Biochemistry 673 Lecture 2

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Introduction to steroid hormone receptor (nuclear receptor) signalling

Resources:

Latchman

Lodish chapter 10, 20

Helmreich, chapter 11

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

stke.sciencemag.org, Estrogen receptor pathway

Mangelsdorf et al., 1995, Cell vol 83

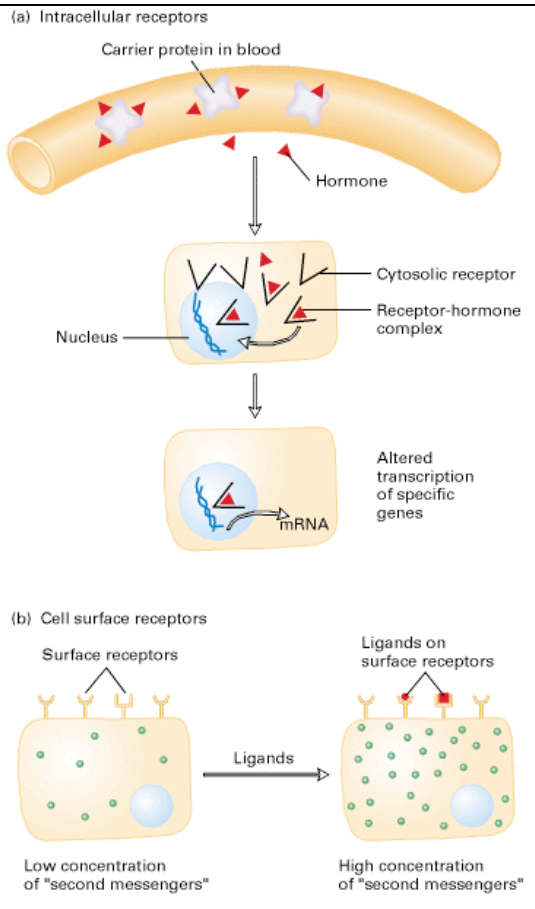
http://www.nuclear-receptor.com

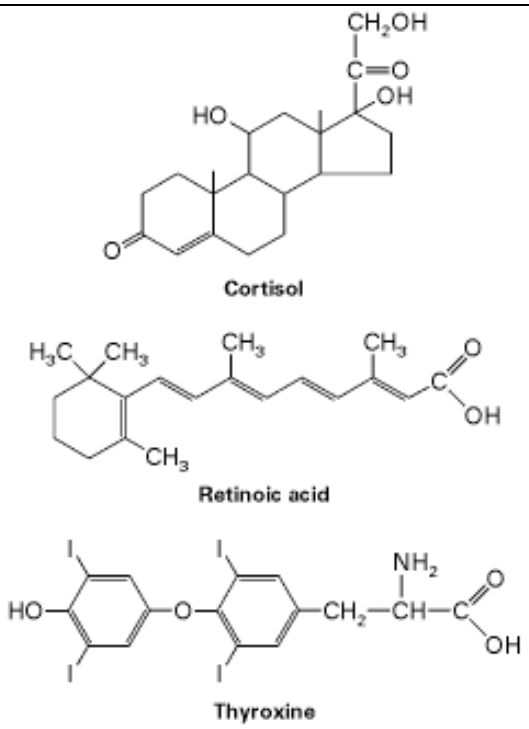
Hsp90 info DL'd from www.arches.uga.edu/~algraves/ bcmb8010/

This is an extremely active field, including professional web sites and journals.

The nuclear receptors are a large family of proteins that bind to lipophilic hormones, including steroid hormones, and activate or repress gene expression. They regulate development (ecdysones in insects), differentiation, and physiology. Being lipophilic, these hormones circulate in the blood bound to carrier proteins but then can go through the lipid bilayer (or be transported?). They bind to intracellular receptors, and conformational changes in the receptors lead to changes in gene expression.

Figure 10-63 of Lodish, showing comparison between SHR's and cell surface receptors.



MCB Figure 20-2. Some hormones bind to intracellular receptors; others, to cell-surface receptors. (a) Steroid hormones, thyroxine, and retinoids, being lipophilic, are transported by carrier proteins in the blood. After dissociation from these carriers, such hormones diffuse across the cell membrane and bind to specific receptors in the cytosol or nucleus. The receptor-hormone complex then acts on nuclear DNA to alter transcription of specific genes.	

Receptors were isolated by purifying proteins that bound to radioactive ligands and were cloned starting in the mid-80's.

There are now >150 members of the nuclear receptor superfamily in eukaryotes. The two main types are Type I, which are cytoplasmic until they bind ligand, at which point they exchange ligand for inihibitory Hsp90, are translocated to the nucleus, homodimerize and bind inverted repeat DNA recognition elements. The classic type I receptor is the glucorcorticoid receptor (GR), which responds to cortisone, an adrenal hormone that regulates inflammation. The Type II receptors are typically nuclear all the time, like the thyroid hormone receptor and retinoic acid receptor. They bind nonsteroidal hormones like retinoic acid (imp. in development), typically bind as heterodimers with RXR being one partner, and bind different direct repeat recognition elements; heterodimerization allows for more versatility in DNA sequence recognition. [RXR binds to 9-cis-retinoic acids whereas the RAR's bind both cis and trans isomers.] There are also a lot of orphan nuclear receptors, meaning that no ligand has been identified; some may not have ligands, may be regulated by post-transcriptional modifications.

Domain structure of steroid hormone receptors: (Figure from Mangelsdorf)

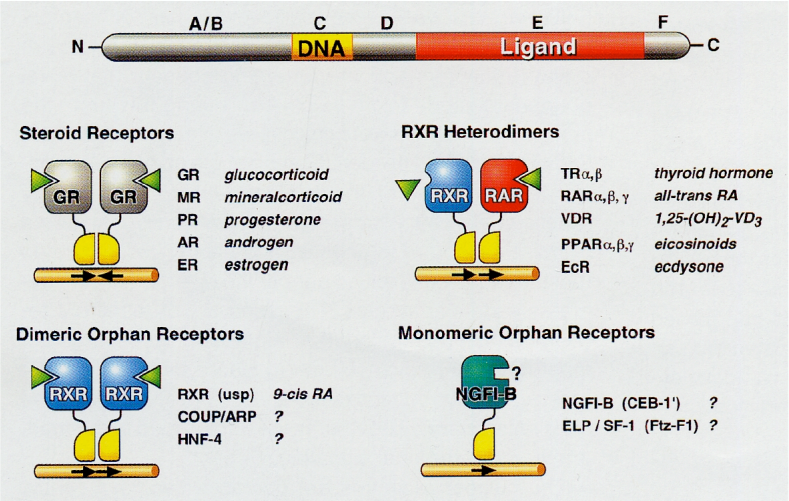
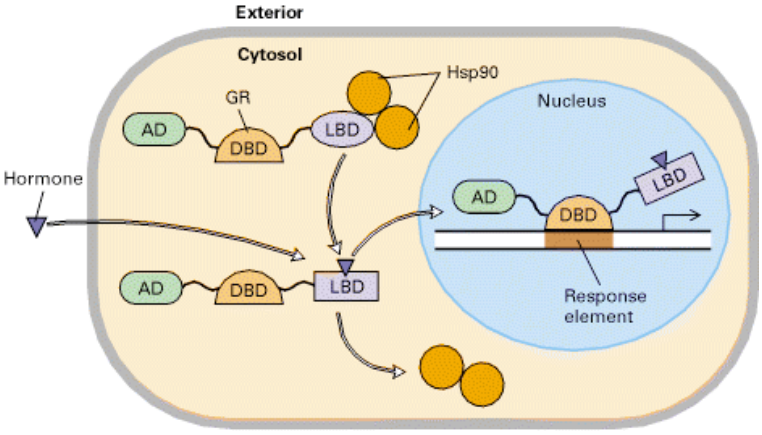


Figure 2. Nuclear Receptors Share Common Structure/Function Domains

A typical nuclear receptor contains a variable N-terminal region (A/B), a conserved DBD (C), a variable hinge region (D), a conserved LBD (E), and a variable C-terminal region (F). Nuclear receptors can be grouped into four classes according to their ligand binding, DNA binding, and dimerization properties: steroid receptors, RXR heterodimers, homodimeric orphan receptors, and monomeric orphan receptors. Shown are representative receptors for each group. A complete listing is given in Figure 3. Question marks refer to orphan receptors for which ligands are not known. See accompanying reviews for details.

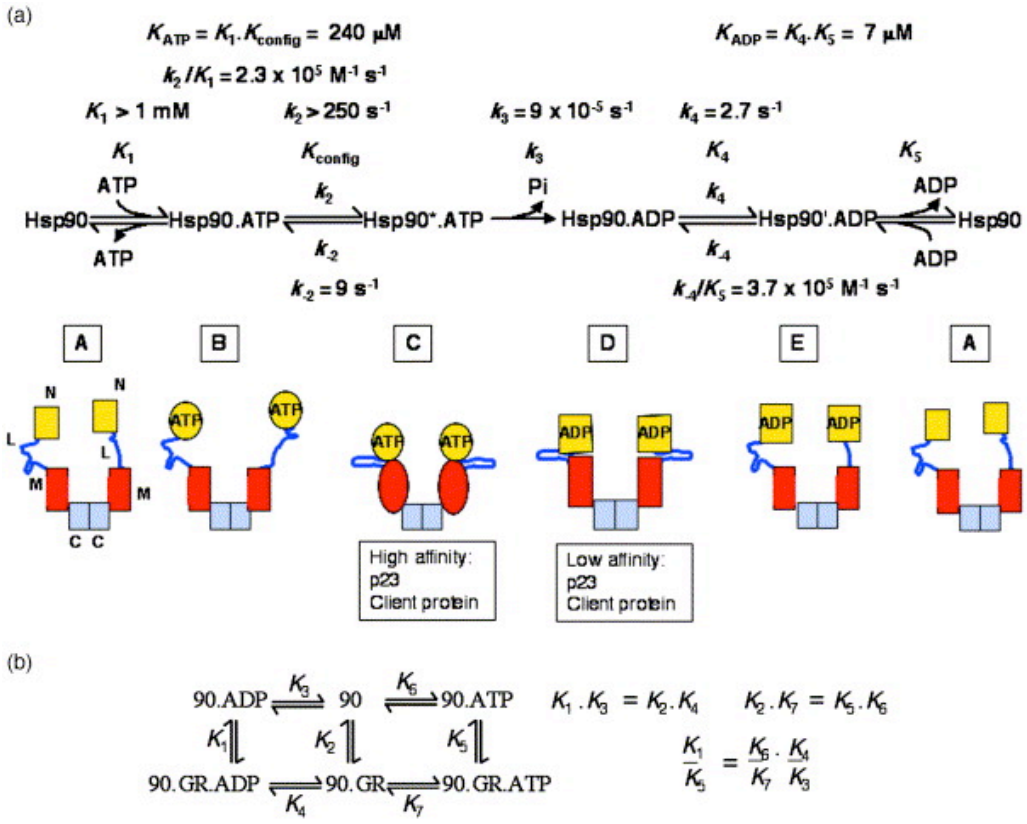
C-terminal activation domain of variable sequence. Conserved DNA binding domain (DBD), hinge, ligand binding domain that determines hormone specificity, and an N-terminal variable activation domain. DBD's are zinc-dependent but they are different from the classic zinc finger domain that we covered last semester. We will cover structural aspects in more detail later.

MCB Figure 10-67. Model of hormone-dependent gene activation by the glucocorticoid receptor (GR). In the absence of hormone, GR is bound in a complex with Hsp90 in the cytoplasm via its ligand-binding domain (light purple). When hormone is present, it diffuses through the plasma membrane and binds to the GR ligand-binding domain, causing a conformational change in the ligand-binding domain that releases the receptor from Hsp90. The receptor with bound ligand is then translocated into the nucleus where its DNA-binding domain (orange) binds to response elements, allowing the activation domain (green) to stimulate transcription of target genes.



Hsp90 is an essential heat shock protein. Present all the time but up-regulated by elevated temperature. It protects unfolded proteins from aggregation and degradation. Thus the naïve expectation would be that deletion of Hsp90 should lead to constitutive activity of the GR, but in fact decreased responsiveness is seen because the receptor is degraded. Hsp90 is the target of geldanamycin, which blocks its protein binding site and hence inactivates Hsp90 partners including nuclear receptors and kinases.

Hsp90 is an ATPase that apparently cycles so as to release and rebind substrate proteins. If the ligand binds the substrate, then it is no longer competent to bind Hsp90.



McLauglin et al. JMB 2004. Figure 8. Kinetic model of human Hsp90 ATPase cycle. (a) ATP binds rapidly to Hsp90 under diffusion control (A to B). The binding of ATP in state B is weak with an equilibrium constant in the millimolar range. ATP binding induces a conformational change, possibly involving the "ATP-lid" in the N-terminal domain and/or contact between the N and M domain (B to C). However, the precise conformational changes that occur are unknown at present. The ATP-bound

conformation of Hsp90 has a high affinity for client proteins and the co-chaperone p23. The rate-limiting step is the hydrolysis of ATP (C to D) possibly involving re-arrangement of a catalytic loop in the M domain. The ADP-bound state D has a lower affinity for both client and p23. Rapid dissociation of ADP allows recycling of Hsp90. (b) Thermodynamic linkage of client protein binding and nucleotide affinity allows the effect of nucleotide on the Hsp90–client protein affinity to be calculated.

Hsp90 is viewed as a "genetic capacitor" allowing variations to accumulate that don't have phenotypes except upon loss or strain on Hsp90.

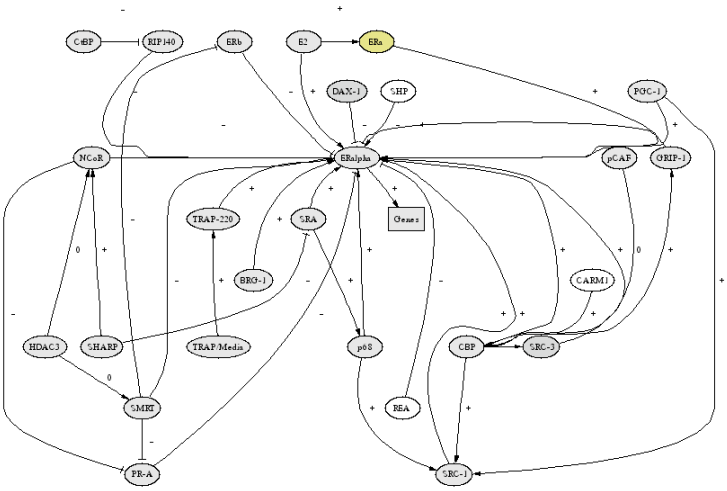
Ligand binding unmasks nuclear localization signals that allow the steroid hormone receptors (class I receptors) to be translocated to the nucleus. SHR is also phosphorylated on several serines. Hormone binding also potentiates DNA binding and/or interaction with coactivators: for example, tamoxifen is an estrogen antagonist that allows DNA binding but not transcription activation.
They act by binding coactivators such as CBP, SRC-1 that have histone acetyltransferase (HAT) activity, therefore opening up chromatin structure and allowing increased transcription. Allows access to the DNA by constitutive transcription factors such as NF1 that otherwise cannot access targets in chromatin. GR may also target SWI/SNF, ATPase motor complex that helps make nucleosomes more "fluid". Finally the SHR and/or associated coactivators may act to recruit the RNA pol II basal machinery. We will see more of this in the reading next week. Presumably when hormone diffuses away there is a machinery for recycling the receptor to the cytoplasm, or destroying it.

Class II receptors are typically nuclear all the time, and ligand binding converts them from repressors into activators. In the absence of ligand, they recruit nuclear corepressor (N-CoR) and SMRT (silencing mediator for RAR and THR), which have histone deacetylase activity (HDAC). V-erbA is a viral oncogene that encodes a dominant-negative thyroid hormone receptor that can repress but not activate transcription. Then upon ligand binding, the RAR/RXR receptors recruit HAT and other coactivators to activate transcription.

Steroid receptors are part of complex signalling networks:

From STKE of estrogen pathway:

ERalpha and ER beta independently interact with estrogen.



Finally, coactivator levels can be regulated and different complexes may compete with each other, providing an additional level of regulation/tissue specificity.

(from O'Malley review at NURSA)