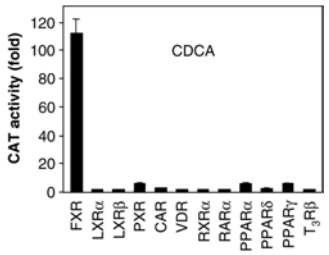
High cholesterol intake leads to reduced cholesterol synthesis and increased bile acid synthesis

Both of these changes represent changes in expression of rate limiting enzymes
- **eg. HMG CoA reductase (down)**
- **Cyp7a1 (up)**

In addition, bile acids also regulate gene expression, in particular of
- **Cyp7a1 (down) and the**
- **intestinal bile acid binding protein, I-BABP (up)**

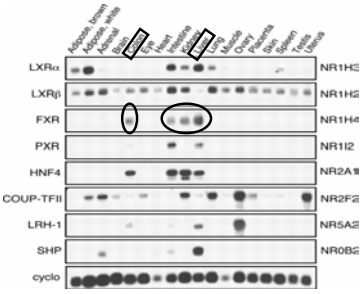
Do cholesterol and/or bile acids act as ligands for orphan receptors?

The orphan receptor, FXR is activated by bile acids



Ligand: chenodeoxycholic acid (CDCA, a primary bile acid)

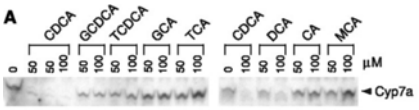
FXR expression pattern makes sense



Bile Acids regulate endogenous gene expression

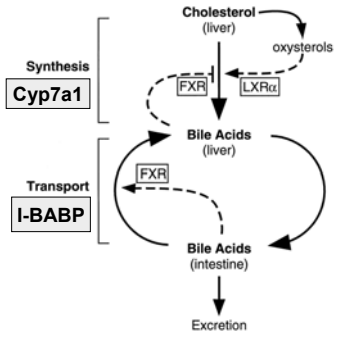
a) immunoblot of Cyp7a1

b) RT-PCR of cyp7a1 mRNA



B

Bile Acid	Control	CDCA	GCDCA	TCDCA	GCA	TCA	DCA	CA
Percent Expression	100	0.4	43	11	43	67	10	56



Small lipophilic molecule

↓

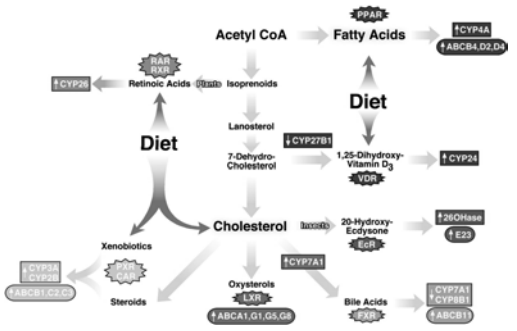
Nuclear Receptor

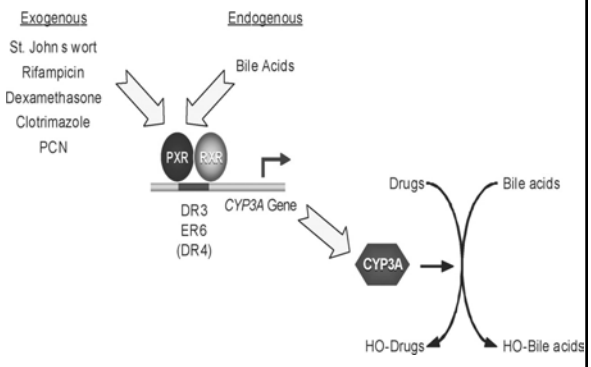
↓

Responsive genes:

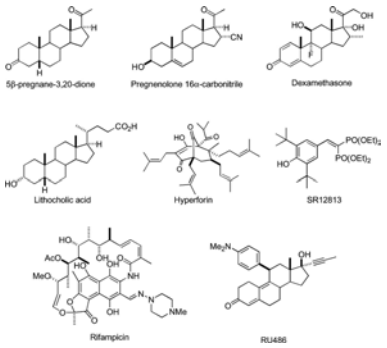
major phenotypic response	homeostatic response
-	cytochrome P450 enzymes (CYP x)
	Transporters (ABCs)
	Cellular binding proteins

Receptor	Ligand	CYP	ABC transporter
LXR	oxysterols	CYP7A1	ABCA1 etc.
FXR	bile acids	CYP7A1 ↓	ABCB11
PPAR	fibrates	CYP4A1	ABCB4
	fatty acids	CYP4A3	
RAR	retinoic acid	CYP26A1	?
SXR/PXR	steroids/ xenobiotics	CYP3A CYP2C	ABCB1, C2





A sampling of ligands for PXR:



Although SXR/PXR ligand specificity is loose (very), it is also species specific.

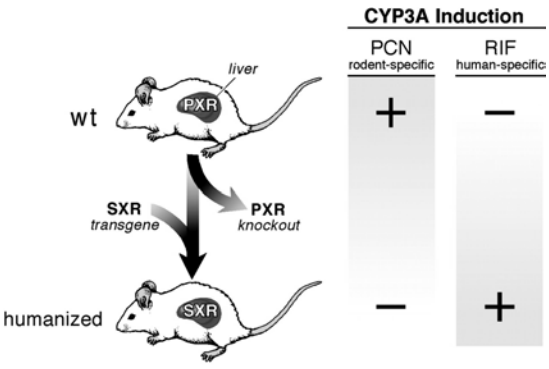
For example:

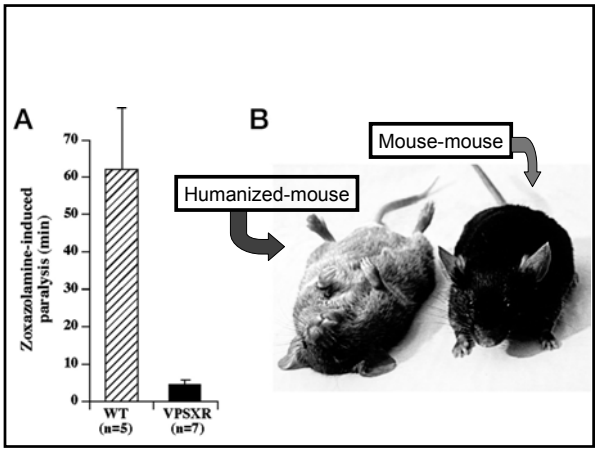
PCN activates mPXR not hPXR

Rifampcin activates hPXR(SXR) but not mPXR.

This becomes problematic when studying pharmokinetics and drug-drug interactions using rodent models

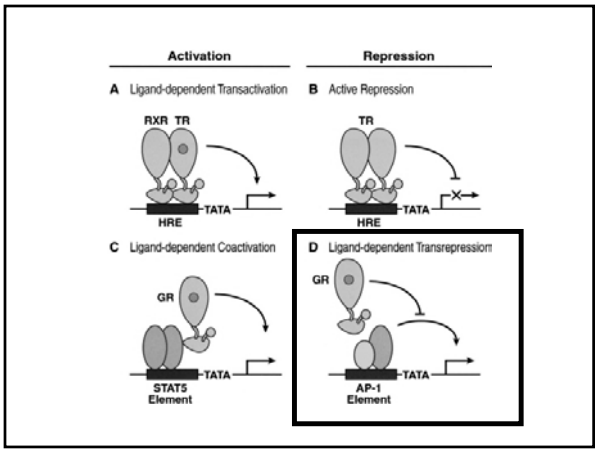
Possible solution: make a "humanized" mouse





Non-traditional functions of nuclear receptors:

- **Trans-repression**
- **Non-genomic actions**



TRANS-REPRESSION

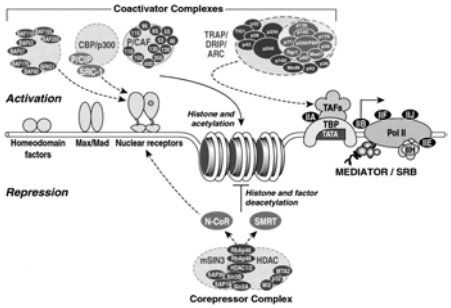
Signal X

Factor X
(eg. NFkB, AP-1)

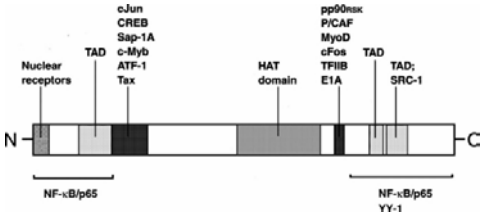
Target Gene

NHR + Ligand

Transcription Factors recruit large, multi-protein co-activator complexes to specific sites on chromatin

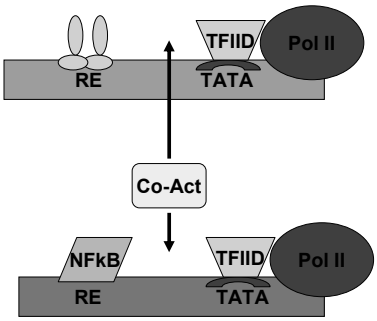


Co-activators are seemingly non-discriminatory

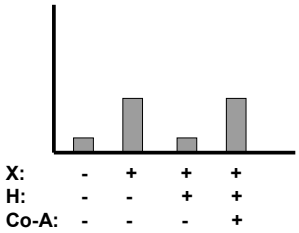


CBP/p300

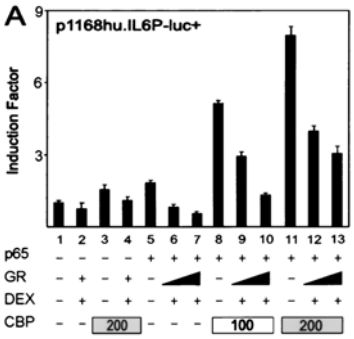
Trans-repression might result from competition for limiting amounts of co-activators:



If trans-repression results from competition for limiting co-activator, then adding more should relieve repression:

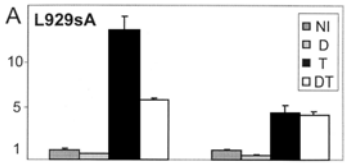


But GR trans-repression of IL6 promoter not reversed



De Bosscher et al PNAS 97:3919,2000

…and trans-repression is promoter specific



NI : no inducer
D = dexamethasone
T = TPA

IL6kB-luc **pNFkB-luc**

De Bosscher et al PNAS 97:3919,2000

Trans-repression---is it real?

Best evidence for:

GR^{Dim}/GR^{Dim} mice have point mutation preventing dimerization and DNA binding:

Mutant mice are viable (GR^{-}/GR^{-} mutants are not!)

No evidence of transactivation or of active repression (via nGRE in promoter of POMC or prolactin genes)

Non-genomic functions

Some ligands have actions that are not dependent on transcriptional regulation:

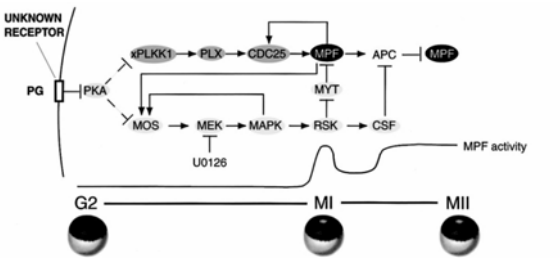
Examples:

•Progesterone induced maturation of oocytes
•Estrogen activation of:

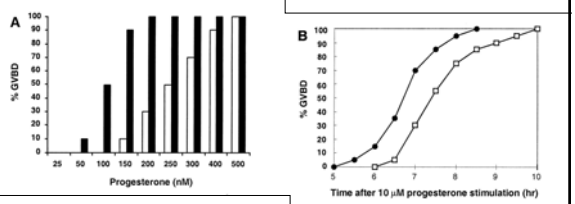
G-proteins/ Calcium channels / NO synthesis
cAMP and cGMP synthesis / K channels
PKC, PKA, PI3K/PKB, ERK, p38, IGF-1R…
and so on.

- Many of these events occur within seconds to minutes
- Many occur in the presence of actinomycin D and/or cycloheximide (i.e. in the absence of RNA or protein synthesis!)
- Many can be induced using BSA-E_2 conjugates (i.e. forms of E_2 that can not enter cells!!)
- Therefore, there has to be additional receptors, additional functions for the known receptors, or both

The Progesterone Receptor mediates hormone-induced oocyte maturation

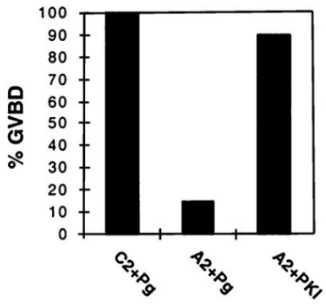


The Progesterone Receptor mediates hormone-induced oocyte maturation - I



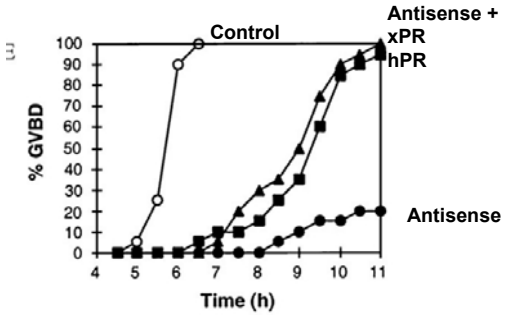
Open bars: H_2O
Filled bars: XPR-1 mRNA

The Progesterone Receptor mediates hormone-induced oocyte maturation - II

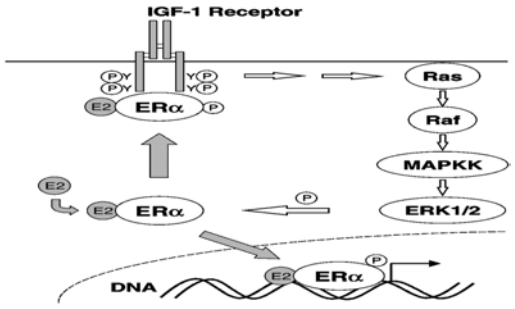


A2 = antisense oligos
PKI = inhibitor of PKA

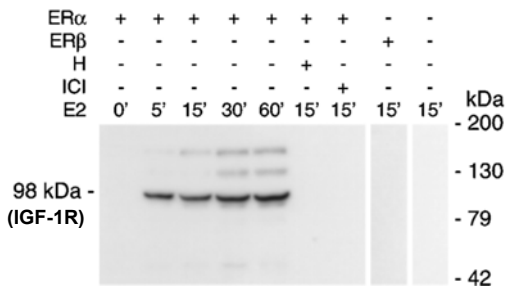
The Progesterone Receptor mediates hormone-induced oocyte maturation - III



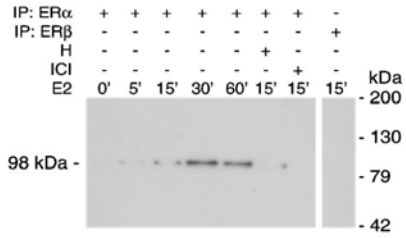
Cytoplasmic estrogen receptor-α, physically interacts with components of kinase signaling cascades



E2:ERα activates IGF-1R



E2 induces physical interactions between ERα and IGF-1R



Phosphorylation regulates receptor function

Relevant kinases include:
PKA, PKC, ERK, JNK

Phosphorylation directly:

Modulates ligand-dependent activation
(PKA and RARα in F9 cells, ERK and PPARγ in adipocytes)

Is required for function
(TFIIH and RARγ / RARα in F9 cells)

Activates receptors in the absence of ligand
(ERK and ER, PKA and PR)

Phosphorylation (continued)

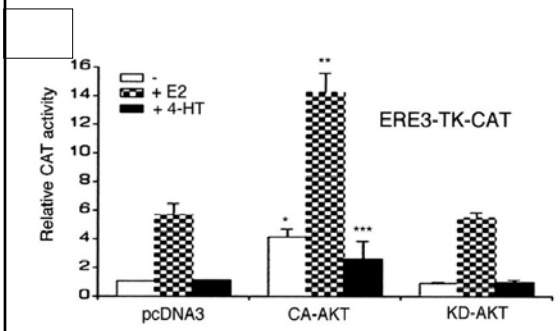
Limits duration of ligand activation
(TFIIH and RARγ and the proteosome, PKC and RARα and RXR dimerization)

Phosphorylation indirectly:

Alters subcellular localization
(PKA and RARα in Sertoli cells)

Inhibits receptor function
(RARα/γ and ERKs)

PKB/Akt Can Activate ERα



PKB/Akt Activates the AF1 Domain of ERα