Estrogen-related Receptor α Is a Repressor of Phosphoenolpyruvate Carboxykinase Gene Transcription

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The orphan nuclear receptor estrogen-related receptor (ERR) α is a downstream effector of the transcriptional coactivator PGC-1α in the regulation of genes important for mitochondrial oxidative capacity. PGC-1α is also a potent activator of the transcriptional program required for hepatic gluconeogenesis, and in particular of the key gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK). We report here that the regulatory sequences of the PEPCK gene harbor a functional ERRα binding site. However, in contrast to the co-stimulating effects of ERRα and PGC-1α on mitochondrial gene expression, ERRα acts as a transcriptional repressor of the PEPCK gene. Suppression of ERRα expression by small interfering RNA leads to reduced binding of ERRα to the endogenous PEPCK gene, and an increase in promoter occupancy by PGC-1α, suggesting that part of the ERRα function at this gene is to antagonize the action of PGC-1α. In agreement with the in vitro studies, animals that lack ERRα show increased expression of gluconeogenic genes, including PEPCK and glycolytic kinase, but decreased expression of mitochondrial genes, such as ATP synthase subunit β and cytochrome c-1. Our findings suggest that ERRα has opposing effects on genes important for mitochondrial oxidative capacity and gluconeogenesis. The different functions of ERRα in the regulation of these pathways suggest that enhancing ERRα activity could have beneficial effects on glucose metabolism in diabetic subjects by two distinct mechanisms: increasing mitochondrial oxidative capacity in peripheral tissues and liver, and suppressing hepatic glucose production.

Nuclear receptors mediate the effects of many hormonal and dietary signals. These receptors bind to specific genomic sequences, recruit coactivators or corepressors of transcription, and regulate accordingly the expression of genes important for a wide range of biological processes, including development, reproduction, and metabolism (for reviews, see Refs. 1 and 2 and references therein). The estrogen-related receptor (ERR)α is a nuclear receptor with high sequence similarity to the estrogen receptors, and the founding member of a small family of orphan receptors that also includes ERRβ and ERRγ (3, 4). Despite their similarity to estrogen receptors, ERRs are not activated by estrogens or other known natural agonists (reviewed in Refs. 5 and 6). Structural studies of ERRα and ERRγ indicate that these receptors can achieve a transcriptionally active conformation in the absence of a ligand, and suggest that ERR activity may not be subject to regulation by small lipophilic molecules (7, 8). As an alternative mechanism of regulation, ERRα activity is controlled by the availability of specific coactivators that act as protein ligands (9–11). Notably, the transactivation function of ERRα is weak in many cells where other nuclear receptors are active, and is greatly enhanced by expression of the transcriptional coactivators PGC-1α or PGC-1β (9–11). PGC-1α not only activates the transcriptional function of ERRα but also induces the expression of this receptor (9). Consistent with the ability of PGC-1α to induce ERRα activity and expression, PGC-1α and ERRα show similar spatial and temporal expression patterns in vivo. They are co-expressed at high levels in tissues with high energy demands, and are co-induced in a tissue-specific manner in response to signals like fasting, exposure to cold, and physical exercise (9, 12, 13).

Several recent studies support a role for ERRα in the regulation of mitochondrial oxidative capacity. Binding sites for ERRα (ERRα response elements) are present in genes with roles in fatty acid oxidation (PPARA and ACOX1), mitochondrial biogenesis (NRF2 and NRF1), the citric acid cycle (IDH3A), the respiratory chain (CYCS and ATP5B), and mitochondrial dynamics and function (MFN2) (13–18). At these targets, ERRα co-operates with PGC-1α to induce gene expression (16–18). Consistent with the ERRα functions observed in cell culture systems, ERRα null mice show defects in lipid metabolism and decreased expression of genes coding for fatty acid oxidation enzymes and oxidative phosphorylation components (18, 19).

The transcriptional coactivator PGC-1α coordinates, in a signal- and tissue-specific manner, the induction of genes important for multiple adaptive metabolic responses (reviewed in Ref. 20). PGC-1α functions in adaptive thermogenesis in brown adipose tissue (21–23), and fiber-type specification in skeletal muscle (24), two processes that involve the regulation of mitochondrial biogenesis and function. PGC-1α also regulates gluconeogenesis in liver (25–27). The induction of hepatic gluconeogenesis during periods of fasting is an essential adaptive response that requires the enhanced transcription of genes encoding rate-determining gluconeogenic enzymes, such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (28–31). Transcriptional regulation of PEPCK is achieved by the transcriptional repressor ERRα, and the suppression of ERRα expression by small interfering RNA leads to reduced binding of ERRα to the endogenous PEPCK gene, and an increase in promoter occupancy by PGC-1α, suggesting that part of the ERRα function at this gene is to antagonize the action of PGC-1α. In agreement with the in vitro studies, animals that lack ERRα show increased expression of gluconeogenic genes, including PEPCK and glycolytic kinase, but decreased expression of mitochondrial genes, such as ATP synthase subunit β and cytochrome c-1. Our findings suggest that ERRα has opposing effects on genes important for mitochondrial oxidative capacity and gluconeogenesis. The different functions of ERRα in the regulation of these pathways suggest that enhancing ERRα activity could have beneficial effects on glucose metabolism in diabetic subjects by two distinct mechanisms: increasing mitochondrial oxidative capacity in peripheral tissues and liver, and suppressing hepatic glucose production.

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2 The abbreviations used are: ERR, estrogen-related receptor; PEPCK, phosphoenolpyruvate carboxykinase; ATP5B, ATP synthase subunit β; CYCS, cytochrome c somatic; PGC-1, peroxisome-proliferator activator receptor γ coactivator-1; siRNA, small interfering RNA; GFP, green fluorescent protein; gaF3, glucocorticoid accessory factor 3; ERR, ERR response element; COUP-TF, chicken ovalbumin upstream promoter transcription factor; HNF-4, hepatic nuclear factor 4.
tion of the **PEPCK** gene is tightly regulated by hormones, including glucagon, insulin, and glucocorticoids, via complex mechanisms that involve several transcription factors, such as HNF-4α, COUP-TFI, forkhead factors, the glucocorticoid receptor, C/EBPs, cAMP-response element-binding protein, and AP-1 (32–35). PGC-1α, whose hepatic expression is increased upon fasting, contributes to the induction of the gluconeogenic genes **PEPCK** and glucose-6-phosphatase, via its interactions with HNF-4α, glucocorticoid receptor, and the forkhead factor FOXO1 (26, 36–38). Importantly, increased expression of PGC-1α in the liver of fed animals is sufficient to inappropriately induce gluconeogenic enzymes and elevate blood glucose levels, a finding that underscores the possible involvement of this coactivator in the elevations of hepatic glucose production in the diabetic state (26).

Whereas a positive role of ERRα in PGC-1α-induced expression of mitochondrial genes has been established, the role of ERRα in the regulation of genes involved in hepatic glucose metabolism, including PGC-1α-stimulated gluconeogenesis, is not clear. ERRα is induced in the liver in response to fasting, but has been proposed to either be a repressor of general PGC-1α transcriptional activity (12) or to have no effect on gluconeogenic enzymes (17). Notably, the **PEPCK** gene promoter contains a sequence that resembles an ERRE and that overlaps with a site (glucocorticoid accessory factor 3 (gAF3)) critical for the activation of **PEPCK** transcription by PGC-1α (45). In this study, we address in vitro and in vivo the role of ERRα in the regulation of **PEPCK** gene expression. Our findings demonstrate that ERRα inhibits **PEPCK** gene expression, at least in part by restricting the interaction of PGC-1α with the **PEPCK** promoter. Furthermore, the repressive effect of ERRα is specific for gluconeogenic genes, whereas genes involved in mitochondrial respiratory function are activated by hepatic ERRα. The potential benefits of an ERRα role in reducing hepatic glucose production while increasing oxidative capacity are discussed.

### MATERIALS AND METHODS

**Animals**—Generation of ERRα null mice in the C57BL/6 background has been described elsewhere (19). Mice were housed at 21 °C on a 12-h light-dark cycle and fed ad libitum with a standard diet containing 5% fat (Harland Tekland LM-485, Indianapolis, IN). For gene expression analysis, adult (8–10 weeks) ERRα null females and wild type littermates were fasted for 24 h and then fed for 5 h with standard diet. After the refeeding period, mice were euthanized, and tissues were removed and stored for subsequent analysis. All procedures were performed in accordance with the guidelines for animal care and use of The Scripps Research Institute.

**Plasmids and Adenoviral Constructs**—Luciferase reporter constructs for wild type and mutant (AF3mβ and AF3mγ) **PEPCK** promoter, and expression plasmids for ERRα–VP16 and PGC-1α have been described previously (9, 39). pSG5/mERRα for the expression of full-length mouse ERRα was a gift of J.-M. Vanacker (40). Adenoviral vectors expressing GFP, ERRα, PGC-1α, ERRα–VP16, and small interfering RNA (siRNA) for human ERRα have been described (9, 16). The adenoviral vector expressing siRNA for rat ERRα was generated by CRE-lox-mediated recombination in CREB8 cells (41), and targets the sequence 5′-GAGCATCCAGGCCTTCC-3′ of mouse and rat ERRα.

**Cell Culture, Transfections, Luciferase Assays, and Adenovirus Infections**—H4IIE rat hepatoma cells were maintained as described (42). HepG2 and COS-7 cells were grown in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum. H4IIE cells were transfected with calcium phosphate-precipitated DNA (42). COS-7 and HepG2 cells were transfected using the FuGENE 6 reagent (Roche) according to the manufacturer’s instructions. Luciferase reporter activities were determined ~18 h after transfections using the Dual Luciferase Reporter Assay System (Promega). For adenosivirus infection, cells were infected first with the siERRα adenovirus or a control virus in 10-cm dishes and at a multiplicity of infection of 100, and then (3 days later) with GFP or PGC-1α expressing viruses and a second dose of the siERRα or control viruses (multiplicity of infection 50 each). Cells were harvested 24 or 48 h after the second infection.

**Protein Extractions and Western Blot**—Protein lysates were prepared using either the NE-PER reagent (Pierce) (Fig. 1) or by lysis in Nonidet P-40 buffer (Figs. 2 and 4) as previously described (9), and subjected to Western analysis using antibodies against PGC-1α (9) and ERRα (43).

**Electromobility Shift Assay**—Electromobility shift assays were performed as described previously (32). Nuclear extracts from COS-7 cells (about 1.5 μg/sample) transfected with an ERRα expression vector or H4IIE cells (about 8 μg/sample) were incubated with labeled probe and separated on an 8% polyacrylamide gel, in 0.5× TBE buffer at 25 mA (~180 V). Labeled probe and unlabeled oligonucleotides used for competition studies were as follows (5′ to 3′, the ERRα recognition site is shown bold, mutations are underlined): TCCCGGCAGCCTGTC-CTTGAACCCTACTTGAATTAAGG (PEPCK AF3 probe); GCCG-ATTTGTCAAGGTCACACAGCGC (TRα); GCCAGTTTTGCAAG-GTCACAGGCGC (TRαM4); GATCGCCGACCCCAGCTATTGGA-ACCCCAACTCTAGAACATAAGG (AF3mβ); GATCGGCCAGCCC-TGTCCTAACACCCACCTGACAATTAAGG (AF3mγ). Antibodies against ERRα (14) or COUP-TFI (44) were included in the incubation, as indicated in the figure legend.

**Chromatin Immunoprecipitation Assay**—Chromatin immunoprecipitations were performed with antibodies against the glucocorticoid receptor (Santa Cruz, sc-1004), PGC-1α (Santa Cruz, sc-13067), or ERRα (14) as described (45).

**RNA Isolation, Reverse Transcription, and Quantitative PCR**—Total RNA was isolated using the TRIzol reagent (Invitrogen). RNA (400 ng) was reverse transcribed to cDNA using the SuperScript II RNase H-Reverse Transcriptase system (Invitrogen) and specific transcripts were quantitated by real-time PCR using the Chromo4 (MJ Research), gene-specific primers, and the SYBR GREEN system (Applied Biosystems). Sequences of the primers for human CYCS, ATP5B, isocitrate dehydrogenase subunit a, and glyceraldehyde-3-phosphate dehydrogenase have been published (16). Other primers used in this study are as follows (gene, forward primer/reverse primer, 5′ to 3′): human ERRα, TTCTCATCCTGGTCGTGCTCT/CAAGCTCCACCTAGATTTG; mouse and rat Errα, ATCTGCTGTTGGTTGAAACCTTG/AGAAGCTGCTGG-ATGCTCTTTG; human PEPCK, GAAAAAACCTTGGGCCACAT/TT-GCTTTCAAGGCCAGATCT; mouse Pepck, ATCTTTTGGTGGC-CTAGACCT/GCCAGTGCCGCACTAGTT; mouse and rat ERRα, TCTCTCTCTTCTCT/CAAGCTCCACCTAGATTTG; mouse and rat ERRα, TCTCTCTCTTCTCT/CAAGCTCCACCTAGATTTG; mouse and rat ERRα, TCTCTCTCTTCTCT/CAAGCTCCACCTAGATTTG; mouse and rat ERRα, TCTCTCTCTTCTCT/CAAGCTCCACCTAGATTTG; mouse and rat ERRα, TCTCTCTCTTCTCT/CAAGCTCCACCTAGATTTG; mouse and rat ERRα, TCTCTCTCTTCTCT/CAAGCTCCACCTAGATTTG; mouse and rat ERRα, TCTCTCTCTTCTCT/CAAGCTCCACCTAGATTTG.
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FIGURE 1. ERRα binds to the gAF3 element of the PEPCK regulatory sequences. A, alignment of the consensus ERRE, ERRE-like sequences in the regulatory regions of PEPCK and glycerol kinase (GlyK) genes, and other characterized ERREs (16, 46). The position (5′ end) of the ERRα relative to the transcription start site of the genes is indicated in parentheses. The nucleotides that have been mutated in the ERREs of the PEPCK gAF3 and TRα (in AF3 m/AF3mβ and TRαM4, respectively) are underlined. The PEPCK gene sequences are shown in the reverse-complementary orientation. B, end-labeled PEPCK AF3 oligonucleotides, containing the sequences from nucleotide −345 to −304 of the rat Pepck gene, were incubated with nuclear extracts from COS-7 transfected with the ERRα expression vector (pSG5/mERRα), in the presence or absence of antibodies or of 50-fold molar excess of competitor oligonucleotides, as indicated. The DNA-protein complexes were separated by electrophoresis. The complexes supershifted by the antibodies are indicated by the arrow. D, H4IIE cells were treated for 2 h with vehicle (−) or 100 μM dexamethasone (+), and occupancy of glucocorticoid receptor (GR) and ERRα at the PEPCK regulatory regions was determined by chromatin immunoprecipitation. PEPCK sequences harboring the gAF3/ERR site (nucleotides −486 to −268, relative to the transcription start site) were amplified by PCR.

RESULTS

ERRα Binds to the Regulatory Sequences of the PEPCK Gene—The sequence of the gAF3 binding site in the PEPCK gene promoter shows high degeneracy with known response elements for ERRα (14, 16, 46). This putative ERRE is conserved in the mouse, rat, and human PEPCK regulatory sequences (Fig. 1A). To test whether ERRα binds to this site, we used gel mobility shift assays. Incubation of nuclear extracts from COS-7 cells expressing ERRα with a labeled oligonucleotide containing the gAF3 sequence gave rise to a complex that could be supershifted with an anti-ERRα antibody but not with a nonspecific antibody (Fig. 1B). A 50-fold molar excess of an oligonucleotide containing a known ERRE from the TRα promoter (TRα) or the gAF3 sequence (AF3) effectively competed for the formation of the complex. However, oligonucleotides with mutations in the TRα-ERRE (TRαM4) or the gAF3-sequence (AF3mβ and AF3mγ) failed to compete for the formation of the complex, demonstrating that ERRα binds specifically to the gAF3 site (Fig. 1B). Next, we asked if endogenous ERRα in H4IIE rat hepatoma cells recognizes the gAF3 site. Incubation of nuclear extracts from H4IIE cells with the gAF3 probe led to the formation of multiple complexes (Fig. 1C). As shown previously, an antibody against COUP-TFI, which also binds the gAF3 site, resulted in a supershift (39). An antibody specific for ERRα also caused a supershift (Fig. 1C), whereas a control antibody had no effect, demonstrating that endogenous ERRα binds the gAF3 site of the PEPCK promoter. Finally, we tested whether ERRα binds the endogenous PEPCK promoter in its chromatin context, using chromatin immunoprecipitation assays. H4IIE cells were treated with no hormone or dexamethasone for 2 h. Cross-linked and sheared chromatin fragments were precipitated with either no antibody as control, an antibody against the glucocorticoid receptor or the anti-ERRα serum. The PEPCK promoter sequences containing the gAF3 site could be amplified from the precipitates with the ERRα-specific antibody but not from the control sample (Fig. 1D). In contrast to the binding of the glucocorticoid receptor, which requires the presence of hormone, ERRα binds to the PEPCK promoter independent of glucocorticoid treatment.

ERRα Inhibits the Induction of PEPCK Gene Expression by PGC-1α—ERRα mediates the stimulatory effect of PGC-1α on the expression of several genes involved in mitochondrial function (16, 17). Because PGC-1α also stimulates the expression of PEPCK (25, 26), we tested whether ERRα could mediate the enhancing effect of PGC-1α on the PEPCK gene. We used adenoviral vectors to express ERRα and PGC-1α in HepG2 hepatoma cells. Expression of ERRα alone had no effect on basal PEPCK expression in these cells and PGC-1α-induced PEPCK expression as expected (26), however, coexpression of ERRα with PGC-1α repressed the stimulatory effect of PGC-1α (Fig. 2A). The decreased induction of PEPCK expression could not be accounted for by changes in PGC-1α expression (Fig. 2B). Next, we used a PEPCK luciferase reporter to test if ERRα acts through the promoter sequence present in this construct. Expression of PGC-1α in H4IIE hepatoma cells enhanced the expression of a PEPCK luciferase reporter, as shown previously (26, 45) (Fig. 2C). Increasing the cellular levels of ERRα, by transfecting increasing amounts of an ERRα expression plasmid, repressed the activity of the PEPCK luciferase reporter in a dose-dependent manner (Fig. 2C). These results indicate that ERRα exerts an inhibitory effect on the expression of PEPCK, and show that the regulatory sequence that drives the expression of the PEPCK luciferase reporter is sufficient to mediate both the induction by PGC-1α and the inhibition by ERRα.

A Constitutively Activating Form of ERRα Induces PEPCK Gene Expression—The observed inhibition of PEPCK expression by ERRα could be due to the ability of ERRα to bind the PEPCK promoter in a mode that supports repression of transcription. Alternatively, ERRα could be acting indirectly, e.g. by inducing a repressor of PEPCK expression or of PGC-1α function. We reasoned that, if ERRα acted as a bona fide repressor when bound to the PEPCK promoter, modification of the
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![Graph A](image1.png)

**Figure 2.** ERRα represses PEPCK gene transcription. A, ERRα and/or PGC-1α were overexpressed by means of adenovirus infection in HepG2 cells, as indicated. RNA was isolated 40 h after infection and PEPCK mRNA levels were determined by quantitative RT-PCR. Data are expressed as fold induction over control samples (cells infected with GFP-expressing virus) and represent the mean ± S.D. of two experiments performed in duplicate or triplicate. B, the levels of ERRα and PGC-1α protein in HepG2 cells of panel A were determined by Western blot analysis (+, non-specific bands). C, H4IE cells were transfected with 5 μg of wild type PEPCK luciferase reporter gene construct, 1 μg of PGC-1α expression plasmid, and increasing amounts (62 ng to 2 μg) of ERRα expression plasmid, as indicated. Reporter gene activity was measured and normalized to Renilla luciferase activity. Data are expressed relative to the control sample (reporter without PGC-1α and ERRα) and represent the average of two independent experiments.

A constitutively activating variant of ERRα induces PEPCK gene transcription, acting through the AF3/ERRE site. A, HepG2 cells were infected with adenoviruses expressing GFP or ERRα-VP16, and the mRNA levels of the endogenous PEPCK were determined 40 h later by quantitative RT-PCR. Data (expressed as in Fig. 2) are the mean ± S.D. of three experiments performed in duplicate. B, COS-7 cells were transfected with reporter constructs that contain wild type PEPCK gene promoter sequence (WT) or two different mutations in the ERRE (AF3β and AF3γ), and expression constructs for VP16 alone (white bars) or ERRα-VP16 (black bars). Reporter gene activity was measured 16 h after transfection and normalized to Renilla luciferase activity. Data are expressed as the fold induction compared with control samples (VP16 alone), and represent the average ± S.D. of five independent experiments.

Suppression of Endogenous ERRα Leads to an Enhancement of PEPCK Gene Expression—To test the role of endogenous ERRα on the regulation of the PEPCK gene, we used an adenovirus that expresses a siRNA for ERRα and leads to efficient suppression of endogenous ERRα expression (Fig. 4A). Expression of PGC-1α in HepG2 cells led to the induction of endogenous ERRα, as has been shown in other cell types (9, 17, 47), and the siRNA for ERRα effectively suppressed this induction (16) (Fig. 4, A and B). Suppression of ERRα expression in HepG2 cells had no significant effect on basal PEPCK expression, but led to an increased induction of PEPCK mRNA by PGC-1α (Fig. 4C). The increased induction of PEPCK mRNA was not because of changes in PGC-1α protein levels, which were not affected by the siRNA for ERRα (Fig. 4B), suggesting that ERRα antagonizes PGC-1α action on the PEPCK gene promoter. These findings demonstrate that endogenous ERRα functions as a repressor of PEPCK gene expression. Furthermore, they suggest a possible feedback regulation loop, with PGC-1α inducing ERRα, and ERRα antagonizing the action of PGC-1α.

**Figure 3.** A constitutively activating variant of ERRα induces PEPCK gene transcription, acting through the AF3/ERRE site. A, HepG2 cells were infected with adenoviruses expressing GFP or ERRα-VP16, and the mRNA levels of the endogenous PEPCK were determined 40 h later by quantitative RT-PCR. Data (expressed as in Fig. 2) are the mean ± S.D. of three experiments performed in duplicate. B, COS-7 cells were transfected with reporter constructs that contain wild type PEPCK gene promoter sequence (WT) or two different mutations in the ERRE (AF3β and AF3γ), and expression constructs for VP16 alone (white bars) or ERRα-VP16 (black bars). Reporter gene activity was measured 16 h after transfection and normalized to Renilla luciferase activity. Data are expressed as the fold induction compared with control samples (VP16 alone), and represent the average ± S.D. of five independent experiments.

Protein so that it functions as a constitutive activator of transcription should alter the regulatory effect on PEPCK. To test this, we expressed a chimeric protein consisting of the ERRα receptor and the potent activation domain VP16 (ERRα-VP16) (14). The expression of ERRα-VP16 induced endogenous PEPCK gene expression about 6-fold, compared with control GFP (Fig. 3A). These results support the notion that a transcripational repressor function of wild type ERRα is required for the down-regulation of PEPCK expression. Moreover, ERRα-VP16 activated the PEPCK luciferase reporter gene, suggesting that it acts through the regulatory sequences present in this construct (Fig. 3B). Finally, in this context, we tested the role of the gAF3 site, by introducing two different mutations that prevent ERRα binding to the gAF3 site (Fig. 1B). In contrast to the wild type reporter, ERRα-VP16 did not enhance reporter activity from either one of these mutant reporter constructs. The inability of ERRα-VP16 to act on PEPCK promoter with the mutated AF3 site supports the notion that ERRα regulation of the PEPCK promoter depends on this promoter element.
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FIGURE 4. Inhibition of endogenous ERRα enhances the induction of PEPCk expression by PGC-1α. HepG2 cells were infected with an adenovirus expressing siRNA for ERRα (siERRα) or the control virus Super, and a PGC-1α expressing virus or the control virus expressing GFP, as described under “Materials and Methods.” A, endogenous ERRα mRNA levels were determined by quantitative RT-PCR. B, protein levels of PGC-1α and ERRα in HepG2 cells were determined by Western blot analysis of cell lysates, using antibodies specific to PGC-1α and ERRα. Samples for PGC-1α expressing cells (with and without siRNA for ERRα) are shown in duplicate. C, endogenous PEPCk mRNA levels were determined by quantitative RT-PCR. Data in A and C are expressed as the fold change, relative to control samples (Super, GFP), and represent the mean ± S.D. of two experiments performed in duplicates or triplicates.

results are consistent with a model where the presence of ERRα antagonizes the recruitment of PGC-1α to the PEPCk gene promoter.

Endogenous ERRα Is Required for the Induction of Mitochondrial Gene Expression by PGC-1α in Hepatocytes—ERRα mediates the PGC-1α-induced expression of genes involved in mitochondrial function in SAOS2 and C2C12 cells (16, 17). Next we asked if the repressive effect of ERRα on PEPCk gene expression in hepatocytes reflects a distinct function of ERRα in different cell types (e.g. liver versus muscle) or at different types of genes (e.g. gluconeogenic versus mitochondrial genes). To address this point we determined the effect that suppression of ERRα expression in HepG2 cells had on genes encoding mitochondrial proteins of the citric acid cycle (IDH3A encoding isocitrate dehydrogenase subunit α) or with a role in oxidative phosphorylation (CYCS and ATPSB, encoding cytochrome c, somatic, and the β subunit of ATP synthase). As shown in Fig. 6, the ability of PGC-1α to induce the expression of these 3 genes depends on the expression of endogenous ERRα. Suppression of ERRα levels led to a diminished induction by PGC-1α, despite the fact that PGC-1α was expressed comparably in the presence and absence of ERRα (protein levels for PGC-1α and ERRα shown in Fig. 4). These findings suggest that in the same cell type, ERRα mediates the enhancing effects of PGC-1α on one set of genes, while repressing a distinct set of PGC-1α-regulated genes.

ERRα Has Distinct Functions on the Two Different Sets of PGC-1α-regulated Genes in Vivo—To test the notion that ERRα acts as a downstream effector of PGC-1α on some targets, such as genes that encode mitochondrial proteins, while repressing other targets, such as genes encoding critical enzymes in hepatic gluconeogenesis, we compared the expression of representative genes in the liver of mice lacking ERRα (ERRα knock-out) and their wild type littermates. Consistent with the in vitro experiments, the expression of several genes involved in gluconeogenesis, including PEPCk and glycerol kinase,3 was markedly increased in the liver of fed ERRα knock-out mice (Fig. 7A). The expression of glucose-6-phosphatase, another gluconeogenic gene, was also enhanced in ERRα knock-out animals, although the increase did not reach statistical significance (data not shown). Interestingly, there was no effect of ERRα on the induced levels of these genes in fasted animals, suggesting that the repressive function of ERRα is most apparent when PGC-1α levels are low⁴ (data not shown). In contrast, genes important for oxidative phosphorylation, including ATPSB and cytochrome c-1,

3 A putative EBRE is present in the regulatory sequence of the glycerol kinase gene (Fig. 1A).

4 PGC-1α protein is detectable in hepatic nuclear extracts from fed animals, albeit at lower levels than in ones from fasted mice (48). Consistent with ERRα repression being most apparent when PGC-1α levels are low, the repressive function of ERRα on PEPCk gene transcription in HepG2 cells can be overcome by expressing PGC-1α at 5–8-fold higher levels than the ones shown in Fig. 4 (data not shown).
were expressed at reduced levels in the liver of ERRα knock-out, compared with wild type animals, supporting the positive role of ERRα for their expression (Fig. 7B). These results support a view in which ERRα represses gluconeogenic gene expression parallel to enhancing mitochondrial gene expression in liver. Moreover, we noticed that ERRα null animals also express higher levels of hepatic PGC-1α in the fed state (Fig. 7C). These differences in PGC-1α expression, which have not been seen in the in vitro systems (Fig. 4 and data not shown), suggest that the repressive effects of ERRα on gluconeogenic gene expression in vivo may be exerted at multiple levels, and not only by direct binding to the promoters of PGC-1α target genes like PEPCK.

**DISCUSSION**

Hepatic glucose production is tightly regulated by signals that relay the metabolic state of the organism. Gluconeogenesis is promoted at times of fasting (by glucagon and glucocorticoids), and suppressed during the fed state (by insulin and high levels of glucose). The stimulatory effects of glucagon (mediated by cAMP) and glucocorticoids on the transcription of key genes involved in gluconeogenesis have been studied in great detail (25, 29, 49). In contrast, much less is known about the mechanisms that repress the expression of gluconeogenic genes in the fed state (50, 51). PEPCK, a rate-determining enzyme of gluconeogenesis, is tightly regulated at the level of gene expression by the signals that regulate gluconeogenesis (28). Consequently, PEPCK has served as a valuable model for dissecting the signal transduction pathways and transcription factors that control gluconeogenesis in liver (25–27).

Here we show evidence of a mechanism involving ERRα that suppresses PEPCK gene expression. Our findings suggest that one physiological role of ERRα in liver is to limit the expression of the gluconeogenic program in the fed state, possibly by antagonizing the stimulatory effects of PGC-1α. Several lines of evidence support the repressive function of ERRα on PEPCK expression. First, ERRα binds to the PEPCK gene promoter. Second, overexpression of ERRα represses the induction of PEPCK by PGC-1α, whereas inhibition of ERRα expression results in enhanced PEPCK gene expression. Third, disruption of the ability of ERRα to repress, by combining it with a potent viral transcriptional activation domain, converts ERRα to an activator of PEPCK expression. Finally, animals that lack ERRα show increased PEPCK gene expression in the liver, compared with wild type littermates.

Previous studies have shown that ERRα interacts with an inhibitory domain of PGC-1α and represses the transcriptional activity of PGC-1α in transfection assays (12). ERRα was proposed to affect the subnuclear distribution of PGC-1α, and render PGC-1α less accessible to target promoters in general (12). Our findings in this study are consistent with these earlier observations in that ERRα indeed antagonizes the PGC-1α
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It is possible that hormonal signals (e.g., glucocorticoid receptors, ERRα) represses in a promoter-specific manner, and is not a general inhibitor of PGC-1α activity.

Consistent with gene-specific repression by ERRα, we find that ERRα binds at the previously characterized gAF3 site of the PEPCK promoter. It is not yet clear how the ERRα binding site at the PEPCK gene is distinct from the similar ERRα binding sites at the promoters of ATPSB and CYCS (Fig. 1A). Notably, despite the elevated expression of several rate-determining gluconeogenic genes, ERRα represses in a promoter-specific manner, and is not a general inhibitor of PGC-1α activity.

It is not yet clear how the ERRα binding site at the PEPCK gene promoter is recognized by other nuclear receptors as well (e.g. RAR/RXR, COUP-TFs), and is important for responsiveness to both glucocorticoids and PGC-1α (39, 45). It is possible that ERRα bound at the gAF3 site fails to cooperate properly with adjacent transcription factors, as well as prevents the binding of another factor, perhaps a nuclear receptor, that promotes the formation of a transcriptionally active and as well as prevents the binding of another factor, perhaps a nuclear receptor, that promotes the formation of a transcriptionally active and represses, such as at the PEPCK gene. Because ERRα regulates its own expression in an autoregulatory loop (17, 47), putative agonist ERRα ligands are likely to increase ERRα expression (54), and thus may enhance the native functions of ERRα, including enhancement of mitochondrial genes and suppression of gluconeogenic gene expression. If so, agonists of ERRα may exert beneficial effects in the treatment of diabetes by two mechanisms: (i) increasing mitochondrial function and oxidative capacity (16–18), and (ii) inhibiting hepatic gluconeogenesis and lowering plasma glucose levels.

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