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Review

Recent Progress in Orphan Nuclear Hormone Receptors

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The nuclear hormone receptor superfamily currently includes approximately equal numbers of conventional receptors and orphan receptors, which do not have known ligands. Here, we review recent progress from this laboratory on three orphans, two of which are moving from orphan to conventional receptor status. Perhaps the most unusual is CAR, which is a constitutive transactivator in the absence of ligands but becomes transcriptionally inactive in the presence of its ligands, which are androgen metabolites. The response of CAR to its ligands is thus opposite to that of the conventional receptor paradigm. RIP14 (also known as FXR) is activated by both all-*trans* retinoic acid and a synthetic retinoid previously thought to specifically target the retinoic acid receptors (RARs), and thus appears to be a novel retinoid receptor. Finally, SHP is a novel orphan that lacks a DNA binding domain and interacts with a number of other receptor superfamily members. While it generally inhibits its targets, including CAR, the retinoid X receptor (RXR), and the estrogen receptor (ER), it stimulates transactivation by the orphan SF-1.

Keywords: CAR, FXR, Nuclear hormone receptor, Orphan receptor, RIP14, SHP.

Introduction

The nuclear hormone receptor superfamily (Mangelsdorf *et al.*, 1995) is a group of ligand dependent transcription factors that includes the receptors for steroids, thyroid hormone, vitamin D_3, and other potent biologic regulators. In addition to these conventional receptors, the family contains a number of proteins that have no known ligands, termed orphan receptors (Enmark and Gustafsson, 1996). The nuclear receptors exert their effects by binding to specific DNA sequences called response elements and either positively or negatively regulating transcription. The conventional receptors are generally transactivators in the presence of ligand, and can either be active repressors or transcriptionally inert in the absence of ligand. Some of the orphans seem to be transcriptionally inert, and thus behave as if they need a ligand. Others, however, behave as either constitutive activators or repressors. These orphans are either under the control of ubiquitous ligands that have not been identified, or do not need to bind ligands to exert these activities.

The nuclear receptors can be separated into several classes based on their DNA binding properties. The classical steroid hormone receptors generally bind as homodimers to response elements composed of a palindromic arrangement of a hexameric motif. The majority of the members of the superfamily belong to a much larger group (referred to as the class II receptors) which includes the all-*trans* retinoic acid receptors (RARs), thyroid hormone receptors (TRs), the vitamin D_3 receptor (VDR), and a number of other conventional or orphan receptors (Mangelsdorf and Evans, 1995). The members of this group bind as heterodimers to response elements composed of direct repeats (DR) of a distinct hexameric sequence (RGGTCA), or to inverted palindromic (IR; head-to-head) or everted palindromic (ER; tail-to-tail) arrangements of this hexamer. In these heterodimers, a monomer of one of the retinoid X receptors (RXRs) interacts with a monomer of any of a number of other superfamily members. A much smaller group of orphans, including HNF-4, bind direct repeats of the RGGTCA hexamer as homodimers (Sladek *et al.*, 1990). The fourth class includes several unusual orphans that bind DNA as monomers, rather than dimers. These orphans generally recognize several base-pairs upstream of the RGGTCA, with different proteins recognizing different sequences (Wilson *et al.*, 1993). The orphan NGFI-B, which was the first member of this class, also exemplifies the need for caution in this type of categorization, since it is also capable of binding as a heterodimer with RXR to direct repeats of the RGGTCA hexamer (Perlmann and

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Jansson, 1995). Finally, there are two known members of the nuclear receptor superfamily [DAX-1 (Zanaria *et al.*, 1994) and SHP (Seol *et al.*, 1996)] that lack the conserved DNA binding domain entirely.

Here, we review recent advances in the characterization of several nuclear receptor superfamily members isolated in this laboratory, including CAR, RIP14 (which is also known as FXR), and SHP.

CAR: the Protein

The orphan receptor, CAR, is a novel murine member of the steroid/thyroid receptor superfamily. CAR is mainly expressed in the liver and it exists as two isoforms (Choi *et al.*, 1997). The main isoform, CAR1, and its close relative, the human orphan MB67 (Baes *et al.*, 1994), both contain an unusually short N-terminal or A/B domain, a conserved DNA binding region (C domain), a short hinge region (D domain) and a ligand binding/transactivation/ heterodimerization (E domain). The truncated variant, CAR2, lacks the C-terminal portion of the ligand binding/ transactivation/ heterodimerization domain.

Evolutionarily, CAR is closer to the nonsteroidal receptors than the classical steroid receptors. It shares the highest degree of similarity with its human relative MB67, or hCAR, and then with the Vitamin D_3 Receptor, VDR, the insect Ecdysone Receptor, EcR, and the Pregnane Activated Receptor, PXR.1.

CAR forms heterodimers with the receptor for 9-*cis* RA/RXR that bind to specific response elements in the promoters of target genes (Choi *et al.*, 1997). One such target is the gene for the Retinoic Acid Receptor $\beta 2$ isoform, RAR$\beta 2$. The RARβ promoter contains a Retinoic Acid Response Element (RARE) composed of two direct repeats of the RGGTCA motif with 5 bp in the spacer region (DR-5). This RARE binds both RAR/RXR heterodimers and CAR/RXR heterodimers with equal affinity (Baes, 1994 #402; Choi *et al.*, 1997). Both heterodimers bind on the βRARE with the RXR partner occupying the upstream-half site and the RAR or mCAR partner occupying the downstream-half site.

Other targets of the CAR/RXR heterodimer are the DR-5 motif found on the Alcohol Dehydrogenase III promoter and the DR-2 motif (2 base-pair spacer) found on the CRBPI promoter. Finally, binding studies of other direct repeats and mutational analysis suggested that the binding of the CAR/RXR heterodimer is highly restricted and depends on the nature of the spacer region inbetween the direct repeats.

Functional Analysis of mCAR

CAR transactivates target genes in an apparently constitutive manner. When transfected into HepG2 cells, for example, the CAR/RXR complex activates a βRARE-TKluc reporter construct in the absence of any exogenous ligand. The complex is not responsive to 9-*cis*-RA, a natural ligand for the RXR partner, an observation in agreement with the positioning of the two partners on the βRARE. The apparent activity of CAR is observed in both mammalian and yeast systems, and depends on an intact AF-2 domain in Helix 12, since deletions or substitution mutations in the core region of AF-2 abolish transactivation (Choi *et al.*, 1997).

Since CAR contains all the sequence characteristics of a hormone binding receptor, it was hypothesized that a natural ligand might exist for CAR. Therefore, a large number of small hydrophobic molecules were tested in transfection experiments in order to identify such a ligand, and two molecules were found to modulate the constitutive activity of mCAR. Surprisingly, these two androstane metabolites, called androstanol (5α-androstan 3α-ol) and androstenol (5α-androstan-16-en-3α-ol), were found to inhibit this constitutive activity. In transient transfection experiments with mCAR and βRARE-TKluc reporter, for example, 1 μM androstanol reduces transactivation to 15%. Both compounds display half-maximal inhibition at approximately 400 nM (EC_{50} = 400 nM). The effect of the androstan metabolites is stereospecific, since only the 3α-hydroxy, 5α-reduced compounds are inhibitory, and is observed in both mammalian and yeast systems. Additional studies showed that the presence of androstanol does not affect protein expression or nuclear localization of the mCAR protein and it does not inhibit formation of the CAR/RXR heterodimer or binding to DNA.

In order to test the hypothesis that CAR is a true constitutive activator and that the androstane metabolites act as retroactivators by blocking this constitutive activity, mutations were introduced in two highly conserved positions in the ligand binding pocket of mCAR, amino acids F171 and I174. These amino acids are part of the ligand binding pocket in both the TR and RAR, as shown in their crystal structures. So, if the constitutive activity of mCAR depends on an unidentified endogenous ligand, mutations that block ligand binding should also block constitutive activity. In contrast, if this activity is ligand independent, these mutations should have no effect other than rendering the protein resistant to androstanol. Indeed, the F171A, the I174A, and a double mutant render the CAR protein resistant to androstanol at a wide range of concentrations, but have little effect on its constitutive activity (Fig. 1). This data demonstrates that the constitutive activity of CAR is ligand independent, and that androstanol blocks activity by acting directly on the ligand binding domain of CAR.

Model for Mode of Action of a Retroligand

In general, transcriptional activation by the classical hormone nuclear receptors involves a ligand-dependent

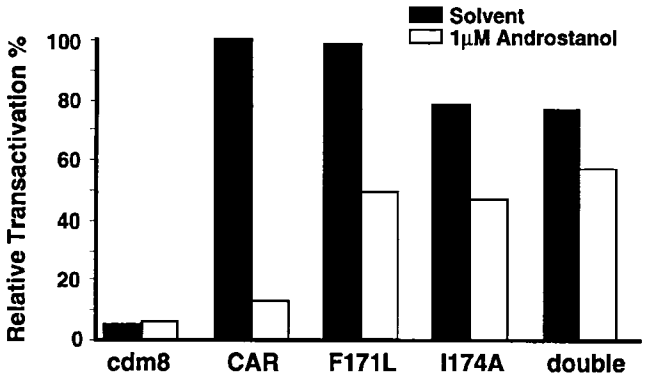

Fig. 1. Androstanol resistance of helix 3 mutants. Cells were transiently transfected with either wild-type CAR, the indicated single mutants, a double mutant, or vector alone, as indicated along with a previously described CAR responsive reporter (Choi *et al.*, 1997). Relative transactivation is shown in either the presence or absence of androstanol. The mutants retain constitutive activity, but become resistant to androstanol.

conformational change of the receptor that promotes the release of corepressors and/or recruitment of coactivators at the site of transcription. Thus, the constitutive activity of CAR could reflect a ligand-independent recruitment of coactivators, whereas binding of androstanol could cause release of a coactivator and/or recruitment of a corepressor. In order to test this hypothesis, we performed reverse CARLA assays (Krey *et al.*, 1997). These are *in vitro* assays that make use of bacterially-expressed glutathione-S-transferase (GST) fusion proteins, ^{35}S-labeled proteins (coactivators), and potential ligands. As predicted by the hypothesis, a GST–CAR fusion interacts well with the ^{35}S-labeled coactivator SRC-1 in the absence of androstanol. In the presence of androstanol, dissociation of the CAR-SRC-1 complex is observed. This data demonstrates that the hormone nuclear receptor mCAR is a constitutive activator that is inhibited in the presence of its ligand, androstanol. This inhibition involves the androstanol-induced release of the coactivator SRC-1 from mCAR.

In classical receptors, ligand binding induces a conformational shift in the ligand binding domain that positions Helix 12, which is crucial for the ligand-dependent AF-2 transactivation function, in an active conformation. This promotes release of corepressors and allows interaction with coactivators. Thus, our data suggests that the CAR helix 12 may already exist in an active conformation that allows interaction with coactivators in the absence of any ligand. In contrast, in the presence of androstanol, we predict that helix 12 will adopt an inactive conformation that will promote the release of coactivators and inhibition of transactivation (Fig. 2).

Overall, these results demonstrate that mCAR is a novel receptor that responds to a classical steroid molecule. CAR is therefore a Constitutive Androstane Receptor that functions in a manner opposite to that of the conventional receptor paradigm.

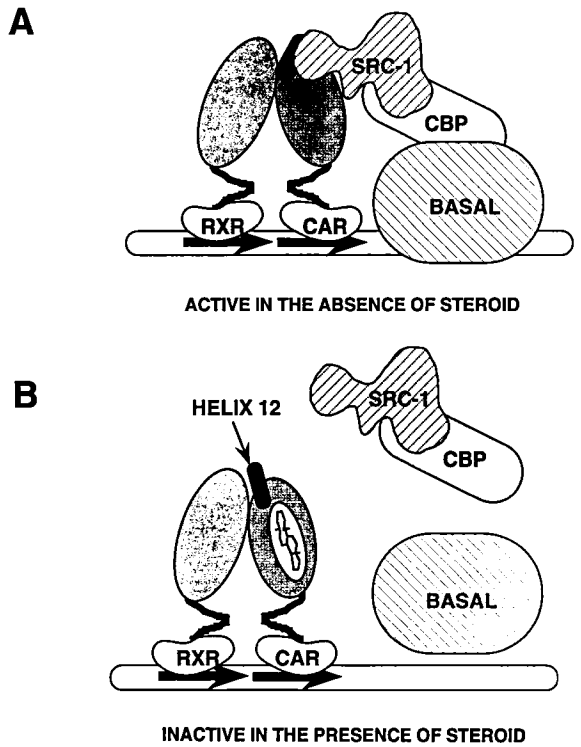

Fig. 2. Model for retroactivator function. A. In the absence of its ligand, CAR is a transcriptional activator that recruits coactivators. B. Helix 12, which carries the core of the AF-2 transactivation function, is displaced from the active position when the steroid ligand is bound. The CAR/SRC-1 interaction is disrupted, and the complex is transcriptionally inactive.

RIP14, a Retinoid Activated Receptor

RIP14 was originally isolated in a yeast two-hybrid screen using RXR as "bait" to clone RXR interacting proteins (Seol *et al.*, 1995). One of a number of proteins identified, RIP14 (RXR interacting protein 14), was a novel orphan receptor with 82% amino acid sequence identity with the *Drosophila* ecdysone receptor (EcR) in the DNA binding domain, and approximately 30–40% identity to other RXR heterodimer partners in the ligand binding domain. Northern analysis indicated that RIP14 is expressed primarily in the liver and kidney (Seol *et al.*, 1995).

Isolation of a series of distinct cDNA clones indicates that there are at least two isoforms of the RIP14 protein. As has been commonly observed for other receptors, RIP14 isoforms differ at the amino terminus by the addition of unique segments of 22 and 36 amino acids. These isoforms are referred to as RIP14-1 and RIP14-2, respectively. This N-terminal divergence is likely to be a consequence of utilization of distinct promoters, but could be due to alternative mRNA splicing patterns, or a combination of both mechanisms. RIP14-2 also differs in that it contains a 4 amino acids insertion just downstream of the conventionally defined DNA binding domain, in a region associated with effects on heterodimerization (Wilson

et al., 1992; Kurokawa *et al.*, 1994; Zechel *et al.*, 1994; Rastinejad *et al.*, 1995). The cDNA sequences suggest that this divergence is a consequence of alternative 5′ splice site utilization. Both isoforms are found in kidney and liver, and mRNA based PCR demonstrates that transcripts with and without the 4 amino acids insert are expressed at comparable levels in liver (Seol *et al.*, 1995). Whether isoforms of RIP 14-1 plus the 4 amino acids insertion and 14-2 minus the 4 amino acids insertion exist *in vivo* is unknown, but it is certainly a distinct possibility.

Gel mobility shift analysis using either direct repeats (DR) or inverted repeats (IR) of the consensus hexamer AGGTCA separated by 0–5 bp demonstrates that RIP14-1 can bind as a heterodimer with RXR to DR-2, DR-4, and DR-5 elements, and also IR-0 or IR-1 sites (Seol *et al.*, 1995). As expected from the high degree of DNA binding domain similarity with the EcR, RIP14-1/RXR heterodimers also bind with high affinity to an IR-1 ecdysone response element (EcRE) from the *Drosophila* heat shock protein 27 (hsp27) promoter. RIP14-2/RXR heterodimers bind with somewhat weaker affinity to both the EcRE and a DR-5 RARE (Seol *et al.*, 1995).

FXR is the Rat Homolog of RIP14

The rat homolog of RIP14 was also isolated by Forman *et al.* by screening a rat liver library using a degenerate probe derived from the highly-conserved P box DNA recognition helix of the nuclear receptor superfamily (Forman *et al.*, 1995). This clone, called FXR, is 97% identical to RIP14-2, with the exception that the 4 amino acids insertion present in RIP14-2 is absent in FXR. As expected, Northern blots confirm FXR expression in rat liver and kidney, and *in situ* hybridization studies also demonstrate expression in gut and adrenal cortex. FXR/RXR heterodimers were also shown to bind both the hsp27 EcRE and a synthetic IR-1 element (Forman *et al.*, 1995).

The name FXR (farnesoid X-activated receptor) refers to the ability of this rat orphan to transactivate a reporter construct, containing six copies of the hsp27 EcRE, approximately 10-fold in the presence of high (50 μM) concentrations of farnesol. Since no direct binding of farnesol to FXR has been observed, it is thought that a farnesol metabolite could be the true activator of FXR. We have been unable to duplicate this result using RIP14-1, but we have been able to obtain a 3–5 fold response to farnesol using either FXR or an FXR-like version of the RIP14-2 receptor in which the 4 amino acids insertion after the DNA binding domain has been deleted. Thus, it is possible that the differing responses of RIP14-1 and FXR to farnesol may be localized to their amino termini. Still, this slight response to farnesol contrasts greatly to the potent response observed in the presence of retinoids discussed below.

RIP14 — the Seventh Retinoid Receptor?

Retinoids are crucial regulators of a wide variety of processes in both developing and adult animals. Six retinoid receptors have been thought to mediate these effects. The three types of retinoic acid receptors (RXRα, β, γ) are activated by either all-*trans* retinoic acid (tRA) or 9-*cis* retinoic acid (9-*cis*-RA), while the three types of retinoid X receptors (RXRα, β, γ) are activated specifically by 9-*cis*-RA.

Surprisingly, RIP14 can also be activated by high doses of either tRA, or the synthetic retinoid TTNPB [(*E*)-4-(2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)propen-1-yl)benzoic acid], previously thought to be a specific agonist for RARs (Crettaz *et al.*, 1990). Thus, transfections of RIP14-1 plus RXR with a previously described luciferase reporter containing several IR-1 RIP14/RXR binding sites (Zavacki *et al.*, 1997) showed a greater than 200-fold induction in response to TTNPB (Fig. 3) or tRA. These responses are dependent on the presence of RIP14, since cotransfection of either the expression vector or RXR alone did not confer responsiveness to any of these compounds. Cotransfected RARβ was also unable to activate this reporter in the presence of either TTNPB or tRA, and the IR-1 binding site was not bound by RAR alone, or by combinations of RAR + RXR or RIP14 + RAR (Zavacki *et al.*, 1997). Thus, the observed responses cannot be attributed to activation of endogenous RARs by TTNPB or tRA.

RIP14-1 + RXR was also responsive to the RXR specific agonist LG1069 (Boehm *et al.*, 1994), and significantly greater activation than seen with either compound alone was observed when doses of LG1069 saturating for the RXR receptor (1 μM) were added in addition to TTNPB. Similar results were observed using tRA and 9-*cis*-RA (Zavacki *et al.*, 1997). These results are most consistent with a model in which RXR is specifically

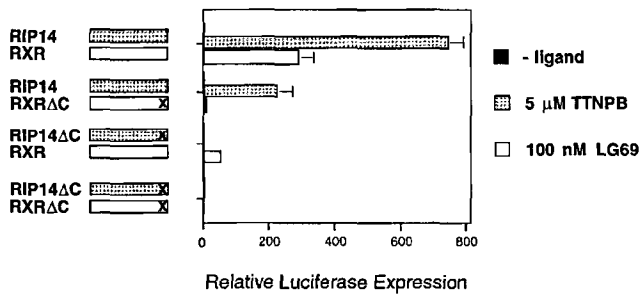

Fig. 3. RIP14 is a direct target for activation by TTNPB. Either wild-type RIP14 and RXR or C-terminal truncations removing the helix 12 AF-2 domain were cotransfected with a luciferase reporter containing multiple copies of an IR-1 RIP14/RXR binding site, in the presence or absence of TTNPB or LG1069, as indicated. Mutation of the RIP14 AF-2 function completely blocks response to TTNPB, while mutation of the RXR AF-2 function completely blocks response to LG1069.

activated by LG1069, while RIP14 is specifically activated by TTNPB.

To confirm and extend this conclusion, the C-terminal helix 12 AF-2 domains of RIP14 and RXR were deleted. The RXRΔ19C deletion was previously demonstrated to block ligand-dependent transactivation of RXR itself, but to retain both heterodimerization function and ligand activation of heterodimer partners (Zhang *et al.*, 1994). A similar mutation was introduced into RIP14, deleting the last 9 amino acids. If RIP14 is the target for TTNPB activation and RXR is the target for LG1069 activation, TTNPB activation should be lost in the RIP14 mutant RIP14Δ9C, while the RXR mutant RXRD19C should lose LG1069 signaling. This is exactly what was observed (Fig. 3). In both cases, response associated with the mutated receptor was completely absent. The other response was maintained, but decreased, which is consistent with several previous reports (Leng *et al.*, 1994; Zhang *et al.*, 1994; Schulman *et al.*, 1996), presumably reflecting a contribution by the unliganded AF-2 domain to the overall activation associated with the ligand-bound partner. As expected, experiments using the AF-2 receptors confirmed that 9-*cis*-RA was activating through RXR, while tRA was acting through both RIP14 and RXR, presumably activating RXR through metabolism to 9-*cis*-RA (Zavacki *et al.*, 1997). Overall, these results confirm that RIP14 is a direct target of activation by retinoids, and that RIP14/RXR heterodimers belong to the growing class of RXR heterodimers permissive for RXR signaling (Mangelsdorf and Evans, 1995).

These results raise the intriguing possibility that TTNPB or tRA could be ligands for RIP14. Dose response experiments using TTNPB show that in the absence of 9-*cis*-RA, the EC_{50} for cotransfected RIP14 + RXR is reproducibly greater than 1 μM, but is decreased to approximately 0.5–1 μM in the presence of 9-*cis*-RA. Similar results were observed using tRA. These apparent affinities are approximately 100–1000 fold lower than those observed for RAR in the same experiment, suggesting that TTNPB and tRA are not high affinity RIP14 ligands and may require metabolic conversion to generate a proximal ligand. Furthermore, these high concentrations required for RIP14 activation provide an additional confirmation that endogenous RARs are not involved in this process.

Two other approaches have been taken to test the possibility that either TTNPB or tRA are RIP14 ligands. A number of receptors, including RARs, TR, and RXRs have been found to be stabilized against protease cleavage upon ligand binding (Leng *et al.*, 1993; 1995). Under several different conditions where RARs show significant stabilization in response to TTNPB or tRA binding, RIP14 shows no such effects. Similar results were observed in the presence or absence of either 9-*cis*-RA, RXR, or both, and with additional proteases. Secondly, the interaction of RIP14 with several proteins that show ligand-dependent interaction with other known receptors in GST pull-down experiments was also evaluated. While the coactivators SRC-1 (Onate *et al.*, 1995) or RAC-3/ACTR (Chen *et al.*, 1997) show an increased interaction with RAR in the presence of tRA and TTNPB, neither show any increased interaction with RIP14 in the presence of these compounds. Based on these results, we believe that the true ligand for RIP14 may be an as yet unidentified retinoid metabolite.

SHP

The major functional domains of the nuclear hormone receptors are the DNA binding domain and the ligand binding domain. Although these domains are highly conserved between subtypes of receptors, a small group of only two orphans missing the conventional DNA binding domain has recently emerged. One of these is SHP (small heterodimer partner), which was isolated in this laboratory as a CAR interacting protein using the yeast two-hybrid system (Seol *et al.*, 1996). A rat homolog of SHP was independently isolated as a PPARα interactor (Masuda *et al.*, 1997). Interestingly, SHP contains a very short N-terminal domain that lacks the conventional DNA binding domain but shows a modest similarity with the N-terminal domain of the orphan DAX-1 (Zanaria *et al.*, 1994), which is the other orphan that also lacks a conventional DNA binding domain. Within the putative ligand binding domain, DAX-1 is the closest relative of SHP with approximately 40% amino acid sequence identity.

SHP interacts not only with CAR and PPAR, but also with a broad range of other nuclear hormone receptors, including RXR, TR, and RAR, as manifested by the GST pull-down assay and yeast two-hybrid system (Seol *et al.*, 1996; 1997). This broad interaction ability is comparable to that of RXR, which heterodimerizes with a number other nuclear receptors such as TR, RAR, PPAR, etc, and plays a key role in a wide variety of hormone-regulating gene expressions during development and differentiation (Mangelsdorf and Evans, 1995). In contrast to the positive effects of RXR heterodimerization, however, interaction with SHP inhibits receptor transactivation, as demonstrated by transient transfection assays.

Since addition of SHP to RXR/RAR heterodimers blocks their ability to bind an RARE, it was thought that the inhibitory effects of SHP on transactivation might be simply due to its inhibitory effects on DNA binding (Seol *et al.*, 1996). Recently, however, an additional potential mechanism for inhibition was provided by the identification of a novel SHP repressor domain (Seol *et al.*, 1997). Thus, studies with GAL4–SHP and GAL4-VP16–SHP fusion proteins indicate that SHP can function directly as a transcriptional repressor, and deletion analysis mapped

this repression function to a C-terminal portion of the putative LBD (Seol *et al.*, 1997). N-CoR, the well known ligand-dependent corepressor that interacts with several other nuclear receptors (Horlein *et al.*, 1995), failed to interact with the repressor domain of SHP in GST pull-down and yeast two-hybrid systems, suggesting that SHP may interact with a novel corepressor.

The existence of this SHP domain suggests two possible additional mechanisms for SHP-dependent repression. One is that SHP acts directly at as yet unidentified DNA binding sites of its own. The other is that SHP could be recruited to DNA by the interaction with nuclear hormone receptors that did not lose DNA binding activity upon interaction with SHP. This latter mechanism is supported by recent results with SHP and ER (Seol *et al.*, 1998). Thus, SHP inhibits ER transactivation, and also interacts directly with ER in a manner that appears quite similar to its interactions with RXR and other receptors (Seol *et al.*, 1998). In contrast to results with other receptors, however, SHP does not decrease DNA binding by ER homodimers. This result is consistent with the fact that ER homodimerization is primarily mediated by the DNA binding domain, while SHP targets the ligand binding domain. Based on this lack of inhibition of DNA binding, a likely mechanism for the inhibitory effect of SHP is recruitment of its repressor activity to DNA in an ERE/ER/SHP complex, as diagrammed in Fig. 4A, although there are alternative possibilities.

This mechanism of inhibition by recruitment of a repressor function is similar to that recently proposed for the functional interaction of the SHP relative, DAX-1, with the orphan SF-1. SF-1 is required for genesis of both gonads and adrenals and is also an activator of a number of genes that encode steroidogenic enzymes (Wong *et al.*, 1997). It is also one of the best characterized of the monomer binders. Ito *et al.* have reported that DAX-1 and SF-1 can interact and, as expected, that this interaction does not block SF-1 DNA binding (Ito *et al.*, 1997). As a consequence of the recruitment of the C-terminal repressor domain in DAX-1, this interaction results in an inhibition of SF-1 mediated transactivation.

An excellent chance to extend the analysis of effects on SF-1 to include SHP was provided by our recent observation that both the mouse and human SHP promoters contain several SF-1 binding sites. As expected from the presence of these multiple sites, SF-1 transactivates SHP promoter driven luciferase reporter constructs 2–10-fold, depending on cell types transfected. In contrast to the expected inhibition, however, coexpression of SHP increased SF-1 dependent transactivation. This stimulatory effect was observed not only with the SHP promoter constructs, but also with synthetic reporter constructs containing SF-1 binding sites. Direct interaction between SHP and SF-1 was detected by GST pull-down assays and the yeast two-hybrid system. Like nearly all other nuclear

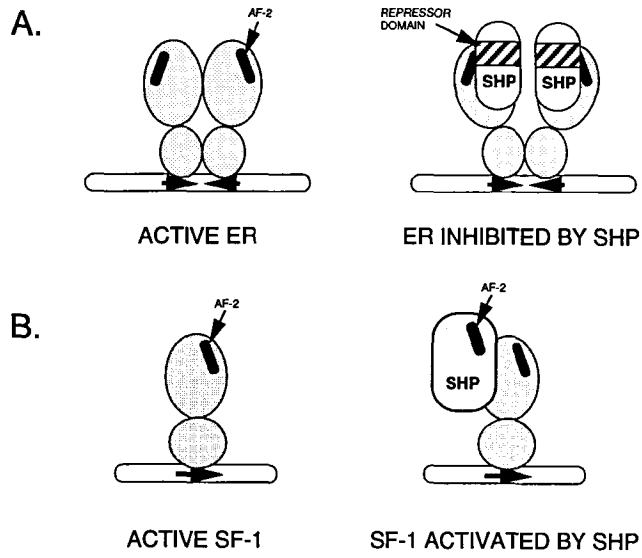

Fig. 4. Models for inhibition of ER transactivation and activation of SF-1 transactivation by SHP. A. In the presence of estrogen but the absence of SHP, ER binds DNA and is a transactivator. When SHP is present, it interacts with the LBD of the activated ER. This brings the SHP repressor domain to the DNA, which results in the loss of transactivation. B. In the absence of SHP, SF-1 is also a transactivator. When SHP binds SF-1, however, its repressor function is lost and it becomes a transactivator. SF-1 may also become a more potent transactivator.

receptors, SHP and SF-1 both contain a copy of the conserved AF-2 motif in their putative helix 12, and both of these motifs must be present for the stimulatory effect to be observed. When this AF-2 motif is deleted from SHP, the mutant protein has no effect on SF-1 transactivation. Interestingly, when the transactivation motif of SF-1 is removed, SHP acts as a repressor of the residual transcriptional activity by the mutant SF-1. Overall, these functional results suggest that interaction with SF-1 results in a loss of the repressor function of SHP. This interaction may also cause a conformational change in SF-1 that results in stabilization of its interactions with coactivators or otherwise increases its transactivator function. However, the requirement for the SHP helix 12 indicates that the increased transactivation may be a consequence of conversion of SHP into a direct transactivator, as diagrammed in Fig. 4B. Particularly since several coactivators for SF-1 have been reported in recent studies, these hypotheses can be directly tested in the near future.

Conclusion

Over the last decade, the functional roles of the nuclear hormone receptors and their mechanisms of action have become increasingly complex. The results described here have added several new wrinkles, and there is certainly no reason to assume that they will be the last. Thus, while the results with CAR indicate that androstanol is a true ligand,

it seems quite possible that additional, higher affinity ligands will be found among the pool of steroid metabolites. It also seems reasonable to assume that higher affinity retinoid ligands will be identified for RIP14, and results with farnesol raise the possibility that other compounds may act as RIP14 ligands. By analogy with recent results with the PPARs, both low and high affinity compounds with diverse structures may play such roles (Krey *et al.*, 1997). It also seems possible that ligands will be identified for SHP, although their nature and their actions are impossible to guess. Overall, we can anticipate with confidence that these and other receptors will continue to lead to surprising new insights.

References

Baes, M., Gulick, T., Choi, H.-S., Martinoli, M. G., Simha, D. and Moore, D. D. (1994). A new orphan member of the nuclear receptor superfamily that interacts with a subset of retinoic acid response elements. *Mol. Cell. Biol.* **14,** 1544–1552.

Boehm, M. F., Zhang, L., Badea, B. A., White, S. K., Mais, D. E., Berger, E., Suto, C. M., Goldman, M. E. and Heyman, R. A. (1994). Synthesis and structure-activity relationships of novel retinoid X receptor-selective retinoids. *J. Med. Chem.* **37,** 2930–2941.

Chen, H., Lin, R. J., Schiltz, R. L., Chakravarti, D., Nash, A., Nagy, L., Privalsky, M. L., Nakatani, Y. and Evans, R. M. (1997). Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. *Cell* **90,** 569–580.

Choi, H. S., Chung, M., Tzameli, I., Simha, D., Lee, Y. K., Seol, W. and Moore, D. D. (1997). Differential transactivation by two isoforms of the orphan nuclear hormone receptor CAR. *J. Biol. Chem.* **272,** 23565–23571.

Crettaz, M., Baron, A., Siegenthaler, G. and Hunziker, W. (1990). Ligand specificities of recombinant retinoic acid receptors RAR alpha and RAR beta. *Biochem. J.* **272,** 391–397.

Enmark, E. and Gustafsson, J. A. (1996). Orphan nuclear receptors — the first eight years. *Mol. Endocrinol.* **10,** 1293–1307.

Forman, B. M., Goode, E., Chen, J., Oro, A. E., Bradley, D. J., Perlmann, T., Noonan, D. J., Burka, L. T., McMorris, T., Lamph, W. W., Evans, R. M. and Weinberger, C. (1995). Identification of a nuclear receptor that is activated by farnesol metabolites. *Cell* **81,** 687–693.

Hörlein, A. J., Näär, A. M., Heinzel, T., Torchia, J., Gloss, B., Kurokawa, R., Ryan, A., Kamei, Y., Söderström, M., Glass, C. K. and Rosenfeld, M. G. (1995). Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. *Nature* **377,** 397–404.

Ito, M., Yu, R. and Jameson, J. L. (1997). DAX-1 inhibits SF-1-mediated transactivation via a carboxy-terminal domain that is deleted in adrenal hypoplasia congenita. *Mol. Cell. Biol.* **17,** 1476–1483.

Krey, G., Braissant, O., L'Horset, F., Kalkhoven, E., Perroud, M., Parker, M. G. and Wahli, W. (1997). Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay. *Mol. Endocrinol.* **11,** 779–791.

Kurokawa, R., DiRenzo, J., Boehm, M., Sugarman, J., Gloss, B., Rosenfeld, M. G., Heyman, R. A. and Glass, C. K. (1994). Regulation of retinoid signalling by receptor polarity and allosteric control of ligand binding. *Nature* **371,** 528–531.

Leng, X., Blanco, J., Tsai, S. Y., Ozato, K., O'Malley, B. W. and Tsai, M. J. (1994). Mechanisms for synergistic activation of thyroid hormone receptor and retinoid X receptor on different response elements. *J. Biol. Chem.* **269,** 31436–31442.

Leng, X., Blanco, J., Tsai, S. Y., Ozato, K., O'Malley, B. W. and Tsai, M. J. (1995). Mouse retinoid X receptor contains a separable ligand-binding and transactivation domain in its E region. *Mol. Cell. Biol.* **15,** 255–263.

Leng, X., Tsai, S.-Y., O'Malley, B. W. and Tsai, M. J. (1993). Ligand-dependent conformational changes in thyroid hormone and retinoic acid receptors are potentially enhanced by heterodimerization with retinoic X receptor. *J. Steroid Biochem. Mol. Biol.* **46,** 643–661.

Mangelsdorf, D. J. and Evans, R. M. (1995). The RXR heterodimers and orphan receptors. *Cell* **83,** 841–850.

Mangelsdorf, D. J., Thummel, C., Beato, M., Herrlich, P., Schütz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., Chambon, P. and Evans, R. M. (1995). The nuclear receptor superfamily: the second decade. *Cell* **83,** 835–839.

Masuda, N., Yasumo, H., Tamura, T., Hashiguchi, N., Furusawa, T., Tsukamoto, T., Sadano, H. and Osumi, T. (1997). An orphan nuclear receptor lacking a zinc-finger DNA-binding domain: interaction with several nuclear receptors. *Biochim. Biophys. Acta* **1350,** 27–32.

Onate, S. A., Tsai, S. Y., Tsai, M. J. and O'Malley, B. W. (1995). Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. *Science* **270,** 1354–1357.

Perlmann, T. and Jansson, L. (1995). A novel pathway for vitamin A signaling mediated by RXR heterodimerization with NGFI-B and NURR1. *Genes & Dev.* **9,** 769–782.

Rastinejad, F., Perlmann, T., Evans, R. M. and Sigler, P. B. (1995). Structural determinants of nuclear receptor assembly on DNA direct repeats. *Nature* **375,** 203–211.

Schulman, I. G., Juguilon, H. and Evans, R. M. (1996). Activation and repression by nuclear hormone receptors: hormone modulates an equilibrium between active and repressive states. *Mol. Cell. Biol.* **16,** 3807–3813.

Seol, W., Choi, H.-S. and Moore, D. D. (1995). Isolation of proteins that interact specifically with the retinoid X receptor: two novel orphan receptors. *Mol. Endocrinol.* **9,** 72–85.

Seol, W., Choi, H. S. and Moore, D. D. (1996). An orphan nuclear hormone receptor that lacks a DNA binding domain and heterodimerizes with other receptors. *Science* **272,** 1336–1339.

Seol, W., Chung, M. and Moore, D. D. (1997). Novel receptor interaction and repression domains in the orphan receptor SHP [In Process Citation]. *Mol. Cell. Biol.* **17,** 7126–7131.

Seol, W., Hanstein, B., Brown, B. and Moore, D. D. (1998). Inhibition of estrogen receptor action by the orphan receptor SHP. *Mol. Endocrinol.* in press.

Sladek, F. M., Zhong, W., Lai, E. and Darnell, J. E. (1990). Liver-enriched transcription factor HNF-4 is a novel member of the steroid hormone receptor superfamily. *Genes & Dev.* **4,** 2353–2365.

Wilson, T. E., Fahrner, T. J. and Milbrandt, J. (1993). The orphan receptors NGFI-B and steroidogenic factor 1 establish

monomer binding as a third paradigm of nuclear receptor-DNA interaction. *Mol. Cell. Biol.* **13,** 5794–5804.

Wilson, T. E., Paulsen, R. E., Padgett, K. A. and Milbrandt, J. (1992). Participation of non-zinc finger residues in DNA binding by two nuclear orphan receptors. *Science* **2546,** 107–110.

Wong, M., Ikeda, Y., Luo, X., Caron, K. M., Weber, T. J., Swain, A., Schimmer, B. P. and Parker, K. L. (1997). Steroidogenic factor 1 plays multiple roles in endocrine development and function. *Recent Prog. Horm. Res.* **52,** 167–182; discussion 182–184.

Zanaria, E., Muscatelli, F., Bardoni, B., Strom, T. M., Guioli, S., Guo, W., Lalli, E., Moser, C., Walker, A. P., McCabe, E. R. B., Meitinger, T., Monaco, A. P., Sassone-Corsi, P. and Camerino, G. (1994). An unusual member of the nuclear hormone receptor superfamily responsible for X-linked adrenal hypoplasia congenita. *Nature* **372,** 635–641.

Zavacki, A. M., Lehmann, J. M., Seol, W., Willson, T. M., Kliewer, S. A. and Moore, D. D. (1997). Activation of the orphan receptor RIP14 by retinoids. *Proc. Natl. Acad. Sci. USA* **94,** 7909–7914.

Zechel, C., Shen, X. Q., Chen, J. Y., Chen, Z. P., Chambon, P. and Gronemeyer, H. (1994). The dimerization interfaces formed between the DNA binding domains of RXR, RAR and TR determine the binding specificity and polarity of the full-length receptors to direct repeats. *EMBP J.* **13,** 1425–1433.

Zhang, X.-K., Salbert, G., Lee, M.-O. and Pfahl, M. (1994). Mutations that alter ligand-induced switches and dimerization activities in the retinoid X receptor. *Mol. Cell. Biol.* **14,** 4311–4323.