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Steroid Hormone Receptors: Evolution, Ligands, and Molecular Basis of Biologic Function

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Abstract The characterization of the superfamily of nuclear receptors, in particular the steroid/retinoid/thyroid hormone receptors, has resulted in a more complete understanding of how a repertoire of hormonally and nutritionally derived lipophilic ligands controls cell functions to effect development and homeostasis. As transducers of hormonal signaling in the nucleus, this superfamily of DNA-binding proteins appears to represent a crucial link in the emergence of multicellular organisms. Because nuclear receptors bind and are conformationally activated by a chemically diverse array of ligands, yet are closely related in general structure, they present an intriguing example of paralogous evolution. It is hypothesized that an ancient prototype receptor evolved into an intricate set of dimerizing isoforms, capable of recognizing an ensemble of hormone-responsive element motifs in DNA, and exerting ligand-directed combinatorial control of gene expression. The effector domains of nuclear receptors mediate transcriptional activation by recruiting coregulatory multisubunit complexes that remodel chromatin, target the initiation site, and stabilize the RNA polymerase II machinery for repeated rounds of transcription of the regulated gene. Because some nuclear receptors also function in gene repression, while others are constitutive activators, this superfamily of proteins provides a number of avenues for investigating hormonal regulation of gene expression. This review surveys briefly the latest findings in the nuclear receptor field and identifies particular areas where future studies should be fruitful. J. Cell. Biochem. Suppls. 32/33:110–122, 1999.

Key words: nuclear receptor phylogeny; steroid biosynthesis; hormonal control of gene transcription; zinc-finger proteins; hormone-responsive elements

Nuclear receptors comprise a superfamily of gene regulatory, DNA binding proteins that numbers over 300 recognized members [Laudet, 1997; Sluder et al., 1999]. Receptors in this superfamily are known to play crucial roles in development, homeostasis, and other biological processes in metazoic animals. A subset of these receptors binds specific steroids and related compounds to mediate the effects of these hormonal ligands by influencing transcription of

Abbreviations used: $1,25(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; ACTH, adrenocorticotropic hormone; ACTR, activator of thyroid and retinoid receptors; AF1, activation function-1; AF2, activation function-2; AR, androgen receptor; BRG-1, *brahma*-related gene-1; CARs, constitutive androstane receptors α and β; CBP/p300, CREB binding protein; CeNHR, *Caenorhabditis elegans* nuclear hormone receptor; COUP-TF, chicken ovalbumin upstream promoter-transcription factor; CTE, C-terminal extension; DHR, *Drosophila* hormone receptor; DRIPs, vitamin D-receptor interacting proteins; EcR, ecdysone receptor; ErbA, avian erythroblastosis virus oncogene A; ERs, estrogen receptors α and β; ERR1,2, estrogen-related receptors 1 and 2; FXR, farnesoid X receptor; GR, glucocorticoid receptor; GRE, glucocorticoid-responsive element; GRIP1, glucocorticoid receptor interacting protein 1; h9, heptad nine; HAT, histone acetyl transferase; HMG-CoA, hydroxymethylglutaryl coenzyme A; HNF4, hepatocyte nuclear factor 4; LBD, ligand binding domain; LRH-1, liver receptor homolog-1; LXRs, liver X receptors α and β; MR, mineralocorticoid receptor; NCoA-62, nuclear receptor coactivator-62; NOR1, neuron-derived orphan receptor; NURR1, nuclear receptor related 1; pCAF, CBP/p300-associated factor; PPARs, peroxisome proliferator activated receptors α, β, and γ; PR, progesterone receptor; PXR/ONR, pregnane X receptor/*Xenopus* orphan nuclear receptor; RARs, (all-*trans*) retinoic acid receptors α, β, and γ; RevErbA, reverse ErbA; ROR, RAR-related orphan receptor; RXRs, retinoid X receptors α, β, and γ; SF-1, steroidogenic factor-1; SMAD, Sma- and Mad-related protein; SRC-1, steroid receptor coactivator-1; SWI/SNF, mating type switch/sucrose nonfermenting complex; TAFs, TATA-binding protein-associated factors; TFIIB, transcription factor IIB; TGFβ, transforming growth factor β; TLX, *Drosophila* tailless gene homologue; TRs, thyroid hormone receptors α and β; TR2, testicular receptor 2; TR4, testicular receptor 4; USP, ultraspiracle; VDR, (1α,25-dihydroxy) vitamin D_3 receptor; VDRE, vitamin D-responsive element.

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target genes. Other receptors in this superfamily bind hormonal ligands that are terpene-derived molecules [Moore, 1990], or even amino acid derivatives, as is the case for thyroid hormone. However, the majority of superfamily members described to date have no observed ligand, and are therefore termed "orphan" receptors. As this name implies, these proteins may bind unknown compounds, so an important future goal will be to "reunite" the orphan receptors with their cognate ligands, in the process likely uncovering novel regulatory circuits involving bioactive lipid metabolites [Gustafsson, 1999].

NUCLEAR RECEPTOR LIGANDS DERIVED FROM THE CHOLESTEROL BIOSYNTHETIC PATHWAY

Figure 1 displays most of the recognized ligands for nuclear receptors, which consist mainly of compounds derived from acetyl CoA (e.g., fatty acids), and especially those derived from isoprene units, such as retinoids, sterols/steroids, and vitamin D (a seco-steroid). Thyroid hormone is omitted from this diagram due to its entirely different metabolic origin, from iodine and the amino acid tyrosine. The common feature displayed by all nuclear receptor ligands, including thyroid hormone, is their lipophilicity, enabling them to readily penetrate the plasma membrane and enter the cytoplasm and nucleus. Retention of hormonal ligands in the target cell nucleus is facilitated by specific, high-affinity binding to receptors that are expressed in a tissue selective manner. Thus, the distribution of a particular nuclear receptor determines the pattern of cellular responsiveness to the hormonal ligand within multicellular organisms.

The classic steroid hormones bind their cognate receptors to constitute an ensemble of regulators that control, for example, sodium reabsorption and blood pressure (aldosterone), gluconeogenesis plus lung surfactant biosynthesis (cortisol), as well as reproduction and sexual development (progesterone, estrogens, and androgens). Other nuclear receptors that associate with sterol ligands in vertebrates include PXR, which binds pregnenolone; LXR, that recognizes 22-hydroxycholesterol; FXR, which binds bile acids such as chenodeoxycholic acid; CARs, that associate with androgen metabolites like androstenol; and VDR, the receptor for the renal $1,25(OH)_2D_3$ hormone that stimulates intestinal calcium absorption and bone remodeling. In arthropods, ecdysone and its receptor, EcR, are key regulators of molting [Thummel, 1997].

Beyond recognizing and binding sterol ligands, nuclear receptors also participate in the regulation of sterol metabolism. Interestingly, the pivotal initial pathway in Figure 1, namely the biosynthesis of the fused four ring structure of cholesterol from acetyl CoA via HMG-CoA and isoprene units, is not feedback controlled by nuclear receptors. Instead cholesterol biosynthesis is regulated transcriptionally at the HMG-CoA reductase step by a mechanism involving an endoplasmic reticulum-produced, basic-helix-loop-helix leucine zipper protein, termed sterol regulatory element binding protein. However, the subsequent metabolic fate of cholesterol in liver does appear to be governed by steroid receptors [Russell, 1999]. Two prominent examples are FXR and its bile acid ligands that function to feedback repress the rate limiting cholesterol 7α-hydroxylase enzyme in bile acid synthesis and, conversely, LXR binding its 22-hydroxycholesterol ligand and inducing 7α-hydroxylase gene transcription [Makishima et al., 1999]. In addition, the classic steroid hormone receptor ligands (in the lower part of Fig. 1), which are quantitatively minor but important products of cholesterol metabolism, feedback regulate their own biosynthesis from cholesterol by binding to their cognate nuclear receptors and effecting short and long endocrine loops. A well-known example is cortisol suppression of ACTH release from the anterior pituitary, which then reduces the conversion of cholesterol to pregnenolone in the adrenal cortex.

As discussed below, nuclear receptors appear to have a long evolutionary history, implying that they have assumed a wide variety of important physiologic roles. Targeted disruptions of receptor genes in mice, as well as the clinical syndromes of hormone resistance in humans, indeed confirm this conclusion. Ablation of certain receptor genes has lethal consequences; for example, knockout of MR causes mice to die of salt loss and dehydration, and GR null neonatal mice perish from respiratory distress syndrome. Knockout of specific pairs of RARs and RXRs leads to a large spectrum of fetal defects

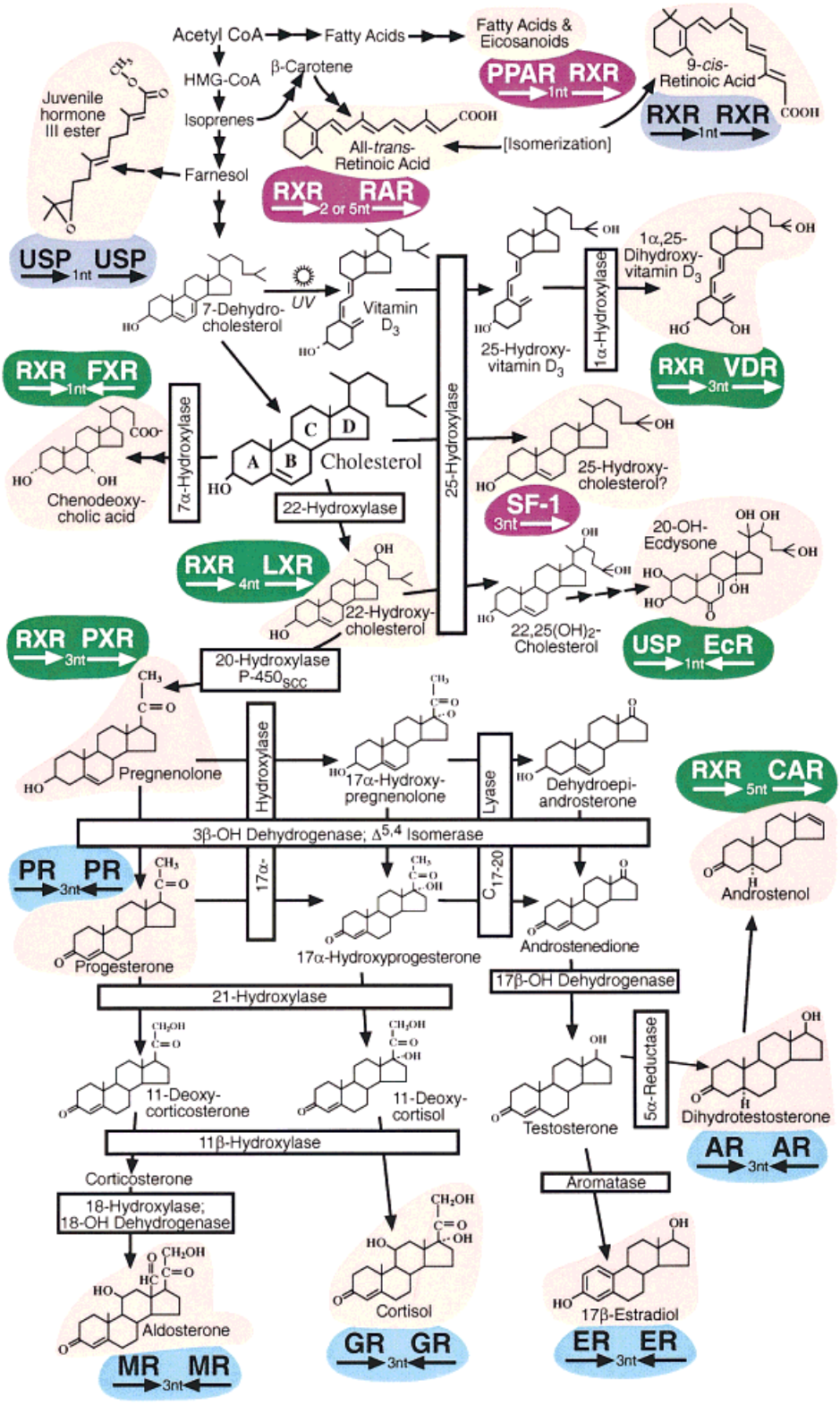

Fig. 1. Lipophilic ligands derived from acetyl CoA. Compounds serving as specific, high-affinity ligands for nuclear receptors are highlighted with a salmon colored background. For simplicity, only one ligand is shown per receptor; the actual physiologic ligand in a given species may vary. The ligand for SF-1 is a tentative assignment [Lala et al., 1997]. Key metabolic steps from acetyl CoA are depicted, and the enzymes catalyzing steroid biosynthesis from the central cholesterol intermediate are boxed. Receptors are shown near their cognate ligand against a background color signifying a proposed evolutionary grouping (see Fig. 2). Most liganded receptors are configured as dimers on DNA. Bold arrows represent the DNA hexanucleotide half-elements, usually arranged as either tandem or inverted repeat motifs, separated by a spacer of 1–5 nucleotides (nt), forming the complete hormone-responsive element. For homodimers, both receptors are likely liganded, whereas for heterodimers, liganding of the primary receptor residing on the 3′ half-element is usually sufficient for activity.

that recapitulates the fetal vitamin A deficiency syndrome [Sapin et al., 1997]. Similarly, VDR null mice [Li et al., 1997] exhibit rickets indistinguishable from that caused by nutritional vitamin D deficiency, and also mimic the alopecic phenotype of vitamin D resistant rachitic patients with certain inactivating mutations in VDR [Whitfield et al., 1996; Haussler et al., 1998]. In a particularly instructive clinical condition, mutant ARs that elicit androgen insensitivity cause individuals with a male genotype to develop as phenotypic females. These genetic disorders in nuclear receptors are mimicked in part by parallel metabolic defects that affect the generation of the cognate ligands. One example of an inherited defect in steroid hormone biosynthesis is congenital adrenal hyperplasia, wherein a defective 21-hydroxylase or 17α-hydroxylase enzyme results in deficient production of mineralocorticoids/glucocorticoids or glucocorticoids/androgens, respectively (Fig. 1). Also, a type of familial vitamin D resistance has been characterized by the presence of inactivating mutations in the 1α-hydroxylase enzyme that produces the $1,25(OH)_2D_3$ hormone [Fu et al., 1997]. The general similarity between absence of a given hormone and a defect in its receptor gene argues that these nuclear receptors mediate most, if not all of the major biological effects of their cognate ligands.

DIMERIZATION AND DNA BINDING MOTIFS FOR THE NUCLEAR RECEPTORS

Typically, the response of nuclear receptors to ligand binding at the molecular level entails first dimerizing, either as a homodimer or a heterodimer with RXR, followed by high-affinity binding to specific hexanucleotide half-elements arranged in a particular motif. Most receptors bind DNA according to one of five recurring patterns: (1) as heterodimers with RXR (or, in arthropods, with its USP equivalent) on directly (i.e., tandemly) repeated half-elements separated by a spacer of 1–5 bp; (2) as heterodimers on inverted (palindromic) responsive elements separated by 1 bp; (3) as homodimers on direct repeats separated by 1 bp; (4) as homodimers on inverted repeats separated by 3 bp; or (5) as monomers on a single half-site, often containing a 3 bp 5′-extension. Examples of all five patterns are found in Figure 1. Monomeric DNA binding is commonly observed among orphan receptors [Laudet, 1997; Gustafsson, 1999]. Should SF-1, which is required for adrenal cortex and gonadal development [Sadovsky et al., 1995], prove to recognize an oxysterol ligand (Fig. 1), as suggested by Lala et al. [1997], it would become the only example of a true monomeric receptor with a known ligand. The common feature that is conserved in the DNA binding scenarios illustrated schematically in Figure 1 is the sequence of the hexanucleotide half-element, which is usually some variation of AGGTCA, although four of the classic steroid hormone receptors (GR, MR, PR, and AR) deviate from this consensus, binding an AGAACA half-site. After binding to DNA, receptors then influence the transcription of nearby target genes, as discussed later (see Fig. 4).

EVOLUTIONARY CLASSIFICATION OF NUCLEAR RECEPTORS

On the basis of sequence similarities, nuclear receptors are believed to form a coherent group of evolutionarily related proteins, presumably all derived from a common ancestral gene via gene duplication, and exon shuffling in certain cases [Laudet, 1997; Sluder et al., 1999]. Figure 2 displays a proposed grouping of representative receptors expanded beyond those shown in Figure 1. This synthesis includes the subgroupings of Laudet [1997], but also incorporates the position of an intron-exon junction in the region of the gene encoding the DNA binding domain [Detera-Wadleigh and Fanning, 1994]. The position of an intron in this highly conserved segment presumably would be relatively stable over the course of evolution, and thus is likely to be informative concerning ancient phylogenetic relationships [Detera-Wadleigh and Fanning, 1994]. Following this reasoning, the nuclear receptors can be sorted into four categories depending upon the location of the intron/exon boundary in the gene encoding the region of the P-box, a cluster of amino acids in the C-terminal portion of the first zinc-finger motif [Detera-Wadleigh and Fanning, 1994] (Fig. 2, left). Two of the four divisions presented in Figure 2 are consistent with other analyses, namely the top (a) and bottom (d) groupings, which correspond to group 2 and group 3 of Laudet [1997], respectively, while the middle two sets (b and c) represent novel, speculative reclassifications.

In the top group shown in Figure 2, five receptors are known to have an intron/exon

junction at position (a), 5′ of the P-box codons.[1] The other two genes in this group with known intron/exon breaks, those for jellyfish RXR and human COUP-TF, lack an intron in the entire portion of the gene coding for the DNA binding region as indicated by a superscripted (e). Nonetheless, a consensus of phylogenetic analyses supports the assignment of all these receptors to a single group based on sequence similarity [Detera-Wadleigh and Fanning, 1994; Laudet, 1997; Sluder et al., 1999]. Functional considerations also provide support for this categorization. RXR and USP are unique among nuclear receptors for their ability to heterodimerize with a variety of other nuclear receptors outside their own phylogenetic subgroup (see Fig. 1 for several examples), while RXR/USP along with COUP-TF and TR2 all share the property of being able to homodimerize on a direct repeat responsive element, a feature proposed to be primitive for nuclear receptors [Laudet, 1997].

From an evolutionary perspective, it is noteworthy that jellyfish and *C. elegans* represent ancient metazoic groups (Fig. 2, inset, lower right). Both the jellyfish phylum (Cnidaria) and the Nematoda diverged from other animal phyla before the split between protostomes (e.g., arthropods) and deuterostomes (e.g., vertebrates), more than 600 million years ago [Ayala and Rzhetsky, 1998]. Jellyfish RXR nonetheless possesses both the ligand binding and heterodimerization properties distinctive of RXRs [Kostrouch et al., 1998]. Strikingly, in *C. elegans*, nuclear receptors comprise the single largest simple class of transcription factors encoded in the entire genome [Sluder et al., 1999]. Thus, the top group of nuclear receptors in Figure 2, along with their ligands, may represent an especially ancient signaling mechanism in metazoans.

The second grouping of receptors in Figure 2, headed by TR, is distinguished by the intron at position (b) within the P-box. This subclass is noteworthy for its unusually diverse ligands and is also heterogeneous in terms of DNA binding characteristics, containing examples of receptors that form heterodimers with RXR (TRs, RARs, and PPARs), as well as many receptors that bind DNA as monomers (SF-1, RORs, RevErb, and LRH-1). The assignment of the 5A and 5B receptors to this second grouping differs from that of other analyses [Detera-Wadleigh and Fanning, 1994; Laudet, 1997], and is based solely on the intron/exon junction at position (b). This grouping must therefore remain speculative, awaiting more exhaustive phylogenetic analyses (preferably taking sequence and intron/exon organization into account), as well as the cloning of nuclear receptors from a greater variety of animal phyla.

The third grouping in Figure 2, headed by the LXRs, is again based on the intron/exon boundary, in this case at position (c), 3′ of the P-box; the exact break position varies ±1 bp in this group. While nine of these receptors have introns at this location, three lack introns in the entire gene region encoding the DNA-binding domain. The repeated observation that one or more receptors in a given grouping is missing the expected intron suggests that certain taxa, notably *Drosophila*, may have a tendency to lose introns over the course of evolution [Da Lage et al., 1996]. Most receptors in the third (c) grouping in Figure 2 heterodimerize with RXR/USP on DNA, and where the ligand has been identified, it is a sterol. However, some members of group (c) are orphan receptors (e.g., NOR1) that may bind DNA as monomers, and the properties of the *C. elegans* receptors remain undefined.

The final evolutionary grouping (d), shown in the lower left of Figure 2 and headed by the ERs, contains classic steroid hormone receptors that bind DNA as homodimers on inverted repeat elements separated by 3 bp. This grouping is of relatively recent evolutionary origin, having been found to date only in vertebrates, and has the added distinction that it alone contains

[1]Receptor gene sequences used in the determination of intron/exon boundaries are provided in alphabetical order and are human unless otherwise noted. The 6–8 character accession numbers can be used to obtain these sequences and/or their corresponding references at <http://www.ncbi.nlm.nih.gov/Entrez/nucleotide.html>: AR (M27423), CARα (mouse, AF009326), COUP-TF (NM_005654) [Qiu et al., 1995], DHR3 (*Drosophila*, M90806) [Koelle et al., 1992], DHR38 (*Drosophila*, Y15606), E75 (*Drosophila*, X51548), E78 (*Drosophila*, U01087), EcR (*Drosophila*, M74078), ERα (AF123494), ERα (Atlantic salmon, AF047895), ERα (tilapia fish, X93558), GR (U78508), MR (AF068617), NHR-6 (*C. elegans*, Z36237), NHR-23 (*C. elegans*, Z71258, listed as "CHR3"), NHR-25 (*C. elegans*, CAA91028), NURR1 (AB019433), NURR1 (mouse, AAC53153), PPARγ (AB005523), PR (X69071), PR (chicken, M32726), RARα (AF088890), RARγ (M38258), RORγ (mouse, AF019657), RXR (jellyfish, AF091121) [Kostrouch et al., 1998], RXRβ (AF065396), RXRβ (mouse, D21830), RXRγ (mouse, S62948) [Liu and Linney, 1993], SF-1 (S65878), TRα (X55070), TRα (Fugu fish, AJ012380), TRβ (mouse, U15544), TRβ (*Xenopus*, U04675) [Shi et al., 1992], VDR (AB002162).

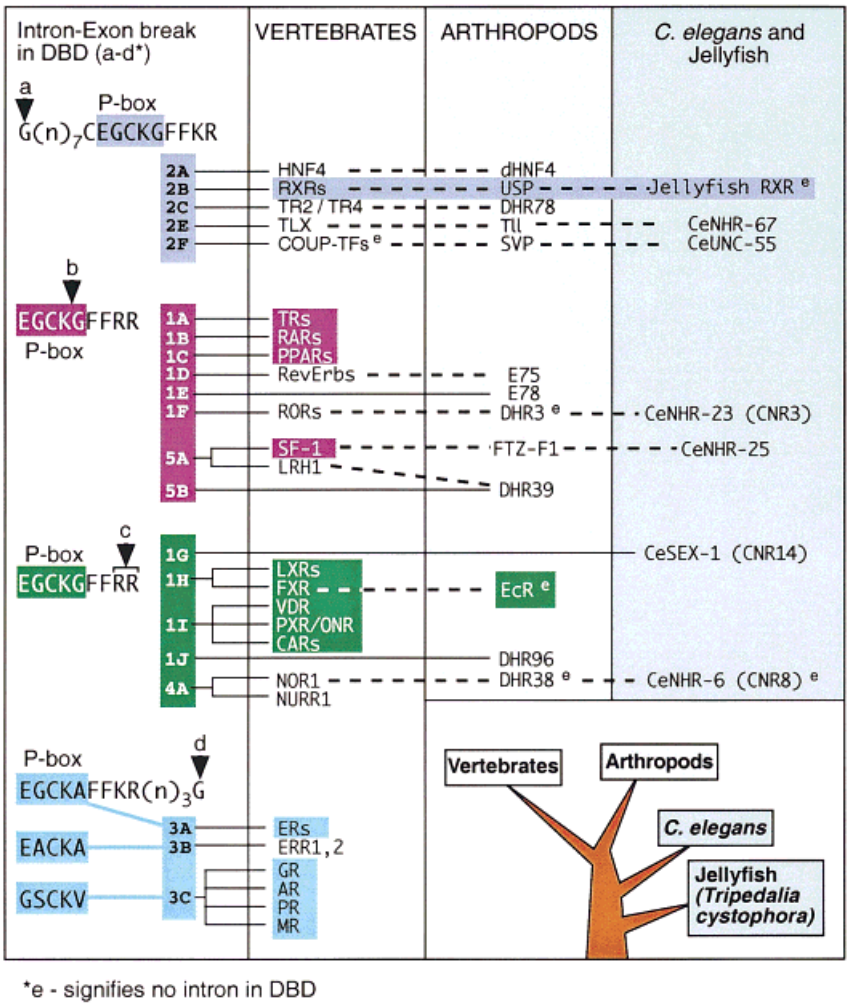

Fig. 2. Proposed evolutionary grouping of a representative selection of nuclear receptors. A prominent feature of this speculative classification is the location of an intron-exon boundary in the DNA encoding the region of the highly conserved P-box in the DNA binding domain [Detera-Wadleigh and Fanning, 1994]. Four major positions of the intron-exon break (**a–d**) are found in the selected receptors and are illustrated in the left panel; these four groupings are assigned different color backgrounds, which are also used in Figures 1 and 3. Subgroup designations 1A–5B follow a suggested unified nomenclature for nuclear receptors [Laudet et al., 1999] and are listed adjacent to the proposed new groupings. Receptors are arranged in columns by phylogenetic category (partial receptor sequences are not included). Only those receptors with a known or proposed ligand are highlighted in color; others represent orphan receptors. Dotted horizontal lines indicate evolutionary subgroupings according to Laudet [1997]; the position of some *C. elegans* receptors follows Sluder et al. [1999]. Intron positions were deduced from genomic sequences obtained from Genbank.[1] Those receptors for which the genes lack an intron in the relevant region of the DNA binding domain are indicated by a superscripted (**e**). Tentative placement of these receptors into one of the four groups (**a–d**) is explained in the text. Lower right, simplified evolutionary tree, depicting separate terminal branches for arthropods, vertebrates, *C. elegans*, and jellyfish.

receptors in which the P-box amino acid sequence differs significantly from the superfamily consensus of EGCKx. As a result, GR, AR, PR, and MR, which possess a unique GSCKV P-box (Fig. 2), bind an AGAACA half-element that is divergent from the AGGTCA consensus (differences from consensus are underlined) [Zilliacus et al., 1995].

Despite the evolutionary and functional differences outlined above, nuclear receptors still retain many similarities, especially in their structural organization (Fig. 3; see also later discussion). Since, for the most part, their ligands belong to classes of compounds that are related by origin in isoprene-like units [Moore, 1990], it is tempting to seek potential evolutionary correlations between the receptors and the class of ligand they bind. For example, the subset of receptors containing RXR and USP (group a, with a lavender background in Figs. 1

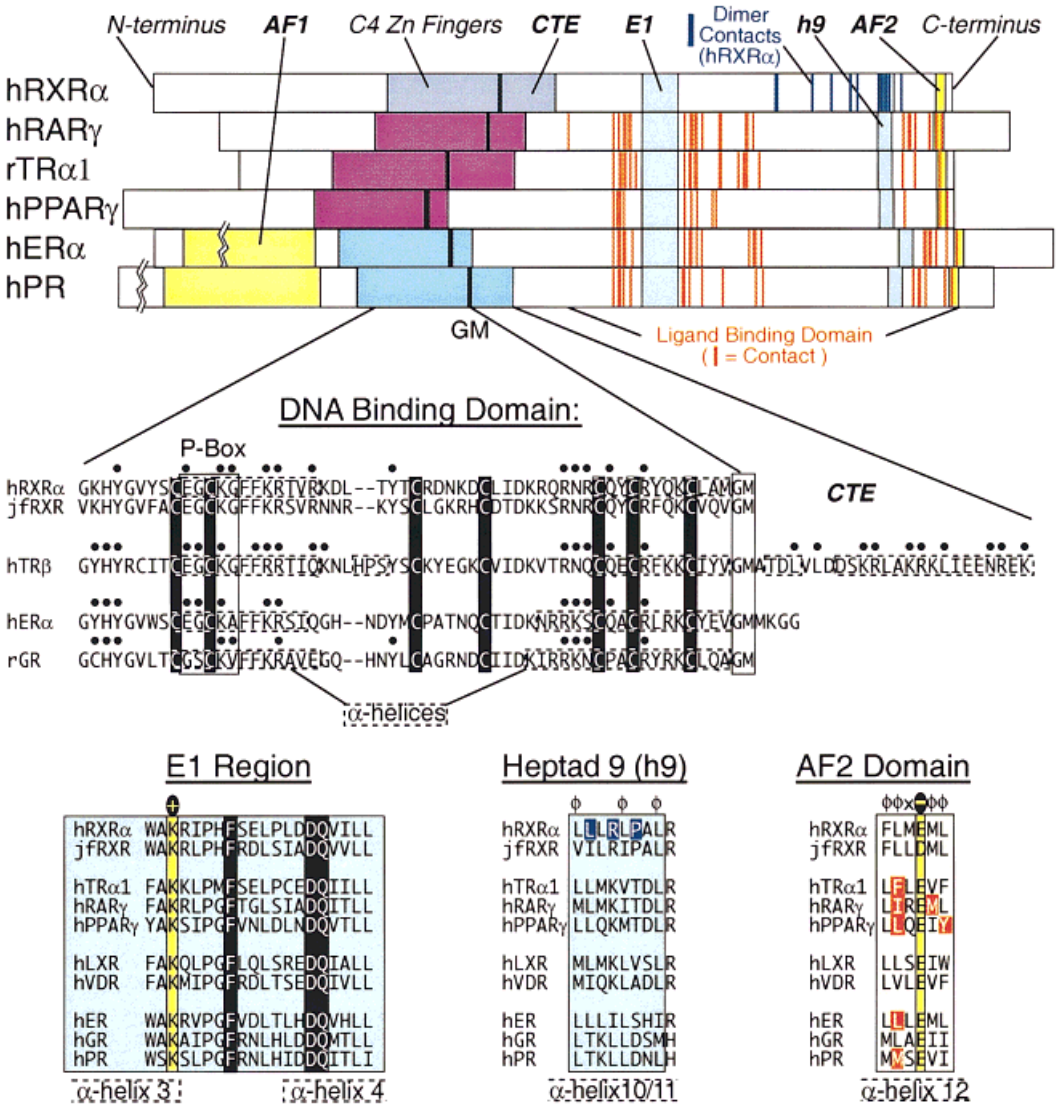

Fig. 3. Schematic view of nuclear receptor functional domains. The modular diagrams in the top panel are drawn to scale and aligned at the conserved E1 domain. The DNA-binding region consists of two $(Cys)_4$-type zinc-finger motifs (C4 Zn fingers), followed by a CTE of varying length. A selected portion of this domain is shown for several receptors in the center panel,[2] with solid circles indicating DNA contacts as determined by X-ray crystallography for human RXRα, human TRβ, human ERα, and rat GR; the jellyfish RXR sequence is added for comparison. C-terminal of the DNA-binding domain/CTE lies the large LBD that extends approximately to the C-terminus. Actual ligand contacts determined via X-ray crystallography (vertical rust colored bars in the top panel) are depicted for human RARγ [Renaud et al., 1995], rat TRα [Wagner et al., 1995], human PPARγ [Nolte et al., 1998], human ERα [Brzozowski et al., 1997], and human PR [Williams and Sigler, 1998]. The LBD also contains a dimerization interface, and the dimer contacts for a human RXRα homodimer determined by X-ray crystallography [Bourguet et al., 1995] are displayed as vertical dark blue bars (top). The lowest portion details three subregions of the LBD in several nuclear receptors,[2] including the conserved E1 domain (residues absolutely conserved in the entire superfamily are highlighted) that supports dimerization and participates in transactivation, h9, which mediates dimerization (in part), and the AF2 region that contains ligand contacts and effects transactivation.

[2]Receptor protein sequences used in Figure 3 are listed in alphabetical order and are human unless otherwise noted. The accession numbers provided can be used to obtain these sequences at: <http://www.ncbi.nlm.nih.gov/Entrez/protein.html>: ERα (P03372), GR (NP_000167), GR (rat, P06536), LXRα (NP_005684), PPARγ (P37231), PR (NP_000917), RARγ (AAA52692), RXRα (CAA36982), RXR (jellyfish, AAC80008), TRα1 (CAA38749), TRα1 (rat, AAA42238), TRβ (TVHUAR), VDR (NP_000367).

and 2), known to include particularly ancient receptors, binds terpene derivatives with predominantly linear structures such as 9-*cis*-retinoic acid and juvenile hormone. At the other extreme, the evolutionarily more recent subgrouping (d) including the ER, GR, and other traditional steroid hormone receptors (shown with a medium blue background in Figs. 1 and

2), binds ligands that represent terminal derivatives of cholesterol devoid of either the C_{22-27} or C_{20-27} side chain. Extending this theme, one of the intermediate groupings, (c) (green background in Figs. 1 and 2), containing LXR, FXR, VDR, PXR, etc., binds ligands with a steroid or seco-steroid ring structure including several that retain the cholesterol side chain. Further circumstantial evidence for the presence of an evolutionary relationship between receptor and cognate hormone is the expanding realization, discussed above, that nuclear receptors regulate the synthesis of their sterol ligands.

The postulated evolutionary correlation between ligand and nuclear receptor becomes rather tenuous, however, when applied to the remaining large (b) subclass (shown with a magenta background in Figs. 1 and 2) containing TRs, RARs, PPARs, and SF-1. Ligands for this group encompass a wide variety of compounds, not only sterols (SF-1), nearly linear terpenes (RARs), fatty acids and eicosanoids (PPARs), but also an amino acid derivative (TRs). The recent revelation that all these nuclear receptor ligands share a similar molecular volume [Bogan et al., 1998] provides, in principle, the basis for an alternative relationship between ligands and receptors, whereby lipophilic ligands may have been evolutionarily "adopted" by various receptors based on limitations of size and surface features, irrespective of their metabolic origin. A third possibility is that nature has utilized both coevolution of ligand and receptor, as well as fortuitous adoption of unrelated ligands, in the genesis of the complex nuclear receptor superfamily of biologic regulators.

NUCLEAR RECEPTOR STRUCTURE AND FUNCTIONAL DOMAINS

The functional domains within nuclear receptors are depicted schematically in Figure 3 in the context of the entire primary sequence of several representative superfamily members for which X-ray crystallographic data are available. The top panel of Figure 3 highlights the following characteristic domains: (1) an N-terminal extension of widely varying length, which is large and known to harbor a transactivation function (AF1) in the group (d) receptors (human ERα and human PR are shown as examples); (2) a well-conserved central DNA binding region, containing two zinc-finger DNA binding motifs of the $(Cys)_4$ type; (3) the variable C-terminal extension (CTE) flanking the zinc fingers, which also participates in DNA binding by some receptors (e.g., TR); (4) a large C-terminal ligand binding domain (LBD), with ligand contacts occurring in three distinct clusters, separate from receptor dimerization contacts (depicted for hRXRα) that also occur in the LBD; (5) a conserved E1 region, so named for its existence within the E (LBD) segment of nuclear receptors [Haussler et al., 1997; Laudet, 1997]; (6) a less well-conserved heptad nine (h9) region; and (7) a second transactivation domain (AF2) near the extreme C-terminus of the receptor.

Figure 3 (middle) shows that portion of the DNA binding region and CTE that contains DNA contacts as determined for human RXRα and human TRβ [Rastinejad et al., 1995], as well as human ERα and rat GR [Zilliacus et al., 1995]. The first two conserved cysteines in the zinc-finger domain are omitted, and the remaining seven are depicted in white lettering on a black background. Note that the DNA binding contacts for hRXRα, hERα and rGR are confined to the zinc-finger region, predominantly within the base-pair recognizing α-helix containing the P-box, and the more C-terminal phosphate backbone-binding α-helix. By contrast, the DNA contacts for hTRβ extend well into the additional α-helices of the CTE [Rastinejad et al., 1995]. While these observations may reflect artifactual binding of truncated receptors used in the crystallographic analyses, an attractive alternative explanation is that the length and participation of the CTE in DNA binding may be related functionally to the size of the spacer separating the two half-sites of the responsive element. Thus, it has been postulated that the lengthy CTE of hTRβ may function not only to assist in DNA binding, but also to exclude interaction of TR with direct repeat responsive elements containing spacers of less than 4 bp [Rastinejad et al., 1995].

The hormonal ligand binding domains of all nuclear receptors share a relatively similar tertiary structure, consisting of a sandwich of 11–13 α-helices and several small β-strands organized around a lipophilic binding cavity [Williams and Sigler, 1998], with the specificity of ligand-receptor interaction dictated primarily by the occurrence of unique amino acid side chains within the context of the general hormone binding pocket. When the ligand contact residues are mapped onto a linear representa-

tion of the hormone binding domains of several receptors (top right portion of Fig. 3), there is reasonable positional correspondence of these unique amino acids within the primary sequence. This similarity of ligand contact positions is consistent with the nearly superimposable 3-dimensional structures of the ligand binding domains of nuclear receptors.

Figure 3 (bottom) details three subregions of the ligand binding domain. All three participate in functions other than ligand binding, although the AF2 does contain one or two known ligand contact amino acids (shown with rust colored background in the lower right box). The h9 region is thought to function mainly in dimerization [Bourguet et al., 1995]. A cluster of dimer contacts occurs in this LBD subregion, at least for hRXRα (Fig. 3, top). Sequences in h9 of selected receptors (center panel, bottom) are characterized by three hydrophobic residues (ϕ) spaced 3–4 amino acids apart in the α-helical framework. However, the actual dimerization contact residues (highlighted with a dark blue background) within h9 for hRXRα differ from the conserved hydrophobic amino acids. This indicates that the conserved hydrophobic side chains likely execute intramolecular interactions required to maintain the α-helix in the proper configuration for dimerization [Bourguet et al., 1995].

Residues in the E1 region also appear to be crucial for supporting receptor dimerization, as concluded from point mutagenesis and protein chimera experiments [Haussler et al., 1997]; curiously, none of these residues is a direct dimer contact in the hRXRα homodimer LBD co-crystal [Bourguet et al., 1995]. Thus, either the hRXRα homodimer contacts do not reflect the dimer interface in other receptors, or the E1 region, which encompasses the junction between two α-helices in the structures of all six LBD crystals analyzed to date, may simply comprise a crucial part of the structural scaffold for dimerization [Bourguet et al., 1995]. Near the N-terminus of the E1 region resides a highly conserved lysine residue (boxed in yellow) that is essential for transactivation by nuclear receptors [Whitfield et al., 1995; Feng et al., 1998; Nolte et al., 1998].

Another obligatory region for transactivation is the AF2 domain [Jurutka et al., 1997]. As shown in the bottom right of Figure 3, the AF2 has preserved the following characteristic motif: a negatively charged residue (usually glutamate, highlighted in deep yellow), flanked on each side by pairs of hydrophobic amino acids (ϕ). The actual participation of this negatively charged AF2 residue, along with the conserved lysine in the E1 region, in contacting a transcriptional coactivator has been confirmed by X-ray crystallographic analysis of human PPARγ [Nolte et al., 1998]. Finally, the fact that the AF2 also possesses ligand binding residues has profound implications for the design of agonist/antagonist analogs that are able to conform the receptor for selective recruitment of coactivators/corepressors.

MECHANISM OF TRANSCRIPTIONAL CONTROL BY NUCLEAR RECEPTORS

Once nuclear receptors have bound their cognate ligands, dimerized and achieved high-affinity association with specific responsive elements in DNA, how do their transactivation domains function together at the molecular level to bring about gene activation? While the full sequence of events remains unknown, a large number of proteins have been identified that interact with nuclear receptors during the transactivation process. Figure 4 illustrates simplified models of ligand-elicited transactivation by receptors from two distinct groups: VDR, an RXR heterodimerizing receptor in the (c) evolutionary classification, and GR, a homodimerizing receptor in the (d) group. The diagrams represented in Figure 4 are not intended to be comprehensive and contain only a representative selection of transcriptional coregulators. The reader is referred to recent treatises [McKenna et al., 1999; Xu et al., 1999] on nuclear receptor coactivator networks for a more extensive discussion.

An early step in the activation of genes by both types of receptors is the attraction of coactivator proteins with intrinsic HAT activity, including the p160 class (SRC-1, GRIP1, and ACTR), pCAF, and the CBP/p300 cointegrator. Histone acetylation is thought to derepress chromatin to allow for binding and assembly of the transcription machine [Kornberg and Lorch, 1999]. However, in the case of VDR, uncontrolled histone hyperacetylation (elicited by butyrate) abrogates $1,25(OH)_2D_3$-stimulated transactivation of the osteocalcin gene [Montecino et al., 1999]. This observation indicates that a precise level of chromatin acetylation in the promoter region of a regulated gene may be crucial for transcriptional control.

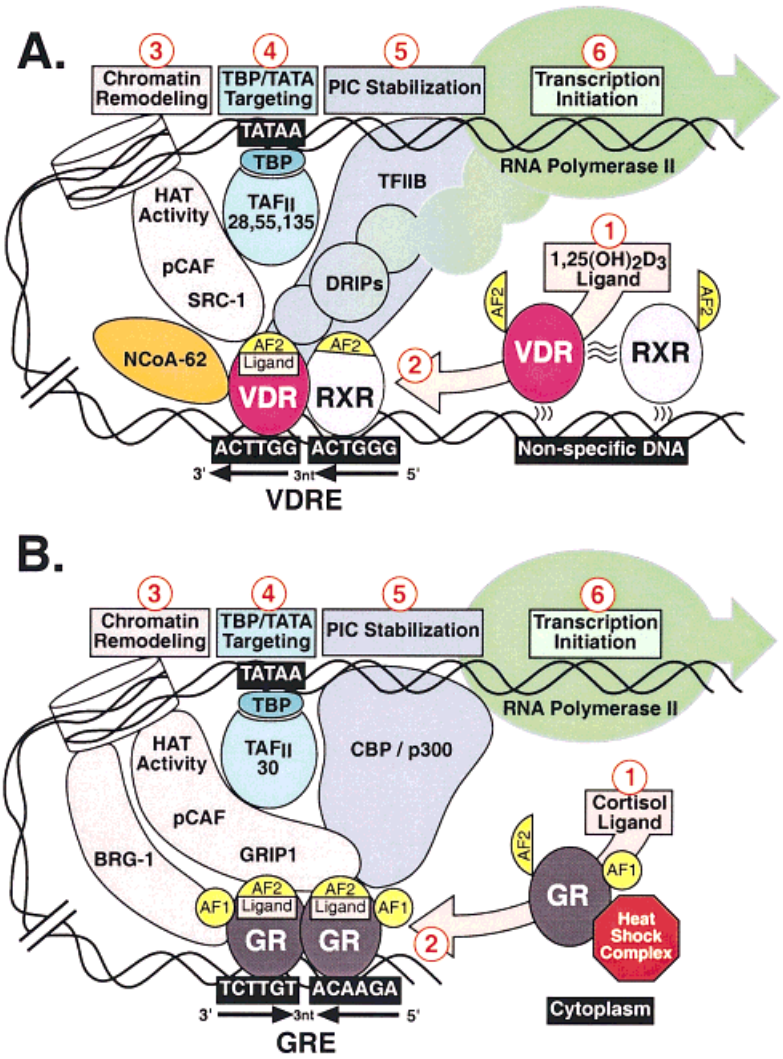

Fig. 4. Simplified models of transactivation by heterodimerizing and homodimerizing receptors. **A:** Unliganded heterodimerizing receptors, exemplified here by VDR, exist as weakly associated heterodimers with RXR, presumably bound nonspecifically to DNA [Haussler et al., 1998]. Binding of the $1,25(OH)_2D_3$ ligand to VDR (1) promotes high-affinity heterodimerization with RXR accompanied by binding of the heterodimer to its direct repeat VDRE (2). Ligand also reconfigures the AF2 region of VDR to allow for recruitment of SRC-1, DRIPs, and possibly other coactivators [reviewed in Xu et al., 1999], while the AF2 of RXRs is likely also positioned for contact with additional coactivators (not shown). Some coregulatory proteins possess HAT activity (e.g., SRC-1 and pCAF), which catalyzes local chromatin remodeling (3) as a prelude to transactivation [Kornberg and Lorch, 1999]. Subsequent steps include attraction of TAFs [Haussler et al., 1998] that target TBP/TATA (4), and binding of TFIIB and DRIPs to stabilize the preinitiation complex (PIC) (5), and allow for transcription initiation by RNA polymerase II (6). **B:** Unliganded GR, like other receptors in group (d) (see Fig. 2), exists as a complex with heat shock proteins in the cytoplasm. Upon binding its cortisol ligand (1), GR dissociates from the cytoplasmic complex, translocates to the nucleus and forms a homodimer on its palindromic GRE (2). Triggered by a ligand-mediated change in GR conformation, the AF1 and AF2 domains then synergize to promote a series of events (3–6) involving the recruitment of coregulatory complexes similar to those described for the VDR-RXR heterodimer, but with some distinctive features. For example, direct contact with TFIIB has not been reported for GR. Other differences, such as the participation of an AF1, the action of BRG-1 to modify chromatin, and the dual role of the CBP/p300 cointegrator as a HAT and a link to RNA polymerase II, are discussed in the text.

After HAT activity has catalyzed the appropriate reorganization of nucleosome architecture, several other coactivators (or, more likely, coactivator complexes) promote actual transcriptional initiation. For VDR (Fig. 4A), prominent reported examples include various TAFs [Haussler et al., 1998], as well as a large preformed complex containing multiple DRIPs that bridge to RNA polymerase II [Rachez et al., 1999]. VDR has also been shown to bind TFIIB and, likely after $1,25(OH)_2$ D_3 and VDRE binding, supplies this factor to promote and/or stabilize

the formation of the transcription preinitiation complex [Haussler et al., 1998]. The binding interface between TFIIB and VDR does not involve the AF2 region [Jurutka et al., 1997], indicating that other transactivation domains must exist in heterodimerizing receptors. One such domain appears to include helix 3 (Fig. 3, bottom left), shown to form a coactivator cleft for SRC-1 and its related p160 coactivators by associating with the helix 12 AF2 in human TRs [Feng et al., 1998]. Other domains may contact novel coactivators like NCoA-62 [Baudino et al., 1998], a VDR-interacting protein for which the exact role in transactivation has not been elucidated.

For GR (Fig. 4B), a similarly elaborate scenario has been developed. Chromatin is thought first to be derepressed by HAT activity such as that found in GRIP1 and pCAF, with likely additional participation of CBP/p300. Chromatin is also modified by BRG-1 [McKenna et al., 1999], which has intrinsic ATPase activity and remodels nucleosomes by uncoupling ionic interactions between histones and DNA. Subsequently (or possibly as part of the same large complex including the HATs), TAFs and other factors, such as CBP/p300, target the TATA-box as well as recruit and stabilize the preinitiation complex for enhanced RNA polymerase II activity. This picture for GR molecular action thus resembles that for VDR and other heterodimerizing receptors, yet important differences exist. First, GR and other group (d) classic steroid hormone receptors form what is in principle a symmetrical homodimeric complex on a palindromic responsive element. Second, as shown in Figures 3 and 4, receptors in group (d) possess an N-terminal AF1 region, which differs in amino acid composition from the more extensively characterized and highly conserved AF2 regions. Finally, there are some indications that certain coactivators may exhibit receptor specificity [Xu et al., 1999], implying that steroid hormonal regulation entails not only complex and selective interactions between ligand, receptor, and hormone-responsive element within each unique target gene promoter, but also encompasses participation by differing combinations of coregulator(s).

There are reports that some coregulatory proteins, in fact, do display cell- or promoter-specificity, thereby explaining the need for multiple transactivation domains and associated factors. For example, the HAT activities of CBP/p300 and pCAF display different substrate specificities [Xu et al., 1999], and studies of mice whose SRC-1 genes have been ablated suggest that signaling by some, but not all, nuclear receptors is affected [Weiss et al., 1999]. An added complexity is that chromatin remodeling and transactivation may occur in many stages, analogous to an enzyme pathway [Xu et al., 1999], requiring a factor or a complex of factors for each step. Indeed, in the case of VDR-RXR-mediated regulation of osteocalcin transcription, the osteoblast specific factor Osf-2/CbfA1 binds sequences flanking the VDRE to maintain chromatin in a permissive configuration for $1,25(OH)_2D_3$ induction [Lian et al., 1999]. An important question to be answered is whether multiple coregulatory complexes interact simultaneously with the activated receptor dimer and its target gene promoter, or whether they instead are recruited in a sequential manner.

The two examples illustrated in Figure 4 represent ligand-dependent activation of gene transcription. While this is perhaps the most common means of gene regulation by nuclear receptors, other modulatory paradigms have been discovered, some of which have important physiologic consequences. For example, VDR and GR are also ligand-dependent repressors of certain genes, and ligand-independent repression of transcription is known to occur for other receptors, such as unoccupied TR [McKenna et al., 1999]. An unusual example is CARβ, which is a constitutive activator that becomes a repressor upon binding its ligand [Forman et al., 1998]. Finally, the role of protein phosphorylation in modulating receptor function appears to range from modest quantitative alterations of activity, to a complete qualitative activation of the transcriptional potential of a receptor in the absence of ligand [reviewed in Haussler et al., 1997]. Therefore, the complete picture of nuclear receptor molecular function is far more complicated than that shown in Figure 4, and includes the recruitment of corepressor complexes by unliganded as well as liganded receptors. Finally, nuclear receptors interact with other signal transduction pathways initiated at the cell surface, such as the TGFβ/SMAD system in the case of VDR [Yanagisawa et al., 1999].

CONCLUDING REMARKS

The steroid/retinoid/thyroid hormone receptors, a subset of a large superfamily of evolution-

arily ancient nuclear receptors, are the primary mediators of the biological actions of a vast array of lipophilic ligands derived from acetyl CoA, isoprenoids, and cholesterol. The last 15 years have witnessed the cloning of key members of the superfamily, and the dawning of the new millennium will no doubt usher in advances in the nuclear hormone receptor field, likely culminating in the purification and reassembly of functional units capable of supporting hormone-dependent transcription by nuclear receptors, in vitro. Significant developments have occurred in the X-ray crystallographic analyses of nuclear receptor domains, permitting the visualization of contact surfaces between nuclear receptors and coactivators. These structural insights have confirmed the crucial roles played by conserved charged residues in the E1/helix 3 and AF2/helix 12 regions [Feng et al., 1998; Nolte et al., 1998] and are allowing the elucidation of the mechanism whereby ligand binding promotes these contacts, as well as the manner by which hormone antagonists subvert this process [Brzozowski et al., 1997]. Extension of these investigations likely will facilitate the rational design of superagonists, more effective antagonists, and cell- and/or target promoter-specific analogs as potential pharmaceuticals that operate at the level of nuclear receptors. Finally, it is anticipated that the cloning and characterization of nuclear receptors from a greater variety of metazoic taxa will extend our appreciation for the evolutionary history of this remarkable superfamily of transcriptional regulators.

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