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Novel Receptor Interaction and Repression Domains in the Orphan Receptor SHP

WONGI SEOL,† MIRRA CHUNG,‡ AND DAVID D. MOORE*

Department of Molecular Biology, Massachusetts General Hospital, Boston, Massachusetts 02114

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SHP (short heterodimer partner) is a novel orphan receptor that lacks a conventional DNA binding domain and interacts with other members of the nuclear hormone receptor superfamily. We have characterized the SHP sequences required for interaction with other superfamily members, and have defined an SHP repressor domain. In the mammalian two-hybrid system, a fusion of full-length SHP to the GAL4 DNA binding domain shows 9-*cis*-retinoic acid-dependent interaction with a VP16-retinoid X receptor α (RXRα) fusion. By deletion analysis, sequences required for this RXR interaction map to the central portion of SHP (amino acids 92 to 148). The same region is required for interaction with RXR in vitro and in the yeast two-hybrid system, and results from the yeast system suggest that the same SHP sequences are required for interaction with other members of the nuclear hormone receptor superfamily such as thyroid hormone receptor and retinoic acid receptor. In mammalian cells, a GAL4-SHP fusion protein shows about 10-fold-decreased transcriptional activation relative to GAL4 alone, and fusion of SHP to the C terminus of a GAL4-VP16 fusion to generate a triple chimera also results in a strong decrease in transactivation activity. Sequences required for this repressor function were mapped to the C terminus of SHP. This region is distinct from that required for corepressor interaction by other members of the nuclear hormone receptor superfamily, and SHP did not interact with N-CoR in either the yeast or mammalian two-hybrid system. Together, these results identify novel receptor interaction and repressor domains in SHP and suggest two distinct mechanisms for inhibition of receptor signaling pathways by SHP.

The members of the nuclear hormone receptor superfamily play a variety of important roles in development and differentiation by regulating transcription of specific target genes (3, 20, 24, 25). In general, these proteins bind DNA with high affinity as dimers. While the members of the steroid hormone receptor subfamily bind to their response elements as homodimers, the majority of the non-steroid hormone receptors, such as the thyroid hormone receptor (TR), retinoic acid receptor (RAR), or the orphan receptor MB67, require heterodimerization with retinoid X receptor (RXR) for high-affinity binding to their response elements (20, 24, 25). In these heterodimeric complexes, RXR can either be silent or hormone (9-*cis*-retinoic acid [9-*cis*-RA]) responsive (21, 24). For example, in RXR-PPAR, RXR-LXR and RXR-RIP14 (FXR) complexes, the RXR partner can be activated by 9-*cis* RA, but in RXR-RAR and RXR-TR complexes, it functions only as a heterodimerization partner. RXR can apparently also function as a homodimer, and both homodimerization and transcriptional activation by the homodimer are dependent on 9-*cis*-RA.

Receptor superfamily members have dimerization interfaces in both their DNA binding domain (DBD) and ligand binding domain (LBD). Though the DBD interfaces can be quite different for different receptors, the LBD interface is primarily based on a conserved motif referred to as the 9th heptad (13, 14) or the I-box (28). For the various RXR partners, this motif is required for heterodimerization, and it is also involved in homodimerization of both RXR (4) and estrogen receptor (ER) (10, 23).

The activities of the orphan receptor SHP (short heterodimer partner) are in some ways opposite to those of RXR. This orphan was initially isolated by yeast two-hybrid screening (33) based on its interaction with mCAR, a murine homolog of an orphan receptor, MB67 (2). Isolation of full-length cDNA clones revealed that SHP, like the orphan DAX-1 (43), lacks a conventional DBD (33). Both direct biochemical results and results with the yeast two-hybrid system demonstrated that SHP can interact with TR, RAR, RXR, and other members of the receptor superfamily. As expected from its lack of a DBD, addition of SHP inhibits in vitro DNA binding by RAR-RXR heterodimers, for example. In mammalian cell cotransfections, SHP also inhibits transactivation by RAR, mCAR and other receptor superfamily members with which it interacts.

To further understand the mechanism by which SHP interacts with other superfamily members, we have characterized the sequences required for such interaction. The mammalian and yeast two-hybrid systems, as well as direct biochemical studies, map such sequences to the N-terminal region of the putative LBD of SHP. This region is distinct from the more C-terminal domain associated with heterodimerization in other receptor superfamily members.

In addition, studies with GAL4-SHP and GAL4-VP16-SHP fusion proteins indicate that SHP can function directly as a transcriptional repressor. The SHP sequences required for this repressor function map the C-terminal portion of the putative LBD. For several other members of the receptor superfamily, repressor activity is thought to be mediated by interaction with corepressor proteins, such as N-CoR/RIP13 (17, 34) and SMRT/TRAC (7, 30). However, the sequences required for SHP repressor function do not overlap previously identified target regions for corepressor interaction, and SHP does not

* Corresponding author. Present address: Department of Cell Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030. Phone: (713) 798-3313. Fax: (713) 798-8005.

† Department of Adult Oncology, Dana-Farber Cancer Institute, Boston, MA 02115.

‡ Present address: Department of Gerontology, Beth Israel Hospital, Boston, MA 02115.

interact with N-CoR/RIP13 in either the yeast or mammalian two-hybrid assay.

Overall, these results identify novel receptor interaction and repressor domains within SHP and suggest two distinct mechanisms for inhibition of receptor signaling pathways by SHP.

MATERIALS AND METHODS

Plasmids. pT7lac vector containing His and Myc tags (pT7lacHisMyc) was used for construction of pT7lac-SHP, by insertion of a PCR product corresponding to the mouse SHP cDNA using *Sal*I and *Not*I sites. After sequence confirmation, a glutathione *S*-transferase (GST)-SHP fusion plasmid was made by insertion of the SHP sequence from pT7lac-SHP into a modified pGEX2T vector (Pharmacia, Piscataway, N.J.) with *Sal*I and *Not*I sites.

The GAL4-SHP full-length fusion vector was constructed by insertion of the SHP wild-type cDNA digested with *Sal*I and *Bam*HI from pT7lac-SHP into pCMX-GAL4 DBD treated with *Sal*I and *Bam*HI.

The GAL4-VP16 fusion was constructed by insertion of a PCR product of the VP16 activator domain (78 amino acids) into pCMX-GAL4 DBD with *Bam*HI and *Nhe*I (34). Nine amino acids derived from the polylinker region of the vector are present between the GAL4 DBD and VP16 activator domain. GAL4-VP16 containing full-length SHP was made by insertion of a blunt-ended SHP fragment into blunt-ended, *Nhe*I-digested vector. There are five amino acids derived from linker region of the vector between VP16 activator domain and the SHP gene.

The various deletion mutants of SHP were made either by PCR or by digestion with suitable restriction endonucleases. Fragments were inserted into the pCMX-GAL4 DBD or CDM8 mammalian expression vectors. After confirmation of their sequences, these mutant constructs were inserted into pL202PL (44), the yeast LexA expression vector, pCMX-GAL4-VP16, the mammalian two-hybrid vector, or pT7lac with His and Myc tags, the bacterial expression vector. GAL4-SHPΔN-148 and Lex-SHPΔN-148 have 11 and 19 additional amino acids at their C termini, respectively, derived from the polylinkers.

In vitro interaction. Full-length human RXRα was inserted into a modified version of the pGEX2T GST fusion vector (Pharmacia), expressed in *Escherichia coli*, and purified by glutathione-Sepharose affinity chromatography as suggested by the vendor. [^{35}S]methionine-labeled proteins were prepared by in vitro translation with pT7lacHisMyc vectors containing cDNAs coding for full-length SHP and deletion mutants and the TNT-coupled transcriptional translation system under the conditions described by the manufacturer (Promega, Madison, Wis.). In vitro protein-protein interaction assays were carried out as described previously (33).

Yeast two-hybrid assay. For the yeast two-hybrid system (11, 16, 44), LexA-mSHP full-length and deletion mutants and B42-receptor fusion plasmids were cotransformed into *Saccharomyces cerevisiae* EGY48 containing the β-galactosidase reporter plasmid, 8H18-34. All B42-receptor fusions except B42-mCAR contain sequences extending from the carboxyl-terminal portions of the DNA binding domains to the carboxyl termini of the receptors (22, 32). Characterization of β-galactosidase expression on plates was carried out as described previously (22). Similar results were obtained in more than two similar experiments with independently isolated transformants.

Cell culture and transfections. HepG2 and JEG-3 cells were propagated in Dulbecco's modified Eagle's medium plus 10% fetal bovine serum. Cells were grown in 12-well plates with medium supplemented with 10% charcoal-stripped serum for 24 h and transfected as described previously (1) by the DEAE-dextran–chloroquine method followed by dimethyl sulfoxide (DMSO) treatment with the indicated amount of plasmids expressing proteins of interest and 0.5 μg of both the internal control plasmid pTKGH (31) and reporter plasmids containing a luciferase gene and an appropriate response element per well. Approximately 16 h after DMSO treatment, appropriate ligands were added with fresh medium, and cells were incubated for 1 day. Luciferase was assayed as described by the manufacturer (Promega), and the results were normalized by using growth hormone expression from the internal TKGH control. Similar results were obtained in more than two similar experiments.

RESULTS

The receptor interaction domain of SHP is distinct from other heterodimerization domains. The ability of SHP to interact with multiple members of the nuclear receptor superfamily was originally identified by the yeast two-hybrid system (33). A series of deletion mutants (Fig. 1) was used to determine the SHP sequences necessary for such interactions with RXR, RAR, TR, and the orphan receptor mCAR, which was the bait used for the original isolation of SHP. As shown in Table 1, deletion of C-terminal sequences required by other receptors for heterodimeric interactions did not block interaction of SHP with RXR, RAR, or mCAR. Thus, RXR, RAR, and mCAR showed interaction not only with full-length SHP, but also with the C-terminal deletion mutants W160X and ΔN-148. In contrast, interaction with all of these receptors was lost with the mutant ΔN, missing the N-terminal extension, and with the more extensive N-terminal mutant Δ120. However, interaction was observed with the mutant ΔN-148, which is missing both C- and N-terminal sequences. While it is unclear why the loss of additional C-terminal sequences relative to the ΔN deletion results in interaction with this construct, it identifies SHP residues 72 to 148 as a minimal receptor interaction domain. Overall, these data suggest that the primary receptor interaction domain in SHP is located near the N terminus of the putative LBD.

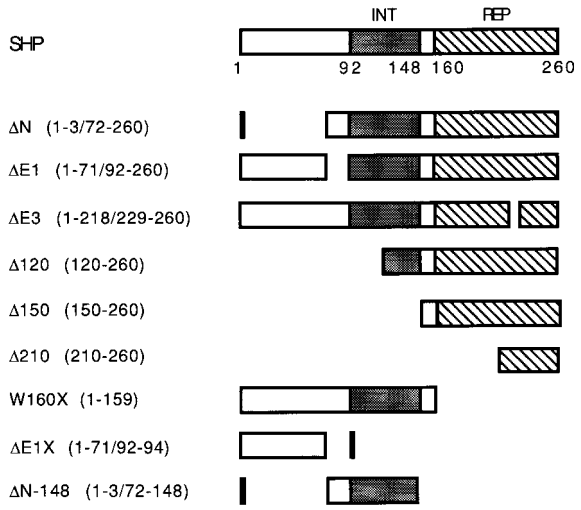

FIG. 1. Deletion mutants of SHP. The receptor-interaction (INT) and repressor (REP) domains are indicated by shaded and hatched boxes, respectively. The deletion constructs were generated as described in Materials and Methods, and the numbers of their first and last amino acids are indicated.

As previously reported, SHP also interacts in vitro with a variety of receptor superfamily members, and the SHP deletion mutants used in yeast were also examined for such in vitro interactions. For simplicity, these studies focused on RXR, which shows relatively strong interaction with SHP in yeast and in mammalian cells (see below). When a GST fusion to full-length human RXRα was incubated with radiolabeled full-length SHP, specific interaction was observed (Fig. 2). As expected from previous results with GST-SHP and in vitro-translated RXRα (33), this interaction was strongly dependent on the presence of 9-*cis*-RA, confirming its specificity. In general, the binding of ^{35}S-SHP to GST-RXR was comparable to, but somewhat less than the binding of ^{35}S-TR, though the latter binding was not dependent on 9-*cis*-RA, as expected (data not shown). A similar interaction was also observed with several SHP mutants, including the W160X mutant lacking the I-box or the 9th heptad, as well as internal deletions of this region (ΔE3), or of the more highly conserved signature motif located near the N terminus of the LBD (ΔE1). In agreement with the results in yeast, the SHP N-terminal deletion Δ120 did not show specific interaction in vitro with GST-RXR relative to that with GST alone (data not shown). Reproducible in vitro interaction was also retained with the ΔN-148 mutant, as observed in yeast, but this in vitro interaction was significantly weaker than that observed with full-length SHP or W160X (data not shown). Overall, these results support the conclusion that the C terminus of SHP is not required for interaction and,

TABLE 1. Interaction of full-length SHP and mutants with other receptors in yeast[a]

Protein	Interaction with receptor								
	Lex	Lex-SHP							Lex-RXR
		WT	ΔE1	ΔN	ΔN-148	Δ120	ΔE1X	W160X	
B42	−	−	−	−	−	−	−	−	−/−
B42-RXR	−/−	3+/3+	2+/3+	−/−	3+/3+	−/−	−/−	3+/3+	3+/3+
B42-TR	−/−	−/2+	−/−	−/−	−/2+	−/−	NT	−/−	3+/3+
B42-RAR	−/−	2+/2+	+/+	−/−	2+/2+	−/−	−/−	+/+	3+/3+
B42-mCAR	−	3+	−	−	+	−	−	+	3+

[a] 3+, strongly blue colonies after 2 days of incubation and strong interaction; 2+, light blue colonies after 2 days of incubation and intermediate interaction; +, light blue colonies after more than 2 days of incubation and weak interaction; −, white colonies and no interaction; NT, not tested. For RXR, TR, and RAR, 100 μl of a 10^{-6} M stock of the appropriate ligand was added to the plate prior to plating, as previously described (22). Results are indicated in the absence (−) and presence (+) of ligand (−/+). The LBDs of human RXRα and rat TRβ were used for construction of B42 fusions. B42-RAR starts at the middle of the second zinc finger of mouse RARα, and B42-mCAR starts at the first methionine of mCAR.

based on the results with ΔE1, suggest that sequences required for interaction lie between SHP residues 92 and 148.

To further confirm the yeast and biochemical results, the mammalian two-hybrid assay was used. Fusions of the GAL4 DBD to intact SHP and various deletion mutants (Fig. 1) were cotransfected into HepG2 cells with a fusion of the VP16 transactivation domain to the hinge and LBD of human RXRα. In this system, the VP16-RXR LBD fusion showed relatively modest, but significant 9-*cis*-RA-dependent interaction with GAL4-SHP (Fig. 3). Much more striking ligand-dependent interaction was observed with GAL4 fusions to two other SHP mutants previously shown to retain interaction in yeast and in vitro, ΔN-148 and W160X. Interaction was lost with the N-terminal Δ120 deletion that also blocked interaction in yeast and in vitro. The mammalian system seems somewhat more stringent than the in vitro system, since interaction was also lost with the internal deletions ΔE3 and ΔE1, which show only decreased interaction in vitro. Very similar results were obtained when the same set of transfections was carried out in JEG3 cells or when a fusion of VP16 to full-length RXR was used in HepG2 cells, although the full-length fusion showed a somewhat lower level of activity than the LBD fusion.

Repressor activity of SHP. Two aspects of the results with the mammalian two-hybrid system suggested that SHP might have inherent transcriptional repressor activity. The strong increase in apparent interaction observed with the GAL4 fusions to the two C-terminal mutants is reminiscent of previous studies of RIP13–N-CoR interaction (34), in which similar increases were observed upon deletion of the RIP13/N-CoR repressor domains from one of the hybrids. Perhaps more directly, the transcriptional activity observed with GAL4-SHP is much lower than that observed with GAL4 alone. As indicated in an independent experiment in Fig. 4A, GAL4-SHP shows only approximately 10% of the activity of GAL4 alone.

To further examine its repression function, intact SHP was added to the C terminus of a GAL4 DNA fusion to the potent VP16 transcriptional activation domain. As expected, the GAL4-VP16 fusion protein alone (GV) exhibits about 1,000-fold activation of the reporter gene, compared to GAL4 alone (Fig. 4B). However, addition of full-length SHP to GV, generating GV-SHP, resulted in an approximately 10-fold repression of this activity. Various deletions of SHP (Fig. 1) were also

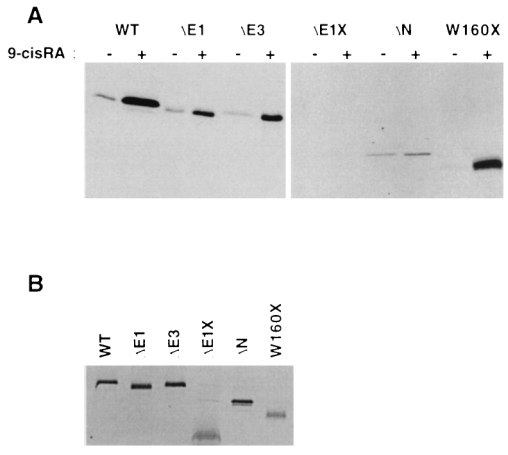

FIG. 2. In vitro interaction of GST-RXR with SHP. Full-length murine SHP (wild type [WT]) and various mutants were radiolabeled with [^{35}S]methionine by in vitro translation with pT7lacHisMyc vectors containing the appropriate cDNAs. SHP products were tested for interaction with a GST fusion to full-length human RXRα (A) in the absence and presence of 1 μM 9-*cis*-RA. (B) Ten percent of the total input of labeled proteins. No interaction was observed with GST alone except with SHPΔN, which showed interaction comparable to that of GST-RXR in the absence of 9-*cis*-RA. Panel A was exposed for 4 days, and panel B was exposed overnight. The overall binding efficiency of SHP and the deletion mutants was less than 5% of the total input in this experiment.

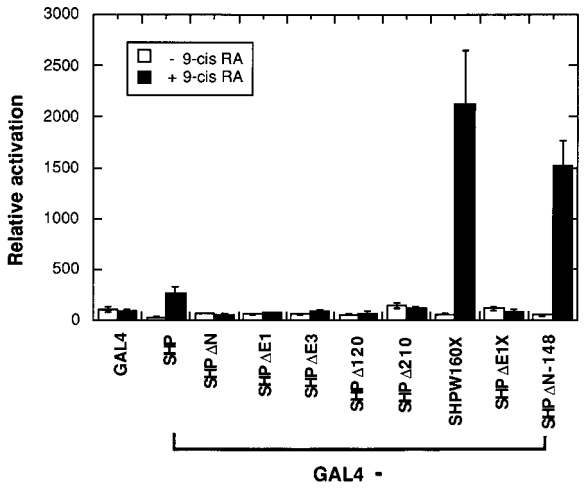

FIG. 3. Mapping of the RXR-interaction domain of SHP. GAL4-SHP and deletion mutants (0.5 μg/well) were cotransfected with VP16-hRXRα LBD (0.5 μg/well) into HepG2 cells. 9-*cis*-RA (1 μM) was added 16 h after DMSO. Results are expressed relative to luciferase expression with GAL4 alone plus VP16-RXR, in the absence of 9-*cis*-RA (1,523 relative light units = 100%).

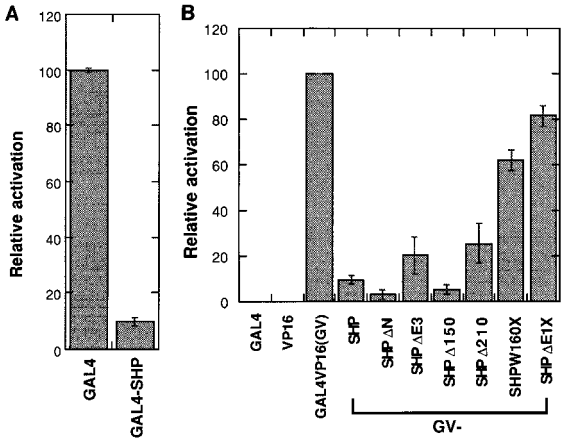

FIG. 4. (A) Repression by SHP. Plasmids containing GAL4 alone or the GAL4-SHP fusion (0.5 μg/well) were cotransfected with TKGH and a reporter plasmid containing three copies of the GAL4 binding site into HepG2 cells. Results are expressed relative to luciferase activity with GAL4 alone (1,315 relative light units = 100%). (B) Mapping of the repressor domain of SHP by GAL4-VP16 (GV) fusion constructs. GAL4VP16SHPWT (wild type) and fusion constructs of deletion mutants (0.5 μg/well) were cotransfected with TKGH and a reporter plasmid containing three copies of GAL4 binding sites into HepG2 cells. Results are expressed relative to luciferase activity with GAL4-VP16 alone (2.1×10^6 relative light units = 100%). The luciferase activities of GAL4 alone and VP16 alone were 2,256 and 1,237 relative light units, respectively.

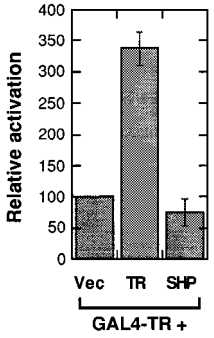

FIG. 5. Cotransfection of GAL4-TR with intact SHP did not restore repression activity of GAL4-TR. GAL4-TR (0.1 μg/well) was cotransfected with 2 μg of intact TR, SHP, or empty vector (Vec) per well into HepG2 cells. GAL4-TR showed about 10-fold repression compared to transfection of GAL4 alone (data not shown). Results are expressed relative to luciferase activity with GAL4-TR plus empty expression vector (93 relative light units = 100%).

fused to GV and tested for repression activity. The majority of the repression was lost with GV-SHPW160X, which has a deletion of 101 amino acids at the C terminus of SHP that does not affect receptor interaction function. This indication of the existence of a C-terminal repressor domain was confirmed by GV-SHPΔ150, which contains only the C-terminal 111 amino acids of SHP and shows even stronger repression than GV-SHP. Interestingly, a further deletion of 60 amino acids, GV-SHPΔ210, exhibited some repression activity, suggesting that the repressor domain may consist of at least two functional regions. A very similar pattern of repression was observed in transfections of JEG-3 cells.

Although there were minor variations in expression, Western analysis with lysates of cells transfected with the GV-SHP wild type and mutants showed that the differences in luciferase activity are not due to differential expression of GV-SHP fusions (data not shown). For example, GV-SHPΔ150 and -Δ210 showed indistinguishable levels of expression, but they differ markedly in their repression activity.

SHP does not interact with N-CoR/RIP13. N-CoR/RIP13 (17, 34) and SMRT/TRAC (7, 30) are corepressors that interact with TR and RAR and repress transcription in the absence of their ligands (5, 29). These corepressors also mediate the repression function of the orphan receptors Rev-Erb and COUP-TF (8, 9, 35, 42). Thus, we used several approaches to test whether SHP could interact with N-CoR/RIP13 or other corepressors.

The first was based on previous results indicating that the TR LBD can compete with GAL4-TR fusions for interaction with corepressors (5, 29). As shown in Fig. 5, cotransfection with TR partially relieved the repression observed with GAL4-TR, as indicated by increased basal expression. In contrast, no such increase was observed in cotransfections of GAL4-TR with SHP. Previous results also demonstrate that the TR LBD can decrease the repression activity observed with GAL4-TR-VP16 fusions similar to the GV-SHP fusions studied here (5). However, coexpression of the TR LBD did not increase expression observed with such GV-SHP fusions (data not shown).

We also tested whether SHP can interact with RIP13/N-CoR in the mammalian or yeast two-hybrid systems or in vitro. Strong interactions were previously observed between VP16-TR and VP16-RAR fusions and a series of GAL4 fusions of RIP13/N-CoR containing either one or both of the two identified receptor interaction domains (34). However, none of these GAL4-RIP13 fusions showed significant interaction with VP16-SHP in HepG2 cells (data not shown). In the yeast two-hybrid system, a LexA fusion to full-length SHP also failed to interact with B42-RIP13ΔN2, which contains both of the receptor interaction domains and shows strong interaction with other LexA-receptor fusions (34), although the LexA-SHP fusion did show weak interaction with a full-length B42-RIP13a fusion (data not shown). Finally, GST-SHP also failed to interact in vitro with radiolabeled RIP13ΔN4, which contains both of the interaction domains and was previously shown to interact with both TR and RAR (34). Although negative, these consistent results strongly suggest that SHP is unable to interact with at least the C-terminal portion of RIP13/N-CoR that is present in the constructs used.

DISCUSSION

The ninth heptad (13, 14) or the I-box (28), is a conserved motif in the 10th helix of the LBD of receptor superfamily members (41) that is required for heterodimerization with RXR and is also involved in homodimerization of both RXR (4) and ER (10, 23). Surprisingly, the region corresponding to the ninth heptad in SHP is not required for interaction with its various targets. Instead, the minimal interaction domain was mapped to the region of helices 5 to 7 (41). In alignments of superfamily sequences, this segment of SHP shows an unusual insertion of approximately 18 amino acids at the C terminus of helix 5 (41), as indicated by box I in Fig. 6. The only other receptor superfamily members that share this feature are two mammalian orphans, DAX-1 and GCNF (6), and the insect RXR homolog USP. The sequence of the DAX-1 insertion, but not the others, shares some similarity with the SHP sequence in this region, which is consistent with the fact that DAX-1 is the closest relative of SHP within the superfamily (Fig. 6). Interestingly, DAX-1 also does not require the ninth heptad for interaction with the orphan SF-1 (19). Initial deletion mapping results indicate that the region of DAX-1 includ-

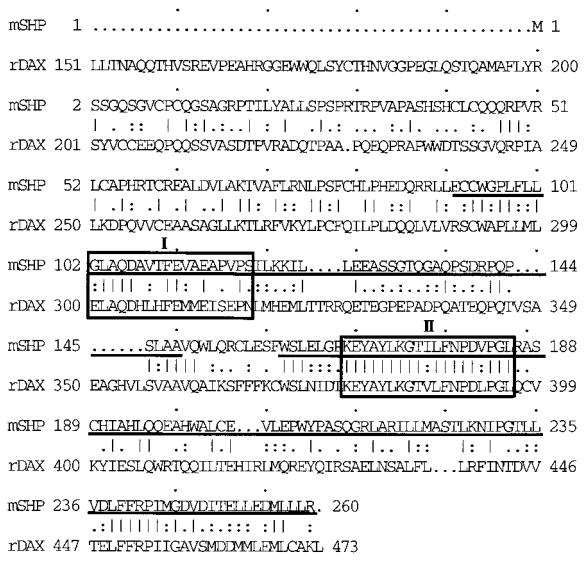

FIG. 6. Sequence comparison between murine SHP and rat DAX1. The N-terminal receptor-interaction and C-terminal repressor domains are underlined. Regions of 18 amino acids at the C terminus of helix 5 and 19 amino acids at the N terminus of the repressor domain are also shown as boxes I and II, respectively.

ing the insertion is neither necessary nor sufficient for this interaction. However, more-detailed mutagenesis studies in the context of the intact proteins will be required to determine whether this conserved insertion is involved in receptor-receptor interactions in SHP or DAX-1.

SHP inhibits DNA binding by the superfamily members with which it interacts, and this inhibition was assumed to account for its inhibition of transactivation in appropriate mammalian cell cotransfections (33). In this context, the relevance of the direct repressor activity of SHP described here seems uncertain. However, there are at least two situations where this activity could be important. DAX-1, which lacks a conventional receptor DBD like SHP, has been reported to bind a retinoic acid response element via its novel N-terminal domain. Although we have been unable to detect SHP binding to retinoic acid response elements, or any of a number of other DNA sequences tested, it remains possible that the N-terminal domain of SHP is a DBD. Thus, SHP repressor function may be directed toward its own, as yet unidentified, DNA binding sites. An alternative scenario for recruitment of SHP to DNA is provided by the subset of nuclear hormone receptors that bind DNA as monomers. The interaction of SHP with one of these proteins would not be expected to prevent DNA binding. However, the inherent SHP repressor activity could result in the repression of transactivation by such monomeric receptors via a mechanism analogous to that recently proposed for the inhibition of SF-1 transactivation by DAX-1 (19). Among the limited number of monomer binders tested to date, we have observed only a modest inhibitory effect of SHP on the potent monomeric activator RORα (reference 15 and data not shown).

The mechanism responsible for repression by SHP remains unknown. Several reports have linked the repressor activity of unliganded TR, RAR, and the orphan receptors COUP-TF and Rev-Erb to the recruitment of the corepressors N-CoR/RIP13 and SMRT/TRAC. The consequences of these interactions have recently been ascribed to the recruitment of histone deacetylase activity by the corepressors (for review, see references 27, 39, and 40). We have been unable to detect any interaction of SHP with N-CoR/RIP13 by a variety of approaches. Although it remains possible that SHP interacts directly with the N-terminal repressor domains, or with the related corepressor SMRT, these results suggest that SHP may act by recruiting an as-yet-unidentified corepressor. Alternatively, SHP could interact directly with a histone deacetylase (36) or Sin3-like protein (38), independently of the corepressors. Another possibility is that SHP may interfere with the activity of general transcription factors, and thereby inhibit formation of an active preinitiation complex. This could be similar to the reported inhibitory interaction of unliganded TR with two components of general transcription machinery, the TATA box binding protein (TBP) and TFIIB (12, 37). It was also reported that Dr1 acts as a repressor of class II gene transcription (18) by formation of a strong complex with DRAP1 (Dr1-associated polypeptide), TBP, and the TATA motif and precludes the entry of TFIIA and/or TFIIB into preinitiation complexes (26).

Although the mechanism of repression by SHP remains unknown, there is also an interesting similarity between SHP and DAX-1 in this region. Overall, the SHP repressor domain shows 41% sequence identity to the corresponding region of DAX-1, but within the N-terminal portion of this domain, a stretch of 19 amino acids shows only two conservative changes between SHP and DAX-1 (box II in Fig. 6). This striking conservation of sequence in the repressor domain clearly suggests a conserved function for this motif in repression, although more-detailed mutagenesis studies will be required to test this prediction.

In conclusion, we have characterized a novel receptor interaction domain near the N terminus of the LBD of SHP and a novel repressor domain near its C terminus. The results described here suggest that SHP may inhibit nuclear hormone repressor signaling pathways by two mechanisms: directly, via its inherent repressor function, and indirectly, by blocking DNA binding as previously described (33). The ligand dependence of the interaction suggests that SHP specifically targets activated receptors and acts to decrease or dampen expression of hormone-induced genes. The range of receptor interactions observed for SHP suggests a very broad range of potential targets for such inhibition. However, there are several factors that may limit these effects. It seems likely that the large number of potential targets would compete for SHP interaction, with only its highest-affinity partners significantly affected at limiting concentrations. The relatively narrow expression of SHP in liver and a limited number of other tissues also suggest that the inhibitory effects may be cell-type specific. Thus, detailed studies of its levels of expression in various cell types and its ability to interact with various receptors will be required to define the most likely targets for SHP. The fact that SHP includes a putative AF-2 transactivation domain suggests that further characterization of its basic activities will also be required for a full understanding of its functional role.

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REFERENCES

1. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.).** 1997. Current protocols in molecular biology. Greene Publishing Assoc., New York, N.Y.
2. **Baes, M., T. Gulick, H.-S. Choi, M. G. Martinoli, D. Simha, and D. D. Moore.** 1994. A new orphan member of the nuclear hormone receptor superfamily that interacts with a subset of retinoic acid response elements. Mol. Cell. Biol. **14:**1544–1552.

3. **Beato, M., P. Herrlich, and G. Schutz.** 1995. Steroid hormone receptors: many actors in search of a plot. Cell **83:**851–857.
4. **Bourget, W., M. Ruff, P. Chambon, H. Gronemeyer, and D. Moras.** 1995. Crystal structure of the ligand binding domain of the human nuclear receptor RXRalpha. Nature **375:**377–382.
5. **Casanova, J., E. Helmer, S. Selmi-Ruby, J.-S. Qi, M. Au-Fliegner, V. Desai-Yajnik, N. Koudinova, F. Yarm, B. M. Raaka, and H. H. Samuels.** 1994. Functional evidence for ligand-dependent dissociation of thyroid hormone and retinoic acid receptors from an inhibitory cellular factor. Mol. Cell. Biol. **14:**5756–5765.
6. **Chen, F., A. J. Cooney, Y. Wang, S. W. Law, and B. W. O'Malley.** 1994. Cloning of a novel orphan receptor (GCNF) expressed during germ cell development. Mol. Endocrinol. **8:**1434–1444.
7. **Chen, J. D., and R. M. Evans.** 1995. A transcriptional co-repressor that interacts with nuclear hormone receptors. Nature **377:**454–457.
8. **Downes, M., L. J. Burke, P. J. Bailey, and G. E. Muscat.** 1996. Two receptor interaction domains in the corepressor, N-CoR/RIP13, are required for an efficient interaction with Rev-erbA alpha and RVR: physical association is dependent on the E region of the orphan receptors. Nucleic Acids Res. **24:**4379–4386.
9. **Downes, M., L. J. Burke, and G. E. Muscat.** 1996. Transcriptional repression by Rev-erbA alpha is dependent on the signature motif and helix 5 in the ligand binding domain: silencing does not involve an interaction with N-CoR. Nucleic Acids Res. **24:**3490–3498.
10. **Fawell, S. E., J. A. Lees, R. White, and M. G. Parker.** 1990. Characterization and colocalization of steroid binding and dimerization activities in the mouse estrogen receptor. Cell **60:**953–962.
11. **Fields, S., and O. Song.** 1989. A novel genetic system to detect protein-protein interaction. Nature **340:**245–246.
12. **Fondell, J. D., F. Brunel, K. Hisatake, and R. G. Roeder.** 1996. Unliganded thyroid hormone receptor α can target TATA-binding protein for transcriptional repression. Mol. Cell. Biol. **16:**281–287.
13. **Forman, B. M., and H. H. Samuels.** 1990. Minireview: interactions among a subfamily of nuclear hormone receptors: the regulatory zipper hypothesis. Mol. Endocrinol. **4:**1293–1302.
14. **Forman, B. M., C.-R. Yang, M. Au, J. Casanova, J. Ghysdael, and H. H. Samuels.** 1987. A domain containing a leucine zipper like motif mediates novel in vivo interactions between the thyroid hormone and retinoic acid receptors. Mol. Endocrinol. **3:**1610–1626.
15. **Giguere, V., M. Tini, G. Flock, E. Ong, R. M. Evans, and G. Otulakowski.** 1994. Isoform-specific amino-terminal domains dictate DNA-binding properties of RORα, a novel family of orphan hormone nuclear receptors. Genes Dev. **8:**538–553.
16. **Gyuris, J., E. Golemis, H. Chertkov, and R. Brent.** 1993. Cdi1, a human G_1 and S phase protein phosphatase that associates with Cdk2. Cell **75:**791–803.
17. **Horlein, A. J., A. M. Naar, T. Heinzel, J. Torchia, B. Gloss, R. Kurokawa, A. Ryan, Y. Kamei, M. Soderstrom, C. K. Glass, and M. G. Rosenfeld.** 1995. Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. Nature **377:**397–404.
18. **Inostroza, J. A., F. H. Mermelstein, I. Ha, W. S. Lane, and D. Reinberg.** 1992. Dr1, a TATA-binding protein-associated phosphoprotein and inhibitor of class II gene transcription. Cell **70:**477–489.
19. **Ito, M., R. Yu, and J. L. Jameson.** 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.
20. **Kastner, P., M. Mark, and P. Chambon.** 1995. Nonsteroid nuclear receptors: what are genetic studies telling us about their role in real life. Cell **83:**859–869.
21. **Leblanc, B. P., and H. G. Stunnenberg.** 1995. 9-cis Retinoic acid signaling: changing partners causes some excitement. Genes Dev. **9:**1811–1816.
22. **Lee, J. W., H.-S. Choi, J. Gyuris, R. Brent, and D. D. Moore.** 1995. Two classes of proteins dependent on either the presence or absence of thyroid hormone for interaction with the thyroid hormone receptor. Mol. Endocrinol. **9:**243–254.
23. **Lees, J. A., S. E. Fawell, R. White, and M. G. Parker.** 1990. A 22-amino-acid peptide restores DNA-binding activity to dimerization-defective mutants of the estrogen receptor. Mol. Cell. Biol. **10:**5529–5531.
24. **Mangelsdorf, D. J., and R. M. Evans.** 1995. The RXR heterodimers and orphan receptors. Cell **83:**841–850.
25. **Mangelsdorf, D. J., C. Thummel, M. Beato, P. Herrlich, G. Schutz, K. Umesono, B. Blumberg, P. Kastner, M. Mark, P. Chambon, and R. M. Evans.** 1995. The nuclear receptor superfamily: the second decade. Cell **83:**835–839.
26. **Mermelstein, F., K. Yeung, J. Cao, J. A. Inostroza, H. Erdjument-Bromage, K. Eagelson, D. Landsman, P. Levitt, P. Tempst, and D. Reinberg.** 1996. Requirement of a corepressor for Dr1-mediated repression of transcription. Genes Dev. **10:**1033–1048.
27. **Pazin, M. J., and J. T. Kadonaga.** 1997. What's up and down with histone deacetylation and transcription? Cell **89:**325–328.
28. **Perlmann, T., K. Umesono, P. N. Rangarajan, B. M. Forman, and R. M. Evans.** 1996. Two distinct dimerization interfaces differentially modulate target gene specificity of nuclear hormone receptors. Mol. Endocrinol. **10:**958–966.
29. **Qi, J.-S., V. Desai-Yajnik, M. E. Greene, B. M. Raaka, and H. H. Samuels.** 1995. The ligand-binding domains of the thyroid hormone/retinoid receptor gene subfamily function in vivo to mediate heterodimerization, gene silencing, and transactivation. Mol. Cell. Biol. **15:**1817–1825.
30. **Sande, S., and M. L. Privalsky.** 1996. Identification of TRACs (T3 receptor-associating cofactors), a family of cofactors that associate with, and modulate the activity of, nuclear hormone receptors. Mol. Endocrinol. **10:**813–825.
31. **Selden, R. F., K. B. Howie, M. E. Rowe, H. M. Goodman, and D. D. Moore.** 1986. Human growth hormone as a reporter gene in regulation studies employing transient gene expression. Mol. Cell. Biol. **6:**3173–3179.
32. **Seol, W., H.-S. Choi, and D. D. Moore.** 1995. Isolation of proteins that interact specifically with the retinoid X receptor: two novel orphan receptors. Mol. Endocrinol. **9:**72–85.
33. **Seol, W., H. S. Choi, and D. D. Moore.** 1996. An orphan nuclear hormone receptor that lacks a DNA binding domain and heterodimerizes with other receptors. Science **272:**1336–1339.
34. **Seol, W., M. J. Mahon, Y. K. Lee, and D. D. Moore.** 1996. Two receptor interacting domains in the nuclear hormone receptor corepressor RIP13/N-CoR. Mol. Endocrinol. **10:**1646–1655.
35. **Shibata, H., Z. Nawaz, S. Y. Tsai, B. W. O'Malley, and M. J. Tsai.** 1997. Gene silencing by chicken ovalbumin upstream promoter-transcription factor I (COUP-TFI) is mediated by transcriptional corepressors, nuclear receptor-corepressor (N-CoR) and silencing mediator for retinoic acid receptor and thyroid hormone receptor (SMRT). Mol. Endocrinol. **11:**714–724.
36. **Taunton, J., C. A. Hassig, and S. L. Schreiber.** 1996. A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. Science **272:**408–411.
37. **Tong, G. X., M. R. Tanen, and M. K. Bagchi.** 1995. Ligand modulates the interaction of thyroid hormone receptor beta with the basal transcription machinery. J. Biol. Chem. **270:**10601–10611.
38. **Vidal, M., and R. F. Gaber.** 1991. *RPD3* encodes a second factor required to achieve maximum positive and negative transcriptional states in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11:**6317–6327.
39. **Wade, P. A., and A. P. Wolffe.** 1997. Histone acetyltransferases in control. Curr. Biol. **7:**R82–R84.
40. **Wolffe, A. P.** 1997. Transcriptional control. Sinful repression. Nature **387:**16–17.
41. **Wurtz, J. M., W. Bourguet, J. P. Renaud, V. Vivat, P. Chambon, D. Moras, and H. Gronemeyer.** 1996. A canonical structure for the ligand-binding domain of nuclear receptors. Nat. Struct. Biol. **3:**87–94.
42. **Zamir, I., J. Zhang, and M. A. Lazar.** 1997. Stoichiometric and steric principles governing repression by nuclear hormone receptors. Genes Dev. **11:**835–846.
43. **Zanaria, E., F. Muscatelli, B. Bardoni, T. M. Strom, S. Guioli, W. Guo, E. Lalli, C. Moser, A. P. Walker, E. R. B. McCabe, T. Meitinger, A. P. Monaco, P. Sassone-Corsi, and G. Camerino.** 1994. An unusual member of the nuclear hormone receptor superfamily responsible for X-linked adrenal hypoplasia congenita. Nature **372:**635–641.
44. **Zervos, A. S., J. Gyuris, and R. Brent.** 1993. Mxi1, a protein that specifically interacts with Max to bind Myc-Max recognition sites. Cell **72:**223–232.