*Proc. Natl. Acad. Sci. USA*
Vol. 92, pp. 2096–2100, March 1995
Biochemistry

# OR-1, a member of the nuclear receptor superfamily that interacts with the 9-*cis*-retinoic acid receptor

M. TEBOUL*†, E. ENMARK*, Q. LI‡, A. C. WIKSTRÖM‡, M. PELTO-HUIKKO§¶, AND J.-Å. GUSTAFSSON*‡‖

*Center for Biotechnology and ‡Department of Medical Nutrition, Karolinska Institute, NOVUM, S-141 86 Huddinge, Sweden; and §Department of Biomedical Sciences, University of Tampere, P.O. Box 607, FIN-33101 Tampere, Finland; and ¶Department of Neurology, University of Kuopio, Kuopio, Finland

*Communicated by E. E. Baulieu, Institut National de la Santé et de la Recherche Médicale, Bicêtre Cedex, France, November 16, 1994 (received for review November 25, 1993)*

ABSTRACT **We have cloned a member of the nuclear receptor superfamily. The cDNA was isolated from a rat liver library and encodes a protein of 446 aa with a predicted mass of 50 kDa. This clone (OR-1) shows no striking homology to any known member of the steroid/thyroid hormone receptor superfamily. The most related receptor is the ecdysone receptor and the highest homologies represent <10% in the amino-terminal domain, between 15–37% in the carboxyl-terminal domain and 50–62% in the DNA binding domain. The expression of OR-1 appears to be widespread in both fetal and adult rat tissues. Potential DNA response elements composed of a direct repeat of the hexameric motif AGGTCA spaced by 0–6 nt were tested in gel shift experiments. OR-1 was shown to interact with the 9-*cis*-retinoic acid receptor (retinoid X receptor, RXR) and the OR-1/RXR complex to bind to a direct repeat spaced by 4 nt (DR4). In transfection experiments, OR-1 appears to activate RXR-mediated function through the DR4. Therefore OR-1 might modulate 9-*cis*-retinoic acid signaling by interacting with RXR.**

Cellular responsiveness to retinoic acid (RA), vitamin $D_3$, steroid hormones, and thyroid hormone is conferred through a superfamily of nuclear receptors (1). In addition to the members that respond to characterized ligands, the superfamily comprises a large collection of so-called orphan receptors that lack identified ligands (1, 2). Members of this superfamily activate and/or repress gene transcription through binding to discrete cis-acting elements termed hormone response elements (HREs). These HREs are composed of repeats of consensus hexanucleotide DNA motifs, with specificity determined through the orientation and the spacing between the half-sites (3). Specific DNA binding is mediated by a distinct domain, containing two zinc fingers, which is conserved among all family members (1). Three amino acids at the carboxyl-terminal base of the first zinc finger (known as the P box) are important for recognition of the half-site nucleotide sequence. Members of the nuclear receptor superfamily have been classified into different groups on the basis of the amino acid sequence within the P box.

We have cloned a member of the nuclear receptor superfamily by low-stringency screening of a rat liver cDNA library.** According to the P-box sequence, this orphan receptor (OR-1) belongs to a subfamily also including RA receptor (RAR), vitamin $D_3$ receptor (VDR), and thyroid hormone receptor (TR). The natural response element for these receptors consists of a direct repeat of a binding site related to the hexamer AGGTCA. VDR, RAR, and TR bind to their response elements as heterodimers with retinoid X receptor (RXR) (4, 5), whose ligand is 9-*cis*-RA (6, 7). The spacing of the direct repeat determines the binding preference: 3 bp for VDR, 4 bp for TR, and 5 bp for RAR (3–4–5 rule) (3). However, recent results suggest that, in fact, more than one parameter controls the selectivity of the interaction between response elements and nuclear receptors, and some elements may be recognized by multiple receptor species (8). To investigate the function of OR-1, we have determined in which tissues it is expressed. Since OR-1 appears to be a member of the RAR/TR subfamily of nuclear receptors, we also wanted to investigate whether OR-1 would bind to a HRE formed by an AGGTCA repeat, and to determine whether OR-1—like VDR, RAR, and TR—forms a heterodimeric complex with RXR. We have found that OR-1 interacts with RXR and that this complex binds to a direct repeat element with 4-bp spacing (DR4 element). In transfection experiments, coexpression of OR-1 with RXR confers a constitutive activation of a reporter gene under the control of a DR4-containing promoter. This activation is enhanced by 9-*cis*-RA. This suggests that OR-1 may act as a helper of RXR on a DR4-containing promoter.

## MATERIALS AND METHODS

**Cloning and Expression of OR-1.** Rat OR-1 was cloned from a Sprague–Dawley rat liver cDNA library in the λ ZAP vector (Stratagene) by low-stringency screening with a mixture of synthetic oligonucleotide probes derived from known steroid receptors (9).

***In Situ* Hybridization.** Fetal and adult rat tissues were excised after decapitation and frozen on dry ice. Cryostat sections were hybridized to 51-mer oligonucleotides complementary to OR-1 mRNA (nt 800–850 and 850–900) as described previously (10). Several unrelated oligonucleotides were used as controls, and addition of a 100-fold excess of the respective nonlabeled oligonucleotide abolished all labeling observed with the OR-1 probes.

**Plasmids.** OR-1 cDNA was subcloned in pGEM-3Z (Promega) as an *Eco*RI fragment, to give the plasmid pROR-1-Sp6, or in the multiple cloning site of pCMV5 (11), to give the plasmid pCMV-OR-1. The reporter constructs pDR4-AF, pDR1-AF, pDR3-AF, and pDR5-AF (kindly provided by Stefan Nilsson, Karo Bio, Huddinge, Sweden) contain an *Sph* I–*Xho* I fragment of the cDNA for a secreted form of human placental alkaline phosphatase (12) under the control of DR4-MMTV, DR1-MMTV, DR3-MMTV, and DR5-MMTV-containing promoters, respectively, where MMTV represents the long terminal repeat of mouse mammary tumor virus. pRRXR-T7 and pCMV-RXR were described previously (13).

**Antibody Production and Immunoprecipitation.** A rabbit antiserum was raised to a peptide corresponding to aa 55–71

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: HRE, hormone response element; RA, retinoic acid; RAR, RA receptor; VDR, vitamin $D_3$ receptor; TR, thyroid hormone receptor; RXR, retinoid X receptor; TRE, thyroid hormone response element; DR*n*, direct repeat with *n*-bp spacing.

†Present address: Laboratoire de Biologie Moléculaire et Cellulaire, UMR49CNRS, ENS Lyon, 46 Allée d'Italie, 69364 Lyon Cedex, France.

‖To whom reprint requests should be addressed.

**The sequence reported in this paper has been deposited in the GenBank database (accession no U20389).

of OR-1. After one-step glutaraldehyde coupling of the peptide to keyhole limpet hemocyanin (KLH) (Pierce), rabbits were immunized with 0.5 mg of peptide–KLH in complete Freund's adjuvant. After several booster injections, sera were tested against the peptide by ELISA. The antiserum was purified on a cyanogen bromide-activated Sepharose 4B column (Pharmacia) to which the peptide had been coupled as recommended by the manufacturer. The bound antibodies were eluted with 0.1 M citrate buffer (pH 3). The crude as well as the affinity-purified antiserum specifically precipitated OR-1 expressed in a programmed reticulocyte lysate and also gave rise to a signal corresponding to the [$^{35}$S]methionine-labeled OR-1 in Western immunoblotting experiments (unpublished results). Control experiments including the exclusion of primary antiserum as well as substitution of the primary antiserum with rabbit normal serum did not give rise to any signal. Detection was with horseradish peroxidase-labeled sheep anti-rabbit immunoglobulins revealed by Amersham's ECL system.

Fifty microliters of antiserum was incubated with 50 $\mu$l of protein A-Sepharose CL-4B (Pharmacia) in a total volume of 400 $\mu$l for 1 hr and then washed three times with phosphate-buffered saline. The *in vitro* synthesized proteins were added as indicated and the mixture was incubated for 1 hr, washed three times with phosphate-buffered saline and boiled in SDS sample buffer before separation in a SDS/7.5% polyacrylamide gel.

**DNA Binding Studies.** Gel shift assays were performed with *in vitro* translated OR-1 and RXR prepared with the TNT coupled reticulocyte lysate system (Promega). Proteins were incubated on ice for 15 min with 4 $\mu$g of poly(dI-dC) and with unlabeled competitor DNA, where indicated, in 100 mM KCl/10 mM Hepes, pH 7.6/1 mM dithiothreitol/1 mM EDTA/10% (wt/vol) glycerol, before addition of 0.5 ng of a $^{32}$P-end-labeled oligonucleotide probe. The reaction mixtures were incubated for a further 10 min at 22°C before electrophoresis at 200 V and 4°C in pre-run 4% polyacrylamide/0.25× TBE gels (1× TBE is 90 mM Tris/64.6 mM boric acid/2.5 mM EDTA, pH 8.3). The following oligonucleotides and their complements were used as probes: DR0, 5′-AGCTTCAGGTCAAGGTCAGGTTCA-3′; DR1, 5′-AGCTTCAGGTCACAGGTCAGTTCA-3′; DR2, 5′-AGCTTAGGTCACCAGGTCAGTTCA-3′; DR3, 5′-AGTCC AGGTCACTCAGGTCAGTCA-3′; DR4, 5′-AGTCAGGTCA CTCGAGGTCAGTCA-3′; DR5, 5′-AGTCAGGTCACTCGT AGGTCAGTCA-3′; and DR6, 5′-AGTCAGGTCACTCGTTA GGTCAGTCA-3′.

**Cells and Transfection.** CHO-K1 cells were cultured in Ham's F-12 medium supplemented with 10% fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 $\mu$g/ml). Cells were plated in duplicate in 35-mm Petri dishes and transfected at 30% confluence by using Lipofectin reagent (BRL) according to the recommendations of the supplier. After 12 hr, the medium was changed, supplemented or not with 100 nM 9*cis*-RA (gift of Hoffmann–La Roche) as indicated, and incubated for an additional 36 hr. Cell culture supernatants were then heated to 65°C for 30 min. Alkaline phosphatase activity was determined as the increase in $A_{405}$ at 30°C in a 1-ml reaction mixture containing 0.75 ml of supernatant, 200 nM Tris (pH 8.8), 275 mM NaCl, 0.5 mM $MgCl_2$, and 5 mM *p*-nitrophenyl phosphate. All transfections were repeated at least three times.

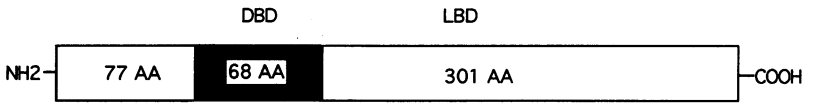
## RESULTS

**Cloning of OR-1.** We have cloned a member of the nuclear receptor superfamily by low-stringency screening of a rat liver cDNA library with a mixture of synthetic oligonucleotide probes derived from known steroid receptors (9). This clone spans 1940 bp, including a 55-bp-long poly(A) tail, and contains an open reading frame starting with an ATG codon

```
   1 GGAATTCCAAGTGCTGTGGAGGAGCAATCACCGGTGCGGACACAGAGCTCCCGCCTCCCA
  61 CAGCCATTTCCAGGGTAACGAAGTAGGAGACCCCCTCCTGCGACCCCCTCACGATCGCCG
 121 GTGCAGTCATGAGCCCCGCCTCCCCCTGGTGCACGGAGAGGGGCGGGGCCTGGAACGAGG
 181 CTGCTTCGTGACCCACTATGTCTTCCCCCACAAGTTCTCTGGACACTCCCTTGCCTGGGA
           * P T M S S P T S S L D T P L P G N
 241 ATGGTTCTCCCCAGCCCAGTACCTCCTCCACTTCACCCACTATTAAGGAGGAGGGACAGG
       G S P Q P S T S S T S P T I K E E G Q E
 301 AGACTGATCCACCTCCAGGCTCTGAAGGGTCCAGCTCTGCCTACATCGTGGTCATCTTAG
       T D P P P G S E G S S S A Y I V V I L E
 361 AGCCAGAGGATGAACCTGAGCGCAAGCGGAAGAAGGGTCCGGCCCCGAAGATGCTGGGCC
       P E D E P E R K R K K G P A P K M L G H
 421 ATGAGCTGTGCCGCGTGTGCGGGGACAAGGCCTCGGGCTTCCACTACAATGTGCTCAGTT
       E L C R V C G D K A S G F H Y N V L S C
 481 GTGAAGGCTGCAAAGGCTTCTTCCGGCGTAGCGTGGTCCATGGTGGGGCCGGGCGCTATG
       E G C K G F F R R S V V H G G A G R Y A
 541 CCTGTCGGGGCAGCGGAACCTGCCAGATGGATGCCTTCATGCGGCGCAAGTGCCAGCTCT
       C R G S G T C Q M D A F M R R K C Q L C
 601 GCAGACTGCGCAAGTGCAAGGAGGCTGGCATGCGGGAGCAGTGCGTGCTTTCTGAGGAGC
       R L R K C K E A G M R E Q C V L S E E Q
 661 AGATTCGGAAGAAAAAGATTCAGAAGCAGCAACAGCAGCAGCCACCGCCCCCGACTGAGC
       I R K K K I Q K Q Q Q Q Q P P P P T E P
 721 CAGCATCCGGTAGCTCAGCCCGGCCTGCAGCCTCCCCTGGCACTTCGGAAGCAAGTAGCC
       A S G S S A R P A A S P G T S E A S S Q
 781 AGGGCTCCGGGGAAGGAGAGGGCATCCAGCTGACAGCGGCTCAGGAGCTGATGATCCAAC
       G S G E G E G I Q L T A A Q E L M I Q Q
 841 AGTTAGTTGCCGCGCAGCTGCAGTGCAACAAGCGATCTTTCTCCGACCACGCCTAAAGTCA
       L V A A Q L Q C N K R S F S D Q P K V T
 901 CGCCCTGGCCCTTGGGTGCAGACCCTCAGTCCCGAGACGCTCGTCAGCAACGCTTTGCCC
       P W P L G A D P Q S R D A R Q Q R F A H
 961 ACTTCACTGAGCTAGCCATCATCTCAGTCCAGGAGATCGTGGACTTCGCCAAGCAGGTGC
       F T E L A I I S V Q E I V D F A K Q V P
1021 CAGGGTTCCTGCAGCTGGGCCGGGAGGACCAGATCGCCCTCCTGAAGGCATCCACCATCG
       G F L Q L G R E D Q I A L L K A S T I E
1081 AGATCATGTTGCTAGAGACAGCCAGACGCTACAACCACGAGACAGAGTGCATCACGTTCC
       I M L L E T A R R Y N H E T E C I T F L
1141 TGAAGGACTTCACCTACAGCAAGGACGACTTCCACCGTGCAGGCTTGCAGGTGGAGTTCA
       K D F T Y S K D D F H R A G L Q V E F I
1201 TCAATCCCATCTTTGAGTTCTCTCGGGCTATGCGTCGGCTGGGCCTAGACGATGCAGAGT
       N P I F E F S R A M R R L G L D D A E Y
1261 ATGCCTTGCTCATTGCCATCAACATCTTCTCAGCGGACCGGCCTAATGTGCAGGAGCCCA
       A L L I A I N I F S A D R P N V Q E P S
1321 GCCGTGTGGAGGCTCTGCAGCAGCCCTATGTGGAGGCCCTCCTCTCCTACACGAGGATCA
       R V E A L Q Q P Y V E A L L S Y T R I K
1381 AGCGGCCGCAGGACCAGCTGCGCTTCCCACGAATGCTCATGAAGCTGGTGAGCCTGCGCA
       R P Q D Q L R F P R M L M K L V S L R T
1441 CCCTCAGCTCCGTGCACTCGGAGCAGGTTTTCGCATTGCGTCTCCAGGACAAGAAGCTGC
       L S S V H S E Q V F A L R L Q D K K L P
1501 CGCCTTTGCTGTCCGAGATCTGGGATGTGCATGAGTAGGGGCCGCCACAAGTGCCCCAGC
       P L L S E I W D V H E *
1561 CTTGGTGGTGTCTACTTGCAGATGGACGCTTCCTTTGCCTTTCCTGGGGTGGGAGGACAC
1621 TGTCACAGCCCAGTCCCCTGGGCTCGGGCTGAGCGAGTGGCAGTTGGCACTAGAAGGTCC
1681 CACCCCACCCGCTGAGTCTTCCAGGAGTGGTGAGGGTCACAGGCCCTAGCCTCTGATCTT
1741 TACCAGCTGCCCTTCCTCCCGAGCTTACACCTCAGCCTACCACACCATGCACCTTGAGTG
1801 GAGAGAGGTTAGGGCAGGTGGCTCCCCACAGTTGGGAGACCACAGGCCCCCTCTTCTGCC
1861 CCTTTTATTTAATAAAAAAATAAAATAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
1921 AAAAAAAAAAAAAAAAAAAA
```

FIG. 1. Sequence of the rat OR-1 cDNA and predicted amino acid sequence. The clone contains an open reading frame that starts with an initiation methionine (M in bold type in the sequence) preceded by a well-conserved Kozak consensus sequence and ends with a TAG stop codon (asterisk). The predicted DNA-binding domain is underlined and the cysteines are in bold type. The putative polyadenylylation signals are also underlined.

corresponding to a protein of 446 aa with a predicted molecular mass of 50 kDa (Fig. 1). This orphan receptor (OR-1) shows no striking homology to any known members of the nuclear receptor superfamily. The most related receptor is the ecdysone receptor, and the highest homologies represent <10% amino acid identity in the amino-terminal domain, about 50–62% identity in the DNA-binding domain, and 20–37% identity in the putative ligand-binding domain (Fig. 2). The amino-terminal domain of OR-1 is 77 aa long and is to a large extent constituted of a PEST sequence—i.e., an amino acid sequence rich in proline, glutamic acid, serine, threonine, and aspartic acid. Such sequences are claimed to be characteristic of proteins with short half-lives (14), and have also been observed in other members of the steroid receptor superfamily (e.g., NGFIB; refs. 15–17). The DNA-binding domain consists of 68 aa including the nine invariable cysteines characteristic of the members of the nuclear receptor superfamily, as well as the other amino acids that are found to be




| | | | |
|---|---|---|---|
| Ecd R | < 10 % | 61.8 % | 37.1 % |
| E 75-A | < 10 % | 57.4 % | 23.5 % |
| seven-up | < 10 % | 54.4 % | 28.5 % |
| r ER | < 10 % | 54.4 % | 19.9 % |
| r HNF-4 | < 10 % | 52.9 % | 25.1 % |
| m RARγ | < 10 % | 52.9 % | 31.8 % |
| USP | < 10 % | 52.9 % | 28.7 % |
| h ARP-1 | < 10 % | 51.1 % | 30 % |
| h COUP-TF | < 10 % | 51.1 % | 29 % |
| r VDR | < 10 % | 50 % | 34.7 % |
| h RXR α | < 10 % | 50 % | 23.6 % |
| r TR β | < 10 % | 50 % | 29.7 % |
| r PPAR | < 10 % | 50 % | 28.5 % |
| r MR | < 10 % | 50 % | 18.3 % |
| r NGFIB | < 10 % | 48.5 % | 30.3 % |

FIG. 2. Amino acid sequence comparison of rat OR-1 and other members of the nuclear receptor superfamily. DBD, DNA-binding domain; LBD, ligand-binding domain; Ecd R, ecdysone receptor; r, rat; h, human; ER, estrogen receptor; HNF-4, hepatocyte nuclear factor 4; USP, upstream stimulatory protein; ARP-1, COUP-TF-II; PPAR, peroxisome-proliferator-activator receptor; MR, mineralocorticoid receptor.

conserved in all members of this protein family. Most orphan receptors cloned so far belong to the RAR/TR subfamily, including our isolate OR-1, which has the same P box as TR and RAR (EGCKG) (18). In addition, the number of amino acids separating the two zinc fingers is the same as in TR. The D box, which is thought to play a role in receptor dimerization, spans 5 aa as is the case in most nuclear receptors. It shows identity in three out of five positions to the orphan receptor TR2 (19) but no striking similarity to any other receptor.

**Tissue Distribution of OR-1.** To analyze the tissue distribution of OR-1 transcripts, we performed *in situ* hybridization on fetal and adult rat tissues. Labeling for OR-1 was found in several tissues of both fetal and adult rats. Prominent expression in fetus was observed in thymus, brown fat, spleen, gastrointestinal tract, salivary gland, thyroid gland, pituitary gland, and retina. Moderate levels were seen in developing cerebrum and cerebellum, in perichondrium around developing bones, liver, lung, heart, and skin, and low levels of OR-1 mRNA were present in skeletal muscle (Fig. 3). In adult rats, strong labeling was seen in lymph node, prostate, and adrenal cortex. Moderate levels were seen in liver, testis, salivary gland, thyroid, and parathyroid gland, adrenal medulla, anterior pituitary, intermediate lobe of the pituitary, and kidney. In the brain, a moderate signal was observed in neurons in the granular cell layer of cerebellum and in hippocampus.

**OR-1 Interacts with RXR *in Vitro* and Binds to a DR4 Motif.** A set of potential HREs predicted by the 3–4–5 rule was synthesized (DR0 through DR6) and assayed in gel shift experiments using *in vitro* translated OR-1 alone or in combination with RXR. *In vitro* translated OR-1 alone was unable to bind to any of the direct repeats, but OR-1 combined with RXR, also translated *in vitro*, formed a specific complex on the DR4 element (Fig. 4*A*). A very weak shift was also observed on DR3 and DR5 when the film was overexposed. The DR4 sequence is usually described as a thyroid hormone response element (TRE) (3). However, OR-1 alone or in combination with RXR did not recognize another TRE consisting of a palindromic sequence of the motif AGGTCA (TRE pal) (data not shown) and did not recognize any other sequence consisting of a palindromic or an inverted repeat of the motif AGGTCA spaced by 0 to 6 nt (data not shown).

To determine whether OR-1 and RXR could dimerize in solution in the absence of DNA, we carried out a coimmuno-

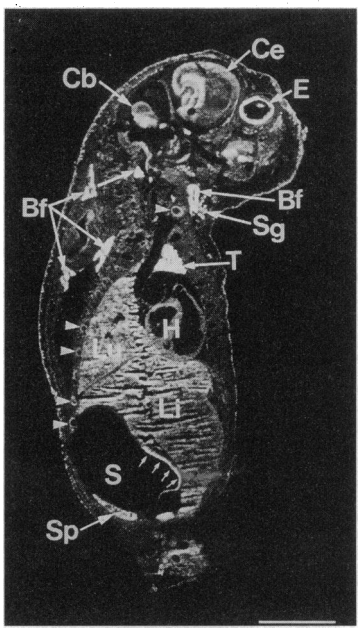



FIG. 3. *In situ* hybridization analysis of the distribution of OR-1 mRNA in a 21-day rat fetus. Strong signal is detected in brown fat (Bf), thymus (T), spleen (Sp), the retina of the eye (E), and the columnar epithelium (arrows) of the stomach (S). Moderate intensity is present in lung (Lu), liver (Li), heart (H), submandibular gland (Sg), cerebrum (Ce), cerebellum (Cb), and the periosteal collar of developing bones (arrowheads). (Bar = 450 μm.)

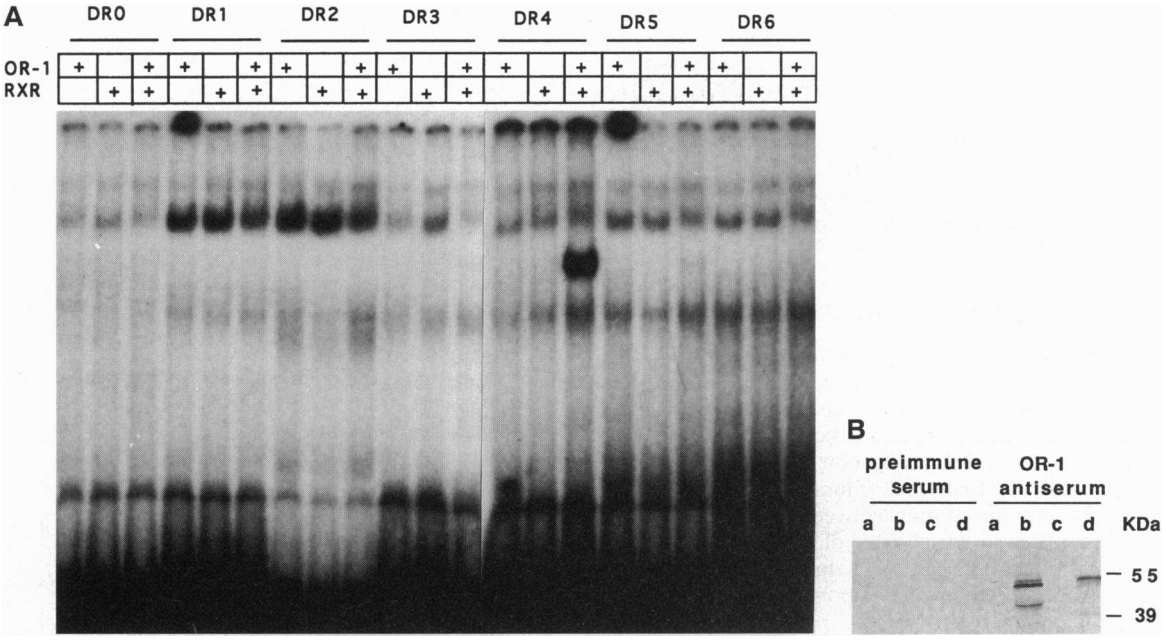



FIG. 4. (*A*) Interaction of OR-1 and RXR on DR4. *In vitro* translated OR-1 and RXR were incubated with $^{32}$P-end-labeled DR0–DR6 oligonucleotide as indicated and then subjected to electrophoretic gel mobility-shift assay. (*B*) OR-1 interacts with RXR in solution in the absence of DNA. *In vitro* synthesized proteins (6 μl) were incubated in a total volume of 12 μl of lysate for 10 min at room temperature as indicated and then subjected to immunoprecipitation. Lanes: a, $^{35}$S-labeled RXR; b, $^{35}$S-labeled OR-1; c, nonradioactive OR-1 plus free [$^{35}$S]methionine, d, nonradioactive OR-1 plus $^{35}$S-labeled RXR.

precipitation experiment using antibodies raised against OR-1. When we mixed [$^{35}$S]methionine-labeled *in vitro* synthesized RXR with nonradioactive *in vitro* synthesized OR-1, the labeled RXR was coprecipitated by anti-OR-1 antibody but not by nonspecific serum (Fig. 4*B*). Thus OR-1 heterodimerizes with RXR in solution in the absence of DNA.

**OR-1 Acts as a Helper of RXR on a DR4-Containing Promoter.** Since OR-1 and RXR formed a specific complex on the DR4 sequence *in vitro*, we tested whether coexpression of OR-1 with RXR in CHO cells could affect the activity of a reporter gene under the control of a DR4-containing promoter. RXR has been shown to be an auxiliary factor heterodimerizing with nuclear receptors, thereby controlling their ligand responsiveness (4, 5). In addition, 9-cis-RA can induce RXR homodimer formation, and RXR homodimers bind and activate several RA response elements (RAREs), but not natural TREs (20). In agreement with earlier reports (21), our transfection studies showed almost no effect of RXR on a

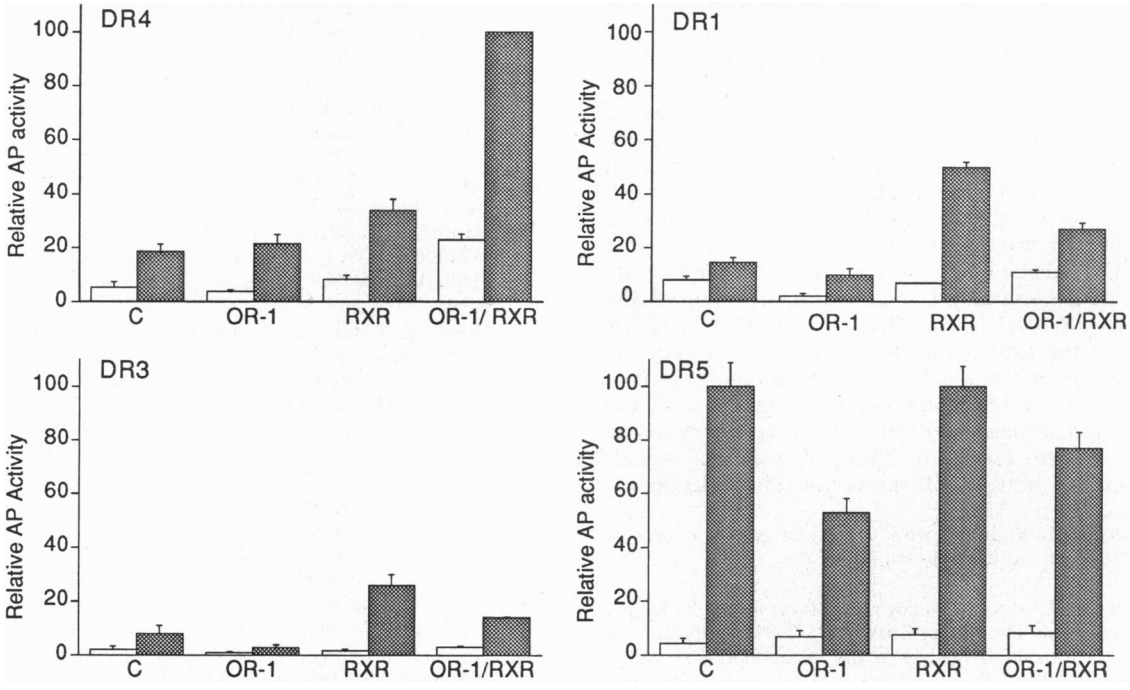



FIG. 5. OR-1 enhances RXR activation on a DR4-containing promoter. CHO cells were cotransfected with the reporter (1 μg) and with 10 ng of expression vector, where indicated. C, control (no expression vector). Cells were then exposed to either vehicle alone (open bars) or 100 nM 9*cis*-RA (cross-hatched bars). Alkaline phosphatase (AP) activity (mean and SD) is plotted as the percent maximal response observed in these experiments.

reporter gene under the control of a DR4-containing promoter (Fig. 5). Interestingly, coexpression of OR-1 increased RXR action, both in normal medium and in medium supplemented with 9-*cis*-RA. Thus, acting as a helper of RXR, OR-1 appears to enhance 9-*cis*-RA signaling on DR4-containing promoters. This effect, although slight, was reproducible and specific for the DR4 element. On other response element tested, expression of OR-1 was found to inhibit the basal response caused by endogenous factors, perhaps by titrating out RXR (22) (Fig. 5).

## DISCUSSION

The present study describes the cloning and functional analysis of OR-1, a member of the nuclear receptor superfamily. Although OR-1 shows no striking homology to other members of the nuclear receptor superfamily, it appears to be more related to the ecdysone receptor. By screening a collection of theoretical target sequences, we have shown that OR-1 interacts with RXR on a DR4 sequence. Heterodimerization between OR-1 and RXR also occurred in solution in the absence of DNA. Transfection experiments suggest that OR-1 acts as a helper of RXR, increasing the level of transactivation of a reporter gene under the control of a DR4-containing promoter.

RXR has been described to have a central role as an auxiliary protein for several classes of hormone receptors, controlling the ligand response of receptors with which it forms heterodimers (4, 5). It is possible that a physiological role of OR-1 is to act as a helper of RXR and that OR-1 might lack a natural ligand. Although the steroid/thyroid hormone receptor superfamily was originally identified as a family of ligand-activated transcription factors, recent evidence suggests that ligand modulation may not be required for the activity of all the members of the superfamily. First, several orphan receptors (e.g., Nurr 77) have been shown to be active transcription factors that do not require exogenously added ligand for their activity (15, 16, 24). Second, several members have been shown to be phosphoproteins (25–29) that may be activated in the absence of ligand by phosphorylation (25, 30, 31). Another possible mechanism would be that OR-1 has a ligand and acts both via its ligand and 9-*cis*-RA through the OR-1/RXR heterodimer on DR4. The expression of OR-1 appears to be widespread in fetal as well as in adult rat tissues, suggesting that OR-1 mediates several effects. 9-*cis*-RA plays a role in thymocyte development, being a potent negative regulator of activation-induced T-cell apoptosis (32). Since OR-1 is expressed at a high level in the thymus during the fetal stages, it may play a role in regulating T-cell development. Although the DR4 sequence was initially described to be a TRE, it has been shown that another receptor, COUP-TF, can bind to this DR4 element, though its preferential binding site is DR1 (23). Thus, by sharing a common response element, the heterodimers OR-1/RXR, TR/RXR, and COUP-TF/RXR could regulate the expression of the same set of genes in response to different hormonal signals. The promoter context could be a major determinant for selecting the response pathway used in a particular cell type. Such a regulatory system would allow cells to respond to diverse extracellular signals through a complex network of transcriptional regulation.

**Note Added in Proof.** While this work was under review, papers on some related receptors were published (33–35).

We thank Dr. S. Nilsson for providing plasmid vectors and for helpful discussions. We thank Drs. Anja Crickmore, Olivier Lahuna, and Franziska Wiebel for critical reading of the manuscript. The skillful technical assistance of Mrs. Hannele Ylitie is greatly appreciated. This work was supported by a fellowship from the Institut National de la Santé et de la Recherche Médicale, by the Swedish Medical Research Council (M.T., CS/MCC-806), and by grants from the Swedish Cancer Society and the Swedish Medical Research Council (13x2819).

1. Evans, R. M. (1988) *Science* **240,** 889–895.
2. O'Malley, B. (1990) *Mol. Endocrinol.* **4,** 363–369.
3. Umesono, K., Murakami, K. K., Thompson, C. C. & Evans, R. M. (1991) *Cell* **65,** 1255–1266.
4. Yu, V. C., Delsert, C., Andersen, B., Holloway, J. M., Devary, O. V., Näär, A. M., Kim, S. Y., Boutin, J. M., Glass, C. K. & Rosenfeld, M. G. (1991) *Cell* **67,** 1251–1266.
5. Bugge, T. H., Pohl, J., Lonnoy, O. & Stunnenberg, H. G. (1992) *EMBO J.* **12,** 1409–1418.
6. Heyman, R. A., Mangelsdorf, D. J., Dyck, J. A., Stein, R. B., Eichele, G., Evans, R. M. & Thaller, C. (1992) *Cell* **68,** 397–406.
7. Levin, A. A., Sturzenbecker, J., Kazmer, S., Bosakowski, Y., Huselton, C., Allenby, G., Speck, J., Kratzeisen, C. I., Rosenberger, M., Lovey, A. & Grippo, J. F. (1992) *Nature (London)* **355,** 359–361.
8. Mader, S., Leroy, P., Chen, J.-Y. & Chambon, P. (1993) *J. Biol. Chem* **268,** 591–600.
9. Göttlicher, M., Widmark, E., Li, Q. & Gustafsson, J.-Å. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 4653–4657.
10. Dagerlind, Å, Friberg, K., Bean, A. J. & Hökfeld, T. (1992) *Histochemistry* **98,** 39–49.
11. Andersson, S., Davis, D. L., Dahlback, H., Jörnvall, H. & Russel, D. W. (1989) *J. Biol. Chem,* **264,** 8222–8229.
12. Berger, J., Hauber, J., Geiger, R. & Cullen, B. R. (1988) *Gene* **66,** 1–10.
13. Gearing, K. L., Göttlicher, M., Teboul, M., Widmark, E. & Gustafsson, J.-Å. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 1440–1444.
14. Rogers, S., Wells, R. & Rechsteiner, M. (1986) *Science* **234,** 364–368.
15. Davis, I. J., Hazel, T. G. & Lau, L. F. (1991) *Mol. Endocrinol.* **5,** 854–859.
16. Hazel, T. G., Nathans, D. & Lau, L. F. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8444–8448.
17. Nakai, A., Kartha, S., Sakurai, A., Toback, F. G. & DeGroot, L. J. (1990) *Mol. Endocrinol.* **4,** 1438–1443.
18. Forman, B. M. & Samuels, H. H. (1990) *Mol. Endocrinol.* **4,** 1293–1301.
19. Chang, C. & Kokontis, J. (1968) *Biochem. Biophys. Res. Commun.* **155,** 971–977.
20. Zhang, X. K., Lehman, J., Hoffman, B., Dawson, M. I., Cameron, J., Graupner, G., Hermann, T., Tran, P. & Pfahl, M. (1992) *Nature (London)* **358,** 587–591.
21. Hallenbeck, P. L., Phyillaier, M. & Nikodem, V. M. (1993) *J. Biol. Chem.* **268,** 3825–3828.
22. Barettino, D., Bugge, T. H., Bartunek, P., Vivanvo Ruiz, M. M., Sonntag-Buck, V., Beug, H., Zenke, M. & Stunnenberg, H. G. (1993) *EMBO J.* **12,** 1343–1354.
23. Cooney, A. J., Tsai, S. Y., O'Malley, B. W. & Tsai, M. J. (1992) *Mol. Cell. Biol.* **12,** 4153–4163.
24. Lydon, J. P., Power, R. F. & Conneely, O. M. (1992) *Gene Expression* **2,** 273–283.
25. Denner, L. A., Weigel, N. L., Maxwell, B. L., Schrader, W. T. & O'Malley, B. W. (1990) *Science* **250,** 1740–1743.
26. Singh, V. B. & Moudgil, V. K. (1985) *J. Biol. Chem.* **260,** 3684–3690.
27. Goldberg, Y., Glimeur, C., Gesquiere, J. C., Ricourt, A., Sap, J., Vennström, B. & Ghysdael, J. (1988) *EMBO J.* **7,** 2425–2433.
28. Hsieh, J. C., Jurutka, P. W., Galligan, M. A., Terpening, C. M., Haussler, C. A., Samuels, D. S., Shimizu, Y., Shimizu, N. & Haussler, M. R. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 9315–9319.
29. Lin, K. U., Ashizawa, K. & Cheng, S. Y. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 7737–7741.
30. Power, R. F., Lydon, J. P., Conneely, O. M. & O'Malley, B. W. (1991) *Science* **252,** 1546–1548.
31. Power, R. F., Mani, S. K., Codina, J., Conneely, O. M. & O'Malley, B. W. (1991) *Science* **254,** 1636–1639.
32. Yang, Y., Vacchio, M. S. & Ashwell, J. D. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 6170–6174.
33. Shinar, D. M., Endo, N., Rutledge, S. J., Vogel, R., Rodan, G. A. & Schmidt, A. (1994) *Gene* **147,** 273–276.
34. Song, C., Kokontis, J. M., Hiipakka, R. A. & Liao, S. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 10809–10813.
35. Apfel, R., Benbrook, D., Lernhardt, E., Ortiz, M. A., Salbert, G. & Pfahl, M. (1994) *Mol. Cell. Biol.* **14,** 7025–7035.