

# The Coactivator PGC-1 Cooperates with Peroxisome Proliferator-Activated Receptor $\alpha$ in Transcriptional Control of Nuclear Genes Encoding Mitochondrial Fatty Acid Oxidation Enzymes

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**Peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ) plays a key role in the transcriptional control of genes encoding mitochondrial fatty acid  $\beta$ -oxidation (FAO) enzymes. In this study we sought to determine whether the recently identified PPAR gamma coactivator 1 (PGC-1) is capable of coactivating PPAR $\alpha$  in the transcriptional control of genes encoding FAO enzymes. Mammalian cell cotransfection experiments demonstrated that PGC-1 enhanced PPAR $\alpha$ -mediated transcriptional activation of reporter plasmids containing PPAR $\alpha$  target elements. PGC-1 also enhanced the transactivation activity of a PPAR $\alpha$ -Gal4 DNA binding domain fusion protein. Retroviral vector-mediated expression studies performed in 3T3-L1 cells demonstrated that PPAR $\alpha$  and PGC-1 cooperatively induced the expression of PPAR $\alpha$  target genes and increased cellular palmitate oxidation rates. Glutathione *S*-transferase “pull-down” studies revealed that in contrast to the previously reported ligand-independent interaction with PPAR $\gamma$ , PGC-1 binds PPAR $\alpha$  in a ligand-influenced manner. Protein-protein interaction studies and mammalian cell hybrid experiments demonstrated that the PGC-1-PPAR $\alpha$  interaction involves an LXXLL domain in PGC-1 and the PPAR $\alpha$  AF2 region, consistent with the observed ligand influence. Last, the PGC-1 transactivation domain was mapped to within the NH<sub>2</sub>-terminal 120 amino acids of the PGC-1 molecule, a region distinct from the PPAR $\alpha$  interacting domains. These results identify PGC-1 as a coactivator of PPAR $\alpha$  in the transcriptional control of mitochondrial FAO capacity, define separable PPAR $\alpha$  interaction and transactivation domains within the PGC-1 molecule, and demonstrate that certain features of the PPAR $\alpha$ -PGC-1 interaction are distinct from that of PPAR $\gamma$ -PGC-1.**

The peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ) is a fatty acid-activated nuclear receptor that plays a key role in the transcriptional regulation of genes involved in cellular lipid and energy metabolism. PPAR $\alpha$  together with PPAR $\delta$  and PPAR $\gamma$  form a subgroup within the nuclear receptor superfamily (12, 17). In contrast to PPAR $\alpha$  which is involved in the control of cellular lipid utilization, PPAR $\gamma$  has been shown to be a necessary component of the adipocyte differentiation program (22, 36). The biological function of PPAR $\delta$  is unknown. A diverse group of compounds can act as activating ligands for PPAR $\alpha$  including several prostaglandin derivatives, eicosanoids, and long-chain unsaturated fatty acids (8, 18, 39). To date, the majority of PPAR $\alpha$  target genes identified are involved in cellular fatty acid oxidation (FAO) (22). We and others have previously demonstrated that PPAR $\alpha$  mediates fatty acid-induced transcriptional control of several nuclear genes encoding mitochondrial FAO enzymes, including medium-chain acyl coenzyme A (acyl-CoA) dehydrogenase (MCAD) (9) and muscle carnitine palmitoyltransferase I (M-CPT I or CPT I $\beta$ ) (2, 9, 26, 41). PPAR $\alpha$  is enriched in tissues with high oxidative energy demands that depend on mitochondrial FAO as a primary energy source such as heart and liver (17). PPAR $\alpha$  is also expressed at high levels in brown adipose tissue (BAT), a specialized tissue in which mitochondrial FAO provides the reducing equivalents necessary for the generation of heat via

the uncoupling of oxidative phosphorylation. Consistent with its regulatory role in mitochondrial FAO, the expression of PPAR $\alpha$  is much higher in BAT than in white adipose tissue, which is a lipid storage tissue (15, 36). Recent studies of PPAR $\alpha$ -null mice have confirmed that PPAR $\alpha$  is necessary in vivo for high-level expression of mitochondrial and peroxisomal FAO enzyme genes in heart and liver under basal and stimulated conditions (1, 7, 24).

Evidence has emerged that nuclear receptors regulate transcription, in large part, via interactions with coactivator (e.g., CBP/p300, SRC-1, GRIP1, pCIP) or corepressor (e.g., N-CoR, SMRT) molecules (4, 5, 10, 11, 14, 20). Nuclear receptor interacting proteins regulate transcriptional activity by affecting chromatin structure through changes in the acetylation status of histones. Most coactivators are recruited to nuclear receptors upon ligand binding. Several coactivators such as SRC-1, which possesses intrinsic histone acetylase activity, also serve as adaptor molecules to link nuclear receptors to multiprotein complexes containing larger pleiotropic activator proteins such as CBP or p300 (35, 37, 40). The ligand-mediated activation of PPARs also involves coactivator networks (28, 44). Crystallographic studies have demonstrated that the binding of ligand to PPAR stabilizes the position of an alpha-helical domain (the AF2 helix) forming a “charge clamp” that interacts with an LXXLL motif within coactivator molecules (28). Indeed, SRC-1 has been shown to interact with the PPARs upon ligand binding leading to transcriptional activation (44). However, in vivo disruption of SRC-1 does not appear to impair PPAR $\alpha$ 's ability to respond to its ligand activators (32). Accordingly,

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other coactivator molecules must exist to mediate transactivation by PPAR $\alpha$ .

Recently the PPAR gamma coactivator 1, or PGC-1, was cloned based on its BAT-enriched expression and ability to bind and coactivate PPAR $\gamma$  (31). In contrast to most nuclear receptor-coactivator interactions, PGC-1 was shown to interact with PPAR $\gamma$  in a ligand-independent manner. Interestingly, PGC-1 exhibits a tissue-enriched expression pattern with abundant levels in tissue types with high capacity for mitochondrial FAO such as BAT and heart (31). In addition, PGC-1 was shown to be markedly induced in BAT upon cold exposure (31), suggesting that it transduces physiologic stimuli to the transcriptional control of genes involved in thermogenesis. Given the key role of mitochondrial FAO in the thermogenic process in BAT and its importance in cardiac energy production, we hypothesized that PGC-1 cooperates with PPAR $\alpha$  to regulate mitochondrial FAO enzyme gene expression. We show here that PGC-1 interacts with PPAR $\alpha$  to coactivate target genes involved in mitochondrial FAO. Surprisingly, in contrast to the PGC-1-PPAR $\gamma$  interaction, PGC-1 binds PPAR $\alpha$  in a ligand-influenced manner and the PPAR $\alpha$  binding domains within the PGC-1 molecule are at least partially distinct from that reported for PPAR $\gamma$ . Moreover, we show that PGC-1 domains critical for PPAR $\alpha$  interaction and transcriptional activation are distinct and separable. These results establish a role for PGC-1 as a PPAR $\alpha$  coactivator in the control of cellular FAO.

#### MATERIALS AND METHODS

**Plasmid constructs.** (i) **Mammalian expression vectors.** CDMRXR $\alpha$  and CDMPPAR $\alpha$  have been described elsewhere (9). A *Hind*III fragment from pSVSport.PGC-1 (a gift from Bruce Spiegelman, Harvard Medical School) was cloned into pcDNA3.1myc/his (Invitrogen) to give myc/his.PGC-1, which contains amino acids 1 to 794 of PGC-1. PGC-1<sub>LXXFF</sub> was created by PCR-based mutagenesis and cloned into pcDNA3.1myc/his at the *Hind*III site to give a construct identical to the wild type except for the LKKLL-to-LKKFF mutation described in Results. The PGC-1 deletion series (PGC<sub>338</sub>, PGC<sub>284</sub>, and PGC<sub>120</sub>) was created by PCR which introduced a *Bgl*II site at the start of the coding sequence and a stop sequence at codons 339, 285, and 120. The resultant PCR products were cloned into pCMV-Tag1 (Stratagene) which fuses a FLAG epitope at the 5' end of the PGC-1 deletions. The PGC-1 deletions were then subcloned into pcDNA3.1 (Invitrogen) and used for subsequent transfection studies. The Gal4-PGC-1 constructs were created by subcloning a *Bam*HI fragment from the pcDNA3.1-PGC-1 plasmids into pCMX-Gal4 (a gift from David D. Moore, Baylor University). PPAR $\alpha$  $\Delta$ AF2 was generated by PCR which introduced a stop codon at codon 445, deleting the carboxy-terminal 18 amino acids. PPAR $\alpha$  and PPAR $\alpha$  $\Delta$ AF2 were subcloned into the *Eco*RI site of pCMX-Gal4 to yield constructs expressing amino acids 25 to 462 and 25 to 444 of PPAR $\alpha$ , respectively, fused to the Gal4 DNA binding domain (Gal4DBD).

(ii) **Reporter constructs.** MCPTL.Luc.781 has been described elsewhere (2). (PPRE)<sub>3</sub>TKLuc contains three copies of a known PPAR $\alpha$  response element derived from the peroxisomal acyl-CoA oxidase gene promoter (5'-TTCCGAA CGTGACCTTTGTCTCCGGTCCCTTTA-3') cloned into the *Bam*HI site of a construct containing the herpes simplex virus thymidine kinase promoter linked to the luciferase gene (TKLuc). The TKLuc and Gal4TKLuc vectors were gifts from David D. Moore.

(iii) **Retroviral expression vectors.**  $\Delta$ U3nlsLacZ (a gift from Daniel S. Ory, Washington University School of Medicine) has been described previously (29). PPAR $\alpha$  and PGC-1 were cloned into the *Nco*I/*Bam*HI sites of the  $\Delta$ U3 vector to give  $\Delta$ U3-PPAR $\alpha$  and  $\Delta$ U3-PGC-1, respectively.

(iv) **Bacterial expression vectors.** A *Bam*HI/*Not*I fragment was isolated from pcDNA-PGC<sub>338</sub>, -PGC<sub>284</sub>, and -PGC<sub>120</sub> and subcloned into pGEX4T-3 (Pharmacia) to give GST-PGC<sub>338</sub>, GST-PGC<sub>284</sub>, and GST-PGC<sub>120</sub>, respectively. An *Eco*RI fragment from either pcDNA-PGC-1 or pcDNA-PGC-1<sub>LXXFF</sub> was subcloned into pGEX4T-3 to yield the GST.PGC<sub>190</sub> or GST.PGC<sub>LXXFF</sub> construct, respectively.

**Protein-protein interaction studies.** The glutathione *S*-transferase (GST) fusion proteins were produced in bacteria according to the manufacturer's instructions (Pharmacia). <sup>35</sup>S-labeled PPAR $\alpha$ ,  $\Delta$ AF2,  $\Delta$ EF, and  $\Delta$ DEF were produced in the TNT T7 Quick coupled in vitro transcription/translation system (Promega). For the "pull-down" assays, 50  $\mu$ l of a 50% slurry of GST fusion protein bound to glutathione beads was resuspended in 500  $\mu$ l of binding buffer (20 mM Tris [pH 7.5], 100 mM KCl, 0.1 mM EDTA, 0.05% Nonidet P-40, 10% glycerol, bovine serum albumin [BSA] [1 mg/ml], and 0.1 mM phenylmethylsulfonyl flu-

oride [PMSF]). Ligand was added where indicated. <sup>35</sup>S-labeled PPAR $\alpha$  or PPAR $\alpha$  $\Delta$ AF2 was added to the resuspended GST fusion proteins and incubated at room temperature for 1 h. The beads were spun down and washed three times in binding buffer alone or binding buffer with ligand. An equal volume of sample reducing buffer was added and boiled for 3 min. The samples were then resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

**Cell culture and transfection studies.** 3T3-L1 cells preadipocytes were maintained in Dulbecco's modified Eagle's medium (DME) and 10% calf serum as described previously (3). At confluency, the medium was changed to DME containing 10% fetal calf serum supplemented with 10  $\mu$ g of insulin per ml, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), and 1  $\mu$ M dexamethasone for the first 48 h. Thereafter, cells were placed in DME containing 10% fetal calf serum supplemented with 2.5  $\mu$ g of insulin per ml every 48 h. CV-1 cells were maintained and transfected as previously described (23). Cells were transfected by the calcium phosphate coprecipitation method. Briefly, 4  $\mu$ g of reporter construct and 500 ng of each expression construct or expression construct without insert was used. The day after transfection, oleic acid complexed to BSA (250  $\mu$ M), 5,8,11,14-eicosate traynoic acid (ETYA) (10  $\mu$ M), or vehicle control was added to the cells. The cells were harvested 24 h later in cell lysis buffer (Promega), and luciferase activity was measured as previously described. All transfection data are presented as means ( $\pm$  standard errors of means) of at least three separate transfection experiments done in triplicate.

**Retroviral infection.** Production of recombinant Moloney murine leukemia virus (MMLV) was used for retroviral transfer as described elsewhere (29). Expression of the cDNA is driven by a promoter derived from the MMLV long terminal repeat region. Briefly, virus production was carried out in the packaging 293GP cell line. Retroviral expression constructs were transiently transfected into the cells by use of Lipofectamine (Gibco BRL) per the manufacturer's instructions. Virus produced from this transient transfection was used to infect 293GP cells to produce a stable population of virus-producing cells. Virus produced from these cells was concentrated by centrifugation at 25,000  $\times$  g for 90 min. Concentrated virus was resuspended in 3T3-L1 growth medium. A single 8-h exposure of subconfluent cells to virus resulted in greater than 95% infection efficiency as judged by 5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactopyranoside (X-Gal) staining of cells infected with virus producing LacZ. Infected cells were grown to confluency and induced to differentiate as described above. At day 6 after the addition of differentiation media, ETYA or dimethyl sulfoxide (DMSO) vehicle was added to the cells.

**RNA blot analyses.** Total RNA was isolated from virus-infected cells 48 h later using the RNazol (Tel-Test, Inc.) method. Northern blot analysis was performed with QuikHyb (Stratagene) using random-primed <sup>32</sup>P-labeled cDNA probes. cDNA probes encoding mouse MCAD, rat long-chain acyl-CoA dehydrogenase (LCAD) (a gift from Bryan Hainline, Indiana University), rat L-CPT I, mouse PPAR $\alpha$ , and mouse PGC-1.

[<sup>14</sup>C]palmitate oxidation studies. Measurements of palmitate oxidation were performed as described elsewhere (9). Briefly, 3T3-L1 preadipocytes seeded in 25-cm<sup>2</sup> flasks were infected as described above with recombinant retroviral particles encoding a LacZ control, PPAR $\alpha$ , PGC-1, or PPAR $\alpha$  and PGC-1. Seventy-two hours later, [<sup>14</sup>C]palmitate (American Radiolabeled Chemicals, St. Louis, Mo.) was added to a final concentration of 200 nCi/ml. The flasks were sealed and fitted with a center well containing a piece of Whatman no. 1 filter paper (1 in. by 1.5 in.). After 6 h, the <sup>14</sup>CO<sub>2</sub> was released from the culture medium by acidification with 2 ml of 6 N HCl. The <sup>14</sup>CO<sub>2</sub> was collected overnight by alkalization of the filter paper with 250  $\mu$ l of 2 N NaOH. <sup>14</sup>CO<sub>2</sub> was then measured by scintillation counting of the filters. The measurements presented are a compilation of three separate experiments performed in duplicate or triplicate. Statistical analysis was performed using analysis of variance coupled with the Scheffe test.

#### RESULTS

**PGC-1 enhances PPAR $\alpha$ -mediated transcriptional activation.** To examine the effect of PGC-1 on PPAR $\alpha$  activity, cotransfection studies were performed in the CV-1 cell line. A target reporter plasmid containing a PPAR $\alpha$  response element (PPRE) derived from the peroxisomal acyl-CoA oxidase gene promoter multimerized upstream of the thymidine kinase minimal promoter [(PPRE)<sub>3</sub>TKLuc] was employed in these studies. Cotransfection of expression vectors for RXR $\alpha$ /PPAR $\alpha$  or PGC-1 had no effect on a reporter plasmid lacking the PPRE (TKLuc) (Fig. 1). As expected, cotransfection of expression plasmids for RXR $\alpha$  and PPAR $\alpha$  activated (PPRE)<sub>3</sub>TKLuc more than sixfold, an effect that was further enhanced by addition of the PPAR $\alpha$  ligand, oleic acid (Fig. 1A). Cotransfection of a PGC-1 expression vector with the RXR $\alpha$ /PPAR $\alpha$  expression plasmids increased the level of PPAR $\alpha$ -mediated activation of the reporter both in the presence and absence of

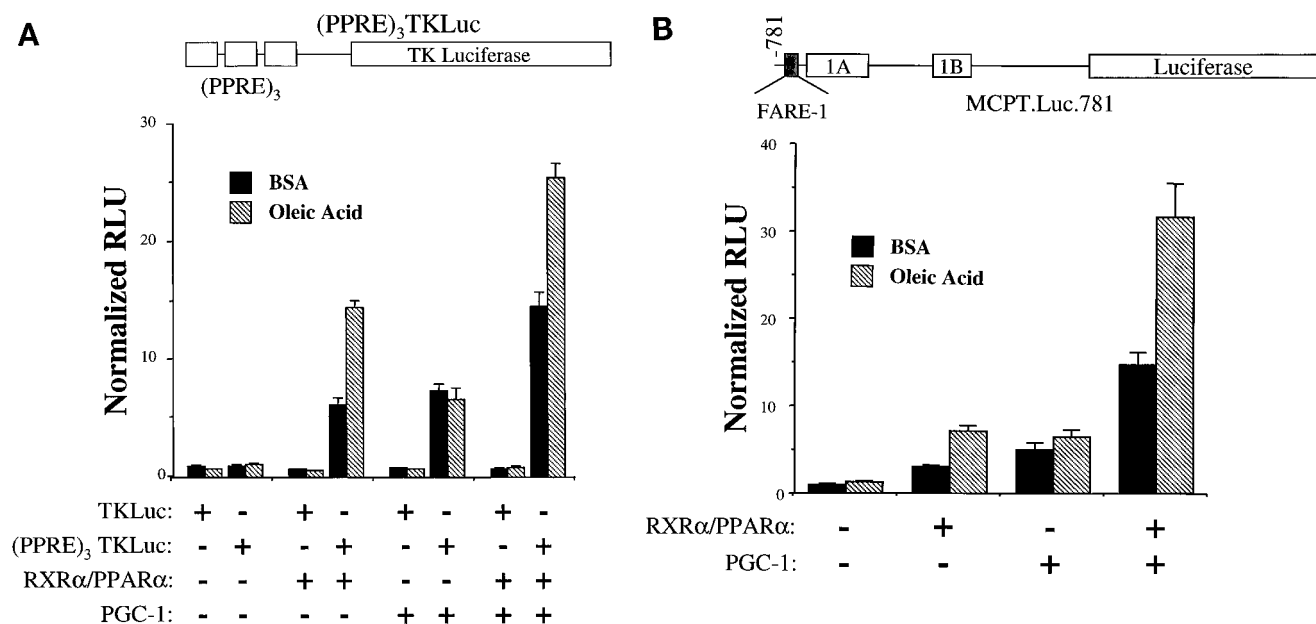


FIG. 1. PGC-1 enhances PPAR $\alpha$ -mediated transactivation. The heterologous promoter reporter construct (PPRE)<sub>3</sub>TKLuc (A) or the homologous promoter reporter MCPT.Luc.781 (B) was transiently transfected into CV-1 cells. Expression constructs encoding RXR $\alpha$ /PPAR $\alpha$  and/or PGC-1, were cotransfected in the presence of BSA vehicle or oleic acid (250  $\mu$ M) complexed to BSA as indicated. Bars represent mean ( $\pm$  standard error) relative luciferase units (RLU) normalized (=1.0) to the activity of (PPRE)<sub>3</sub>TKLuc (A) or MCPT.Luc.781 (B) cotransfected with expression vector backbone in the absence of ligand. All transfection data represent the means of at least three independent experiments.

oleic acid (Fig. 1A). Cotransfection of PGC-1 in the absence of cotransfected RXR $\alpha$ /PPAR $\alpha$  also resulted in a reproducible albeit lower magnitude activation of (PPRE)<sub>3</sub>TKLuc, an effect that may be mediated through interactions with endogenous nuclear receptors other than PPAR $\alpha$  because oleic acid had no effect on this activity (Fig. 1A).

The enzyme carnitine palmitoyltransferase I (CPT I) catalyzes the rate-limiting step in the import of long-chain fatty acids into the mitochondrion prior to entering the FAO cycle. We and others have shown that the gene encoding muscle CPT I (M-CPT I) is a PPAR $\alpha$  target (2, 26, 41). A reporter construct containing a portion of the human M-CPT I gene promoter (MCPT.Luc.781) containing the known PPAR $\alpha$  response element, FARE-1 (2), was employed to further examine the coregulatory effect of PGC-1 with PPAR $\alpha$  on a bona fide target gene. As expected, RXR $\alpha$ /PPAR $\alpha$  activated the transcription of MCPT.Luc.781, an effect that was enhanced by the addition of oleic acid (Fig. 1B). As with the heterologous PPAR $\alpha$  target construct, cotransfection of PGC-1 activated transcription of MCPT.Luc.781 in the absence or presence of exogenous RXR $\alpha$ /PPAR $\alpha$ . However, in this case, a clear synergistic effect was seen between PGC-1 and RXR $\alpha$ /PPAR $\alpha$  in the presence of PPAR $\alpha$  ligand.

**PPAR $\alpha$  and PGC-1 cooperate to increase mitochondrial FAO enzyme gene expression and cellular FAO rates.** To determine whether PGC-1 coactivates the PPAR $\alpha$ -mediated up-regulation of mitochondrial FAO enzyme gene expression, PPAR $\alpha$  and PGC-1 were ectopically overexpressed alone or together in the 3T3-L1 cell line, using a retroviral expression system. Upon differentiation, 3T3-L1 cells most closely resemble the white adipocyte, a cell with inherent low expression of mitochondrial FAO enzymes. 3T3-L1 preadipocytes were infected with recombinant retroviral particles encoding LacZ (control), PPAR $\alpha$ , PGC-1, or PPAR $\alpha$  and PGC-1. Each condition was evaluated in the presence or absence of the known PPAR $\alpha$  activator, ETYA. Following infection, the cells were

switched to differentiation media (see Materials and Methods), and RNA blot analysis was performed to examine the level of expression of PPAR $\alpha$ , PGC-1, and several mitochondrial FAO enzyme genes (MCAD, LCAD, and CPT I). The retrovirus-mediated expression of PGC-1 and PPAR $\alpha$  mRNA and proteins was documented by RNA blot analysis (Fig. 2) and immunoblotting studies (data not shown), respectively. Independent expression of either PPAR $\alpha$  or PGC-1 led to a modest increase in the levels of mRNAs encoding MCAD, LCAD, and CPT I compared to cells infected with the retroviral backbone alone (Fig. 2). The induction conferred by PPAR $\alpha$  was greatest with the addition of the exogenous PPAR $\alpha$  ligand, ETYA. However, coexpression of both PPAR $\alpha$  and PGC-1 led to a marked coordinate increase in the levels of the FAO enzyme mRNAs in the absence or presence of PPAR $\alpha$  ligand (Fig. 2). The expression of the PPAR $\gamma$  gene was downregulated in cells overexpressing PGC-1, indicating that the observed effects were not a result of a PGC-1-PPAR $\gamma$  interaction. These results demonstrate that PPAR $\alpha$  and PGC-1 cooperatively induce PPAR $\alpha$  target genes involved in mitochondrial FAO.

To determine whether cellular FAO rates were increased by the cooperative action of PPAR $\alpha$  and PGC-1, palmitate oxidation studies were performed in cells infected with the retroviral vectors described above. Flux through the mitochondrial FAO pathway was determined by measurement of the rate of release of <sup>14</sup>CO<sub>2</sub> from 3T3-L1 preadipocytes following incubation with [1-<sup>14</sup>C]palmitate. For these studies, 3T3-L1 preadipocytes were used to avoid dilution of labeled palmitate with intracellular long-chain fatty acid known to be present in lipid droplets within adipocytes. The amount of <sup>14</sup>CO<sub>2</sub> produced following a 6-h incubation with [1-<sup>14</sup>C]palmitate was significantly greater in cells overexpressing both PGC-1 and PPAR $\alpha$  compared to either alone (Fig. 3). These results are consistent with that of the gene expression studies shown in Fig. 2 and

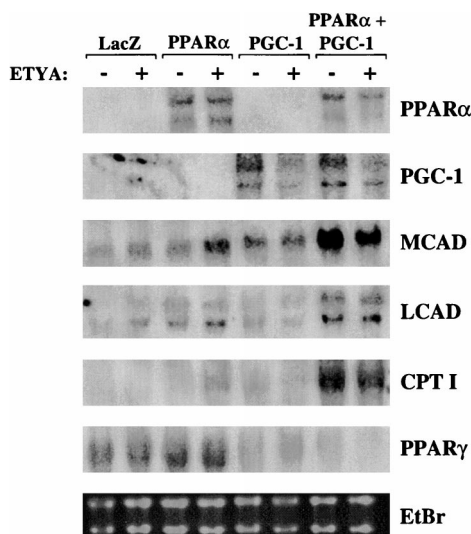


FIG. 2. PPAR $\alpha$  and PGC-1 cooperate to induce PPAR $\alpha$  gene target markers of the mitochondrial FAO pathway. Autoradiographs of Northern blot analysis performed with total RNA (15  $\mu$ g) isolated from 3T3-L1 preadipocytes infected with recombinant retroviral particles encoding LacZ, PPAR $\alpha$ , PGC-1, or PPAR $\alpha$  and PGC-1 as indicated at the top are shown. Cells were grown to confluence and induced to differentiate as described in Materials and Methods. Six days after addition of differentiation media, ETYA (+) or vehicle control (-) was added. RNA was isolated 48 h after addition of ligand or vehicle. The blot was hybridized with the radiolabeled cDNA probes indicated on the right. The ethidium bromide (EtBr)-stained RNA is included as a control for loading and RNA integrity.

indicate that PPAR $\alpha$  and PGC-1 cooperatively increase cellular long-chain FAO capacity.

**PPAR $\alpha$  binds PGC-1 in a ligand-influenced manner.** A series of in vitro GST pulldown assays were performed to confirm a direct interaction between PPAR $\alpha$  and PGC-1 and to map the corresponding interaction domains. A recent study demonstrated that PGC-1 interacts with PPAR $\gamma$  in a ligand-independent manner via a region located between amino acids 292 and 338 (31). Based on this information, we constructed several GST-PGC-1 fusion proteins (Fig. 4A) to be used in

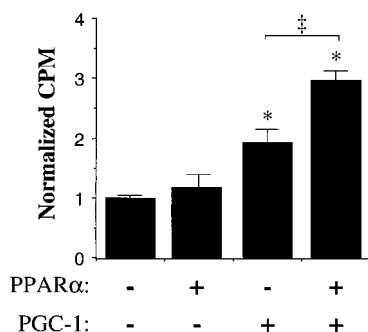


FIG. 3. PPAR $\alpha$  and PGC-1 increase cellular palmitate oxidation rates. Palmitate oxidation studies were performed on 3T3-L1 preadipocytes in culture infected with retroviral vectors expressing LacZ (control), PPAR $\alpha$ , PGC-1, or PPAR $\alpha$  and PGC-1 as described in Materials and Methods. Following incubation of the cells with [ $^{14}$ C]palmitate for 6 h, the amount of  $^{14}$ CO $_2$  liberated from the cells was measured by scintillation counting. The bars represent mean ( $\pm$  standard error)  $^{14}$ CO $_2$  (in counts per minute) normalized (=1.0) to the mean value obtained with the LacZ-infected control cells. An asterisk denotes a significant difference ( $P < 0.01$ ) compared to the control value. The double dagger denotes a significant difference ( $P < 0.01$ ) between the values for PPAR $\alpha$  without and with PGC-1.

pulldown studies with  $^{35}$ S-methionine-labeled, in vitro-translated PPAR $\alpha$ . Initial experiments were performed with a GST-PGC fusion protein containing 338 amino acids of PGC-1 including the PPAR $\gamma$  binding domain (GST.PGC $_{338}$  [Fig. 4A]). A modest interaction between GST.PGC $_{338}$  and PPAR $\alpha$  was detected in the absence of ligand (Fig. 4A). However, addition of the PPAR $\alpha$  ligand ETYA significantly increased the PPAR $\alpha$ -GST-PGC $_{338}$  interaction (Fig. 4A). Surprisingly, strong, ligand-influenced PPAR $\alpha$  binding was also observed with a PGC-1 deletion mutant lacking the PPAR $\gamma$  binding domain (GST.PGC $_{284}$ ), indicating that in contrast to PPAR $\gamma$  (31), the region between amino acids 284 and 338 does not play a significant role in the interaction between PPAR $\alpha$  and PGC-1. However, further deletion from amino acids 284 to 120 (GST.PGC $_{120}$ ), abolished the PGC-1-PPAR $\alpha$  interaction in the presence or absence of ligand. These results indicate that a critical PPAR $\alpha$  binding domain exists in a region located between amino acids 120 and 284 of the PGC-1 molecule. Moreover, these data identify two stark differences between the nature of the interaction between PGC-1 and PPAR $\alpha$  compared with that of PPAR $\gamma$ . First, whereas the PGC-1-PPAR $\gamma$  interaction is ligand independent (31), the binding of PPAR $\alpha$  by PGC-1 is increased by ligand. Second, the PGC-1 domains required for the interaction with PPAR $\gamma$  and PPAR $\alpha$  are distinct.

As demonstrated in Fig. 4A, ligand potentiated the interaction between PPAR $\alpha$  and PGC-1. The AF2 domain of nuclear receptors has been shown to mediate ligand-responsive interactions with coactivators through LXXLL motifs present in the latter. PGC-1 contains one such LXXLL sequence located at amino acids 142 to 146 (LKKLL). As shown in Fig. 4A, deletion of a region (amino acids 120 to 184) of the PGC-1 molecule which contains an LKKLL motif abolished the interaction between PPAR $\alpha$  and PGC-1. Given these results, we predicted that the PPAR $\alpha$ -PGC-1 interaction required the PPAR $\alpha$  AF2 domain. To explore this possibility, GST pulldown experiments were performed with a PPAR $\alpha$  deletion mutant lacking the AF2 helical domain ( $\Delta$ AF2). GST-PGC $_{284}$  was used in these experiments. In contrast to the full-length PPAR $\alpha$ , the interaction of GST-PGC-1 $_{284}$  with the  $\Delta$ AF2 PPAR $\alpha$  protein was markedly diminished and was not enhanced by ligand (Fig. 4B). Further removal of the ligand binding domain ( $\Delta$ EF) of PPAR $\alpha$  did not change the PGC-1 interaction binding pattern compared to that of the  $\Delta$ AF2 protein. However, removal of the D domain ( $\Delta$ DEF) abolished the residual, ligand-independent PGC-1 binding. Interestingly, the D domain of PPAR $\gamma$  was shown previously to be required for binding to PGC-1 (31). These results demonstrate a key role for the PPAR $\alpha$  AF2 domain in the interaction with PGC-1 and identify both ligand-influenced and ligand-independent PGC-1 interaction regions within the PPAR $\alpha$  molecule.

To explore further the role of the LXXLL domain of PGC-1 in binding with PPAR $\alpha$ , protein-protein interaction studies were repeated with a mutant PGC-1 protein fragment in which the LXXLL motif was mutated. For these experiments, PPAR $\alpha$  pulldowns were performed with a PGC-1-GST fusion protein containing the amino-terminal 190 amino acids, including the LXXLL domain (GST.PGC $_{190}$ ), or a mutant GST.PGC $_{190}$  fragment in which LXXLL was changed to LXXFF (GST.PGC $_{LXXFF}$ ). As shown in Fig. 4C, the interaction of GST.PGC $_{LXXFF}$  with  $^{35}$ S-labeled PPAR $\alpha$  was markedly reduced compared with that of GST.PGC $_{190}$ . Moreover, ligand did not increase the GST.PGC $_{LXXFF}$ -PPAR $\alpha$  interaction. These results, which are consistent with the data shown in Fig. 4A and B, demonstrate that the PGC-1 LXXLL motif is a critical participant in the PGC-1-PPAR interaction.

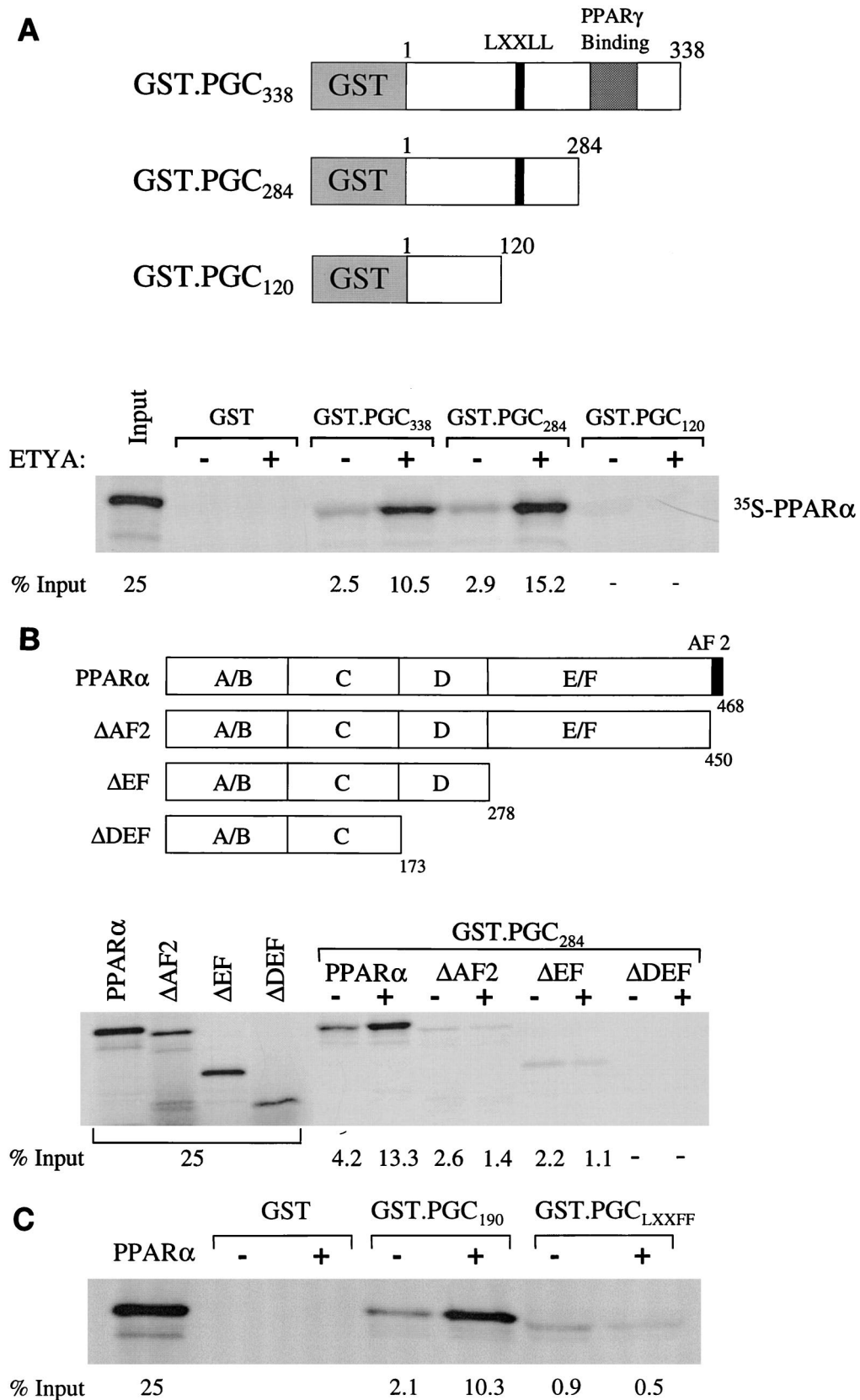


FIG. 4. PGC-1 interacts with PPAR<sub>α</sub>. (A) The GST-PGC-1 fusion proteins used for PPAR<sub>α</sub> pull-down assays are shown schematically at the top with numbers corresponding to the amino acids within the PGC-1 molecule (31). The locations of the domain necessary for PPAR<sub>γ</sub> binding (31) and the single LXXLL domain are also shown. Autoradiographs depicting the results of GST pull-down assays performed with <sup>35</sup>S-labeled PPAR<sub>α</sub> and several GST-PGC-1 fusion proteins or GST alone in the presence of the DMSO vehicle (-) or the PPAR<sub>α</sub> ligand ETYA (+) are shown at the bottom of each panel. The numbers below each pull-down product shown in the autoradiographs indicate the percent total input as determined by phosphorimager analysis. 25% of the input is shown for comparison. (B) Pull-down studies using <sup>35</sup>S-labeled PPAR<sub>α</sub> deletion mutant proteins (shown at the top) and GST-PGC<sub>284</sub>. (C) The results of pull-down studies performed with the GST.PGC<sub>190</sub> fusion protein and the LXXLL mutant, GST.PGC<sub>LXXFF</sub>.

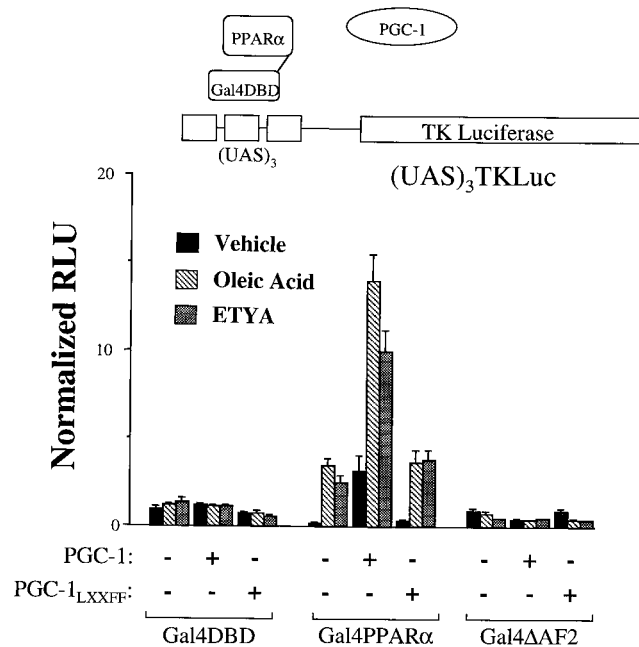


FIG. 5. Coactivation of PPAR $\alpha$  by PGC-1 requires intact AF2 and LXXLL motifs. To examine functional correlates of the GST pull-down interaction studies, a mammalian cell hybrid system was employed (shown schematically at the top). PPAR $\alpha$  or PPAR $\Delta$ AF2 was fused to the Gal4 DNA binding domain (DBD) and cotransfected with an expression plasmid encoding PGC-1 or a mutant PGC-1 in which the LXXLL motif was mutated (PGC<sub>LXXFF</sub>). A plasmid containing the Gal4 upstream activating sequence (UAS) multimerized upstream of TK luciferase [(UAS)<sub>3</sub>TKLuc] was used as a reporter in these experiments. Transfections were performed in the presence of the PPAR $\alpha$  ligands (oleic acid or ETYA) or vehicle controls. Bars represent mean RLU normalized (=1.0) to the value obtained with Gal4DBD cotransfected with expression plasmid backbone in the presence of vehicle.

**The AF2-LXXLL interaction is necessary for the coactivation of PPAR $\alpha$  by PGC-1.** To establish functional correlates of the protein-protein interaction studies shown in Fig. 4, a mammalian cell protein hybrid system was employed. This system also allowed a functional assessment of the PGC-1-PPAR $\alpha$  interaction in the absence of the background, PPAR $\alpha$ -independent, PGC-1-mediated activation observed in the cotransfection studies shown in Fig. 1. In these experiments we took advantage of the transcriptional activation properties of PGC-1. An expression vector for the full-length PGC-1 was cotransfected with an expression vector encoding PPAR $\alpha$  fused, in frame, to the Gal4 DNA binding domain (Gal4-PPAR $\alpha$ ) or the Gal4 DNA binding domain alone (Gal4DBD), along with a reporter construct containing three copies of the Gal4 binding site upstream of the TK minimal promoter, [(UAS)<sub>3</sub>TKLuc]. Gal4-PPAR $\alpha$ , in the absence of ligand, modestly repressed (UAS)<sub>3</sub>TKLuc (Fig. 5). Addition of the PPAR $\alpha$  ligands (oleic acid or ETYA) activated (UAS)<sub>3</sub>TKLuc only in the presence of Gal4-PPAR $\alpha$ . Addition of PGC-1 markedly increased the transcriptional activation by Gal4-PPAR $\alpha$  in the presence or absence of ligand (Fig. 5), whereas PGC-1 had no effect on the Gal4DBD alone.

To determine whether as predicted by the results of the GST pull-down experiments, the PPAR $\alpha$ -PGC-1 interaction was mediated by the AF2 domain of PPAR $\alpha$  and the LXXLL motif of PGC-1, the effect of deleting or mutating each of these domains on the PGC-1-mediated coactivation of PPAR $\alpha$  was evaluated. As expected, a Gal4-PPAR $\alpha$  fusion lacking the AF2 activation domain (Gal4- $\Delta$ AF2) was unresponsive to PPAR $\alpha$

ligand (Fig. 5) and was unable to confer the coactivation by PGC-1. Conversely, mutation of the LXXLL motif within the full-length PGC-1 (PGC-1<sub>LXXFF</sub>) prevented the PGC-1-mediated activation of PPAR $\alpha$ , either in the presence or absence of ligand (Fig. 5). Taken together with the data shown in Fig. 4, these results demonstrate that the coactivation of PPAR $\alpha$  by PGC-1 involves an AF2-LXXLL interaction.

**Identification of a potent transactivation domain within the PGC-1 molecule.** In order to delineate the region of PGC-1 critical for its transactivating function, we evaluated the PPAR $\alpha$  coactivating function of a series of carboxy-terminal PGC-1 deletion mutants (Fig. 6A). Surprisingly, removal of the C-terminal region (PGC<sub>338</sub>) actually enhanced the PGC-1-mediated increase in ligand-dependent activation of Gal4-PPAR $\alpha$  (Fig. 6B). The portion of the molecule deleted contains a serine-arginine rich (SR) domain and a region which has similarity to RNA binding domains of other proteins. Further carboxy-terminal deletions, which included removal of the region shown previously to be involved in binding to PPAR $\gamma$  (PGC<sub>284</sub>), also increased PGC-1 activity but not to the same level as PGC<sub>338</sub>. A vector containing only the NH<sub>2</sub>-terminal 120 amino acids of PGC-1 (PGC<sub>120</sub>), however, had no effect on PPAR $\alpha$  activity. Parallel immunoblotting experiments performed with an antibody to the FLAG epitope confirmed that each of the PGC protein fragments was expressed (data not shown). These results indicate that a transactivation function is conferred by the NH<sub>2</sub>-terminal 284 amino acids of the PGC-1 molecule.

The data shown in Fig. 6B identify a transactivating function in the NH<sub>2</sub>-terminal region of PGC-1 but do not distinguish between the action of a distinct transactivation domain and the PPAR $\alpha$  binding function which was mapped to this region. Accordingly, to further delineate the PGC-1 transactivation domain, PGC<sub>797</sub>, PGC<sub>284</sub>, and PGC<sub>120</sub> were fused to the Gal4DBD and were evaluated in a one-hybrid assay in the CV-1 cell line. This strategy allowed us to evaluate the PGC-1 transactivating function independent of the interaction with PPAR $\alpha$ . Whereas PGC<sub>120</sub> had no activity in the mammalian two-hybrid system (Fig. 6B), Gal4-PGC<sub>120</sub> exhibited a potent transcriptional activation function (Fig. 6C), despite the fact that this region lacks the PPAR $\alpha$  binding domain. These data demonstrate that the NH<sub>2</sub>-terminal 120 amino acids of PGC-1 comprises a potent activation region which is distinct and separable from the PPAR $\alpha$  interacting domain.

## DISCUSSION

Coactivator molecules play a critical role in the transcriptional activation of nuclear receptor target genes. A current challenge in the understanding of nuclear receptor biology is to elucidate the mechanisms involved in the receptor-coactivator interaction. PGC-1 is a coactivator molecule identified recently based on its ability to interact with PPAR $\gamma$  (31). In this report, we extend the role of PGC-1 by demonstrating that it is a bona fide coactivator for PPAR $\alpha$  in the transcriptional control of genes involved in mitochondrial FAO. We also demonstrate that the interaction between PPAR $\alpha$  and PGC-1 is influenced by ligand and involves domains distinct from that described previously for the PPAR $\gamma$ -PGC-1 interaction. Finally, a potent transactivation domain separable from the PPAR $\alpha$  interaction domains has been identified within the PGC-1 molecule.

PGC-1, a new member of the rapidly growing list of nuclear receptor coactivators, has several unique characteristics. First, in contrast to most coactivators reported to date, PGC-1 exhibits a tissue-restricted expression pattern. Second, PGC-1 expression is induced by physiologic stimuli; PGC-1 mRNA

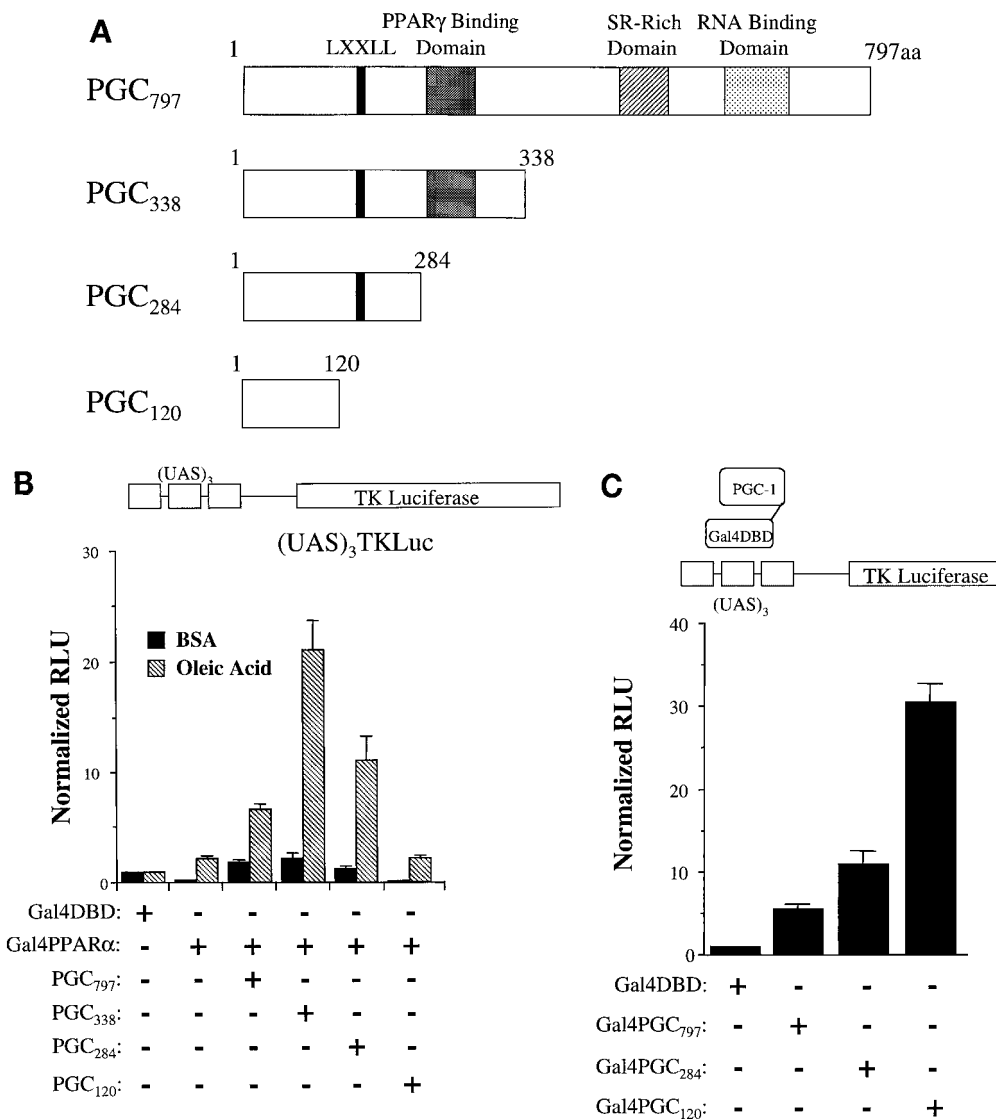


FIG. 6. The NH<sub>2</sub>-terminal region of PGC-1 is required for transactivation function. (A) Schematic representations of PGC-1 deletion mutants used in the transactivation studies shown in Fig. 6B and 7. aa, amino acids. A region homologous with known RNA binding domains and a serine-arginine (SR)-rich domain are shown. (B) Gal4-PPAR $\alpha$  was cotransfected with expression vectors into CV-1 cells for each of the PGC-1 deletion mutants shown in panel A in the presence or absence of oleic acid. Bars represent RLU normalized (=1.0) to the activity of the (UAS)<sub>3</sub>TKLuc reporter cotransfected with Gal4DBD and empty PGC-1 expression vector. (C) Expression vectors encoding PGC-1 deletion mutants (Fig. 6A), fused to the Gal4DBD, were cotransfected with the (UAS)<sub>3</sub>TKLuc reporter plasmid (one-hybrid assay). The values represent RLU normalized (=1.0) to that of the Gal4DBD alone.

levels increase dramatically upon cold exposure in tissues with a role in heat production, namely, BAT and skeletal muscle (31). We have also found that fasting induces PGC-1 gene expression in heart (J. J. Lehman, T. C. Leone, and D. P. Kelly, unpublished data). These observations suggest that PGC-1 transduces extracellular stimuli to the transcriptional control of genes involved in cellular energy metabolism. The observation that PGC-1 expression is induced by fasting and cold exposure, physiologic conditions known to increase cellular lipid utilization, suggested that PGC-1 may function as a regulator of mitochondrial  $\beta$ -oxidation. Accordingly, we explored the possibility that PGC-1 could serve as a coactivator for PPAR $\alpha$ , a key factor in the transcriptional control of the mitochondrial FAO pathway (2, 9, 21). Our results indicate that PGC-1 is indeed capable of enhancing PPAR $\alpha$ -mediated transactivation based on the following observations. (i) PGC-1

interacts directly with PPAR $\alpha$  in GST pulldown assays and mammalian protein hybrid studies. (ii) PGC-1 coactivates PPAR $\alpha$ -mediated transactivation of known PPAR $\alpha$  target elements in homologous and heterologous promoter contexts. (iii) Ectopic overexpression of PPAR $\alpha$  and PGC-1 expression in 3T3-L1 cells, which have an inherently low capacity for FAO, cooperatively induces the expression of mitochondrial FAO enzyme genes and increases cellular palmitate oxidation rates. The coactivating effect of PGC-1 on PPAR $\alpha$  in the 3T3-L1 cells was independent of its known interaction with PPAR $\gamma$  given that the expression of the latter was downregulated in the PGC-1 overexpressing cells. The reason for the lower expression of PPAR $\gamma$  in the PGC-1-expressing cells is unclear but could be related to a squelching effect or a biologically relevant feedback inhibition. These results establish PGC-1 as a PPAR $\alpha$  coactivator in the control of mitochondrial

FAO enzyme gene expression. The high-level expression of PGC-1 in heart and BAT (31), tissues with high expression of mitochondrial FAO enzymes, is consistent with a role for this coactivator in the control of the FAO pathway.

Recently, PGC-1 was shown to induce the expression of genes encoding mitochondrial proteins involved in electron transport, mitochondrial number, and cellular mitochondrial DNA content (31, 38). These results are indicative of mitochondrial biogenesis. In further support of a role for PGC-1 in the control of mitochondrial oxidative capacity, the results of our cellular oxidation studies demonstrated a significant increase in the oxidation of palmitate to CO<sub>2</sub> in cells overexpressing both PPAR $\alpha$  and PGC-1, consistent with an increase in both FAO and tricarboxylic acid cycle flux, two key mitochondrial pathways. We propose that PGC-1 serves as the elusive link between the gene regulatory pathway involved in the transcriptional control of nuclear genes encoding mitochondrial FAO enzymes and the broad program of mitochondrial biogenesis in tissues with high fatty acid utilization rates such as heart and BAT.

Our results demonstrate several surprising differences in the nature of the interaction of PGC-1 with PPAR $\alpha$  compared to that of PPAR $\gamma$ . In contrast to the ligand-independent interaction of PGC-1 with PPAR $\gamma$  (31), we found that ligand influences the PPAR $\alpha$ -PGC-1 interaction in GST pulldown studies. Cell cotransfection experiments demonstrated that the AF-2 domain of PPAR $\alpha$  and the LXXLL domain of PGC-1 were required for the cooperative PPAR $\alpha$ -PGC-1 interaction. We also found that the previously defined PPAR $\gamma$  interaction domain within the PGC-1 molecule was dispensable for the PPAR $\alpha$  interaction, consistent with the differences in ligand dependence. However, we did not observe a strong dependence on exogenous PPAR ligand in the cotransfection experiments shown in Fig. 1. This discrepancy may be explained by the presence of endogenous ligand in the cell culture media or in the CV-1 cells. Alternatively, the PGC-1-PPAR $\alpha$  interaction could occur via both ligand-independent and ligand-influenced mechanisms.

The primary structure of PGC-1 provides few clues as to how it activates transcription. Many coactivator molecules contain histone acetylase (HAT) activity which is thought to be critical to the transcriptional activation function. However, the PGC-1 molecule does not contain significant amino acid sequence similarity with any known histone acetylase. PGC-1 does contain an SR-rich region juxtaposed to a second region with homology to RNA binding domains of other proteins. These two domains are seen in the SR family of splicing factors as well as a group of proteins which interact with the C-terminal domain of RNA polymerase II (25, 30, 42). However, these latter regions were not necessary for transactivation function in our one-hybrid assay experiments. Rather, a separate domain comprised of the NH<sub>2</sub>-terminal 120 amino acids of the PGC-1 molecule was responsible for transactivation. The mechanism whereby the transactivation domain of PGC-1 exerts its effects is unknown. There are no similarities between this region of the molecule and any other published protein sequence. It is possible that this region contains HAT activity or binds to a protein with this activity such as CBP/p300. However, other proteins that interact simultaneously with nuclear receptors and other coactivators (e.g., SRC-1) usually contain multiple LXXLL motifs which mediate interactions between both sets of proteins (27). PGC-1 contains only one LXXLL sequence mitigating against the possibility that it interacts with both PPAR $\alpha$  and p300/CBP, although interaction with other coactivator proteins via novel domains is possible. Recently, several nuclear receptor coactivators have been found to exist in large

preformed complexes containing multiple proteins (13, 33). These complexes are likely involved in mediating activation from a variety of transcription factors in addition to nuclear receptors including p53, VP16, and NF- $\kappa$ B. The delineation of the precise mechanism involved in the PGC-1 transactivation function and its relationship to the recently described transcriptional regulatory complexes are important avenues for future studies.

Recent studies have indicated that PPAR $\alpha$  plays a pivotal role in the control of cellular fatty acid utilization pathways in response to diverse physiologic conditions including fasting (16, 19, 23), nutritional alterations (16), and aging (6). The PPAR $\alpha$  regulatory pathway has also been implicated in disease states including cardiac hypertrophy (34), obesity (6), and diabetes mellitus (19, 43). The factors involved in the modulation of PPAR $\alpha$  activity are largely unknown. Availability of ligand has been considered one potential key regulator of PPAR $\alpha$  activity. The identification of a PPAR $\alpha$  coactivator molecule that is induced by physiologic stimuli adds a new layer of regulatory complexity for PPAR $\alpha$  as well as for the entire nuclear receptor superfamily. We speculate that certain nuclear receptor coactivators, such as PGC-1, serve to transduce physiologic input to changes in gene expression. Our results identify the mitochondrial FAO enzyme genes as one candidate group of targets regulated by such a mechanism.

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#### ADDENDUM IN PROOF

During the final review of this paper, Puigserver et al. (Science 286:1368-1371, 1999) demonstrated that the PGC-1 transactivation domain interacts with the coactivator SRC-1.

#### REFERENCES

- Aoyama, T., J. M. Peters, N. Iritani, T. Nakajima, K. Furihata, T. Hashimoto, and F. J. Gonzalez. 1998. Altered constitutive expression of fatty acid-metabolizing enzymes in mice lacking the peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ). *J. Biol. Chem.* 273:5678-5684.
- Brandt, J., F. Djouadi, and D. P. Kelly. 1998. Fatty acids activate transcription of the muscle carnitine palmitoyltransferase I gene in cardiac myocytes via the peroxisome proliferator-activated receptor  $\alpha$ . *J. Biol. Chem.* 273:23786-23793.
- Cao, Z., R. M. Umek, and S. L. McKnight. 1991. Regulated expression of three C/EBP isoforms during adipose conversion of 3T3-L1 cells. *Genes Dev.* 5:1538-1552.
- Cavaillès, V., S. Dauvois, P. S. Danielian, and M. G. Parker. 1994. Interaction of proteins with transcriptionally active estrogen receptors. *Proc. Natl. Acad. Sci. USA* 91:10009-10013.
- Chen, J. D., K. Umeson, and R. M. Evans. 1996. SMRT isoforms mediate repression and anti-repression of nuclear receptor heterodimers. *Proc. Natl. Acad. Sci. USA* 93:7567-7571.
- Costet, P., C. Legendre, J. Moré, A. Edgar, P. Galtier, and T. Pineau. 1998. Peroxisome proliferator-activated receptor  $\alpha$ -isoform deficiency leads to progressive dyslipidemia with sexually dimorphic obesity and steatosis. *J. Biol. Chem.* 273:29577-29585.
- Djouadi, F., C. Weinheimer, J. E. Saffitz, C. Pitchford, J. Bastin, F. J. Gonzalez, and D. P. Kelly. 1998. A gender-related defect in lipid metabolism and glucose homeostasis in peroxisome proliferator-activated receptor  $\alpha$ -deficient mice. *J. Clin. Invest.* 102:1083-1091.
- Forman, B. M., J. Chen, and R. M. Evans. 1997. Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors  $\alpha$  and  $\delta$ . *Proc. Natl. Acad. Sci. USA* 94:4312-4317.



9. Gulick, T., S. Cresci, T. Cairra, D. D. Moore, and D. P. Kelly. 1994. The peroxisome proliferator activated receptor regulates mitochondrial fatty acid oxidative enzyme gene expression. *Proc. Natl. Acad. Sci. USA* **91**:11012–11016.
10. Halachmi, S., E. Marden, G. Martin, H. MacKay, C. Abbondanza, and M. Brown. 1994. Estrogen receptor-associated proteins: possible mediators of hormone-induced transcription. *Science* **264**:1455–1458.
11. Hong, H., K. Kohli, M. J. Garabedian, and M. R. Stallcup. 1997. GRIP1, a transcriptional coactivator for the AF-2 transactivation domain of steroid, thyroid, retinoid, and vitamin D receptors. *Mol. Cell. Biol.* **17**:2735–2744.
12. Issemann, I., and S. Green. 1990. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature* **347**:645–650.
13. Ito, M., C. X. Yuan, S. Malik, W. Gu, L. D. Fondell, S. Yamamura, Z. Y. Fu, X. Zhang, J. Qin, and R. G. Roeder. 1999. Identity between TRAP and SMCC complexes indicates novel pathways for the function of nuclear receptors and diverse mammalian activators. *Mol. Cell* **3**:361–370.
14. Janknecht, R., and T. Hunter. 1996. Transcription. A growing coactivator network. *Nature* **383**:22–23.
15. Jones, P. S., R. Savory, P. Barratt, A. R. Bell, T. Gray, N. A. Jenkins, D. J. Gilbert, N. G. Copeland, and D. R. Bell. 1995. Chromosomal localisation, inducibility, tissue-specific expression and strain differences in three murine peroxisome proliferator-activated receptor genes. *Eur. J. Biochem.* **233**:219–226.
16. Kersten, S., J. Seydoux, J. M. Peters, F. J. Gonzalez, B. Desvergne, and W. Wahli. 1999. Peroxisome proliferator-activated receptor  $\alpha$  mediates the adaptive response to fasting. *J. Clin. Invest.* **103**:1489–1498.
17. Kliewer, S. A., B. M. Forman, B. Blumberg, E. S. Ong, U. Borgmeyer, D. J. Mangelsdorf, K. Umeson, and R. M. Evans. 1994. Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. *Proc. Natl. Acad. Sci. USA* **91**:7355–7359.
18. Kliewer, S. A., S. S. Sundseth, S. A. Jones, P. J. Brown, G. B. Wisely, C. S. Koble, P. Devchand, W. Wahli, T. M. Willson, J. M. Lenhard, and J. M. Lehman. 1997. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors  $\alpha$  and  $\gamma$ . *Proc. Natl. Acad. Sci. USA* **94**:4318–4323.
19. Kroetz, D. L., P. Yook, P. Costet, P. Bianchi, and T. Pineau. 1998. Peroxisome proliferator-activated receptor  $\alpha$  controls the hepatic *CYP4A* induction adaptive response to starvation and diabetes. *J. Biol. Chem.* **273**:31581–31589.
20. Kurokawa, R., M. Soderstrom, A. Horlein, S. Halachmi, M. Brown, M. G. Rosenfeld, and C. K. Glass. 1995. Polarity-specific activities of retinoic acid receptors determined by a co-repressor. *Nature* **377**:451–454.
21. Lee, S. S. T., T. Pineau, J. Drago, E. J. Lee, J. W. Owens, D. L. Kroetz, P. M. Fernandez-Salguero, H. Westphal, and F. J. Gonzalez. 1995. Targeted disruption of the  $\alpha$  isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. *Mol. Cell. Biol.* **15**:3012–3022.
22. Lemberger, T., B. Desvergne, and W. Wahli. 1996. Peroxisome proliferator-activated receptors: a nuclear receptor signaling pathway in lipid physiology. *Annu. Rev. Cell Dev. Biol.* **12**:335–363.
23. Leone, T. C., S. Cresci, M. E. Carter, Z. Zhang, D. S. Lala, A. W. Strauss, and D. P. Kelly. 1995. The human medium chain acyl-CoA dehydrogenase gene promoter consists of a complex arrangement of nuclear receptor response elements and Sp1 binding sites. *J. Biol. Chem.* **270**:16308–16314.
24. Leone, T. C., C. J. Weinheimer, and D. P. Kelly. 1999. A critical role for the peroxisome proliferator-activated receptor alpha (PPAR $\alpha$ ) in the cellular fasting response: the PPAR $\alpha$ -null mouse as a model of fatty acid oxidation disorders. *Proc. Natl. Acad. Sci. USA* **96**:7473–7478.
25. Manley, J. L., and R. Tacke. 1996. SR proteins and splicing control. *Genes Dev.* **10**:1569–1579.
26. Mascaró, C., E. Acosta, J. A. Ortiz, P. F. Marrero, F. G. Hegardt, and D. Haro. 1998. Control of human muscle-type carnitine palmitoyltransferase I gene transcription by peroxisome proliferator-activated receptor. *J. Biol. Chem.* **273**:8560–8563.
27. McInerney, E. M., D. W. Rose, S. E. Flynn, S. Westin, T.-M. Mullen, A. Kronen, J. Inostroza, J. Torchia, R. T. Nolte, N. Assa-Munt, M. V. Milburn, C. K. Glass, and M. G. Rosenfeld. 1998. Determinants of coactivator LXXLL motif specificity in nuclear receptor transcriptional activation. *Genes Dev.* **13**:3357–3368.
28. Nolte, R. T., G. B. Wisely, S. Westin, J. E. Cobb, M. H. Lambert, R. Kurokawa, M. G. Rosenfeld, T. M. Willson, C. K. Glass, and M. V. Milburn. 1998. Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor  $\gamma$ . *Nature* **395**:137–143.
29. Ory, D. S., B. A. Neugeboren, and R. C. Mulligan. 1996. A stable human-derived packaging cell line for production of high titer retrovirus/vesicular stomatitis virus G pseudotypes. *Proc. Natl. Acad. Sci. USA* **93**:11400–11406.
30. Patturajan, M., X. Wei, R. Berezney, and J. L. Corden. 1998. A nuclear matrix protein interacts with the phosphorylated C-terminal domain of RNA polymerase II. *Mol. Cell. Biol.* **18**:2406–2415.
31. Puigserver, P., Z. Wu, C. W. Park, R. Graves, M. Wright, and B. M. Spiegelman. 1998. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* **92**:829–839.
32. Qi, C., Y. Zhu, J. Pan, A. V. Yeldandi, M. S. Rao, N. Maeda, V. Subbarao, S. Pulikuri, T. Hashimoto, and J. K. Reddy. 1999. Mouse steroid receptor coactivator-1 is not essential for peroxisome proliferator-activated receptor  $\alpha$ -regulated gene expression. *Proc. Natl. Acad. Sci. USA* **96**:1585–1590.
33. Rachez, C., B. D. Lemon, Z. Suldan, V. Bromleigh, M. Gamble, A. M. Naar, H. Erdjument-Bromage, P. Tempst, and L. P. Freedman. 1999. Ligand-dependent transcription activation by nuclear receptors requires the DRIP complex. *Nature* **398**:825–828.
34. Sack, M. N., D. L. Disch, H. A. Rockman, and D. P. Kelly. 1997. A role for Sp and nuclear receptor transcription factors in a cardiac hypertrophic growth program. *Proc. Natl. Acad. Sci. USA* **94**:6438–6443.
35. Spencer, T. E., G. Jenste, M. M. Burcin, C. D. Allis, J. Zhou, C. A. Mizzen, N. J. McKenna, S. A. Onate, S. Y. Tsai, M. J. Tsai, and B. W. O'Malley. 1997. Steroid receptor coactivator-1 is a histone acetyltransferase. *Nature* **389**:194–198.
36. Tontonoz, P., E. Hu, and B. M. Spiegelman. 1994. Stimulation of adipogenesis in fibroblasts by PPAR  $\gamma$ 2, a lipid-activated transcription factor. *Cell* **79**:1147–1156.
37. Torchia, J., D. W. Rose, J. Inostroza, Y. Damei, S. Westin, C. K. Glass, and M. G. Rosenfeld. 1997. The transcriptional co-activator p/CIP binds CBP and mediates nuclear-receptor function. *Nature* **387**:677–684.
38. Wu, Z., P. Puigserver, U. Andersson, C. Zhang, G. Adelmant, V. Mootha, A. Troy, S. Cinti, B. Lowell, R. C. Scarpulla, and B. M. Spiegelman. 1999. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* **98**:115–124.
39. Xu, H. E., M. H. Lambert, V. G. Montana, D. J. Parks, S. G. Blanchard, P. J. Brown, D. D. Sternbach, J. M. Lehmann, G. B. Wisely, T. M. Willson, S. A. Kliewer, and M. V. Milburn. 1999. Molecular recognition of fatty acids by peroxisome proliferator-activated receptors. *Mol. Cell* **3**:397–403.
40. Yao, T. P., G. Ku, N. Zhou, R. Scully, and D. M. Livingston. 1996. The nuclear hormone receptor coactivator SRC-1 is a specific target of p300. *Proc. Natl. Acad. Sci. USA* **93**:10626–10631.
41. Yu, G.-S., Y.-C. Lu, and T. Gulick. 1998. Co-regulation of tissue-specific alternative human carnitine palmitoyltransferase  $\beta$  gene promoters by fatty acid enzyme substrate. *J. Biol. Chem.* **273**:32901–32909.
42. Yuryev, A., M. Patturajan, Y. Litingtung, R. V. Joshi, C. Gentile, M. Gebara, and J. Corden. 1996. The C-terminal domain of the largest subunit of RNA polymerase II interacts with a novel set of serine/arginine-rich proteins. *Proc. Natl. Acad. Sci. USA* **93**:6975–6980.
43. Zhou, Y. T., M. Shimabukuro, M. Y. Wang, Y. Lee, M. Higa, J. L. Milburn, C. B. Newgard, and R. H. Unger. 1998. Role of peroxisome proliferator-activated receptor  $\alpha$  in disease of pancreatic  $\beta$  cells. *Proc. Natl. Acad. Sci. USA* **95**:8898–8903.
44. Zhu, Y., C. Qi, C. Calandra, M. S. Rao, and J. K. Reddy. 1996. Cloning and identification of mouse steroid receptor coactivator-1 (mSRC-1), as a coactivator of peroxisome proliferator-activated receptor  $\gamma$ . *Gene Expr.* **6**:185–195.