Receptors and Signalling - 2

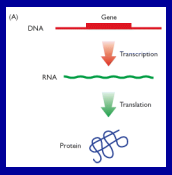
Learning Objectives

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

Gene expression is the process by which the final, active product of a particular gene is produced. Protein or an RNA (rRNA or tRNA).

There are 30,000 genes in a human cells.



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:

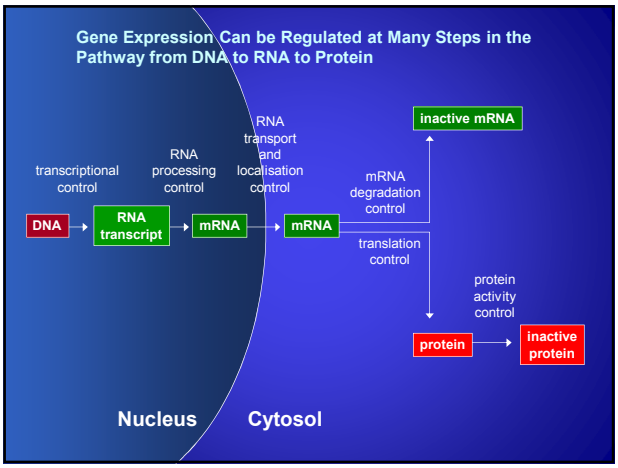
correct genes are expressed at:

- 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).

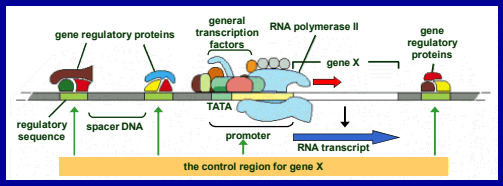


Protein-coding genes

- **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

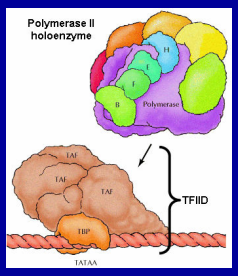


The promoter is the DNA sequence where the general transcription factors and the polymerase assemble.

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.

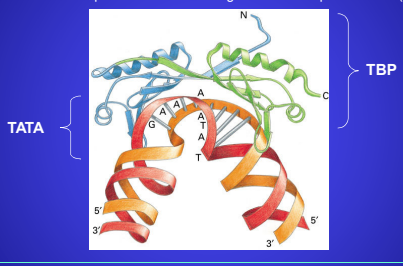
RNA polymerase II holoenzyme



- 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).



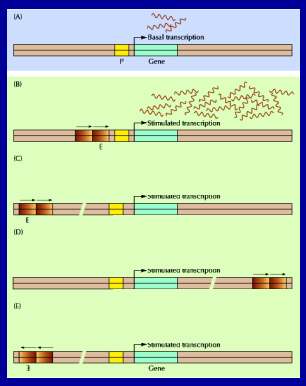
TFIIH Enzymatic Activity

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

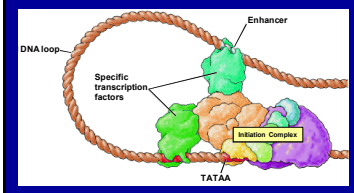
- contain short consensus sequences
- not fixed in location but usually within 200 bp upstream of the transcription start site
- a single element is usually sufficient to confer a regulatory response
- can be located in a promoter or an enhancer
- assumed that a specific protein binds to the element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

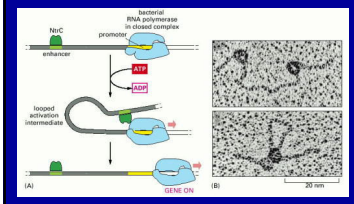
Action of Enhancers



- 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.

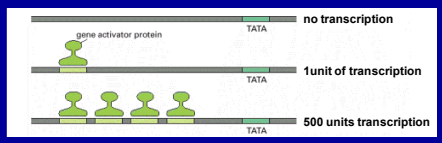
Gene Activation at a Distance: DNA Looping





- 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.
- NtrC facilitates the transition between the initial binding of RNA polymerase to the promoter and the formation of an initiating complex.
- Requires the energy produced by ATP hydrolysis.
- The interaction of NtrC and RNA polymerase is visualised with the intervening DNA looped out.

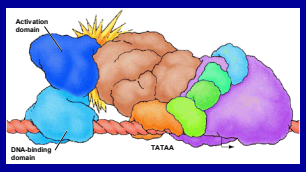
Synergistic Action of Transcriptional Activators



Transcriptional synergy:

- Greater than additive effect of the activators.
- Typically observed between different gene activator proteins 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

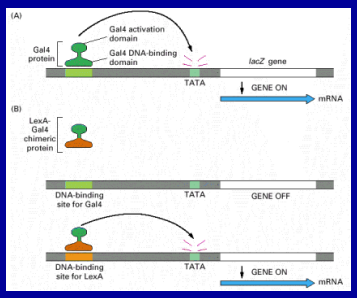


Transcriptional activators consist of two independent domains.

The DNA-binding domain recognises a specific DNA sequence.

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

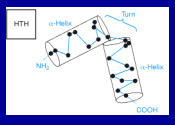
The modular structure of a gene activator protein

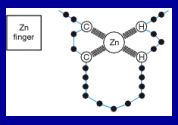


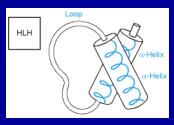
A functional activator can be reconstituted from the C-terminal portion of the yeast Gal4 protein if it is attached to the DNA-binding domain of a bacterial gene regulatory protein (the LexA protein).

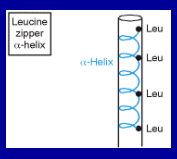
The resulting hybrid protein activates transcription from genes provided that the specific DNA-binding site is present.

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









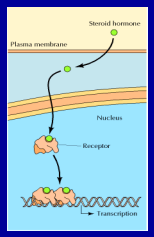
(HTH) Helix-turn-helix domains consist of 2 α helices connected by short chain of amino acids. C-terminal helix fits into major groove of DNA. N-terminal helix – structural component helps position the recognition helix.

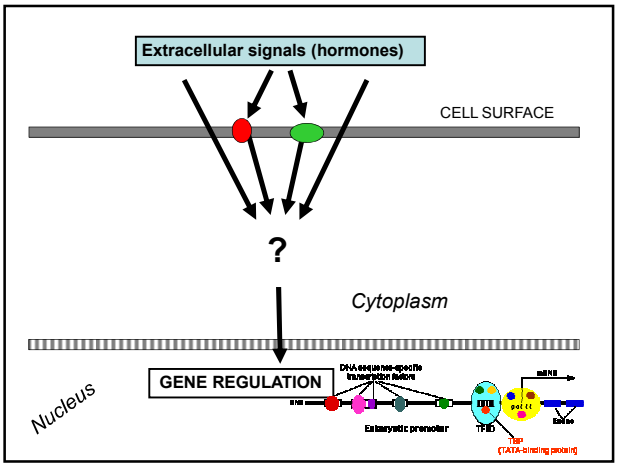
The leucine zipper monomer is *amphipathic* [i.e. has hydrophobic residues (leucines) consistently on one face of the helix]. Interactions between the hydrophobic side chains of leucine residues exposed on one side of a helical region are responsible for dimerization. Two α helices form an inverted Y-shape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

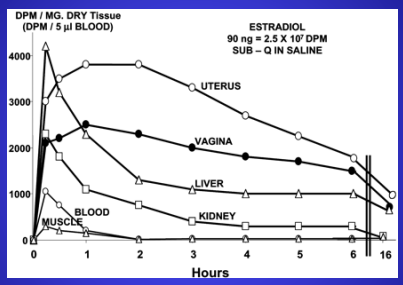
- function as **transcription factors**
- bind to specific DNA sequences in the **promoters** or **enhancers** of target genes
- regulate **gene expression**

There are 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.

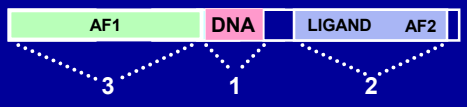
Tissue-specific concentation of ^{3}H Estradiol

Elwood Jensen 1962



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

Nuclear Receptors (NR)

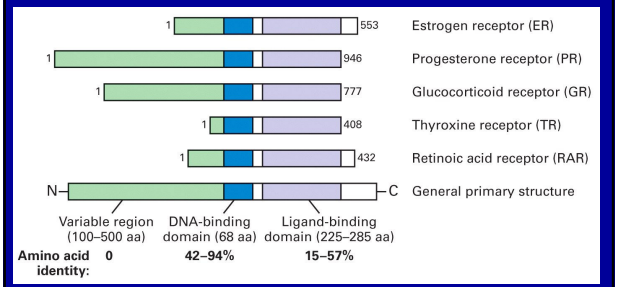


3 major 'domains' in each protein.

1. **DBD** A highly conserved DNA binding domain (DBD)*
2. **LBD** A moderately conserved ligand binding domain (LBD) containing a transcriptional activation function **AF2**
3. **AF1** A highly variable N-terminal domain that contains a second transcriptional activation function (**AF1**)

*DAX1 and SHP lack DBD

Domain structure of Nuclear Receptors



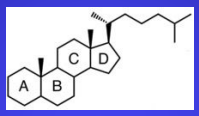
How can Nuclear Receptors be classified?

LIGAND BINDING

DNA BINDING & DIMERISATION

EXPRESSION PATTERN

Steroids



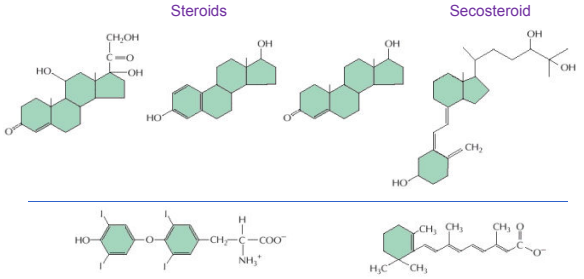
A **steroid** is composed of four fused carbon rings: three cyclohexane rings (A, B, C) and one cyclopentane ring (D) that determine the characteristic **sterane** core.

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

Natural ligands of nuclear receptors include molecules such as steroids, thyronines and retinoids

Steroids

Secosteroid



Secosteroid: Structure with one of the steroid rings broken.

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.

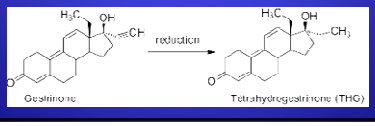
Although diverse in origin, NR ligands are all similar in mass and molecular size.

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

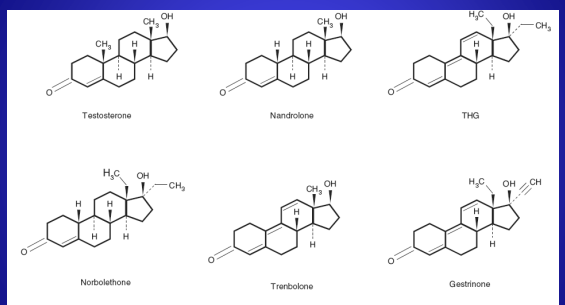
Designer Androgens in Sport

Dwain Chambers

Marion Jones



Designer Androgens in Sport

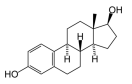


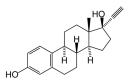
THG and related natural and synthetic androgen structures. Note the structural similarities between the two designer androgens norbolethone and THG with THG's parent gestrinone (differing by only a side chain reduction) and the known potent androgens nandrolone and trenbolone.

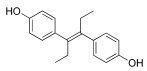
Endocrine Disrupting Chemicals

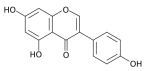
Endocrine disruptors are exogenous substances that act like hormones in the endocrine system.

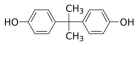
They disrupt the physiologic function of endogenous hormones.

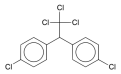

17β-Estradiol


Ethinylestradiol

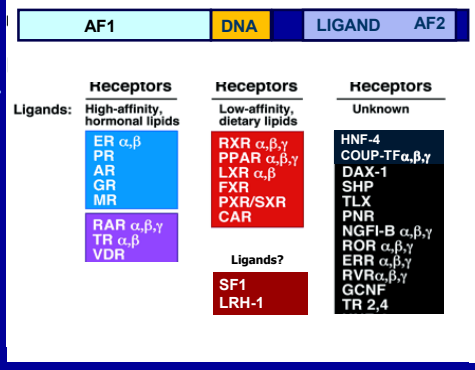

Diethylstilbestrol (DES)


Genistein


Bisphenol A

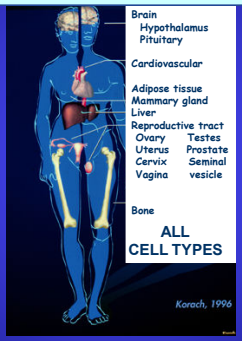

DDT

Nuclear Receptor Family

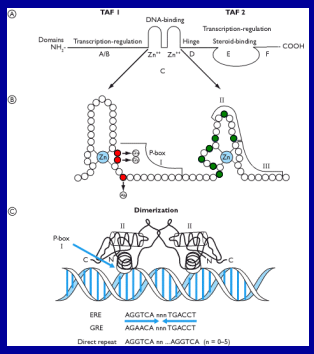


Adapted and modified from (Chawla *et al* Science 294 p1866 2001)

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

Generalised Nuclear Receptor Structure



- Amino Acid residues that confer Response Element Binding Specificity
- Amino Acid residues that are important for receptor dimerisation

Diagram showing dimerization of two receptors and helix I of each receptor slotting into the helix of the DNA.

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

AGGTCA$_{n_{1-5}}$AGGTCA

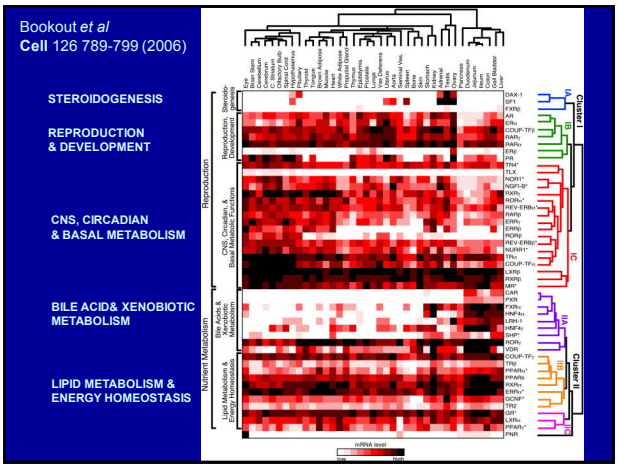
RXR, COUP, HNF-4

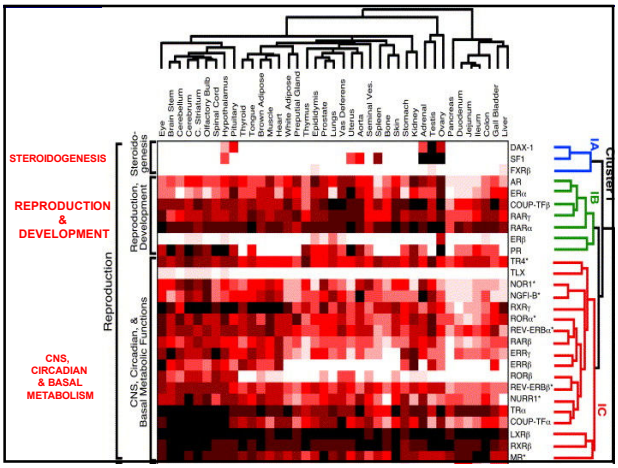
AGGTCAnAGGTCA

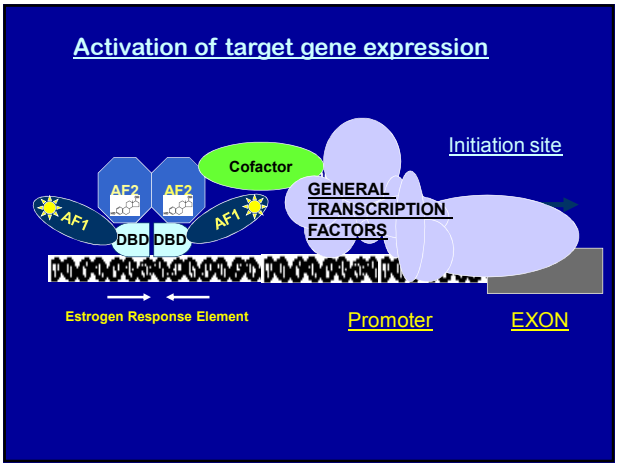
NGFI-B, SF-1

AGGTCA

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


Bookout *et al*
Cell 126 789-799 (2006)
STEROIDOGENESIS
REPRODUCTION & DEVELOPMENT
CNS, CIRCADIAN & BASAL METABOLISM
BILE ACID& XENOBIOTIC METABOLISM
LIPID METABOLISM & ENERGY HOMEOSTASIS

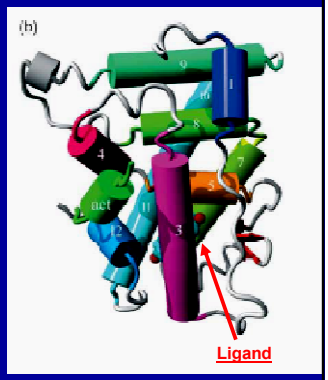

STEROIDOGENESIS
REPRODUCTION & DEVELOPMENT
CNS, CIRCADIAN & BASAL METABOLISM
Reproduction
Steroido-genesis
Reproduction, Development
CNS, Circadian, & Basal Metabolic Functions
DAX-1
SF1
FXRβ
AR
ERα
COUP-TFβ
RARγ
RARα
ERβ
PR
TR4*
TLX
NOR1*
NGFI-B*
RXRγ
RORα*
REV-ERBα*
RARβ
ERRγ
ERRβ
RORβ
REV-ERBβ*
NURR1*
TRα
COUP-TFα
LXRβ
RXRβ
MR*
Cluster I
IA
IB
IC


Activation of target gene expression
Initiation site
Cofactor
AF2
AF2
AF1
AF1
DBD
DBD
GENERAL TRANSCRIPTION FACTORS
Estrogen Response Element
Promoter
EXON

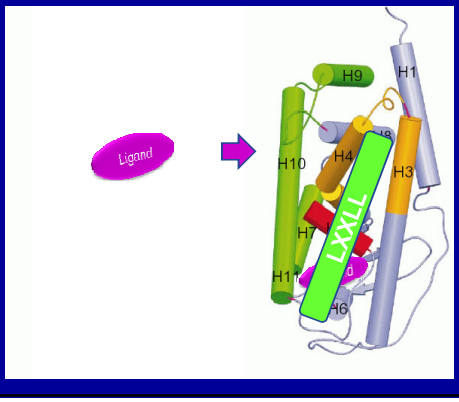
Structure of the LBD

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

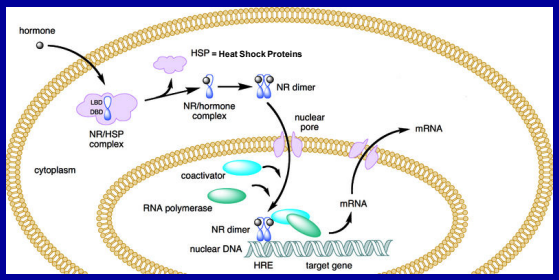
- 12 α-helices (H1-H12) folded to form an "α-helical sandwich"
- Helix 11 required in receptor dimerisation
- Helices 3, 5, 6 and 11 form the ligand binding pocket



Ligand binding recruits LXXLL- motif containing coregulators to Nuclear Receptors



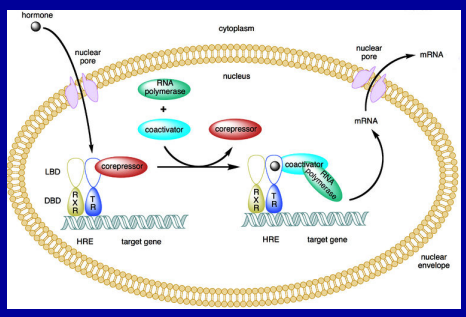
The Mechanism of Nuclear Receptors Action



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.

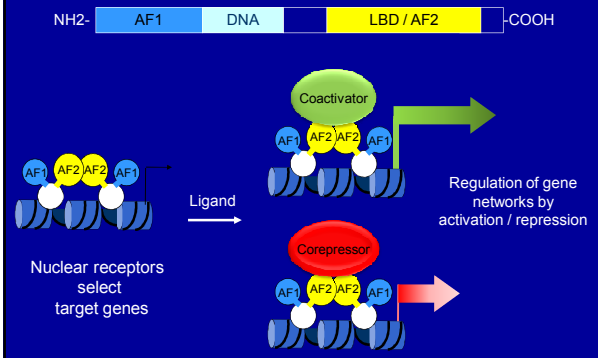
The Mechanism of Nuclear Receptors Action



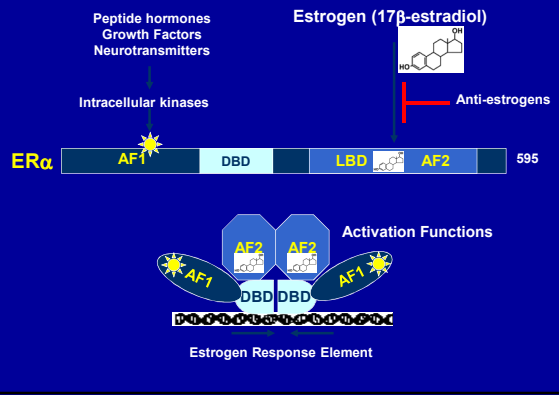
Nuclear receptors such as retinoic acid receptor, retinoid X receptor and thyroid hormone receptor are retained in the nucleus regardless of ligand availability.

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

Transcription by Nuclear Receptors is determined by cofactor recruitment



Regulation of Nuclear Receptor activity



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

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