

Carcinogenesis & Nuclear Receptors

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Nuclear Receptor Super-Family

Distinction based to:

(α) Activating ligand

(β) Structure

(γ) DNA binding domain

1. Steroid Nuclear Receptors

- ERs, PRs, ARs, GRs
- Homo-dimers
- DNA binding domain with reverse nucleotide sequence

2. Non-Steroid Nuclear Receptors

- RARs, RXRs, TRs, VDRs, PPARs, FXRs
- Hetero-dimers (mainly with RXR)
- DNA binding domain with continuous nucleotide sequence

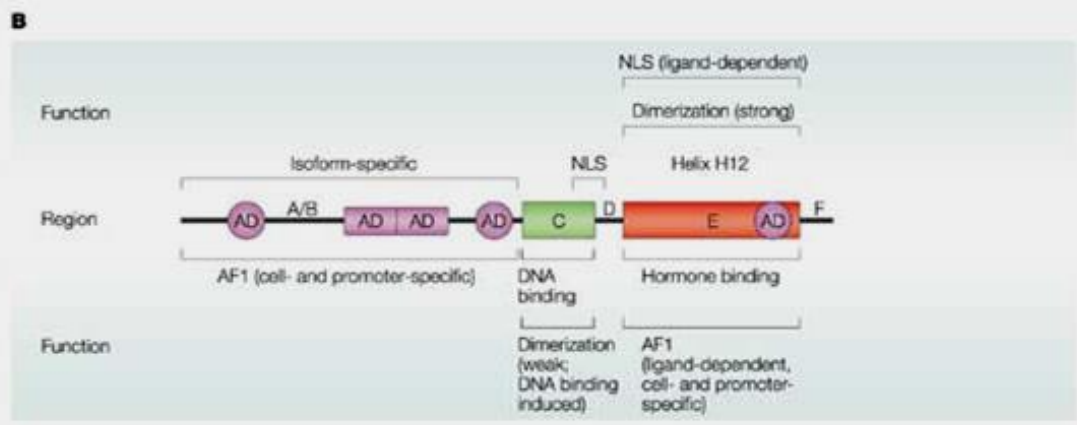
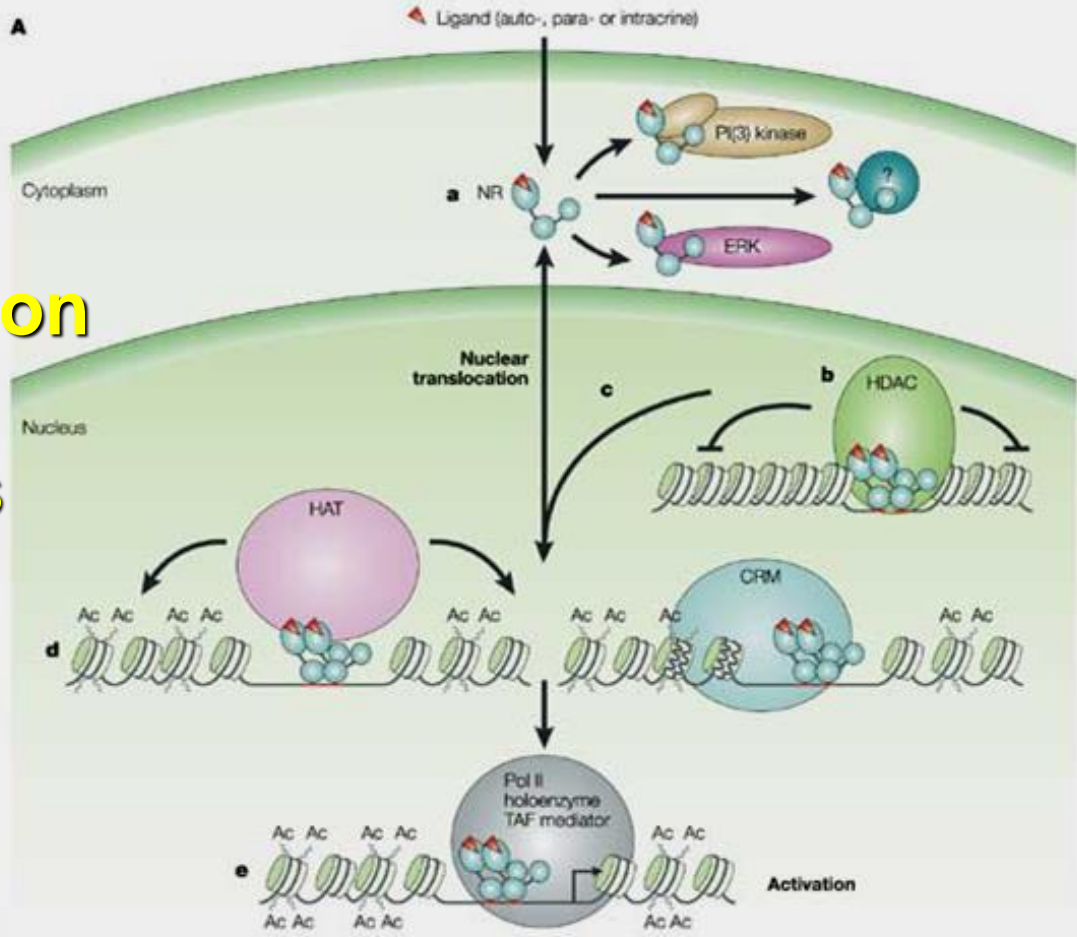
3. “Orphan” Nuclear Receptors

Nuclear Receptor Super-Family

DNA Binding Domain Receptor	(Hormone Response Element-HRE) HRE
AR (Androgen Receptor)	GG(A/T)ACAN ₂ TGTTCT
GR (Glycocorticoid Receptor)	GGTACAN ₂ TGTTCT
RAR (Retinoic Acid Receptor)	AGGTCAN ₂ AGGTCA
VDR (Vitamin D Receptor)	AGGTCAN ₂ AGGTCA
TR (Thyroid Receptor)	AGGTCAN ₂ AGGTCA

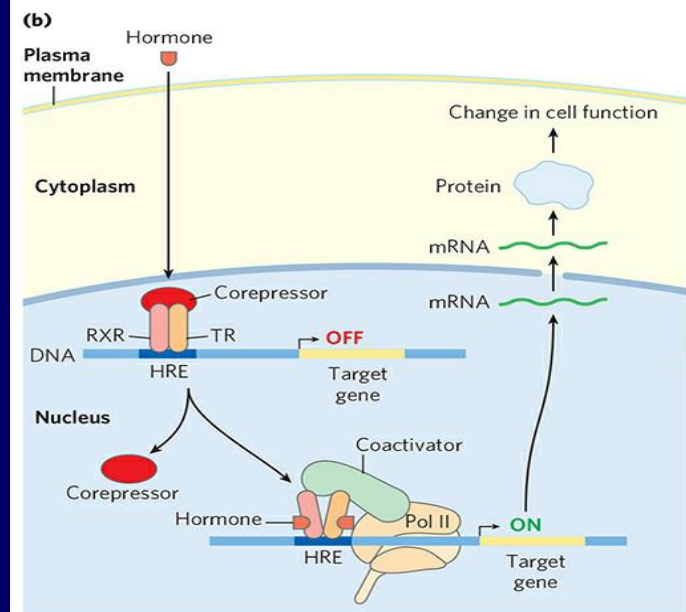
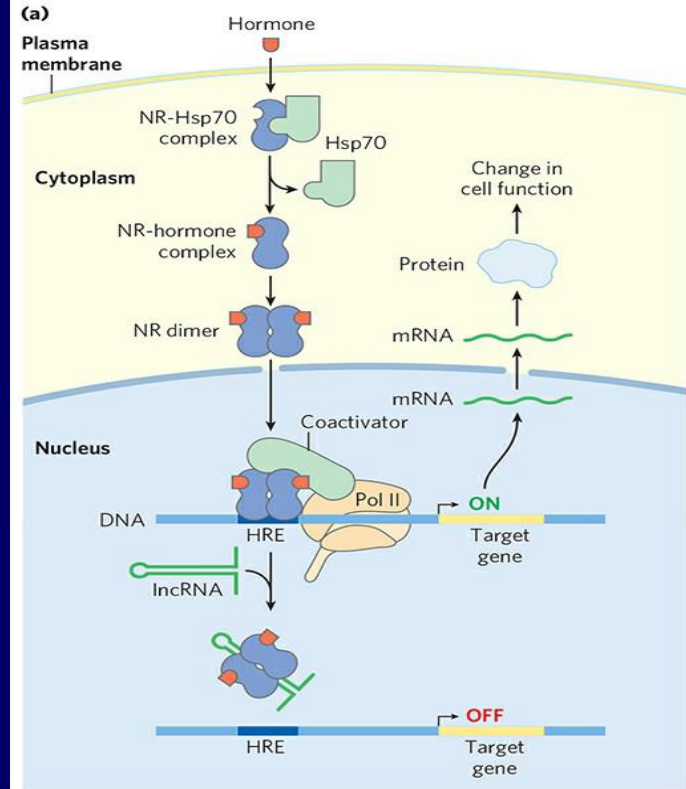
<https://www.youtube.com/watch?v=T0-fRSvNWjE>

Mode of Action Nuclear Receptors



Nuclear Receptors

FIGURE 28-33 Mechanisms of steroid hormone receptor function. There are two types of steroid-binding nuclear receptors. (a) Monomeric type I receptors (NR) are found in the cytoplasm, in a complex with the heat shock protein Hsp70. Receptors for estrogen, progesterone, androgens, and glucocorticoids are of this type. When the steroid hormone binds, the Hsp70 dissociates and the receptor dimerizes, exposing a nuclear localization signal. The dimeric receptor, with hormone bound, migrates to the nucleus, where it binds to a hormone response element (HRE) and acts as a transcription activator. The activity of the receptor can be repressed by binding to an lncRNA (such as GAS5), which competes directly with binding to the HRE. (b) Type II receptors, by contrast, are always in the nucleus, bound to an HRE in the DNA and to a corepressor that renders the receptor inactive. The thyroid hormone receptor (TR) is of this type. The hormone migrates through the cytoplasm and diffuses across the nuclear membrane. In the nucleus it binds to a heterodimer consisting of the thyroid hormone receptor and the retinoid X receptor (RXR). A conformation change leads to dissociation of the corepressor, and the receptor then functions as a transcription activator.



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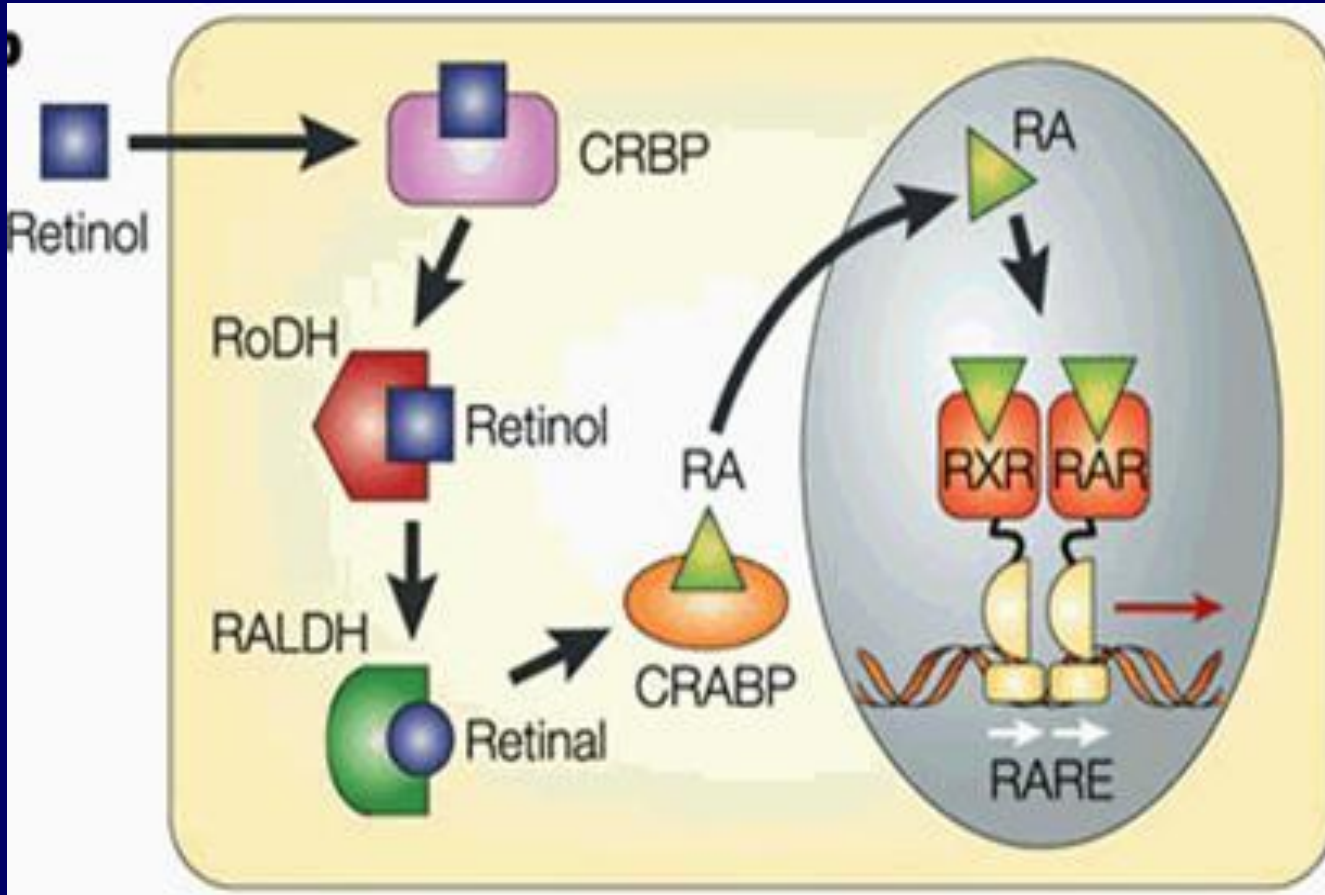
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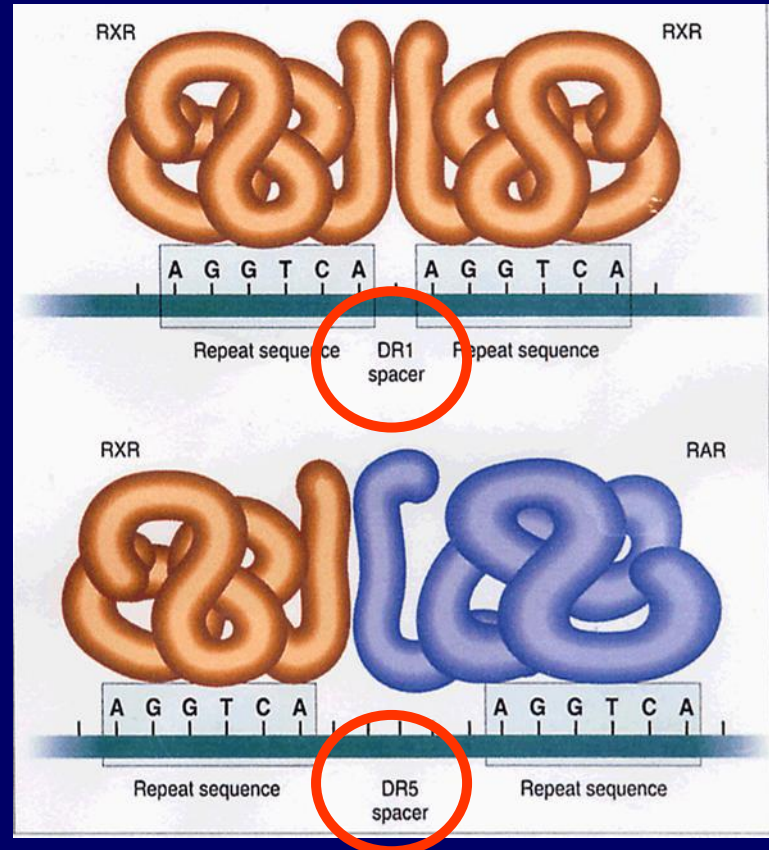
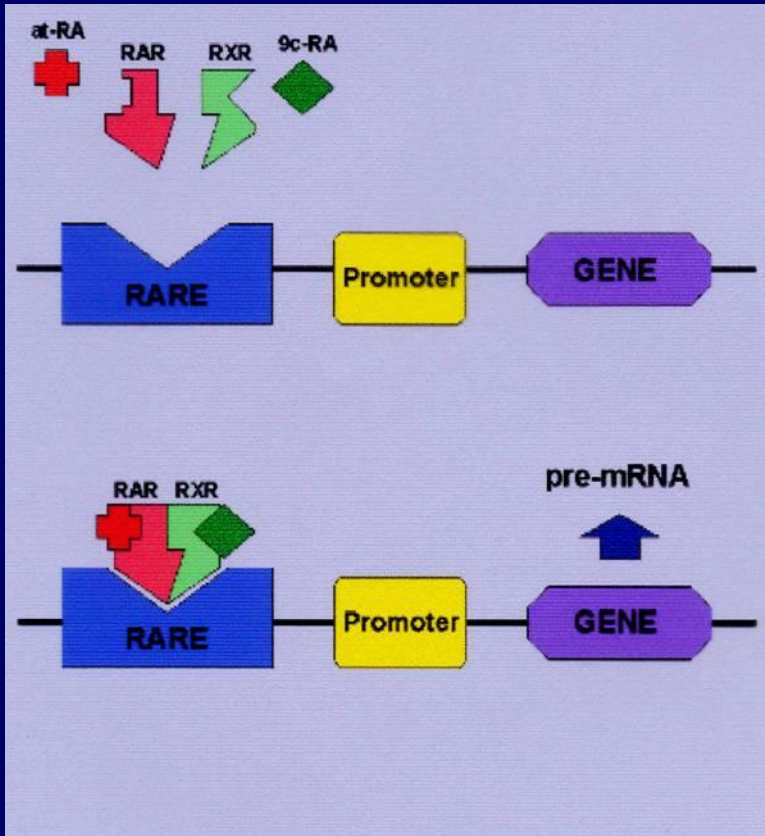
3. “Orphan” Nuclear Receptors

Retinoids' mode of action



Pierre Chambon & Ronald M. Evans (1987)
Retinoids Receptors

Retinoids' mode of action



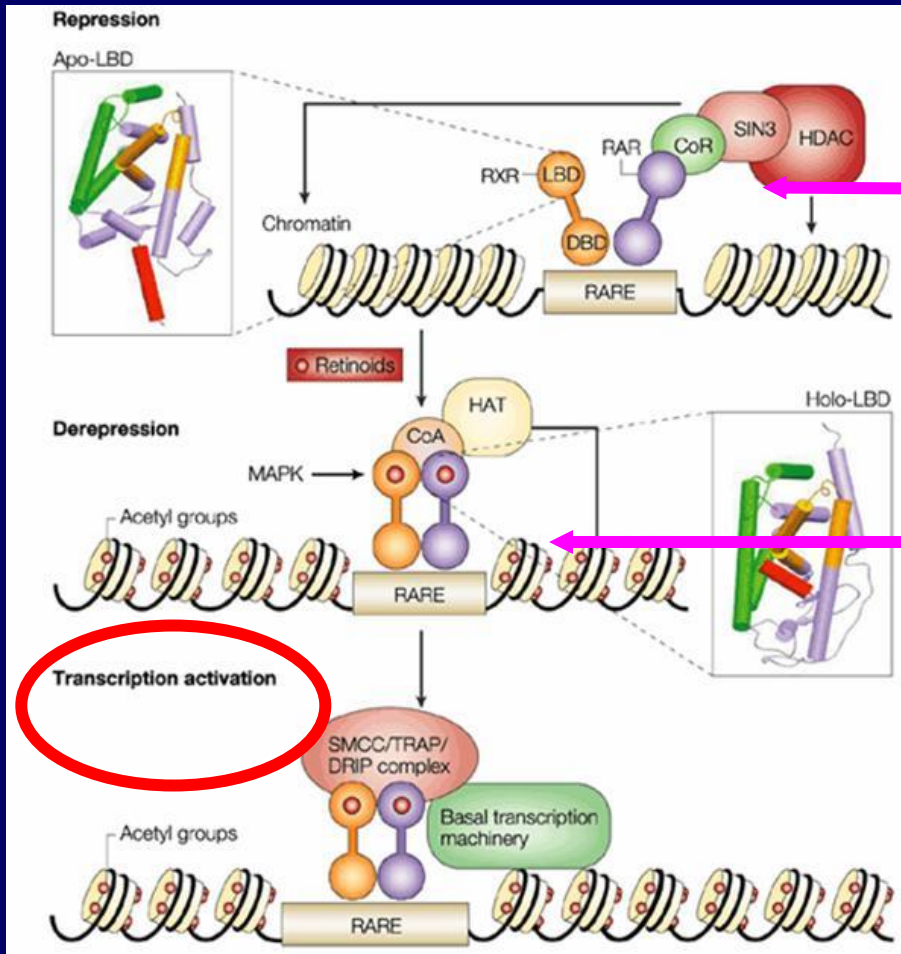
RAR (α , β , γ)

RXR (α , β , γ)



encoded by different genes
at least 2 isoforms for each isotype
(differential usage of promoters and/or alternative splicing)

Positive Transcriptional Activity



Co-repressors

Co-activators

Nuclear Receptor Super-Family

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1. Steroid Nuclear Receptors

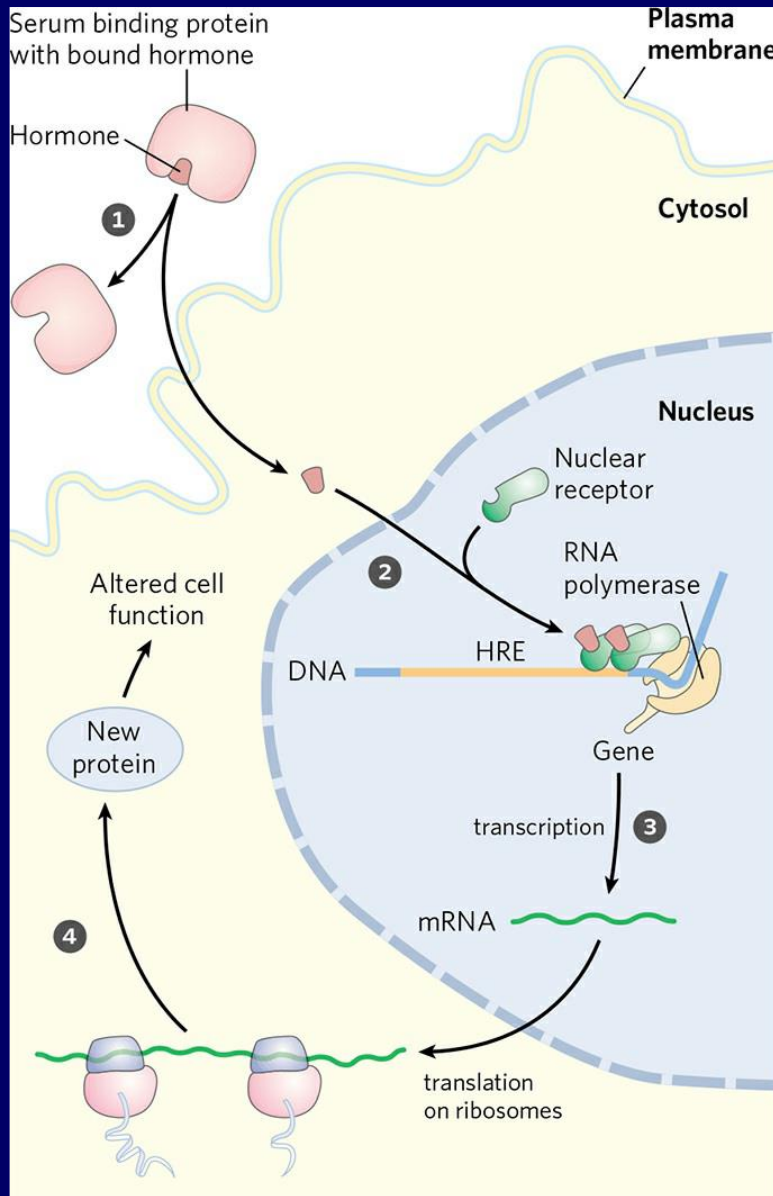
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Steroid Nuclear Receptors



1 Hormone, carried to the target tissue on serum binding proteins, diffuses across the plasma membrane and binds to its specific receptor protein in the nucleus.

2 Hormone binding changes the conformation of the receptor; it forms homo- or heterodimers with other hormone-receptor complexes and binds to specific regulatory regions called hormone response elements (HREs) in the DNA adjacent to specific genes.

3 Receptor attracts coactivator or corepressor protein(s) and, with them, regulates transcription of the adjacent gene(s), increasing or decreasing the rate of mRNA formation.

4 Altered levels of the hormone-regulated gene product produce the cellular response to the hormone.

FIGURE 12-30 General mechanism by which steroid and thyroid hormones, retinoids, and vitamin D regulate gene expression. Some steroids also act through plasma membrane receptors by a completely different mechanism.

Structure Nuclear Receptors

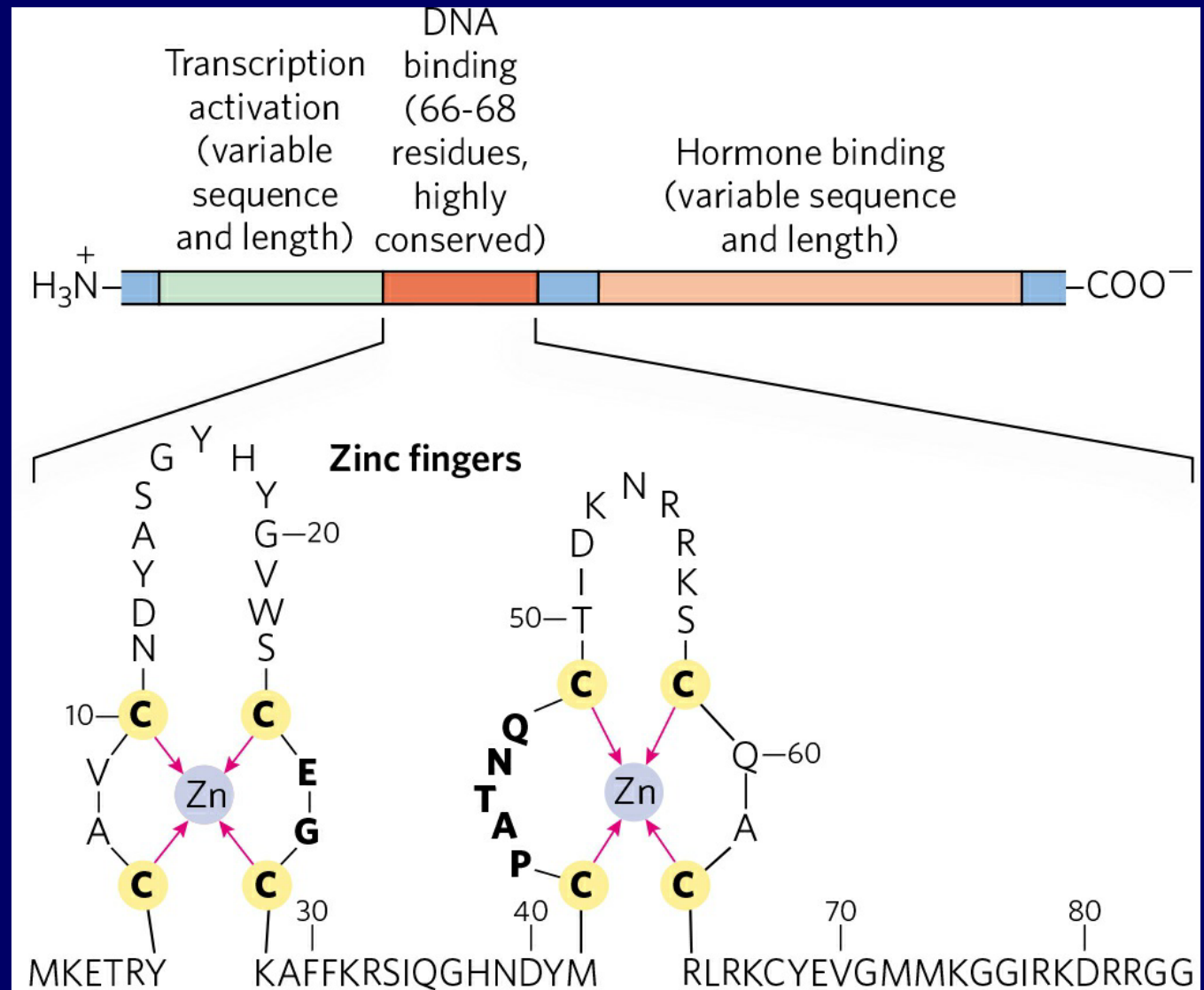
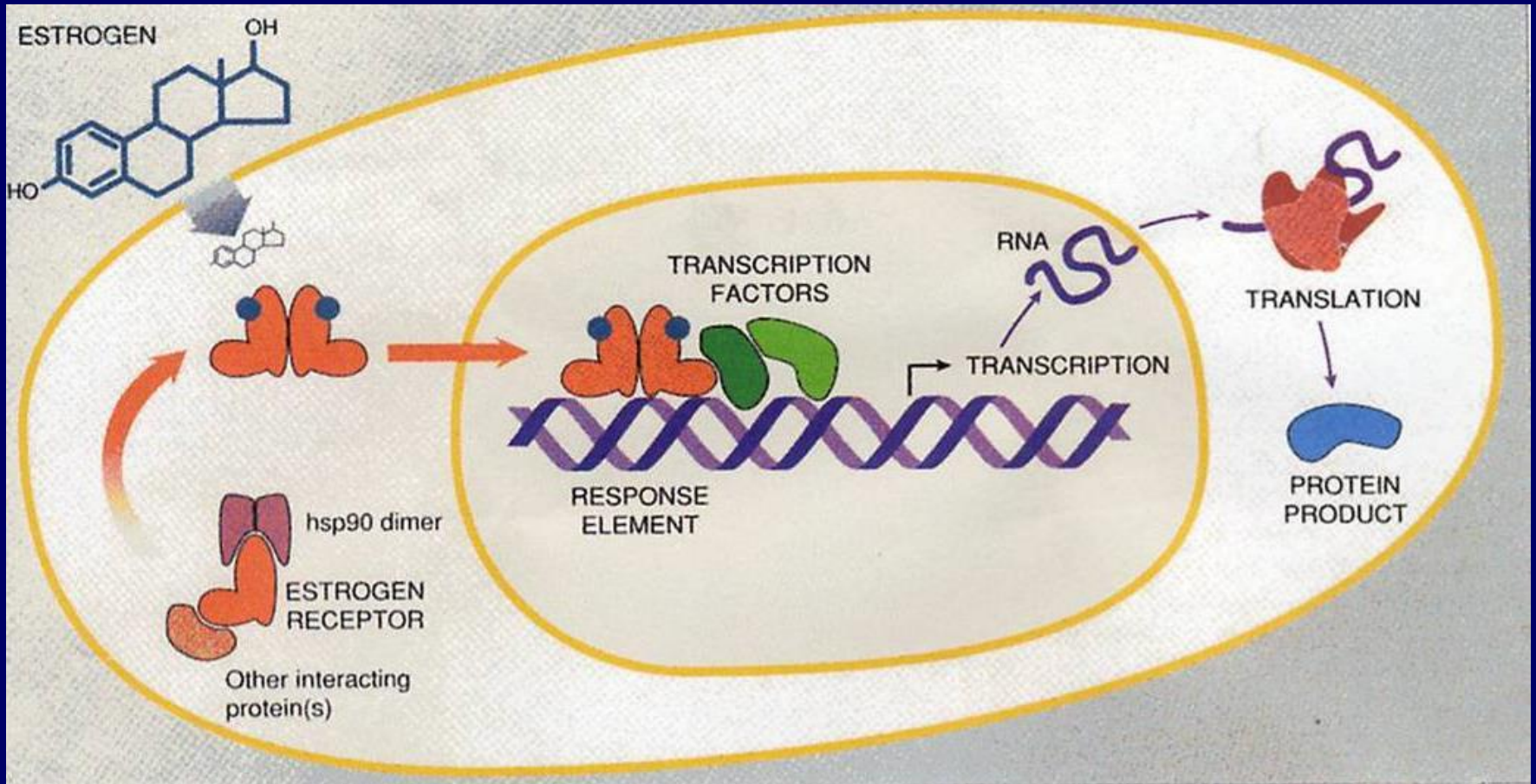
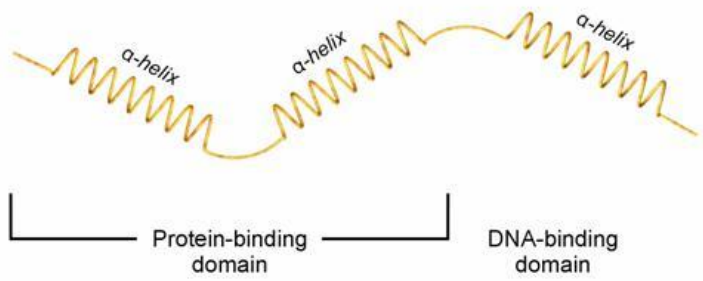


FIGURE 28-34 Typical steroid hormone receptors. These receptor proteins have a binding site for the hormone, a DNA-binding domain, and a region that activates transcription of the regulated gene. The highly conserved DNA-binding domain has two zinc fingers. The sequence shown here is that for the estrogen receptor, but the residues in bold type are common to all steroid hormone receptors

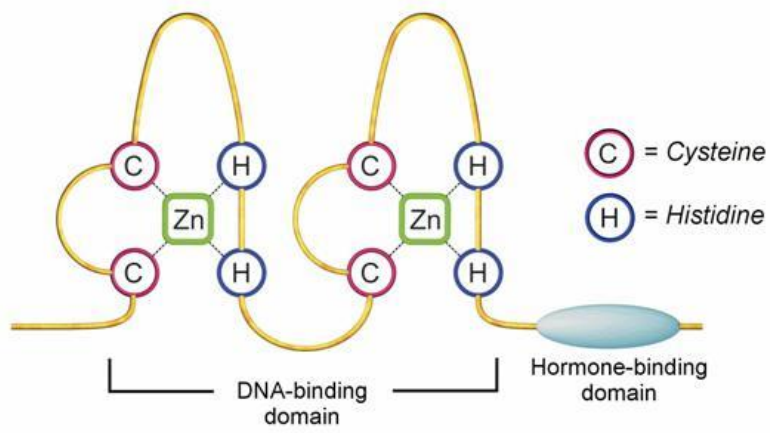
Action Model of Hormone Receptors



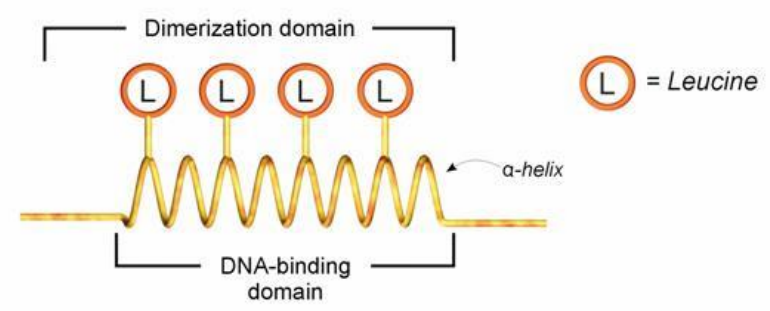
https://www.youtube.com/watch?v=xGtF77c_Ui4



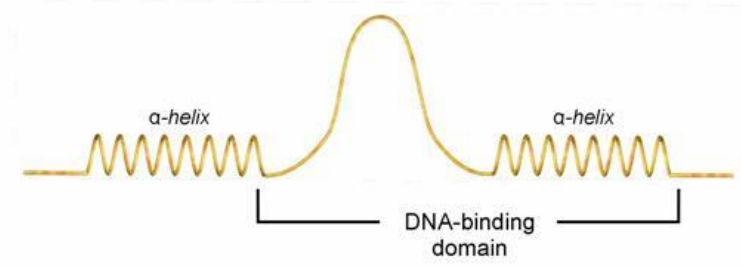
Helix-turn-helix Motif
(e.g. Oct-1)



Zinc Finger Motif
(e.g. estrogen receptor)



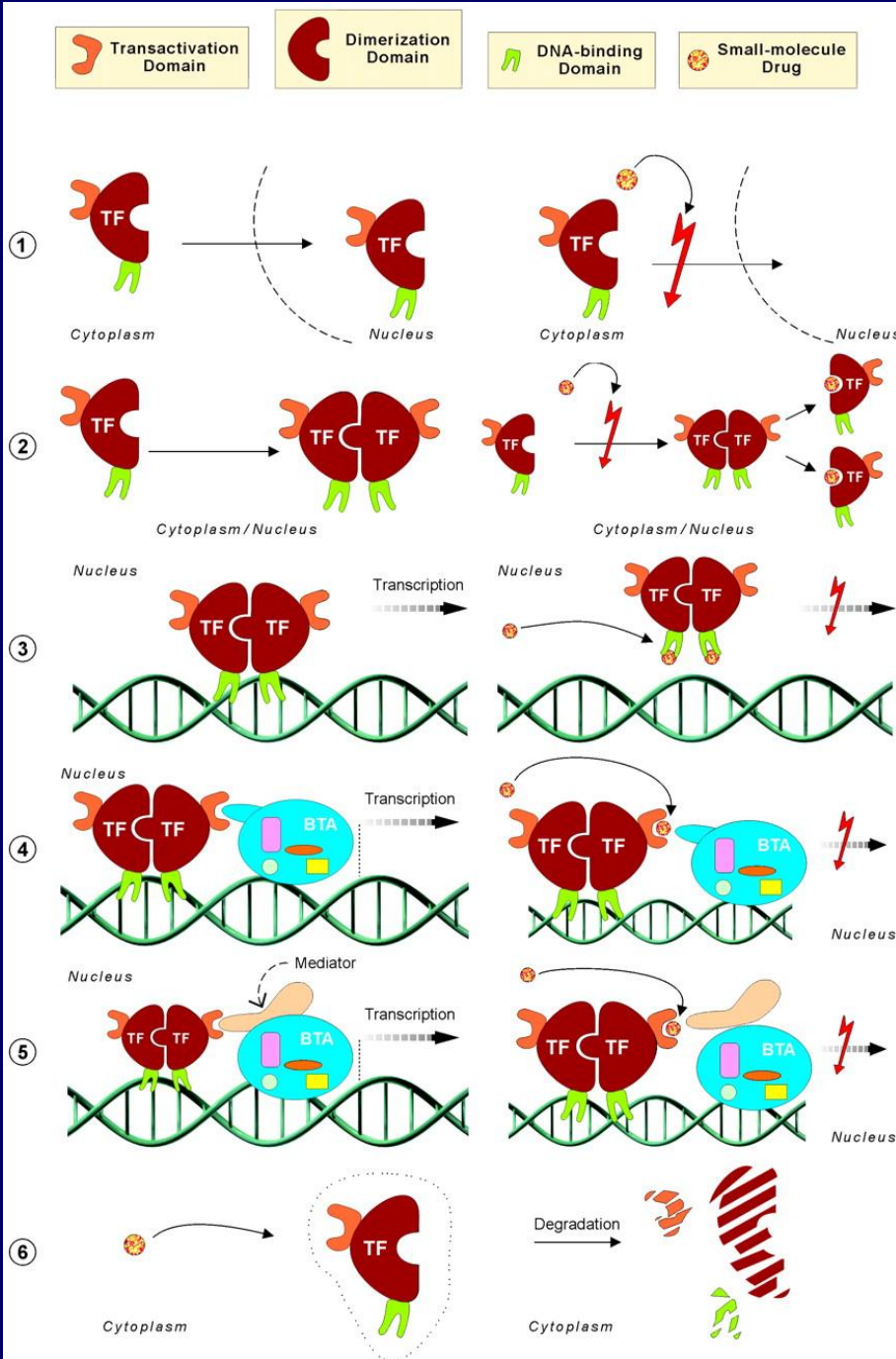
Leucine Zipper Motif
(e.g. c-Jun)



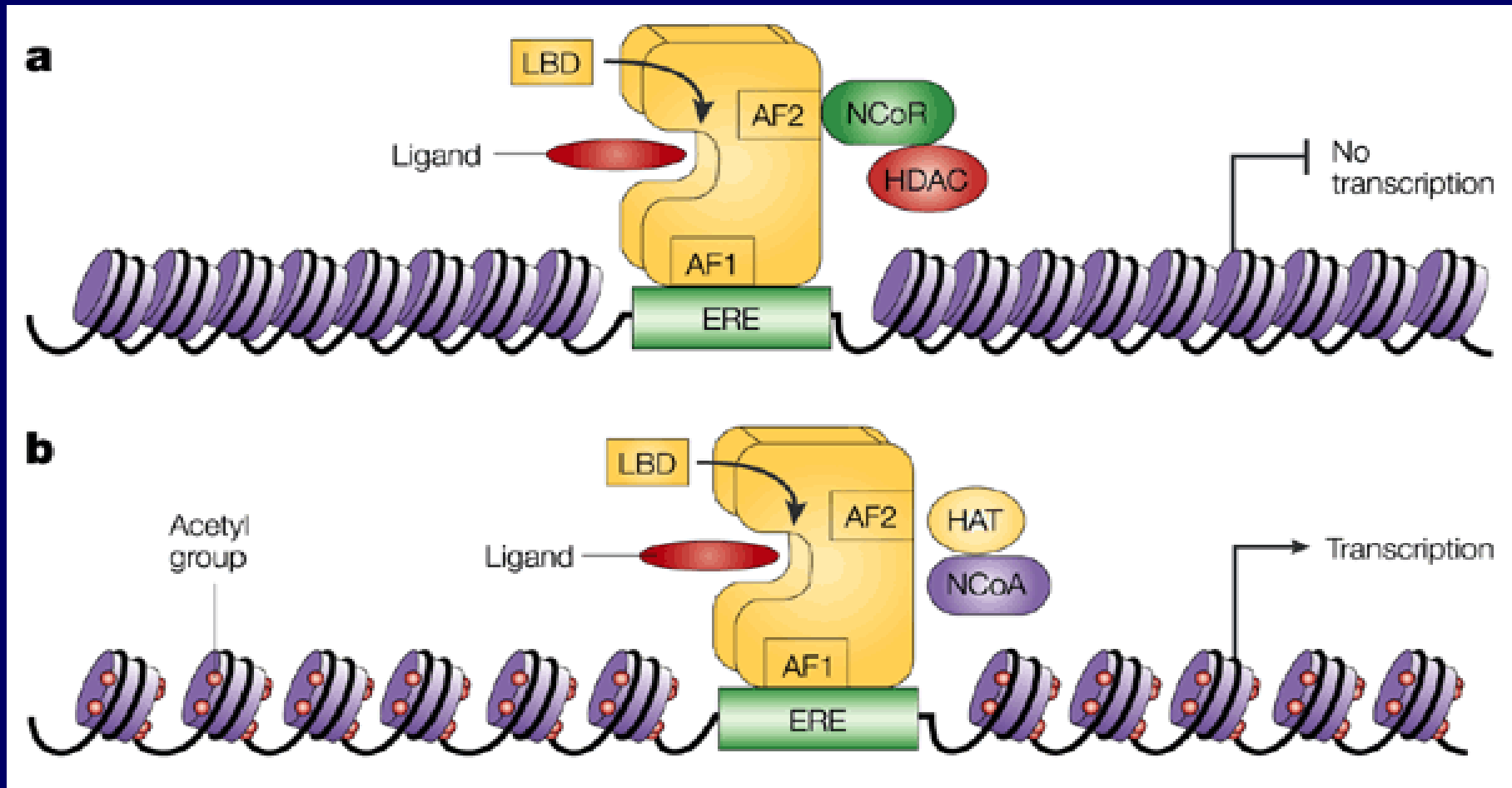
Helix-loop-helix Motif
(e.g. Myc)

Specific Transcription Factors

Specific Transcription Factors & Carcinogenesis



Action Model of Hormone Receptors



Breast Cancer Treatment

1. Selective Modulators of Estrogen Receptors (SERMs)

Tamoxifen

Raloxifen

2. Estrogen Receptors Competitors

Fulvestrant

3. Aromatase Inhibitors

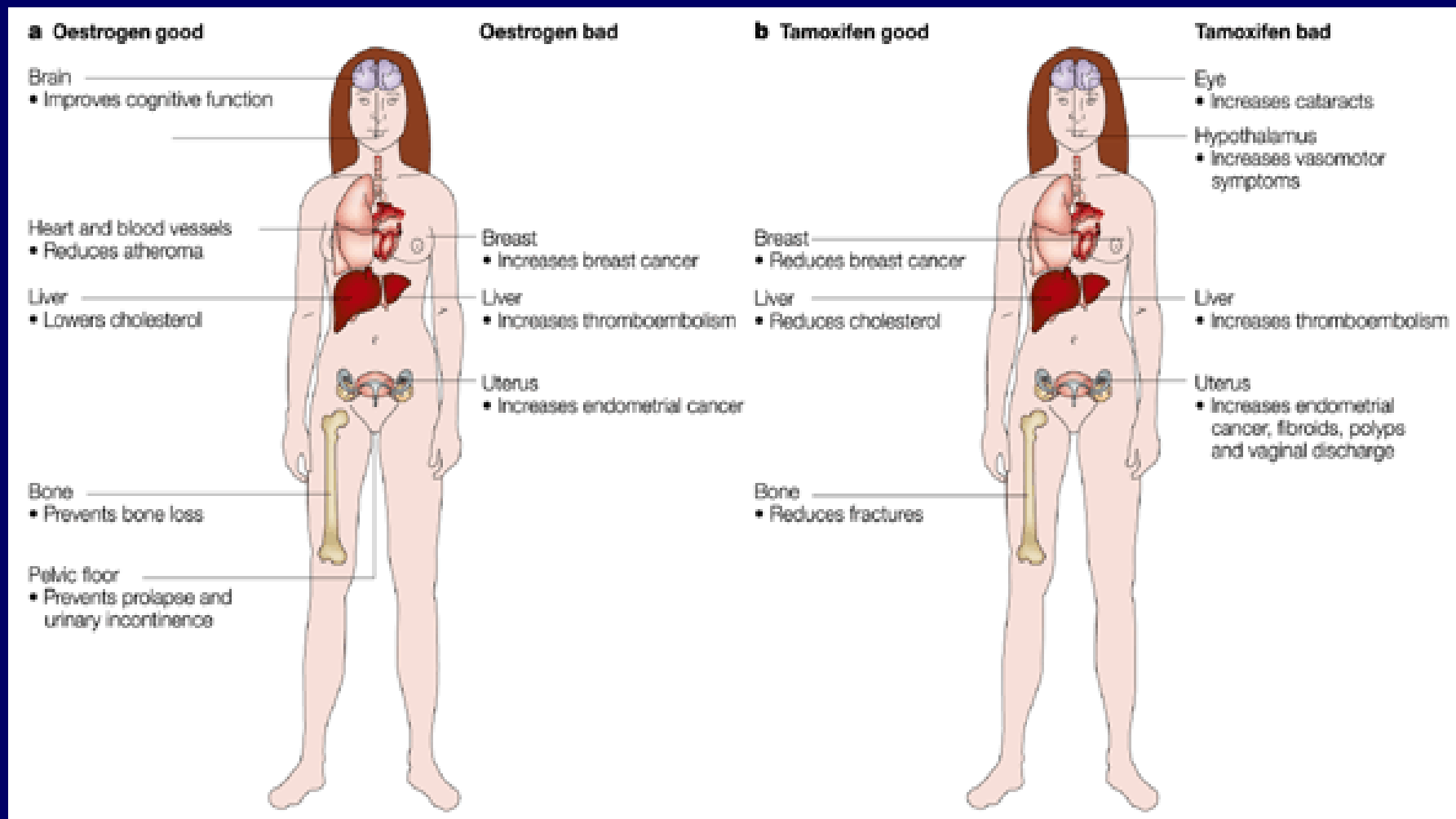
Anastrozole

Letrozole

Exemestane

Breast Cancer Treatment

Tamoxifen



Breast Cancer Treatment

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3. Aromatase Inhibitors

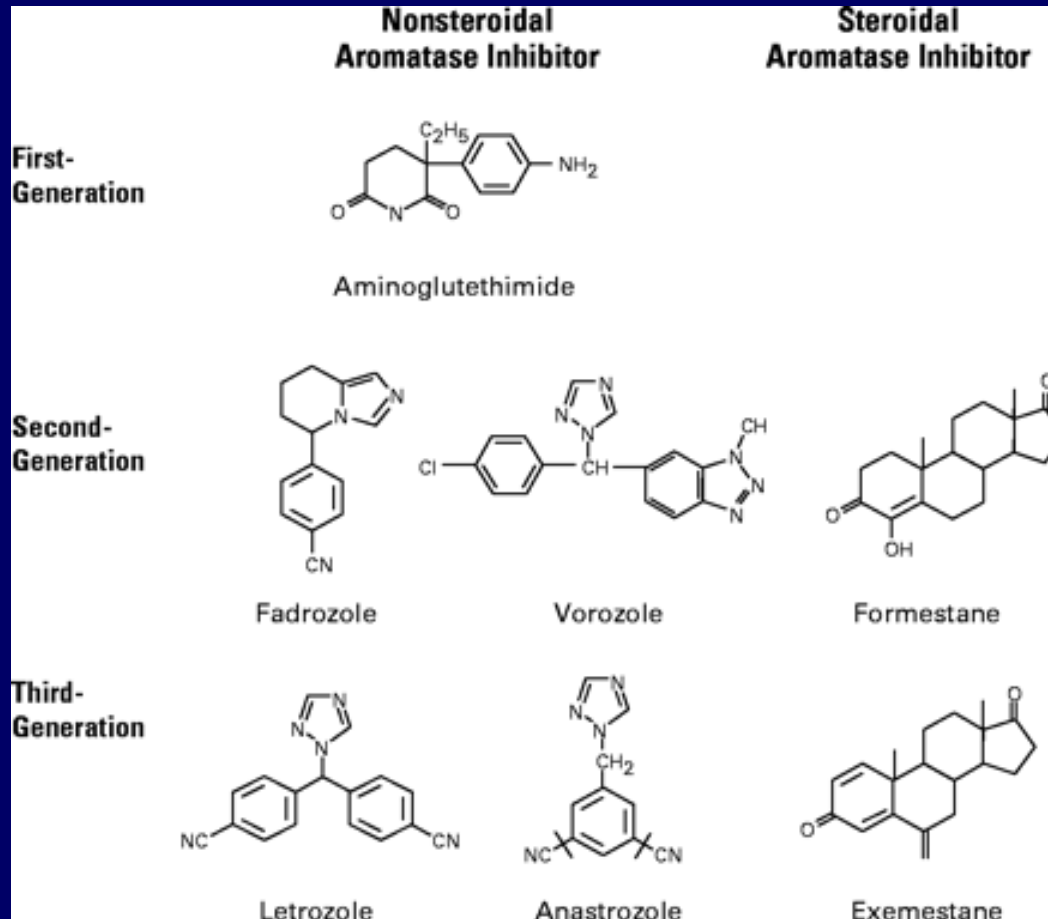
Anastrozole

Letrozole

Exemestane

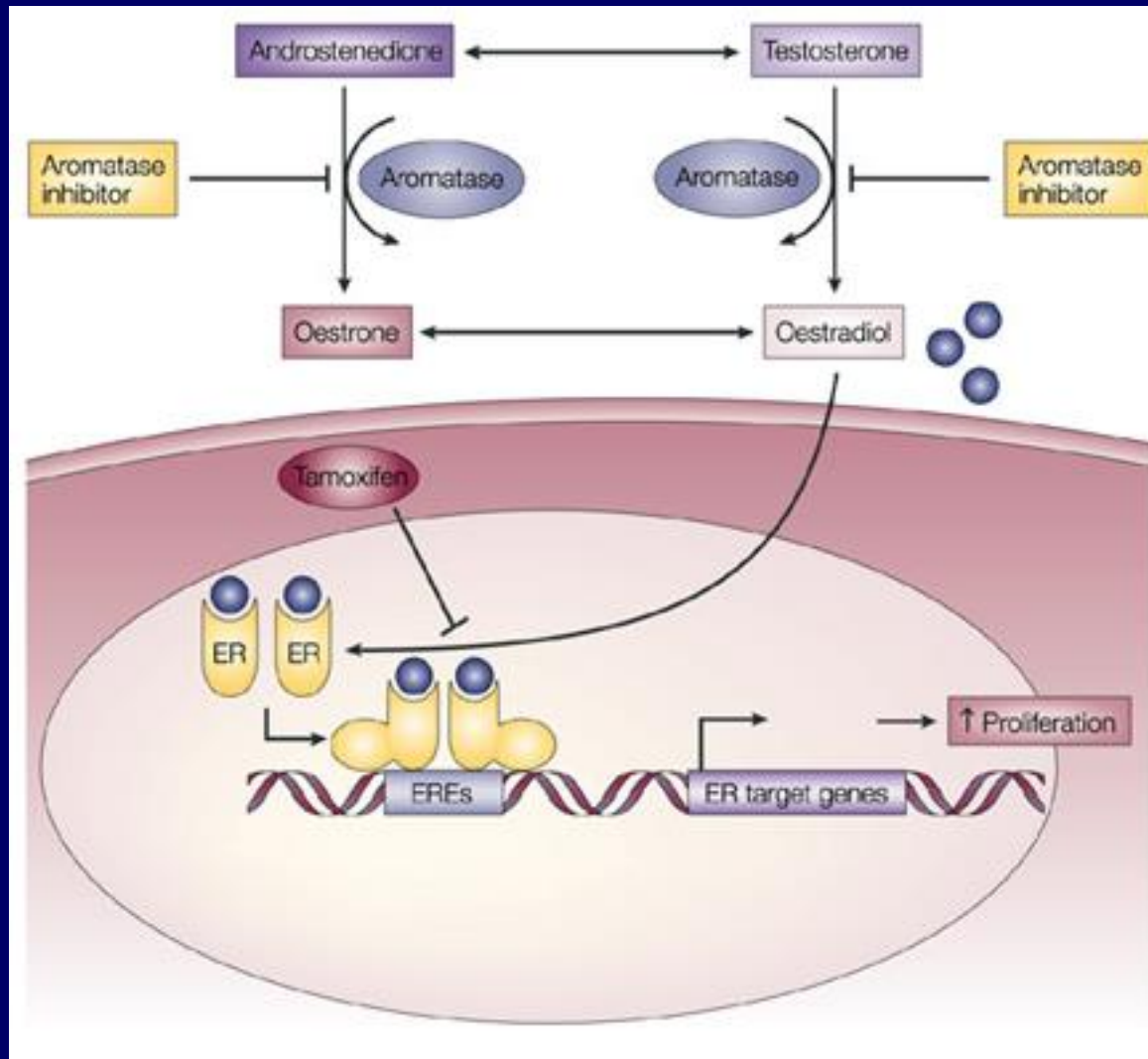
Breast Cancer Treatment

Aromatase Inhibitors



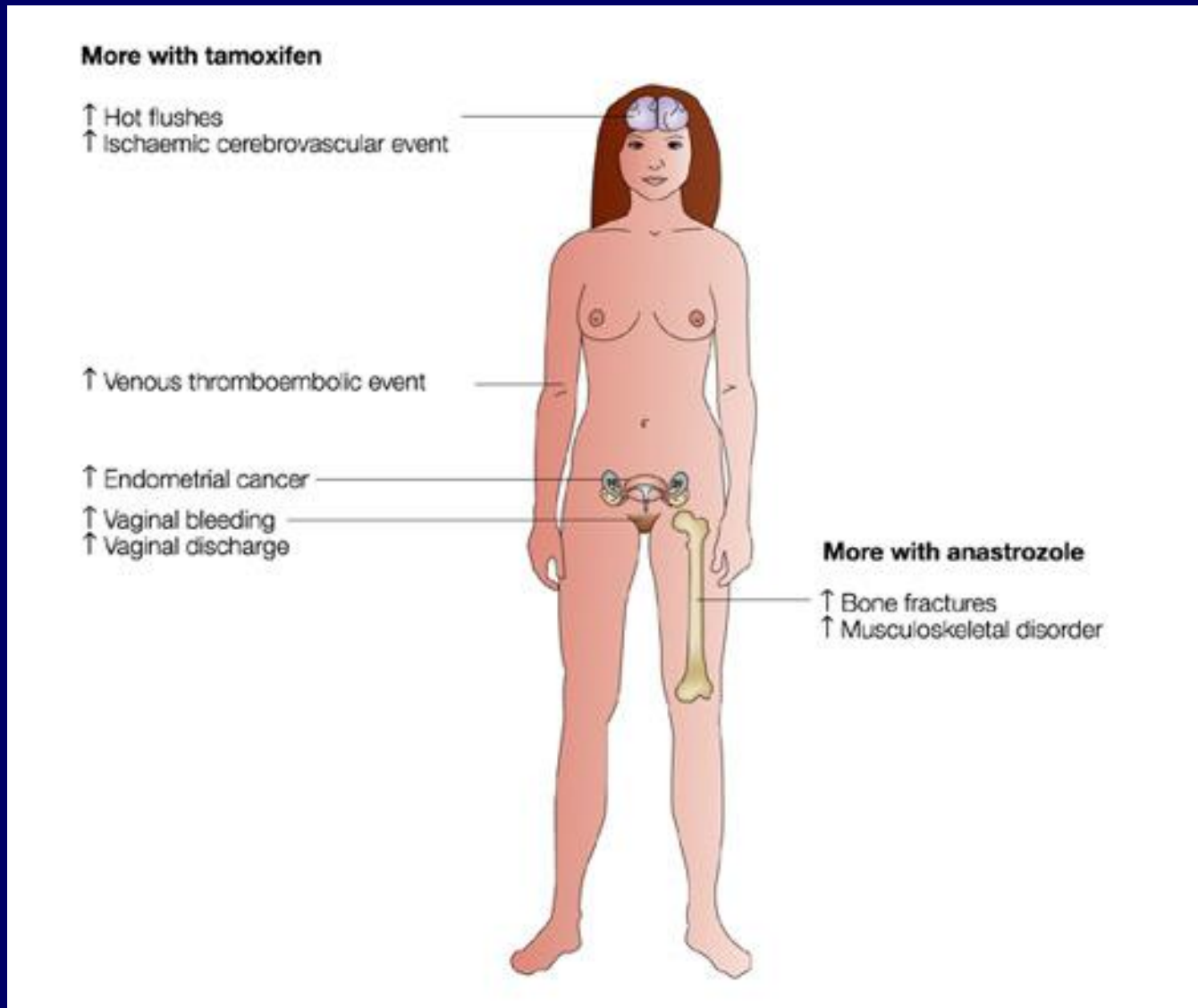
Breast Cancer Treatment

Aromatase Inhibitors



Breast Cancer Treatment

Aromatase Inhibitors vs Tamoxifen



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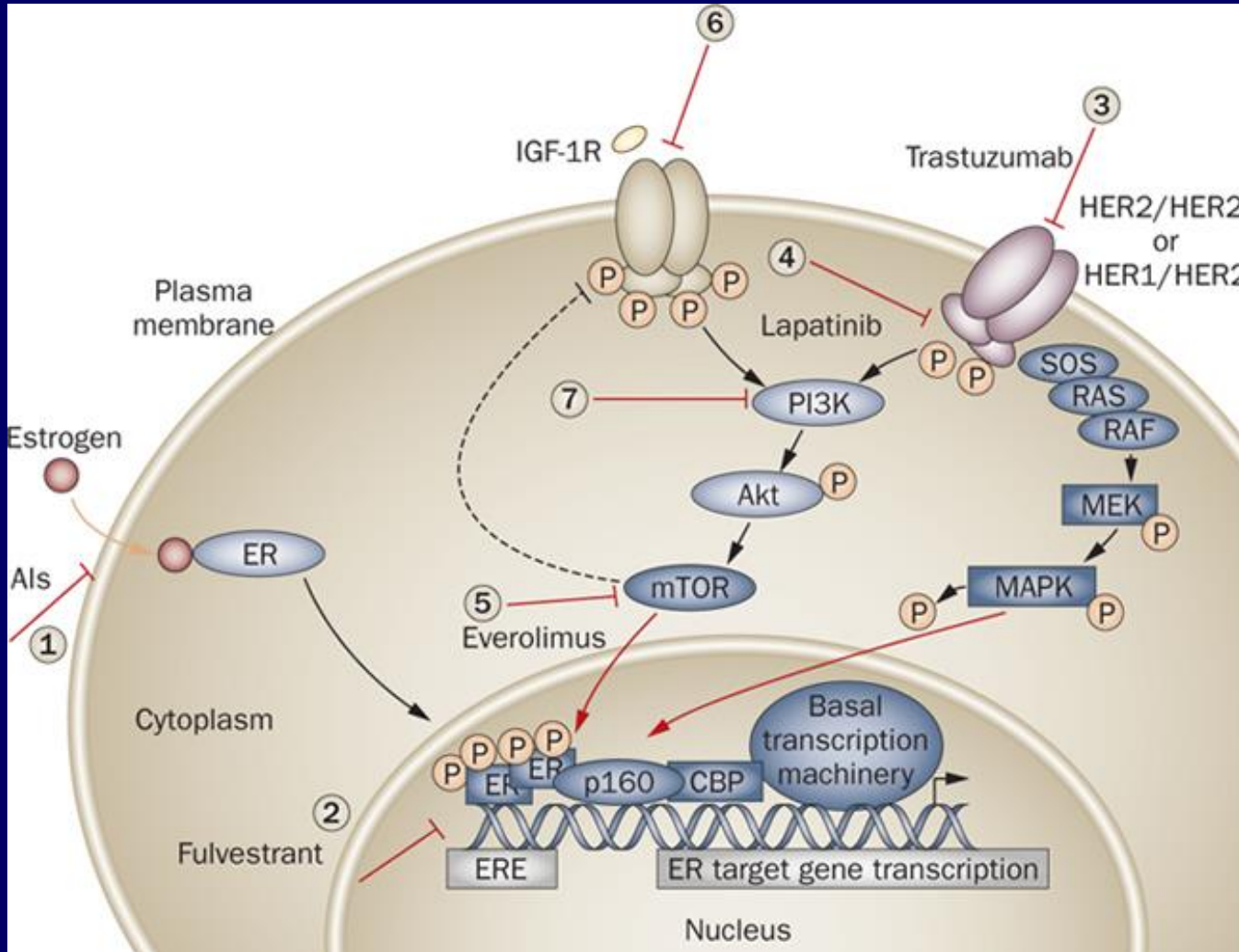
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3. “Orphan” Nuclear Receptors

Resistance in Hormone Tx



Nuclear Receptor Super-Family

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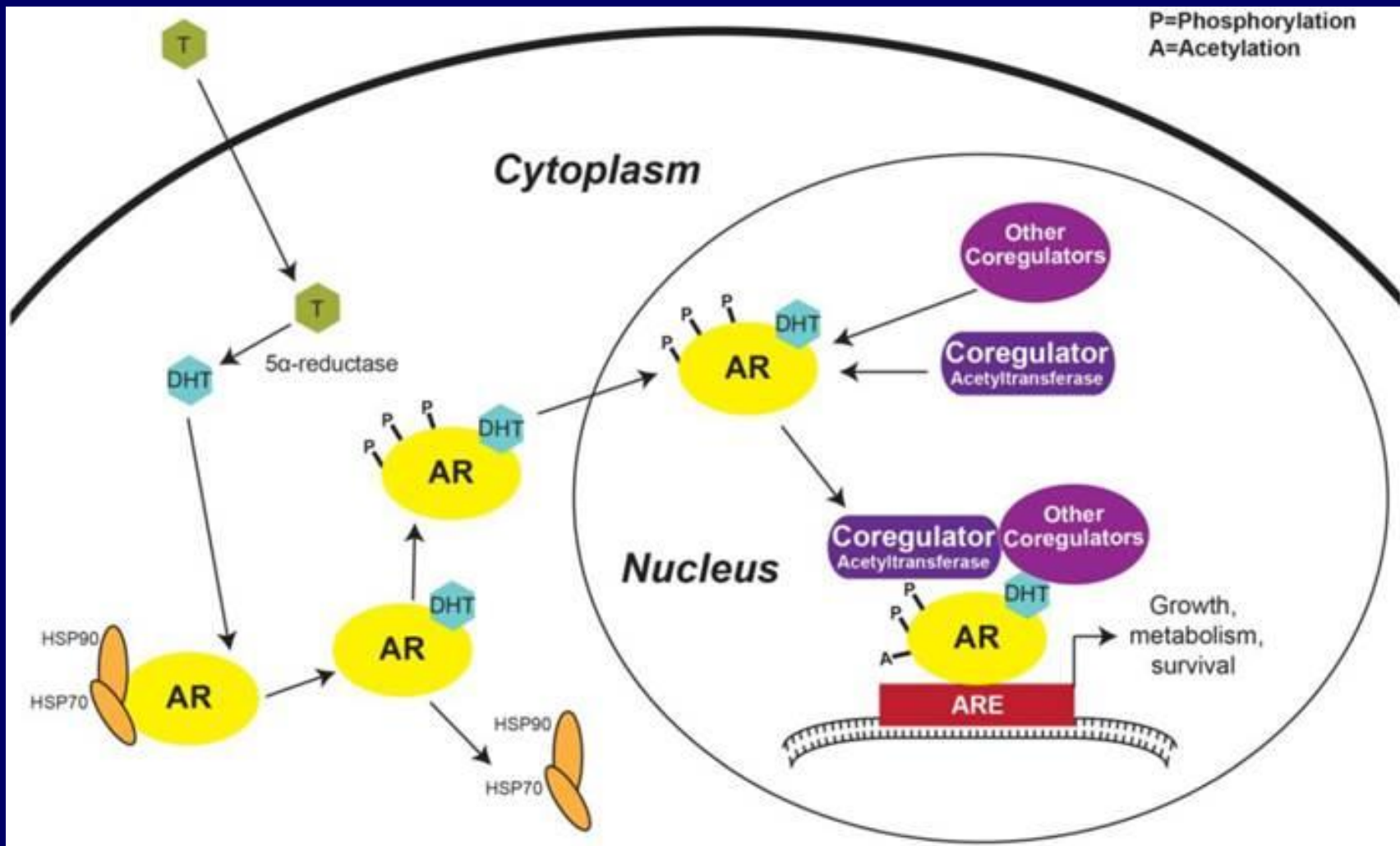
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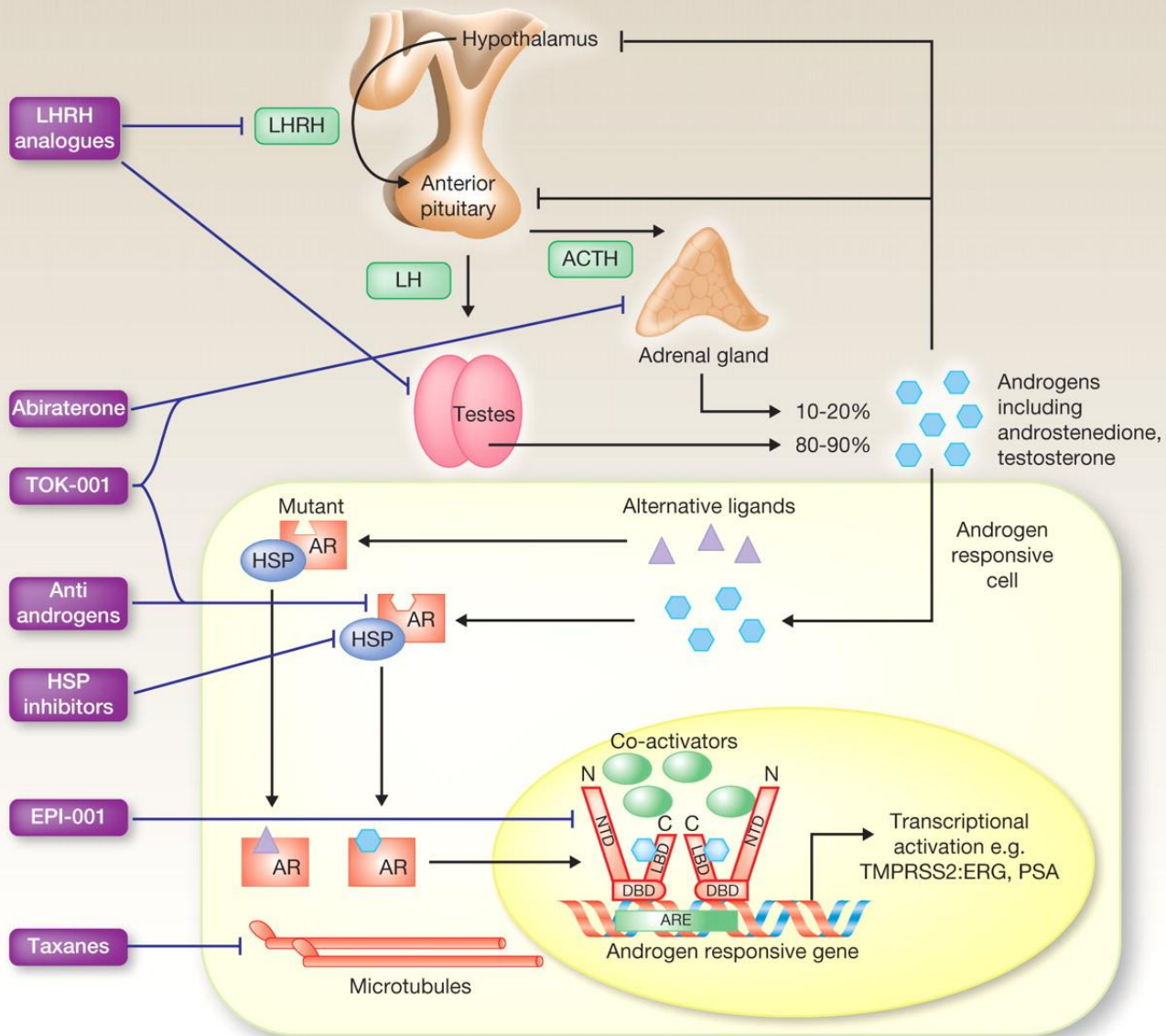
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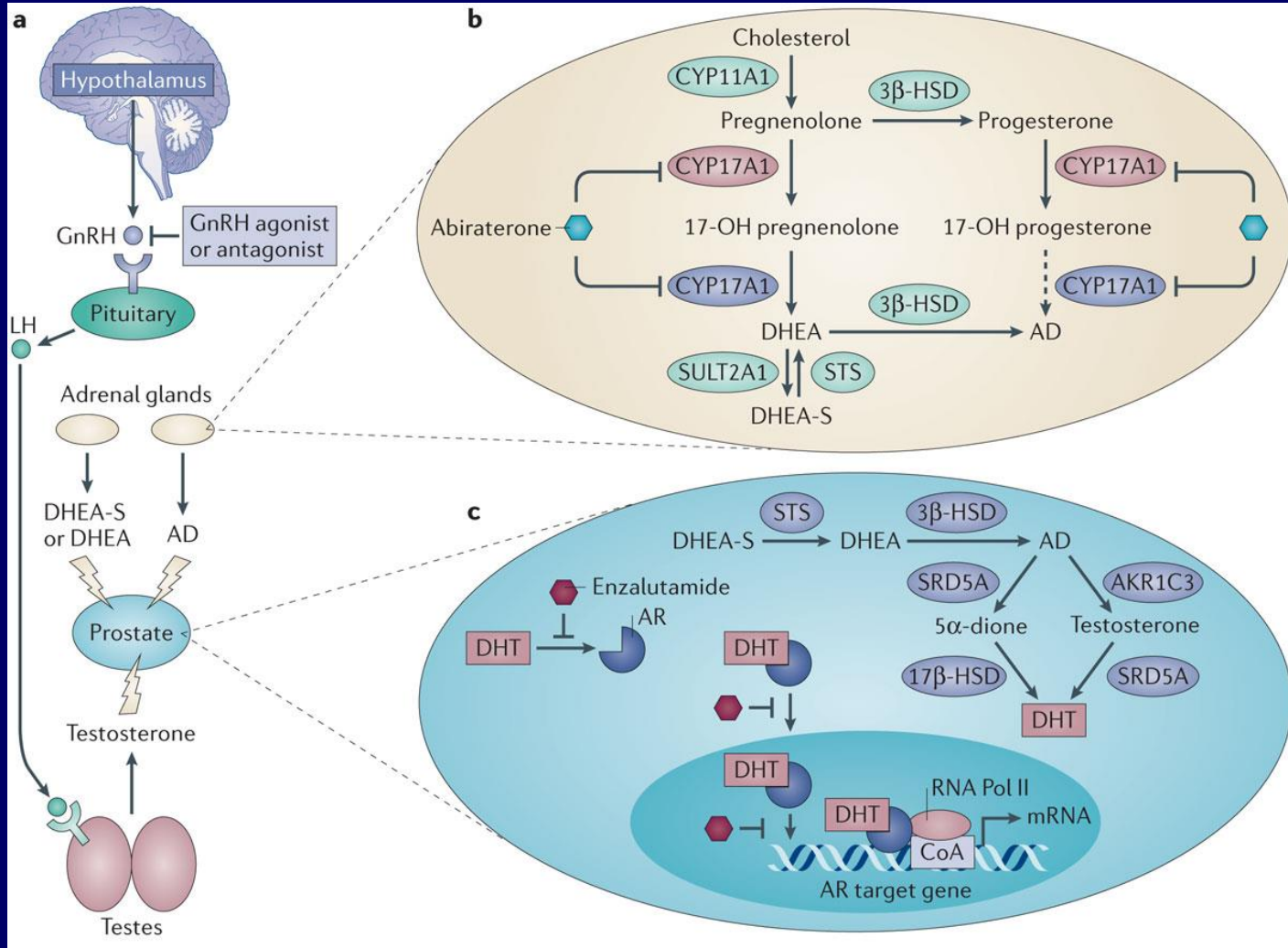
Action Model of Androgen Receptors



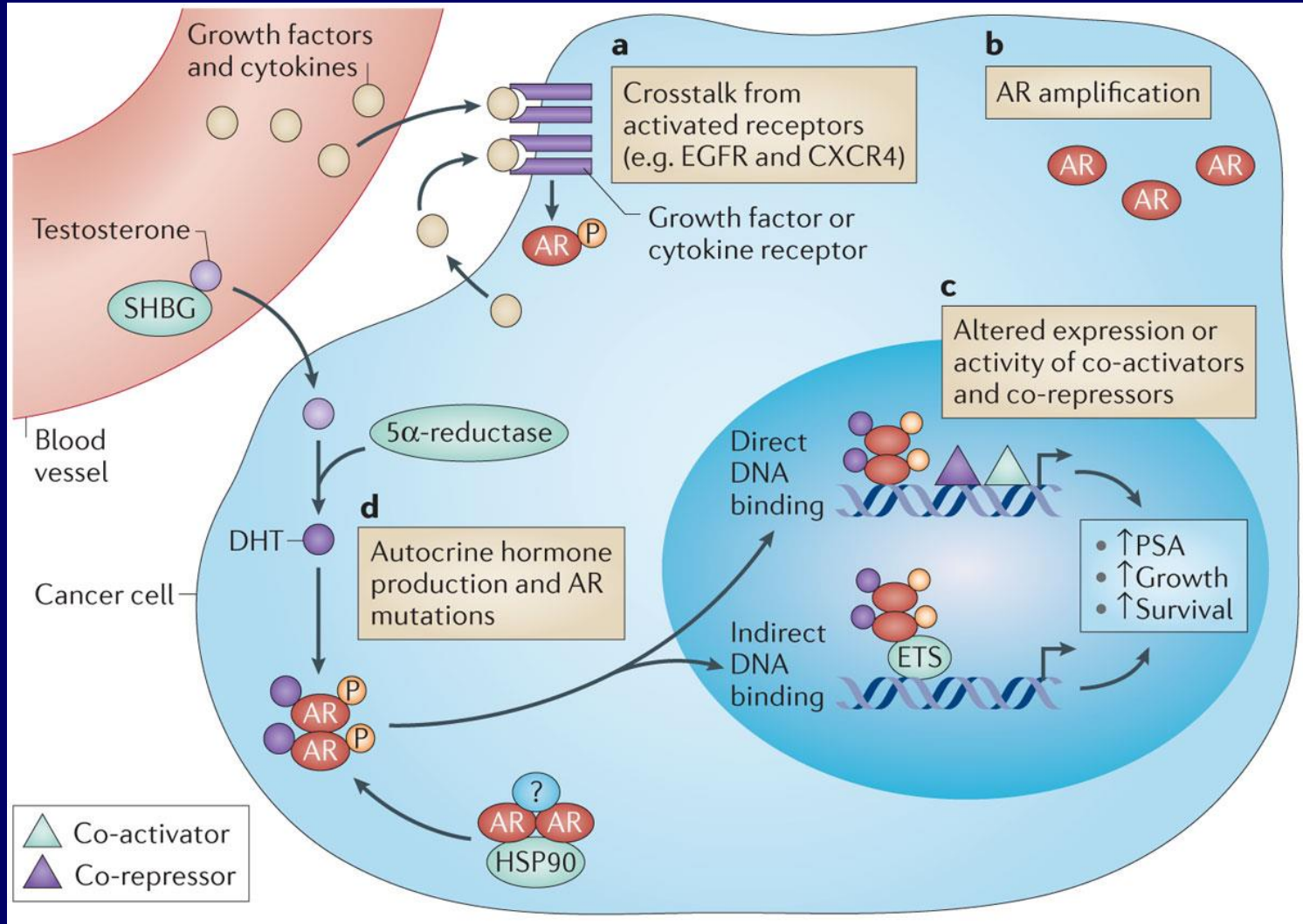
Androgen Receptor



Castration-Resistant Prostate Cancer (CRPC)



Molecular Mechanisms of CRPC

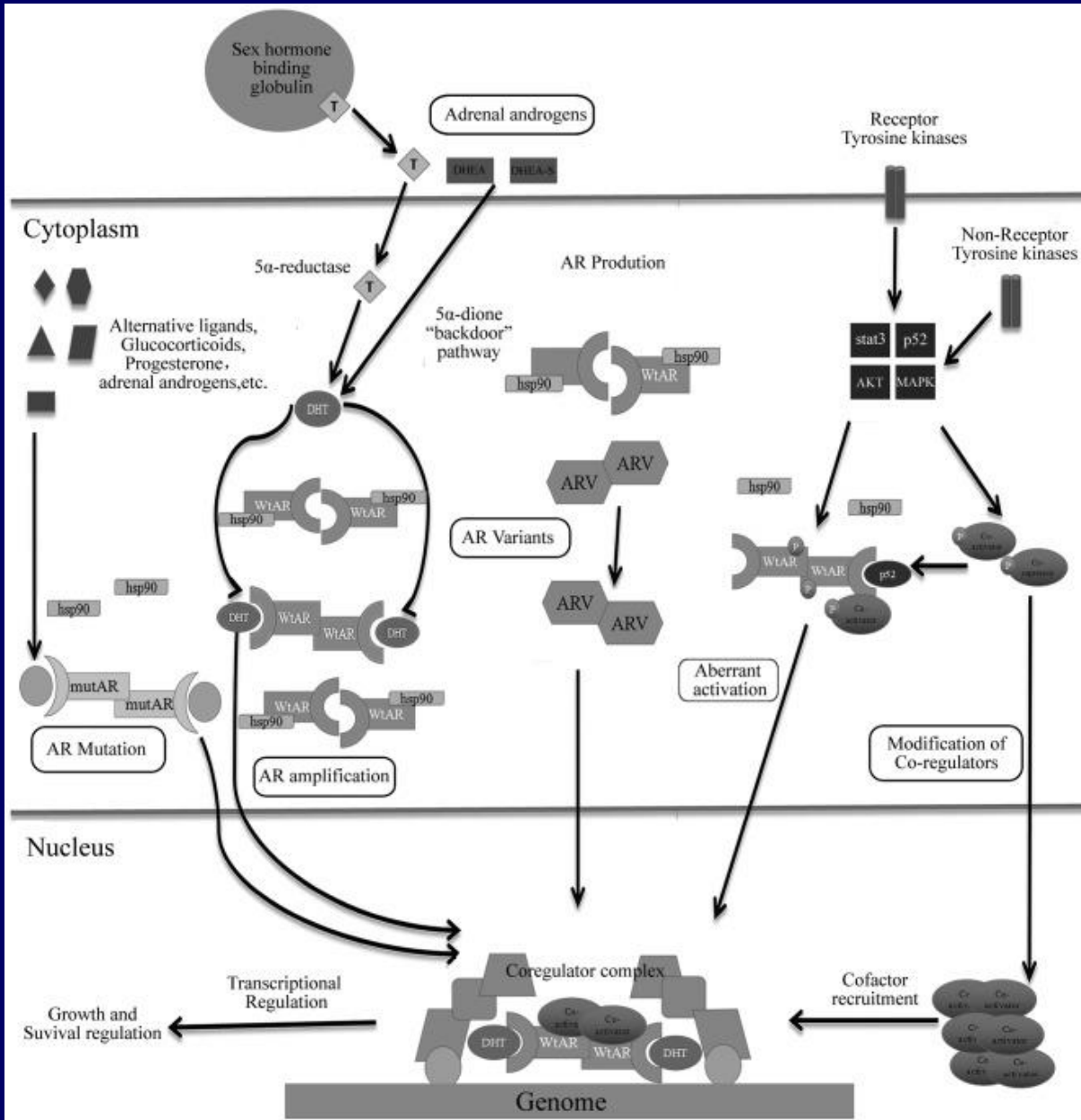


Molecular Mechanisms of CRPC

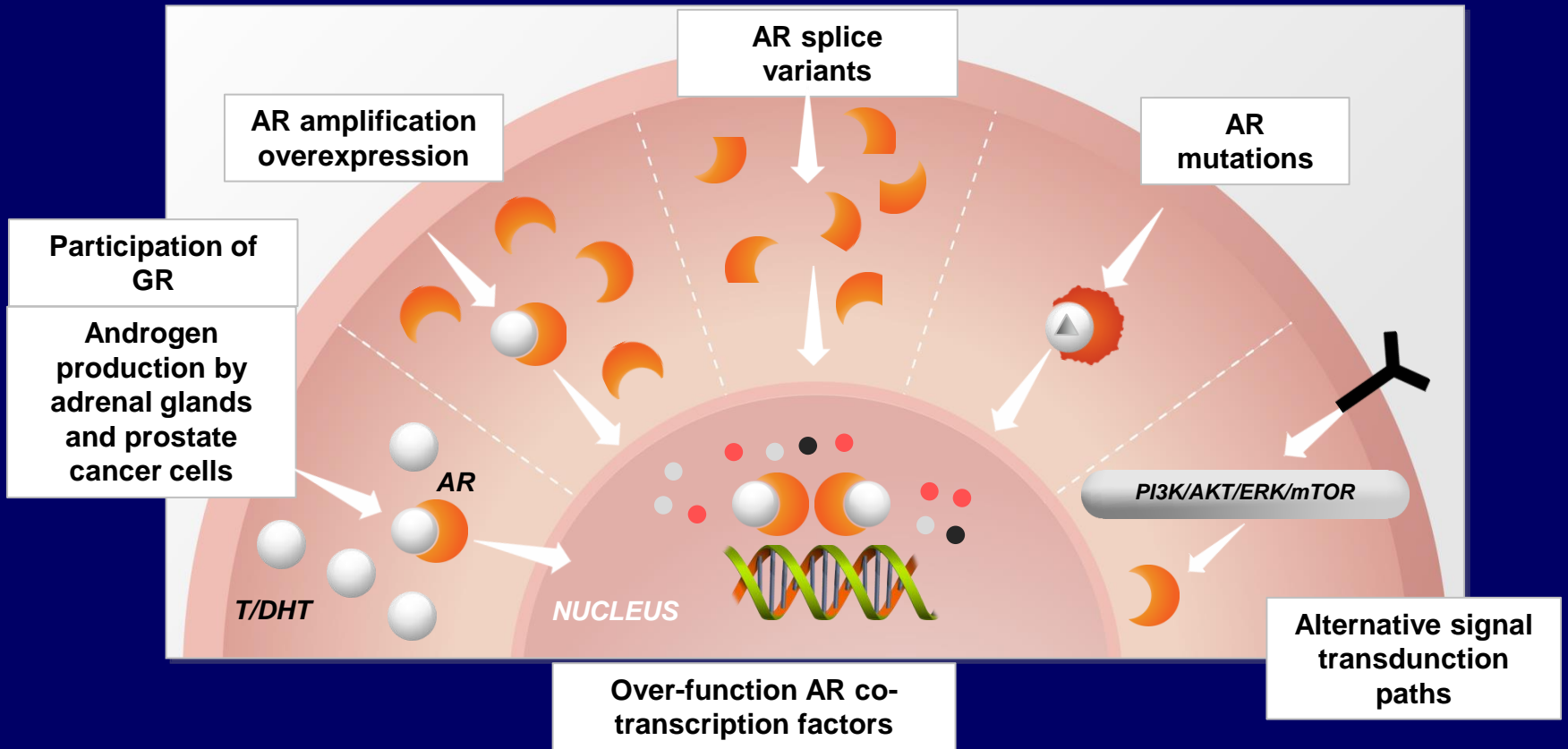
- ❖ AR-dependent pathways
- ❖ AR-independent pathways

Molecular Mechanisms of CRPC

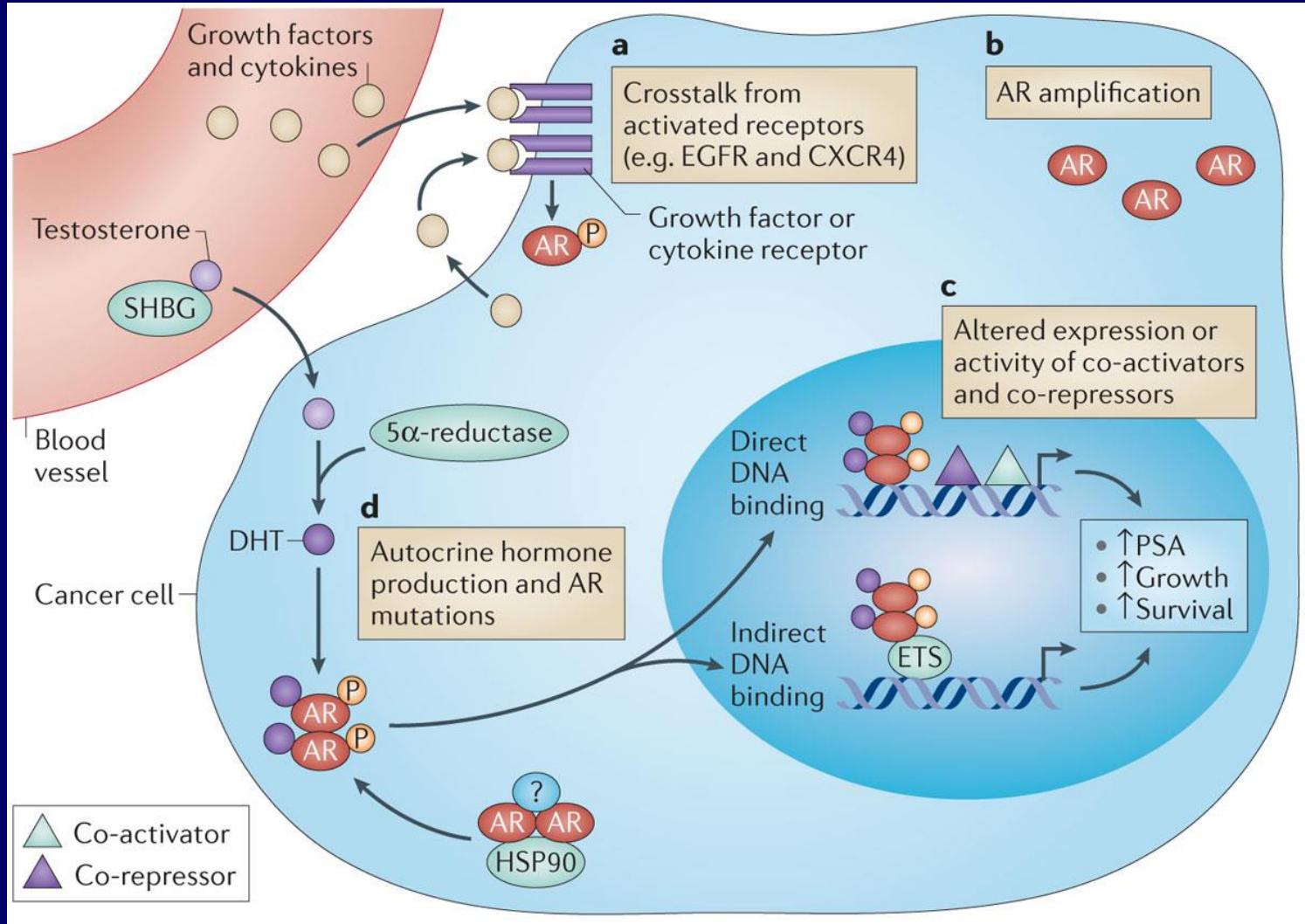
AR-dependent pathways



Molecular Mechanisms of CRPC

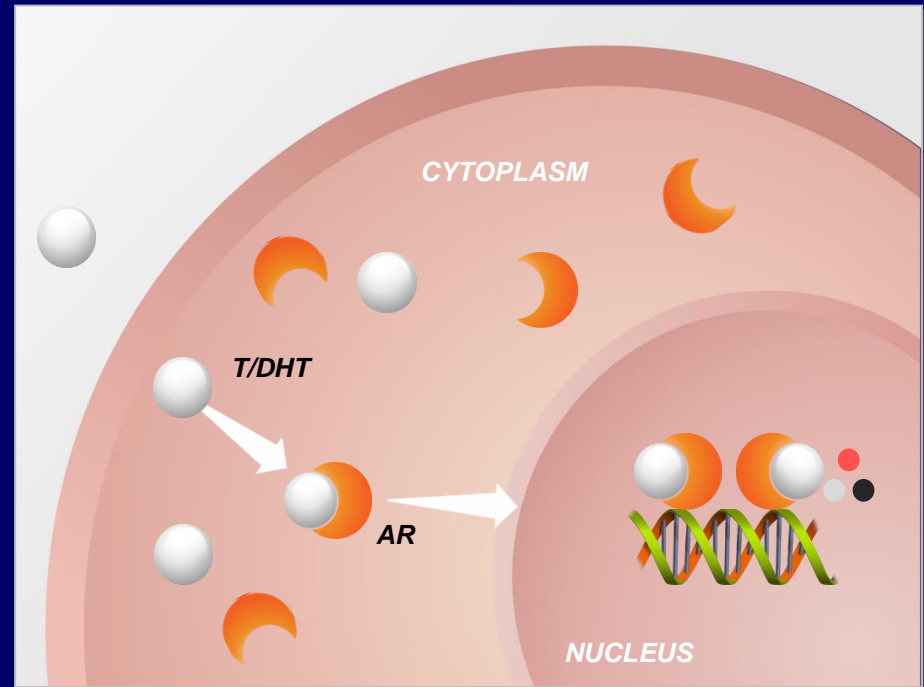


Molecular Mechanisms of CRPC



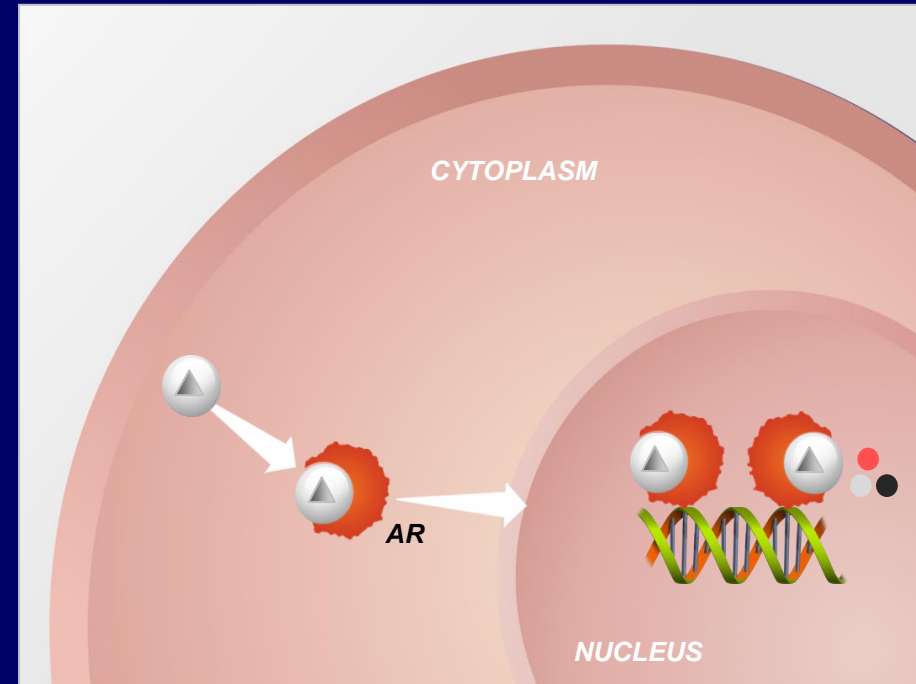
Molecular Mechanisms of CRPC

- ❖ Amplification of AR gene has been identified in **50–85%** of CRPC patients
- ❖ AR over-expression results in:
 - Increased levels of AR protein
 - Enhanced response in low concentration of androgens



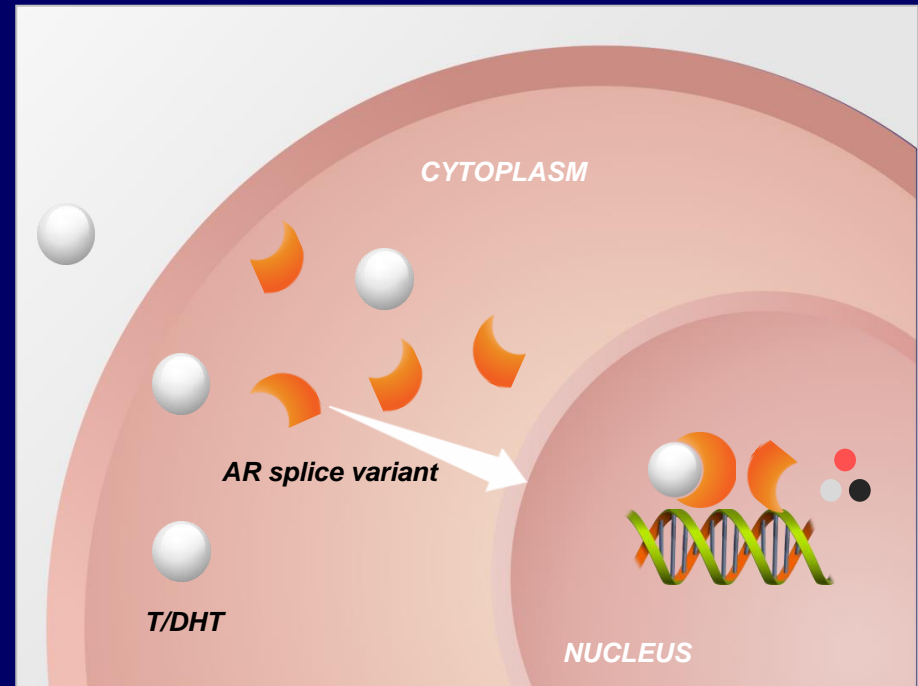
Molecular Mechanisms of CRPC

- ❖ The incidence of AR mutations increases with more advanced disease stages
 - Androgen deprivation enhances the chance of AR mutations
 - Almost **10%** of CRPC have mutations
- ❖ Mutations in ligand-binding domain of AR increases the number of ligands that can activate it
- ❖ AR mutations may allow its' activation also from anti-androgen substances (agonist action)!!!



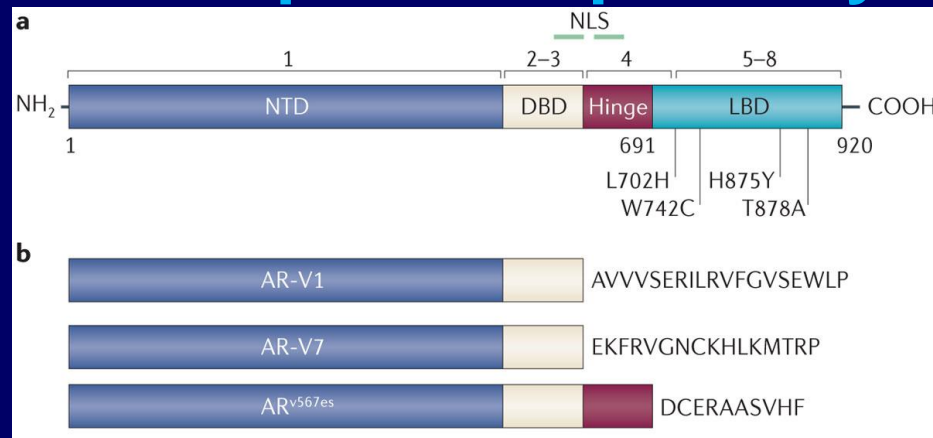
Molecular Mechanisms of CRPC

- ❖ Increased levels of **AR splice variants** have been found in CRPCs
- ❖ AR splice variants do not have ligand-binding domain and can be constantly activated
- ❖ AR signal transduction is active irrespective of androgen serum levels



Molecular Mechanisms of CRPC

AR-dependent pathways



Variant

ARV7 (AR3)

ARv567es

ARV12

AR1/2/2b (ARV3)

AR1/2/3/2b
(ARV4, AR5)

ARV1 (AR4)

ARV9

ARV13

ARV2

ARV5/V6

ARV8/10/11

ARV14

Protein Regions

NTD, DBD

NTD, DBD, Hinge

NTD, DBD, Hinge

NTD, partial DBD

NTD, DBD

NTD, DBD

NTD, DBD

NTD, DBD, Hinge, partial LBD

NTD, DBD

NTD, DBD

NTD, DBD

NTD, DBD, Hinge, partial LBD

Activity

Ligand-independent, Nuclear

Ligand-independent, Nuclear

Ligand-independent, Nuclear

Ligand-independent

Ligand-independent

LNCaP: Ligand-independent, PC3: Inactive,
Cytoplasmic

LNCaP: Ligand-independent, PC3: Inactive,
Cytoplasmic

Inactive

Not determined

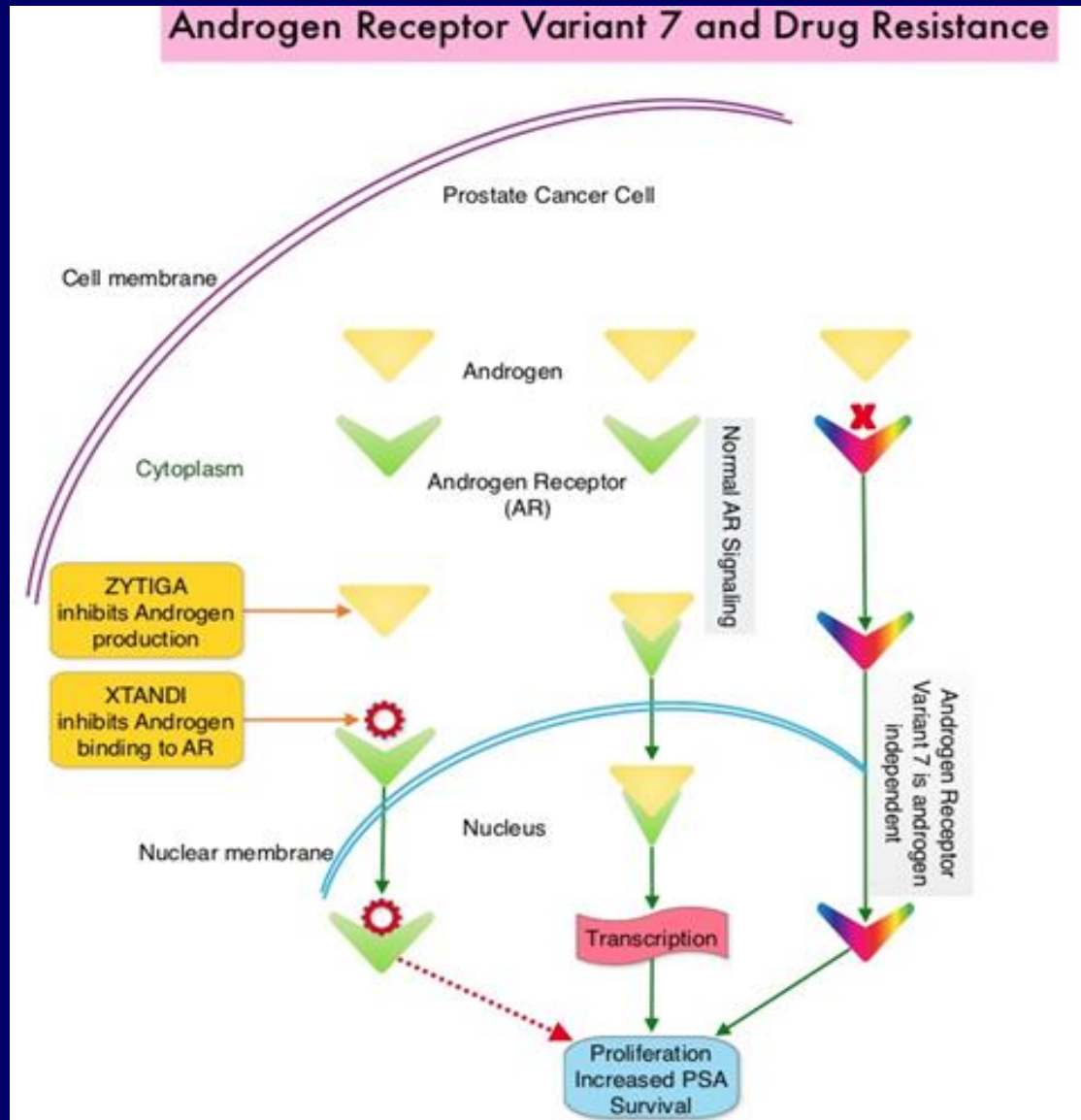
Not determined

Not determined

Not determined

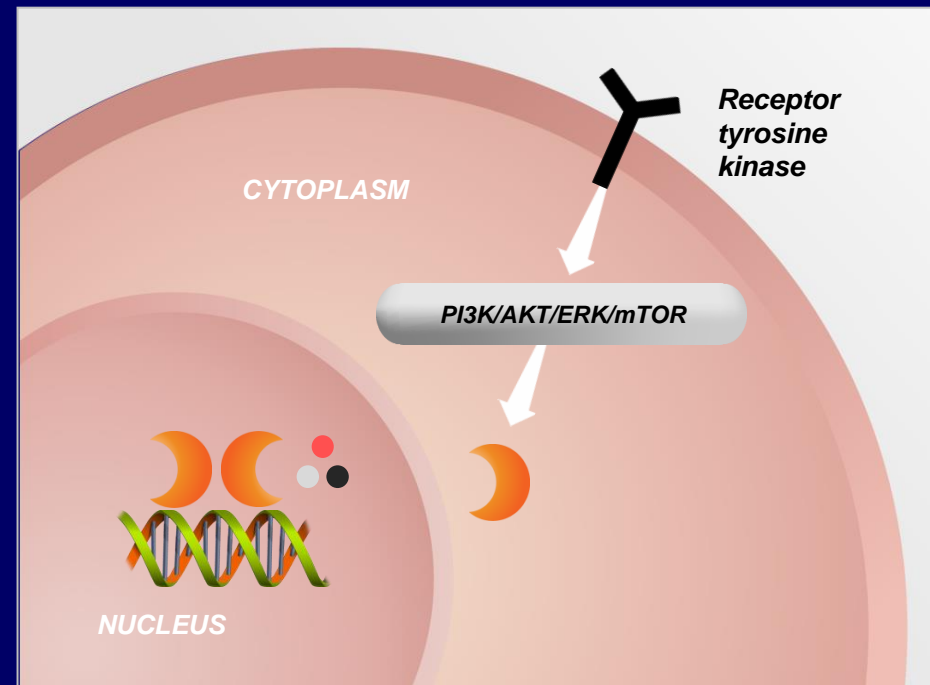
AR Splice Variants

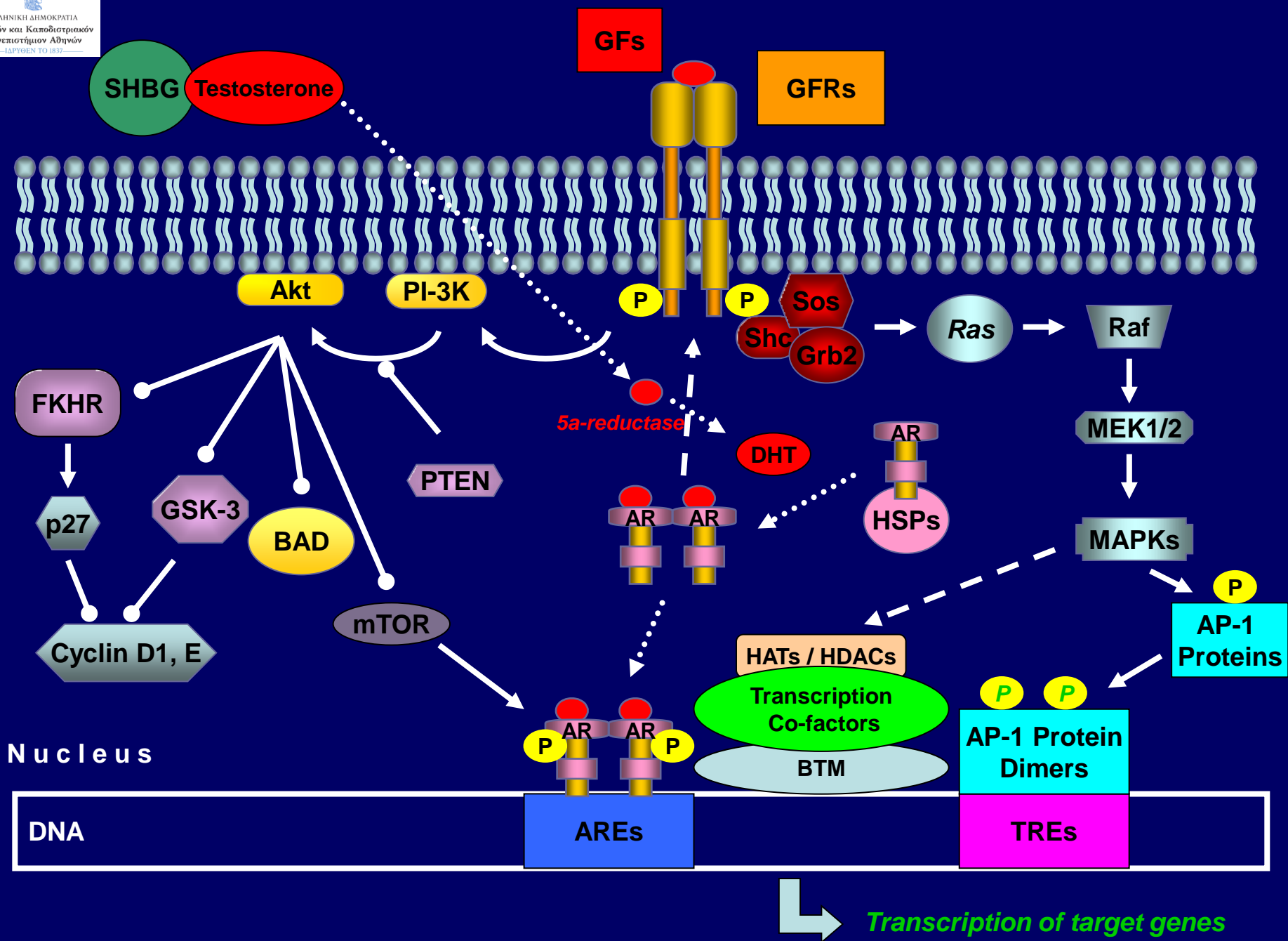
AR-dependent pathways



Molecular Mechanisms of CRPC

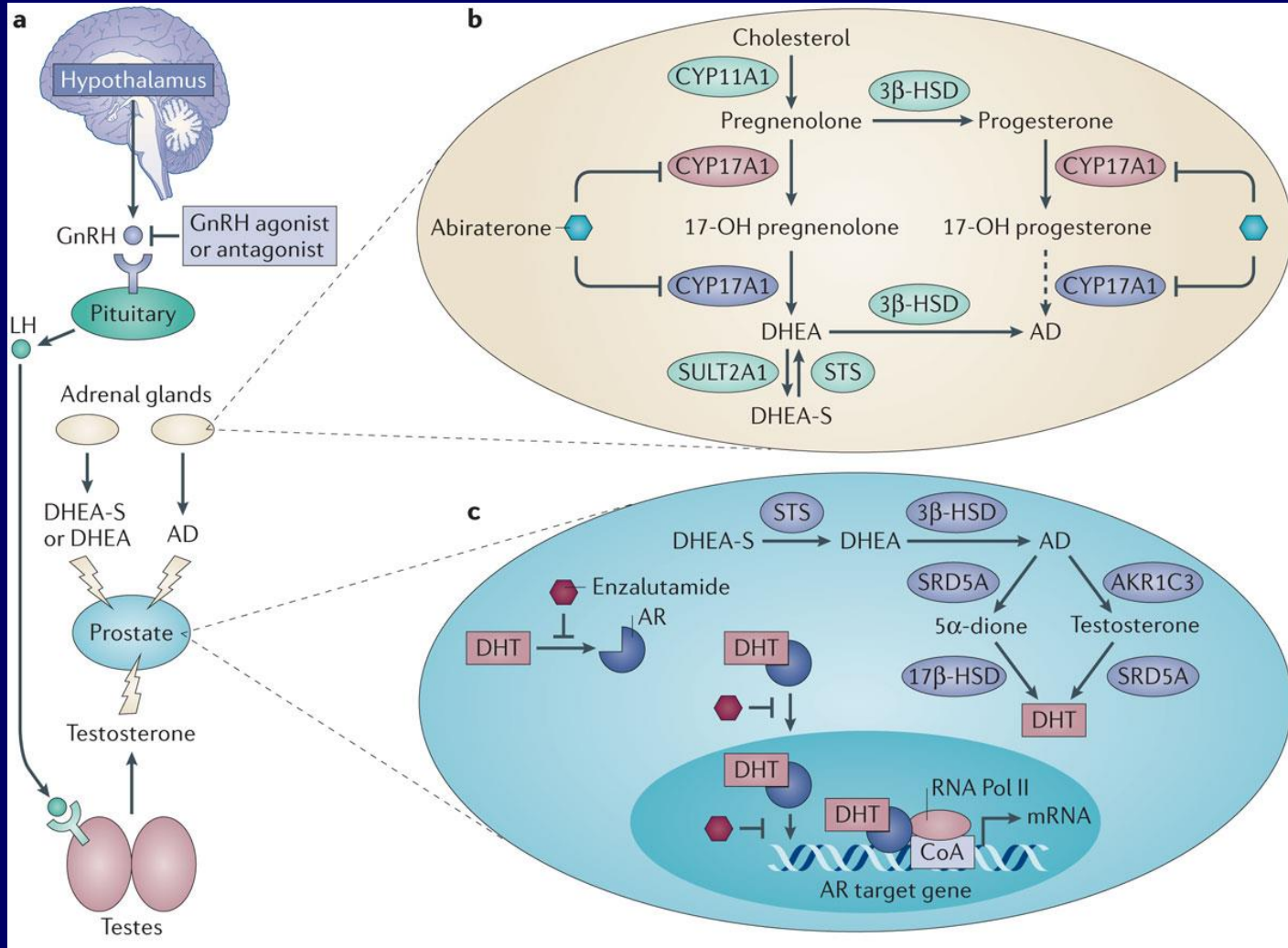
- ❖ Cross-talk between different signal transduction pathways can activate AR
- ❖ Frequent involved pathways:
 - MAP-kinases
 - AKT - PI3K - mTOR





↳ **Transcription of target genes**

Castration-Resistant Prostate Cancer (CRPC)



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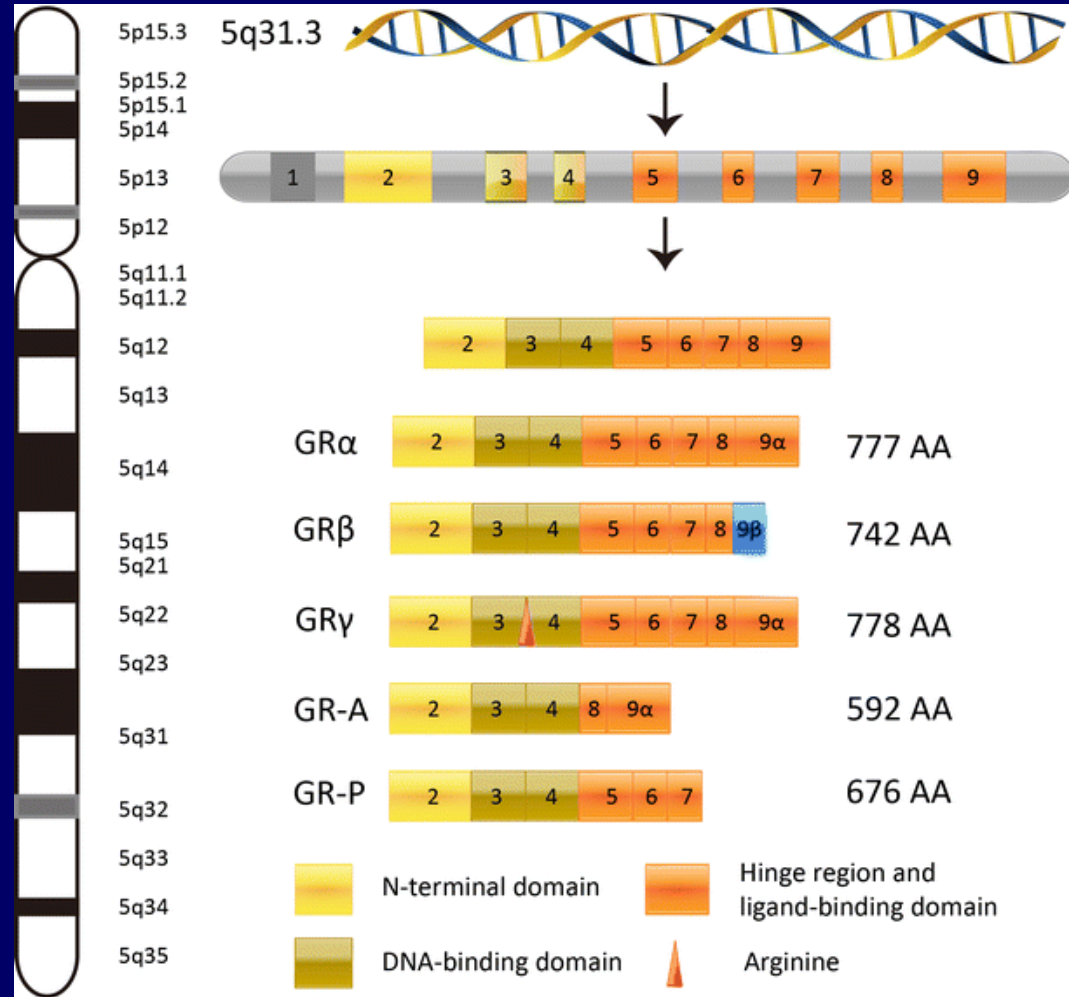
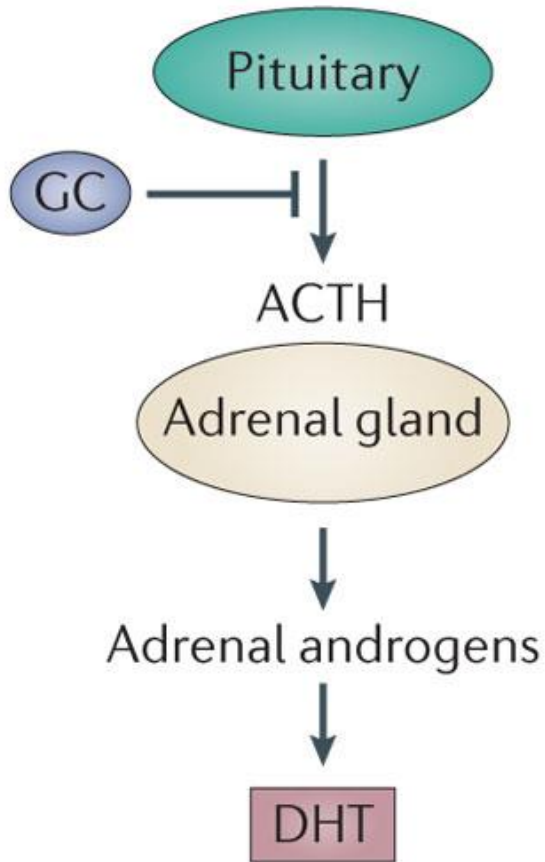
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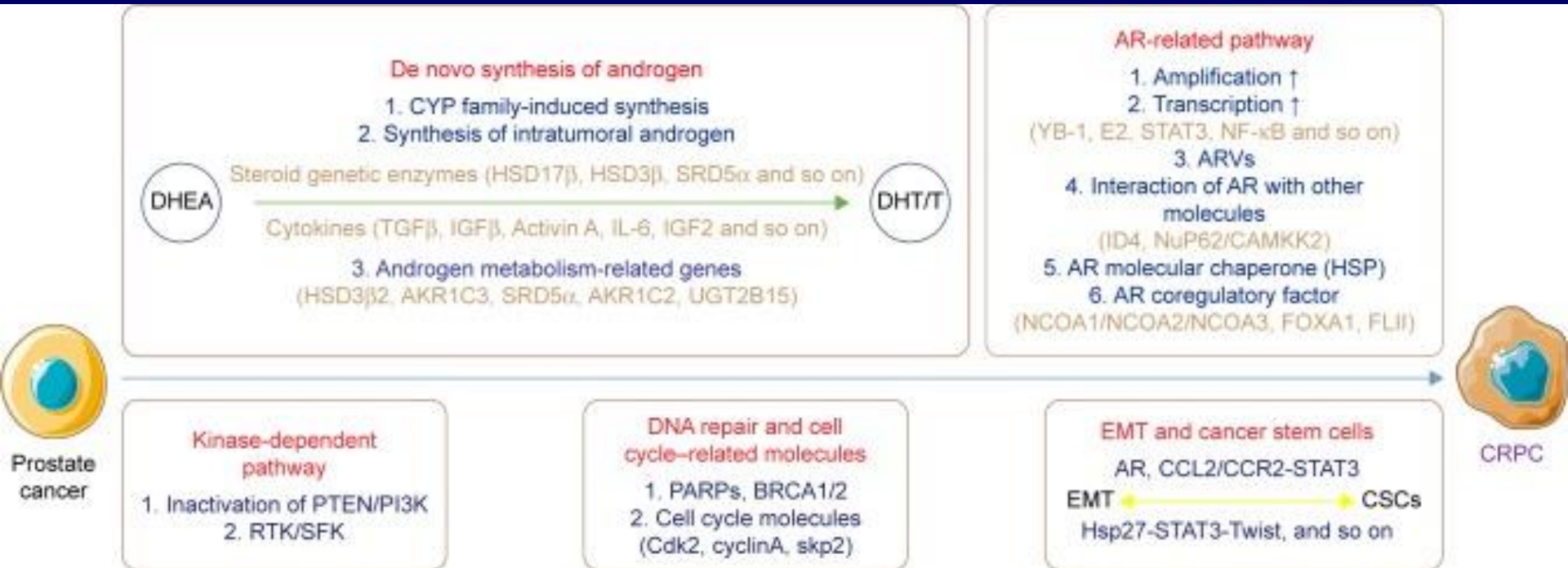
Molecular Mechanisms of CRPC

AR-independent pathways

a Endocrine antitumour effects



Molecular Mechanisms of CRPC



Molecular Characteristics of CRPC clinical evolution

