

# Nuclear Hormone Receptors and Gene Expression

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**Aranda, Ana, and Angel Pascual.** Nuclear Hormone Receptors and Gene Expression. *Physiol Rev* 81: 1269–1304, 2001.—The nuclear hormone receptor superfamily includes receptors for thyroid and steroid hormones, retinoids and vitamin D, as well as different "orphan" receptors of unknown ligand. Ligands for some of these receptors have been recently identified, showing that products of lipid metabolism such as fatty acids, prostaglandins, or cholesterol derivatives can regulate gene expression by binding to nuclear receptors. Nuclear receptors act as ligand-inducible transcription factors by directly interacting as monomers, homodimers, or heterodimers with the retinoid X receptor with DNA response elements of target genes, as well as by "cross-talking" to other signaling pathways. The effects of nuclear receptors on transcription are mediated through recruitment of coregulators. A subset of receptors binds corepressor factors and actively represses target gene expression in the absence of ligand. Corepressors are found within multicomponent complexes that contain histone deacetylase activity. Deacetylation leads to chromatin compactation and transcriptional repression. Upon ligand binding, the receptors undergo a conformational change that allows the recruitment of multiple coactivator complexes. Some of these proteins are chromatin remodeling factors or possess histone acetylase activity, whereas others may interact directly with the basic transcriptional machinery. Recruitment of coactivator complexes to the target promoter causes chromatin decompactation and transcriptional activation. The characterization of corepressor and coactivator complexes, in concert with the identification of the

specific interaction motifs in the receptors, has demonstrated the existence of a general molecular mechanism by which different receptors elicit their transcriptional responses in target genes.

## I. INTRODUCTION

Small lipophilic molecules such as steroid and thyroid hormones or the active forms of vitamin A (retinoids) and vitamin D play an important role in the growth, differentiation, metabolism, reproduction, and morphogenesis of higher organisms and humans. Most cellular actions of these molecules are mediated through binding to nuclear receptors that act as ligand-inducible transcription factors. Almost two decades have gone by since the cloning of the first nuclear receptor for a steroid hormone. Since then, other nuclear hormone receptors were rapidly cloned and their target sequences on DNA identified. Our knowledge on regulation of gene expression by nuclear receptors has grown spectacularly during the last years, mainly due to the realization that not only the interaction of the receptors with DNA was important for transcriptional responses, but also that many coregulators (coactivators and corepressors) were crucial in transmitting the hormonal signal to the transcriptional machinery. On the other hand, crystal structures of ligand-binding domains of nuclear receptors have been solved, and this has allowed the definition of the structural basis for their transcriptional functions. Another major breakthrough in the study of nuclear receptors has been the targeted disruption of receptor genes in mice that allows an analysis of the relevance of particular receptors and receptor isoforms on mammalian physiology and development. These studies, which have shown the complexity of the mechanisms by which hormones elicit their role *in vivo*, and the existence of both redundant and specific

mechanisms for particular receptor isoforms are not described in this review.

Cloning of the receptors for steroid and thyroid hormones demonstrated that they share an extensive homology, and this observation led to a search for new proteins with similar structure. During the course of the last decade, the identification and characterization of close to 40 vertebrate receptors has led to the discovery of new hormonal responses and to the novel concept of "reverse endocrinology" in which the characterization of the receptor precedes the study of its physiological function. Regulatory ligands for many of these receptors have not yet been identified, and they have been called "orphan receptors." In the last years ligands have been found for several of these orphan receptors. Some of these ligands are products of lipid metabolism, and it is now known that compounds such as fatty acids, leukotrienes, prostaglandin and cholesterol derivatives, bile acids, pregnanes, or even benzoate derivatives can regulate gene expression through their binding to nuclear receptors. Therefore, as opposed to classic hormones, other ligands are intracellularly originated as metabolic products, which may explain why their role as regulators of nuclear receptors was not previously identified by physiological experimentation. Many other orphan receptors may have a still unidentified ligand, but others may act in a constitutive manner or could be activated by other means, *i.e.*, phosphorylation (Fig. 1). That orphan receptors also play key roles in development, homeostasis, and disease has been proven by targeted deletion in mice and by their association with different diseases including atherosclerosis,

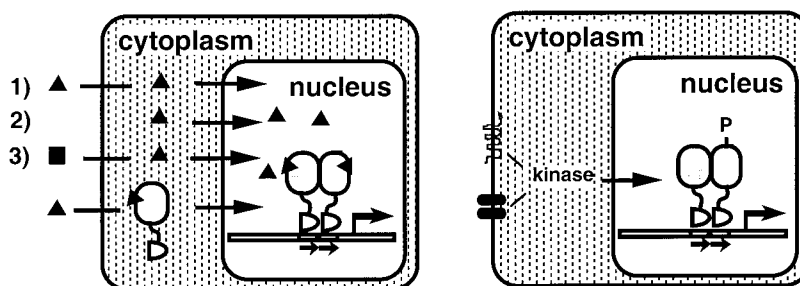


FIG. 1. Mechanism of action of nuclear receptors. *Left*: the ligand can be generated in three different ways: 1) an active ligand or hormone is synthesized in a classical endocrine organ and enters the cell, 2) the ligand may be generated from a precursor or prohormone within the target cell, and 3) the ligand may be a metabolite synthesized within the target cell. The unliganded receptor may have a nuclear location. However, some steroid receptors are cytoplasmic in the absence of ligand due to their association with a large multiprotein complex of chaperones, including Hsp90 and Hsp56. Ligand binding induces dissociation of the complex and nuclear translocation. Once in the nucleus, the receptors regulate transcription by binding, generally as dimers, to hormone response elements (HREs) normally located in regulatory regions of target genes. *Right*: alternative ligand-independent pathways for activation of nuclear receptors exist. Some receptors may be constitutively active, and the activity of others is modulated by other means, for instance, phosphorylation mediated by hormones and growth factors that stimulate diverse signal transduction pathways.

cancer, diabetes, or lipid disorders. These findings have opened new strategies for treatment of these diseases, and orphan receptors at this point, together with the search for new agonist and antagonist ligands for classical receptors, constitute important targets for drug discovery.

The goal of this work is to review the progress in the field of transcriptional regulation by nuclear receptors. We start by describing the domain structure of nuclear receptors and the characterization of DNA hormone response elements to which they bind in general as homo- or heterodimers. A brief description of the existence of mechanisms involved in non-DNA binding-dependent regulation by cross-talk with other signal transduction pathways follows. Some of the problems facing this field are the elucidation of mechanisms of transcriptional activation, mechanisms of transcriptional repression, and characterization of coactivator and corepressor complexes that are described with more detail.

To limit the references to a reasonable number, it is impossible to make a comprehensive analysis of all that has been published on nuclear receptors signaling. Instead, we try to highlight the more recent discoveries and to summarize the present knowledge on the mechanisms by which nuclear receptors regulate gene expression. To facilitate understanding we include a table that summarizes the more representative mammalian receptors, and we have created figures that schematically explain the mechanisms involved in transcriptional regulation. Because it is not possible to cite all relevant articles, we are including many up-to-date reviews on specific topics. We apologize to our colleagues when an original reference is not mentioned due to lack of space.

## II. THE NUCLEAR RECEPTOR SUPERFAMILY

Nuclear receptors are grouped into a large superfamily and are thought to be evolutionarily derived from a common ancestor. A list of classical and orphan hormone receptors and their ligands is shown in Table 1. Evolutionary analysis of the receptors has led to a subdivision in six different subfamilies (145). One large family is formed by thyroid hormone receptors (TRs), retinoic acid receptors (RARs), vitamin D receptors (VDRs) and peroxisome proliferator-activated receptors (PPARs) as well as different orphan receptors. Ligands for some of these receptors have been recently identified (see Table 1). The second subfamily contains the retinoid X receptors (RXRs) together with chicken ovalbumin upstream stimulators (COUPS), hepatocyte nuclear factor 4 (HNF4), testis receptors (TR2) and receptors involved in eye development (TLX and PNR). RXRs bind 9-*cis*-retinoic acid and play an important role in nuclear receptor signaling, as they are partners for different receptors that bind as

heterodimers to DNA. Ligands for other receptors have not been identified, whereas long-chain fatty acid acyl-CoA thioesters may be endogenous ligands for HNF4. The third family is formed by the steroid receptors and the highly related orphan receptors estrogen-related receptors (ERRs). The fourth, fifth, and sixth subfamilies contain the orphan receptors NGFI-B, FTZ-1/SF-1, and GCNF, respectively (for a recent comprehensive review in function and recently identified ligands for nuclear orphan receptors see Ref. 84). Most subfamilies appear to be ancient since they have an arthropod homolog, with the exception of steroid receptors that have no known homologs. It has been suggested that the ancestral receptors were constitutive homodimeric transcription factors that evolved to independently acquire the ability to bind a ligand and to heterodimerize. However, the possibility that the ancestral receptor was ligand dependent and that mutations changed the ligand-binding specificity or led to loss of ligand binding during evolution cannot be ruled out.

### A. Domain Structure

Like other transcriptional regulators, nuclear receptors exhibit a modular structure with different regions corresponding to autonomous functional domains that can be interchanged between related receptors without loss of function. A typical nuclear receptor consists of a variable NH<sub>2</sub>-terminal region (A/B), a conserved DNA-binding domain (DBD) or region C, a linker region D, and a conserved E region that contains the ligand binding domain (LBD). Some receptors contain also a COOH-terminal region (F) of unknown function. A scheme of a nuclear receptor is shown in Figure 2. The receptors also contain regions required for transcriptional activation. The hypervariable A/F region of many receptors contains an autonomous transcriptional activation function, referred to as AF-1, that contributes to constitutive ligand-independent activation by the receptor. A second transcriptional activation domain, termed AF-2, is located in the COOH terminus of the LBD, but unlike the AF-1 domain, the AF-2 is strictly ligand dependent and conserved among members of the nuclear receptor superfamily (see sect. vA).

#### 1. The A/B region

This modulatory region is the most variable both in size and sequence and in many cases contains an AF-1 domain. Multiple receptor isoforms generated from a single gene by alternative splicing or by the use of alternative promoters diverge in their A/B regions in most cases. This is the case for the TR isoforms TR $\beta$ 1 and TR $\beta$ 2 or for the various isoforms generated from the RAR genes, which are identical in their DBD and LBD, but differ in their

TABLE 1. *Subfamilies of mammalian nuclear receptors*

	Receptor	Subtype	Denomination	Ligand	Response Element	Monomer, Homodimer, or Heterodimer
Class I	TR	$\alpha, \beta$	Thyroid hormone receptor	Thyroid hormone ( $T_3$ )	Pal, DR-4, IP	H
	RAR	$\alpha, \beta, \gamma$	Retinoic acid receptor	Retinoic acid	DR-2, DR-5	H
	VDR		Vitamin D receptor	1-25(OH) $_2$ vitamin D $_3$	Pal, IP	H
	PPAR	$\alpha, \beta, \gamma$	Peroxisome proliferator activated receptor	Benzotriene B4; Wy 14.643 Eicosanoids; thiazolidinediones (TZD $_s$ ); 15-deoxy-12,41-prostaglandin J $_2$ ; polyunsaturated fatty acids	DR-3, IP-9 DR-1	H
	PXR		Pregnane X receptor	Pregnanes; C21 steroids	DR-3	H
	CAR/MB67	$\alpha, \beta$	Constitutive androstane receptor	Androstanes; 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene	DR-5	H
	LXR	$\alpha, \beta$	Liver X receptor	Oxysterols	DR-4	H
	FXR		Farnesoid X receptor	Bile acids	DR-4, IR-1	H
	RevErb	$\alpha, \beta$	Reverse ErbA	Unknown	DR-2, Hemisite	M, D
	RZR/ROR	$\alpha, \beta, \gamma$	Retinoid Z receptor/retinoic acid-related orphan receptor	Unknown	Hemisite	M
Class II	UR		Ubiquitous receptor	Unknown	DR-4	H
	RXR	$\alpha, \beta, \gamma$	Retinoid X receptor	9- <i>Cis</i> -retinoic acid	Pal, DR-1	D
	COUP-TF	$\alpha, \beta, \gamma$	Chicken ovalbumin upstream promoter transcription factor	Unknown	Pal, DR-5	D, H
	HNF-4	$\alpha, \beta, \gamma$	Hepatocyte nuclear factor 4	Fatty acyl-CoA thioesters	DR-1, DR-2	D
	TLX		Tailles-related receptor	Unknown	DR-1, Hemisite	M, D
Class III	PNR		Photoreceptor-specific nuclear receptor	Unknown	DR-1, Hemisite	M, D
	TR2	$\alpha, \beta$	Testis receptor	Unknown	DR-1 to DR5	D, H
	GR		Glucocorticoid receptor	Glucocorticoids	Pal	D
	AR		Androgen receptor	Androgens	Pal	D
	PR		Progesterone receptor	Progestins	Pal	D
	ER	$\alpha, \beta$	Estrogen receptor	Estradiol	Pal	D
	ERR	$\alpha, \beta, \gamma$	Estrogen-related receptor	Unknown	Pal, Hemisite	M, D
Class IV	NGFI-B	$\alpha, \beta, \gamma$	NGF-induced clone B	Unknown	Pal, DR-5	M, D, H
Class V	SF-1/FTZ-F1	$\alpha, \beta$	Steroidogenic factor 1 Fushi Tarazu factor 1	Oxysterols	Hemisite	M
Class VI	GCNF		Germ cell nuclear factor	Unknown	DR-0	D
Class 0	SHP		Small heterodimeric partner	Unknown		H
	DAX-1		Dosage-sensitive sex reversal	Unknown		

M, monomer; D, homodimer; H, heterodimer; NGF, nerve growth factor; DR, direct repeat; Pal, palindrome; IP, inverted palindrome.

NH $_2$ -terminal regions. The A/B domain shows promoter- and cell-specific activity, suggesting that it is likely to contribute to the specificity of action among receptor isoforms and that it could interact with cell type-specific factors. On the other hand, the modulatory domain is the target for phosphorylation mediated by different signaling pathways, and this modification can significantly affect transcriptional activity (for a review see Ref. 243). There are several reports indicating that RARs and other receptors can be phosphorylated by cyclin-dependent kinases and that this phosphorylation is important for ligand-dependent and -independent transactivation (222, 223, 259). Furthermore, other nuclear receptors such as the estrogen receptors (ERs) are phosphorylated at serine or threonine residues by the mitogen-activated protein kinase (MAPK) *in vitro*, and in cells treated with growth factors that stimulate the Ras-MAPK cascade, and this

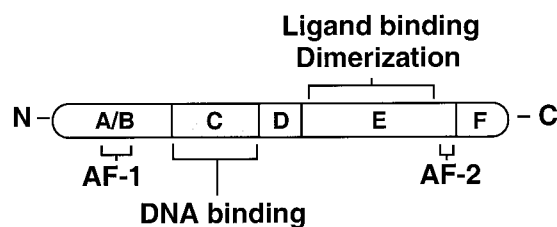


FIG. 2. Schematic representation of a nuclear receptor. A typical nuclear receptor is composed of several functional domains. The variable NH $_2$ -terminal region (A/B) contains the ligand-independent AF-1 transactivation domain. The conserved DNA-binding domain (DBD), or region C, is responsible for the recognition of specific DNA sequences. A variable linker region D connects the DBD to the conserved E/F region that contains the ligand-binding domain (LBD) as well as the dimerization surface. The ligand-independent transcriptional activation domain is contained within the A/B region, and the ligand-dependent AF-2 core transactivation domain within the COOH-terminal portion of the LBD.

phosphorylation enhances transcriptional activity (131, 197). A specific tyrosine phosphorylation site located at the COOH-terminal region of the receptor is involved in ligand-independent activity and may be a target for a different signaling pathway (285). A strong AF-1 domain in PPAR $\alpha$  is also modulated by phosphorylation by MAPK, and this phosphorylation enhances transcriptional activity (124). However, phosphorylation of the A/B domain of PPAR $\gamma$  by the same kinase negatively regulates its transcriptional functions. Interestingly, this modification reduces ligand binding to the receptor, showing that binding can be regulated by intramolecular communication between the modulatory domain and the COOH-terminal LBD (244). MAPK-dependent phosphorylation of the RXR can also alter biological actions of a partner receptor (250).

## 2. The DBD

The DBD, the most conserved domain of nuclear receptors, confers the ability to recognize specific target sequences and activate genes (Fig. 3). The DBD contains nine cysteines, as well as other residues that are conserved across the nuclear receptor superfamily and are required for high-affinity DNA binding. This domain comprises two "zinc fingers" that span ~60–70 amino acids and a COOH-terminal extension (CTE) that contains the so-called T and A boxes. In each zinc finger, four of the invariable cysteines coordinate tetrahedrally one zinc ion, and both zinc finger modules fold together to form a compact, interdependent structure as determined by nuclear magnetic resonance and crystallographic studies

(160, 239). Amino acids required for discrimination of core DNA recognition motifs are present at the base of the first finger in a region termed the "P box," and other residues of the second zinc finger that form the so-called "D box" are involved in dimerization. The core DBD contains two  $\alpha$ -helices: the first one beginning at the third conserved cysteine residue (the recognition helix) binds the major groove of DNA making contacts with specific bases, and the second one that spans the COOH terminus of the second zinc finger forms a right angle with the recognition helix (see Fig. 3). The nuclear magnetic resonance structure of the RXR DBD identified a third helix in the CTE that packs against helix 1 (148).

## 3. The hinge region

The D domain is not well conserved among the different receptors and serves as a hinge between the DBD and the LBD, allowing rotation of the DBD. The D domain in many cases harbors nuclear localization signals and also contains residues whose mutation abolishes interaction with nuclear receptor corepressors (see sect. vi).

## 4. The LBD

The LBD is a multifunctional domain that, in addition to the binding of ligand, mediates homo- and heterodimerization, interaction with heat-shock proteins, ligand-dependent transcriptional activity, and in some cases, hormone reversible transcriptional repression. The LBDs contain two well-conserved regions: a "signature motif" or Ti and the COOH-terminal AF-2 motif responsible for ligand-dependent transcriptional activation (294).

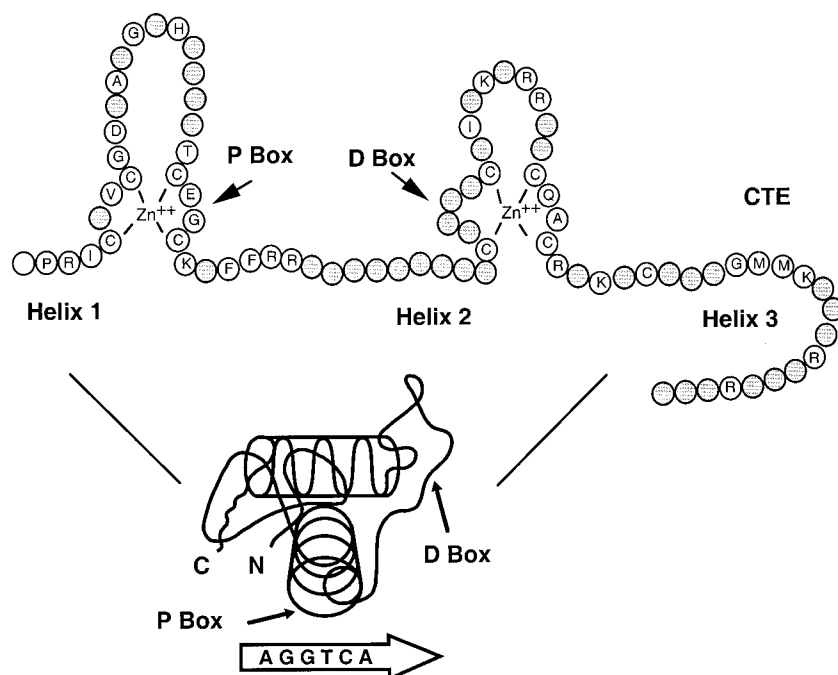


FIG. 3. The DNA binding domain of the nuclear receptors. A diagram of the two zinc fingers and the COOH-terminal extension (CTE). In the zinc fingers, four conserved cysteines coordinate a zinc ion. Other conserved residues are shown and designated by the corresponding letter. Helix 1 contains P box residues involved in the discrimination of the response element. Residues in the second zinc finger labeled as D box form a dimerization interface. The CTE contains the T and A boxes critical for monomeric DNA binding. As shown in the *bottom panel*, helix 1 and helix 2 cross at right angles to form the core of the DBD that recognizes a hemi-site of the response element. [*Bottom panel* from Glass (87). Copyright the Endocrine Society.]

The crystal structures of the LBDs of multiple nuclear receptors have been solved. These studies have demonstrated that overall structures of the different receptors are rather similar, suggesting a canonical structure for the different members of the nuclear receptor superfamily (for a review see Ref. 178). Figure 4 shows a schematic representation of the crystal structure of a receptor LBD. The LBDs are formed by 12 conserved  $\alpha$ -helical regions numbered from H1 to H12. A conserved  $\beta$ -turn is situated between H5 and H6. However, PPAR $\gamma$  is unique in its overall structure and contains an extra helix designed H2', and the VDR contains a poorly structured insertion between helices H1 and H3 for which no functional role has been defined (221). The LBDs are folded into a three-layered, antiparallel helical sandwich. A central core layer of three helices is packed between two additional layers to create a cavity, the ligand-binding pocket, which accommodates the ligand. This domain is mainly hydrophobic and is buried within the bottom half of the LBD. Contacts with the ligand can be extensive and include different structural elements through the LBD. The size of the ligand binding pocket varies among the different receptors, being for instance very large in PPAR $\gamma$ , which allows binding of very differently sized ligands (273). Several differences are evident when comparing unliganded and ligand-bound receptors. The liganded structures are more compact than the unliganded

ones, demonstrating that upon ligand binding the receptors undergo a clear conformational change.

## B. Hormone Response Elements

Nuclear receptors regulate transcription by binding to specific DNA sequences in target genes known as hormone response elements or HREs. These elements are located in regulatory sequences normally present in the 5'-flanking region of the target gene. Although often the HREs are found relatively close to the core promoter, in some cases they are present in enhancer regions several kilobases upstream of the transcriptional initiation site. The analysis of a large number of naturally occurring as well as synthetic HREs revealed that a sequence of 6 bp constitutes the core recognition motif. Two consensus motifs have been identified: the sequence AGAACA is preferentially recognized by steroid class III receptors, whereas AGG/TTCA serves as recognition motif for the remaining receptors of the superfamily (17). It should be noted that these motifs represent consensus idealized sequences and that naturally occurring HREs can show significant variation from the consensus. Although some monomeric receptors can bind to a single hexameric motif, most receptors bind as homo- or heterodimers to HREs composed typically of two core hexameric motifs.

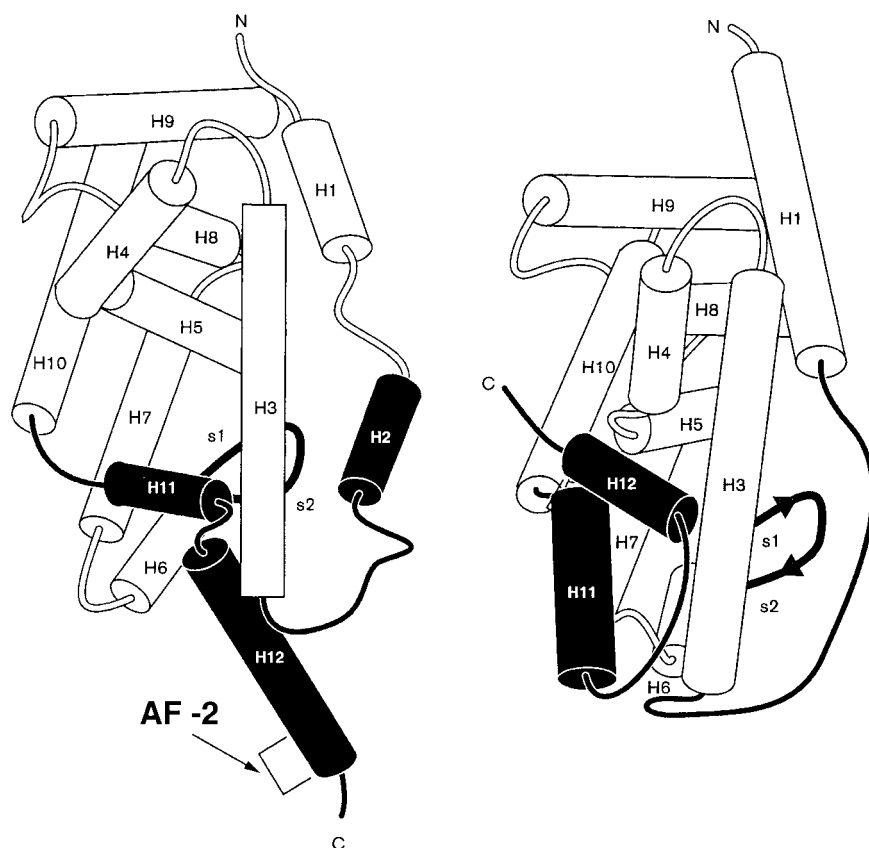


FIG. 4. Schematic drawing of the nuclear receptor ligand-binding domain (LBD). On the *left*, the LBD from the crystal structure of the unliganded RXR $\alpha$  is shown. On the *right*, the ligand-bound LBD of the RAR $\gamma$  is shown. Cylinders represent  $\alpha$ -helices that are numbered from 1 to 12. Note the different position of the COOH-terminal helix 12 that contains the core AF-2 domain in both situations. [From Wurtz et al. (294), reprinted by permission from Nature, Macmillan Magazines Ltd.]

For dimeric HREs, the half-sites can be configured as palindromes (Pal), inverted palindromes (IPs), or direct repeats (DRs).

Steroid hormone receptors typically bind to palindromes of the AGAACA sequence separated by three nucleotides, with the exception of the ERs that recognize the consensus AGGTCA motif with the same configuration. On the basis of the analysis of glucocorticoid receptor/ER chimeras, the first zinc finger has been identified as the one responsible for the discrimination of the DNA motif (91). Further studies have shown that mutation of three residues in the P box, which are identical in the glucocorticoid, progesterone, androgen, and mineralocorticoid receptors that recognize the same HRE, was sufficient to switch the sequence recognized by glucocorticoid receptors and ERs. Furthermore, cocrystal structures of receptor DBDs with DNA have shown that P box residues, which are contained within the recognition helix 1 of the DBD, were indeed involved in interaction with specific bases of the recognition motifs (for a detailed review on the interaction of receptors with the HREs, see Ref. 87).

In contrast to steroid receptors that almost exclusively recognize palindromic elements, nonsteroidal receptors can bind to HREs with different configurations (Fig. 5). In this case, the arrangement as well as the spacing between the motifs are determinant to confer selectivity and specificity. Some of these response elements are capable of mediating transcriptional responses to more than one ligand. This is the case of the palindromic element AGGTCATGACCT that confers regulation by both thyroid hormones and retinoic acid (271). As a consequence, both ligands can control overlapping gene networks as demonstrated by the regulation of the rat growth hormone gene by the two hormones via a common HRE (19). Similarly, IPs can also mediate transcriptional responses to both ligands as well as to vitamin D. However, a careful analysis of natural and synthetic HREs has shown that the most potent HREs for nonsteroid receptors are configured as DRs. Analysis of variably spaced DRs suggested that the length of the spacer region was an important determinant of the specificity of hormonal responses. Thus DRs separated by 3, 4, and 5 bp (i.e., DR3, DR4, and DR5) mediate preferential regulation by vitamin D, thyroid hormone, and retinoic acid, respectively (183, 272). The subsequent demonstration that DR1 serves as the preferred HRE for the RXR or for the PPAR and that RARs can also activate transcription through a DR2, expanded the model from a 3-to-5 rule to a 1-to-5 rule (reviewed in Ref. 163). Furthermore, a DR0 sequence can also act as a receptor binding site, and widely spaced DRs can act as promiscuous response elements for different nonsteroid receptors and even for ERs (132). The configuration of the preferred HREs for different classical and orphan receptors has been included in Table 1.

More recent results have shown that in addition to

spacing, small differences in the half-site sequence and the sequence of the flanking extension of the response elements also appear to be important parameters in determining receptor binding efficiency (162).

### C. Monomers, Homodimers, and Heterodimers

Several orphan nuclear receptors can bind DNA with high affinity as monomers (84). For monomeric HREs, a single AGG/TTCA half-site is preceded by a 5'-flanking A/T-rich sequence. Monomeric nuclear receptors utilize the CTE of the LBD to recognize that sequence (37, 85, 86, 289). The "A box" in this region is critical for the recognition of the amino acids at positions -1 and -2 of the core recognition motif (290). The third helix formed by the CTE can make extensive contacts with the minor groove of DNA and effectively extends the surface contact of the receptor DBD to beyond the consensus half-site recognition sequence providing additional receptor-DNA contacts in monomeric sites necessary for specific and high-affinity binding. Although other nuclear receptors generally do not bind with high affinity to DNA as monomers, it is likely that residues of the A and T boxes in the CTE also contribute to sequence specificity and affinity of binding to DNA.

Steroid receptors almost exclusively bind as homodimers to the HRE. Two steroid hormone receptor monomers bind cooperatively to their response elements, and dimerization interfaces have been identified both in the LBD and in the DBD. In ER, the dimer interface in the LBD contains residues from helices 7, 8, and 9 as well as the loop between helices 8 and 9 but is dominated by a conserved hydrophobic region at the NH<sub>2</sub> terminus of helix 10/11 (260). Other nuclear receptors use similar dimer interfaces because the corresponding residues are highly conserved in different receptors and have been implicated in dimerization by mutagenesis. It has been recently shown that the PPAR $\gamma$ /RXR $\alpha$  heterodimer is asymmetric and that the heterodimeric interface is composed of conserved motifs that form a coiled-coil along helix 10 with additional charge interactions from helices 7 and 9 (80). In contrast, the RAR/RXR heterodimer is not asymmetrical (30).

Palindromic DNA repeats impose a symmetrical structure that results in a head-to-head arrangement of the DBDs with each DBD of the homodimer making analogous contacts with one half-site. Crystallographic analysis of the DBD of the glucocorticoid receptor DBD-DNA complexes has demonstrated that the dimerization interface in the DBD involves amino acids of the D box. The formation of this interface is responsible for the selection of the spacing distance between the two halves of the palindrome, but it does not appear to function as an effective dimerization interface in the absence of DNA

since isolated DBDs do not dimerize in solution (100, 274).

Although several nonsteroidal nuclear receptors also bind DNA as homodimers, many nonsteroidal receptors bind to their HREs preferentially as heterodimers. In this case, the RXR is the promiscuous partner for different receptors (33, 135, 150, 163, 165, 307, 317). Typical heterodimeric receptors such as TR, RAR, or VDR can bind to their response elements as homodimers, but heterodimerization with RXR strongly increases the efficiency of DNA binding and transcriptional activity.

Some monomeric receptors (for instance NGFI-B) can also form heterodimers with RXR, and the heterodimers then recognize DRs rather than the monomeric extended sequence. Homodimeric receptors such as COUP-TF can also form heterodimers with RXR (84, 87). Furthermore, there are receptors that can bind as monomers, homodimers, and heterodimers to different response elements. In Table 1, the receptors that bind as monomers, homodimers, and heterodimers to their HREs are indicated.

Because DRs are inherently asymmetric, heterodimeric complexes may bind to them with two distinct polarities. Indeed, it has been established that on DR3, DR4, and DR5, RXR occupies the upstream half-site, and the heterodimeric partner (e.g., VDR, TR, or RAR) occupies the downstream motif (141, 151, 201, 235, 312). RAR/RXR heterodimers can bind to DR1 elements, and under these conditions, the heterodimer exhibits no response to RAR activating ligands. Interestingly, a reversed polarity is found in the case of a RAR/RXR heterodimer bound on a DR1 element in which RXR occupies the 3' half-site (140). However, this orientation not always results in inactivity, since the PPAR/RXR heterodimer activates transcription by binding to DR1 elements with the same polarity (68).

A two-step model for heterodimeric binding to DNA has been proposed. First, RXR would form heterodimers in solution with its partner through their dimerization interfaces contained in the LBDs, and in a second step, the DBDs would be able to bind with affinity to the DNA (163). The ability of heterodimeric receptors to bind to palindromes, IPs, and DR elements implies that the DBDs must be rotationally flexible with respect to the LBD dimerization interface (see Fig. 5). In contrast to the head-to-head arrangement of steroid receptor DBDs on DNA, the X-ray structure of the RXR/TR heterodimer reveals a polar head-to-tail assembly of the two proteins on a DR4, with RXR indeed occupying the upstream motif (212). Selective binding of heterodimers to their appropriate DRs appears to be a consequence of a cooperative dimer interaction within the DBDs. In a DR, a different region of the DBD of each receptor is used to create the dimerization interface. The heterodimeric DBD interface that is responsible for the cooperative binding of RXR/

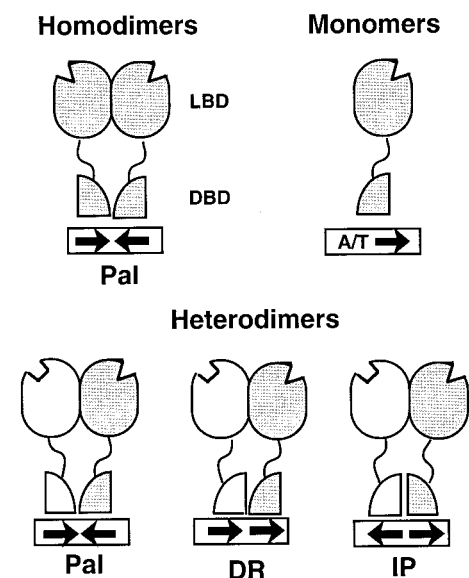


FIG. 5. Binding of receptors to the hormone response elements (HREs). Receptors can bind as monomers, homodimers, or RXR heterodimers to DNA. Dimerization is mediated by a strong dimerization interface (composed of hydrophobic heptad repeats) present in the LBD, and cooperative binding of receptor dimers is facilitated by a DNA-dependent interface that forms between the DBDs. Steroid receptors bind as homodimers to palindromic elements spaced by three nucleotides in a symmetrical way. Monomeric binding requires the half-core motif preceded by a 5'-flanking A/T-rich sequence. Heterodimers can recognize diverse HREs in which half-core motifs can be arranged as palindromes (Pal), direct repeats (DRs), or inverted palindromes (IPs). The ability of binding to these different motifs implies that the DBDs can rotate with respect to the LBDs that are held together through the dimerization interface.

RAR to DR5 elements involves the D box of RXR and the tip of the RAR first zinc finger (311). Similar interfaces would be used for binding of RXR/TR to a DR4 (201). A second type of dimerization surface, which specifically implicates the RAR T box and the second zinc finger of RXR, determines selective binding of RXR/RAR to DR2 elements (312). The same type of dimerization interface (RXR T box and second zinc finger) is responsible for the cooperative binding of RXR homodimers to DR1 elements. In all cases the DBD contributing the second zinc finger has to be positioned 5' to its cooperatively bound partner resulting in polarity of the heterodimer. In the case of RAR/RXR bound to the DR1 element with the reverse polarity, the heterodimeric partners associate in a DNA-dependent manner using the T box of RXR and the second zinc finger of RAR. The protein-DNA contacts, the dimerization interface, and the DNA curvature in the RAR/RXR complex are different from those of the RXR homodimer bound to the same element (213).

Theoretically, four different states of heterodimer occupancy can be predicted: both receptors unoccupied, only RXR occupied, only the partner receptor occupied, and both receptors occupied. However, three types of heterodimeric complexes exist: unoccupied

heterodimers, nonpermissive heterodimers that can be activated only by the partner's ligand but not by an RXR ligand alone (77, 140), and permissive heterodimers that can be activated by ligands of either RXR or its partner receptor and are synergistically activated in the presence of both ligands (117, 135, 287) (Fig. 6). Nonpermissive heterodimers include RXR/TR, RXR/VDR, or RXR/RAR heterodimers. In the nonpermissive heterodimers the ligand-induced transcriptional activities for RXR are suppressed when complexed with VDR, TR, and RAR, and the formation of the heterodimer actually precludes binding of ligand to RXR. Thus, in these instances, RXR is said to be a "silent partner." However, in the case of RXR/RAR, although a RXR ligand alone cannot activate the heterodimer, binding of the RAR ligand allows the subsequent binding of the ligand of RXR, that then enhances the transcriptional response to the RAR ligand (174).

PPAR/RXR, FXR/RXR, or NGFI-B/RXR are permissive heterodimers. The LXR-RXR complex also belongs to the class of permissive heterodimers as demonstrated by the finding that an RXR ligand stimulates the transcriptional activity of the heterodimer (286, 287). However, RXR occupies the upstream half-site of the HRE, a polarity that inhibits binding of the ligand to RXR in other heterodimers, demonstrating that permissivity does not depend exclusively on the polarity of the heterodimer.

Interestingly, stimulation by *9-cis*-retinoic acid requires the LXR but not the RXR AF-2 domain, demonstrating that binding of the RXR ligand results in a conformational change in LXR that leads to transcriptional activation. This phenomenon has been referred to as the "phantom ligand" effect (286). A synthetic retinoid specific for RXR also behaves as a phantom ligand and mimics exactly the effects of an RAR ligand without occupying the RAR ligand binding pocket (238).

The ligands could play a role in dimerization and binding to DNA. For instance, thyroid hormone inhibits binding of homodimers but not heterodimers to DNA, thus promoting formation of heterodimeric complexes on the HRE (219). In contrast *9-cis*-retinoic acid in some instances can increase binding of RXR homodimers to a DR1 (318), which can lead to unavailability for heterodimer formation with other receptors and to decreased levels of transcription for genes depending on heterodimers.

The above-mentioned observations demonstrate that RXR plays a dual role in nuclear receptor signaling. On one hand, this receptor binds to a DR1 as a homodimer and activates transcription in response to *9-cis*-retinoic acid (164), and on the other hand serves as a heterodimer partner for other nuclear receptors. Experiments with knock-out mice have clearly shown that the RXR/RAR heterodimer is responsible for different biological effects

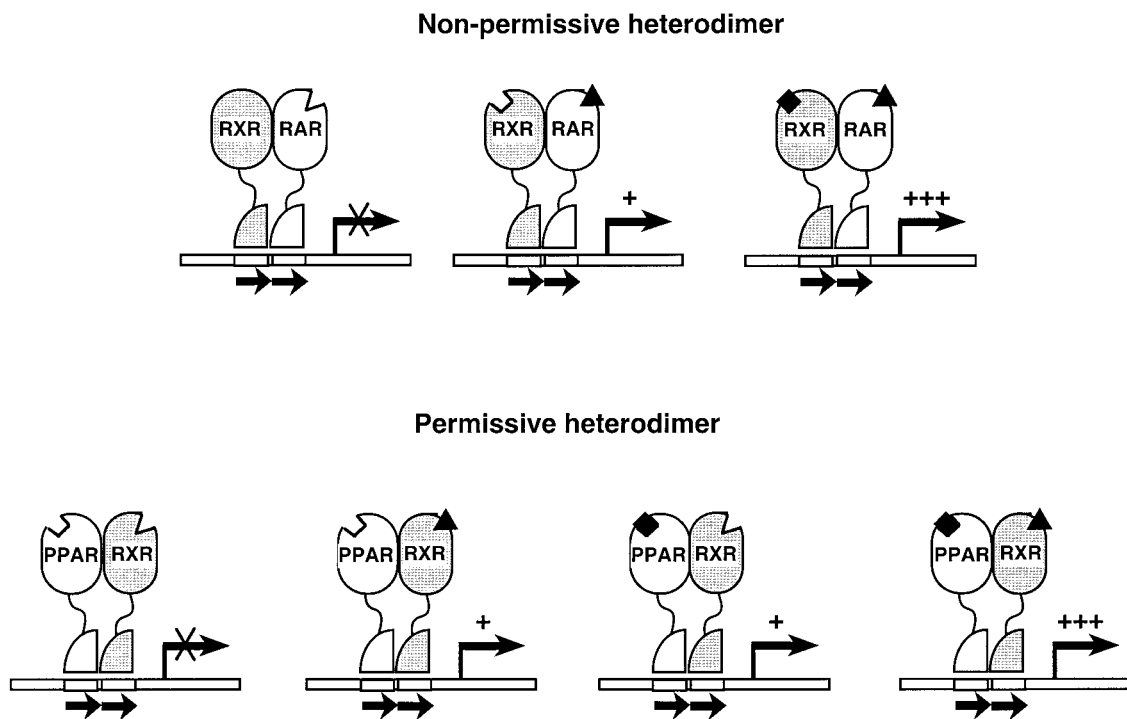


FIG. 6. Permissive and nonpermissive heterodimers. In nonpermissive heterodimers, such as RXR/RAR, heterodimerization precludes binding of the RXR ligand. Binding of ligand to the RAR moiety causes receptor activation and allows binding of the RXR ligand resulting in synergism. Permissive heterodimers, such as PPAR/RXR, can be activated by ligands of either RXR or its partner receptor and are synergistically activated in the presence of both ligands.

of retinoids on development (129, 130). However, there is still no evidence of a role for RXR in signaling by other heterodimeric receptors and, in fact, double TR-RXR knock-outs do not have a stronger phenotype than that shown by the TR knock-out alone (14).

### III. TRANSACTIVATION AND TRANSREPRESSION

#### A. Positive and Negative Response Elements

Although most of the attention has been focused on transcriptional activation by binding of nuclear receptors to positive HREs, nuclear receptors can also repress gene expression in a ligand-dependent manner. In some cases repressive effects may be due to passive inhibition, which can occur due to competition for DNA sites with other transactivators or to formation of transcriptionally inactive heterodimers. However, there are also the so-called “negative HREs” that bind the receptors and mediate negative regulation by the ligand. These elements have been identified for glucocorticoids in the proopiomelanocortin gene (POMC) and for thyroid hormones in the thyrotropin (TSH) and thyrotropin-releasing hormone (TRH) genes, indicating an important role of these sites in feedback mechanisms in the pituitary (28, 38, 70, 71, 106). In the case of the TRs, several other negative elements have been identified. Some of these elements have been shown to preferentially bind a TR homodimer in the absence of hormone, and a RXR/TR heterodimer in the presence of 3,3',5-triiodothyronine ( $T_3$ ). However, other negative HREs essentially bind only heterodimers both in the presence and absence of ligand. A rather common finding is that on negative HREs the unoccupied receptor increases transcription and the ligand reverses this stimulation. Although at the present time the properties of the negative HREs are not totally understood, location of the element may play a role. Negative HREs are generally very close to the transcription initiation sites, and some are positioned downstream of the TATA box (20, 199, 226) or even have an unusual location at the 3'-untranslated region (24). This sequence can have properties that depend on its localization, exhibiting negative responses only when placed downstream of the transcription initiation site, suggesting that the HRE could affect the transcriptional activity of the target gene by regulating the rate of release of RNA polymerase II from the promoter.

In addition to ligand-dependent gene activation and inhibition, a subset of nuclear receptors represses basal transcription in the absence of ligand when bound to a positive HRE. This silencing activity is due to the binding

of corepressors to the unliganded receptors and is reviewed in detail in section VI.

#### B. Transcriptional Antagonism and “Cross-Talk” With Other Signaling Pathways

Although as described in section II C, the orientation and spacing of the half-sites can determine selective transcriptional responses to nuclear receptors, specificity is not total, and some HREs can bind different heterodimers with high affinity. However, only a subset of receptor DNA binding elements function as response elements. As described above, the heterodimer RAR/RXR binds to a DR1 in a transcriptionally inactive form and antagonizes the response mediated by the active RXR homodimers (140). Equally, VDR/RXR can bind retinoic acid and thyroid hormone response elements in a transcriptionally inactive form, and under these circumstances vitamin D can inhibit the response to those ligands (81, 120). However, although competition for DNA binding by transcriptionally inactive VDR/RXR heterodimers may contribute to this inhibitory response, mutants lacking the A/B domain and the DNA-binding domain also display a dominant negative activity, suggesting that titration of coactivators could be involved in the inhibitory effect of vitamin D (121). Similarly, mutant or truncated transcriptionally inactive receptors in some syndromes of hormone resistance can compete binding of wild-type receptors to DNA, presenting a dominant-negative activity and reducing hormone-mediated transcriptional responses.

In the case of heterodimeric receptors, competition for limiting concentrations of RXR may also represent a mechanism for modulating transcriptional responses to several partner receptors (12). Thus COUPs can act as transcriptional repressors antagonizing activation mediated by different receptors, and this antagonism may involve competition for DNA binding sites, competition for RXR, and formation of inactive complexes with other receptors (269). An unusual receptor, the small heterodimer partner (SHP), lacks a typical DBD and can heterodimerize with different nuclear receptors leading to inhibition of binding to DNA and transcriptional inactivation (122, 240, 241).

Nuclear receptors can also modulate gene expression by mechanisms independent of binding to an HRE. Thus they can alter expression of genes that do not contain an HRE through positive or negative interference with the activity of other transcription factors, a mechanism generally referred to as “transcriptional cross-talk” (90). The ERs utilize protein-protein interactions to enhance transcription of genes that contain AP-1 sites (83). The AP-1 complex that is composed of dimers of Jun family proteins and preferentially of Jun/Fos heterodimers plays an important role in cell proliferation. ER $\alpha$  and ER $\beta$

have been shown to signal in opposite ways at AP-1 sites. ER $\alpha$  activates transcription in the presence of estradiol, whereas with ER $\beta$  estradiol inhibits AP-1-dependent transcription. Furthermore, antiestrogens can act as agonists of ER action at AP-1 sites. This is particularly evident in the case of ER $\beta$ , which enhances AP-1-dependent transcription in the presence of antiestrogens but not estrogens (194).

One of the best known examples of the cross-talk between nuclear receptors and AP-1 complexes is the finding that several receptors, such as TR, RAR, or GR, can act as ligand-dependent transrepressors of AP-1 (Jun/Fos) activity, and reciprocally, that AP1 can inhibit transactivation by nuclear receptors (203). It is believed that many of the antiproliferative effects of ligands of nuclear receptors could be mediated by their anti-AP-1 activity. Similarly, some nuclear receptors, specifically GR, can also mutually interfere with NF- $\kappa$ B activity, which could be involved in the anti-inflammatory and immunosuppressive effects of glucocorticoids.

In some cases the cross-talk between the receptors and AP-1 can involve binding to a "composite element" that can bind both the receptors and the AP-1 complex, and depending on the composition of the AP-1 complexes they can either cooperate or antagonize transcription by nuclear receptors (for a review see Ref. 203). However, the receptors can negatively regulate target gene promoters that carry AP-1, NF- $\kappa$ B, or CREB binding sites, without binding to these DNA elements themselves. It was originally proposed that the receptors directly contact the basic leucine zipper region of c-Jun or the *rel* homology domain of the p65 subunit of NF- $\kappa$ B and that this interaction inhibits binding to their corresponding cognate sites (291). However, more recent evidence suggested that competition for common transcriptional mediators could be involved in the antagonism observed (90; see also sect. vC). Additional mechanisms have been suggested, including an induction of the I $\kappa$ -B $\alpha$  factor that sequesters NF- $\kappa$ B in the cytoplasm (5), or an inhibition of the Jun-NH<sub>2</sub>-terminal kinase (JNK) activity by the receptors that would prevent phosphorylation of c-Jun (35).

A most interesting finding is that receptor-mediated transactivation and transrepression can be separated: mutations that impair transactivation, retain their ability to antagonize AP-1 or NF- $\kappa$ B activity. Interestingly, it has been possible to generate synthetic ligands of GR and retinoid receptors that dissociate transactivation from transrepression (217). These ligands are largely devoid of the ability to activate target genes containing HREs, but they retain *in vivo* anti-inflammatory or antiproliferative activity (53, 159, 275). These "dissociated" ligands have a large potential as pharmacological tools in the treatment of a variety of diseases including cancer and inflammatory diseases.

That transrepression plays a very important role *in vivo* has been demonstrated in a "knock-in" mouse in

which the wild-type glucocorticoid receptor has been replaced by a mutant receptor containing a substitution in the DBD that results in a dimerization-defective receptor (GR<sup>dim</sup>). This mutation allows transrepression, but the mutant receptor no longer binds with high affinity to the glucocorticoid response element. Whereas GR "knock-out" mice die at birth as a result of a failure in lung maturation, the GR<sup>dim</sup> survives despite impairment of several physiological functions of glucocorticoids (214).

The cross-talk between nuclear receptors and other signaling pathways is not restricted to the transcriptional antagonism described above (198). Phosphorylation of nuclear receptors provides an important link between signaling pathways. As already stated in section II A, multiple kinases activated by extracellular signals that bind to surface receptors, including for instance MAPKs, cell cycle-dependent kinases (CDKs), casein kinase, and protein kinase A, affect receptor activity through phosphorylation events (243). Depending on the receptor and in the residue involved, in some cases phosphorylation can inhibit ligand-dependent activation by nuclear receptors due to a reduction in ligand binding or in DNA binding affinity. However, in other cases, the receptors can be activated in the absence of its cognate ligand by phosphorylation through signals originated in membrane receptors.

Contrary to the antiproliferative effects of some nuclear receptor ligands, ovarian hormones stimulate growth of breast cancer cells. It has been reported that estrogens activate the Src/Ras/MAPK signal transduction pathway and that this cross-talk could be crucial for their growth-promoting effect in these cells. MAPK activation occurs very rapidly and is receptor mediated, but appears to represent a nongenomic action of the steroid (172). A direct interaction of ER with c-Src could be involved in this phenomenon, and the progesterone receptor (PR) that does not interact with c-Src can activate this pathway by association with ER (173).

A novel mechanism of cross-talk between nuclear receptors, specifically VDR, and transforming growth factor- $\beta$  (TGF- $\beta$ ) has been recently reported (300). Smad3, one of the proteins downstream in the TGF- $\beta$  signaling pathway, was found to act as a coactivator for VDR by forming a complex with a nuclear receptor coactivator. These interactions are potentially important in the control of cell proliferation and differentiation by vitamin D and the growth factor.

#### IV. RECEPTOR-INTERACTING PROTEINS

##### A. Interaction With General Transcription Factors

Promoters transcribed by RNA polymerase II are recognized by two types of transcription factors: the basal or general transcription factors (GTFs) that interact with the

core promoter elements, and the sequence-specific transcription factors, among which nuclear receptors are included, which generally interact with sequences located further upstream. The core promoter may contain the TATA box close to an initiator sequence that spans the transcriptional start site where the RNA polymerase II binds. Most of the factors involved in formation of the transcriptional initiation complex have been characterized. In addition to RNA polymerase II (which is composed of at least 12 subunits), these include TFIID, TFIIB, TFIIA, TFIIF, TFIIIE, and TFIIF (for a review see Ref. 288).

TFIID, whose binding to the promoter is thought to be a rate-limiting step in transcriptional initiation, is composed of TBP (or TATA binding protein) and TBP-associated factors (TAF<sub>II</sub>s) forming several complexes (18, 224). TBP is a highly conserved protein that binds to the minor groove of DNA over the TATA region causing a drastic bend of DNA and also contacts the largest subunit of RNA polymerase II. TFIID is comprised of at least two different subpopulations, one containing TAF<sub>II</sub>250, TAF<sub>II</sub>135, TAF<sub>II</sub>100, and TAF<sub>II</sub>28, present in all TFIID complexes, and the other containing additional TAFs, such as TAF<sub>II</sub>30, TAF<sub>II</sub>20, or TAF<sub>II</sub>18. After TFIID binding to DNA, recruitment of TFIIB is a critical step in the formation of the preinitiation complex. TFIIB contacts DNA upstream and downstream of the TATA box on the concave side of the bend induced by TFIID binding. It was formerly believed that the preinitiation complex was assembled in an ordered fashion, with binding of TFIID to the TATA box followed by sequential binding of TFIIB, the polymerase, and other factors. An alternative to the sequential recruitment of individual GTFs is the existence of preformed complexes, including the RNA polymerase II and GTFs, that could be directly recruited to the promoter by sequence-specific transcription factors. These complexes, that contain RNA polymerase II, TFIIB, TFIIF, TFIIF, SRBs (suppressor of RNA polymerase B), and several other proteins, have been isolated from yeast and mammalian cells and are termed the holoenzyme (45, 92). The current hypothesis is that transcription factors will finally cause their effect on gene expression by influencing the rate of assembly of these complexes to the regulated promoter.

As with other transcriptional regulatory proteins, one aspect of the mechanisms by which nuclear receptors affect the rate of RNA polymerase II-directed transcription likely involves the interaction of receptors with components of the transcription preinitiation complex. This interaction may be direct, or it may occur indirectly through the action of coregulators (coactivators and corepressors, see sects. v and vi) which act as bridging factors. Nuclear receptors seem to be able to interact with several components of the general transcriptional machinery. It has been shown that TBP can interact with several receptors and that overexpression of TBP enhances ligand-

dependent transactivation in transfection assays (22, 227, 237). TAF<sub>II</sub>s have been also identified as potential targets for hormone receptors. Thus TAF<sub>II</sub>30 is required for transactivation by ER (116), whereas expression of TAF<sub>II</sub>135 strongly potentiates transcriptional stimulation by RAR, TR, or VDR, but does not affect the responses to ER or RXR (171). Therefore, TAF<sub>II</sub>s can act as coactivators of nuclear receptors. An interaction with TFIIB has been well documented for TR and VDR as well as for other receptors (8, 27, 167).

Although the functionality of direct protein to protein interactions of receptors with the basal transcriptional machinery is yet to be determined, it is likely that these interactions could cause the recruitment of the basal components to the promoter and the enhancement of transcription.

## B. Interaction With Sequence-Specific Transcription Factors

In natural promoters HREs are located close to recognition sequences for other transcription factors, and interaction between the receptors and these factors, which can result in functional synergism or repression, can play an important role in determining transcriptional rates. Early observations demonstrated that HREs can synergize with many different transcription factors in artificial promoters (236). In some cases HREs have been shown to be dependent on cooperative interactions with adjacent transcription factors. Such interactions may serve to restrict a hormonal response to cell types that express the appropriate set of transcription factors. Expression of pituitary genes appears to be a good example of these interactions. Transcription of the growth hormone and prolactin genes is stimulated by a number of ligands for nuclear receptors, and this stimulation requires binding of the pituitary-specific homeodomain factor GHF-1/Pit-1 to its recognition sites in the promoters (40, 55, 65, 262). A direct protein to protein interaction between the receptors and these factors appears to be involved in this synergism (40, 195). Similarly, on the mouse mammary tumor virus (MMTV) promoter, the transcription factors NF-1 and Oct-1 are required for a normal induction by glucocorticoids or progesterone, and a direct interaction of GR and PR with Oct-1 has been described (31).

## C. Interaction With Coactivators and Corepressors

Modulation of the assembly of preinitiation complexes by transcriptional activators involves not only direct actions but also indirect actions on components of the basal transcriptional machinery. Experimental evidence supports the existence of bridging molecules, also termed coactivators or transcription intermediary factors (TIFs), that are thought to mediate the interactions of

transcription factors with the basal transcriptional machinery. Conversely, corepressors can bind transcriptional activators and inhibit the formation of transcriptionally active complexes. The original indication of the existence of coactivators for nuclear receptors comes from the existence of transcriptional interference or “squenching” between different receptors in transient transfection assays in which the presence of a second receptor represses transactivation of a promoter regulated by a given receptor. The clear inference from those results is that titration of putative coactivators, which are commonly utilized by both receptors, is responsible for the transcriptional interference observed.

## V. NUCLEAR RECEPTOR COACTIVATORS

### A. The AF-2 Domain of Nuclear Receptors

Early studies suggested that the most COOH-terminal part of nuclear receptors, termed the AF-2 domain (13, 62, 72, 281), was involved in ligand-dependent transactivation “in vivo,” and mutation analysis has shown that this region is also involved in transcriptional interference. This domain possesses a high homology over a very short region from which the consensus motif  $\phi\phi XE\phi\phi$  ( $\phi$  being a hydrophobic amino acid) can be derived, preceded by a loop of length varying from 8 to 12 amino acids that is variable in sequence and composition. The region comprising the conserved sequence adopts an amphipathic  $\alpha$ -helical conformation with the two well-conserved pairs of hydrophobic residues pointing toward the core of the LDB and negatively charged residues exposed on its surface (313). This motif is conserved in most members of the nuclear receptor superfamily, with the exception that it is absent in Rev-erbA and the viral oncogene *v-erbA*, contains a conservative substitution of aspartic for glutamic acid in COUP-TF, and a positive charged amino acid substitutes for the highly conserved central glutamic acid residue in NGFI-B. This residue is important for transactivation but is not required for ligand binding, and its mutation in different receptors generates dominant-negative mutants that are transcriptionally silent (72). Remarkably, one of the “hot spots” for mutations in the TR $\beta$  gene that cause the syndrome of generalized resistance to thyroid hormone, maps to the COOH-terminal region (46, 137), and mutations in this region of PPAR $\gamma$  are associated with severe insulin resistance, diabetes mellitus, and hypertension (15).

Although the COOH-terminal region, that is located in helix 12 of the LBD, contains the core AF-2 activity, this domain comprises other dispersed elements brought together upon ligand binding. One such element is a region whose sequence is also extremely well conserved. This region, which has been called the nuclear receptor “sig-

nature motif,” encompasses the COOH-terminal half of helix 3, helix 4, and the loop between them. Mutations in this region affect neither ligand binding nor dimerization, but impair ligand-dependent transactivation. Specifically, a highly conserved lysine in the COOH terminus of helix 3 that is exposed to the solvent in the receptor crystals is important for transcriptional activity of several receptors (104, 119). Furthermore, natural mutations in the signature region have been identified in patients with androgen insensitivity syndrome (208) and also in thyroid hormone-resistant patients (57). Crystal structure of nuclear receptors has provided an explanation for the importance of the COOH-terminal AF-2 domain and this residue in ligand-dependent transactivation. The most striking difference observed in the receptors upon ligand binding is the position of helix 12, which contains the core AF-2 domain. Helix 12 projects away from the body of the LBD in unliganded RXR (29). However, in liganded receptors, this helix moves in a “mouse-trap” model being tightly packed against helix 3 or 4 and making direct contacts with the ligand (216, 280) (see Fig. 4). Because both the charged residues in helix 12 and residues in the signature region, including the lysine residue in helix 3, are contiguous and exposed in the surface of the LBD, they probably generate a hydrophilic surface responsible for coactivator interactions (178). Reinforcing this model, it has been recently demonstrated that in ER LBD bound to the antagonists raloxifen or dihydroxytamoxifen the position of helix 12 is different from that shown by the agonist-bound LBD (32, 245). In the antagonist-bound receptor, helix 12 is rotated and shifted with respect to its position when bound to estrogen. As a result, helix 12 lies in a groove formed by helix 5 and the COOH-terminal end of helix 3. This position overlaps with the surface of coactivator interaction, thus precluding coactivator binding and consequently transcriptional activity.

### B. Coactivator Families

Initial biochemical studies demonstrated that several proteins interact with the nuclear receptors. The most abundant of these were proteins of a molecular mass of 140 and 160 kDa (p140 and p160) designated as ER-associated proteins (ERAPS) (98), receptor-interacting proteins (RIPs) (41, 42), glucocorticoid receptor interacting proteins (GRIPs) (74), or TR-associated proteins (TRAPs) (75). A potential role for these proteins as coactivators for the nuclear receptors was suggested by the ligand dependence for their interaction with the receptors and by the finding that they failed to interact with transcriptionally inactive receptor mutants or with antagonist-bound receptors. Different cloning strategies have led to the identification of numerous receptor-interacting proteins. Some of them have been demonstrated to play a

role as bona fide receptor coactivators, whereas others could play different roles in modulating nuclear receptor function (for recent reviews, see Refs. 88, 89, 169, 220, 264, 297). To date, the following families of coactivators have been characterized.

### 1. The p160 family

Cloning of cDNAs encoding the biochemically identified p160 proteins has yielded three distinct but related family members from different species, with each family member having a number of splice variants. These include SRC-1/NCoA-1, TIF-2/GRIP-1/NCoA-2, and p/CIP/ACTR/AIB1/TRAM1/RAC3.

The first coactivator, identified using a yeast two-hybrid screen of a human B-lymphocyte library using PR as bait, was SRC-1 (192). This protein interacts with the receptor in an agonist and AF-2-dependent manner and acts as a prototypic coactivator for different nuclear receptors including other steroid receptors such as GR or ER, and nonsteroid group II receptors such as VDR, PPAR, TR, or RXR, stimulating the transcriptional activity of the corresponding ligands both in mammalian cells and in yeasts. In parallel studies, the mouse homolog of SRC-1 was identified by screening bacteriophage-based expression libraries with the LBD of ER in the presence of estrogen and was denominated NCoA-1 (265). This protein was highly related to human SRC-1 at the COOH terminus but encoded an extended NH<sub>2</sub> terminus, suggesting that the initially identified SRC-1 was either a partial clone or a splice variant of the full-length protein.

Immunoprecipitation experiments showed that SRC-1/NCoA-1 only accounted partially for the p160 proteins, suggesting that other coactivators with the same size might also exist. This was demonstrated by the cloning of a second set of p160 coactivators (SRC-2), termed TIF-2 in humans (278) and GRIP-1 or NCoA-2 in mice (107, 265). Truncated versions of these proteins exhibit dominant negative activity and can inhibit ligand and coactivator responses (108, 118). Both types of coactivators share not only considerable sequence similarity, but also many functional characteristics. Apart from interacting with various receptors and enhancing ligand-dependent transcriptional responses, they are also capable of relieving squelching, showing that they constitute common limiting factors recruited by the liganded receptors (278). Loss of function studies using microinjected antibodies against the coactivators also suggest that they are required for nuclear receptors function. Furthermore, these coactivators contain two major transactivation domains that retain their activity when fused with the DBD of the yeast GAL4 activator.

A third member of the p160 family of proteins was subsequently characterized. It was independently isolated as p/CIP in mice (265) and ACTR, AIB1, RAC3, or TRAM-1

in humans (4, 48, 152, 257). This coactivator has been generically named SRC-3. Although many properties of this coactivator are similar to those of the other p160 proteins, a major difference is that it also enhances the transcriptional activity of a number of different transcription factors including signal transducers and activators of transcription (STAT-1) and cAMP response element binding protein (CREB) (265). It should be noted that although SRC-1 was initially considered as a nuclear receptor-specific coactivator, more recently it has been demonstrated that it can also function as a coactivator for NF- $\kappa$ B, serum response factor, or p53 (134, 149) and that it is even required for muscle cell differentiation mediated by the helix-loop-helix transcription factor MEF-2 (54).

The three members of the p160 family of coactivators show a sequence similarity of 40%. Conservation is maximal in their NH<sub>2</sub>-terminal domains that contain the nuclear localization signal, and bHLH and PAS domains. These domains mediate protein to protein homo- and heterodimeric interactions, suggesting that these coactivators could interact with other PAS proteins. A serine/threonine-rich region and a COOH-terminal glutamine-rich region are also well conserved in these coactivators, which contain three nuclear receptor-interacting domains (see sect. vD) in their central region. Both activation domains are also located at the COOH terminus. The stronger transactivation domain is indistinguishable from the region of interaction with the cointegrator CREB binding protein (CBP), and a weaker transactivation domain located in the far COOH terminus of the coactivators has been recently shown to interact with an arginine methyltransferase (47, 97). The p160 coactivators possess histone acetyltransferase activity that maps also to the COOH-terminal region (48, 253). A diagram of the structure of a p160 coactivator is shown in Figure 7.

A possible application derived from the ligand-dependent recruitment of coactivators by the receptors is the identification of new ligands. An assay termed coactivator-dependent receptor ligand assay (CARLA) using SRC-1 has served for the identification of naturally occurring fatty acids and metabolites as well as hypolipidemic drugs as bona fide ligands for PPARs (139). This technique, which only identifies agonist ligands, is also applicable to the identification of ligands for orphan receptors.

### 2. PPAR $\gamma$ coactivator-1

PPAR $\gamma$  coactivator-1 (PGC-1), which was isolated in a yeast two-hybrid screen using a PPAR $\gamma$  fragment as the bait and a brown fat cDNA library, was demonstrated to interact with this receptor, as well as with other members of the nuclear receptor superfamily. PGC-1 is a coactivator that plays a major role in the regulation of adaptive thermogenesis, an important component of energy homeostasis (207). PGC-1 mRNA expression is dramatically

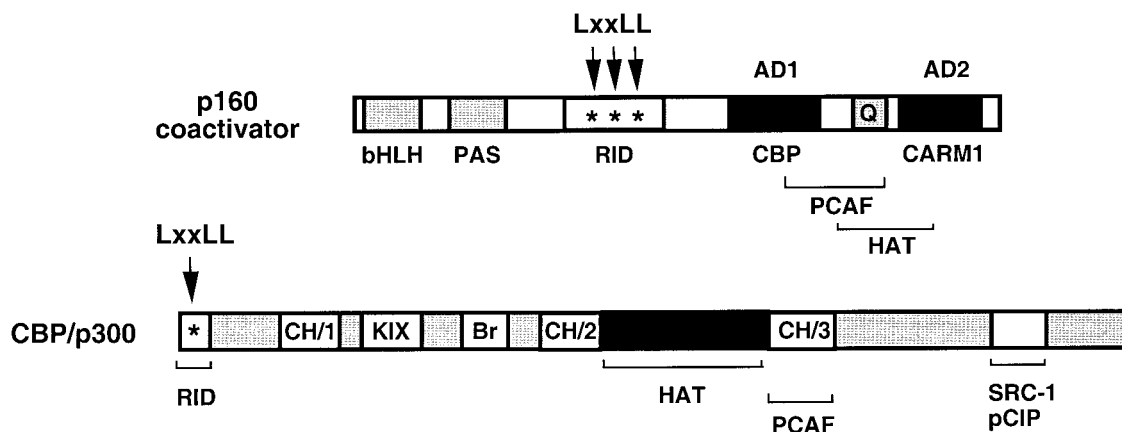


FIG. 7. Domains of p160 and CBP/p300 families of receptor coactivators. General features based on sequence homology. p160 coactivators contain a basic helix-loop-helix (bHLH) motif and a Per-Arnt-Sim (PAS) homology region at the NH<sub>2</sub> terminus. The nuclear receptor interacting domain (RID) contains three LXXLL motifs indicated by asterisks. Two activation domains (AD1 and AD2) with an intervening glutamine-rich (Q) region are located at the COOH terminus. Below the sequence, brackets refer to the functional region histone acetyltransferase (HAT) activity and to the regions of interaction with CBP/p300, the HAT PCAF or the arginine methyltransferase CARM1. In CBP/p300 the RID, indicated by an asterisk, is located in the NH<sub>2</sub> terminus. CBP/p300 also contains the KIX domain (of interaction with CREB), and a bromodomain (Br) as well as three zinc finger regions (C/H1, C/H2, and C/H3) of interaction with various transcription factors and components of the basal transcriptional machinery. The HAT domain, as well as the regions of interaction with PCAF and p160 coactivators are also indicated.

elevated upon cold exposure of mice in both brown fat and skeletal muscle, two key thermogenic tissues. PGC-1 greatly increases the transcriptional activity of PPAR $\gamma$  and the thyroid hormone receptor on the uncoupling protein (UCP-1) promoter. PGC-1 also stimulates mitochondrial biogenesis and respiration in muscle cells through an induction of uncoupling protein 2 (UCP-2) and through regulation of the nuclear respiratory factors (NRFs), which are transcription factors that regulate genes involved in mitochondrial DNA replication and transcription (293). PGC-1 has been shown to have a low inherent transcriptional activity when it is not bound to a transcription factor. The docking of PGC-1 to PPAR $\gamma$  stimulates an apparent conformational change in PGC-1 that permits binding of SRC-1 and the cointegrator CBP/p300, resulting in a large increase in transcriptional activity. Thus transcription factor docking can serve to switch on the activity of coactivators (206).

### 3. An RNA coactivator

A surprising finding has been the identification of an RNA coactivator for steroid receptors (144). This RNA, denominated SAR, works exclusively through the NH<sub>2</sub>-terminal AF-1 domain and can be detected in a large complex of 600–700 kDa which contains several proteins and specifically SRC-1. It has been suggested that SAR might serve as part of a ribonucleoprotein scaffold through which SRC-1 is recruited, and whether or not this RNA could possess intrinsic catalytic activity is still unknown.

### 4. Other coactivators

To date, many other proteins have been demonstrated to enhance transactivation by nuclear receptors. A list of these proteins with a description of their characteristics can be found in excellent recent reviews (169, 220) and references therein. Some of these proteins such as E6-AP, ARA70, NCoA62, or NRIF3 interact with the receptors in a ligand-dependent manner and require the AF-2 domain. However, other coregulators, including p68, PGC-1, or PGC-2 interact with the AF-1 domain. Other coactivators such as TLS, Trip-1/Sug-1, or TSC-2 could be involved in protein degradation pathways, RNA stability, or nuclear transport. Future studies will surely clarify the role of each protein in transcriptional regulation by nuclear receptors. Recent studies also suggest that cell-specific coactivators may play an important role in gene-specific transcriptional activation. In addition, some coactivators exhibit a relative preference for a determined group of nuclear receptors. For instance, ARA70 specifically enhances androgen receptor transcriptional responses (304), and FHL2, which has a unique tissue-specific expression pattern, selectively increases the transcriptional activity of this receptor, but not that of other nuclear receptors, in an agonist- and AF-2-dependent manner (180).

## C. Cointegrators

### 1. CBP/p300

CBP and p300 are large evolutionary conserved proteins that serve coactivator roles for different types of

transcription factors. CBP was originally identified on the basis of its association with CREB in response to cAMP-mediated phosphorylation (142), and the highly related protein p300 was isolated by its interaction with the viral E1A protein (73). Further studies have demonstrated that CBP/p300 interacts with a large variety of transcription factors including AP-1, myoD, Jun, Fos, NF- $\kappa$ B, Pit-1, STATs, and Ets and serves a coactivator role for these factors potentiating their transcriptional activity (247). The finding that these proteins can function as coactivators for different transcription factors has led to the notion that they serve as cointegrators of extracellular and intracellular signaling pathways. CBP/p300 also interacts with TBP, TFIIB, or YY1 and might serve to link the receptors to the basal transcriptional machinery.

In vitro studies, coimmunoprecipitation experiments, and yeast and mammalian two-hybrid assays have also demonstrated an interaction of different nuclear receptors with CBP. This interaction is ligand dependent and AF-2 dependent, and CBP/p300 appears to function as an essential coactivator for the receptors (43, 126). This conclusion comes from the observations that overexpression of CBP/p300 potentiates ligand-dependent transcriptional activation by different nuclear receptors and that, more importantly, microinjection of anti-CBP antibodies blocks ligand-dependent activation by GR, RAR, and RXR. In addition, retinoic acid-dependent transcription is markedly blunted in fibroblasts from p300 knock-out mice (303), supporting the notion that CBP/p300 are key components of hormonal regulation of transcription in vivo.

The interaction between CBP and the receptors maps to the NH<sub>2</sub> terminus of the coactivator (126) (Fig. 7). CBP/p300 contains several other functional domains, including the CREB interaction domain (KIX) to which other transcription factors such as Jun or Myb also associate, and the three zinc finger regions (C/H1, C/H2, and CH3) that bind many other factors. The histone acetylase PCAF, an ortholog of yeast GCN5, also associates with the CH3 region. A bromodomain is present between KIX and the second zinc finger, and a domain exhibiting intrinsic histone acetyltransferase (HAT) activity is found between the bromodomain and the third zinc finger (11, 190). Removal or mutation of the HAT domain results in loss of function for many transcription factors, indicating the importance of this activity.

CBP/p300 not only directly binds the nuclear receptors, but also associates with the p160 family of coactivators through a different, COOH-terminal, region (126, 265, 277). This interaction has been identified both in vivo and in vitro and provides the receptors with two different ways of interacting with CBP/p300, one through a direct interaction with the NH<sub>2</sub>-terminal domain, and other through interaction with the p160 coactivators. As different regions of CBP are involved in interaction with receptors and coactivators, it is possible that they may form a

ternary complex. That these complexes are indeed formed in the cells is suggested by the finding that CBP synergizes with SRC-1 in PR- and ER-mediated transactivation (249). As in the case of CBP, microinjection of anti-p/CIP antibodies blocks ligand-dependent activation by different receptors, and activity can only be restored when both p/CIP and CBP expression vectors are coinjected (265). These results, as well as coimmunoprecipitation experiments demonstrating that a significant portion of endogenous CBP/p300 associates with p/CIP, also suggest that they form a functional complex.

ACTR also interacts with PCAF (26, 138), the first identified mammalian HAT. Thus ACTR might independently interact with both CBP/p300 and their associated factor PCAF, serving as a docking platform to bridge this protein complex to DNA-bound nuclear receptors. There is also evidence that PCAF is a nuclear receptor coactivator. It has been shown that retinoid receptors directly recruit PCAF from mammalian cell extracts in a ligand-dependent manner and that increased expression of PCAF leads to enhanced retinoid-dependent transcription. Direct interaction of PCAF with multiple receptors suggests that its recruitment may be a universal property of nuclear receptors. In contrast to CBP/p300 and the p160 coactivators which require the AF-2 receptor domain for binding, the receptor DBD has a critical role in binding to PCAF, although other regions of the receptors may have auxiliary roles. PCAF binds directly and independently to both nuclear receptors (26, 138) and CBP (302). These interactions may occur sequentially with PCAF first associating with receptors followed by interaction with CBP/p300. Ligand binding may stimulate independent recruitment of both coactivators to the receptor dimers, which is then followed by a cooperative multipoint interaction between these molecules (26). It has also been shown that SRC-1 family members may exist in vivo in heteromultimeric forms with each other and that liganded PR is present in stable complexes containing SRC-1 and TIF2 in vivo. These results suggest that the assembly of large, modular transcriptional complexes by recruitment of distinct subclasses of preformed coregulator subcomplexes may be involved in transcriptional regulation by activated nuclear receptors.

Despite their similarities, p300 and CBP could have distinct functions in cells. Thus, using hammerhead ribozymes that reduce either p300 or CBP mRNAs, F9 cells expressing a p300-specific ribozyme became resistant to retinoic acid-induced differentiation, whereas cells expressing a CBP-specific ribozyme were unaffected. Similarly, retinoic acid-induced transcriptional upregulation of the cell cycle inhibitor p21Cip1 required normal levels of p300, but not CBP, whereas the reverse was true for p27Kip1 (133).

The competition for limiting concentrations of CBP/p300 in cells could be, at least in part, responsible for the

antagonistic actions of nuclear receptor ligands on AP-1 activity, based in the requirement of CBP/p300 for both classes of transcription factors. This is suggested by the finding that the inhibitory effects of RAR and GR on AP-1-dependent activation are significantly reversed by cotransfection of vectors expressing CBP or p300 (126). This reversal could reflect either an allosteric effect whereby binding of nuclear receptors could preclude binding of the AP-1 complex to CBP, or a competition between ligand-bound nuclear receptors and phosphorylated AP-1 for limiting amounts of CBP. The first possibility appears unlikely because AP-1 can effectively bind to RAR-associated CBP (126). A similar mechanism could be involved in the antagonism between nuclear receptors and CRE-mediated transcription (126, 230), where the limiting amount of CBP factors would be partitioned between the liganded receptors and phosphorylated CREB. A hypothetical explanation for glucocorticoid repression of NF- $\kappa$ B-dependent gene expression was also the competition between GR and these transcription factors for limiting amounts of CBP and/or other coactivators. However, glucocorticoids have been demonstrated to maintain their repressive capacity irrespective of the amount of coactivator levels in the cells, suggesting the existence of alternative mechanisms for this transcriptional antagonism (64).

## 2. The TRAP/DRIP complex

Multiprotein complexes denominated TRAP and DRIP that interact with TR (75, 76) or VDR (210, 211) in a ligand-dependent manner have been recently isolated. Both complexes are probably identical and are composed of 14–16 proteins that range in size from 70 to 230 kDa. The complexes are recruited to the core AF-2 receptor region in response to ligand binding through a single subunit (DRIP205/TRAP220) via a receptor interacting motif identical to that found in the p160 coactivators (209). This subunit anchors the other proteins comprising the TRAP/DRIP complex, which is presumably preformed in the cell. The components of this complex are different from those in p160 coactivators, or other previously characterized coactivators, although DRIP205/TRAP220 is identical to the independently characterized protein PBP/PPARBP (322). The DRIP/TRAP complex was shown to enhance the ligand-dependent activity of TR and VDR in a cell-free transcription system with chromatin templates, which has only been demonstrated very recently for SRC-1 (157). Several DRIP/TRAP components are also present in Mediator (211), a complex that together with SRB proteins associates with the large subunit of RNA polymerase II. This has suggested that the DRIP/TRAP complex could act by recruiting the polymerase to the target promoter. Most interestingly, it has been found that other transcriptional activators, different from nuclear

receptors, are able to bind and to recruit similar complexes. Thus many of the DRIP/TRAP components are present in two similar, if not identical, SRB-interacting complexes, ARC, NAT, and SMCC (94, 114, 182) that enhance transcriptional activity of SRBP1- $\alpha$ , the p65 subunit of NF- $\kappa$ B, VP16, or p53. Furthermore, a complex called CRSP (225), which serves as a coactivator for the transcription factor SP-1, is a subcomplex of TRAP/DRIP. These observations suggest that many classes of activators are likely to recruit coactivator complexes and subcomplexes containing the shared subunits of DRIP/TRAP and SRB/Mediator. In the case of nuclear receptors, inducibility would be imposed by ligand binding, which is required for the recruitment of the complex by the receptor through the DRIP205/TRAP220 (78). In the case of other activators, the complexes can be recruited through different subunits and, in fact, p53 and VP16 activation domains interact directly with TRAP80 (114).

The functional interaction of TRAP/DRIP complexes and the p160/CBP/PCAF system is presently unclear. As both complexes interact with the same receptor region they should not bind simultaneously. Imaging of live cells and fluorescence resonance energy transfer (FRET) analysis indicates that ligand-dependent transcriptional activity of nuclear receptors requires recruitment of p160 and DRAP/TRIP-containing coactivator complexes (158). They may act independently and, in this case, the levels of each complex as well as the relative affinity for individual receptors could determine their functional importance in a given hormonal response, or they may act consecutively. If the later is true, the factors that determine the dissociation of the first complex and the binding of the second are still unknown (78).

## D. The LXXLL Motif

The different members of p160 family of coactivators possess a nuclear receptor interacting domain in their central region. This domain contains three highly conserved LXXLL motifs, where L is leucine and X is any amino acid, and is necessary and sufficient to mediate association of coactivators to ligand-bound receptors (67, 102, 265). An additional motif is present in the COOH terminus of the human SRC-1 (125). Mutations in these boxes abolish interaction with the receptor and coactivator activity but do not affect interaction with CBP (102). However, two related motifs harboring acidic and leucine residues, located in a more COOH-terminal position, appear to be responsible for the functional and physical interaction of p160 coactivators with CBP, potentially interacting with a hydrophobic binding pocket. A variable number of LXXLL boxes have been identified in the different coactivators and coregulators that bind the liganded receptors, including CBP, TIF1, RIP140, or

DRIP205/TRAP220 (102, 146, 211, 265, 308). Analysis of the LXXLL motifs has revealed that they form amphipathic  $\alpha$ -helices with the leucines forming a hydrophobic surface on one face of the helix. The structure of these receptor interacting motifs is reminiscent of that of helix 12 in the receptors, which is required for ligand-dependent interaction with coactivators, and also forms an amphipathic  $\alpha$ -helix. It is conceivable that these motifs have evolved to provide a critical mode of assembling the ligand-dependent nuclear receptor-coactivator complexes.

Although the different receptors bind the common LXXLL motif in the p160 coactivators, there is receptor-specific differential utilization of these motifs. Whereas a single motif of the SRC-1 coactivator is sufficient for activation by ER, different combinations of two, appropriately spaced, motifs are required for activation by TR, RAR, androgen receptor (AR), or PPAR. This specificity appears to be dictated by specific residues COOH-terminal to the core LXXLL motifs (168). Surprisingly, different LXXLL motifs are required for PPAR $\gamma$  function in response to specific ligands, suggesting distinct configuration of assembled complexes. The finding that sequences flanking the core LXXLL motif play a role in determining receptor sensitivity has suggested that it may be possible to target receptor-LXXLL interactions to develop receptor-specific antagonists. Thus peptides containing these sequences could interact with a particular receptor in an agonist-dependent manner and disrupt ligand-dependent transcriptional activity. Using combinatorial peptide libraries a peptide was discovered which when overexpressed in cells selectively inhibited ER $\beta$  but not ER $\alpha$ -mediated transcription (44).

The cocrystal structure of the PPAR $\gamma$  LBD and a SRC-1 fragment containing two LXXLL motifs, and ER and TR $\beta$  with a peptide containing an LXXLL motif of GRIP-1, has been recently solved (187). The structures obtained indicate that a conserved glutamic acid residue in helix 12 and the conserved lysine residue at the COOH terminus of helix 3 make hydrogen bonds to leucines 1 and 5. The length and orientation of the LXXLL helical motif is vital for proper backbone interactions with both residues. These contacts form a charge clamp that orients and positions the receptor interacting motif of the coactivator and allows packing of the leucine residues into the hydrophobic pocket formed by helices 3, 4, and 5 (in PPAR $\gamma$ ) or 3, 5, and 6 (in TR $\beta$ ). Specifically, V284 of H3, F293 of H4, and L305 of H5 in TR $\beta$  are part of the groove that forms the binding surface of coactivators and directly contact leucine residues of the signature motif (63). The structure of the cocrystals also indicates that two LXXLL motifs from a single coactivator molecule interact with the AF-2 domains of both dimer partners and that each member of the homo- or heterodimer can cooperatively recruit one molecule of coactivator (187).

Specific partners of RXR in heterodimers, including TR and RAR, allosterically inhibit the binding of ligands to RXR (77). Whereas RAR-RXR heterodimers can be activated by RAR ligands only, RXR ligands can potentiate the transcriptional effects of RAR ligands on cells (52). In parallel, RXR-specific ligands can only potentiate the binding of SRC-1 in the presence of a RAR-specific ligand. This could be, at least in part, due to the interaction of the RXR helix 12 with the coactivator binding pocket of its dimeric partner. Crystal structure of receptor dimers indicates that the AF-2 helix of an apo receptor can interact with the LBD of a second receptor (283). Allosteric inhibition of RXR appears to result from a rotation of the RXR AF-2 helix that places it in contact with the RAR coactivator binding site. In the absence of ligand, the AF-2 domain of RXR is docked to the RAR coactivator-interaction site. Under these conditions, the conformation of RXR could prevent closure of the ligand binding pocket and consequently ligand binding. Recruitment of an LXXLL motif of SRC-1 to RAR in response to ligand displaces the RXR AF-2 domain from RAR, allowing ligands to bind to RXR. Binding of the second ligand can then promote the interaction with a second LXXLL motif from the same SRC-1 molecule, stabilizing the complex. The fact that RAR and PPAR show different affinities for the RXR AF-2 domain could explain why RAR allosterically inhibits RXR, whereas PPAR does not.

### **E. Role of Coactivators on AF-1 and Ligand-Independent Activity**

In contrast to ligand-dependent AF-2 activity, the mechanisms responsible for constitutive AF-1 activity are poorly understood. However, recent evidence suggests a participation of the coactivators that bind to the AF-2 domain on AF-1 activity. It has been shown that p160 coactivators also interact with the NH<sub>2</sub>-terminal AF-1-containing domain of several steroid receptors (1, 161, 191) and the TR $\beta$ 2 TR isoform (189). This interaction appears to be involved in the cooperativity observed between AF-1 and AF-2 domains. Interaction of coactivators with the AF-1 domain appears to be particularly important in the case of the AR in which most of its activity is mediated by the A/B domain. This region appears to be sufficient to recruit SRC-1 to the receptor. Binding of coactivators to the AF-1 domain does not involve LXXLL motifs, but rather the glutamine-rich region of the coactivator (23).

On the other hand, the AF-1 domain can be modulated by kinase signaling pathways. For instance, phosphorylation of two serine residues in the AF-1 domain of ER $\beta$  by MAPK leads to the recruitment of SRC-1. Phosphorylation-mediated recruitment of coactivators provides the molecular basis for ligand-independent activa-

tion of this receptor by the MAPK pathway (266). Phosphorylation also causes binding of coactivators to the ligand-independent AF-1 domain of the orphan receptor steroidogenic factor-1 (SF-1) (99). Furthermore, the estrogen receptor can also be stimulated in a ligand-independent manner by association with cyclin D1 (324). Cyclin D1 interacts with p160 coactivators (323) and also with the acetylase PCAF (170), and by acting as a bridging factor between ER and coactivators, cyclin D1 can recruit p160 coactivators to ER in the absence of ligand (323).

Components of the DRIP/TRAP complex can also participate in AF-1 activity. It has been shown that DRIP150 interacts specifically with the glucocorticoid receptor AF-1 surface in the A/B domain and enhances AF-1-mediated transactivation. Thus glucocorticoid receptor AF-1 is capable of recruiting regulatory factors that regulate transcriptional enhancement. In addition, another member of the DRIP complex, DRIP205, interacts with the AF-2 domain of the glucocorticoid receptor in a hormone-dependent manner and facilitates transactivation in concert with DRIP150. These results suggest that DRIP150 and DRIP205 functionally link glucocorticoid receptor AF-1 and AF-2 and represent important mediators in glucocorticoid receptor transcriptional activity (105).

## VI. NUCLEAR RECEPTOR COREPRESSORS

In addition to ligand-dependent gene activation, selected receptors including TR and RAR repress basal transcription in the absence of ligand. Binding of hormonal ligand to the receptor releases the transcriptional silencing and leads to gene activation. Bahniahmad et al. (9) first demonstrated the existence of active silencing domains in TR and showed that these domains functioned as repressors when fused to a heterologous DBD. Squelching experiments also suggested the existence of inhibitory cellular factors necessary for transcriptional silencing that dissociated from TR and RAR in a ligand-dependent manner (10, 39, 263). Therefore, the current model of gene regulation by these receptors assumes that the unliganded receptors are bound to the HRE and that under these conditions are associated with corepressors responsible for the silencing activity. After ligand binding, the conformational changes in the receptors described in section vA would cause the dissociation of corepressors and the recruitment of coactivator complexes responsible for transcriptional activation. This model would apply to class II receptors, but not to other receptors, such as the unliganded steroid receptors that do not bind corepressor proteins.

This model assumes that unliganded receptors should bind to their response elements *in vivo*. However, definitive proof for this model is still missing, and it has

been shown that RA is required for the *in vivo* occupancy of the response element present in the RAR $\beta$ 2 promoter (65). If this is a general phenomenon, binding of corepressors by the unliganded receptors does not easily explain transcriptional silencing.

### A. Nuclear Corepressor and Silencing Mediator for Retinoic Acid and Thyroid Hormone Receptors

Biochemical studies of cellular proteins associated with unliganded TR and RAR led to the identification of a 270-kDa cellular protein named nuclear corepressor (NCoR) (109) or RIP-13 (242). In parallel, silencing mediator for retinoic and thyroid hormone receptors (SMRT) was isolated by a yeast two-hybrid screening of a human lymphocyte cDNA library (50). The receptor-interacting protein named TRAC2 (231) was demonstrated to be identical to SMRT. Several lines of evidence demonstrate an essential function of these corepressors on ligand-independent repression by nuclear receptors. 1) Unliganded TR and RAR interact strongly *in vitro* with NCoR and SMRT, and addition of ligand induces dissociation from the corepressors. 2) Strong ligand-reversible interaction of the LBDs of TR and RAR with the corepressors has been observed in yeast and mammalian two-hybrid studies. 3) Recruitment of SMRT or NCoR to a promoter by fusion with a heterologous DBD results in a strong repression of basal promoter activity. 4) Microinjection of neutralizing antibodies against NCoR and SMRT results in relief of repression of hormone responsive reporter genes.

NCoR and SMRT are related both structurally and functionally. Figure 8 shows a diagram of the domain structure of these corepressors. Several isoforms of SMRT and NCoR have been reported (51). These include the SMRT dominant-negative form TRAC1 that contains only the COOH-terminal receptor interacting domain and the NCoR/RIP13 form that is similar in size and structure to the originally identified SMRT. The latter was substantially shorter than NCoR, lacking an NH<sub>2</sub>-terminal domain of ~1,000 amino acids. More recently, an extended SMRT isoform has been identified (196), revealing a striking homology to the NH<sub>2</sub> terminus of NCoR. Functional studies demonstrate the existence of multiple repression domains in this extension.

It has been reported that receptor stoichiometry is a crucial determinant of transcriptional repression mediated by NCoR and SMRT. Corepressors appear to bind to TR homodimers but not to TR monomers on DNA. This provides a molecular explanation for the observation that receptors repress transcription as dimers but not as monomers. Additionally, corepressor function appears to be restricted by steric effect related to DNA binding. Although corepressors are capable of binding to several

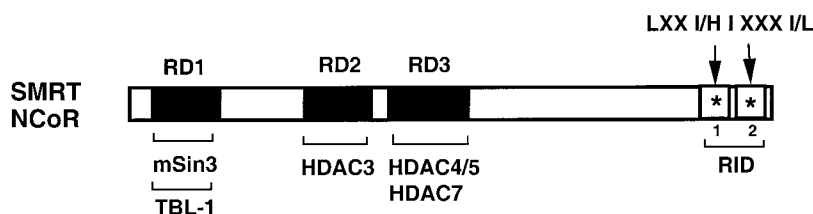


FIG. 8. Structure of the nuclear receptor corepressors SMRT and NCoR. Schematic representation of a corepressor showing the location of the repressor domains (RD1, RD2, and RD3) and the two receptor interacting domains (RID). The RIDs located at the COOH terminus contain the extended helical motif LXX I/H I XXX I/L indicated by asterisks. The RD1 interacts with mSin3A, which in turn recruits class I deacetylases (HDAC1 and -2). The protein TBL1 contacts both the corepressor and mSin3A. Class II deacetylases bind at RD3 without Sin3 as a mediator. HDAC3 has been shown to interact directly with RD2. Class II deacetylases also bind mSin3A through a region different from the HDAC1 interacting domain.

receptors in solution, they are highly selective about receptor binding on DNA, a context that reflects their *in vivo* function more accurately. Thus PPAR $\gamma$  interacts strongly with NCoR and SMRT in solution but not on a PPRE, which is consistent with the observation that PPAR does not repress transcription on this site (310).

Cell-specific repression by nuclear receptors correlates with levels of NCoR. mSiah2, a mammalian homolog of *Drosophila* Seven in absentia, targets NCoR for proteasomal degradation. mSiah2 expression is cell type specific and differentially regulates the repressive activities of nuclear receptors. These findings establish targeted proteolysis of transcriptional coregulators as a mechanism for cell-specific regulation of gene transcription (314).

Ligand binding by itself is not sufficient to induce dissociation of corepressors. Rather, it appears that the AF-2 region serves to trigger the release of corepressors from the receptors. Helix 12 containing the core AF-2 domain is fully inhibitory for corepressor binding to most nuclear receptors. Nuclear receptors lacking this AF-2 region act as constitutive transcriptional repressors. Natural examples include the retroviral oncoprotein *v-erbA*, which is a mutated TR (61, 181, 232), as well as the orphan receptor RevErb that represses transcription when bound as a dimer to a specific subset of DR2 sites (101). Furthermore, RXR does not bind corepressors and does not have a repressive effect, but deletion of this region enhances corepressor interaction and *in vivo* repression (315). In the case of TRs and RARs, mutation or deletion of the AF-2 domain increases interactions with corepressors and reduces the release of the corepressors after ligand binding, indicating again that helix 12 is inhibitory and that the conserved glutamic acid residue of AF-2, critical for coactivator binding and function, is not required in the case of corepressor binding (154).

Recent studies have indicated a role of corepressors in mediating transcriptional silencing properties of several orphan receptors including COUP-TFs and DAX-1 (60, 246), and in addition to nuclear receptors, SMRT and NCoR have been shown to interact with other nonnuclear

receptor transcriptional regulators such as the homeodomain proteins Rpx2 and Pit-1 (298), the transcription factor CBF/RBp-Jk (128), or MyoD (7). Other corepressor-interacting proteins include the promyelocyte zinc-finger protein (PLZF), which is found in the translocation t(11:17) present in acute promyelocytic leukemia (APL) and the acute myeloid leukemia fusion partner ETO involved in t(8:21). For a review, see Reference 176.

Corepressors could also play a role in the syndrome of generalized resistance to thyroid hormone. Patients with mutations in TR $\beta$  manifest various degrees of delay of bone development, hearing defects, and mental retardation. In these cases receptor mutants have reduced or completely lost the hormone-dependent activation but retain silencing function. Different mutations in the TR $\beta$  gene that cause the syndrome result in an abnormally strong constitutive retention of the corepressors SMRT and NCoR by the mutant receptors (56, 228, 305).

Mutational analysis of the TR LBD identified a domain, the CoR box, located in helix 1 of the LBD within the hinge region, which is essential for interaction of receptors with the corepressors (50, 109). Some residues in this box were well conserved in the nuclear receptors that act as ligand-independent transcriptional repressors, and their mutation causes the loss of repression. This led to the hypothesis that the NCoR box could belong to the interaction surface of the receptors with the corepressors. However, X-ray crystal structure demonstrated that the conserved residues are buried within the LBD and could not constitute a surface for corepressor binding. Furthermore, modeling of the corepressor interacting motifs indicated that they cannot make contact with helix 1, and the positions of these residues are more consistent with the idea that they affect the placement of other helices sufficiently to facilitate corepressor binding.

Initial mapping studies with the corepressor proteins revealed that the receptor interaction and the repression function are separable, with the receptor interacting domain (RID) located toward the COOH terminus. Further studies revealed that the interaction domain could be subdivided into two subdomains that can interact with the

receptors when isolated from the rest of the protein. In analogous fashion with coactivators, corepressors contain two motifs related to the LXXLL sequences (110, 185, 200). These motifs (17 and 19 amino acids long, respectively), which exhibit a consensus sequence of LXX I/H I XXX I/L, have been also named CoRNR boxes and are conserved in both position and sequence between NCoR and SMRT. Secondary structure prediction suggests that they are also likely to adopt an amphipathic  $\alpha$ -helical conformation. However, compared with the LXXLL motif, the CoRNR motif presents an NH<sub>2</sub>-terminal extended helix. This extension appears to be required for effective binding to the unliganded receptor. Any mutation of the hydrophobic core abolishes interaction of the corepressor with the receptors, and additional flanking sequences are needed for the corepressor-receptor interaction, consistent with additional contacts to stabilize binding.

The finding that corepressor and coactivator binding motifs are both amphipathic  $\alpha$ -helices suggests that they may bind to similar or overlapping sites on the receptor LBD. Interestingly, mutations in the residues of helices 3, 4, and 5 of TR $\beta$  that are part of the groove responsible for coactivators binding, also diminish interaction with corepressors as well as active repression (110, 185, 200). The extended helical CoRNR motif appears to be prevented from binding to the coactivator pocket in the presence of ligand because it is too long to be accommodated by the charge clamp that accommodates the LXXLL motif. Perissi et al. (200) have suggested that the extended helix displaces the AF-2 helix out of the pocket and makes contact with the receptor coactivator pocket. Thus the NH<sub>2</sub>-terminal extension of the  $\alpha$ -helix constitutes a critical distinction in the alternative ligand-independent binding of corepressors and ligand-dependent recruitment of coactivators to nuclear receptors.

Although steroid hormone antagonists induce receptor dimerization and DNA binding, the resulting dimer is unable to stimulate transcription. These compounds can act as pure antagonists or partial agonists depending on the cell type and the promoter context. A number of studies suggest that cellular corepressors could be responsible for downregulating the transcriptional activity of antagonist-bound steroid receptors. Recent results indicate that although agonist liganded steroid hormone receptors do not appear to interact effectively with NCoR or SMRT, clear interactions both in vivo and in vitro were observed with receptor-bound antagonists (115, 248, 316). It is possible that the repositioning of helix 12 by the estrogen antagonist tamoxifen permits corepressor binding into the hydrophobic pocket. This suggests that steroid receptors occupied by antagonists are not intrinsically inactive and that their transactivating functions may be masked by binding of cellular corepressors.

## B. Other Corepressors

Other proteins different from NCoR and SMRT that also act as corepressors of nuclear receptors have been isolated. Small ubiquitous nuclear corepressor (SUN-CoR) which shows no homology to NCoR or SMRT, is a highly basic 16-kDa nuclear protein that is expressed at high levels in adult tissues and is induced during adipocyte and myogenic differentiation. SUN-CoR potentiates transcriptional repression by TR and RevErb in vivo, represses transcription when fused to a heterologous DNA binding domain, and interacts with both receptors in vitro. SUN-CoR also interacts with NCoR and SMRT in vitro and with endogenous NCoR in cells. Thus SUN-CoR may function as an additional component of the complex involved in transcriptional repression by unliganded and orphan nuclear hormone receptors (309).

A novel corepressor, Alien, has been identified recently. This corepressor has a 90% identity with the Alien protein of *Drosophila*. Alien interacts with a subset of receptors such as TR, the *Drosophila* homolog of COUP-TF-1, the ecdysone receptor or DAX-1, but not with RAR, RXR, or its *Drosophila* homolog Ultraspiracle. Lack of interaction with RAR and the absence of significant sequence homologies with SMRT and NCoR indicate that Alien belongs to a different class of corepressors, which interact in a hormone-sensitive manner (69).

## C. RIP 140: a Coactivator or a Corepressor?

Cloning of the cDNA for the protein initially identified as p140 demonstrated the existence of a receptor interacting protein (RIP 140) that is widely expressed in mammalian cells and interacts with ER and other receptors, but not with transcriptionally defective mutants, in an agonist-dependent manner. This suggested that RIP 140 could act as a bona fide coactivator (42). However, although this protein contains several strong receptor interacting domains, it only minimally potentiates hormonal responses, and when transfected in increasing amounts produces a biphasic effect, decreasing transcriptional responses at high concentrations. This has led to the hypothesis that RIP 140 can function as a corepressor rather than as a coactivator. Nevertheless, RIP140 can act as a coactivator and enhance ligand-dependent transactivation in vivo in yeast (123). The general consensus is that this protein may function as a regulatory factor by influencing binding of endogenous coactivators. In fact, in transient transfection assays, RIP 140 can antagonize SRC-1-mediated activation possibly through competition with the coactivator for binding to the AF-2 receptor domain (267).

## VII. NUCLEAR RECEPTORS AND CHROMATIN

### A. Acetylation and Deacetylation

Nucleosomes, which fold chromosomal DNA, contain two molecules each of the core histones H2A, H2B, H3, and H4. Almost two turns of DNA are wrapped around this octameric core, which provides a major impediment to transcription. Two different mechanisms can alleviate this repression: histones can be posttranslationally modified to destabilize chromatin, and nucleosomes can be disrupted through activation of ATP-driven machines that can remodel chromatin (198, 276). Histones can be posttranslationally modified, and this modification alters chromatin structure and function. Of these modifications, histone acetylation has generated most interest because it provided an early link between transcription and chromatin modification (3). Increased acetylation of lysines in histones tails has been correlated with transcriptional activity, whereas hypoacetylation has been associated with transcriptional repression (254, 292). Acetylation neutralizes the positively charged lysine residues of the histone NH<sub>2</sub> termini decreasing their affinity for DNA, which causes nucleosome unfolding and can increase access of transcription factor to the promoter. It is also probable that the acetylation of specific lysine residues in the core histones provides novel recognition surfaces to promote the association of positive regulators in the transcription process. Additionally, it has been shown that histone tails are involved in nucleosomal-nucleosomal contacts, and this suggests that acetylation may also disrupt higher order chromatin structure (218).

Recent studies have revealed a strong link between histone acetylation, chromatin remodeling, and gene regulation (254, 270). The relative levels of histone acetylation are known to be determined by the enzymatic activities of both HATs and histone deacetylases (HDACs), and it has been demonstrated that transcriptional activators such as GCN5 (the yeast homolog of PCAF) are HATs. Conversely, the mammalian histone deacetylase HDAC1 (or HD1) (261) is related to the yeast RPD3 repressor, and the deacetylase HDAC2, which is homologous with the yeast HDA1 (93), has been found to repress transcription (301). HDACs and HATs are enzymes with no observable preference for a specific DNA sequence. However, they associate with coactivators and corepressors, which in turn are brought to DNA through binding to sequence-specific transcription factors.

There is evidence that binding of hormone receptors locally alters the chromatin structure. Thus the glucocorticoid receptor can disrupt nucleosomes in the absence of DNA replication, and the disrupted nucleosome can also rapidly reassemble after hormone withdrawal (215). In other models, binding of receptors does not remove the

histone octamer from the promoter but produces more subtle changes that lead to greater accessibility of other transcription factors (205). This is the case of the mouse mammary tumor virus (MMTV) promoter that is regulated by steroid hormones through a hormone-responsive region that is organized in a positioned nucleosome. Hormone induction leads to a structural change of this nucleosome, which makes its DNA more sensitive to cleavage by DNase I and enables simultaneous binding of all relevant transcription factors. In fact, hormone activation of the MMTV promoter leads to the establishment of specific translational positioning of nucleosomes *in vivo* despite the lack of significant positioning in the inactive state (21). Moderate acetylation of core histones, generated by treatment with low concentrations of the histone deacetylase inhibitors sodium butyrate or trichostatin A, potentiates transactivation of the MMTV promoter by either glucocorticoids or progestins (16). Analogously, small increases in histone acetylation are able to significantly increase the activity of thyroid hormone and retinoic acid-responsive promoters, existing a good correlation between the accumulation of hyperacetylated histones upon inhibition of histone deacetylases and the potentiation of the transcriptional response to ligands (82). In the MMTV model, inducing inhibitor concentrations led to the same type of nucleosomal DNase I hypersensitivity as hormone treatment, suggesting that moderate acetylation of core histone activates the promoter by mechanisms involving chromatin remodeling similar to that generated by the inducing hormones. Although these observations suggested that histone acetylation could play an important role in the transcriptional responses to nuclear receptors, they did not demonstrate that gene activation results from this modification.

#### 1. Coactivators and histone acetylation

The notion that core histone acetylation facilitates nuclear receptor-mediated gene expression has gained further support as not only PCAF but also CBP/p300 (11), TAFII250 (177) and the p160 coactivators SRC-1 (253) and pCIP/ACTR (48) have been shown to possess HAT activity. These proteins can acetylate both free histones and nucleosomal histones *in vitro*. In addition, it has been recently shown that CBP/p300 and PCAF are able to acetylate nonhistone proteins, including p53, E2F, and MyoD and components of the general transcriptional machinery such as TFIIE (95, 112, 166, 233). Acetylation can increase binding of p53 and MyoD to DNA, and acetylation of the latter appears to be required for MyoD-mediated muscular differentiation, showing its functional importance. Furthermore, CBP can acetylate the orphan receptor HNF-4 at lysine residues within the nuclear localization sequence (251, 252), and this acetylation appears to be critical for target gene activation.

As described in section *vB*, the p160 coactivators have a COOH-terminal domain responsible for HAT activity and interaction with CBP/p300. On the other hand, the action of CBP/p300 appears to require both its intrinsic HAT activity and its role as a platform for a large number of transcription factors, coactivators, and components of the basal transcriptional machinery (89, 169). Furthermore, PCAF possesses intrinsic HAT activity (302) and can acetylate free histones and nucleosomal H3. The COOH-terminal region contains the HAT domain and the sequences responsible for interaction with p/CIP, whereas the NH<sub>2</sub>-terminal region interacts with CBP, SRC-1, and the nuclear receptors (26, 138). Thus PCAF could have a dual role. First as a HAT it has the direct capacity to modify chromatin to reverse repression, and second, via its CBP/p300 and p160-interaction domains it can serve to recruit additional activators with acetylase activity to the vicinity of the target promoter.

The fact that the receptors can recruit complexes in which each of their components has HAT activity suggests a functional redundancy. It is possible that different classes of transcription factors functionally require distinct components of the coactivator complex. In this respect, RAR, CREB, and Stat-1 appear to require different HAT activities to activate transcription: the HAT activity of CBP is required for CREB and STAT-1 function, whereas that of PCAF appears to be indispensable for nuclear receptor activation (138). Thus alternative interaction interfaces appear to be used in recruitment of specific factors into the coactivator complex.

In vivo evidence that ligand binding causes the recruitment of complexes with HAT activity has been obtained. It has been demonstrated that in vivo histone acetylation levels of nuclear receptor target genes is strongly induced upon treatment with the corresponding ligand (49). Hyperacetylation is only triggered by agonists and is AF-2 dependent. These results confirm that histone acetylation is a critical step in nuclear receptor-mediated hormone signaling. The same authors have demonstrated that CBP/p300 HAT is particularly critical for hormone-induced hyperacetylation of H3 and H4 histones. Unexpectedly, hormone-induced histone hyperacetylation at the target promoter is transient and coincides with attenuation of hormone-induced gene activation. The underlying mechanism for this observation appears to be that the acetylase ACTR is acetylated by p300/CBP and acetylation neutralizes the positive charges of two lysine residues adjacent to the core LXXLL motif and disrupts the association of HAT coactivator complexes with promoter-bound receptors (49). Most likely, this mechanism also operates with other p160 coactivators such as SRC-1 or TIF-2.

## 2. Corepressors and histone deacetylation

Transcriptional repression by a sequence specific DNA-binding factor can be mediated by the recruitment of a deacetylase to the promoter region. A milestone in establishing histone deacetylation as an important mechanism of mammalian transcriptional repression was the identification of a mammalian homolog of the yeast repressor RPD3 as a histone deacetylase (HDAC1) (261). Subsequently, a second repressor, HDAC2, highly homologous to HDAC1 was identified (301). These enzymes are found in the cells in large multiprotein complexes. There are two major complexes each containing HDAC1 and HDAC2. The first is conserved from yeast to mammals and comprises the HDACs and the histone binding proteins RbAp46/48 (the core HDAC complex) associated with the yeast corepressor Sin3P, or its mammalian homologs mSin3A and mSin3B. The second complex Mi-2/NuRD (nucleosome remodeling histone deacetylase complex) lacks mSin3 but contains an ATP-dependent chromatin remodeling activity in addition to HDAC activity (136). A clear function of this complex in transcriptional repression by nuclear receptors has not been identified to date, but immunodepletion of NURD or Mi-2 relieves transcriptional repression by unliganded receptors, suggesting that these proteins can regulate access of nuclear receptor corepressor complexes to promoters (299).

It appears that one of the mechanisms for the recruitment of HDACs to target genes involves their association with Sin3 proteins tethered to DNA through interaction with sequence-specific, DNA binding factors (136). Sin3 corepressor complexes contain at least eight different polypeptides (319). Sin3 itself is a large multidomain protein that most likely forms the scaffold upon which the rest of the complex assembles (6). Several interaction domains mediate binding with the proteins, which in turn facilitates targeting of the complex. Another conserved region binds components of the HDAC core complex that appear to be critical for Sin3-mediated repression. mSin3-HDAC complexes are abundant and stable and could be available for binding and recruitment by the repressors. The nuclear receptor corepressors SMRT and NCoR are among the factors that associate with mSin3 (103, 184). mSin3 and associated HDACs are required for repression by unliganded receptors, suggesting that the corepressors function by recruiting the mSin3-HDAC complex. The interaction between unliganded receptors and mSin3A is therefore not direct but is mediated by NCoR and SMRT whose function would be to link the receptors to the HDAC/mSin3 complex. Sin3 proteins contain four imperfect repeats of a paired amphipathic helix (PAH) motif postulated to mediate protein-protein interactions. NCoR interacts with both PAH1 and a second region centered around PAH3 of mSin3 by means of a COOH-terminal

region (amino acids 1829–1940) and an NH<sub>2</sub>-terminal repression domain (amino acids 254–312) (2, 103, 184). This regulation is evolutionarily conserved since repression mediated by the *Drosophila* ecdysone receptor requires orthologs of the corepressors Sin3 and HDAC (268). SAP30, another component of the Sin3 complex, associates with HDAC and Sin and additionally with one of the repression domains of NCoR (143).

Different transcription factors may associate with mSin3 complex simultaneously. For instance, the repressor Mad appears to be present not only in complexes involved in repression at Mad-1 binding sites but also in complexes mediating repression by nuclear receptors (188).

Until very recently, the corepressors were thought to act exclusively through the above-described indirect recruitment of HDAC1 or -2 (class I deacetylases), via the adaptor mSin3 protein. Surprisingly, however, numerous biochemical studies have not detected NCoR or SMRT in mSin3- and HDAC1-containing complexes. A new family of histone deacetylases (class II deacetylases) including HDAC4 and HDAC5 has been identified (93), and two-hybrid screen on SMRT-interacting proteins has led to the isolation of other acetylase, a new family member termed HDAC7 (127). Corepressors contain multiple nonredundant repression domains, and one of them, which is conserved in NCoR and SMRT, has been demonstrated to repress transcription by directly interacting with class II HDACs. Endogenous NCoR and SMRT each associates with class II HDACs in a complex that does not contain mSin3A or HDAC1 (111, 127). Therefore, a single core-

pressor could use distinct domains to engage class I HDAC complexes in a Sin3A-dependent manner and class II HDAC complexes in a Sin3A-independent manner. Furthermore, a novel SMRT-containing complex has been isolated from cells. This complex contains HDAC3 and transducin beta-like protein 1 (TBL1), a protein that interacts with histone H3 and is associated with human sensorineural deafness. In vivo, TBL1 is bridged to HDAC3 through SMRT and can potentiate repression by TR (96). Intriguingly, loss-of-function TR $\beta$  mutations cause deafness in mice and humans.

The above observations suggest that compaction of chromatin structure due to recruitment of histone deacetylases complexes by the corepressors is involved in transcriptional silencing by the unliganded receptors. Ligand binding would allow the release of corepressors and enable the receptors to recruit coactivators and stimulate transcription (Fig. 9).

There is evidence that corepressors and deacetylase activity could be also involved in ligand-dependent negative regulation by nuclear receptors. In contrast to positively regulated genes, it is known that thyroid hormone receptors increase basal activity of negatively regulated promoters, and addition of ligand reverses this stimulation. It has been reported that corepressors are involved in basal activation of the TSH $\alpha$  and TRH promoters by unliganded TRs, since overexpression of NCoR or SMRT paradoxically enhances rather than suppresses basal activity (255). In the TSH $\alpha$  promoter, the recruitment of CoRs by TR is associated with transcriptional stimulation and an increase in histone acetylation. Expression of

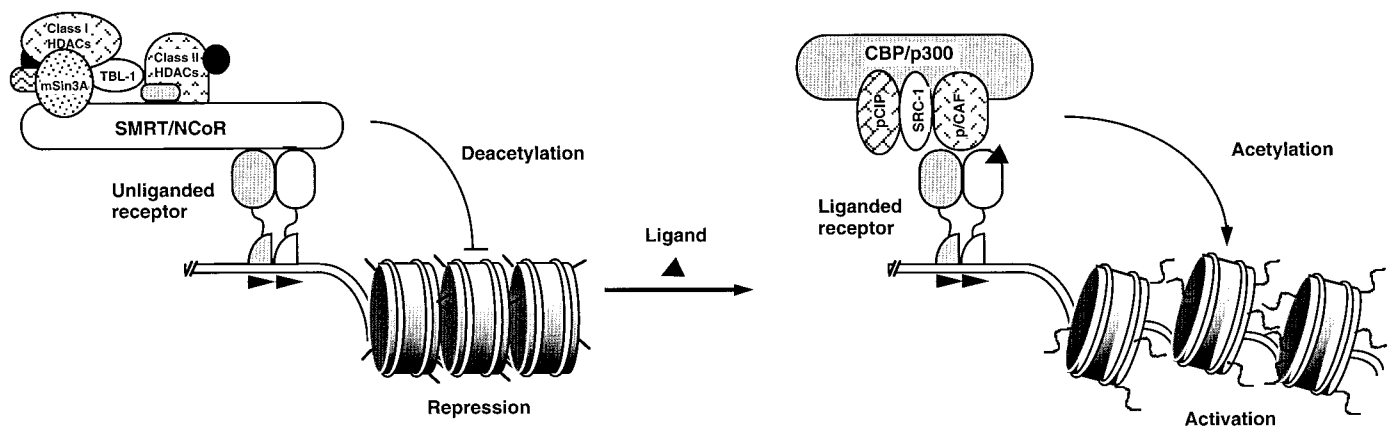


FIG. 9. Coactivator and corepressor complexes and histone acetylation. In the absence of ligand, the nuclear hormone receptor heterodimer is associated with corepressor complexes. The corepressors (SMRT/NCoR) recruit histone deacetylases (HDACs) either directly or through their interaction with Sin3. Many other proteins must belong to these complexes, whose exact composition is still unknown. Deacetylation of histone tails leads to chromatin compaction and transcriptional repression. Ligand binding causes the release of the corepressor complex and the AF-2-dependent recruitment of a coactivator complex that contains at least p160 coactivators (such as p/CIP or SRC-1), CBP/p300, and PCAF. All of these proteins possess histone acetyltransferase (HAT) activity that allows chromatin decompaction and gene activation. Multiple protein-protein interactions exist among the different components: CBP/p300 contacts the receptor, the p160 coactivators, and PCAF through independent domains. Similarly, the receptor binds CBP/p300, p160 coactivators, and PCAF, and PCAF can also bind directly to CBP/p300, p160 coactivators, and the receptor.

HDAC1 reverses the stimulation caused by the unliganded receptor and the corepressor, whereas ligand results in transcriptional repression and loss of histone acetylation (256). Ligand-independent activation could then result from withdrawal of HDAC from other target sites, such as the basal promoter, resulting in a net increase of histone acetylation and transcriptional stimulation. In another study (234), it has been demonstrated that  $T_3$  induces recruitment of HDAC2 and TR to a negative response element in the TSH $\beta$  promoter. This is contrary to the expectation based on positive response elements in which HDACs are dissociated from the receptors upon ligand binding.

## B. Other Chromatin Modifications

The histone NH<sub>2</sub> termini extend from the nucleosomal core and can be modified not only by acetylation, but also by phosphorylation, methylation, and ADP-ribosylation. These modifications affect their charge and function and alter chromatin structure and gene expression. Although ADP-ribosylation could participate in transcriptional activation by nuclear receptors (229), the role of histone modifications other than acetylation is not well known. However, an arginine methyltransferase (CARM1) that binds to the COOH-terminal region of the p160 family of coactivators has been recently identified (47). This protein enhances transcriptional activation by nuclear receptors by functioning as a secondary coactivator through its association with p160 coactivators. CARM1 can methylate histone H3 *in vitro*, and a mutation in the *S*-adenosylmethionine binding domain greatly reduces coactivator activity, suggesting that, in addition to histone acetylation, coactivator-mediated methylation of histones or other proteins in the transcriptional machinery may also contribute to transcriptional regulation by nuclear receptors. That histone phosphorylation could also have an important impact in hormonal responses is suggested by the finding that the MMTV promoter becomes refractory upon prolonged glucocorticoid exposure, due to ligand-induced global dephosphorylation of linker histone H1 (147).

## C. Chromatin Remodeling

Two major mechanisms alleviate the block of transcription caused by the nucleosomal structure: histones can be posttranslationally modified to destabilize chromatin, especially by acetylation as described above, and nucleosomes can be disrupted through the activity of ATP-driven machines (276). The fundamental function of these remodeling factors is the mobilization of nucleosomes via the alteration of histone-DNA contacts. Chromatin remodeling factors comprise an ATPase subunit

with a conserved NTP-binding motif along with other polypeptides. There are at least three subfamilies of ATPase subunits: SWI2/SNF2, Mi-2/CDH, and ISWI/SNFL2, and the existence of other proteins closely related to the ATPase subunits suggests that there are still other chromatin remodeling complexes that remain to be discovered (270). Remodeling factors use the energy derived from ATP hydrolysis to catalyze nucleosome mobilization, which is a net change in the position of the histone octamer relative to DNA. This change is believed to facilitate the access and function of key components of the transcriptional apparatus. Some nucleosome remodeling factors appear to be targeted to specific regions of the genome. Activators that can bind to DNA in chromatin could recruit a remodeling complex to the promoter that could facilitate binding of other factors whose binding sites were not previously exposed in the nucleosomes.

The yeast SWI/SNF complex is well characterized, and there are mammalian homologs for both proteins (brahma and brahma-related gene-1, respectively) (279). This complex appears to be implicated in transcriptional regulation by nuclear receptors (79, 179), and it has been shown that GR and ER can interact with the SWI/SNF complex in a ligand-dependent manner (186). Furthermore, this complex appears to be important for transcriptional stimulation by GR, at least in yeasts, since a yeast strain bearing mutations in the *swi* genes is unable to support GR-dependent transactivation, whereas in a wild-type strain, glucocorticoids can transactivate a reporter gene in the presence of cotransfected receptor (306).

On the other hand, it is known that receptors can bind to their response elements packaged into chromatin in mammalian cells and that receptor binding to DNA indeed facilitates factor access to chromatin in an ATP-dependent manner (66). However, although bound receptors are known to facilitate SWI/SNF-mediated disruption of MMTV mononucleosomes (193), the chromatin remodeling activity caused by PR on the MMTV promoter does not seem to be related to the SWI/SNIF complex, and the activity involved seems to be ISWI. It has been found that PR causes recruitment of ISWI to chromatin and, as this protein is only found in complexes with other proteins (58), the receptor should cause recruitment of an ISWI-containing complex. One of the complexes could contain ISWI and the inorganic pyrophosphatase NURF38, but the SNF-2-related ATPases have not been identified in the remodeling complex recruited (66).

Histone modification and HAT activity might be required either to disrupt chromatin or to act at a step subsequent to chromatin disruption. In this respect, results in yeast suggest that the SWI-SNF complex acts before HATs to activate transcription (59). That chromatin remodeling by ATP-driven machines might precede the action of HATs in transcriptional responses to nuclear receptors has also been suggested. It has been shown that

ligand-bound TR targets chromatin disruption independently of gene activation and that the acetyltransferase p300 neither disrupts chromatin nor activates transcription from a disrupted template in the presence or absence of ligand-bound receptor. However, the acetyltransferase activity of p300 facilitates transcription from the previously disrupted chromatin template (153).

Interestingly, chromatin remodeling can also facilitate transcriptional repression: the SWI/SNF complex and the related RSC complex appear to be important not only for transcriptional activation, but also for transcriptional repression (25, 136). Thus the NuRD/Mi-2 complex has both chromatin remodeling and histone deacetylation activities. The nucleosome remodeling activity of NuRD is required for deacetylation, which suggests that chromatin remodeling facilitates the access of the NuRD HDACs to the histone tails. This complex may be recruited to specific promoters by direct interaction between the Mi-2 ATP subunit and sequence specific transcriptional repressors. NuRD appears also to interact with methylated DNA and could participate in inactivation of gene expression due to CpG methylation (270).

## VIII. PHYSIOLOGICAL ROLE OF COACTIVATORS AND COREPRESSORS

Although biochemical and functional data strongly suggest that coactivators and corepressors play a key role in transcriptional regulation by nuclear receptors, one of the most important questions to be answered concerns the function of each of these coregulators *in vivo*. Although different coactivators and corepressors possess similar properties in terms of interaction with nuclear receptors and they are widely expressed, relative expression levels are dependent on tissue types. The physiological role of specific coactivators on the *in vivo* function of the receptors has been proven by targeted deletion in mice. The results obtained have shown that different coactivators can indeed play a distinct but overlapping role in nuclear receptor function. On the other hand, the characterization of different human diseases in which the organization or expression of nuclear receptor coregulators is altered has further demonstrated the important physiological role of coactivators and corepressors.

### A. Genetic Disruption of Coregulators

#### 1. *CBP/p300*

CBP appears to be present in limiting concentrations in most cells. This is suggested by the finding that embryos lacking a single CBP allele present abnormal skeletal patterning (258) with similarities to patients suffering from the Rubinstein-Taybi syndrome, a disorder charac-

terized by mental retardation and various physical defects caused by heterozygous mutations of the CBP gene in humans (202). On the other hand, targeted deletion of the p300 gene causes embryonic lethality, indicating that expression of CBP is not sufficient to prevent the defects due to p300 loss (303). These observations show that although CBP and p300 are very similar and share many functional properties, the functional redundancy of these factors is not complete.

#### 2. *SRC-1*

The *in vivo* importance of SRC-1 in hormone-dependent gene transcription has been assessed in mice in which the SRC-1 gene has been inactivated by gene targeting. These knock-out mice do not exhibit a dramatic phenotype. However, although they are viable and fertile, they exhibit a decreased growth of sexual steroid target organs in response to hormonal stimulation (296) and display pituitary resistance to thyroid hormone as evidenced by the elevated levels of serum thyrotropin in the presence of elevated free thyroid hormone levels (282). Interestingly, there is a compensatory overexpression of TIF-2 in the SRC-1 null mutants, suggesting the existence of redundancy between both coactivators.

#### 3. *SRC-3*

Genetic disruption of SRC-3 in mice results in a pleiotropic phenotype showing dwarfism, delayed puberty, reduced female reproductive function, and blunted mammary gland development. Some of these anomalies are secondary to reduced production of estrogens, and growth retardation in mice lacking SRC-3 is most likely due to deficiency in insulin-like growth factor I (295). With the exception of the deficiency in mammary gland development, the phenotype obtained in SRC-3 knock-out mice is not observed in SRC-1 null mice. This indicates that members of the p160 family of coactivators have distinct roles *in vivo* and that SRC-3 may play a more critical role in overall growth and sexual maturation.

#### 4. *TRAP220/DRIP205*

The critical function of this coactivator in embryonic development has been demonstrated by targeted inactivation of the gene in mice. Lack of TRAP220 results in lethality during early gestation. The null mutants present heart failure and exhibit impaired neuronal differentiation with extensive apoptosis. In addition, haploinsufficient animals show growth retardation, dysfunction of the pituitary/thyroid axis, and a generalized transcriptional impairment of organs such as testis or brain. Primary embryonic fibroblasts (MEFs) derived from the knock-out mice show a marked decrease of thyroid hormone receptor function. Surprisingly, they show no defect in activa-

tion by RAR/RXR. This suggests a stronger contribution of alternative coactivators in retinoid signaling than in thyroid hormone signaling (113). On the other hand, the syndrome of X-linked dementia and hypothyroidism in humans has been assigned to mutations in the TRAP230 subunit of the TRAP/DRIP complex (204). These observations suggest that the TRAP/DRIP complex plays a crucial role in maintaining thyroid hormone homeostasis.

### 5. RIP 140

Null mice for RIP 140 are viable, but female mice are infertile because mature follicles fail to release the oocyte at ovulation (284). In contrast, luteinization proceeds normally, resulting in a phenotype closely resembling that of luteinized unruptured follicle syndrome, often associated with infertility in women. Therefore, whereas the preovulatory surge of luteinizing hormone induces both ovulation and luteinization, the ability to suppress the action of nuclear receptors is essential for the coordinated control of ovarian function with the essential process of oocyte release dependent on the activity of RIP 140.

## B. Implication of Coactivators and Corepressors in Clinical Disorders

### 1. Steroid-dependent tumors

The nuclear receptor coactivator AIB1 (SRC-3) was identified because of its overexpression in primary breast tumors and in ovarian and breast cancer cell lines, suggesting that aberrant expression of receptor coactivators could play a role in the development of steroid-dependent tumors. High levels of expression are specific for this isoform, since SRC-1 and GRIP-1/NCoA2 are expressed at normal levels in these tumors (4). However, another recently identified ER coactivator (PBP/PPARBP), identical to the TRAP220/DRIP205 subunit of the TRAP/DRIP complex, is also amplified and overexpressed in breast tumors, indicating again a potential role of coactivators in breast carcinogenesis (321).

### 2. Leukemias

Nuclear receptor coactivators with HAT activity appear to play a role in malignant transformation and more specifically in APL. The genes for monocytic leukemia zinc finger protein (MOZ) and the coactivator TIF2 are involved in the inv(8)(p11q13) that causes leukemia. The inversion creates a fusion between MOZ and TIF-2. The predicted fusion protein contains the zinc finger domains, the nuclear localization domains, the HAT domain, and a portion of the acidic domain of MOZ, coupled to the CBP interaction domain and the activation domains of TIF2. Other translocation in acute monocytic leukemia also fuses MOZ with CBP. Leukemia cell phenotype observed

in both cases could arise by recruitment of CBP, resulting in modulation of the transcriptional activity of target genes by a mechanism involving abnormal histone acetylation (36). Another example of the importance of histone acetylation in the pathogenesis of APL is demonstrated by the fact that PML and Tif-1 $\alpha$ , a protein that associates with the receptor, are fused to RAR $\alpha$  and B-Raf, respectively, resulting in the production of PML-RAR $\alpha$  and Tif1 $\alpha$ -B-Raf (T18) oncoproteins. It has been shown that PML, Tif1 $\alpha$ , and RXR $\alpha$ /RAR $\alpha$  function together in a transcription complex that is dependent on retinoic acid. PML interacts with Tif1 $\alpha$  and CBP, and the oncoproteins T18 and PML-RAR $\alpha$  disrupt the retinoic acid-dependent activity of this complex in a dominant-negative manner resulting in a growth advantage (320).

Several observations indicate the importance of repression in hormone action and disease. For instance, v-ErbA gives rise to erythroleukemia in chickens, and a single amino acid substitution, P160A, that abolishes the ability of the oncogenic protein to repress basal transcription and to interact with SMRT also abolishes oncogenic transformation (34). Furthermore, mutant forms of RAR $\alpha$ , which retain the receptor DBD and LBD, created by chromosomal translocations with either the promyelocytic leukemia (PML) or the PLZF locus are oncogenic and result in APL. PML-RAR $\alpha$ -APL patients achieve complete remission after treatment with pharmacological doses of retinoic acid, and the recruitment of nuclear receptor corepressors is critical for the oncogenic function of PML-RAR $\alpha$ . In contrast, PLZF-RAR $\alpha$  patients do not respond to retinoic acid treatment. These differences have been attributed to differences in corepressor release after retinoid binding to these fusion proteins. A direct binding of SMRT to PLZF, as well as RAR has been demonstrated (156), an interaction that is maintained after ligand binding to the RAR moiety. The PML-RAR fusion protein, however, binds corepressors only through the RAR LBD, and upon ligand binding, the corepressor dissociates, allowing retinoic acid-mediated differentiation processes to occur. Mutation of the NCoR binding site abolishes the ability of PML-RAR to block differentiation. Therefore, the differential effects of RAR on the stability of the PML-RAR and PLZF-RAR corepressor complexes determine the differential response of APLs to retinoic acid. The ability of PML-RAR $\alpha$  to form homodimers is responsible for its increased binding efficiency to the corepressors and impaired hematopoietic differentiation, illustrating oligomerization of transcription factors as a novel mechanism of oncogenic activation (155, 175).

## IX. CLOSING COMMENTARIES

During the course of the last years, our knowledge of the molecular mechanisms by which nuclear receptors

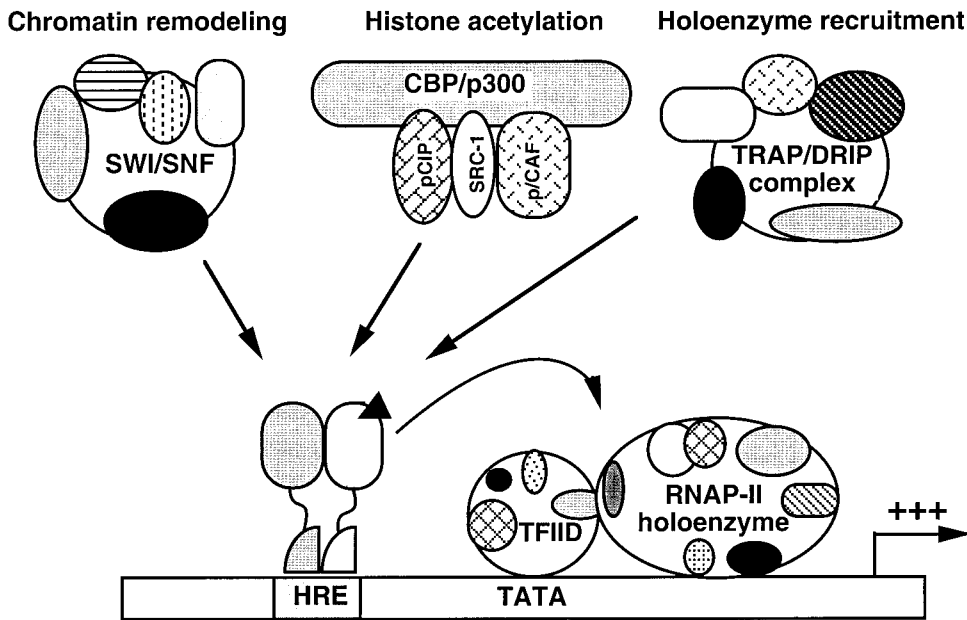


FIG. 10. Ligand-dependent recruitment of multiple coactivator complexes. Upon ligand binding, the receptors recruit different coactivator complexes. The complex CBP/p160/PCAF possesses histone acetyltransferase activity, the SWI/SNF complex possesses ATP-dependent chromatin remodeling activity, and the TRAP/DRIP complex may recruit the RNA polymerase II (RNAP II) holoenzyme. Recruitment of the complexes may be sequential or combinatorial. It is conceivable that chromatin remodeling complexes are initially recruited to the promoter. These factors may relieve the repression imposed by high-order chromatin structure and allow a second acetylation-dependent step on gene activation. Activation would require the combinatorial of subsequent action of additional complexes that include the TRAP/DRIP complex.

and their ligands elicit their actions in cells has grown spectacularly. However, the exact biochemical mechanisms by which these receptors stimulate transcription are still unclear. As discussed in this review, the liganded receptors recruit three types of coactivator complexes with different activities (Fig. 10): the TRIP/DRAP complexes, which may favor the recruitment of components of the basal transcriptional machinery including RNA polymerase to the target promoter; the PCAF/CBP/p160 complexes, which function as enzymes that modify chromatin structure by acetylation; and the ATP-dependent chromatin remodeling factors. Despite the astonishing amount of information gathered recently, many important questions remain to be solved. Thus whether these complexes act independently or in a coordinated or sequential manner remains unclear. Also, the individual contribution of each complex to the final transcriptional response is still unknown. In addition, many aspects of the mechanisms by which corepressor complexes mediate silencing activity of nuclear receptors are yet to be determined. The near future undoubtedly will provide new insights into the complex combinatorial actions by which coregulators mediate specific transcriptional functions of nuclear receptors.

We thank the members of the laboratory for helpful discussions. We also thank Mario Vallejo for critical reading of the manuscript and Ana Pascual for her help.

The work of the authors' laboratory has been supported by Spanish Ministry of Education Grants PM97-0135 and SAF97-0183 and Comunidad de Madrid Grants 08.1/0032 and 08.5/0036.

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