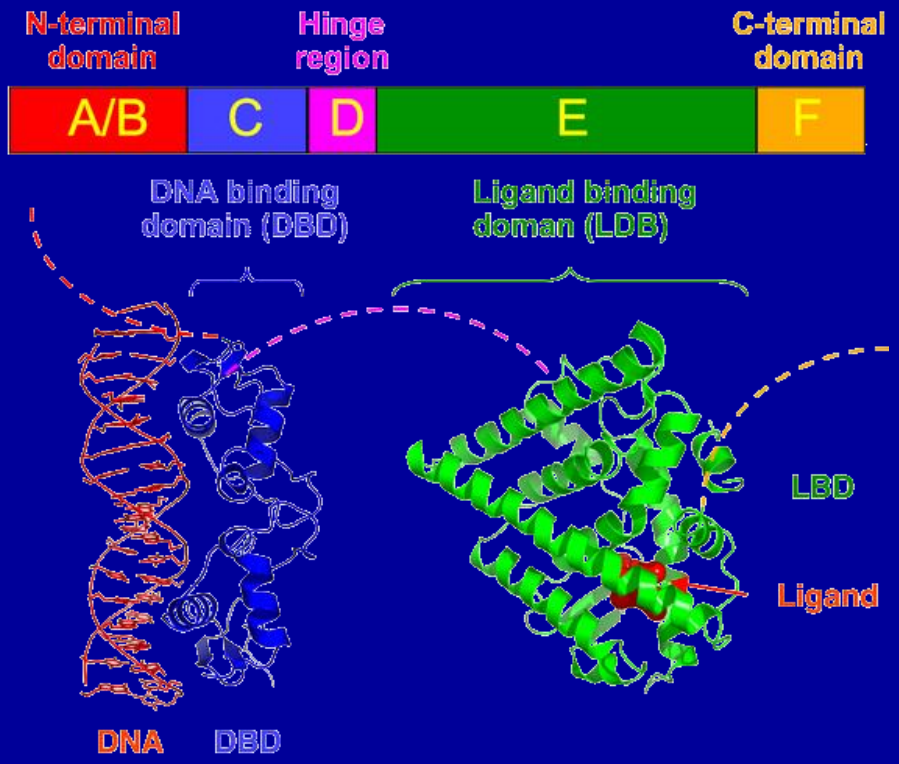
Receptor & Signalling II



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Learning Outcomes

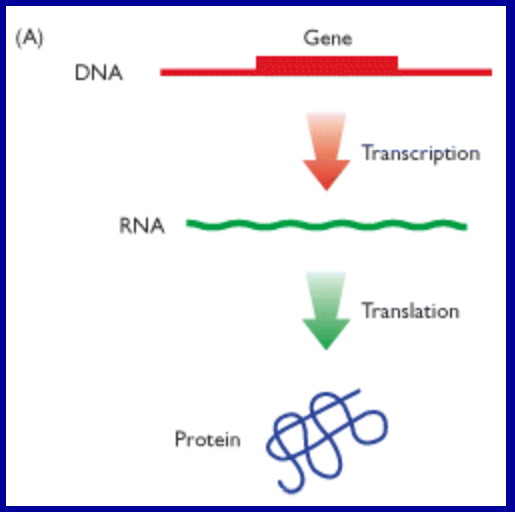
1. To list three key features of regulatory elements.
2. To list two functional domains of transcription factors.
3. To list the basic structural requirements for Nuclear Receptor (NR) action.
4. To classify the NRs as a ‘superfamily’ of proteins.
5. To classify the different types of ligands that modulate the activity of NRs.
6. To explain how NRs regulate the expression of a target gene.

Gene Regulation in Eukaryotes

Constitutive expression

Some of these genes expressed in all cells all the time.

House keeping genes are responsible for the routine metabolic functions common to all cells e.g respiration, basic metabolism, proteins of the cytoskeleton and chromosomes.



Regulated expression

Some genes are only expressed in specialised cells. Other differences in gene expression between cell types may be more subtle.

Transcription Factors Regulate Gene Expression

Why does gene expression need to be controlled anyway?

Primary purpose in multicellular organisms is to execute precise developmental decisions so that:

correct genes are expressed at:

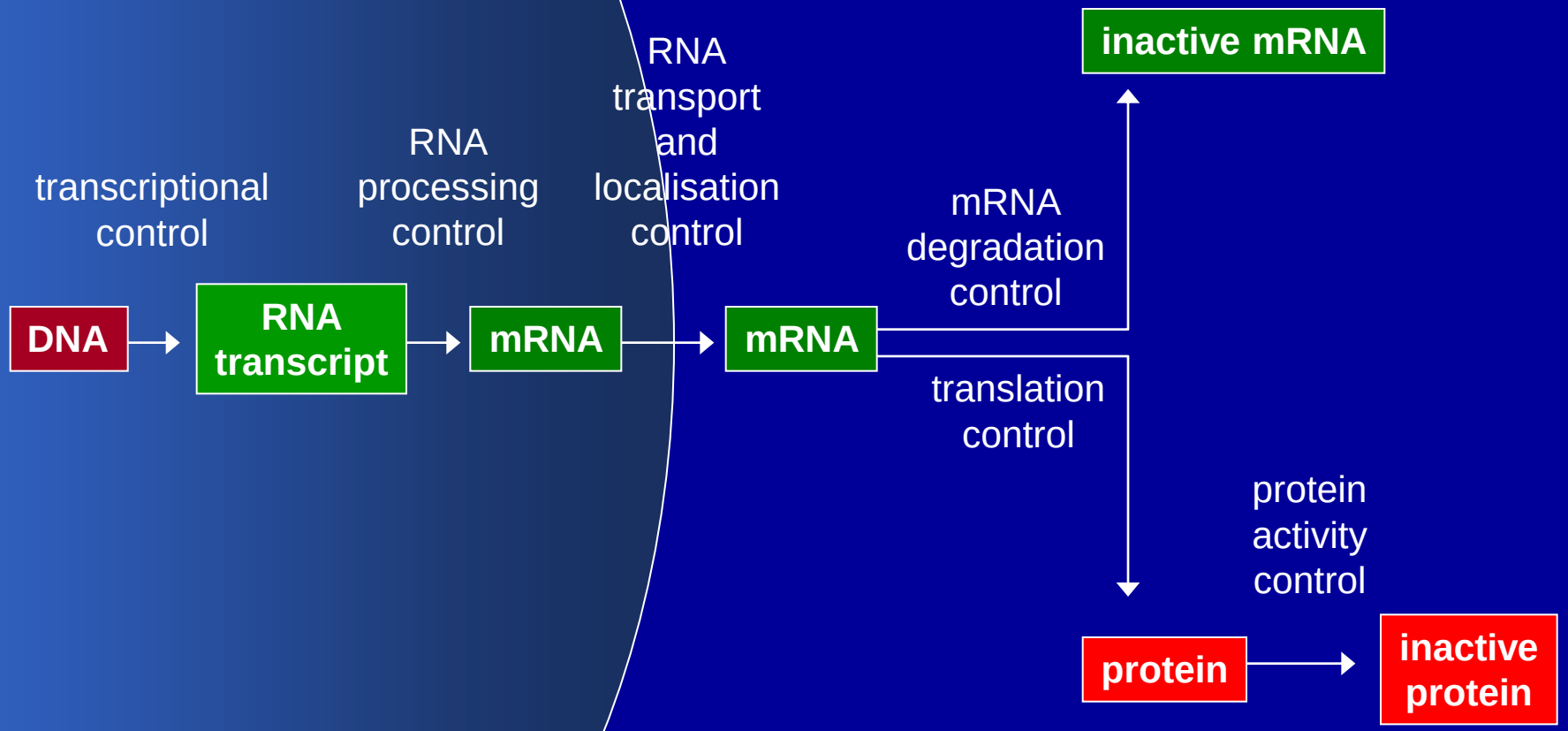
- appropriate time
- correct place
- at the required levels

so that development, growth and differentiation proceed correctly.

Stimuli cause alterations in gene expression.
Some genes are expressed in response to nutritional/chemical stimuli (sugar, amino acid, hormone signal).

Environmental signal (light, temperature).

Gene Expression Can be Regulated at Many Steps in the Pathway from DNA to RNA to Protein

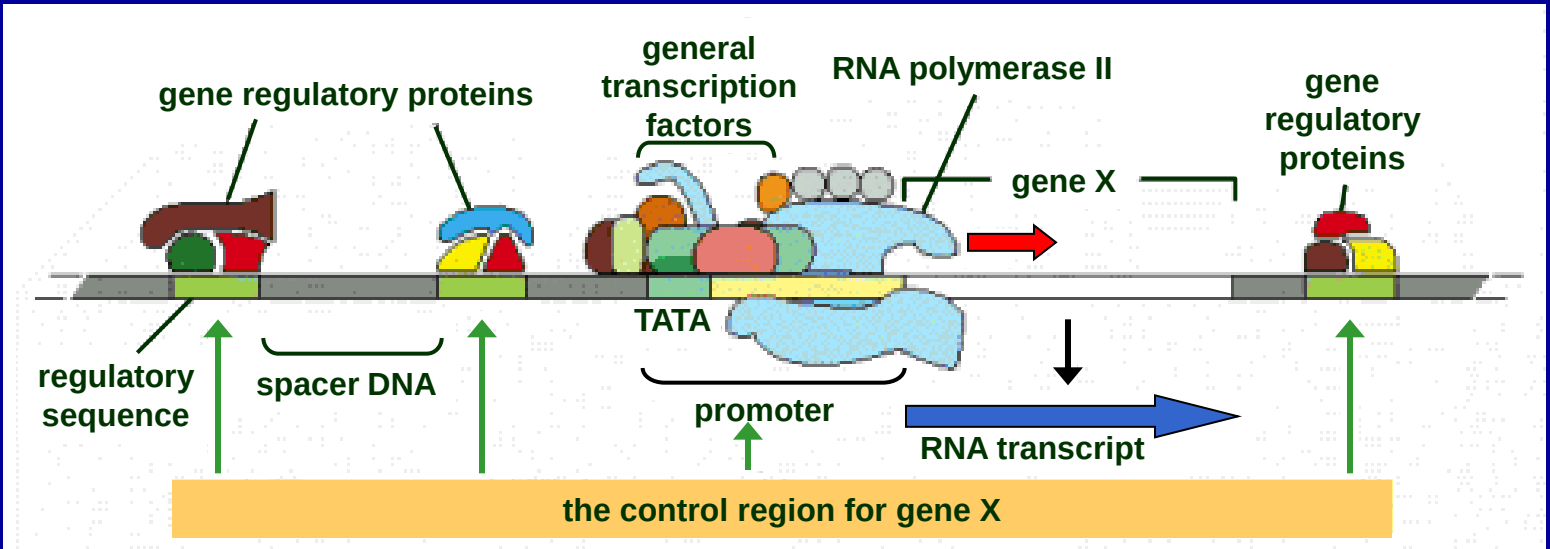


Protein-coding genes

- **Exons** whose sequence encodes the polypeptide
- **Introns**
- **Transcription start site**
- **RNA polymerase II** synthesizes precursors of mRNAs.

- **Proximal Promoter**.
  i) **Basal** or **core** promoter located within 40bp of the start site.
  ii) “Upstream” promoter which may extend over as many as 200bp upstream.
- **Enhancers**
- **Silencers**

The Gene Control Region of a Typical Eukaryotic Gene



The promoter is the DNA sequence where the general transcription factors and the polymerase assemble.

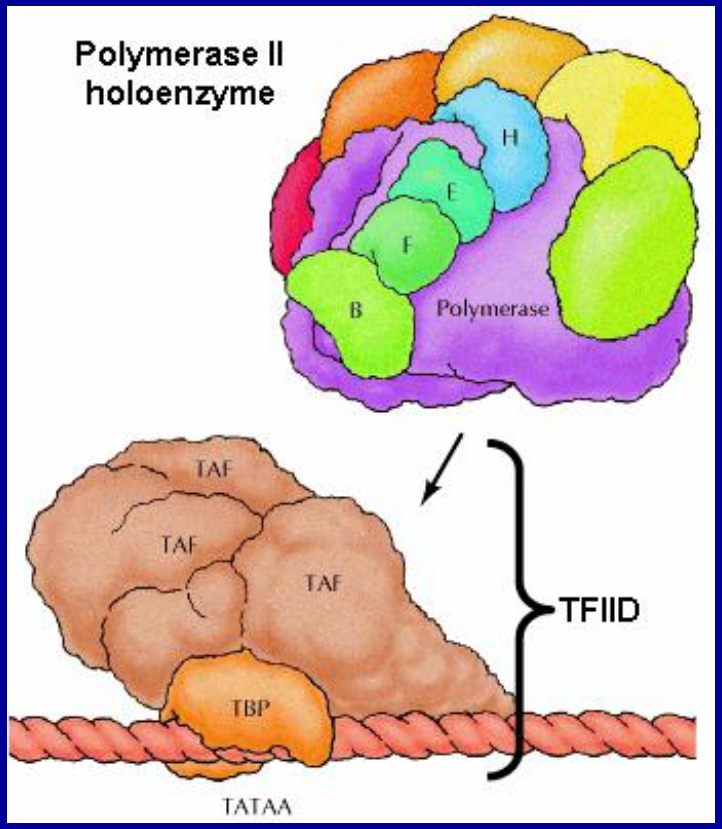
The regulatory sequences serve as binding sites for gene regulatory proteins:

affects the rate of transcription initiation

can be located adjacent to the promoter, far upstream, within introns or downstream of the gene.

Whereas the general transcription factors that assemble at the promoter are similar for all polymerase II transcribed genes, the gene regulatory proteins and the locations of their binding sites relative to the promoter are different for each gene.

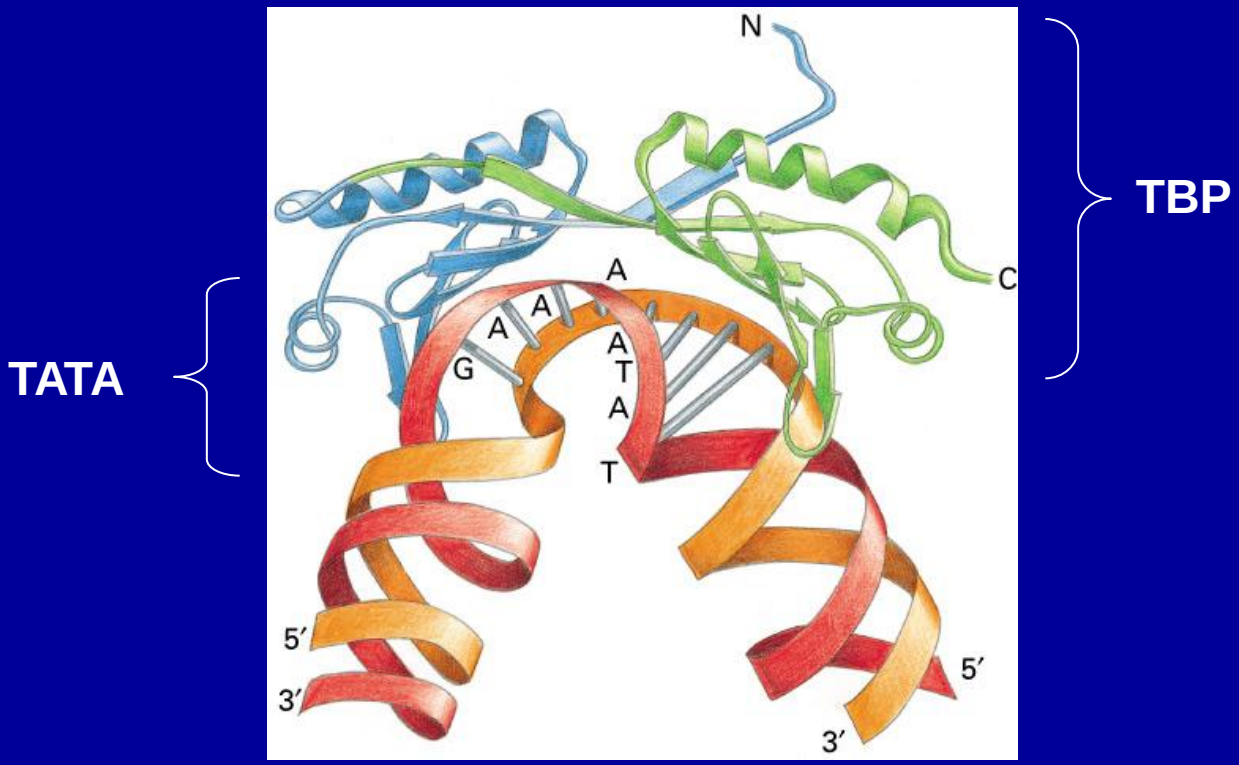
RNA polymerase II holoenzyme



- RNA Polymerase II Requires General Transcription Factors
- The holoenzyme consists of a complex of RNA polymerase II, the general transcription factors TFIIB, TFIIE, TFIIF, and TFIIH, and several other proteins that activate transcription. (TFII = transcription factor for polymerase II)
- This complex can be recruited directly to a promoter via interaction with TFIID.
- TFIID is composed of TBP (TATA Binding Protein), and TAFs (TBP-assocatiated Factors).

TBP Induces Bending of DNA

The unique DNA bending at the TATA box caused by TBP generates two kinks in the double helix separated by partly unwound DNA may serve as a landmark for an active promoter that helps to attract the other general transcription factors (TFIIB).



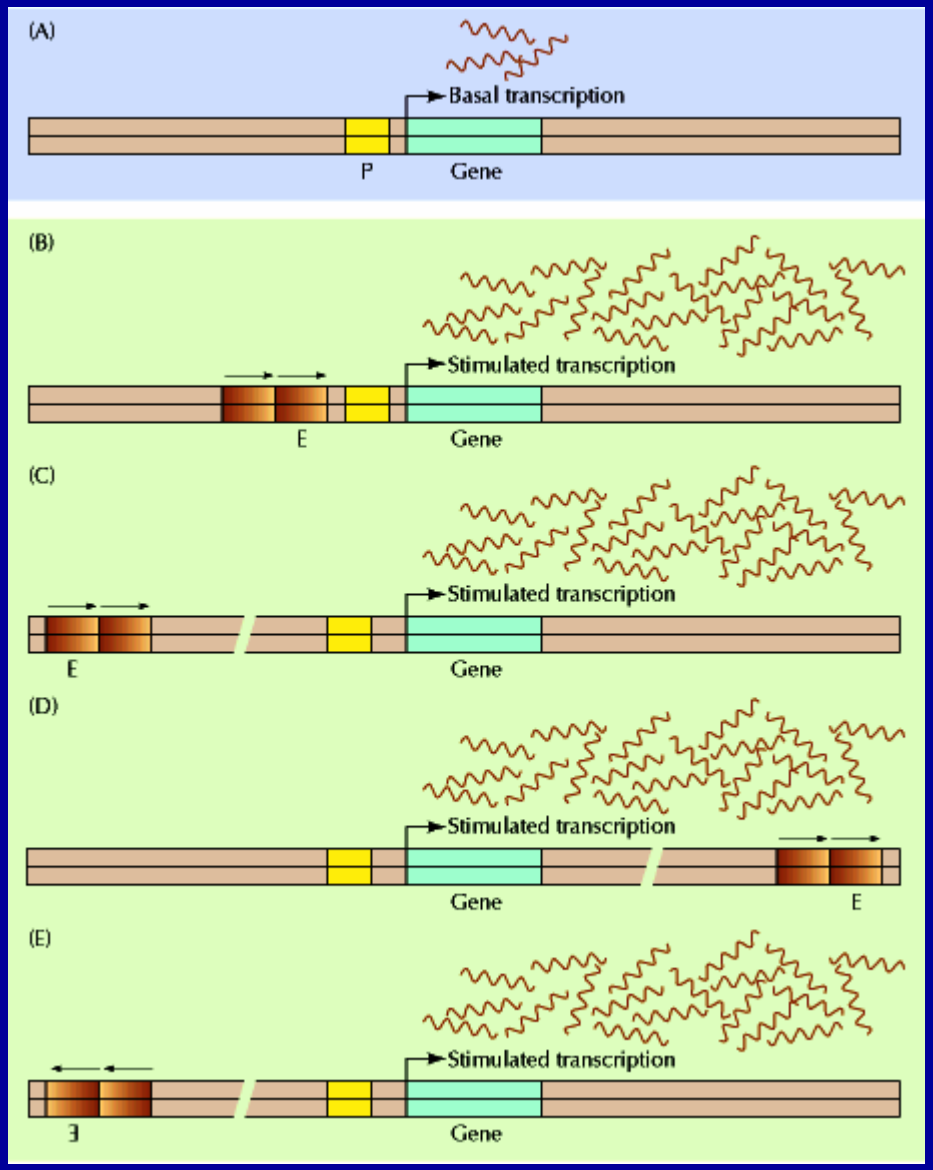
TFIIH Enzymatic Activity

TFIIH - has helicase activity (ATP-dependent) and protein kinase activity - phosphorylation of the C-terminus of Pol II is required for its activity.

Features of Response Elements

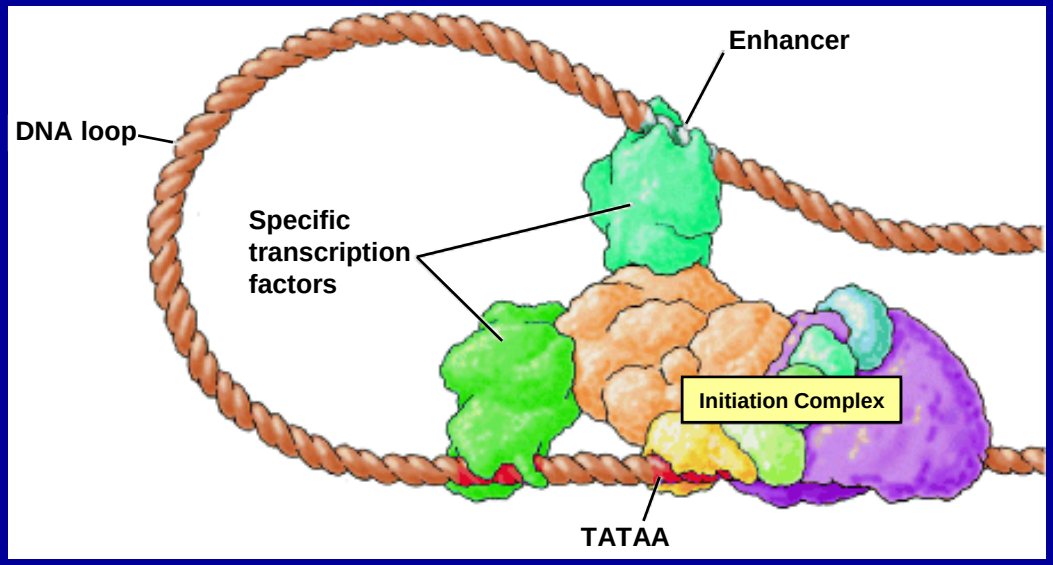
- contain short consensus sequences
- not fixed in location but usually within 200 bp upstream of the transcription start site
- a single element is usually sufficient to confer a regulatory response
- can be located in a promoter or an enhancer
- a specific protein (transcription factor) binds to the element and the presence of that protein is developmentally regulated

Action of Enhancers



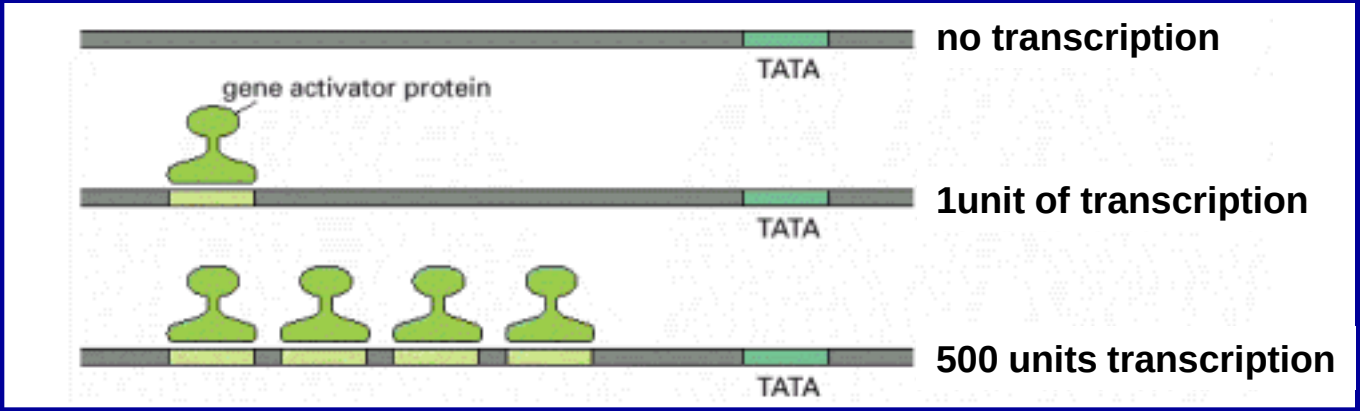
- Without an enhancer, the gene is transcribed at a low basal level.
- Addition of an enhancer, E for example, stimulates transcription.
- The enhancer is active not only when placed just upstream of the promoter, but also when inserted up to several kilobases either upstream or downstream from the transcription start site.
- In addition, enhancers are active in either the forward or reverse orientation.

Gene Activation at a Distance: DNA Looping



- Transcription factors bound at distant enhancers are able to interact with general transcription factors at the promoter.
- The intervening DNA can form loops.
- There is therefore no fundamental difference between the action of transcription factors bound to DNA just upstream of the promoter and to distant enhancers.

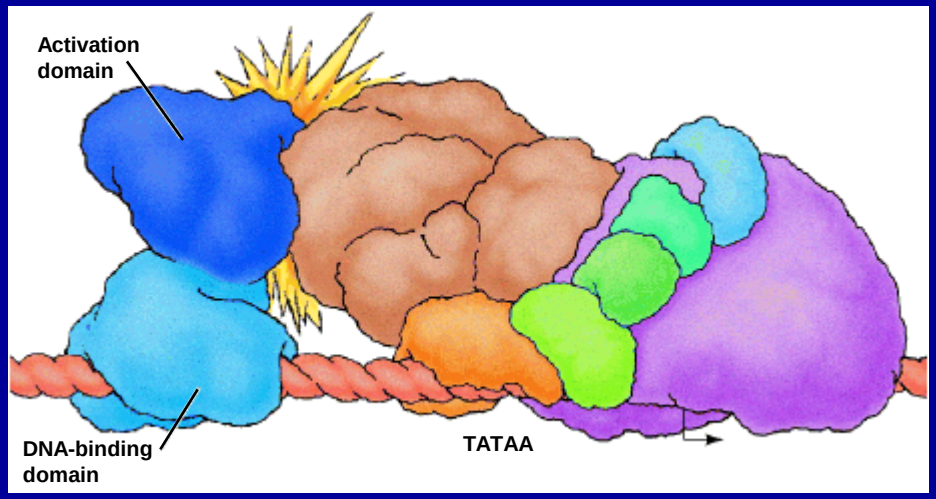
Synergistic Action of Transcriptional Activators



Transcriptional synergy:

- Greater than additive effect of the activators.
- Typically observed between different gene activator proteins (transcription factors) from the same organism.
- Also between activator proteins from widely different eukaryotic species when they are experimentally introduced into the same cell (The transcriptional machinery is highly conserved).

Structure of Transcriptional Activators

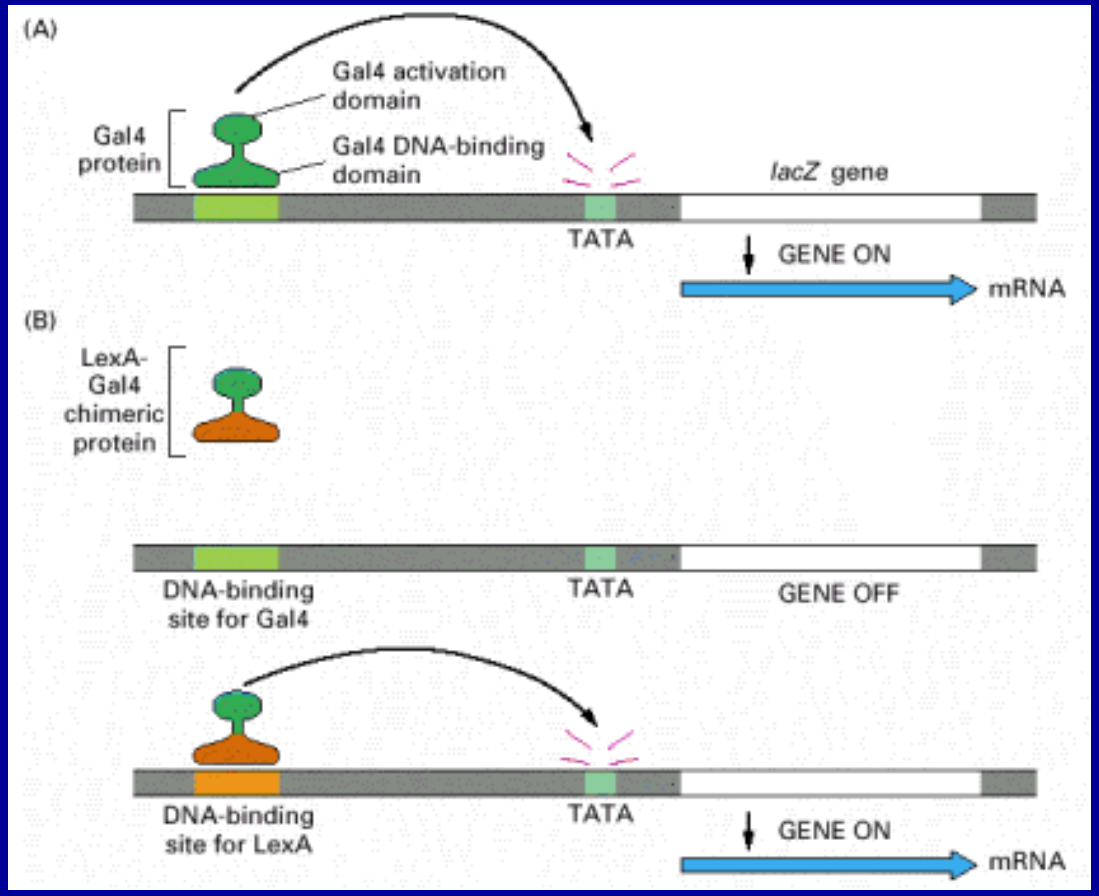


Transcriptional activators consist of two independent domains.

The DNA-binding domain recognises a specific DNA sequence.

The activation domain interacts with other components of the transcriptional machinery.

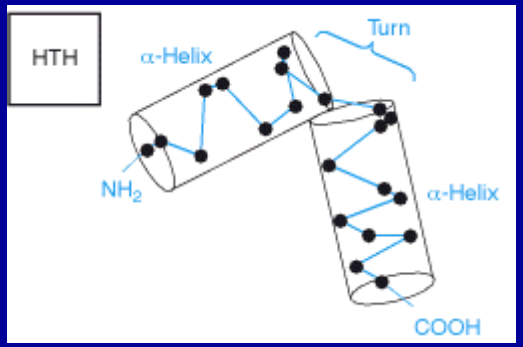
The modular structure of a gene activator protein

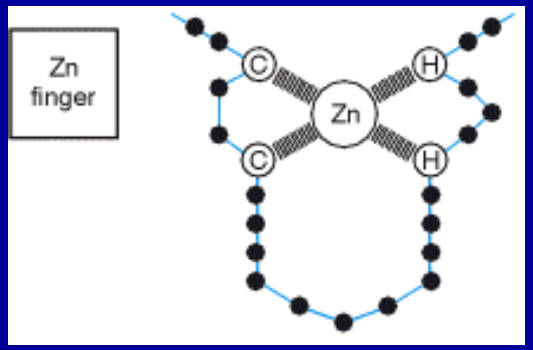


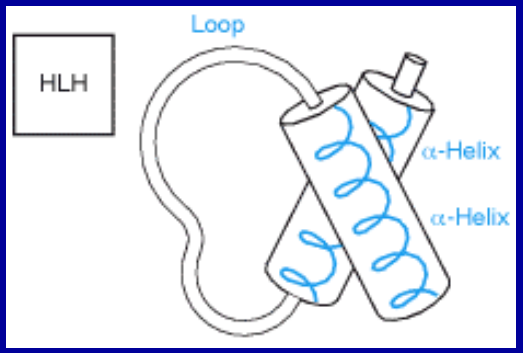
A functional activator can be reconstituted from the C-terminal portion of the yeast Gal4 protein if it is attached to the DNA-binding domain of a bacterial gene regulatory protein (the LexA protein).

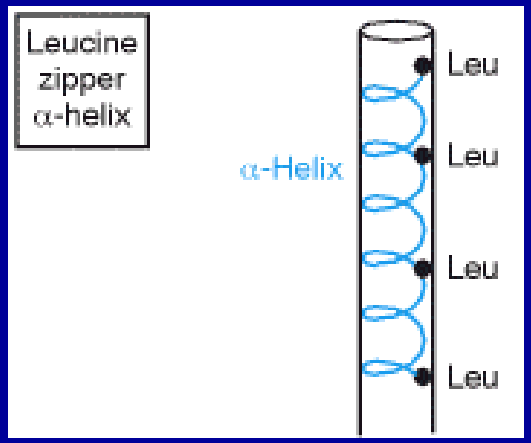
The resulting hybrid protein activates transcription from genes provided that the specific DNA-binding site is present.

Structural motifs commonly found in transcription factors and DNA-binding proteins.









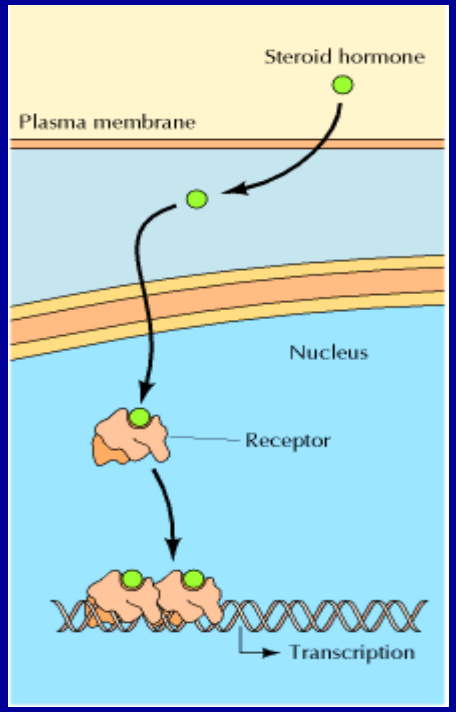
(HTH) Helix-turn-helix domains consist of 2 α helices connected by short chain of amino acids. C-terminal helix fits into major groove of DNA. N-terminal helix – structural component helps position the recognition helix.

The leucine zipper monomer is *amphipathic* [i.e. has hydrophobic residues (leucines) consistently on one face of the helix]. Interactions between the hydrophobic side chains of leucine residues exposed on one side of a helical region are responsible for dimerization. Two α helices form an inverted Y-shape structure that grips the DNA helix.

Zinc finger domains consist of loops in which an α helix and a β sheet coordinately bind a zinc ion (via Cysteine-Cysteine-Histidine-Histidine). Clusters of Zn fingers mediate strong and sequence specific DNA binding.

(HLH) Helix-loop-helix domains are similar to leucine zippers, except that the dimerization domains of these proteins each consist of two helical regions separated by a loop.

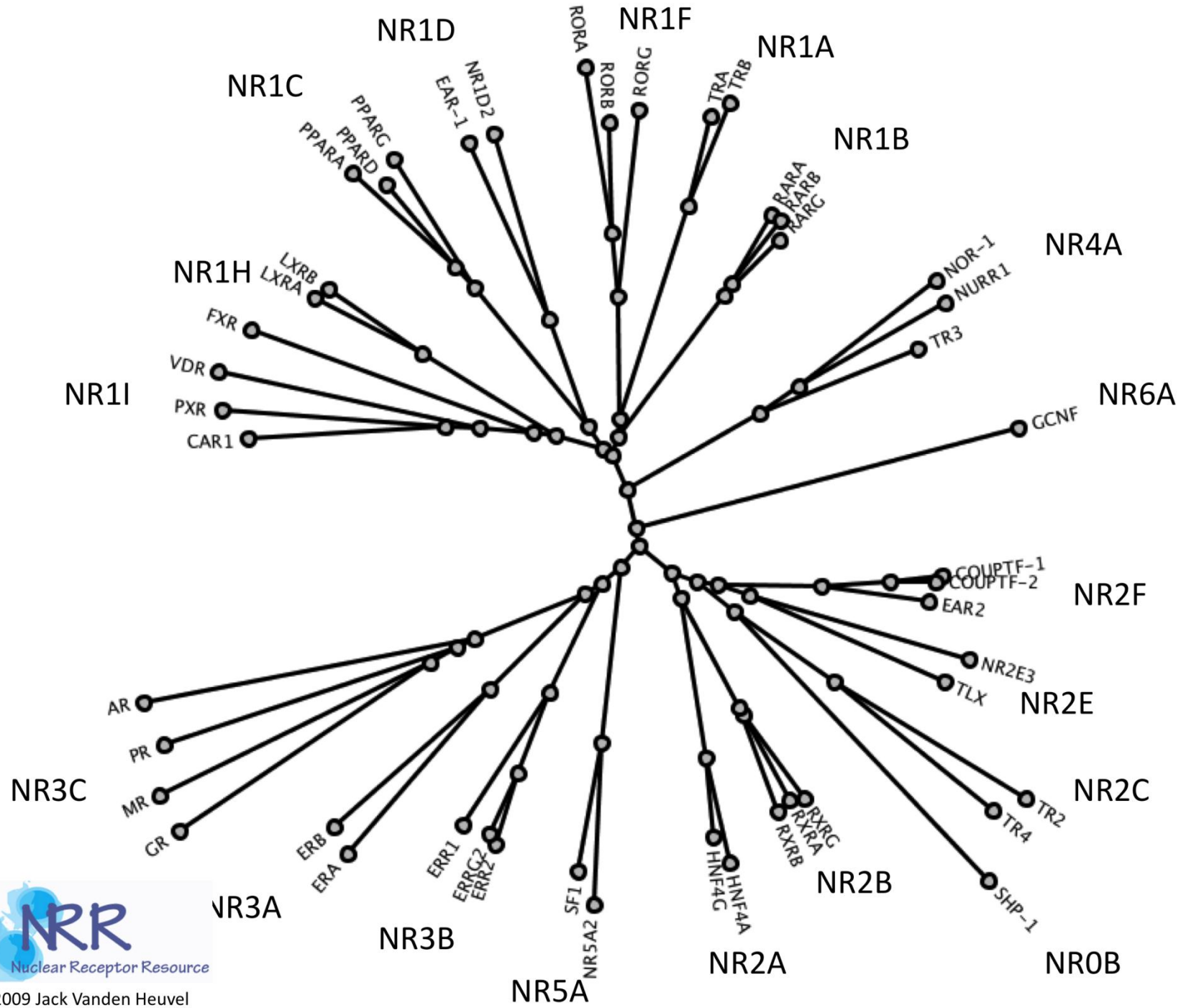
Nuclear Receptors - Transcription Factor Family



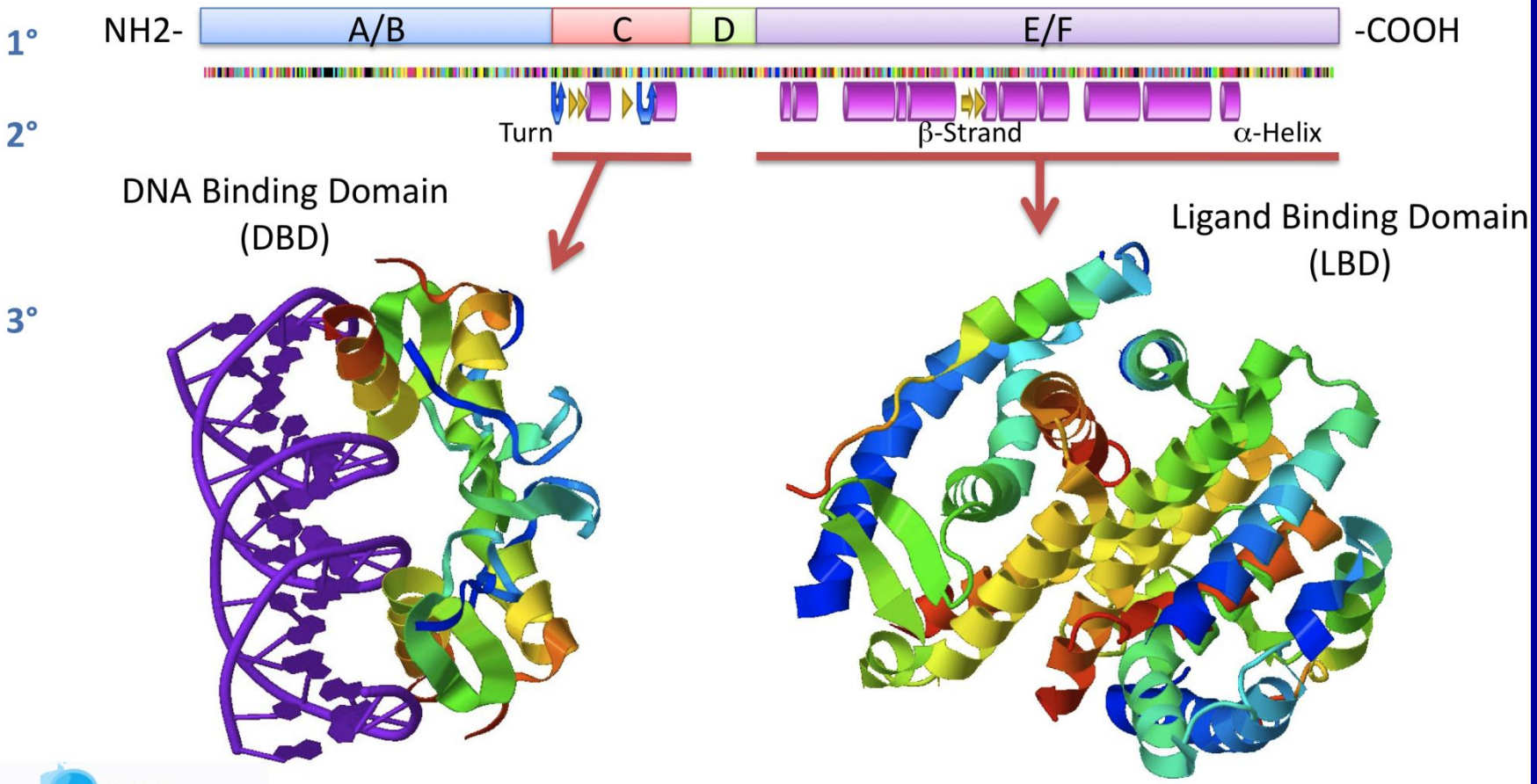
The Nuclear Receptors are intracellular proteins that:

- **function as transcription factors**
- **bind to specific DNA sequences in the promoters or enhancers of target genes**
- **regulate gene expression**
- **48 genes coding for nuclear receptors in the human genome.**
- **Some are widely expressed while others are restricted to very specific tissues and cell types.**

Phylogeny and Nomenclature

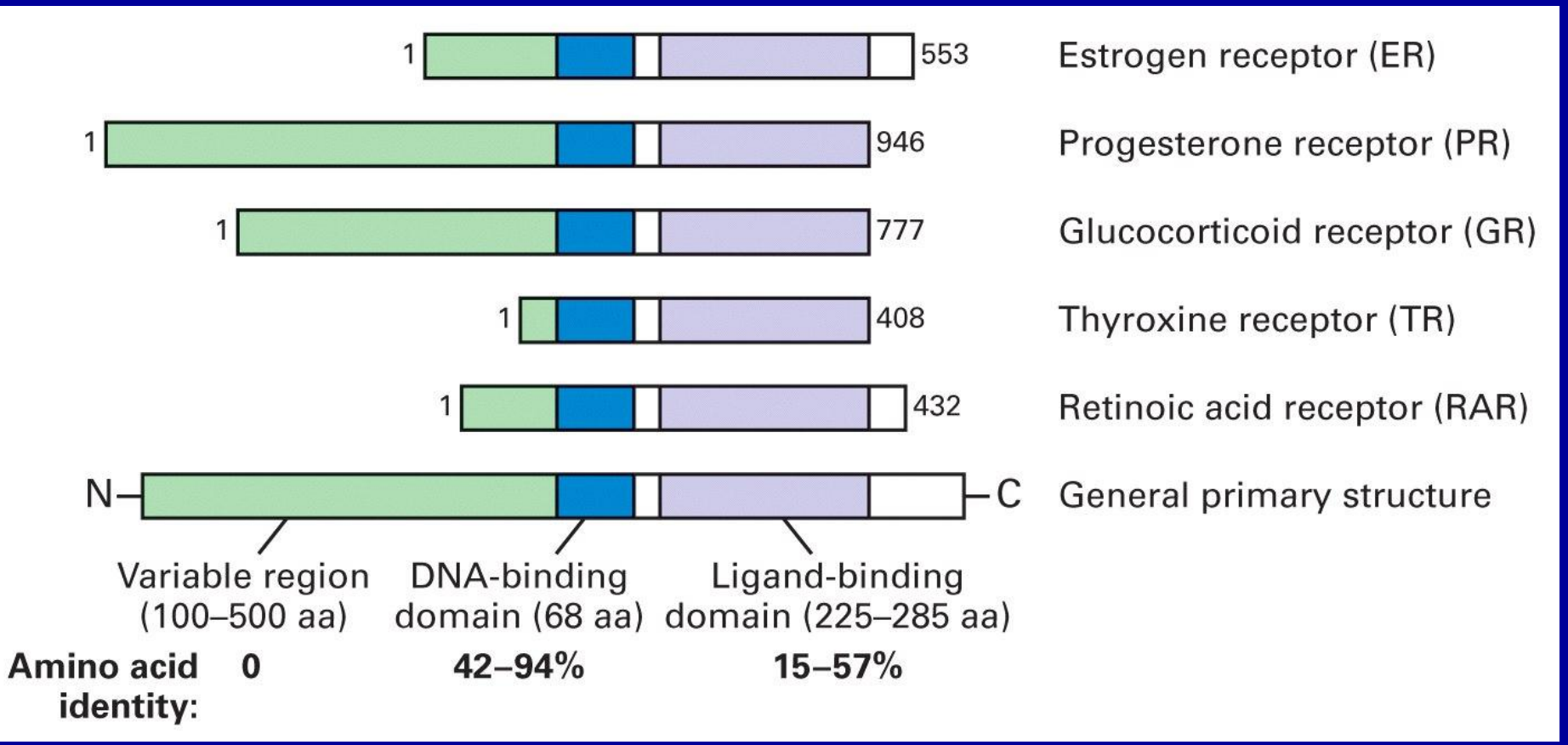


Structure of NRs



NRR
Nuclear Receptor Resource

Domain structure of Nuclear Receptors



How can Nuclear Receptors be classified?

LIGAND BINDING

DNA BINDING & DIMERISATION

EXPRESSION PATTERN

Ligands for Nuclear Receptors

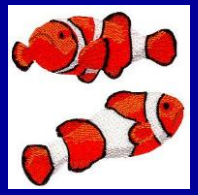
Task:

List 4 different substances that can act as ligands for NRs.

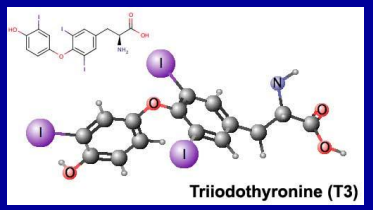
The biochemical origins of Nuclear Receptor ligands are varied:

Cholesterol is the biosynthetic source of the steroid hormones

Retinoic acids are produced from β-carotene.



The eicosanoid, prostaglandin J_2, is a product of fatty acid metabolism.

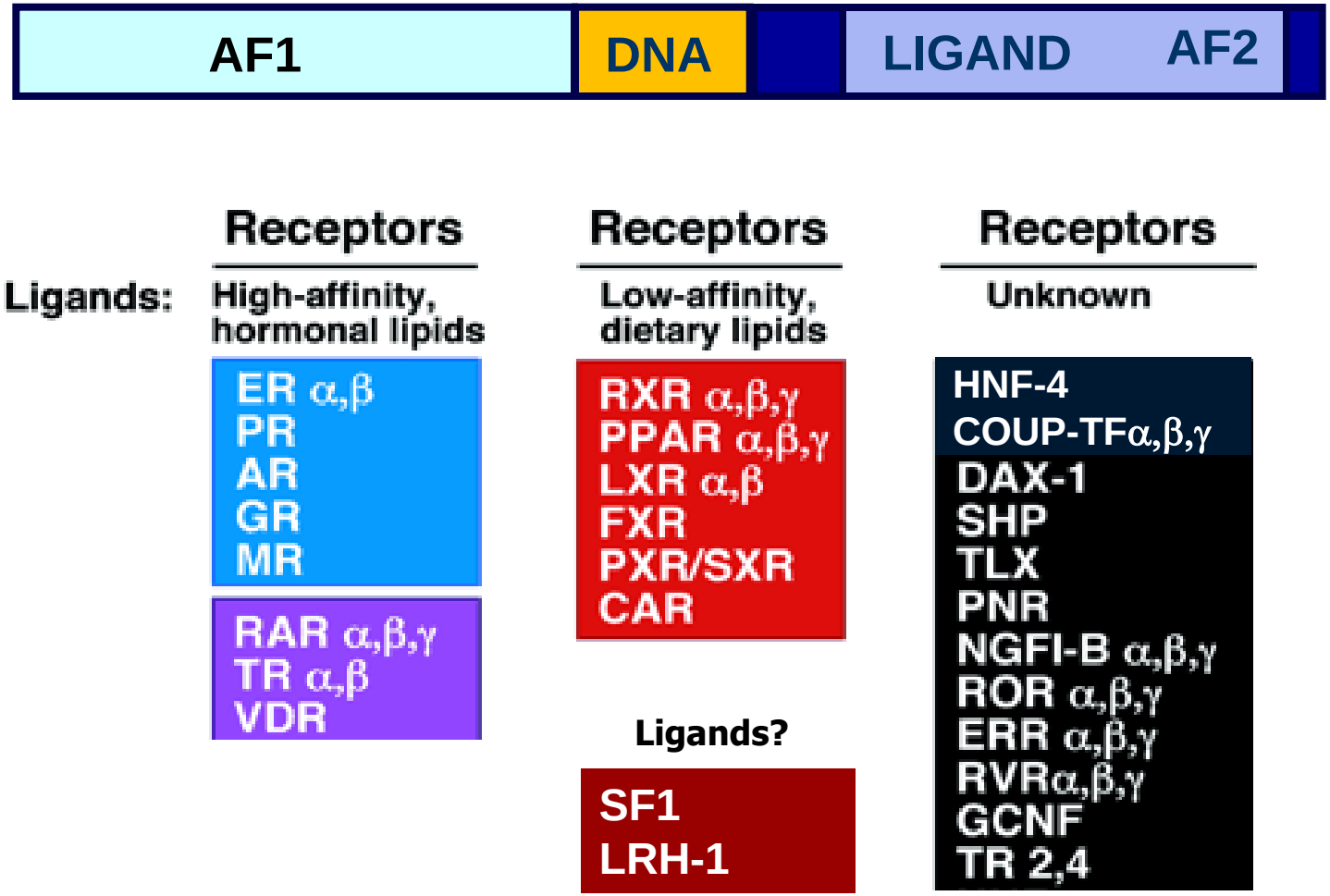


Thyroid hormone is a tri-iodinated thyronine made as a degradation product of crosslinked iodinated tyrosines in the protein thyroglobulin.

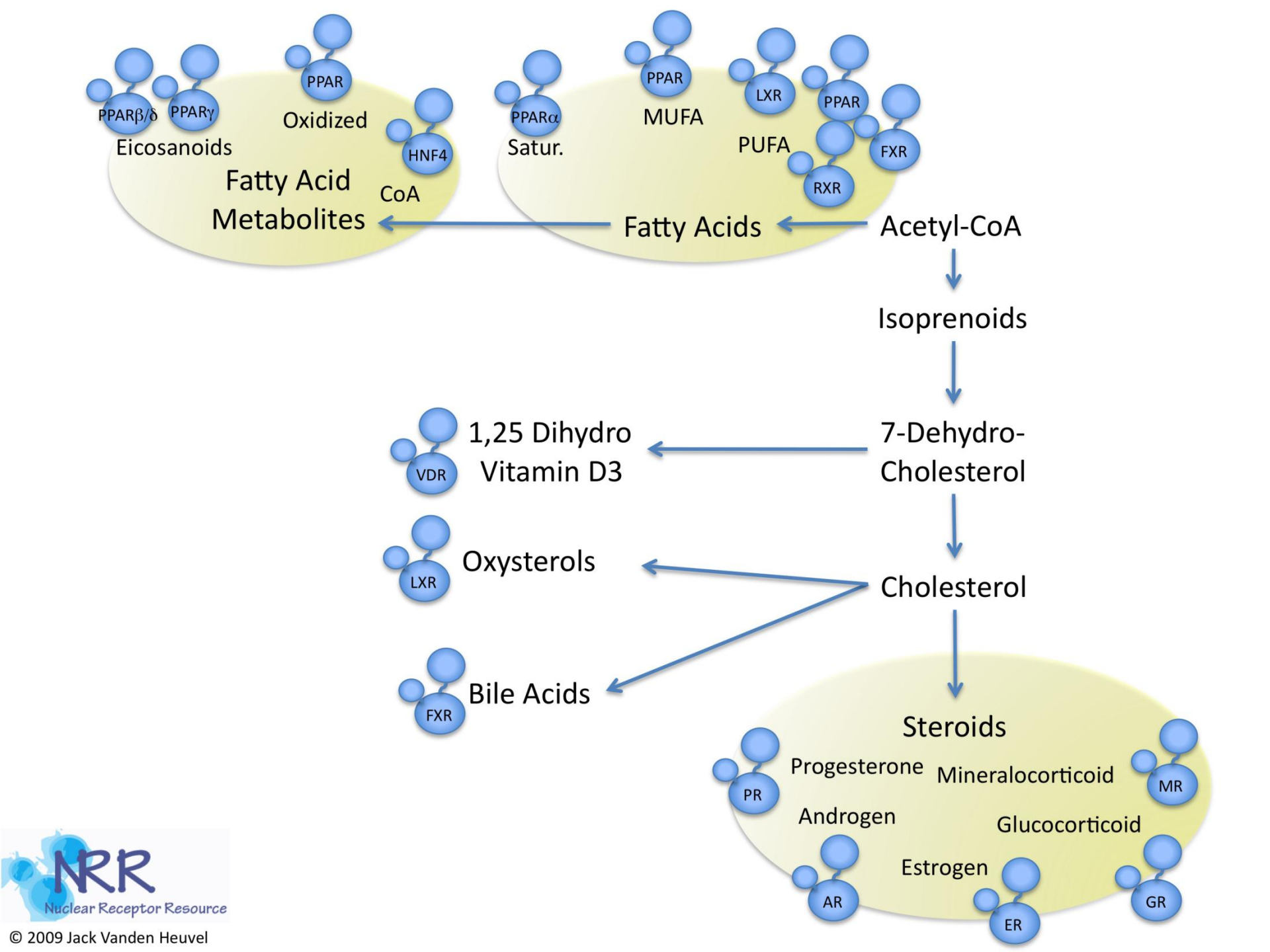
Although diverse in origin, NR ligands are all similar in mass and molecular size.

Bogan et al : Nature Structural Biology **5**, 679 - 681 (1998)

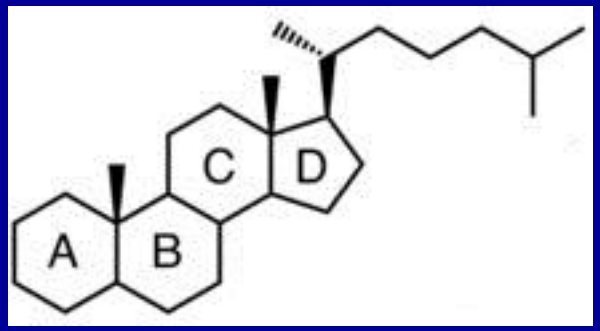
Nuclear Receptor Family



Adapted and modified from (Chawla *et al* Science 294 p1866 2001)



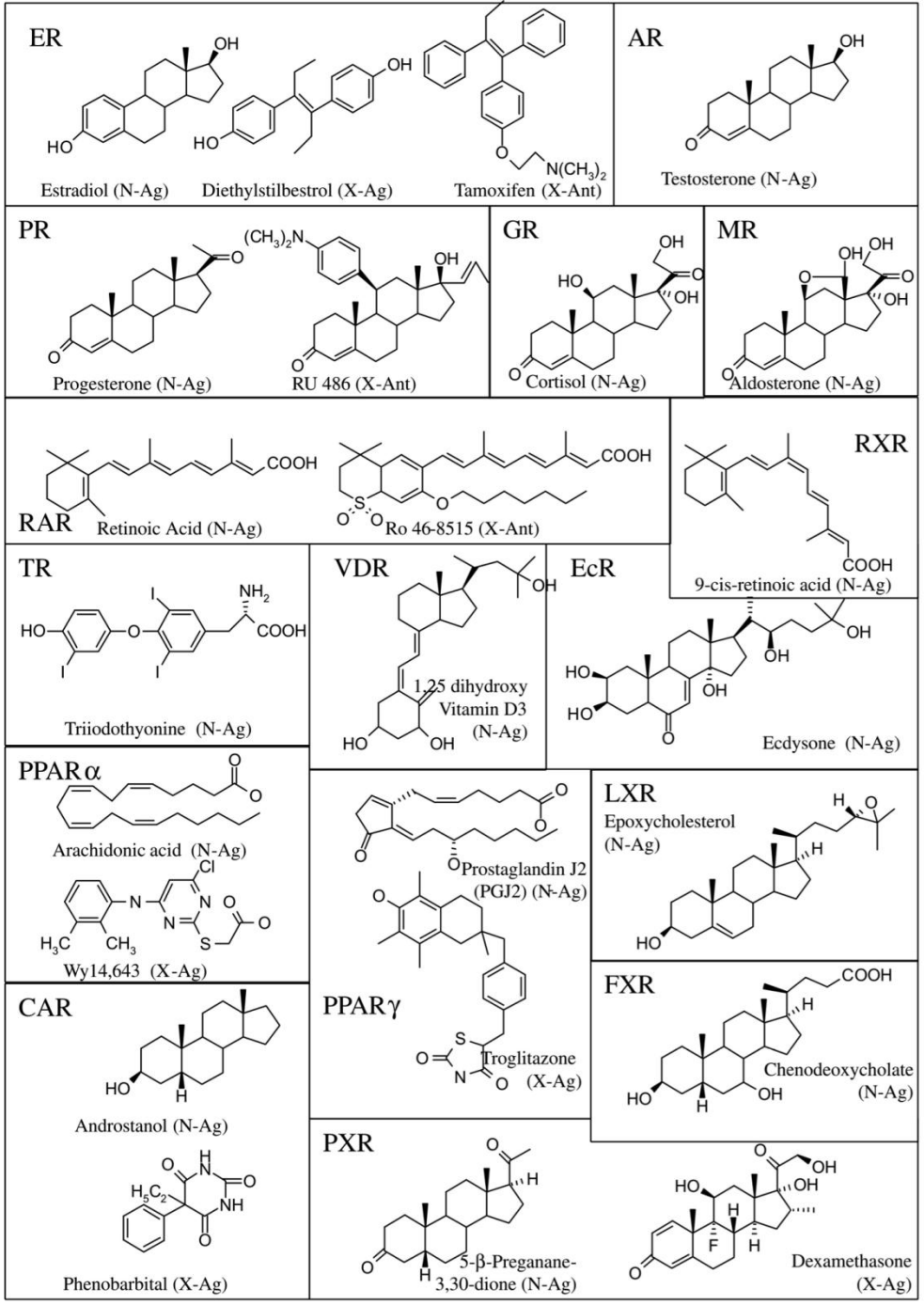
Steroids



A **steroid** is composed of four fused carbon rings: three cyclohexane rings (A, B, C) and one cyclopentane ring (D) that determine the characteristic **sterane** core.

Steroids vary due to additional functional groups attached to the carbon rings and oxidation states of the rings.

Representative NR Ligands

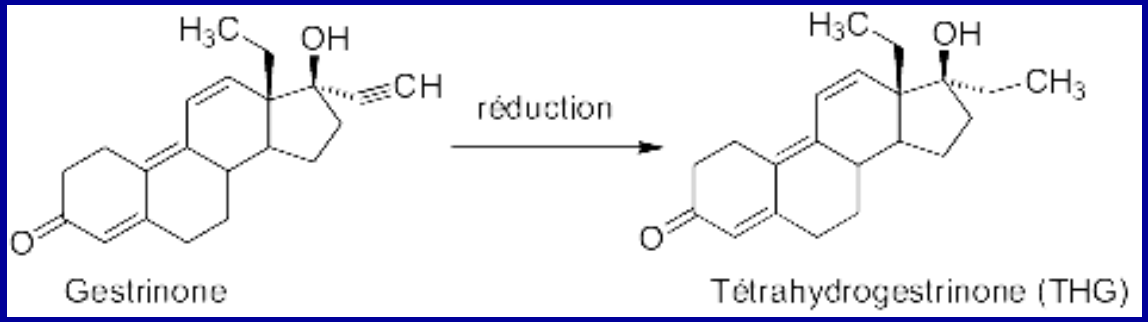


Nuclear Receptor Resource

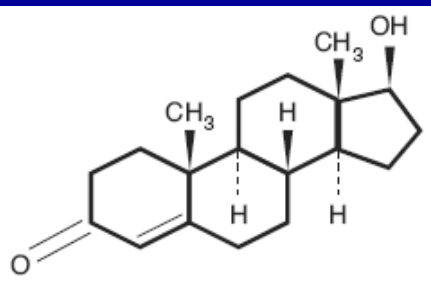
Designer Androgens in Sport

Dwain Chambers

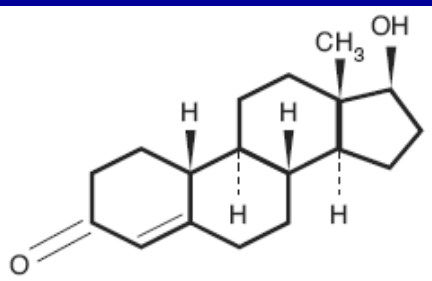
Marion Jones



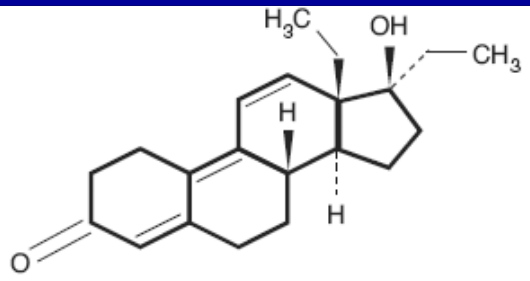
Designer Androgens in Sport



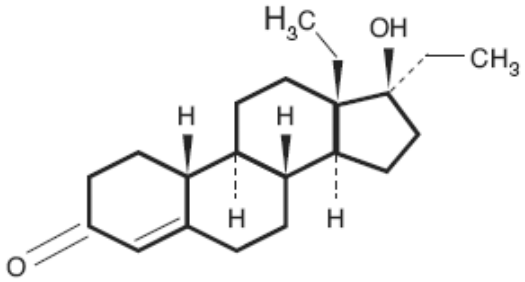
Testosterone



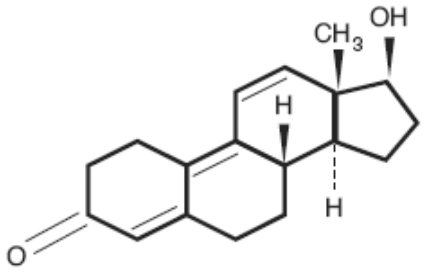
Nandrolone



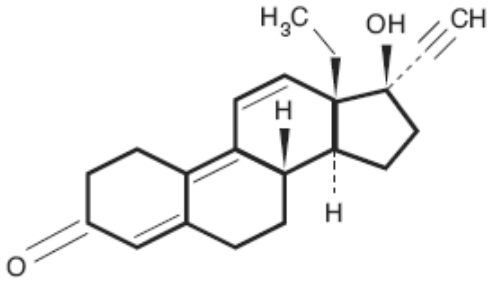
THG



Norbolethone



Trenbolone



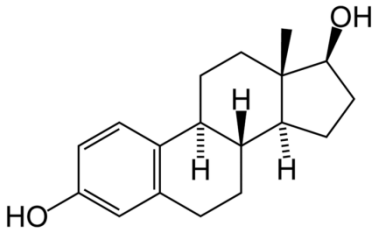
Gestrinone

THG and related natural and synthetic androgen structures. Note the structural similarities between the two designer androgens norbolethone and THG with THG's parent gestrinone (differing by only a side chain reduction) and the known potent androgens nandrolone and trenbolone.

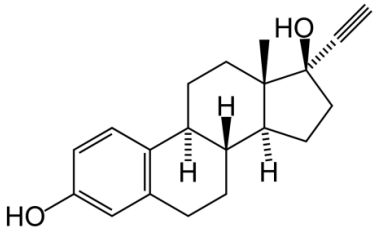
Endocrine Disrupting Chemicals

Endocrine disruptors are exogenous substances that act like hormones in the endocrine system.

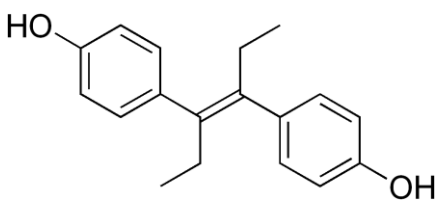
They disrupt the physiologic function of endogenous hormones.



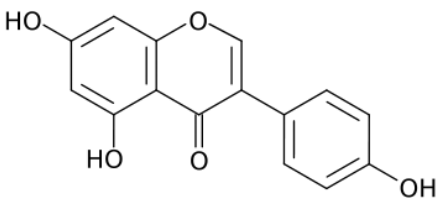
17β-Estradiol



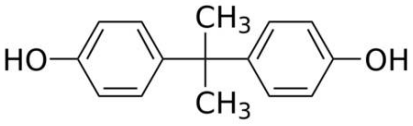
Ethinylestradiol



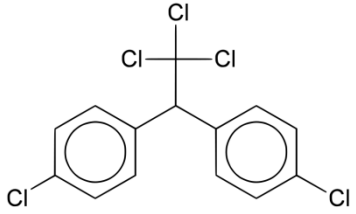
Diethylstilbestrol (DES)



Genistein



Bisphenol A

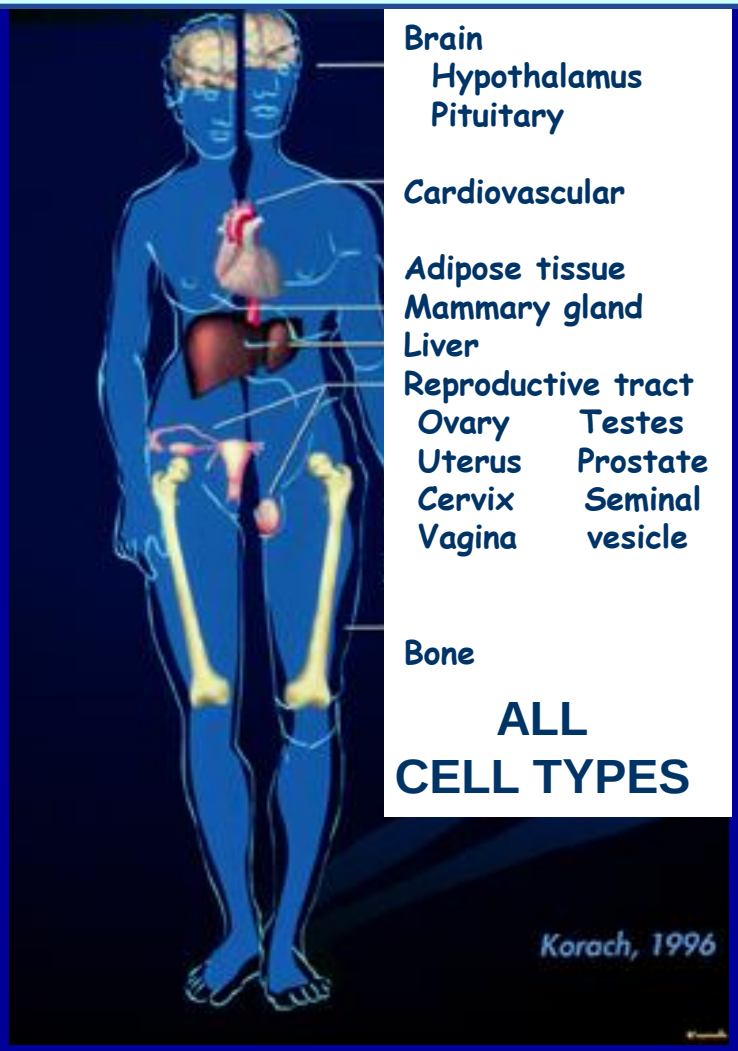


DDT

What are the functions of Nuclear Receptors ?

LIGANDS/HORMONES	TARGET TISSUES	PROCESS

- Steroids (androgens, estrogens, progestins, mineralocorticoids, glucocorticoids).
- Retinoids
- Thyroid hormone
- Vitamin D
- Fatty acids
- Bile acids
- Phospholipids
- *Environmental compounds (eg plasticisers)*



Reproduction

Cell growth

Cell differentiation

Homeostasis

Inflammation

Apoptosis

Aberrant signalling

⇩

Clinical disorders

Current and Potential Therapeutic Indications

TRβ	Obesity, dyslipidemia, hypothryroidism
PPARα	Dyslipidemia, atherosclerosis, inflammation
PPARγ	Diabetes, obesity, cancer, inflammation, osteoporosis
LXRα/β	Dyslipidemia, atherosclerosis, diabetes
VDR	Osteoporosis, psoriasis, cancer, inflammation
GR	Arthritis, asthma, immunosuppression, obesity, diabetes
MR	Hypertension, CHF
PR	Contraception, cancer, osteoporosis
AR	Frailty, prostate cancer, sexual dysfunction, osteoporosis
ERα	Breast cancer, osteoporosis, cardiovascular disease, gynecological disorders, Alzheimer's
ERβ	Prostate cancer, osteoporosis, obesity, cardiovascular disease, Alzheimer's
HNF4α	Diabetes, dyslipidemia
RAR(α,β,γ)	Cancer, psoriasis
RXR(α,β,γ)	Diabetes, cancer

Possible Therapeutic Indications

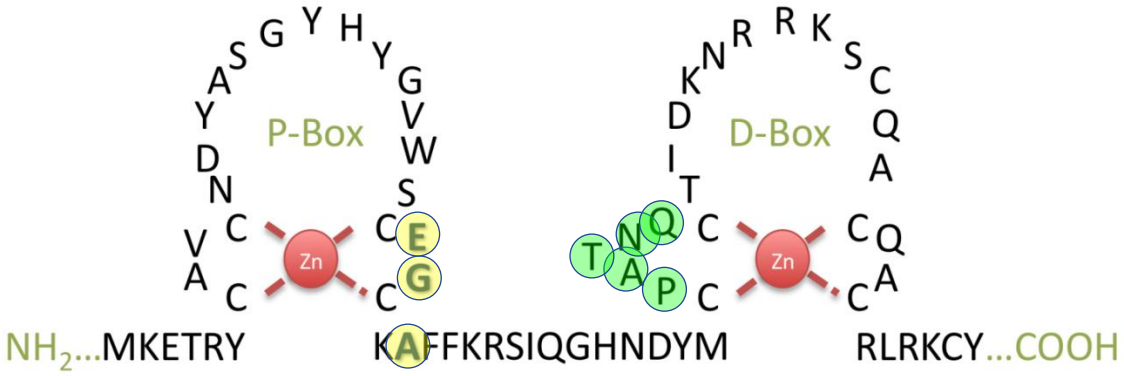
- Rev-Erb — Circadian rhythym
- (α,β) — atherosclerosis
- RORα — Atherosclerosis, dyslipidemia, inflammation, rheumatoid arthritis, osteoporosis, neurodegeneration
- RORγ — Osteoporosis, immunosuppression
- FXR — Dyslipidemia, liver disease
- PXR, CAR — Xenobiotic metabolism
- TR2, TR4 — Cancer, male fertility
- TLX — Neurodegeneration
- PNR — Retinal degeneration
- COUP-TFI — Breast cancer, neural development
- COUP-TFII — Cancer, angiogenesis
- EAR2 — Uterine/gynecological disorders

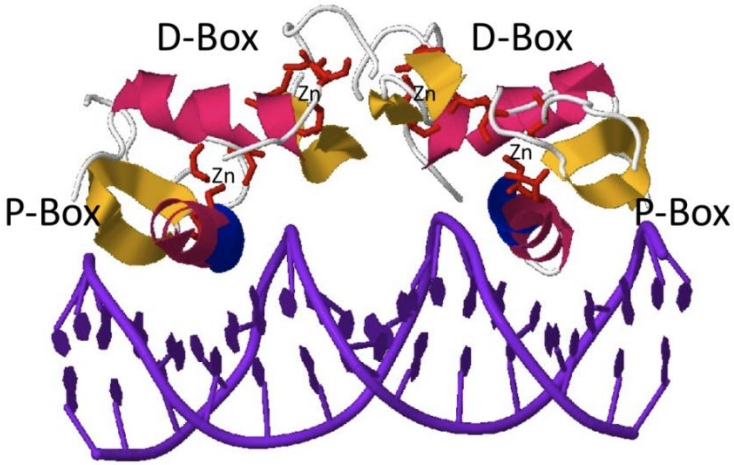
- NGFI-B — Drug abuse, cancer, schizophrenia, manic-depression, psychoses, neurodegeneration, immunomodulation
- NURR1 — Parkinson's, schizophrenia, manic-depression, cancer
- NOR1 — Drug abuse, cancer, immunomodulation
- SF-1 — Adrenal disease, disorders of steroid metabolism
- LRH — Breast cancer, fertility, dyslipidemia
- GCNF — Fertility/contraception
- DAX-1 — Adrenal disease, fertility, gynecologic disorders
- SHP — Obesity, dyslipidemia, diabetes, liver disease, cancer
- ERRα — Osteoporosis, dyslipidemia
- ERRβ — Fertility

Nuclear Receptor Resource

Sources: Lifespan Database
Giguere, V. *Endocrine Rev*. 1999, *20*, 689-725
Willson, T. M.; Moore, J. T. *Mol. Endocrinology* 2002, *16*, 1135-1144

Nuclear Receptor Structure and DNA Binding





Amino Acid residues that confer Response Element Binding Specificity

Amino Acid residues that are important for receptor dimerisation

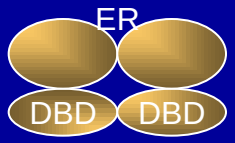
RECEPTORS	P-BOX	HALF-SITE	RESPONSE ELEMENT
ER	c**EG**ck**A**	AGGTCA	AGGTCAnnnTGACCT
GR, MR, PR, AR	c**GS**ck**V**	TGTTCT	AGGACAnnnTGTCCT
PPAR, RAR, VDR, PPAR	c**EG**ck**G**	AGGTCA	AGGTCAnAGGTCA

Nuclear Receptor Resource

Consensus binding sites for Nuclear Receptors (AGGTCA)

Inverse palindromes

ER	Estrogen
GR	Glucocorticoid
MR	Mineralocorticoid
PR	Progesterone
AR	Androgen



AGGTCAnnnTGACCT

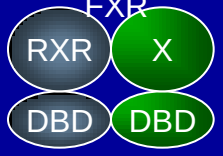
AGAACAnnnTGTTCT

Direct repeats

TR	Thyroid Hormone
VDR	Vitamin D3
RAR	Retinoic Acid
PPAR	Peroxisome proliferator

DR-1	AGGTCAnAGGTCA	RXR/RXR, PPAR/RXR, RAR/RXR, COUP/RXR
DR-2	AGGTCAnnAGGTCA	RAR/RXR
DR-3	AGGTCAnnnAGGTCA	VDR/RXR
DR-4	AGGTCAnnnnAGGTCA	TR/RXR
DR-5	AGGTCAnnnnnAGGTCA	RAR/RXR

RAR, TR, VDR, PPAR, PXR, LXR, FXR



AGGTCAn$_{1-5}$AGGTCA

RXR, COUP, HNF-4

AGGTCAnAGGTCA

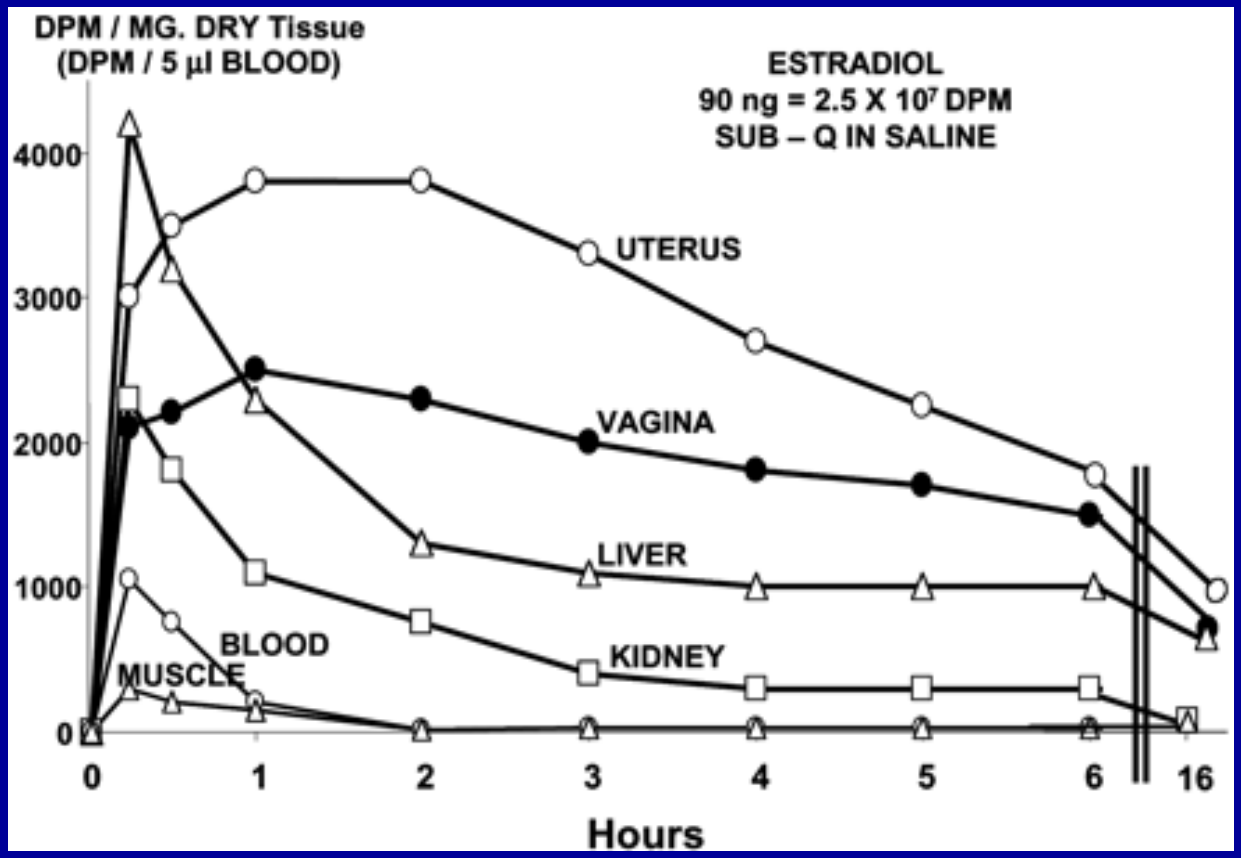
NGFI-B, SF-1

AGGTCA

Which Nuclear Receptors are important for the normal function of reproductive tissues?

Tissue-specific concentration of ^{3}H Estradiol

Elwood Jensen 1962



The concentration of radioactivity in immature rat tissues after a single s.c. injection of ^{3}H estradiol.

Bookout *et al*
Cell 126 789-799 (2006)

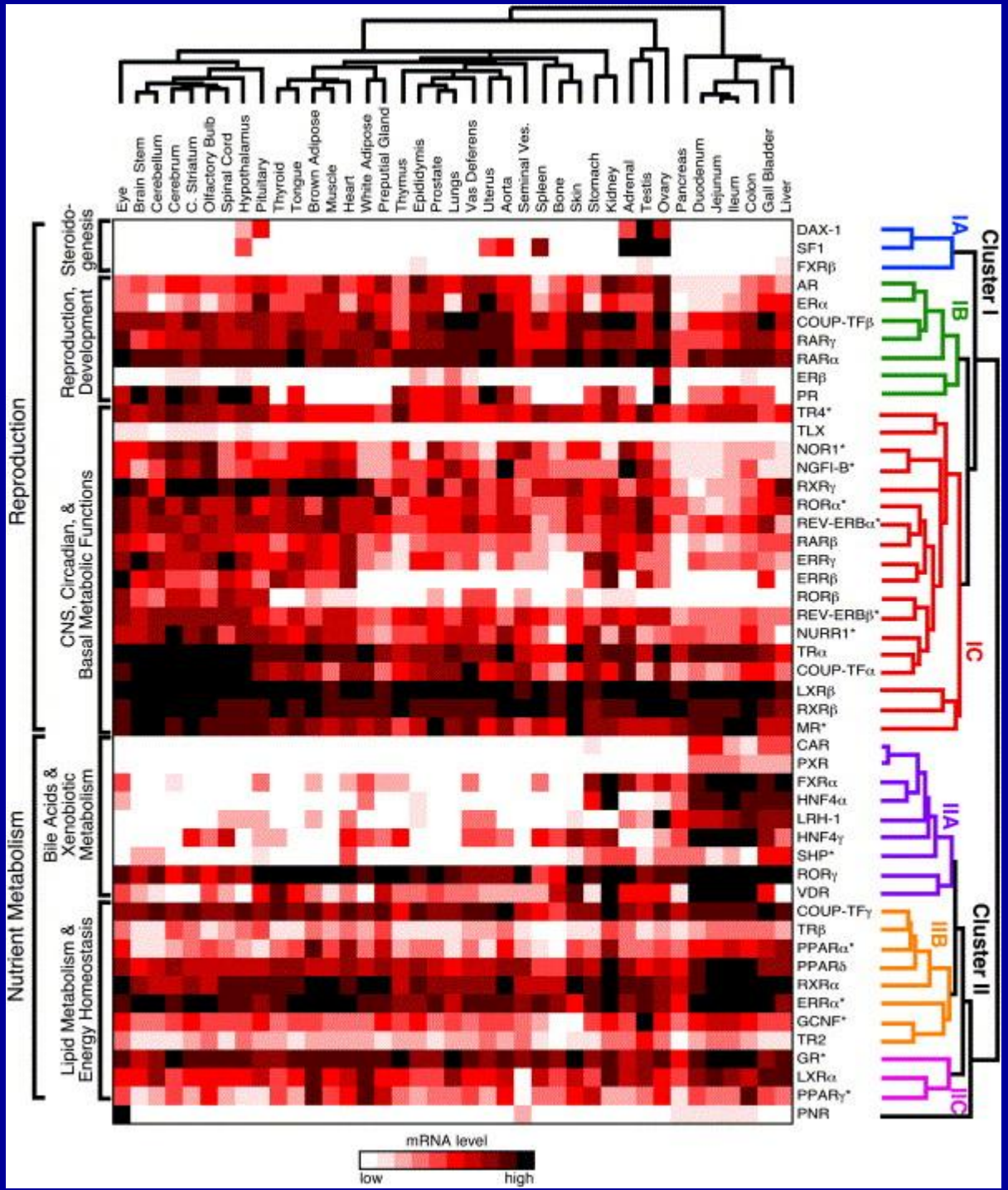
STEROIDOGENESIS

REPRODUCTION & DEVELOPMENT

CNS, CIRCADIAN & BASAL METABOLISM

BILE ACID& XENOBIOTIC METABOLISM

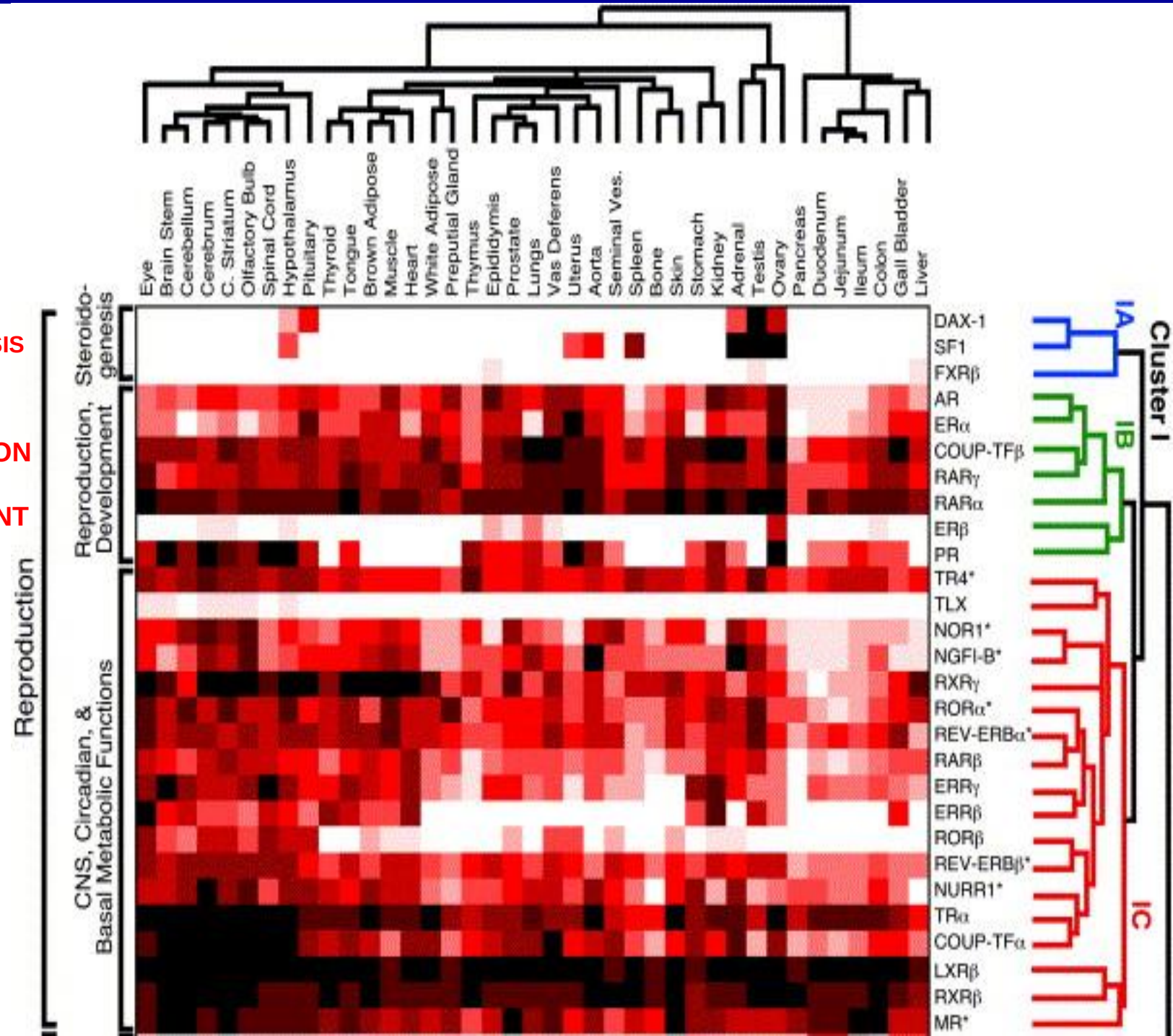
LIPID METABOLISM & ENERGY HOMEOSTASIS



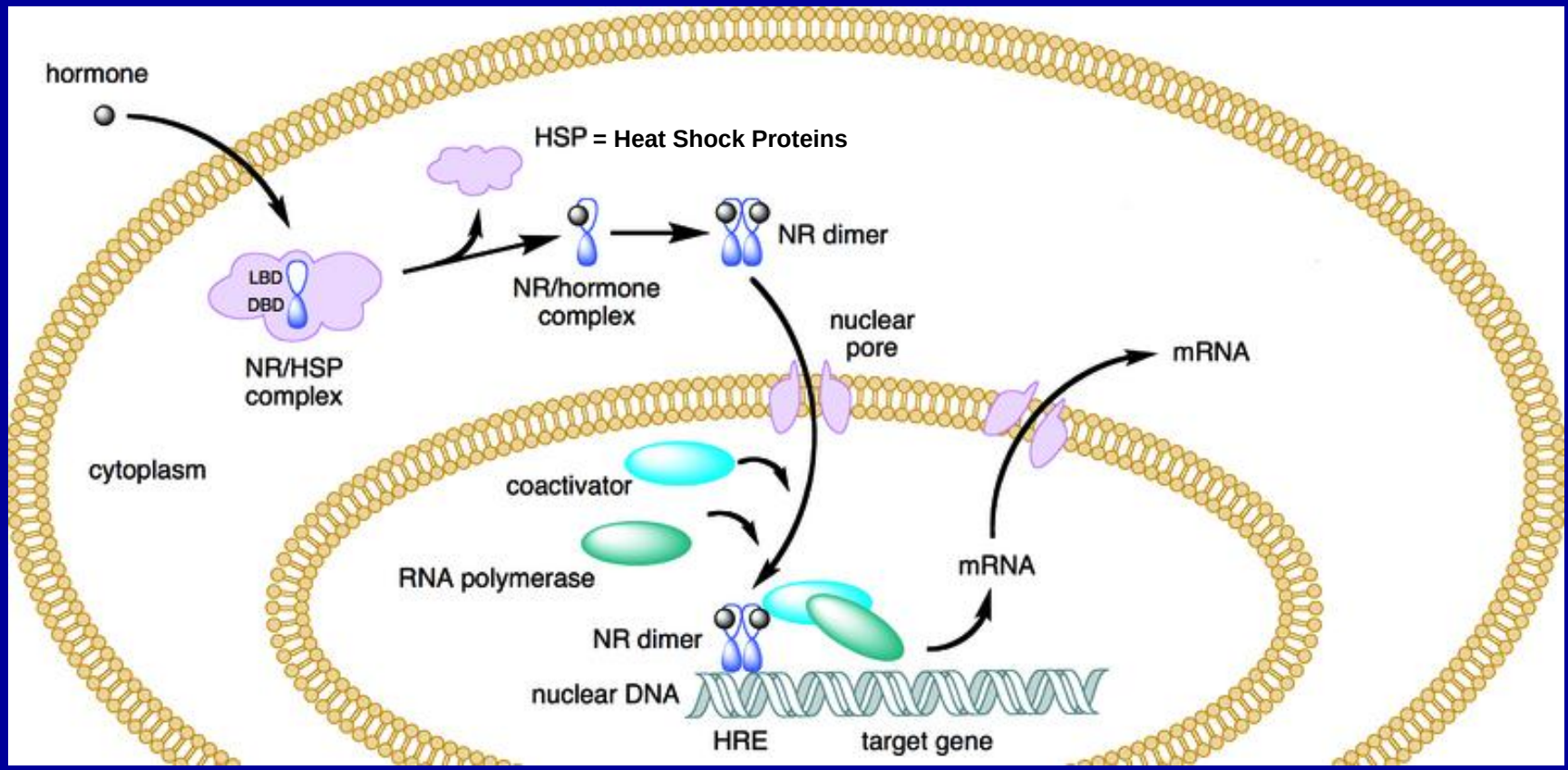
STEROIDOGENESIS

REPRODUCTION & DEVELOPMENT

CNS, CIRCADIAN & BASAL METABOLISM



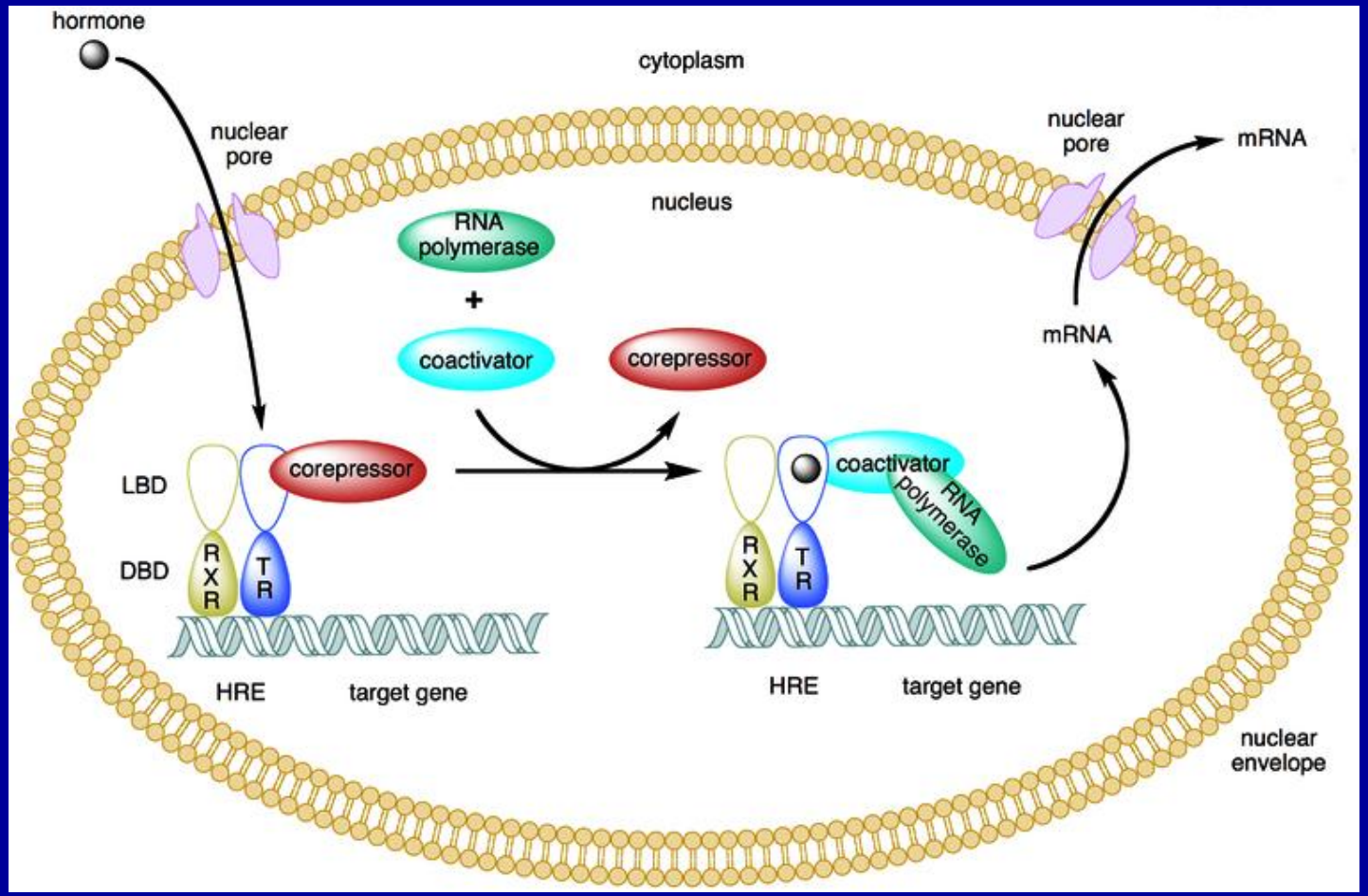
The Mechanism of Nuclear Receptors Action



Following ligand binding NR dissociated from HSP complex, dimerizes and translocates to the nucleus.

Occurs for steroid receptors such as Androgen, Estrogen, Progesterone and Glucocorticoid Receptors (some receptor may be nuclear in absence of ligand).

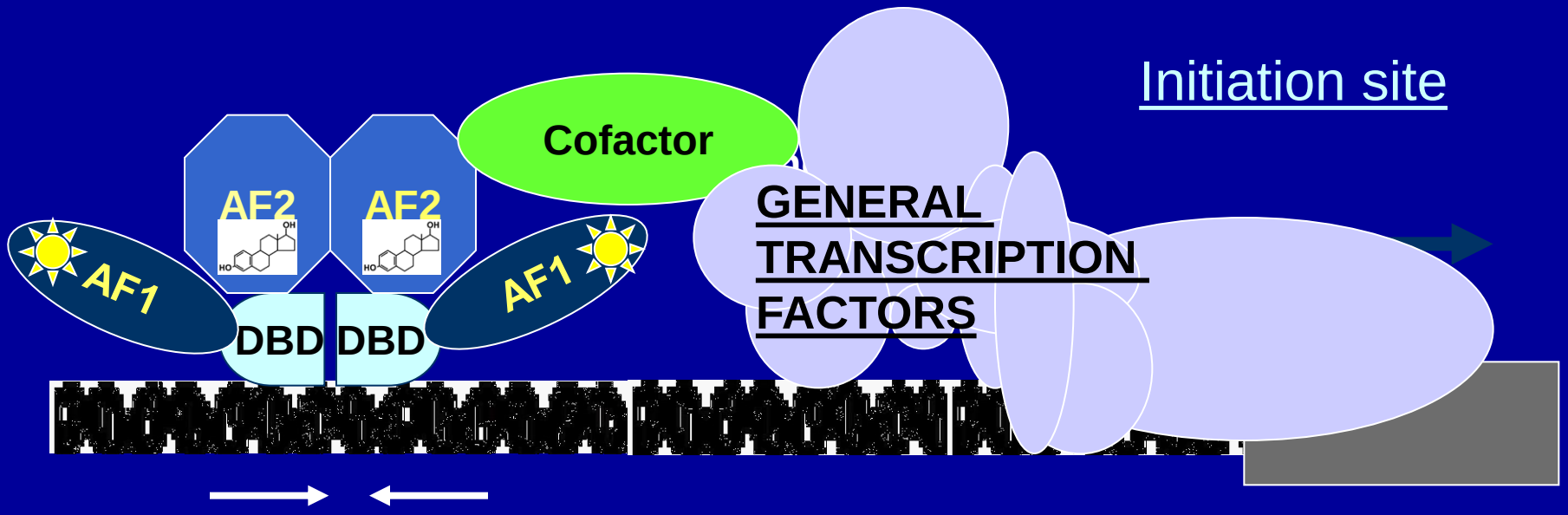
The Mechanism of Nuclear Receptors Action



Nuclear receptors such as retinoic acid receptor, retinoid X receptor and thyroid hormone receptor are retained in the nucleus regardless of ligand availability.

Following ligand binding corepressors dissociate and coactivators bind to the NR.

Activation of target gene expression



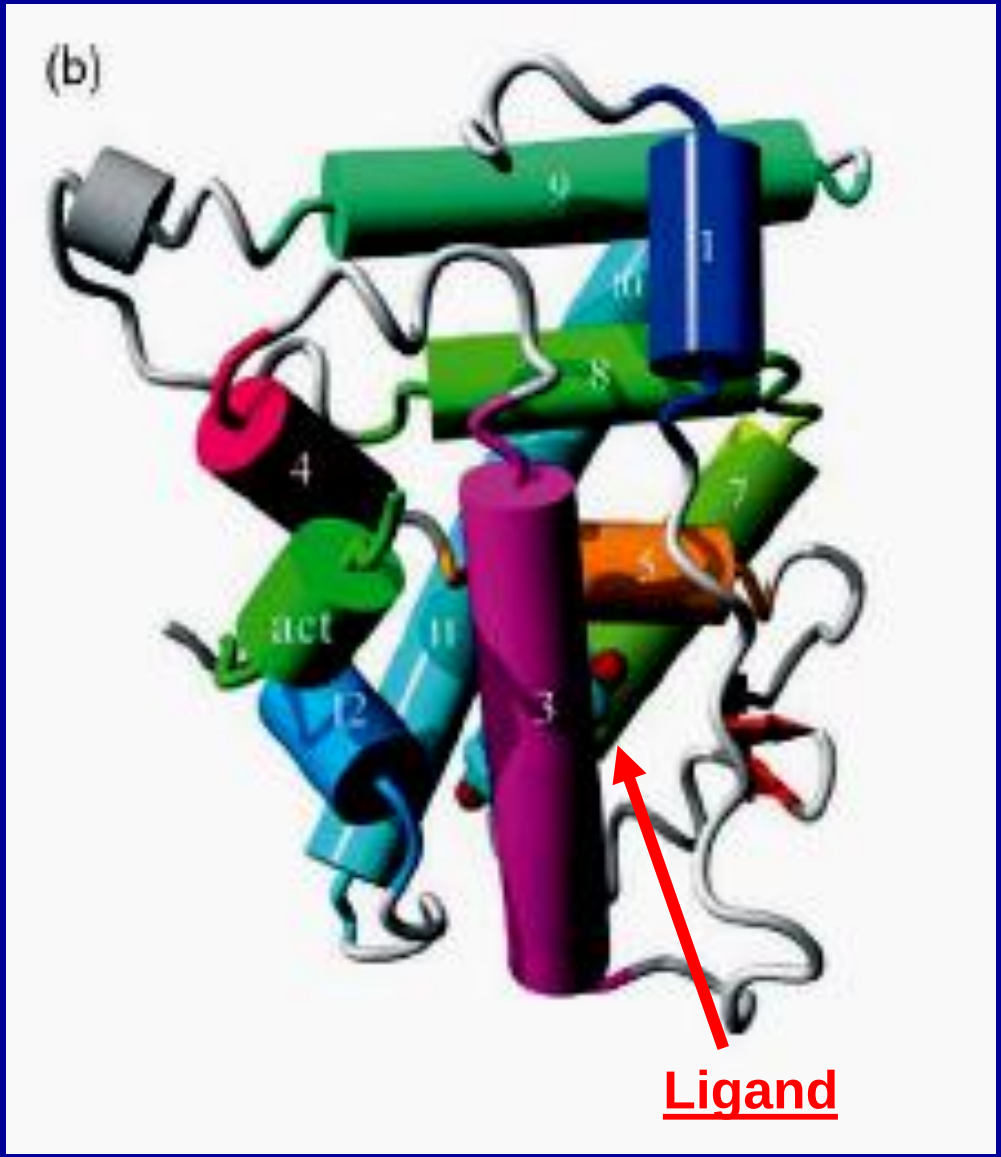
Structure of the LBD

Molecular structures have been determined for the LBD's of many NR

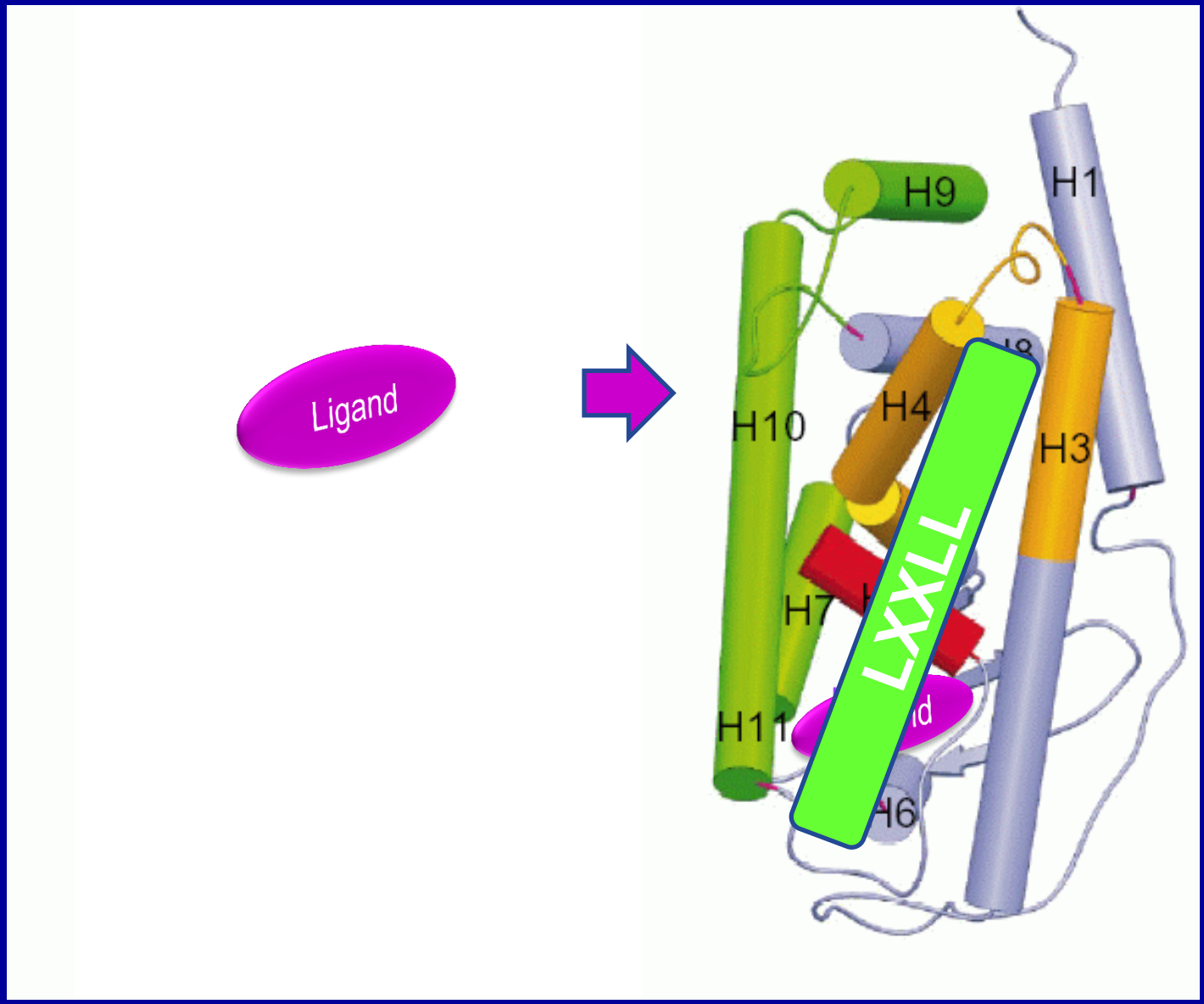
12 α-helices (H1-H12) folded to form an "α-helical sandwich"

Helix 11 required in receptor dimerisation

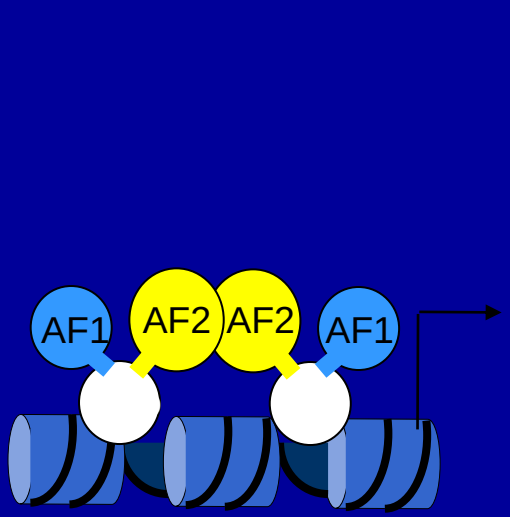
Helices 3, 5, 6 and 11 form the ligand binding pocket



Ligand binding recruits LXXLL- motif containing coregulators to Nuclear Receptors

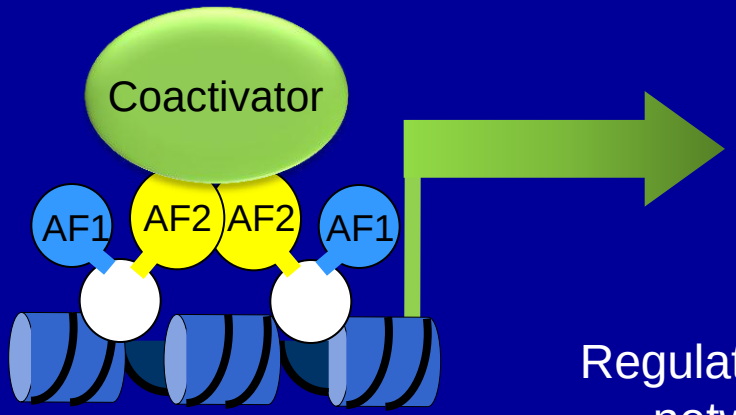


Transcription by Nuclear Receptors is determined by cofactor recruitment

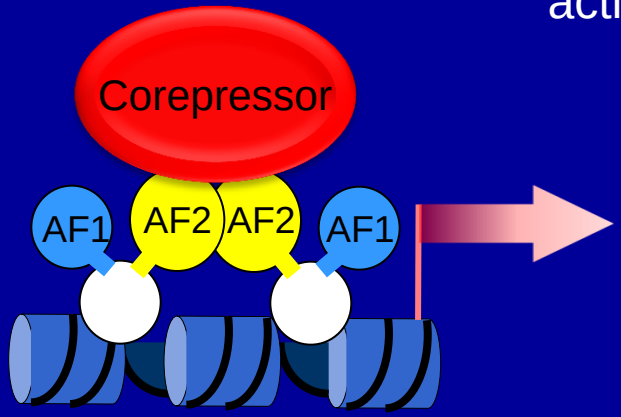


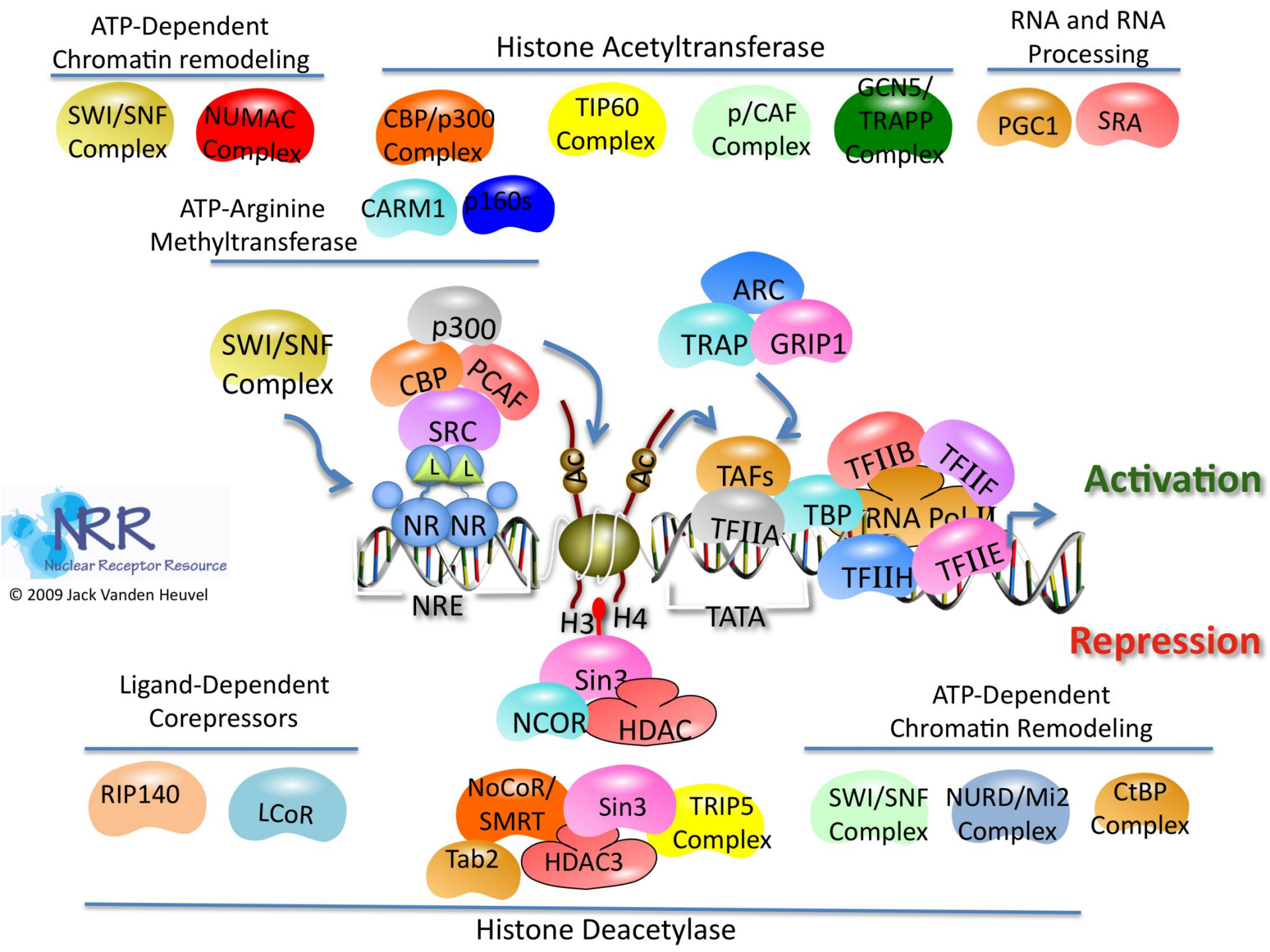
Nuclear receptors select target genes

Ligand



Regulation of gene networks by activation / repression



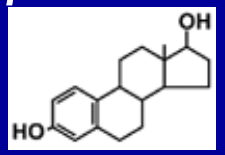

ATP-Dependent Chromatin remodeling
SWI/SNF Complex
NUMAC Complex
Histone Acetyltransferase
CBP/p300 Complex
TIP60 Complex
p/CAF Complex
GCN5/ TRAPP Complex
RNA and RNA Processing
PGC1
SRA
ATP-Arginine Methyltransferase
CARM1
p160s
SWI/SNF Complex
p300
CBP
PCAF
SRC
L
L
NR NR
NRE
Ac
Ac
H3
H4
ARC
TRAP
GRIP1
TAFs
TFIIA
TBP
TFIIB
TFIIF
RNA Pol II
TFIIH
TFIIE
TATA
Activation
Repression
NRR
Nuclear Receptor Resource
© 2009 Jack Vanden Heuvel
Ligand-Dependent Corepressors
RIP140
LCoR
Sin3
NCOR
HDAC
NoCoR/ SMRT
Sin3
TRIP5 Complex
Tab2
HDAC3
ATP-Dependent Chromatin Remodeling
SWI/SNF Complex
NURD/Mi2 Complex
CtBP Complex
Histone Deacetylase

Regulation of Nuclear Receptor activity

Peptide hormones
Growth Factors
Neurotransmitters

Intracellular kinases

Estrogen (17β-estradiol)



Anti-estrogens

ERα

AF1 | DBD | LBD | AF2

595

Activation Functions

AF2 AF2

AF1 AF1

DBD DBD

Estrogen Response Element

Summary

The basic modular structure of a Nuclear Receptor includes DBD, LBD/AF2 and AF1.

The Nuclear Receptor Superfamily can be classified according to:

1) Ligand binding
2) DNA binding and dimerization
3) Expression Pattern

Ligands for NRs include steroids, fatty acids, pharmaceuticals, environmental chemicals.

The Nuclear Receptor DBD contains two Zinc Fingers and is the most highly conserved domain.

Activated Nuclear Receptors recruit coregulators to facilitate the regulation of target gene expression.

Further Reading

http://www.nursa.org/flash/gene/nuclearreceptor/start.html

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