Nuclear Receptors
(intracellular receptors)

لا حول ولا قوة إلا بالله

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The mechanism of steroid hormone action. Steroid hormones are lipid-soluble and thus readily diffuse through the plasma membrane of cells. They bind to receptor proteins in either the cytoplasm or nucleus (not shown). If the steroid binds to a receptor in the cytoplasm, the hormone-receptor complex moves into the nucleus. The hormone-receptor complex then binds to specific regions of the major groove of DNA, stimulating the production of messenger RNA (mRNA).

• **Nuclear receptors** (NRs) are ligand-inducible transcription factors that specifically regulate the expression of target genes involved in metabolism, development, and reproduction

• The nuclear receptor superfamily include **nuclear hormone receptors** (NHRs) and **orphan nuclear receptors.**

• NHRs are receptors for which hormonal ligands have been identified,

orphan receptors are so named because their ligands or stimulaters are unknown

• Often the hormones together with nuclear receptors function as inducers or repressors of gene expression.
• In the case of the cytosolic receptors, the hormone binding induces translocation into the nucleus where the hormone-receptor complex binds a DNA element and alters the transcription of the target gene.
• Transcription factor, defined as any regulatory protein that directly influences gene transcription.

• Nuclear Receptors groups
• Based on the receptor activation mechanism the nuclear receptors can be divided into two basic groups:

1- Nuclear hormone receptors localized in the **cytoplasm**.

2- Nuclear hormone receptors localized in the **nucleus** bonded with **nuclear DNA**.

• The *steroid hormone* receptor receives the hormonal signal in the *cytosol*, becomes activated by hormone binding, at which point it enters the *nucleus* to regulate the *transcription initiation of cognate genes*

General structure of nuclear receptor

1- The A/B region (N-terminal region). This region is the **most variable** both in **size and sequence** and in many cases contains a *ligand-independent* **transcription activation functions 1 (TAF-1)** domain.
• The A/B domain shows *promoter* and *cell-specific* activity and is the target for **phosphorylation** mediated by different signaling pathways, and this modification can significantly affect transcriptional activity.
• Phosphorylation may increase or decrease transcription

2- Region C or DNA binding domain (DBD):

functions

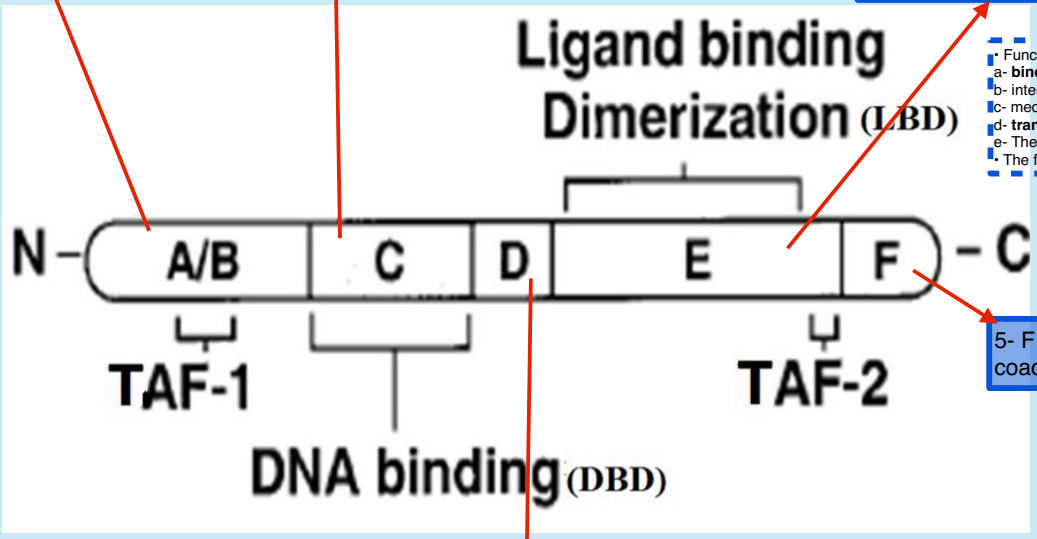
1- mediate **specific recognition** of the **hormone response elements** (HRE) by two zinc fingers besides stabilizing binding to these DNA sequence

- Hormone Response Elements (HRE)
- HRE is a **specific region in DNA** which in average is made up of **6 bp.**
- HRE are often located **close to the core promoter**, in some cases they are present **in enhancer regions**.
- The DBD in the receptor of the hormone recognize the HRE and binds to it.
- **Nuclear receptors** regulate transcription by binding to specific HREs

2- **dimerization** of the **receptor** on the HRE.

3- The DNA binding domain also contain the "**nuclear localization signal domain**" directing the hormone/receptor complex to the HRE acceptor is accomplished by which are few amino acid

4- E region contains the **ligand (hormone) binding domain** (LBD) where the shape of the ligand-binding pocket (hydrophobic nature) matches that of the ligand (also hydrophobic).



- Functions:
a- **binding site for the hormone,**
b- interaction with **heat-shock proteins,**
c- mediate **dimerization** of the receptors
d- **transactivation and transrepression** through binding of **coactivators and corepressors**.
e- The LBDs contain the **TAF-2 motif** responsible for ligand- dependent transcriptional activation.
- The function both TAF1 and TAF2 is to activate transcription.

5- F region: its function is not clear but it thought to play a role in coactivator recruitment to the E domain

3- Linker region D, serves as a hinge between the DBD and the LBD, **allowing rotation of the DBD**. It might allow the DBDs and LBDs to adopt several different conformations **without creating steric hindrance problems**

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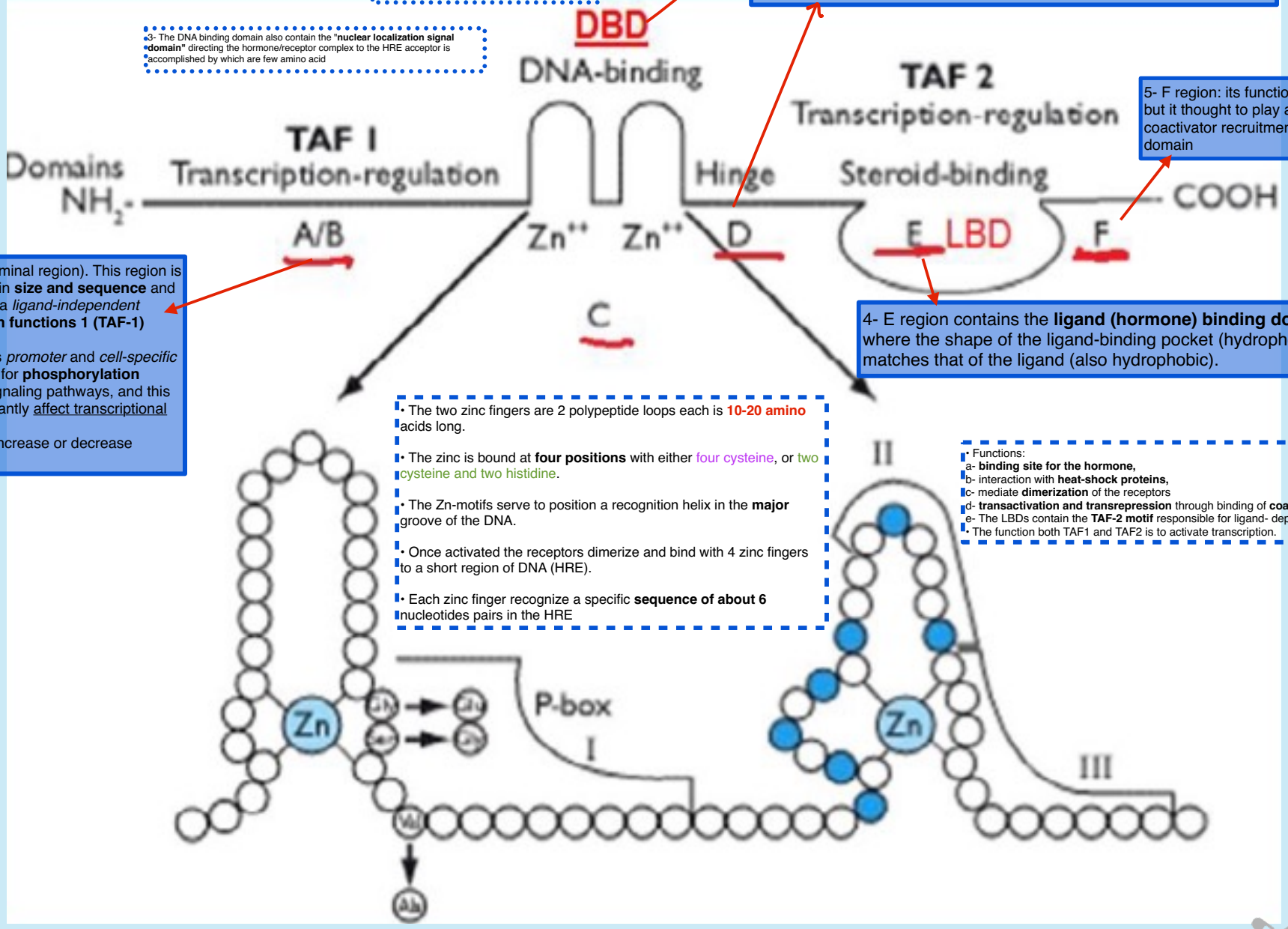

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4- E region contains the **ligand (hormone) binding domain** (LBD) where the shape of the ligand-binding pocket (hydrophobic nature) matches that of the ligand (also hydrophobic).

• The two zinc fingers are 2 polypeptide loops each is **10-20 amino** acids long.

• The zinc is bound at **four positions** with either four cysteine, or two cysteine and two histidine.

• The Zn-motifs serve to position a recognition helix in the **major** groove of the DNA.

• Once activated the receptors dimerize and bind with 4 zinc fingers to a short region of DNA (HRE).

• Each zinc finger recognize a specific **sequence of about 6** nucleotides pairs in the HRE

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• Homo and hetero-dimerization

• Receptors can bind as monomers, homodimers, and heterodimers to different HRE.

• In **dimerizations two hormone receptor monomers** bind cooperatively to their response elements, and dimerization interfaces have been identified both in the **ligand binding domain (LBD)** and in the **DNA binding domain (DBD).**

• In general hormone receptor resides in the cytoplasm will dimerise as homodimers while the one in the nucleus as heterodimers.

• The retinoid X receptor (RXR) is a member of nuclear hormone receptor family proteins.

• Three retinoid X receptors (RXR α, -β, and -γ), members of the nuclear hormone receptor superfamily, act as ligand-inducible transcription factors.

• RXRs are dimerization partners for a large number of nuclear receptors.

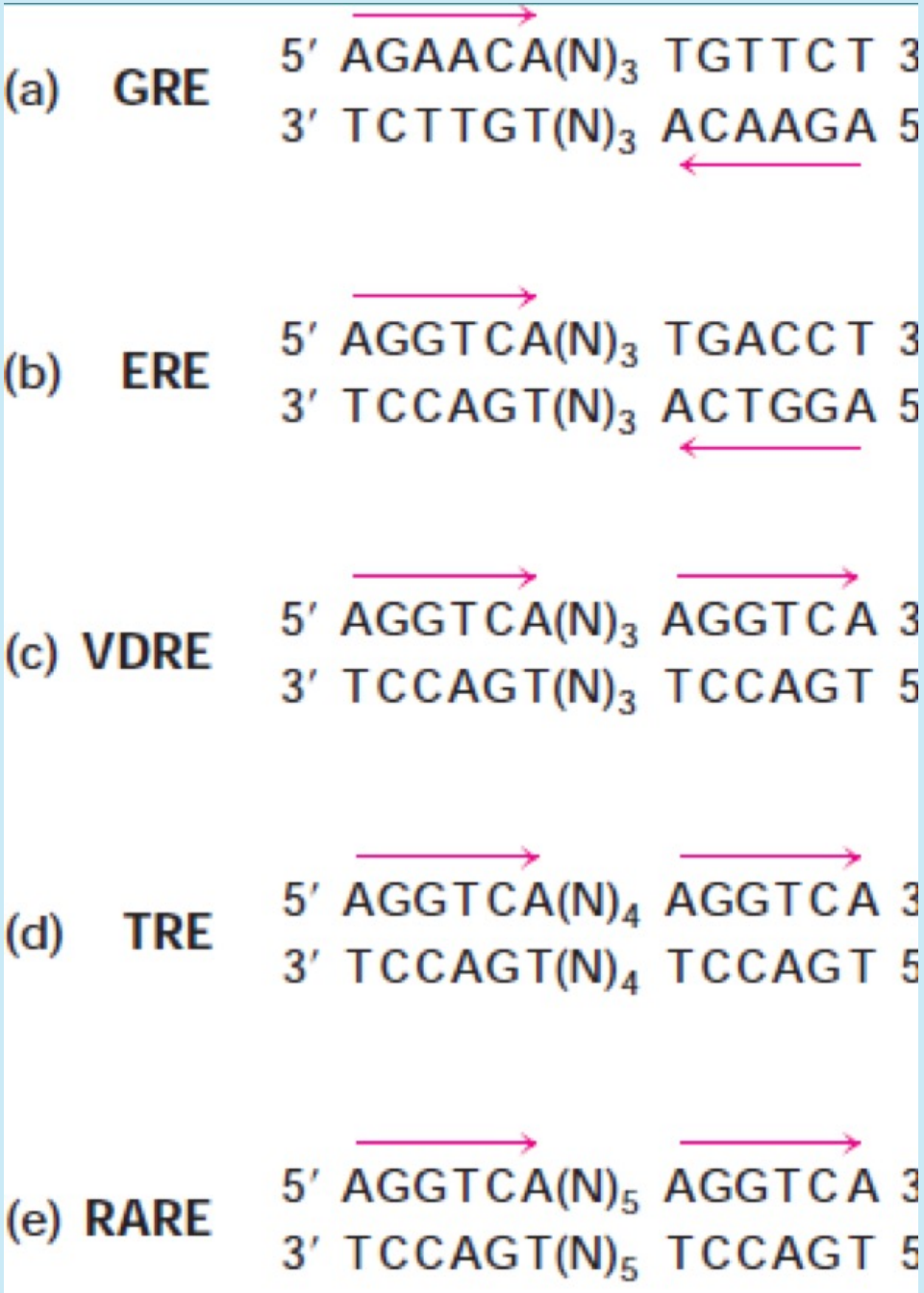
• Different binding partner of RXR causes a different DNA-binding specificity of the heterodimer.

• Dimerization is a general mechanism to **increase binding site affinity, specificity, and diversity.**

•**Nuclear receptors** are divided according to the way they bind to their respective DNA elements into:

1- The response elements for Glucocorticoid receptor (GRE) estrogen receptor (ERE), mineralocorticoids (MR), androgens (AR), and progestins (PR) bind as **homodimeric** to inverted repeats and normally found in the cytoplasm

2- The response elements for vitamin D3 receptor (VDRE), thyroid hormone receptor (TRE), and retinoic acid (vitamin A-derived) receptor (RARE) bind as **heterodimeric** to direct repeat separated by three to five base pairs and normally found bonded with nucleus DNA



• **Activation of the Cytoplasmic Apo-Receptor Complexes**

• In the absence of hormones the cytoplasmic receptors remain in an inactive complex, designated the apo-receptor complex.

• In the aporeceptor complex the receptor is bound to proteins belonging to the heat shock protein (Hsp).

• The heat shock proteins (example chaperones) are used as tools in this system for regulation of activity of the steroid hormone receptors and to fix the receptor in a conformation which allows high affinity binding to the hormone.

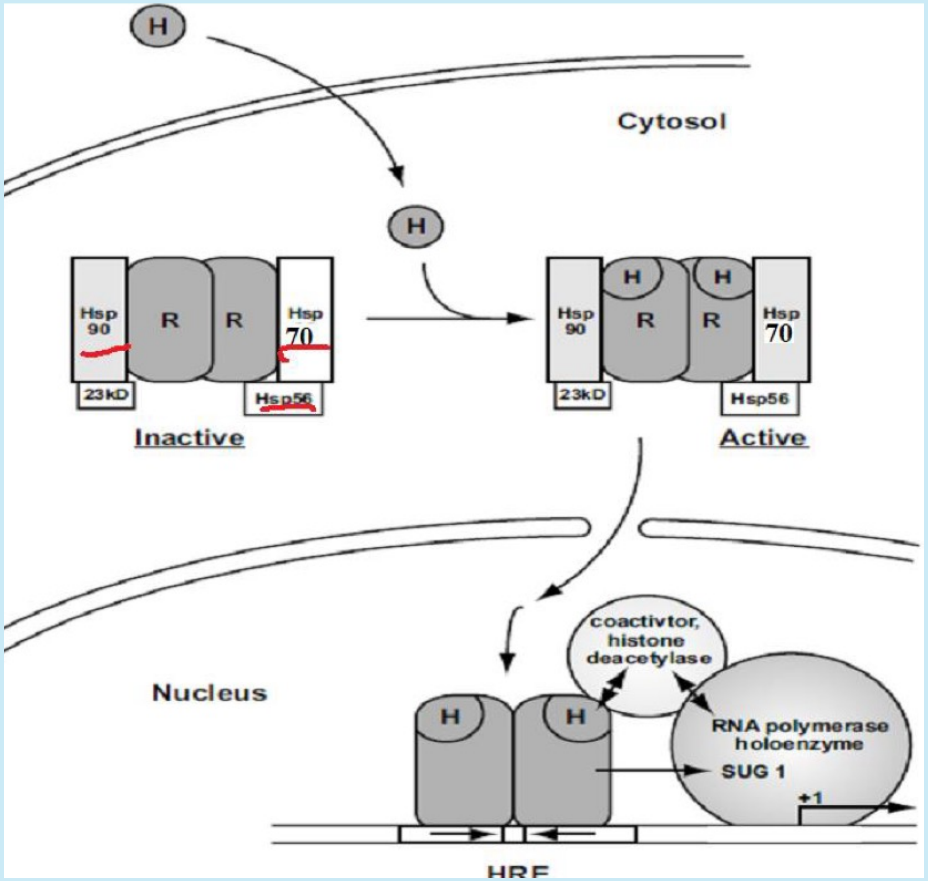
• The **cytoplasmic receptors** interact with at **least three heat shock protein Hsp90, Hsp70 and Hsp56.**

• The binding of the hormone to the aporeceptor complex leads to *conformational change* in the receptor and the release of heat shock proteins.

• **The receptor activation initiates** the translocation of the ligand-receptor into the nucleus frequently as a homodiamer and bind to DNA.

• Receptors dimerization is required before the activated receptor can bind to their HRE.

• The now activated receptor moves into the nucleus and binds with high affinity to a specific HRE



2- Activations of receptors located within the nucleus (in association with chromatin)

• Most intracellular receptors are **gene-specific transcription factors**, proteins that bind to DNA and **regulate the transcription of certain genes.**

• Normally hormone receptors localized in the *nucleus* most often found *bounded with DNA* and a **corepressor protein**.

Therefore they act as repressors of gene activity.

• The binding of hormones to their nucleus receptor causes the dissociation of the corepressor and binding of coactivator proteins which **attract the RNA polymerase** and the activation of gene expression is usually observed.

• Also in *rare examples the binding of the ligand has an inhibitory effect on* gene activation

• Coactivators, corepressors, and other mediator proteins **do not bind directly** to DNA but generally **bind to components of the receptor complex** and mediate its assembly at the promoter.
They can be specific for a given gene transcription factor or general and bind many different gene-specific transcription factors.

• Nuclear receptor coactivators influence receptor transcription through a variety of mechanisms, including **acetylation, methylation, phosphorylation and mRNA splicing**

• The function of *corepressors* is to *suppress or silence gene transcription*

Nuclear Receptors as Ligand-Dependent Transcription Factors.

A: in the absence of hormone, the TAF2-domain conformation promotes receptor interaction with **corepressors**. The multiple- subunit corepressor complex stabilizes repressive local chromatin structure and **blocks** access of the transcription machinery (red X) to the promoter.

B: Hormone binding triggers a conformational change in the TAF2 domain, which destabilizes corepressor interaction and promotes **coactivator** binding.
Multiple-subunit coactivator complexes **activate** local chromatin structure and recruit the transcription machinery to the promoter, where target-gene transcription commences.