Ligand-Dependent Coactivation of the Human Bile Acid Receptor FXR by the Peroxisome Proliferator-Activated Receptor γ Coactivator-1α

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Received June 2, 2004; accepted August 24, 2004

ABSTRACT

Peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) has been shown to play an important role in energy metabolism by coordinating transcriptional programs involved in mitochondrial biogenesis, adaptive thermogenesis, gluconeogenesis, and fatty acid oxidation. PGC-1α also plays a crucial role in cholesterol metabolism by serving as a coactivator of the liver X receptor-α and inducing the expression of cholesterol 7-α-hydroxylase. Here, we demonstrate that PGC-1α also functions as an effective coactivator of farnesoid X receptor (FXR), the bile acid receptor. Transient cotransfection assays demonstrate that PGC-1α enhances ligand-mediated FXR transcription when either full-length FXR or Gal4 DNA binding domain-FXR-ligand binding domain chimeras were analyzed. Mammalian two-hybrid analyses, glutathione S-transferase affinity chromatography and biochemical coactivator recruitment assays demonstrate ligand-dependent interaction between the two proteins both in vivo and in vitro. PGC-1α-mediated coactivation of FXR was highly ligand-dependent and absolutely required an intact activation function-2 (AF-2) domain of FXR and the LXXLL motif in PGC-1α. The integrity of the charge clamp was required, further illustrating the role of the ligand binding domain of FXR in PGC-1α recognition. Together, these results indicate that PGC-1α functions as a potent coactivator for FXR and further implicates its role in the regulation of genes that are involved in bile acid and lipid metabolism.

The enterohepatic conversion of cholesterol to bile acids is a highly regulated process. The rate-limiting step in this process is the conversion of cholesterol into 7-α-hydroxycholesterol, which is catalyzed by the enzyme cholesterol 7-α-hydroxylase (CYP7A1) (Russell, 2003). In rodents, the expression of the gene encoding CYP7A1 is activated by oxysterols and inhibited by bile acids. Positive regulation of CYP7A1 is mediated by the nuclear receptor (NR) liver X receptor (LXRα; NR1H3), which binds to and is activated by oxysterols (Janowski et al., 1996; Lehmann et al., 1997; Peet et al., 1998). LXRα is predominantly expressed in the liver, adipose tissue, and macrophages and is involved in the transcriptional control of a number of genes involved in cholesterol and fatty acid metabolism, including CYP7A1 (Lehmann et al., 1997; Peet et al., 1998), the sterol response element binding protein-1c (SREBP-1c) (Repa et al., 2000a; Schultz et al., 2000), a number of ATP-binding cassette transporters (ABCA1, ABCG5, and ABCG8) (Repa et al., 2000b, 2002), and the phospholipid transfer protein (PLTP) (Cao et al., 2002; Mak et al., 2002).

Conversely, bile acids exhibit their inhibitory effect on CYP7A1 expression by activating another NR, the farnesoid X receptor (FXR; NR1H4) (Bramlett et al., 2000; Goodwin et al., 2000). Several studies have demonstrated that FXR is the sensor of physiological levels of enterohepatic bile acids (Makishima et al., 1999; Parks et al., 1999) and a major regulator of bile acid homeostasis. The role of FXR as a bile acid receptor is supported by its selective pattern of expression in the tissues that are exposed to high concentrations of bile acids: the liver, kidney, and ileum (Forman et al., 1995;
Seol et al., 1995). Recent in vivo studies also suggest that FXR ligands could be potentially useful in the treatment of cholestatic liver disease (Goodwin et al., 2000) and dyslipidemia (Urizar et al., 2002).

Like other members of the NR superfamily, FXR is comprised of a conserved functional domain structure (Burris, 2001). The central region contains the highly conserved DNA-binding domain (DBD) harboring two zinc fingers, whereas the C-terminal ligand-binding domain (LBD) contains a ligand-dependent activation function-2 (AF-2) and a receptor dimerization interface (Burris, 2001). Binding of ligands to the receptor induces a conformational change within the LBD, resulting in the release of existing corepressor proteins and permitting the association of coactivator proteins that mediate a series of events leading to transcriptional activation of the target genes. FXR functions as a heterodimer with the retinoid X receptor (RXR) and augments transcription of its target genes upon binding to an FXR response element located in the promoters of its target genes (Grober et al., 1999). The role of FXR in bile acid, cholesterol, and lipid homeostasis is mediated by transcriptional regulation of a diverse subset of target genes, including the intestinal bile acid-binding protein (I-BABP) (Makishima et al., 1999; Parks et al., 1999), PLTP (Urizar et al., 2000), small heterodimer partner (SHP) (Goodwin et al., 2000; Lu et al., 2000), bile salt export pump (BSEP) (Ananthaarayan et al., 2001; Yu et al., 2002), apolipoprotein C-II (Kast et al., 2001), and apolipoprotein E (Mak et al., 2002).

Recently, it has been demonstrated that PGC-1α plays a crucial role in cholesterol metabolism and bile acid biosynthesis, where it serves as a coactivator of LXRα (Oberkofler et al., 2003) and activates CYP7A1 expression (Shin et al., 2003). The PLTP-tk-Luc reporter plasmid was generated by inserting two copies of an IR1 response element derived from the human PLTP gene (Urizar et al., 2000) upstream of the minimal thymidine kinase (TK) promoter linked to a luciferase gene (pTA-Luc; BD Biosciences Clontech). The pG5-Luc reporter plasmid, containing five copies of the DNA-binding site of the yeast Gal4 transcription factor cloned upstream of the minimal TK promoter linked to a luciferase gene, was purchased from Promega (Madison, WI). The sequence of all plasmids was verified by DNA sequencing.

**Cell Culture and Transient Cotransfection Assays.** HEK293 cells were maintained in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin at 37°C under 5% CO2. Before transfection, cells were plated into 96-well plates at a density of 1 × 10^4 cells/well. Each transfection contained 25 ng of the appropriate luciferase reporter plasmid and various combinations of other expression plasmids as described in the figure legend using FuGENE 6 (Roche Molecular Biochemicals, Indianapolis, IN). Sixteen hours post-transfection, fresh media containing either 0.1% DMSO or chenodeoxycholic acid (CDCA) (50 μM in 0.1% DMSO) was added as indicated. Twenty hours post-treatment, the luciferase activity was measured using the standard luciferase substrate reagents (BD Biosciences PharMingen, San Diego, CA). CDCA was purchased from Sigma-Aldrich (St. Louis, MO). Expression of mutant receptors and coactivators (full-length and chimeric) were compared with wild type by Western blots and showed no significant variation in expression (data not shown). Hormone binding capacity of mutant receptors [FXRΔAF-2, FXR(E^17↓→A), and FXR(K307^A→A)] was assessed by limited protease digestion and determined to be within ~35% of wild-type FXR (data not shown).

**Adenovirus Infections and Gene Expression.** Full-length murine PGC1α and green fluorescent protein (GFP) adenovirus constructs were generated as described previously (Lehman et al., 2000). HuH7 human hepatoma cells were plated into 96-well plates at a density of 2 × 10^5 cells/well. Cells were infected at an approximate multiplicity of infection of 10 to achieve 100% infection. Sixteen hours post-infection, virus-containing media were removed, and cells were stimulated with various concentrations of CDCA, as indicated, for 24 h. RNA and cDNA were prepared using ABI 6100 reagents and methodologies and ABI high-capacity cDNA synthesis kit (Applied Biosystems, Foster City, CA). BSEP mRNA was quantitated using standard quantitative-PCR techniques as described previously (Bramlett et al., 2003).

**GST Affinity Chromatography Analysis.** In vitro protein-protein interactions were assessed by performing GST affinity chromatography assays as described previously (Wu et al., 2003) using in vitro transcribed/translated [35S]-labeled FXR or FXRΔAF-2, in the absence or presence of ligand. Beads were extensively washed with the binding buffer, and the bound proteins were analyzed by SDS-polyacrylamide gel electrophoresis and visualized by autoradiography.

**Immunoprecipitation Assay.** In vivo interaction of PGC-1α and FXR was assessed using an immunoprecipitation assay as described previously for PGC-1α and RXR (Delerive et al., 2002) with the
following modifications. HEK293 cells were transfected with amino-terminal myc-his-tagged PGC-1α and full-length FXR. Controls were performed that lacked cotransfection with myc-his-tagged PGC-1α. After 24 h of treatment in the presence or absence of ligand (50 μM CDCA or 0.1% DMSO), cells were lysed followed by immunoprecipitation with a monoclonal myc antibody (9E10; Santa Cruz Biotechnology, Inc., Santa Cruz, CA). The samples were then subjected to Western analysis using a polyclonal FXR antibody (H130; Santa Cruz Biotechnology, Inc.).

**Coactivator Recruitment Assay.** Biochemical interaction between FXR-LBD and the coactivator PGC-1α was assayed using AlphaScreen (amplified luminescent proximity homogenous assay) technology (PerkinElmer Life and Analytical Sciences, Boston, MA). The assay was performed in white, low-volume, 384-well plates using a final volume of 15 μl containing final concentrations of 20 nM *Escherichia coli*-expressed polyhistidine-tagged FXR-LBD protein (encompassing amino acids 226–476 of FXR), 5 nM GST-PGC-1α fusion protein (encoding amino acids 100–411 of PGC-1α), and 10 μg/ml of both Ni⁶⁺-chelate donor beads and anti-GST acceptor beads (PerkinElmer Life and Analytical Sciences). The assay buffer contained 25 mM HEPES (pH 7.0), 100 mM NaCl, 0.1% bovine serum albumin, and 2 mM dithiothreitol. CDCA dissolved in DMSO was added as indicated. The concentration of DMSO in each well was maintained at a final concentration of 1%. All manipulations involving assay beads were done in ambient light. Assay plates were covered with a clear seal and incubated in the dark for 2 h after which the plates were read for 1 s/well in a PerkinElmer Fusion microplate analyzer using the manufacturer’s standard AlphaScreen detection protocol. Dose-response curves were analyzed in GraphPad Prism (GraphPad Software Inc., San Diego, CA) permitting the calculation of EC₅₀.

**Results**

**PGC-1α Activates FXR-Mediated Transcription in a Ligand-Dependent Manner.** We analyzed the relative effectiveness of several classes of coactivators in terms of their ability to augment the transcriptional activity the LBD of FXR using a Gal4 DBD-FXR LBD chimera. As shown in Fig. 1, neither pCAF nor SRC-1 displayed much coactivator activity in the context of this chimeric receptor; however, both p300 and PGC-1α exhibited particularly strong activity. Particularly apparent was the activity of PGC-1α relative to the other coactivators examined, leading us to investigate the nature of this potential interaction. To confirm this observation, a transient transfection assay was performed with plasmids expressing full-length FXR and a luciferase reporter gene driven by the minimal TK promoter preceded by two repeats of an IR1 FXR response element derived from the human *PLTP* gene (Urizar et al., 2000). Luciferase activity was measured in the absence or presence of the FXR ligand CDCA (Fig. 2A). Cotransfection of a plasmid expressing PGC-1α in the absence of ligand resulted in a 3- to 4-fold activation of the reporter gene at the highest concentration of the transfected plasmid (50 ng). Addition of CDCA resulted in an approximate 5-fold activation of the reporter gene in the absence of PGC-1α. Increasing amounts of the PGC-1α expression plasmid resulted in ~60-fold activation of the reporter in the presence of CDCA. Thus, although subtle effects of PGC-1α-mediated coactivation are observed in the absence of ligand, efficacious coactivation of FXR by PGC-1α is highly dependent on the presence of the ligand.

Since the interaction between PGC-1α and other NRs has been mapped to the LBD of the NRs in many cases, we again used the chimeric Gal4 DBD-FXR LBD. As in Fig. 1, this Gal4-DBD/FXR-LBD fusion protein (Gal4-FXR-LBD) was co-expressed with a cognate Gal4-responsive reporter gene in the absence or presence ligand. Overexpression of PGC-1α had no effect on the basal activity of the reporter gene, even at the highest concentration of the expressed PGC-1α. However, in the absence of transfected PGC-1α, activation of the reporter gene was increased 10-fold in the presence of CDCA. Maximal overexpression of PGC-1α resulted in a further increase in reporter expression to 400-fold in the presence of ligand (Fig. 2B). Thus, the dose-responsive induction of the chimeric Gal4-FXR-LBD-mediated transcription by PGC-1α confirms that PGC-1α serves as a coactivator for FXR-mediated transcription in a ligand-dependent manner. Furthermore, our results also demonstrate that the LBD of FXR (encompassing amino acids 226–476) is sufficient for the coactivation to occur.

To determine whether increasing PGC-1α levels would affect the expression of a FXR target gene in a cell line expressing endogenous FXR, an adenoviral vector directing the expression of either PGC-1α or GFP was used to infect HuhH7 cells. After infection, cells were treated with increasing doses of CDCA for 16 h followed by assessment of expression of BSEP mRNA. As shown in Fig. 2C, CDCA treatment results in a dose-dependent increase in BSEP mRNA expression ranging from 8-fold (1 μM CDCA) to 31-fold (50 μM CDCA) in control cells (GFP-adenovirus-infected cells). In cells where PGC-1α was overexpressed, the levels of BSEP mRNA were enhanced at every dose of CDCA examined (2–4-fold), consistent with our observation that PGC-1α functions as a coactivator of FXR. PGC-1α overexpression alone, without ligand, did not result in enhancement of BSEP mRNA expression confirming the ligand dependence of the coactivation.
The AF-2 Domain of FXR and the LXXLL Motif of PGC-1α Are Essential for Coactivation of FXR by PGC-1α. Previous studies demonstrated that the C-terminal LBD of FXR that contains the AF-2 is required for its response to ligands (Zavacki et al., 1997). To determine whether the AF-2 domain was necessary for the coactivation of FXR by PGC-1α, cotransfection assays were performed using either a full-length FXR expression plasmid lacking the AF-2 or a Gal4-FXR-LBD plasmid lacking the AF-2 in combination with their cognate luciferase reporter plasmid. Wild-type FXR-LBD-mediated transcriptional activity was increased by CDCA, and activation was further enhanced by overexpression of PGC-1α (Fig. 3A). However, deletion of helix 12 of FXR abolished transcriptional activity in response to ligand and to overexpression of PGC-1α. These results were reiterated when Gal4-FXR-LBD and the corresponding helix 12 deletion constructs were assessed with ligand and overexpression of PGC-1α (Fig. 3B). Hence, an intact AF-2 domain is necessary for FXR-mediated transcription in response either to ligand or to PGC-1α coactivation.

Binding of a ligand to the LBD of NRs induces a conformational change that permits the recognition of specific motifs contained within coactivator proteins. This motif, commonly referred to as the NR box or LXXLL motif, is necessary and sufficient to permit most NR-coactivator interactions. Previous studies have demonstrated that an intact LXXLL motif in PGC-1α is required for its coactivation of several NRs (Tcherepanova et al., 2000; Vega et al., 2000; Knutti et al., 2003; Schreiber et al., 2003). To ascertain whether the NR box of PGC-1α mediates a ligand-dependent interaction with FXR, cotransfection assays were performed with the wild type, full-length FXR and plasmids expressing either wild-type PGC-1α or LXXLL→AXXAA mutated PGC-1α. Co-expression of wild-type PGC-1α resulted in enhancement of transcriptional activity in the presence of CDCA (Fig. 4A). However, mutation of the central LXXLL (L2) motif of PGC-1α resulted in the inability of the protein to coactivate FXR as indicated by the levels of activity reverting to the levels comparable with FXR alone. To determine whether the LXXLL motif of PGC-1α mediated ligand-dependent interaction with the Gal4-FXR-LBD protein, cotransfection assays were performed as described above. Indeed, the PGC-1α,AXXAA mutant was also unable to coactivate the chimeric FXR (Fig. 4B). The observation that the L2 LXXLL domain of FXR and PGC-1α is the critical determinant in receptor recognition is consistent with our previous studies with TRβ, RXR, and PPARγ (Delerive et al., 2002; Wu et al., 2002, 2003). Thus, the AF-2 region of FXR and the NR box of PGC-1α were required for coactivation of FXR by PGC-1α.

FXR and PGC-1α Directly Interact Both in Vivo and in Vitro. To determine whether the coactivation of FXR by PGC-1α is a result of a direct interaction between the two proteins in vivo, a mammalian two-hybrid assay was performed using plasmids expressing a Gal4-DDB-full-length FXR chimera (Gal4-FXR) and a VP16-PGC-1α chimera (spanning amino acids 100–411 of PGC-1α) along with the luciferase reporter plasmid (Fig. 5A). Wild-type Gal4-FXR-mediated transcriptional activity was increased upon addition of CDCA. This activation was further enhanced by expression of VP16-PGC-1α, indicating that the region of PGC-1α encompassing amino acids 100 to 411, spanning the...
LXXLL motif, was sufficient to mediate the ligand-dependent interaction with FXR. However, deletion of helix 12 of FXR abolished the interaction with PGC-1α, demonstrating that the AF-2 domain of FXR was required for the interaction. To examine whether this interaction also occurs in vitro, a GST affinity chromatography assay was performed using in vitro transcribed/translated 35S-labeled full-length FXR and a fragment of PGC-1α containing the LXXLL motif (amino acids 100–411) expressed as a GST fusion protein immobilized on glutathione-Sepharose 4B beads. The immobilized GST-PGC-1α interacts strongly with the in vitro-translated FXR in a ligand-dependent manner (Fig. 5B). Consistent with our previous observations (Fig. 4), mutation of the leucines in the LXXLL motif of PGC-1α to alanines (AXXAA) resulted in a substantial reduction in this interaction. Furthermore, deletion of the AF-2 domain of FXR abolished the interaction with PGC-1α, confirming that the AF-2 domain of FXR and the NR box of PGC-1α are required for the interaction. The interaction between PGC-1α and FXR was also assessed by coimmunoprecipitation. Amino-terminal myc-
immunoprecipitated with a myc antibody, and the presence of FXR in the immunoprecipitate was revealed by Western blotting with a polyclonal FXR antibody. The results shown in Fig. 5C indicate that FXR and PGC-1α interact in vivo; however, in contrast to other results, the interaction is ligand-independent. It is unclear why the results of the immunoprecipitation assay conflict with the ligand dependence of the interaction detected with the two-hybrid assay, GST-pull down assay, and the AlphaScreen assay (described below). Nonetheless, the functional assays using full-length and Gal4-FXR LBD cotransfections as well as overexpression of PGC-1α in Huh7 cells expressing endogenous FXR suggest the coactivation is ligand-dependent.

To confirm the in vitro interaction between the two proteins, a biochemical coactivator recruitment assay using the AlphaScreen technology was performed using a GST-PGC-1α fusion protein and the LBD of FXR that was expressed as a polyhistidine-tagged fusion protein in E. coli. We have used this methodology previously to examine the coactivator binding activity of LXRα, LXRβ, vitamin D receptor, and RXRα (Bettoun et al., 2003; Bramlett et al., 2003). As illustrated in Fig. 5D, CDCA induced interaction between the FXR LBD and PGC-1α. The half-maximal effective concentration (EC_{50}) of 16 ± 11 μM for CDCA is consistent with the activity observed in cell-based assays (Parks et al., 1999; Bramlett et al., 2000). Results from the coactivator recruitment assay and the cotransfection experiments using the Gal4-FXR-LBD chimera also demonstrate that the LBD of FXR is sufficient for PGC-1α-mediated coactivation of FXR both in vitro and in vivo. Thus, coactivation of FXR by PGC-1α is mediated via direct interaction that requires an intact AF-2 domain of FXR and the LXXLL motif in PGC-1α.

**Requirement of the Charge Clamp Residues of FXR for PGC-1α-Mediated Coactivation.** Recognition of the LXXLL motif by NRs is mediated by a “charge clamp” that is comprised of two highly conserved residues in the NR LBD. A lysine residue (present in helix 3) and a glutamic acid residue (in helix 12) have been shown to clamp the NR box. The basic portion of the charge clamp, lysine 307 within helix 3 (FXR(E471K)) virtually eliminated the basal transcriptional activity of full-length FXR as well as ligand-activated activity. PGC-1α was ineffective in coactivation of this mutant (Fig. 6A). A similar pattern was observed in the context of the Gal4 DBD-FXR LBD chimera (Fig. 6B). Mutation of the basic portion of the charge clamp, lysine 307 within helix 3 [FXR(K307−A)] also resulted in near elimination of the transcriptional activity of either the full-length or chimeric receptor (Fig. 6). As observed with mutation of the glutamic acid component of the charge clamp, mutation of the lysine component also resulted in the inability of PGC-1α to coactivate the receptor. Thus, the charge clamp residues are required for PGC-1α-mediated coactivation of FXR, which is
consistent with our observations that the interaction is a ligand-dependent interaction requiring helix 12 of FXR and the NR box of PGC-1α.

**Discussion**

PGC-1α has been shown to play an important role in energy metabolism by coordinating transcriptional programs involved in mitochondrial biogenesis, adaptive thermogenesis, gluconeogenesis, and fatty acid oxidation. Recent studies suggesting additional roles for PGC-1α in cholesterol and bile acid metabolism via coactivation of LXRα and regulation of CYP7A1 (Oberkofler et al., 2003; Shin et al., 2003) led us to investigate the potential coregulatory role of this coactivator on the activity of the bile acid receptor FXR. In this study, we demonstrate that PGC-1α functions as an efficient ligand-dependent coactivator of FXR. Our results indicate that PGC-1α plays an additional role in cholesterol and bile acid metabolism by directly modulating the activity of the bile acid receptor. Thus, the array of FXR target genes that regulate bile acid and lipid metabolism are likely under the influence of PGC-1α, whose expression is regulated in an environmental- and metabolic state-dependent manner.

The interaction of PGC-1α with PPARγ is primarily a ligand-independent interaction (Puigserver et al., 1998); however, in certain circumstances a significant ligand-dependent component can be dissected (Wu et al., 2003). Other receptors such as estrogen-related receptor α also display a strong ligand-independent mode of interaction with PGC-1α (Schreiber et al., 2003), but the majority of receptors that have now been examined seem to display a significant ligand-dependent component. For example, TRα, TRβ, estrogen-related receptor α, RXR, and PPARα all exhibit ligand-dependent interaction with PGC-1α with some of these receptors still retaining a limited element of context-dependent ligand independency (Tcherepanova et al., 2000; Vega et al., 2000; Delerive et al., 2002; Wu et al., 2002). Our results

**Fig. 5.** FXR and PGC-1α directly interact both in vivo and in vitro. A, cotransfection and measurement of luciferase activity was performed as described in Fig. 2 but with 25 ng of plasmids expressing either the Gal4-FXR or Gal4-FXRΔAF2 mutant, 50 ng of a VP16-PGC-1α (amino acids 100–411) expressing plasmid, and 25 ng of the pG5-Luc reporter plasmid in the absence or presence of ligand (CDCA; 50 μM). All experiments were completed in triplicate, and the data are displayed as the mean ± S.D. of a single experiment, representative of at least three independent experiments. B, E. coli expressed GST, GST-PGC-1α (amino acids 100–411), or its mutant GST-PGC-1α AXAA immobilized on glutathione-Sepharose 4B beads, was incubated with in vitro translated 35S-labeled full-length FXR or the FXRΔAF2 mutant for 1 h at room temperature. After washing extensively, the proteins bound on the beads were analyzed by SDS-polyacrylamide gel electrophoresis and visualized by autoradiography. C, HEK293 cells were transfected with amino-terminal myc-his-tagged PGC-1α and full-length FXR. A control was performed that lacked cotransfection with the myc-his tagged PGC-1α. After 24 h of treatment in the presence or absence of ligand (50 μM CDCA or 0.1% DMSO), cells were lysed followed by immunoprecipitation with a monoclonal myc antibody. The samples were then subjected to Western analysis using a polyclonal FXR antibody. D, E. coli expressed GST-PGC-1α (amino acids 100–411) was incubated with E. coli expressed polyhistidine-tagged FXR-LBD (amino acids 226–476) protein in 384-well plates using a final volume of 15 μl in the presence of increasing concentration of CDCA. Assay plates were covered with a clear seal and incubated in the dark for 2 h, after which the plates were read for 1 s/well in a PerkinElmer Fusion microplate analyzer using the manufacturer’s standard AlphaScreen detection protocol. All experiments were completed in triplicate, and the data are displayed as the mean ± S.E. of a single experiment, representative of at least three independent experiments.
demonstrate that PGC-1α functions primarily as a ligand-dependent coactivator of FXR. PGC-1α coactivation of FXR activity in either a full-length or Gal4-FXR-LBD chimeric protein context is dependent on the presence of the ligand. Both systems demonstrate that coactivation absolutely requires the AF-2 domain and the charge clamp residues of FXR as well as the NR box of PGC-1α, and occurs via a direct interaction between the two proteins. It is interesting to note the requirement of the E471 charge clamp residue of FXR for PGC-1α-mediated coactivation given our initial observations with TR and PPARγ that the class III NR box of PGC-1α does not require this component of the charge clamp (Wu et al., 2002, 2003). However, we have observed, at least in the case of PPARγ, that the identity of the ligand determines whether the glutamic acid component of the charge clamp is required for recognition of PGC-1α (Wu et al., 2003). It is possible that CDCA-ligated FXR requires the E471 residue, whereas another FXR ligand may induce a conformation that recognizes PGC-1α independent of this residue. Thus, our data are consistent with the observations that binding of a ligand to the LBD of the receptor stabilizes the AF-2 domain in an active conformation that permits the LBD to recruit coactivator proteins and activate target genes.

Most recently, Zhang et al. (2004) published a study describing a role for PGC-1α in regulation triglyceride metabolism by regulating FXR expression as well as coactivating this receptor. This study clearly illustrates the in vivo significance of the interaction between PGC-1α and FXR. However, these investigators describe an unusual mode of interaction responsible for coactivation. In contrast to our results, the LBD of murine FXR is not involved in the interaction with PGC-1α; rather, the interaction occurs via the DNA binding domain of FXR and does not require the LXXLL motif of PGC-1α, leading to a complete ligand-independent mode of interaction. However, our results clearly demonstrate that the LBD of FXR is necessary and sufficient for PGC-1α interaction and that coactivation is strictly ligand-dependent. We demonstrate this with a variety of assays, including cotransfection assays using either the full-length FXR or the Gal4-FXR-LBD chimera, the two-hybrid system, GST-pull down assays, and a direct interaction between recombinant FXR-LBD and PGC-1α in an AlphaScreen format. Consistent with our observations that the coactivation is ligand-dependent, the interaction and coactivation require an intact FXR AF-2 domain and the NR box of PGC-1α. In addition, mutation of either amino acid component of the charge clamp of FXR responsible for coactivator NR box recognition results in the inability of PGC-1α to coactivate FXR transcriptional activity. It is unusual that such discrepancies are observed in the mode of interaction between FXR and PGC-1α, but these differences may be due to the fact that Zhang et al. (2004) used the mouse receptor, whereas we studied the human receptor.

These investigators show a clear role for FXR in mediation of fasting-induced effects on triglyceride metabolism. FXR null mice were unresponsive in terms of fasting-induced decreases in triglycerides, and ectopic expression of PGC-1α resulted in decreased triglyceride synthesis from hepatocytes derived from wild-type mice but not from hepatocytes derived from FXR null mice (Zhang et al., 2004). However, as described above, in this model the pathway would not be expected to be regulated by bile acid ligands. In contrast, our data describes a more classical mode of coactivation of FXR by PGC-1α that displays an absolute requirement for bile acid ligands. Thus, PGC-1α modulation of FXR-dependent regulation of triglyceride metabolism is likely sensitive to bile acid levels, providing a link between bile acid signaling and PGC-1α-dependent effects on triglyceride metabolism.
In summary, these results indicate that PGC-1α functions as a potent ligand-dependent coactivator for FXR and further defines its role in the regulation of genes that are involved in bile acid and lipid metabolism. Furthermore, these observations provide evidence for linkage between bile acid signaling and the numerous metabolic pathways regulated by PGC-1α.

References


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