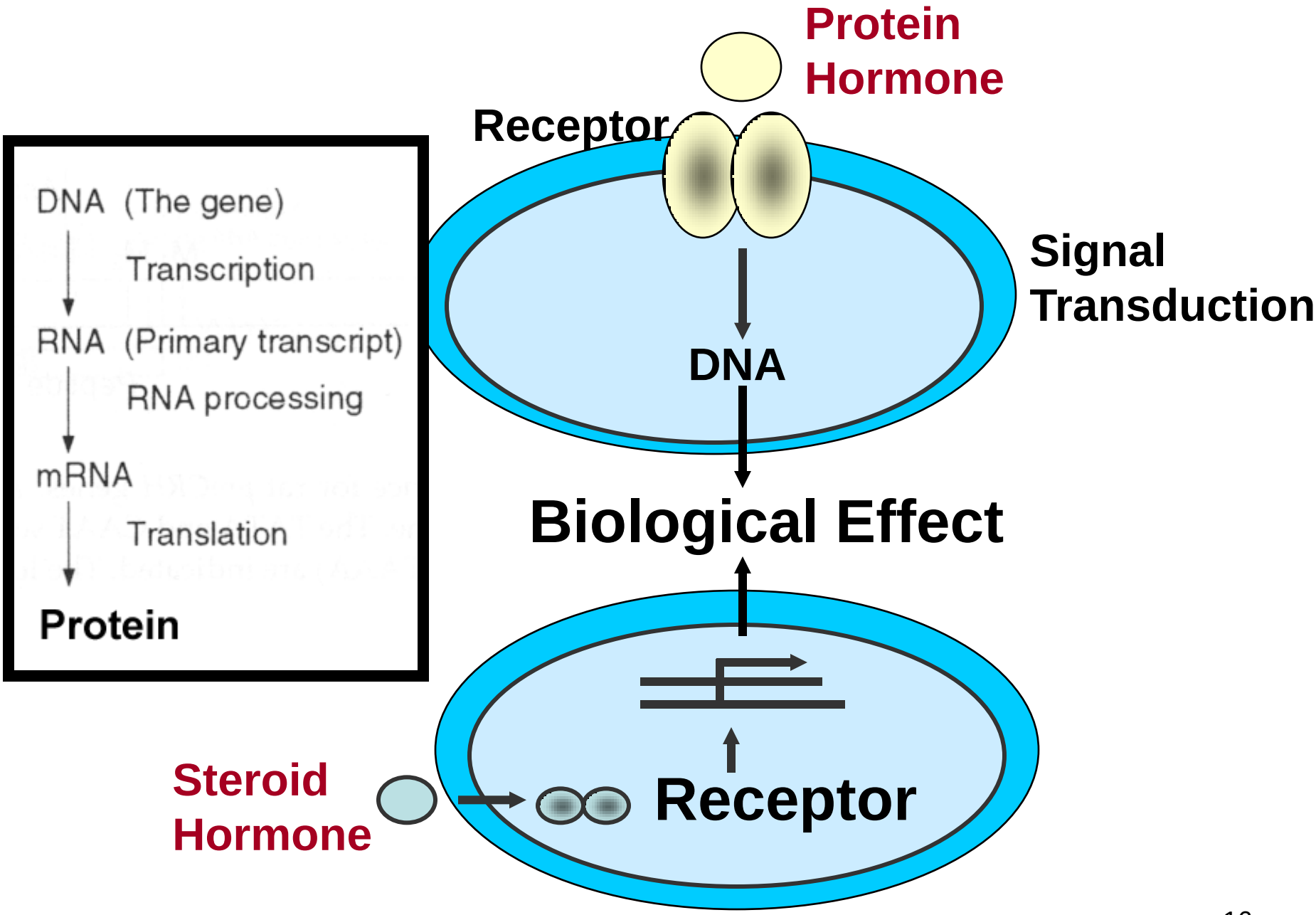
## Stimulus for Secretion



# Classic Feedback Systems




Protein Hormone
Receptor
DNA (The gene)
Transcription
RNA (Primary transcript)
RNA processing
mRNA
Translation
Protein
Signal Transduction
DNA
Biological Effect
Steroid Hormone
Receptor

# Steroid Hormone Action Overview

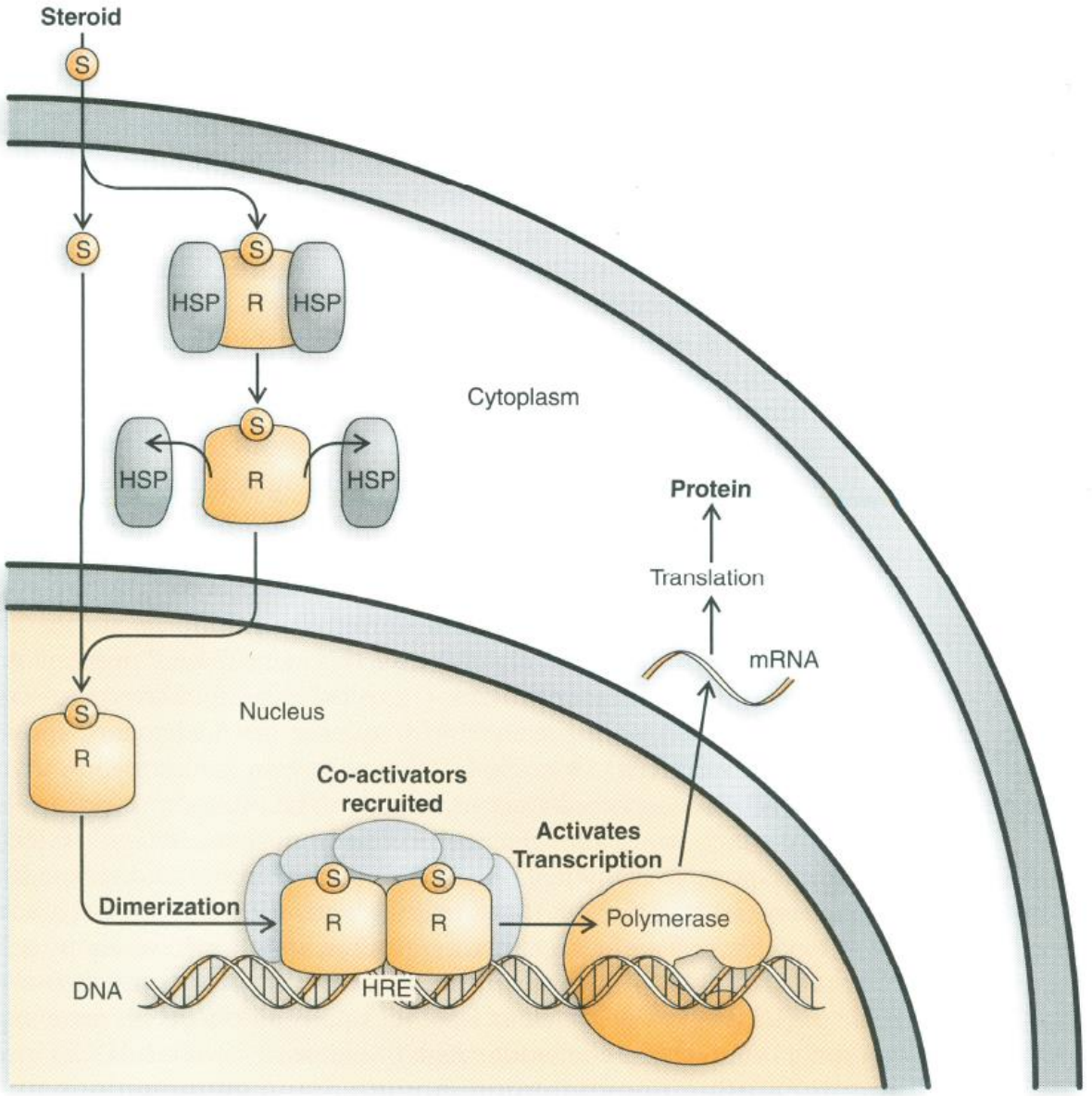



**Figure 3.15**
Mechanisms of steroid hormone action.

# Genomic and Non-genomic Actions of Steroid Receptors

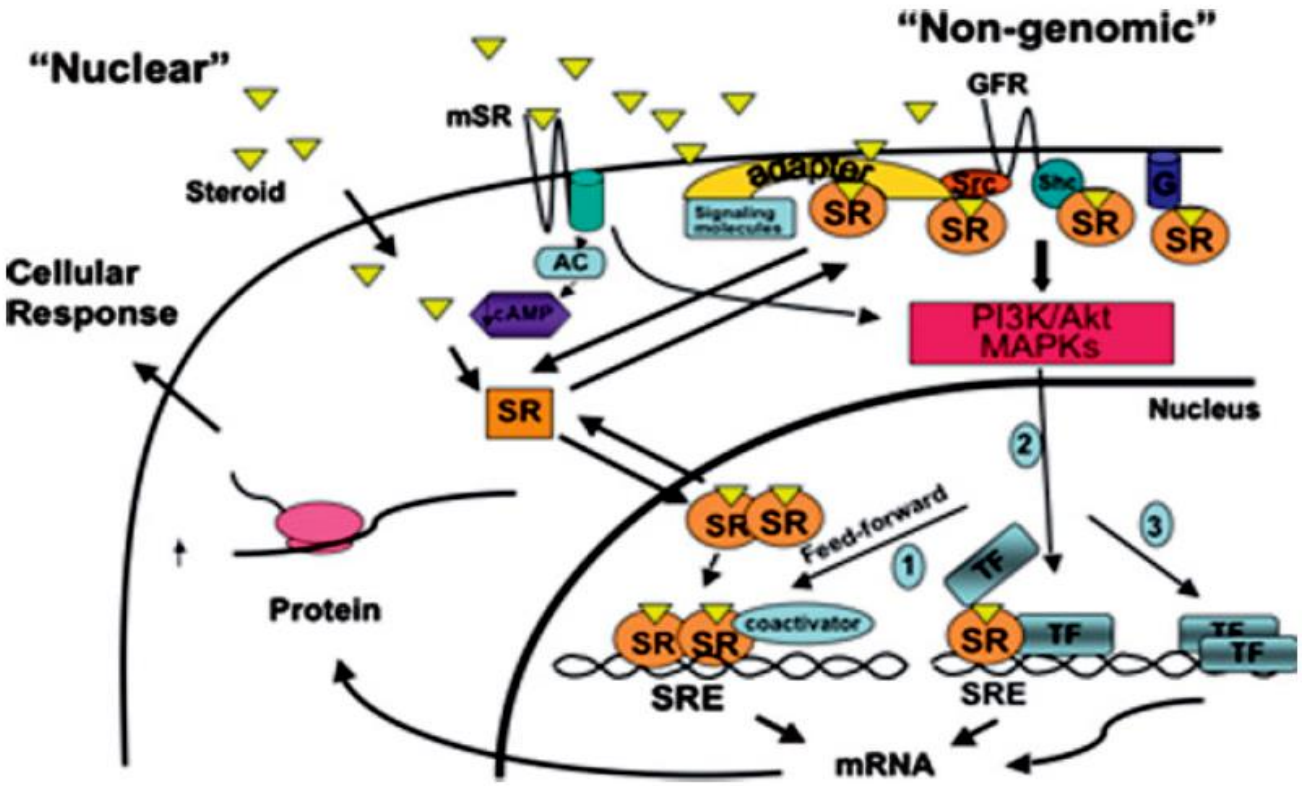



**Figure 2** Nuclear transcriptional and non-genomic signaling pathways activated by sex steroid hormones. Sex steroids activate nuclear steroid hormone receptors (SR) by inducing receptor conformational changes, nuclear translocation, dimerization, and binding to steroid hormone response elements (SREs) in promoters/enhancers of target genes. Alternatively, a subpopulation of nuclear steroid receptors localized in cytoplasm/membrane can associate transiently with other signaling molecules including G protein–coupled receptor (GPCR; G), c-Src, Shc, adapter proteins (adapters), or membrane targeting proteins leading to activation of mitogen-activated protein kinase (MAPK) or phosphatidylinositol 3-kinase (PI3K)/Akt signaling cascades. Novel GPCR membrane receptors (mSR) unrelated to nuclear steroid receptors have been reported to also mediate rapid non-genomic effects of steroid hormones through inhibition of adenylate cyclase (AC) and cyclic adenosine monophosphate (cAMP) production and activation of MAPK. A biological consequence of sex steroid–induced activation of cytoplasmic signaling cascades is ultimately to influence gene transcription by three possible mechanisms. (1) A feed-forward regulatory loop whereby the nuclear transcriptional activity of SR or coactivators are enhanced by phosphorylation. (2) Signaling pathways that converge upon and activate target genes that require other transcription factors (TF) to cooperate with SRs either by tethering or by binding on composite SRE promoters. (3) Activation of other transcription factors independent of direct SR binding to DNA.

# Hormone (Ligand) Receptors

- **Hormones act on specific target tissues.**
- **How are target tissues "selected" by specific hormones?**
  - **Determined through receptors on target cells that provide the specificity for hormone-cell interactions**
- **Receptors may be components of the cell membrane, cytosolic, or nuclear elements.**
- **They are hormone-specific binding proteins.**

# Principles of Receptor Binding

- **1) Hormone specificity**
  - **Receptors interact with (bind) a specific hormone**
  - **Hormones have a primary receptor, but may interact with less affinity with other receptors**
    - **Insulin receptor will interact with Insulin-Like Growth Factor 1**
    - **Androgen receptor (AR) will bind progestins**
    - **Glucocorticoid receptor (GR) will bind aldosterone**
    - **Testosterone will bind estrogen (ESR1) and glucocorticoid receptors (GR)**
- **2) High affinity**
  - **Receptor affinity is related to concentration of hormone which is requisite for specificity of receptors. The dissociation constant, $K_D$ is the reciprocal of the affinity constant, $K_A$ and is usually very low (1 nM to 10 pM)**

# Principles of Receptor Binding

- **3) Tissue Specificity**
  - **Target tissues contain receptors that respond specifically to a hormone after it binds to its receptor.**
  - **The effector system must be operational and coupled to the receptor to elicit a response. That means that there must be a receptor to bind the ligand and that a secondary signal acts at the level of gene expression to elicit a response.**
  - **Some non-specific binding of hormone to a receptor may occur, but this type of binding is generally of low affinity with no hormonal or tissue specificity.**

# Principles of Receptor Binding

- **4) Saturable**
  - **Usually one specific binding site per molecule**
  - **Should be a finite number of receptors**

- **5) Reversible**
  - **Hormone binding must be reversible.**
  - **The "on/off" rate is dependent on binding affinity**


- **6) Must be related to biological effect**
  - **Binding of hormone to receptor must elicit a cellular response.**

# Numbers of Receptors

- **Receptors are not static.**
- **Numbers change with cellular development or differentiation**
- **Hormones regulate their own (homospecific) or other (heterospecific) receptors.**
  - **Prolactin (PRL) upregulates prolactin receptors (PRLR).**
  - **Chronic exposure of lymphocytes to insulin decreases binding due to down-regulation of insulin receptor which decreases the biological effect of insulin**
  - **Long-term exposure to progesterone down-regulates progesterone receptors (PGR) which then allows up-regulation of estrogen receptors (ER) in the uterus.**
  - **Estrogen, on the other hand, up-regulates expression of several receptors such as PGR, ESR1, and Oxytocin Receptor (OXTR)**

# Spare Receptors

- **Most maximal biological responses are achieved when only a small percentage of receptors is occupied, perhaps 10%.**

- **Remaining receptors:  spare or excess receptors**

- **The spare receptors may increase the sensitivity of target cells to activation by low levels of hormone**
  - **Maximal stimulation of steroidogenesis by Leydig cells occurs when only 1% of LHCGR receptors are occupied.**

# Principles of Receptor Binding

- **4) Saturable**
  - **Usually one specific binding site per molecule**
  - **Should be a finite number of receptors**

- **5) Reversible**
  - **Hormone binding must be reversible.**
  - **The "on/off" rate is dependent on binding affinity**


- **6) Must be related to biological effect**
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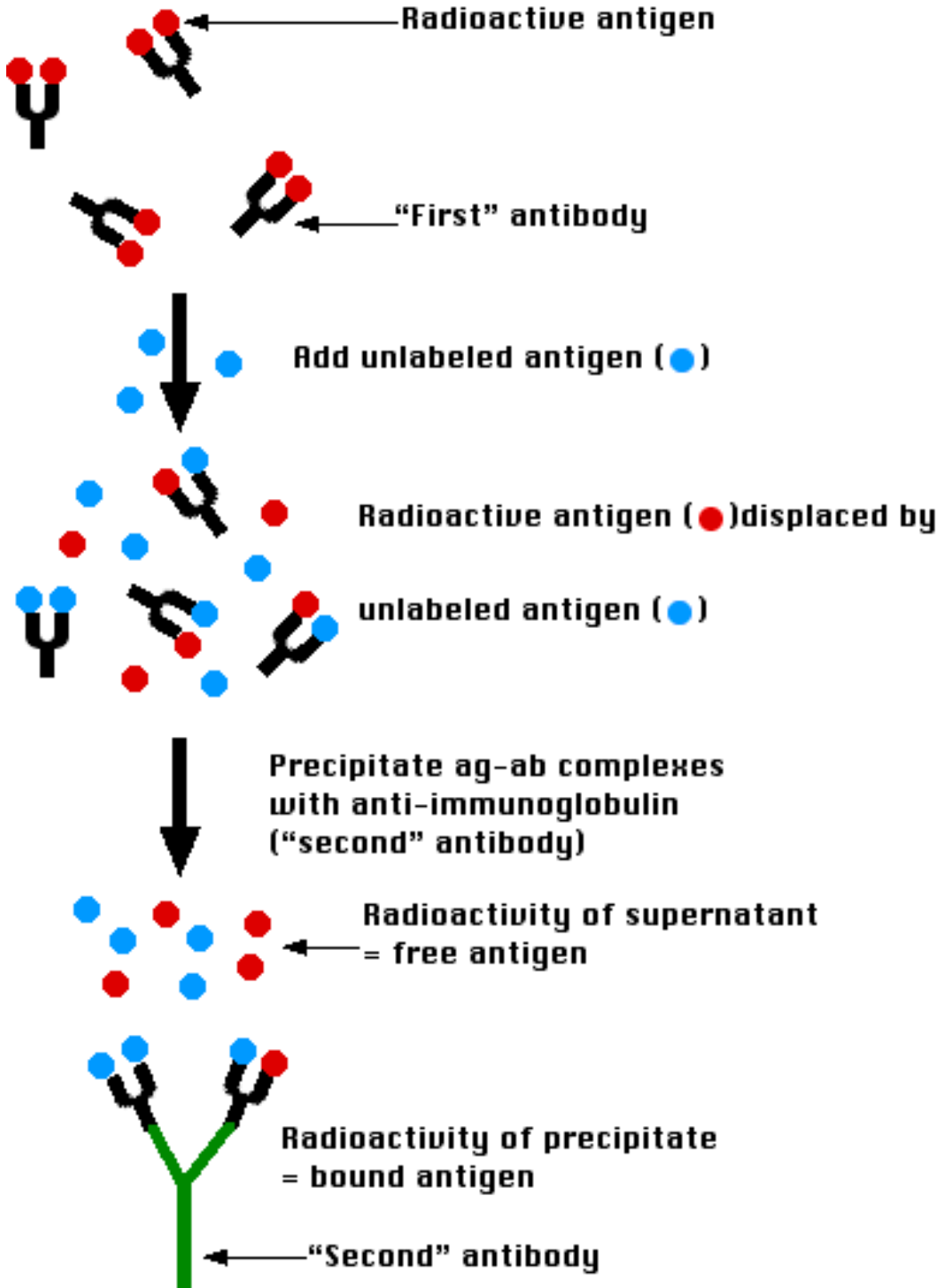
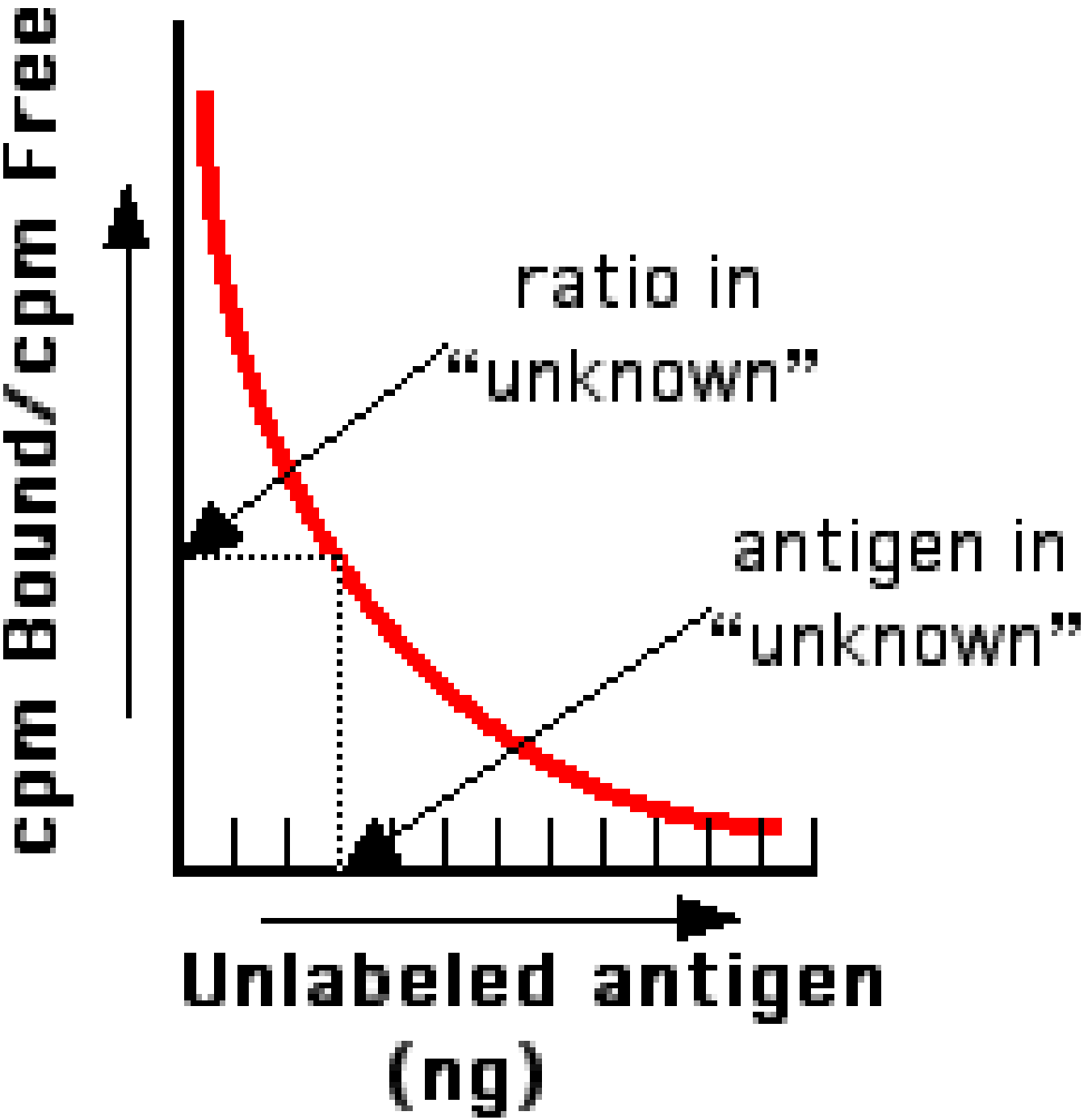
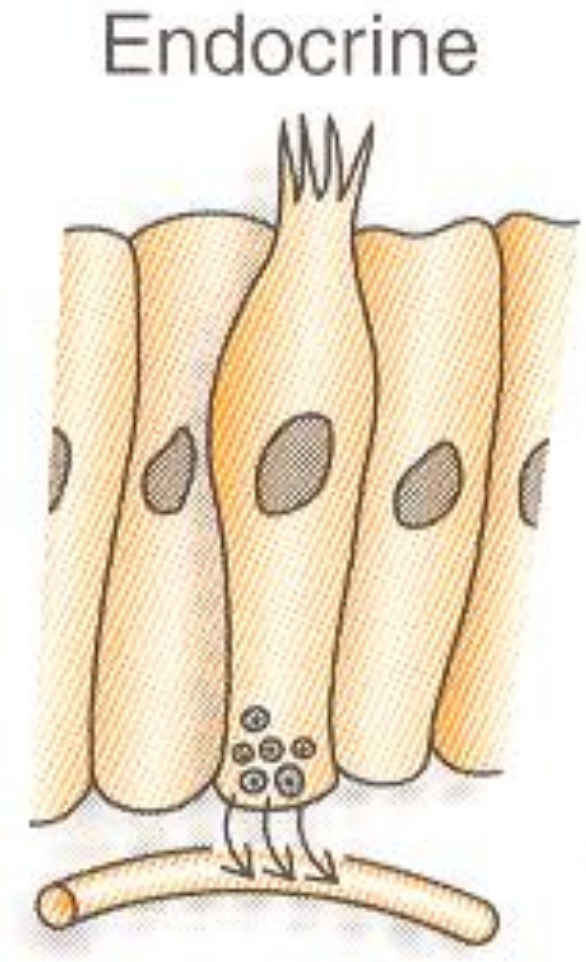

Radioactive antigen
"First" antibody
Add unlabeled antigen ( )
Radioactive antigen ( )displaced by
unlabeled antigen ( )
Precipitate ag-ab complexes
with anti-immunoglobulin
("second" antibody)
Radioactivity of supernatant
= free antigen
Radioactivity of precipitate
= bound antigen
"Second" antibody


cpm Bound/cpm Free
ratio in
"unknown"
antigen in
"unknown"
Unlabeled antigen
(ng)

# Modes of hormone action

- **<u>Endocrine: hormone is bloodborne</u>**

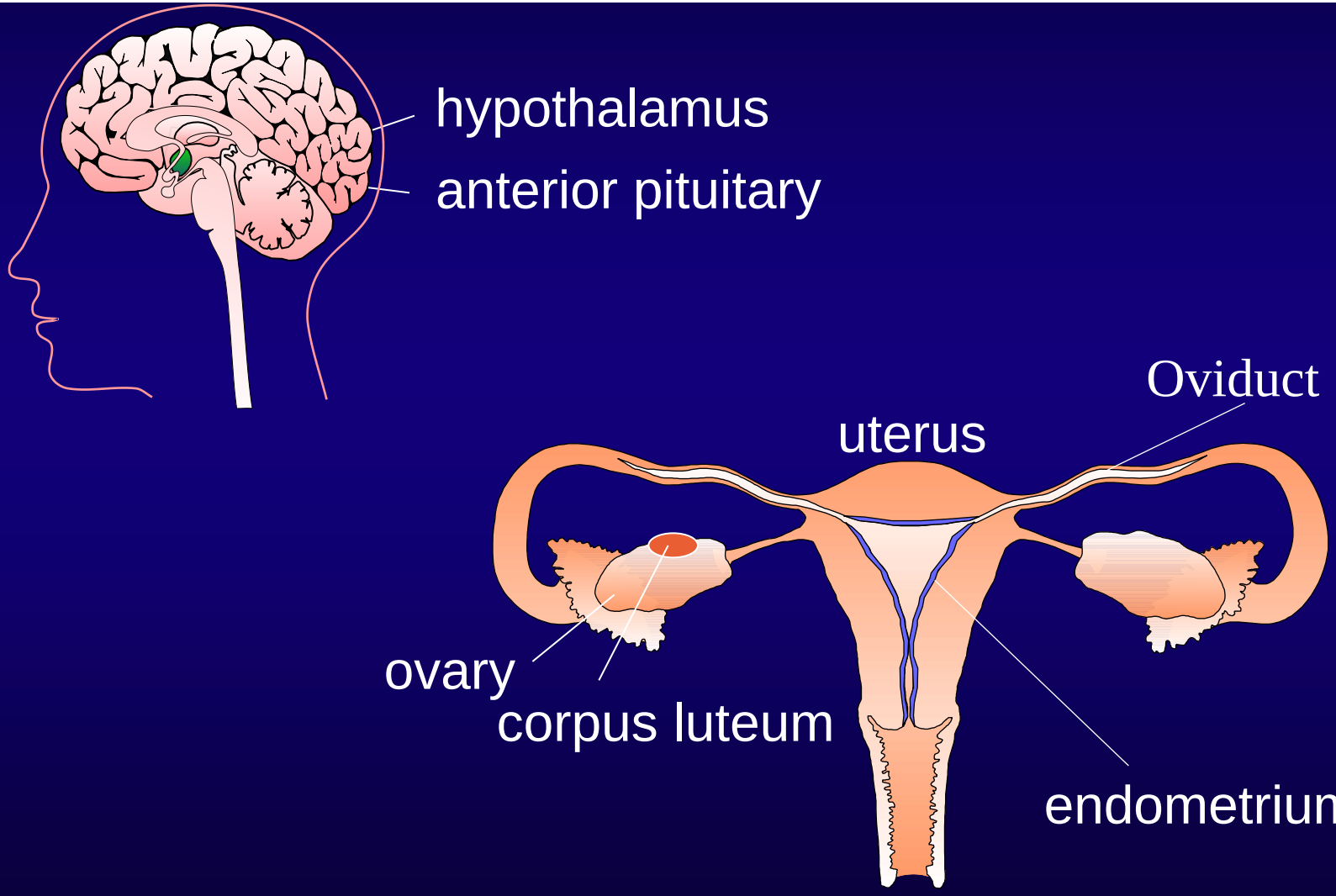



Hadley and Levine, 2007

# Transport and Metabolism of Hormones

- Circulate freely (water soluble)
  - Amines, peptides, proteins
- Bound to a carrier protein
  - Steroids and thyroid hormones
  - IGFs
- Albumin non-selectively transports low MW hormones
- Globulins are specific transport proteins that have high affinity, saturable binding sites for hormones
  - TBG (thyroid hormone-binding globulin)
  - TeBG (testosterone-binding globulin)
  - CBG (cortisol-binding globulin)
- Binding proteins and globulins affect half-life and clearance rate
- Liver and kidneys perform the bulk of hormone clearance via hydrolysis, oxidation, hydroxylation, methylation, decarboxylation, sulfation and glucoronidation.
  - Less than 1% of any hormone is excreted intact in urine or feces

# Organs involved in female reproductive cycle

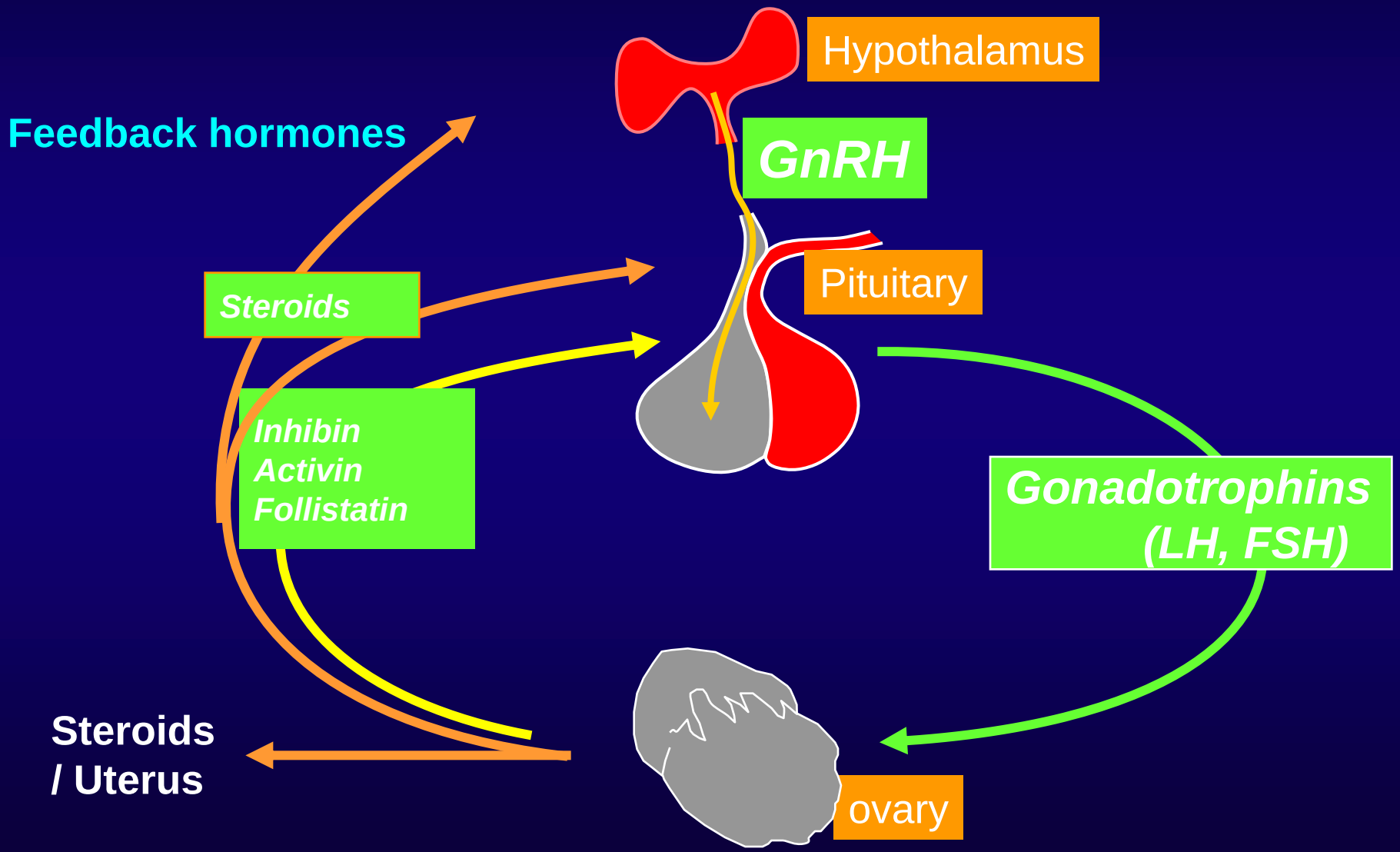


# Major hormones regulating the female reproductive cycle

| Hormone | Site of production |
| --- | --- |
| gonadotropin-releasing hormone - GnRH | hypothalamus |
| luteinizing hormone - LH | anterior pituitary (gonadotroph) |
| follicle stimulating hormone - FSH | anterior pituitary (gonadotroph) |
| estradiol 17β (E2) | ovarian follicle |
| Progesterone (P4) | corpus luteum |

After ovulation, cells of dominant follicle give rise to the CL

# The female hypothalamo-pituitary ovarian axis



# Estrous cycles consist of two major phases

## Follicular phase

Ovarian FOLLICLES - dominant structures in the ovary

ESTROGEN is the dominant hormone

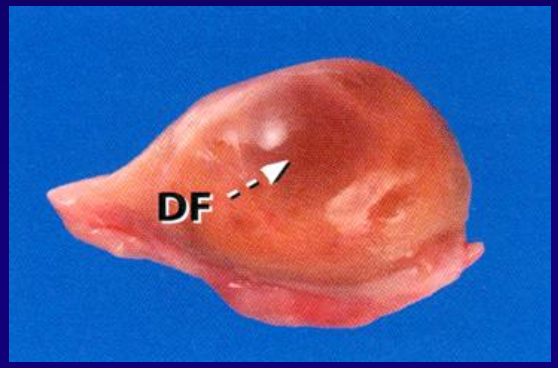
## Luteal phase

CORPORA LUTEA – dominant ovarian structures

PROGESTERONE is the dominant hormone

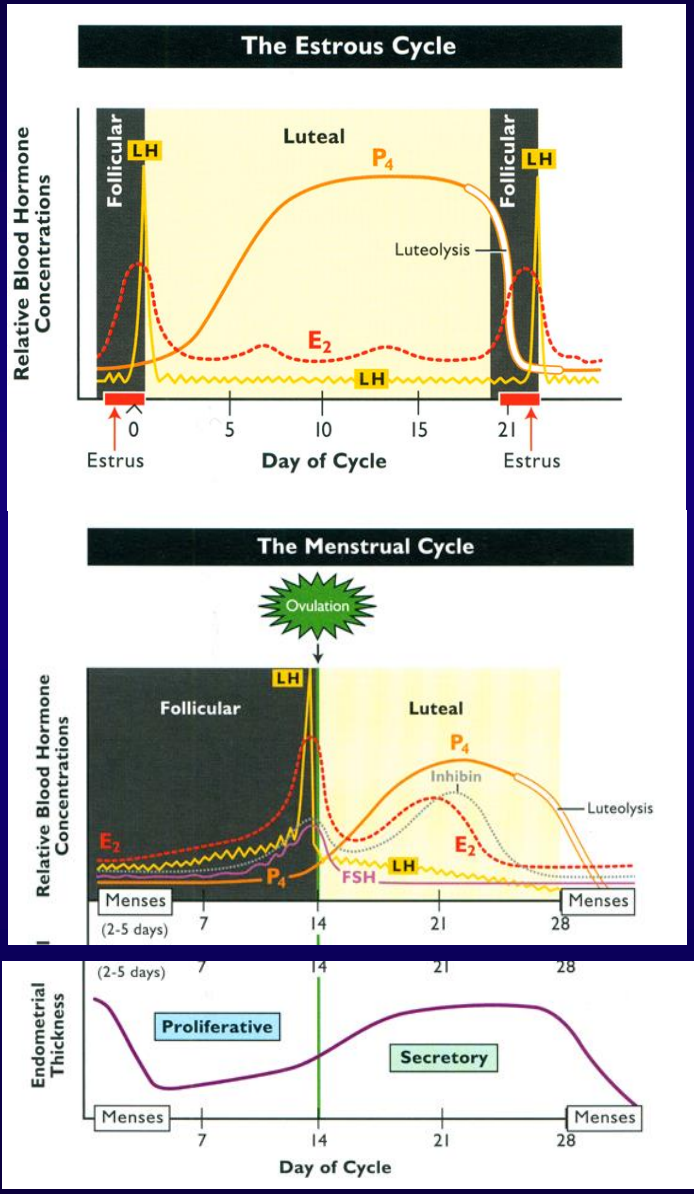
# The estrous cycle has 4 stages

- Pro-estrus – formation of ovulatory follicles + E2 secretion

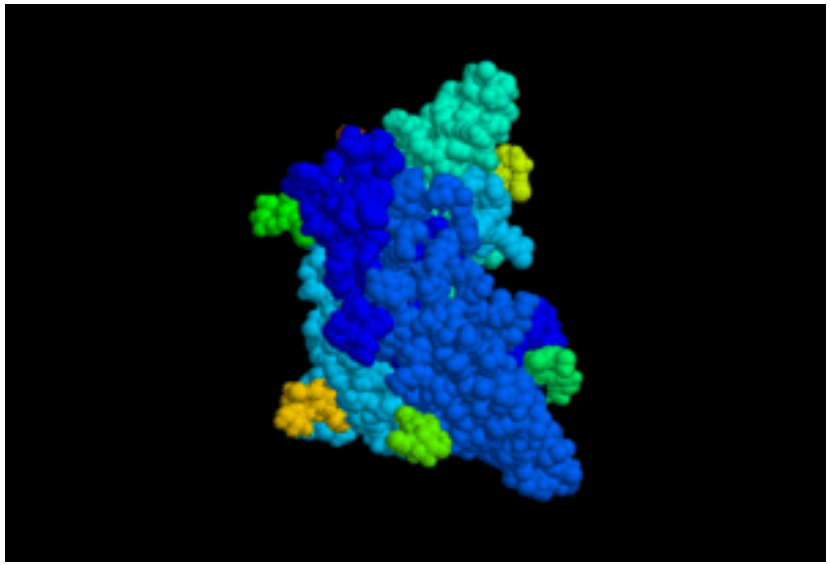



- Estrus – sexual receptivity + peak E2 secretion + ovulation
- Metestrus – CL formation + early P4 secretion
- Diestrus – substantial secretion of P4

- In women, the two phases are of equal length
- The phases are named for the changes that occur in the endometrium
- Follicular phase = proliferative phase
- Luteal phase = secretory phase
- Subprimates: Protestrus, Estrus, Metestrus and Diestrus



# FSH

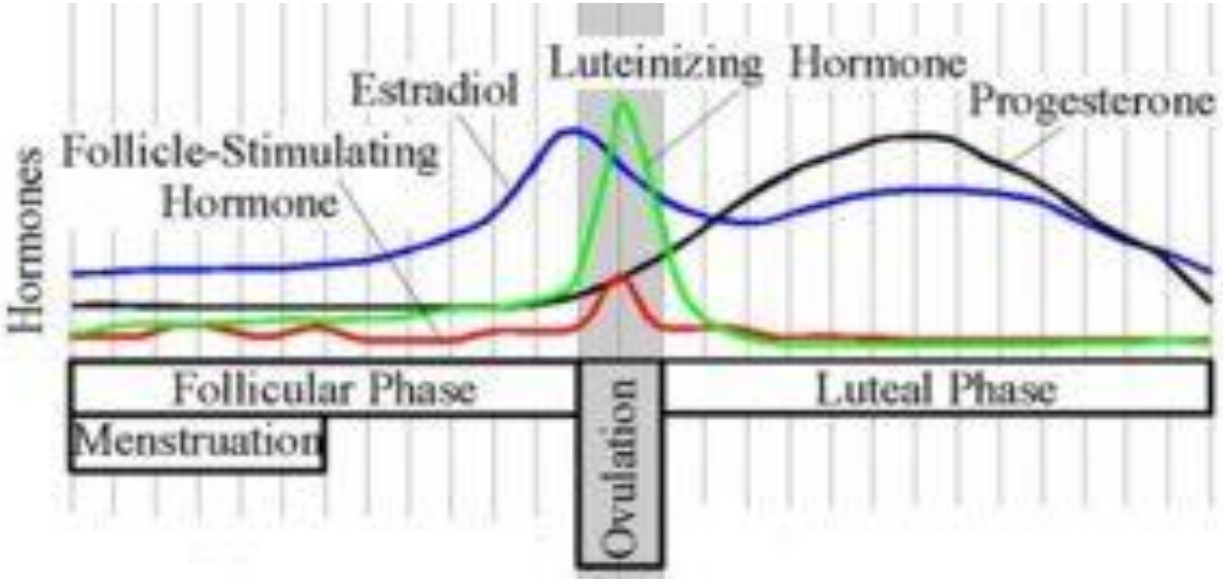


FSH is a glycoprotein. Each monomeric unit is a protein molecule with a sugar attached to it; two of these make the full, functional protein. Its structure is similar to LH, TSH, and hCG. The protein dimer contains 2 polypeptide units, labelled alpha and beta subunits. The alpha subunits of LH, FSH, TSH, and hCG are identical, and contain 92 amino acids. The beta subunits vary. FSH has a beta subunit of 118 amino acids (FSHB) that confers its specific biologic action and is responsible for interaction with the FSH-receptor.The sugar part of the hormone is composed of fucose, galactose, mannose, galactosamine, glucosamine, and sialic acid, the latter being critical for its biologic half-life. The half-life of FSH is 3-4 hours.

# Functions of FSH in Female and Male

- In both *males* and *females*, FSH stimulates the maturation of germ cells
    - FSH -/- mice have no phenotype
- In *males*, FSH induces Cells of Leydig to Secrete Inhibin
- Inhibin stimulates the formation of sertoli-sertoli tight junctions (zonula occludens) to form blood-testis barrier
- In *females*, FSH initiates follicular growth and maturation and stimulates granulosa cells to secrete inhibin and follistatin

# LH




LH is a dimeric glycoprotein with 2 polypeptide units, alpha and beta, connected by two disulfide bridges

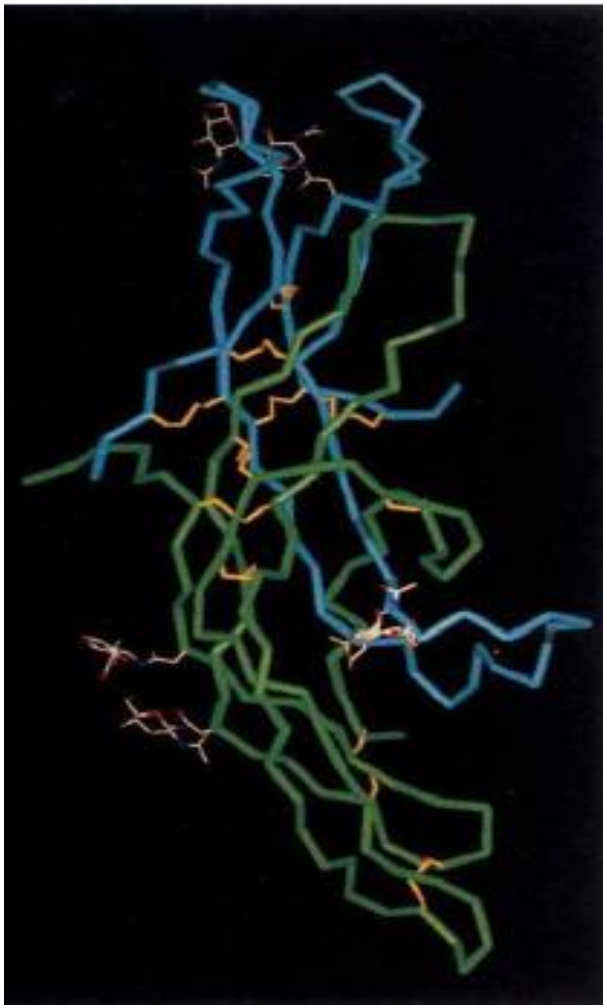
*alpha subunits* of LH, FSH, TSH, and hCG are identical, and contain 92 amino acids.

*beta subunits*: LH beta subunit of 121 amino acids confers specific biologic action and binding to LH receptor. This beta subunit identical to beta sub unit of hCG and both bind LH receptor, but hCG beta subunit contains an additional 24 amino acids

half-life of LH is 20 minutes, shorter than that of FSH (3-4 hours) or hCG (24 hours).

# Classes and chemical structures of hormones

- **Proteins – dimeric structure**
  - Glycoprotein hormones (LH, FSH, hCG, TSH)



- Common alpha, but specific beta chain
- Product of distinct genes

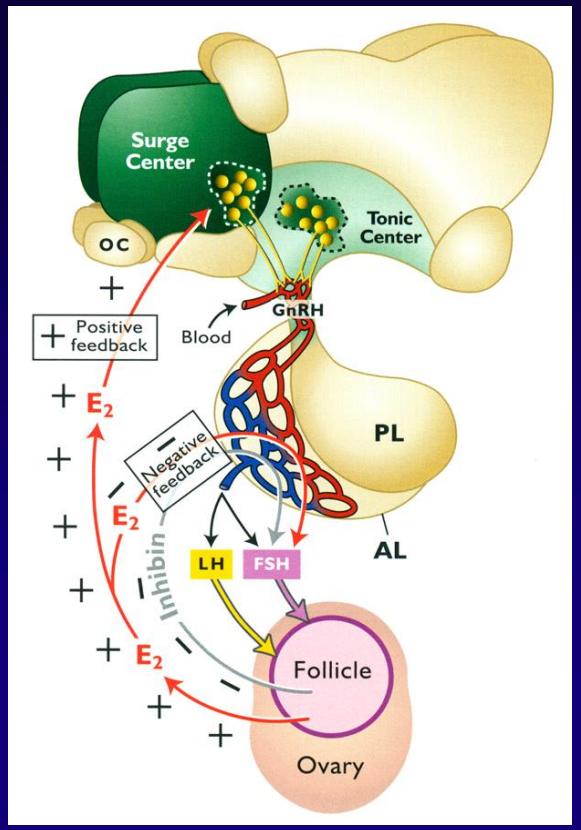
**Crystal structure of human chorionic gonadotropin (hCG).** The alpha subunit is shown in blue and the beta subunit in green. (hydrogens bonds - red dotted lines, and disulfide atoms and bonds – yellow are shown). Lapthorn et al. (1994) Nature 369: 338.

# Roles of LH

- LH induces ovulation of mature Graffian folliles on the ovaries of females to:
  - Release oocyte into oviduct for fertilization
  - Induce luteinization of granulosa and theca cells of the ovarian follicle which form a corpus luteum and produce progesterone
  - Stimulate production of progesterone by corpus luteum
  - Progesterone essential for endometrium to support implantation of blastocyst and maintain pregnancy
  - Chorionic gonadotrophin produced by trophectoderm of primate conceptuses has LH activity and is the pregnancy recognition signal in primates

# Primary steps required for the pre-ovulatory LH surge

- In follicular phase GnRH pulse frequency increases to increase secretion of FSH and LH
- Increase in Estrogen (E2) production
- E2 stimulates:
  - increases in GnRH Receptors on Gonadotrophs;
  - increase in GnRH pulse frequency
  - surge in GnRH responsible for the ovulatory surge of LH
- Granulosa cells of follicles secrete inhibin to suppress secretion of FSH



# HORMONES FROM GRANULOSA CELLS OF FOLLICLE AND SERTOLI CELLS OF TESTES THAT REGULATE FSH SECRETION

**Inhibin: a peptide inhibitor of FSH synthesis and secretion participates in regulation of estrous and menstrual cycles.**

**Structure**: contains an alpha and beta subunit linked by disulfide bonds. Two forms of inhibin differ in their beta subunits (A or B), while alpha subunits are identical. Inhibin belongs to the transforming growth factor-β (TGF-β) superfamily.

**********

**Activin: a peptide stimulator of FSH synthesis and secretion participates in regulation of estrous and menstrual** cycles

**Structure**: two beta subunits identical to the two beta subunits (A or B) of inhibin, allowing for the formation of three forms of activin: A, AB, and B; linked by a single covalent disulfide bond.

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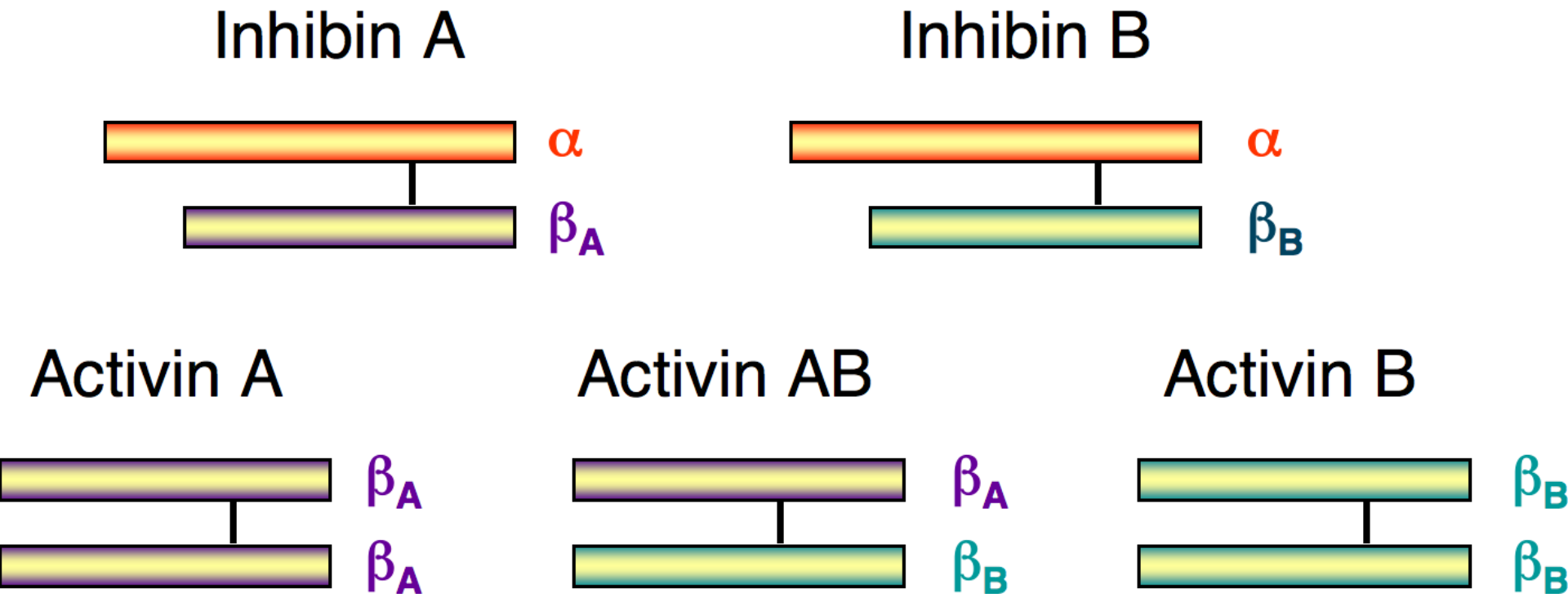
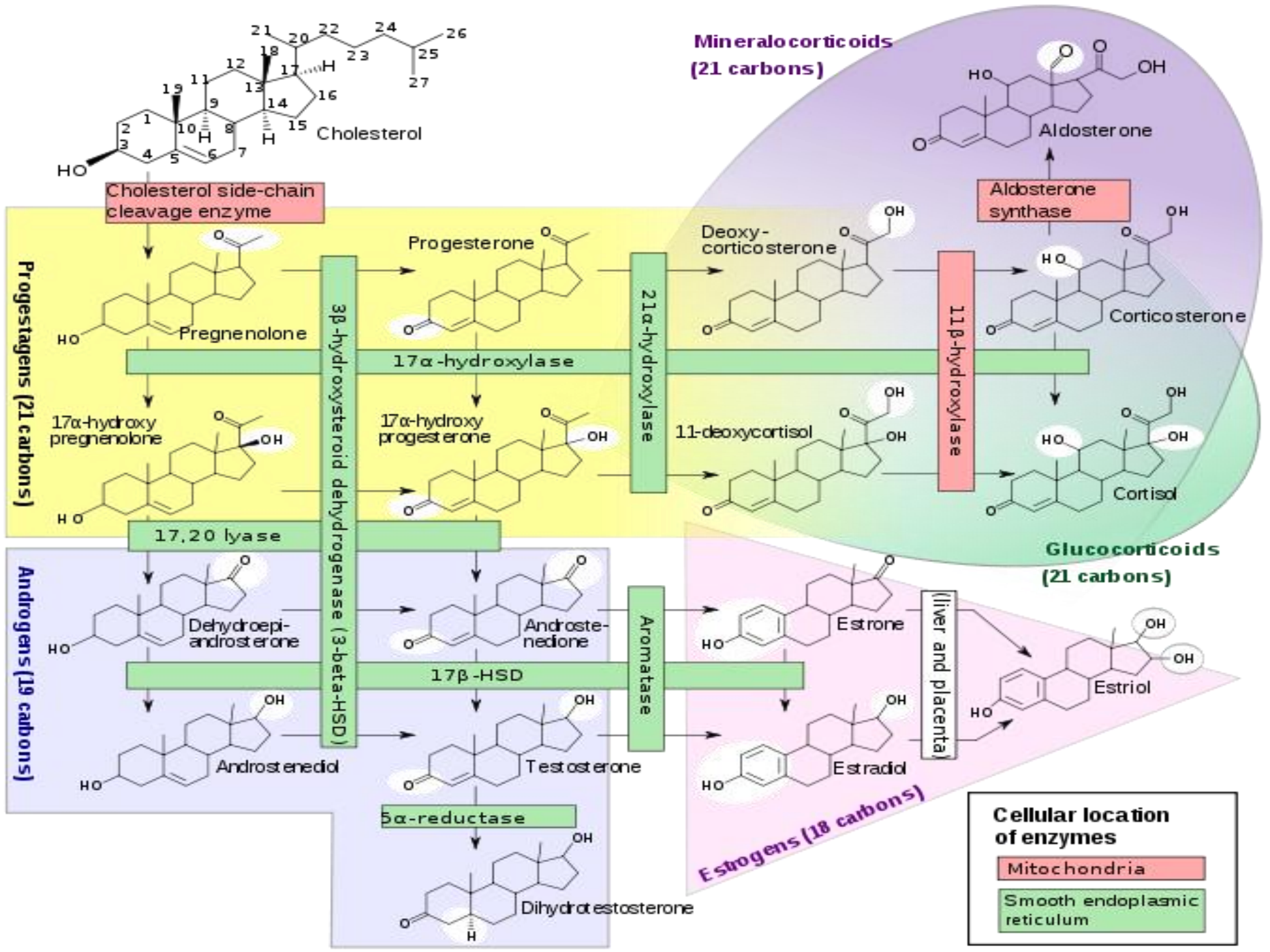
**Follistatin: a single chain gonadal protein that inhibits FSH synthesis and release by binding and antagonizing Activin**.


Inhibin A
α
βA
Inhibin B
α
βB
Activin A
βA
βA
Activin AB
βA
βB
Activin B
βB
βB


Cholesterol
Cholesterol side-chain cleavage enzyme
Progestagens (21 carbons)
Pregnenolone
Progesterone
17α-hydroxy pregnenolone
17α-hydroxy progesterone
3β-hydroxysteroid dehydrogenase (3-beta-HSD)
17α-hydroxylase
17,20 lyase
Androgens (19 carbons)
Dehydroepi-androsterone
Androste-nedione
Androstenediol
Testosterone
17β-HSD
5α-reductase
Dihydrotestosterone
21α-hydroxylase
Aromatase
Mineralocorticoids (21 carbons)
Deoxy-corticosterone
11-deoxycortisol
11β-hydroxylase
Aldosterone synthase
Aldosterone
Corticosterone
Cortisol
Glucocorticoids (21 carbons)
Estrone
Estradiol
(liver and placental)
Estriol
Estrogens (18 carbons)
Cellular location of enzymes
Mitochondria
Smooth endoplasmic reticulum

# Steroid Hormone Action: Classical

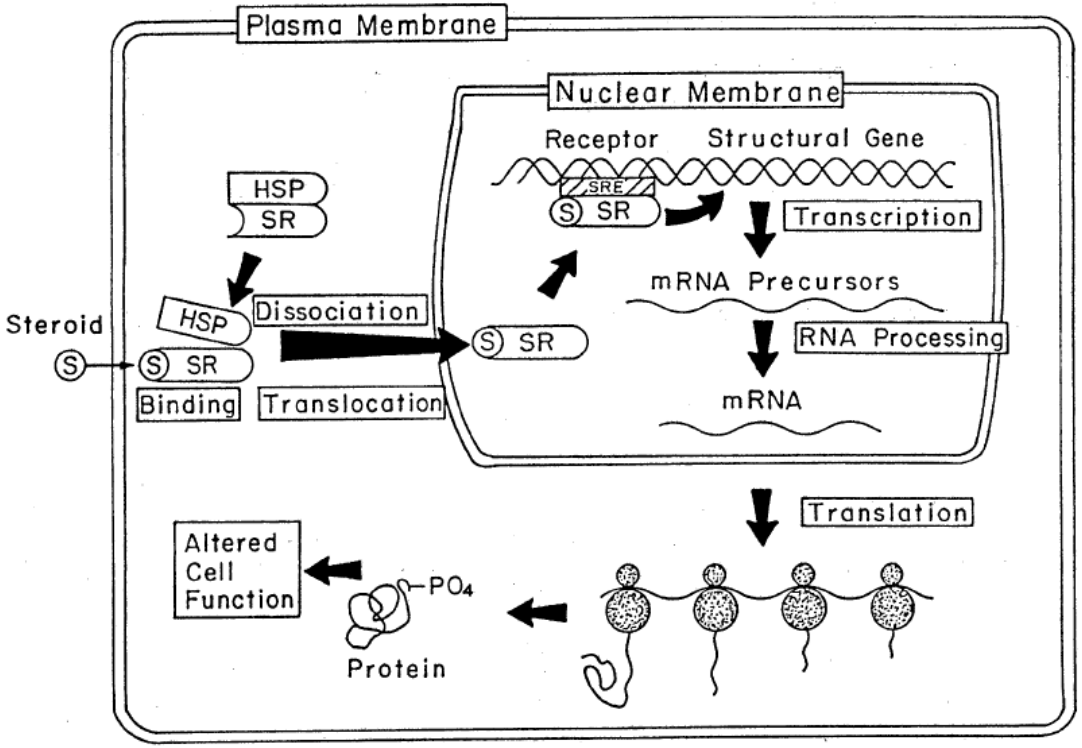



**Figure 2–19.** Proposed mechanism of action of steroids (glucocorticoids, estrogens, and progesterone) in activation of specific gene transcription. In this model the steroid (S) readily diffuses across the plasma membrane and binds to a cytosolic receptor (SR). In the absence of steroid, the receptor resides in the cytoplasm as an inactive complex with heat shock protein (hsp). When the steroid binds to the receptor, the hsp dissociates from it. The steroid-receptor complex is translocated to the nucleus, where it binds to a chromatin receptor consisting of the steroid receptor response DNA element (SRE), thereby activating the transcription of specific genes involved in steroid hormone action. RNA transcripts are translated into proteins that mediate changes in cell function. Some evidence suggests an alternative model in which steroid receptor resides in the nucleus and not in the cytoplasm. In this model, presumably, steroid diffuses through the cytoplasm into the nucleoplasm, where it binds to the receptor before gene activation occurs. (Adapted from Chan L, O'Malley BW. Mechanism of action of the sex steroid hormones. Reprinted by permission of The New England Journal of Medicine 1976; 294:1322–1328, 1372–1382, 1429–1437.)

# Steroid Hormone Action Overview

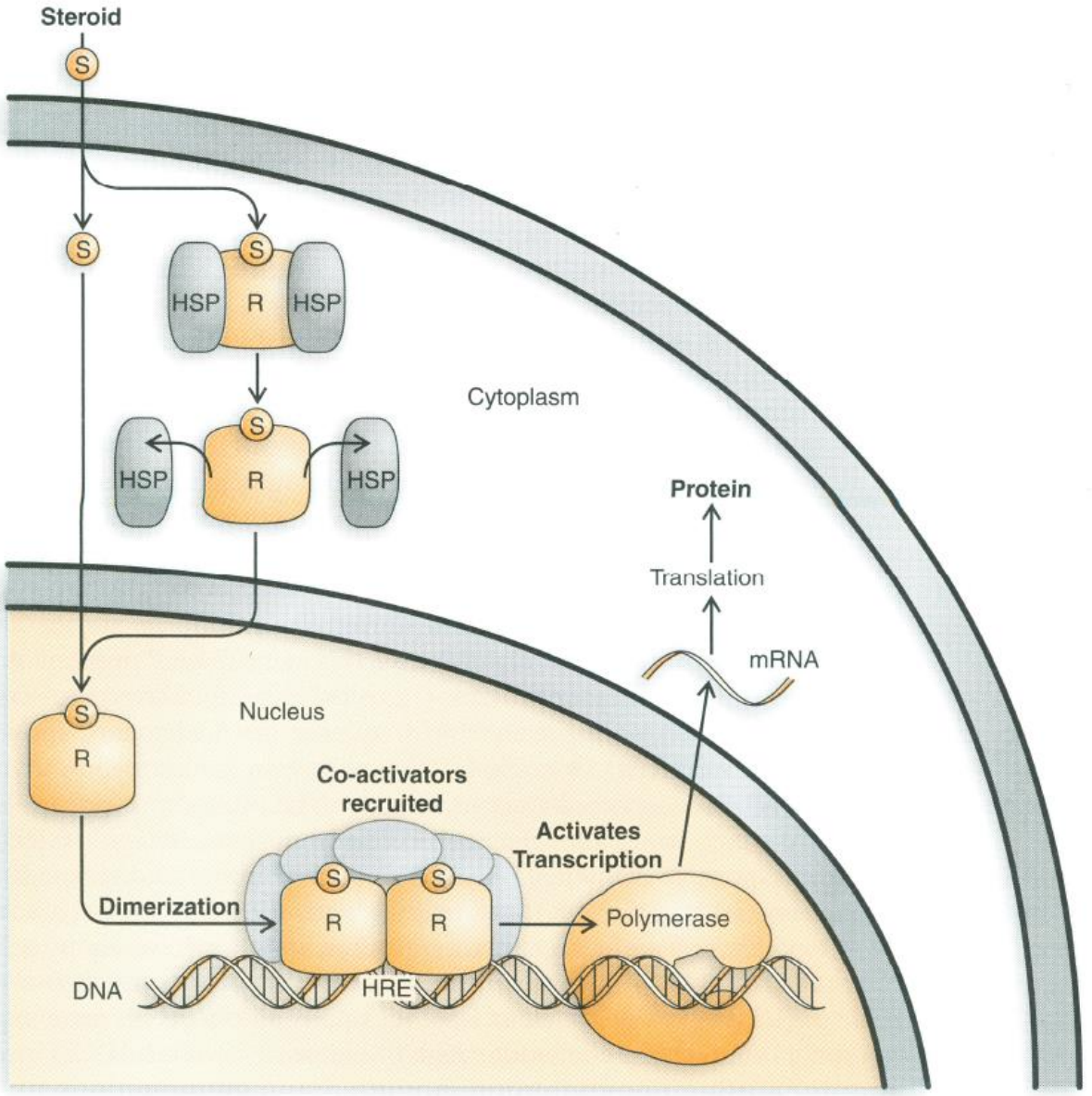
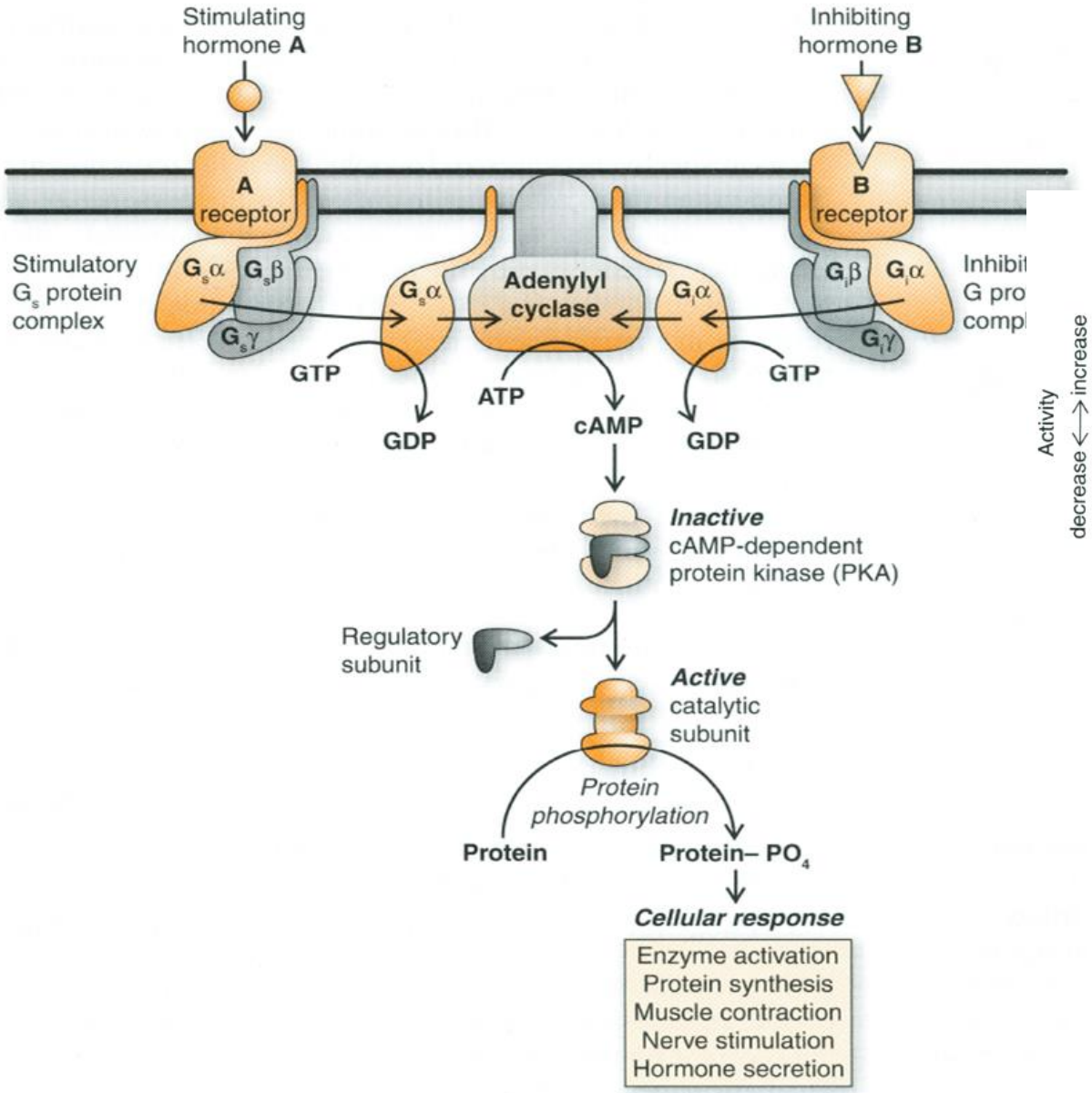



**Figure 3.15**
Mechanisms of steroid hormone action.

# Cyclic AMP Production and Action



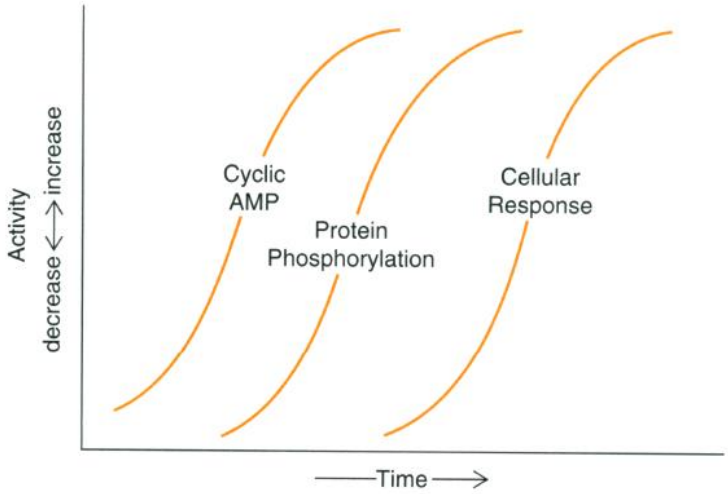
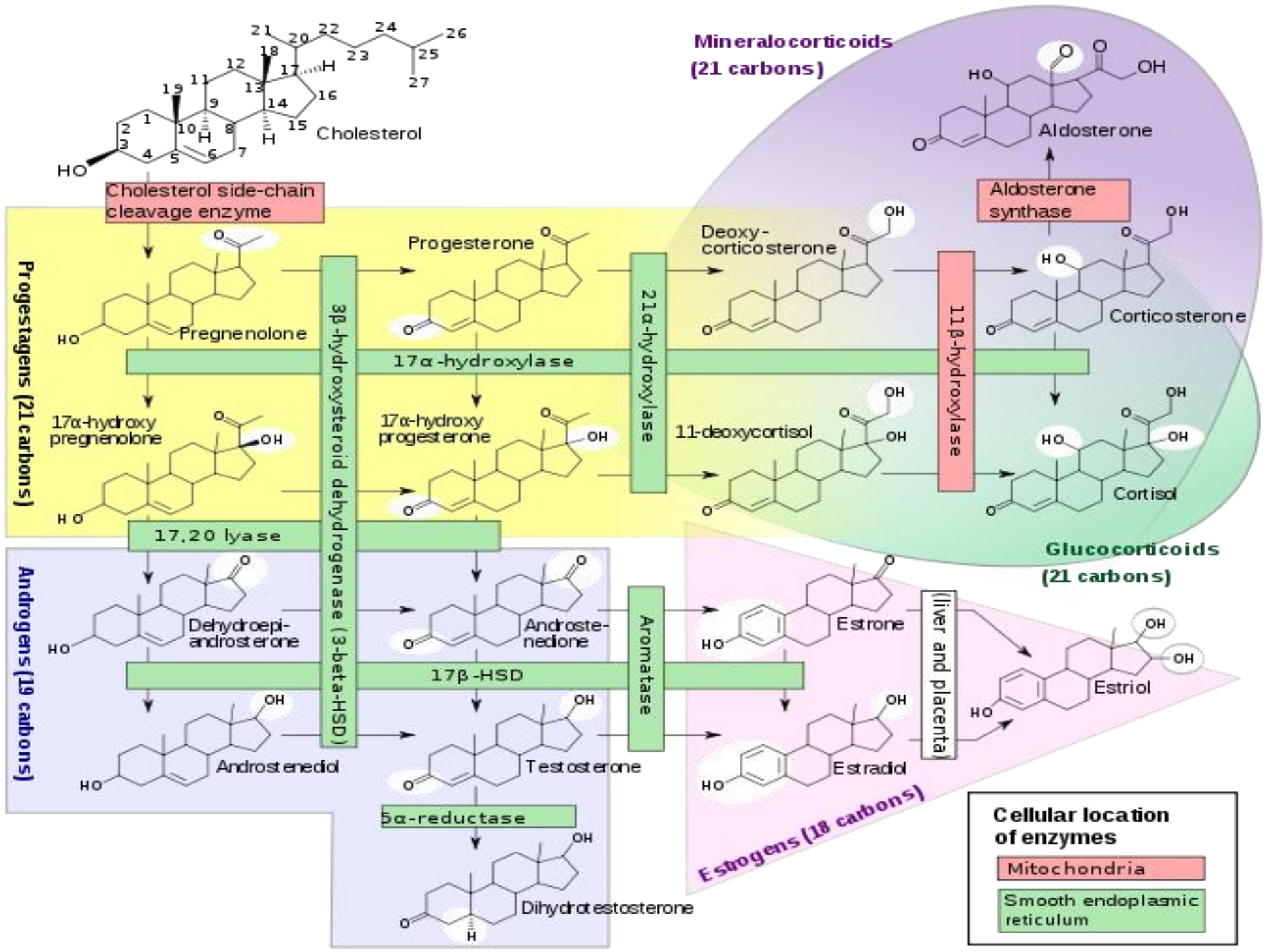
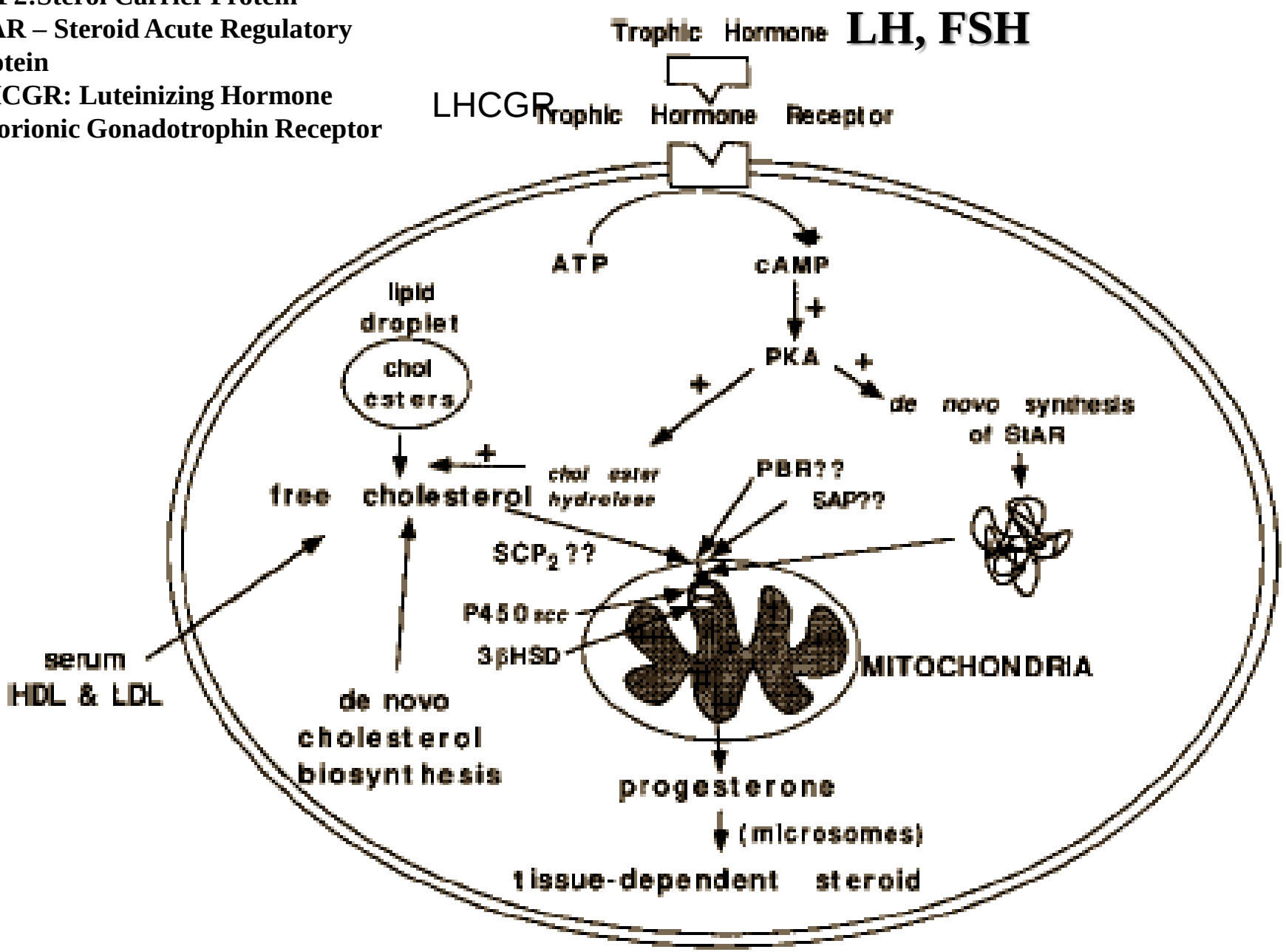



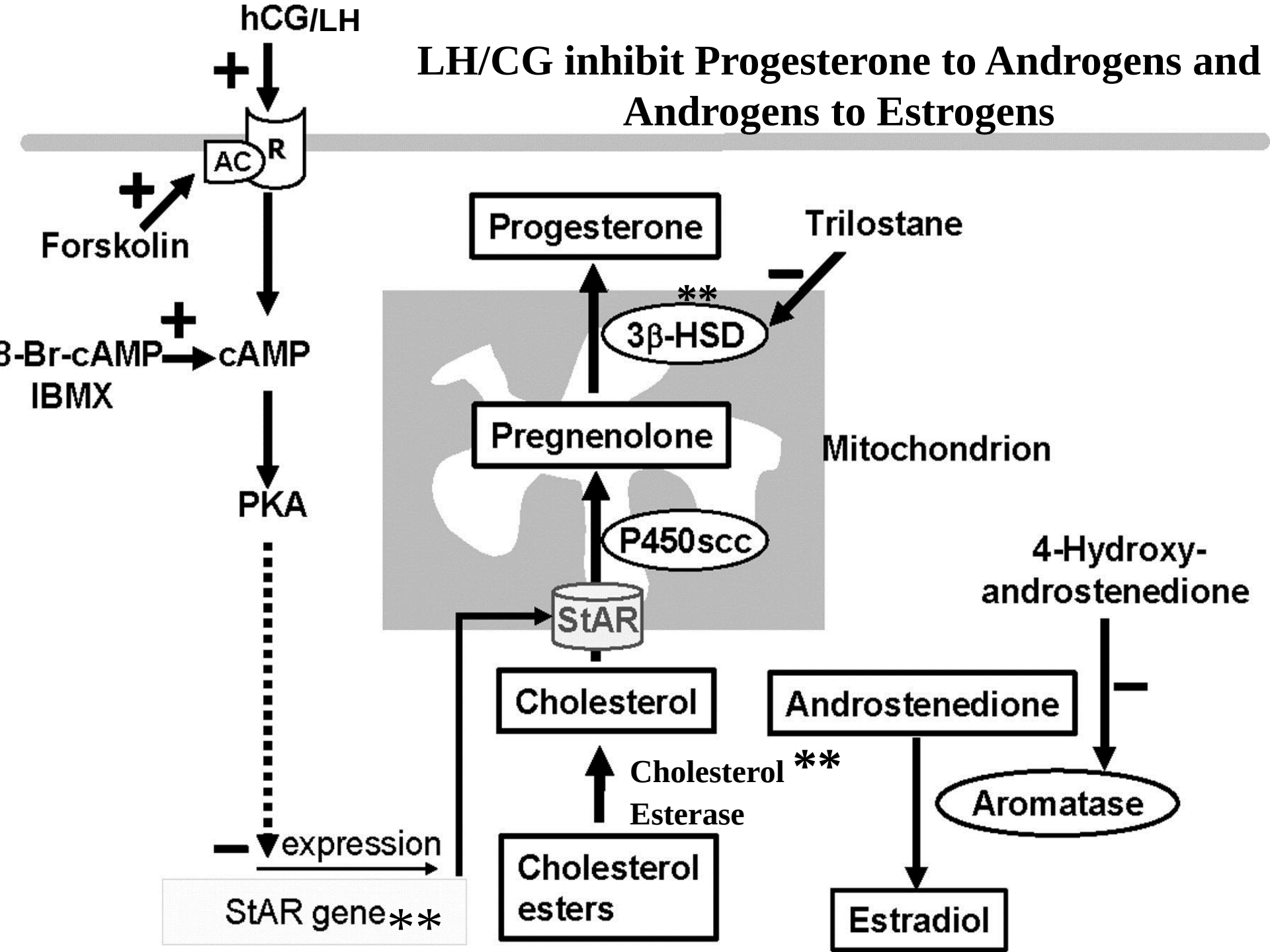

**Figure 3.8** Temporal cellular events in homone-mediated cyclic AMP production and action.


Cholesterol
Cholesterol side-chain cleavage enzyme
Progestagens (21 carbons)
Pregnenolone
Progesterone
17α-hydroxy pregnenolone
17α-hydroxy progesterone
3β-hydroxysteroid dehydrogenase (3-beta-HSD)
17α-hydroxylase
17,20 lyase
Androgens (19 carbons)
Dehydroepi-androsterone
Androste-nedione
Androstenediol
Testosterone
17β-HSD
5α-reductase
Dihydrotestosterone
21α-hydroxylase
Aromatase
Mineralocorticoids (21 carbons)
Deoxy-corticosterone
11-deoxycortisol
11β-hydroxylase
Aldosterone synthase
Aldosterone
Corticosterone
Cortisol
Glucocorticoids (21 carbons)
Estrone
Estradiol
(liver and placental)
Estriol
Estrogens (18 carbons)
Cellular location of enzymes
Mitochondria
Smooth endoplasmic reticulum

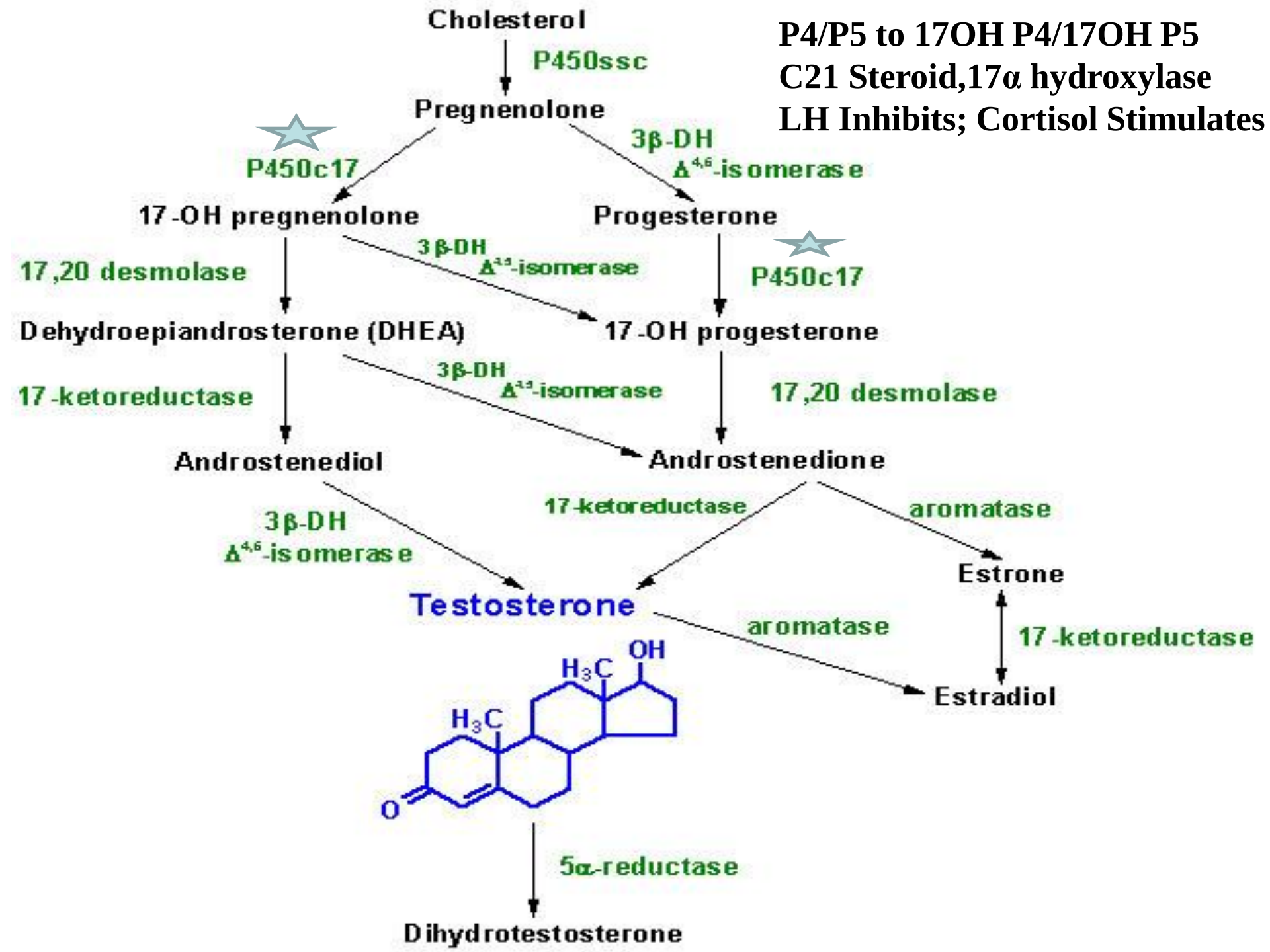
**SCP2:Sterol Carrier Protein**
**StAR – Steroid Acute Regulatory Protein**
**LHCGR: Luteinizing Hormone Chorionic Gonadotrophin Receptor**



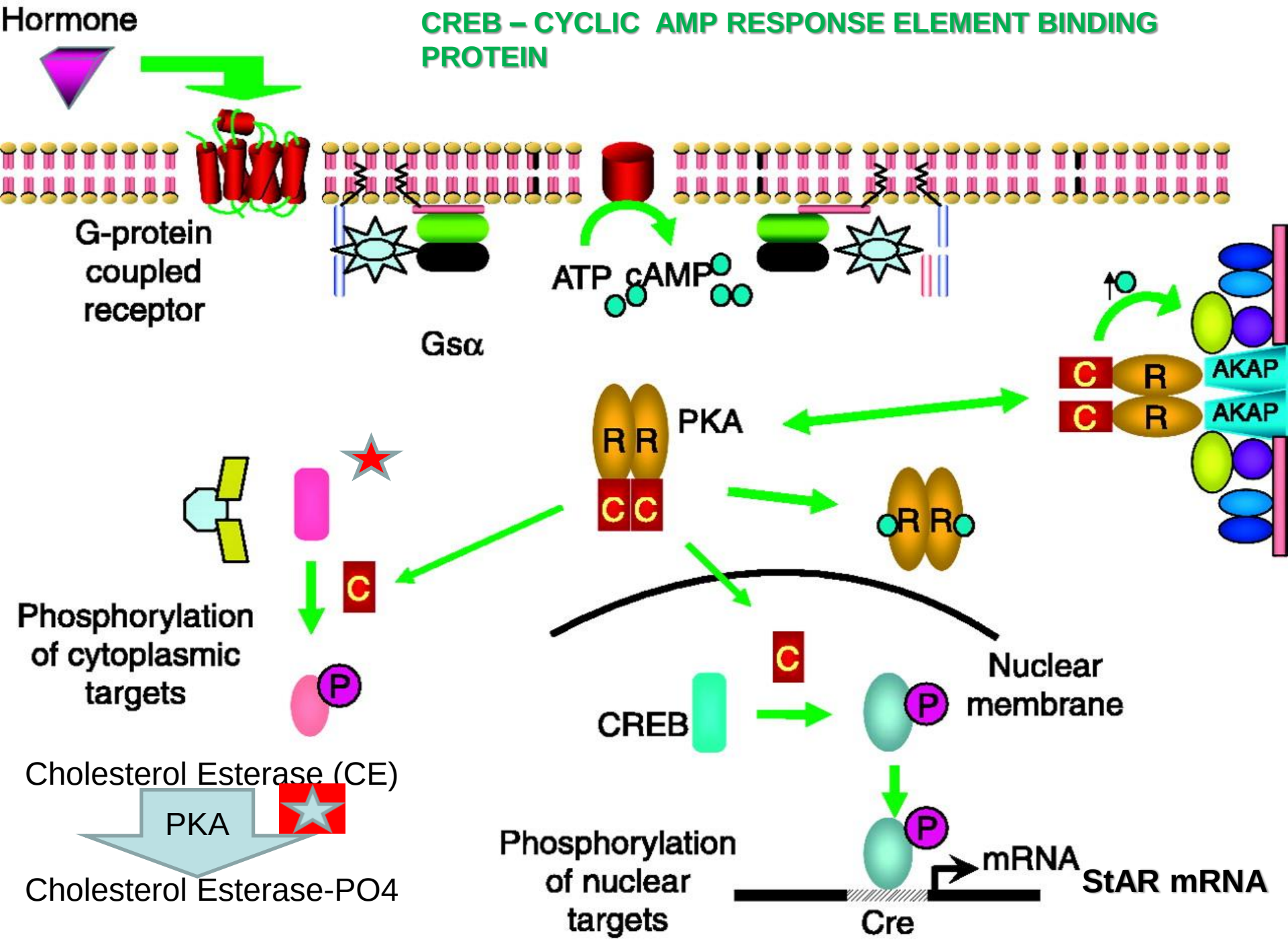
# LH/CG inhibit Progesterone to Androgens and Androgens to Estrogens



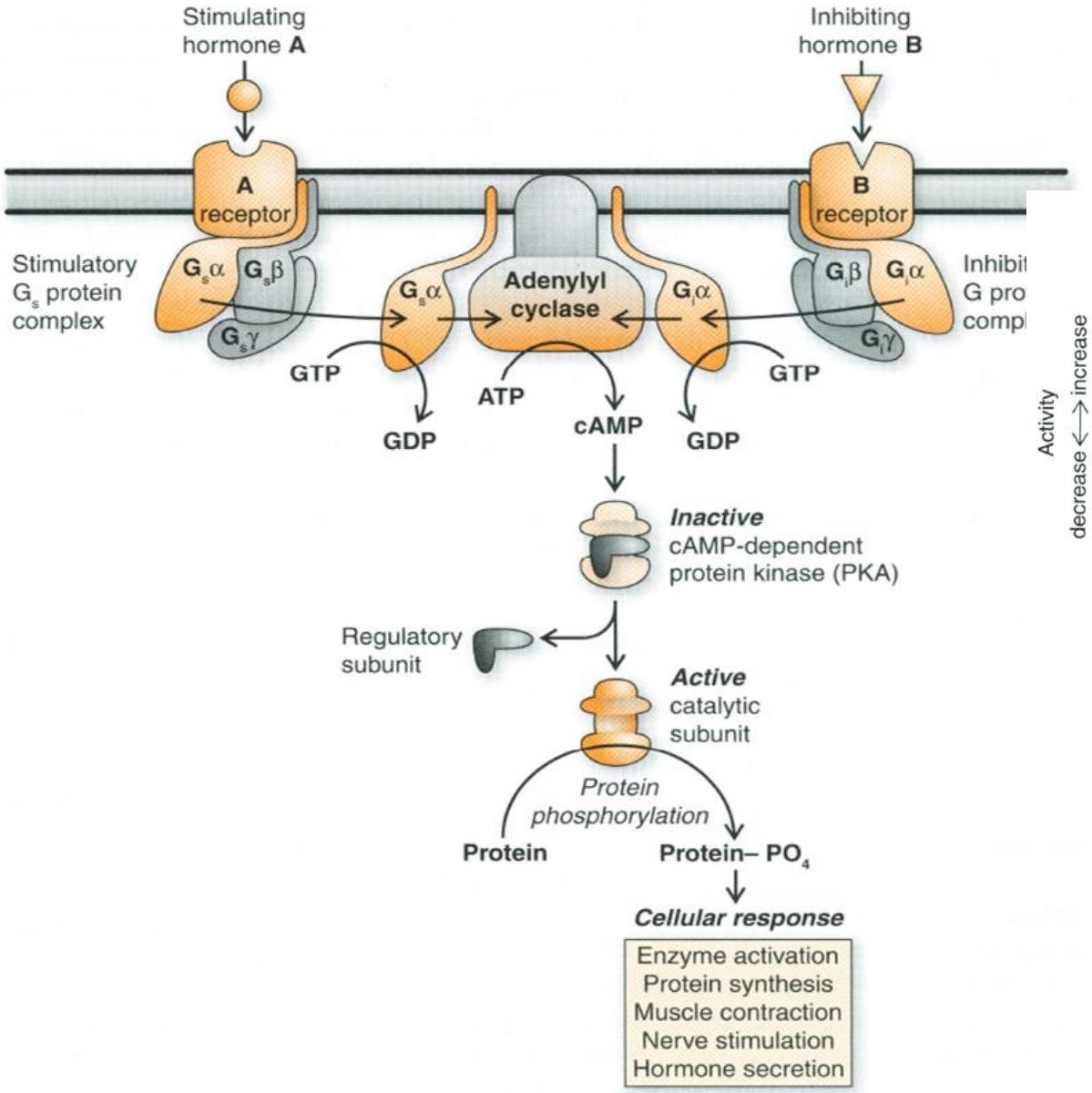
**P4/P5 to 17OH P4/17OH P5**
**C21 Steroid,17α hydroxylase**
**LH Inhibits; Cortisol Stimulates**



# CREB – CYCLIC AMP RESPONSE ELEMENT BINDING PROTEIN



# Cyclic AMP Production and Action



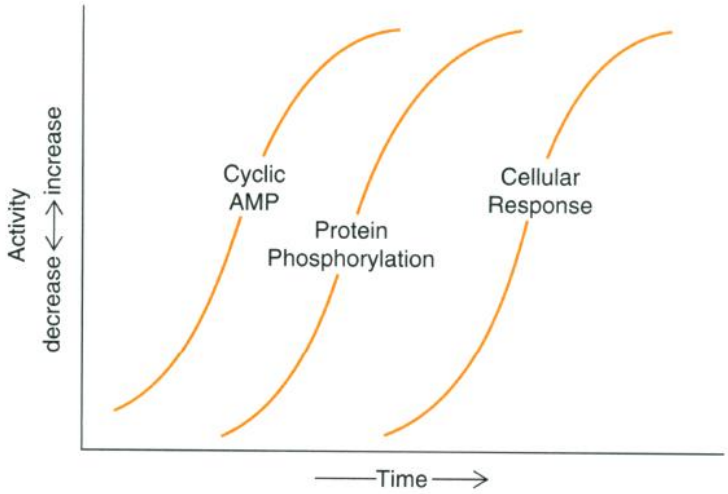
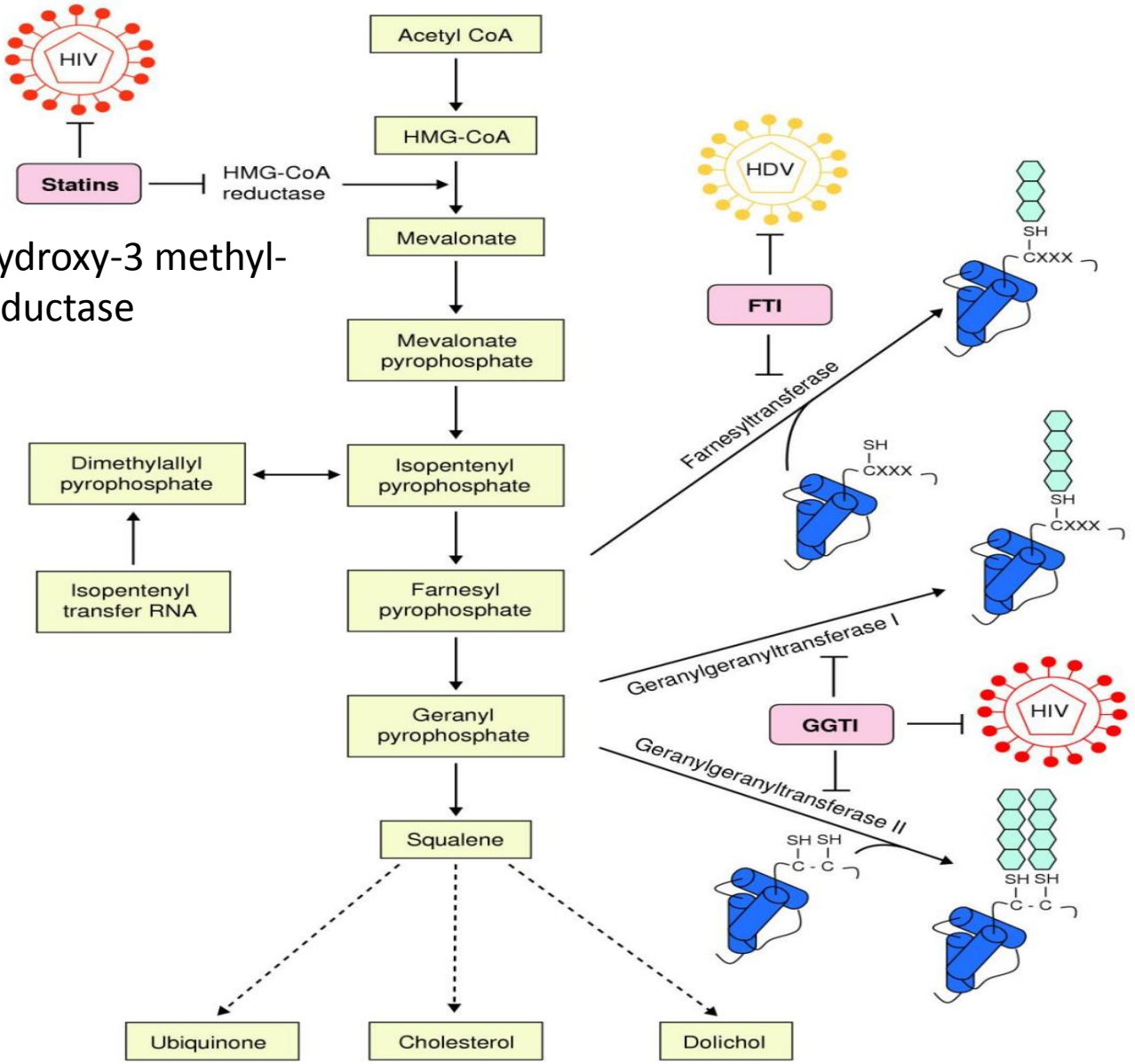



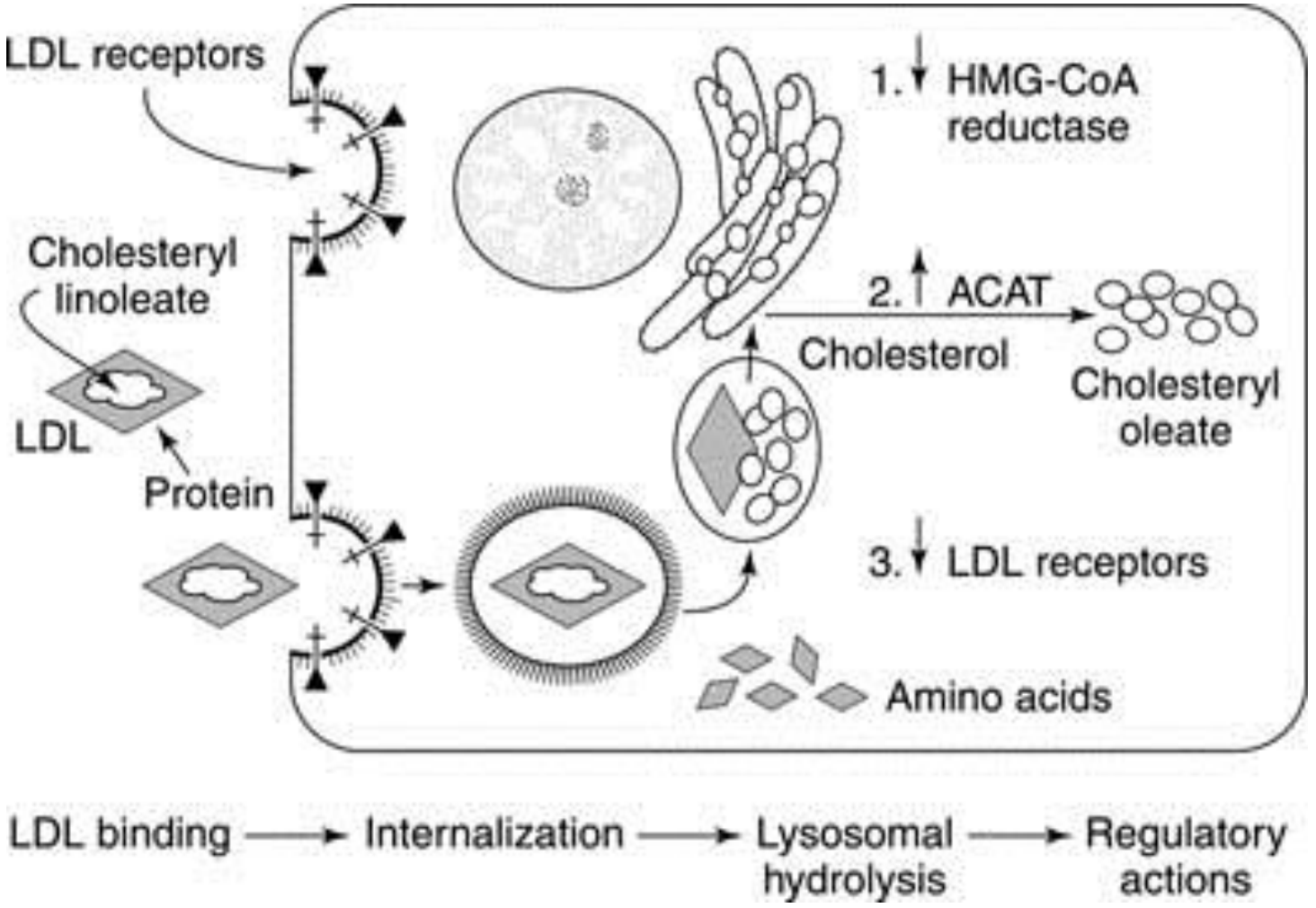

**Figure 3.8** Temporal cellular events in homone-mediated cyclic AMP production and action.

# STEROID BIOSYNTHESIS

HMG-CoA: 3-hydroxy-3 methyl-Glutaryl-CoA reductase

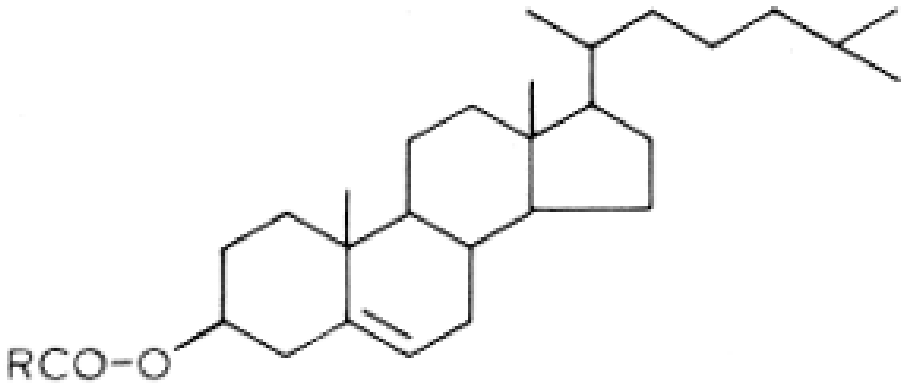


# INTERNALIZATION OF CHOLESTEROL INTO CELLS




ACAT: Acetyl-Coenzyme A acetyltransferase – cholesterol to cholesterol esters

*cholesteryl esters*

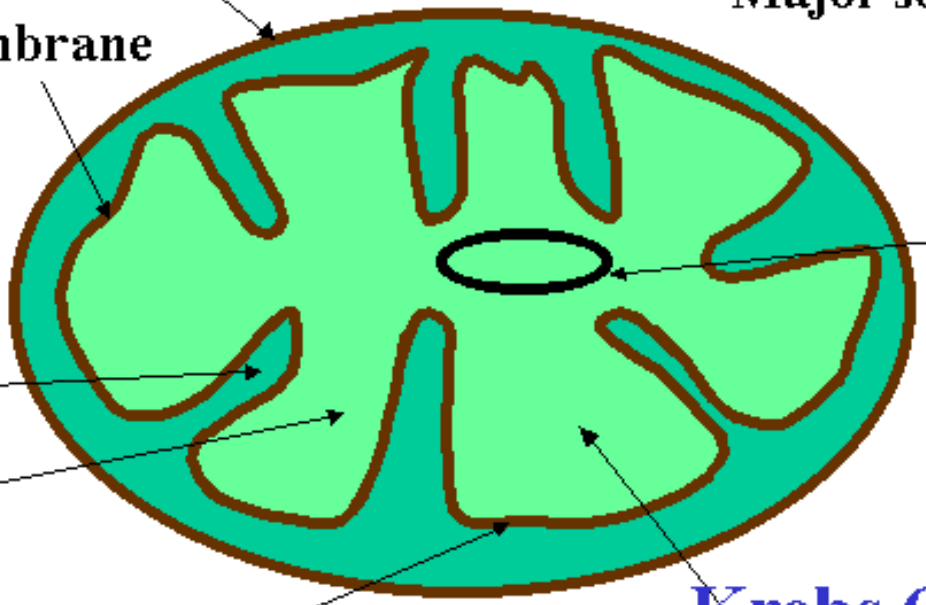



3-*O*-acyl-cholest-5-en-3$\beta$-ol

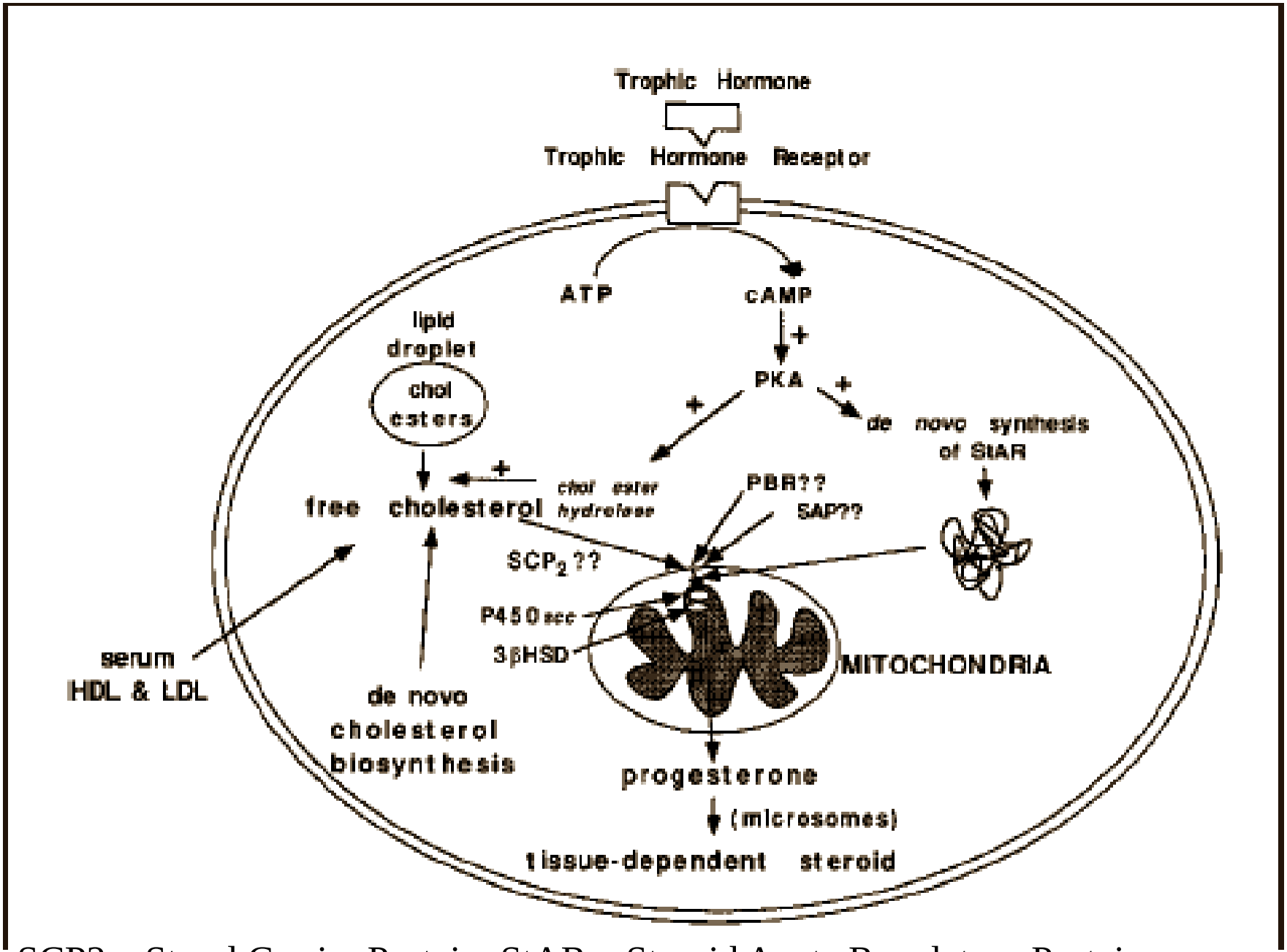
RCO = linoleic acid, palmitic acid, oleic acid etc

# Mitochondria

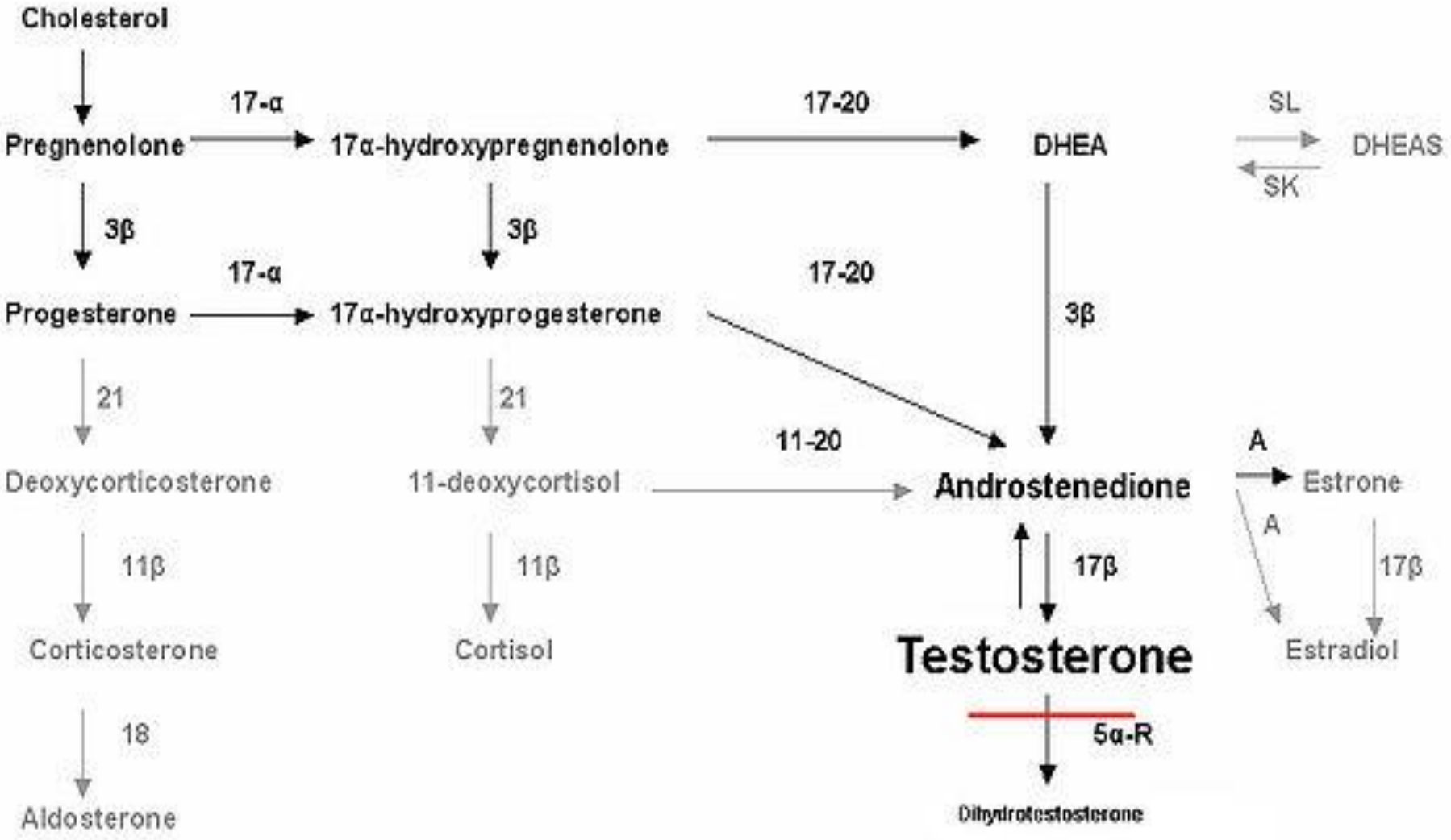
**Function:**
**Synthesis of ATP**
**Major source of energy**




$$\text{Sucrose} + 6\ O_2 + 38\ \text{ADP} + 38\ \text{Pi} \rightarrow 6\ CO_2 + 6\ H_2O + 38\ \text{ATP}$$

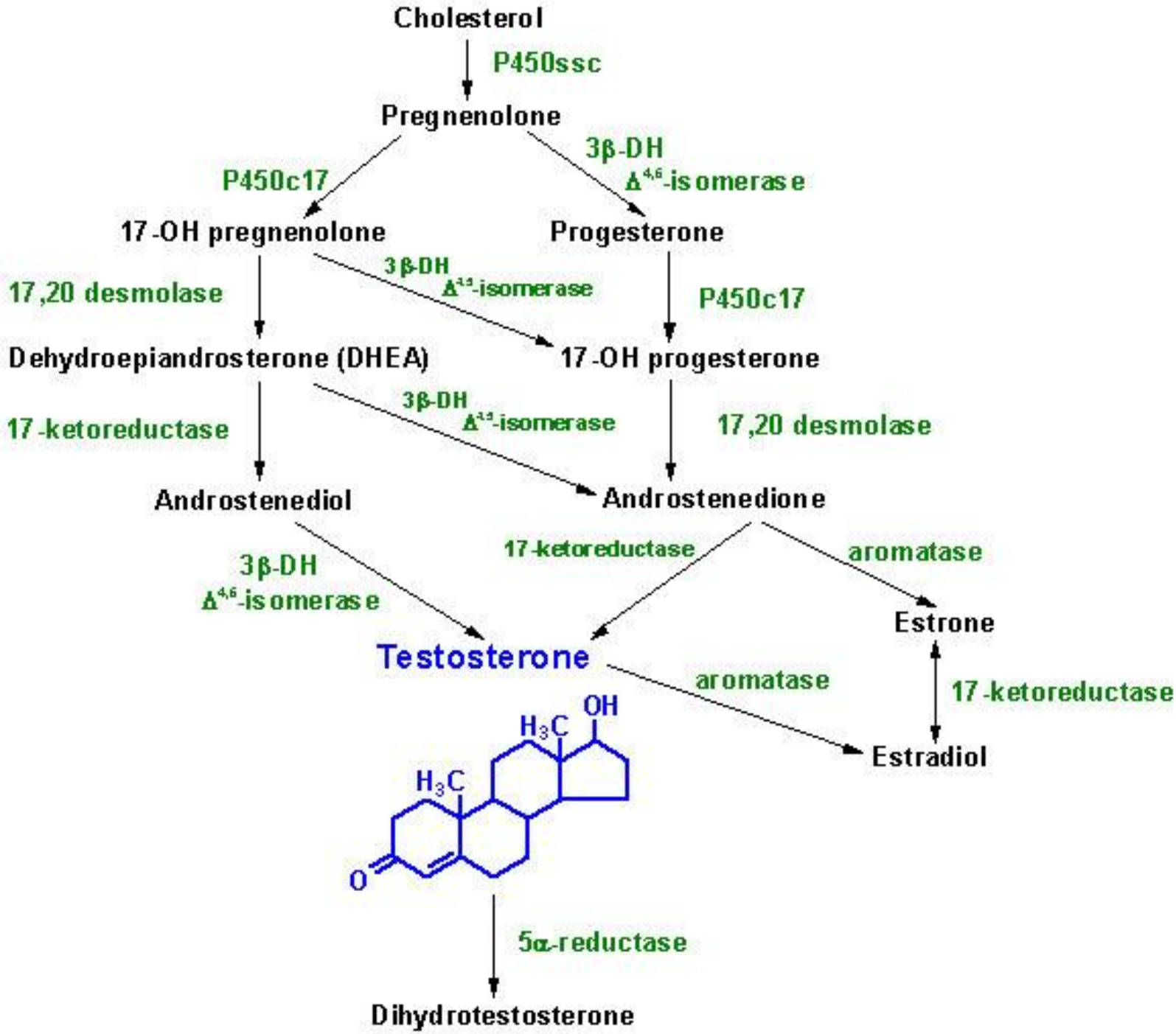



SCP2 – Sterol Carrier Protein; StAR – Steroid Acute Regulatory Protein

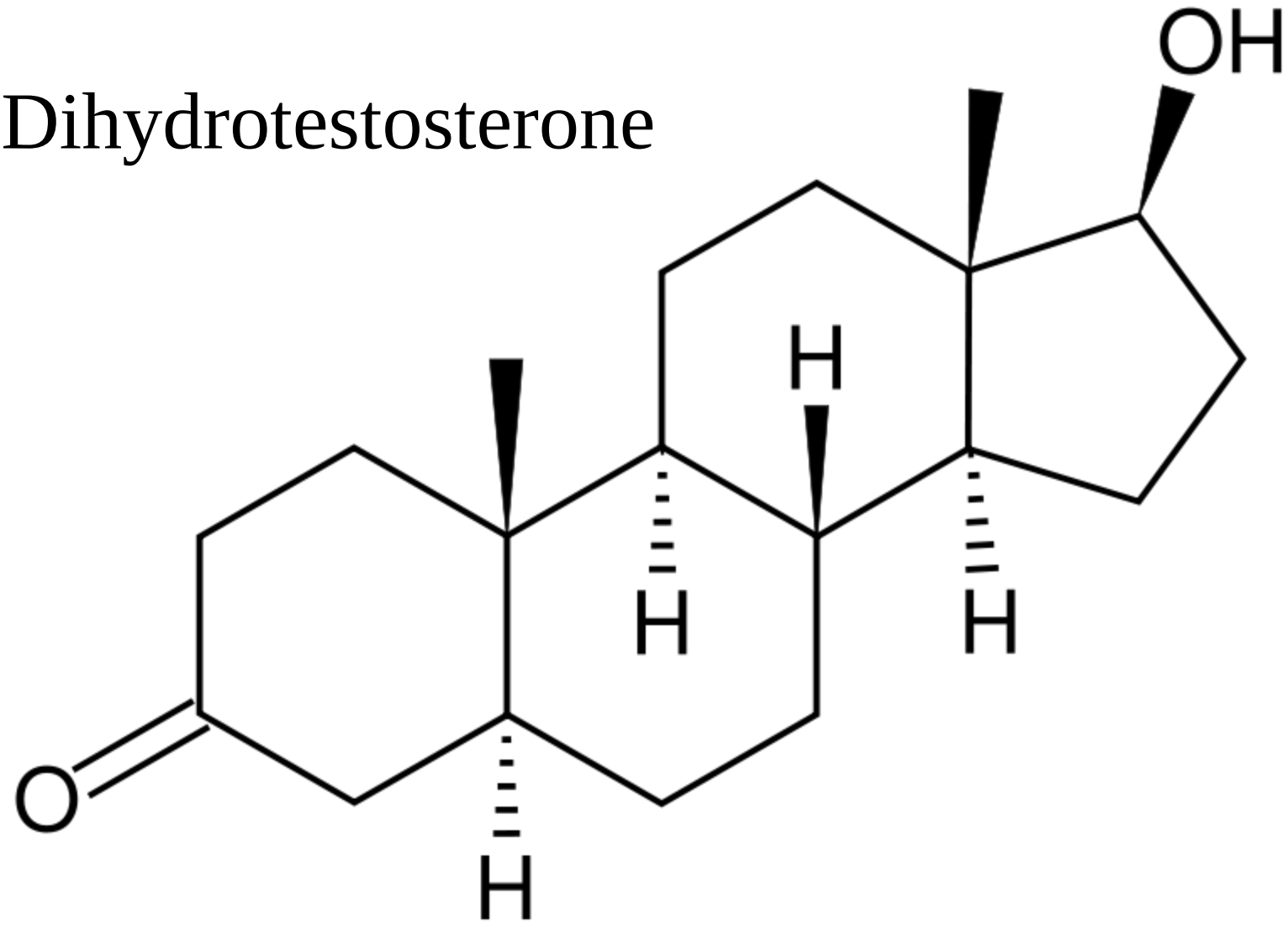



## LH Inhibits and Cortisol Stimulates 17α-hydroxylase

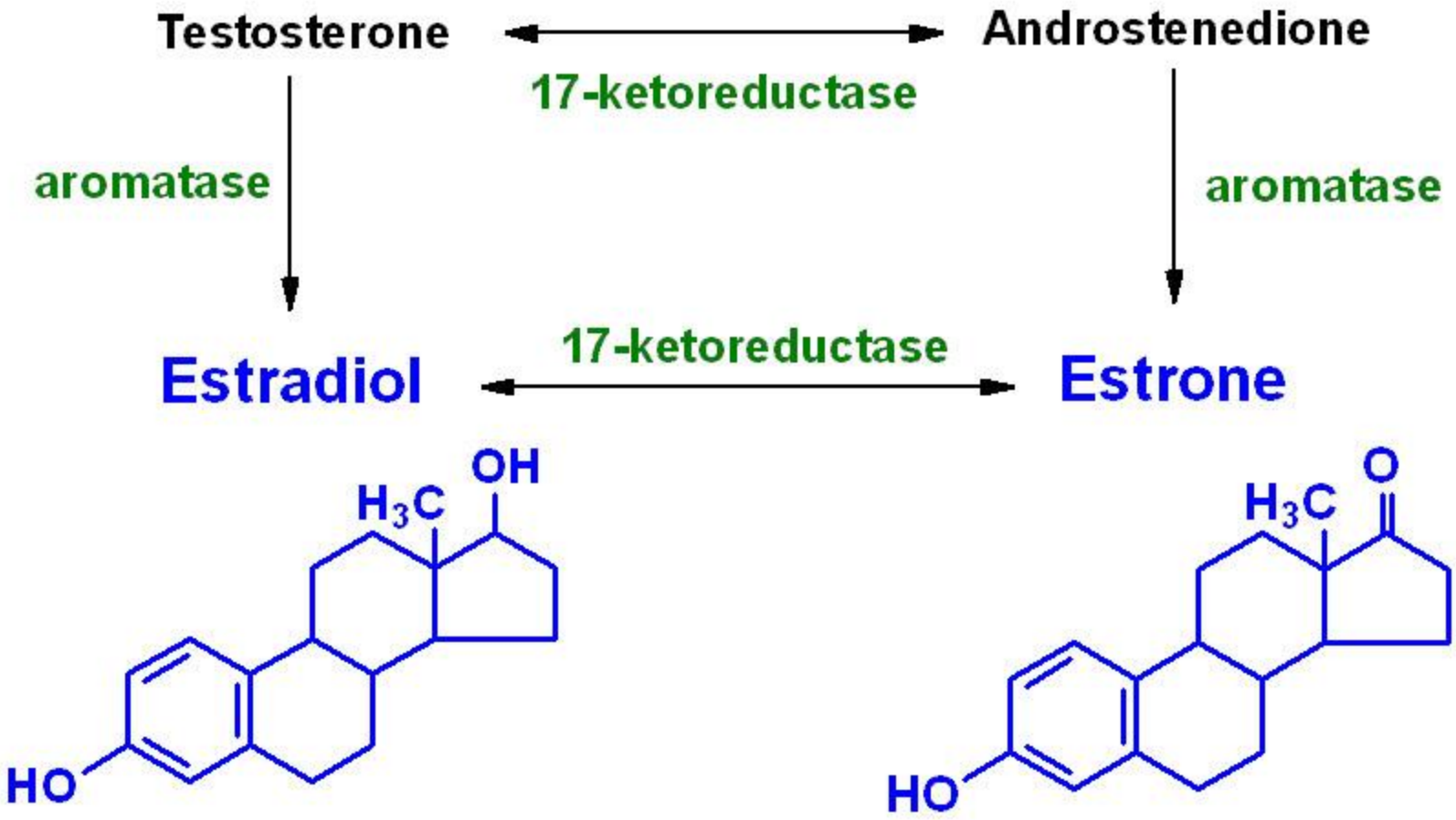
17α : 17α-hydroxylase
17,20 : 17,20-lyase
21 : 21-hydroxylase
3β : 3-HSD (hydroxysteroid dehydrogenase)
17β : 17β-HSD (hydroxysteroid dehydrogenase)
5α-R : 5α-reductase


Cholesterol
P450ssc
Pregnenolone
3β-DH
Δ4,6-isomerase
P450c17
17-OH pregnenolone
Progesterone
3β-DH
Δ5,4-isomerase
17,20 desmolase
P450c17
Dehydroepiandrosterone (DHEA)
17-OH progesterone
3β-DH
Δ5,4-isomerase
17-ketoreductase
17,20 desmolase
Androstenediol
Androstenedione
3β-DH
Δ4,6-isomerase
17-ketoreductase
aromatase
Estrone
Testosterone
aromatase
17-ketoreductase
Estradiol
OH
H3C
H3C
O
5α-reductase
Dihydrotestosterone

# Dihydrotestosterone




NON-AROMATIZABLE TO ESTROGENS
MALE SECONDARY SEX CARACHTERISTICS


Testosterone
Androstenedione
17-ketoreductase
aromatase
aromatase
17-ketoreductase
Estradiol
Estrone
OH
H3C
HO
O
H3C
HO

# STEROID BIOSYNTHESIS

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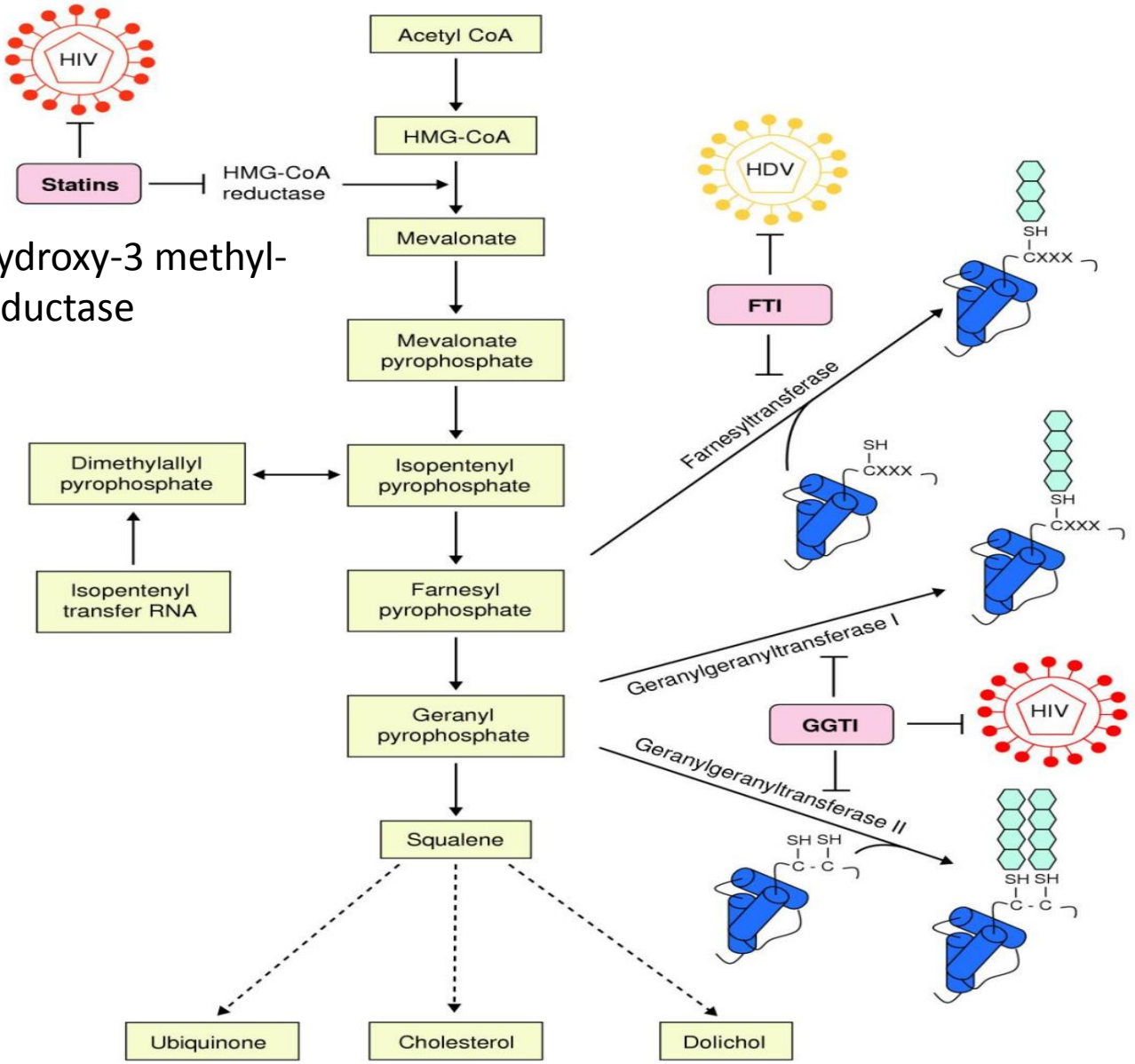

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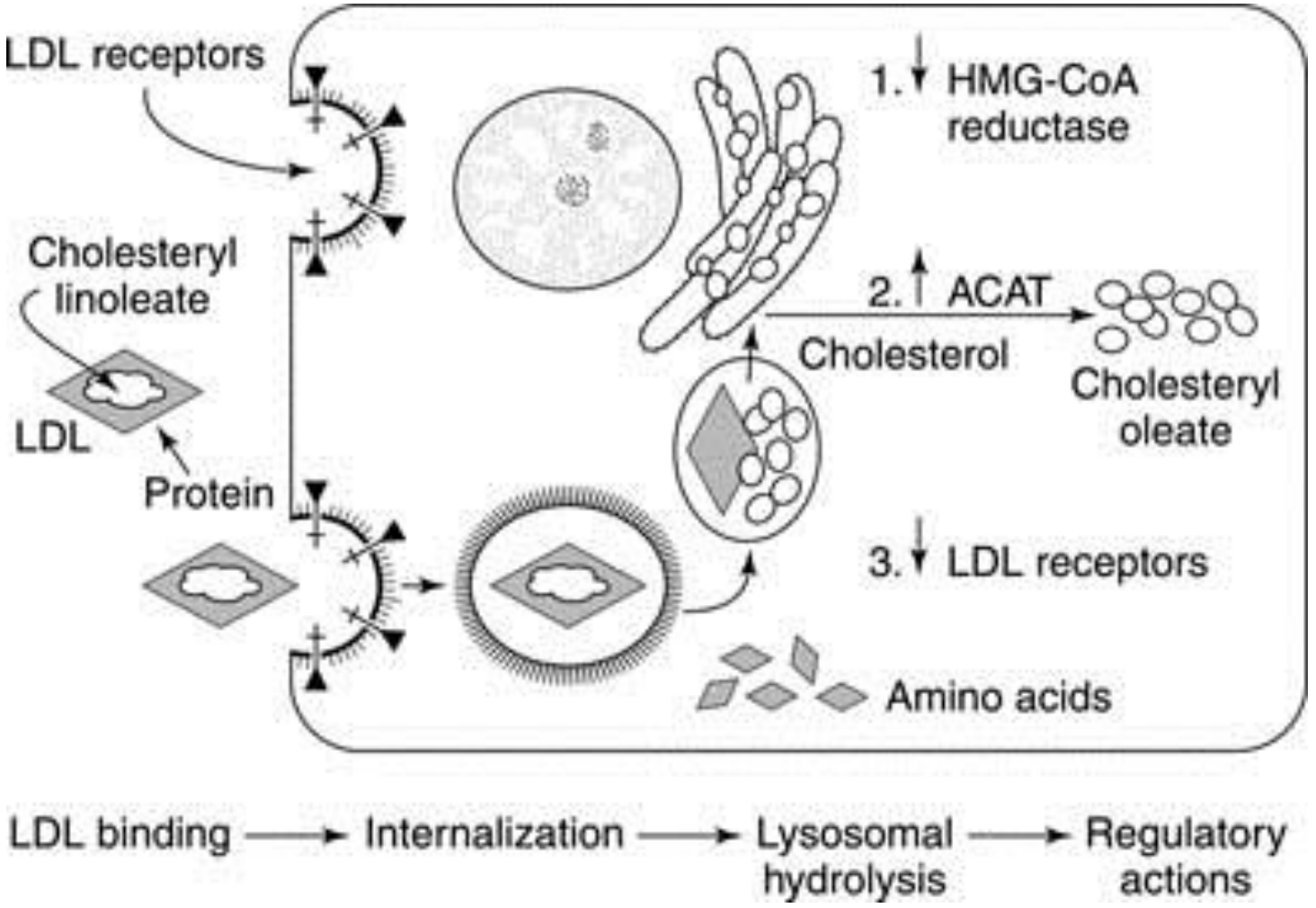


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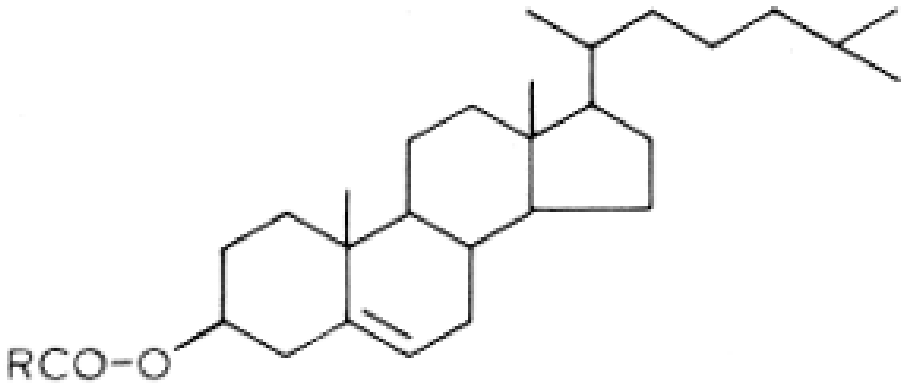
*cholesteryl esters*

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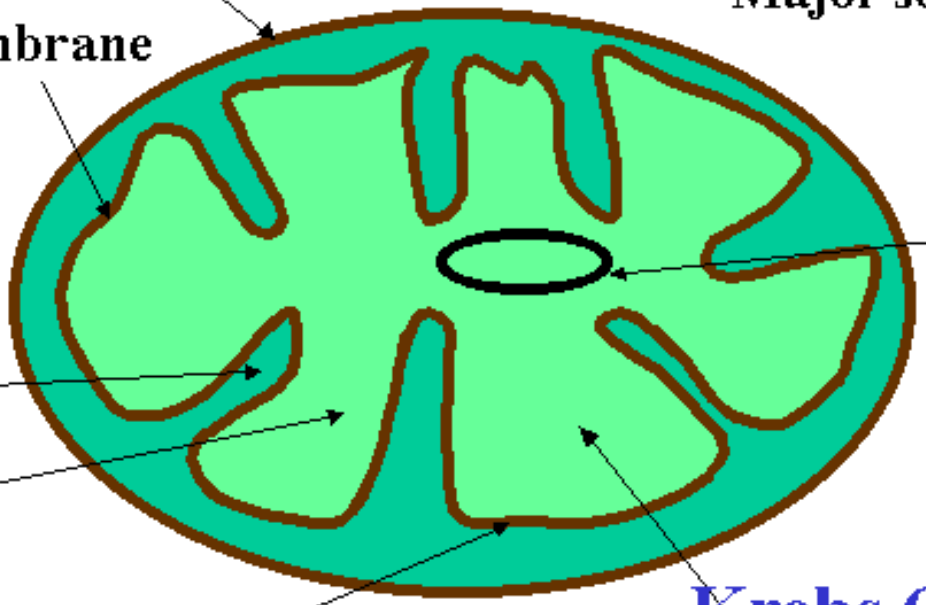


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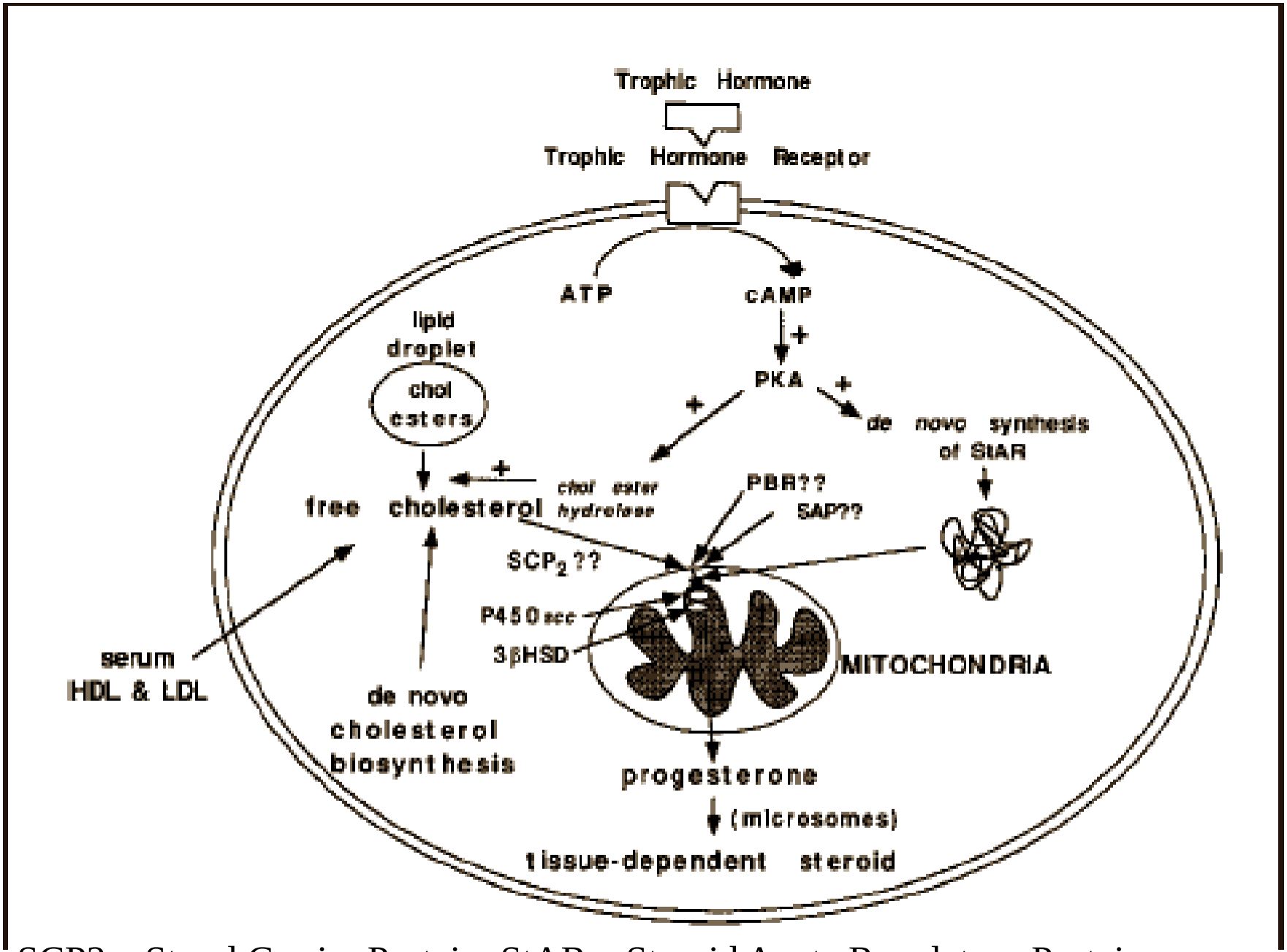


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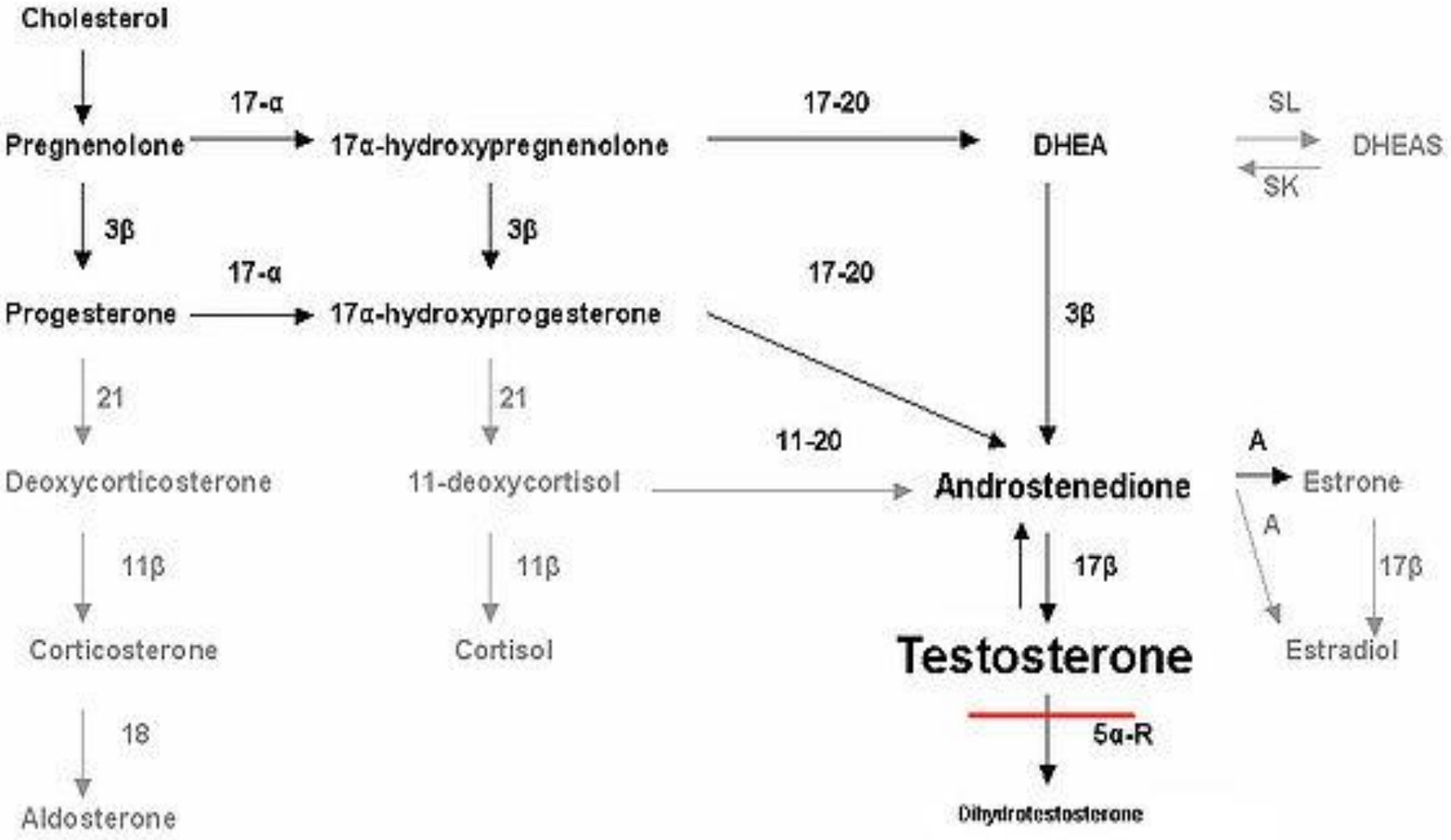


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17,20 : 17,20-lyase
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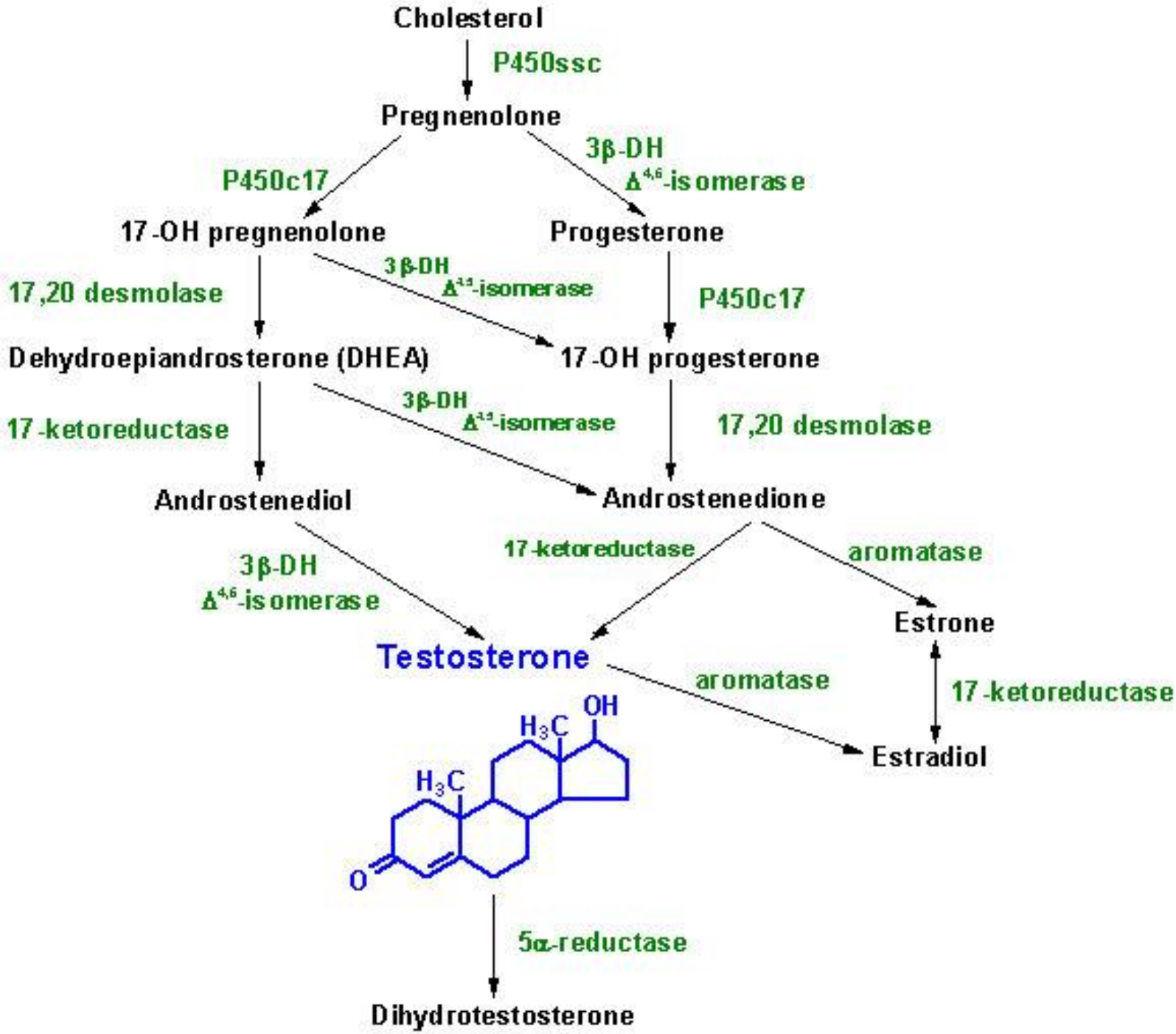
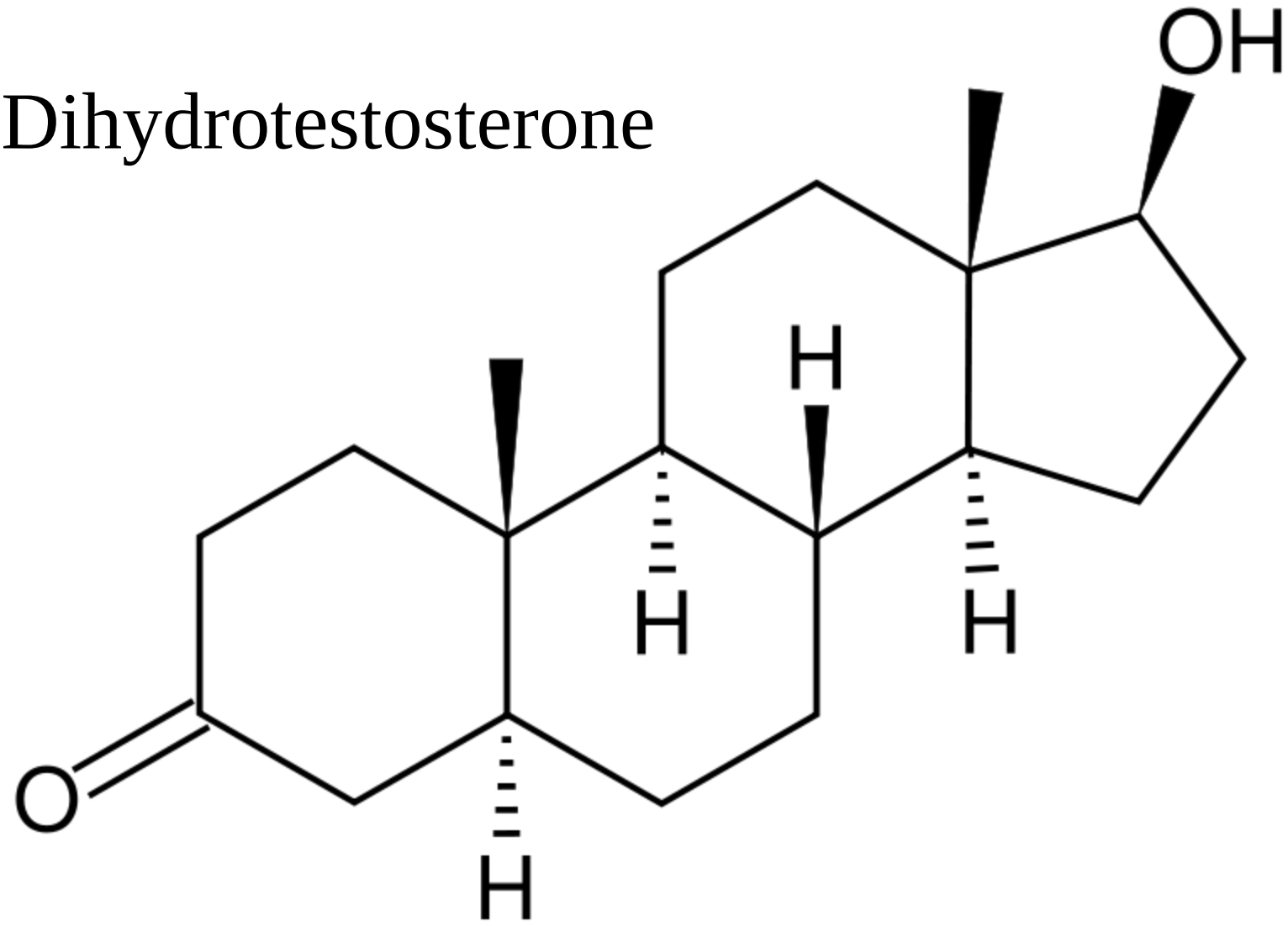
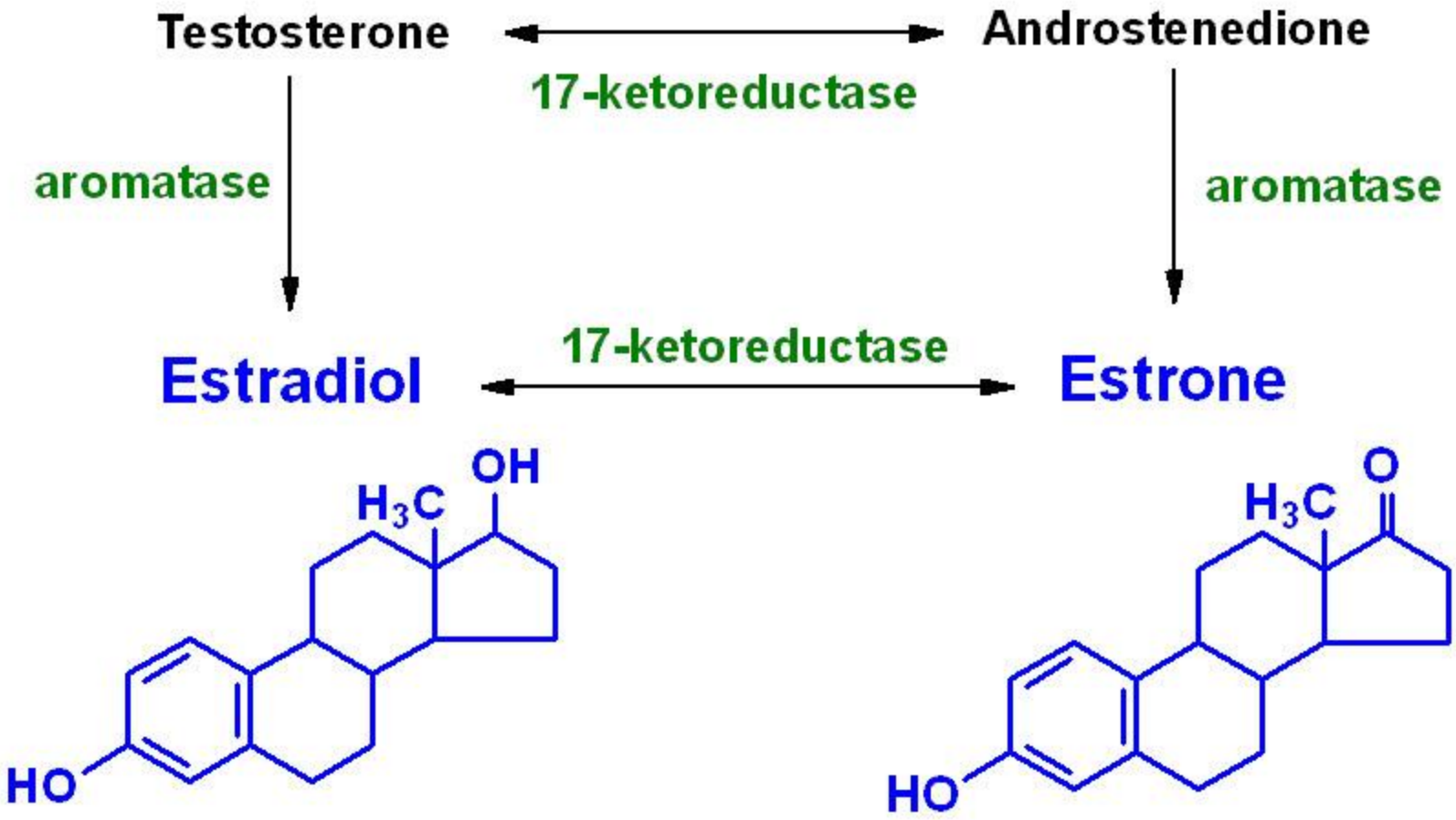

Cholesterol
P450ssc
Pregnenolone
3β-DH
Δ4,6-isomerase
P450c17
17-OH pregnenolone
Progesterone
3β-DH
Δ5,4-isomerase
17,20 desmolase
P450c17
Dehydroepiandrosterone (DHEA)
17-OH progesterone
3β-DH
Δ5,4-isomerase
17-ketoreductase
17,20 desmolase
Androstenediol
Androstenedione
3β-DH
Δ4,6-isomerase
17-ketoreductase
aromatase
Estrone
Testosterone
aromatase
17-ketoreductase
Estradiol
OH
H3C
H3C
O
5α-reductase
Dihydrotestosterone

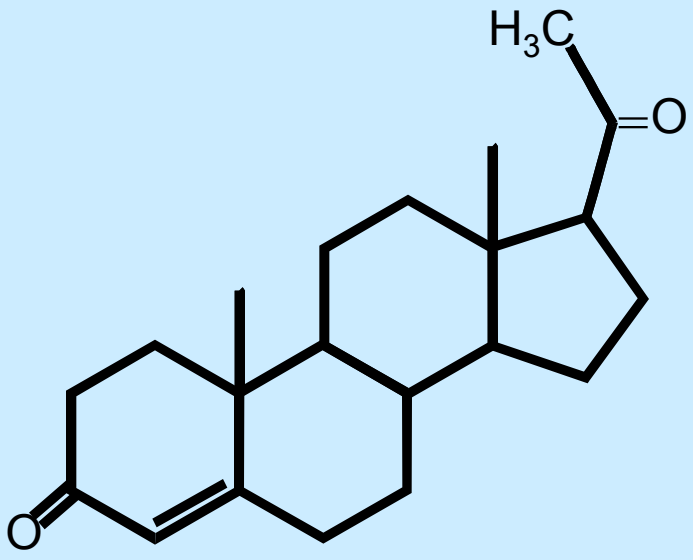
# Dihydrotestosterone




NON-AROMATIZABLE TO ESTROGENS
MALE SECONDARY SEX CARACHTERISTICS


Testosterone
Androstenedione
17-ketoreductase
aromatase
aromatase
17-ketoreductase
Estradiol
Estrone
OH
H3C
HO
O
H3C
HO

# 20α-Hydroxysteriod Dehydrogenase
# 20α-HSD

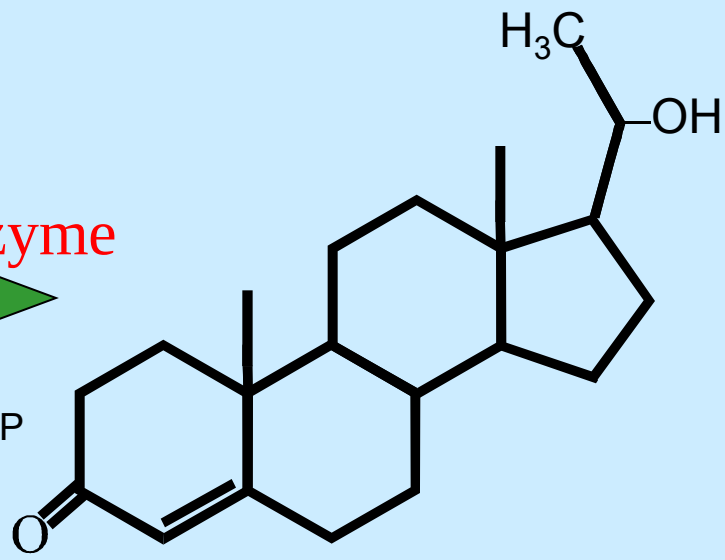



Progesterone

Prolactin
Inhibits Enzyme

20α-HSD

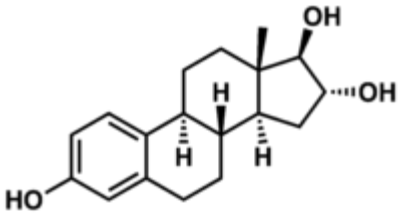
NADPH NADP



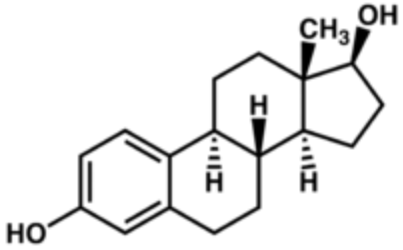

20α-dihydroprogesterone

Does not support pregnancy
or decidualization in rodents

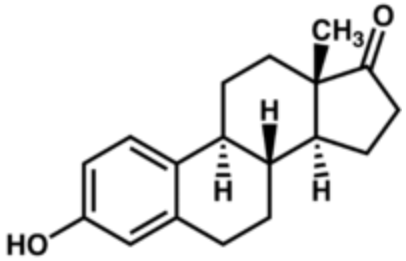
ESTRIOL, E3




ESTRADIOL, E2

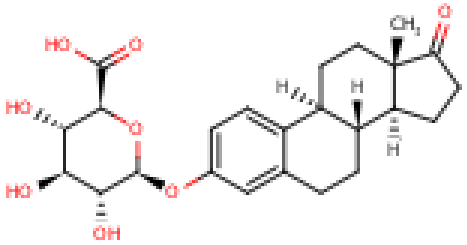


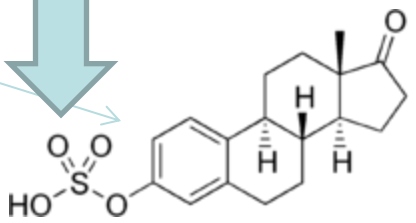

ESTRONE, E1



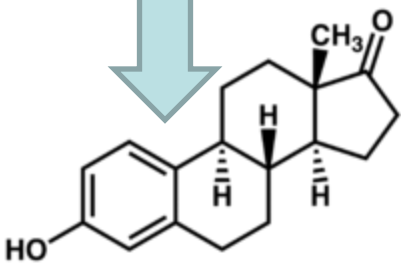

UDP glucuronyltransferase

Sulfotransferase




Sulfatase



# Nuclear Hormone Receptor Functional Domains

FIGURE 9

GENERAL STRUCTURE AND FUNCTIONAL ORGANIZATION OF THE NUCLEAR HORMONE RECEPTORS

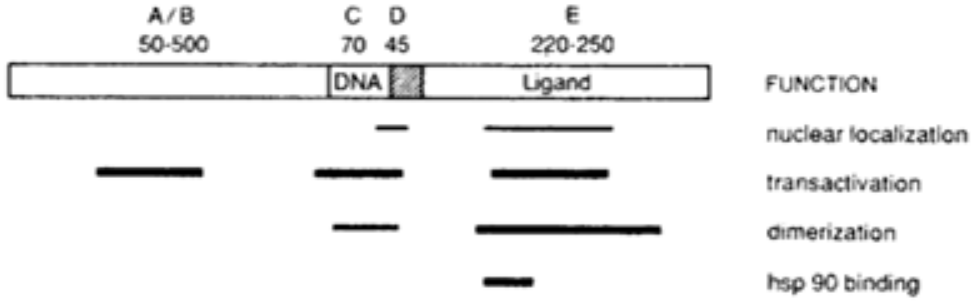
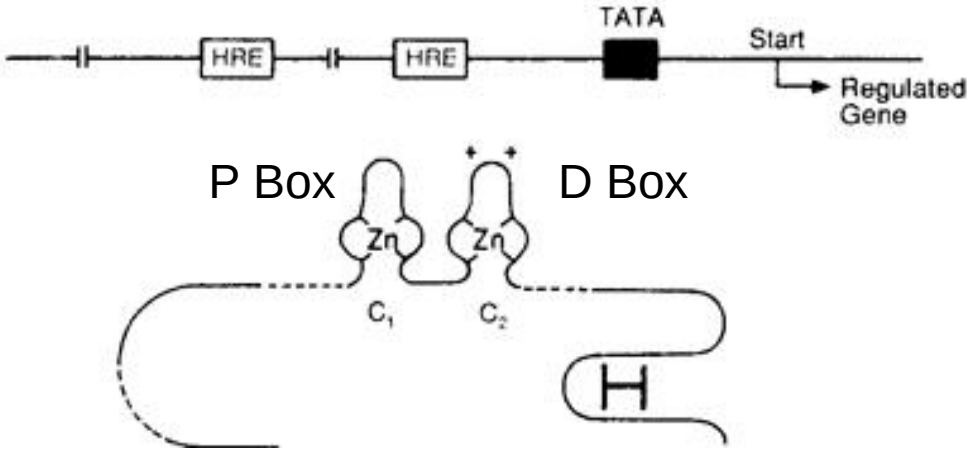


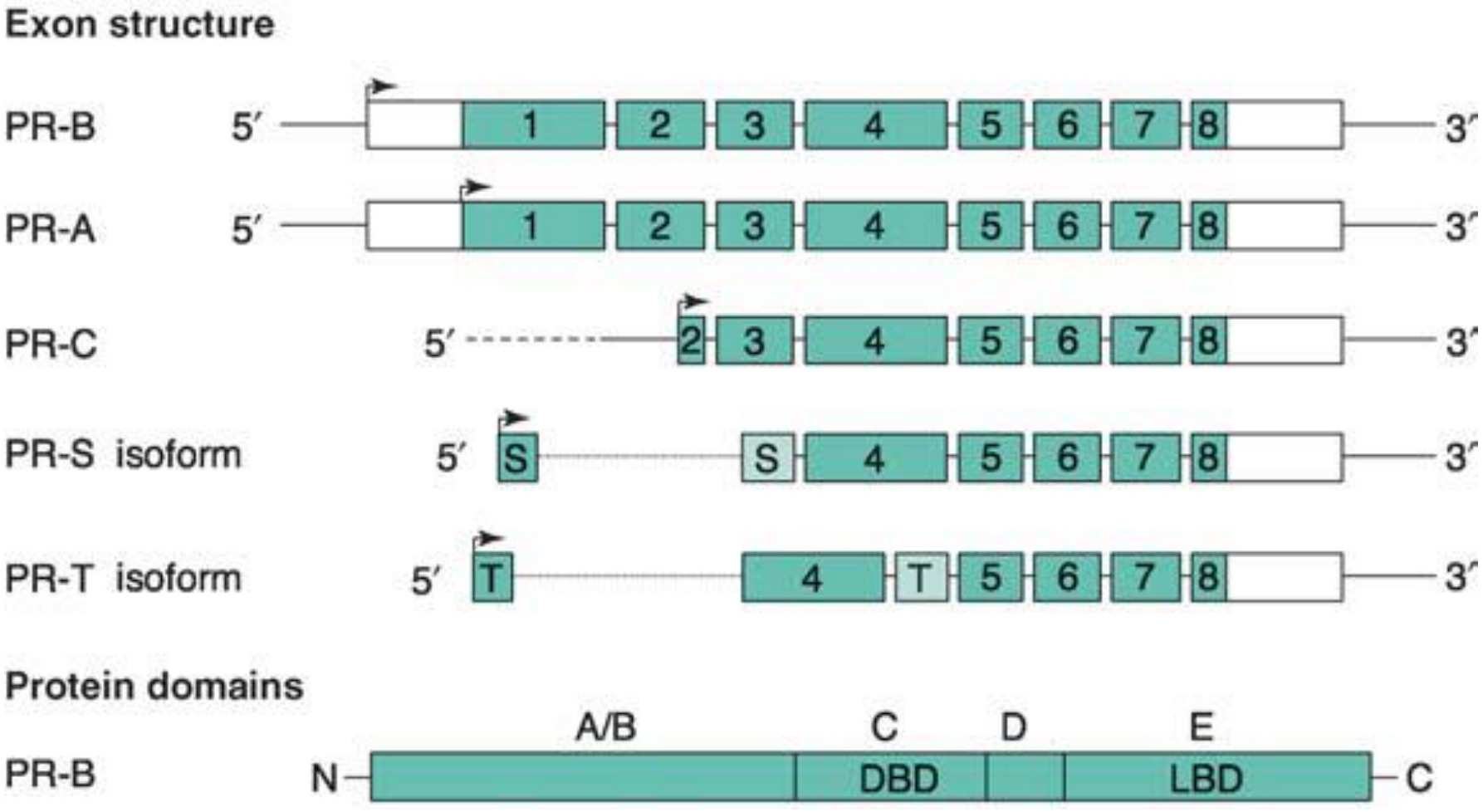

FIGURE 10

CONFORMATION OF THE DNA BINDING DOMAIN



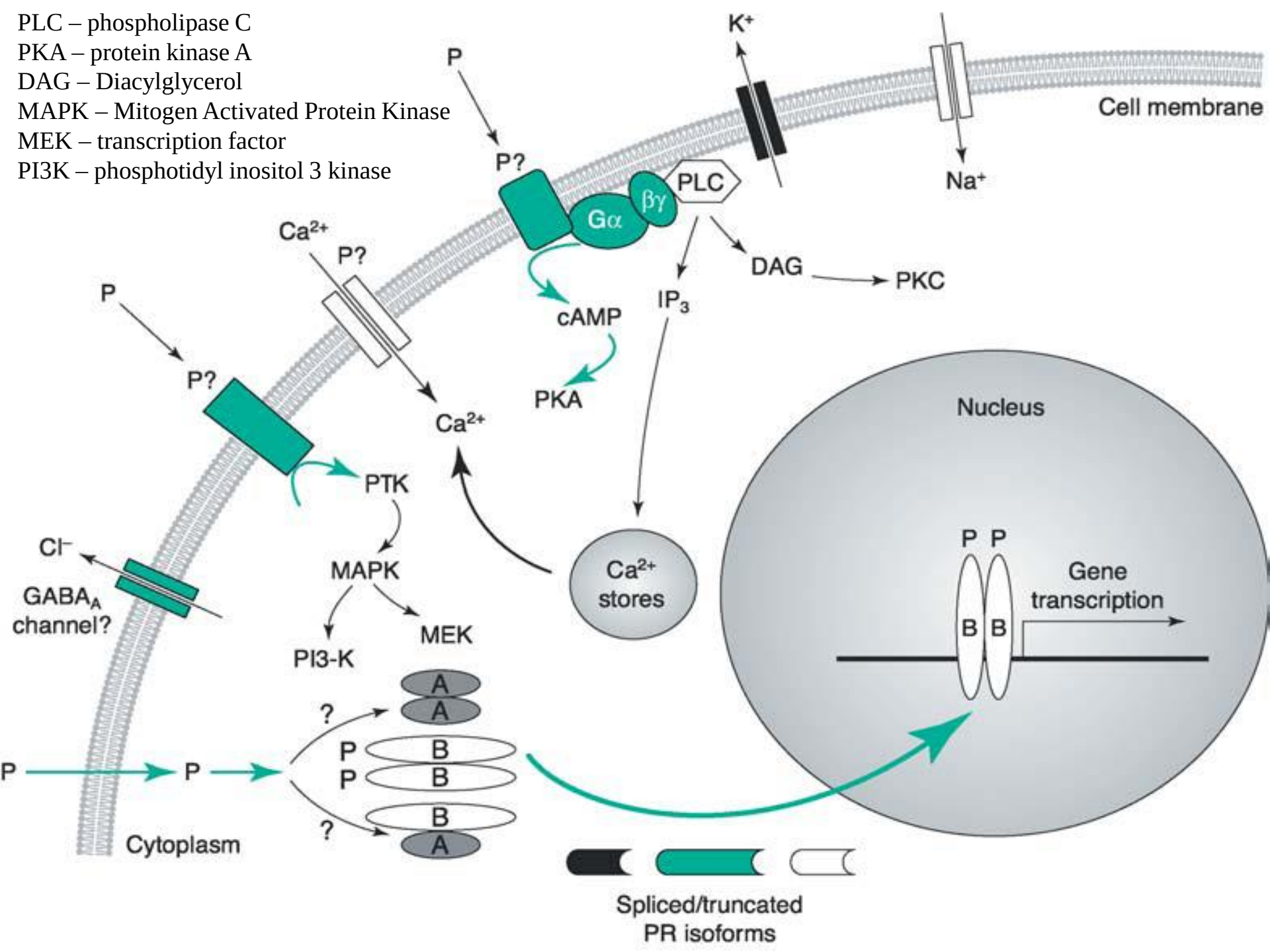
# Progesterone Receptors (PGR)

- Progesterone receptor (hPGR), a member of the steroid-receptor superfamily of nuclear receptors.
- Single-copy PGR gene uses separate promoters and translational start sites to produce two primary isoforms
  - PGRA and PGRB
    - identical except for an additional 165 amino acids present only in the N terminus of PGRB
    - PGRB shares important structural domains with PGRA, but they are two functionally distinct transcription factors.
    - Selective ablation of PGRA in mice revealed that PGR-B stimulated, rather than inhibited epithelial cell proliferation in response to estrogen alone and to progesterone and estrogen.
    - PGRA isoform opposes estrogen-induced proliferation and PGRB-dependent proliferation.

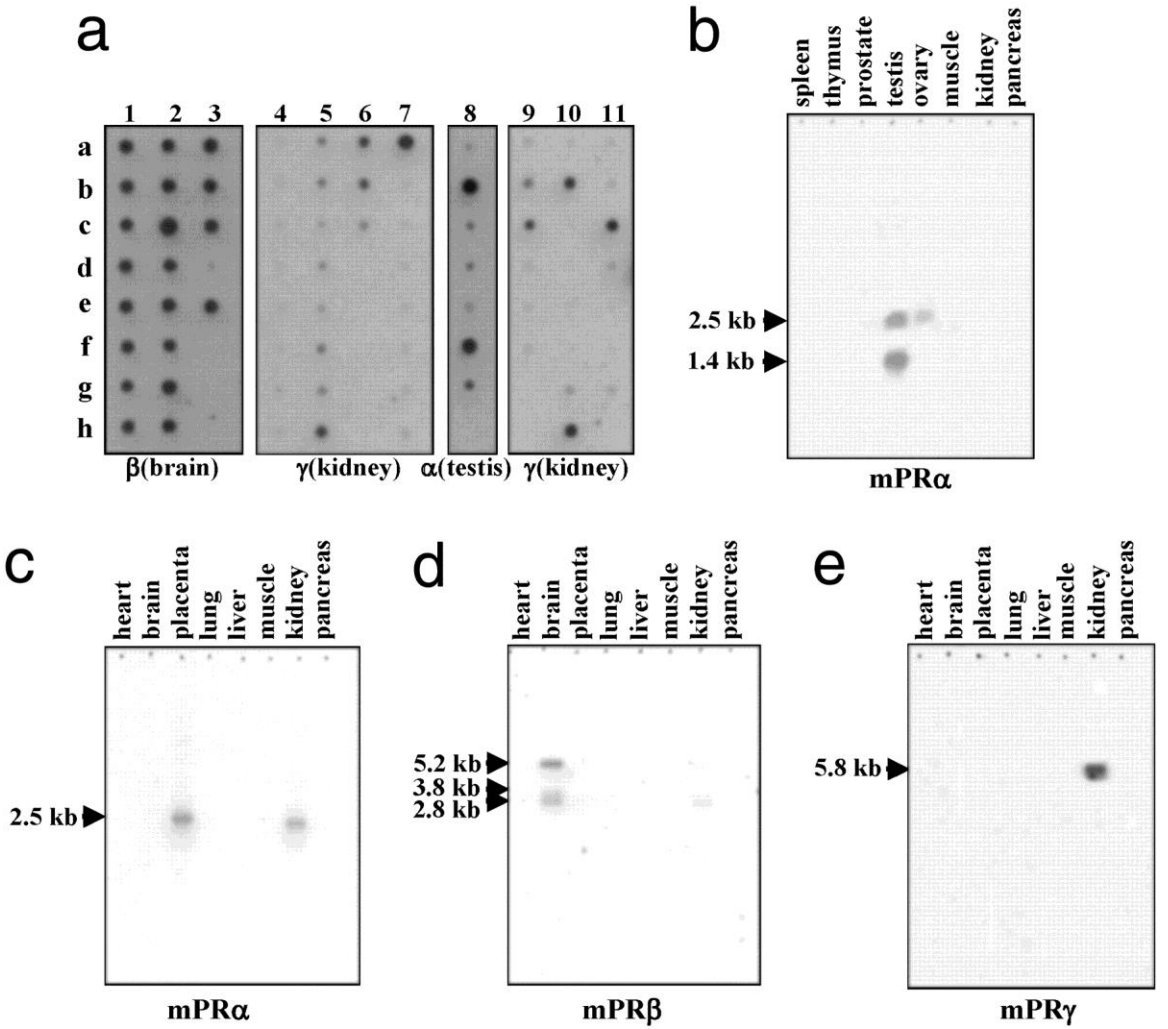



A/B: Activation Domains
C: DBD or DNA Binding Domain
D: Hinge Region for binding certain regulatory elements such as RU486
E: LBD or ligand Binding Domain

PLC – phospholipase C
PKA – protein kinase A
DAG – Diacylglycerol
MAPK – Mitogen Activated Protein Kinase
MEK – transcription factor
PI3K – phosphotidyl inositol 3 kinase

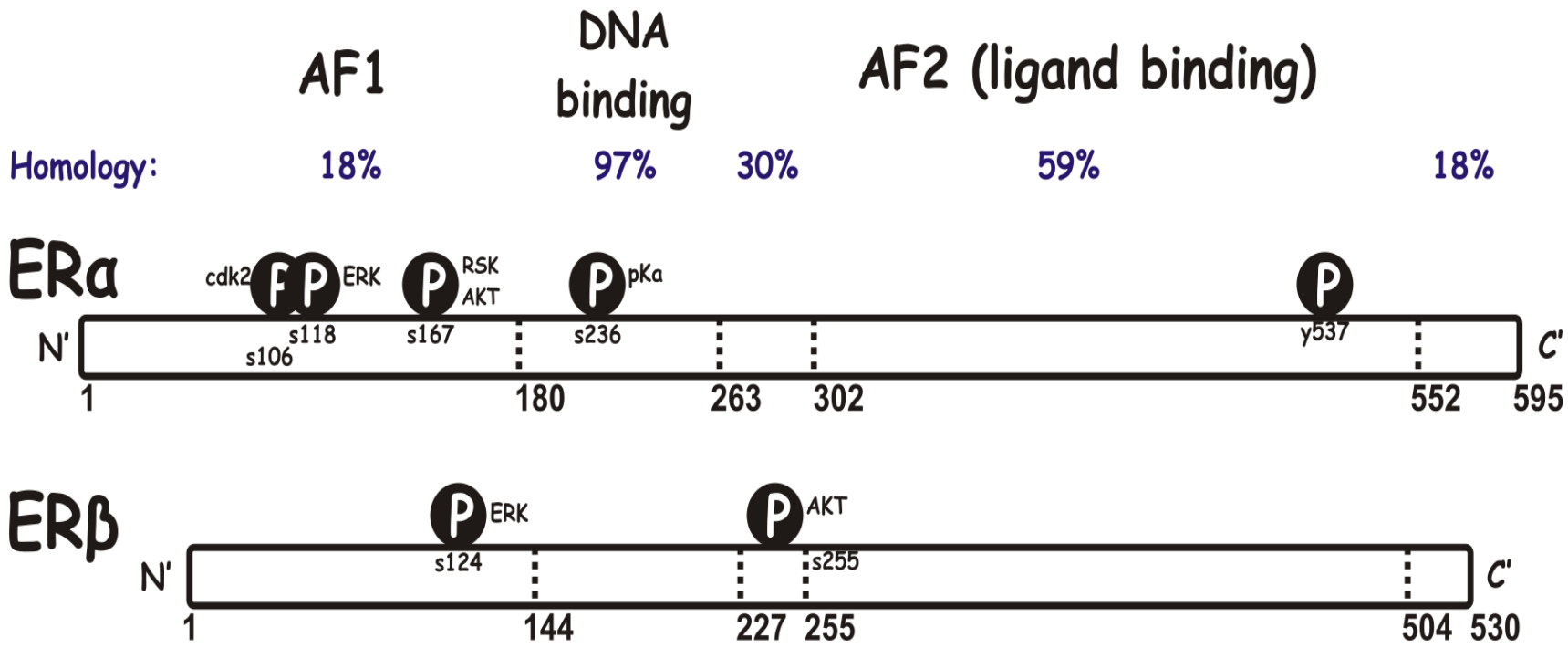


**(a) Dot blot hybridization of human mPR α (testicular), β (brain), and γ (kidney) mRNA probes with human multiple tissue arrays (CLONTECH).**




Zhu Y et al. PNAS 2003;100:2237-2242

# ESTROGEN RECEPTORS (ESR1 and ESR2)




AF1 = A/B Domain
DNA Binding Domain = C Domain
263-302 = D – Hinge Region
AF2 = E Ligand Binding Domain

# TISSUE DISTRIBUTION OF ESR1 AND ESR2

- Both ESR1 and ESR2 are widely expressed in different tissue types with some notable differences

- ESR1 is found in uterus, breast cancer cells, ovarian stroma cells and hypothalamus.

- ESR2 is found in kidney, brain, bone, heart, lungs, intestinal mucosa, prostate, and endothelial cells.

# Membrane ER Action

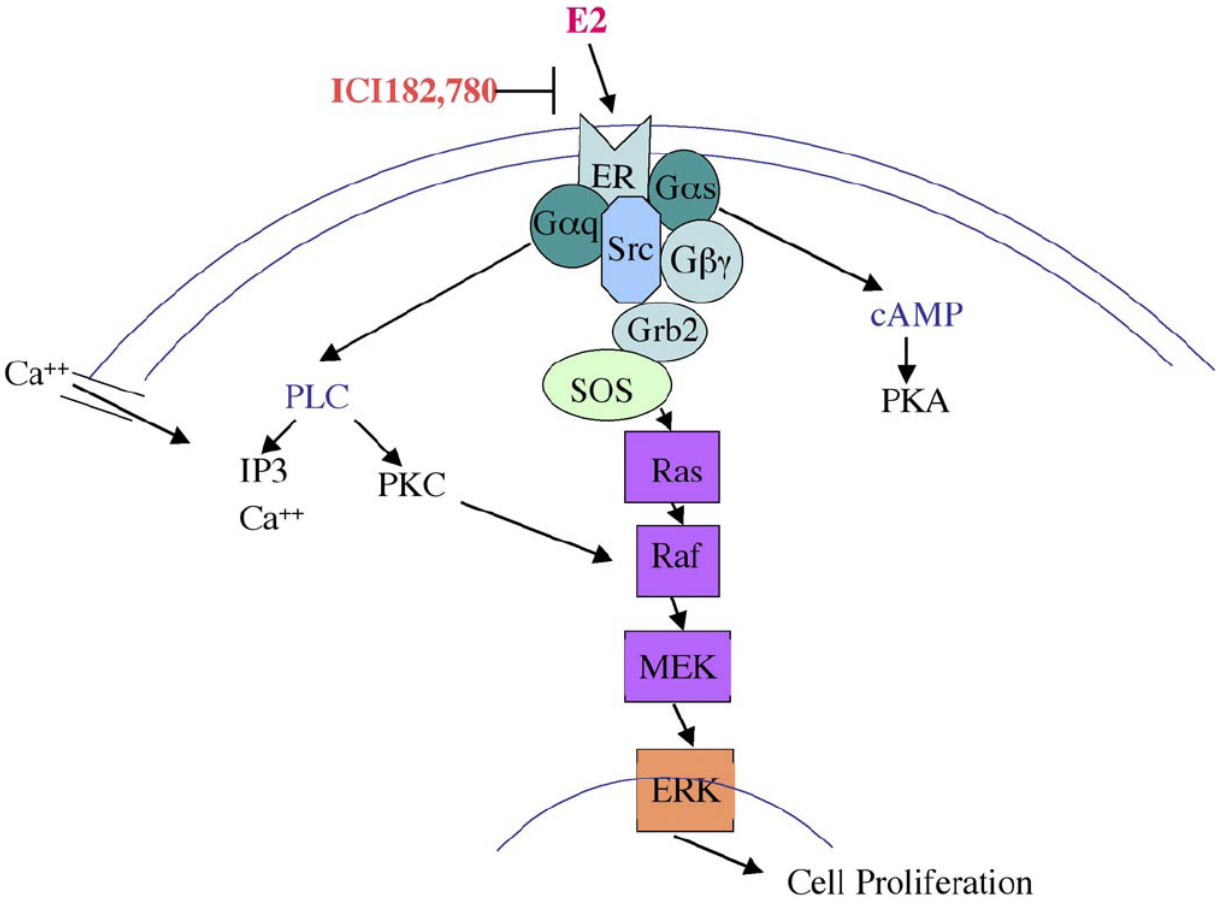



Fig. 1. Cartoon of membrane estrogen receptor (ER)-α rapid signaling in breast cancer cells. Membrane-translocated ER-α dimerizes in response to 17β-estradiol ($E_2$), resulting in rapid G protein subunit activation. G protein activation results in other rapid signals generated, leading to kinase cascades and resulting cell biology. MEK, mitogen-activated protein kinase/extracellular regulated kinase; IP3, inositol 1,4,5-trisphosphate.



*Am J Physiol Regul Integr Comp Physiol* 295: R1425–R1430, 2008.

# Genomic and Non-genomic Actions of Steroid Receptors

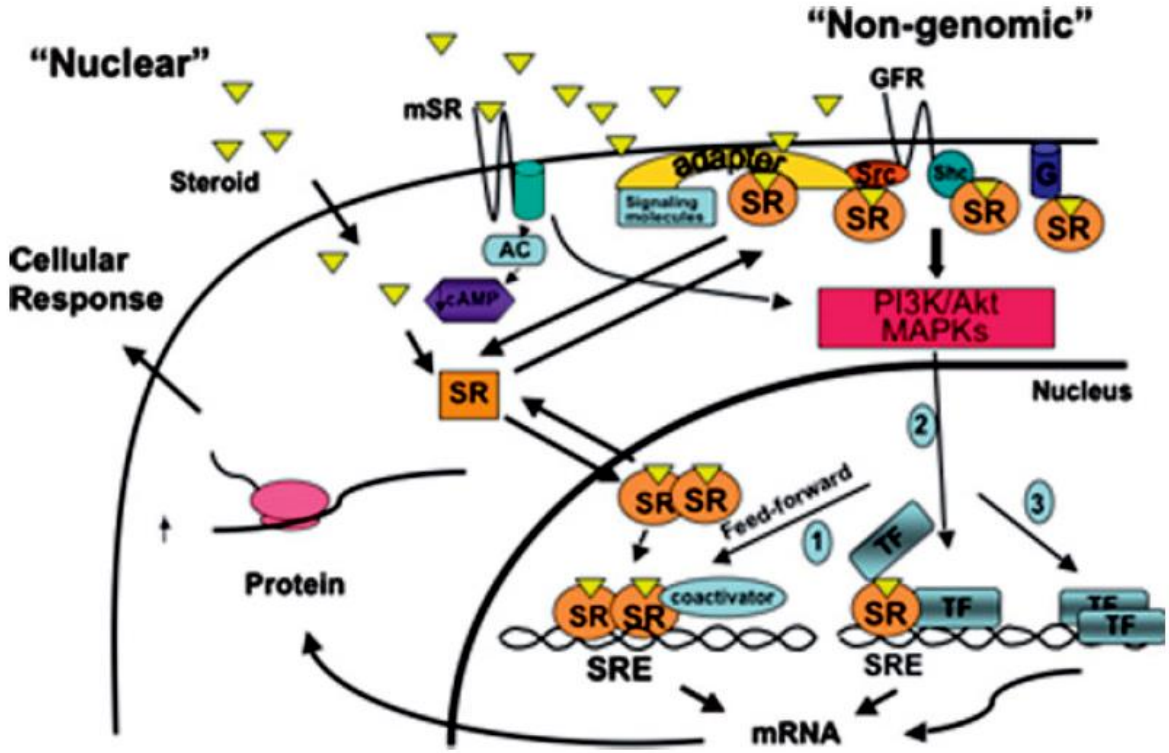



**Figure 2** Nuclear transcriptional and non-genomic signaling pathways activated by sex steroid hormones. Sex steroids activate nuclear steroid hormone receptors (SR) by inducing receptor conformational changes, nuclear translocation, dimerization, and binding to steroid hormone response elements (SREs) in promoters/enhancers of target genes. Alternatively, a subpopulation of nuclear steroid receptors localized in cytoplasm/membrane can associate transiently with other signaling molecules including G protein–coupled receptor (GPCR; G), c-Src, Shc, adapter proteins (adapters), or membrane targeting proteins leading to activation of mitogen-activated protein kinase (MAPK) or phosphatidylinositol 3-kinase (PI3K)/Akt signaling cascades. Novel GPCR membrane receptors (mSR) unrelated to nuclear steroid receptors have been reported to also mediate rapid non-genomic effects of steroid hormones through inhibition of adenylate cyclase (AC) and cyclic adenosine monophosphate (cAMP) production and activation of MAPK. A biological consequence of sex steroid–induced activation of cytoplasmic signaling cascades is ultimately to influence gene transcription by three possible mechanisms. (1) A feed-forward regulatory loop whereby the nuclear transcriptional activity of SR or coactivators are enhanced by phosphorylation. (2) Signaling pathways that converge upon and activate target genes that require other transcription factors (TF) to cooperate with SRs either by tethering or by binding on composite SRE promoters. (3) Activation of other transcription factors independent of direct SR binding to DNA.

# Gene Structure

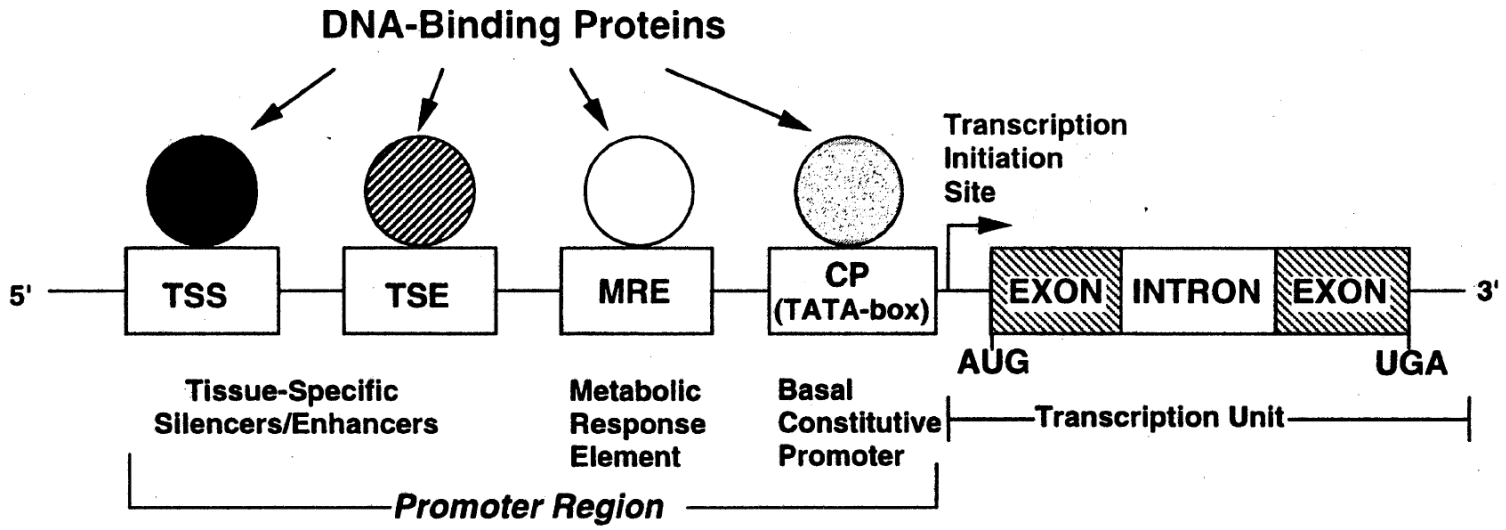



**Figure 2–8.** Diagrammatic structure of a "consensus" gene encoding a prototypical polypeptide hormone. Such a gene typically consists of a promoter region and a transcription unit. The transcription unit is the region of DNA composed of exons and introns that is transcribed into a mRNA precursor. Transcription begins at the cap site sequence in DNA and extends several hundred bases beyond the poly(A) addition site in the 3′ region. During post-transcriptional processing of the RNA precursor, the 5′ end of mRNA is capped by addition of methylguanosine residues. The transcript is then cleaved at the poly(A) addition site approximately 20 bases 3′ to the AATAAA signal sequence, and the poly(A) tract is added to the 3′ end of the RNA. Introns are cleaved from the RNA precursor, and exons are joined together. Dinucleotides GT and AG are invariably found at the 5′ and 3′ ends of introns. Translation of mRNA invariably starts with the codon ATG for methionine. Translation is terminated when the polyribosome reaches the stop codons TGA, TAA, or TAG. The promoter region of the gene located 5′ to the cap site contains numerous short regulatory DNA sequences that are targets for interactions with specific DNA-binding proteins. These sequences consist of the basal constitutive promoter (TATA box), metabolic response elements that modulate transcription, e.g., in response to cAMP, steroid hormone receptors, and thyroid hormone receptors, as well as tissue-specific enhancers and silencers that permit or prevent transcription of the gene, respectively. The enhancer and silencer elements direct expression of specific subsets of genes to cells of a given phenotype. Whether a gene will or will not be expressed in a particular cellular phenotype depends on complex interactions of the various DNA-binding proteins among themselves and, most important, with the TATA box proteins of the basal constitutive promoter.

# Anatomy of Nuclear Receptors and Typical Gene Structure

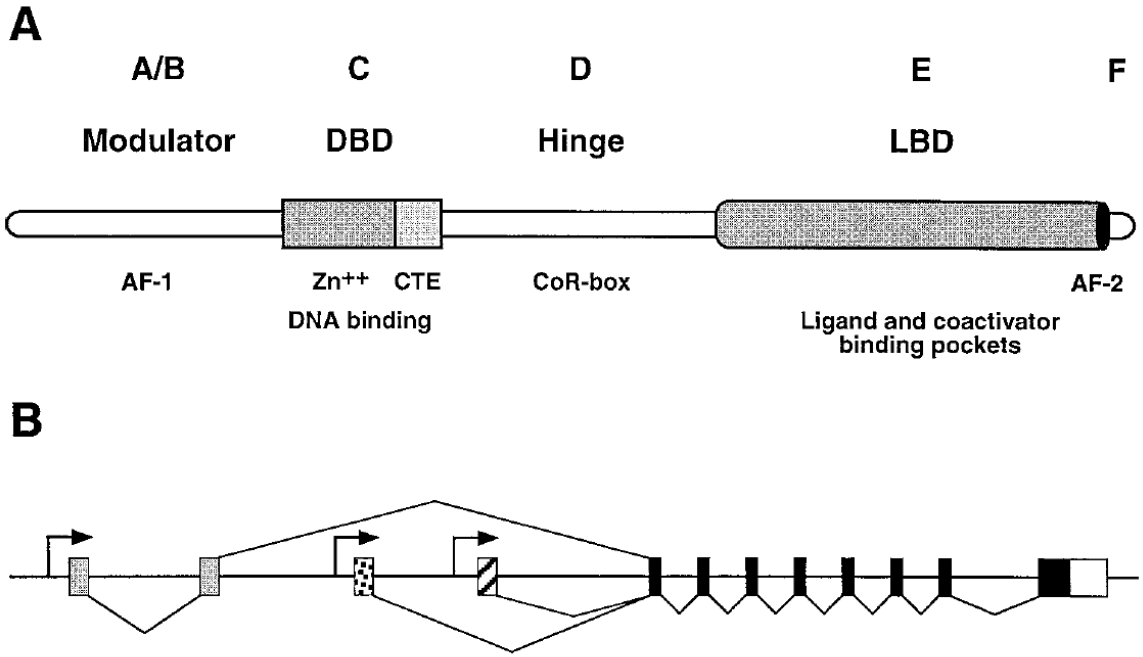



FIG. 2. Anatomy of nuclear receptors and typical gene structure. A, Nuclear receptors are composed of independent functional domains that include the DBD and LBD, the primary functions of which are to recognize specific DNA sequences and ligands, respectively. Nuclear receptors generally possess two transcription activation functions (AF-1 and -2) located at the amino and carboxy termini. The division of nuclear receptors into domains A–F is based on the degree of amino acid sequences conservation between the same receptor in different species. B, Schematic representation of the exon-intron organization of a typical nuclear receptor gene. The modulator domain is usually encoded by one or two exons. Distinct modulator domains can be generated by alternative promoter usage (*arrows*) and splicing (*linked exons*). The two zinc finger modules are generally encoded by distinct exons while the hinge and LBD are encoded by 6 to 10 exons. Additional alternative splicing may generate nuclear receptors with modified LBDs.

AF = activation function DBD = DNA binding domain LBD = ligand binding domain
CoR = coregulator interaction box

# Translocation of ESR1

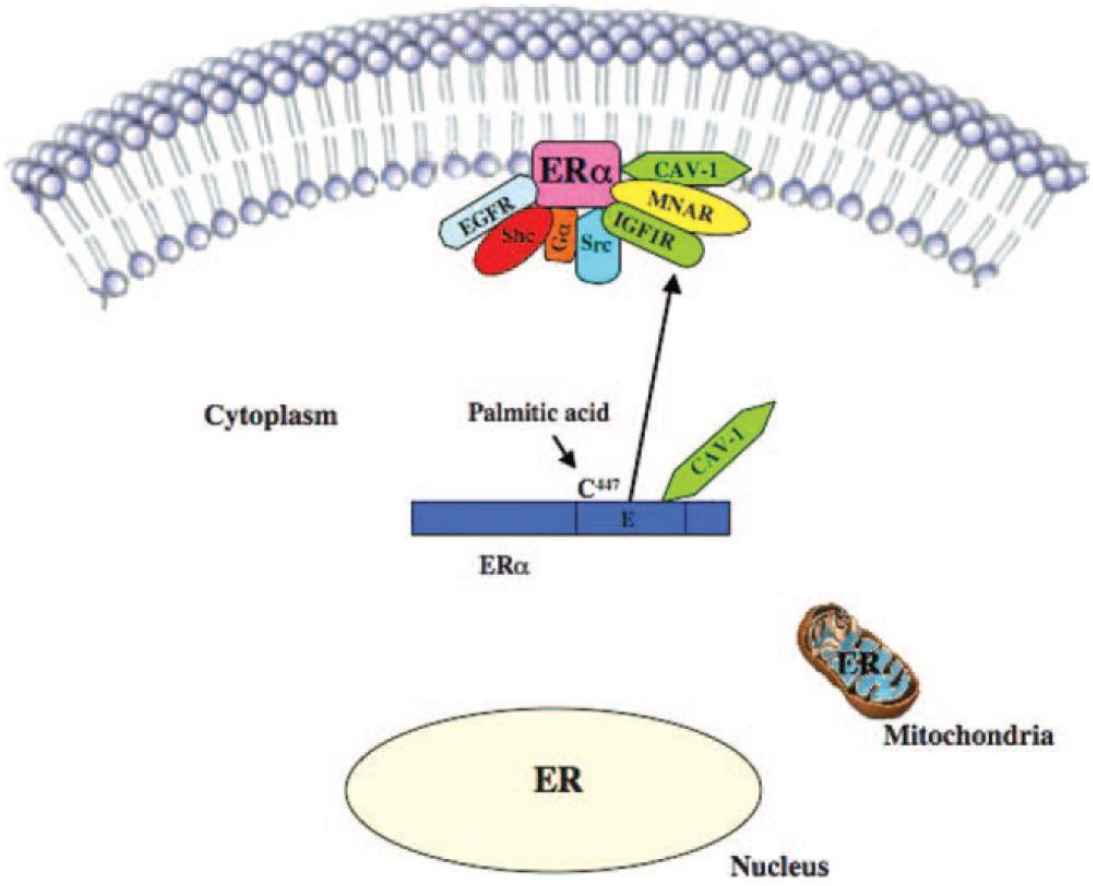
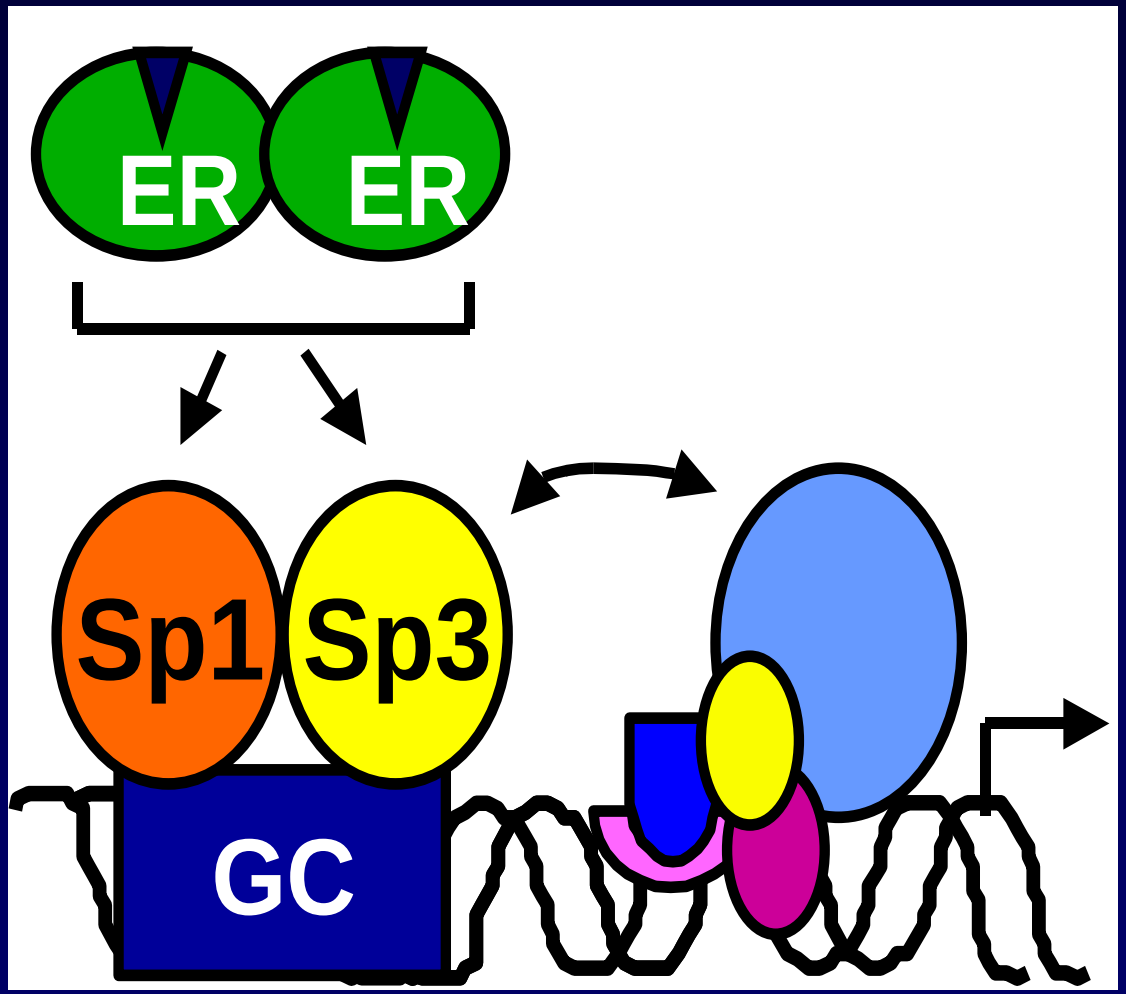



FIG. 1. Translocation of ERα to the plasma membrane. Palmitoylation at cysteine 447 of ERα promotes the association of the steroid receptor with caveolin 1. The scaffolding domain of caveolin-1 (amino acids 80–100) then facilitates the translocation of caveolin and ER to the caveolae rafts in the membrane. Here, ER associates with a large protein complex, changing in nature depending upon the cell and signal context, to effect G protein activation and downstream signaling to cell biology.

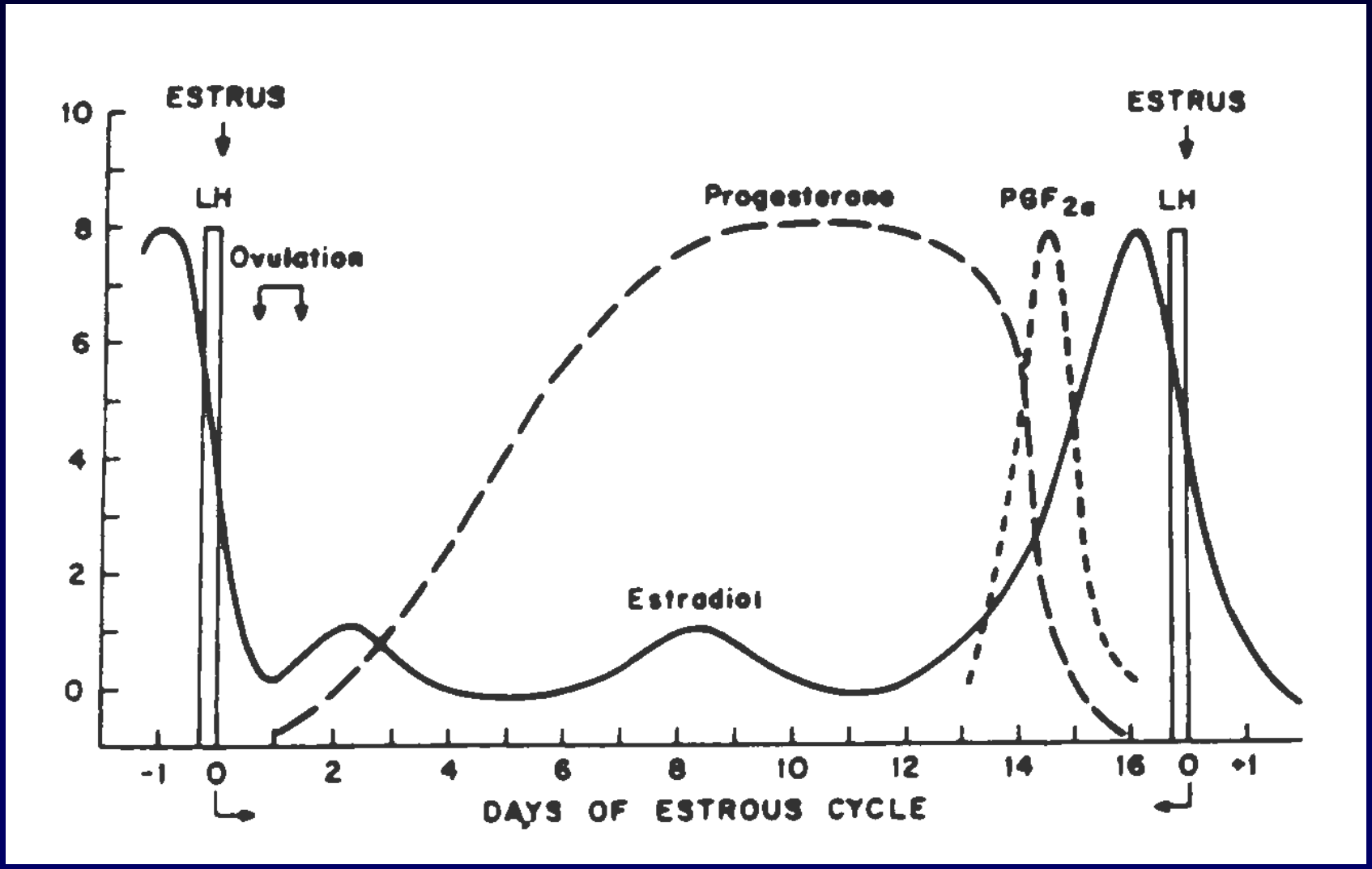
Hammes & Levin, Endo Rev 2007; 28:726

# DNA-INDEPENDENT ER/Sp1 ACTION TRANSACTIVATION



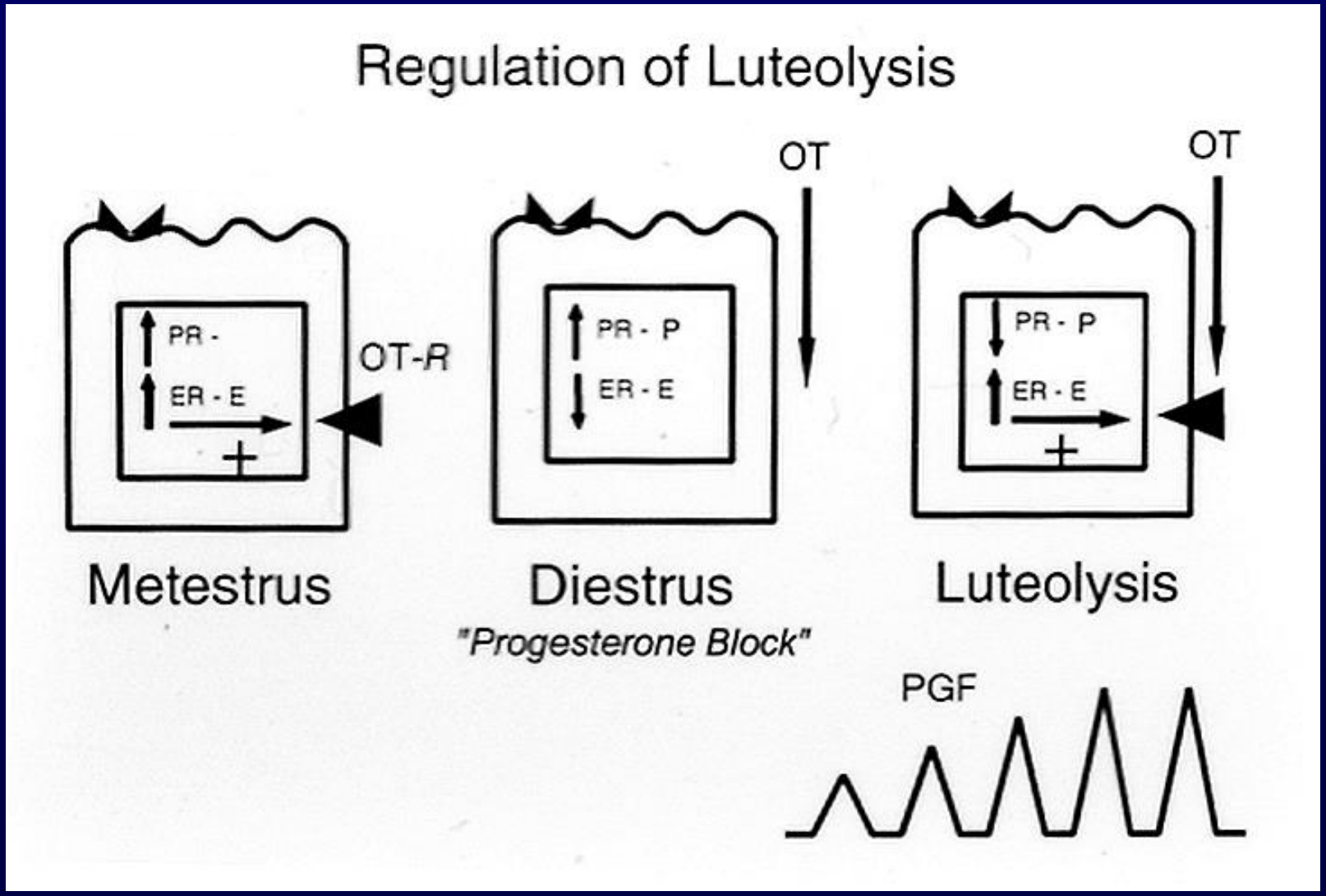

- ERα/Sp1 or ERα/Sp3 upregulation
- Enhances cofactor recruitment

# Hormone Profile in Cyclic Ewes



# McCracken Hypothesis

**(Anim Reprod Sci 1984; 7:31-55)**



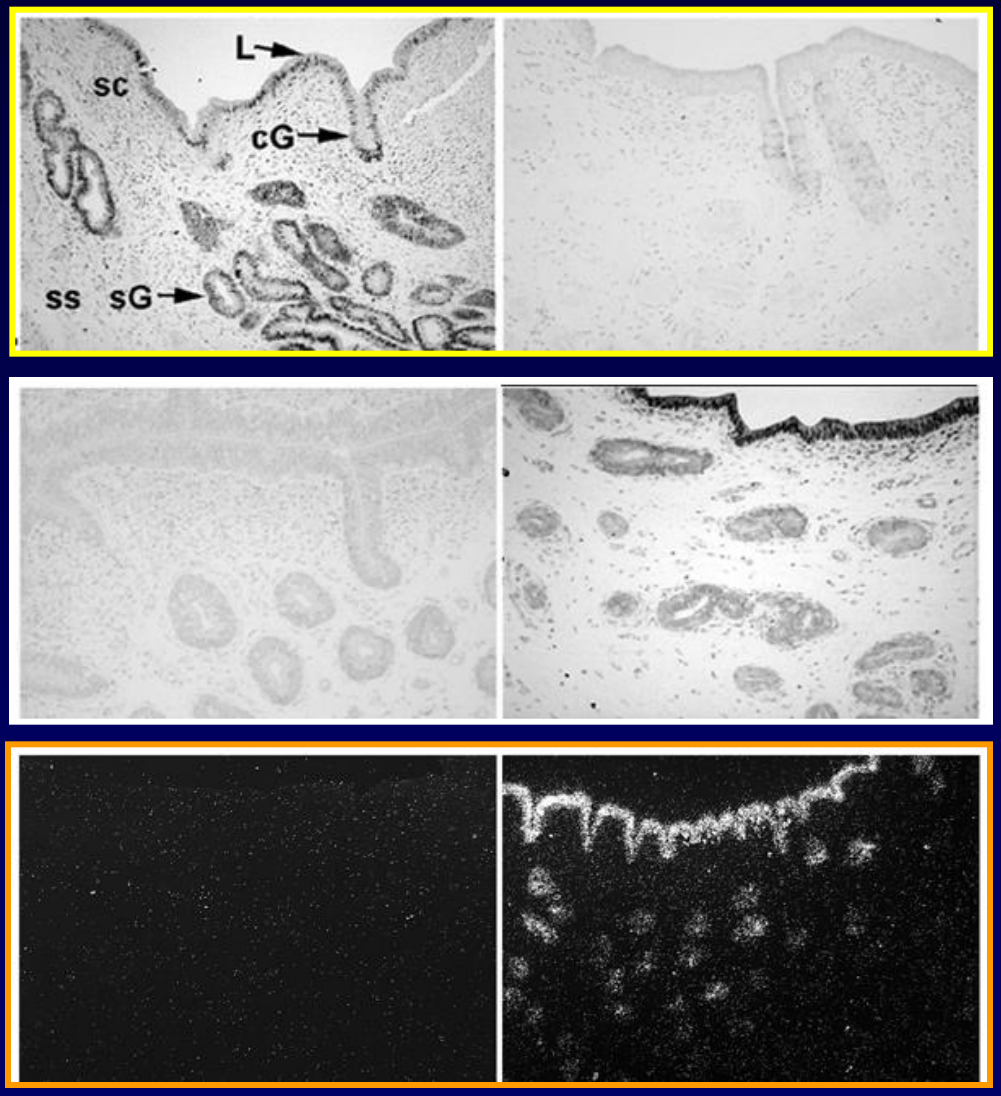
# Hormone Receptors During Development of the Endometrial Luteolytic Mechanism in Cyclic Ewes

**Day 9** **Day 15**

**Progesterone Receptor (PGR)**

**Estrogen Receptor α (ESR1)**

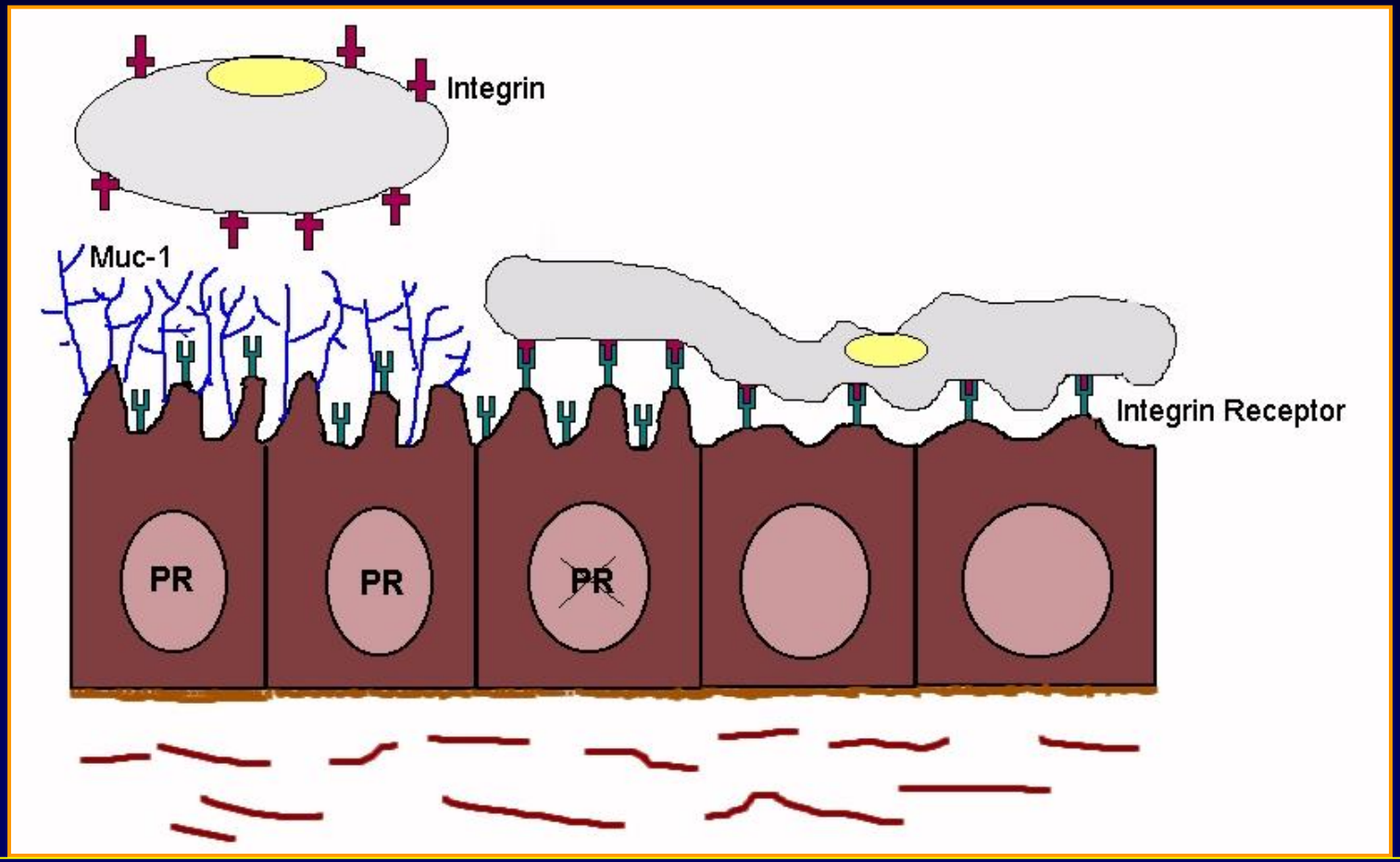
**Oxytocin Receptor (OXTR)**




*Wathes & Hamon, J Endocrinol 1993; 138:479* *Spencer & Bazer, Biol Reprod 1995; 53:1*

# Common Feature of Pregnancy

***Loss of PGR is universal event in rodents, pig, ruminants, ferret, skunk, shrew and human***




- **Decline in anti-adhesive Muc-1**
- **Increase in certain adhesive integrins**
- **Change in patterns of epithelial gene expression**

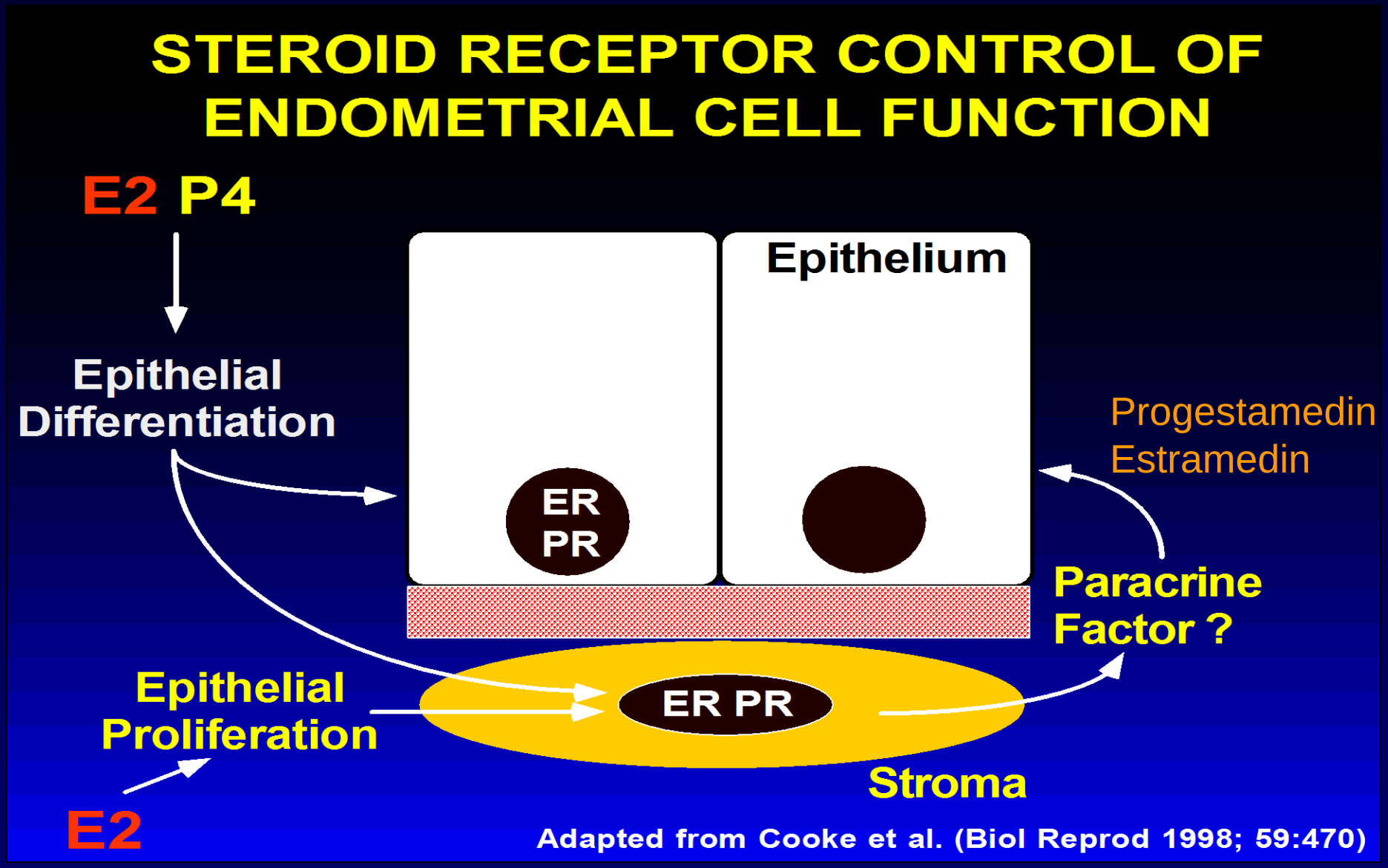
# Fibroblast Growth Factors 7 and 10: Progestamedins

- Fibroblast growth factor-7 (FGF-7)
- 25 - 30 kDa monomeric polypeptide
- Binds to KGFR (FGFR2IIIb) for signaling
- Cell proliferation, differentiation, morphogenesis, anti-apoptosis
- Mesenchymal origin in skin, lung, ovary, prostate, uterus
- **Paracrine growth factor in epithelial-mesenchymal interaction(EMI)**

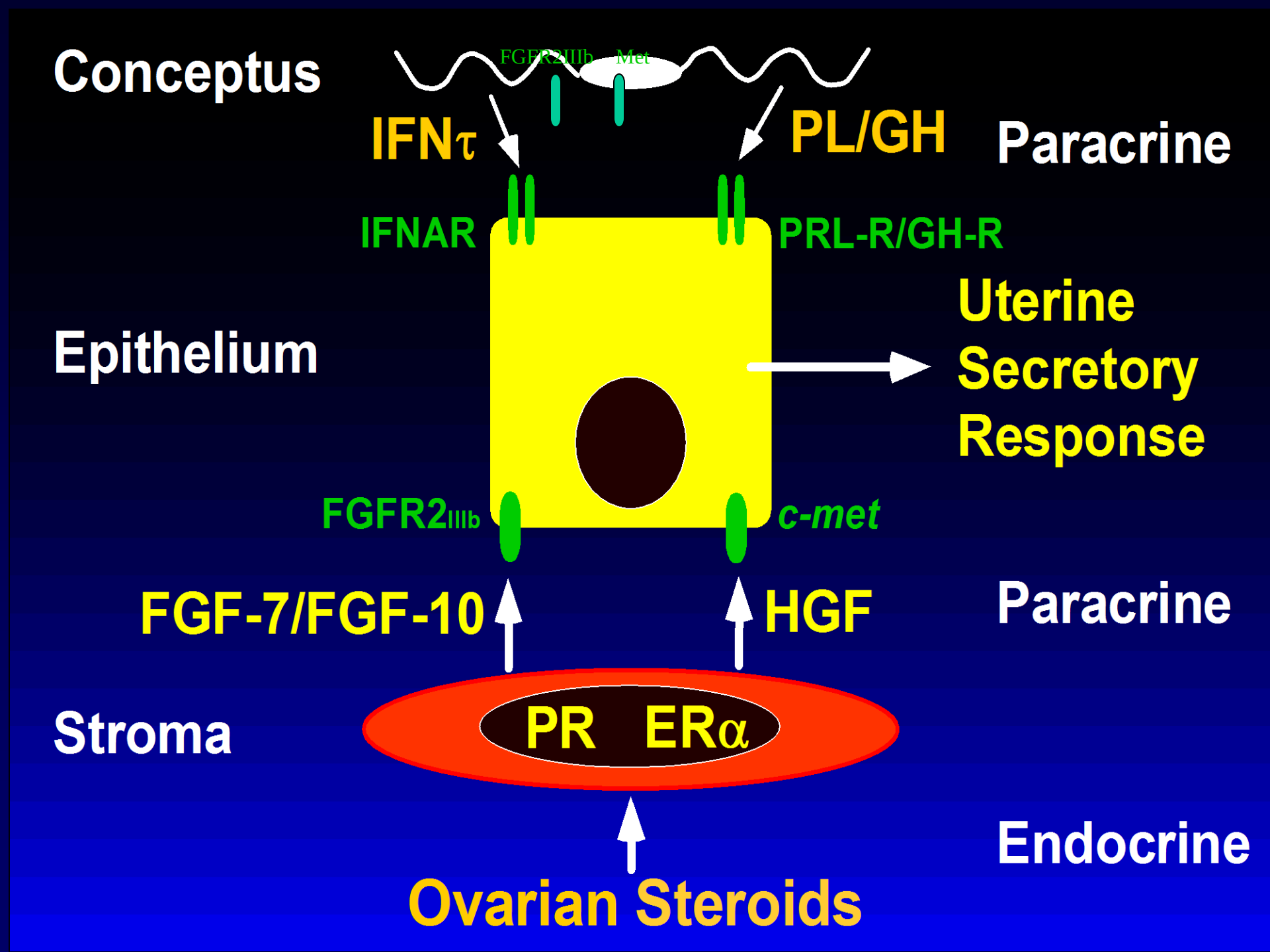
# Hepatocyte Growth Factor(HGF, Progestamedin/Estramedin

- **728 amino acid heparin binding protein**
- **Isolated from rat platelets as a mitogen for hepatocytes in primary culture**
- **Pro-HGF is converted to heterodimer**
- **Multi-function: mitogenesis, morphogenesis, angiogenesis, motogenesis**
- **Widely expressed (testis, prostate, ovary, uterus, placenta)**
- **Estrogen up-regulates HGF mRNA in mouse ovary and primate uterus**
- **Paracrine Growth Factor that mediates stromal-epithelial cell signaling**

# COMMON FEATURES OF PREGNANCY



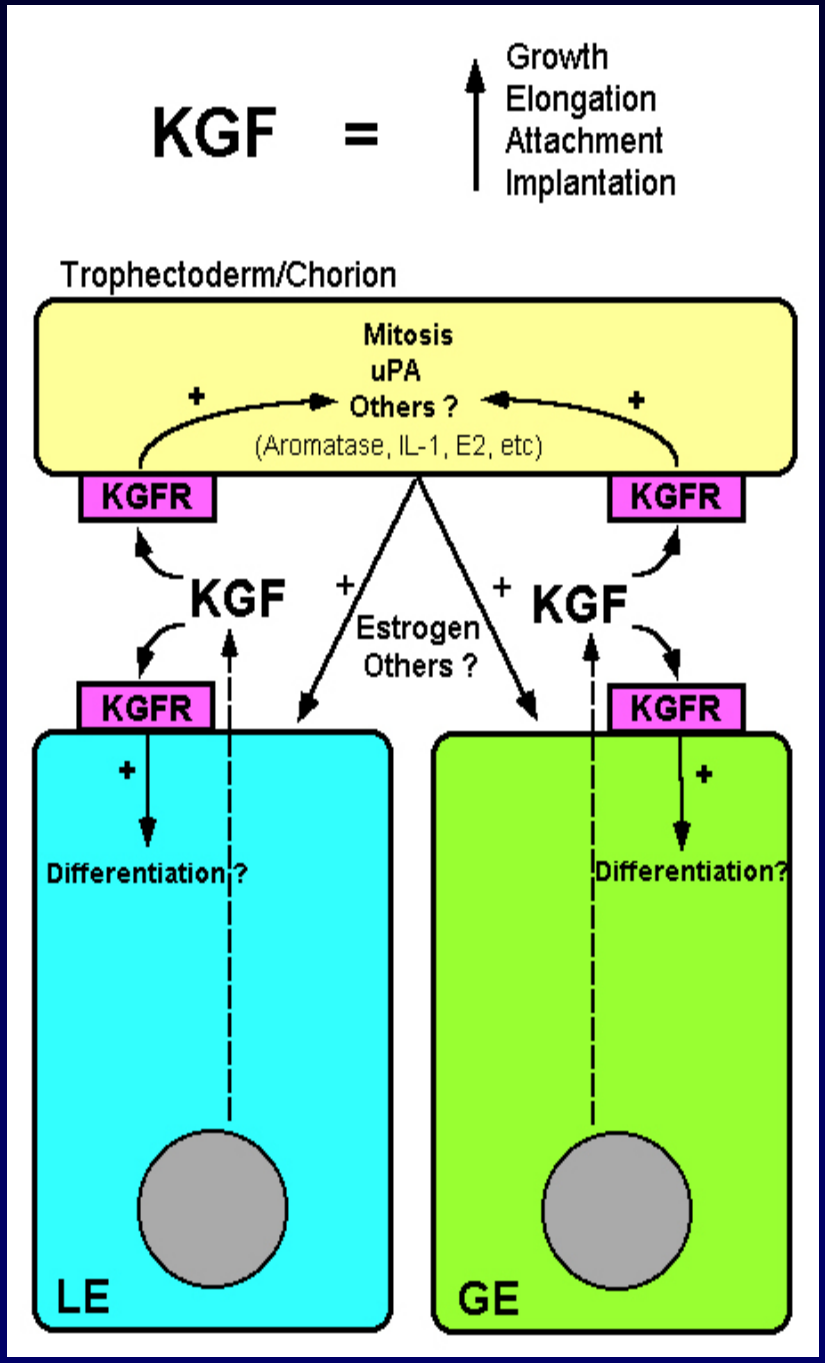

Why Do SC Remain PGR Positive? Does P4 regulate progestamedins?


Conceptus
FGFR2IIIb
Met
IFNτ
PL/GH
Paracrine
IFNAR
PRL-R/GH-R
Epithelium
Uterine
Secretory
Response
FGFR2IIIb
c-met
FGF-7/FGF-10
HGF
Paracrine
Stroma
PR
ERα
Endocrine
Ovarian Steroids

**Working Model of FGF7/KGF Expression and Function in the Porcine Uterus**

**FGF7 Expressed by LE to Day 20 and then by GE**

**FGFR2IIIb Expressed by LE, GE and Tr**



**Responses to target cell to E2:**

1. Histamine mobilization
2. Hyperemia

a.growth of blood vessels

b.vasodilation

3. Lysosome labilization (lysosomal membrane becomes more fragile.
4. in RNA and protein synthesis
5. in lipid metabolism because Ca mobilization and arachadonic acid production
6. in secretion-due to release of secretory vesicles (stimulus-secretion coupling)-some pancreatic proteins can be release this way.
7. precursor uptake-amino acid production and glucose
8. _mitotic activity
9. cell hypertrophy
10. in membrane excitability
11. **in OXTR** (parturition, luteolytic mechanism)
12. in Ca mobilization
13. water inhibition of tissue

**Responses to target cells to progesterone:**

1. RNA and protein synthesis
2. growth of uterine glands
3. water inhibition
4. membrane potential (smooth muscle cell relaxed) LD50 -because can cause relaxation of the diaphragm and kill rat
5. phospholipid stores
6. PG synthesis (PG synthase and phopholipid stores must be present)
7. substrate (AA and glucose) uptake
8. mitotic activity

# Biological Effects of Progesterone

- Converts uterine endometrium to secretory stage for implantation and pregnancy.
- Increases thickness of vaginal epithelium and cervical mucus to form cervical “plug”
- Prepares uterus to produce prostaglandin F2-alpha to regress CL in subprimate mammals
  - Increase phospholipids in uterine epithelia that yield arachadonic acid
  - Increase Prostaglandin Synthase II that converts arachidonic acid to prostaglandins, e.g., PGF2α

# Biological Effects of Progesterone

- Stimulates development of uterine glands for pregnancy
- Stimulates uterine secretions to support development of conceptus to term
- Decreases contractility of uterine myometrium
- Inhibits lactation during pregnancy: decrease in P4 necessary for milk production.
- Decreases to allow myometrial contractions
- Precursor for placental estrogens and adrenal cortisol

# Biological Effects of Progesterone

- Down-regulates estrogen receptors in uterine epithelia
- Down-regulates progesterone receptors in uterine epithelia
  - A prerequisite for implantation
  - A prerequisite for gene expression by uterine epithelia
- Induces expression of progestamedins by uterine stromal cells, e.g., FGF7, FGF10, HGF

# Biological Effects of Progesterone

- Neuroprotective - protects nerve myelination
- Improves memory and cognitive functions
- Suppresses apoptotic (cell death) genes
- Mood stabilizer with analgesic effects
- LD50 due to relaxation of neuronal inputs into smooth muscle such as diaphragm
- Increases endorphins, enkephalins and dynorphins to decrease pain

# Other Biological Effects of Progesterone

- Increase epidermal growth factor to induce cell proliferation and sustain stem cells
- Increases core body temperature
- Anti-inflammatory and regulates immune cells
- Normalizes blood clotting and vascular tone
- Prevents uterine cancer, perhaps by ensuring down-regulation of progesterone and estrogen receptors in uterine epithelia
- Prevents mammary tumors, perhaps by ensuring down-regulation of progesterone and estrogen receptors in mammary epithelia

# Biological Effects of Estrogen
# Female Sex Hormone

- Promote development of female secondary sexual characteristics
  - breasts, uterus, recovery of endometrium after menses
  - thickening of the endometrium during follicular phase of menstrual cycle
  - regulate menstrual cycle via effects on GnRH and uterine production of PGF2α

# Biological Effects of Estrogen
# Female Sex Hormone

- Induces closure of epiphyseal plate on long bones
- accelerate metabolism (burn fat)
- reduce muscle mass
- stimulate endometrial growth
- increase uterine growth
- increase vaginal lubrication
- thicken the vaginal wall
- maintenance of blood vessels and skin
- reduce bone resorption, increase bone formation – supresses acid phosphatase 5/uteroferrin

# Biological Effects of Estrogen

- protein synthesis
  - Increase production of steroid binding proteins in liver
- coagulation
  - increase circulating level of factors 2, 7, 9, 10, plasminogen
  - decrease antithrombin III
  - increase platelet adhesiveness
- Lipid
  - increase HDL, triglyceride
  - decrease LDL, fat deposition
- Fluid balance
  - Sodium and water retention

# Biological Effects of Estrogen

- Hormones
  - increase cortisol, Steroid Hormone Binding Globulin
- Gastrointestinal tract
  - reduce bowel motility
  - increase cholesterol in bile
- Melanin
  - increase pheomelanin, reduce eumelanin (skin pigments)
- Cancer
  - support hormone-sensitive breast cancers
- Lung function
  - promotes lung function by supporting alveoli
- Sexual desire is dependent on androgen levels and estrogen levels

# Biological Effects of Estrogen

- In mice, estrogens (which are locally aromatized from androgens in the brain) play an important role in psychosexual differentiation
- Estrogen withdrawal:  mood lowering and depression - recovery from postpartum, perimenopausal, and postmenopausal depression
- Negative feedback on hypthalamus and anterior pituitary to reduce circulating levels of FSH and LH
- Estrogen during proestrus increases GnRH Receptors in Anterior Pituitary (gonadotrophs) and release of ovulatory surge of LH

# Biological Effects of Estrogen

- Increase receptors for progesterone and estrogens in reproductive tissues and mammary tissues
- Induces expression of genes, e.g., oxytocin
- Stimulates cell proliferation
- Increases mobilization of histamines and lipids, such as lysophosphatidic acid that stimulates migration of embryos in pigs and rodents and perhaps other species
- Induces increases in vasodilation of blood vessels
- Key component of uterine luteolytic mechanism

# Half-Life of Protein, Amine and Steroid Hormones in Plasma

**Table 1-3** Half-Life of Protein, Amine, and Steroid Hormones in Plasma

| Hormone | Half-life |
|---|---|
| Amines | 2–3 minutes |
| Thyroid hormones | |
| $T_4$ | 6.7 days |
| $T_3$ | 0.75 day |
| Polypeptides | 4–40 minutes |
| Proteins | 15–170 minutes |
| Steroids | 4–120 minutes |

# Hormone metabolism

- **Inactivation**
  - Regulatory mechanism
  - Intracellular and extracellular
  - Enzymatic
    - Carboxypeptidases, aminopeptidases, endopeptidases
    - Deamination
    - Reduction of dissulfide bonds

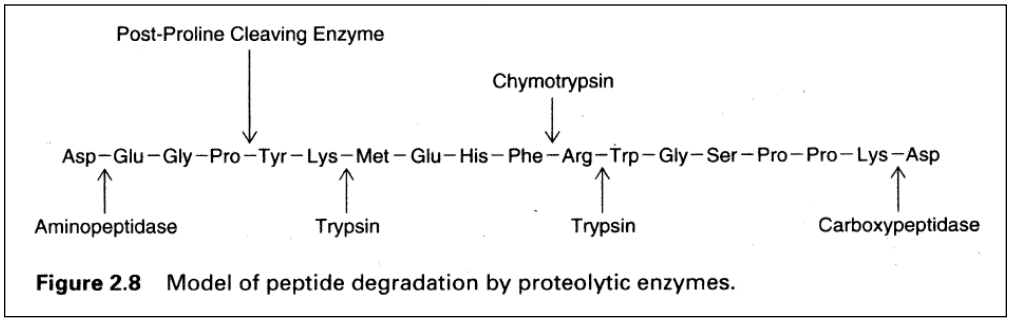
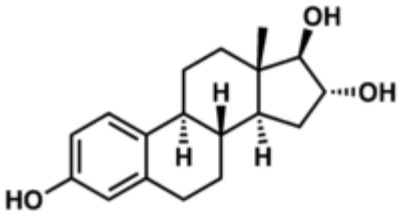


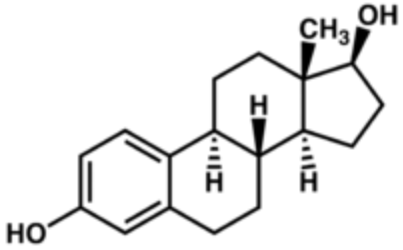
**Figure 2.8** Model of peptide degradation by proteolytic enzymes.

  - **Conjugation (steroid hormones – sulfate and glucuronide forms)**
  - Deiodination (thyroid hormone)

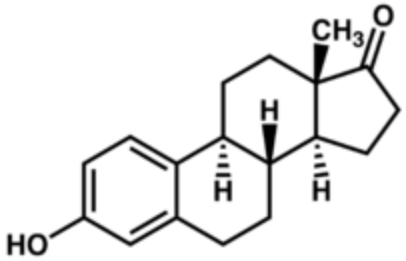
ESTRIOL, E3



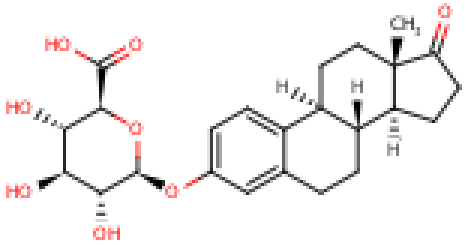

ESTRADIOL, E2



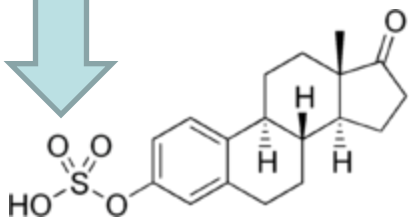

ESTRONE, E1



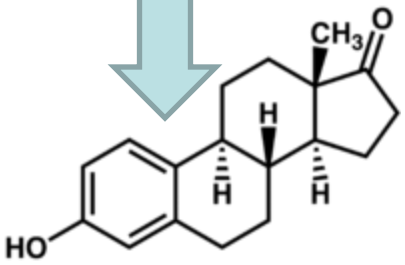

UDP glucuronyltransferase




Sulfotransferase




Sulfatase



# PROLACTIN

Prolactin is a single chain polypeptide of 199 amino acids with a molecular weight of about 24,000 daltons. Its structure is similar to that of growth hormone and placental lactogen. The molecule is folded due to the activity of three disulfide bonds.

# Major Functions of Prolactin

- Mammogenesis and Lactogenesis
- Formation and Function of Corpus Luteum, particularly in rodents
- Immunological Competence
- Uterine Secretory Activity
- Lung Maturation in Fetus
- Activates Janus Kinase – Signal Transducer and Activator of Transcription Cell Signaling
- Transport of Water and Electrolytes Across Membranes
- Rapid Eye Movement (REM) Sleep

# Lactogenic Hormones

- PROLACTIN - PRL
- PLACENTAL LACTOGEN - PL
- DECIDUAL PROLACTINS -dPRL

**ALL SIGNAL VIA HOMODIMER OF PROLACTIN RECEPTORS OR HETERODIMER OF PROLACTIN RECEPTOR AND GROWTH HORMONE RECEPTOR**

# Rodents

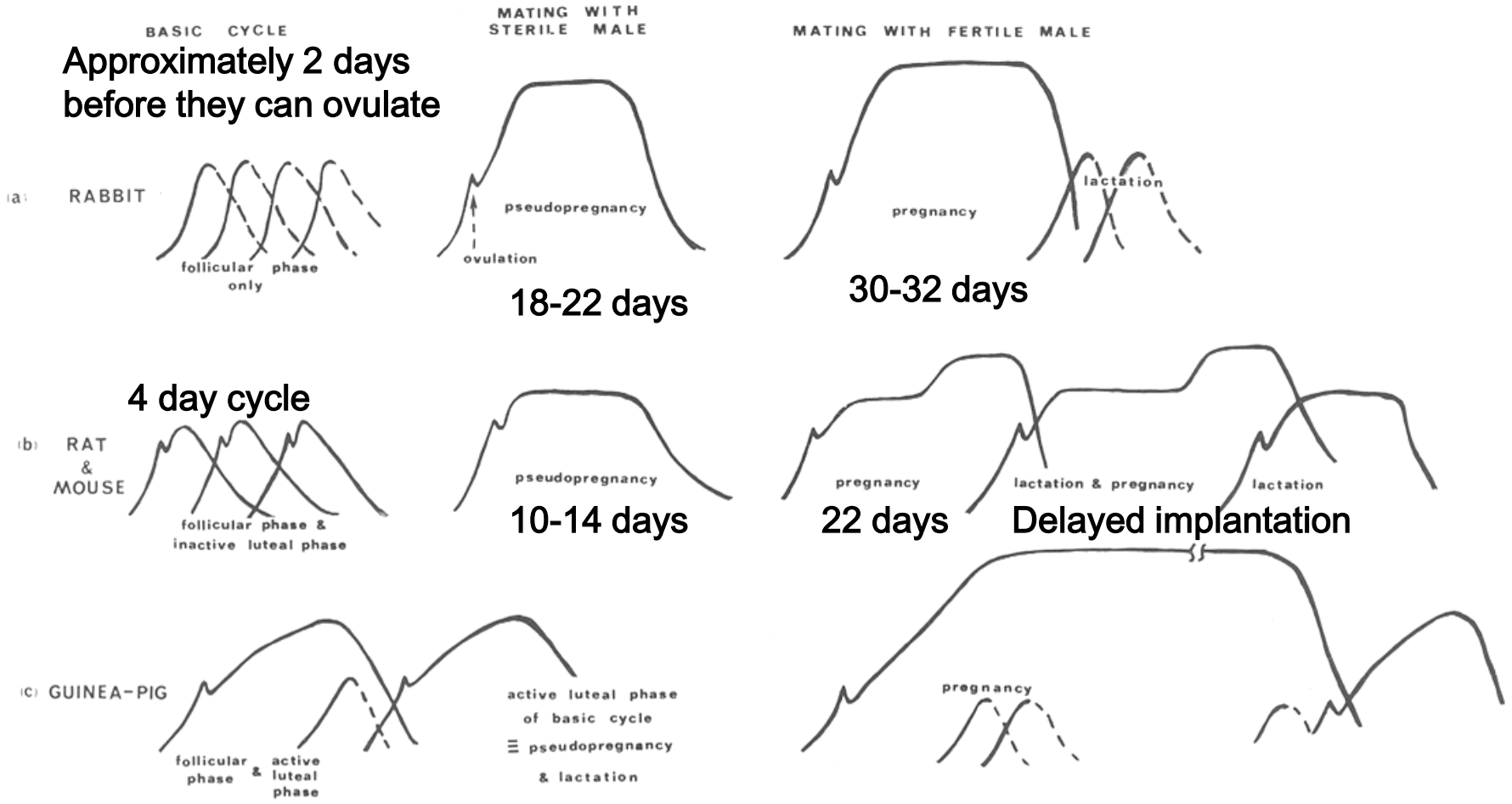
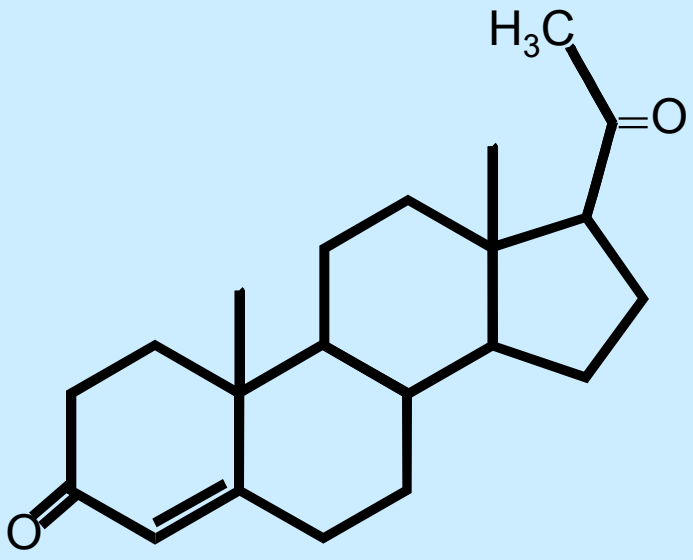



**Fig. 7.1** Diagram of various types of ovarian cycle depicting the growth of the follicles and the corpora lutea as modified in various reproduction states in (a) reflexly and (b, c) spontaneously ovulating mammals. (From Rowlands & Weir 1977)

Marshalls Physiology of Reproduction, p460

# 20α-Hydroxysteriod Dehydrogenase
# 20α-HSD

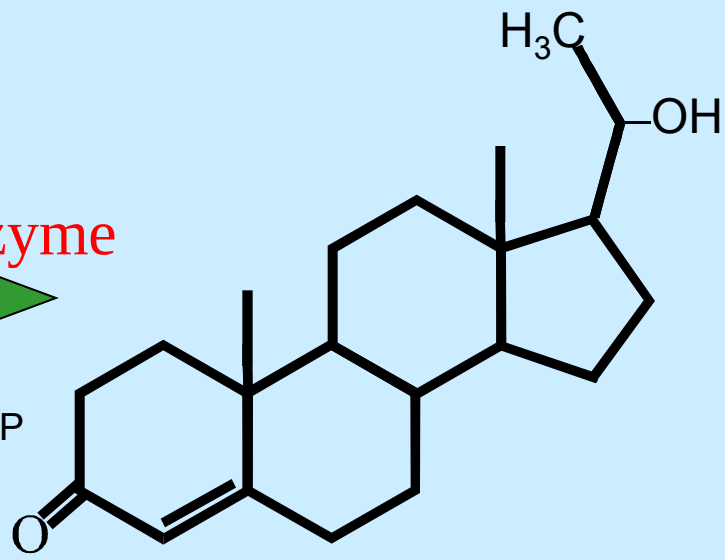



Progesterone

Prolactin
Inhibits Enzyme

20α-HSD

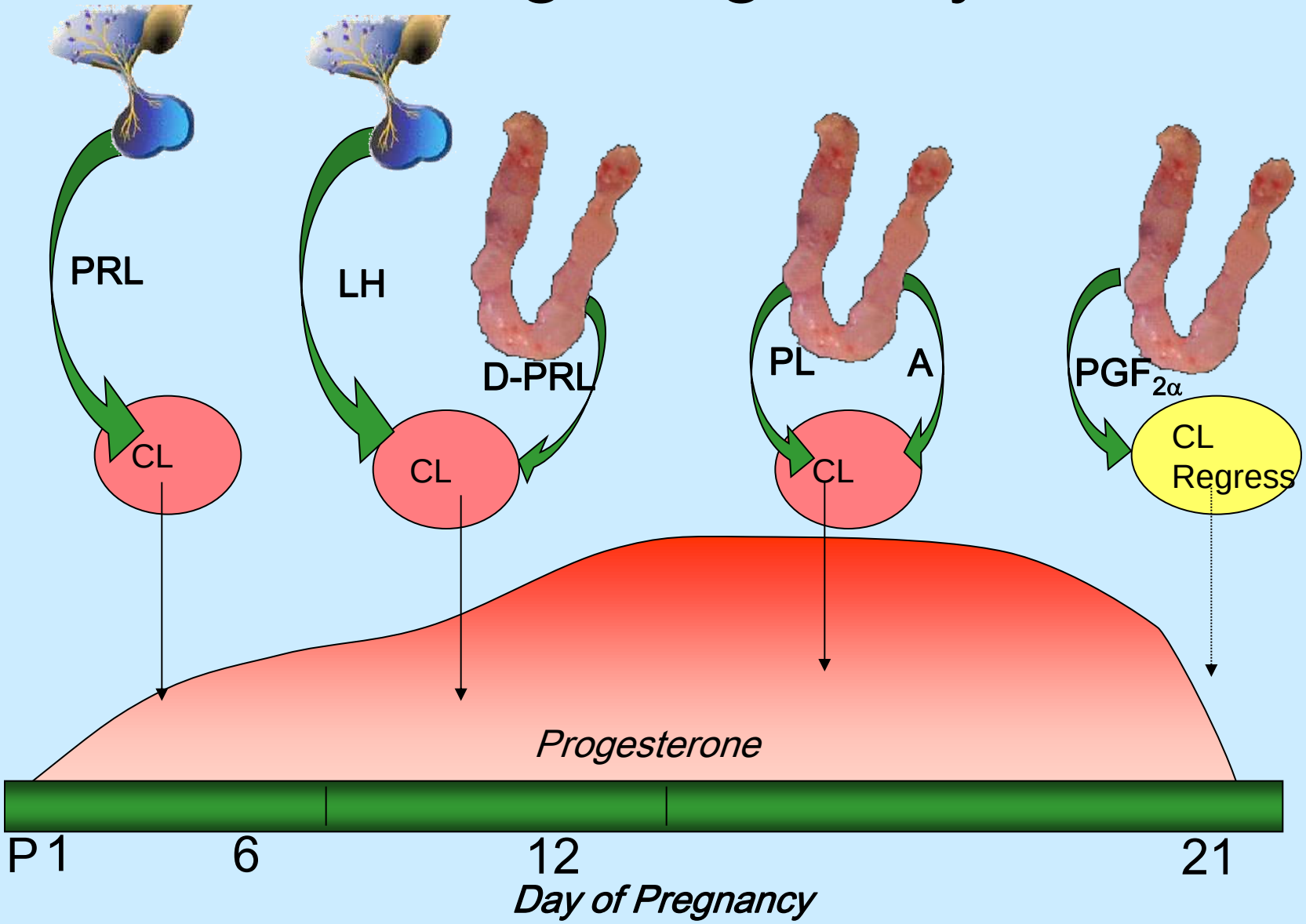
NADPH NADP




20α-dihydroprogesterone

Does not support pregnancy
or decidualization in rodents

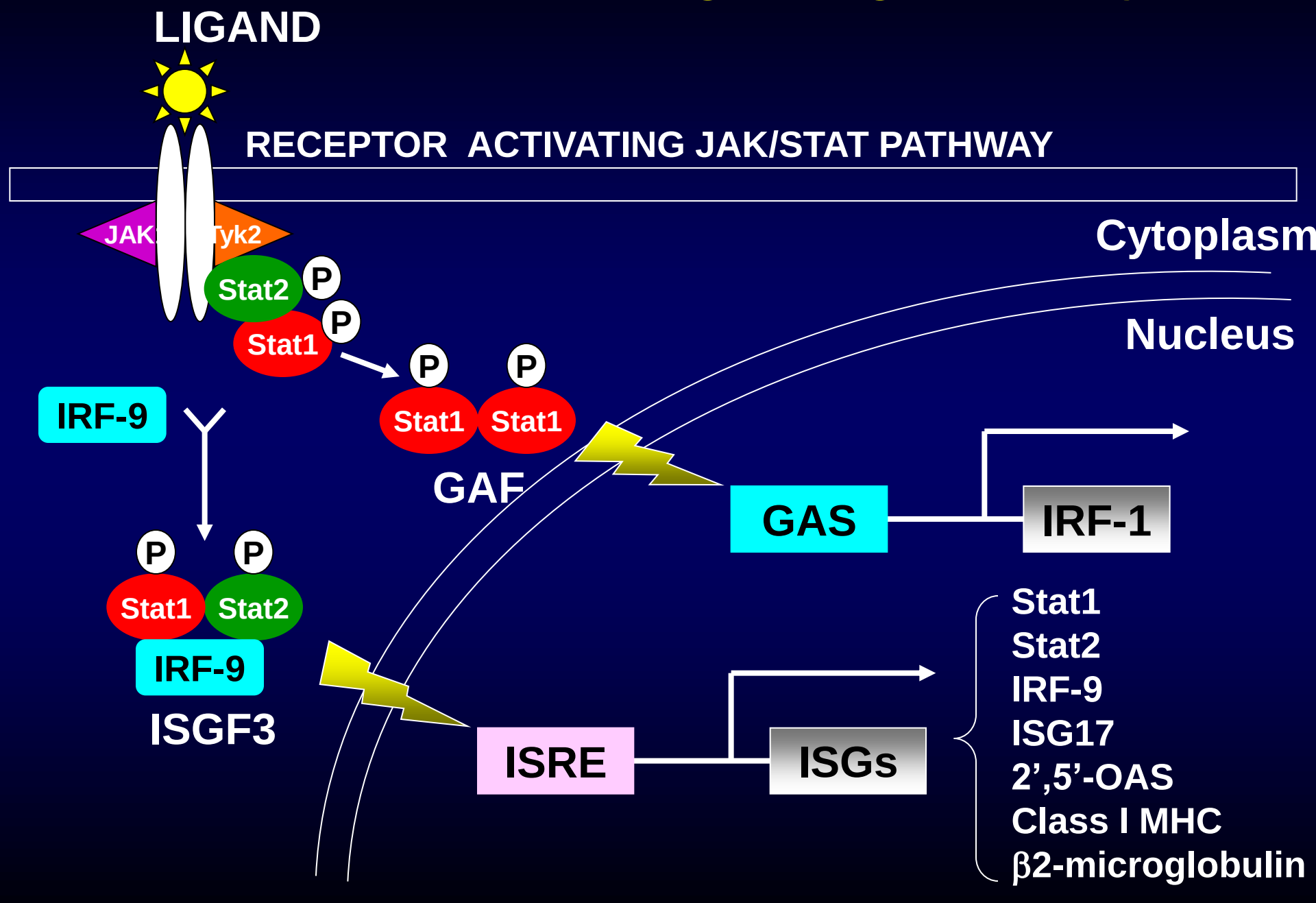
# Regulation of Rat Corpus Luteum during Pregnancy

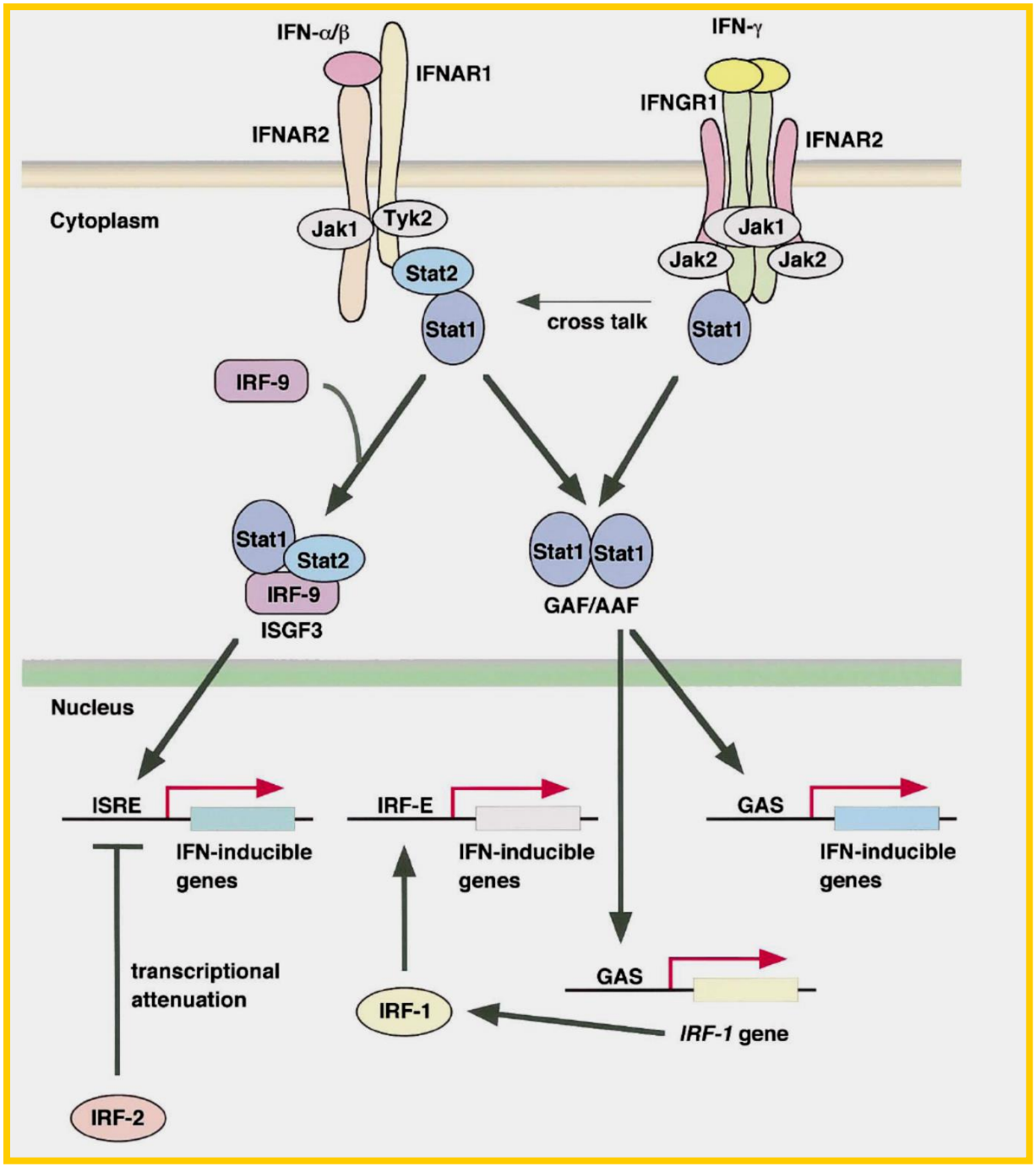


# NOMENCLATURE

- PKA:  protein kinase A –
- JAK:  Janus Kinase
- STAT: Signal Transducer and Activtor of Transcription
- GAF:  Gamma Activation Factor
- GAS:  Gamma Activation Sequence
- IRF:  Interferon Regulatory Factor
- IRE:  Interferon Response Element
- ISRE:  Interferon Stimulatory Response Element
- ISGF3:  Interferon Stimulated Gene Factor 3

# JAK/STAT CELL Signaling Pathway






**Type I Interferon (IFN)**
Alpha
Beta
Omega
Tau

**Type II IFN**
Gamma

**PROLACTIN**

**GROWTH HORMONE**

IFNAR = IFNA receptor

IFNGR = IFNG receptor

IRF= IFN regulatory factor

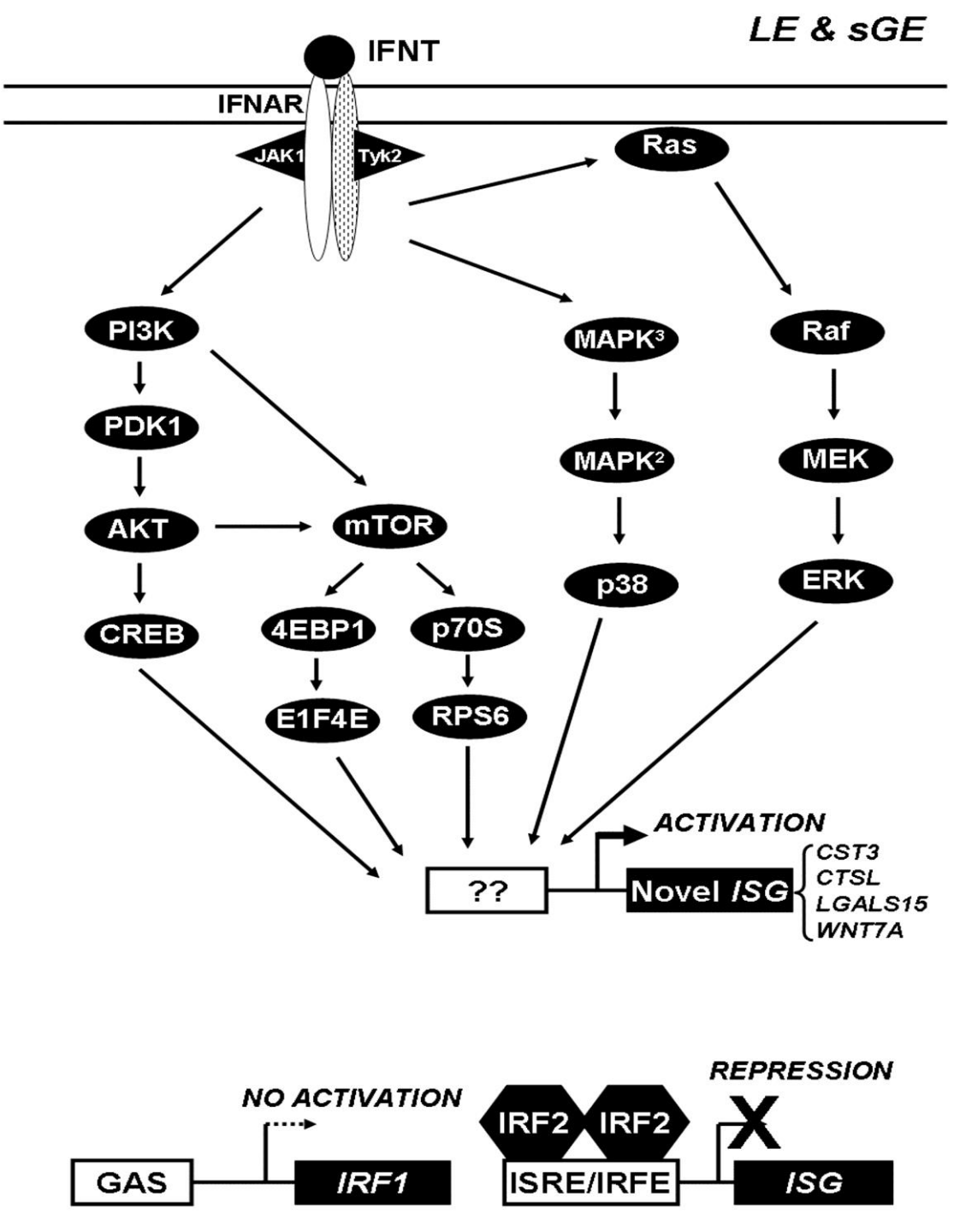
GAS= gamma activation sequence

ISRE = IFN-stimulated response element

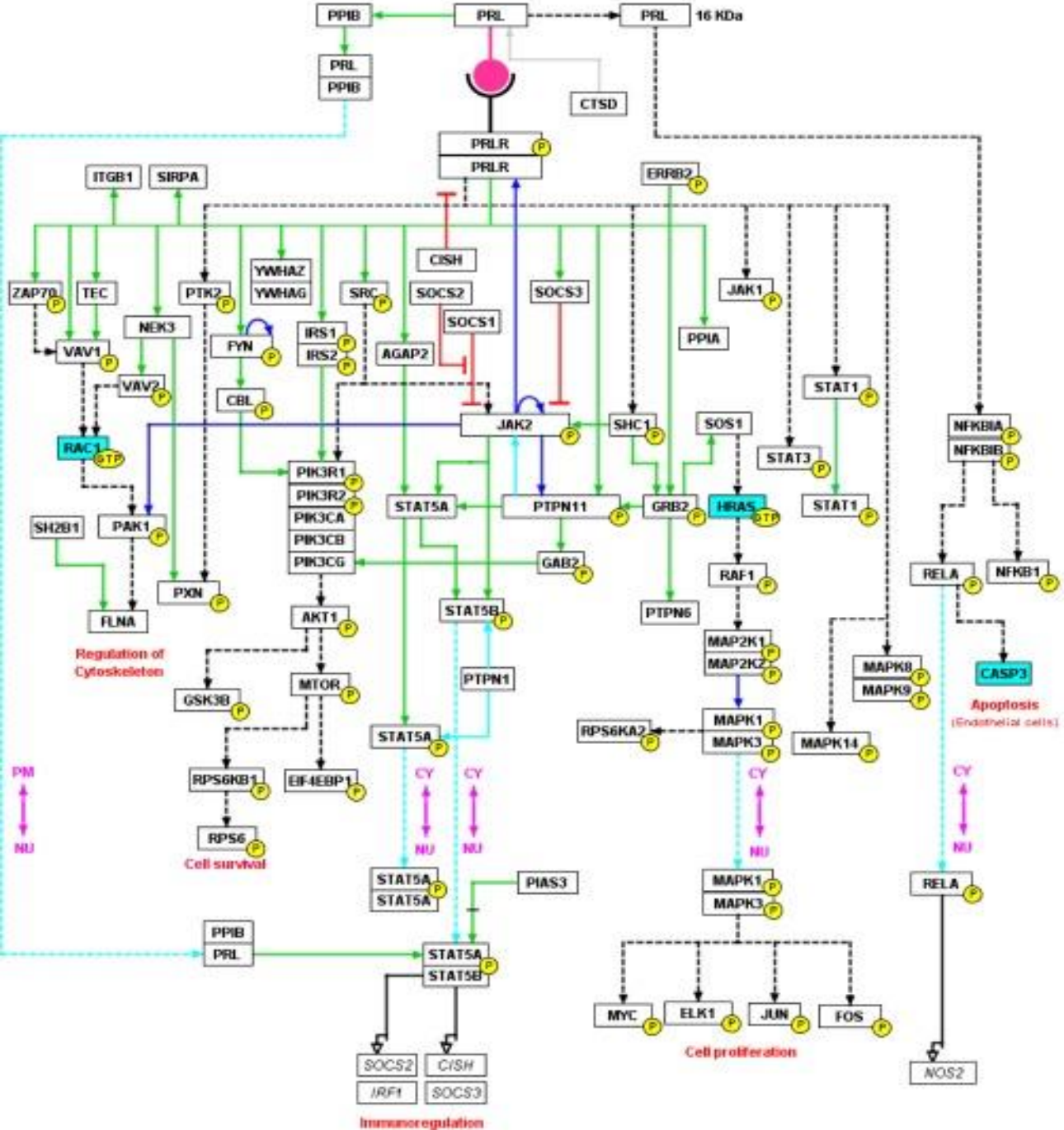
# Alternate Type I IFN Cell Signaling Pathways

Platanias LC, Nature Reviews Immunology 2005: 5:375-386

Cross-Talk: IFN Cell Signaling and Stromal Cell Derived Progestamedin(s) Cell Signaling



# Prolactin Signaling Pathway



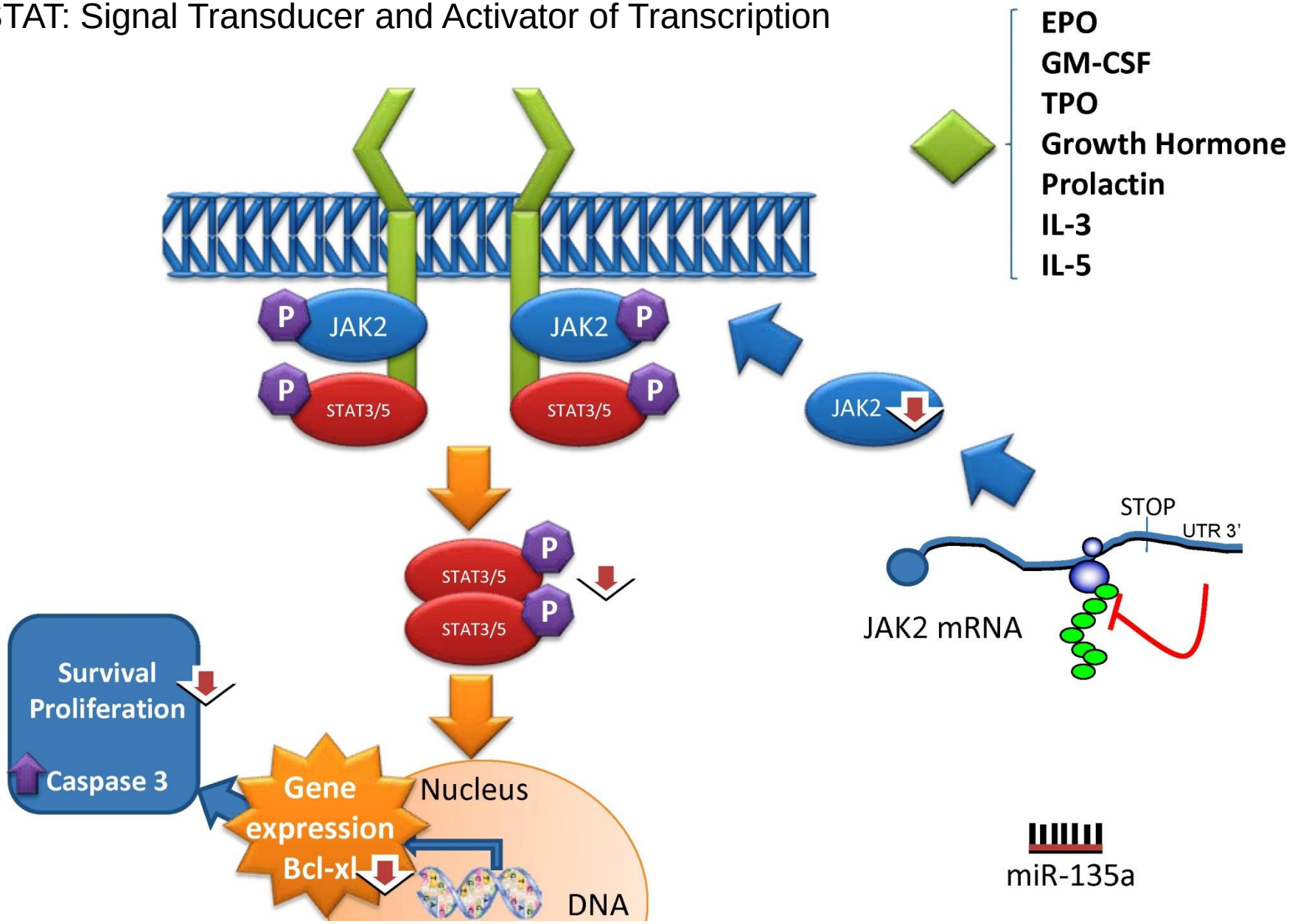

## LEGEND

Ligand
Receptor
Protein
mRNA
Enzyme complex

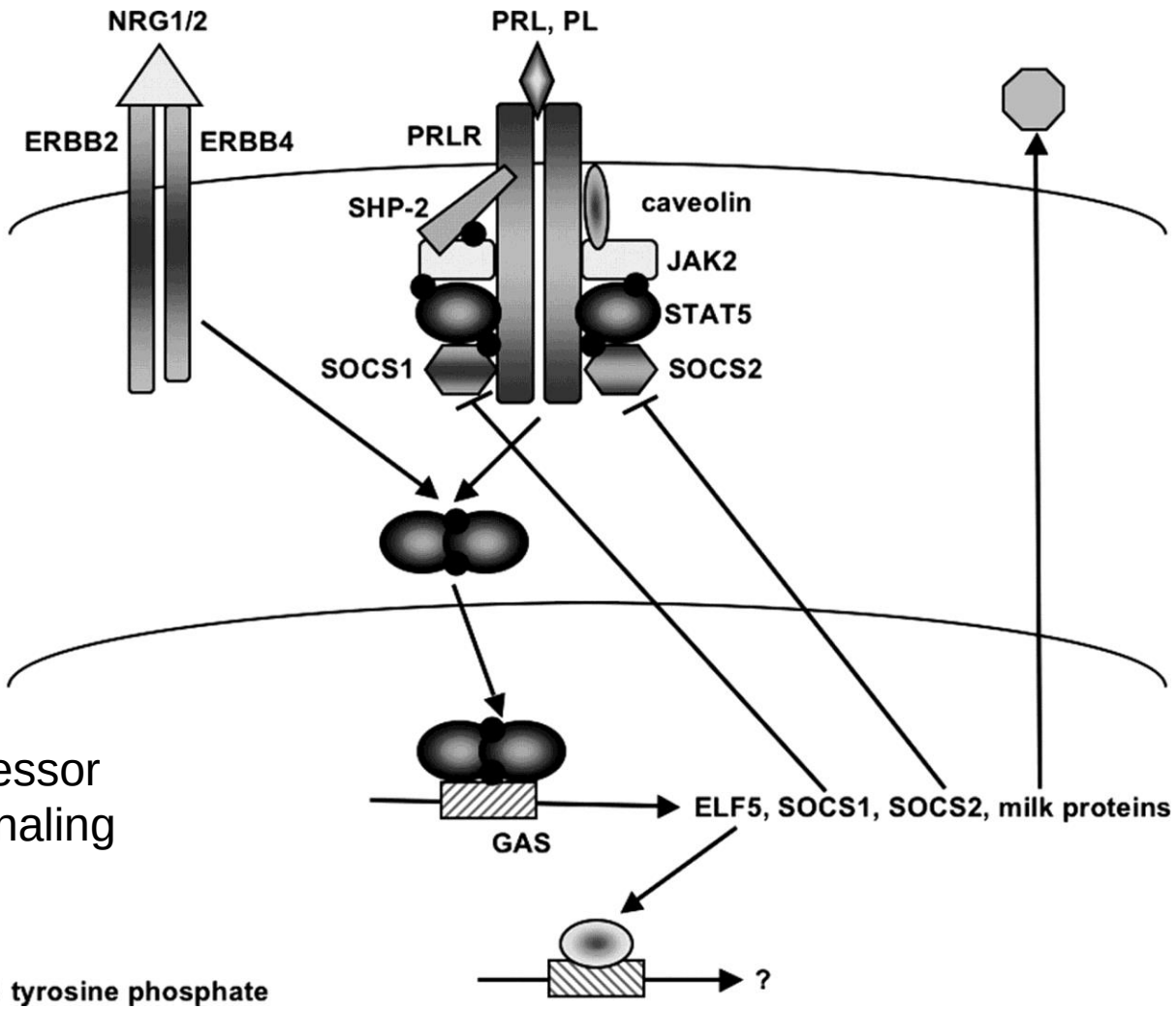
- Protein-protein interaction
- Protein-protein dissociation
- Leads to through unknown mechanism
- Positive regulation of gene expression
- Negative regulation of gene expression
- Auto catalysis
- Acetylation
- Deacetylation
- Phosphorylation
- Dephosphorylation
- Sumoylation
- Desumoylation
- Ubiquitination
- Deubiquitination
- Methylation
- Demethylation
- Palmitoylation
- Proteolytic cleavage
- Inhibition
- Transport
- Protein — Induced catalysis
- Protein — Induced activation
- Translocation

PM Plasma membrane
CY Cytoplasm
EC Extracellular
EN Endosome
ER Endoplasmic reticulum
GO Golgi apparatus
MT Mitochondrion
NU Nucleus

JAK: Janus Kinase
STAT: Signal Transducer and Activator of Transcription



**Regulation of STAT signaling in mammary epithelial cells.**



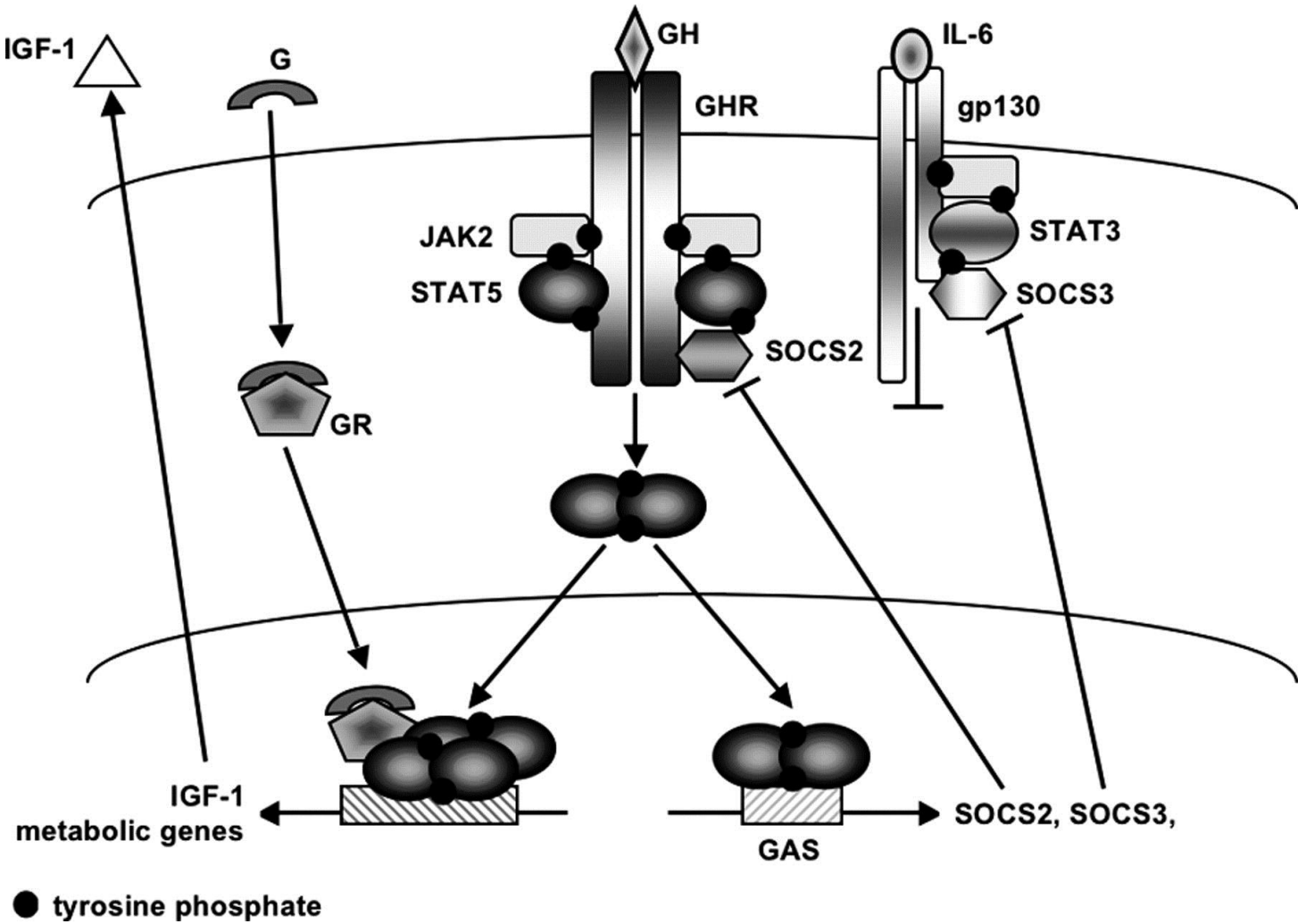

SOCS – Suppressor of Cytokine Signaling

**Hennighausen L , and Robinson G W Genes Dev. 2008;22:711-721**

GAS: Gamma Activation Sequence

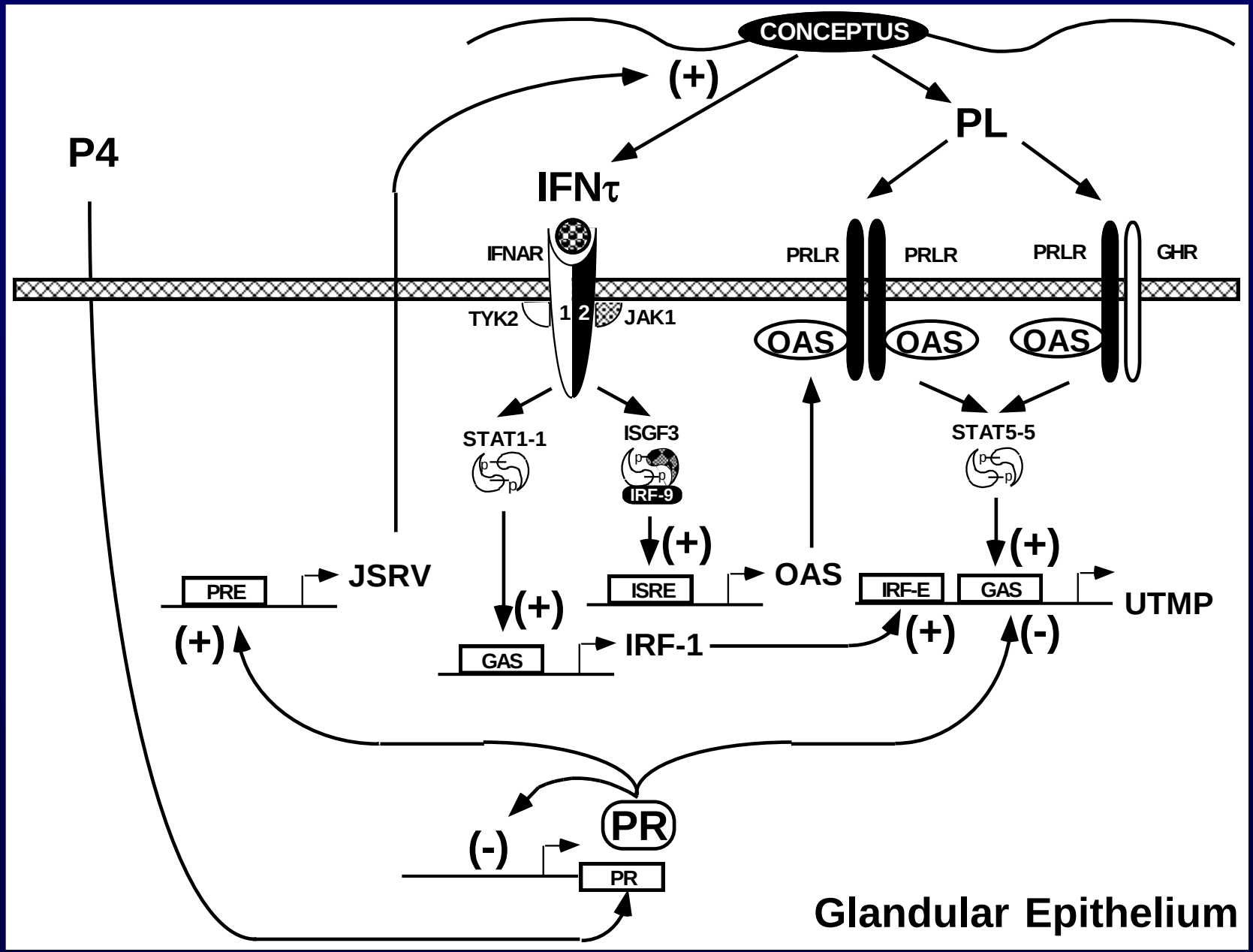
ETS: Epithelium Specific Transcription Factor

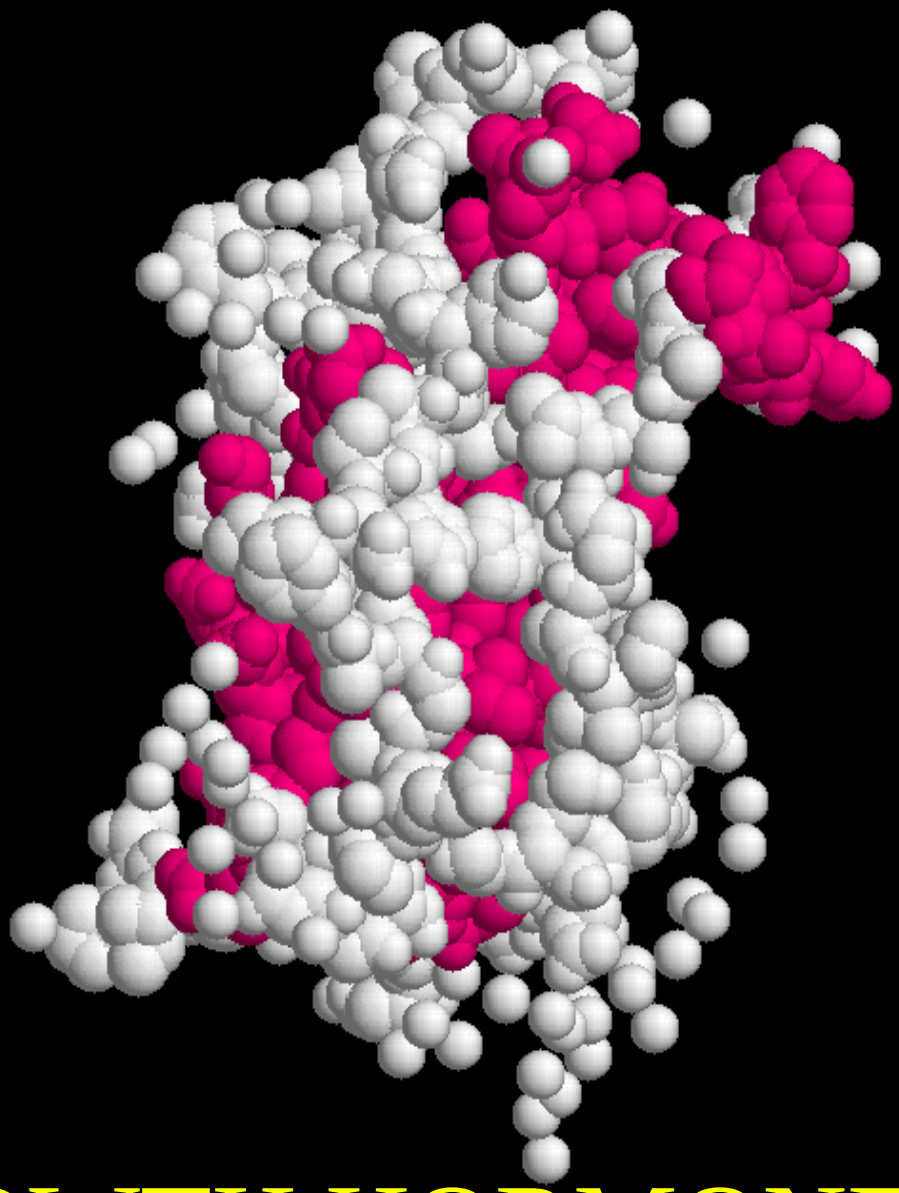
# Interaction of GHR and GR signaling through STAT5 in hepatocytes.




Hennighausen L , and Robinson G W Genes Dev.
2008;22:711-721

# Servomechanism of Pregnancy Hypothesis





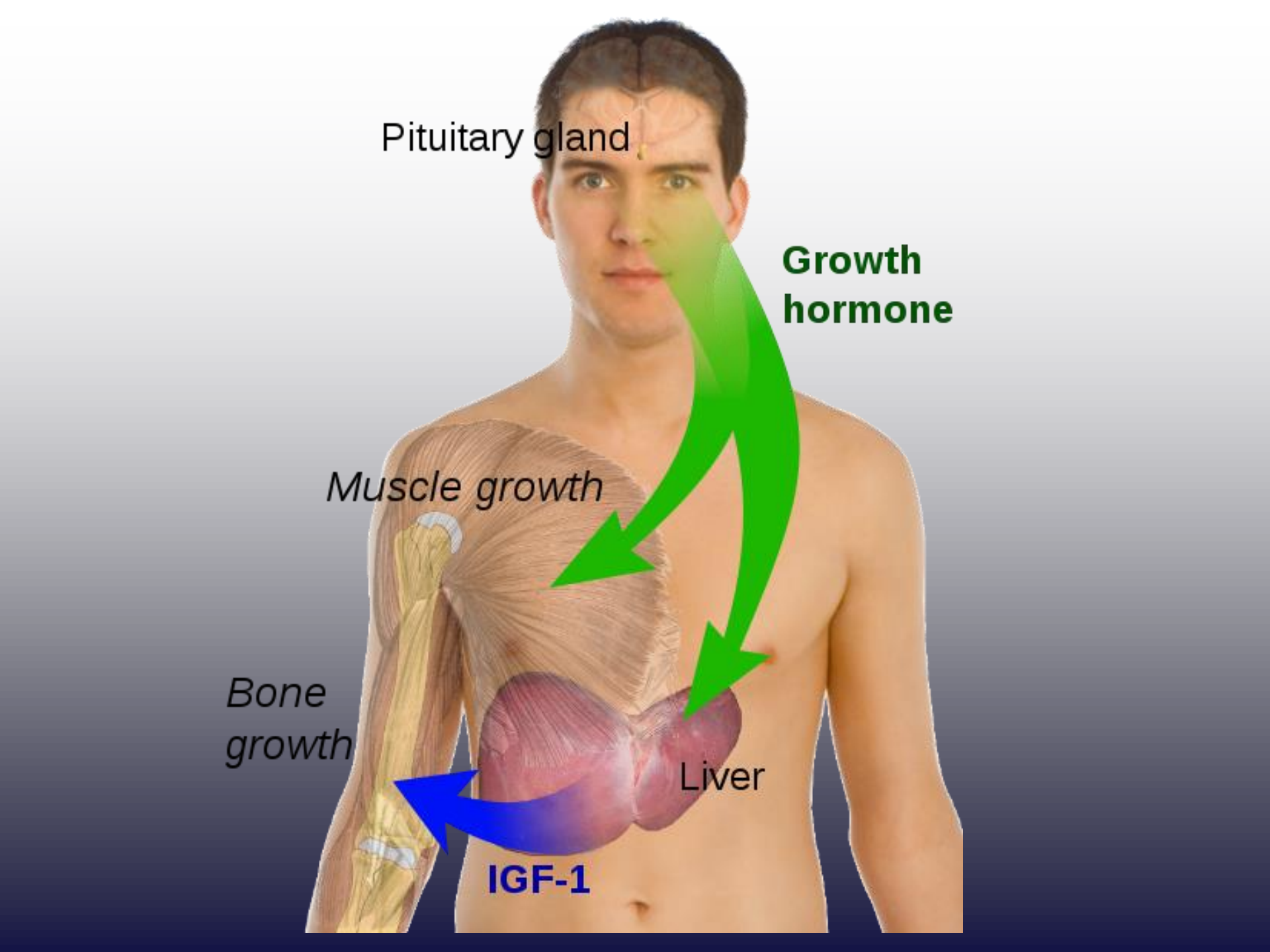
GROWTH HORMONE

# Growth Hormone (GH)

- **A 191-amino acid, single-chain polypeptide hormone that is synthesized, stored, and secreted by the somatotroph cells within the anterior pituitary gland.**
- ***Stimulators* of GH secretion include:**
  - **GH releasing hormone (GHRH) or somatocrinin)**
  - **Androgens from adrenal cortex and testes**
  - **Estrogen**
  - **L-DOPA stimulates**
  - **Arginine by inhibiting somatostatin release**
  - **vigorous exercise**

# Growth Hormone

- *Inhibitors* of GH secretion include:
  - somatostatin from the periventricular nucleus
  - circulating concentrations of GH and IGF-1 (negative feedback on the pituitary and hypothalamus)
  - hyperglycemia
  - glucocorticoids
  - dihydrotestosterone


Pituitary gland
Growth hormone
Muscle growth
Bone growth
Liver
IGF-1

# Functions of Growth Hormone

- stimulates division and multiplication of chondrocytes of cartilage.
- stimulates production of insulin-like growth factor 1 by liver and IGF-1 has:
  - growth-stimulating effects on a wide variety of tissues. stimulatory effects on osteoblast and chondrocyte activity to promote bone growth.
  - Increases calcium retention, and strengthens and increases the mineralization of bone
- Increases muscle mass through sarcomere hyperplasia

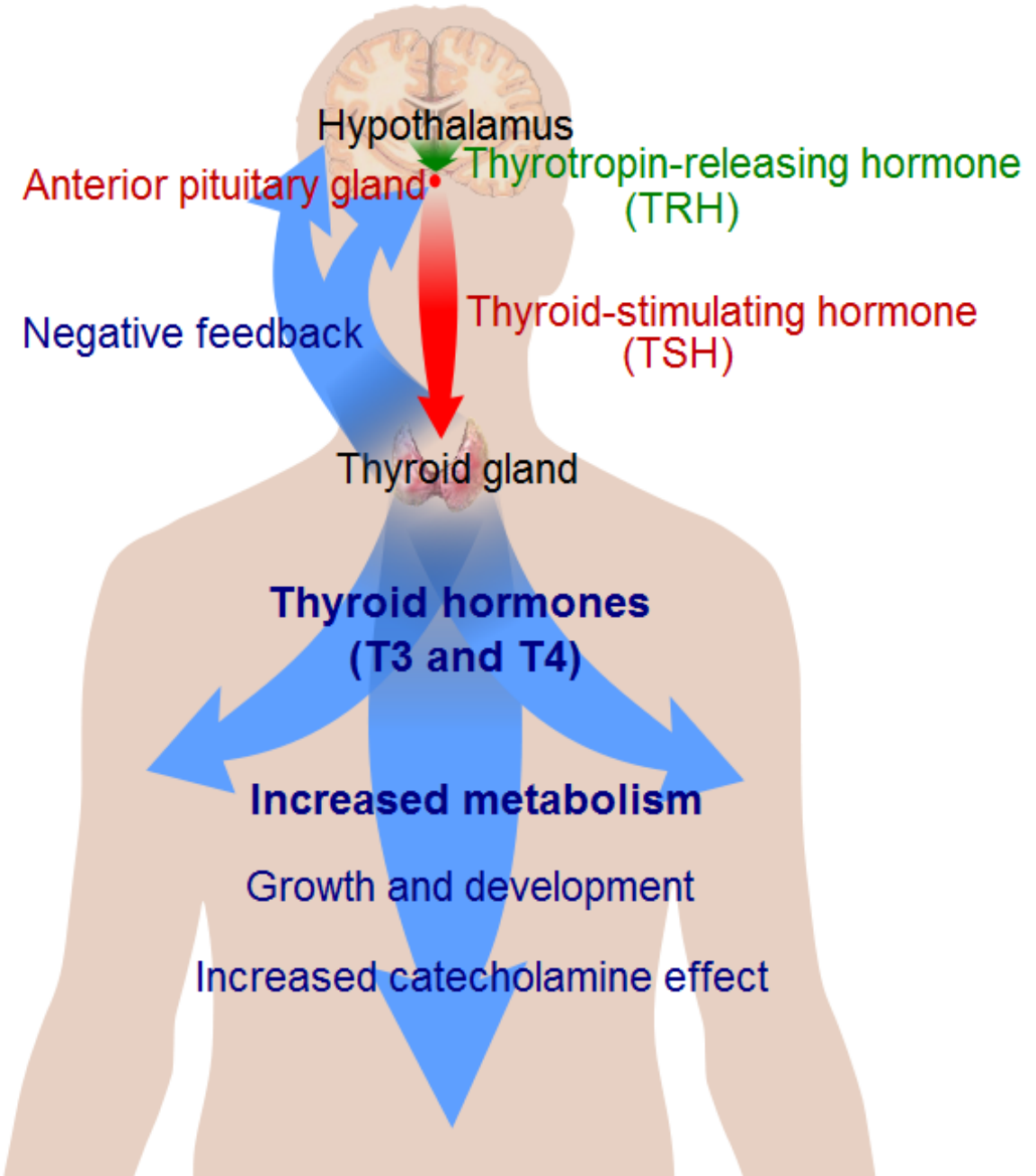
# Functions of GH

- Promotes lipolysis
- Increases protein synthesis
- Stimulates growth of all internal organs excluding the brain
- Reduces liver uptake of glucose
- Promotes gluconeogenesis in the liver
- Contributes to maintenance and function of pancreatic islets
- Stimulates the immune system

# Thyroid Stimulating Hormone

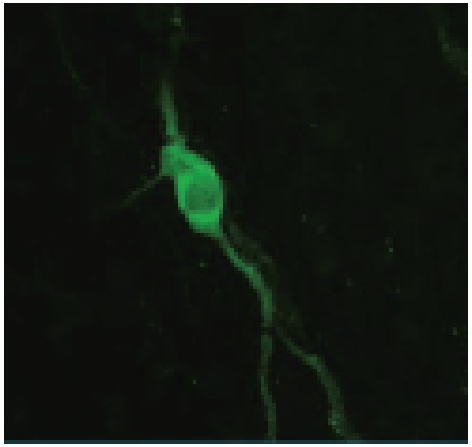
- **TSH is a glycoprotein and consists of two subunits, the *alpha* and the *beta* subunit.**
- **The α (*alpha*) subunit is identical to that of HCG, LH, and FSH .**
- **The β (*beta*) subunit (TSHB) is unique to TSH, and therefore determines its function.**
- **<u>The TSH receptor</u>**
- **found mainly on thyroid follicular cells and stimulates $T_3$ and $T_4$ production and secretion.**

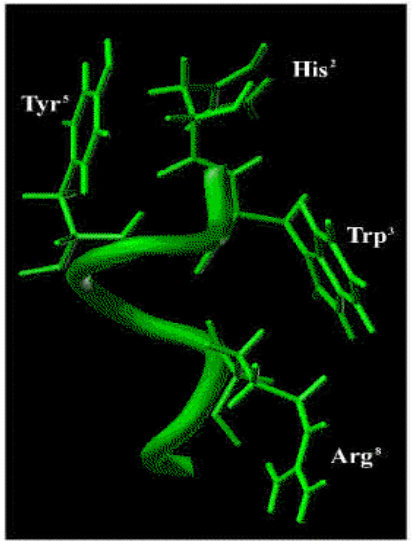
# Thyroid system



# *Gonadotropin-Releasing Hormone (GnRH) and GnRH network*

## GnRH Neuron: common pathway for the central control of reproduction






Watts et al. 2001. J Biomol Struct Dyn 18: 733

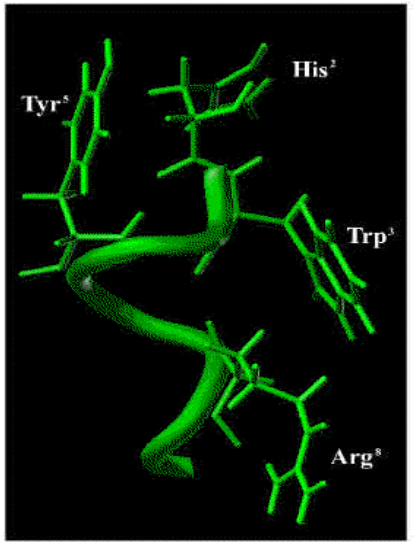
(with permission)

# *Functions of various GnRH isoforms*

- **Release of gonadotropins**
- **Release of growth hormone (GH) in some fish species**
- **Gonadal activation in tunicates**
- **Cell growth (placenta, breast and prostate cancer)**
- **Neurotransmitter in central nervous system**
  - **Sensory (pheromones in bony fish)**
  - **Reproductive behavior**
  - **Stimulation of GnRH firing activity**

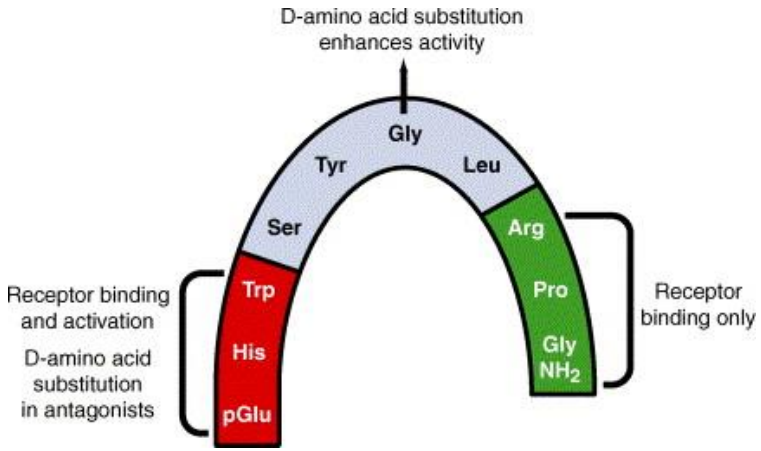
# GnRH1

- Conserved $NH_2$ and COOH terminus
  - Receptor binding and activation
- Non-conserved residues
  - Receptor specificity?



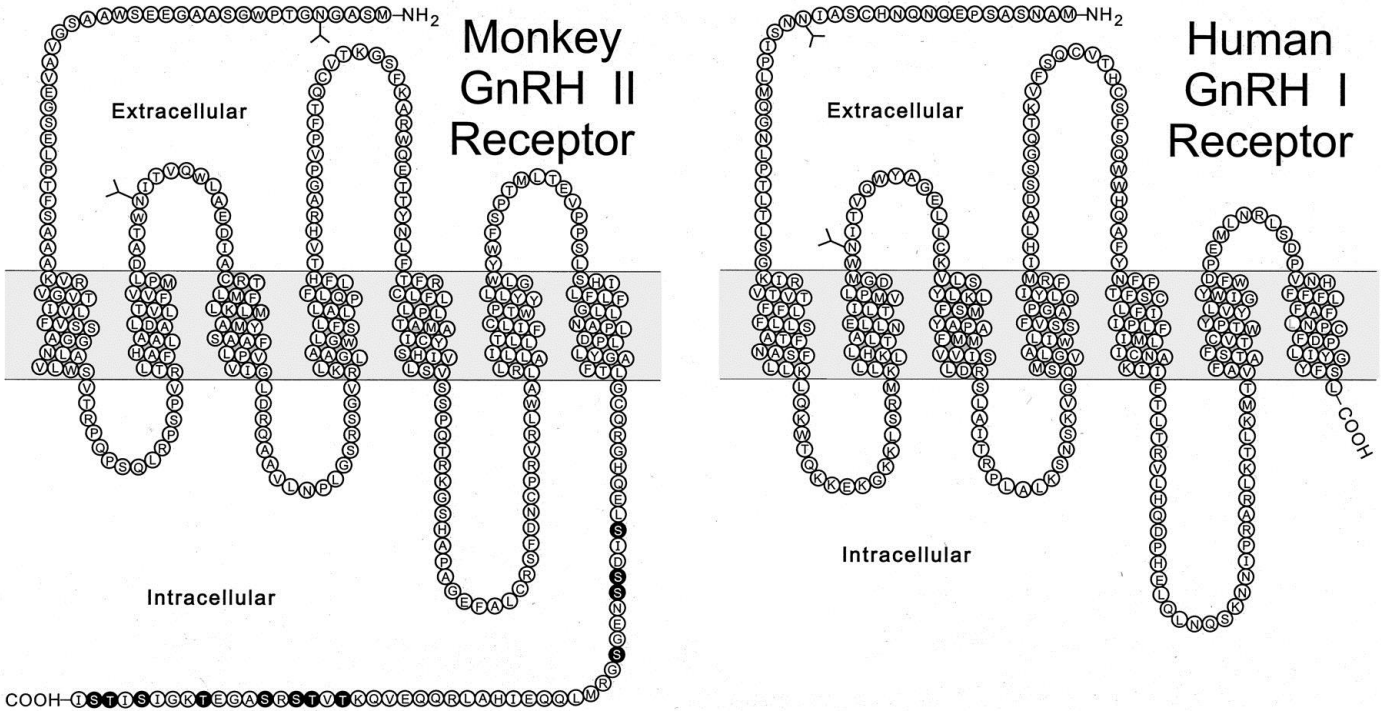

Watts et al. 2001. J Biomol Struct Dyn 18: 733

(with permission)




Millar 2005 Anim Reprod Sci 88 5

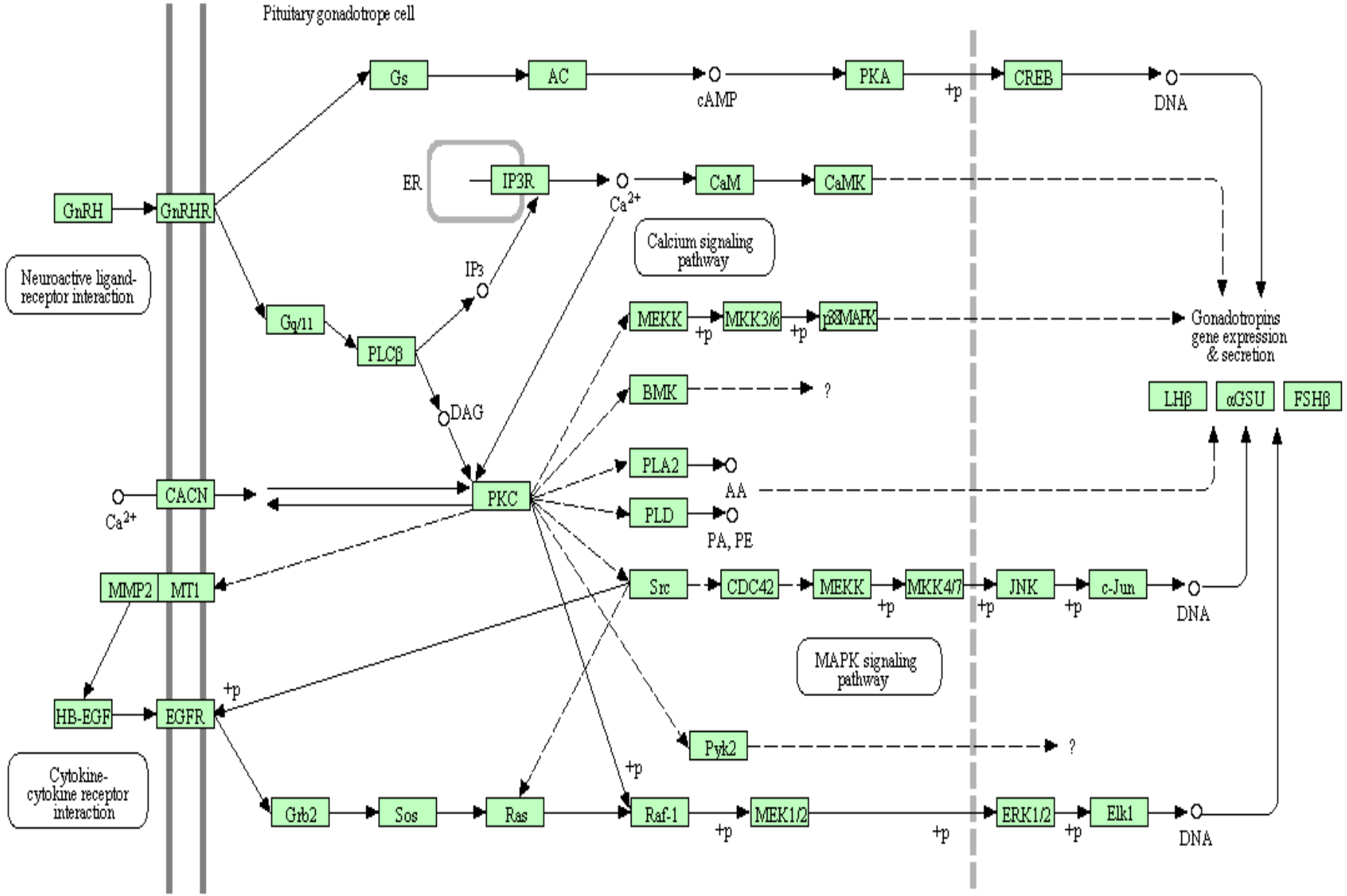
# GnRH Receptors




Neill 2002 Endocrinology 143:737-743

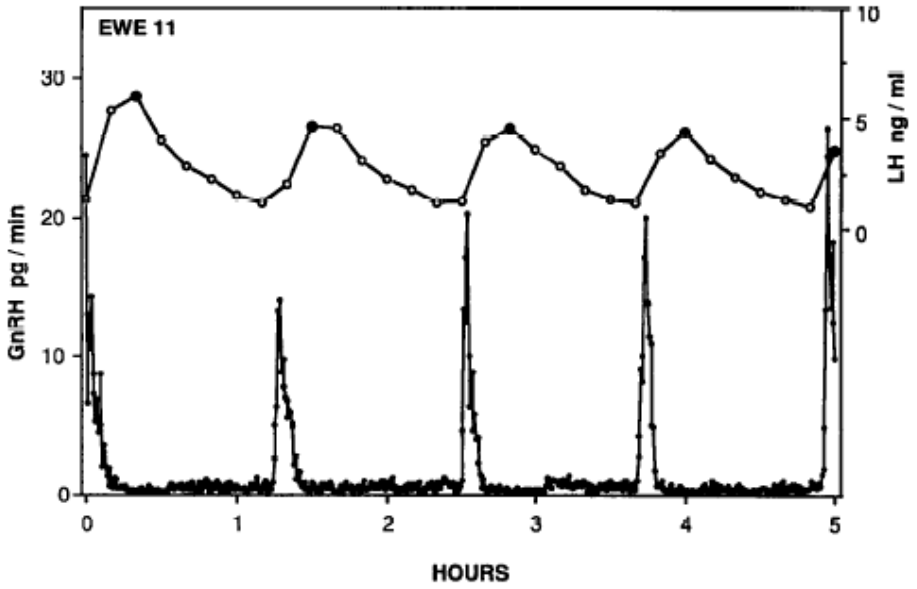
- 7 transmembrane, G protein-coupled receptor
- Cytoplamic C-terminus

# GnRH SIGNALING PATHWAY



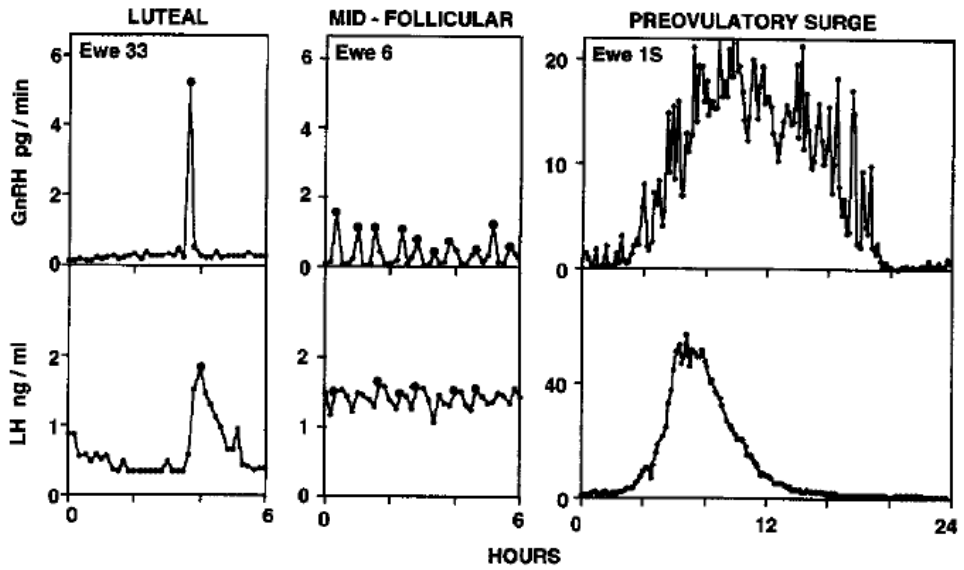
# *What is the functional significance of episodic nature of GnRH/LH release?*

- **Functional characteristics associated with release of LH**
  - **Frequency of pulses**
  - **Amplitude of pulses**
  - **Mean concentrations**
  - **Area under the curve**




Moenter et al., 1992. Endocrinology 130: 503.

# *Changes in Episodic Release of LH During the Estrous Cycle*




Karsch et al 1997 Biol Repro 56 303

- **Preovulatory surge of GnRH**
  - **"Continuous dampening" of GnRH into portal vasculature**

**What is the physiological function of the GnRH surge?**

# *Preovulatory Surge of GnRH*

**Monkey**

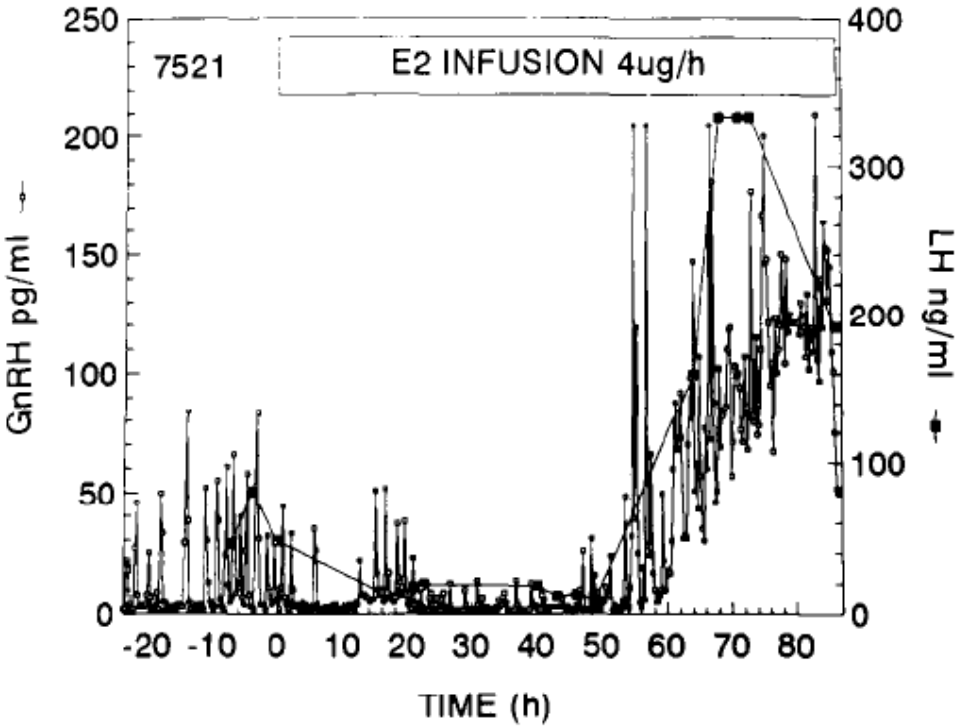
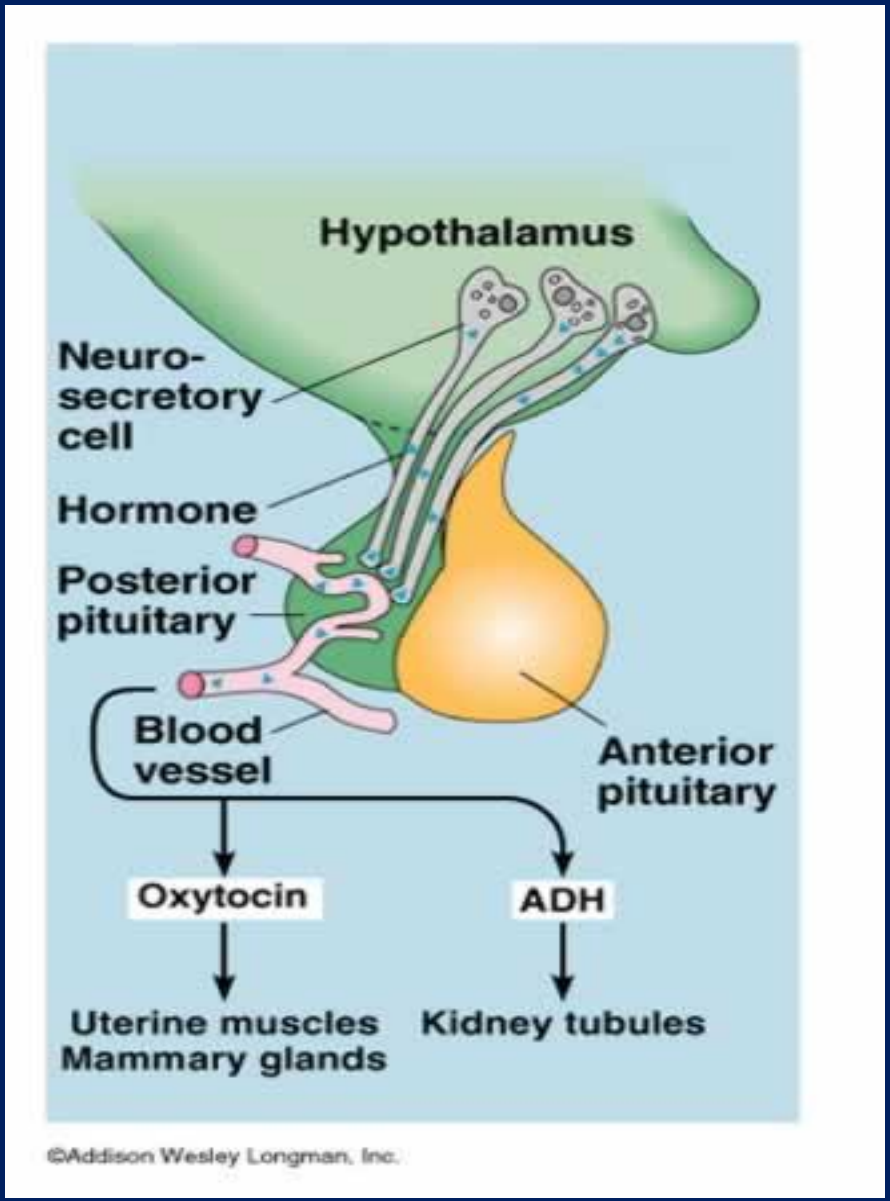


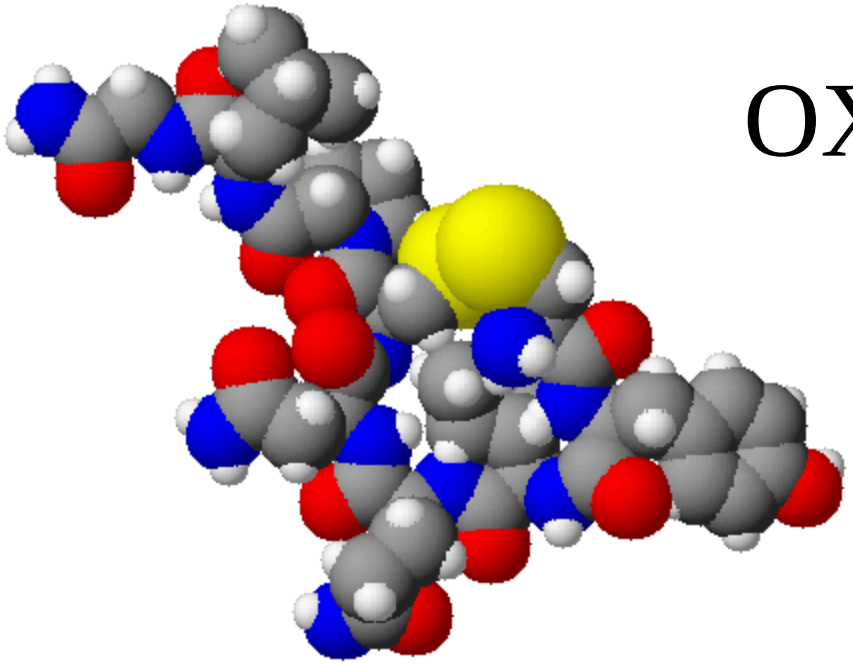

FIG. 4. GnRH and LH responses to estradiol-17$\beta$ ($E_2$) iv infusion in one OVX monkey. Time 0 denotes the start of the $E_2$ infusion, which lasted throughout the experimental period. The animal was tethered within its cage.

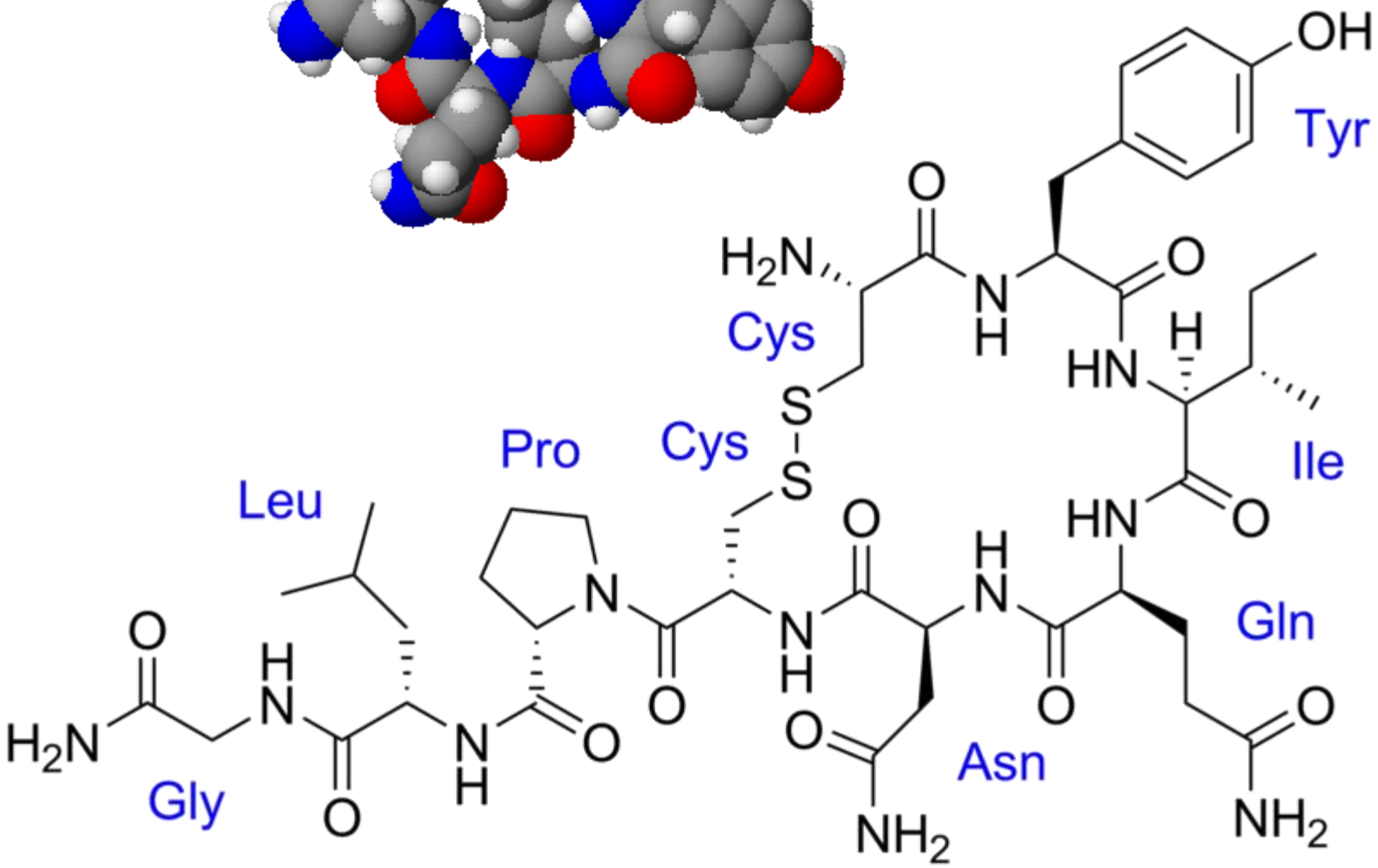
**Xia et al. 1992 Endocrinology 131 2812**

# THE PARS NERVOSA OR PARS DISTALIS OR POSTERIOR PITUITARY GLAND



# OXYTOCIN




OH
Tyr
O
$H_2N$
N
H
O
Cys
HN
H
S
S
Pro
Cys
Ile
Leu
HN
O
O
N
H
N
Gln
$H_2N$
N
H
O
O
O
O
Asn
Gly
O
$NH_2$
$NH_2$

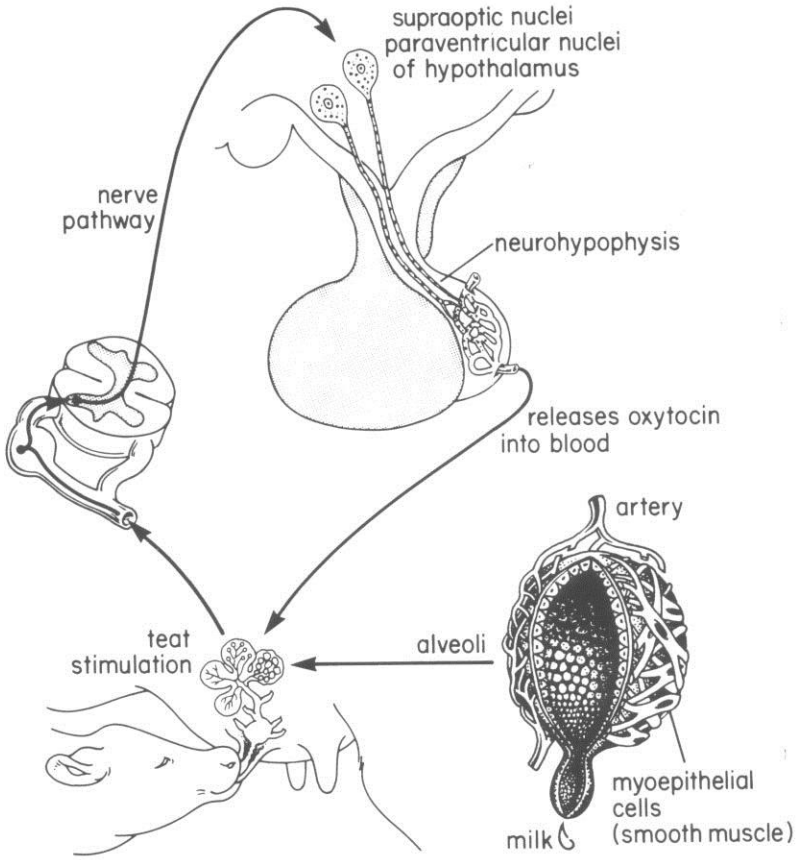
# Neuronal Sources of Oxtocin

- **Neural sources**
- **Magnocellular neurons of paraventricular nuclei** in hypothalamus and stored in axon terminals in the posterior pituitary as oxytocin-neurophysin I.
- Released from axon terminals after cleavage from neurophysin I as free oxytocin by the enzyme maturase.
- Secretion of oxytocin from the neurosecretory nerve endings by exocytosis is regulated by the electrical activity of the axon terminals upon depolarizataion.

# Hormones of the Neurohypophysis

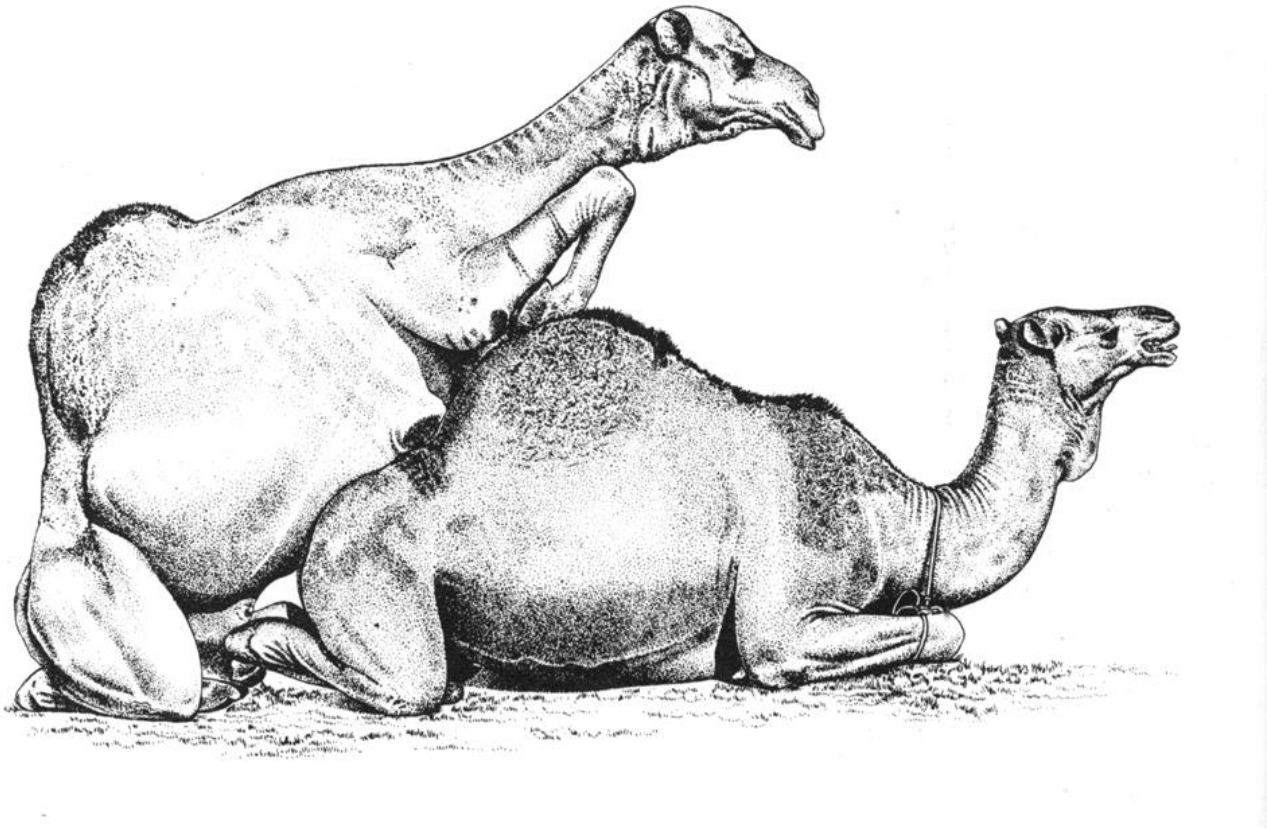
- Oxytocin
  - Smooth Muscle Contractions
    - Female Reproductive Tract
      - Sperm Transport
      - Parturition
    - Mammary Gland
      - Milk Ejection
  - Brain
    - Bonding
      - Sexual Partners
      - Mother and Offspring
      - Erection in male mice when injected into CSF

# Neuro-Endocrine Reflex



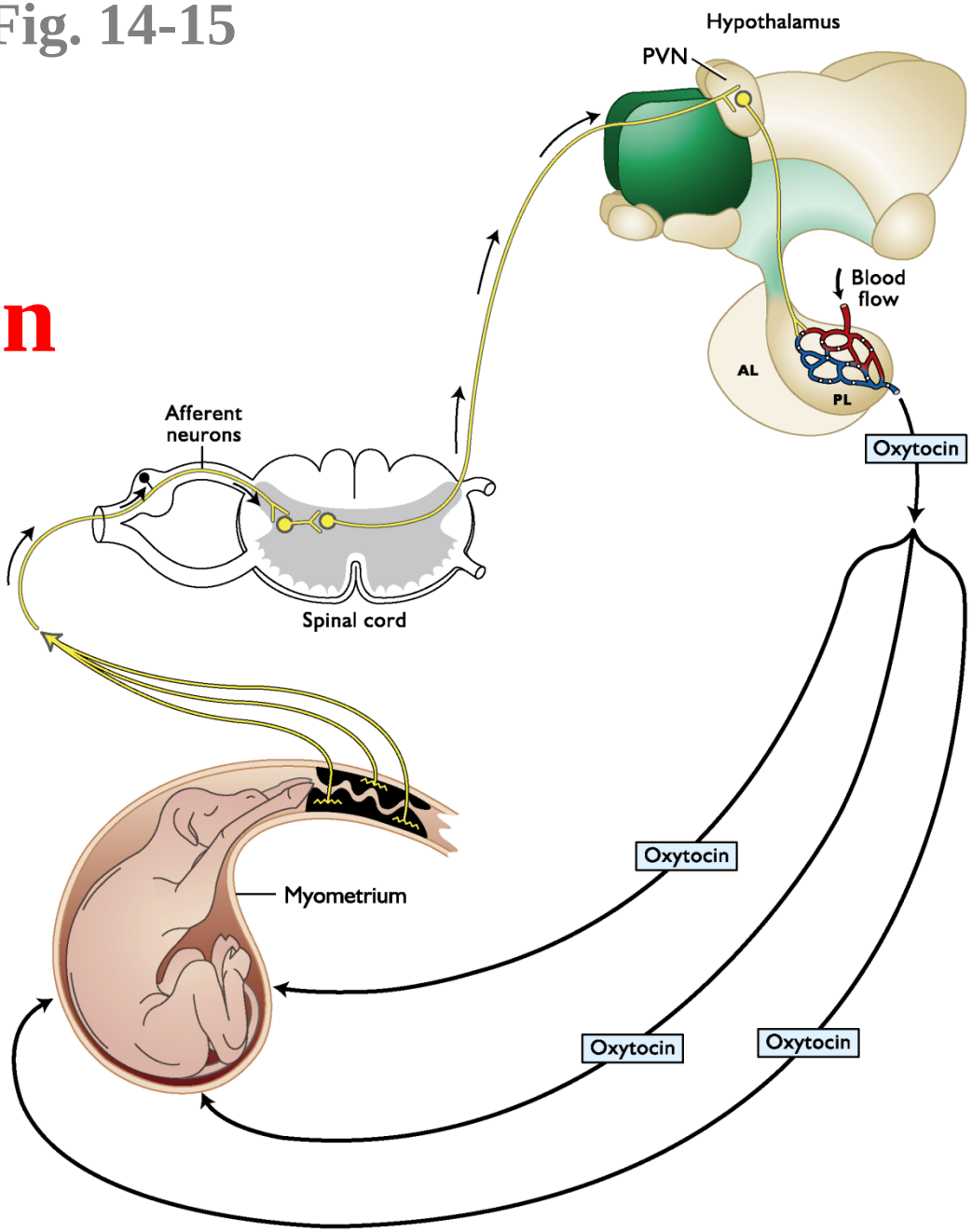
Estrus = heat = period of sexual receptivity

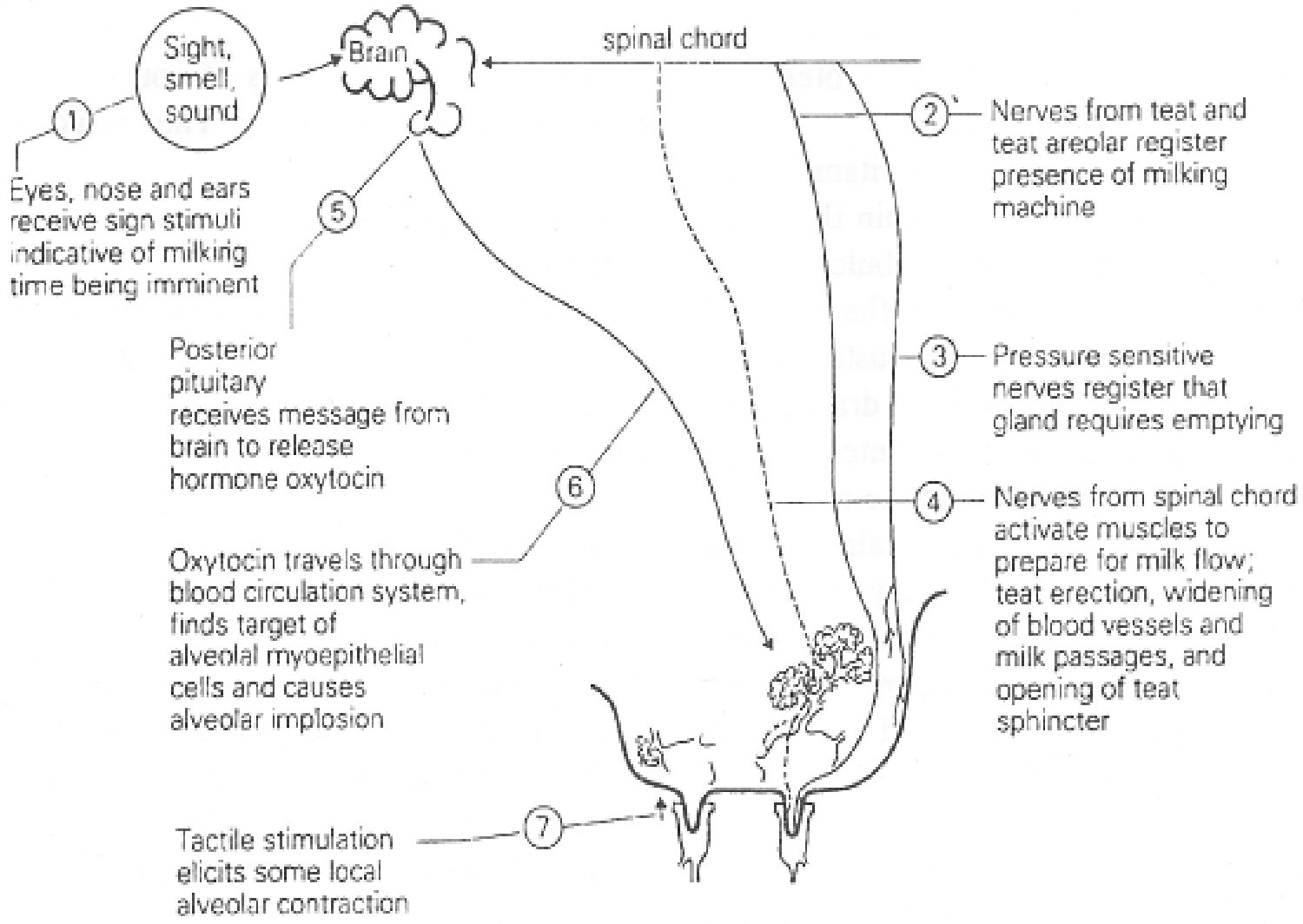
Mating – Stimulation of Female Genitalia – Oxtocin Release – Vaginal/Cervical/Uterine/Oviductal Contractions for Sperm Transport



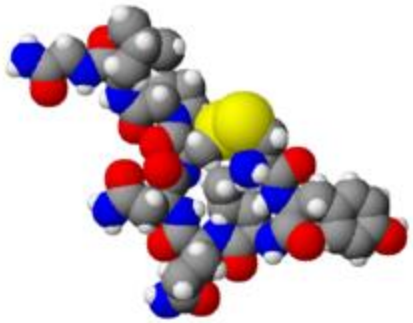
# Ferguson Reflex

Fig. 14-15




Sight, smell, sound
Brain
spinal chord
1
Eyes, nose and ears receive sign stimuli indicative of milking time being imminent
2
Nerves from teat and teat areolar register presence of milking machine
3
Pressure sensitive nerves register that gland requires emptying
4
Nerves from spinal chord activate muscles to prepare for milk flow; teat erection, widening of blood vessels and milk passages, and opening of teat sphincter
5
Posterior pituitary receives message from brain to release hormone oxytocin
6
Oxytocin travels through blood circulation system, finds target of alveolal myoepithelial cells and causes alveolar implosion
7
Tactile stimulation elicits some local alveolar contraction

# OXYTOCIN

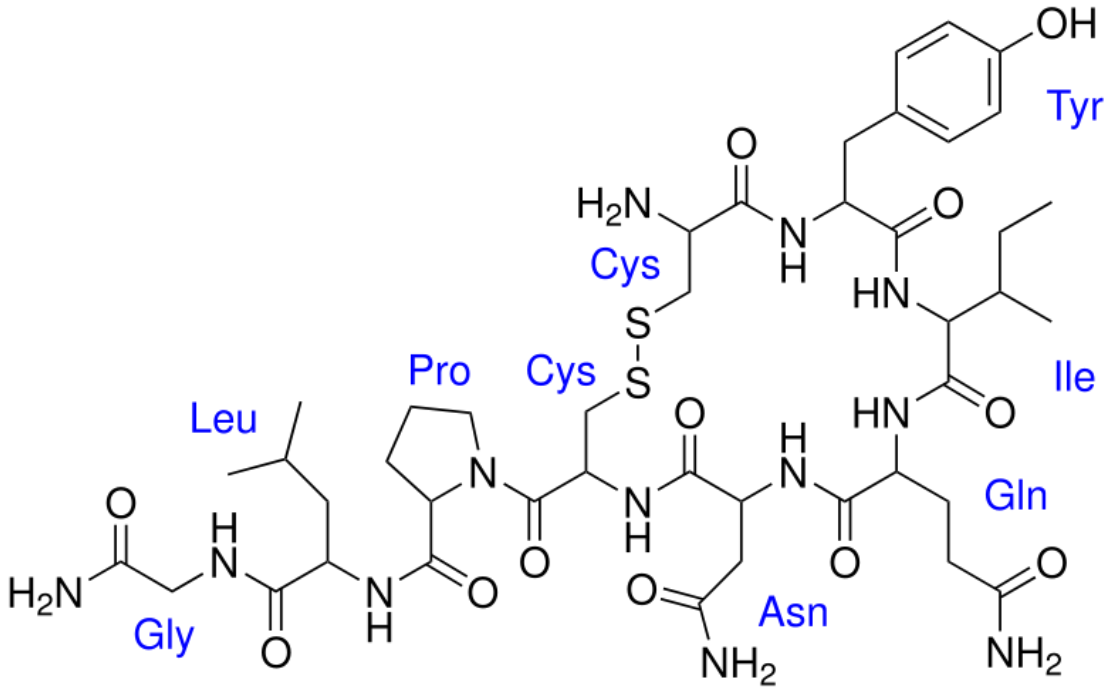


Oxytocin-Neurophysin

Maturase

Oxytocin + Neurophysin

Circulation
To Target
Tissues




**Oxytocin**

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|
| Cys | Tyr | **Ile** | Gln | Asn | Cys | Pro | **Leu** | Gly-$NH_2$ |

S–S

**ADH** (Vasopressin)

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|
| Cys | Tyr | **Phe** | Gln | Asn | Cys | Pro | **Arg** | Gly-$NH_2$ |

S–S

# Non-Neuronal Sources of Oxytocin

## These Sources Vary Among Species

- Corpus luteum of ruminants and humans
- Interstitial Cells of Leydig in the testis
- Retina
- Adrenal medulla
- Placenta
- Thymus
- Pancreas

# Oxytocin Receptor

- **Oxytocin receptor polymorphism exists in humans with those having the G allele being less prone to stress and to have better parenting skills.**

# Inositol Phosphate and Receptor Signal Transduction

DG – Diacylglycerol
PLC- Phospholipase C
PKC-Protein Kinase C

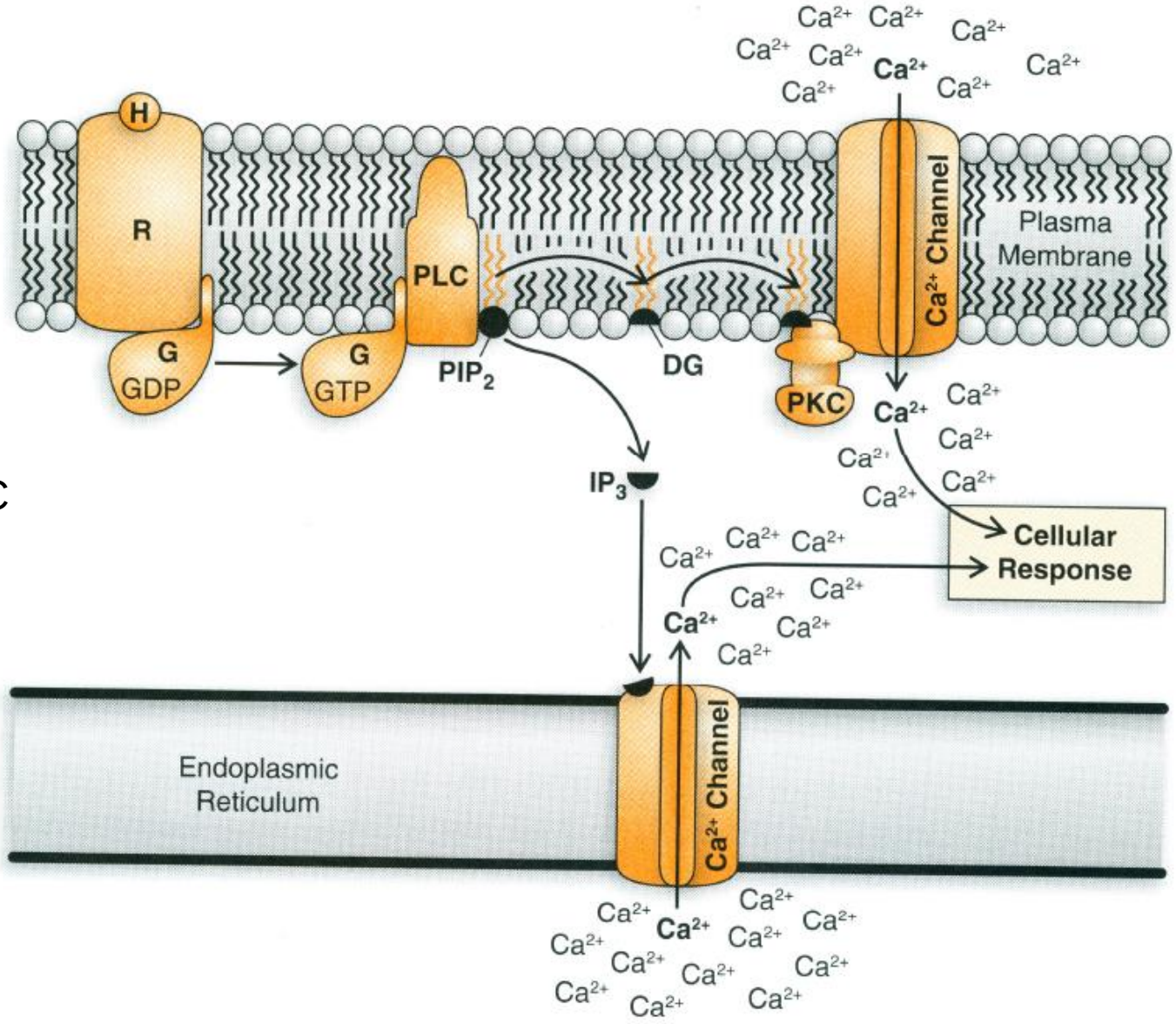
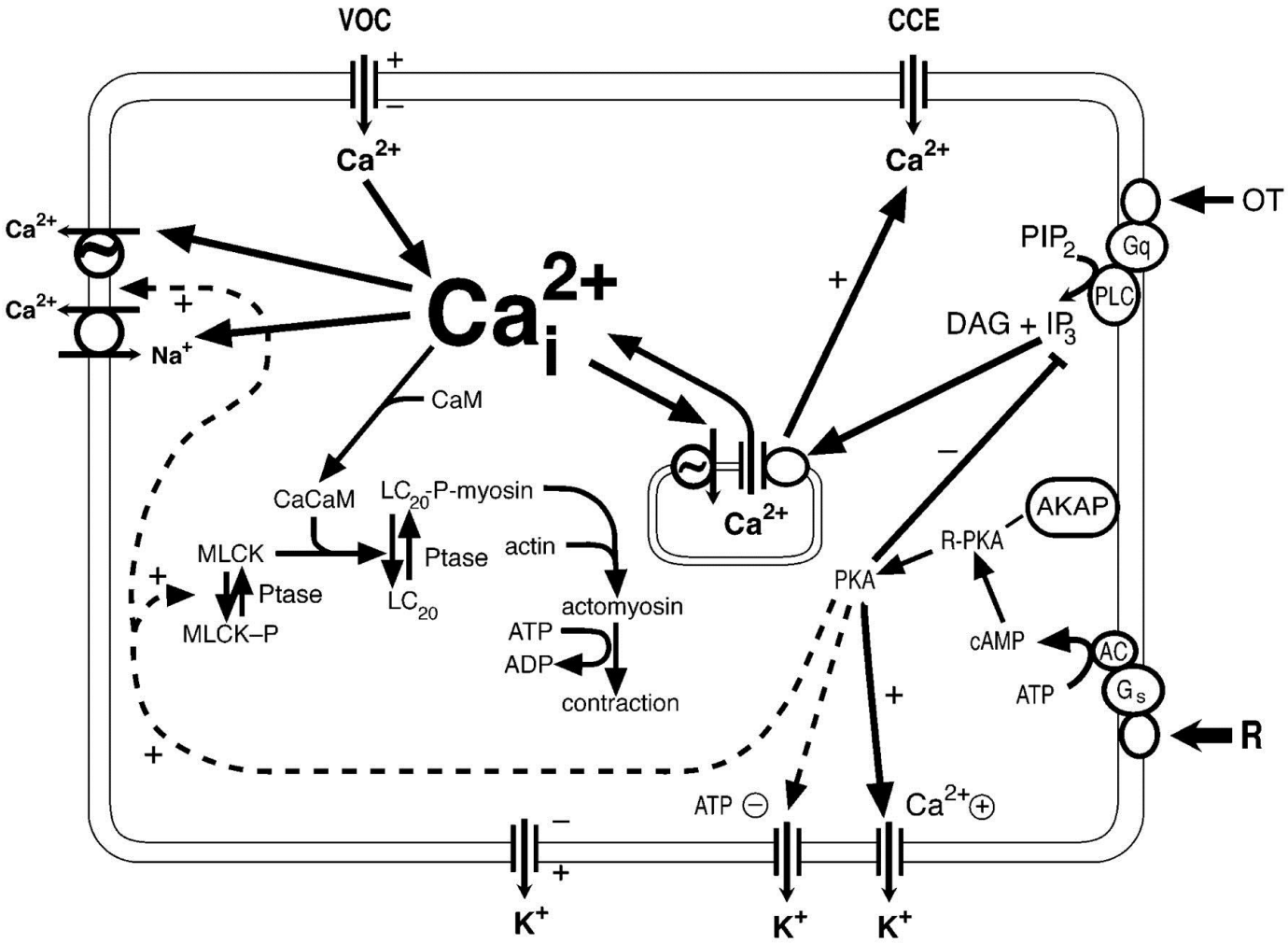



**Figure 3.12** Inositol phosphate and receptor signal transduction.

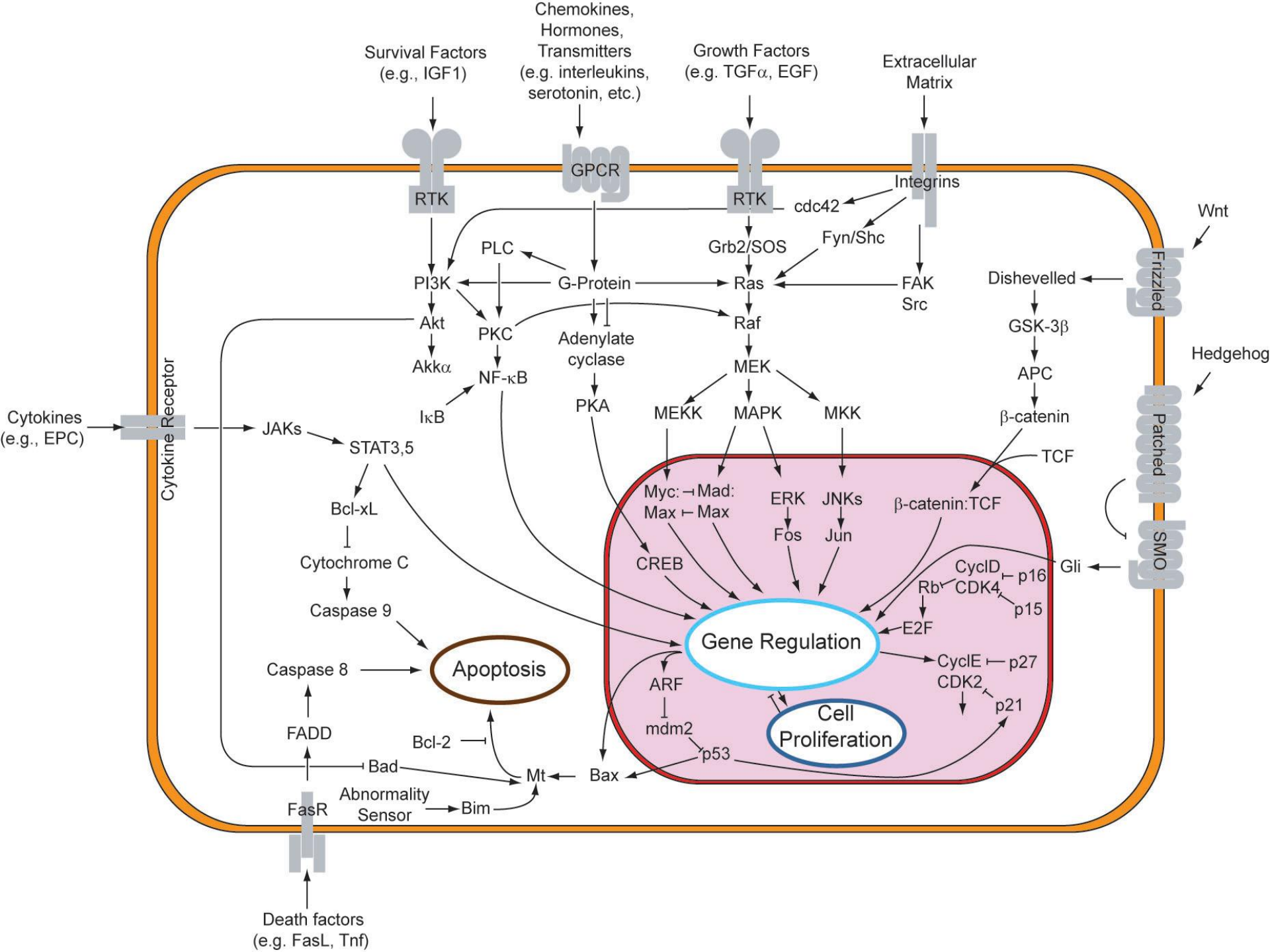
# Intracellular mechanisms whereby relaxin and oxytocin regulate contractions of uterine myometrial cells

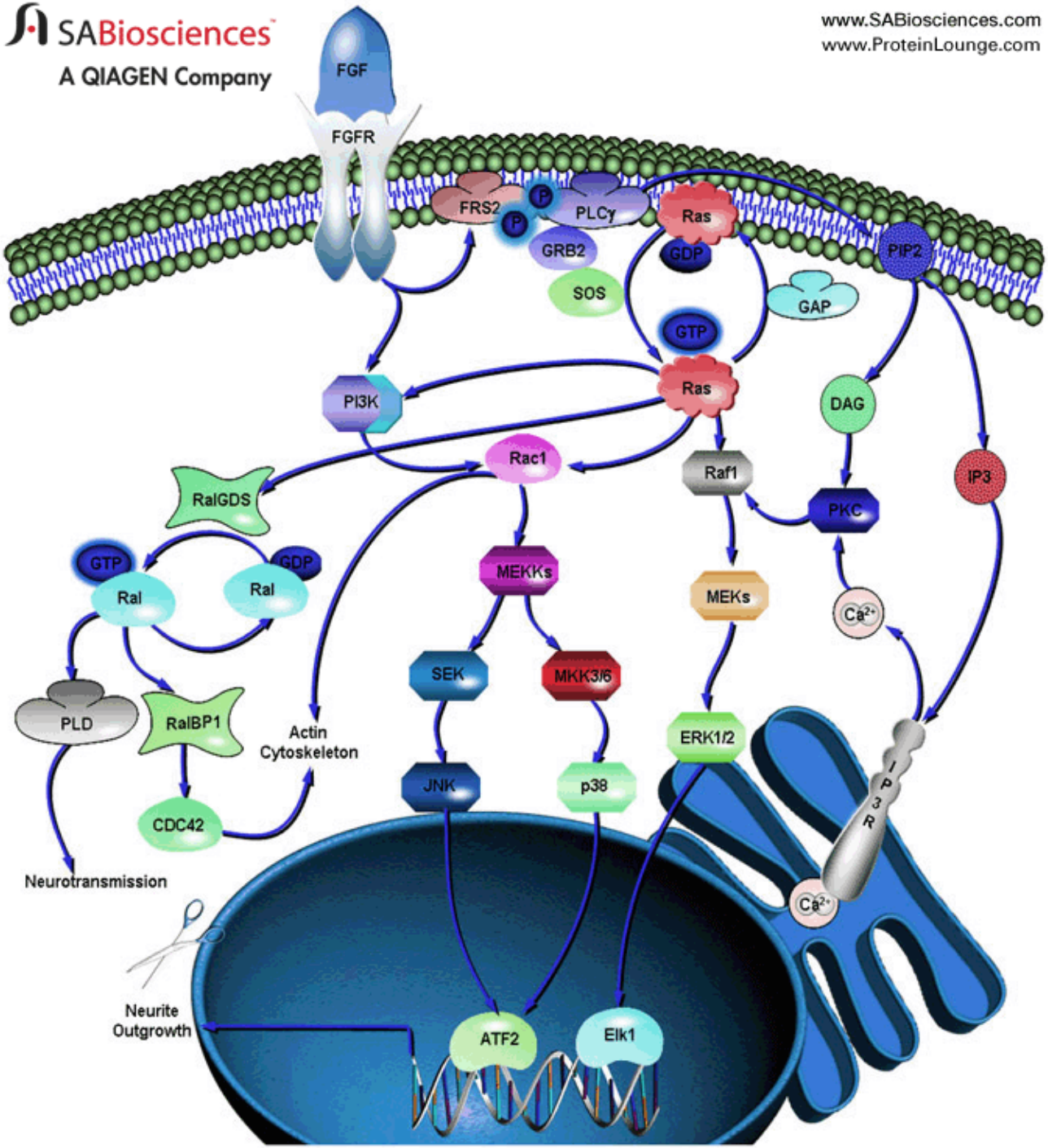
Sherwood, O. D. Endocr Rev 2004;25:205-234




**MLCK – Myosin Light Chain Kinase; DAG – diacylglycerol**
**CaM-Calmodulin; CaCaM – Calcium Calmodulin Kinase**

ENDOCRINE REVIEWS


Survival Factors
(e.g., IGF1)
Chemokines,
Hormones,
Transmitters
(e.g. interleukins,
serotonin, etc.)
Growth Factors
(e.g. TGFα, EGF)
Extracellular
Matrix
RTK
GPCR
RTK
Integrins
cdc42
Wnt
PLC
Grb2/SOS
Fyn/Shc
PI3K
G-Protein
Ras
FAK
Src
Dishevelled
Frizzled
Akt
PKC
Adenylate
cyclase
Raf
GSK-3β
Hedgehog
Akkα
NF-κB
MEK
APC
Cytokines
(e.g., EPC)
Cytokine Receptor
IκB
PKA
MEKK
MAPK
MKK
β-catenin
Patched
JAKs
STAT3,5
TCF
Myc: ⊣ Mad:
Max ⊢ Max
ERK
JNKs
β-catenin:TCF
Bcl-xL
Fos
Jun
CREB
Gli
CyclD
p16
Cytochrome C
Rb
CDK4
p15
Caspase 9
E2F
Gene Regulation
Caspase 8
Apoptosis
CyclE
p27
ARF
CDK2
p21
FADD
Cell
Proliferation
Bcl-2
mdm2
p53
Bad
Mt
Bax
FasR
Abnormality
Sensor
Bim
SMO
Death factors
(e.g. FasL, Tnf)


SABiosciences
A QIAGEN Company
www.SABiosciences.com
www.ProteinLounge.com
FGF
FGFR
FRS2
P
P
PLCγ
Ras
GDP
GRB2
SOS
GAP
PIP2
GTP
Ras
PI3K
DAG
Rac1
Raf1
IP3
RalGDS
PKC
GTP
Ral
GDP
Ral
MEKKs
MEKs
Ca2+
SEK
MKK3/6
PLD
RalBP1
Actin
Cytoskeleton
ERK1/2
JNK
p38
IP3R
CDC42
Neurotransmission
Neurite
Outgrowth
ATF2
Elk1
Ca2+