Mechanism of hormone actions I Intracellular (nuclear) Receptors

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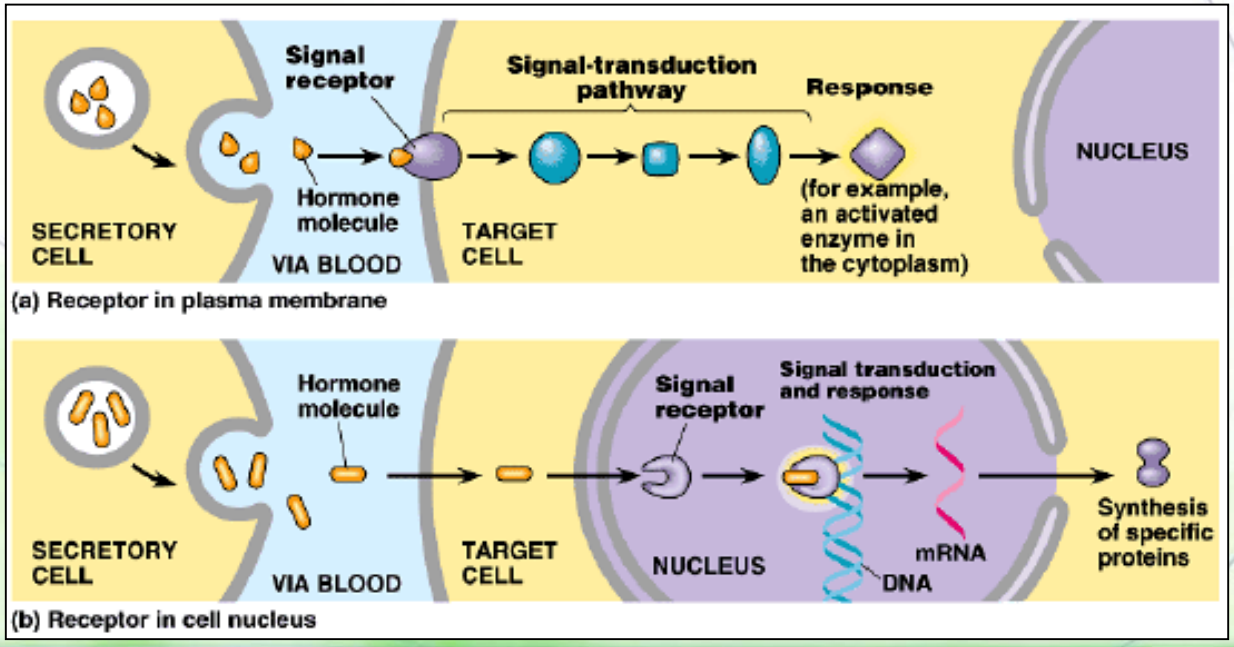
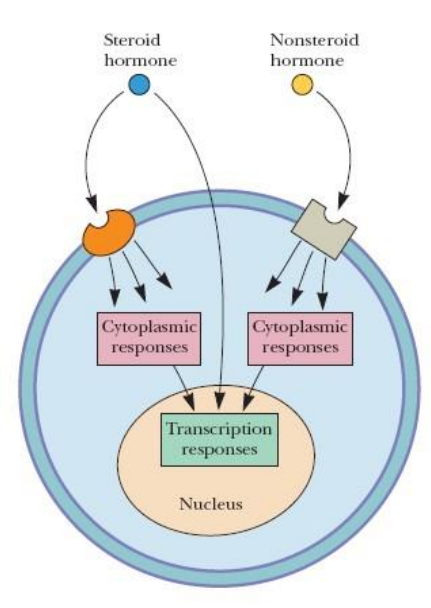
Hormone receptors

- Hormones cause cellular alterations via receptors
- The cellular localization of hormonal receptors depends on the type of hormones
  - Lipid-soluble: intracellular
  - Water-soluble: extracellular



General classification of hormones

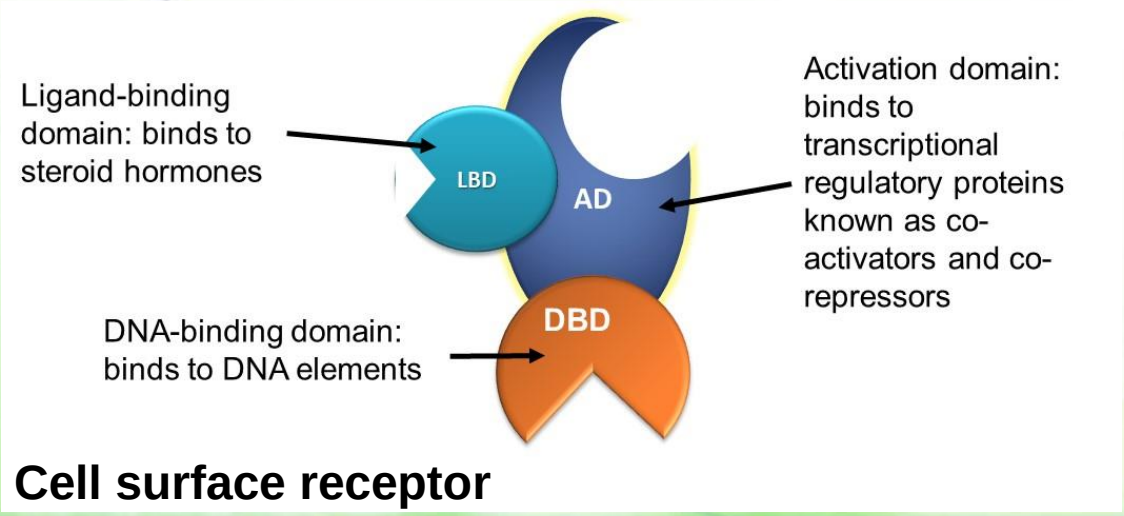
	Group I	Group II
Types	Steroids, iodothyronines, calcitriol, retinoids	Polypeptides, proteins, glycoproteins, catecholamines
Action	Slow	Fast
Solubility	Lipophilic	Hydrophilic
Transport proteins	Yes	No
Plasma $t_{1/2}$	Long (hrs - days)	Short (minutes)
Receptor	Intracellular	Plasma membrane
Mediator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



Functional Domains

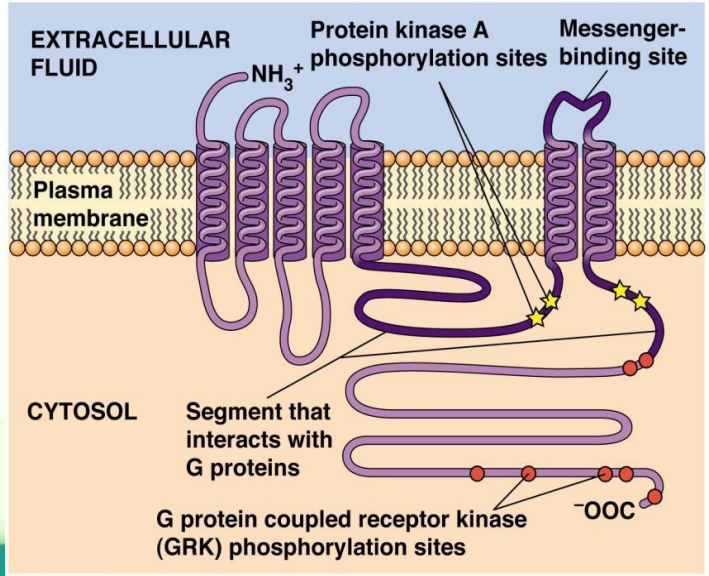
- All receptors have at least 2 functional domains:
  - Recognition domain binds the hormone (hormone-binding domain)
  - Coupling domain generates a signal that couples hormone recognition to some intracellular function (activation domain or functional domain.

Different from plasma carrier proteins that bind hormone, but do not generate a signal



Cell surface receptor

Membrane receptor



Regulation of Receptors

- Down-regulation: Receptor-mediated endocytosis:
  - Insulin, glucagon, TRH, growth hormone, LH, FSH, and catecholamines down regulate their receptors.
- Covalent modification of the receptor:
  - Phosphorylation without a change in receptor number
- Upregulation:
  - Angiotensin II and prolactin increase their receptor number as receptors on the cell surface become occupied
- Interaction with modulators: intracellular receptors can bind to other protein corepressors or coactivators modulating receptor function
  - Steroid receptors
- Modulation of an intermediary signaling molecule

Spare receptors

- Most maximal biological responses are achieved when only a small percentage of receptors is occupied.
- The remaining free receptors are known as spare receptors.
- These fully functional spare receptors would compensate in two situations:
  - a low affinity binding of the hormone to the receptor
  - a low level of hormone concentration in the system
- In both situations, spare receptors increase the sensitivity of target cells to the hormone.
- The greater the proportion of spare receptors:
  - the more sensitive the target cell to the hormone
  - the lower concentration of hormone required to achieve half-maximal response

Examples

- A maximal stimulation of steroidogenesis by Leydig cells when only 1% of LH receptors are occupied.
- The requirement of 10% occupancy of receptors for a full steroid-induced transcriptional response.
- A maximum glucose oxidation in adipocytes by insulin bound to only 2-3% of receptors.

Types of receptors

- Three different types of receptors
  - intracellular (nuclear) receptors
  - G protein-coupled receptors
  - receptor tyrosine kinases

Group I. HORMONES THAT BIND TO INTRACELLULAR RECEPTORS

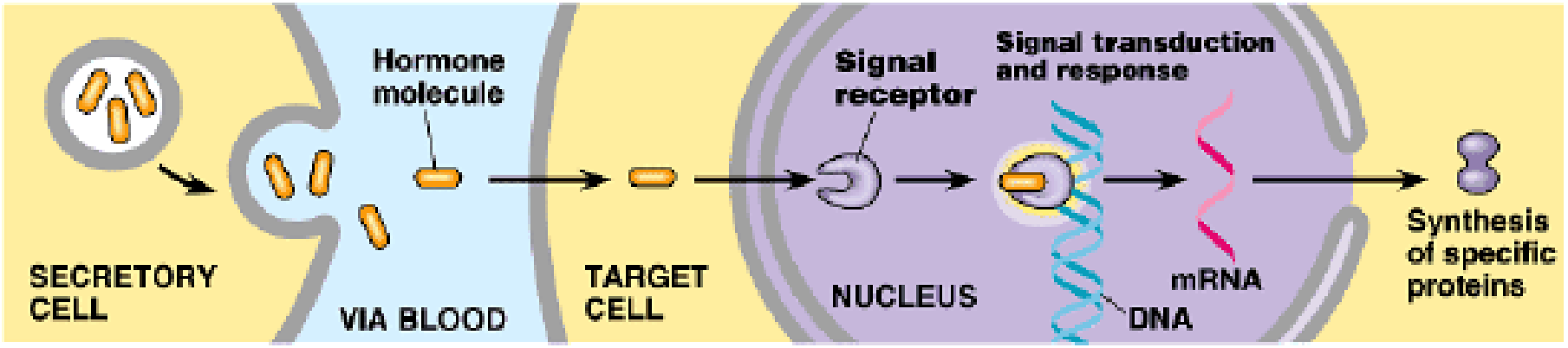
Estrogens	Calcitriol (1,25 $[OH]_2$-D_3)
Glucocorticoids	Androgens
Mineralocorticoids	Thyroid hormones (T_3 and T_4)
Progestins	Retinoids (Vit A)

Group II. HORMONES THAT BIND TO CELL SURFACE RECEPTORS

A. The second messenger is cAMP	
Adrenocortropic hormone (ACTH)	Parathyroid hormone (PTH)
Angiotensin II	Opioids
Antidiuretic hormone (ADH)	Acetylcholine
Follicle-stimulating hormone (FSH)	Glucagon
Human chorionic gonadotropin (hCG)	α_2-Adrenegic catecholamines
Lipotropin (LPH)	Corticotropin-releasing hormone (CRH)
Luteinizing hormone (LH)	Calcitonin
Melanocyte-stimulating hormone (MSH)	Somatostatin
Thyroid-stimulating hormone (TSH)	β-Adrenergic catecholamines
B. The second messenger is calcuim or phosphatidylinositides (or both)	
α_1-Adrenergic catecholamines	Acetylcholine (muscarinic)
Cholecystokinin	Substance P
Gastrin	Angiotensin II
Thyrotropin-releasing hormone (TRH)	Gonadotropin-releasing hormone(GnRH)
Vasopressin	
C. The intracell messenger is a protein kinase cascade (started by tyr phosphorylation)	
Growth hormone (GH)	Oxytocin
Insulin	Nerve growth factor (NGF)
Insulin-like growth factors (IGF-1, IGF-II)	Epidermal growth factor(EGF)
Prolactin (PRL)	Platelet-derived growth factor
	Fibroblast growth factor (FGF)

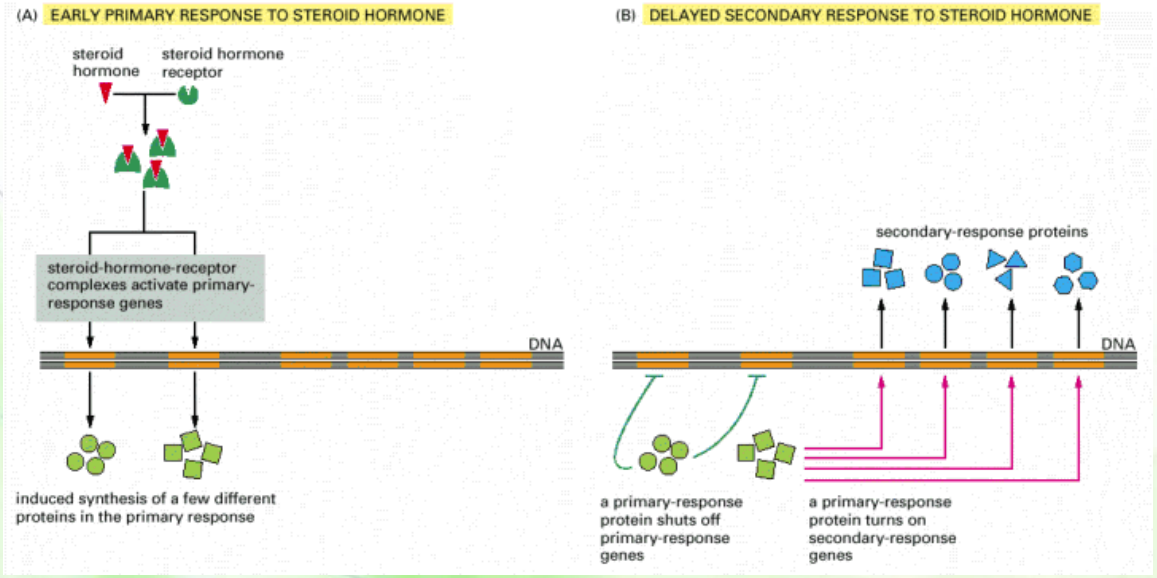
Nuclear Receptors

- The receptors to which lipophilic steroid hormones bind are ligand-activated proteins that regulate transcription of selected genes
- They are found in the cytosol and the nucleus
- Upon hormonal binding, the hormone-receptor complex bind to specific DNA promoter/enhancer sequences



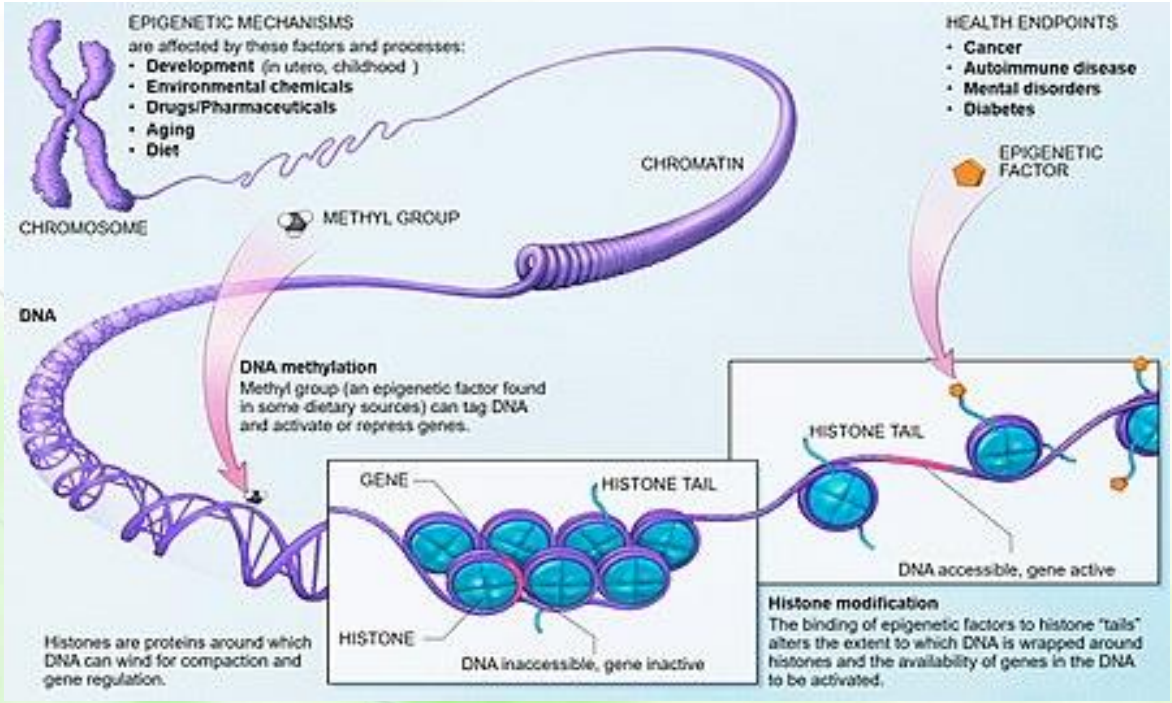
Types of response

- These genes can themselves be transcriptional factors that, in turn, activate other genes to produce a delayed, secondary response; and so on.
  - Primary response: direct activation of a small number of specific genes (30 minutes).
  - Secondary response: the protein products of active genes in the primary response in turn activate other genes.



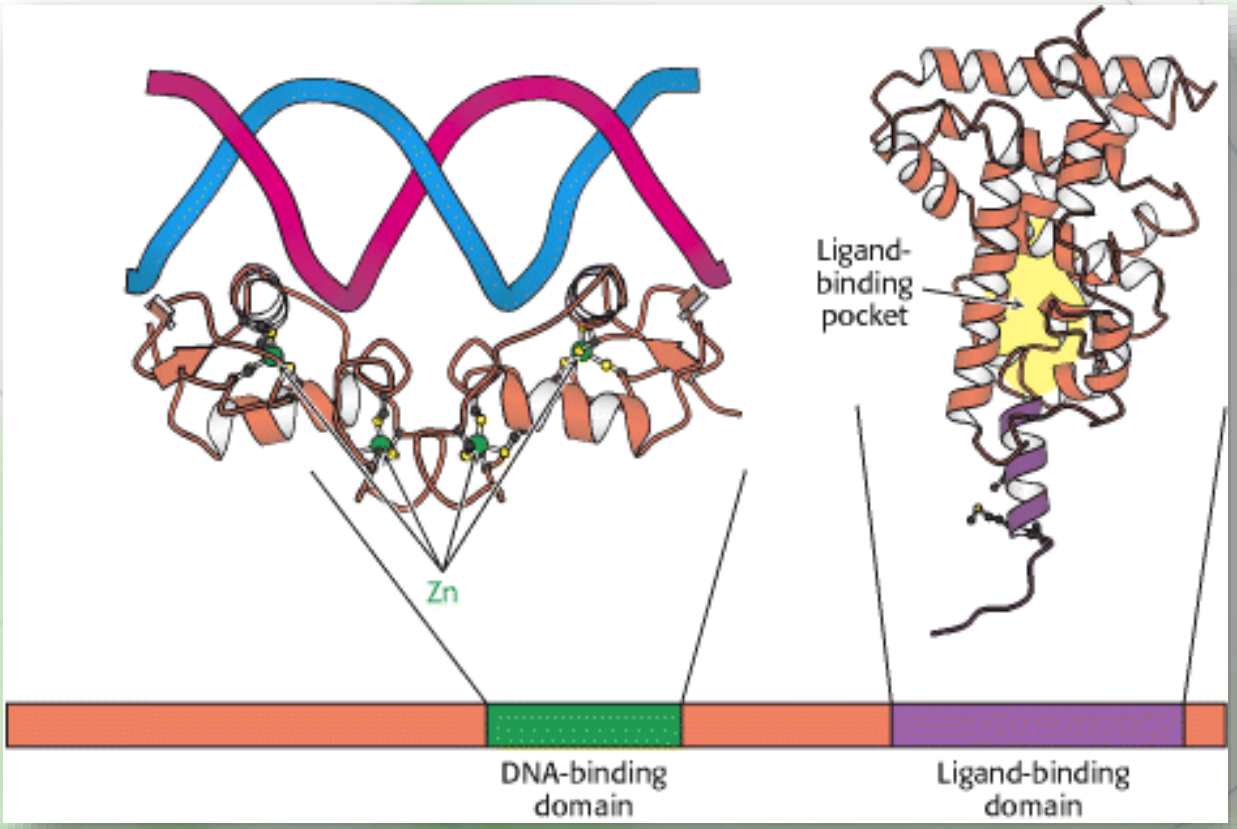
Cell-specific response

- Only certain types of cells have receptors for the hormones
- Even if cells have identical intracellular receptor, each cell type contains a different combination of other cell-type-specific gene regulatory proteins that influence the gene transcription
- Epigenetic regulation (DNA packaging and modification)



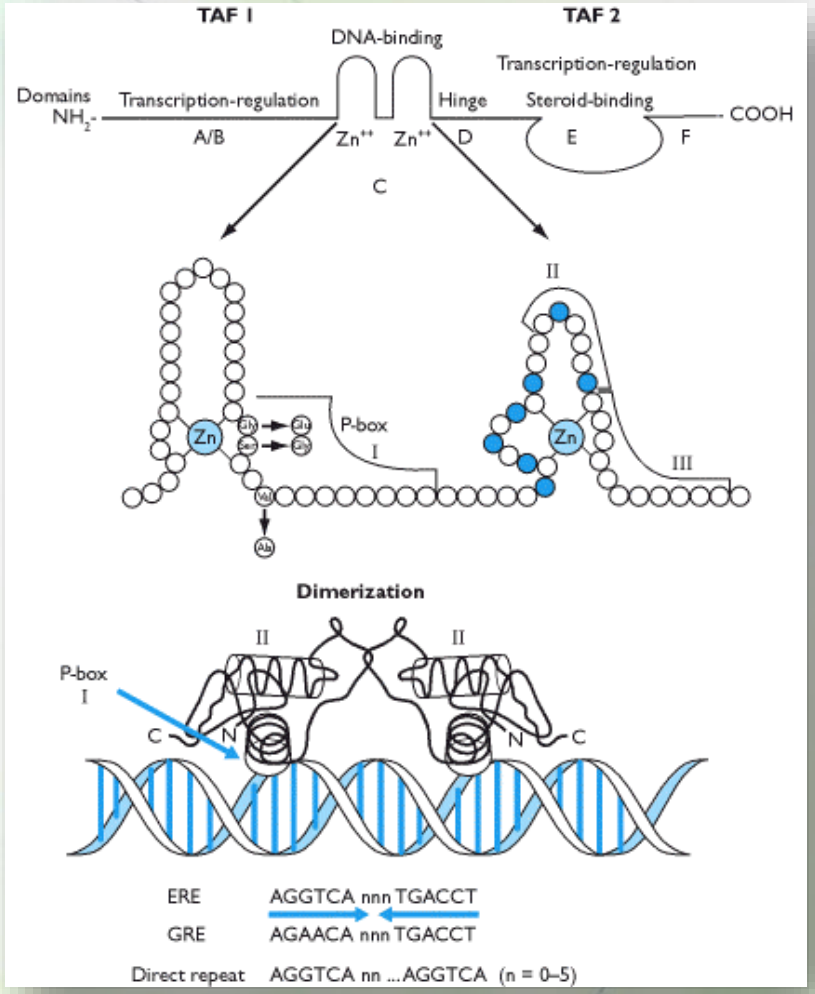
Structure of receptors

- All intracellular hormone receptors have at least two domains: and hormone-binding domain and a DNA-binding domain



Zinc finger domain

- A special DNA-binding domain is known as zinc finger
- The specific amino acid sequence of the zinc fingers in the DNA binding domain is important for determining the bases in the DNA helix to which the receptor binds and, thus, the specificity of the transcriptional activity of the receptor



Mechanisms of control of gene expression

- There are two ways by which steroid hormones can alter gene expression:
- Mechanism 1: Direct binding to DNA sequences known as hormone response elements
  - a glucocorticoid response element (GRE)
  - estrogen response element (ERE)
- Mechanism 2: Binding to and activating/repressing other transcription factors that recognize a particular site on DNA.

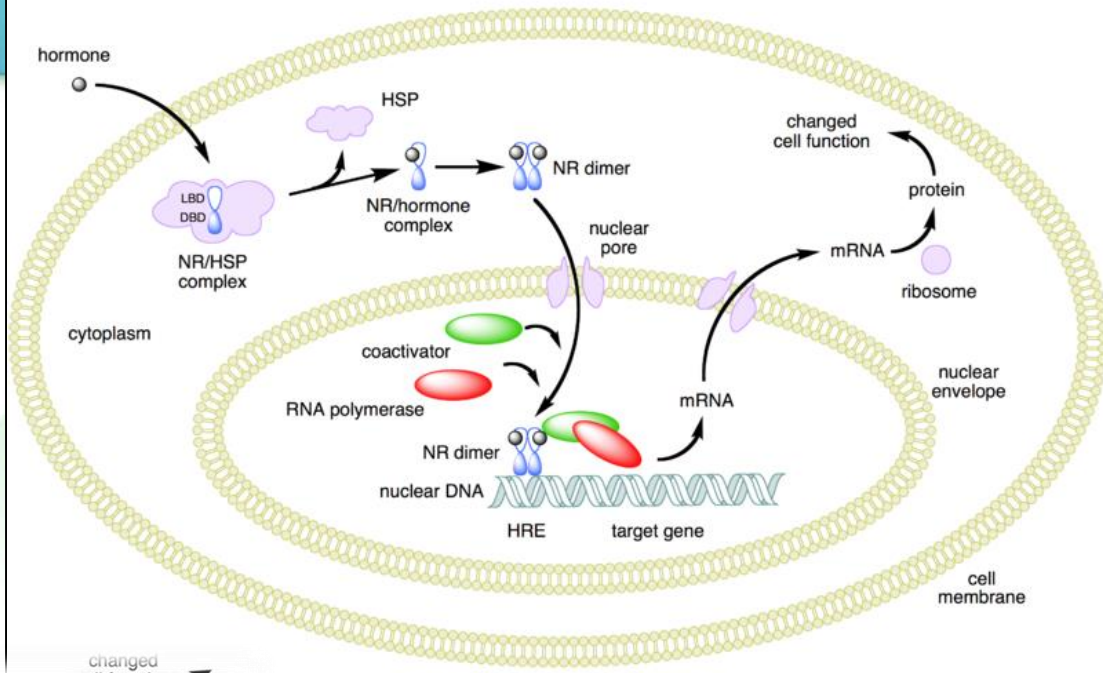
Non-genomic cellular effects

- Many steroids and thyroid hormones can stimulate rapid responses by interaction with cell surface receptors.
- Such receptors may initiate the opening of ion channels or activate classical second messenger systems.

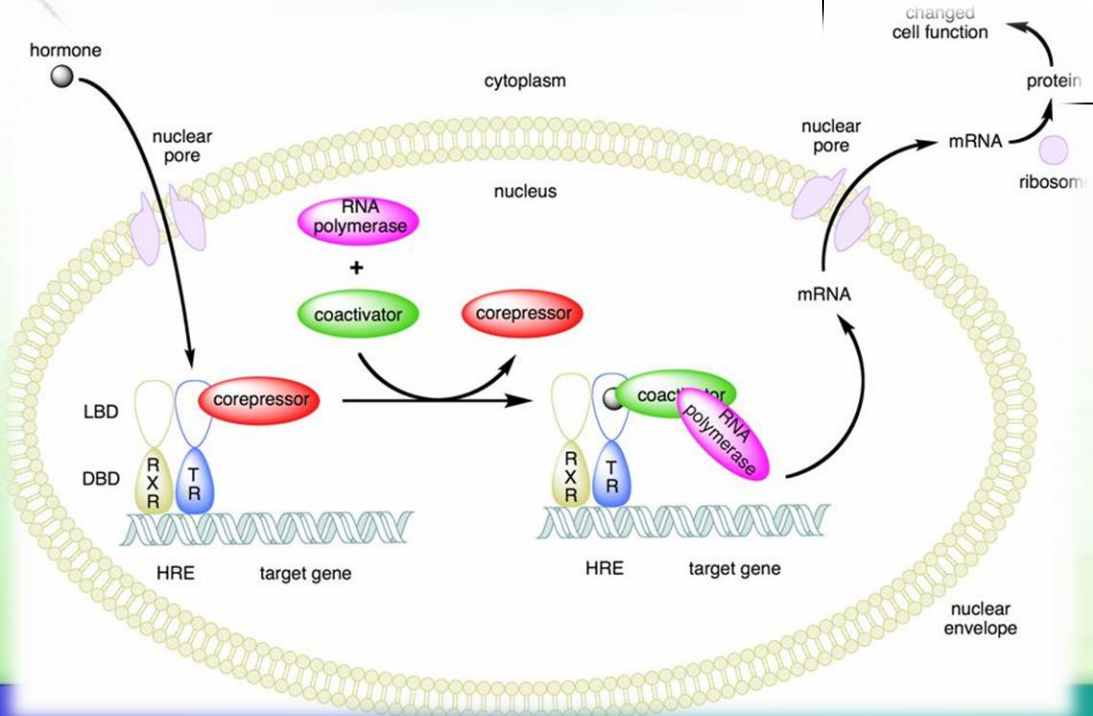
Hormone classification (location of intracellular receptors)

- Type 1 receptors
  - Predominantly cytoplasmic
  - Examples: the glucocorticoid, mineralocorticoid, estrogen, androgen and progesterone receptors
  - They are bound to heat shock proteins, but, once bound to the steroid hormone, is released, dimerizes, is translocated into the nucleus, and binds to DNA.
- Type 2 receptors
  - They are nuclear and may be bound to DNA with corepressors, but, when bound to hormone, they replace corepressors by coactivators.
  - They characterstically form heterodimers (e.g. thyroid hormone receptor and retinoid X receptor).

Type I receptors



Type II receptors



Glucocorticoid receptors

- Mineralocorticoids and glucocorticoids
  - The physiological mineralocorticoid is aldosterone
  - The physiological glucocorticoid is cortisol
- They are synthesized in the adrenal cortex of mammals.
- Ligand-bound corticosteroid receptors form complexes with other transcription factors, such as the jun protein. Such interactions are responsible for activation of AP-1 sites and for the glucocorticoid-mediated suppression of transcription (e.g. pro-opiomelanocortin gene).

Non-genomic effects

- Cortisol may exert effects via membrane receptors.
- The serum protein that transports cortisol, cortisol-binding globulin (CBG), can bind to cell surface receptors.
- Cortisol may then bind to the CBG-receptor complex and activate adenylate cyclase.

Types of estrogen receptors

- Two forms of the estrogen receptor have been identified: α and β
- These receptors can form different dimers - α/α, α/β and β/β
- Both receptors bind estrogen with high affinity and bind to ERE
- They also share high degree of amino acid homology
- ERβ has an additional repressor domain that that is inhibitory to ERα transcriptional activity.
  - This inhibition occurs when ERαβ heterdimer forms.
- However, they have different distributions in target tissues indicating they may have different biological effects
  - For example, whereas ERα induces the expression of progesterone receptor in glandular epithelia cells of uterine tissues, ERβ downregulates the expression of the same receptor in luminal epithelial cells.

Progesterone receptor

- The progesterone receptor can form heterodimers and the progesterone-bound receptors bind to GRE on DNA similar to that of glucocorticoids.

Androgen receptor

- In many target tissues, testosterone is rapidly converted to DHT by the 5α-reductase enzyme before it interacts with the androgen receptor.
- When activated, it binds to androgen-response element.

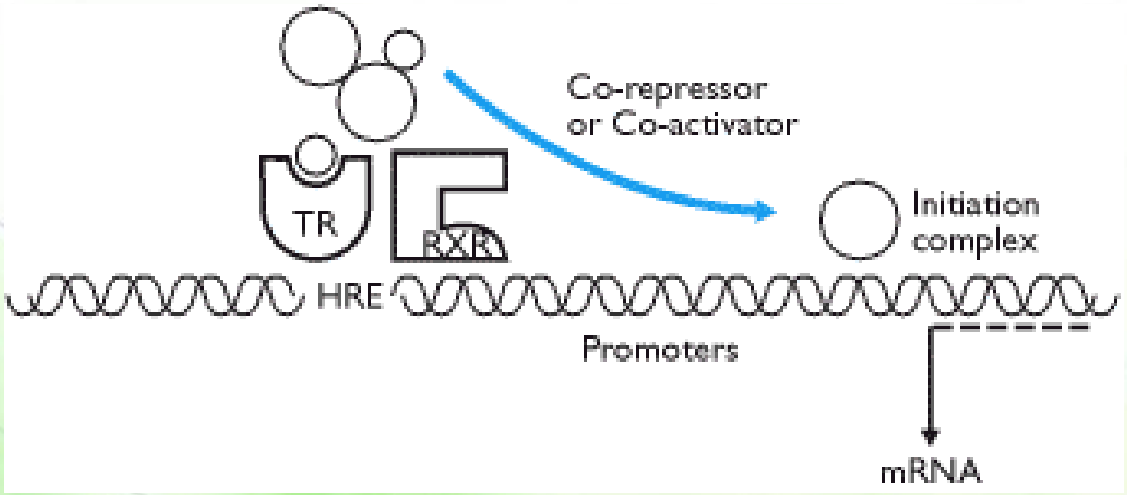
- As with other steroid hormones there is evidence that testosterone may exert non-genomic effects on certain cells via cell surface molecules.

Thyroid hormone receptors

- Unlike some steroid receptors, thyroid hormone receptors exist in the nucleus and free of HSP.
- The receptors may remain bound to DNA in the absence of hormone binding.
- Many of the actions of thyroid hormones are mediated by binding to nuclear receptors that have a preferential affinity for triiodothyronine (T3).
- Once inside the nucleus, T3 binds to its receptor
- This dimerizes with another T3 receptor (to form a homodimer) or with a different receptor, particularly the retinoic acid receptor, to form a heterodimer

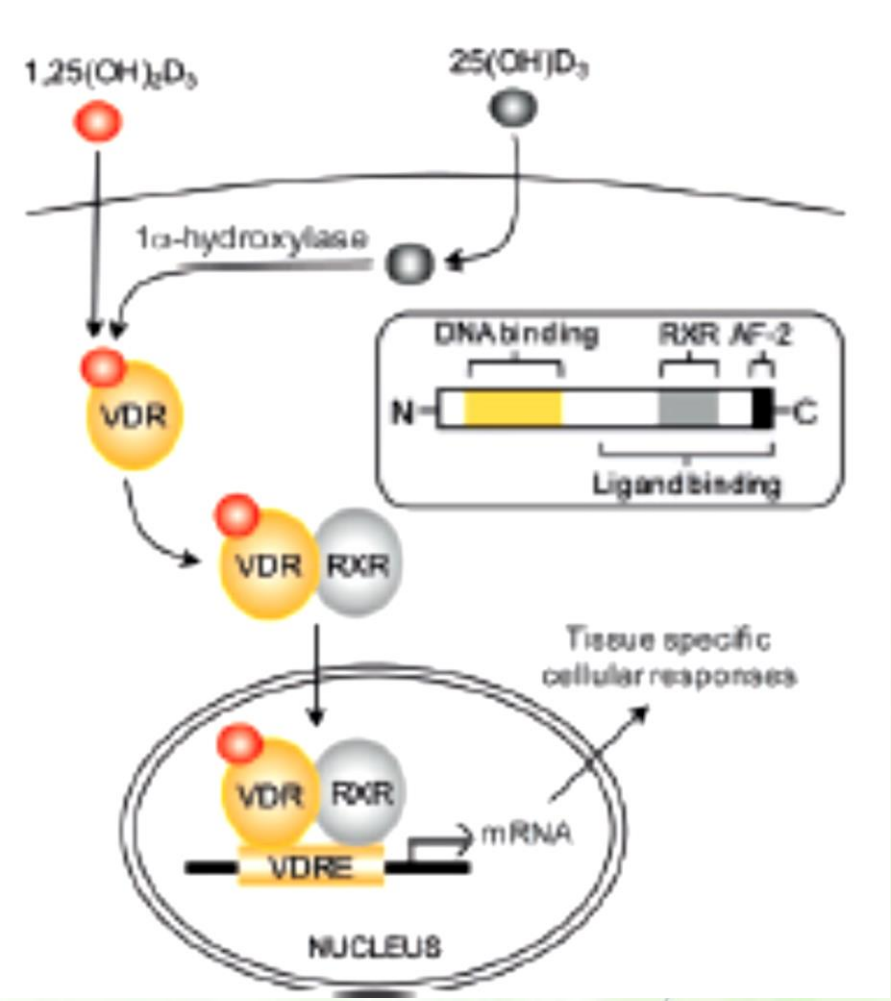
DNA binding

- As dimers, the zinc fingers of the DNA binding domain bind into a hormone response elements (HRE) on the DNA helix
- Along with other transcription factors (co-activators/repressors), they regulate gene expression



Vitamin D

- Binding of VDR to its ligand, 1,25(OH)2D3, enables dimerization of VDR and RXR, allowing nuclear translocation and binding of the VDR-RXR complex to VDREs in the promoter region of responsive gene.



Other nuclear receptors

- Retinoid X receptors (RXRs)
- The peroxisome proliferator-activated receptors (PPARs)
- The liver X receptors (LXRs)
- The farnesoid X receptors (FXRs)
- The pregnane X receptor (PXR)

Retinoid X receptors (RXRs)

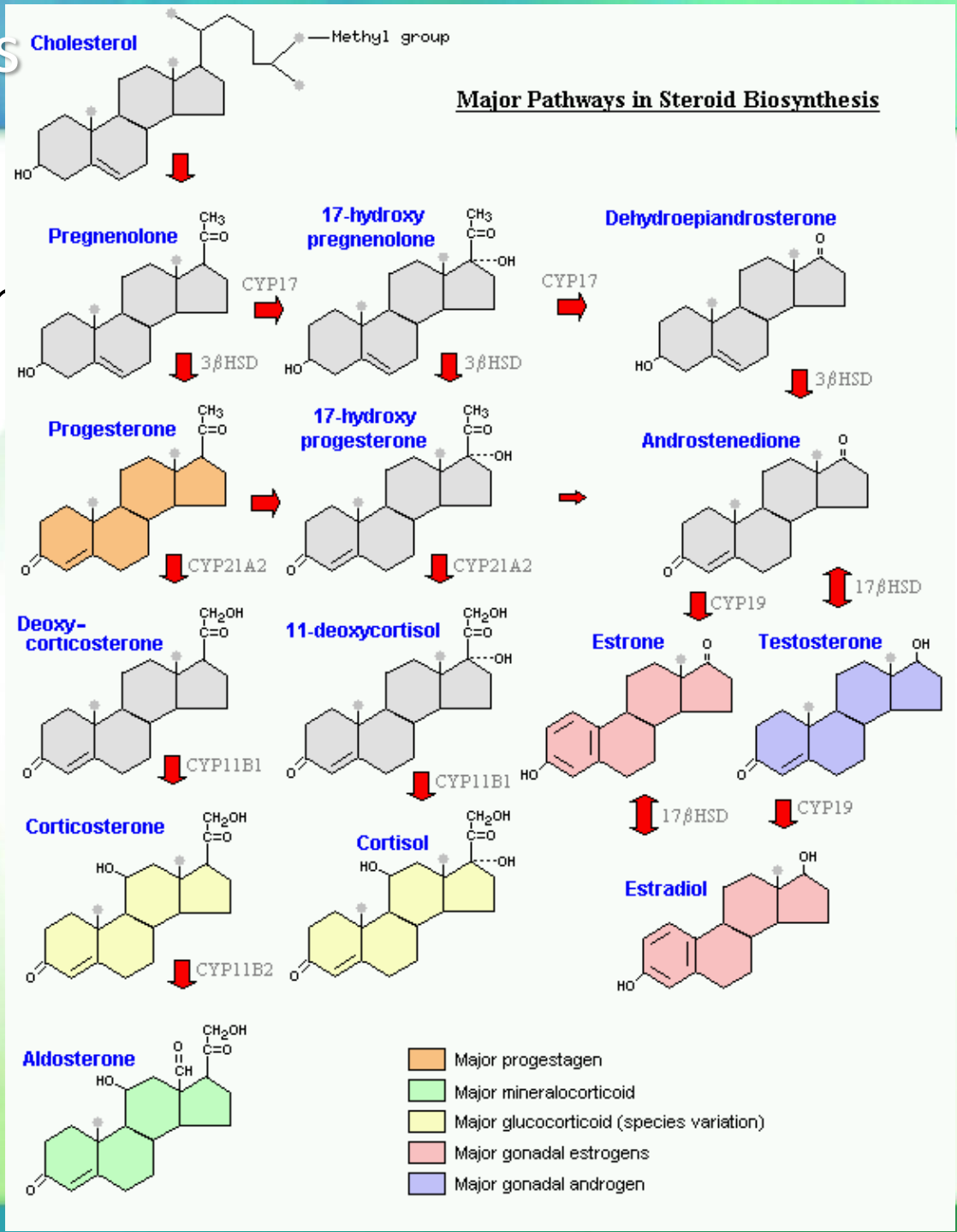
- Three isotypes: RXRα, RXRβ, and RXRγ
- Each isotype is composed of several isoforms.
- The RXRs serve as obligatory heterodimeric partners for numerous members of the nuclear receptor family.
- In the absence of a heterodimeric binding partner, the RXRs are bound to hormone response elements (HREs) in DNA and are complexed with co-repressor proteins including a histone deacetylase (HDAC).

The peroxisome proliferator-activated receptors (PPARs)

- Three family members: PPARα, PPARβ/δ, and PPARγ
- Each of these receptors forms a heterodimer with the RXRs.
- PPARα is the receptor for polyunsaturated fatty acids.
  - It induces hepatic peroxisomal fatty acid oxidation during periods of fasting.
- PPARγ is a master regulator of adipogenesis and is most abundantly expressed in adipose tissue.
- PPARδ is expressed in most tissues and is involved in the promotion of mitochondrial fatty acid oxidation, energy consumption, and thermogenesis.

Steroid hormone synthesis

- C21:
  - Progesterone: directly from pregnenolone
  - Cortisol & Aldosterone: from progesterone
- C19
  - Testosterone
  - from progesterone or pregnenolone
  - 2C shortage
- C18 (estrogen):
  - Aromatase
  - Cleaves C18
  - Reduction



Synthesis, metabolism and transport of testosterone

Testicular hormones

- The testis secretes over 95% of the circulating testosterone.
- Most of the potent androgen, dihydrotestosterone (DHT) and estradiol circulating in men is derived from peripheral conversion of testosterone.
- Only about 2% of circulating testosterone is in the free form and the rest is either bound to albumin (approximately 40%) or, mostly, to sex-hormone-binding globulin (SHBG) and is in equilibrium with the free form.
- Most circulating testosterone is converted in the liver to metabolites after conjugation with glucuronide or sulfate and are excreted.

Synthesis, metabolism and transport of estrogen and progesterone

Estradiol

- Estradiol, the most important steroid secreted by the ovary, is transported bound to albumin (approximately 60%) and about 30% to SHBG.
- Metabolites are conjugated with sulfate or glucuronide before excretion by the kidney.

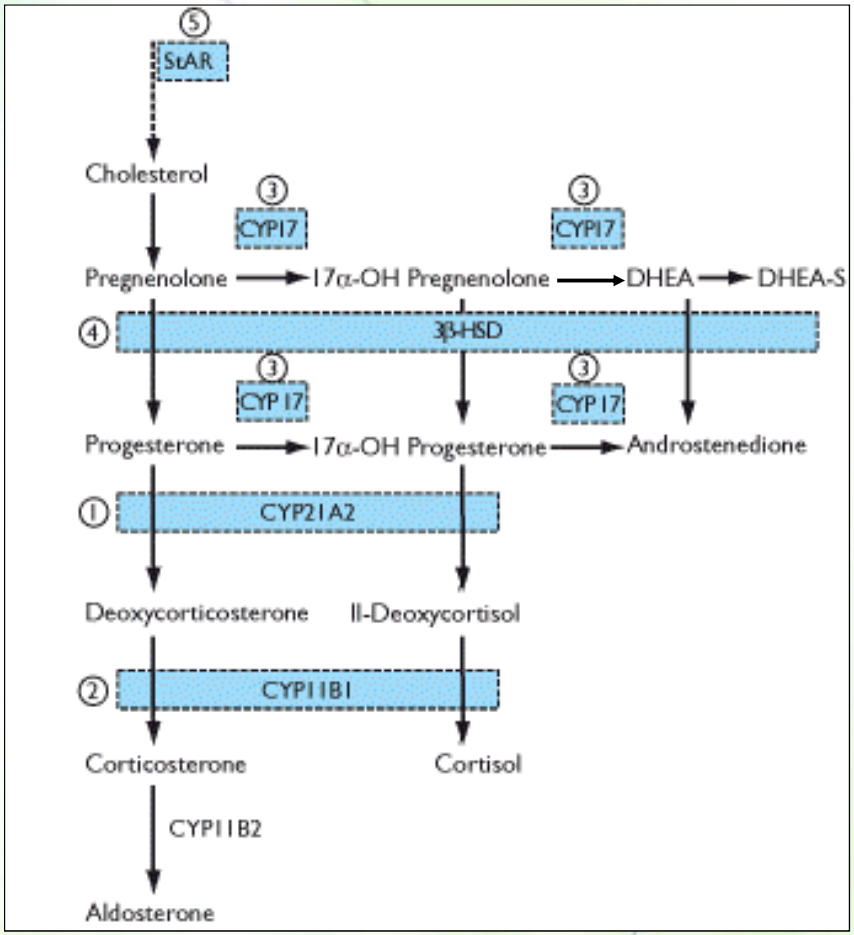
Progesterone

- Progesterone is mainly bound to albumin in the circulation and, to a lesser extent, cortisol-binding globulin.
- It is rapidly cleared from the circulation and is conjugated with glucuronic acid in the liver in which form it can be excreted.

Critical enzymes

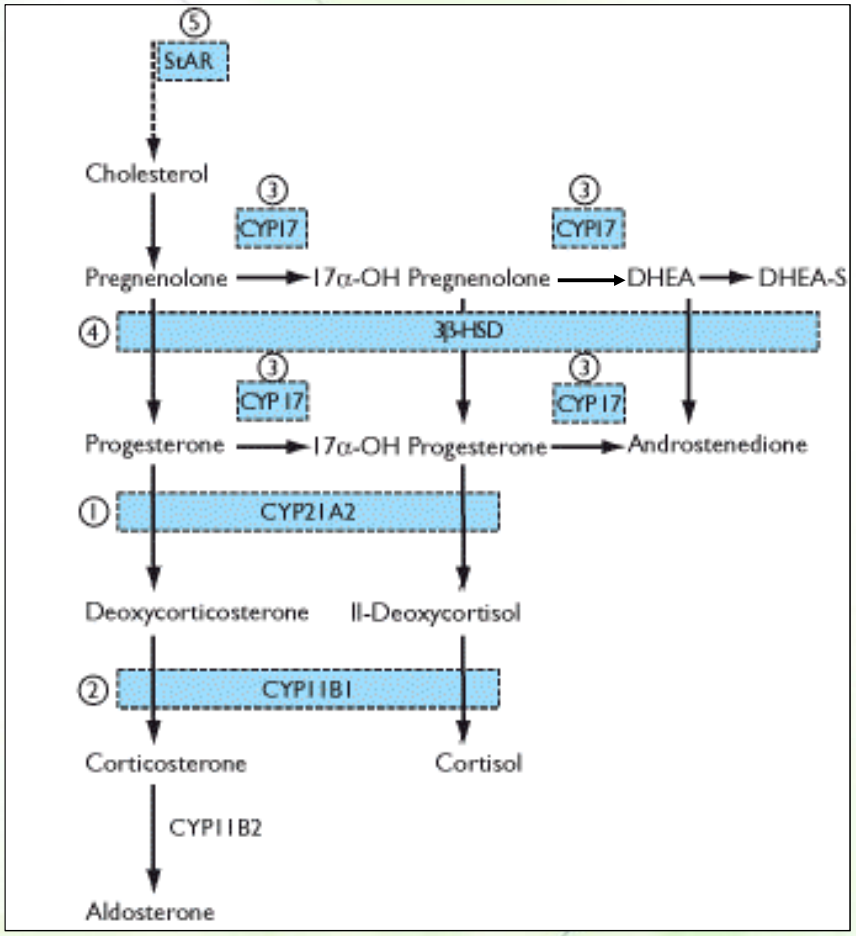
3β-hydroxysteroid dehydrogenase

- Conversion of pregnenlone into progesterone
- Deficiency in this enzyme would result in the formation of female genitalia in all patients.
- No glucocorticoids and mineralocorticoids



17α-hydroxylase (CYP17)

- 17α-hydroxylase (CYP17):
  - formation of 17α-hydroxypregnenlone
  - formation 17α-hydroxyprogesterone
- Both of these can be used to make androstenedione, a precursor of testosterone and estrogens.

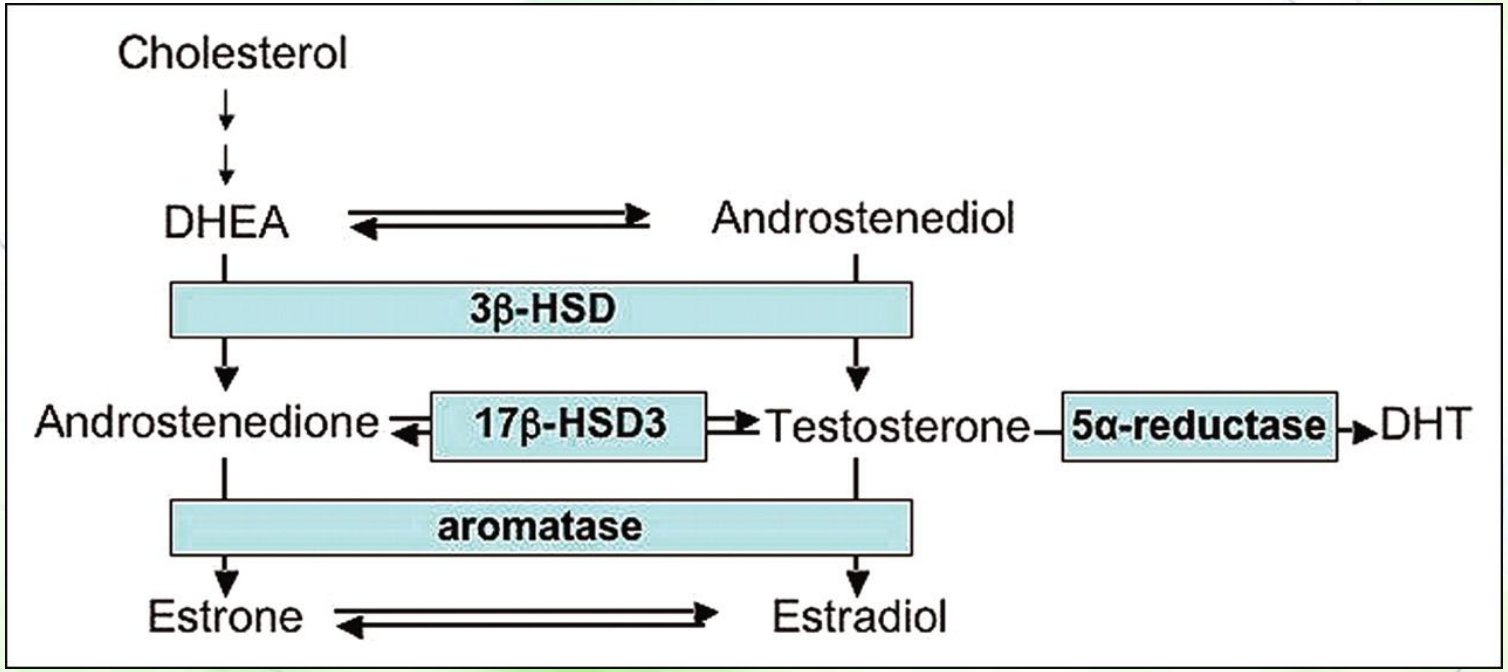


Deficiency of 17α-hydroxylase

- Deficiency in this enzyme leads to the formation of female genitalia in all patients .
- In addition, no cortisol or sex steroid would be produced.
- On the other hand, mineralocorticoids will be overproduced, resulting in higher retention of Na+ and, consequently, hypertension.

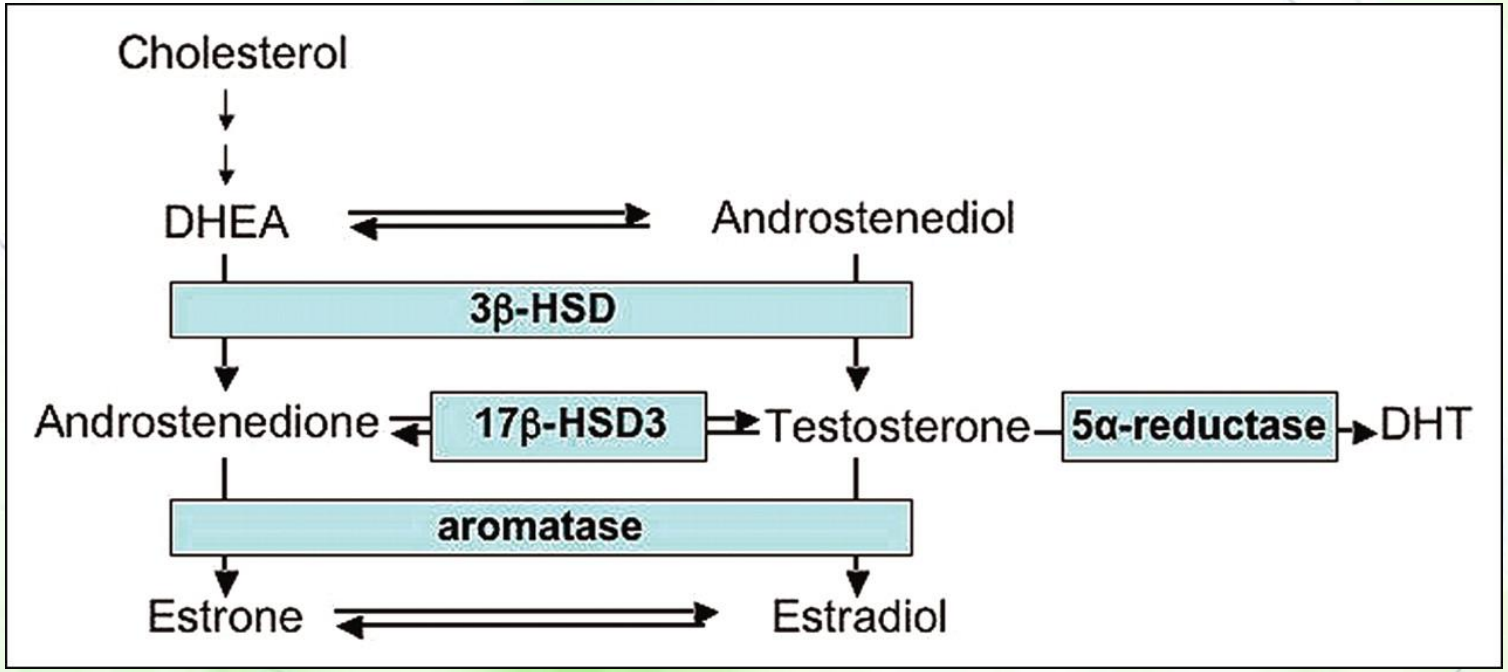
17β-hydroxysteroid dehydrogenase

- Testes and ovaries contain the enzyme, 17β-hydroxysteroid dehydrogenase, which enables androgens to be converted to testosterone.



5α-reductase

- In Sertoli cells and other target cells, testosterone is rapidly converted to DHT by the 5α-reductase enzyme before it interacts with the androgen receptor.



Aromatase

- Aromatase converts androstenedione and testosterone into the estrogens.