Signaling by Cytosolic Receptors

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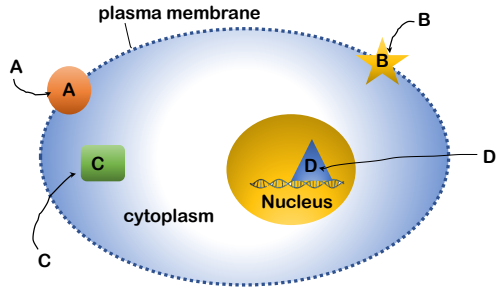
Objectives

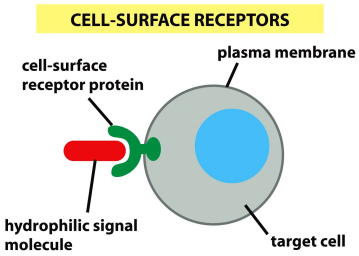
- Classification of nuclear receptors
- Classification of ligands for nuclear receptors
- Where are the ligands come from?
- Common structural & functional organization of nuclear receptors
- Mechanisms of action of nuclear receptors
- Implications of nuclear receptors

Cells contain an array of receptors

1. Localized to plasma membrane (A & B)
2. Localized intracellulary (C & D)

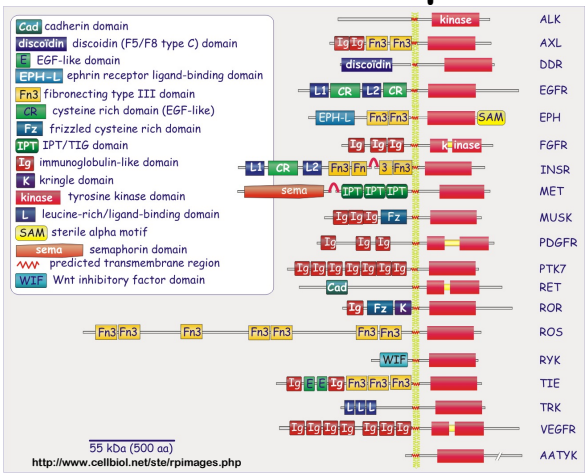


Cell-Surface Receptors

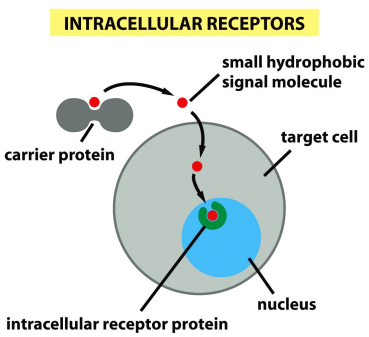


- E.g., Water-soluble ligands: growth factors
- Could be a fast response via second messenger signals
- or cascade of signaling responses that eventually acting in the nucleus

Cell-Surface Receptors



Intracellular Receptors



- Lipid-soluble ligands: e.g., hormones, thyroxine, retinoids
- Ligands are unknown for some receptors.
- Need a "Carrier protein" to bring the hydrophobic ligand to the target cell.
- Genomic action: Act as a transcription factor in the nucleus.
- Regulate the transcription of target genes.

Seven families of nuclear receptors

Endocrine or paracrine receptors	Nutritional sensors	Receptor w/ structural ligand	Orphan receptors	Endogenous ligand Not identified
Steroid-hormone receptors ER α, β PR AR GR MR	PPAR α, β, γ LXR α, β FXR PXR RXR α, β, γ? CAR	HNF4 α, γ ROR α	Empty LBP LRH-1 ERR α, β, γ ROR β, γ	Ligand Binding Pocket
Non-steroid-hormone receptors RAR α, β, γ TR α, β VDR RXR α, β, γ?	Peroxisome proliferator-activated receptors (Prostaglandins) Liver X receptor (cholesterol deriv.) Farnesoid X receptor (bile acid) Pregnan X receptor (hormones, bile acid) Constitutive androstane receptor (androstane)	Hepatocyte Nuclear Factor 4 (linoleic acid) RAR-related orphan receptor (cholesterol deriv.)	No LBP NGFI-B Nurr1 Nor1 RevErb α, β?	
Retinoid X receptor (retinoic acid)			Unknown COUP-TF α, β, γ SF-1 GCNF DAX-1 SHP TLX PNR TR2 TR4	

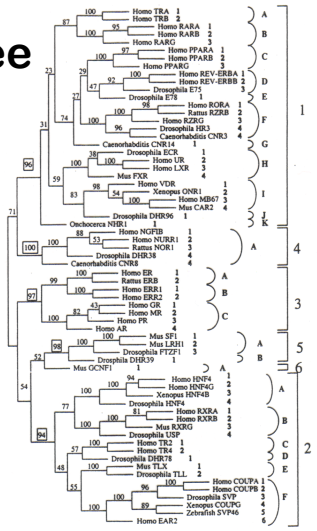
Full list can be found:
http://www.guidetopharmacology.org/GRAC/NHRListForward

Benoit *et al* 2004 Trends in Cell Biology 14:369

Phylogenetic Tree

Evolutionarily conserved - according to the DNA sequences

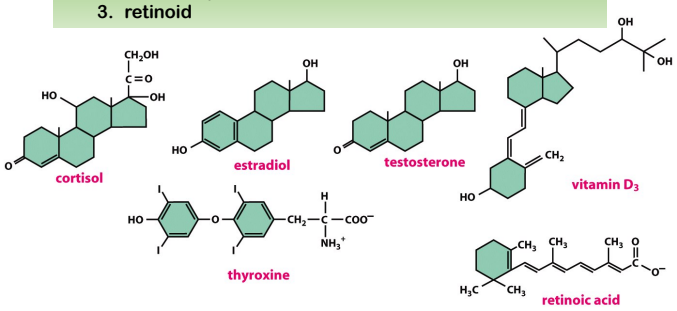
- Human
- Mouse
- Rat
- Drosophila
- C. elegans
- Xenopus
- Zebrafish



Small Lipophilic Ligands

Cholesterol-derived steroids:
1. sex steroids (estradiol, androgens, progestins)
2. adrenal steroid (glucocorticoids, mineralocorticoids)

Non-steroids:
1. thyroid hormone
2. vitamin D_3
3. retinoid



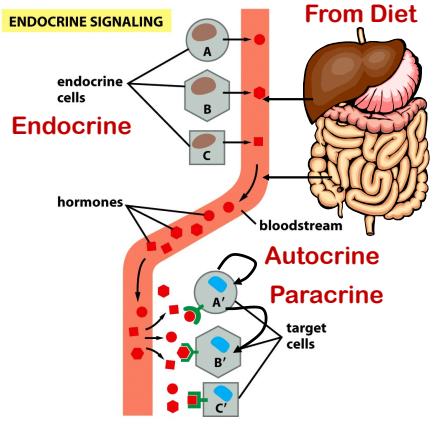
Other Lipid Ligands for NRs

Fatty Acids	PALMITIC ACID	CLOFIBRIC ACID	
Eicosanoids	OLEIC ACID	Wy 14, 643	
Prostagladins	ARACHIDONIC ACID	NAFENOPIN	
Fibrates	ETYA	MEHP	
Phthalate esters	PGJ_2		

Practical classification of NRs

	Endocrine receptors	Adopted Orphan Receptors	Orphan Receptors
LIGANDS:	High-affinity hormonal lipids	Low-affinity dietary lipids	Unknown
	ER α,β PR AR GR MR	RXR α,β,γ PPAR α,β,γ LXR α,β FXR PXR/SXR CAR	SF-1 LRH-1 DAX-1 SHP TLX PNR NGFI-B α,β,γ ROR α,β,γ ERR α,β,γ RVRα,β,γ GCNF TR 2,4 HNF-4 COUP-TFα,β,γ
	RAR α,β,γ TR α,β VDR EcR	NR1, NR2	
	NR3 - sex, adrenal NR1, NR2	$\sim10^{-5}$ -10^{-6} M dietary lipid sensors	
	$\sim10^{-8}$ -10^{11} M hormonal lipids Evidence of negative feedback		NR0, NR4, NR5, NR6
			No ligands

Where are the ligands come from?

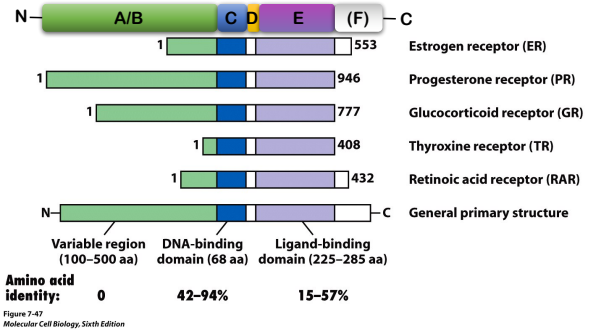


Transport through bloodstream

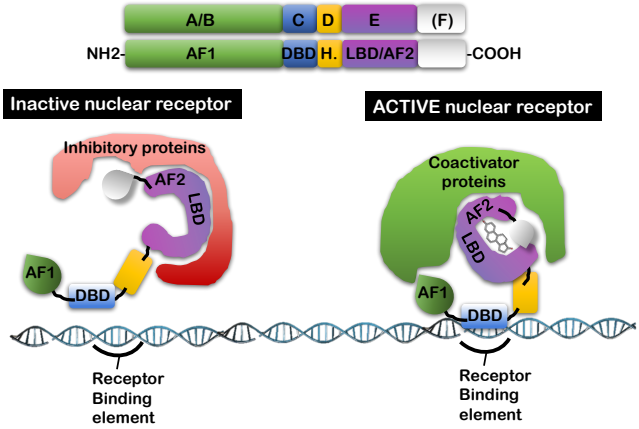
Requires some serum binding proteins
- Pre-albumin
- Albumin
- Transcortin (for steroids)
- Retinol binding protein
- Transthyretin
- Thyroxine-binding globulin

NRs: Common structural & functional organization: A-F domains

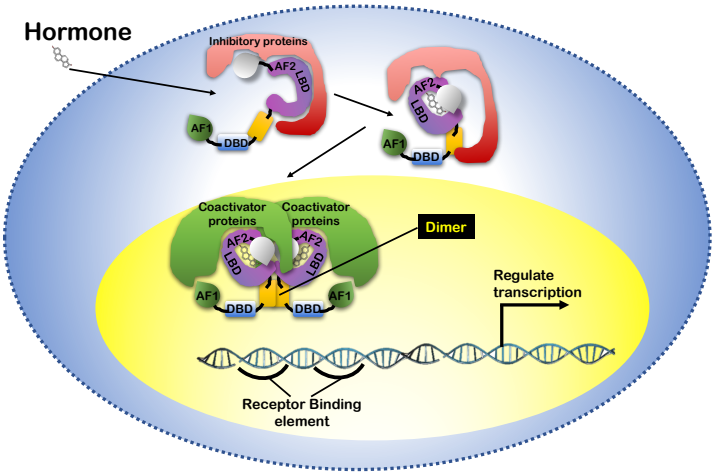
- The length of A/B domain varies. A/B domain is hypervariable.
- Even receptor isoforms have different sequences in A/B domain.
- DNA binding domain (DBD) is most conserved; length is conserved.
- Ligand binding domain (LBD) is conserved among isoforms; length is conserved
- Some receptors lack F domain.



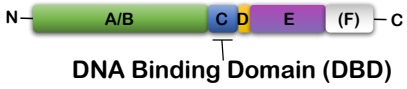
Binding of ligand causes dissociation of inhibitory proteins & binding of coactivators



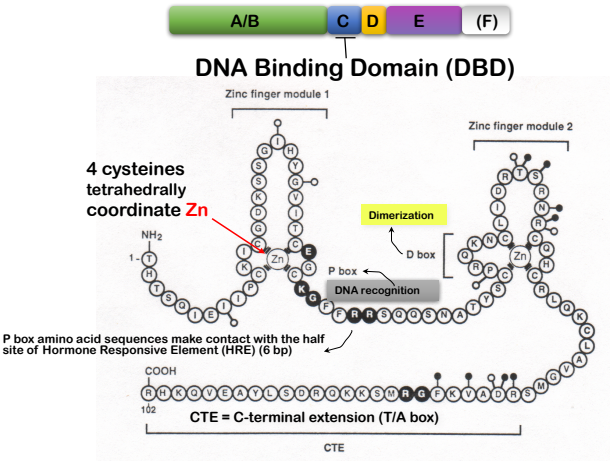
Hormone Action through Nuclear Receptor



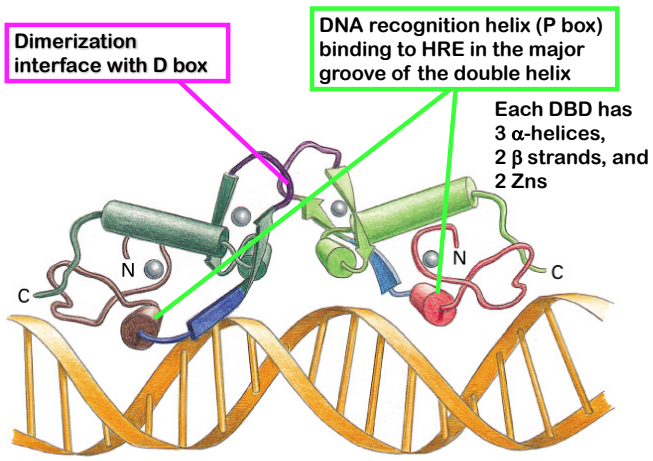
NRs: Common structural & functional organization: A-F domains



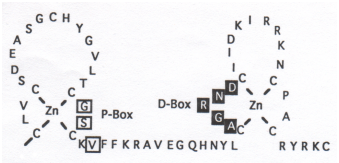
DNA Binding Domain of NRs



Dimerization is stabilized by the P box binding to HRE



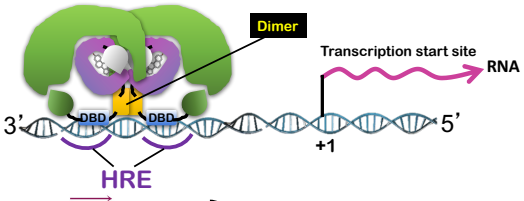
HRE and the P-box amino acid sequences



Receptors	P-Box amino acids	DNA sequence on HRE (half site)
ER	CEGCKA	AGGTCA
GR,MR,PR,AR	CGSCKV	TGTTCT
TR, RAR, VDR, RXR, PPAR, NGF1-B, NURR-1, TR2, TR2R1, EAR1, REVERBa	CEGCKG	AGGTCA
HNF3	CDGCKG	AGGTCA
EAR2	CEGCKS	AGGTCA
SF1	CESCKG	AGGTCA
ERR1	CEACKA	AGGTCA

DNA half site sequences are very similar: Where is the diversity coming from?

Strong HRE usually within -1 kb upstream (3') of the transcriptional start site



(a) GRE 5' AGAACA(N)3 TGTTCT 3' / 3' TCTTGT(N)3 ACAAGA 5'

(b) ERE 5' AGGTCA(N)3 TGACCT 3' / 3' TCCAGT(N)3 ACTGGA 5'

Indirect repeat (IR)-3 steroid hormone receptors

(c) VDRE 5' AGGTCA(N)3 AGGTCA 3' / 3' TCCAGT(N)3 TCCAGT 5'

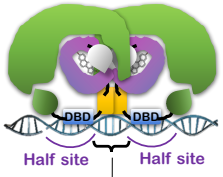
(d) TRE 5' AGGTCA(N)4 AGGTCA 3' / 3' TCCAGT(N)4 TCCAGT 5'

(e) RARE 5' AGGTCA(N)5 AGGTCA 3' / 3' TCCAGT(N)5 TCCAGT 5'

Direct repeats (DR)-1 to DR5

Half sites spaced by 1 to 5 nucleotides

"1-to-5 Rule"



spacer

n = any nucleotide

Table 2. DNA sites for nuclear receptors.

DNA-binding sites	Receptors
Steroid response elements:	
AGAACAnnnTGTTCT GRE	AR, GR, MR, PR
AGGTCAnnnTGACCT ERE	ER
Direct repeats:	
AGGTCAnAGGTCA DR-1	RXR/RXR, PPAR/RXR, RAR/RXR, COUP/RXR
AGGTCAnnAGGTCA DR-2	RAR/RXR
AGGTCAnnnAGGTCA DR-3	VDR/RXR
AGGTCAnnnnAGGTCA DR-4	TR/RXR
AGGTCAnnnnnAGGTCA DR-5	RAR/RXR

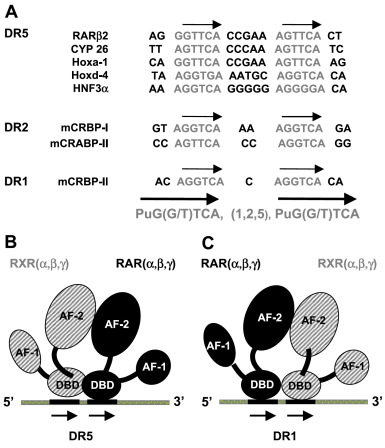
IR-3

n = 3 (IR-3)
3 nucleotides spacer

DR1-5

GRE, glucocorticoid response element; ERE, oestrogen response element; n, any nucleotide; palindromic sequence; direct repeat.

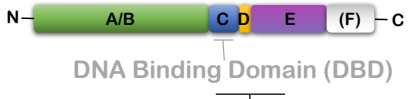
Position of the left and right receptors



Changes by the number of the nucleotides in between the half sites

Bastien, Rochette-Egly, Gene 328, 1-15 (2004) Review

NRs: Common structural & functional organization: A-F domains



DNA Binding Domain (DBD)

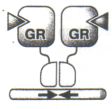
Dimerization region (Hinge-region)

Dimerization Region of NRs

Dimerization region (Hinge-region)

Steroids, IR, Homodimer

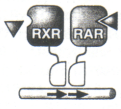
Steroid Receptors



GR *glucocorticoid*
MR *mineralcorticoid*
PR *progesterone*
AR *androgen*
ER *estrogen*

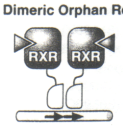
Non-steroids, DR, RXR Heterodimer

RXR Heterodimers



TRα,β *thyroid hormone*
RARα,β,γ *all-trans RA*
VDR *1,25-(OH)2-VD3*
PPARα,β,γ *eicosinoids*
EcR *ecdysone*

Dimeric Orphan Receptors



RXR (usp) *9-cis RA*
COUP/ARP ?
HNF-4 ?

Non-steroids, DR, RXR homodimer

Monomeric Orphan Receptors

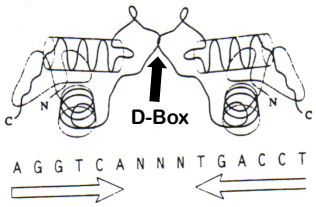
NGFI-B (CEB-1') ?
ELP / SF-1 (Ftz-F1) ?

No ligand known, NR - Half site & the adjoining 5' sequence, Monomer

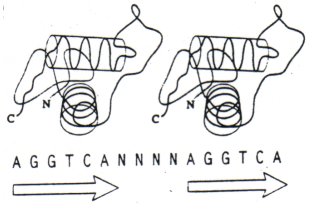
Dimerization depends on:

1. IR or DR
2. head-to-head or head-to-tail arrangement

Indirect Repeat
- Head to Head arrangement (mirror images)



Direct Repeat
- Head to Tail arrangement



Potential for diverse biological functions!

Heterodimerization means a possibility of a diverse combination of receptors

Homodimers 5 dimers, if n = 5

1/1	2/2	3/3	4/4	5/5

Different way to think:
- Also, allows some to have a similar function.
- Safety net called "Functional redundancy"
- No phenotype with one gene knockout mice

Homo- and Heterodimers 15 dimers, if n=5

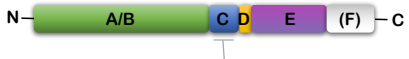
1/1	1/2	1/3	1/4	1/5
	2/2	2/3	2/4	2/5
		3/3	3/4	3/5
			4/4	4/5
				5/5

n = # of receptors

$\frac{n^2 + n}{2}$

If n = 5, (25 + 5)/2 = 15

NRs: Common structural & functional organization: A-F domains



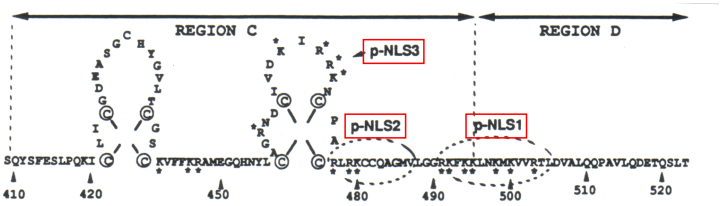
DNA Binding Domain (DBD)

Dimerization region (Hinge-region)

Nuclear localization signals (NLS)

Nuclear Localization Signals of NRs

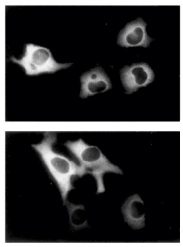
Nuclear localization signals (NLS)



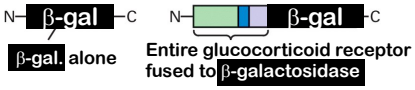
- NLS is a one or more clusters of positively charged amino acids (*K, *R).
- Three NLS in the C and D domains of estrogen receptor.

Induction of nuclear receptor localization in the presence of NLS-domain

Dexamethasone (Dex) = ligand for glucocorticoid receptor



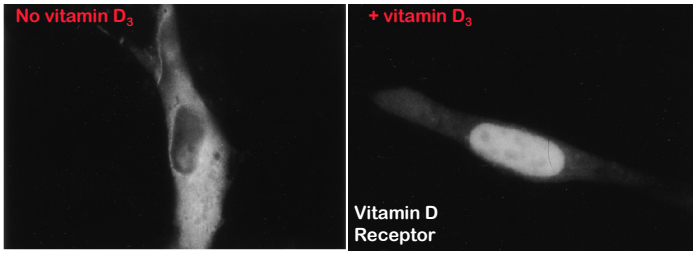
Protein expressed



Entire glucocorticoid receptor fused to β-galactosidase

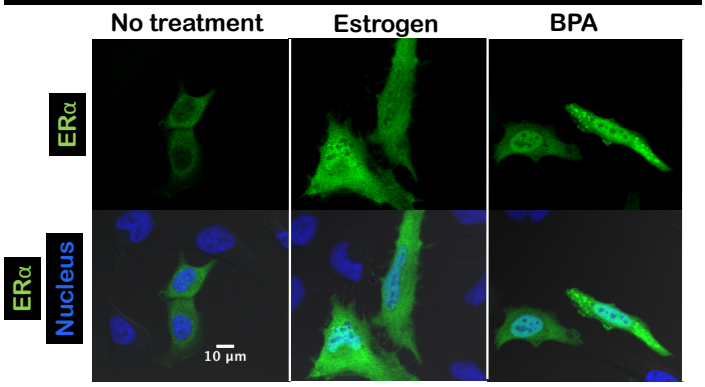
Nuclear receptors are shuttling in and out of nucleus in the absence of ligand

Nuclear retention is stabilized by ligand binding.



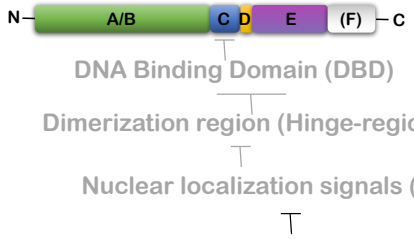
Tool to test estrogenicity of chemicals

Estrogen receptor (ERα) -tagged with green fluorescent protein



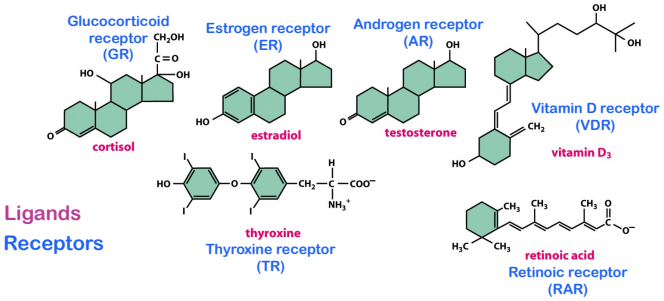
Estrogen & BPA induce ESR1 to localize in the nucleus.

NRs: Common structural & functional organization: A-F domains



Ligand Binding Domain of NRs

Ligand Binding Domain (LBD)



Ligand Binding Domain of NRs

LBD AF2 Transcriptional Activation Function 2

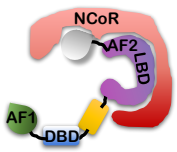
AF2

NCoA

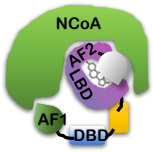
NCoR

NCoA and NCoR Binding sites overlapping

INACTIVE nuclear receptor

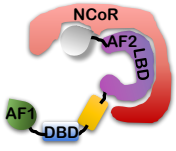


ACTIVE nuclear receptor

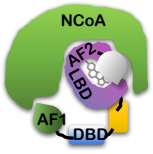


Nuclear Receptors: What are the implications?

INACTIVE nuclear receptor



ACTIVE nuclear receptor



Selective Estrogen Receptor Modulators

A ligand binds to estrogen receptor, behaves like estrogen agonist in certain tissues, but antagonist in other tissues.

Tamoxifen for breast cancer treatment therapy

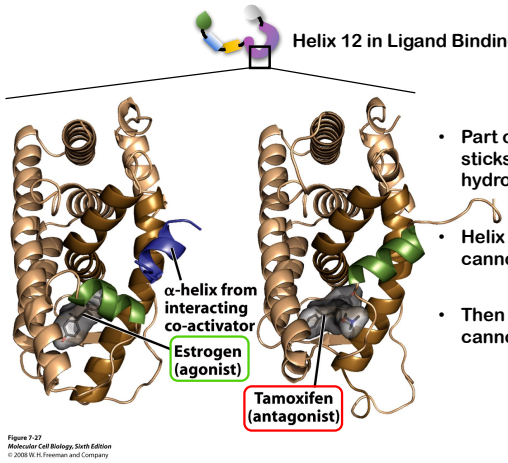
- Antagonist for breast tissues
- Agonist for uterine tissues

How Selective Estrogen Receptor Modulators work

1 Different ligands

	Agonists			Antagonists	
ER	Estradiol	PPT (ERα agonist)	DPN (ERβ agonist)	Tamoxifen	ICI 182780 Fulvestrant
GPER			G1 (GPER agonist)	G15 (GPER antagonist) (and enantiomer)	
PR	Progesterone	R5020 Promgesterone		RU486 Mifepristone	
AR	Testosterone	Dihydrotestosterone	R1881 Methyltrienolone	Hydroxyflutamide	Cyproterone acetate
GR	Hydrocortisone	Dexamethasone			

2 Different Estrogen Receptor Conformational changes



- Part of tamoxifen sticks out from the hydrophobic pocket.
- Helix 12 of LBD cannot shut properly.
- Then co-activators cannot bind helix 12.

Figure 7-27
Molecular Cell Biology, Sixth Edition
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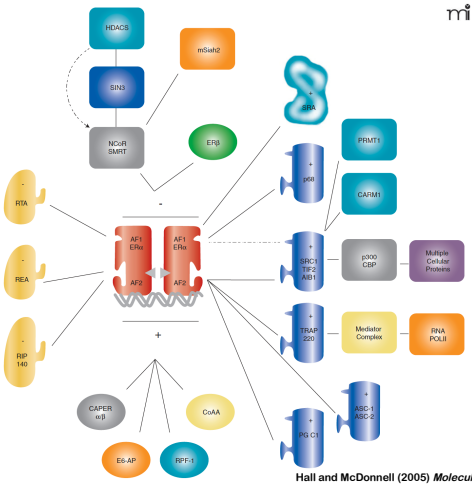
3 Different co-activators/co-repressors in different tissues



Hall and McDonnell (2005) *Molecular Interventions* 5:343.

Coactivators for Nuclear Receptors

- Discovered in early 1990's; now nearly 300 coregulators;
- Some are overexpressed in cancer cells. Thought to be responsible for tissue-specificity of NR.
- They are responsible for virtually "all" of the reactions needed for control of TF-dependent gene expression."

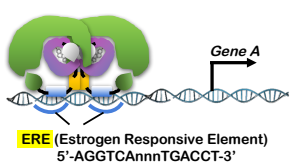
Coactivators for Nuclear receptors	Function
SRC/p160s; p300/CBP; P/CAF, pCIP	Acetyltransferase (HAT)
E6-AP	Ubiquitin ligases
BRG-1	Chromatin redmodeling ATPases
CARM-1; PRMT-1	Protein Methyltransferases
SRA	RNA transcripts
Cdc25B	Cell cycle regulators
p72	RNA helicases
TRAP/DRIP/Mediators	Proteins that make direct contact with basal transcription factors

(O'Malley and Kumar, Cancer Res, 69, 8217-8221)

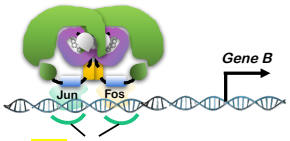
How Selective Estrogen Receptor Modulators work

1. Different ligands
2. Different Estrogen Receptor Conformational changes
3. Different co-activators/co-repressors in different tissues
4. Different responsive elements on DNA sequences

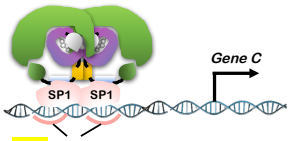
4 Different responsive elements on DNA sequences



ERE (Estrogen Responsive Element)
5'-AGGTCAnnnTGACCT-3'



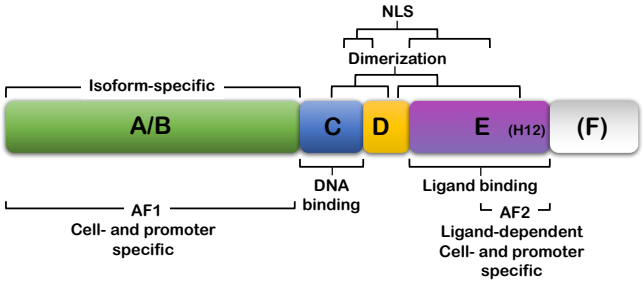
AP1 binding site 5'-TGACCA-3'



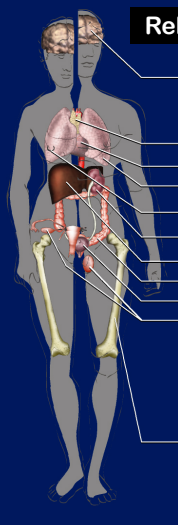
SP1 binding site 5'-CCGCCCnnnnCCGCCC-3'

Target gene promotor specificity

Different domains of nuclear receptors are crucial for cellular function



Relative tissue expression of ERα & ERβ



	ERα	ERβ
Brain		
Hypothalamus	ERα	ERβ
Pituitary	ERα	ERβ
Thymus	ERα	ERβ
Cardiovascular System	ERα	ERβ
Lung	ERα	ERβ
Mammary gland	ERα	ERβ
Kidney	ERα	ERβ
Liver	ERα	–
Gastrointestinal tract	ERα	ERβ
Bladder	ERα	ERβ
Reproductive tract		
Uterus	ERα	ERβ
Testes	ERα	ERβ
Ovary	ERα	ERβ
Prostate	ERα	ERβ
Bone	ERα	ERβ

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What'd happened when NR does not function?

Estrogen receptor mutations

18-year-old female patient

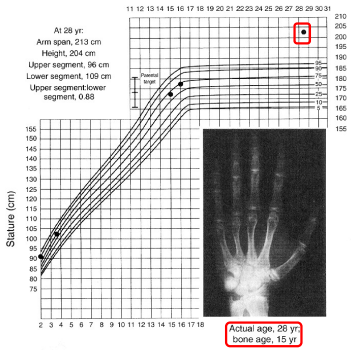
Homozygous mutation
Gln375His (LBD)

No breast development
No menstrual cycle

Quaynor SD, *et al* N Engl J Med 2013; 369:164-171

28-year-old male patient

T157C mutation → replacement of a CGA with a premature stop codon (TGA)
Lacking the DBD & LBD



Smith EP, *et al* N Engl J Med 1994; 331:1056-1061

Think about the implications

How do different ligands bind the same receptor but exhibit different type of physiological responses?

- Differential cell-type, tissue-specific, developmental-specific expression of NR.
- Nuclear localization of receptors
- Different HRE's: organized, but not much diversity
- Dimerization creates diversity: head-to-head or head-to-tail arrangement.
- Master regulator: crosstalk and competition; applies to RXR and some orphan receptors.
- Coregulators/Corepressors are tissue-specific; crosstalk and competition possible.
- Rational Drug design: Agonist vs. Antagonist

Questions?

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