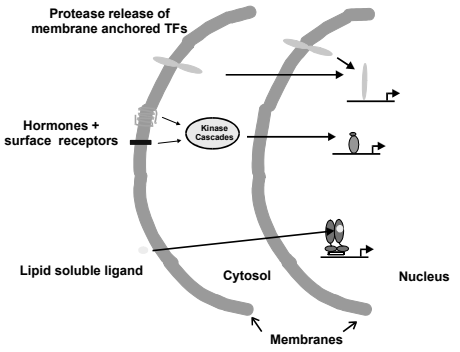
Different Strategies for Activating Transcription Factors



NUCLEAR RECEPTORS:

Hormone (+) receptors that bind ligand and act in the cell nucleus rather than at the cell surface

Classical examples are the steroid hormone receptors

Recent data demonstrates that these are the prototypes of a large family of receptors for small lipophilic signaling molecules including steroid hormone, fat soluble vitamins fatty acid metabolites and cholesterol metabolites

Nuclear Receptor Family is Large but not ubiquitous:

- **mammals have ~50-60 genes**
- **flies 21**
- **worms 270 (!!!)**
- **plants 0**
- **yeast 0**

Only a handful of physiological ligands have been identified, (despite many genes, worms lack any known lipid based endocrine system)

Modular structure provides means to identify novel ligands for orphan receptors (more on this later)

The Nuclear Receptor superfamily can be subdivided based on many different structural and functional criteria:

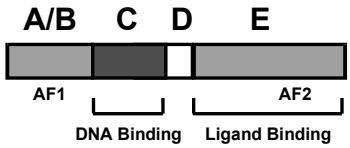
nuclear vs cytosolic localization in absence of ligands (RAR/VDR/PPAR etc vs GR/AR/PR/MR)

half site recognition (AGAACA vs RGGTCA)

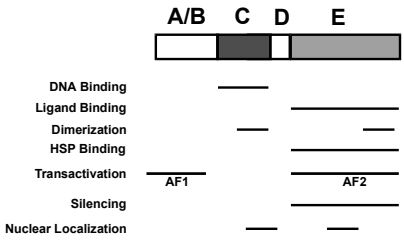
homodimers vs heterodimers (vs monomers)

sequence similarity in DBD
(basis of standardized nomenclature)

Modular Structure of Nuclear Receptors



Nuclear Receptor



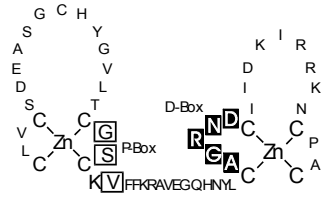
AF1 and AF2 are trans-activation functions; AF1 is ligand-independent and AF2 is ligand-dependent

The DNA binding domains of the NHR contain two Zinc fingers.

The first (more N-terminal) binds DNA

The second provides a dimerization interface (probably DNA dependent)

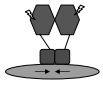
Small primary sequence determinants in the "P-Box" confer specificity of DNA binding



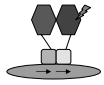
Receptor	P-Box	Half Site
GR/MR/PR/AR	CGSCKV	TGTTCT
ER	CEGCKA	GGTCA
TR/RAR/VDR/RXR	CEGCKG	GGTCA

NHRs differ in dimerization and DNA binding properties

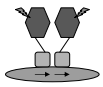
Steroid Receptors



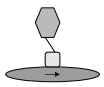
RXR Heterodimers



Dimeric Orphans



Monomeric Orphans



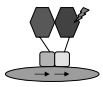
Steroid hormone receptors form homodimers and bind inverted repeats. In absence of ligand they are monomeric but complexed with a number of other proteins, notably HSP90. Ligand binding allows dissociation from this complex, exposure of NLS and dimerization.

All other NHR for which ligands have been identified form heterodimers with RXR and bind to direct repeats. They are present in the nucleus in the absence of ligand. The classic model has them forming dimers, binding to response elements and either being inactive or repressing transcription (but this is probably not correct). These include the RARs, the TRs, VDR, the PPARs, FXR the LXRs and the RXRs.

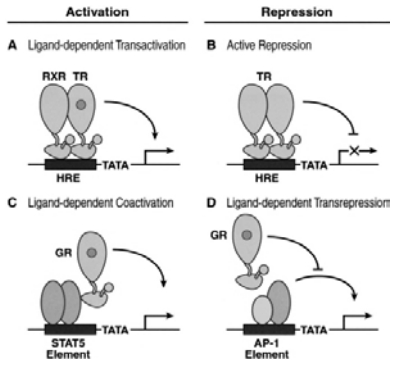
THE SPACING RULE

- **RXR heterodimers bind direct repeats of specific half sites.**
- **The direct repeats are separated by different numbers of nucleotides**
  - **n=1; DR-1**
  - **n=2; DR-2**
  - **etc.**
- **Different heterodimers bind to different HREs depending on the value of n**

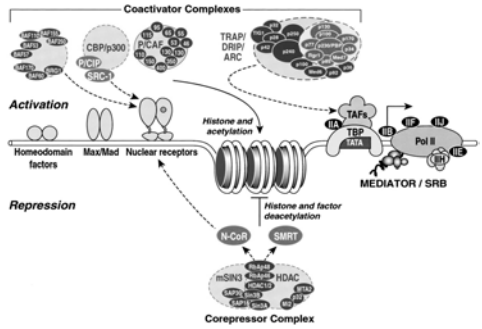
RXR Heterodimers



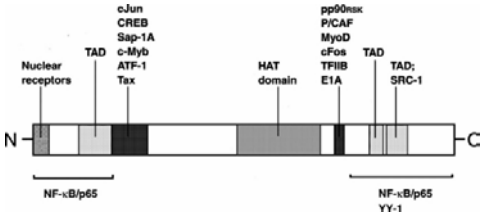
RXR Partner:	HRE Type
RXR	DR-1
PPAR	DR-1
RAR	DR-1*
	DR-2, DR-5
VDR	DR-3
TR	DR-4



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

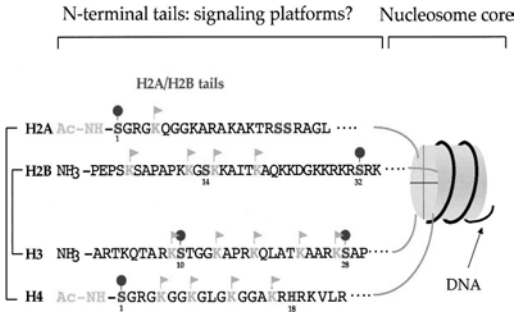


Co-activators are seemingly non-discriminatory



CBP/p300

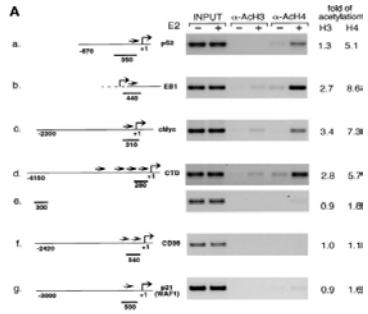
Histones are targets for co-activator modifications



CHIP Assay -- Chromatin Immunoprecipitation

- Cross-link protein and DNA with formaldehyde
- Shear DNA
- Using antibody against protein (or modification) of interest, immunoprecipitate protein-DNA complex
- Use heat to reverse cross-link
- Amplify specific DNA by PCR

Ligand bound ER recruits HATs to target promoters



Chen et al 1999 Cell 98:675

The above model assumes that nuclear hormone receptors are always present on DNA, presumably bound to HRE

However, at least 3 experiments contradict this model

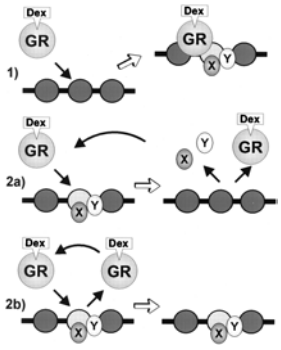
- **in vivo footprinting of the RARβ2 promoter +/-RA**
- **CHIP time course experiments on EREs**
- **photo-bleaching of live nuclei containing GFP-GRs**

Hormone binds receptor, then

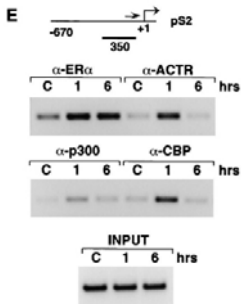
Model 1:
Ligand-bound receptor stably associates with HRE

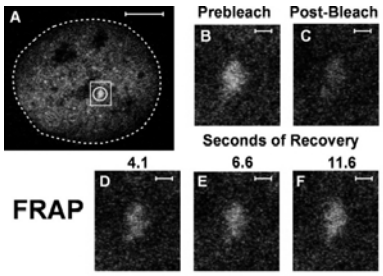
Model 2:
Ligand-bound receptor binds, recruits co-activators, remodeling complex and then is recycled (either alone(2b), or along with co-factors(2a)).

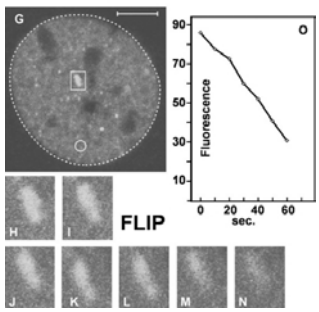
McNally et al. 2000 Science 287:1262



Ligand bound ER recruits HATs - II







How to find a ligand for an orphan receptor:

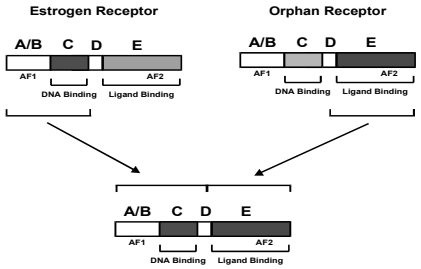
•Take advantage of modular structure to swap domains Test in transient transfections

•Demonstrate physical binding

•Demonstrate ligand and receptor present in same cell (at appropriate concentrations!!!)

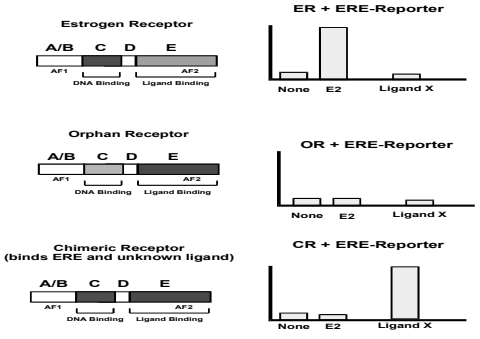
•Find target genes and show ligand and receptor dependent regulation in vivo

Domain swaps allow identification of new ligands

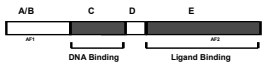


Chimeric Receptor (binds ERE and unknown ligand)

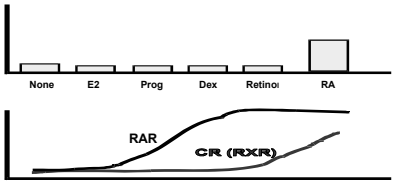
Domain swaps - II



Chimeric Receptor
(binds ERE and unknown ligand)



Transfect cells with CR expressing plasmid + ERE-Reporter plasmid,
treat with various test ligands,
and measure reporter gene expression



Difference in dose response curve similar to Retinol vs RA activating RAR

Is RA a precursor of RXR ligand?

Transfect cells with RXR expression plasmid,
Treat with 3H-RA
Isolate nuclei, purify RXR and identify what (if anything) is bound

All radioactivity is in form of 9-cisRA, not as all transRA