The Nuclear Receptor Superfamily: The Second Decade

Overview

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Differential control of gene expression has become a central theme in modern molecular biology. The lipophilic hormones, because they are able both to diffuse from a source and permeate to a target, are ideal candidates to serve as regulators of this process. These hormones, including the steroids, retinoids, thyroid hormones, and vitamin D_3, are potent regulators of development, cell differentiation, and organ physiology (Figure 1). Historically, they were isolated in the early part of this century based on their abilities to affect development, differentiation, metamorphosis, and physiology. In addition, many of these hormones were associated with known human diseases, and by the middle of the century glucocorticoids had gained popularity as a therapeutic agent. While the mediators of the action of these hormones remained elusive, development of radiolabeled ligands allowed the identification of

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binding proteins that were shown to translocate from the cytoplasm to the nucleus, leading to a suggested link between transcriptional control and physiology (Jensen et al., 1966). This concept was elaborated upon by the work of Ashburner et al. (1974), which demonstrated that ecdysteroids, the metamorphic hormones of insects, trigger chromosomal puffing at specific sites in the Drosophila polytene chromosome. In the mid-1970s, steroids were shown to be targeted to their responsive tissues by the presence of specific high affinity receptor proteins. Unlike the water-soluble peptide hormones and growth factors, which bind to cell surface receptors, the fat-soluble steroid hormones can pass through the lipid bilayer of the cell membrane and interact with their cognate receptors. The subsequent identification of hormonally responsive target genes within these tissues completed the initial characterization of a steroid hormone signaling pathway. Together, these biochemical studies (reviewed by Yamamoto, 1985) provided the classic model of steroid hormone action. In this model it was postulated that the binding of hormone to its receptor induces an allosteric change that enables the hormone–receptor complex to bind to high affinity sites in chromatin and modulate transcription. The cloning of the steroid receptors was an essential prerequisite for ultimately understanding the molecular basis of this model.

It has now been 10 years since the isolation of cDNAs encoding the glucocorticoid and estrogen receptors, which were the first cellular transcription factors for RNA polymerase II to be cloned (Hollenberg et al., 1985; Miesfeld et al., 1986; Green et al., 1986). The homology of these receptors to the v-erbA oncogene led to the discovery of the c-erbA locus as the thyroid hormone receptor (Sap et al., 1986; Weinberger et al., 1986). The cloning of these first receptors represented a critical advance and demonstrated that chemically distinct ligands interact with structurally related receptors. The discovery in 1987 of the receptors for the vitamin A metabolite known as retinoic acid solidified the view of the existence of a nuclear receptor superfamily (Giguère et al., 1987; Petkovich et al., 1987). Following this work came the identification of receptors for all known nuclear hormones as well as a myriad of orphan receptors and the emerging concept that novel hormonal ligands might exist. Also during this time, the first insect receptors were discovered (Nauber et al., 1988; Oro et al., 1988). The identification of the ecdysone receptor as a member of the nuclear receptor superfamily (Koelle et al., 1991) suggested the likely universal nature of these receptors in animals and clearly demonstrated that the receptors evolved prior to the divergence of vertebrates and invertebrates. The discovery of retinoid X receptor (RXR) (Mangelsdorf et al., 1990) represented another important advance both in identifying the mediator of receptor heterodimerization and the novel orphan ligand 9-cis retinoic acid. Together these findings led to our modern view of hormonal signaling.

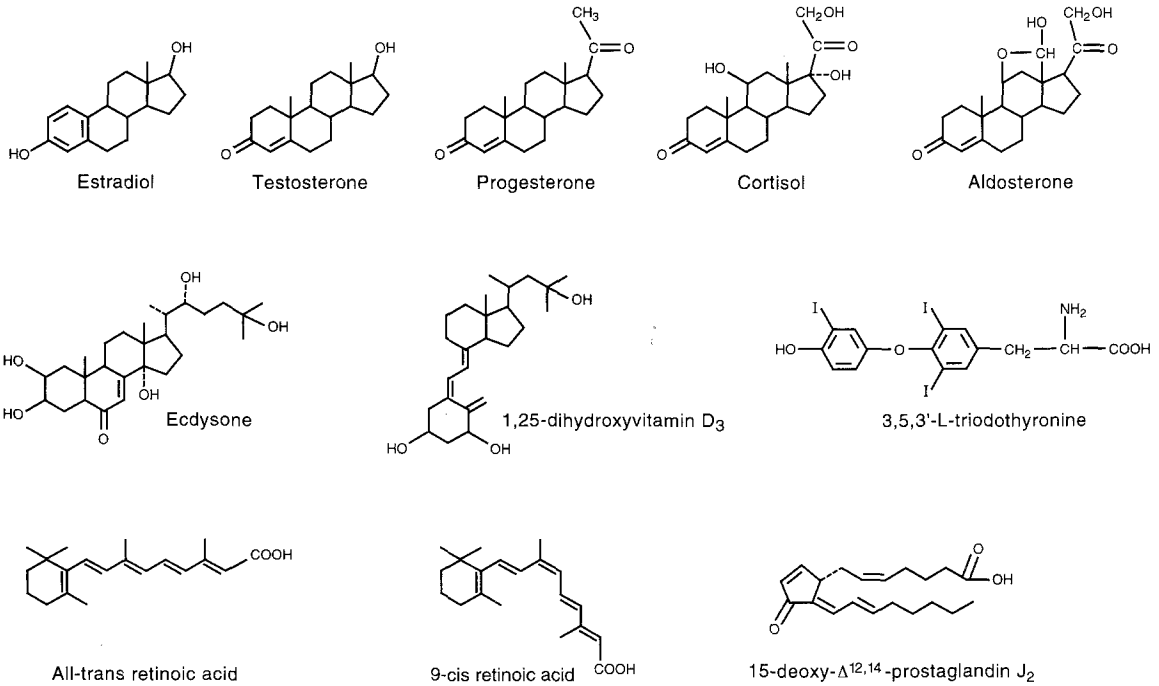The nuclear receptors are characterized by a central

Figure 1. Structures of the Known Ligands for Nuclear Hormone Receptors

DNA-binding domain (DBD) (Figure 2), which targets the receptor to specific DNA sequences known as hormone response elements. The DBD is composed of two highly conserved zinc fingers that set the nuclear receptors apart from other DNA-binding proteins (Berg, 1989; Klug and Schwabe, 1995). The C-terminal half of the receptor encompasses the ligand-binding domain (LBD), which possesses the essential property of hormone recognition and ensures both specificity and selectivity of the physiologic response. In its simplest terms, the LBD can be thought of as a molecular switch that, upon binding ligand, shifts the receptor to a transcriptionally active state. It is unknown whether these domains evolved independently of

each other or whether the first members of the family were simply constitutive transcription factors. Nonetheless, the ancient association of an LBD with a DBD has proven to be a highly successful union. Indeed, the success of this family is reflected by the observation that evolution has selected nuclear receptors as the dominant mediator of organ physiology as well as the regulator of insect morphogenesis. Because there are other intracellular receptor signaling pathways, such as sterol-regulatory element–binding protein for cholesterol (Wang et al., 1994) and the arylhydrocarbon receptor for dioxin (Burbach et al., 1992), the discovery of a single family of receptors for steroids, retinoids, and thyroid hormones was unexpected. Appar-
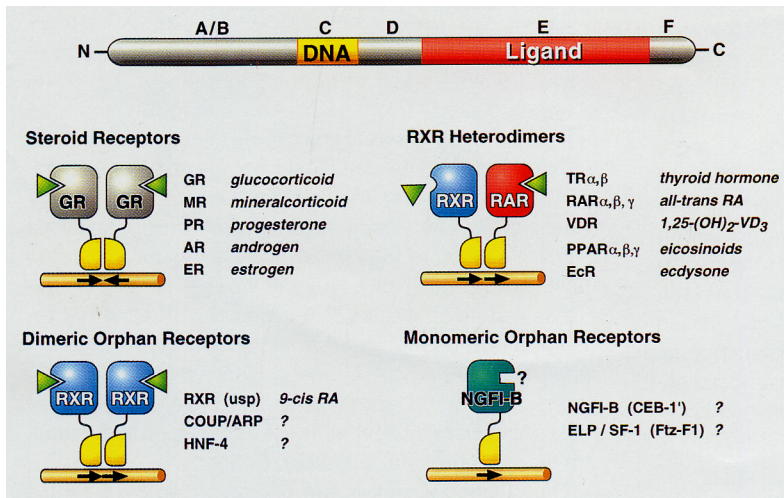

Figure 2. Nuclear Receptors Share Common Structure/Function Domains

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.

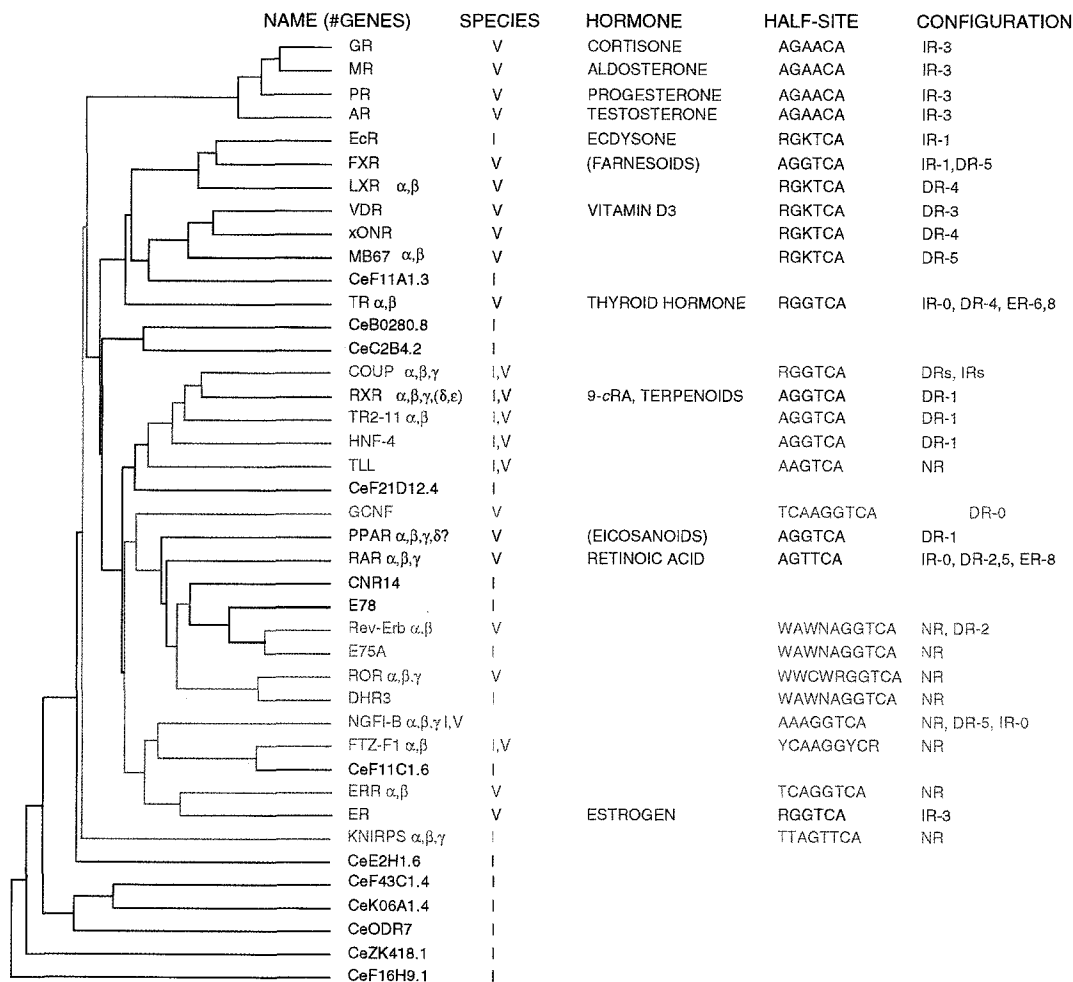| NAME (#GENES)	SPECIES	HORMONE	HALF-SITE	CONFIGURATION
GR	V	CORTISONE	AGAACA	IR-3
MR	V	ALDOSTERONE	AGAACA	IR-3
PR	V	PROGESTERONE	AGAACA	IR-3
AR	V	TESTOSTERONE	AGAACA	IR-3
EcR	I	ECDYSONE	RGKTCA	IR-1
FXR	V	(FARNESOIDS)	AGGTCA	IR-1,DR-5
LXR α,β	V		RGKTCA	DR-4
VDR	V	VITAMIN D3	RGKTCA	DR-3
xONR	V		RGKTCA	DR-4
MB67 α,β	V		RGKTCA	DR-5
CeF11A1.3	I			
TR α,β	V	THYROID HORMONE	RGGTCA	IR-0, DR-4, ER-6,8
CeB0280.8	I			
CeC2B4.2	I			
COUP α,β,γ	I,V		RGGTCA	DRs, IRs
RXR α,β,γ,(δ,ε)	I,V	9-cRA, TERPENOIDS	AGGTCA	DR-1
TR2-11 α,β	I,V		AGGTCA	DR-1
HNF-4	I,V		AGGTCA	DR-1
TLL	I,V		AAGTCA	NR
CeF21D12.4	I			
GCNF	V		TCAAGGTCA	DR-0
PPAR α,β,γ,δ?	V	(EICOSANOIDS)	AGGTCA	DR-1
RAR α,β,γ	V	RETINOIC ACID	AGTTCA	IR-0, DR-2,5, ER-8
CNR14	I			
E78	I			
Rev-Erb α,β	V		WAWNAGGTCA	NR, DR-2
E75A	I		WAWNAGGTCA	NR
ROR α,β,γ	V		WWCWRGGTCA	NR
DHR3	I		WAWNAGGTCA	NR
NGFI-B α,β,γ I,V			AAAGGTCA	NR, DR-5, IR-0
FTZ-F1 α,β	I,V		YCAAGGYCR	NR
CeF11C1.6	I			
ERR α,β	V		TCAGGTCA	NR
ER	V	ESTROGEN	RGGTCA	IR-3
KNIRPS α,β,γ	I		TTAGTTCA	NR
CeE2H1.6	I			
CeF43C1.4	I			
CeK06A1.4	I			
CeODR7	I			
CeZK418.1	I			
CeF16H9.1	I			

Figure 3. Known Members of the Nuclear Receptor Superfamily

The dendrogram represents the relationship among known nuclear hormone receptors prepared from a multiple sequence alignment (Feng and Doolittle, 1987); it is not an evolutionary tree. Amino acid sequences from the entire DBD (including 16 flanking C-terminal residues) were aligned using the University of Wisconsin Genetics Computer Group program PILEUP. Receptors classified according to the scheme in Figure 2 are highlighted here in color: steroid receptors in red, RXR heterodimers in blue, homodimeric orphan receptors in green, and monomeric orphan receptors in brown. Receptors for which a classification scheme is lacking are colored black. Gene designations in parenthesis, e.g., RXR (δ, ε), indicate that these subtypes do not bind hormone. Species designations refer to whether the receptor has been identified in invertebrates, vertebrates, or both. Hormone activators given in parenthesis have not yet been proven to bind receptor. Hormone response elements are configured as direct repeats (DR), inverted repeats (IR), everted repeats (ER), or nonrepeats (NR) and are spaced by the given number of nucleotides. A maximum of two GenBank accession numbers (listed below) were allowed for each receptor type and species; we apologize to authors whose accession numbers could not be cited owing to this space limitation.

For accession numbers and receptor types, species designations are as follows: f, zebrafish or other fish; a, Xenopus or other amphibian; c, chick or other avian; m, mouse; r, rat; h, human.

Vertebrate receptors are GR (X72211, a; X04435, m; M14053, r; X03225, h); MR (U15135 and U15133, a; M36074, r; M16801, h); AR (X58955, a; L25901, c; X53779 and X59592, m; M20133, r; M20132 and M21748, h); PR (M37518, c; M68915, m; L16922, r; M15716, h); ER (M31559 and D28954, f; L20736, a; X03805, c; M38651, m; X61098, r; X03635 and M11457, h); RARα (L03398 and L03399, f; L11445 and S53907, a; M60909, m; S77802, r; X06614 and X06538, h); RARβ (X59473 and X57340, c; X57528 and M60909, m; Y00291 and X07282, h); RARγ (S74156 and L03400, f; L11444 and X59396, a; X73973, c; X15848 and M34475, m; S77804, r; M24857 and M57707, h); TRα (D16461 and D16462, f; M35343 and M35344, a; Y00987, c; X51983 and X07750, m; M18028 and M31174, r; M24748 and M24899, h); TRβ (D45245, f; M35359 and M35361, a; M65207 and X17504, c; S62756, m; J03819 and J03933, r; M26747, h); VDR (U12641, c; D31969, m; J04147, r; J03258, h); PPARα (M84161, a; X57638, m; M88592, r; L02932 and S74349, h); PPARβ/δ (M84162, a; U10375 and L28116, m; U10375, h); PPARγ (M84163, a; U10374 and U09138, m; L40904, h); LXRα (U11685, r; U22662, h); LXRβ (U09419, m; U14533 and U20389, r; U14534 and U07132, h); MB67 (Z30425 and L29623, h); FXR (U09416 and U09418, m; U18374, r); xONR (X75163, a); NGFI-Bα (X70700, a; J04113 and X60132, m; U17254, r; L13740 and D49728, h); NGFI-Bβ (S53744, m; L08595, c; X75918 and S77154, h); NGFI-Bγ (D38530, r; U12767, h); RORα (U22437, m; L14611 and U04897, h); RORβ (L14160, r; T26966, h); RORγ (H33887, r; U16997, h); REV-ERBα (X86010, m; M25804, r; M24898 and X72631, h); REV-ERBβ (U12142 and U09504, m; U20796 and X82777, r; L31785, h); RXRα (U29940, f; L11446, a; M84817 and X66223, m; L06482, r; X52773, h); RXRβ (S73269, a; X66224 and D21831, m; M81766, r; M84820 and X63522, h); RXRγ (U29894, f; L11443, a; X58997, c; X66225 and M84819, m; S77808, r); RXRδ (U29941, f); RXRε (U29942, f); COUPα (X70299 and X70300, f; X63092, a; U07625 and X74134, m; U10995, r; X12795 and X16155, h); COUPβ (L25674 and X76654, m; X12794, h); COUPγ (U00697, c; X76653, m; M64497 and M62760, h); TR2α (M29960, h); TR2β (L27513, r; L27586 and U10990, h); TLX (S72373, c; S77482, m); HNF4 (D29015, m; X57133, r; X76930, h); ERRα (X51416, h); ERRβ (X51417, h); GCNF (U14666 and U09563, m); Ftz-F1α (S65876, m; D42152, r; U32592, h); Ftz-F1β (U05001 and U05002, m; M81385, m).

Drosophila receptors are ECR (M74078); USP (X53417); SVP (M28863); XR78E/F (U31517); E78 (U01807); TLL (M34639); E75A (X51548); DHR38 (X89246); FTZ-F1α (M63711); FTZ-F1β (L06423); dHNF4 (PIR accession number S36218); DHR3 (M90806); Knirps (X13331); KNRL (X14153); EGON (X16631).

C. elegans receptors are CeF11C1.6 (Z54270); CeC2B4.2 (Z50004); Ce21D12.4 (U23518); Ce2H1.6 (Z47075); CeF11A1.3 (Z50857); CeZK418.1 (U00047); CeK06A1.4 (U23449); CeF21D12.4 (U23518); CeF16H9.1 (Z50005); CNR-14 (P41830); CHR-3 (P41828); CNR-8 (P41829); CeF43C1.4 (Q09565); CeODR7 (P41933); CeB0280.8 (P41999).

ently the evolutionary drive for a single receptor family came from the suitability of the receptors to control physiology rather than the chemistry of the regulators.

By 1990 a total of 15 members of the superfamily had been identified as receptors for all the known fat-soluble hormones. Today, there are more than 150 different members of the family, spanning a large diversity of animal species from worm to insect to human. While the availability of purified hormones and antibodies enabled the discovery of the first receptors, low stringency hybridization screening and genetic and molecular cloning techniques have allowed the identification of numerous members of the family for which there are no apparent ligands. These so-called orphan receptors are found in every metazoan species, and it can be expected that some may interact with novel ligands, while others may represent constitutive activators/repressors or factors whose activity is modulated by posttranslational modification.

Figures 2 and 3 are diagrammatic representations of the structure/function and sequence relationships among the members of the nuclear hormone receptor superfamily. We use the term superfamily to encompass all of the known nuclear hormone receptors. The superfamily is often further divided into the steroid receptor family and the thyroid/retinoid/vitamin D (or nonsteroid) receptor family. Each type of receptor constitutes a subfamily (e.g., the retinoic acid receptor [RAR] subfamily). Receptor subtypes are the products of individual genes (e.g., RARα, RARβ, RARγ), and receptor isoforms are the products of alternate splicing, promoter usage, or both (e.g., RARα1, RARα2).

The nuclear receptor superfamily can be broadly divided into four classes based on their dimerization and DNA-binding properties (Figure 2; see also Stunnenberg, 1993). Class I receptors include the known steroid hormone receptors, which function as ligand induced homodimers and bind to DNA half-sites organized as inverted repeats. Class II receptors heterodimerize with RXR and characteristically bind to direct repeats (although some bind to symmetrical repeats as well). Exclusive of the steroid hormones, this group includes all other known ligand-dependent receptors. Class III receptors bind primarily to direct repeats as homodimers. Class IV receptors typically bind to extended core sites as monomers. Most of the orphan receptors fall into class III and IV categories.

The dendrogram in Figure 3 summarizes the similarity among the receptors by sequence. It was constructed by selecting a single member of each receptor subfamily as representative and then producing a multiple sequence alignment based on their DBD sequences (for an alternative evolutionary comparison of the receptors, see Gronemeyer and Laudet, 1995). Several themes emerge from a consideration of Figure 3. For example, when the different functional classes of receptors described in Figure 2 are highlighted in color in Figure 3, a clear relationship between receptor function and sequence can be seen. Interestingly, most class II receptors (shown in blue in Figure 3) are ligand dependent, and it seems likely that the remaining members of this family may also have ligands.

Another potentially significant point is the great diversity in the rapidly expanding list of Caenorhabditis elegans receptors (shown in black in Figure 3). Although little is known about these receptors, one might speculate that some of these new receptor subtypes will be represented in vertebrates and that the nuclear receptor superfamily will continue its explosive growth in the future.

The purpose of the ensuing reviews will be systematically to discuss the major discoveries as well as the emerging concepts that have come from the last 10 years of study on the members of this superfamily. Beato et al. review the steroid hormone receptor family, which, although they are taken as the archetype for this superfamily, are in fact a specialized branch that has only recently evolved in vertebrates. The review by Mangelsdorf and Evans focuses on the RXR heterodimers and orphan receptors, both from a structural and mechanistic point of view. Kastner et al. review the recent efforts to characterize receptor function genetically via homologous recombination, which supports many earlier studies on hormone deficiency syndromes as well as revealing many unanticipated developmental and physiologic effects. Finally, Thummel reviews advances in nonvertebrate Drosophila receptors, which establish a clear unity between the vertebrates and invertebrates and point out common genetic and mechanistic features underlying animal development and physiology. We have come to realize in the reviewing process that the progress of the field is so great that it is not possible to review any one area in exhaustive detail. The readers will be referred to many other excellent reviews that deal with portions of these subjects in more detail. Rather, the goal of this series is to present an overview of the progress of the last 10 years and to chart the course for future research. Finally, we hope this series of reviews provides a realization that we are in the midst of an explosion in our understanding of hormonal signaling and that the next 10 years is likely to be as exciting and dramatic as the first.

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