Isolation and Characterization of Peroxisome Proliferator-activated Receptor (PPAR) Interacting Protein (PRIP) as a Coactivator for PPAR*

Received for publication, December 15, 1999, and in revised form, February 3, 2000

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We previously isolated and identified steroid receptor coactivator-1 (SRC-1) and peroxisome proliferator-activated receptor (PPAR)-binding protein (PBP/PPARBP) as coactivators for PPAR, using the ligand-binding domain of PPARγ as bait in a yeast two-hybrid screening. As part of our continuing effort to identify cofactors that influence the transcriptional activity of PPARs, we now report the isolation of a novel coactivator from mouse, designated PRIP (peroxisome proliferator-activated receptor interacting protein), a nuclear protein with 2068 amino acids and encoded by 13 exons. Northern analysis showed that PRIP mRNA is ubiquitously expressed in many tissues of adult mice. PRIP contains two LXXL signature motifs. The amino-terminal LXXL motif (amino acid position 892 to 896) of PRIP was found to be necessary for nuclear receptor interaction, but the second LXXL motif (amino acid position 1496 to 1500) appeared unable to bind PPARγ. Deletion of the last 12 amino acids from the carboxyl terminus of PPARγ resulted in the abolition of the interaction between PRIP and PPARγ. PRIP also binds to PPARα, RARs, RXRs, ER, and TRβ1, and this binding is increased in the presence of specific ligands. PRIP acts as a strong coactivator for PPARγ in the yeast and also potentiates the transcriptional activities of PPARα and RXRs in mammalian cells. A truncated form of PRIP (amino acids 786–1132) acts as a dominant-negative repressor, suggesting that PRIP is a genuine coactivator.

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily that regulates the expression of target genes, in particular those associated with lipid metabolism (1–3). PPARs, which derive the designation by virtue of their ability to mediate predictable pleiotropic effects in response to peroxisome proliferators (2, 4), consist of three isotypes, namely PPARα, PPARδ (also called PPARβ), and PPARγ, which are products of separate genes (1, 5–7). These PPAR isotypes appear to exhibit distinct patterns of tissue distribution and differ considerably in their ligand-binding domains, implying that they possibly perform different functions in different cell types (5–9). Of the three isotypes, PPARα expression is relatively high in hepatocytes, enterocytes, and the proximal tubular epithelium of kidney when compared with other cell types (8), and evidence derived from mice with PPARα gene disruption indicates that this receptor is essential for the pleiotropic responses induced by peroxisome proliferators (10). Also worth noting is that the fatty acyl-CoA oxidase, the first and the rate-limiting enzyme of the peroxisomal fatty acid β-oxidation system, plays a critical role in the metabolism of biological/natural ligands of PPARα in that mice with fatty acyl-CoA oxidase gene disruption exhibit sustained spontaneous up-regulation of PPARα-responsive genes (11). PPARδ isotype is ubiquitously expressed in the adult tissues and binds the same ligands as other PPARs (7, 12, 13). Recent evidence suggests that nonsteroidal anti-inflammatory drugs inhibit colon carcinogenesis through inhibition of PPARα and that PPARβ functions as a target for adenomatous polyposis coli (14). PPARγ, which is expressed predominantly in adipose tissue, as well as in certain epithelial cells such as those of the colon mucosa, mammary epithelium, and urinary bladder (8, 9), plays a pivotal role in adipocyte development and lipid homeostasis (15–17). Although a great deal is known about genes regulated by PPARα, especially in conjunction with peroxisome proliferator-induced pleiotropic responses (2), there is very little information on PPARγ- and PPARδ-regulated genes in specific cell types, despite the fact that many of the ligands identified to date interact with one or more PPAR isotypes (12, 13).

Evidence accumulated so far indicates that the transcriptional activation of nuclear receptors after ligand binding involves the participation of cofactors termed nuclear receptor corepressors and coactivators (18, 19). The current model suggests that unliganded receptors are maintained in a repressed state by nuclear receptor corepressors such as N-CoR (20) and SMRT (21). Upon ligand binding, the corepressor(s) dissociates from the nuclear receptor, thus enabling the liganded nuclear receptor to recruit a complex of proteins, called nuclear receptor coactivators, that bridge the nuclear receptors with basal transcription machinery (18). The coactivators identified in recent years include the SRC-1 family, which has three member (SRC-1 (22–24), TIF-2 (SRC-2, GRIP1) (25, 26), p/CIP (ACTR, AIB1, RAC3, and SRC-3) (26–28, 30); CBP/p300 (31,
32; RIPC140 (33) PBPl (34); and PGC-1 (35) among others (18). All of these nuclear receptor coactivators contain the conserved LXXLL (L, leucine; X, any amino acid) motif that mediates recognition of nuclear receptor (26, 36). Some of the coactivators, including SRC-1 family and CBP/p300, contain intrinsic histone acetyltransferase activities that modify the chromatin structure (37–40).

The identification and functional analysis of many of the known nuclear receptor coactivators resulted from studies utilizing the yeast two-hybrid screening (22, 24, 34, 35). Alternative strategies that focused on the identification and purification of the putative transcriptional complexes yielded valuable information regarding the transcriptional activation of thyroid hormone receptor (TR), vitamin D receptor, and other transcriptional units (41–44). The thyroid hormone receptor-associated proteins (TRAPs) can increase the transactivation activity of the liganded TR (42). Likewise, multiple protein complexes such as DRIP (vitamin D3 receptor interacting protein) and ARC (activator-recruited cofactor) are identified and found to be required for the transcriptional activation of multiple transcription factors including SP1, p53, NF-xb, vpx16, and nuclear receptors (43, 44). It is of special interest that this complex acts on the nuclear receptors by binding to PBP (PPARBP), a nuclear receptor coactivator we originally identified as a coactivator for PPAR (34). More recently, we demonstrated that the PBP gene (PPARBP) is overexpressed in about 50% of breast cancers examined, and the PBP gene amplification was noted in about 25% of breast tumors (45). These observations underscore the importance of nuclear receptor coactivators in transcriptional activation and also point to their possible role in neoplastic conversion. Here we report the characterization of a new nuclear receptor coactivator, designated PRIP, identified during yeast two-hybrid screening. PRIP contains two LXXLL signature motifs at amino acid positions 892–896 and 1496–1500, but the latter motif did not appear to reflect its ability to bind PPAR and to distinguish it from PBP (34). The PRIP cDNA fragment containing two LXXLL motifs spans amino acids 786–1132 functions as a dominant-negative repressor, suggesting that PRIP is a genuine coactivator.

EXPERIMENTAL PROCEDURES

Plasmids—GALA-PPAR-α, GALA-PPAR-β12, GST-PPAR-α, GSTD-PPAR-β, GSTD-PPAR-γ, PPRE-LUC, and pS-vp16-PPAR-α, as well as the vectors for in vitro translation of RARs, TRβ1, and ER, have been described elsewhere (34). PCMX-RRα and IXXRE-TK-LUC were provided by Dr. R. Evans (Salk Institute). PCMV-PPAR-γ was constructed by inserting the full-length coding region of PRIP cDNA into the HindIII/Xbal site of PCDNA 3.1 (+) (Invitrogen). PCMV-PPAR-α was produced in the form of a fusion protein with two HA epitopes at the carboxyl terminus into the HindIII site of PCDNA 3.1 (+). PGAD10-PRIP was generated by inserting the full-length PRIP cDNA into the HindIII site of PGAD10. The PFLAG-PRIP-T and GST-PRIP were generated by subcloning the fragment released from PGAD10-PRIP-T clone isolated by the yeast two-hybrid screening into the EcoRI site of a modified PCMV-FLAG2 containing a nuclear targeting signal peptide PKKRRRKV and PGEX-5X-2, respectively.

Isolation of Mouse PRIP cDNA—A partial cDNA encoding a peptide that interacts with PPARα was isolated by yeast two-hybrid screening of a mouse liver cDNA library as described previously (24, 34). We then obtained the full-length cDNA from a ZAP newborn mouse kidney cDNA library with RACE PCR using a kit from Life Technologies, Inc. according to the manufacturer’s protocol. The PCR products were cloned into pGEM-T (Promega), and the independent clones were sequenced. The full-length cDNA that we cloned has been designated PRIP, or peroxisome proliferator activated receptor interacting protein to reflect its ability to bind PPARs and to distinguish it from PBP (PPARBP), which we cloned earlier (34).

Northern Blot Analysis—Mouse multiple tissue Northern blot (CLONTECH) containing 2 μg of poly(A) RNA in each lane was probed with 32P-labeled PRIP full-length cDNA according to the conditions outlined by the manufacturer.

Characterization of Mouse PRIP Gene—By screening a BAC mouse library (Genome Systems, St. Louis, MO) with PCR primers 5-AGAGATTGACTTGAGACG-3' and 5'-TTTGTCTGGAGTTCCACCTT-3', one BAC clone (#21803) containing the PRIP gene was obtained. The BAC clone was digested with the appropriate restriction enzymes and subcloned as necessary. The intron sizes and exon/intron boundaries were determined by Southern blotting, restriction enzyme mapping, PCR, sequencing, and a comparison of sequences between the cDNA and genomic DNA.

Results

Quantitative β-Galactosidase Assays—Appropriate plasmids were cotransfected into yeast HTFC, plated on selective media plates in the presence or absence of 10-5 M RBL49653, a PPARγ ligand, and then incubated for 4 days at 30 °C. For each assay, five isolates were suspended in 150 μl of buffer Z (60 mM Na2HPO4, 40 mM NaH2PO4, 10 mM KCl, 1 mM MgSO4, 35 mM 2-mercaptoethanol). Equal number of cells in suspension was pelleted by centrifugation, and β-galactosidase activity was determined by a chemiluminescent reporter protocol (Galacto-light kit, Tropix, Bedford, MA). GST Pull-down Assays—The GST alone and GST-fusion proteins GST-PRIP, GST-RXRα, GST-RARα, and GST-PPARγ were produced in Escherichia coli DH5α and bound to glutathione-Sepharose beads according to the manufacturer’s instructions (Amersham Pharmacia Biotech). The GST alone and GST-fusion proteins were produced in Escherichia coli DH5α and bound to glutathione-Sepharose beads according to the manufacturer’s instructions (Amersham Pharmacia Biotech). The GST alone and GST-fusion proteins were produced in Escherichia coli DH5α and bound to glutathione-Sepharose beads according to the manufacturer’s instructions (Amersham Pharmacia Biotech). The GST alone and GST-fusion proteins were produced in Escherichia coli DH5α and bound to glutathione-Sepharose beads according to the manufacturer’s instructions (Amersham Pharmacia Biotech). The GST alone and GST-fusion proteins were produced in Escherichia coli DH5α and bound to glutathione-Sepharose beads according to the manufacturer’s instructions (Amersham Pharmacia Biotech).
known sequences in GeneBank™, it showed 96% homology to KIAA0181 except that the first 58 amino acids were missing in the KIAA0181 cDNA. The KIAA0181 cDNA was isolated from human myeloid KG-1 cells (46). The function of this protein has not been investigated. Mouse PRIP contains a stretch of 31 glutamine residues from amino acids Glu261-Glu291, whereas its human homologue has 25 contiguous glutamines. The PRIP was expressed as an 8.5-kb transcript in all tissues examined including blood, spleen, prostate, thymus, colon, testis, ovary (Fig. 2), liver, and kidney, among others (data not shown). A high level of expression of PRIP mRNA has been noted in the testis. A second; 4-kb transcript is detected in the testis, which may represent an isoform for PRIP. Transfection of a plasmid containing two HA epitopes linked to the carboxyl-terminal portion of PRIP protein into COS-1 cells revealed the nuclear localization of the expressed PRIP protein (Fig. 3).

Genomic Organization of the Mouse PRIP Gene—To isolate the PRIP gene, a BAC mouse library was first screened by PCR using a pair of oligonucleotide primers. One clone covered the entire PRIP gene. The size of the introns and the exon/intron boundaries were determined by XL-PCR and by sequencing. The PRIP gene consists of 13 exons and spans about 47.5 kb (Table I). Similar to the gene structures of mSRC-1 and PBP-1, about half of the PRIP cDNA is encoded by a single large exon (exon 9). The sequences of all of the splice acceptor and donor sequences conformed to the GT-AG consensus rule for splicing (47).

Interaction of PRIP with PPARγ in Yeast—We investigated the influence of PPARγ ligand BRL49653 on the binding of PRIP to PPARγ in yeast. PGADH10-PRIP-T (amino acids 786–1132), which was isolated by yeast two-hybrid screening and expressed as the fusion protein with the GAL activation domain, was cotransformed with Gal-PPARγ into yeast HF7c and the β-galactosidase activity measured as an indication of the relative strength of interaction in the presence or absence of ligand. In the absence of ligand, we observed an interaction between PRIP and PPARγ, which resulted in an ~20-fold increase in the β-galactosidase activity (Fig. 4). The presence of the ligand BRL49653 further potentiated this interaction between PRIP and PPARγ, leading to an ~8-fold increase in the β-galactosidase activity (Fig. 4).

The extreme carboxy-terminal region of the ligand-binding domain conserved among the nuclear receptors has been shown to be essential for the ligand-dependent transcriptional activation (48). To determine whether this region is important for the binding of PPARγ to PRIP, GAL-PPARγΔ12, which lacks the last 12 amino acids from the carboxyl terminus of PPARγ, was cotransformed with PGADH10-PRIP-T into HF7c. The presence of PRIP did not lead to an increase in β-galactosidase activity either in the presence or absence of the ligand, indicating that the mutation eliminates the ability of PPARγ to bind PRIP (Fig. 4). As LXXLL is the signature motif for the binding of cofactor to nuclear receptors, we tested whether the second LXXLL in the PRIP can bind to PPARγ. Plasmid PGADH10-PRIP-M2, expressing a fusion protein between GAL activation domain and the partial PRIP cDNA (amino acids...
1440–1556) containing the second LXXLL, was cotransformed with GAL-PPARα into HF7C. The presence of the second LXXLL motif did not increase the activity of β-galactosidase (Fig. 4). Therefore, we conclude that the second LXXLL motif is not necessary for the interaction between PRIP and PPARα, but the first LXXLL motif appears necessary and sufficient.

Interaction of PRIP with Nuclear Receptor in Vitro—The direct interaction between PRIP and PPARα was tested further by an in vitro GST binding assay with bacterially generated GST-PPARα fusion protein and in vitro translated PRIP. Although the immobilized GST-PPARα, but not GST alone, retained the [35S]methionine-labeled PRIP in the presence and absence of PPARα ligand BRL49653, the addition of the ligand strongly enhanced this interaction (Fig. 5). Moreover, PRIP also showed the ligand-dependent interaction with RXRα, RXRα, ER, and TRβ1, as well as PPARα (Fig. 5).

Interaction between PRIP and PPARα in Intact Cells—To determine whether PPARα and PRIP form a complex in the context of intact cells, a vector encoding PRIP with a carboxyl-terminal HA epitope was constructed and expressed in 293 cell line derived from primary embryonal human kidney (ATCC CRL 1573). The potential complex was immunoprecipitated with anti-PPARα and analyzed by immunoblotting using anti-HA. This study established the ligand-dependent interaction between PPARα and PRIP in vivo (Fig. 6). A similar study revealed that PRIP-T (amino acids 786–1132) containing the first LXXLL but not PRIP-M2 (amino acids 1440–1556), which contains the second LXXLL motif, interacts with PPARα in vivo (Fig. 6).

PRIP Acts as a Strong Coactivator for PPARα in Yeast—To elucidate if PRIP acts as a coactivator in the yeast, the GAL-PPARα was coexpressed with full-length PRIP in yeast strain HF7c. PRIP moderately increases the transcriptional activity of PPARα in the absence of ligand (~3-fold), whereas the increase is ~22-fold in the presence of ligand BRL49653 (Fig. 7).

PRIP Potentiates the Transcriptional Activities of PPARα and RXRα in Mammalian Cells—To determine whether PRIP serves as a coactivator for PPARα in mammalian cells, we overexpressed PRIP in CV-1 cells along with PPARα and monitored the transcriptional activity of PPARα with expression of the peroxisome proliferator response element-linked reporter luciferase gene. PRIP moderately increased the transcription of the luciferase gene by about 1.9-fold in the presence of BRL49653 (Fig. 8). When PRIP was co-expressed with RXRα, it increased the transactivation capacity of RXRα by 3.9-fold.

Truncated PRIP with the First LXXLL Motif Functions as a Dominant-negative Form—To further confirm PRIP as a coactivator for PPARα, we overexpressed in CV-1 cells, a truncated form of PRIP (PRIP-T, amino acids 786–1132), which contains the first LXXLL and has been shown to interact with PPARα. Cotransfection of PRIP-T resulted in a decrease in PPARα-mediated transcription of reporter in the presence of ligand, whereas no significant change was detected in the absence of the ligand (Fig. 9A). PRIP-M2 (amino acids 1440–1556), which contains the second LXXLL motif that does not bind PPARα, cannot inhibit the transcriptional activity of PPARα. Moreover, the suppressive effect of PRIP-T can be reversed by cotransfection with wild type PRIP. When tested with RXRα, the inhibitory effect of truncated PRIP on the transcriptional activity was stronger in comparison with PPARα (Fig. 9B).

DISCUSSION

Using a yeast two-hybrid system with Gal4-PPARα as bait to screen a mouse liver cDNA library, we isolated a cDNA designated as PRIP, which is a nuclear protein with 2068 amino acids. In this report, we present data on the initial characterization of this coactivator protein, which reveals a strong ligand and AF-2-dependent interaction with PPARα and several other nuclear receptors (Fig. 5). The entire PRIP gene consists of 13 exons and extends ~47.5 kb. Northern analysis showed that

**TABLE I**

| Exon (bp) | Donor | Intron | Acceptor | Exon |
|-----------|-------|--------|----------|------|
| 1 (248)   | C, ATG, G | gtaattaac | cgtgaccaag | AA, TCC | 2 |
| 2 (154)   | T, GAA, G | gttggttact | tcctctctac | GG, GAA | 3 |
| 3 (122)   | T, CCA, G | gttctact | cttctctcag | GT, CTA | 4 |
| 4 (128)   | G, TCA, G | gttggttct | ctttttacag | AT, GCA | 5 |
| 5 (899)   | A, GGA, G | gtaggaac | cttctact | GA, ATG | 6 |
| 6 (146)   | C, GCAG | gttgcctact | cttctact | GT, CTA | 7 |
| 7 (1116)  | CTA, AA | gtaagttatg | tgtgttttag | C, ACC, C | 8 |
| 8 (121)   | A, CCA, G | gtaaagtgat | cttctttttag | GT, TAT | 9 |
| 9 (2975)  | C, ATG, G | gtaaggtact | cttgctctag | CC, CTT | 10 |
| 10 (78)   | A, CCA, G | gtaaggtgcc | ttttttacag | AA, CTT | 11 |
| 11 (36)   | CAA, AG | gtaggacccct | tcctgctagag | C, TGT, G | 12 |
| 12 (149)  | T, AAA, G | gtagcagtg | ttctactag | AT, ATA | 13 |
The bound proteins were eluted, analyzed using 10% SDS-polyacrylamide gel electrophoresis, and autoradiographed.

The ligands used were: BRL49653, the ligand for PPARγ; BRL49653, the ligand for RXRα; cis-1,2,3-retinoic acid, the ligand for RARα; T3, the ligand for TRα; or TRβ1, generated by in vitro translation, was incubated with glutathione-Sepharose beads bound with either purified E. coli expressed GST-PPARγ, GST-RARα, GST-RXRα, or with GST, in the presence (+) or absence (−) of ligand. The ligand for PPARγ was BRL49653, and the ligand for RXRα and RARα was 9-cis-retinoic acid. B, [35S]methionine-labeled PPARγ, GST-PRIP, GST, or TRβ1, generated by in vitro translation, was incubated with glutathione-Sepharose beads bound with either purified E. coli-expressed GST-PPARγ or GST in the presence (+) or absence (−) of ligand. The ligands used were: PPARγ, Wy-14643; ER, estrogen; and TRβ1, T3. The bound proteins were eluted, analyzed using 10% SDS-polyacrylamide gel electrophoresis, and autoradiographed.

CAG repeats, detected in a number of proteins, are critical to inherent neurodegenerative conditions such as Huntington disease (49). In these conditions, the number of CAG repeats is more than 40, and the abnormal proteins accumulate as aggregates forming intranuclear inclusions. Also of interest is the fact that a continuous stretch of 29 CAG repeats has been detected in ACTR/AIB1, another nuclear receptor coactivator (27, 28). Likewise, TATA-binding protein and androgen receptor reveal continuous stretches of 38 and 22 glutamines, respectively (50, 51). It is possible that whereas the LXXLL motifs facilitate interaction between coactivators and nuclear receptors, the polyclutamine tracts might promote high affinity interactions between polypeptides carrying polyclutamides, especially with TATA-binding protein (50).

We found that PRIP functions as a much stronger coactivator in the yeast when compared with its coactivator activity in the mammalian cells used in this study. This finding may be explained in part by the attenuation of the coactivator function by other factors involved in mammalian cell transcription and the relative lack of abundance of these factors in yeast; it could also be due to the fact that mammalian cells already contain endogenous PRIP, whereas data bank searches failed to reveal a PRIP homologous protein in yeast. We also found no PRIP homologue in the Caenorhabditis elegans, although this organ-
PRIP moderately enhances PPARγ and RXRα-mediated transactivation of reporter expression in CV-1. CV-1 cells were cotransfected with 1.5 μg of reporter construct PPRE-TK-LUC, 0.25 μg of PCMV-mPPARγ, 0.5 μg of PCMV-PRIP, and 0.5 μg of PCMVβ in the absence (−) or presence (+) of 10^-5 M BRL49653. Transfection without PCMV-PBP was compensated by adding the same amount of pcDNA3.1. The activity obtained on transfection of the PPRE-TK-LUC without exogenous PRIP in the absence of ligand was taken as 1. Results are the mean of four independent transfections normalized to the internal controls of β-galactosidase expression. Transfection analysis for RXRα was performed as for PPARγ except using BARE-TK-LUC and PCMX-RXRα.

FIG. 8. PRIP moderately enhances PPARγ and RXRα-mediated transactivation of reporter expression in CV-1. CV-1 cells were cotransfected with 1 μg of reporter construct PPRE-TK-LUC, 0.25 μg of PCMV-mPPARγ, 0.5 μg of PCMV-PRIP, and 0.5 μg of PCMVβ in the absence (−) or presence (+) of 10^-5 M BRL49653. Transfection without PCMV-PBP was compensated by adding the same amount of pcDNA3.1. The activity obtained on transfection of the PPRE-TK-LUC without exogenous PRIP in the absence of ligand was taken as 1. Results are the mean of four independent transfections normalized to the internal controls of β-galactosidase expression. Transfection analysis for RXRα was performed as for PPARγ except using BARE-TK-LUC and PCMX-RXRα.

In CV-1 cells, PRIP was able to increase the transcriptional activity of RXRα to a higher degree than that of PPARγ. Consistent with this observation is the finding that the truncated form of PRIP exerts a stronger inhibitory effect on the transcriptional activation of RXRα as compared with PPARγ. Therefore, it is possible that PRIP may contribute more to the RXR transcriptional activation than to PPARγ under certain circumstances. As PRIP also binds to PPARα, RARα, RXRα, ER, and TRβ1, and as this binding is increased in the presence of specific ligands, we expect PRIP to function as a coactivator for a variety of nuclear receptors. The effectiveness of the coactivator function may, to some extent, depend on the nature of a given nuclear receptor and the cell type involved.

There is now increasing evidence to support the contention that the transcriptional activation of nuclear receptors involves at least two classes of nuclear receptor cofactors, termed corepressors and coactivators (1). Corepressors are associated with unliganded receptors to mediate repression; the ligand binding causes the dissociation of corepressor from the nuclear receptor. Subsequently, the liganded nuclear receptors actively recruit coactivators to facilitate transcription. The coactivators identified so far can be broadly categorized into two groups. The first category of coactivators, such as those belonging to the p300/CBP and SRC-1 family, possess histone acetyltransferase activities and as a result are involved in the modification of chromatin (37–40). The second group of coactivators, which are devoid of histone acetyltransferase activity, appear to serve as the facilitators linking the receptor complex to the basal transcription machinery (1). In this context, PBP (PPARBP), which we cloned and characterized earlier as a nuclear receptor coactivator (34), has been found to be a critical component of the TRAP/DRIP/CRSP/ARC complexes (41–44) and appears more than likely to be involved in the second step of coactivation, i.e., linking the receptors with the basal transcription machinery. Whether PRIP, which we have now cloned, is part of these, or other, multiprotein transcriptional complexes needs to be ascertained.

The reason for the existence of a multitude of coactivators remains elusive. One possibility is that different coactivators may preferentially participate in the transcription of specific target genes, as exemplified by the finding that CBP and p300 tend to exhibit target gene preference (52). It is also possible that specific nuclear receptors use only a distinct subset of coactivators for optimal transcriptional activity and that such a subset of coactivators may not function effectively for other members of the nuclear receptor superfamily because of novel sequence determinants in peroxisome proliferator signaling (53) and other complex cross-talk mechanisms that control transcription (54). For example, SRC-1 null mice are viable and fertile and exhibit only a subtle, or no, phenotypic alterations when evaluated for certain nuclear receptor functions (29, 55), suggesting functional redundancy among coactivators. On the other hand, mice with PBP (PPARBP) gene ablation exhibited embryonic lethality (56). Recently, we also found overexpression and amplification of the PBP gene in breast cancer (45). Another nuclear receptor coactivator, AIB1/ACTR (27, 28), was also found amplified and overexpressed in breast cancer (28). These observations raise interesting possibilities regarding the role of coactivators in cell proliferation, differentiation, and
neoplastic change. Additional studies are needed to determine the functional role of PRIP in embryonic development and whether this gene is also amplified and overexpressed in certain neoplasms.

Acknowledgments—We thank Dr. Ronald M. Evans for plasmids. We also appreciate the technical assistance of W. Cao and the secretarial support of Nancy Starks.

Note Added in Proof—After revision of our manuscript, we found that PRIP is identical to human nuclear receptor coactivator RAP250 (Caira, F., Antonson, P., Pello-Huikki, M., Treuter, E., and Gustafsson, J.-A. (2000) J. Biol. Chem. 275, 5308–5317) and ASC-2 (Lee, S.-K., Anzick, S. L., Choi, J.-E., Bubendorf, L., Guan, X.-Y., Jung, Y.-K., Kallioniemi, O. P., Kononen, J., Trent, J. M., Azorsa, D., Jhun, B.-H., Cheong, J. H., Lee, Y. C., Meltzer, P. S., and Lee, J. W. (1999) J. Biol. Chem. 274, 34283–34293).

REFERENCES
1. Issmann, J., and Green, S. (1990) Nature 347, 645–650
2. Chu, R., and Reddy, J. K. (1996) Ann. N. Y. Acad. Sci. 780, 176–201
3. Matsuzaki, D. J., Thummel, C., Beato, M., Herrlich, P., Schultz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., C., and Evans, R. M. (1993) Cell 73, 835–839
4. Reddy, J. K., and Lavalani, N. D. (1988) CRC Crit. Rev. Toxicol. 12, 1–58
5. Dreyer, C., Krey, G., Keller, H., Givel, F., Helftenbein, G., and Wahli, W. (1992) Cell 68, 879–887
6. Zhu, Y., Qi, C., Kurokawa, R. J., Chen, X.-N., Noya, D., Rao, M. S., and Reddy, J. K. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 7921–7925
7. Kliewer, S. A., Forman, B. M., Blumberg, B., Ong, E. S., Bergmeyer, U., Mangelsdorf, D. J., Umesono, K., and Evans, R. M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 7355–7359
8. Braissant, O., Foulle, F., Scotto, C., Dauma, M., and Wahli, W. (1996) Endocrinology 137, 354–366
9. Jain, S., Pulikuri, S., Zhu, Y., Qi, C., Kanwar, Y. S., Yeldandi, A. V., Rao, M. S., and Reddy, J. K. (1996) Am. J. Pathol. 148, 439–454
10. Lee, S.-S. T., Pineau, T., Drago, J., Lee, E., Owens, J. W., Kroetz, D. L., Forman, B. M., Chen, J., and Evans, R. M. (1997) Cell 88, 829–839
11. Fan, C.-Y., Pan, J., Usuda, N., Yeldandi, A. V., Rao, M. S., and Reddy, J. K. (1998) J. Biol. Chem. 273, 15639–15645
12. Forman, B. M., Chen, J., and Evans, R. M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 4133–4137
13. Kliewer, S. A., Sundet, S. S., Jones, S. A., Brown, P. J., Wisely, G. B., Koble, C. S., De Vechand, P., Wahl, W., Wilson, T. M., Lenhard, M. J., and Lehman, J. M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 4318–4323
14. He, T.-C., Chan, T. A., Vogelstein, B., and Kinzler, K. W. (1999) Cell 99, 335–345
15. Tsutsumi, T. H., Hu, E., and Spiegelman, B. M. (1994) Cell 79, 1147–1156
16. Barak, Y., Nelson, M. C., Ong, E. S., Jones, Y. Z., Ross, J. W., and Reddy, J. K. (1996) Am. J. Pathol. 148, 439–454
17. Katabi, N. T., Heine, M. J. S., Chambon, P., and Gronemeyer, H. (1990) Mol. Cell. Biol. 10, 187–204
18. Horlein, A. J., Naar, A. M., Heinzel, T., Torchia, J., Gershon, E., Kurokawa, R., Kloth, O., Kurokawa, R., and Reddy, J. K. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 3474–3479
19. Voegel, J. J., Heine, M. J. S., Zechel, C., Chambon, P., and Gronemeyer, H. (1999) Cell 97, 345–347
20. Palmer, C. N. A., Hsu, M.-H., Griffin, J. K., and Johnson, E. F. (1995) Science 268, 289–292
21. Matsuzaki, D. J., Thummel, C., Beato, M., Herrlich, P., Schultz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., C., and Evans, R. M. (1993) Cell 73, 835–839
22. Lee, Y. C., Meltzer, P. S., and Lee, J. W. (1999) J. Biol. Chem. 274, 34283–34293.
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Yijun Zhu, Lixin Kan, Chao Qi, Yashpal S. Kanwar, Anjana V. Yeldandi, M. Sambasiva Rao and Janardan K. Reddy

J. Biol. Chem. 2000, 275:13510-13516.
doi: 10.1074/jbc.275.18.13510

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