URF13 to that membrane. Alternatively, if factor X is an aldehyde, it could be inactivated through oxidation. Either of these events could alter the toxicity associated with the accumulation of URF13 in the mitochondria without affecting URF13 accumulation (consistent with the behavior of *Rf2*).

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18. On the basis of their pedigrees, five of the *rf2-m* alleles isolated in the *Mutator* transposon-tagging experiment (*rf2-m8110, rf2-m8122, rf2-m9323, rf2-m9390*, and *rf2-m9437*) would be expected to have been derived from *Rf2-Q66, Rf2-Q67, Rf2-B77*, or *Rf2-B79*. Their wild-type progenitor alleles were determined by comparing the restriction fragment length polymorphism (RFLP) fingerprints through the use of 3′ *sus1* and *umc153* markers that flank the *rf2* locus [R. P. Wise and P. S. Schnable, *Theor. Appl. Genet.* **88**, 785 (1994)] and *rf2* intron sequences of DNA samples carrying mutant alleles with those carrying the potential progenitor alleles.
19. The inbred lines W22 and B73 carry *Rf2* alleles. The 1.2- and 2.2-kb *Rf2* cDNAs were isolated from the 2ts library prepared by the Dellaporta laboratory [A. Delong, A. Calderon-Urrea, S. L. Dellaporta, *Cell* **74**, 757 (1993)] and from a library prepared by A. Barkan, respectively (A. Barkan, personal communication).
20. Total RNA was isolated from immature tassels according to the method of Dean *et al.* [*EMBO J.* **4**, 3055 (1985)]. About 10 μg of total RNA was loaded in each lane of the RNA gel [H. Lehrach *et al.*, *Biochemistry* **16**, 4743 (1977)]. RNAs were transferred onto a Magna Charge nylon membrane (Micron Separations) and hybridized with an *rf2* cDNA probe at 68°C. RNA loading was quantified by stripping the membrane and then rehybridizing it to a tubulin cDNA probe (*tub1*) [R. Villemur *et al.*, *J. Mol. Biol.* **227**, 81 (1992)].
21. Each strand of the 1.2- and 2.2-kb cDNAs was sequenced at least once at the Iowa State University Nucleic Acid Facility.
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An Orphan Nuclear Hormone Receptor That Lacks a DNA Binding Domain and Heterodimerizes with Other Receptors

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SHP is an orphan member of the nuclear hormone receptor superfamily that contains the dimerization and ligand-binding domain found in other family members but lacks the conserved DNA binding domain. In the yeast two-hybrid system, SHP interacted with several conventional and orphan members of the receptor superfamily, including retinoid receptors, the thyroid hormone receptor, and the orphan receptor MB67. SHP also interacted directly with these receptors in vitro. In mammalian cells, SHP specifically inhibited transactivation by the superfamily members with which it interacted. These results suggest that SHP functions as a negative regulator of receptor-dependent signaling pathways.

The yeast two-hybrid system (*1*, *2*) has proved useful in both the characterization of the heterodimeric interactions of members of the nuclear hormone receptor superfamily and the isolation and characterization of receptor-interacting proteins (*3*–*9*). We used this system to isolate proteins that interact specifically with mCAR, a close murine relative of the human orphan receptor MB67 (*10*). Among several mCAR-interacting proteins, one isolate included the ligand-binding and dimerization domain of a previously uncharacterized orphan member of the receptor superfamily. On the basis of its small size and its ability to interact with several superfamily members, we called this protein SHP (small heterodimer partner).

SHP is encoded by an ~1.3-kb transcript expressed in liver and at lower levels in heart and pancreas (Fig. 1A). Extensive screening of two murine liver cDNA libraries produced a number of cDNA clones (*11*). Two lines of evidence suggested that these clones were full length. The first was simply that the 5′ ends of 10 of 12 independent clones fell within a short 32-nucleotide (nt) segment located upstream of the initiator methionine. More importantly, their length [~1120 base pairs (bp)] corresponded precisely to that of the SHP transcript without its presumably 100- to 300-nt polyA tract. A similar clone was isolated from a human liver cDNA library.

The human and murine SHP proteins are well conserved (Fig. 1B). Both include complete ligand-binding and dimerization domains, with sequences similar to those associated with heterodimerization in other superfamily members, but neither contains a conventional DNA binding domain. Addition of the at least 200 nt necessary to encode the DNA binding domain would increase the predicted size of the mRNA to between 1.4 and 1.6 kb, substantially greater than that observed.

The closest relative of SHP in the receptor superfamily is DAX-1 (Fig. 1C), the only other family member that lacks a conventional DNA binding domain (*12*). These two proteins are approximately as

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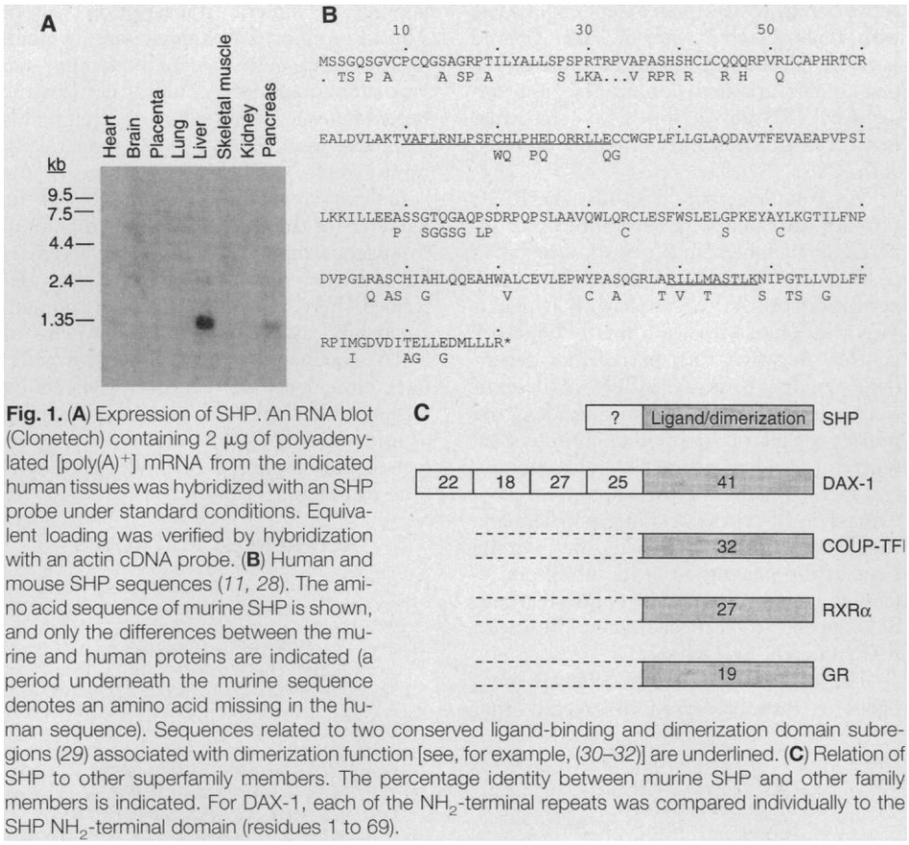

Fig. 1. (**A**) Expression of SHP. An RNA blot (Clonetech) containing 2 μg of polyadenylated [poly(A)$^+$] mRNA from the indicated human tissues was hybridized with an SHP probe under standard conditions. Equivalent loading was verified by hybridization with an actin cDNA probe. (**B**) Human and mouse SHP sequences (*11, 28*). The amino acid sequence of murine SHP is shown, and only the differences between the murine and human proteins are indicated (a period underneath the murine sequence denotes an amino acid missing in the human sequence). Sequences related to two conserved ligand-binding and dimerization domain subregions (*29*) associated with dimerization function [see, for example, (*30–32*)] are underlined. (**C**) Relation of SHP to other superfamily members. The percentage identity between murine SHP and other family members is indicated. For DAX-1, each of the NH_2-terminal repeats was compared individually to the SHP NH_2-terminal domain (residues 1 to 69).

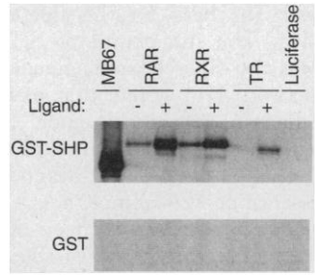

Fig. 2. Direct interaction of SHP with other superfamily members in vitro. Either a GST-SHP fusion protein or GST alone was bound to beads and incubated under stringent conditions (*33*) with the indicated superfamily members or luciferase labeled with [^{35}S]methionine by in vitro translation. Beads were washed, and specifically bound material was eluted with reduced glutathione and resolved by SDS–polyacrylamide gel electrophoresis.

similar to each other as the thyroid hormone receptors (TRs) are to the retinoic acid receptors (RARs) and are divergent from the rest of the superfamily. The NH_2-terminus of DAX-1, which consists of three complete copies and one truncated copy of an ~70–amino acid motif, apparently binds the βRARE, the retinoic acid response element from the RARβ2 promoter (*12*). Although the NH_2-terminal region of SHP shows a limited amount of similarity to this DAX-1 motif, SHP did not bind the βRARE or any of a number of other synthetic direct-repeat or inverted-repeat hormone response elements tested. However, both the arrangement and conservation of six cysteines in the NH_2-terminal region of the human and murine SHP proteins suggest that this region could be a metal-binding domain involved in protein-DNA or protein-protein interactions.

In the yeast two-hybrid system, a fusion protein with SHP joined to the B42 transcriptional-activation domain interacted with LexA fusions including the ligand-binding and dimerization domain of either mCAR or MB67. MB67 (*10*) and mCAR are retinoid X receptor (RXR) heterodimer partners, and both of these LexA hybrids also interacted with a previously described B42-RXR hybrid (*3*). However, the B42-SHP hybrid also interacted with analogous LexA fusions to several conventional receptors, including RARα, RXRα, and TRβ (Table 1). The SHP-TR interaction was strongly dependent on the presence of thyroid hormone. Retinoids were not required for SHP interaction with RAR and RXR, although they did have a small stimulatory effect in quantitative β-galactosidase assays (*13*). The B42-SHP fusion did not interact with LexA alone or with a LexA fusion to the orphan receptor RIP14, an RXR heterodimer partner isolated through the use of LexA-RXR as a bait in the two-hybrid system (*4*). Overall, the similarities in the pattern of interactions observed with B42-SHP and B42-RXR suggest strongly that SHP, like the RXRs, is a common heterodimer partner for members of the receptor superfamily.

To confirm the interactions observed in yeast, we incubated either a glutathione S-transferase (GST)–SHP fusion protein or GST alone with several different receptor proteins labeled with [^{35}S]methionine by in vitro translation. MB67, RARα, RXRα, and TRα bound specifically to GST-SHP (Fig. 2). Similar results were obtained with in vitro–translated SHP protein and GST fusions to TR and RXR. As observed in yeast, the interaction of SHP with RAR, RXR, and TR was increased by the ligand. In the case of RAR and RXR, however, the effect of the ligand on this in vitro interaction was much stronger than that observed

Table 1. SHP interacts with multiple superfamily members in yeast. The yeast interaction trap (*2*) was used to define interactions of the various fusion proteins as described previously for the RXR, RAR, TR, and RIP14 hybrids (*3, 4*). The LexA and B42 fusions include sequences from the COOH-terminus of the DNA binding domain of the various receptors to their COOH-termini, except the B42-SHP hybrid, in which the full-length murine SHP protein is fused to the B42 transcriptional activation domain. A plus indicates a specific interaction as demonstrated by the formation of blue colonies on an X-Gal indicator plate; a minus indicates white colonies and thus no interaction. None of the B42 fusion proteins interacted with LexA alone. The respective ligands for RXR, RAR, and TR were added to the plates as described (*3, 4*). The interaction of SHP with TR in the presence of ligand was somewhat weaker than the other interactions when scored on indicator plates but was comparable with the other SHP-receptor interactions in quantitative β-galactosidase assays of cells grown in liquid culture. The expected stimulatory effect of 9-*cis*-retinoic acid on the RXR-RXR interaction was also confirmed by such quantitative β-galactosidase assays as described previously (*3, 4*).

B42 fusion protein	LexA-MB67 (no ligand)	LexA-RXR		LexA-TR		LexA-RAR		LexA-RIP14 (no ligand)
		No ligand	Ligand	No ligand	Ligand	No ligand	Ligand	
B42-SHP	+	+	+	−	+	+	+	−
B42-RXR	+	+	+	+	+	+	+	+
B42-TR	−	+	+	−	−	−	−	−
B42 alone	−	−	−	−	−	−	−	−

in yeast. The basis for this difference is unknown. The interaction of SHP with RXR was also observed in mammalian cells with a two-hybrid system that reproduces heterodimeric receptor-receptor interactions (*14, 15*). As with the in vitro binding results, this interaction was dependent on the presence of 9-*cis*-retinoic acid.

The dominant-negative effects of a variety of mutant receptors unable to bind DNA predict that SHP should inhibit both DNA binding and transactivation by those receptors with which it interacts. Our results confirm the expected inhibitory effect of SHP on binding of the βRARE by RAR-RXR heterodimers (Fig. 3A). In contrast to its distant relative DAX-1 (*12*), SHP did not bind this element. Addition of increasing amounts of SHP, however, specifically inhibited binding by RAR-RXR heterodimers.

SHP alone had no effect on a βRARE-containing reporter in mammalian cell cotransfections. However, SHP coexpression specifically inhibited transactivation of this reporter by either mCAR or RARβ (Fig. 3B). In additional experiments, SHP also inhibited 9-*cis*-retinoic acid–dependent transactivation by RXRα to an extent comparable with that observed with mCAR. Thyroid hormone–dependent transactivation of the palindromic triiodothyronine response element by TRβ was inhibited to a somewhat lesser extent, comparable to that observed with RAR.

We conclude that SHP, like the RXRs (*16–20*), interacts with several receptor superfamily members. In contrast to the broad positive effects of RXR, SHP inhibits transactivation by the receptors with which it interacts. Thus, we suggest that SHP plays a central, negative role in receptor-dependent signaling pathways in liver and potentially in other tissues. The magnitude of the impact of SHP on function of an individual receptor would clearly be dependent on several factors, including the relative levels of expression of potential dimer partners and their relative affinity for each other. On the basis of the magnitude of its inhibitory effect in mammalian cell cotransfections, SHP appears to show the highest affinity for RXR, mCAR, and MB67.

The inhibitory effects of SHP are analogous to those observed in several other families of dimeric transcription factors. The Id proteins, for example, are members of the basic helix-loop-helix family that lack a functional DNA binding domain and heterodimerize with a subset of other family members, resulting in a broad range of inhibitory effects (*21–23*). However, the complexity apparent in the functions of the members of the nuclear receptor superfamily suggests that SHP could have activities beyond the simple negative effects described here. Its NH_2-terminal domain could bind as yet unidentified proteins or DNA sequences, for example, and it could have either positive or negative effects on receptors that bind DNA with high affinity as monomers (*24–26*). Additionally, its effects could be altered by binding of a specific ligand.

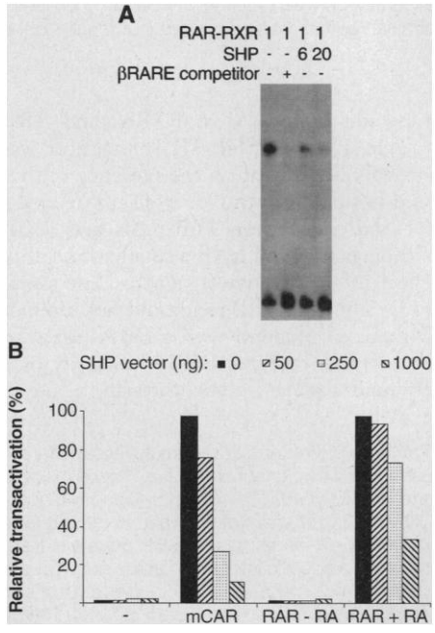

Fig. 3. Functional interaction of SHP with other superfamily members. (**A**) SHP inhibits DNA binding by RAR-RXR heterodimers. A ^{32}P-labeled oligonucleotide containing the βRARE (*34, 35*) was incubated with RAR and RXR, with or without SHP as indicated. Specific complexes were resolved by gel electrophoresis. The amounts of RAR and RXR were constant, and either a 6-fold or 20-fold molar excess of SHP protein was added, as indicated. A specific oligonucleotide competitor was added to the incubation in the second lane. No binding was observed with RAR, RXR, or SHP alone, or with combinations of SHP with either RAR or RXR. SHP was produced by in vitro translation, and the total amount of reticulocyte lysate was kept constant in all lanes by addition of unprogrammed lysate as needed. His-tagged derivatives of RAR and RXR were expressed in *Escherichia coli* and purified with nickel-agarose. Protein amounts were estimated by direct measurement for RAR and RXR and by incorporation of [^{35}S]methionine for SHP. Electrophoretic mobility–shift assays were as described (*10*), except that components were preincubated in the absence of probe for 10 min on ice. After the addition of probe, reactions were further incubated for 20 min at room temperature. (**B**) SHP inhibits transactivation by mCAR and RAR. The indicated amounts (50 ng to 1 μg) of SHP expression vector were added to cotransfections that included 50 ng of either mCAR or RARβ expression vectors, 1.5 μg of a thymidine kinase (TK)–luciferase reporter containing three copies of the βRARE, and 2.0 μg of an internal-control reporter in which the same TK promoter directs human growth hormone (hGH) expression (*36, 37*). Total amounts of expression vector were kept constant by adding decreasing amounts of the CDM8 expression vector to transfections containing increasing amounts of the SHP vector. Like MB67 (*10*), mCAR transactivates this βRARE-luciferase reporter in the absence of any exogenously added ligand. The absolute transactivation mediated by mCAR is about two to four times as great as that described previously for MB67 but is less than half of that directed by RAR. SHP did not significantly affect hGH expression in the presence or absence of mCAR or RAR. HepG2 cells grown in 35-mm wells were incubated with Dulbecco's minimum essential medium plus 10% charcoal-stripped fetal bovine serum and transfected. Luciferase and growth hormone assays were as described (*10*). For both mCAR and RAR, normalized luciferase expression from duplicate samples is presented relative to the transactivation observed in the absence of SHP. Similar results were obtained in two similar experiments.

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11. The initial isolate of SHP was one of several clones obtained from an appropriate mouse liver cDNA library (*4*) through use of the interaction trap (*2*) with a LexA fusion to the hinge and ligand-binding and dimerization domains of mCAR as a bait. Interaction-trap screening was as described for isolation of proteins that interact with TR (*6*) and RXR (*4*). mCAR is a close murine relative of the orphan MB67 (*10*) that is also an apparently constitutive transactivator of a subset of RAREs (H.-S. Choi, D. Simha, D. D. Moore, unpublished data). Twelve independent murine SHP clones were isolated from two different mouse liver cDNA libraries through the use of conventional screening. The 5′ ends of 10 of the 12 clones fall within a 32–bp segment upstream of the first methionine codon, which includes a good match to the consensus for translation initiation. One additional clone starts within the coding region, and the other contains 16 bp that diverge from those of the majority class upstream of the eighth amino acid. Despite extensive screening, no clones were isolated that corresponded to a larger SHP transcript present at very low levels in liver. It remains unclear whether this minor species represents a larger mRNA or a nuclear precursor not present in the cytoplasmic RNA used to make the libraries. One human cDNA clone was isolated by screening a human liver library with a fragment of the mouse clone as a probe. The human clone begins at the second position of the putative initiator methionine codon and includes a 318–bp intron with apparent 5′ and 3′ splice sites between amino acids 178 and 179. The murine (L76567) and human (L76571) SHP cDNA sequences have been deposited in GenBank.
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33. The intact SHP clone was fused to a derivative of the pGEX series of GST expression vectors, and the GST-SHP fusion protein or GST alone was expressed in *Escherichia coli* as described (*27*). Receptors and luciferase were expressed by in vitro translation, and protein interaction tests were done as described (*6*), except that ^{35}S-labeled proteins were precleared by incubation with GST alone bound to glutathione–Sepharose-4B beads. After centrifugation, the supernatant was incubated with GST-SHP or GST protein. Specifically bound proteins were eluted from beads with 40 mM reduced glutathione in 50 mM tris (pH 8.0).
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PKD2, a Gene for Polycystic Kidney Disease That Encodes an Integral Membrane Protein

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A second gene for autosomal dominant polycystic kidney disease was identified by positional cloning. Nonsense mutations in this gene (*PKD2*) segregated with the disease in three PKD2 families. The predicted 968–amino acid sequence of the *PKD2* gene product has six transmembrane spans with intracellular amino- and carboxyl-termini. The PKD2 protein has amino acid similarity with PKD1, the *Caenorhabditis elegans* homolog of PKD1, and the family of voltage-activated calcium (and sodium) channels, and it contains a potential calcium-binding domain.

Autosomal dominant polycystic kidney disease (ADPKD) accounts for 8 to 10% of all end-stage renal disease worldwide (*1*). Its principal clinical manifestation is bilateral renal cysts that result in chronic renal failure in about 45% of affected individuals by age 60 (*1*). Hypertension and liver cysts are common, and the involvement of other organ systems (*2*) lends support to the view that polycystic kidney disease is a systemic disorder (*1*). Genetic heterogeneity in ADPKD has been demonstrated after linkage was initially discovered for the gene on chromosome 16p13.3 (*PKD1*) (*3*, *4*). The second gene, *PKD2*, has been localized on chromosome 4q21-23 and accounts for approximately 15% of affected families (*5*, *6*). At least one more gene for ADPKD is known to exist (*4*). Clinical studies have demonstrated a milder phenotype for the non-PKD1 forms (*7*). This report describes the positional cloning (*8*) of a candidate gene in which truncating mutations have been identified in three PKD2 families (*9*).

The *PKD2* genetic interval is flanked by the polymorphic markers D4S231 and D4S414/423 (*5*). We constructed a yeast artificial chromosome (YAC) contig and high-density sequence tag site (STS) map of this region (*10*) (Fig. 1). Genetic studies in affected families using physically ordered polymorphic markers led to several progressive refinements of the *PKD2* interval (*11*, *12*). The closest unambiguous flanking genetic markers are AFMa059xc9 proximally and AICA1 distally (Fig. 1, A and B) (*10*). We constructed a cosmid- and P1-based (*13*) contig extending over ~680 kb from AICA1 to the region centromeric to the polymorphic marker JSTG3 (Fig. 1C) (*14*). This contig contains a single gap of less than 40 kb. Complementary DNAs corresponding to genes in this region were isolated with inserts from the genomic clones used to screen either a human fetal brain or adult kidney cDNA library (*15*). The mapping of the cDNA clones identified was confirmed, and the clones were sequenced. These sequences were analyzed to identify open reading frames (ORFs), and database searches with the BLAST algorithms (*16*) were performed.

One group of cDNA clones, collectively termed cTM-4, were initially isolated with insert DNA from cosmid c44a9 from the chromosome 4–specific cosmid library (Fig. 1D) (*13*, *15*). None of the cTM-4 clones have homology at the nucleotide level to any known genes, although two randomly sequenced cDNA clones were identified (Fig. 1E). Northern (RNA) blot hybridization with the cTM-4B3-3 insert (Fig. 1E) revealed a ~5.4-kb transcript expressed in most fetal and adult tissues (Fig. 2). cTM-4 is strongly expressed in ovary, fetal and adult kidney, testis, small and large intestine, and fetal lung. Peripheral blood leukocytes were the only tissue tested in which expression was not detected.

Initial database searches with the six translated reading frames obtained from the sequence of clone cTM-4B3-3 revealed homology at the amino acid level with the *PKD1* gene product also called polycystin (*17*, *18*). On the basis of its map location, pattern of expression, and the observed homology, the cTM-4 gene was further investigated as a candidate for *PKD2*. Nine over-

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