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Ubiquitous receptor: A receptor that modulates gene activation by retinoic acid and thyroid hormone receptors

(nuclear receptor/transcriptional regulation/anti-receptor antibody/orphan receptor/prostate cancer)

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ABSTRACT **The cDNA for a member of the nuclear receptor family was cloned and named ubiquitous receptor (UR), since UR protein and mRNA are detected in many cell types. Rat UR/human retinoid X receptor α (hRXRα) heterodimers bound preferentially to double-stranded oligonucleotide direct repeats having the consensus half-site sequence AGGTCA and 4-nt spacing (DR-4). Coexpression of UR in COS-1 cells inhibited the stimulation of chloramphenicol acetyltransferase (CAT) reporter gene expression by hRXRα and human retinoic acid receptor α in the presence of all-*trans*-retinoic acid when DR-4 (but not DR-5) was present upstream of the promoter of a CAT reporter gene (DR-4–CAT). UR expression also inhibited the activation of a DR-4–CAT reporter gene by hRXRα and 9-*cis*-retinoic acid or by thyroid hormone receptor β in the presence of thyroid hormone. However, in the absence of 9-*cis*-retinoic acid, UR in combination with hRXRα stimulated DR-4–CAT expression. Coexpression of thyroid hormone receptor markedly reduced this stimulation in the absence of thyroid hormone. UR may play an important role in normal growth and differentiation by modulating gene activation in retinoic acid and thyroid hormone signaling pathways.**

In vertebrates, cellular responses to vitamin D_3, retinoids, and steroid and thyroid hormones are dependent on a group of specific nuclear receptors belonging to a superfamily of ligand-activated transcription factors (1). Nuclear receptors modulate the transcription of specific genes by interacting with specific DNA sequences termed hormone response elements (HREs), often located upstream of target genes (2). Whereas steroid hormone receptors interact as homodimers with their cognate HREs arranged as palindromic repeats of 6 nt with 3-nt spacing, members of the thyroid hormone/retinoid receptor subfamily bind most efficiently to an HRE as heterodimers with the retinoid X receptor (RXR) and utilize response elements arranged as direct repeats (DRs) (2, 3). Different receptor/RXR heterodimers have different preferences for the spacing of these DRs (4, 5). The nature of the DNA flanking these half-sites also is important in determining the specificity of a response element (6). Homodimers of the thyroid hormone/retinoid receptor family are also able to modulate transcriptional activity in different ways than their heterodimeric forms (7). The effect of these homo- and heterodimeric receptors on transcriptional activity also depends on their occupancy by specific ligands (8). The complexity of this network of interacting factors is increasing with the discovery of new members of this superfamily of nuclear receptors, many of which are called orphan receptors, since they lack known ligands. An interplay of receptors, ligands, response elements, and yet-to-be-discovered factors may ultimately control the activity of these transcriptional factors and ensure the appropriate cellular responses during development and in the adult.

We report here our discovery of a member of the nuclear receptor family of transcription factors that we have named ubiquitous receptor (UR),† because of its wide tissue distribution. UR is not an isoform of any known receptor and interacts with the response elements and network of receptors in the thyroid receptor (TR)/retinoic acid receptor (RAR) subfamily.

MATERIALS AND METHODS

Cloning and Sequencing of cDNA. A cDNA library was constructed in λZAPII (Stratagene) with poly(A)$^+$ RNA from rat vagina and screened with 5′-^{32}P-end-labeled oligodeoxynucleotide probes highly homologous to the DNA-binding domain (DBD) of known rat nuclear receptors. These probes were 5′-TT(A/G)AAGAA(A/T)AC(C/T)TTRCAGCT(T/C/G)CCACA-3′, 5′-CT(A/G)AAGAAICCCTTGCAGCCITCACAGGT-3′, 5′-TT(A/G)AAGAA(A/T)AC(C/T)TTGCAGCT(A/T)CCACAIGT-3′, 5′-C(C/T)CC(A/G)TA(A/G)TG(A/G)CAICC(A/T)GA(A/G)GCITCATC-3′, and 5′-AGTGI(A/T)A(A/G)CCIGTGGC(C/T)(C/T)GGTCICCACA-3′ (where I is deoxyinosine). A clone that hybridized to these probes contained a 1.9-kb cDNA insert that coded for a full-length nuclear receptor. A λZAPII cDNA library constructed with poly(A)$^+$ RNA from PC-3 human prostate cancer cells was screened with the 1.9-kb cDNA insert of the rat UR (rUR) clone to identify human UR (hUR) cDNA clones. Sequences were aligned by use of GENEWORKS software (IntelliGenetics). Similarity searches were performed with the BLAST (9) algorithm and data banks at the National Center for Biotechnology Information (Bethesda, MD).

Northern Blot Analysis. Northern blot analysis of poly(A)$^+$ RNA was carried out (10) using a 1.6-kb rUR cDNA fragment (nt 368–1899) labeled with ^{32}P by random priming (11) as the probe.

Gel Shift DNA-Binding Assays. rUR and hRXRα were synthesized *in vitro* by rabbit reticulocyte lysate programmed with RNA transcribed from pSG5 vectors (Stratagene) containing the appropriate cDNAs. Lysate containing rUR and/or hRXRα was incubated with the indicated ^{32}P-labeled double-stranded oligonucleotide in the absence or presence of unlabeled oligonucleotides containing AGGTCA repeats with variable spacing and orientation. The sequences of the sense strand of these nucleotides (5′-to-3′ with response element

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Abbreviations: r, rat; h, human; T_3, 3,3′,5-triiodo-L-thyronine; RA, retinoic acid; *t*-RA, all-*trans*-RA; 9*c*-RA, 9-*cis*-retinoic acid; RAR, RA receptor; RXR, retinoid X receptor; TR, thyroid hormone receptor; UR, ubiquitous receptor; DBD, DNA-binding domain; LBD, ligand-binding domain; DR, direct repeat; HRE, hormone response element; CAT, chloramphenicol acetyltransferase.

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†The sequences reported in this paper have been deposited in the GenBank data base [accession nos. U14533 (rat) and U14534 (human)].

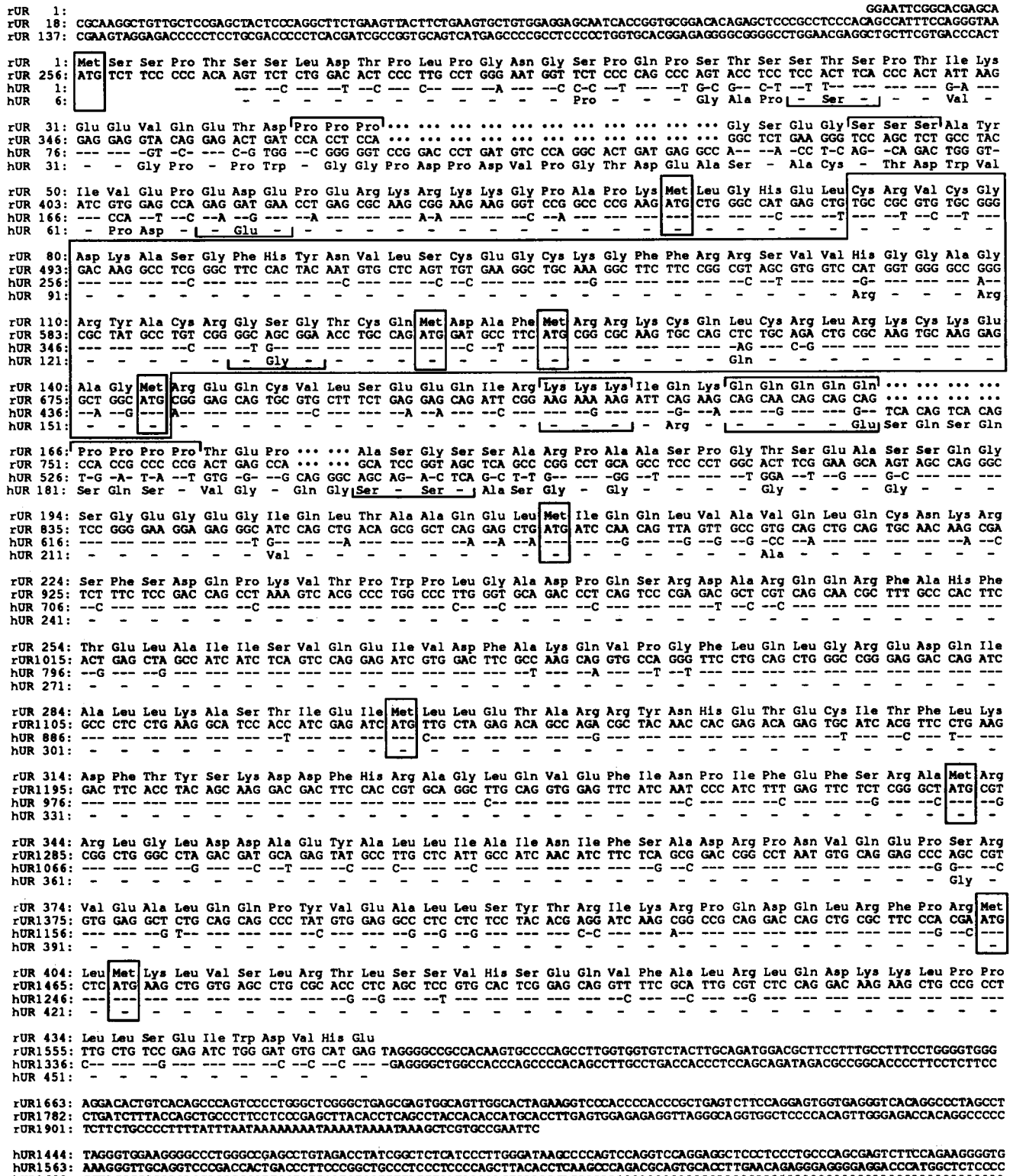

FIG. 1. cDNA sequences and deduced amino acid sequences of rUR and hUR. hUR residues that are identical with rUR residues are shown as hyphens. Gaps introduced to maximize match are indicated by dots. All ATG codons (methionine residues) are boxed. Oligo- or polyamino acid regions are shown by boldface brackets. The putative DBD is boxed.

half-site underlined) were as follows: DR-0, GATCCTCAGGTCAAGGTCAGAAGCT; DR-1, GATCCTCAGGTCAGAGGTCAGAAGCT; DR-2, GATCCTCAGGTCAAGAGGTCAGAAGCT; DR-3, GATCCTCAGGTCAAGGAGGTCAGAAGCT; DR-4, GATCCTCAGGTCACAGGAGGTCAGAAGCT; DR-5, GATCCTCAGGTCACCAGGAGGTCAGAAGCT; DR-6, GATCCTCAGGTCACCAAGGAGGTCAGAAGCT; and pal, GATCAGCTTCAGGTCATGACCTGAGAGCTGATC. For the gel mobility-shift analysis, 2 μl of programmed lysate was mixed with 20 μl of binding buffer [10 mM Hepes, pH 7.9/50 mM KCl/2.5 mM $MgCl_2$/2 mM dithiothreitol/10% (vol/vol) glycerol containing poly(dI-dC) (50 μg/ml) and sonicated salmon testis DNA (250 μg/ml)] and incubated for 20 min on ice. Labeled probe (20 ng) and

(when indicated) competitors were then added and the mixture was incubated again for 10 min on ice. The DNA–protein complexes were resolved by electrophoresis in 5% polyacrylamide gels containing 23 mM Tris, 23 mM boric acid, and 0.5 mM EDTA, pH 8.0. The gels were run at constant power of 6 W for 3 hr at 4°C, dried, and exposed to XAR-5 Kodak film at −80°C overnight.

Mammalian Cell Transfection and Chloramphenicol Acetyltransferase (CAT) Assay. COS-1 cells were plated (10^6 cells per 100-mm plate) in Dulbecco's modified Eagle's medium supplemented with either 10% dextran-coated charcoal-stripped or 10% AG1-X8 resin-treated fetal bovine serum (12). The next day, cells were transfected by the calcium phosphate precipitate method (11) with 4 μg of pCH110 (Pharmacia) for β-galactosidase expression, 8 μg of Δ56c-fosCAT (13) reporter plasmid with four tandem copies of DR-3, DR-4, or DR-5 response elements inserted at the *Hin*dIII site, and 4 μg of each kind of nuclear receptor expression vector. For DR-1, the CRBPII-tk CAT vector (14) was used. The pSG5 vector was used for rUR, hRXRα, and hRARα expression, and the pCDM8 vector (Invitrogen) was used for hTRβ expression. Cell transfection, hormone treatments, and CAT assays were performed as described (15). Transfection efficiency was normalized to β-galactosidase activity from cotransfected pCH110 (11). Acetylation of [^{14}C]chloramphenicol was quantified after thin-layer chromatography by the AMBIS radioanalytic imaging system (AMBIS Systems). The data shown are the average of duplicates from representative experiments.

Production of Anti-UR Antibodies. Antigens were 15-aa peptides from either the amino (UN-15) or the carboxyl (UC-15) terminus of rUR conjugated with keyhole limpet hemocyanin, and an *Escherichia coli* TrpE–rUR (full-length) fusion protein. Polyclonal antibodies were produced in rabbits and affinity purified (16).

RESULTS AND DISCUSSION

Structure of rUR. The nucleotide and deduced amino acid sequences of hUR and rUR cDNAs are shown in Fig. 1. A clone from a rat vagina cDNA library had a 1959-bp insert containing an open reading frame that coded for a protein of 443 aa. The ATG codon at nt 256–258 of the rUR cDNA is in the appropriate context for translation initiation (17), and an in-frame stop codon TGA is present upstream of this codon. The putative protein, with a calculated molecular mass of 49,448 Da, contains a cysteine-rich region, which has a zinc finger motif typical of the DBD of members of the nuclear receptor family.

Sequence comparison with the various receptors of this nuclear receptor family indicates that rUR is most closely related to the *Drosophila* ecdysone receptor, with which it shares 62% amino acid identity in the DBD. The 5 aa (P box) responsible for HRE half-site recognition in other receptors (18, 19) are identical to those of TR and other members of the TR/RAR subfamily. The putative ligand-binding domain (LBD) of rUR is not highly homologous to any other members of the nuclear receptor family.

Structure of hUR. All hUR cDNA clones were truncated in the 5′ coding region and lacked DNA coding for 5 aa that are present at the amino terminus of rUR (Fig. 1). Sequence analysis of hUR genomic clones suggests the presence of an intron in this region, based on potential 5′ and 3′ splice sites that conform to consensus splice sites (20). hUR genomic clones were also used as probes to localize the hUR gene to chromosome 19, band q13.3 by fluorescence *in situ* hybridization of normal human metaphase chromosomes (M. M. LeBeau, E. M. Davis, C.S., J.M.K., R.A.H., and S.L., unpublished observation).

The deduced hUR protein sequence (assuming the presence of the missing 5 aa at the amino terminus) has 460 aa with a calculated molecular mass of 50,482 Da and shares nearly 90% homology with rUR (Fig. 1). The percent identity in the DBD as well as the LBD between rUR and hUR is 94%. Most of the amino acid sequence differences lie in the amino-terminal portion of the LBD. There is only one amino acid difference (Ser^{372} of rUR is replaced by Gly at position 389 of hUR) in the 227 aa at the carboxyl-terminal end of the LBD of the two URs.

UR mRNA Expression and UR Localization in Organs and Cells. A rUR cDNA probe hybridized to a single prominent 2-kb RNA species on Northern blots of poly(A)$^+$ RNA from heart, liver, kidney, brain, testis, ovary, adrenal, uterus, prostate, vagina, lung, and spleen (Fig. 2). A single band of ≈2 kb was also detected in cultured cells, including the human prostate carcinoma cell lines PC-3 and LNCaP, human skin fibroblasts, BALB/c 3T3 (murine fibroblast line), RPMI 1788 and BJAB (human B-cell lines), and WEHI-231 (a murine immature-B-cell line) (data not shown).

On Western blots UR antibodies reacted with a 50-kDa component in nuclear (but not cytosolic) extracts of rat liver, heart, kidney, prostate, seminal vesicle, small intestine, ovary, and skin. UR was detected predominantly in nuclei by immunocytochemical staining in all organs and cultured cells examined.

UR Binding to Specific DNA Sequences. Since the P-box amino acids of the UR DBD were identical to those of TR and other nuclear receptors that bind to AGGTCA half-sites, we analyzed the ability of *in vitro* expressed rUR to bind to oligonucleotides with AGGTCA repeats of different spacing using a gel shift assay. Both rUR monomer and dimer complexes were detected on a series of DRs with 0- to 6-nt spacing. Monomers were present in greatest amount on DR-2 and DR-3, with lesser amounts on DR-1, -4, -5, and -6. Dimer complexes were detected only on DR-3, -4, -5, and -6, with DR-4 having the highest level of bound dimer. Little or no rUR was detected on palindromatic repeats with zero spacing.

Members of the thyroid hormone/retinoid receptor subfamily of nuclear receptor bind to response elements with greater affinity and transactivate genes more effectively when they heterodimerize or are coexpressed with RXR (4,

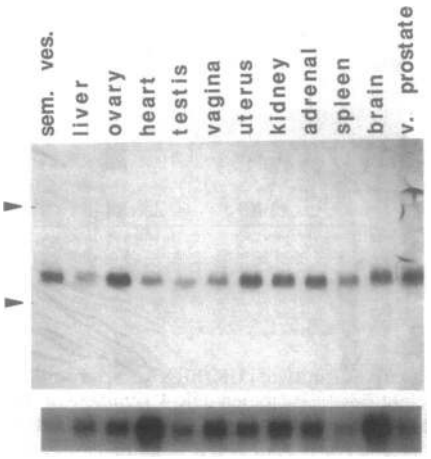

FIG. 2. rUR mRNA expression in various rat tissues. (*Upper*) A Northern blot of poly(A)$^+$ RNA (8 μg) from adult Sprague–Dawley rat tissues was hybridized under stringent conditions with a ^{32}P-labeled rUR cDNA probe. Arrowheads represent the positions of 28S and 18S ribosomal RNA. sem. ves., Seminal vesicles; v. prostate, ventral prostate. (*Lower*) The membrane was also probed with ^{32}P-labeled rat glyceraldehyde-3-phosphate dehydrogenase cDNA after the UR probe was stripped off.

21–24). We also found that rUR/hRXRα heterodimers bound to DR sequences with higher affinity than homodimers of rUR or hRXRα. Binding of rUR/hRXRα heterodimers to ^{32}P-labeled DR-4 or DR-5 was inhibited by the addition of nonradioactive DR oligonucleotides (Fig. 3), and DR-4 was the best competitor. rUR/hRXRα heterodimers, therefore, appeared to bind to DR-4 preferentially. Antibodies against the amino terminus of rUR (UN-15) supershifted rUR/hRXRα heterodimers without reducing the total amount of rUR bound to DR-4. Antibodies against the carboxyl terminus of rUR (UC-15) also supershifted some dimers but significantly inhibited the formation of rUR heterodimers bound to DR-4. Therefore, a heptad-repeat leucine zipper structure in the carboxyl-terminal LBD of rUR which is believed to be important in nuclear receptor heterodimerization may be involved in UR heterodimerization and the UC-15 antibody may have blocked this process.

Reporter Gene Expression in COS-1 Cells Transfected with UR, TR, and RXR. Expression vectors containing cDNAs for hRXRα, hTRβ, and rUR were cotransfected alone or in combination into COS-1 cells along with the DR-4 reporter plasmid, in which four tandemly arranged DR-4 elements were inserted upstream of a heterologous promoter linked to a CAT reporter gene. The CAT activity in cells coexpressing rUR and hRXRα was 4- to 5-fold greater than that in COS-1 cells expressing either rUR or hRXRα alone (Fig. 4*A*). This activity was independent of T_3 or 9*c*-RA. Whether rUR/hRXRα activation of the CAT gene required a ligand that was present in culture medium or produced by COS-1 cells is not clear. The rUR/hRXRα-dependent CAT activity was markedly reduced by coexpression of hTRβ in the absence but not the presence of T_3. TR in the absence of ligand acts as a transcriptional repressor (8), which may explain the reduction observed. Also, the formation of hTRβ/hRXRα heterodimers may have reduced the formation of rUR/hRXRα heterodimers and utilization of the DR-4 promoter. In the presence of T_3, hTRβ expression alone gave high CAT activity, probably utilizing endogenous RXR (6) as a binding partner (Fig. 4*A*). rUR/hRXRα transactivation of DR-4–CAT, however, required the exogenous coexpression of rUR and hRXRα. Addition of 9*c*-RA and T_3 to cells coexpressing

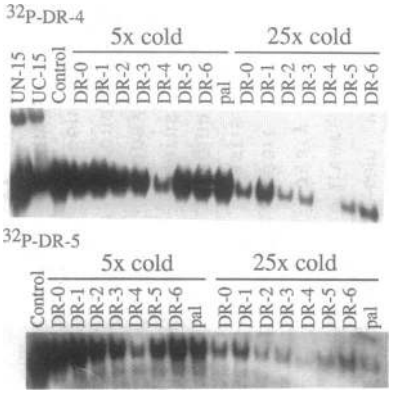

FIG. 3. Relative affinity of rUR/hRXRα heterodimers for various DR oligonucleotides. ^{32}P-labeled DR-4 (*Upper*) or DR-5 (*Lower*) oligonucleotides were used as probes in a gel shift DNA-binding assay. Nonradioactive ("cold") DR oligonucleotides (5- or 25-fold molar excess over ^{32}P-DR-4 or ^{32}P-DR-5) were added as competitors. The control mixture had no competitor. If used, 1 μg of the indicated antibodies (UN-15 or UC-15) was incubated for 30 min on ice with receptors before the addition of the probe. Only bands representing shifted heterodimer and antibody-supershifted complexes (*Upper*, left two lanes) are shown. The amount of monomer bound to DR oligonucleotides was <20% of the amount of dimer bound to the DR probes. hRXRα alone did not form any protein/DR oligonucleotide complexes under the conditions used.

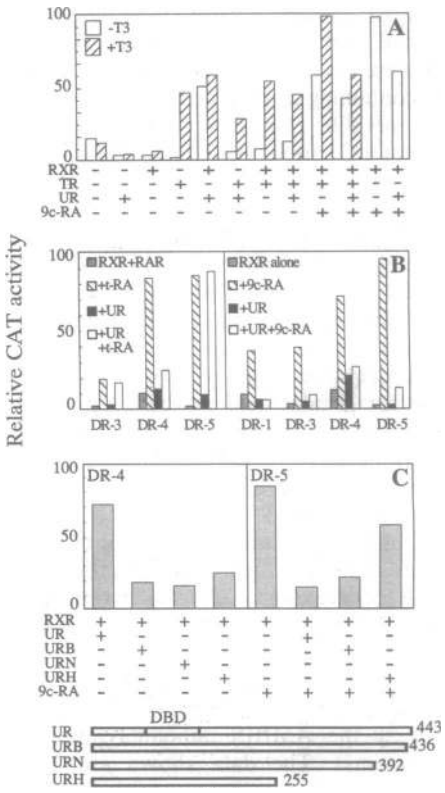

FIG. 4. rUR modulation of hRXRα, hRARα, and hTRβ-dependent transactivation of reporter genes. (*A*) Transcriptional activation of a DR-4–CAT reporter plasmid in COS-1 cells by transiently expressed rUR, in combination with hRXRα and hTRβ. 3,3',5-Triiodo-L-thyronine (T_3, 100 nM) and/or 9-*cis*-retinoic acid (9*c*-RA, 50 nM) was added to cell cultures as indicated. (*B*) Selective inhibition by rUR of gene transactivation by hRXRα/hRARα heterodimer (*Left*) and hRXRα homodimer (*Right*) in COS-1 cells. All-*trans*-RA (*t*-RA, 1 μM) t-RA or 9*c*-RA (50 nM) was added to cell cultures as indicated. (*C*) Transcriptional activation and inhibition by carboxyl-terminal truncation mutants of rUR in COS-1 cells cotransfected with a DR-4 or DR-5 reporter gene. Expression vectors containing cDNAs encoding full-length rUR or one of the three carboxyl-terminal deletion mutants shown at the bottom were cotransfected with a hRXRα expression vector.

hRXRα and hTRβ further induced CAT activity, presumably through the additive activities of hRXRα homodimers and hTRβ/hRXRα heterodimers, although similar high activity was generated with hRXRα and 9*c*-RA alone. This increase in CAT activity was repressed by expression of rUR to the level observed in cells coexpressing only hRXRα and rUR, suggesting that rUR repressed the 9*c*-RA/RXR-dependent CAT activity through the formation of rUR/hRXRα heterodimers, which transactivate the reporter gene less effectively. Also, rUR homodimers bound to DR-4 in gel shift assays (data not shown) but showed little transcriptional activity (Fig. 4*A*) and may have competed with hTRβ/hRXRα heterodimers for the DR-4 response element. Coexpression of rUR repressed T_3-dependent hTRβ stimulation of CAT activity (Fig. 4*A*). This may have been due to the formation of rUR/hTRβ heterodimers, since we also have observed rUR/hTRβ (but not rUR/hTRα) heterodimers complexed to DR-4 by gel shift analysis.

UR Modulation of RAR- and RXR-Dependent Transactivation. Coexpression of hRARα and hRXRα activated CAT expression from reporter plasmids containing DR-3, DR-4, and DR-5 response elements, although the level of transactivation with DR-3 was only about 20% of that on DR-4 and DR-5 (Fig. 4*B Left*). This *t*-RA-dependent CAT gene activation by hRARα/hRXRα was virtually abolished by coexpres-

sion of rUR in cells transfected with the DR-4 reporter plasmid, but not in cells transfected with the DR-3 or DR-5 reporter plasmids. This specificity may reflect the response element-binding affinity and transcriptional activity of the various homo- and heterodimers present in the transfected cell. rUR homo- and heterodimers bind best to DR-4 but have relatively poor transcriptional activity compared with hRARα/hRXRα, whereas hRARα/hRXRα binds best to DR-5 and has high transcriptional activity. CAT activity induced by 9*c*-RA in cells transfected with a hRXRα expression vector and DR-1, DR-3, DR-4, or DR-5 reporter plasmids was also inhibited by coexpression of rUR (Fig. 4*B Right*). This inhibition might have been due to the formation of rUR/hRXRα heterodimers, which have lower transcriptional activity, and reduction in the level of RXR homodimers which have higher transcriptional activity.

RAR/RXR heterodimers have been shown to transactivate reporter gene expression through DR elements with various nucleotide spacings (25). DR-1 and DR-2 elements have also been reported to be RAR response elements (26). However, the most potent natural RAR response elements are most similar to DR-5 (4). The ability of rUR to inhibit the transcriptional activity of hRARα/hRXRα heterodimers on DR-4 but not DR-5 elements is potentially significant, since this selective inhibition may enable *t*-RA-induced RXR/RAR-dependent transactivation to occur only in genes under the control of a DR-5 element when UR is present.

Evidence for the Involvement of the Carboxyl Terminus of UR in the Regulation of RXR-Dependent CAT Expression. Three rUR carboxyl-terminal deletion mutants (URB, URN, and URH; see Fig. 4) were constructed and tested for their ability to modulate gene expression (Fig. 4*C*). The three truncation mutants, including URB, which lacked only 7 aa at the carboxyl terminus, were not as effective as the full-length UR in stimulating CAT gene transactivation in conjunction with RXR. When cells were transfected with the DR-5–CAT reporter plasmid, both UR and URB inhibited 9*c*-RA/hRXRα-dependent induction of CAT activity. This inhibition was much less when URH was used. URH lacks the LBD, which may be important for receptor dimerization.

Biological Significance. Although the makeup of the natural response elements for UR, RXR, RAR, and TR in the control regions of various genes is undoubtedly more complex than the synthetic DR sequences used in this study, the interaction of UR with RXR as well as UR modulation of gene transactivation by TR and RAR suggest a mechanism in which a number of nuclear receptors of this subfamily, possibly including some yet to be discovered, interact in a composite fashion to yield a net transcriptional activity in the cell nucleus for a given response element. This net transcriptional activity would additionally be dependent upon the presence of receptor ligands and the particular structure of the response element. The ability of UR to selectively inhibit gene transactivation by RAR/RXR on select response elements is similar to the effect of the orphan receptor COUP-TF, which also acts as a negative regulator of the RA response pathway with certain response elements (27). However, in contrast with UR, COUP-TF does not form heterodimers with RXR in gel shift assays but forms homodimers that compete for binding to response elements. Since UR has the potential to modulate the thyroid hormone signal pathway, it is reasonable to consider whether abnormality in UR function is responsible for some cases of thyroid hormone dysfunction. The ability of UR to restrict transactivation by RAR to specific response elements may indicate a potentially important physiological function for UR.

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