Cloning and Identification of Rat Deoxyuridine Triphosphatase as an Inhibitor of Peroxisome Proliferator-activated Receptor α*

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Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily that transcriptionally regulate responsive genes by binding to the peroxisome proliferator response elements. Proteins interacting with PPAR isoforms (α, δ, and γ) may modulate the PPAR-mediated transcriptional activation. Using a yeast two-hybrid system to screen a rat liver cDNA library, we have identified rat deoxyuridine-triphosphatase (dUTPase, EC 3.6.1.23) as a PPARα-interacting protein. This cDNA encodes a polypeptide of 203 amino acids; the C-terminal 141-amino acid segment of this protein corresponds to the full-length human enzyme, which exhibits 92% identity with human dUTPase; the N-terminal extra 62-amino acid residue region is arginine-rich. In vitro binding assays indicate that rat dUTPase interacts with all three isoforms of mouse PPAR, but not with retinoid X receptor and thyroid hormone receptor. Interaction of PPARα with dUTPase is with the N-terminal 62-amino acid segment of rat dUTPase. Full-length rat dUTPase prevents PPAR-retinoid X receptor heterodimerization resulting in an inhibition of PPAR activity in a ligand-independent manner. Immunostaining of human kidney tsA201 cells, transiently expressing dUTPase showed that this protein is present predominantly in the cytoplasm but translocates into the nucleus with PPARα when PPARα is co-expressed with dUTPase. Northern blot hybridization shows that rat dUTPase is encoded by an abundant 1-kilobase mRNA species present in all rat tissues. The identification of dUTPase as a PPAR-interacting protein suggests a possible link between tumorigenic peroxisome proliferators and the enzyme system involved in the maintenance of DNA fidelity.

Peroxisomes are cytoplasmic organelles widely distributed in most animal and plant cells. Although their number and volume density remain fairly constant under various physiological and pathological conditions, they increase dramatically in the liver cells associated with transcriptional activation of genes encoding for the peroxisomal β-oxidation enzymes (3), the cytochrome P-450 CYPA4 isoforms (4), and the fatty acid binding protein (5), among others (6). Chronic administration of peroxisome proliferators leads to the development of liver tumors in rats and mice (7). To date, all the compounds with peroxisome-proliferative effect that have been tested in long term studies have been found to be tumorigenic despite the fact that these chemicals neither bind covalently to DNA nor produce somatic mutations directly or after metabolic activation (7). It has been postulated that H2O2 overproduced by the sustained increases in H2O2-generating peroxisomal fatty acid β-oxidation system, results in oxidative stress that contributes to hepatocarcinogenesis in rodents (8).

The induction of peroxisome proliferation is mediated by members of the nuclear receptor superfamily, termed peroxisome proliferator-activated receptors (PPARs)† that are closely related to the thyroid hormone receptors and retinoid receptors (9, 10). To date, three isoforms of PPARs have been identified in amphibians, rodents, and humans: PPARα, PPARδ (also called β or NUC-1), and PPARγ (11, 12). PPAR isoforms display distinct patterns of tissue distribution and appear to have different functions (12, 13). PPARα is highly expressed in hepatocytes, cardiomyocytes, enterocytes, and the proximal tubular epithelium of kidney (14) and plays a crucial role in the peroxisome proliferator-induced pleiotropic responses (6, 10). Corroborative evidence for the functional role of PPARα in peroxisome proliferator-induced signal transduction comes from the observation that disruption of the mPPARα gene results in the abolishment of the pleiotropic effects of peroxisome proliferators in mice (15). PPARδ is expressed ubiquitously and often at higher levels than PPARα and PPARγ (12, 14). Instead of activation, the human PPARδ has been found to repress the activation of PPARα, as well as thyroid hormone receptor (16). PPARγ is expressed predominantly in adipose tissue and the immune system (14, 17) and is activated by prostaglandin 15dΔ12,14 PGJ2 (18, 19) and thiazolidinediones (20). Expression and activation of PPARγ in fibroblasts is sufficient to trigger the adipocyte differentiation cascade, implying that PPARγ plays a key role in adipogenesis (21, 22).

Tissue and species responses to peroxisome proliferators may depend on pharmacokinetics, the relative abundance of the PPAR isoforms and their auxiliary proteins, the nature of peroxisome proliferator response element (PPRE), and to some

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The abbreviations used are: PPAR, peroxisome proliferator-activated receptor; dUTPase, deoxyuridine-triphosphatase; RXR, retinoid X receptor; TR, thyroid hormone (T3) receptor; GST, glutathione S-transferase; kb, kilobase(s); h, human; r, rat; m, mouse; PPRE, peroxisome proliferator response element.
extent, hormone levels (23). PPARs regulate target genes by forming a heterodimer with RXRs, another subfamily of nuclear receptors (24). RXRs also form heterodimers with other members of the nuclear receptor superfamily, and these interactions appear to influence the PPAR-regulated gene activation because of the competition among various RXR heterodimerization partners for RXR (23). Nuclear receptor actions are also modulated by factors referred to as co-activators or co-repressors (25). Co-activators for glucocorticoid receptors (26), protegerone receptor (27), and TR-RXR (28) identified so far show no common structural features, implying that different nuclear receptors contact with the transcription machinery through receptor specific co-activators. Recently, two structurally related proteins, designated as N-CoR (nuclear receptor co-repressor) and SMRT (silencing mediator for retinoid and thyroid hormone receptors), have been shown to interact with TR and RAR and repress basal transcription in the absence of ligand (29, 30). Also, the finding that human RNA polymerase II complex contains transcription factors as well as repair proteins suggests that the transcriptional activation and DNA repair functions are coordinated (31). In an attempt to identify co-activators or co-repressors for PPARs, we utilized a yeast two-hybrid system to screen rat liver cDNA library and identified deoxyuridine-triphosphatase (dUTPase) as a PPARα-interacting protein. We show that the rat dUTPase interacts with all three PPAR isoforms and blocks the formation of PPAR-RXR heterodimers, causing repression of PPAR-mediated transcriptional activation. The identification of rat dUTPase as a PPAR-interacting protein not only provides an additional factor that participates in the PPAR-mediated gene regulation but also suggests a novel link between the tumorogenic peroxisome proliferators and the DNA fidelity enzyme system.

EXPERIMENTAL PROCEDURES

Plasmids—A yeast two-hybrid system was purchased from Clontech. cDNAs encoding amino acids 26–468, 112–467, and 32–461 of PRα, RXRα, and hTRβ, respectively, were inserted into pGBT9 vector downstream of the GAL4 DNA binding domain. The resulting plasmids were verified by restriction enzyme digestion and sequencing. GAL4 DNA binding domain and murine PPAR chimera expression constructs described by Smith and Johnson (33). After transformation into yeast SFY526, the isolated plasmids were reintroduced into the yeast strain SFY526 (MATa, ura3–52, his3–200, ade2–101, lys2–801, trpl–901, leu2–3, 112, can1–100, gal4–542, gal80–538, URA3::GAL1-lacZ) with pbGT9, pbGT9-rPPARα, pbGT9-rRXRα, and pbGT9-hTRβ, respectively, and tested for β-galactosidase activity.

Screening of dUTPase-encoding PPARα Proteins—After sequencing, one of the activation domain plasmids encoding a polypeptide showing homologies with the human dUTPase was digested with HindIII and blunt-ended by filling with Klenow and dNTPs. The plasmid was further cut with BamHI, and the insert was subcloned into the BamHI and Smal sites of the prokaryotic expression vector pGEX-2T (Pharmacia Biotech Inc.). The same fragment was also cloned into the BamHI and SmaI sites of a mammalian expression vector pSG5 (Stratagene). The full-length cDNA was further truncated into 5′ and 3′ fragment by polymerase chain reaction amplification with two sets of primers: 5′-AAATCCACTACAATGGATTGA-TGCATAT-3′/S-AGATTCGATCCATGCTTTCGCTGACGC-CC-3′ and 5′-GAATTCGGATCCATGCTTTCGCTGACGC-CC-3′ and 5′-GAATTCGGATCCATGCTTTCGCTGACGC-CC-3′. The truncated cDNA was subcloned into the pMAM-hTRβ, pMAM-RxRα, and pMAM pSG5 plasmids. The authenticity and orientation of the amplified fragment in these vectors were confirmed by restriction digestion and sequencing.

Expression and Purification of Rat dUTPase in E. coli—The expression and purification of GST fusion proteins were essentially as described by Smith and Johnson (33). After transformation into E. coli JM109 and induction by isopropyl-1-thio-β-D-galactoside, bacteria were collected by centrifugation, resuspended 1:10 in NETN (0.5% Nonidet P-40/1 mM EDTA/20 mM Tris, pH 8.0/0.1 M NaCl) containing 2 mM phenylmethylsulfonyl fluoride, sonicated, and centrifuged. Fusion proteins were then purified on glutathione-agarose beads. 10 μl of supernatant with 1 ml of beads (1.1 in NETN plus 0.5% powdered milk) for 30 min. After extensive washes with NETN, the beads were used for GST pull-down assay as described (27) or further eluted by 5 mM reduced glutathione for gel retardation assay (23) and for antibody generation in goat using standard immunization procedures (34).

In Vitro Protein-Protein Interaction Assays—The plasmids pMAM-RxRα, pMAM-RxRβ, and hTRβ were achieved by in vitro translation using 35S-methionine (Amersham Corp.) and a TNT Coupled Wheat Germ Extract System (Promega) according to manufacturer’s instructions, and 50 μl of labeled receptors were preincubated twice for 1 h at 4°C on 25 μl of beads preloaded with GST. The receptors were then washed with 25 μl of beads preloaded with GST-dUTPase for 1 h at 4°C. After three washes with NETN, an equal volume of SDS-polyacrylamide gel electrophoresis loading buffer was added into the beads and boiled for 5 min. Samples were resolved by SDS-polyacrylamide gel electrophoresis (35). Binding of RfPPARα to truncated forms of GST-dUTPase was carried out using the same procedure except an in vitro 35S-methionine-labeled rPPARα was used (32).

Binding of rPPARα to truncated GST fusion proteins—The human embryonic kidney cells (a gift from Dr. Richard Horn, Thomas Jefferson University) were maintained in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) containing 10% hormone-depleted fetal bovine serum (36) and 1% penicillin/streptomycin at 37°C in 5% CO2. Transfections were done using the calcium liposome-mediated (21,2,3-dioleyloxy)propyl-N,N,N′,N′-trimethylammonium methysulfate) method according to the manufacturer’s instructions (Boehringer Mannheim) in 24-well plates. Unless otherwise indicated, each transfection contained a total of 200 μg of reporter and receptor/dUTPase expression plasmid DNA balanced with pSG5 and an additional 50 μg of β-galactosidase expression vector pCMVβ (Clontech). The cells were incubated with DNA for 6 h and washed with phosphate-buffered saline before adding fresh medium containing the indicated ligand. After 40 h of incubation, the cells were processed to assess luciferase activity, and the activity obtained for individual transfections was expressed relative to the pSG5-galactosidase activity obtained for the E. coli leuB mutation according to the manufacturer’s instructions. The isolated plasmids were reintroduced into the yeast strain SFY526 (MATa, ura3–52, his3–200, ade2–101, lys2–801, trpl–901, leu2–3, 112, can1–100, gal4–542, gal80–538, URA3::GAL1-lacZ) with pbGT9, pbGT9-rPPARα, pbGT9-rRXRα, and pbGT9-hTRβ, respectively. Screening of a rat liver cDNA Library—Screening a GAL4ad rat liver cDNA hybrid library with the two-hybrid system was done according to the manufacturer’s instructions (Clontech). Yeast strain Hef7c (MATa, ura3–5, his3–200, lys2–801, ade2–101, trpl–901, leu2–3, 112, gal4–542, gal80–538, LYS2::GAL1-HIS3, URA3::GAL1-lacZ) were transformed with pGT9, pGT9-rPPARα, pGT9-hTRβ, and pGT9-rRXRα. Transfection of the target protein was checked by immunoblot analysis with an antibody against RfPPARα (32). To select for colonies containing interacting hybrid plasmids, transformants were spread on a synthetic minimal dropout agar medium (0.67% w/v) yeast nitrogen base, 2% (w/v) glucose/10% appropriate auxotroph supplements, 2% (w/v) agar) lacking leucine, tryptophan, and histidine but supplemented with 10 μg 3-aminol-1,2,4-triazole (β-galactosidase activity of HIS3-positive yeast transformants was assayed on VWR grade 410 filter replicas. Transformants activating both the HIS3 and β-galactosidase reporter genes were isolated, replate and retested for β-galactosidase activity. The activation domain plasmids encoding rPPARα-interacting proteins were isolated by transforming Escherichia coli HB101 for complementation of the E. coli leuB mutation according to the manufacturer’s instructions. The isolated plasmids were reintroduced into the yeast strain SFY526 (MATa, ura3–52, his3–200, ade2–101, lys2–801, trpl–901, leu2–3, 112, can1–100, gal4–542, gal80–538, URA3::GAL1-lacZ) with pbGT9, pbGT9-rPPARα, pbGT9-rRXRα, and pbGT9-hTRβ, respectively, and tested for β-galactosidase activity.

Other Methods—Plasmid isolation, endonuclease digestion, ligaton, and DNA sequencing were performed according to standard protocols (37). dUTPase enzyme assay was performed as described (38) with minor modifications. Protein concentration was determined by the method of Bradford (39).
RESULTS

Identification of Rat Liver cDNA Clone(s) That Interact with rPPARα in the Yeast Two-hybrid Screen—The rPPARα, expressed in S. cerevisiae strain HF7c, was used as the bait in a two-hybrid screen. Two-hybrid interactions in this strain activate transcription of the HIS3 and lacZ genes. A rat liver cDNA library in pGAD10 was screened for proteins that interact with the pGBT9-rPPARα bait. Approximately 1.5 million transformants were plated on His^2^ plates, and surviving colonies were further screened by β-galactosidase assay for lacZ expression.

Atotal of 41 clones, which activate both the HIS3 and β-galactosidase reporter genes, were isolated and reintroduced into yeast strain SFY526 with pGBT9, pGBT9-rPPARα, pGBT9-rRXRα, and pGBT9-hTRβ, respectively. Among these 41 clones, six exhibited strong and specific interaction with rPPARα (data not shown). These positive clones were digested with restriction enzymes and partially sequenced. Clone number 4, designated PIP4 (PPAR-interacting protein 4), exhibited strongest interaction with rPPARα and was chosen for further analysis.

**PIP4 Encodes Deoxyuridine-triphosphatase**—Sequence analysis of the 1-kb cDNA insert of PIP4 revealed the presence of an open reading frame encoding a peptide of 203 amino acids with the predicted molecular mass of the protein of 23.9 kDa. The start of the coding sequence was defined by the first ATG, with the surrounding sequences (AGCGCCATGCCC) conforming to a consensus sequence for the translation initiation site (40). The 3' untranslated region includes a putative polyadenylation signal sequence AATAA 17 base pairs upstream of a short poly(A) stretch of 18 nucleotides (Fig. 1A). A GenBank® data base search revealed that the deduced 203-amino acid sequence has significant homology with the human dUTPase (41). The rat dUTPase is 62 amino acids longer than the human enzyme with an identity of 92%. The rat dUTPase also contains five distinct amino acid sequence motifs (motifs 1–5, Fig. 1B) that are common to all the dUTPases identified so far (42). The N-terminus (residues 1–62) is arginine-rich (10 of 62 residues) and is composed of two putative protein kinase C phosphorylation sites (RRKQRS and RTRS) (43). The portion of the protein between amino acid residues 24 and 49 has significant homology with mouse RNA-DNA binding protein (57%, X70067), human RNA-binding protein (57%, L37368), and DNA-binding nucleocapsid protein (56%, X77048). These proteins are known to bind with single strand DNA and RNA (48), implying that this domain of dUTPase may exhibit similar functions. Thus, on the basis of the degree of identity shared with these proteins, we conclude that the PPARα-interacting PIP4 is a dUTPase.

![Fig. 1. Characterization of the rat dUTPase cDNA. A, nucleotide and deduced amino acid sequences of rat dUTPase. B, multiple sequence alignment of rat, human (41), tomato (44), vaccinia virus (45), yeast (46), and E. coli (47)]. dUTPase. Rat dUTPase is 62 amino acids longer than the human enzyme. The position of motifs 1–5 are shown in boldface type. These five motifs found in all dUTPases identified so far are functionally important (42).
more dUTPase activity than the untransformed control (Table I), indicating that PIP4 did, in fact, encode dUTPase.

**Specific Interaction of Rat dUTPase with all Three Marine PPAR Isoforms**—The interaction of rat dUTPase with PPARs was confirmed by an in vitro GST pull-down assay. For this purpose, rat dUTPase was first expressed in E. coli as a GST fusion protein. After induction with isopropyl-thio-β-D-galactoside, the fusion protein, with an apparent molecular mass of 47 kDa, accumulated in the induced E. coli cells. When glutathione-coupled agarose beads were incubated with the induced E. coli lysate and washed extensively, a 47-kDa protein was the main component retained with the beads. Two other minor bands, with an apparent molecular mass of ~30 kDa, were observed, and these may represent some other bacterial proteins associated with the GST-dUTPase fusion protein (see “Discussion”) (Fig. 2A). Incubation of preloaded glutathione beads (i.e. beads with attached GST-dUTPase fusion protein) with [35S]methionine-labeled mPPARα, mPPARγ, rRXRα, or hTRβ revealed specific interaction of all three PPAR isoforms with the GST-dUTPase fusion protein. Both rRXR and hTRβ failed to bind with the GST-dUTPase fusion protein (Fig. 2B). No binding of PPAR isoforms to GST was observed (data not shown), confirming that PPAR binding to GST-dUTPase preloaded beads is due to the interaction of PPAR with dUTPase.

Arginine-rich N-terminal dUTPase Domain Interacts with PPARs—Rat dUTPase was truncated at the junction of arginine-rich N-terminal domain (1–62 residues) and the C-terminal domain (63–203 residues) by amplification of the full-length cDNA using two sets of primers as described under “Experimental Procedures.” These amplified cDNA fragments were first cloned into the BamHI site of pGAD424 plasmid and tested for interaction with rPPARα, rRXRα, and hTRβ in vivo using yeast strain SFY526. In this in vivo assay, the N-terminal domain (1–62 residues) exhibited strong interaction with rPPARα similar to that observed with the full-length dUTPase, whereas the C-terminal domain (63–203 residues) did not interact with rPPARα. Neither the full-length nor the N-terminal 62-amino acid segment of dUTPase was able to interact with rRXRα and hTRβ (Fig. 3A). The interaction was further confirmed in vitro in experiments using [35S]methionine-labeled recombinant rPPARα produced in insect Sf9 cells and bacterially expressed truncated forms of dUTPase. Among the labeled insect cell proteins, only rPPARα bound to the full-length dUTPase and to the N-terminal 62-amino acid segment (Fig. 3B). The rPPARα failed to bind to the C-terminal (63–203 residues) dUTPase segment. These results indicate that the interaction is specific and the N-terminal 62-amino acid domain is responsible for interacting with PPARα.

**dUTPase Prevents Formation of PPAR-RXR Heterodimers**—PPARs heterodimerize with RXRs and bind to PPRE to regulate target gene transcription (24). To test whether dUTPase interacts with the PPAR-RXR-PPRE complex, bacterially expressed full-length and truncated GST-dUTPase fusion proteins were used for gel mobility shift assays (23). Recombinant rPPARα and rRXRα form a heterodimer on rat peroxisomal fatty acyl-CoA oxidase PPRE (Fig. 4, lane 1), whereas full-length dUTPase alone did not bind to this DNA element (Fig. 4, lane 11). Combination experiments were performed with increasing amounts of GST-dUTPase (Fig. 4, lanes 2–4), GST-Δ1–62-dUTPase (Fig. 4, lanes 5 and 6), or GST-Δ1–62-dUTPase (Fig. 4, lanes 7–9) added to an equal amount of rPPARαrRXRα-PPRE binding reaction mixture. The results revealed that the full-length dUTPase blocked the formation of PPAR-RXR-PPRE complex, but the two truncated dUTPase fusion proteins showed no effect. We also noted that dUTPase incubated with either rPPARα or rRXRα failed to bind to PPRE (data not shown). These results show that dUTPase, instead of interacting with the PPAR-RXR-PPRE complex to induce a supershift, actually blocks the formation of PPAR-RXR-PPRE complex. Accordingly, this dUTPase-PPARα interaction may lead to inhibition of transcription.

**Rat dUTPase Is a Ligand-independent PPAR Inhibitor**—To investigate the functional significance of the interaction of dUTPase with PPARs, transient transfection assays were carried out in mammalian cells using an established chimeric system, in which the ligand-binding domains of murine PPARα, PPARγ, and PPARγ were fused to the DNA binding domain of yeast transcription factor Gal4 (20). Expression plasmid for GAL4-PPARα chimera was first used to transfect human kidney tsA201 cells together with a luciferase reporter construct containing five copies of the GAL4-binding site upstream of the minimal thymidine kinase promoter, and various amounts of dUTPase-expression plasmid (Fig. 5A). As expected, the luciferase reporter gene was efficiently transactivated by GAL4-PPARα chimera in the presence of 10−5 μM Wy 14, 643, a potent peroxisome proliferator. Co-transfection with dUTPase caused a reduction in the reporter gene activity, and this reduction correlated with increases in the amounts of dUTPase-expressing plasmid added. In contrast, both truncated forms (Δ1–62, and Δ63–203) did not exhibit inhibitor activity. Inhibition of PPAR transactivation by dUTPase was further investigated using different concentrations of ligand (Fig. 5B). The PPARα was optimally activated by Wy 14, 643 at a concentration of 1 × 10−6 μM, whereas PPARγ had highest transcriptional activity in the presence of 5 × 10−5 μM 15d-12,14PGJ2, the PPARγ-specific ligand. These activities were significantly reduced in the presence of dUTPase at different concentrations of

| Table I | dUTPase activity expressed from PIP4 |
|---------|-----------------------------------|
| Transfectant | Units of dUTPase activity |
| SFY526 | 10.3 ± 1.0 |
| SFY526/pGAD10 | 11.2 ± 1.2 |
| SFY526/PIP4 | 112.0 ± 3.1 |

**Fig. 2. Interaction of rat dUTPase with PPARs in vitro.** A, expression and purification of rat dUTPase in E. coli. A BamHI-BglII fragment from the rPPARα-interacting clone PIP4 was inserted into the BamHI site of pGEX-2T plasmid (Pharmacia) in a right orientation for expressing GST-dUTPase fusion protein. E. coli cells transformed with dUTPase-expressing plasmid were grown in LB medium (lane 1) and subsequently induced with 0.2 mM isopropyl-thio-β-D-galactoside for 2 h (lane 2). The induced proteins were purified by glutathione-agarose beads (lane 3). Purified GST protein (lane 4) was used as a control. The lane marked M contains molecular mass markers for sizes indicated. B, GST pull-down assay using TNT-translated[35S]methionine labeled receptors. 50 μl of [35S]methionine-labeled mPPARα, mPPARγ, rRXRα, and hTRβ receptors were first precleared for 1 h at 4°C on 25 μl of GST-beads and then incubated with 25 μl of beads preloaded with GST-dUTPase for 1 h at 4°C. The beads were then washed three times with NETN and analyzed using 10% SDS-polyacrylamide gel electrophoresis, on lanes referred to as bound (b). In lanes referred to as input (i), 5 μl of translated receptor proteins were used as control.
Rat dUTPase interacts with PPARα

FIG. 3. Mapping of the interaction domain of rat dUTPase. A, mapping of the interaction domain in vitro. The cDNA fragment encoding for amino acid residues 1–62 and 63–203 were obtained by polymerase chain reaction amplification of the full-length dUTPase cDNA using specific primers as described in the text. Fragments were than cloned into a GAL4 activation domain vector pGAD424 (Clontech) in the right orientation. The resulting plasmids together with the original PIP4 clone (full-length dUTPase) and pGAD424 plasmid were transformed into yeast SFY 528 strain with the GAL4 DNA binding domain vector (pGBT9) containing rPPARα, rRXRα, hTRβ, and the empty vector pGBT9, respectively, as indicated. The transformants were subject to β-galactosidase filter assays according to manufacturer’s instructions (Clontech). B, mapping of the interaction domain of dUTPase in vivo. a, expression of truncated dUTPase in E. coli cells. The same cDNA fragments encoding C-terminal 141 amino acids (63–203) and N-terminal 62 amino acids (1–62) were cloned into pGEX-2T vector as described in the text. The procedures for fusion protein induction and purification were the same as for the full-length dUTPase described in Fig. 2. Lane 1, GST fused with full-length dUTPase; lane 2, GST fused with the C-terminal 141-residue (63–203) segment; lane 3, GST fused with the N-terminal 62-residue (1–62) domain; lane 4, GST alone. b, [35S]methionine labeling of insect Sf9 cells infected with a recombinant baculovirus expressing rPPARα (32). Standard protocols were used for insect cell culture, infection, and in vitro labeling. c, GST pull-down assay. Glutathione-agarose beads preloaded with GST fusion proteins from a were incubated with [35S]methionine-labeled proteins as shown in b using the same procedure as described in Fig. 2.

DISCUSSION

Using a GAL4 DNA-binding domain-PPARα fusion in a yeast two-hybrid screen, we isolated several cDNA clones, and one of these clones, PIP4 (peroxisome proliferator-activated receptor-interacting protein-4), has been found to interact strongly with unliganded full-length PPARα but not with other receptors such as RXRa and TRβ. This PPARα-interacting protein prevented the formation of PPAR-RXR heterodimers and inhibited the transcriptional activity of all three isoforms of PPAR (α, δ, and γ). Furthermore, when PPARα and PIP4 are transiently coexpressed in human embryonic kidney cells, these proteins appeared to colocalize within the nucleus. Thus it appears that PIP4 interacts with PPAR and that this interaction results in the functional repression of PPAR activity.

Nucleotide sequence of PIP4 showed that it encodes a polypeptide of 203 amino acids with an estimated relative molecular mass of 23.9 kDa. This protein has an arginine-rich N-terminal 62-amino acid segment that has been shown to interact strongly with the PPARα. Homology search revealed that the C-terminal 141 amino acid sequence of this protein corresponds to the full-length human dUTPase (141 residues) and exhibits significant identity with this human enzyme (41). The other dUTPases cloned so far, such as the yeast (147 residues) and E. coli (150 residues), are also shorter in length when compared with the rat dUTPase. Evidence also includes the pseudoprotease domains of some retrovirus-encoded dUTPases (42). The presence of dUTPase in widely divergent organisms (i.e., from humans to the retroviruses) suggests that this enzyme may generally perform a vital role in DNA replication (49).
In vivo, dUTPase hydrolyzes dUTP to dUMP and pyrophosphate ion and prevents accumulation of cellular dUTP. It is widely speculated that dUTPase is essential for cell viability because this enzyme is believed to maintain extremely low levels of intracellular dUTP and thus prevent the synthesis of highly uracil-substituted DNA during replication (50). Although deletion of the dUTPase from herpes simplex virus type 1 did not affect the viral DNA replication, the loss of dUTPase activity resulted in viruses with 5-fold increased relative mutant frequency, indicating that the herpes simplex virus type 1 dUTPase has an antimutator function (51). Mutants of E. coli deficient in dUTPase demonstrate an apparent increase in the amount of short, Okazaki-like DNA fragments, which are intermediates of DNA synthesis and are explained by the misincorporation of uracil in DNA as a result of an increase in available dUTP, followed by its rapid excision and repair (52). The presence of high levels of dUTPase mRNA in various normal and developing tissues reflects that dUTPase functions in these tissues to keep low levels of dUTP in order to prevent misincorporation of uracil into DNA and maintain DNA fidelity.

The observation that rat dUTPase interacts with PPARα (and other isoforms of this receptor) and functions as a repressor raises some interesting questions. Rat PPARα, when interacting with dUTPase, cannot form heterodimers with RXRα to bind to PPRE (Fig. 4). Activation of target gene expression requires PPARs to heterodimerize with RXR, and that dUTPase acts as a negative regulator is confirmed by transfection assays. PPARα interacts with rat dUTPase with the 62-amino acid N-terminal region, but it appears that the C-terminal dUTPase domain is also necessary for the inactivation as seen in the gel mobility shift assay and transfection assays. The 62-amino acid sequence of rat dUTPase is arginine-rich, consists of two putative protein kinase C phosphorylation sites (43) and has significant homology with single-strand DNA-RNA binding proteins and viral DNA-binding nuclear capsid pro...
tines (48). The mouse mammary tumor virus contains a dUTPase domain derived from the pro-open reading frame and a nucleoscapid protein domain at its N-terminal region (53). dUTPase (pseudoproteases) encoded by poxviruses contains 370 residues (42). The functional significance of the extra domain present in these dUTPases remains to be fully elucidated, but our results demonstrate that the extra domain of rat dUTPase is responsible for interaction with PPARs. The human dUTPase lacks this extra 62-amino acid portion (41), implying that it may not interfere with PPAR action. Because the rat dUTPase is only the second mammalian dUTPase cDNA cloned to date, cloning of dUTPases from other species that are responsive to peroxisome proliferators, such as the mouse, will enhance the understanding of the significance of this additional arginine-rich N-terminal sequence present in the rat but not in the human dUTPase.

Although PPARs serve as transcription factors, the interaction of PPARs with dUTPase suggests the possibility that peroxisome proliferators may also modulate dUTPase activity. In T4 phage-infected E. coli, dUTPase is found in a multienzyme complex in a total mass of about 1500 kDa, and it functionally interacts with other dNTP-synthesizing enzymes (54). Whether PPAR-dUTPase interaction modulates the dNTP-synthesizing enzymes remains speculative at this time. The intricate interrelationships between transcriptional machinery and DNA-repair/synthesis processes are becoming increasingly evident from recent observation (31). dUTPase is a crucial enzyme involved in the maintenance of DNA fidelity (50–52). During the early Drosophila development, dUTPase activity is modulated by interaction with a regulatory 61-kDa protein (55). The interaction of PPARs with dUTPase suggests the possibility that peroxisome proliferators affect the DNA fidelity by modulating dUTPase activity. In T4 phage-infected E. coli, dUTPase is found in a multienzyme complex in a total mass of about 1500 kDa, and it functionally interacts with other dNTP-synthesizing enzymes (54). Whether PPAR-dUTPase interaction modulates the dNTP-synthesizing enzymes remains speculative at this time. The intricate interrelationships between transcriptional machinery and DNA-repair/synthesis processes are becoming increasingly evident from recent observation (31). dUTPase is a crucial enzyme involved in the maintenance of DNA fidelity (50–52). During the early Drosophila development, dUTPase activity is modulated by interaction with a regulatory 61-kDa protein (55). The interaction of PPARs with dUTPase suggests the possibility that peroxisome proliferators affect the DNA fidelity by modulating dUTPase activity. In T4 phage-infected E. coli, dUTPase is found in a multienzyme complex in a total mass of about 1500 kDa, and it functionally interacts with other dNTP-synthesizing enzymes (54). Whether PPAR-dUTPase interaction modulates the dUTPase-synthesizing enzymes remains speculative at this time. The intricate interrelationships between transcriptional machinery and DNA-repair/synthesis processes are becoming increasingly evident from recent observation (31). dUTPase is a crucial enzyme involved in the maintenance of DNA fidelity (50–52).