

## UNIT VI: Nuclear Receptors: Zn Finger proteins and their roles in Promotion and Induction

### Assigned Reading:

Mangelsdorf, et al. (1995). The Nuclear receptor Superfamily: The Second decade. *Cell* 83:835.

Bojes et al. (1997) Antibodies to TNF $\alpha$  prevent increases on cell replication in liver due to the potent peroxisome proliferator, WY-1463. *Carcinogenesis*, 18:669-674.

### General comments about nuclear receptors

Many tumor promoters can be described in the following way. First, they often have mitogenic activity in normal tissue. Second, many have been shown to induce proliferative responses in altered hepatic foci (AHF) in preference to surrounding tissue. Third, they are often ligands for receptors (e.g., dioxins, peroxisome proliferators, sex steroids, Phenobarbital). Fourth they are often inducers of cytochrome P450 gene batteries. In an effort to make a mechanistic connection between receptor binding, induction and tumor promotion in liver, we will discuss prototype signal transduction pathways that are suspected to mediate the activity of important classes of tumor promoters in the liver. Be forewarned that the connection will not be as strong as it is for genotoxic carcinogens.

### Useful Definitions:

1. Superfamily: a set of gene families that all descended from a distant (common) ancestor. Members of gene families are most often identified by sequence similarity.
2. Receptor: stereospecific binding sites for a molecule (e.g., promoter, ligand, hormone, drug) that mediates the biological effects of that compound. Contrast this with binding proteins that do not mediate the signal but may still be important biologically (e.g. albumin).
3. Nuclear receptor: A signal transduction molecule that binds a ligand and acts in the nucleus through interactions with genomic response elements. Nuclear receptors are not always found in the nucleus. They may be cytosolic during some signaling steps.
4. Orphan receptors: Common usage of the term "orphan receptor" refers to any poorly characterized receptor that has been cloned usually by sequence homology. Many of these orphans probably not receptors at all, many appear to be constitutively active.

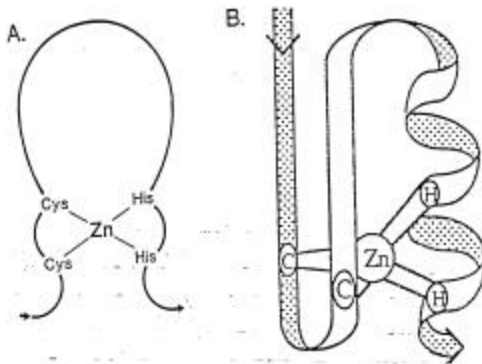
The term "nuclear receptor" is commonly used to denote signal transduction molecules that have the following characteristics; 1) Ligand activated transcription factors, 2) Bind small lipophilic ligands, 3) Members of "superfamilies," 4) Modular domain structure, 5) Bind to enhancers upstream of target genes, 6) Active gene expression through homo- and heterodimers, 7) Partners bind to characteristic DNA half-sites, 8) DNA sequence specificity through alpha helices in major groove, 9) Often associated with heat shock proteins.

### Zn Finger receptor superfamily

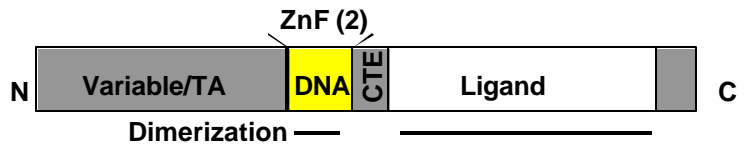
In most literature, the term "nuclear receptor superfamily" is referring to those receptors that have Zn Finger DNA binding motifs. We will take a broader view and use the term nuclear receptors to include both Zn Finger receptor and PAS receptors (to be discussed later). The Zn Finger receptor superfamily has also been referred to as the "steroid/thyroxin receptor superfamily." To avoid confusion, it is probably best to think of them as Zn Finger Receptors. There are currently more than 150 Zn finger proteins of this superfamily known to exist. This superfamily is characterized by a particular amino acid sequence within the Zn Finger region. Also this superfamily always has two adjacent Zn Fingers. Not all Zn finger proteins are receptors. It has been estimated that as much as 5% of the genome is dedicated to encoding Zn Finger proteins of one type or another. Only a small fraction are receptors of this superfamily.

### What is a Zn Finger?

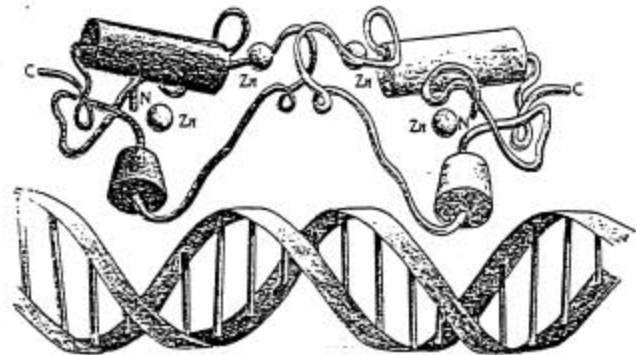
A Zn finger is a common DNA binding motif found in all members of this family of proteins. This domain coordinates a Zinc through cysteines and histidines. Zinc finger receptors have two "fingers". The helices of the first zinc finger (C terminal) lie in the major groove of DNA and provide sequence binding specificity. The second finger can act as a dimerization surface to stabilize certain homodimers (Steroid receptors only?).



### Domain Structure of Zn Finger Receptor



Typical Zn finger receptors contain a variable N-termini involved in transactivation (TA), "Zinc fingers (ZnF)" DNA binding domain and C-terminal ligand binding domain. A C-terminal extension (CTE) can provide flexibility between the Zn finger and ligand binding domains. Two dimerization domains can exist but both are not always in use. Proteins range from 60-100 kDa.



Two subclasses of this receptor superfamily are particularly relevant to our recent discussions.

- ?? Homodimeric steroid receptors (glucocorticoid, progesterone, mineralocorticoid, estrogen, androgen)
- ?? Heterodimeric receptors with RXR (peroxisome proliferators, thyroxine, retinoic acid)

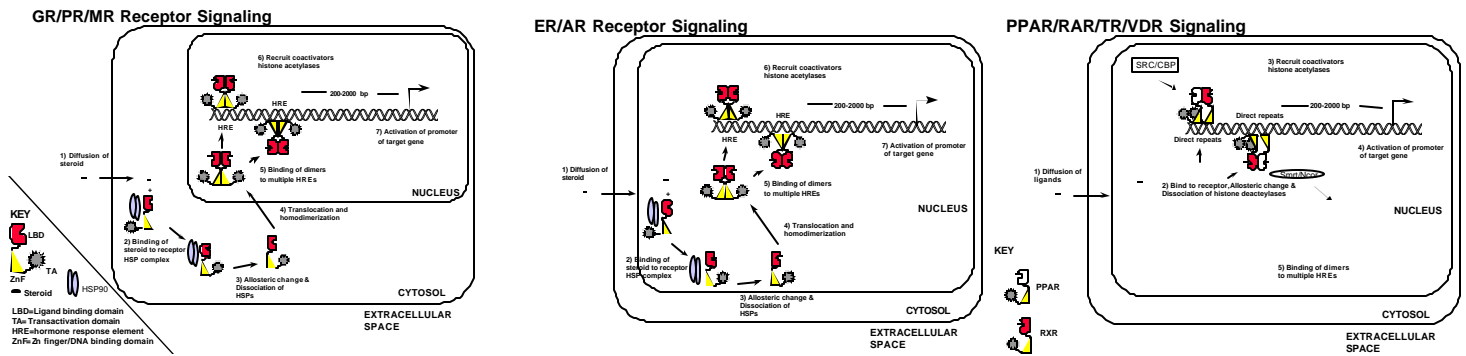
### What distinguishes steroid receptors from other Zn finger proteins?

- ?? Steroid receptors bind as symmetrical homodimers. Each partner recognizes a hexad-half-site sequence (A/GGA/GA/TCA) Thus, their hormone response elements (HREs) are often degenerate inverted repeats with a spacer in between (palindromes).
- ?? Steroid receptors interact with the 90 kDa heat shock protein (HSP90). This protein keeps the receptors in a form that binds ligand and that cannot bind DNA. Ligand binding leads to HSP90 dissociation
- ?? Steroid receptors use dimerization surfaces in their ligand binding domains and in one Zn finger.
- ?? Most, if not all, steroid receptors have been cloned.

### The PPAR is a member of a class of Zn finger receptors that form heterodimers with RXR

- ?? RXR ("retinoid X-receptor") is a Zn finger receptor that binds 9-cis-retinoic acid.
- ?? RXR also forms heterodimers with a number of Zn finger receptors that don't bind steroids. For example, the receptors for thyroxine, retinoic acid (vitamin A), vitamin D and peroxisome proliferators.

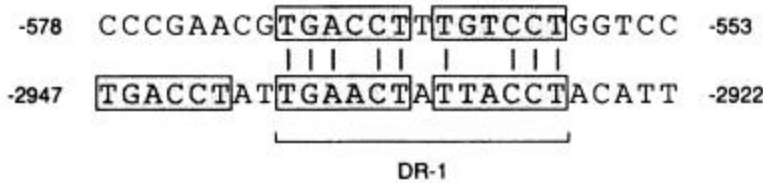
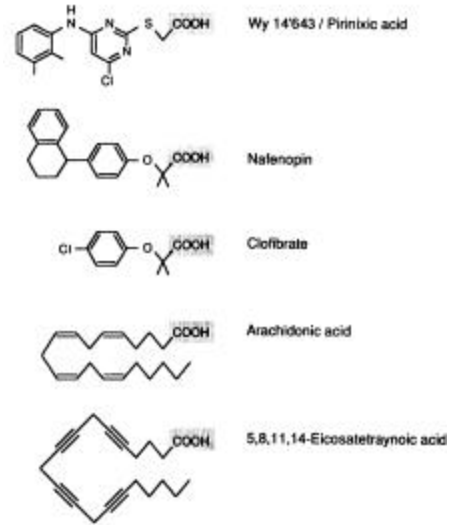
The Zn Finger receptors can signal in a few different ways. GR, PR and MR appear to be cytosolic in the absence of ligand bound to Hsp90. Upon binding, they dissociate a repressor protein (Hsp90) and translocate to the nucleus where the heterodimerize and bind to palindromic sequences in DNA. Estrogen receptor (ER) and Androgen receptor (AR) appear to reside in the nucleus associated with Hsp90. In response to ligand, the proteins form heterodimers that bind to palindromic sequences in DNA. Evidence from the thyroxine receptor system (TR) suggests that TR, peroxisome proliferator activated receptor (PPAR) and vitamin D receptor (VDR) are all bound to chromatin as a heterodimer with retinoid X receptor (RXR). When ligand binds, the complexes release histone acetylases (that are locally repressing the region) and recruit histone deacetylases.



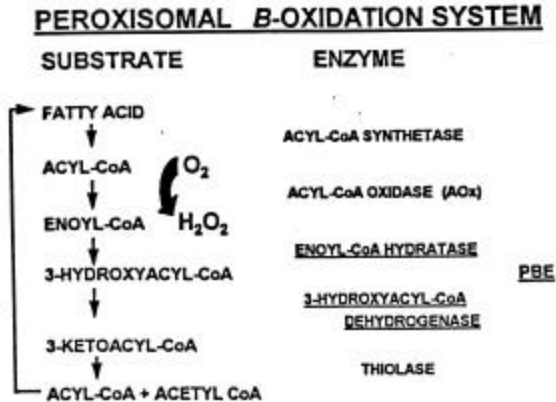
**Tumor promotion by peroxisome proliferators:**

Peroxisome proliferators include a large group of structurally diverse drugs (Clofibrate, Nafenopin) and a wide variety of environmental contaminants (phthalate esters that are often used as plasticizers). These compounds induce high yields of HCC in rodents that are exposed to chronic dosing. These compounds are also negative for mutagenicity in the Ames Assay and do not covalently interact with DNA.

These compounds activate the PPAR, which heterodimerizes with RXR and binds DR-1 sequences (direct repeats with a 1 nucleotide spacer).



The activated complex upregulates a number of genes including a battery of P450s (e.g., CYP4A1). Its endogenous biological function is believed to be as a regulator of beta oxidation and adipocyte differentiation. A considerable amount is known about regulation of beta oxidation. Many of the PPAR regulated genes have been cloned, they are upregulated by PP by as much as 40-fold. **Is there anything about upregulation of this pathway that you might suspect has an impact on tumor yield, initiation, promotion? What is your interpretation of the mechanism proposed in the assigned reading from "Carcinogenesis". If you hadn't read that paper, what other hypothetical model might you put forth?**



**Additional Reading:**

Peroxisome Proliferator-Activated Receptors and Lipid Metabolism (1996) 157-173. Ann. NY Acad. Sci.