Endocrine, vol. 4, no. 2, 91-105, April 1996 0969-711X/96/4:91-105/$8.75

REVIEW

The Vitamin D_3 Receptor in the Context of the Nuclear Receptor Superfamily

The Central Role of the Retinoid X Receptor

Carsten Carlberg

Clinique de Dermatologie, Hôpital Cantonal Universitaire, Genève, Switzerland

The nuclear hormone 1α,25-dihydroxyvitamin D_3 (VD) is an important regulator of calcium homeostasis and is also a modulator of the cell cycle. The genomic actions of the hormone are mediated by a single transcription factor, the vitamin D_3 receptor (VDR). On the majority of the known VD response elements, VDR binds as heterodimeric complex with the retinoid X receptor (RXR), which is a member of the nuclear receptor superfamily like VDR. RXR supports not only the DNA binding affinity and specificity of VDR, but allosterically also its transactivation properties. Moreover, RXR is a partner in other hormone response systems, which supports the idea that the different nuclear hormone signaling pathways are functionally linked.

Key Words: Regulation of transcription; vitamin D receptor; retinoid X receptor; nuclear receptor heterodimers; transactivation; network of nuclear receptors.

Introduction

Vitamin D_3 is a biologically inert seco-steroid with cleaved B-ring, which is either obtained from diet or synthesized in the skin from 7-dehydrocholesterol. It is converted into its biologically active form, 1α,25-dihydroxyvitamin D_3, by hydroxylation at carbon 25 in the liver and by hydroxylation at carbon 1 in the kidney. In the 1920s, VD had been classified as vitamin because it could be given as a supplement to the diet to correct deficiency that produce diseases. However, VD was reclassified as a hormone through the discovery of its carefully regulated production in kidney (Fraser and Kodicek, 1970) and the purification of a nuclear receptor for VD from intestine (Haussler and Norman, 1969). As a small hydrophobic molecule, VD is able to cross passively biological membranes, so that the molecular cloning of VDR from chicken (McDonnell et al., 1987), humans (Baker et al., 1988), and rat (Burmester et al., 1988) about 8 yr ago and its classification as a member of the superfamily of ligand-activated transcription factors (Evans, 1988; Green and Chambon, 1988; O'Malley, 1990) made clear that VD is a nuclear hormone and a direct regulator of gene transcription.

VDR has first been identified in the intestine, kidney, and bone (for review, *see* DeLuca et al., 1990; Pike, 1991; Walters, 1992), and its classical action is to maintain calcium homeostasis by coordinating calcium transport from intestine and kidney to the bloodstream and supporting bone calcium mineralization. Involved in this events are the VDR-regulated proteins, e.g., calbindin D_{9k}, calbindin D_{28k}, osteocalcin, osteopontin, and carbonic anhydrase II.

Within the last 20 yr, VDR was also found in numerous other (nonclassical) tissues, e.g., in the hematopoietic system (T-, and B-cells, macrophages, and monocytes), in the reproductive system (uterus, testis, ovary, prostate, placenta, and mammary gland), in the endocrine system (adrenal cortex, pancreas, pituitary, and thyroid), in skeletal, smooth, and heart muscle, in the brain, in the skin, and in the liver (for review, *see* Walters, 1992). Therefore, VDR is nearly ubiquitously expressed, which expands the concept of the role of VD from that of a hormone of calcium metabolism to that of a more general regulatory agent. The endocrinology of VD gained a new perspective with the findings that VD can induce the differentiation of monocytes (Mangelsdorf et al., 1984) into macrophages, granulocytes, and osteoclasts, and that it can inhibit the proliferation of cancer cells (Eisman et al., 1987). This indicates that VD, similar to retinoids, is a modulator of the cell cycle. The antiproliferative effect of VD was first observed in vitro, but had been confirmed later on in vivo, and provided the hormone with an interesting therapeutical potential. In contrast to retinoids that have strong side effects, for VD, only the problem of hypercalcemia has to be controlled.

Received December 18, 1995; Accepted January 23, 1996.

Author to whom all correspondence and reprint requests should be addressed: Carsten Carlberg, PhD, Clinique de Dermatologie, Hôpital Cantonal Universitaire, CH-1211 Genève 14, Switzerland, E-mail: carlberg-carsten@diogenes.hcuge.ch

The purpose of this article is to summarize recent reports on the actions of VDR that provide a new understanding of the role of VD in molecular endocrinology. The retinoid X receptor, which is also a member of the nuclear receptor superfamily, appears to play a central role in VD signaling. However, RXR is also a partner in other hormone response systems, which supports the idea that the different nuclear hormone signaling pathways are functionally linked. Heterodimerization of nuclear receptors produces novel complexes that bind DNA with higher affinity or altered specificity, and a key question is: how much does each receptor monomer contribute to the transcriptional properties of the complex? Thus, the action of VDR-RXR heterodimers will be discussed in the context of the complex network of nuclear receptor superfamily.

The Nuclear Receptor Superfamily

In addition to VDR, the cDNAs of virtually all nuclear receptors for steroid hormones (glucocorticoids, GR [Hollenberg et al., 1985; Miesfeld et al., 1986]; mineralocorticoids, MR [Arriza et al., 1987]; estrogen, ER [Green et al., 1986]; progesterone, PR [Gronemeyer et al., 1987], and androgen, AR [Chang et al., 1988]), thyroid hormone (T_3, T_3R. [Sap et al., 1986; Thompson et al., 1987]) and all-trans retinoic acid (RA, RAR [Giguère et al., 1987; Petkovich et al., 1987]) have been cloned in the second half of the 1980s. Comparison of the amino acid sequences of these receptors showed that they are structurally related and comprise a superfamily of nuclear receptors (Evans, 1988; Green and Chambon, 1988; O'Malley, 1990). All family members are composed of modular domains (Giguère et al., 1986; Kumar et al., 1987) (Fig. 1), which are the highly conserved DNA-binding domain (DBD) of 66–70 amino acids in the center region or close to the amino-terminus, the less-well-conserved ligand-binding domain (LBD) of more than 200 amino acids in the hydrophobic carboxy- terminal half and the, in sequence and size, highly variable amino-terminal domain. The DBD allows protein–DNA as well as protein–protein interactions (Freedman, 1992; Freedman and Luisi, 1993), and in addition to ligand recognition, the LBD encodes receptor dimerization and transactivation or repressor functions. The DBD and the LBD are separated by a hinge region that allows free rotation of the two domains relative to each other. With 427 amino acids, VDR is one of the smallest members of the nuclear receptor superfamily; in particular, with only 23 amino acids, its amino-terminal domain is very condensed. In contrast to T_3Rs (Lazar, 1993) and RARs (Giguère, 1994), only one subtype and also only one isoform of VDR have been identified so far.

Based on different cloning strategies, e.g., low stringency crosshybridization using previously identified cDNAs as probes or biochemical purification, an ever-growing number of novel members of the nuclear receptor superfamily have been identified over the past years (O'Malley and Conneely, 1992). Consequently, when initially identified, these receptors had no known ligand and usually no known function; therefore, they are called orphan nuclear receptors. Most prominent orphans are RXR (Hamada et al., 1989; Mangelsdorf et al., 1990), chicken ovalbumin upstream promoter transcription factor (COUP-TF; Wang et al., 1989), peroxisome proliferator-activated receptor (PPAR; Issemann and Green, 1990), nerve growth factor I-B (NGFI-B; Milbrandt, 1988),*fushi tarazu* factor 1 (FTZ-F1; Lavorgna et al., 1991), reverse ErbA (Rev-ErbA; Lazar et al., 1989; Miyajima et al., 1989), and retinoid Z receptor (RZR/ROR; Becker-André et al., 1993; Carlberg et al., 1994a; Giguère et al., 1994).

The DBD of each nuclear receptor contains eight conserved cysteine residues that form two modules that tetrahedrally each coordinate a zinc ion, resulting in the formation of two so-called zinc fingers, more properly referred to as class II zinc finger-binding motifs (Freedman, 1992; Freedman and Luisi, 1993). Structural analysis demonstrated that the zinc fingers form structures that contact the major grove of a hexameric sequence called core binding motif (Luisi et al., 1991; Schwabe et al., 1993). Most of the hormone response elements, which have been identified in the promoter of hormone responding genes, consist of two such core binding motifs. In the remaining response elements, either only one or even multiple core binding motifs have been found (Glass, 1994). This suggests that nuclear receptors can bind to DNA either as monomers, dimers, or even multimers. However, multimeric nuclear receptor complexes have not yet been very extensively studied, and it might be possible that its components bind noncooperatively in close vicinity as monomers or dimers. Most of the ligand-activated nuclear receptor do not have sufficient affinity to bind as a monomer to a single core binding motif. Thus, they have to find a partner, which can be either the same or a different type of nuclear receptor, in order to bind cooperatively as homo- or heterodimer to DNA. Therefore, their respective response elements consist of two core binding motifs in a relative orientation, and a distance that provides optimal protein–DNA and protein–protein contacts.

The modes of DNA binding as homodimers, heterodimers, or monomers allow a simple classification of the members of the nuclear receptor superfamily into one of three following groups (Laudet et al., 1992; Yu et al., 1992; Glass, 1994):

1. The first group consists of the classical steroid hormone receptors that form exclusively homodimers as first demonstrated in the cases of ER (Kumar and Chambon, 1988; Farwell et al., 1990) and GR (Tsai et al., 1988). Steroid hormone response elements generally consist of a palindromic arrangements of two core binding motifs. Each

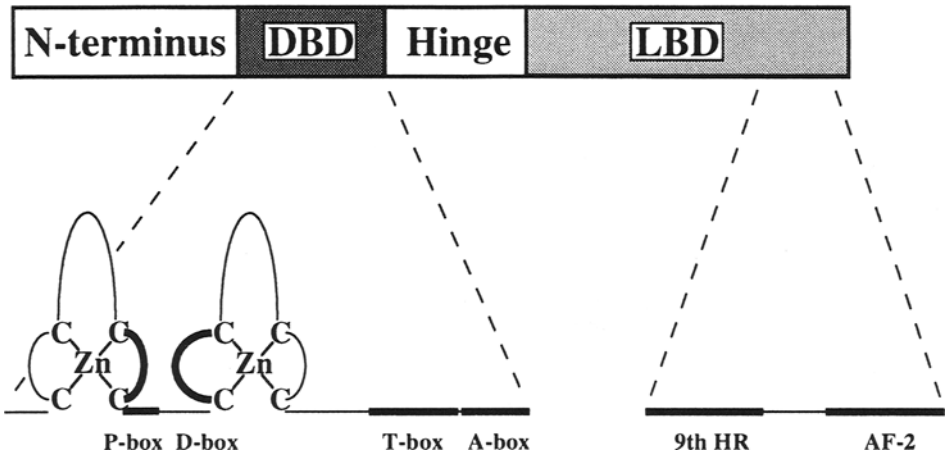

Fig. 1. Modular organization of nuclear receptors. Nuclear receptors are characterized by their highly conserved DBD, which is formed by two zinc finger structures. The less conserved LBD is linked via a hinge region to the DBD. The amino (N)-terminus is highly divergent in sequence and size. The locations of important sequence motifs, in the DBD and LBD are schematically depicted. HR indicates heptad repeat.

DBD of the dimer makes analogous contacts with one of the core binding motifs, resulting in a rotationally symmetric structure (Luisi et al., 1991; Schwabe et al., 1993).

2. The second group consists of the remaining nuclear receptors that have a defined ligand, i.e., RARs, T_3Rs and VDR. These receptors bind as heterodimers with RXR to response elements consisting of two directly repeated core binding motifs (Yu et al., 1991; Bugge et al., 1992; Kliewer et al., 1992b; Leid et al., 1992; Marks et al., 1992; Zhang et al., 1992a), i.e., they form an asymmetric complex.
3. The third group comprises the orphan receptors, of which the majority was initially assumed to bind DNA as monomers (O'Malley and Conneely, 1992; Laudet and Adelmant, 1995).

Recent reports have shown that members of the second and third group are able to form homodimers, and that some orphan receptors also form heterodimers with RXR (Table 1). Consequently, the classification of receptors from the second and third group should be reconsidered.

The RXR

In 1990, Mangelsdorf et al. (1990) observed that an orphan receptor could be activated by micromolar concentrations of all-trans RA. Therefore, they postulated that its natural ligand was likely to be a novel retinoid and called the receptor RXR. This prediction was confirmed by the identification of 9-cis RA as the ligand of RXR (Heyman et al., 1992; Levin et al., 1992). Similar to RARs (Giguère, 1994), three RXR subtypes (RXRα, RXRβ, and RXRγ; Mangelsdorf et al., 1992) have been identified, which are all bound and activated by nanomolar concentrations of 9-cis RA (Mangelsdorf et al., 1992; Allenby et al., 1993, 1994). The three RXR genes are widely expressed in adult tissues, and at least one RXR subtype is present in every tissue examined (Mangelsdorf et al., 1992). 9-cis RA has been detected in low nanomolar concentrations in kidney and liver (Heyman et al., 1992), which strengthens the suggestion that it is a natural hormone. However, 9-cis RA appears to be a bifunctional ligand, since it binds also the three RAR subtypes with even higher affinity than RXRs (Allenby et al., 1993, 1994). The metabolic pathway leading to the synthesis of 9-cis RA is unknown, but in analogy with the visual system that uses 11-cis RA, it could involve a specific isomerase (Bernstein et al., 1987). Interestingly, the cellular all-trans RA-binding proteins CRABP-I and CRABP-II do not bind 9-cis RA (Allenby et al., 1993), which suggests the existence of a metabolic pathway for 9-cis RA that is distinct from that of all-trans RA.

Recently it was reported (Harmon et al., 1995) that methoprene acid, which is used as a pesticide, binds and activates RXRs, but not RARs. Methoprene acid is not a naturally occurring compounds, but it may have a natural counterpart in vertebrates. This opens up again the question concerning whether a natural RXR ligand may exist that is more specific than 9-cis RA.

Hormone Response Elements

The first response elements have been identified for steroid hormone receptors; they are composed of two copies of the hexameric core binding motif AGGAGA or AGGTCA (for ER), which are arranged as palindromes, i.e., mirror symmetric structures (Klock et al., 1987). This symmetric structure suggested that the receptors bind to the element as homodimers. Three amino acids at the base of the first zinc finger of the DBD, referred to as P-box (Danielsen et al., 1989; Mader et al., 1989; Umesono and Evans, 1989) (Fig. 1), specify the core binding motif recognition of a nuclear receptor. Most members of the superfamily recognize the motif RGGTCA (R = A or G); however, naturally occurring core binding motifs usually

Table 1
Homo- and Heterodimeric Interactions
of the Members of the Second and Third Group of the Nuclear Receptor Superfamily
with Direct Repeats Spaced by One to Eight Nucleotides

Response element	Heterodimer I	Homodimer	Heterodimer II
DR1	RAR-RXR[b]	$(RXR)_2$[c]	RXR-PPAR.*[d]
		$(HNF\text{-}4)_2$[e]	RXR-COUP-TF*[f]
			RXR-FXR*[g]
DR2	RXR-RAR[h,i]	$(Rev\text{-}ErbA)_2$[j]	PPAR-T_3R*[k]
DR3	**RXR VDR**[l,m]	$(VDR)_2$[n,o]	RXR-ONR*[p]
	T_3R-VDR[q]		
	VDR-RXR[m]		
DR4	RXR-T_3R[r,s]	$(T_3R)_2$[t]	RXR-RLD-1*[u]
	VDR-T_3R[q]		RXR-UR*[v]
			RXR-OR-1*[w]
			RXR-LXRα*[x]
			RXR-RIP14*[y]
			RXR-RIP15*[y]
DR5	RXR-RAR[h,i,r,s]	$(RAR)_2$[z,aa,bb]	RXR-NGFI-B*[cc]
	VDR-RAR*[dd,ee]		RXR-MB67*[ff]
DR6	RAR-VDR*[dd,ee]	$(VDR)_2$[gg]	
DR8		$(RZR)_2$[hh]	

[a] The heterodimeric interactions of the three classical members of the second group (VDR, T_3R and RAR) with RXR are summarized as heterodimer I, and VDR-RXR heterodimers are indicated in bold. Heterodimer II lists the heterodimeric interaction of members of third group. The order of the heterodimers should indicate the experimentally proven or assumed (*) polarity. Respective references are indicated. [b] Kurokawa, R., et al. (1994). [c] Zhang, X.-K., et al. (1992b). [d] Kliewer, S. A., et al. (1992c). [e] Jiang, G., et al. (1995). [f] Kliewer, S. A., et al. (1992). [g] Forman, B. M., et al. (1995b). [h] Zechel, C., et al. (1994). [i] Predki, P. F., et al. (1994). [j] Harding, H. P. and Lazar, M. A., (1995). [k] Bogazzi, F., et al. (1994). [l] Schräder, M., et al. (1995). [m] Quélo, I., et al. (1994). [n] Towers, T.L., et al. (1993). [o] Cheskis, B. and Freedman, (1994). [p] Smith, D.P., et al. (1994). [q] Schräder, M., et al. (1994c). [r] Kurokawa, R., et al. (1993). [s] Perlmann, T., et al. (1993). [t] Piedrafita, F.J., et al. (1995). [u] Apfel, R., et al. (1994). [v] Song, C., et al. (1994). [w] Teboul, M., et al. (1995). [x] Willy, P.J., et al. (1995). [y] Seol, W., et al. (1995). [z] Carlberg, C., et al. (1993b). [aa] Carlberg, C. (1993). [bb] Schräder, M., et al. (1993b). [cc] Perlmann, T. and Jansson, L. (1995). [dd] Schräder, M., et al. (1993a). [ee] Schräder, M., et al. (1994). [ff] Baes, M., et al. (1994). [gg] Carlberg, C., et al. (1993a). [hh] Carlberg, C., et al. (1994a).

exhibit some variation from this consensus sequence. Furthermore, two groups of amino acids, called T-box and A-box (Fig. 1), which have been identified as the carboxy-terminal of the second zinc finger, are critical for the recognition of nucleotides 5'-preceding the core binding motif (Wilson et al., 1992). A 5'-extension of the core binding motif increases the specificity and affinity of DNA binding, and appears to be particularly important for nuclear receptors that bind as monomers to DNA. However, nuclear receptors that typically dimerize also have these conserved amino acid regions.

In analogy to the response elements, which were identified for the first group of the nuclear receptor superfamily, the members of the second group had initially also been believed to bind as homodimers to palindromic arrangements of core binding motifs, e.g., to the idealized synthetic palindromic T_3 response element (TRE_{pal}) (Umesono et al., 1988). However, the first identified natural response elements for VD in the human osteocalcin promoter (Kerner et al., 1989; Morrison et al., 1989), for T_3 in the Maloney murine leukemia virus (Sap et al., 1989), and for all-trans RA in the RARβ2 promoter (de Thé et al., 1990) displayed directly repeated core binding motif arrangements. Sequence comparisons and point mutational analyses of natural and synthetic hormone response elements by Umesono et al. (1991) predicted a code for the DNA-binding specificity of VDR, T_3R, and RAR; their specific response elements should be direct repeats of two RGGTCA motifs spaced by 3, 4 or 5 nucleotides, respectively. This genius and simple 3-4-5 rule is a widely accepted model

and had major influence on the consequent identification of natural response elements.

Four features of a response element regulate the specificity of DNA recognition by nuclear receptors: the precise sequence of the core binding motifs, their relative orientation, their spacing, and their 5'-preceding sequence. To date, we are aware of 19 functional VD response elements that have been identified within the promoter region of primary VD responding genes (Carlberg, 1995). In accordance with the 3-4-5 rule, 11 of them are direct repeats spaced by three nucleotides (DR3-type VD response elements), but it has to be noted that their core binding motifs are in part rather divergent from the RGGTCA consensus. In the regulatory regions of rat Pit-1 (Rhodes et al., 1993) and of mouse calbindin D_{28k} (Gill and Christakos, 1993), VD response elements with a DR4-type structure have been identified. Furthermore, functional VD response elements that display a DR6-type structure have been found in the promoter regions of human osteocalcin (Schüle et al., 1990; Carlberg et al., 1993a), rat 24-hydroxylase (Kahlen and Carlberg, 1994) and human, rat, and mouse fibronectin (Polly et al., 1995). Recently, the first natural VD response elements have been identified that have an inverted palindromic arrangement of two core binding motifs that are spaced by nine nucleotides (IP9-type VD response elements) (Schräder et al., 1995).

Heterodimerization of Nuclear Receptors

In vitro assays for the binding of in vitro translated or purified bacterial- or baculovirus-expressed VDR on VD response elements indicated that they show reasonable affinity only at high protein concentrations (MacDonald et al., 1991; Carlberg et al., 1993a; Cheskis and Freedman, 1994). However, the in vitro DNA-binding affinity of VDR could clearly be enhanced by the addition of nuclear extracts containing a nuclear assessory factor (NAF) (Liao et al., 1990; Sone et al., 1991; Ross et al., 1992). Similar results were obtained with T_3Rs (Murray and Towle, 1989; Burnside et al., 1990), and purification of this cofactor by Yu et al. (1991) and Leid et al. (1992) showed that it is at least in part identical with RXR. Also the NAF for VDR appears to be in most (MacDonald et al., 1993; Munder et al., 1995), but not in all cases (Jääskeläinen et al., 1995), one of the three RXRs.

It was shown that in vitro, VDR, T_3Rs, and RARs form a heterodirmeric complex with RXR that efficiently binds to DNA (Yu et al., 1991; Bugge et al., 1992; Kliewer et al., 1992b; Leid et al., 1992; Marks et al., 1992; Zhang et al., 1992a). During the last 3 yr the formation of heterodimers with RXR was also shown for the orphan receptors PPAR (Kliewer et al., 1992c), ear2/ARP-1/COUP-TF (Kliewer et al., 1992c; Widom et al., 1992), RIP14/FXR (Forman et al., 1995a; Seol et al., 1995), ONR-1 (Smith et al., 1994), RLD-1/UR/OR-1/LXRα/RIP15 (Apfel et al., 1994; Song et al., 1994; Seol et al., 1995; Teboul et al., 1995; Willy et al., 1995), MB67 (Baes et al., 1994), and NGFI-B/Nurr-1 (Forman et al., 1995a; Perlmann and Jansson, 1995) (Table 1). Therefore, heterodimerization with RXR is considered important not only for VDR and other ligand-activated nuclear receptors, but also for more and more orphan receptors.

In general, heterodimerization is an elegant way to generate with a limited number of transcription factors, various, functionally different complexes that may explain the multiplicity of nuclear signaling by each of these factors (Laudet and Stehelin, 1992; Green, 1993). Furthermore, heterodimerization of two ligand-activated nuclear receptor, e.g., VDR and RXR, associates their respective nuclear signaling pathways.

VDR-RXR heterodimers bind to most of the natural DR3-type VD response elements (Carlberg, 1995) to the DR4-type response element of the Pit-1 enhancer (Rhodes et al., 1993), and to the two IP9-type response elements of the rat osteocalcin and the human calbindin D_{9k} gene (Schräder et al., 1995). However, so far no VDR-RXR heterodimers have been detected on DR6-type VD response elements. These structures are bound by VDR homodimers, which in the case of the human osteocalcin and the rat 24-hydroxylase VD response element, appear to compete with VDR-RAR heterodimers (Schräder et al., 1993a; Kahlen and Carlberg, 1994). Moreover, on the DR3-type VD response element of rat calbindin D_{9k} and on the DR4-type element of mouse calbindin D_{28k}, the formation of VDR-T_3R heterodimers was reported (Schräder et al., 1994c).

Taken together, VDR-RXR heterodimers show on most VD response elements strong cooperative interaction and bind DNA with a dissociation constant (K_d) of 0.5–1 n*M* (Schräder et al., 1994b, 1995), i.e., with higher affinity than the three other VDR complexes bind to DNA; they display K_d values of 1.5–5 n*M* (Schräder et al., 1994b and unpublished results of the author's laboratory). This emphasizes the central, but not unique role of VDR-RXR heterodimers in VD signaling.

Dimer Interfaces in the LBD

Within the LBDs of most nuclear receptors, a series of up to nine conserved heptad repeats of leucine and other hydrophobic residues has been identified. The "regulatory zipper model" (Forman and Samuels, 1990) suggests that these heptad repeats form a coiled-coil dimerization surface in analogy with the interface found in the leucine zipper and helix-loophelix transcription factors. In particular, the ninth heptad repeat (Fig. 1) has been shown to be critical for the dimerization with RXR (Zhang et al., 1994; Leng et al., 1995).

In the VDR, already minor carboxy-terminal truncations result in markedly decreased ligand binding, dimerization, and transactivation. Nakajiama et al. (1994) found that a carboxy-terminal truncation of 46 amino acids or point mutations at lysine-382, methionine-383, glutamine-385,

or leucine-390 abolished the ability of VDR to heterodimerize with RXR. This is the region of the ninth heptad repeat (amino acids 382–388). In addition, they found that a truncation of the last 25 amino acids retained heterodimerization, but reduced ligand binding affinity by a factor of 10 and completely abolished transactivation. Nishikawa et al. (1995) confirmed these observations; in their experiments, a carboxy-terminal deletion of more than 21 amino acids abolished ligand binding, but retained most of the dimerization activity, which, however, was lost by truncation of the last 64 amino acids. In addition, they found that homo- and heterodimerization of the VDR-LBD was enhanced by the binding of VD. In summary, these results suggest that transactivation function and high-affinity ligand binding are located between amino acids 407 and 427, which is the region of the AF-2 subdomain (Durand et al., 1994). In addition, Rosen et al. (1993) and Nakajiama et al. (1994) provided evidence that amino acids 244–263 and 325–332 are also important for heterodimerization with RXR. A crystal structure of the VDR-LBD will show whether these two regions directly participate in the dimer interface or whether they only provide architectural support for dimerization.

The crystal structure of the human RXRα LBD has recently been resolved (Bourguet et al., 1995); it is formed by 11 antiparallel α-helices, of which mainly the 10th helix and to a lesser extent also the ninth helix and the loop between helix 7 and helix 8 contribute to the dimer interface. Interestingly, helix 10 encompasses the ninth heptad repeat, but the amino acid residues of the repeat do not directly contribute to the dimer interface. Instead, the residues of all heptad repeats are interspersed throughout the structure to provide architectural support. The high conservation of the heptad repeats within the nuclear receptor superfamily (Forman and Samuels, 1990) indicates that these intramolecular interactions are crucial and suggests that the LBDs of other nuclear receptors are organized similar to that of RXR. In this context, the data about the location of the carboxy-terminal dimer interface of VDR will be reanalyzed and refined.

Dimer Interfaces in the DBD

The primary stabilization of a dimeric nuclear receptor complex is mediated by its carboxy-terminal dimerization interface (Fig. 2), but this appears only to enhance, but not to alter response element selectivity (Gronemeyer and Moras, 1995). Isolated DBDs are not able to dimerize in solution, but their assembly on appropriate response elements is highly cooperative. Bourguet et al. (1995) showed that the two LBDs of an RXR homodimer form a rotationally symmetric structure that has the two amino-terminal ends in close vicinity; this will facilitate the dimerization of the DBDs. Response elements with appropriate core binding motif sequence, spacing, and relative orientation then lead to the formation of highly specific dimer interfaces. However, the binding of the DBDs to all three possible core binding motif arrangements, direct repeats, palindromes, and inverted palindromes can only be achieved by the very flexible hinge region between the DBD and the LBD (Figs. 1 and 2). For homodimers of T_3R, ER, and GR that bound to palindromic, i.e., symmetric, response elements, it had been shown that amino acid residues of the second zinc finger, which encompass the so-called D-box (Umesono and Evans, 1989) (Fig. 1), form a symmetrical dimer interface that sets the different core binding motif spacings.

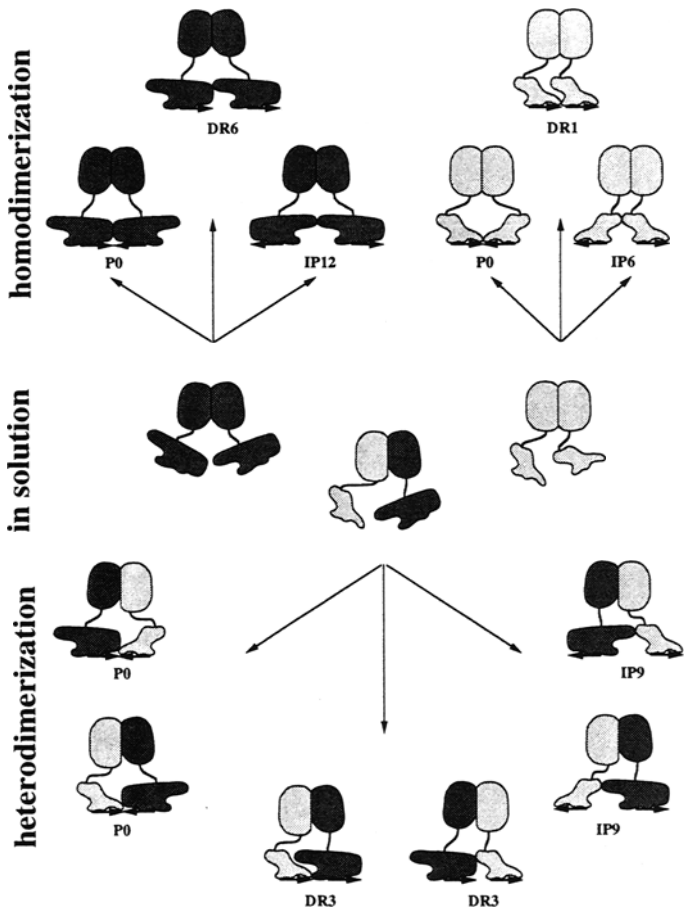

Fig. 2. The spacing-orientation model. The model is exemplified for VDR (dark gray) and RXR (light gray). In solution (center), the receptors form either homodimers (top) or heterodimers (bottom) via interaction of the dimer interface in their LBDs. On appropriate response elements, the complexes are further stabilized via homo- or heterocooperative interactions of their DBDs. The LBD and the DBD are linked via the flexible hinge region that allows free rotation.

The very recently reported crystal structure of T_3R-RXR heterodimer DBDs bound to a DR4-type T_3 response element (Rastinejad et al., 1995) shows how asymmetric dimerization works: the RXR-DBD provides a dimerization surface generated by residues of the second zinc finger, which belong in part to the D-box, and the T_3R-DBD surface comprises residues from a region in front of the first zinc finger (the so-called prefinger), from the first zinc fin-

ger and from the T-box. This study also showed that the specific core binding motif distance of four nucleotides relates to steric hindrance of an α-helix formed by the A-box of T_3R that is strongly involved in DNA binding, but not in the dimer interface. In contrast, through previous nuclear magnetic resonance studies (Lee et al., 1993) an α-helix was discovered in the carboxy-terminal end of the RXR-DBD that encompasses the T-box and appears not to play a role in this heterodimeric complex, but seems to be important for RXR homodimerization on DR1-type response elements.

Further crystallographic and biochemical studies will resolve the details of the homo- and heterodimerization of other nuclear receptor complexes, but it is already obvious that the DBD of each nuclear receptor can provide several surfaces for dimer interfaces. This provides the structural basis for the observed promiscuity in nuclear receptor dimerization (Green, 1993).

The crystal structure of the VDR-DBD has not yet been resolved, but based on the T_3R-RXR heterodimer/DR4-type response element structure, Rastinejad et al. (1995) performed modeling of VDR-RXR heterodimers on a DR3-type response element. They found that the key amino acid residue in the dimerization surface of VDR should be asparagine-37 in the first zinc finger. Interestingly, despite the high conservation of DBDs within the nuclear receptor superfamily, this amino acid is unique for VDR. Lysine-91 and glutamine-92 in the carboxy-terminal T-box contribute also to the contact with RXR. In contrast to T_3R, residues from the prefinger of VDR appear not to interfere in dimerization with RXR. Furthermore, the modeling indicates that, like for T_3R, the spacing requirements of VDR are directed by the steric hindrance of the long α-helix containing the A-box.

Similar predictions had already been made by Towers et al. (1993); by point mutagenesis of the tip of the first zinc finger, of the D-box, and of the T-box, they defined these regions to be important for asymmetric association of VDR-DBDs on direct repeats. The mapping of DNA-independent dimer interfaces in the VDR-DBD performed by Nishikawa et al. (1995) suggested that critical amino acids are contained in the first zinc finger, whereas only for heterodimeric interaction with RXR, but not for homodimerization, the T-box should be important.

The Spacing-Orientation Model

The conclusions of the VDR-RXR heterodimer modeling fit well with a previously proposed, simple two-dimensional model, referred to as "spacing-orientation model" (Carlberg, 1993, 1995; Schräder and Carlberg, 1994; Schräder et al., 1994a) (Fig. 2), which may give a code for homo- and heterocooperative interaction of VDR, RXR, and other nuclear receptors on response elements formed by direct repeats, palindromes, and inverted palindromes. The model is based on the assumption that on direct repeats, the steric hindrance of the receptor monomer on the 3'-motif determines the optimal distance for the interaction with the receptor monomer on the 5'-motif. The T_3R-RXR-DBD crystal structure confirmed this assumption, and indicated that the detailed structure and the relative orientation of the carboxy-terminal α-helix, which contains the A-box, provide each nuclear receptor, once bound to DNA, with a characteristic steric hindrance respective to its partner receptor in 5'-position. In the model, this steric hindrance is called "overhang number." The individual overhang numbers for VDR, RXR, T_3R, and RAR have been determined by in vitro DNA binding and reporter gene assays using a big set of artificial and natural hormone response elements formed by direct repeats, palindromes, and inverted palindromes with various spacing nucleotides. For each receptor, two individual overhang numbers were observed, which are three and six nucleotides for VDR (Carlberg, 1993; Carlberg et al., 1993a; Schräder et al., 1993a, 1994a) (Fig. 2), one and three nucleotides for RXR (Carlberg, 1993; Schräder et al., 1993, 1994a) (Fig. 2), two and four nucleotides for T_3R (Carlberg, 1993; Schräder and Carlberg, 1994), and two and five nucleotides for RAR (Carlberg, 1993; Schräder et al., 1993b; 1994a). The two overhang numbers of each receptor differ either by two or three nucleotides, which corresponds to a relative rotation of the two receptor monomers by 69 or 103°, and suggests that the 3'-receptor presents different DBD surfaces for the formation of the dimer interface. Accordingly, Zechel et al. (1994b) found that in RAR-RXR heterodimers, the RAR- DBD in the 3'-position contacts the RXR-DBD in the 5'-position via the tip of the first zinc finger on DR5-type response elements and via the T-box on DR2-type response elements.

A further assumption of the spacing-orientation model is that the complete DNA-binding unit (DBD and the carboxy-terminal T-box and A-box) binds asymmetrically to DNA, i.e., on direct repeats there should be no steric hindrance toward a 3'-bound receptor. This predicts that on palindromes, where the receptor monomers DBDs have a face-to-face configuration, the core binding motifs do not need spacing nucleotides, whereas on inverted palindromes with a tail-to-tail DBD configuration, the effects of the steric hindrances of both partner receptors should be added (Fig. 2). In accordance with this presumption, binding and functional activity of various dimeric complexes have been observed on P0-type response elements (Umesono et al., 1988; Graupner et al., 1989; Zhang et al., 1992b; Carlberg, 1993; Carlberg et al., 1993a; Schräder and Carlberg, 1994; Schräder et al., 1994a). This points out that palindromic response elements are recognized with high promiscuity, i.e., with low specificity. Therefore, natural VD response elements with a palindromic structure may not have been identified so far, because they were eliminated during evolutionary selection for specificity.

In contrast, response elements with an inverted palindromic structure should display spacings between 2 and 12 nucleotides. The spacing-orientation model does not take into account the helical structure of DNA, i.e., the relative rotation of the receptor monomers in different distances, but it predicted with 9 (= 6 + 3) (Fig. 2) and 7 (= 3 + 4) the right number of intervening nucleotides in inverted palindromes that are optimal for the binding of VDR-RXR and VDR-T_3R heterodimers (Schräder et al., 1994c, 1995).

The binding of T_3R homodimers to inverted palindromes has been reported to have a relaxed spacing preference (Piedrafita et al., 1995), which was generalized as lack of cooperative interaction between DBDs in inverted palindromic arrangements (Glass, 1994). It is clear that in tail-to-tail configuration on inverted palindromes, the DBDs have to use a different dimer interface than in head-to-tail configuration on direct repeats. The mapping of this alternative dimer interface will determine the specificity of interactions with inverted palindromes.

The Influence of Ligand on Dimer Formation

The carboxy-terminal dimer interface colocalizes with the LBD; it is therefore likely that ligand binding and dimerization affect each other. This idea is supported by the observation that 9-cis RA enhances the homodimerization of RXR on palindromic and on DR1-type response elements (Zhang et al., 1992b). In contrast, DNA-bound T_3R and RAR homodimers have been shown to be destabilized by the addition of T_3 (Andersson et al., 1992; Yen et al., 1992; Miyamoto et al., 1993) and 9-cis RA (Carlblerg et al., 1993b), respectively. Cheskis and Freedman (1994) and Nishikawa et al. (1995) obtained slightly different results in their studies on ligand-influenced in vitro dimerization of VDR. In the absence of DNA, Cheskis and Freedman observed VDR independent from the presence or absence of VD predominantly as a monomer, whereas Nishikawa et al. also found in solution VDR homodimers and VDR-RXR heterodimers. On a DR3-type response element, Cheskis and Freedman observed that VD decreased the formation of the VDR homodimer complex, whereas already existing homodimers were destabilized, i.e., VD appears to shift the homodimer/monomer equilibrium toward monomers. This may be a way to liberate VDR monomers to form favorable complexes with RXR and to avoid the competition between a homodimer- and a heterodimer-mediated VD signaling from the same response element. In addition, Cheskis and Freedman suggested that VD may induce a conformational change in VDR that favors the formation of heterodimers with RXR. Conversely, 9-cis RA destabilizes indirectly VDR-RXR heterodimers by enhancing the formation of even more favorable RXR homodimers (MacDonald et al., 1993; Cheskis and Freedman, 1994). However, it is important to note that a VD-induced shift of the VDR homodimer/monomer equilibrium does not imply that VDR homodimers do not exist in the presence of VD, i.e., that VDR homodimers cannot transactivate in a ligand-dependent manner.

The equilibrium between RXR and VDR homodimers, their monomers, and their heterodimeric complex is not only ligand-dependent, but is also influenced by the relative protein amount. MacDonald et al. (1993) observed that the VD-inducibility of a DR3-type response element that binds VDR-RXR heterodimers was decreased by the costimulation with 9-cis RA. This type of ligand-induced squelching at limiting concentrations of RXR had first been described for T_3R-RXR heterodimers (Lehmann et al., 1993) and does depend on the relative amount of RXR. This squelching effect appears not to play a role in cells that have a high RXR/VDR ratio (Schräder et al., 1995). However, the relative expression of nuclear receptors is a major regulatory level not only for VD signaling, but also for other hormone signaling pathways.

Polarity of Heterodimers

The asymmetric binding of heterodirners to direct repeats implies the possibility that the receptor monomers may take two different orders. Heterodimer polarity was first assessed by Perlmann et al. (1993) and Kurokawa et al. (1993), who agreed in their conclusions that in RAR-RXR and T_3R-RXR heterodimers that bind to DR5- and DR4-type response elements, respectively, RXR takes the 5'-position. These results were confirmed by Gronemeyer's (Mader et al., 1993; Zechel et al., 1994a,b) and Giguère's groups (Predki et al., 1994) and extended for RAR-RXR heterodimers on DR2-type response elements, where RXR also takes the 5'-position. The final proof for polarity gave the crystal structure of T_3R-RXR-DBD heterodimers (Rastinejad et al., 1995). For VDR-RXR heterodimers, 5'-RXR-VDR-3' polarity was also assumed, which was confirmed later on for the mouse osteopontin (Quélo et al., 1994) and the rat osteocalcin (Schräder et al., 1995) DR3-type VD response elements. Based on these data, RXR appears to take always the 5'-position and its heterodimeric partner the 3'-position always.

However, contrary to this assumption, 5'-position binding of VDR was observed in VDR-RXR and VDR-T_3R heterodimers on the DR3-type VD response element of carbonic anhydrase II (Quélo et al., 1994) and the DR4-type VD response element of mouse calbindin D_{28k} (Schräder et al., 1994c), respectively. This agrees with the finding of Kurokawa et al. (1994) that RAR takes the 5'-position in RAR-RXR heterodimers that bind to a DR1-type response element. Taken together, there seems not to be a fixed rule for receptor monomer positions in homodimers, but all these experiments demonstrated that each heterodimer binds in a defined polar fashion.

In the spacing-orientation model, the overhang numbers appear to be characteristic for each receptor monomer and seem to provide the optimal distance to the 5'-partner (Carlberg, 1995). Thus, the number of spacing nucleotides

in a direct repeat would predict which receptor should bind to the 5'-motif. Accordingly, on a DR6-type response element, it would be VDR, on a DR5-type element RAR, on a DR4-type element T_3R, on a DR3-type element VDR or RXR, on a DR2-type element RAR or T_3R, and on a DR1-type-element RXR that takes the 3'-position. So far, the model fits with the observations and may be extended for other heterodimer-forming nuclear receptors, e.g., for PPAR, ONR-1, RLD-1/UR/OR-1/LXRα/RIP15, and NGFI-B that should have the overhang numbers 1, 3, 4, and 5, respectively. Thus, they should be found in the 3'-position, when they bind as heterodimers with RXR to DR1-, DR3-, DR4-, and DR5-type response elements (Table 1).

Response elements with an inverted palindromic structure are symmetric *per se*. However, naturally occurring response elements with this structure are owing to sequence variations in the core binding motifs and their 5'-preceding nucleotides not symmetric anymore. Therefore, it is not surprising that a clear directed binding of VDR-RXR (Schräder et al., 1995) and VDR-T_3R heterodimers (Schräder et al., 1994c) on IP9- and IP7-type response elements, respectively, has been reported. Furthermore, a directed binding is an indication that the DBDs of the respective receptor monomers contact each other even over a distance of seven or nine nucleotides. This is necessary for cooperative interaction (Zechel et al., 1994b).

On inverted palindromes, the order of the directed binding of heterodimers cannot be predicted as simply as in the case of direct repeats. Both overhang numbers, i.e., both receptor-specific steric hindrances, contribute to the optimal spacer length (Fig. 2). However, the sequence of the core binding motif and its 5'-preceding nucleotides remarkably contributes to the stabilization of the respective receptor monomers; thus, the analysis of the response element sequence may allow some predictions. Interestingly, the extended core binding motif sequence appears not only to direct the order of heterodimer binding on inverted palindromes (Schräder et al., 1994c, 1995), but also the ratio between homo- and heterodimerization of, e.g., T_3R on DR4-type response elements (Schräder et al., 1994d).

Ligand-Induced Transactivation

The communication between ligand-binding cavity and dimer interface, which appears to result in a quaternary structural change in the dimer, does not only explain the effect of ligand binding on dimerization, but suggests also that dimerization will affect ligand-binding affinity. However, the resulting effect on the transcription of the hormone-responding gene is crucial. Like most other transcription factors, nuclear receptors contact the basic transcriptional machinery through adapter proteins. Very recently, the cloning of coactivators (Cavaillès et al., 1995; Le Douarin et al., 1995; Onate et al., 1995) and corepressors (Burris et al., 1995; Chen and Evans, 1995; Hörlein et al., 1995) has been reported. Ligand-induced transcriptional activation appears to be mediated mainly through the AF-2 subdomain (Fig. 1), which is highly conserved in the nuclear receptor superfamily and has been shown to be essential for transcriptional activation by ER and GR (Danielian et al., 1992) T_3R (Saatcioglu et al., 1993; Barettino et al., 1994), and retinoid receptors (Durand et al., 1994). In the RXRα-LBD crystal structure, AF-2 has been shown to be a part of the last α-helix, which points away from the condensed sandwich structure of the remaining 10 α-helices (Bourguet et al., 1995) and may facilitate the interaction of AF-2 with cofactors. This α-helix is amphipathic, and in the absence of ligand, the residues of AF-2 are exposed only in part on the surface of the receptor, i.e., unliganded RXR contains a masked AF-2. Thus, a ligand-dependent conformational change is necessary to reveal the activation potential of AF-2. For a heterodimeric nuclear receptor complex, this opens the question of whether the AF-2 subdomain of both receptor monomers is presented, i.e., whether both receptors participate in ligand-activated transactivation.

Primary Ligand Activation of Heterodimers

Kurokawa et al. (1994) found that RAR-RXR heterodimers that bind to DR5-type retinoid response elements are only activated through the RAR-LBD; Perlmann and Jansson (1995) and Forman et al. (1995b) confirmed this result. VDR-RXR heterodimers show weak primary induction by 9-cis RA and are more efficiently activated by VD (Carlberg et al., 1993a, 1994b; Quélo et al., 1994; Schräder et al., 1994a,b, 1995); a similar observation was made for T_3R-RXR heterodimers (Schräder et al., 1994d; Schräder and Carlberg, 1994), which according to Forman et al. (1995b), should not respond to 9-cis RA. In contrast, it has recently been shown that NGFI-B-RXR (Perlmann and Jansson, 1995) and LXRα-RXR heterodimers (Willy et al., 1995) are primarily activated through the RXR LBD. Ligands for NGFI-B and LXRα are not known yet. Therefore, their potential effects could not be assessed.

Taken together, all these experiments demonstrate that RXR can be a heterodimer partner that is in primary induction either silent, only weakly active, or fully active. Since in all these heterodimeric complexes RXR binds in the 5'-position, this different inducibility by 9-cis RA appears not to depend on heterodimer polarity. The critical parameter is the identity of the heterodimer partner, which appears to induce a conformational change in RXR that modulates its ligand-binding ability. Kurokawa et al. (1994) reported for RAR-RXR heterodimers that bind in 5'-RAR-RXR-3' polarity to a DR1-type response element an extreme case, in which both heterodimer partners lost their ligand-binding ability. However, more moderate effects of RXR on the inducibility of its heterodimeric partner are also known. For VDR, it was observed that the ligand concentration of half-maximal transactivation, the EC_{50}-value, for its specific ligand is higher in heterodimers with RXR than in homodimers (Carlberg et al., 1994b).

On inverted palindromes, but also on some direct repeats that are bound in a clear polar fashion by VDR-RXR or VDR-T_3R heterodimers, it was observed that the orientation of the response element in relation to the TATA-box influences the EC_{50}-value for induction with VD (Quélo et al., 1994; Schräder et al., 1995): in cases where VDR is closer to the basal transcriptional machinery, lower concentrations of VD are required for the induction of transactivation than in opposite polarity. This may suggest that the efficiency of the ligand-dependent presentation of the AF-2 subdomain for contacts with adapter proteins is orientation-dependent. So far, polarity-directed ligand sensitivity was studied only with heterologous promoters, where the response element is in rather close vicinity to the TATA-box. Therefore, future experiments have to show whether response elements in their natural position, which is usually several hundred base pairs upstream of the transcription start site, are also able to direct ligand sensitivity.

Under physiological conditions, primary ligand activation of a heterodimer should be restricted to one ligand in order to maintain hormone specificity for a given response element. The above-discussed examples demonstrate that heterodimeric partners achieve this demand by their intrinsic property to influence each other allosterically in their contact with ligand. However, different mechanisms seem to have been evolved that can be distinguished in their net effects on ligand-binding affinity and ligand-induced transactivation. Furthermore, additional parameters, e.g., the relative response element orientation, fine regulate the primary ligand responsiveness of a nuclear receptor heterodimer-activated promoter.

The Response of Heterodimers to Two Ligands

Although the primary activation of a heterodimeric complex could more or less only be achieved through one of the two LBDs, a costimulation with both specific ligands leads in most cases to an enhanced transcriptional response. Such a superactivation was observed, e.g., with VDR-RXR heterodimers (Carlberg et al., 1993a, 1994b; Quélo et al., 1994; Schräder et al., 1995). Superactivation demonstrates that the second, initially silent LBD gets back its ligand inducibility, when the first LBD is charged with ligand. Thus, the allosteric reaction between the two LBDs that during complex formation prevents ligand binding of one partner, appears to be taken back, at least to some extent, after primary activation of the heterodimer. Moreover, this second, ligand-dependent allosteric reaction between the two LBDs does not only reactivate the second receptor monomer, but also influences the EC_{50}^{-} value for its specific ligand (unpublished results of the author's laboratory).

Although the stimulation with two ligands results in desired enhancement of the transcriptional response, it may also cause undesired side effects, such as the activation of other hormone signaling pathways. One effect that directly influences transactivation of at least VDR-RXR and T_3R-RXR heterodimers (Lehmann et al., 1993; Schräder et al., 1995), but probably also that of other RXR heterodimers, is the squelching via 9-cis RA-induced RXR homodimers. Thus, in cell lines that express only limiting amounts of RXR, the net effect of costimulation with 9-cis RA may be lower than that with the first ligand alone (MacDonald et al., 1993).

Taken together, allosteric effects between the DBDs and LBDs of a heterodimer regulate transactivation, and depend on ligand concentration, nuclear receptor dimerization, and response element structure. The relative expression of nuclear receptors and, thus, the formation of various homo- and heterodimeric complexes are cell type-specific. Furthermore, the ligand concentration may also be cell type specifically regulated either by intracellular ligand metabolism and catabolism, or by selective and active uptake. Even if the small and lipophilic ligands easily pass the membrane by diffusion, an active transport mechanism would create a concentration gradient that could modulate the access of ligands to their nuclear receptors. Norman's group postulated a membrane receptor for VD (Norman et al., 1992; Dormanen et al., 1994), which they took as evidence for a nongenomic VD signaling pathway, but it could also be a VD pump. For other nuclear hormones also similar evidences accumulate (Thompson, 1995).

The Network of Nuclear Receptors

The crystal structures of the LBD of RXR and the DBDs of GR, ER, RXR, and T_3R provided detailed structural informations, but the mechanisms that control the specificity of homo- and heterodimeric interactions of nuclear receptors remain poorly understood. It is not clear why RXR appears to have a relatively unique ability in forming heterodimers with several other ligand-activated and orphan members of the nuclear receptor family. RXR has a dichotomous function as an independent receptor for 9-cis RA and as a promiscuous partner in multiple signaling pathways. This provides RXR with a central role in integrating the actions of several nuclear receptors. Whether other homo- or heterodimeric complexes, which have been reported for VDR, T_3R, RAR, PPAR, and COUP-TF, can have comparable importance remains to be elucidated.

Within the last years, several crosstalks between nuclear receptors, also termed transcriptional interferences, have been reported, which result from heterodimerization of various receptors, competition between receptors for dimerization with limiting amounts of RXR, activation of the RXR pathway with 9-cis RA or formation of unproductive receptor heterodimers (for review, *see* Pfahl, 1994). Here are some examples:

1. The rather unexpected link of VD and T_3 signaling via VDR-T_3R heterodimers (Schräder et al., 1994b,c);

2. The link of T_3 signaling and the activation of PPARs, which is mediated either directly through T_3R-PPAR heterodimers (Bogazzi et al., 1994) or competition of T_3R and PPAR for RXR (Juge-Aubry et al., 1995);
3. The 9-cis RA-induced squelching of T_3 and VD signaling (Lehmann et al., 1993; Schräder et al., 1995), which occurs at low amounts of RXR;
4. The observations that unliganded T_3R can suppress the all-trans RA induction of a DR5-type RA response element by titrating RXR out of RAR-RXR heterodimers (Barettino et al., 1993); and
5. The finding that overexpression of COUP-TF blunts hormonal responses to VD, T_3, and all-trans RA (Kliewer et al., 1992a; Tran et al., 1992; Widom et al., 1992; Cooney et al., 1993) via the formation of transcriptional nonactive heterodimers.

Most nuclear receptors have similar carboxy-terminal dimerization interfaces and common preferences for core binding motifs; therefore, these few examples already lead to the impression that each nuclear receptor may interfere with the actions of several other members of the superfamily. Thus, one can assume that VDR can also affect other signaling systems either via competition for RXR or by direct protein–protein contacts. However, it is recommended to keep in mind that most of the reports on nuclear crosstalks result from in vitro or receptor overexpression experiments, so that it is not certain whether such complex interactions take place in vivo in the presence of low amounts of endogenous receptors.

VDR-RXR Heterodimers and Synthetic Ligands

The classical and the nonclassical actions of VD are based on the regulation of distinct primary VD-responding genes, i.e., they are all mediated by VDR. Thus, new concepts, that are based on a detailed understanding of the mechanisms of VDR's interaction with RXR in solution and on specific response elements will have a great impact on the development of VD analogs with an optimal therapeutic index. Therapeutically, most potent VD analogs should display high selectivity in VD signaling, i.e., they should avoid upregulation of genes that cause hypercalcemia, but still should be active on specific target genes. Thus far, the screening of the over 1000 different VD analogs, which have been developed during the last 10 yr, provided only a few potent candidates with both high antiproliferative and low calcemic activity (for review, *see* Jones and Calverley, 1993; Pols et al., 1994; Bouillon et al., 1995).

One starting point is the different structure of VD-responding genes. As already outlined, the natural VD response elements known so far can be distinguished into three types of direct repeats (DR3-, DR4-, and DR6-type elements) and inverted palindromes (IP9-type elements). VDR complexes interact in different conformations with these four principal structures, so that VD analogs may be identified that show selectivity only for one of these conformations. The current list of primary VD-responding genes displays a majority of those that are involved in the classical actions of VD. However, VD-regulated proteins, e.g., Pit-1, which is a key transcription factor in pituitary differentiation (Rhodes et al., 1994), suggest the idea that different response element structures may to some extent represent different actions of VD. A response element selectivity, i.e., a promoter selectivity of VD analogs, would activate only a subset of VD-responding genes and may, in parallel, be specific for one of the physiologic actions of VD. The first support for this concept was obtained by the observation that the highly antiproliferative VD analog EB1089 displays selectivity for IP9-type response elements, whereas structurally a rather different analog, KH1230, shows preference for DR3-type response elements (Nayeri et al., 1995).

A second starting point would be the crosstalk between the signaling pathways for VD and retinoids. The observations that RXR can be a transcriptionally active or silent heterodimeric partner of various nuclear receptors strongly indicate that it takes conformations that are accessible differently for 9-cis RA. Therefore, RXR-selective ligands, which in addition show selectivity for VDR-RXR heterodimers, may be identified and could be appropriate tools for the selective modulation of VDR's actions.

However, it has to be noted that for applications in vivo, the pharmacokinetic profile of the synthetic ligand, i.e., the binding to the VD-binding protein and an altered metabolism, may be its most important property.

Concluding Remarks

Several important aspects in VD signaling have not been mentioned in this article, e.g., receptor phosphorylation or other ligand-independent activation mechanisms. However, over the last few years, progress in the understanding of VD signaling was in majority related to the elucidation of the mechanisms of heterocooperative, allosteric interaction of VDR with other nuclear receptors. Among those, the heterodimerization with RXR takes the central role and provides VDR with several advantages:

1. Heterocooperative interaction of the DBDs increases the DNA-binding affinity and specificity for DR3- and IP9-type VD response elements;
2. Depending on RXR expression, 9-cis RA regulates the extent of VD inducibility up (superactivation) or down (RXR homodimer-induced squelching);
3. Polarity-based ligand sensitivities and 9-cis RA-modulated allosteric effects between the LBDs of RXR and VDR fine-regulate the EC_{50} values for stimulation with VD; and
4. The central role of RXR in nuclear hormone signaling links at least indirectly the actions of VDR to the network of other nuclear receptors.

However, there are still various important tasks in nuclear VD signaling that have to be resolved. This is in first priority the crystal structure of the VDR-LBD and DBD, which will give the final proof for models and mechanisms proposed so far. Of similar importance is the identification of more (and finally all) primary VD-responding genes, in particular, of those that are involved in cell growth, cell differentiation, and programmed cell death. This could be achieved by consistent application of powerful methods as the differential RNA display (Liang and Pardee, 1992). In addition, further promoter sequences of VD-responding genes should be analyzed for response elements, in particular, for those that are formed by inverted palindromes. The occupancy of these response elements in a living cell should be assessed under various stimulation conditions by techniques like in vivo footprinting (Breen et al., 1994). Furthermore, the identification and study of all physiological relevant contacts of VDR with other nuclear receptor and structurally unrelated regulatory proteins will confer VDR's adequate position in the context of other transcription factors. Finally, a continuous challenge will be to link the models and mechanisms, which were almost exclusively obtained in vitro or from transfection experiments, with the physiology of VD in vivo.

Acknowledgment

Studies from the author's laboratory were supported by grants from the Swiss National Foundation, the Swiss Cancer Research, and the LEO Foundation.

References

Allenby, G., Bocquel, M.-T., Saunders, M., Kazmer, S., Speck, J., Rosenberger, M., Lovey, A., Kastner, P., Grippo, J. F., Chambon, P., and Levin, A. A. (1993). *Proc. Natl. Acad. Sci. USA* **90,** 30–34.

Allenby, G., Janocha, R., Kazmer, S., Speck, J., Grippo, J. F. and Levin, A. A. (1994). *J. Biol. Chem.* **269,** 16,689–16,695.

Andersson, M. L., Nordstrom, K., Demczuk, S., Harbers, M., and Vennström, B. (1992). *Nucleic Acids Res.* **20,** 4803–4810.

Apfel, R., Benbrook, D., Lernhardt, E., Ortiz, M. A., Salbert, G. and Pfahl, M. (1994). *Mol. Cell. Biol.* **14,** 7025–7035.

Arriza, J. L., Weinberger, C., Cerelli, G., Glaser, T. M., Handelin, B. L., Housman, D. E., and Evans, R. M. (1987). *Science* **237,** 268–275.

Baes, M., Gulick T., Choi, H.-S., Martinoli, M. G., Simha, D., and Moore, D. D. (1994). *Mol. Cell. Biol.* **14,** 1544–1552.

Baker, A. R., McDonnell, D. P., Hughes, M., Crisp, T. M., Mangelsdorf, D. J., Haussler, M. R., Pike, J. W., Shine, J., and O'Malley, B. (1988). *Proc. Natl. Acad. Sci. USA* **85,** 3294–3298.

Barettino, D., Bugge, T. H., Bartunek, P., Vivanco Ruiz, M. M., Sonntag-Buck, V., Beug, H., Zenke, M., and Stunnenberg, H. G. (1993). *EMBO J.* **12,** 1343–1354.

Barettino, D., Vivanco Ruiz, M. M., and Stunnenberg, H. G. (1994). *EMBO J.* **13,** 3039–3049.

Becker-André, M., André, E., and DeLamarter, J. F. (1993). *Biochem. Biophys. Res. Commun.* **194,** 1371–1379.

Bernstein, P. S., Law, W. C., and Rando, R. R. (1987). *Proc. Natl. Acad. Sci. USA* **84,** 1849–1853.

Bogazzi, F., Hudson, L. D., and Nikodem, V. M. (1994). *J. Biol. Chem.* **269,** 11,683–11,686.

Bouillon, R., Okamura, W. H., and Norman, A. W. (1995). *Endocr. Rev.* **16,** 200–257.

Bourguet, W., Ruff, M., Chambon, P., Gronemeyer, H., and Moras, D. (1995). *Nature* **375,** 377–382.

Breen, E. C., van Wijen, A. C., Lian, J. B., Stein, G. S., and Stein, J. L. (1994). *Proc. Natl. Acad. Sci. USA* **91,** 12,902–12,906.

Bugge, T. H., Pohl, J., Lonnoy, O., and Stunnenberg, H. G. (1992). *EMBO J.* **11,** 1409–1418.

Burmester, J. K., Wiese, R. J., Maeda, N., and DeLuca, H. F. (1988). *Proc. Natl. Acad. Sci. USA* **85,** 9499–9502.

Burnside, J., Darling, D. S., and Chin, W. W. (1990). *J. Biol. Chem.* **265,** 2500–2504.

Burris, T. P., Nawaz, Z., Tsai, M.-J., and O'Malley, B. W. (1995). *Proc. Natl. Acad. Sci. USA* **92,** 9525–9529.

Carlberg, C. (1993). *Biochem. Biophys. Res. Commun.* **195,** 1345–1353.

Carlberg, C. (1995). *Eur. J. Biochem.* **231,** 517–527.

Carlberg, C., Bendik, I., Wyss, A., Meier, E., Sturzenbecker, L. J., Grippo, J. F., and Hunziker, W. (1993a). *Nature* **361,** 657–660.

Carlberg, C., Saurat, J.-H., and Siegenthaler, G. (1993b). *Biochem. J.* **295,** 343–346.

Carlberg, C., Hooft van Huijsduijnen, R., Staple, J., DeLamarter, J. F., and Becker-André, M. (1994a). *Mol. Endocrinol.* **8,** 757–770.

Carlberg, C., Mathiasen, I., Saurat, J.-H., and Binderup, L. (1994b). *J. Steroid Biochem. Mol. Biol.* **51,** 137–142.

Cavaillès, V., Dauvois, S., L'Horset, F., Lopez, G., Hoare, S., Kushner, P. J., and Parker, M. G. (1995). *EMBO J.* **14,** 3741–3751.

Chang, C., Kokontis, J., and Liao, S. (1988). *Proc. Natl. Acad. Sci. USA* **85,** 7211–7215.

Chen, J. D. and Evans, R. M. (1995). *Nature* **377,** 454–457.

Cheskis, B. and Freedman, L. P. (1994). *Mol. Cell. Biol.* **14,** 3329–3338.

Cooney, A. J., Leng, X. L., Tsai, S. Y., O'Malley, B. W., and Tsai, M.-J. (1993). *J. Biol. Chem.* **268,** 4152–4160.

Danielian, P. S., White, R., Lees, J. A., and Parker, M. G. (1992). *EMBO J.* **11,** 1025–1033.

Danielsen, M., Hinck, L., and Ringold, G. M. (1989). *Cell* **57,** 1131–1138.

de Thé, H., Vivanco-Ruiz, M. M., Tiollais, P., Stunnenberg, H., and Dejean, A. (1990). *Nature* **343,** 177–180.

DeLuca, H. F., Krisinger, J., and Darwish, H. (1990). *Kidney Int.* **38,** S2–S8.

Dormanen, M. C., Bishop, J. E., Hammonds, M. W., Okamura, W. H., Nemere, I., and Norman, A. W. (1994). *Biochem. Biophys. Res. Commun.* **201,** 394–401.

Durand, B., Saunders, M., Gaudon, C., Roy, B., Losson, R., and Chambon, P. (1994). *EMBO J.* **13,** 5370–5382.

Eisman, J. A., Barkla, D. H., and Tutton, P. J. M. (1987). *Can. Res.* **47,** 21–25.

Evans, R. M. (1988). *Science* **240,** 889–895.

Farwell, S. E., Lees, J. A., White, R., and Parker, M. G. (1990). *Cell* **60,** 953–962.

Forman, B. M. and Samuels, H. H. (1990). *Mol. Endocrinol.* **4,** 1293–1301.

Forman, B. M., Goode, E., Chen, J., Oro, A. E,., Bradley, D. J., Perlmann, T., Noonan, D. J., Burka, L. T., McMorris, T., Lamph, W. W., Evans, R. M., and Weinberger, C. (1995a). *Cell* **81,** 687–693.

Forman, B. M., Umesono, K., Chen, J., and Evans, R. M. (1995b). *Cell* **81,** 541–550.

Fraser, D. R. and Kodicek, E. (1970). *Nature* **288,** 764–766.

Freedman, L. P. (1992). *Endocr. Rev.* **13,** 129–145.

Freedman, L. P. and Luisi, B. F. (1993). *J. Cell. Biochem.* **51,** 140–150.

Giguère, V. (1994). *Endocr. Rev.* **15,** 61–79.

Giguère, V., Hollenberg, S. M., Rosenfeld, M. G., and Evans, R. M. (1986). *Cell* **46,** 645–652.

Giguère, V., Ong, E. S., Segui, P., and Evans, R. M. (1987). *Nature* **330,** 624–629.

Giguère, V., Tini, M., Flock, G., Ong, E., Evans, R. M., and Otulakowski, G. (1994). *Genes and Dev.* **8,** 538–553.

Gill, R. K. and Christakos, S. (1993). *Proc. Natl. Acad. Sci. USA* **90,** 2984–2988.

Glass, C. K. (1994). *Endocr. Rev.* **15,** 391–407.

Graupner, G., Wills, K. N., Tzukerman, M., Zhang, X.-K. and Pfahl, M. (1989). *Nature* **340,** 653–656.

Green, S. (1993). *Nature* **361,** 590,591.

Green, S. and Chambon, P. (1988). *Trends Genet.* **4,** 309–314.

Green, S., Walter, P., Kumar, V., Krust, A., Bornert, J.-M., Argos, P., and Chambon, P. (1986). *Nature* **320,** 134–139.

Gronemeyer, H. and Moras, D. (1995). *Nature* **375,** 190–191.

Gronemeyer, H., Turcotte, B., Quirin-Stricker, C., Bocquel, M. T., Meyer, M. E., Krozowski, Z., Jeltsch, J. M., Lerouge, T., Garnier, J., and Chambon, P. (1987). *EMBO J.* **6,** 3985–3994.

Hamada, K., Gleason, S. L., Levi, B.-Z., Hirschfeld, S., Appella, E., and Ozato, K. (1989). *Proc. Natl. Acad. Sci. USA* **86,** 8289–8293.

Harding, H. P. and Lazar, M. A. (1995). *Mol. Cell. Biol.* **15,** 4791–4802.

Harmon, M. A., Boehm, M. F., Heyman, R. A., and Mangelsdorf, D. J. (1995). *Proc. Natl. Acad. Sci. USA* **92,** 6157–6160.

Haussler, M. and Norman, A. W. (1969). *Proc. Natl. Acad. Sci. USA* **62,** 155–162.

Heyman, R. A., Mangelsdorf, D. J., Dyck, J. A., Stein, R. B., Eichele, G., Evans, R. M., and Thaller, C. (1992). *Cell* **68,** 397–406.

Hollenberg, S. M., Weinberger, C., Ong, E. S., Cerelli, G., Oro, A., Thompson, E. B., Rosenfeld, M. G., and Evans, R. M. (1985). *Nature* **318,** 635–641.

Hörlein, A. J., Näär, A. M., Heinzel, T., Torchia, J., Gloss, B., Kurokawa, R., Ryan, A., Kamei, Y., Söderström, M., Glass, C. K., and Rosenfeld, M. G. (1995). *Nature* **377,** 397–404.

Issemann, I. and Green, S. (1990). *Nature* **347,** 645–650.

Jääskeläinen, T., Itkonen, A., and Mäenpää, P. H. (1995). *Eur. J. Biochem.* **228,** 222–228.

Jiang, G., Nepomuceno, L., Hopkins, K., and Sladek, F. M. (1995). *Mol. Cell. Biol.* **15,** 5131–5143.

Jones, G. and Calverley, M. J. (1993). *Trends Endocrinol. Metabol.* **4,** 297–303.

Juge-Aubry, C. E., Gorla-Bajszczak, A., Pernin, A., Lemberger, T., Wahli, W., Burger, A. G., and Meier, C. A. (1995). *J. Biol. Chem.* **270,** 18,117–18,122.

Kahlen, J.-P. and Carlberg, C. (1994). *Biochem. Biophys. Res. Commun.* **202,** 1366–1372.

Kerner, S. A., Scott, R. A., and Pike, J. W. (1989). *Proc. Natl. Acad. Sci. USA* **86,** 4455–4459.

Kliewer, S. A., Umesono, K., Heyman, R. A., Mangelsdorf, D. J., Dyck, J. A., and Evans, R. M. (1992a). *Proc. Natl. Acad. Sci. USA* **89,** 1448–1452.

Kliewer, S. A., Umesono, K., Mangelsdorf, D. J., and Evans, R. M. (1992b). *Nature* **355,** 446–449.

Kliewer, S. A., Umesono, K., Noonan, D. J., Heyman, R. A., and Evans, R. M. (1992c). *Nature* **358,** 771–774.

Klock, G., Strähle, U., and Schütz, G. (1987). *Nature* **329,** 734–736.

Kumar, V., Green, S., Stack, G., Berry, M., Jin, J.-R., and Chambon, P. (1987). *Cell* **51,** 941–951.

Kumar, V. J. and Chambon, P. (1988). *Cell* **55,** 145–156.

Kurokawa, R., Yu, V. C., Näär, A., Kyakumoto, S., Han, Z., Silverman, S., Rosenfeld, M. G., and Glass, C. K. (1993). *Genes and Dev.* **7,** 1423–1435.

Kurokawa, R., DiRenzo, J., Boehm, M., Sugarman, J., Gloss, B., Rosenfeld, M. G., Heyman, R. A., and Glass, C. K. (1994). *Nature* **371,** 528–531.

Laudet, V. and Adelmant, G. (1995). *Curr. Biol.* **5,** 124–127.

Laudet, V. and Stehelin, D. (1992). *Curr. Biol.* **2,** 293–295.

Laudet, V., Hänni, C., Coll, J., Catzeflis, F., and Stehelin, D. (1992). *EMBO J.* **11,** 1003–1013.

Lavorgna, G., Ueda, H., Clos, J., and Wu, C. (1991). *Science* **252,** 848–851.

Lazar, M., Hodin, R. A., Darling, D. S., and Chin, W. W. (1989). *Mol. Cell. Biol.* **9,** 1128–1136.

Lazar, M. A. (1993). *Endocr. Rev.* **14,** 184–193.

Le Douarin, B., Zechel, C., Garnier, J.-M., Lutz, Y., Tora, L., Pierrat, B., Heery, D., Gronemeyer, H., Chambon, H., Chambon, P., and Losson, R. (1995). *EMBO J.* **14,** 2020–2033.

Lee, M. S., Kliewer, S. A., Provencal, J., Wright, P. E., and Evans, R. M. (1993). *Science* **260,** 1117–1121.

Lehmann, J. M., Zhang, X.-K., Graupner, G., Lee, M.-O., Hermann, T., Hoffmann, B., and Pfahl, M. (1993). *Mol. Cell. Biol.* **13,** 7698–7707.

Leid, M., Kastner, P., Lyons, R., Nakshatri, H., Saunders, M., Zacharewski, T., Chen, J.-Y., Staub, A., Garnier, J.-M., Mader, S., and Chambon, P. (1992). *Cell* **68,** 377–395.

Leng, X., Blanco, J., Tsai, S., Ozato, K., O'Malley, B. W. and Tsai, M.-J. (1995). *Mol. Cell. Biol.* **15,** 255–263.

Levin, A. A., Sturzenbecker, L. J., Kazmer, S., Bosakowski, T., Huselton, C., Allenby, G., Speck, J., Kratzeisen, C., Rosenberger, M., Lovey, A., and Grippo, J. F. (1992). *Nature* **355,** 359–361.

Liang, P. and Pardee, A. B. (1992). *Science* **257,** 967–971.

Liao, J., Ozono, K., Sone, T., McDonnell, D., and Pike, J. W. (1990). *Proc. Natl. Acad. Sci. USA* **87,** 9751–9755.

Luisi, B. F., Xu, W. X., Otwinowski, Z., Freedman, L. P., Yamamoto, K. R., and Sigler, P. B. (1991). *Nature* **352,** 497–505.

MacDonald, P. N., Haussler, C. A., Terpening, C. M., Galligan, M. A., Reeder, M. C., Whitfield, G. K., and Haussler, M. R. (1991). *J. Biol. Chem.* **266,** 18,808–18,813.

MacDonald, P. N., Dowd, D. R., Nakajima, S., Galligan, M. A., Reeder, M. C., Haussler, C. A., Ozato, K., and Haussler, M. R. (1993). *Mol. Cell. Biol.* **13,** 5907–5917.

Mader, S., Kumar, V., DeVerneuil, H.m and Chambon, P. (1989). *Nature* **338,** 271–274.

Mader, S., Chen, J.-Y., Chen, Z., White, J., Chambon, P., and Gronemeyer, H. (1993). *EMBO J.* **12,** 5029–5041.

Mangelsdorf, D. J., Koeffler, H. P., Donaldson, C. A., Pike, J. W., and Haussler, M. R. (1984). *J. Cell. Biol.* **98,** 391–398.

Mangelsdorf, D. J., Ong, E. S., Dyck, J. A., and Evans, R. M. (1990). *Nature* **345,** 224–229.

Mangelsdorf, D. J., Borgmeyer, U., Heyman, R. A., Zhou, J. Y., Ong, E. S., Oro, A. E., Kakizuka, A., and Evans, R. M. (1992). *Genes and Dev.* **6,** 329–344.

Marks, M. S., Hallenbeck, P. L., Nagata, T., Segars, J. H., Appella, E., Nikodem, V. M., and Ozato, K. (1992). *EMBO J.* **11,** 1419–1435.

McDonnell, D. P., Mangelsdorf, D. J., Pike, J. W., Haussler, M. R., and O'Malley, B. W. (1987). *Science* **235,** 1214–1217.

Miesfeld, R., Rusconi, S., Godowski, P. J., Maler, B. A., Okret, S., Wikström, A.-C., Gustafsson, J.-A., and Yamamoto, K. R. (1986). *Cell* **46,** 389–399.

Milbrandt, J. (1988). *Neuron* **1,** 183–188.

Miyajima, N., Horiuchi, R., Shibuya, Y., Fukushige, S.-I., Matsubara, K.-I., Toyoshima, K., and Yamamoto, T. (1989). *Cell* **57,** 31–39.

Miyamoto, T., Suzuki, S., and DeGroot, L. J. (1993). *Mol. Endocrinol.* **7,** 224–231.

Morrison, N. A., Shine, J., Fragonas, J.-C., Verkest, V., McMenemey, M. L., and Eisman, J A. (1989). *Science* **246,** 1158–1161.

Munder, M., Herzberg, I. M., Zierold, C., Moss, V. E., Hanson, K., Clagett-Dame, M., and DeLuca, H. F. (1995). *Proc. Natl. Acad. Sci. USA* **92,** 2795–2799.

Murray, M. B. and Towle, H. C. (1989). *Mol. Endocrinol.* **3,** 1434–1442.

Nakajima, S., Hsieh, J.-C., MacDonald, P. N., Galligan, M. R., Haussler, C. A., Whitfield, G. K., and Haussler, M. R. (1994). *Mol. Endocrinol.* **8,** 159–172.

Nayeri, S., Danielsson, C., Kahlen, J.-P., Schräder, M., Mathiasen, I. S., Binderup, L., and Carlberg, C. (1995). *Oncogene* **11,** 1853–1858.

Nishikawa, J.-I., Kitaura, M., Imagawa, M., and Nishihara, T. (1995). *Nucleic Acids Res.* **23,** 606–611.

Norman, A. W., Nemere, I., Zhou, L.-X., Bishop, J. E., Lowe, K. E., Maiyar, A. C., Collins, E. D., Taoka, T., Sergeev, I., and Farach-Carson, M. C. (1992). *J. Steroid Biochem. Mol. Biol.* **41,** 231–240.

Onate, S. A., Tsai, S. Y., Tsai, M. J., and O'Malley, B. W. (1995). *Science* **270,** 1354–1357.

O'Malley, B. W. (1990). *Mol. Endocrinol.* **4,** 363–369.

O'Malley, B. W. and Conneely, O. M. (1992). *Mol. Endocrinol.* **6,** 1359–1361.

Perlmann, T. and Jansson, L. (1995). *Genes and Dev.* **9,** 769–782.

Perlmann, T., Rangarajan, P. N., Umesono, K., and Evans, R. M. (1993). *Genes and Dev.* **7,** 1411–1422.

Petkovich, M., Brand, N. J., Krust, A., and Chambon, P. (1987). *Nature* **330,** 444–450.

Pfahl, M. (1994). *Semin. Cell Biol.* **5,** 95–103.

Piedrafita, F. J., Bendik, I., Ortiz, M. A., and Pfahl, M. (1995). *Mol. Endocrinol.* **9,** 563–578.

Pike, J. W. (1991). *Annu. Rev. Nutr.* **11,** 189–216.

Polly, P., Carlberg, C., Eisman, J. A., and Morrison, N. A. (1995). *J. Cell. Biochem.*, in press.

Pols, H. A. P., Birkenhäger, J. C., and van Leeuven, J. P. T. M. (1994). *Clin. Endocrinol.* **40,** 285–291.

Predki, P. F., Zamble, D., Sarkar, B., and Giguère, V. (1994). *Mol. Endocrinol.* **8,** 31–39.

Quélo, I., Kahlen, J.-P., Rascle, A., Jurdic, P., and Carlberg, C. (1994). *DNA Cell Biol.* **13,** 1181–1187.

Rastinejad, F., Perlmann, T., Evans, R. M., and Sigler, P. B. (1995). *Nature* **375,** 203–211.

Rhodes, S. J., Chen, R., DiMattia, G. E., Scully, K. M., Kalla, K. A., Lin, S.-C., Yu, V. C., and Rosenfield, M. G. (1993). *Genes Dev.* **7,** 913–932.

Rhodes, S. J., DiMattia, G. E., and Rosenfeld, M. G. (1994). *Curr. Opin. Genet. Devel.* **4,** 709–717.

Rosen, E. D., Beninghof, E. G., and Koenig, R. J. (1993). *J. Biol. Chem.* **268,** 11,534–11,541.

Ross, T. K., Moss, V. E., Prahl, J. M., and DeLuca, H. F. (1992). *Proc. Natl. Acad. Sci. USA* **89,** 256–260.

Saatcioglu, F., Bartunek, P., Deng, T., Zenke, M., and Karin, M. (1993). *Mol. Cell. Biol.* **13,** 3675–3685.

Sap, J., Munoz, A., Damm, K., Goldberg, Y., Ghysdael, J., Leutz, A., Beug, H., and Vennström, B. (1986). *Nature* **324,** 635–640.

Sap, J., Munoz, A., Schmitt, J., Stunnenberg, H., and Vennström, B. (1989). *Nature* **340,** 242–244.

Schräder, M., Bendik, I., Becker-André, M., and Carlberg, C. (1993a). *J. Biol. Chem.* **268,** 17,830–17,836.

Schräder, M., Wyss, A., Sturzenbecker, L. J., Grippo, J. F., LeMotte, P., and Carlberg, C. (1993b). *Nucleic Acids Res.* **21,** 1231–1237.

Schräder, M. and Carlberg, C. (1994). *DNA Cell Biol.* **13,** 333–341.

Schräder, M., Muller, K. M., Becker-André, M., and Carlberg, C. (1994a). *J. Mol. Endocrinol.* **12,** 327–339.

Schräder, M., Muller, K. M., and Carlberg, C. (1994b). *J. Biol. Chem.* **269,** 5501–5504.

Schräder, M., Muller, K. M., Nayeri, S., Kahlen, J.-P., and Carlberg, C. (1994c). *Nature* **370,** 382–386.

Schräder, M., Becker-Andre, M., and Carlberg, C. (1994d). *J. Biol. Chem.* **269,** 6444–6449.

Schräder, M., Nayeri, S., Kahlen, J.-P., Müller, K. M., and Carlberg, C. (1995). *Mol. Cell. Biol.* **15,** 1154–1161.

Schüle, R., Umesono, K., Mangelsdorf, D. J., Bolado, J., Pike, J. W., and Evans, R. M. (1990). *Cell* **61,** 497–504.

Schwabe, J. W. R., Chapman, L., Finch, J. T., and Rhodes, D. (1993). *Cell* **75,** 567–578.

Seol, W., Choi, H.-S., and Moore, D. D. (1995). *Mol. Endocrinol.* **9,** 72–85.

Smith, D. P., Mason, C. S., Jones, E. A., and Old, R. W. (1994). *Nucleic Acids Res.* **22,** 66–71.

Sone, T., Ozono, K., and Pike, J. W. (1991). *Mol. Endocrinol.* **5,** 1578–1586.

Song, C., Kokontis, J. M., Hiipakka, R. A., and Liao, S. (1994). *Proc. Natl. Acad. Sci. USA* **91,** 10,809–10,813.

Teboul, M., Enmark, E., Li, Q., Wikström, A. C., Pelto-Huikko, M., and Gustafsson, J.-A. . (1995). *Proc. Natl. Acad. Sci. USA* **92,** 2096–2100.

Thompson, C. C., Weinberger, C., Lebo, R., and Evans, R. M. (1987). *Science* **237,** 1610–1613.

Thompson, E. B. (1995). *Curr. Biol.* **5,** 730–732.

Towers, T. L., Luisi, B. F., Asianov, A., and Freedman, L. P. (1993). *Proc. Natl. Acad. Sci. USA* **90,** 6310–6314.

Tran, P., Zhang, X.-K., Salbert, G., Hermann, T., Lehmann, J. M., and Pfahl, M. (1992). *Mol. Cell. Biol.* **12,** 4666–4676.

Tsai, S. Y., Carlstedt-Duke, J., Weigel, N. I., Dahlman, K., Gustafsson, J. A., Tsai, M. J., and O'Malley, B. W. (1988). *Cell* **55,** 361–369.

Umesono, K. and Evans, R. M. (1989). *Cell* **57,** 1139–1146.

Umesono, K., Giguère, V., Glass, C. K., Rosenfeld, M. G., and Evans, R. M. (1988). *Nature* **336,** 262–265.

Umesono, K., Murakami, K. K., Thompson, C. C., and Evans, R. M. (1991). *Cell* **65,** 1255–1266.

Walters, M. R. (1992). *Endocr. Rev.* **13,** 719–764.

Wang, L. H., Tsai, S. Y., Cook, R. G., Beattie, W. G., Tsai, M.-J., and O'Malley, B. W. (1989). *Nature* **340,** 163–166.

Widom, R. L., Rhee, M., and Karathanasis, S. K. (1992). *Mol. Cell. Biol.* **12,** 3380–3389.

Willy, P. J., Umesono, K., Ong, E. S., Evans, R. M., Heyman, R. A., and Mangelsdorf, D. J. (1995). *Genes and Dev.* **9,** 1033–1045.

Wilson, T. E., Paulsen, R. E., Padgett, K. A., and Milbrandt, J. (1992). *Science* **256,** 107–110.

Yen, P. M., Sugawara, A., and Chin, W. W. (1992). *J. Biol. Chem.* **267,** 23,248–23,252.

Yu, V. C., Delsert, C., Andersen, B., Holloway, J. M., Devary, O. V., Näär, A. M., Kim, S. Y., Boutin, J.-M., Glass, C. K., and Rosenfeld, M. G. (1991). *Cell* **67,** 1251–1266.

Yu, V. C., Näär, A. M., and Rosenfeld, M. G. (1992). *Curr. Opin. Biotechnol.* **3,** 597–602.

Zechel, C., Shen, X.-Q., Chambon, P., and Gronemeyer, H. (1994a). *EMBO J.* **13,** 1414–1424.

Zechel, C., Shen, X.-Q., Chen, J.-Y., Chen, Z.-P., Chambon, P., and Gronemeyer, H. (1994b). *EMBO J.* **13,** 1425–1433.

Zhang, X.-K., Hoffmann, B., Tran, P. B.-V., Graupner, G., and Pfahl, M. (1992a). *Nature* **355,** 441–446.

Zhang, X.-K., Lehmann, J., Hoffmann, B., Dawson, M. I., Cameron, J., Graupner, G., Herrmann, T., Tran, P., and Pfahl, M. (1992b). *Nature* **358,** 587–591.

Zhang, X.-K., Salbert, G., Lee, M.-O., and Pfahl, M. (1994). *Mol. Cell. Biol.* **14,** 4311–4323.